Proceedings of Multidisciplinary International Conference

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PERSPECTIVES AND CHALLANGES

"Contributions to Sustainable Environment"

JOURNAL OF CURRENT STUDIES (JCS)

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EDITORIAL

I am indeed honoured in welcoming you all at Maliankara, a traditional part of Muziris Project, for the International conference on Green Economy Initiatives:- Perspectives and Challenges being organized on 28 &29 March 2023.I would like to express my gratitude to Kerala State Higher Education Council for giving great support for this International conference.

The theme of the conference was chosen to highlight the importance of Environmental thoughts in the changing scenario of research, new developments and experience related to green economy by the great conference. This conference set a platform for effective interactions and exchange of knowledge on recent developments in the area of the conference.

On behalf of the Organizing committee, it is my pleasure to present before you the compendium of the conference 2023. The contributions presented in this special issue consist of Theme and lead papers. Given the time limit for editing, all submissions were just corrected for typographical errors. The coverage of the contributions are very wide, which is one of the distinguishing features of this conference.

I, sincerely hope that the deliberations made in the International Conference would yield meaningful suggestions/recommendations leading to further strengthening of research. In closing, we would like to thank all authors for submitting their work and all members of the organizing committee for their co-operation. My sincere thanks are due to each and every one, without the unstinted support of whom an event of this scale would not have possible.

Dr. Renjith P.G
Chief Editor

ABOUT THE CONFERENCE

A green economy is an economy that aims at reducing environmental risks and ecological scarcities, that aims for sustainable development without degrading the environment. It is closely related with ecological economics, but has a more politically applied focus. The 2011 UNEP Green Economy Report argues that to be green, an economy must not only be efficient, but also fair. Fairness implies recognizing global and country level equity dimensions, particularly in assuring a just transition to an economy that is low-carbon, resource efficient, and socially inclusive. UN promotes a development path that understands natural capital as a critical economic asset and a source of public benefits, especially for poor people whose livelihoods depend on natural resources. The notion of the green economy does not replace sustainable development, but creates a new focus on the economy, capital, infrastructure, employment and positive social and environmental outcomes across Asia Pacific. The conference titled "GREEN ECONOMY INITATIVES: PERSPECTIVES & CHALLENGES" focuses on many key economic sectors because we see these sectors as driving the defining trends of the transition to a green economy, including increasing human well-being and social equity, and reducing environmental risks and ecological scarcities.

Several problems that had been brought on by the abuse of the idea of the green economy were discussed during the conference. The most significant one has to do with the rate of interest, which is used to account for future generations' consumption (welfare) and environmental harms that are occurring now but won't fully affect economic activity until later. These harms include those caused by climate change, biodiversity loss, and deteriorating water systems, to name a few. The conference provided a platform to propagate ideas on green economy and invited researchers, academicians and industry experts to share their experiences on current and emerging trends in the area of green economy to bring about a balance of theoretical and practical implementation.

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01

Study on Municipal Solid Waste Workers of Mekelle city, Ethiopia with reference to health and socio-economic status

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ABSTRACT

Municipal solid waste workers are those employed by the municipalities to collect, transport, process and finally dispose solid waste to the dump site. A survey on the health and socio economic status of workers including waste collectors and drivers of seven subcities in Mekelle city of Ethiopia was held during 2019. All the randomly selected 61workers were males; 88.52% of them were bachelors and 11.48% were with spouse and children; 83.61% were educated at secondary or primary level; majority (80.33%) belonged to the age group of 18-30 years. All the waste workers worked for 12 hours from 6.00 am to 6.00 pm on all days. The waste collectors received a monthly salary of 1000 Ethiopian birr but the drivers received 4500 to 5000 birr. It was noted that 75.41% of waste workers had suffered from allergy symptoms of cough, asthma, eye sickness and skin rashes; most of them (91.80%) suffered from body injuries like cuts and lacerations; 72.13% faced musculoskeletal problems; 68.85% and 50.82% had respiratory and gastrointestinal problems respectively, and 32.79% had nail infection. The waste workers of higher age group were more affected than the lower age group, and the incidence of health problems was higher among waste collectors than the drivers. Waste workers of Qedamy weyane and Hadnet were the worst affected and those of Quiha and Ayder were the least affected. The study inferred that 73.77% of the waste workers were not satisfied and the rest 26.23% were only partially satisfied with the present job. Waste workers with more than 5 years of service, particularly of lower age group were found declining in the city. The grievances of waste workers need attention by the municipal authorities for improving their socio economic and health status.

KEYWORDS: **Municipal Solid Waste, Waste management, Health issues**

1. Introduction

In Mekelle city of Ethiopia waste is collected from households door to door once per week, however, in some parts of the city twice per week, and daily from business centers. The wastes are stocked without segregation in a single sack, and therefore are likely to be partly decomposed. It creates bad odor, and during collection the wet (liquid) part leaks onto the waste collector's body, exposing them to different health problems. The wastes contain sharp objects like shavers, blades, broken bottles, glasses, needles, and nails etc. which might pierce and injure the worker's body. The waste collectors handle the waste (lifting, carrying, emptying) manually and consequently might develop musculo-skeletal disorders. Work-related risks, symptoms and injuries occur at every stage of their activity from the point of collection to the point of ultimate disposal [1-8]. In addition in the dump site the waste workers are exposed to the toxic smoke which might lead to cough, asthma and other respiratory problems. For want of detailed scientific data, this study on the occupational health problems and socio economic status of waste collectors of Mekelle city was undertaken.

Materials and Methods

The study was carried out in Mekelle. Mekelle is the capital city of the Tigray region, in northern Ethiopia. It is located around 780 km north of the Ethiopian capital Addis Ababa, at a latitude and longitude of 13°29'N 39°28'E, with an elevation of 2084 meters above sea level.

Sample size determination and sampling technique

The sample size was determined using a formula [9]. The sample size was calculated but to increase its precision 15% was further added. Thus the total sample size considered 61 waste workers out of a total population of 133. Waste workers were interviewed using a structured questionnaire in local language during April and May 2019. The questionnaire consisted of 21 questions. These included demographic and socio-economic profile of waste workers, safety and health aspects and job satisfaction.

The collected data were analyzed using SPSS version 20 software. ANOVA test and appropriate significant tests such as student's t-test and tukey test were done for continuous variables. Two-sided p value ≤0.05 was considered significant.

Result and Discussion

The 61 municipal waste workers, all males, were employed as contract employees under the six Private enterprises in the 7 subcities of Mekelle city. Most (75.41%) of them were waste collectors and the rest 24.59% were drivers of the trucks. It was understood that 44.26% of the waste workers were educated up to secondary level and 39.35% up to primary level but 16.39% were illiterate.

About 34.70% of waste workers belonged to age group of 18 to 30 years and had worked for less than 1 year; 83.33% and 61.22% of workers belonged to age groups of 31 to 45 and 18 to 30 years and had worked for 1 to 5 years respectively whereas only 16.67% and 4.08% of workers of age groups of 31 to 45 and 18 to 30 years respectively had worked for more than 5 years.

All (100%) waste workers worked for 12 hours per day from 6.00am to 6.00 pm. All of them worked on all days of the week including Sunday. On Sundays they worked only for 6 hours in the morning.

Wages of waste collectors in all sub cities were uniform. They were paid 1000 Ethiopian birr as monthly salary by the Private enterprises whereas the drivers were given 4500 to 5000 birr per month.

All waste workers except drivers were provided with personal protective gears like cloth uniforms, boots, hand gloves and face masks by the employers. However the workers did not use the protective gears all the time and waste collectors received no training on safety and health from the employers or municipal authorities either before job or on job.

The study revealed that health problems were the main challenges of the waste workers (Table 1)

Type of health issues	Total number and percentage (%) of affected workers
Allergy (Total)	46 (75.41%)
Cough	30 (49.18%)
Asthma	26 (42.62%)
Eye sickness	23 (37.70)
Skin rashes	19 (31.15%)
Injuries (Total)	56 (91.80%)
Cuts and lacerations	53 (86.89%)
Bites by animals	11 (18.03%)
Burns from fire	14 (22.95%)
Health problems (Total)	96.72%
Respiratory problem	42 (68.85%)
Musculoskeletal problem	44 (72.13%)
Joint pain	26 (42.62%
Low back pain	24 (39.34%)
Muscle sprain	5 (8.20%)
Gastrointestinal problem	31 (50.82%)
Dysentery	25 (40.98%)
Nail infection	20 (32.79%)

Table 1 Allergies, injuries and health problems faced by the waste workers of all sub cities of Mekelle city

The health problems, as can be noticed from the table, might be due to manual handling of waste, inhalation of the airborne particles at the collection and dump sites, inhalation of hazardous smoke of the dump site and so on. Similar studies from Addis Ababa and Harar Town, Ethiopia revealed that occupational health symptoms and injuries among waste collectors were (51.2%) and (60.4%) respectively [6,7]. A similar study in Chandrapur, India reported that all (100%) waste workers had different health problems [10]. Majority of waste collectors (91.80%) got injuries. The incidence rate was higher than the study reported from Mekelle and another study of three cities of India (Solan, Mandi and Shimla) [8,11].

The study also revealed that 72.13% of waste collectors were facing musculoskeletal problems. Similar studies [12] and [13] reported that 67.20% and 65.80% of workers had back pain and musculoskeletal problems.

The survey also showed that 68.85% and 50.82% waste workers had respiratory and gastrointestinal problems respectively. This rate was lower than a similar study conducted from Kelantan, Malaysia and higher than Southern Thailand [14,13].

Another observation was that waste workers of higher age group had been more affected by health problems which might be correlated with their longer length of service dealing with waste. Finally, it was noted that the drop outs of waste workers were higher from the lower age group.

Subcity wise differences in health issues of waste workers were also observed. The waste workers of the sub city Qedamy weyane and Hadnet were the worst affected. The ANOVA showed that there were significant differences ($p=0.000$) among the waste workers of seven sub cities.

Almost all the health problems were higher among waste collectors than the drivers. The statistical t-test also proved that waste collectors had significantly ($p=0.000$) higher health issues than the drivers.

The waste workers reported that they had not received any pre- employment medical examination and medical checkup or medical aid for their injuries or health problems at work.

The study further indicated that majority (73.77%) of waste workers was not satisfied and 26.23% were only partially satisfied with their work.

Conclusion

The waste collectors of Mekelle city worked for 12 hours a day for a poor monthly salary of 1000 birr. Exposing themselves to an unhygienic environment they contracted several occupational diseases and they never received any medical help from the employers. Naturally they became dissatisfied with the job and therefore, the number of waste workers with more than 5 years of service, particularly of lower age group was declining in the city. The major recommendations of the study are to provide trucks with hydraulic lifts, extend medical and financial support to the waste workers and promote waste segregation at source.

Acknowledgment

The author is expresses her gratitude to Mekelle city sanitation and beautification administration office staff and the respondents of the survey for their assistance during data collection.

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Conflict of interest: The author declares that there is no conflict of interest.

Ethical Approval: Ethical approval to report this study was obtained from Health Research Ethics Review Committee (ERC 1318/2019) of Mekelle University, Ethiopia.

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02

Effect of Horizontal Canard on tail for Stability and Eco-friendly Airplane

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ABSTRACT

Many researchers have done the extensive study on aircraft stability. In the current investigation an attempt is made to study the static stability of a canard airplane in compared to conventional tail airplane and aerodynamic characteristics such as CL/CD. In general tail is also used as a horizontal stabilizer, whether stability is achieved statically or by fly-by-wire. The canard aircraft whose tail is placed ahead of center of gravity, acts directly to reduce longitudinal static stability (stability in pitch). During the study numerical study was conducted and compared with experimental. In case of canard configuration, the study was extended the location of canard and angle of attach. The analysis is extended even through XFLR. The location of these points played very important pole on stability. The influence of tail volume is also a considerable parameter.

KEYWORDS: **Canard, Wing, XFLR, Stability, Lift, Drag and Efficiency**

1. Introduction

Canard is part of an airplane that functions as a stabilizer or elevator and installed in front of the main wing [10]. High performance aircraft such as military aircraft often require high lift for wide range of angles of attack for high lift for higher maneuverability. Canards are becoming a more common aerodynamic element in the design of aircraft. Depending on the purpose of the aircraft, canards small wings that are situated on the forward fuselage ahead of the main wings can be created in a variety of designs and sizes. A key design factor that influences the aerodynamic performance of the aircraft is the canard's horizontal location.

Numerous studies have looked into how altering the canard's horizontal position affects an aircraft's efficiency and stability. Using computational fluid dynamics (CFD) simulations, Marufuzzaman and Islam [1] examined how altering a canard's horizontal position affected an aircraft's aerodynamics. The lift-to-drag ratio and pitching moment both increased with placing the canard closer to the main wing, indicating improved stability, according to the results. A wind tunnel experiment was done by Rhodes and Lum [2] to determine how altering a canard's spanwise position affected an aircraft's aerodynamics. The results demonstrated that relocating the canard closer to the centerline of the aircraft increased lift-to- drag ratio, decreased pitching moment, and enhanced stability. Using CFD models, Marufuzzaman and Islam [3] examined how altering the canard's aspect ratio affected the aerodynamics of an aeroplane. As a result of raising the aspect ratio, the lift-to-drag ratio improved and the pitching moment decreased, indicating improved efficiency and stability, according to the data.

Despite these studies, further research is still required to determine how adjusting the canard's horizontal position affects an aircraft's efficiency and stability, particularly for various canard designs and operational scenarios. As a result, the objective of this work is to examine aerodynamic characteristics with addition of winglet to the main wing and the impact of horizontal canard location

on aircraft efficiency and stability. The study will concentrate on a particular canard design and explore the results of altering the canard's horizontal location at various angles of attack and Mach numbers. The findings of this study can be extremely helpful in optimizing canard designs for better aircraft performance.

2. Research and Methodology

The inspiration for the current study came from earlier studies that looked at how canard arrangement affected the airflow around it and aerodynamic characteristics. One of this work is done by Dr. Dwivedi [14] in which skin friction drag is examined for canard configuration and another one is low speed aerodynamic characteristics of canard configuration for international vortex flow J. Er-EI and A. Seginer, [12]. This is further continuation to know more about the aerodynamic characteristics with additional winglets. The wake produced by the canard can impact the air foil over the main wing, causing a loss of lift, which is one of the main disadvantages of using canard planes in aircraft.

2.1. Airfoil selection

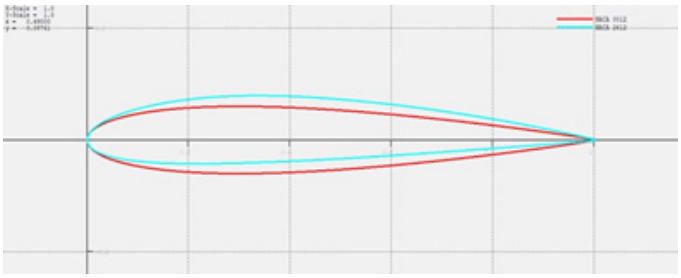

Fig 1: Airfoils NACA 2412 and NACA0012

2.2. Wing design

This method enables us to create the wing, tail, and fuselage with a variety of alternatives. The wing is designed in the XFLR5, utilizing Xfoil design and the wing and plane design processes. The wing used in this experiment with wing span 1.1m as shown in the Fig2

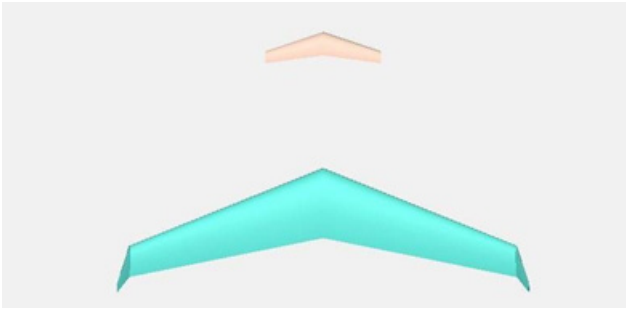

Fig 2: 3D model of canard aircraft

Table 1: Wing Dimension

Wing span	1.100m
Wing area	1.064 m2
Root chord	0.180m
Mac	0.133m
Aspect ratio	8.963
Tapper ratio	0.111
Root tip sweep	25.301 degrees

Table 1 : About the dimensions of the aircraft

2.3. Panel method

Using panel methods, the flow equations for a single wing or a collection of wings are solved. The flow over each of the numerous tiny panels that make up the wing surface is modelled as a potential flow. To determine the lift, drag, and other aerodynamic forces and moments acting on the wing, the resulting system of equations is solved referred these papers [16], the panel visualization can be seen in Fig 3.

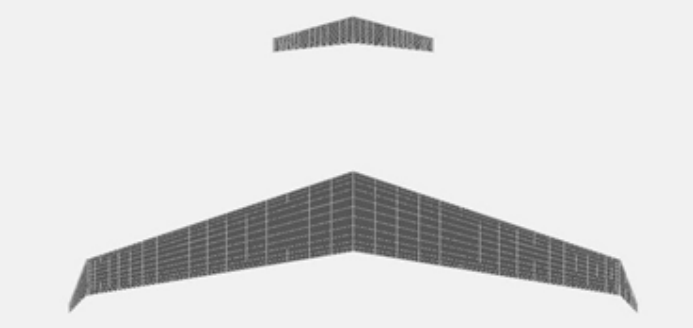

Fig 3: 3D panel model of canard aircraft

3. Theory and Calculations

3.1. XFLR5 Calculations

The XFLR5 is used for computations in this experiment, and aerodynamic and stability studies are performed on the wing-canard and winglet designs. The proposed tapered wing with the chosen airfoil underwent an aerodynamic analysis; the boundary conditions used were those of the wing. The analysis is conducted from -20 to 20 AOA (Angle of Attack), with calculations internally performed by XLR5 using the panel technique and VLM method. Based on Reynolds number, the velocity is assumed to be 35m/s at 00 AOA, the CP and aerodynamic coefficients are recorded.

3.2. General calculations

In order to determine the theoretical values of aerodynamic coefficients, the general formulas CL and CD are utilized. The following is a list of the formulas used in this experiment. Lift and drag are the aerodynamic forces that an airfoil encounters as a result of pressure variations above and below its surface. A fundamental formula for the stability derivative of aero planes is the moment formula, which is the basis for this experiment and was derived from books.

$$lift(L) = \frac{1}{2} * \rho * V^2 * S * Cl$$

$$Drag(D) = \frac{1}{2} * \rho * V^2 * S * Cd$$

$$moment(m) = \frac{1}{2} * \rho * V^2 * S * C\ (XCg - XAc)$$

Where ρ = density of the air, V = velocity, Cl = lift coefficient, S = wing surface area, C = chord length.

4. Results and Discussion

In this the results obtained from this experiment are shown, the analysis performed in XFLR5 gave us the visualizing pressure contours of the canard aircraft, the analysis is started by the selected airfoil NACA 2412 and the wing is designed, the tapered wing and the tapered wing with winglets are designed using wing and plane analysis, the analysis is performed with non-viscous boundary condition and with panel method in XFLRS at velocity of 35 m/s, the results are then compared in between the two wings. Tapered wing is labeled as wing 1 for the better understanding and the

tapered wing with winglet as wing 2

4.1. Aerodynamic analysis of wing1 and wing2

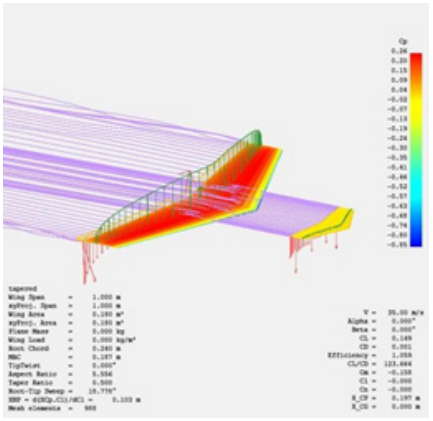

Fig 4: wing l

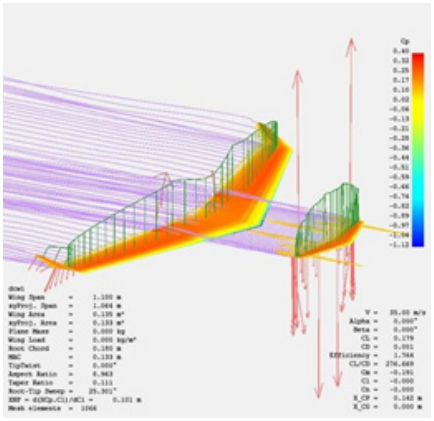

Fig 5: wing 2

As we can see from the fig 4the cl, but in fig 5 we have cl So the wing 2 is selected as it has high better efficiency than the wing l

4.2. Horizontal Canard positions

This result shows that the position of the canard on the aircraft is compared with three different positions, the canard is placed along the axis of the wing, the position is calculated from the leading edge of the wing, here the three positions of canard are at 0.25m, 0.35m and 0.5m from the leading edge of the wing.

The compared results shows that the aircraft with mid canard position i.e., at 0.35 the aircraft has better efficiency and good stability.

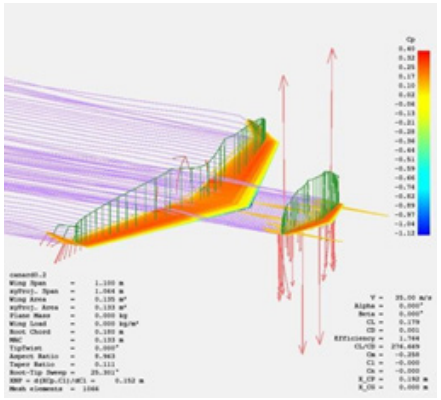

Fig 6: canard position at 0.25m

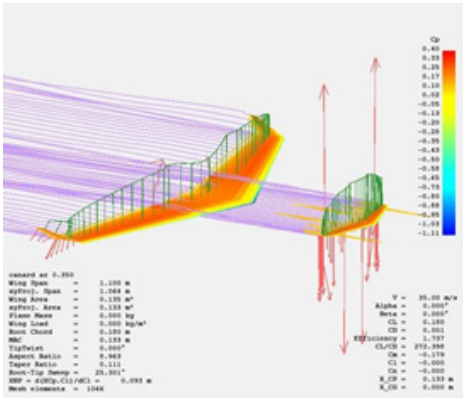

Fig 7: canard position at 0.35m

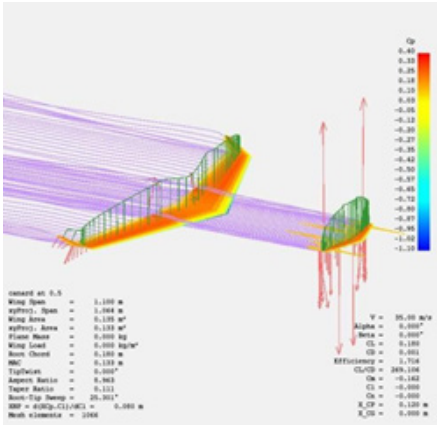

Fig 8: canard position at 0.5m

The above figure 6 is the canard position at 0.25m from the leading edge of the wing, the Fig 7 is the position of canard at 0.35m and the fig 8 is the position of canard art 0.5m

The aerodynamic performances are recorded for these three in the form of graphs in XFLR5 In the fig 9.

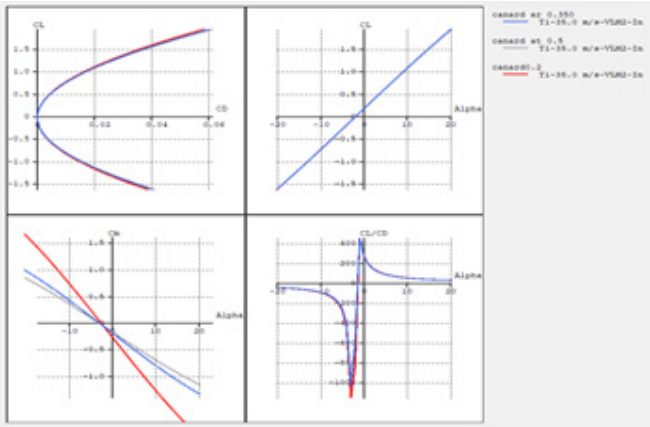

Fig 9: Aerodynamic performances of the three aircrafts

Vertical tail for the canard aircraft

The vertical tail helps in directional stability of the aircraft, so when we add a vertical tail to the canard aircraft the results proved that there will be no change in its aerodynamic performances

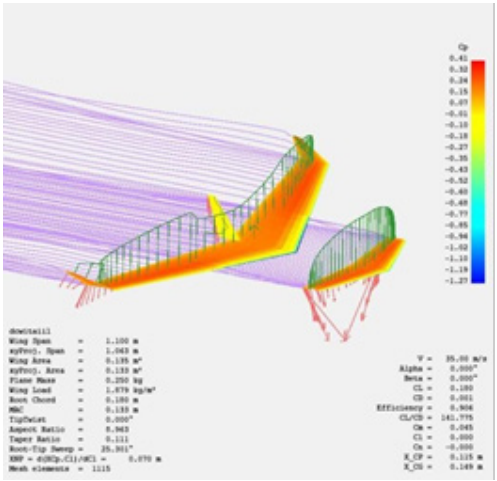

Fig 10: canard aircraft with tail

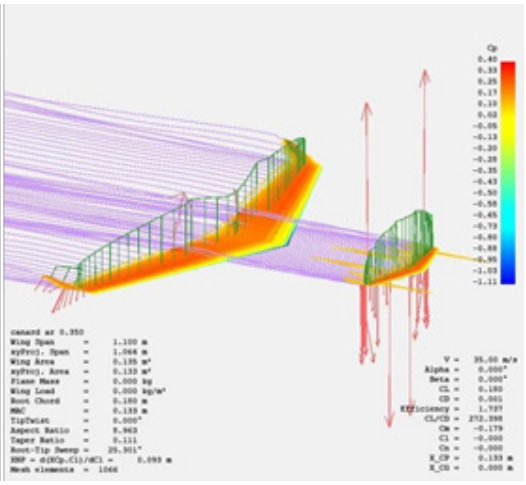

Fig 11: canard without tail

The fig 10 is the canard aircraft with vertical tail has CL= 0.180 and in Fig 11 the picture describes the canard aircraft without tail has CL=0.180, so this proves that vertical tail has no effect in the aerodynamic parameters for canard aircraft.

5. Conclusion

This experiment proves that the canard horizontal position plays a major role in the aircraft stability, a we have changed the canard position horizontally or axially and found from the experiment is that, if the canard position is too far from the wing the aircraft is unstable if the canard is too close from the canard, we cannot produce more lift so we found the exact position where it is close to the mid position where the stability and the aerodynamic performances are high. And also the wing is designed in such a way that winglets added to the main wing of the canard helped to reduce the vortex and increased the flight performance of the aircraft, the NACA 2412 air foil used for the main

wing, the comparison between the tapered wing and the wing with winglets proved that the wing with winglets has better aerodynamic performance, and then the analysis is carried out for the wing with winglets the lift produced by the aircraft with winglets and mid canard position gave us the high stability and high lift and less drag, the canard position from the wing leading edge are 0.25m, 0.35m and 0.5m from these the canard placed at 0.35m from wing leading edge has the high lift coefficient. And when we add a vertical tail to the canard aircraft there will be no change in its aerodynamic performance.

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03

Eggshell as a Cost-effective Material for Photocatalytic Dye Degradation

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ABSTRACT

Eggshell waste is one of the most abundant waste materials coming from food processing technologies. The utilization of eggshells as a primary source for developing value-added materials has received significant attention recently due to its ability as an excellent adsorbent andphotocatalyst which shows better dye degradation to support a sustainable environment. The efficient use of waste resources for the removal of contaminants has the dual benefit of waste disposal and ensuring a more cost-effective and ecofriendly treatment approach. Despite the unique properties that both its components (eggshell, ES, and eggshell membrane, ESM) possess, it is very often discarded without further use. This research work investigated the photo catalytic adsorption property of both chicken and duck egg shells and also their membranes for the degradation of the methylene blue dye. The experiment was conducted in light as well as dark conditions. The effect of different parameters includes initial concentration of adsorbate, adsorbent concentration and types of eggshells and egg membrane were also studied. A colorimetric characterization is used to estimate the adsorption properties. From the results it is concluded that chicken egg shell shows higher adsorption properties than duck egg shells in the light condition.

KEYWORDS: **Egg shell, Eggshell membrane, Photocatalyst, Dye degradation, Decontamination.**

1. Introduction

Water is considered as one of the basic requirements and vital for all forms of life in nature. There has been an extensive exploitation of water resources during the last few decades due to the increasing global population, urbanization, and industrialization.[1]. However, this had a negative impact on water quality because water getting polluted due the release of improperly treated industrial wastes such as textile, dyeing, paper, processing, food, etc. containing various dyes and pigments which makes it toxic to plants and aquatic life[2].Furthermore, Textile dyes and degraded by products of dyes considered toxic and carcinogenic and is one of the one of the main pollutants of freshwater resources [3]. Mostly, the dye waste bearing aromatic structures is difficult to degrade and these kind of dyes are designed to have higher resistance toward environmental influences such as pH and temperature [4]. The reactive dye e.g., methylene blue is an azo-based chromophore and the mechanism of attachment to the textile fiber is through covalent bonds. Therefore, it is highly resistant to traditional wastewater remediation approaches. The decomposition or breaking down of the dye from larger components into smaller, less toxic fragments is a matter of concern and research for the scientific community around the world [3]. To date, the wastewater treatment technology has been depending on different methods of the biological, chemical, physical processes, and its combination[4]. However,

several drawbacks such as high cost, the difficult of regeneration, and the complex separation process, formation of a large number of by-products, scaling up of models for large-scale schemes etc. demands for an alternative cheap method.

To address the above-mentioned disadvantages, an alternativePhotocatalysis method that has received much attention over the years. In photocatalysis approach, pollutants in wastewater can be degraded using semiconductor materials under light irradiation [5] . Photodegradation is an excellent substitute for wastewater treatment techniques. It does not require critical reaction conditions to occur and there is no formation of harmful by-products in the process[6]. Photocatalysis needs less time and a small input of catalyst and can be used for several purposes such as hydrogen generation, liquid phase, solid phase as well as gas-phase treatment. Numerous types of photocatalytic semiconducting materials, including perovskite, titanates, metal oxides of Ti/Zn/Ca/Cu/Fe/Ni/Co, and hetero-composite of carbon nanotubes/ graphene have been widely used for photodegradation of a number of dyes. There is constant search for novel photocatalysts other than traditional TiO2 or ZnO-based catalysts in order to develop catalysts derived from earth-abundant and inexpensive components. Several research have been conducted on the use of calcium oxide, calcium hydroxide, calcium antimony oxide/ hydroxide, etc. for degradation of the dye [7, 8]. In the search for versatile, readily available, non-toxic, and cost-effective resources as potential adsorbent to remove hazardous chemicals from water, eggshells have emerged as a suitable candidate. Studies have shown that eggshell as well as eggshell membranes is promising candidates for removal of heavy metals, organic dyes, Polycyclic aromatic hydrocarbons (PAHs), pharmaceutics etc[11]. A variety of heterogeneous catalysts have also been developed by integrating eggshell powder with polymers and metal oxides which can be used for the dye degradation purpose [9, 10].

The aim of the present study was to prepare and characterize egg shell as well as eggshell membranes of both hen and duck -based adsorbent and to assess its adsorption capacity for methylene blue. The photocatalytic activity of the catalyst was then tested for the photodegradation of the methylene blue dye solution in the natural sunlight irradiation and also compared the dye degradation in dark conditions too.

2. Research Methodology

2.1. Materials

The discarded chicken as well as duck eggshell wastes were collected from the local markets of Moothakunnam, Kerala, India. Methylene blue and acetic acid were procured front Merck Chemicals, India. Distilled water was used for the preparation of methylene blue solution. Molecular structure of methylene blue (MB) dye is given in Figure 1.

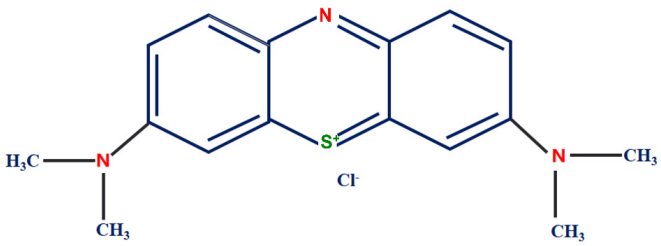

Figure 1: Chemical structure of Methylene blue

Methylene blue is a blue cationic thiazine dye used widely in the field of medicine to treat various illness and disorders. It is a heterocyclic aromatic chemical compound with the molecular formula of C16H18N3SCl and molecular weight of 319.85 g/mol. Its IUPAC name is [7-(dimethylamino) phenothiazin -3-ylidene] – dimethylazanium; chloride.

2.2. Experimental

2.2.1. Preparation of adsorbent

Discarded egg shells of hen and duck were collected. Eggshells were first washed with distilled water thoroughly to remove all the unnecessary materials adhered to the egg shells and then dried at 105°C in a hot air oven for 2 h. The membranes were separated from dried eggshells by hand. The recovered egg shell membrane was kept in acetic acid for hours to remove remnant eggshells. The membrane was then taken from the solution and washed with distilled water till the acetic acid was removed. It is then dried in sunlight and finally dried at 105°C in a hot air oven for 2 h. The dried eggshells and membranes were ground separately using a grinder. The powdered egg shells and membranes were stored in bottles labelled CES (chicken egg shell), DES (duck egg shell), CESM (Chicken egg shell membrane) and DESM (duck egg shell membrane).

2.2.2. Preparation of adsorbate

In this experiment, dye solution was prepared by dissolving methylene blue (MB)powder in distilled water to prevent and minimize possible interference. The dyewas stirred until it was completely dissolved. A stock solution of the dye wasprepared by dissolving 1 gram of dye in 1000 mL distilled water to make a stocksolution of 1000 mg/L. Then, the experimental solution was prepared by diluting definite volume of the stock solution to get the desired concentration. Differentadsorbate concentrations such as 0.005mg/L, 0.007mg/L,0.009 mg/L and0.01mg/L were prepared.

2.2.3. Photocatalytic Experiments

At first the photo catalytic adsorption activity of egg shells was examined by constructing the experiments at light and dark conditions. Four 250 ml beakers were taken and 50 ml 0.11g/250ml methylene blue added to it. Add 0.50g CES powder to two beakers and the same amount of DES powder to the remaining beakers. Place a glass rod in each beaker to stir the content. Two beakers one from each set are kept in sunlight. The remaining two beakers are covered with aluminium foil and black cloth to create a dark condition and placed in a cupboard. They are shake in every minute. And adsorption is checked periodically by colorimeter.

To check adsorption by colorimeter, samples of methylene blue are collected in small test tubes. Adsorption measured using colorimeter after a standard has been checked. The observed values are recorded. Second day experiment is continued to check the effect of adsorbent and adsorbate concentrations. To determine the effect of adsorbent concentration CESpowder is added in different concentrations namely 0.30g, 0.40g, 0.60g and 0.70g to 50ml 0.11g/250ml methylene blue solution. They were kept in sunlight and adsorption checked periodically by colorimeter. Readings are noted. On third day, adsorption property of CESM, DESM and mix were checked. 0.60g of each sample is taken in beaker and 50ml of 0.01/250 ml methylene blue was added to it. They were kept in sunlight and adsorption measured using colorimeter. Observations are tabulated. In each case graph is plotted using the observed values by taking time along the X-axis and colorimeter reading along the Y-axis.

2. Theory

In physical and analytical chemistry, colorimetry is a technique used to determine the concentration of coloured compounds in solution. A colorimeter is a device used to test the concentration of a solution by measuring its absorbance of a specific wavelength of light. To use the colorimeter, different solutions must be made, including a control or reference of known concentration. Before using an electronic automated colorimeter, they must be calibrated with a cuvette containing the control solution. The concentration of a sample can be calculated from the intensity of light before and after it passes through the sample by using the Beer-lambert law.

The colour or wavelength of the filter choses for the colorimeter is extremely important, as the

wavelength of light that is transmitted by the colorimeter has to be the same as that absorbed by the substance being measured. For example, the filter on a colorimeter might be set to red if the liquid is blue.

4. Results and Discussion

4.1. Visual Observation

Photocatalytic degradation of methylene blue was carried out by using two different egg shells and egg shell membrane and were studied at light and dark condition. Dye degradation was initially identified by colour change. Initially, the colour of dye shows deep blue colour changed into light blue after the 1 h of incubation with egg shells and egg shell membrane while exposed to solar light. Finally, the degradation process was completed at after some hours and was identified by the change of reaction mixture colour to colourless. It has been found that sunlight has good effect on adsorption. Adsorption in presence of sunlight gives better results than adsorption at dark condition. Figure 2(a) and (b) represents the adsorption of MB in presence of sunlight by CES and DES respectively. CES showed better results among all the tested samples. CES gave complete dye degradation within 3.5 hrs and are obvious from the colourless solution obtained after 3.5 hr exposure of the sample in sunlight.

From figures 2(a) and (b), it is clear that both the egg shells show a large extend of adsorption in the presence of sunlight. In the case of DES which didn't show complete degradation of the dye solution (fig 2(b)) even at 3.5 hrs of exposure.

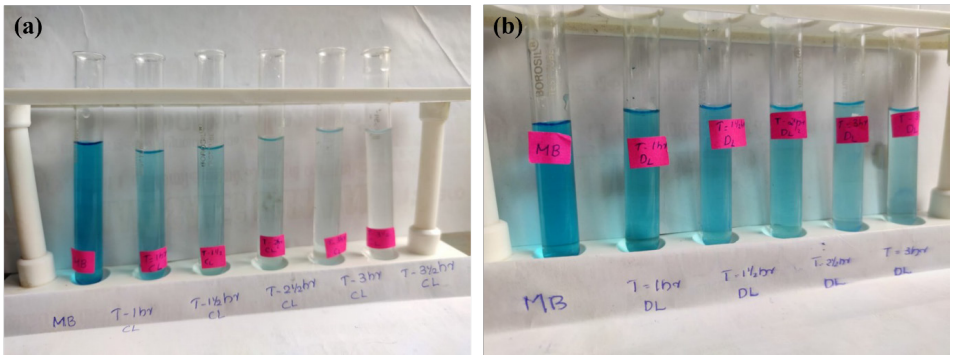

Figure 2: Degradation of MB by (a) CES and (b) DES in sunlight at various time intervals

The dye degradation in dark condition by CES and DES are given in figure 3(a) and (b). Noticeable colour changes are not observed in the results for both CES and DES. When exposed to sunlight both the adsorbent showed an observable fading of blue colour even at 1 hr time period. But only a slight decrease in colour is observed even after 2.5 hrs of experiment by both the adsorbents. This clearly indicates that sunlight has a major role in dye degradation.

It is clear from the visual observation that chicken egg shell CSE shows higher adsorption properties than duck egg shells DES in both conditions.

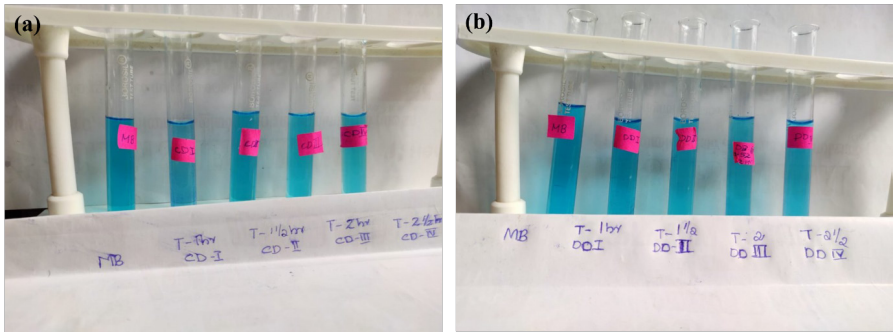

Figure 3: Degradation of MB by (a) CES and (b) DES in dark condition at various time intervals

Figure 4(a) & (b) shows that egg shell membranes also show great adsorption in the presence of sunlight. Also, it is clear that chicken egg shell membrane CESM is better in adsorption than duck egg shell membrane DESM. The colour change obtained after 1 hr and 2 hr exposure to sunlight is shown in the Figure 4 (a) and (b). It is clear from the figure itself that even 2 hr exposure shows a remarkable colour change to the dye solution. A mix of duck egg shell and its membrane also shows high adsorption property.

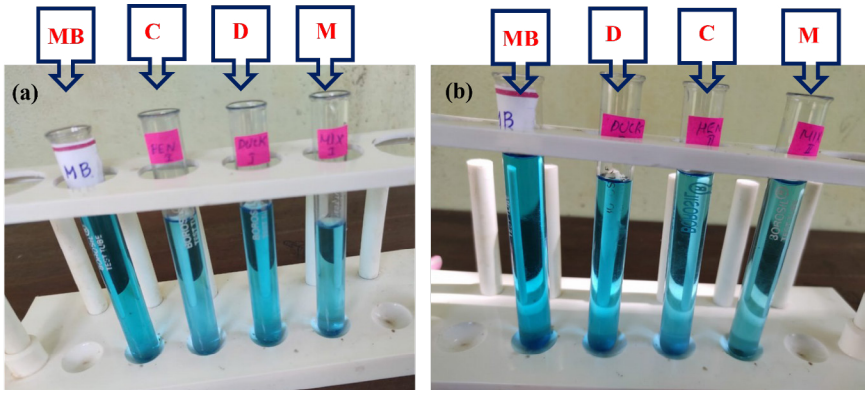

Figure 4: Visual observation of colour changes in light condition by the adsorbents CESM (C), DESM (D) and a mix of DES and DESM (M) at different time intervals (a) 1 hr and (b) 2 hr.

4.2. Effect of light and dark condition

Both the chicken eggshells and duck eggshells are tested for dye degradation both in light and dark conditions. As from the fig. 5 it is clear that the CES showed better dye degradation than the DES in light condition. In the case of dark condition even after 2.5 hr treatment with both the eggshells does not show much adsorption properties. As the contact time increases, both the samples showed almost similar adsorption values. The higher adsorption in presence of sunlight can be explained on the basis of photo catalyst. i.e., adsorption reaction catalysed by light.

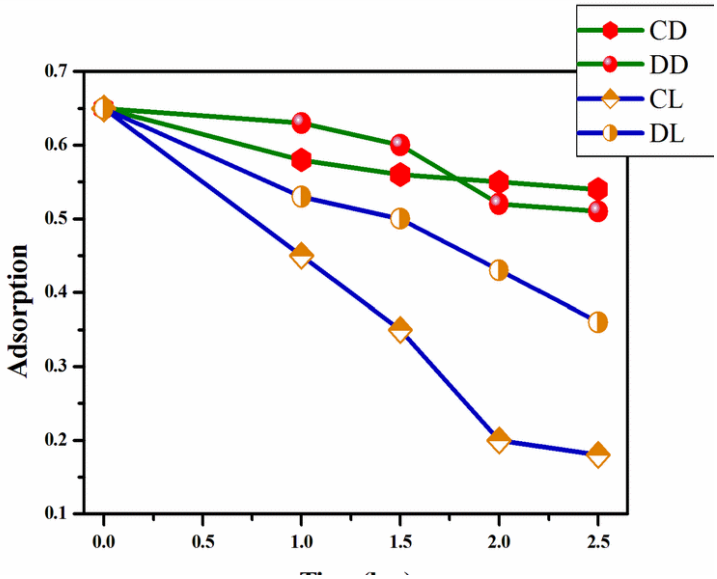

Figure 5: Adsorption characteristics of Chicken (C) & Duck (D) eggshells inlight (L) and dark (D) conditions

4.3. Effect of ESM

Dye solution with ESM of chicken and duck eggshells and also the mix of duck ES and ESM were subjected to sunlight for hours. Chicken ESM showed better photocatalytic activity among the three samples.

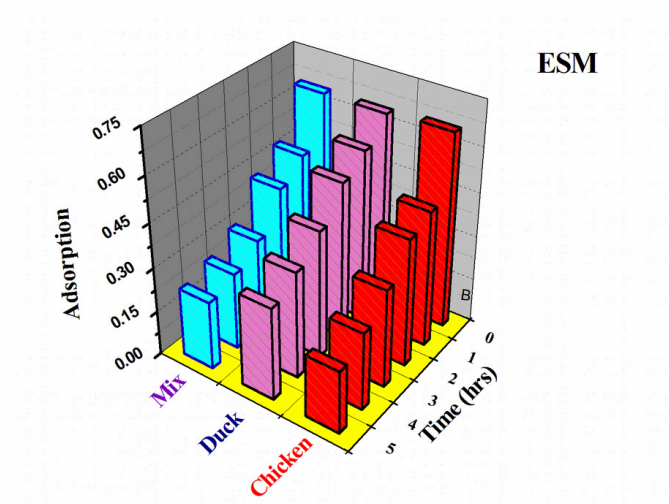

Figure 6: Photocatalytic activity of the ESMs of Chicken, duck and a mix of ES and ESM of duck

4.4. Effect of adsorbent Concentration

Experiment conducted with different adsorbent concentrations of chicken eggshells. From fig.7, we can say that initially adsorption is decreased with the increasing adsorbent concentration. But after several hours all adsorbent concentrations reaches to a minimum point in the adsorption curve. The decrease in adsorption with increase in adsorbent concentration can be explained as following. At lower adsorbent concentrations, the number of active sites is higher. For the higher adsorbent concentration, the formation of aggregation of particles. Since the aggregation of adsorbent particles consequently results in the decrease in adsorption sites.

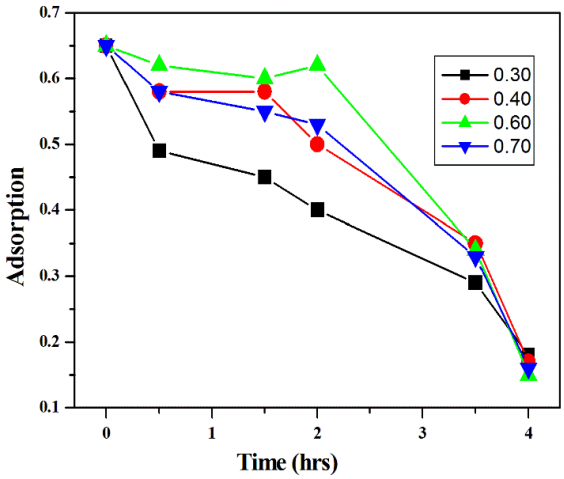

Figure 7: Photocatalytic activity of CES with different adsorbent concentrations

4.5. Effect of adsorbate

Figure 8 indicates the effect of different adsorbate concentrations and it shows that adsorption with lower adsorbate concentration yields better results. As the concentration of adsorbate increases, it is clear that the adsorption rate is decreasing after an increase. It is likely due to most of the sites are already occupied when the concentration is high, and the uptake rate becomes slower. From Figure, it is clear that when the initial concentration is high, the uptake also high up until a plateau but the rate decreases.

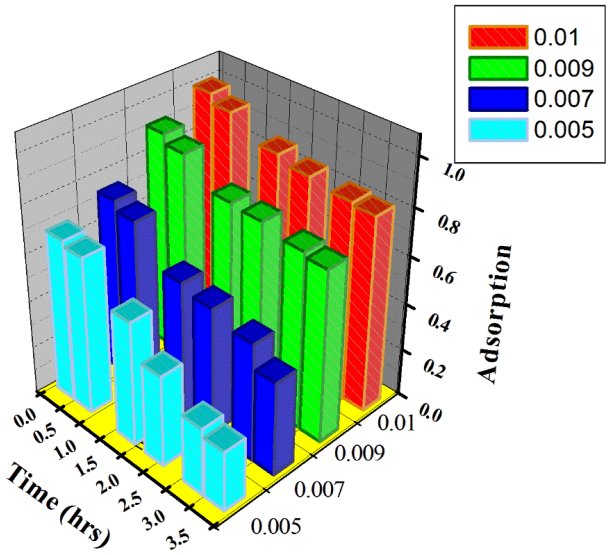

Figure 8 : Photocatalytic activity of CES with different adsorbate concentrations

Conclusion

Egg shells are waste materials often without any reuse. Therefore, this study focused on how waste egg shell can be converted to a photocatalyst to remove toxic dye material. The materials, egg shell (ES) powder and powdered egg shell membrane (ESM) of chicken and duck have been compared for the dye removal efficiency under light and dark conditions. Effect of concentration of adsorbent and adsorbate is also compared. As the concentration of adsorbent increases the adsorption rate decreases initially due to the unavailability of active sites because of the aggregation of adsorbate molecules. The increase in adsorbate concentration decreases the rate of adsorption. This is also due to the unavailability of sites since all the sites are already occupied. The enhanced dye degradation efficiency is observed with CES in the presence of light compared to CES in dark suggesting it is a photocatalytic effect. In conclusion, the CES has enhanced performance for dye degradation compared to DES, DEM and HEM highlighting its potential use as a novel and eco-friendly photocatalyst for dye degradation.

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04

Analysis of Physico-Chemical Parameters and their Corelations to Seasonal Variations in Plankton Diversity of a Selected Pond Ecosystem Emerged by Pamba Irrigation Project, Pathanamthitta

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ABSTRACT

Ponds are among the most significant water assets with multiple human utilization and ecological relevance. This investigation was intended to evaluate the pond water quality, giving importance to the analysis of microscopic pond life in the selected pond of Keekozhoor, Pathanamthitta District, Kerala. In the current examination, an endeavour has been made to study the seasonal variations in the plankton community, their diversity and physico-chemical parameters, viz. pH, dissolved oxygen, nitrite, chloride, sulphate and phosphate. A sum of 40 genera of phytoplankton and 12 genera of zooplankton were identified from various classes during pre-monsoon (March-April) to post-monsoon (May-June) seasons in 2018. Among the distinguished phytoplankton, Chlorophyceae shaped the predominant gathering with 17 genera of the complete phytoplankton network in pre monsoon and 30 genera in post monsoon individually. Among the identified zooplanktons, protozoans established the most predominant gathering with 6 genera of the total zooplankton community in both pre monsoon and post monsoon periods. The information acquired by analysing physico-chemical parameters was utilized to compute the water quality index. The outcomes were assessed and compared with WHO and BIS water quality norms and was found that the samples are falling under very poor category; consequently, not appropriate for local purposes. Hence, the current outcome gives an essential documentation of the plankton community, its diversity and basic understanding of the trophic status of the pond ecosystem.The study is relevant in the scenario that it was conducted during pre-flood period in Kerala. The studies of the same with respect to post-flood season are significant to understand the current status of the plankton diversity in the selected ecosystem in relation to its physico-chemical parameters.

KEYWORDS: **Plankton, physico-chemical parameters, Water Quality Index (WQI), correlation study.**

1. Introduction

Ponds are freshwater biological systems which incorporate biotic and abiotic parts. A solid biological system relies upon these segments. The nature of water is an important parameter, as the low quality of water will antagonistically impact the surrounding life forms in all the frameworks. Both surface and ground water contain some sort of pollutants and suspended materials. Subsequently, it is important to find out the nature of water before its use. Water quality in the aquatic environment is dictated by numerous physical, chemical and biological factors. The physico-chemical parameters, for example, pH, Dissolved oxygen, Chloride, Sulphate and so forth can fluctuate with various environments. These elements decide the water quality which thusly impacts the endurance and presence of the planktonic network.

Phytoplankton adds to the minute algal networks of water bodies. The efficiency of a biological system is straightforwardly identified with the wide variety of phytoplankton. Phytoplankton is a straight pointer of trophic status of an aquatic ecosystem [1]. The seasonal variations of physico-chemical parameters decide the subsequent variations in phytoplankton communities.Zooplankton are little creatures that coasts unreservedly in the water segment basically controlled by water flows. The size of these life forms ranges from a few tens of microns to > 2mm. The different functional aspects of an aquatic system, for example, food chains, food webs, energy flow and cycling of matter are affected by the zooplankton individuals, which are significant biotic segments of an aquatic framework. All the secondary production in aquatic ecosystems legitimately or in a round-about way depends on them. Their conveyance is connected with a complex of components, for example, change of climatic conditions, physical and chemical parameters and vegetation spread. The plankton species have distinctive physiological pre-requisites and accordingly demonstrate varied influences to physico-chemical parameters like pH, Dissolved Oxygen, Nitrite, Chloride, Sulphate, Phosphate and so on. They play vital role as bio indicators for deciding the status of water contamination. Consequently, plankton association, richness, abundance, seasonal variation and diversity can be utilized for the evaluation of water quality. Henceforth, an investigation was led to evaluate the plankton diversity along with their correlationto physico-chemical parameters to analyse the functioning of this important aquatic ecosystem. The point of the current investigation is to choose a disregarded pond ecosystem and study its physical, chemical and biological attributes and to evaluate the trophic status of the pond so as to recommend conservation strategies to save such a vulnerable ecosystem.

2. Materials and Methods

2.1 Study Area

Keekozhoor is a village in Ranni Block in Pathanamthitta District of Kerala State, India which is 9.3523° N, 76.7697° E (Fig.1). It goes under Cherukole Panchayath, having a place with the South Kerala Division. The selected pond lies at the core of the town and the closest milestone to the pond is St. Peter's and St. Paul's Orthodox Church.

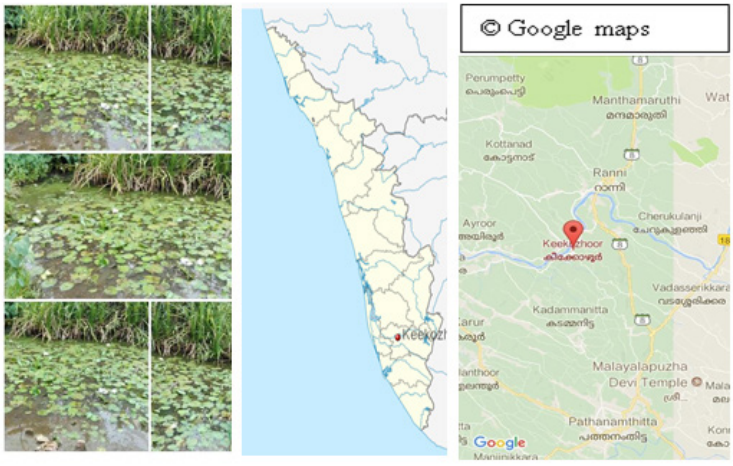


Figure 1: Different views of the selected site-pond at Keekozhoor, emerged by Pamba Irrigation Project, (Left) and Google map of Keekozhoor, Pathanamthitta District (Right) [2]

2.2 Collection of samples

The investigation has been done for a time of four months from March-April (pre-monsoon) and May-June (post-monsoon) in 2018. Five sampling sites were chosen from the pond. The water and algal samples were collected at fixed timespans of 3-4 weeks between 8.00-10.00 am. Water samples were collected in sterile polyethylene bottles, marked and brought to the lab for additional examination.

2.3 Laboratory Analyses

2.3.1 For physicochemical parameters

Physico-chemical parameters viz pH, carbon dioxide,chloride, dissolved oxygen, phosphate, nitrite and sulphate were examined according to the Standard Method for Estimation of Water and Waste Waters [3].

2.3.2 Plankton analysis and theirrelationships with physicochemical parameters

For the qualitative analysis of phytoplankton, the samples were sieved usingplankton net and gathered in clean plastic containers. Epilithic algae were scrapped from the stones at the base of pond soil and the epiphytic algae found attached to floating macrophytes were also collected. For the examination of zooplankton, the examples gathered from soil and those acquired by scratching the underside of floating macrophytes were blended in with distilled water prior to observation under microscope.

Specimens were preserved in buffered formaldehyde (4%) and are stored in ambient conditions until the time of further analysis. Photos of the phytoplankton specimens were taken with exceptional reference to the apical, basal cells and the chloroplast. Phytoplankton were identified up to the genus level following the classifications of Cyanobacteria and Algae-Fritsch[4], Freshwater algae-Prescott[5], Cyanophyta-Desikachary[6] and Freshwater microalgae-Anand[7] and zooplanktons following the classification of Free-Living Protozoa- Patterson[8]. The Observation was carried out with the aid of light microscope (Labovision, MEDSTAR) 10X/40X magnification.

So as to assess the species diversity, all the various genera of plankton were identified and counted individually. The relationship between the planktons and the physico- chemical parameters of their habitat are analysed critically. Paired t-test was done to analyse statistically where there is any significant difference between the estimated values of physicochemical parameters got in the pre-monsoon period with those of the post-monsoon period. Pearson's correlation study was additionally done to decide the relationship among physicochemical parameters and various zooplankton groups by utilizing SPSS programming.Palmer`s pollution index [9] and Nygaard`s topic state indices [10], were utilized for the assessment of tropic status and for analysing the quality of water samples for high or low organic pollution respectively.

2.4 Water Quality Index (WQI)

The computation of WQI was done using a weighted arithmetic index method [11].

qn=[100(Vn-Vi0)/(Sn-Vi0)]

(qn =quality rating for the nth parameter, vn =estimated value of the nth parameter at a given sampling station, S =standard permissible value of nth parameter, Vi0 =ideal value of nth parameter in pure water).

2.4.1 Calculation of Unit Weight (Wn)

The unit weights (wn) for different water quality parameters are inversely proportional to the recommended standards for the corresponding parameters.

Wn=k/Sn; (Wn=unit weight for nth parameter, Sn =standard value for nth parameter, K=constant for proportionality).

2.4.2 Calculation of WQI: WQI=∑q Wn/∑Wn

The calculated WQI is compared with the BIS standards to know the suitability of water for different purposes [12,13].

2.4.3 Statistical analysis

The Pearson correlation coefficient was carried out to find out the significance between the zooplankton groups and physico-chemical parameters. One-way ANOVA was used in the study. The post hoc analysis (Tukey and Duncan) was performed to determine the significant differences between the means of physico-chemical parameters (SPSS-16.0, USA).

3 Results and Discussion

3.1 Phytoplankton analysis: Phytoplankton composition in Keekozhoor pond

Phytoplankton in the open fresh water pond was represented by five classes of algae viz. Chlorophyceae, Cyanophyceae, Bacillariophyceae, Xanthophyceae and Euglenophycae. In the selected pond, 40 genera of phytoplankton members were identified. Among these, Chlorophyceae framed the dominant group (47.06%), Bacillariophyceae (35.30%),Cyanophyceae (5.88%), Euglenophyceae (5.88%) and Xanthophyceae (5.88%) in pre monsoon and Chlorophyceae(70%), Bacillariophyceae (10%), Cyanophyceae (10%), Euglenophyceae (10%) in post monsoon respectively. Chlorophycean diversity is most elevated during the post monsoon period i.e., from May-June. Genus like Oscillatoria, Anabaena andStigonemawere recorded from cyanophyceae. Chlorophyceaewasrepresented by Tetracystis, Botryococcus, Pediastrum, Scenedesmus, Schroederia, Selenastrum, Ulothrix, Oedogonium, Bulbochaete, Netrium, Mougoetia,Sirogonium, Gonatozygon, Closterium,Cosmarium,Micrasterias, Spirogyra, Penium,Spondylosium, Staurastrum, Xanthidium, Ankistrodesmus, Tetrallantos, Nitella, Goniumand Krecheriella. Among the diatomsCyclotella,Cymbella, Fragilaria, Melosira, Navicula,NitzschiaandPinnulariawere found. Euglenophyceae was mostly represented by Euglena, TrachelomonasandPhacus.Xanthophyceae wasmostly represented by Characiopsiswhich demonstrated its presence in the pre-monsoon period as it were.Seasonal distribution of phytoplanktonswas shown in Table1 and Figure 1.

Table 1: Distribution of phytoplankton in a pond ecosystem at Keekozhoor, emerged by PambaIrrigationProject, Pathanamthitta.

Sl. No	Phytoplankton	Pre Monsoon	Post Monsoon
1	Tetracystis	-	-
2	Botryococcus	-	+
3	Pediastrum	+	-
4	Scenedesmus	+	+
5	Schroederia	-	+
6	Selenastrum	-	+
7	Ulothrix	-	+
8	Oedogonium	+	+
9	Bulbochaete	+	-
10	Spirogyra	+	+
11	Netrium	-	+
12	Mougeotia	+	+
13	Sirogonium	+	-

Sl. No	Phytoplankton	Pre Monsoon	Post Monsoon
14	Gonatozygon	-	+
15	Closterium	-	+
16	Cosmarium	-	+
17	Micrasterias	-	+
18	Penium	-	+
19	Spondylosium	-	+
20	Staurastrum	-	+
21	Xanthidium	-	+
22	Ankistrodesmus	-	+
23	Tetrallantos	-	+
24	Krecheriella	-	+
25	Gonium	-	+
26	Nitella	+	-
27	Cyclotella	+	-
28	Cymbella	-	+
29	Fragilaria	+	-
30	Navicula	+	+
31	Melosira	+	-
32	Pinnularia	+	+
33	Nitzschia	+	-
34	Euglena	+	+
35	Phacus	-	+
36	Trachelomonas	-	+
37	Oscillatoria	+	+
38	Anabaena	-	+
39	Stigonema	-	+
40	Characiopsis	+	-
Total no: of species		17	30

Phytoplankton studies are helpful for the identification of physico-chemical and other biological conditions of the water in any aquatic ecosystem. In the course of the most recent couple of decades, there has been discovered more concern about the processes influencing the development of phytoplankton communities, principally according to physico-chemical factors [14, 15]. Phytoplankton are sensitive to the ecological changes and their distribution fluctuates extensively as for seasons, water quality and nutrient concentrations.

(a) Chlorophyceae: In the present study, Chlorophyceae was the dominating group in both the seasons, might be because of high DO, slow water current during this period which are in accordance with the findings of Kaur et al [16]. The higher concentration of nitrate, calcium and phosphate in water favour the growth of certain green algae (Scendesmusand Ankistrodesmus sp.) and henceforth these species are designated as pollution indicators [17]. The higher occurrence of these previously mentioned species at the pond means that they can endure elevated levels of pollution.

(b) Bacillariophyceae: Diatoms are used as water quality indicators as some of the diatoms grow and reproduce quickly while the others get disappeared and help to distinguish the changes in

water quality [18]. The occurrence of Naviculasp, Nitzschia and Synedra ulna indicate pollution in the water body and are observed during the study period which are in accordance with the finding of Tessy& Sreekumar [19].Kaur [16] have recorded an abundance of diatoms where the water was profoundly polluted. The peak of diatoms was recorded during pre-monsoon when the temperature was high and DO contents were comparatively lower.This is in conformity with the observations of Thomas & Deviprasad; Laskar & Gupta [20,21]. Many physico-chemical parameters are necessary for the existence of diatoms. The density of Bacillariophyceae population was found to be closely associated with pH [22]. pH ranges of 7 to 8.25 influence the growth of diatoms, which was evidenced in the present study. Also, there were good supplies of nitrite during the pre-monsoon and have higher growth of diatom indicating the influence of pH and nitrite that favour the growth of diatoms.

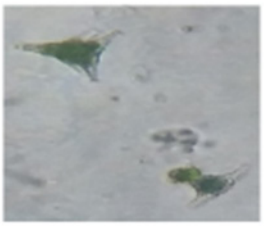
(c) Euglenophyceae: Euglenophyceae species were recorded lowest compared with different classes of algae studied. This is concomitant with the findings of Thomas & Deviprasad; Mahor& Singh; Hosmani who recorded members of Euglenoids in least numbers in studied tropical water bodies [20,23,24]. Abundance of Euglenophyceae members in a water body can be attributed to entry of nutrients through the influx of domestic sewage (an indication of organic pollution) [21]. The presence of the most pollution tolerant Euglena sp and Phacus sp. depicts high organic and sewage contamination. This perception is in concurrence with the findings of several researchers [16, 17, 25].

(d) Cyanophyceae: Temperature, pH, phosphate etc are some of the central components which control the population of Cyanophycean members. Cyanophycean members are seen as more in the post-monsoon period than in the pre-monsoon. The presence of Oscillatoriasp. may propose eutrophication of water.

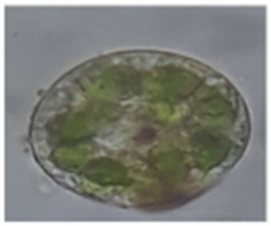
(e) Xanthophyceae: In the present study, Xanthophyceae was represented by just single genera Characiopsis which demonstrated its essence in the pre-monsoon period as it were. They occur often under dystrophic or mesotrophic conditions, showing their diversity in acidic waters enriched with dissolved organic matter [26].

Figure 1: Distribution of phytoplankton in a pond ecosystem at Keekozhoor, emerged by PambaIrrigation Project, Pathanamthitta

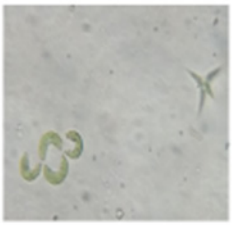
Spondylosium



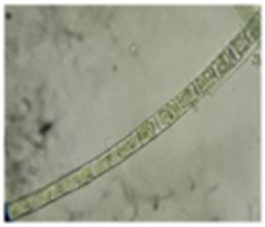
Staurastrum



Tetracystis



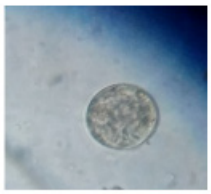
Tetrallantos



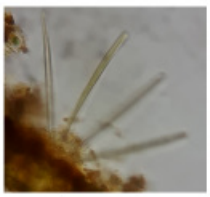
Ulothrix

Xanthium

BACILLARIOPHYCEAE

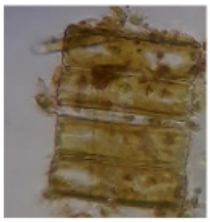


Cyclotella sp.



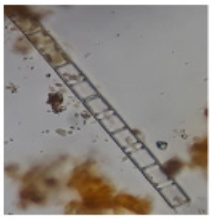
Nitzschia

Navicula



Fragilaria

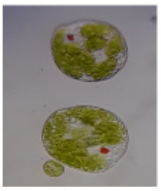
Cymbella



Melosira

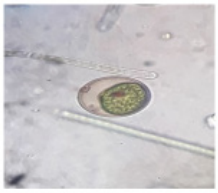
Pinnularia

EUGLENOPHYCEAE



Euglena

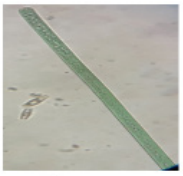
Phacus



Trachelomonas

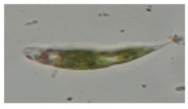
CYANOPHYCEAE

Anabaena



Oscillatoria

Stigonema



Characiopsis
(Xanthophyceae)

3.2 Palmer's Pollution Index: Palmer`s pollution index of algal genera was utilized for rating water samples for high or low organic pollution. Out of the 20 genera recorded by Palmer 10 pollution tolerant genera were recorded from the pond. The total score got is 22 (pre-monsoon) and 20 (post-monsoon) and was more prominent than or equivalent to 20 affirmshigh organic stress and pollution which may be because of the release of domestic sewage into the pond (Table2).

Table 2: Palmer`s Pollution Index of Algal Genera recorded from a pond ecosystem at Keekozhoor,emerged by Pamba Irrigation Project, Pathanamthitta

Sl. No	Genus	Index	Pre-Monsoon	Post-Monsoon
1	Ankistrodesmus	4	-	+
2	Closterium	1	-	+
3	Cyclotella	5	+	-
4	Euglena	1	+	+
5	Melosira	1	+	-
6	Navicula	3	+	+
7	Nitzschia	3	+	-
8	Oscillatoria	5	+	+
9	Phacus	2	-	+
10	Scenedesmus	4	+	+
	Total		22	20

3.3 Nygaard`s Trophic State Index: The calculated values ofMyxophyceae, Chlorophycean, Diatoms, Euglenophycean and Compound indices are 0.439, 0.857, 0.2, 0.333 and 1.857 respectively. The calculated values of Myxophyceae and Diatoms showed oligotrophic nature, while other quotients clearly demonstrated eutrophication in the pond (Table3).

Table 3: Nygaard `s tropic state index of Algal Genera recorded from a pond ecosystem at Keekozhoor, emerged by Pamba Irrigation Project, Pathanamthitta

Sl. No	Index	Calculated Value	Nature
1	Myxophyceae	0.439	Oligotrophic
2	Chlorophycean	0.857	Eutrophic
3	Diatoms	0.2	Oligotrophic
4	Euglenophyte	0.333	Eutrophic
5	Compound	1.857	Eutrophic

ZOOPLANKTON ANALYSIS

3.4 Zooplankton composition in Keekozhoor Pond:

Among the identified zooplankton, Protozoans comprised the most dominating group in both the seasons; of which 16.67% Ciliates, followed by amoeba and Cladocera (33.33%), Tardigrada (16.67%) in pre monsoon and 83.33%Ciliates followed by 16.67% amoeba in post monsoon (Table 4 and Figure 2). Protozoa was represented by Oxytricha,Paramecium, Stylonichia, Vorticella, Stentor, Urostyla which

comes under Ciliates. Arcella, Centropyxis and Difflugia come under Amoebae. Cladocerashaped the second most abundant group of zooplankton and was represented by Daphnia sp, Macrothrixsp and tardigrade by Dactylobiotusschuster.

Table 4: Distribution of Zooplankton in a pond ecosystem at Keekozhoor, emerged by Pamba Irrigation Project, Pathanamthitta

Sl. No	Group	Zooplanktons	Pre-Monsoon	Post- Monsoon
1	Ciliates	Oxytricha	-	+
2		Paramecium	-	+
3		Stentor	+	-
4		Stylonychia	-	+
5		Urostyla	-	+
6		Vorticella	-	+
7	Amoebae	Arcella	-	+
8		Centropyxis	+	-
9		Difflugia	+	-
10	Cladocera	Daphnia	+	-
11		Macrothrix	+	-
12	Tardigrade	Dactyobiotus	+	-
Total no: of species			6	6

(a) Protozoa: They were found to remain high in post-monsoon and low in pre-monsoon seasons. The ascent in protozoan population during post-monsoon season could be related to the fact that the monsoon rain downpours bring a lot of organic matter from the catchment areas which have a huge number of bacteria and accordingly go about as a wellspring of nourishment for protozoans.

(b) Cladocera: After attaining minimum value in pre-monsoon, the Cladoceran density recorded a rise in the post-monsoon season which might be because of variations in pH, luxuriant growth of phytoplankton, favourable temperature and availability of abundant food in the form of bacteria, nanoplankton and suspended detritus.

(c) Tardigrade: Dactylobiotus Schuster was reported from the pre-monsoon period. The literature on tardigrades is relatively limited. Living in various habitats, tardigrades play major roles as consumers and decomposers in trophic networks of terrestrial and fresh water environments and can endure extreme climatic conditions [27].

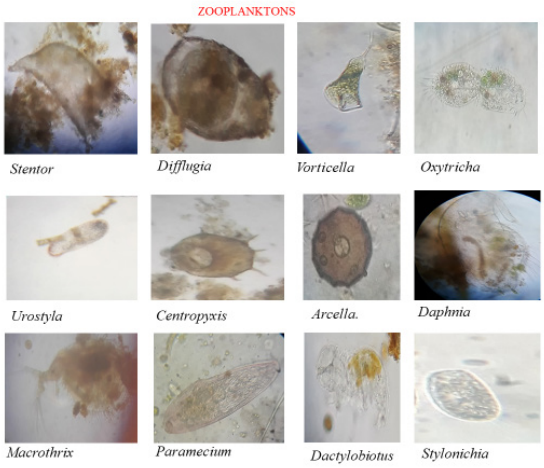


Figure 2:Distribution of Zooplankton in a pond ecosystem at Keekozhoor, emerged by Pamba Irrigation Project, Pathanamthitta

3.5 Analysis of physicochemical parameters:

The Water Quality Index was used to aggregate diverse parameters and their dimensions into a solitary score, showing an image of the water quality of Keekozhoor panchayath. Parameters viz. dissolved oxygen, pH, Sulphate, Phosphate, Nitrite and Chloride were chosen for computing WQI. The physico-chemical environment is critical for the maintenance of the structure and function of the water body. Seasonal variations of physico-chemical parameters in Keekozhoor pond, Pathanamthitta during March-April (pre-monsoon) to May-June 2018 (post-monsoon) are given in Table 5. The drinking water standards and unit weight of each parameter are given in Table 6. The water quality rating (qn) and the calculated sub index (qnwn) values are given in Tables 7 and 8.

Table 5: Seasonal variations of physico-chemical parameters during March-April (pre-monsoon) to May-June 2018 (post-monsoon) recorded from a pond ecosystem a tKeekozhoor, emerged by Pamba Irrigation Project, Pathanamthitta

Physico-Chemical Parameters	Pre-Monsoon Mean± SD	Post-Monsoon Mean± SD
pH	6.57±0.21a	6.92±0.09a
Dissolved Oxygen	3.47±0.16a	3.51±0.1a
Nitrite	5.23±0.15a	3.13±0.15b
Sulphate	0.43±0.08a	0.16±0.03b
Chloride	24.08±0.16a	15.53±0.24b
Phosphate	0.12±0.02a	0.03±0.01a

Along the row, the same superscript letters denote no significance ($p < 0.05$).The statistical analysis of physico-chemical parameters by paired t-test was demonstrated that aside from the estimations for the values of pH, phosphate and dissolved oxygen, other parameters indicated significant differences. The nitrite, sulphate and chloride content were found significantly higher in the pre monsoon period.

Table 6: The drinking water standards and unit weight of each parameter (all values except pH are in mg/l) as per the BIS standards

Sl. No	Parameters	Standards (Sn)	Permissible Values	Unit Weights (Wn)
1	pH	6.5-8.5	7	0.11
2	Dissolved oxygen	5	14.6	0.07
3	Nitrite	3	0.2	1.001
4	Sulphate	200	400	0.0074
5	Chloride	250	1000	0.0074
6	Phosphate	0.025	0.1	1

Table 7: Water quality (qn) and calculated sub index (qnwn) values of different parameters at the siteduring the pre- monsoon and post-monsoon period recorded from a pond ecosystem at Keekozhoor, emerged by Pamba Irrigation Project, Pathanamthitta

Sl. No	Parameters	Pre-Monsoon		Post-Monsoon	
		qn	qnwn	qn	qnwn
1	pH	23.33	2.56	1.33	0.14
2	Dissolved oxygen	114.37	8.00	111.45	7.80
3	Nitrite	182.14	182.32	100	100.10
4	Sulphate	199.78	1.47	199.92	1.47
5	Chloride	130.12	0.96	131.25	0.97
6	Phosphate	26.66	26.66	80	80
Total			222.00		190.49

In this study, the water quality index of pond water samples is found in the range of 86.733 (post-monsoon) to 101.105(pre- monsoon) (table-8). Water Quality Index of water samples depicted in table 8, which was compared to the standard water quality status. This shows that the water sample belongs to the poor category. Along these lines, it tends to be assigned as not reasonable for domestic or irrigation purposes.

Table 8: WQI during the pre-monsoon and post-monsoon period (WQI=∑qnwn/∑wn) calculated using weighted arithmetic index method

Seasons	Water Quality Index (WQI)
Pre-monsoon	101.11a
Post-monsoon	86.73b

Along the column, the same superscript letters denote no significance ($p < 0.05$).

Table 9: Pearson`s correlation coefficient between physico-chemical parameters and various zooplankton groups

Physico-chemical parameters	Zooplankton correlation during pre-monsoon	Zooplanktoncorrelation during post-monsoon
pH	0.998633*	0.488719*
Dissolved Oxygen	0.888527*	-0.88509
Nitrite	0.000148*	-0.8661
Sulphate	-0.67574	-0.8661
Chloride	-1	-0.19952
Phosphate	-0.75583	0.755832*

The correlation coefficient of various physico-chemical parameters and zooplankton groups shows their dependence with one another. It was concluded statistically by Pearson's correlation coefficient analysis, that the physico-chemical parameters such as pH, Dissolved Oxygen and nitrite demonstrated significant positive correlation during the pre-monsoon period whereas pH and phosphate

demonstrated significant positive correlation during the post-monsoon period (Table 9).

(a) pH: For the situation of Keekozhoor pond, the average pH value observed during the investigation time frame was 6.57±0.21 during the pre-monsoon and 6.92±0.09 during the post-monsoon. The norms of pH lower 4.5 and greater than 9.5 as described by RAMP [28] are commonly unsafe to aquatic existence of living beings which demonstrates that the pH of our investigation pond isn't at all perilous to aquatic life. Along these lines, the slight variations in pH worth might be achieved by the downpour during the post-monsoon period. According to Kurbatova [29], the pH extends somewhere in the range of 6.0 and 8.5 shows medium productive nature of a reservoir; more than 8.5 highly productive and under 6.0 low productive nature of a reservoir which demonstrates that our investigation pond has a medium production of plankton population.

(b) Chloride: Temporal patterns of chlorides content in water demonstrated that most extreme chloride content was registered in pre-monsoon (24.08±0.16) contrasted with post- monsoon (15.53±0.24) might be because of high pace of dissipation and organic waste of animal origin. The base estimation of chloride in the post-monsoon period might be because of weakening of pond water by downpour. Thefindings are in agreement with Khabadeetal; Verma & Prakash; Mishra et al. [30,31,32].

(c) Dissolved Oxygen: Dissolved oxygen is a significant parameter of the wetland which is basic to the metabolism of all aquatic organisms that possess aerobic respiration [33]. It as a rule mirrors the physical and biological processes prevailing in water. Additionally, Dissolved oxygen is an important parameter which increases the favourable condition of algal growth during the investigation time frame. In summer (pre-monsoon) with the increase in water temperature, there was reduction in Dissolved oxygen (3.47±0.16), whereas in post-monsoon due to decrease in temperature, the level of Dissolved oxygen increased (3.51±0.1). These results were in conformity with Masood & Krishnamurthy and Srivastava et al.[34,35].

(d) Sulphate: Low estimations of sulphate have been seen in all the sites during the whole investigation time frame,0.43±0.08 during pre-monsoon and 0.16±0.03 during post-monsoon. There is just a slight difference in sulphate concentration between the two periods which might be because of the rot of leaves or other planktons, affidavit of residential squanders and presence of gypsum rocks. Biological oxidation of reduced sulphur species to sulphate likewise increases its concentration [36]. Downpour water has very high concentration of sulphate especially in the zones with high climatic contamination. Analysing the sulphate concentration in the post-monsoon period, values are seen as comparatively low indicating low atmospheric pollution.

(e) Nitrite: The values of nitrite in the present study ranged from 5.23±0.15 mg/l during the pre-monsoon and 3.13±0.15 mg/l during the post-monsoon period.The maximum value was recorded during the pre-monsoon period which might be because of natural squanders, domesticated animals activities, surface spill over and sewage release and the minimum value during the post- monsoon are because of high vegetation that bolsters the growth of plankton [37]. The pH estimations of pre-monsoon and post-monsoon are seen as inversely proportional to the nitrite estimations of similar periods. A comparable outcome was likewise seen by Mayyavan [38].

(f) Phosphate: Phosphate content was less and the greater part of the period it was found underneath the distinguishable level. In the present study, the maximum value was recorded during the pre-monsoon period and least during the post-monsoon. The estimations of phosphate ranged from 0.12±0.02 mg/l during the pre-monsoon because of the high pace of algal growth, aquatic plants growth and rot of vegetation and 0.03±0.01 mg/l during post-monsoon. The estimation of phosphate brought down in the winter season because of expanded take-up of phosphate for the luxuriant growth of macrophytes [39].

4 Conclusion

The present investigation generated important baseline data on the pollution status and plankton community structure of Keekozhoor pond. Pollution indices such as Palmer`s index provides useful information about the pollution load in the water bodies. Calculation of pollution indices showed that the water body is highly organic polluted due to the presence of some algal groups. Zooplanktons are sensitive to environmental changes and their dissemination fluctuates extensively concerning seasons, water quality and nutrient concentrations. Analysis and interpretation of the data on zooplankton and water quality parameters gave the fundamental data to survey the impact of anthropogenic impacts on the hydrobiology of the pond. The present study provides an insight into the distribution, abundance, diversity and ecology of planktons in the selected pond. The current examination was embraced to characterize the quality of water samples with special reference to physicochemical properties to decide its WQI. The water quality indices (WQI) were in the range 86.733-101.105, demonstrating poor water quality in the study area. The results demonstrated that the estimations of physico-chemical parameters were responsible for the diverse group of plankton in the pond. High species assorted variety in the site portrays the favourable conditions in terms of physicochemical conditions and food at all the sites. We can conclude that the pond is moving toward eutrophication and is organically polluted. This study can offer the essential data for the authority to secure and monitor these small water bodies. Consequently, the water body must be protected for their expected use, a reasonable and all-encompassing administration arranging is fundamental for conservation of this pond.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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05

Antimicrobial activity of crude extract of Curcuma longa and Centella asistica against standard microbial strains and antibiotics

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ABSTRACT

Traditionally medicinal plants are regarded as the rich source active components of remedies for many diseases. Most of the medicinal plants were highly nutritional and can be used for effectively treating many diseases. So the good knowledge of active principles of these medicinal plants is important. In the present study two ethnic indigenous medicinal plants such as ; Curcuma longa and Centella asisitica were selected. The objective of the study was to test antibacterial activity of the selected plants using standard protocol of Disc Diffusion Method (DDM). For this the crude extract of the both plants were collected. The main bacteria selected for the study were Salmonella, S.aureus,V. cholerae, B.subtili, P.fluroscence. The tested antibiotics were Amphicilin, Tetracyclin, Pencillin, Srteptomycin and Ciprofloxacin. When comparing the antibacterial activity of these two medicinal plants; Centella asiatica and Curcuma longa ; it is revealed that the crude extract of Centella asistia showed more promising effects. The antibacterial activities were assessed by the presence or absence of inhibition zones .The samples were also subjected to antifungal activity. The main fungal strains under study were Aspergillus flavus and Candida albicans .But the antifungal activity was nil. So the two plants can be recommended for antibacterial processes.

KEYWORDS: **in vitro, antimicrobial activity, Curcuma longa, Centella asiatica, disc diffusion method.**

1 Introduction

In Indian traditional system of medicine, the part of medicinal plants was very important. They possessvarious pharmacological properties and possess various active constituents such as carbohydrates, steroids, alkaloids, flavonoids, glycosides, starch, tannins, and phenolic compounds [1]. Many infectious diseases are known to be treated with herbal remedies. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries [2].The screening of active components of the medicinal plants has been of great interest to scientist for decades. A number of reports concerning the antimicrobial screening of medicinal plants have appeared in the literatures. India's ancient civilization is greatly merged with the goodness of medicinal plants. Most of the ancient books of ayurveda are the treasure knowledge and basis of most of the modern medicine. Charaka Samhita, Ashtanga Hridayam, Sushruta Samhita etc are great classics of ayurveda. Ayurvedic plants play a major role in the human health care around the world. India

has very rich resources of medicinal plants [3,4,5].The concept of ayurveda appeared and developed between 2500 and 5000 BC in India. Literal meaning of ayurveda is "science of life". Medicinal plants are listed in various indigenous systems such as siddha-600, ayurveda-700, amchi-600,unani-700,allopathy-30 in disease management [6].Most of the medicinal property of these herbs is due the presence of secondary metabolites. Recent research is identifying more and more primary roles for these chemicals in plants as signals, antioxidants , and other functions, so "secondary" may not be an accurate description in the future [7]. Most pharmaceuticals are based on plant chemical structures, and secondary metabolites are widely used for recreation and stimulation [8].Curcuma longa and Centella asiatica were two ethnic plants used traditionally in the ailments of many diseases.The main curcuminods were Curcumin, demethoxy curcumin, bisdemethoxycurcumin. Centella asiatica is also native to wetlands of Asian countries with numerous pharmacological importances. It contains numerous terpenoids such as asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose, asiatic, brahmic, centellic and madecassic acids [9]. Asiaticoside and Medacassoside are with excellent wound healing property and have high antioxidant properties [10].Centella asiatica is regarded as the main ingredients in many of the diseases related to nervous sytem. [11] extracted Medecossoside, asiaticoside and Asiatic acid from extract and raw material of C.asiatica. The present study was to screen the antimicrobial of two indigenous medicinal plants of Kerala; such as Centella assitica and Curcuma longa. The antibacterial activity of the selected plants were done using standard protocol of Disc Diffusion Method (DDM). For this the crude extract of the both plants were collected. The main bacteria selected for the study were Salmonella, S.aureus, V.cholerae, B.subtili, P.fluroscence. The tested antibiotics under study were Amphicilin, Tetracyclin, Pencillin, Srteptomycin and Ciprofloxacin. The samples were subjected to antifungal activity. The main fungal strains under study were Aspergillus flavus and Candida albicans.

2 Materials and Methods

Table.1.Shows the brief description of medicinal plans tested for antimicrobial activity.

Collection and processing of plant material

Tubers of Curcuma longa (Zingiberaceae family) and whole plant of Centella asiatica (Apiaceae family) except root were collected from the local stations of Kollam district, Kerala. The collected samples have been identified by Pankaja Kasthuri Ayurveda Research centre, Kattakkada, Trivandrum and samples deposited in herbarium. The rhizomes of Curcuma longa were taken for processing, all debris were washed out, cut into small pieces. Centella asiatica were washed thoroughly to remove the mud and debris. After cutting out the roots, the creepers were chopped into small pieces. Both the samples were allowed for drying in shade. Air dried samples were powdered by electronic mill.

Preparation of extract

The shade dried samples were crushed to get 200 g powder sample and successively extracted with 150 ml of methane in a Soxhlet extractor for 18-20 hrs. Furthermore, the extract was filtered and excess solvent was evaporated using a rotary evaporator.

Preliminary screening techniques[12]

Methanolic extract of sample were prepared in order to process for obtaining major chemical constituents.

Test for carbohydrates

Molisch's test: Dissolved small quantity of 300mg alcoholic and dried extract powder separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution brown color indicated the presence of carbohydrate.

Test for Phenols

Phosphomolybdic acid test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.Blue coloration of the spot indicated the presence of phenols.

Test for flavanoids

Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drug.

Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins

Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for alkaloids

Draggendorf's test: A drop of extract was spotted on a small piece of pre coated TLC plate and the plate was sprayed with modified Draggendorf's reagent. Orange coloration of the spot indicated the presence of alkaloids.

Tests for Glycosides

Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitro prusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.A1cm layer of foam formation indicates the presence of Saponins

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper. Oil stains on the paper indicated the presence of fixed oils.

Plant extracts dilution and preparation of impregnated disc

Plant extracts were diluted in DMSO in a serial two fold dilution across a 96-well plate starting from 200 mg/ml. The concentration was then further diluted to 16 fold in water correspondingly. The final concentration used for the test was from 1 mg/disc to 0.002 mg/disc. The impregnated discs were dried in 37°C incubator for 18 to 24 hours and immediately used for the sensitivity test.

Bacteria Culture

Prior to sensitivity testing, each of the bacteria strains were cultured into blood agar plate and incubated for 18 to 24 hours at 37oC. A single colony was then cultured in 5 ml Mueller Hinton Broth for 4 hours at 37oC. The density of bacteria culture required for the test was adjusted to 0.5 McFarland standard, (1.0 x 108 CFU/ml) measured using the Turbidometer (Oxoid, UK).

Fungal culture

Two fungi Aspergillus flavus,Candida albicans were used.Fungal inoculum was prepared from a week old culture on potato dextrose agar.The fungal spores was scraped from the mother culture and dispensed in sterilized distilled water. Then the spore density was adjusted spectrophotometrically to obtain approximately 105 spores/ml final concentration. Then the inoculums was used for antifungal assays [13].Antifungal activity of solvent extracts was determined using a modified Kirby Bauer disc diffusion method. Briefly, 100 µl of the test fungi was spread into Potato agar plates respectively. The solvent extracts were loaded to the sterilized sterile 6 mm discs, allowed to dry and then the impregnated discs with 50 µl (100 mgml-1 concentration) onto the inoculated plates. The plates were allowed to stand at 4ºC for 2 h before incubation with the test microbial agents. The diameters of the inhibition zones were measured in mm. All the assays were done in triplicate and the results were given in mean ± SD.

Disc Diffusion Method

For testing the antimicrobial test disc diffusion method was carried out[14]. A bacteria culture (which has been adjusted to 0.5 McFarland standard), was used to lawn Muller Hinton agar plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of plant extracts were placed on the Mueller-Hinton agar surface. Each test plate comprises of three discs. One positive control, which is a standard commercial antibiotic disc and two treated discs.The standard antibiotic discs were Salmonella 30 µg and S. aureus 30 µgand V.cholerae 30 µgrespectively, B.subtilis 30 µg P.fluroscence 30µg were used. Besides the controls, each plate had four treated discs placed about equidistance to each other. The plate was then incubated at 37°C for 18 to 24 hours depending on the species of bacteria used in the test. After the incubation, the plates were examined for inhibition zone. The inhibition zone was then measured using calipers and recorded. The test was repeated three times to ensure reliability.

Determination of zone of inhibition (ZIB)

A qualitative method was used to measure antimicrobial resistance activity of plant extracts. Single strain is spread over an agar plate using a sterile swab, and then incubated in the presence of the plant extract. If strain is susceptible to the antimicrobial agent, then a zone of inhibition appears on agar plate after it is incubated for 18-24 h. The diameter of the zone of inhibition is usually related to level of antimicrobial activity present in the plant extract. A larger zone of inhibition usually means that the antimicrobial is more potent.

Statistical analysis

The antimicrobial activities were analyzed using one-way analysis of variance between the methanol extract of two plants and standard drug by SPSS version 4. Significant differences were determined by Turkey's range test.

3 Result and Discussion

Table 2. Shows the main class of compound present in the two medicinal plants. Only C.longa has carbohydrates. Both plants contain phenols. Both plants are rich in flavanoids.C.longa has tannins; not present in C.asiatica. Both plants contain alkaloids. Glycosides present in C.asiatica not in

C.longa. Saponins are found in C.longa not in C.asiatica. Both plants has anthraquinones. Amino acid is not present in both plants. Oils present in C.longa not in C.asiatica.Table.3 and figure.1. Shows the antibacterial activity (Zone of inhibition) of Curcuma longa and Centella asitica on 5 different bacterial strains such as Salmonella, S.aureus, V.cholerae, B.subtilis and P.fluroscence. There were no antibacterial activity were noted in Salmonella and S.aureus. More area of zone wee obtained for B.subtilis(1.0 cm).in the case of V.cholerae and P.fluroscence equal zone were obtained(1.0 cm).when we come to the C. asiatica the activity is more compared to the C.longa. In Salmonella the zone o inhibition was 1.2 cm. In S.aureus the zone is 1.7 cm. in V.cholerae showed highest zone of inhibition it's about 2.0cm. In B.subtilis the zone is about 1.6 cm and in P.fluroscencezone is 1.4cm.Table.4.shows the zone of inhibition of antibiotics on different pathogen. The antibiotics used were Amiphicillin, Tetracycline, Pencillin G, Streptomycin and Ciprofloxacin. Better zone of inhibition were obtained in the Amphicillin against the pathogen Salmonella (4.5cm). Least zone was obtained in the Tetracycline against V.cholerae (1.7 cm).Table 5. showed the antifungal activity of the two medicinal plants with fungal strains. Unfortunately there were no inhibition zone developed for the Aspergillus flavus and Candida albicans.

The present study was to carry out a preliminary investigation on the antibacterial activity of Curcuma longa and Centella asiatica. The plant extracts were checked for and antibacterial and antifungal activity. But the plants showed more antibacterial activity than the antifungal activity. The fungal species were Aspergillus flavus and Candida albicans. The bacterial species selected were Amiphicillin, Tetracycline, Pencillin G, Streptomycin and Ciprofloxacin.Cente lla asiatica showed more antibacterial activity than that of the Curcuma longa. In the case of Curcuma longa Amiphicillin and Tetracycline didn't shown any antibacterial activity. Otherwise all other strains showed better antimicrobial activity. There numerous works are there dealing with the antimicrobial activities traditional medicinal plants[15]. Medicinal plants were regarded as the pool of active ingredients can be used to treat many diseases. [15] investigated the antibacterial and antifungal potential of three Indian medicinal plants. Antimicrobial activity of benzene extracts of three plants namely Abutilon indicum, Plectranthus amboinicus, and Aegle marmelos were determined using agar disc diffusion method at different concentration from 5 to 30 µg/µl against two Gram-positive Staphylococcus aureus, Enterococcus faecalis and two fungal strains Aspergillus niger, Aspergillus fumigatus and compared with standard drugs norfloxacin and fluconazole, respectively. Benzene extract of fruits from A. indicum inhibited S. aureus, E. faecalis at 30 µg/µl, and leaves of P. amboinicus showed considerable inhibiting activity against the A. niger, A. fumigatus at 30 µg/µl.

Curcuma longa and Centella asiatica is one of them with promising medicinal impacts. Both of the plants can be used as food and medicine. C.longa is a common ingredient in the south Indian dishes. In a work by [16] different solvent extracts viz., petroleum ether, chloroform, ethyl acetate and methanol of the medicinal plant Toona ciliata (leaf and flower) were evaluated for photochemical analysis, antimicrobial and antioxidant activities. The study revealed the presence of carbohydrates, proteins, phytosterols, flavonoids, glycosides, tannins and phenolic compounds. Ethyl acetate and methanol extracts showed moderate activity against test phytopathogenic bacteria compared to tetracycline. [17] tested different fractions obtained from rhizome of Curcuma longa was investigated against standard strain and clinical isolates of Staphylococcus aureus. The clinical isolates were found more sensitive for different fractions, than the standard strain of S. aureus. [18] evaluated the antimicrobial activity of C. longa aqueous extract. The aqueous extract of C. longa exhibited antimicrobial activity against Escherichia coli ATCC 25922, Staphylococcus aureus ATCC25923, Krebsilla pneumoniae ATCC 10031 and Staphylococcus epidermidis ATCC 12228 (MIC = 4-16 g L-1.According to [19] Curcuma longa L. (Zingiberaceae family) and its polyphenolic compound curcumin have been subjected to a variety of antimicrobial investigations due to extensive traditional uses and low side effects.Centella asiatica is a herb commonly used in folk medicine, health supplements, and beauty products[20].[21] tested the antifungal activity of methanolic extracts of Centella asiatica and Andrographis paniculata

leaves was observed against fourteen fungi, viz., Alternaria alternata, A. brassicae, A. brassicicola, A. solan~ A. tenuissima, Cercospora blumae, Curvularia lunata,C. penniseti, and Drechslera monoceras, D. oryzae, D. turitica, Fusarium albizziae and F. udum.

5 Conclusion.

In the present study tested the antimicrobial activity of two indigenous medicinal plants ; Centella assitica and Curcuma longa. The antibacterial activity of the selected plants were done using standard protocol of Disc Diffusion Method (DDM). For this the crude extract of the both plants were collected. The main bacteria selected for the study were Salmonella, S.aureus, V.cholerae, B.subtili, P.fluroscence. The tested antibiotics under study were Amphicilin, Tetracyclin, Pencillin, Srteptomycin and Ciprofloxacin. The samples were subjected to antifungal activity. The main fungal strains under study were Aspergillus flavus and Candida albicans. The preliminary screening of the medicinal plants were also done. Only C.longa has carbohydrates. Both plants contain phenols. Both plants are rich in flavanoids.C.longa has tannins; not present in C.asiatica. Both plants contain alkaloids. Glycosides present in C.asiatica not in C.longa. Saponins are found in C.longa not in C.asiatica. Both plants has anthraquinones. Amino acid is not present in both plants. Oils present in C.longa not in C.asiatica. There were no antibacterial activity were noted in Salmonella and S.aureus. More area of zone was obtained for B.subtilis.In the case ofV.cholerae and P.fluroscence equal zone were obtained.when we come to the C. asiatica the activity is more compared to the C.longa. In Salmonella the zone o inhibition was 1.2 cm. In S.aureus the zone is 1.7 cm. in V.cholerae showed highest zone of inhibition it's about 2.0cm. In B.subtilis the zone is about 1.6 cm and in P.fluroscencezone is 1.4cm.Unfortunately there were no inhibition zone developed for the Aspergillus flavus and Candida albicans.

6 Declarations

6.1 Competing interests

Both authors declare that they have no competing interests.

6.2 Acknowledgments

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No. name	Scientific principles	Active names	Local used	Parts uses	Traditional
1.	Curcuma longa	Curcumin,demethoxy curcumin and bisdemethoxy curcumin, volatile oils, sugars, proteins, and resins.	Manjal (malayalam)	Tuber	Antioxidant, hepatoprotective, antiinflammatory, anticarcinogenic, antimicrobial properties (Yu et.al.,2006). Curcumin enhances immunity, exhibits anti-parasitic, antispasmodic, anti-inflammatory and gastrointestinal effects.
2	Centella Asiatica	asiaticoside, centelloside, madecassoside, brahmoside, brahminoside, thankuniside, sceffoleoside, centellose, asiatic, brahmic, centellic and madecassic acids	Kodangal (Malayalam)	Whole plant without root	This is an effective brain tonic and used for various mental disorders. It is a diuretic and local stimulant especially for the cutaneous system.

Table 1: Medicinal plants tested for antibacterial activity

CLASS OF COMPOUNDS	TESTS PERFORMED	C.longa	C.asiatica
Carbohydrates	Molisch's test	++	-
	Fehling's test	++	-
Phenols	Phosphomolybdic acid test	+++	++
Flavanoids	Shinoda test	++	+++
	Lead acetate test	++	+++
Tannins	Braemer's test	+	-
Alkaloids	Draggendrof's test	+++	++
Glycosides	Legal's test	---	+
Saponins	Foam test	++	-
Anthraquinones	Borntragers test	++	+
Amino acid test	Ninhydrin test	---	---
Fixed oils and fats		++	-

Table 2: Preliminary screening techniques on Curcuma longa And Centella asiatica.

Note: the results for the above experiments are noted as follows

- *If the response to the test is high it is noted as +++which indicates that the particular group is present as the major class.*
- *If the response is average is reported it as ++, indicates the presence in moderate quantity.*
- *If the response is very small then it is + indicating the presence of only in traces.*
- *If no response is then negative.*

Sl.No.	Bacterial strains	Zone of inhibition (cm)	
		Curcuma longa	Centella asiatica
1	Salmonella	NZ	1.2 cm
2	S.aureus	NZ	1.7 cm
3	V.cholerae	1.0 cm	2.0 cm
4	B.subtilis	1.2 cm	1.6 cm
5	P.fluroscence	1.0 cm	1.4 cm

Table.3. Table showing Zone of Inhibition of medicinal plants on different pathogens.

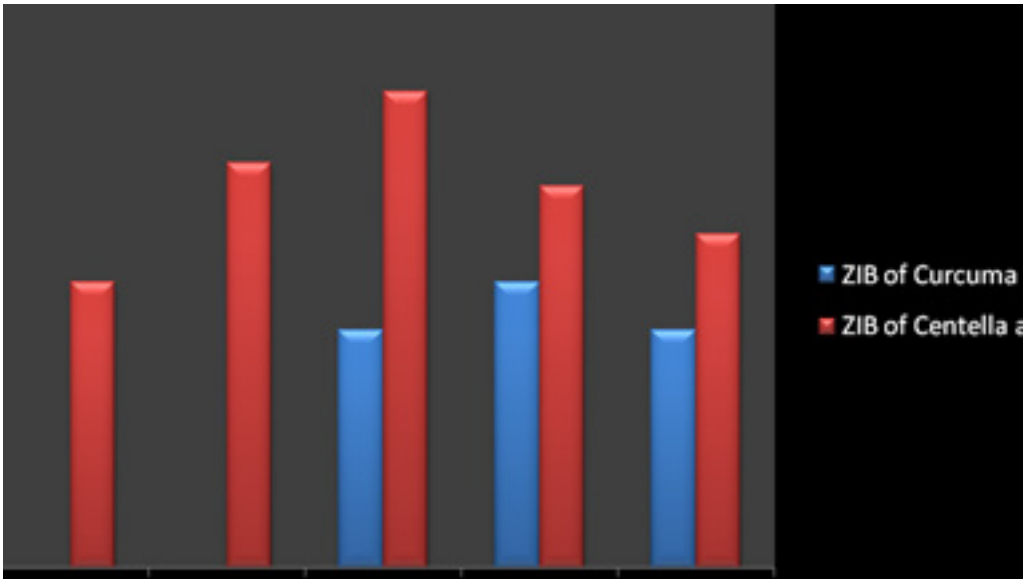

Figure.1: Zone of Inhibition of medicinal plants with different pathogens.

Sl. No.	Sample used	Pathogenic organisms				
		Salmonella	S.aureus	V.cholerae	B.subtilis	p.fluroscence
1.	Antibiotics used and zone developed.	Amiphicillin 4.5 cm	Tetracycline 2.2 cm	Pencillin G 1.7 cm	Streptomycin 2.0 cm	Ciprofloxacin 2.0 cm

Table.4: Table showing Zone of Inhibition of antibiotics on different pathogens

Sl.No.	Fungal strains	Zone of inhibition(cm)	
		Curcuma longa	Centella asiatica
1	Aspergillus flavus	NZ	NZ
2	Candida albicans	NZ	NZ

Table.5: Table showing Zone of inhibition of medicinal plants against different Fungal strains

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06

Green Synthesis of Nano Particles from Economically Viable Plant Material

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ABSTRACT

Nanomaterials are the keystone of nanotechnology and a nano science. The material properties of nano structure are different from the bulk due to high surface area over volume ratio and the possible appearance of quantum effects at the nano scale. There are several methods for creating nanoparticles, including co-precipitation, hydrothermal synthesis, inert gas condensation, ion sputtering scattering, micro-emulsion, microwave, pulse laser ablation, sol-gel, sonochemical, spark discharge, template synthesis, and biological synthesis. Biological synthesis involves the synthesis of nanoparticles by using plant materials such as root, fruit, stem, seed or leaf. The present study focused on the preparation of iron oxide nanoparticles from neem leaf extract. The prepared nano powder was collected and used for further characterisations such as particle size analysis, XRF and XRD. The studies revealed that the synthesized particles were of nanosized and of good quality.

KEYWORDS: **Nanomaterials, Iron oxide, Biological Synthesis, Plant extract.**

1 Introduction

Nano science is a wide area of research that has been growing worldwide from the last few years. Nanomaterials are of important because of their unique magnetic, electric and other properties. These properties have the great impact in industry, machinery and in other fields. The unique properties of nano structured materials are due to their high surface area to volume ratio. Nanomaterials comprise nearly all class of substances like metals, metal oxide, biomolecules etc. They possess high strength, enhanced activity and reduced thermal conductivity. There were literature showing the synthesis of different nano particles for different applications [1-3]. Synthesis of nanoparticles from biomaterials reduce the pollution and reduce the cost. Studies were available for the synthesis of nanoparticles from biomaterials [4-6]. The present study focused on the preparation of iron oxide nanoparticles from neem leaf extract. Basically, this process involves the precipitation of Fe2 + and Fe3 + from aqueous salts solutions (e.g., chlorides, sulphates, and nitrates) by addition of a base (e.g., NaOH).

2 Research Methodology

2.1. Preparation of neem leaf powder

In this work, locally available healthy leaves of Azadirachta indica (neem leaves) were collected. The collected leaves were washed several times with water to remove dust particles and then air dried at room temperature. Then the dried leaves were crushed into fine powder.

2.2. Preparation of neem leaf extract

5 g of finely ground neem leaf powder was weighed and transferred into a conical flask. It is then mixed with 100 mL distilled water and the mixture was heated in a water bath at constant temperature of 800C. Then it was left to cool at room temperature for 1 day and then filtered using Whatman filter paper to obtain leaf extract. The green clear filtered solution of extract was stored for further studies.

2.3. Preparation of iron oxide (Fe3O4) nanoparticles

0.40 g ferrous chloride and 1.10 g of ferric chloride with 1:2 molar ratios were weighed and transferred into a conical flask.100 mL of distilled water was added to it. After that, the resulting mixture was heated for 10 min in oil bath at constant temperature of 800C to obtain homogeneous solution. Then 5 mL of neem leaf extract was added slowly into the hot mixture. Subsequently, prepared 1 M 20 mL of sodium hydroxide (NaOH) was added in to the reaction mixture drop by drop from burette with vigorous stirring. The instantaneous appearance of the precipitate indicated the formation of Fe3O4 nanoparticles. The resulting mixture was cooled at room temperature. After sometime, the intense black precipitate was filtered using Whatman filter paper and dried in air. The obtained black powder was collected and used for further characterisations. All the chemicals used in this study were of analytical grade.

2.4. Characterisation of the prepared Fe3O4 nanoparticle

Elemental composition analysis of the prepared nanoparticle was carried out using the XRF instrument of Elvatech – Elvax - Maxpro of Ukraine. The XRD analysis was performed using the instrument of made Rigaku, Japan.

3 Results and Discussion

3.1. Elemental Analysis

The prepared neem leaf extract was shown in figure 1.

Fig 1: Prepared neem leaf extract

The formation of iron oxide nanoparticle was revealed from figure 2 shown below.

Fig 2: Prepared iron oxide nanoparticles

Elemental studies from figure 3 revealed the presence of iron particles to about 98%.

ie; the prepared nano particles contain least impurities.

Elemental Composition

At. Num	Element	Series	Intensity	Concentration
26	Fe	K	4051163	98.171 ± 0.022%
25	Mn	K	16856	0.507 ± 0.016%
14	Si	K	21835	0.418 ± 0.009%
82	Pb	L	8242	0.215 ± 0.006%
13	Al	K	3322	0.195 ± 0.018%
50	Sn	K	1597	0.186 ± 0.041%
24	Cr	K	9443	0.185 ± 0.015%
28	Ni	K	1659	0.106 ± 0.014%
29	Cu	K	368	0.016 ± 0.007%
33	As	K	0	< 0.005%

Fig 3: Elemental composition of prepared nano particle

3.2. XRD Analysis

The XRD analysis was shown in figure 4.

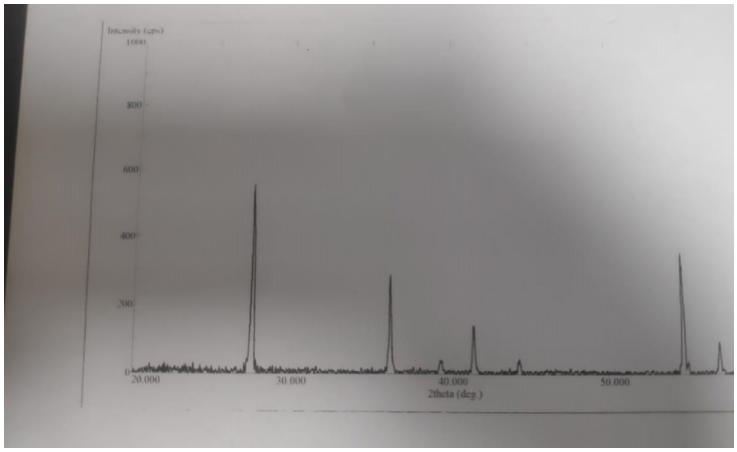

Fig 4: XRD data of the prepared nanoparticle

The peaks were obtained at 2theta values of 290, 370, 400, 420, 440, 520 and 540. These values confirmed the presence of iron oxide particles. The size of the synthesized particles was confirmed from Scherrer equation, $D=K\lambda / \lambda\cos\lambda$ where, D is the size of the particle, K is Scherrer constant (0.94), λ is the X-ray wavelength (1.54178 A0), λ is the full width at half maximum (FWHM) of the diffraction peak. The particle size obtained was 85nm. This confirmed that the synthesized iron oxide particle was of nanometer in size.

4. Conclusion

Nanomaterials are the keystone of nanotechnology and a nano science. The present study focused on the preparation of iron oxide nanoparticles from neem leaf extract. The obtained powder was collected and used for further characterisations. 98% of iron was revealed from the elemental analysis and the particle size obtained was 85nm.

5. Declaration

5.2. Study limitations

This study was a part of the project work and faced shortage of time for doing further investigations of the nanomaterial prepared.

5.3.Acknowledgement

Acknowledgement was given to Department of Chemistry, All Saints' College for providing the facilities for conducting the experiments.

5.4. Funding Source

There is no funding source for doing this work.

5.5. Competing Interest

There is no conflict of interest for this publication.

6. References

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07

Isolation and Characterisation of Amylase Producing Bacteria from Mangrove Soil

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ABSTRACT

Amylase is a digestive enzyme, that could be extracted from different sources such as plants, animals, and microbes. This study was aimed at isolate the amylase producing bacteria from mangrove soil. Traditional serial dilution agar plating method was used for the isolation of amylase producing bacteria. Total six serial dilutions were conducted, from that, 10-3 and 10-4 used for the spread plate method. For the screening of bacteria, selected colonies were placed on to the agar media containing starch. Then the culture was preserved for further protocols. Characterization of the isolated bacteria was done with the help of Bergey's manual. Starch hydrolysis test and Mannitol fermentation test were conducted for the confirmation of amylase producing bacteria. Amylase producing bacteria was isolated from the soil sample. Biochemical tests like Vogues Prosakauer test, Citrate test, Spore staining was also conducted. Amylases are used in microbiology, particularly in process involving starch hydrolysis. The isolates can be used for industrial production of λ-amylase. Microbial enzymes are widely used in industrial processes, due to their productivity and thermostability and λ-amylase is one of the most important industrial enzymes, having applications in industrial processes such as brewing, baking, textiles, pharmaceuticals, starch processing. Microbes like bacteria and fungi are capable of producing amylases but amylases of bactereial origin is preffered over fungi due to their rapid cell growth and fast reproduction.

KEYWORDS: **Amylase, Hydrolysis, Mannitol.**

1 Introduction

The starch degrading enzyme Alpha amylase (λ-1, 4 glucan-glucanohydrolase), is widely distributed in nature which can act as a source for about 30% of the world's enzyme production. This enzyme can hydrolyses λ-1,4 glucosidic linkages present in starch molecule in an endo-fashion forms oligosaccharides and monosaccharides like glucose, maltose and alpha limit dextrin [1,2,3]. Plants, animals and microbes are the different sources amylase enzyme [4]. By acting as an important industrial enzyme amylase can contribute 25% of the enzyme market [4]. Because of their genetic diversity, good enzymatic activity at different physical parameters like extreme pH, temperature, osmolarity, pressure etc, amylases produced from microbes have wide industrial application [6]. One of the important industrial application of amylases is the starch liquefaction [7]. They are widely used in baking and brewing of alcohol [8]. Amylases are the main ingredient for the production of fructose syrups [9]. Another industrial application of amylase includes textile and paper production [10]. There are different sources for amylase production (plants, animals and microorganisms), but amylase obtained from microorganism are considered to be more efficient than any other sources

of amylase production because the method is economical, time saving and consistent [11]. Amylase producing bacteria like Bacillus sp. can be produced from soil samples and can be used in biochemical techniques and starch hydrolysis [12]. Microbes like bacteria and fungi are capable of producing amylases but amylases of bacterial origin is preferred over fungi due to their rapid cell growth and fast reproduction. [13]. Mangroves are complex and dynamic ecosystems varying in salinity, water level and nutrient availability; they also contain diverse and distinct microbial communities. Many marine microorganisms depends on mangroves as their shelter and nurturing sites. Because of the presence of rich source of nutrients mangroves are known as the homeland of microbes. Mangroves are located in the intertidal zone with high salinity so they can tolerate wide range salinities under natural conditions [14]

2. Materials and Methods

Isolation of microbes

Microbes were isolated from the soil sample collected from mangrove area. Traditional serial dilution agar plating method was used for the isolation of amylase producing microbe. The medium used for the isolation of bacteria was nutrient agar with starch at pH 7. The plates were incubated for 48hours at room temperature.

Serial dilution

Label tubes as 10-1,10-2,10-3 up to 10-6. Each tube contains 9mL of sterile water. Prepare 101dilution using appropriate volume of sample (0.1g/1ml). Using a sterile 1ml pipette, transfer 1ml out of the first bottle and add this to the test tube labelled 10-2. Shake well the tubes .by using a new sterile 1ml pipette, transfer 1ml from the 10-2 tube and add it to the 10-3 tube. Repeat the procedure and continue up to 10-6. Inoculate 0.1ml sample from 10-3 and 104 into separate petriplates with medium. Using 'L' shaped glass rod spread the inoculums on the surface. Incubate the plates at room temperature.

Spread plate method

Prepare proper dilutions of the sample. Remove the upper lid of the plate and place about 0.1ml of diluted sample in the centre of the plate. Spread the inoculum over the medium by pushing the glass spreader (L-rod) backward and forward while rotating the plate. Replace the petri dish lid and leave for drying before inverting and incubating. Incubated for 48hours at room temperature.

SCREENING OF BACTERIA

Selected colonies were placed on to agar media containing starch. The plates were incubated for 48 hours. Then the cultures were further inoculated into nutrient broth medium. And the culture was preserved for further protocols.

Gram staining

1. Heat fixes the bacteria smear.
2. Flood the slide with crystal violet staining reagent for one minute.
3. Wash the smear in a gentle and direct stream of tap water for one minute.
4. Flood the slide with Gram Iodine solution for one minute.
5. Wash the slide and decolorize with 95% ethanol for few seconds.
6. Flood the slide with water and then immerses the smear for two minutes in counter stain saffranin
7. Wash the slide, dry and observe under microscope (10X/100X).

Observations were made from cell morphology and color. Gram-positive bacteria will turn purplish blue, while Gram- negative bacteria will turn red.

Vogues proskauer test

This test is used to differentiate the enteric bacteria. These organisms have the capacity to ferment carbohydrates and results in the production of some non-acidic or neutral end products. These products are formed from the organic acids that results from glucose metabolism, which is an important characteristic feature of Enterobacter aerogens.

Citrate test

Citrate test is used to differentiate among enteric bacteria on the basis of their ability to utilize or ferment citrate as the sole carbon source. The utilization of citrate depends on the presence of an enzyme Citrase produced by organisms that breaks down the citrate to oxaloacetic acid and acetic acid. Positive result was indicated by blue colour formation on the line of the organisms. Retention of the original green colour and absence of growth on the line of inoculum indicates negative result.

Spore staining

There are two genes of bacteria that are capable of forming spores. The aerobic spore formers are the genus Bacillus; the anaerobic spore formers are the genus Clostridium. Spore formation is an activity performed by bacteria, not for reproductive purpose, but for survival purposes. The spore formed in the bacteria act as a thick protective wall. By the stage of wall development, these “pre”-spores are called endospores and rest of the bacterium is called sporangium. The wall around the endospore makes it particularly difficult to stain. In order to force a stain to penetrate the endospore wall, it is necessary to heat the slide and then stain. The sporangium is counter-stained for contrast and examined microscopically. The bacterial cells get stained in green due to the presence of spores if Malachite green was added, whereas vegetative cells stained red and lipid granules were unstained.

Mannitol fermentation test

The purpose of this test is to know whether the microbe can ferment the carbohydrate (sugar) mannitol as a carbon source. If mannitol is fermented to produce acid end products, the pH of the medium will drop. A pH indicator in the medium changes colour to indicate acid production. An inoculum from the culture is transferred aseptically to a sterile tube of mannitol broth. The inoculated tube is incubated at 24 hrs and the results are determined. A positive test consists of a colour change yellow, indicating a pH change to acidic.

Starch hydrolysis test

Amylase enzymes are secreted extracellularly by the microorganisms into the medium, which degrade starch primarily into glucose. Hydrolysis of starch can be observed as clear zone around the line of growth. The unhydrolyzed starch gives a blue colour with iodine. Bacterial isolates were screened for amylolytic activity by starch hydrolysis test on starch agar plate. The microbial isolates were streaked on the starch agar plate and incubated at 37°C for 48 hrs. After incubation iodine solution was flooded with dropper for 30 seconds on the starch agar plate. Presence of blue colour around the growth indicates negative result and a clear zone of hydrolysis around the growth indicates positive result. The isolates produced clear zones of hydrolysis were considered as amylase producers and were further investigated.

3. Observation and Result

ISOLATION OF AMYLASE PRODUCING BACTERIA

Upon the performance of serial dilution from soil sample spread plating is done on nutrient agar containing starch.

MACROSCOPICAL EXAMINATION

Fig: 1 Organism produced in nutrient agar plate.

STARCH HYDROLYSIS TEST

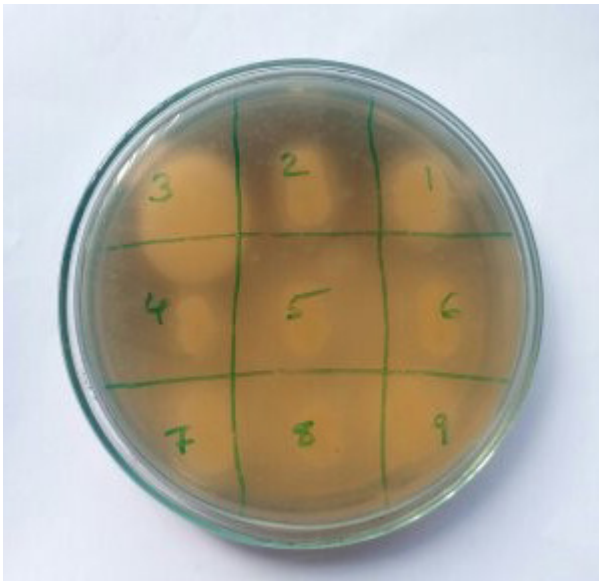

Fig : 2 Organism produced in nutrient agar plate containing starch. Zone of clearance can be seen.

Spot inoculation of pure cultures was carried out on starch agar plate. Bacterial cultures were incubated at 37oC for 24hrs. After incubation the plates were checked for amylase production by addition of gram's iodine solution. The production of amylase was indicated by the clear zone of starch hydrolysis surrounding the colony. Bacteria which have the ability to produce amylase enzymes can form clear zones around bacterial isolates after the addition of iodine solution. These clear zones shows that starch in the media has been degraded by extracellular amylase enzymes produced by bacteria into simple sugars that do not show color reactions with iodine.

MICROSCOPICAL EXAMINATION:

Gram staining

After Gram staining Gram positive bacteria will retain the purple color of t crystal violet, under a microscope it appears purple while Gram negative bacteria cannot maintain the purple color of violet crystals but it can absorb safranin dye on the cell wall and appeared as red

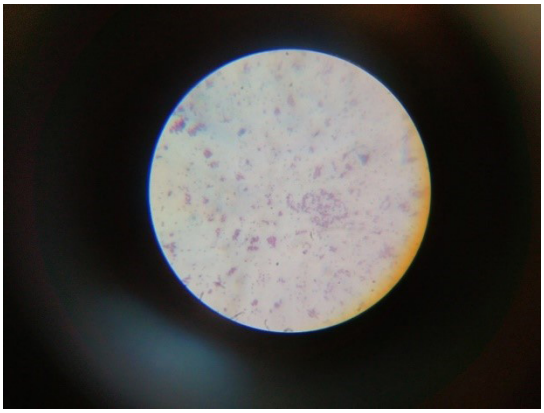

Fig :3 Gram staining showing Gram-positive rod-shaped bacteria.

BIOCHEMICAL TEST

Vogues prosakauer test Citrate test

Vouges Proskauer test – Formation of red colour

Fig: 4: Showing VP Test

Fig :5: Showing Citrate Test.

CITRATE TEST:

Retention of the original green color and absence of growth on the line of inoculums.

Spore staining

The bacterial cells get stained in green due to the presence of spores. Result is positive by the formation of green in bacterial isolates after being viewed under a microscope which shows that the bacteria have endospores.

Mannitol fermentation test

This test consists of a color change yellow, indicating a pH change to acidic.

Fig :7 Showing Mannitol Fermentation Test

BIOCHEMICAL TEST: RESULTS

SL NO	BIOCHEMICAL TEST	OBSERVATION
1	V P Test	Positive
2	Citrate test	Negative
3	Spore staining	Positive
4	Mannitol fermentation	Positive

Table: 1-Results of biochemical test

The main objective of this study was to isolate and characterize the amylase producing bacteria from the mangrove soil. Morphologically all the isolates were colony forming, and all the colonies were gram positive, rod shaped, spore forming organisms. From morphological identification and from biochemical tests (Table:1) it have been tentatively identified that the bacteria present in mangrove soil is Bacillus sp which are able to produce Amylase enzyme.

4. Discussion

The soil sample was collected from the mangrove area and was screened through various screening techniques and finally isolate the amylase producing bacteria. The phenotypic, physiological and biochemical characterization of the soil isolates were performed and the result were noted. All the isolates were found as gram-positive rod-shaped organisms. The isolates were identified as Bacillus species. The importance of amylase can be observed by the great number of published articles recently. In fact, over the last few years, there has been a progressive increase in the number of publications related to the production and industrial application of amylase. Kathiresan & Manivannan (2006) isolated different bacterial strains from the mangrove area. These strains can capable of producing amylase. The strains were identified by morphological and biochemical characters. Growth of the organisms and the enzyme production were measured with varying pH, temperature and various substrate concentrations. The effects of pH, temperature, incubation time, salinity, sources of carbon and nitrogen were tested in submerged fermentation process in production of -amylase by Penicillium fellutanum isolated from coastal mangrove soil.

Amylases could be extracted from different sources such as plant, animal and microbes including bacteria and fungi [15]. These bacteria are screened from natural resources including soil, Biogas plant, Kitchen waste and domestic waste water for its ability to grow on cheap substrates, producing enzymes at high stable rate and no toxic substances. Amylases are among the most important

enzymes used in microbiology, particularly in process involving starch hydrolysis. Though amylases originate from different sources (plants, soil, animals and microorganisms), the microbial amylases are extensively produced and used in industry, due to their productivity and thermo stability.

Microorganism are the most important source of enzyme production, as we know many of enzyme, each with a specific role are required in nature to break down compounds during the biodegradation process[16].

In an interesting way the first enzyme produced industrially was an amylase from a fungal source in 1894, used as a pharmaceutical help for the treatment of Digestive disorders (Pandey et al., 2000). Amylases establish a group of industrial enzymes, which only covers approximately 30% of the enzyme.

In the present investigation amylase producing bacterial strain was isolated from mangrove soil sample. Characteristic feature of the strain indicates that it belongs to the genus Bacillus.

5. Conclusion

In this study amylase enzyme producing bacteria was isolated from mangrove soil. The isolates were identified as Bacillus species and can synthesize amylase enzyme. The amylase enzyme can hydrolyse starch molecule which can be used as important tool in biotechnology. Agriculturally, amylase has been used to develop a more digestible feed for animals. Amylases are known to be produced by a variety of bacteria and fungi and their applications at industrial level have stimulated interest to explore their amylolytic activity in several microbes to be used as bio-resources. Although amylases can extracted from different sources (plants, soil, animals and microorganisms), the bacterial amylases are extensively produced and used in industry, due to their productivity and thermo stability.

6. References

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08

Isolation of laccase-Producing Fungi and its Activity in the Bio-conversion of Textile Dyes

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ABSTRACT

The effluent which is produced by textile industries causes serious problems in the environment and mankind. In the present study, the dye decolorization activity of fungi isolated from decayed wood was monitored. The fungi were cultured on PDA (Potato Dextrose Agar) plates which were supplemented with tannic acid as a substrate for the screening of the laccase-producing fungi. The laccase-positive fungi were morphologically identified and subjected to further analysis. Lignocellulosic material degradation and dye degradation activity of the enzyme were monitored. These degradation properties of laccase lead to its study on the application in textile dyes bioconversion. Due to the laccase dye decolorization activity thus it can be utilized in bioremediation. This is cost-effective and eco-friendly, rendering this technology very attractive.

KEYWORDS: **Laccase, Potato Dextrose Agar, Bioremediation**

1 Introduction

Textile industries produce enormous amounts of contaminated water. Fungi have a strong ability to degrade complex organic compounds by producing extracellular ligninolytic enzymes including laccase. Laccases are enzymes widely distributed in plants, fungi, bacteria and fungi.

Laccase was first described in 1883 from the Japanese lacquer tree Rhus vernicifera [1]. Since then, several laccases have been studied, with respect to their biological function, substrate specificity, copper-binding structures and industrial application. The main purpose of screening is to select fungi with desired characteristics intended for various applications including dye decolorization [2]. The bioremediation process of industrial waste can be made more efficient using laccase enzymes, which are obtained from fungi Laccase have many possible applications in bioremediation nowadays. Bioremediation is the method to destroy or immobilize waste materials with the help of microorganisms [3].

Enzymes are proteins that act as catalysts within the living cells. Various bi-industries require enzymes processing special characteristics for their applications in processing of substrates and raw materials. The special characteristics of enzymes are exploited for their commercial interest and industrial applications.

Environmental pollution with hazardous textile industrial wastes containing recalcitrant xenobiotics has become a major ecological issue. The naturally occurring organic compounds readily degraded up their introduction into the environment. Singh and Gupta [4] examined the microbial effect of laccase and its industrial application. Laccases applied in the removal of large number of hazardous

wastes like alkenes, chlorophenols, dyes, herbicides, benzopyrene, Phenolic pollutants and polycyclic aromatic hydrocarbons from drain water and polluted soil [5].

2. Materials and Methods

2.1 Sample collection and isolation of laccase-producing fungi

Decaying wood samples were collected. The samples were collected in sterile plastic covers and brought to the laboratory without exposing to the external environment for further studies. Decaying wood samples were crushed and directly inoculated onto potato dextrose agar fortified with chloramphenicol (50µg/ml) to prevent bacterial contamination and tannic acid as an indicator for laccase production. The plates were incubated at 24 ˚C for 3 to 5 days. The fungal colonies developed were purified by inoculating each isolate into a fresh potato dextrose agar medium. The cultures were periodically sub-cultured and refrigerated for further use.

2.2 Screening of laccase producers

Screening of Laccase-producing fungus is carried out in potato dextrose agar (pH 5.6) containing 2mM tannic acid as substrate. PDA plates with substrate have to be prepared and sterilized. The organism was inoculated and incubated at 37° C to observe for growth and development of brown-colored precipitate in the tannic acid-containing plate.

2.3 Identification of fungus

The identification of fungus was done on its microscopic, macroscopic and genotypic properties. Morphological characters of the selected fungal cultures were observed. Fungal spores and mycelia were stained by lactophenol cotton blue and observed under light microscope.

2.4 Laccase enzyme production

Inoculate the selected isolate into the production media and incubate in a shaker at 35°C for 48 -72 hours of fermentation period with agitation After termination of fermentation period the fermented broth should be centrifuged at 14000xg for 10 minutes to remove the cells. The clear supernatant thus obtained after centrifugation serves as a crude enzyme source.

2.5 Determination of Laccase activity by enzyme assay

Oxidation of guaiacol is used to measure the enzyme activity of laccase.

2.6 Dye decolorization

Enzyme activity on Methyl red dye can determine by incubating different quantities of laccase with dye solution. For 0.5% methyl red solution made by dissolving 0.125g methyl red dye in 25 ml of distilled water. To conduct the experiment 5 test tubes with 5ml of 0.5% methyl red dye solution were taken and one designated as control, to the other 4 test tubes 0.5 ml,1ml, 1.5ml,2ml of enzyme solution were added respectively. Decolorization of dye was observed regularly. The absorbance of methyl red is 425nm.

Malachite green solution was prepared by adding 16ml malachite green reagent made to one liter using distilled water. 5ml malachite green solution was taken in test tubes and each tubes marked as control, 0.5 ,1 ,1.5, 2 respectively. Control had only dye solution and other test tubes had dye solution with enzymes at different concentration (0.5ml, 1ml,1.5ml,2ml). Decolorization of dye observed at regular intervals and percentage of absorbance can be calculated for the malachite green dye. The disappearance of color by laccase enzyme was monitored at λmax of the respective dye solution. Decolorization was determined as the percentage of absorbance for each dye. Absorbance of malachite green dye was observed in 650nm.

3. Observation and Result

3.1 Isolation and screening of laccase-producing fungi

Fungi colonies were developed on the potato dextrose agar medium (PDA) plates. For screening Laccase-producing fungi, the culture developed were sub-cultured in PDA media with tannic acid as the indicator. The laccase-positive fungi form brown colored zone. (Figure 1 & 2).

White Fungi colonies were observed and they grow the entire plate within 4 days. Using Lactophenol cotton blue staining method cultured fungi identified as Aspergillus flavus and Aspergillus niger which morphological features, conidial heads which were typically radiate, later splitting to form loose columns, biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes were hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia were globose in structure. Mucor genus fungi were also identified in colony that, with sporangiospores, which were simple and form apical, globular sporangia that are supported and elevated by a column-shaped columella. This species were differentiated from other by the shape and insertion of the columella, and the lack of stolons and rhizoids. They form mold with irregular non-septate. (Figure 3,4 &5).

3.2 Decolorization ability of Laccase

The decolorization effect of methyl red using laccase was observed for 3 days at 24 hours intervals. Decolorization potential of laccase showed variation and it depends on the biological source of producing microorganism. Laccase has high efficiency in decolorization of textile dyes

(Table 1 & 2: Figure 6,7,8,9 & 10).

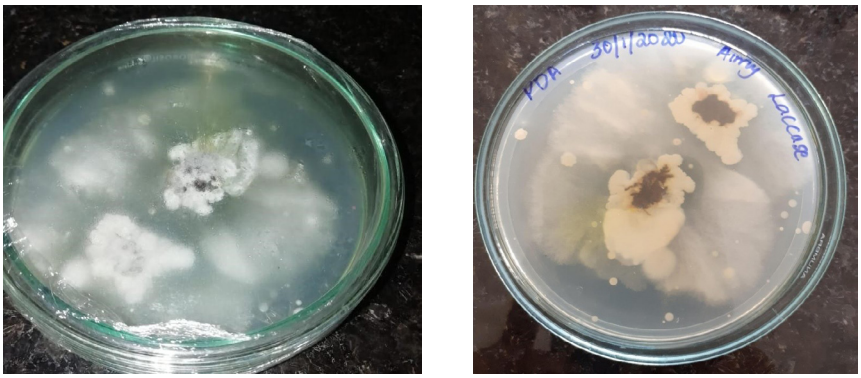
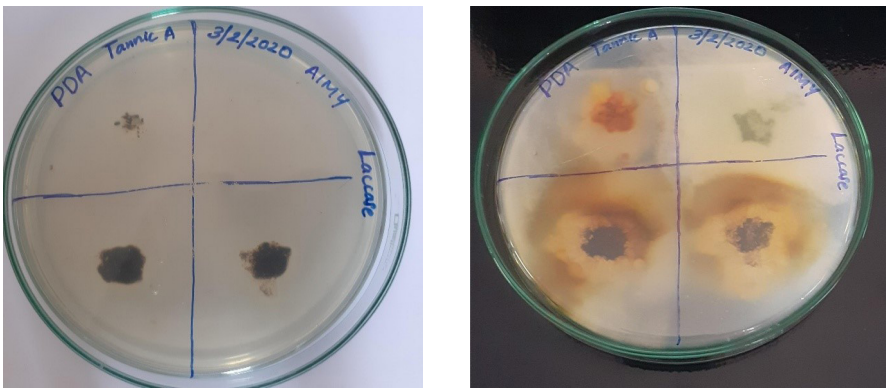
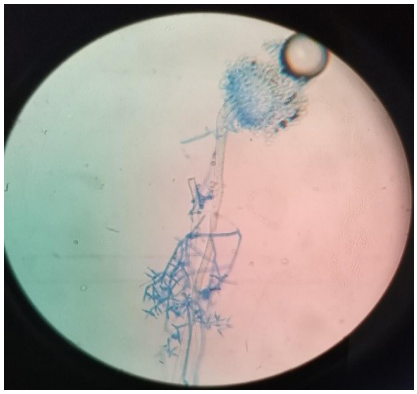


Figure 1. Fungal clnies in PDA cultural medium



Day 1 **Day 4**

Figure 2 Pure Culturing of laccase producing fungi in PDA media with tannic acid



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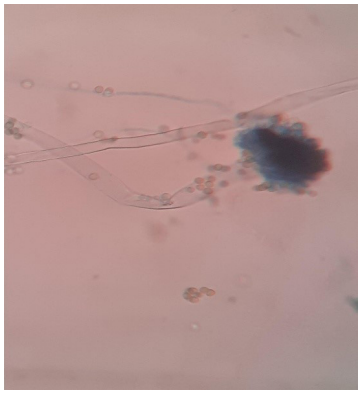

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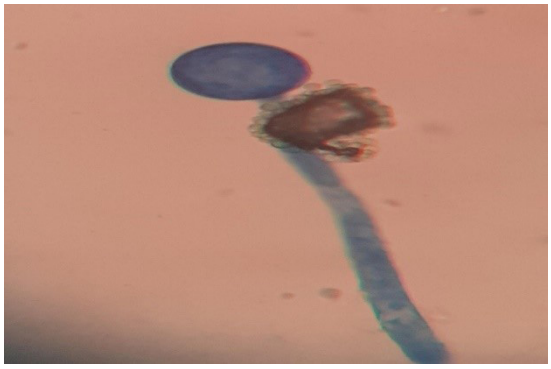

Figure 5

Figure 3,4,& 5: Identification of fungus

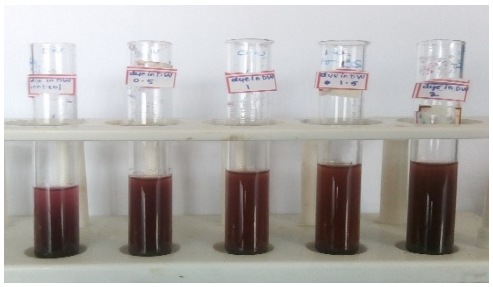

Figure 6

Day 1

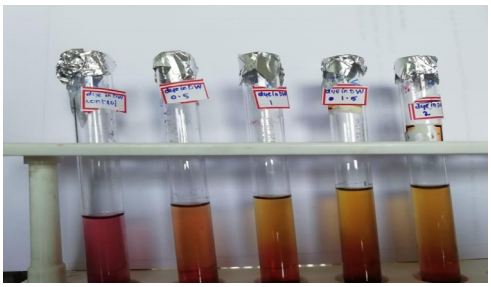

Figure 7

InitialDay 2

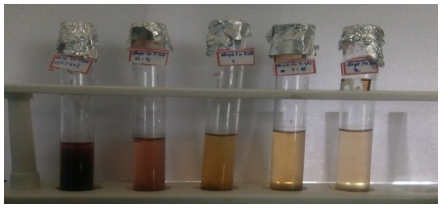

Figure 8

Day 3

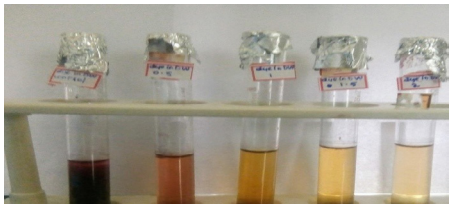

Figure 9

Decolorization of methyl red dye

Figure 6,7, 8 & 9: Application of laccase enzyme (Decolorization of methyl red dye)

Incubation	Absorbance of methyl red at 425nm				
	Control	T0.5	T1	T1.5	T2
Initial	2.5	2.5	2.5	2.5	2.5
Day1	2.410	1.867	1.526	1.323	1.260
Day2	2.301	1.624	0.933	0.802	0.507
Day3	2.286	1.345	0.896	0.672	0398

Table 1 Decolorization of methyl red dye by laccase

Incubation	Percentage of methyl red dye decolourisation(%)				
	Control	T0.5	T1	T1.5	T2
Initial	0	0	0	0	0
Day1	3.6	25.32	38.96	47.08	49.6
Day2	7.96	35.04	62.68	67.92	79.72
Day3	8.56	46.2	64.16	73.12	87.16

Table2 Decolorization percentage of methyl red dye by laccase

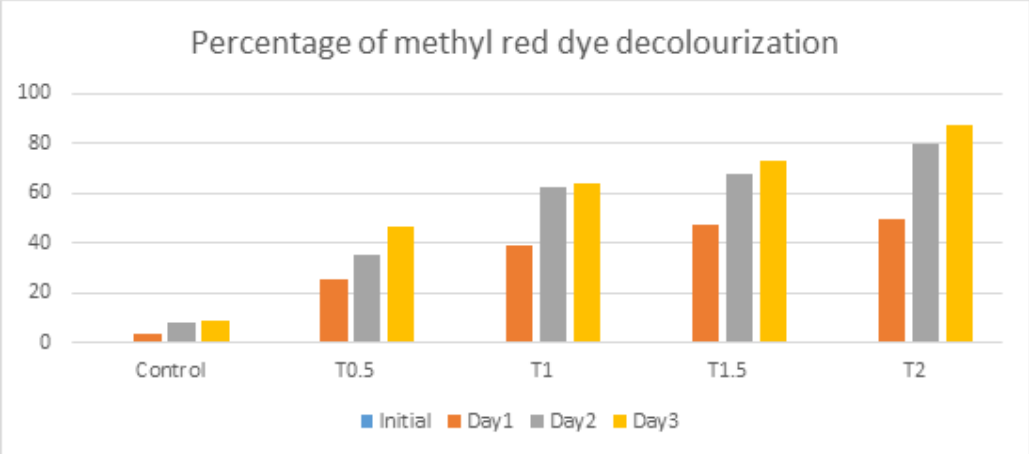

Figure 10. Decolourization percentage of methyl red dye

4. Discussion

Laccases are an interesting group of multicopper enzymes distributed widely in nature. Among the fungi Deuteromycetes, Ascomycetes and a wide range of Basidiomycetes are known producers of laccases, which are exceptionally abundant in many lignin-degrading white-rot fungi [6].

Degrading wood is proved to be a highly interesting source for laccase producers. This is because of the presence of a relatively high concentration of laccase substrate strain lignin. In the present study, laccase-producing fungi were isolated from the declared bark of a tree. It was inoculated on a PDA medium supplemented with tannic acid as a substrate. The brown oxidation zone was developed around the colonies which have the laccase-positive fungi [7].

The laccase-producing fungus is further subjected to morphological identification by continuously

monitoring the spore formation, cultural characteristics, colour, fruiting bodies and spore. The fungal isolated were also stained using the Lactophenol cotton blue staining techniques. [8]. Based on the morphological identification and staining technique it is inferred that the isolated fungus belongs to the Ascomycetes and Zygomycetes. The fungus was identified as Aspergillus flavus, Aspergillus niger, and Mucor mucedo. White rot fungus is one of the main producers of laccase, nevertheless, the brown total fungus is capable of producing laccase too [9]. Aspergillus flavus and Aspergillus niger are brown total fungi which produce laccase with wide industrial and biotechnological applications [10].

Laccase-producing microorganism was subjected to a dye decolorization experiment, it was one of its main applications. Decolorization potential of laccase even on the same dye showed variation and it depends on the biological source of producing microorganism. Moreover, the treatment of dye wastewater has become a matter of great concern and several advanced treatment methods have been suggested. [11]. Due to rapid industrialization and urbanization, a lot of chemicals including dye pigments etc. are extensively used for several industrialization applications. It is an established fact that fungus as biological control agents have a high potential to control specific point pollution and have no effect on the environment [12].

The decolorization effect of methyl red was observed for 3 days at 24-hour intervals. Rodriguez et al., [13] examined the effect of white-rot fungi on the decolorization of 23 industrial dyes. Decolorization of dyes and textile wastewater by various fungal strains [14].5. Conclusion

The laccase-producing fungi isolated from the decayed wood sample has high efficiency in the decolorization of textile dyes and can able to apply in the bioremediation of effluents from the textile industries. The method was eco-friendly and cost-effective.

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09

Phytochemical screening and Antibacterial activity of Artocarpus heterophyllus against Escherichia coli and Staphylococcus aureus.

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ABSTRACT

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Increasing drug resistance of pathogens and negative consequences of antibiotic usage has led to the search for alternative medicines from nature many plants have been exploited to cure infectious diseases from time immemorial. The present investigation evaluated the antimicrobial and phytochemical properties of Artocarpus heterophyllus seed extracts. Distilled water and methanol are used as extraction solvents and test organisms were E. coli and Staphylococcus aureus. Phytochemical screening was carried out by specific chemical identification tests. Phytochemical screening confirmed the presence of carbohydrates, protein, amino acids, alkaloid, terpenoid, saponin, phenol, flavonoid, anthocyanin, steroid and glycoside. Both E. coli and Staphylococcus aureus were cultured. Antimicrobial properties in E. coli and Staphylococcus aureus determined by agar diffusion method. The present study clearly revealed that the presence of various secondary metabolites. It is found that number of colonies of bacteria decreases with increasing concentration of seed extracts. Thus, it provides information about the relationship of concentration of extract and anti-microbial activity.

KEYWORDS: **Artocarpus heterophyllus, E. coli, Staphylococcus aureus, Phytochemicals, Antimicrobial**

1 Introduction

Antibiotic resistance is an emerging problem these days. Multiple drug resistance has developed due to indiscriminate use of antimicrobial drugs which are commonly used in the treatment of infectious diseases. Antibiotics also cause many adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions.

The evolving public health threat of antimicrobial resistance is driven by both appropriate and inappropriate use of anti-infective medicines. According to the world health organization (2020) Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating the process. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researchers to investigate the antimicrobial activity of herbal plants [2].

Artocarpus heterophyllus is commonly called jackfruit in English and Chakka in Malayalam. The jackfruit (Artocarpus heterophyllus) belonging to family Moraceae have been used in traditional

Medicines due to the presence of various flavonoids [4]. Economically, the genus is of appreciable importance as a source of edible fruit, yield fairly good timber and widely used in folk medicines [5].

In addition to the antimicrobial activity of A.heterophyllus, anti-inflammatory, antioxidant, anticholinergic, anti-diabetic, immune modulatory effect, inhibition of protease, oestrogen regulation and inhibition of melanin biosynthesis have also been reported through several pharmacological research investigations of the plant parts[3].

The present study was conducted to analyze phytochemicals and evaluate the antibacterial activities of local jackfruit (Artocarpus heterophyllus) seed against Escherichia coli and Staphylococcus aureus.

2 Research Methodology

Collection and Preparation of Sample

The raw jackfruit (Artocarpus heterophyllus) samples were collected from a local market in North Paravoor. The fruits were cleaned and separated into pulp and seeds. The seeds were rinsed in distilled water, sun dried and ground into a fine powder.

10 g A.heterophyllus powder was dissolved in 100 ml distilled water to make seed extract .After stirring for 12 hours, the solution was sterilized by autoclaving at 121oC for 20 minutes and 4oC for subsequent use.

Phytochemical screening

Presence of phytochemicals were analyzed by following standard procedure which are as follows [3],[4]:

Detection Of Carbohydrates- Benedict's test: 5ml of Benedict's reagent was taken and extract was added to it. Boiled for 2 minutes and cooled for some time. Orange red precipitate indicates the presence of reducing sugars.

Detection Of Protein- Nitric acid Test: 3ml of conc. HNO3 taken in a test tube. The aqueous extract was added slowly along the sides of the test tube. Protein will give a dark brown ring at the zone of contact between nitric acid and the extract.

Detection Of Amino acid- Ninhydrin Test: To the extract of 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicate the presence of amino acids.

Detection Of saponin- Foam Test: 1 ml of extract was shaken with 2ml of water. If foam produced persist for 10 minutes it indicates the presence of saponins.

Detection Of alkaloids- Wagner's Test: Seed extract were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish precipitate indicate the presence of alkaloids.

Detection Of terpenoids- Salkowski test: Seed extract was dissolved in 2ml of chloroform and evaporated to dryness. Add 3ml of concentrated H2SO4 and heated for about 2 minutes. Change of reddish brown colour revealed terpenoids.

Test for phenols- FeCl3 Test: 3 drops of FeCl3 (5%) was added to 5 drops of seed extract taken in a test tube. Development of dark green colour indicate the presence of phenols.

Detection of coumarin- NaOH Test: 3ml of 10% NaOH was added to 2ml aqueous extract. Yellow colour indicated the presence of coumarin.

Detection of tannins- Lead acetate Test: To 2ml of extract few drops of 1% lead acetate was added. Formation of yellow precipitate indicates the presence of tannin.

Test for flavonoids- Alkaline reagent test: 1ml of sample was taken in a test tube and 3ml of dilute NaOH was added. An intense yellow colour was appeared in the test tube. It became colourless when

an addition of a few drops of dilute acid hat indicates the presence of flavonoids.

Test for diterpenes- Copper acetate Test: 2ml of extract were treated with 3 to 4 drops of copper acetate solution. Formation of emerald green indicates the presence of diterpene.

Test for glycoside- Salkowski Test: 1ml extract is mixed with 2ml chloroform. To this solution 2ml concentrated sulphuric acid added carefully and shake gently. Formation of a reddish brown colour indicates the presence of glycosides. Test for steroids: 1ml extract was mixed with 2ml of chloroform and conc.H2SO4 were added along the side of the tube. In the lower chloroform layer, appearance red colour which indicate the presence of steroids.

Test for anthocyanin: Add 2ml of extract with 2ml of 2N HCl. The appearance of a pink-red colour that turns blue after addition of ammonia indicates the presence of anthocyanin.

Antibacterial assay

Test organisms

The pure cultures of bacteria maintained in the microbiology laboratory were used for the microbiological work. The test organisms were maintained on nutrient agar medium. The organism was used for work is Escherichia coli and Staphylococcus aureus. The positive control was Ciprofloxacin (30µg/disk)

Sample preparation

500 g A.heterophyllus powder was soaked in 1000 ml methanol for 5 days to make seed extract .After the extraction process the seed extract was filtered. The filtrate was distilled and concentrated methanol extract was stored in refrigerator [6].

Antibacterial test

The agar diffusion test was used to measure the effect of antimicrobial agent against bacteria grown in culture. Filter paper disk of diameter 0.6mm is prepared. Then disks were soaked with solutions of 20µl of seed extract and dried. The sample disks and the standard disks were placed gently on the marked zones in the agar plates pre-inoculated with test bacteria. The plates were then inverted and kept in an incubator at 37oC for 24 hours. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale [6].

3 Results

Phytochemical analysis

The present study clearly revealed that the presence of various secondary metabolites i.e., Carbohydrates, proteins, amino acid, alkaloid, terpenoid, saponin, phenol, flavonoid, anthocyanin, steroid, glycoside in Artocarpus heterophyllus seed. (Table 1)

Sl.No.	Phytochemical Tests	Test performed	Artocarpus heterophyllus seed
1	Test for carbohydrates	Benedict's test	+
2	Test for proteins	Nitric acid test	+
3	Test for amino acid	Ninhydrin test	+
4	Test for alkaloid	Wagner's test	+
5	Test for terpenoid	Chloroform test	+
6	Test for Saponin	Froath test	+
7	Test for phenol	Fecl3	+
8	Test for coumarin	NaOH	-
9	Test for tannin	Lead acetate test	-
10	Test for flavonoid	NaOH TEST	+
11	Test for diterpene	Copper acetate	-
12	Test for anthocyanin	HCl reaction	+
13	Test for steroid	Chloroform test	+
14	Test for glycoside	Salkowski test	+

Table 1: Summary of phytochemical investigation of Artocarpus heterophyllus seed.

Antibacterial activity on E.coli and Staphylococcus aureus

The study revealed that Artocarpus heterophyllus seed has strong antibacterial activity on E. coli and Staphylococcus aureus. (Table 2)

Name of Bacteria	Zone of inhibition of seed extract (mm)	Positive control (Ciprofloxacin (mm)
E.coli	20 ± 0.8	17 ± 1.4
Staphylococcus aureus	22 ± 0.6	17.5 ± 3.4

Table 2: Showing the zone of inhibition in presence of Artocarpus heterophyllus seed extract.

4 Discussion

The present study revealed that the effect of antibacterial activity of Artocarpus heterophyllus seed extract on E.coli and Staphylococcus aureus. Artocarpus heterophyllus seed is the rich source of secondary metabolites. The phytochemical analysis of Artocarpus heterophyllus fruit showed positive result for Carbohydrates, Proteins, amino acids, alkaloids, terpenoid, saponin, phenol, flavonoid, anthocyanin, steroid, glycoside (Table 1). Phytochemical screening from other research confirmed the presence of phytosterols, anthraquinone, terpenoids, phenols, glycosides, flavonoids and diterpenes in both of the trees i.e., Artocarpus heterophyllus and Artocarpus altilis [1],[3]. Phytochemical studies have shown that jackfruit contains several useful compounds like flavonoids, sterols, and prenylflavones, which may have been responsible for various pharmacological properties. Studies[6], [9], [10] have shown that jackfruit contains many classes of phytochemicals such as carotenoids, flavonoids, volatile acids sterols, and tannins, with varying concentrations depending on the variety. These results are closely related to the results reported by this study.

Antibacterial activity of A. heterophyllus seed was determined by Agar diffusion method (Table 2). Gram positive bacteria, Staphylococcus aureus was found to be maximally inhibited by the methanolic extract of A. heterophyllus with a zone of clearance of 22 mm and zone of inhibition for the gram-negative bacteria, E.coli is 20 mm (Table 2). The methanolic extracts samples were found to produce better antibacterial activity than control ciprofloxacin (Table 2). The screening of antibacterial activity of essential oil of Artocarpus heterophyllus indicated that E. coli was the most sensitive strain tested to the oil of A. Heterophyllus with the strongest inhibition zone (12 mm) followed by K. pneumonia (11 mm). Modest activities were observed against S. aureus (9 mm) and P. aeruginosa (7 mm) [4]. These results are similar to the results reported by this study. Methanolic extracts of A. heterophyllus bark showed the best antibacterial activity against Bacillus subtilis and Pseudomonas fluorescen [7]. Escherichia coli, Pseudomonas and staphylococcus aureus strains were found to be sensitive to essential oils obtained from plant and showed a very effective bactericidal activity [8].

All the above-mentioned facts are revealed that the present study is valid. So, Artocarpus heterophyllus seed has strong antibacterial activity on E. coli and Staphylococcus aureus and it contains various phytochemical compounds that can be used for medicinal field.

5 Conclusions

The demonstration of antibacterial activity of Artocarpus heterophyllus helps to discover medicinally important phytochemical substance that could serve as a selective agent for infectious diseases. Traditionally plants have been used in treatment of ulcers, prevention of night blindness, bone loss etc. It has a numerous chemical value and rich in phytochemicals. The phytochemical analysis of Artocarpus heterophyllus seed showed positive result for Carbohydrates, Proteins, amino acids, alkaloids, terpenoid, saponin, phenol, flavonoid, anthocyanin, steroid, glycoside. Phytochemical analysis showed valuable antibacterial compounds which are ecologically safe and economically viable. As all know that the herbal medicines represent the most important and valuable in the field of traditional medicine all over the world. The antibacterial activity revealed that seed extract showed good. Further study is however needed on the concentration of Artocarpus heterophyllus seed extracts that would be as effective as synthetic antibiotics.

6 References

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10

Pharmacognostic standardization and GC-MS analysis of rhizomes of Kaempferia rotundaL.

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ABSTRACT

Knowledge on traditional plants offers a source of generally recognized natural phytoconstituents for the treatment of wide variety diseases.Quality control is assisting in the improvement of many traditional medicine systems that are becoming increasingly relevant nowadays, as the monetization of medicinal plant-based compositions grows. As a result, the current work was conducted in order to include a detailed report on the quality management and standardization parameters of Kaempferia rotundaL.The aim ofcurrent researchwork was to make establishment of parameters for authentication and standardization of rhizome of K. rotunda for examination. Methods like microscopy and macroscopy, powder analysis, phytochemical analysis were used to establish pharmacognostical standards. Pharmacognostic investigation including powder analysis and microscopic characters with anatomy of rhizome of plant were performed with the regular standard laboratory procedures, and the results were noted. The present study is helpful to supplement the information with regard to its standardization and identification and in carrying out advanced research in ayurvedic system of medicine.

KEYWORDS: **Kaempferia rotunda, Pharmacognostic standardization, Microscopy, Powder analysis.**

1 Introduction

Medicinal plants have been utilised to treat a variety of diseases, since time immemorial. The identification and verification of plants used as a source of medicines require a step-by-step pharmacognostic investigation [1]. The immense potential of medicinal plants used in diverse traditional systems has recently come to light thanks to several evidences [2]. 2000 plant species are identified in Ayurveda as having therapeutic significance, whereas 5700 traditional remedies are recorded in the Chinese Pharmacopoeia, the majority of which are still utilized in western conventional medicine [3]. These are receiving more attention than ever since, particularly in medical and pharmaceutical investigations, they have the potential to have a wide range of positive effects on society or, indeed, on all of humanity. Therefore, based on various phytochemical screening and analysis, it is necessary to analyze phytoconstituents obtained from traditional medicines [4 -7].

To ensure that the natural medicine approach is globally compliant, official documentation and standardization of raw materials used in the herbal treatment are essential steps in this process. The WHO encourages countries to provide traditional treatments and practices in both public

and commercial health care. The development of monographs is primarily intended to improve harmonization in the use of herbal medicines, particularly concerning safety, efficacy, and quality control. Physicochemical evaluation, pharmacognostic standardization, and early phytochemical evaluations have been widely accepted as valid methods for recognizing and confirming the legitimacy of herbal sources [8 - 11].

Kaempferia rotunda is a valued medicinal herb belonging to the family Zingiberaceae, which has been described in Ayurveda for its anti-inflammatory, antiulcer, antitumour, wound healing, stomachic and vulnerary actions. K. rotunda isalso known as Bhumi-Champaka or Hallakam and widely distributedin wet and shaded regions in India[12]. It is possible to ensure the authenticity, effectiveness, safety, and repeatability of herbal medicines by advising a set of standards, constant variables, and clear qualitative and quantitative measurements. The correct identification and quality control of plant materials must be carried out in order to guarantee effective consistency in natural medicines. This will improve the efficacy and safety of natural medicines. The morphological, organoleptic, and physicochemical characteristics of the plant material will be included in the pharmacognostic standardization process. To address the above problems, an attempt has been made to standardize K. rotunda rhizomes based on pharmacognostic and phytochemical analysis which are extensively available and widely used in Ayurvedic system of Medicine.

2. Research Methodology

2.1. Pharmacognostic standardization of Kaempferia rotunda

2.1.1. Microscopic studies

Fine sections of the rhizome were taken using automatic MT3 microtome. The sections were stained with diluted aqueous saffranin, washed thoroughly and mounted in 40% glycerin and observed under the microscope. Trinocular Leica DM 3000 microscope attached with Leica DFC 295 digital camera connected to the computer and Leica application suite software was used for the study. Images obtained were examined thoroughly and compared the anatomical characteristics.

In order to estimate the presence of various cell inclusions like starch grains and oil globules the following methods were adopted:

Starch grains- To examine the presence of starch, the sections were stained with iodine solution. Starch grains got turned blue in colour.

Oil globules- To examine the presence of fixed oil, the sections were stained with sudan red. If present, the oil droplet got coloured orange pink.

2.1.2. Powder analysis

For examining the cell structure in powder form, the rhizomes were powdered, sieved and stained with appropriate stain, mounted in glycerin and observed under microscope. Transferred the images of powder characters to the computer using the computer controlled microscopic system and camera.

2.1.3. SEM analysis of rhizome powder of Kaempferia rotunda

The rhizome powder was subjected to SEM viewing for the detailed surface ornamentation patterns. For SEM analysis, the specimens were (rhizome powder of K. rotunda) placed on aluminium stubs using double sided adhesive tape and sputter coated with gold using a Hummer VII gold coating apparatus. They were observed and photographed under JEOL Model JSM – 6390LV SEM and Gemini SEM 300 under different magnifications.

2.2. Phytochemical analysis of Kaempferia rotunda using GC-MS

2.2.1. Preparation of plant material

The collected rhizomes of K. rotunda were cleaned and washed with distilled water and dried at room temperature. The dried rhizomes were ground to fine powder using a mortar and pestle and the powder was preserved in air sealed plastic covers.

2.2.2. Preparation of samples

Powdered rhizomes of K. rotunda were extracted with methanol by soxhlet extraction method. The extract was filtered. The crude extracts were concentrated by rotary evaporator at 40°C and the concentrated extracts were used for GC-MS analysis[13,14].

2.2.3. GC-MS analysis

Model QP2010S was employed for GC-MS analysis. A cross linked factor four capillary column Rxi-5Sil MS with 30 m × 0.25 mm ID and 0.25 µm film thickness was utilized. Carrier gas used was helium at a flow rate of 1 ml/min. Injection volume was 1 µl. The split ratio was 20.0. The temperature programme for the chromatographic analysis was set at 80°C for 4 min and then heated up at a rate of 5°C min to 280°C. Run time was 50 min. Quantification was done using percentage peak area calculations and identification of individual compounds was done with the help of NIST 11 and WILEY 8 search. The relative concentration of each compound in the methanolic extract was found out based on the peak area integrated by the analysis programme.

2.2.4. Identification of compounds

Interpretation of mass spectrum of GC-MS was carried using the database of NIST 11 and WILEY 8 search. The relative percentage amount of each compound was calculated by comparing its average peak area to the total area.

3. Resultsand Discussion

Pharmacognostic standardization is a method adopted in Ayurveda has become necessary to standardize quality assurance measures so as to safeguard supply of medicinal plants of good quality. It is fundamental to innovate and develop tools that can help in the proper identification of the medicinal plant and also the collected raw materials. Pharmacognostic standardization of plant materials includes its morphological, anatomical and biochemical characteristics [15].

3.1. Microscopic studies

Detailed anatomical study of K. rotunda rhizome showed the presence of epidermal cells followed by an array of parenchyma cells. Vascular bundles were found scattered in the parenchymatous ground tissue. (Fig. 1. A). These parenchyma cells were filled with oval shaped starch granules (SG) and oil globules (OG) (Fig. 1.B-C).

3.2. Powder analysis

Powder microscopic studies are very important in Ayurveda for the proper identification and authentication of plant materials during drug standardization [16,17]. Powder analysis of K. rotunda displayed fragments of vessel with spiral thickening, cortical cells with oleoresin, parenchyma cells and cortical cells with starch grains, fragments of cork cells with underlying cortical cells (Fig. 2.A-F).

3.3. SEM analysis of rhizome powder of Kaempferia rotunda

The surface morphology of the rhizome powder of K. rotunda was investigated using Scanning Electron Microscope (SEM). SEM micrographs of rhizome powder particles taken at room temperature with different magnifications are shown in Fig. 3. Powder particles of different sizes such as 19.68 µm, 16.13

µm, 12.32 µm, 15.36 µm and 8.72 µm are observed under 10 µm magnifications. Powder particles are more or less elliptical in shape with rough surface.

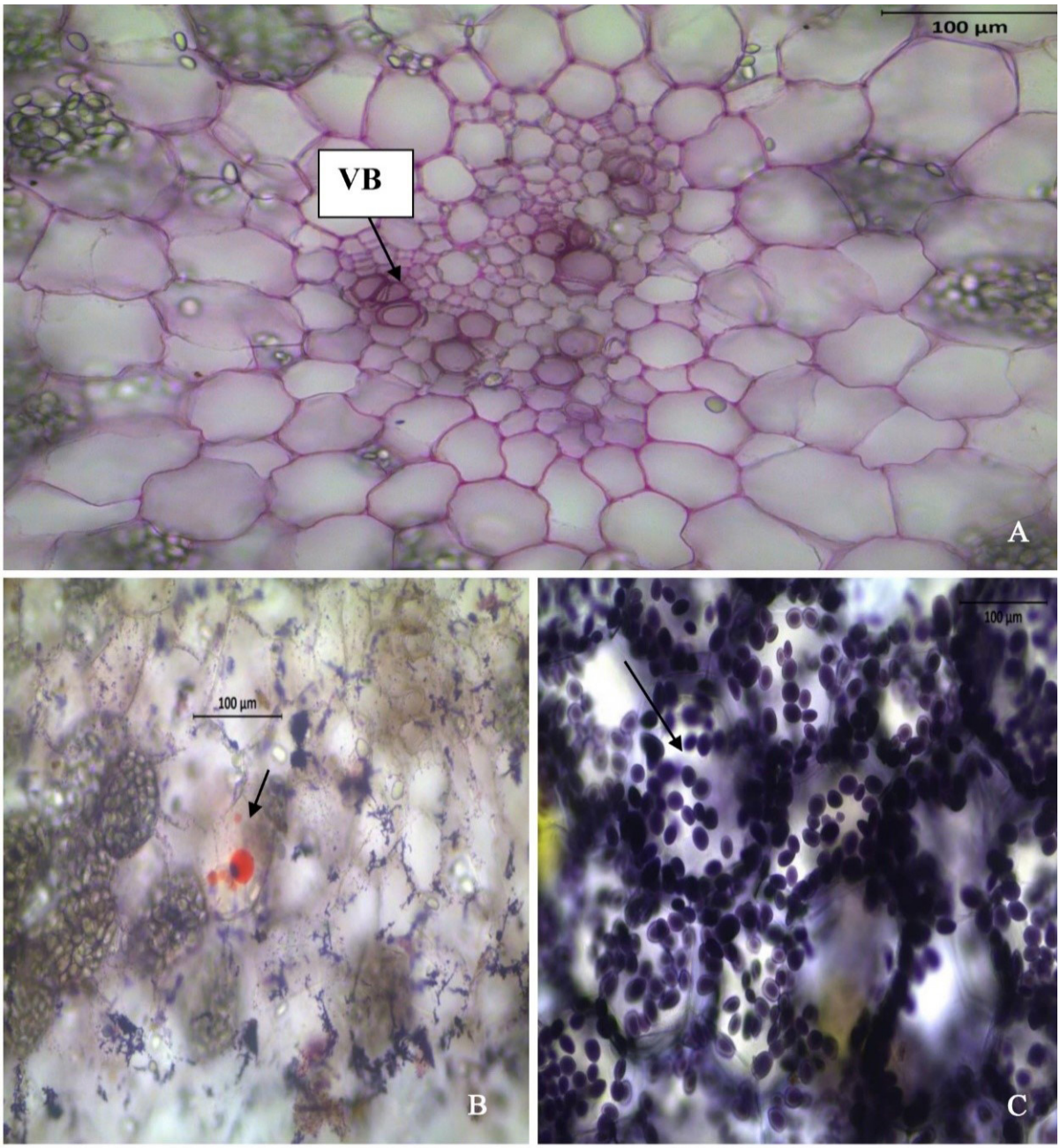

Fig. 1. Microscopic studies of Kaempferia rotundaL.

(A-C) T.S. of Kaempferia rotunda rhizome showing Vascular bundle (A), Oil globules (B) and Starch grains (C)

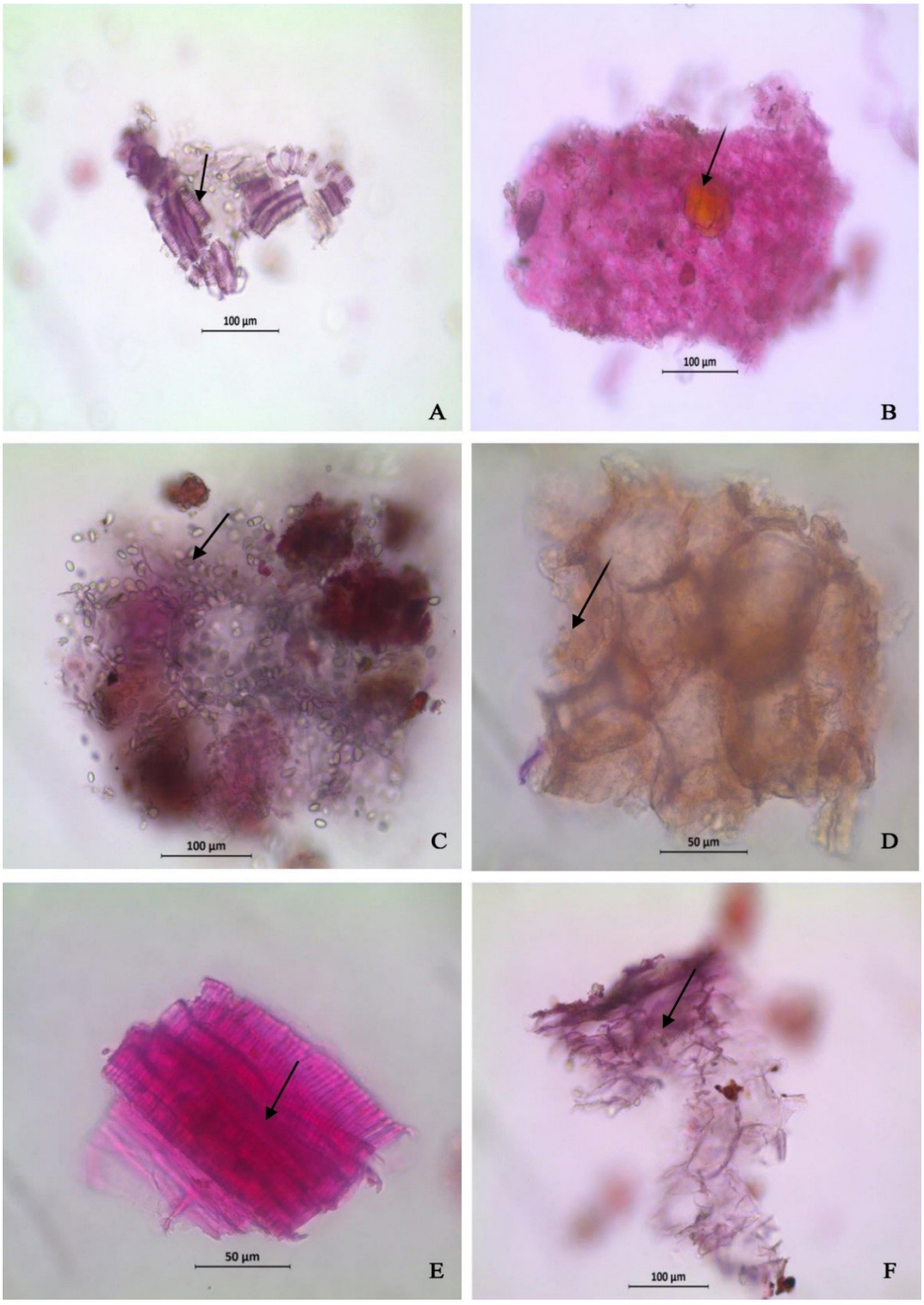

Fig. 2.Powder characteristics of Kaempferia rotunda

(A) Vessel with spiral thickening (B) Cortical cells with oleoresin (C) Parenchyma cells with starch grains (D) Cortical cells with Starch grains (E) Fragments of Vessels (F) Fragments of corkcells and underlying cortical cells

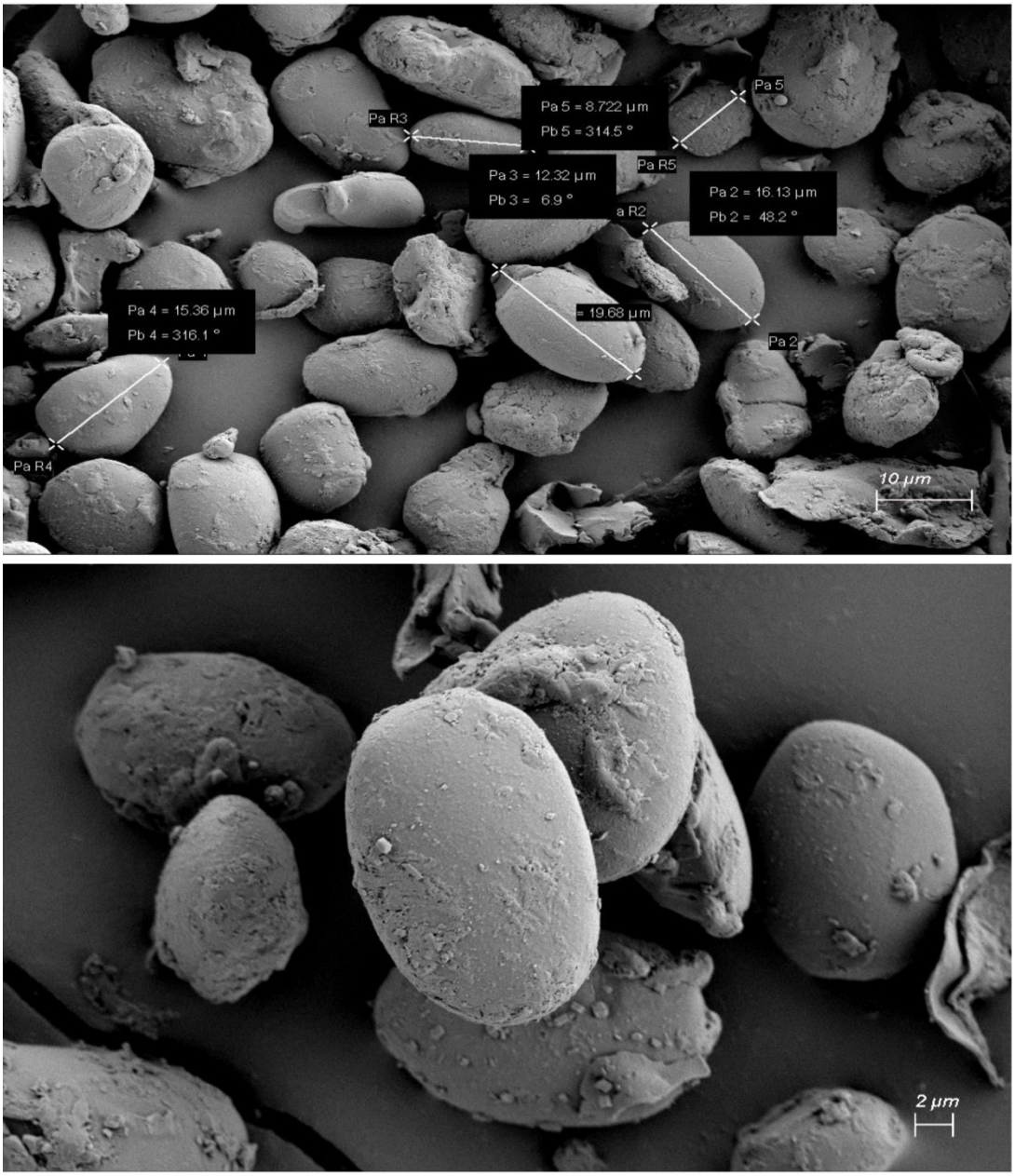

Fig. 3. Scanning electron micrographs obtained from rhizome powder of Kaempferia rotunda under different magnifications

3.4. Phytochemical analysis of Kaempferia rotunda using GC-MS

The result of methanolic extract of K. rotunda rhizome revealed 9 peaks (Fig. 3), with 9 compounds identified (Table 1) representing 100% of the entire extract. The major among them were camphor (1.88%) with retention time (RT) of 9.157 minute, bicyclo [2.2.1] heptan-2-ol,1,7,7-trimethyl-acetate (5.24%) with retention time of 13.054 minute, pentadecane (11.89%) with retention time of 18.765 minute, eicosane (1.90%) with retention time of 23.450 minute, phytol acetate (3.57%) with retention time of 26.196 minute, retinol (4.85%) with retention time of 32.056 minute, (1R-1λ,2λ,4λ,5λ,6λ,7λ))-3,8-dioxa tricycle (5.1.0.0(2,4))octane-5,6-diyl diacetate,4-benzoyl oxy methyl (50.23%) with retention time of 39.041 minute, cholest-5-en-3-yl benzoate (2.40%) with retention time of 46.971 minute and 2λ,9λ-dihydroxyverrucosane (18.03%) with retention time of 48.731 minute. Identification of these compounds in the rhizome extract serves as the basis in determining the possible health benefits of the plant leading to further pharmacological study.

The GC-MS analysis have shown that the terpenoids are the major class of compounds present in the rhizome of K. rotunda. Terpenoids, the most abundant compounds in natural products, are a set of important secondary metabolites in plants with diverse structures. Terpenoids play key roles in plant growth and development, response to the environment, and physiological processes. As raw materials, terpenoids were also widely used in pharmaceuticals, food, and cosmetics industries. Terpenoids possess antitumor, anti-inflammatory, antibacterial, antiviral, antimalarial effects, promote transdermal absorption, prevent and treat cardiovascular diseases, and have hypoglycemic activities[11],[18-[20].

Peak Number	RT (min)	Name of identified compounds	Area %	Class of compound	Base m/z value
1	9.157	Camphor	1.88	Terpenoid	95.20
2	13.054	Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-acetate	5.24	Monoterpene	95.10
3	18.765	Pentadecane	11.89	Acyclic alkanes	57.10
4	23.450	Eicosane	1.90	Acyclic alkanes	57.05
5	26.196	Phytol acetate	3.57	Diterpene	57.05
6	32.056	Retinol	4.85	Vitamin A	123.15
7	39.041	(1R-1α,2β,4α,5β,6α, 7α))-3, 8-dioxa tricycle (5.1.0.0(2,4)) octane-5,6-diyl diacetate,4-benzoyl oxy methyl	50.23	Diterpene	105.05
8	46.971	Cholest-5-en-3-yl benzoate	2.40	Ester of cholesterol and benzoic acid	147.10
9	48.731	2β,9α-dihydroxyverrucosane	18.03	Diterpene	187.10

Table 1.Phytochemical compounds identified in the methanolic extract of Kaempferia rotunda rhizome using GC-MS analysis

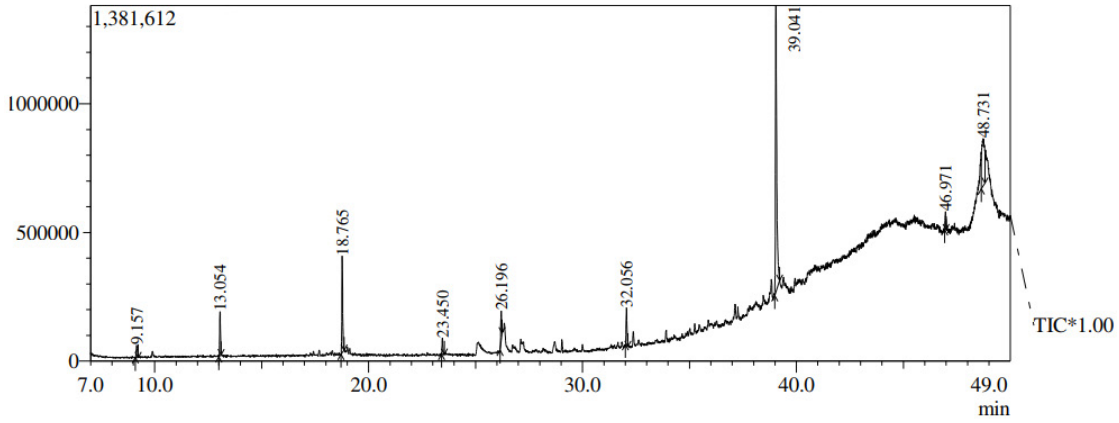

Fig4. GC chromatogram of methanolic extract of Kaempferia rotunda rhizome

4. Conclusion

From the above discussion, it is concluded that the pharmcognostic study is a necessity for ensuring the identity of the herbal drugs.They are important for assuring quality and effectiveness of therapeuticalformulations that use herbal source as raw materials. Systems such as Ayurveda, Siddha, and others that use medications primarily made from herbal sources must have the raw ingredients tested using pharmacognostic methods. A source of generally acknowledged natural phytoconstituents for the treatment of a wide range of ailments is available from knowledge of traditional plants. Despite its considerable medical usefulness, little is known about the specifications for standardizing the species. In order to include a thorough report on the quality management and standardization characteristics of K. rotunda., the current work was carried out. The objective of current study was to establish criteria for authenticating and standardizing K. rotunda rhizome for analysis. Pharmacognostical standards were established using techniques like microscopy and macroscopy, which use physicochemical factors. The results of a pharmacognostic inquiry that included powder analysis, microscopic character analysis, and phytochemical analysis were recorded using normal laboratory practices[21-[23].

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6. Conflict of Interest

The authors have no conflict of interest regarding this investigation

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11

Bio-Inspired Green Concept of Albatross Bird Wing for High Fuel Efficiency

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ABSTRACT

The albatross is the earth's largest sea bird wing with high wing span (2.9 – 3.3 meters) and it can for miles without flapping its wings. The albatross bird have high gliding ratio, it will use the sea breeze to fly for days. Study of this bird will provide better understanding how the bird is able to save the power during flight. This paper deals about albatross wing, to find its efficiency by performing aerodynamics and stability analysis of the wing with different airfoils in XFLR5 software. The albatross wing is designed with airfoils S1223RTL, RAF-19 and E-16 at Reynolds number 500000 and then with two types of tail configurations (convectional and twin tail). The longitudinal and lateral stability of the albatross wing with two tail configurations was recorded. The results proved that the wing with S1223RTL airfoil gave high lift coefficient and with twin tail it is longitudinally static stable. This study proved that the albatross wing has more aerodynamic efficiency than the tapered wing configuration. The results from albatross wing are compared to the tapered wing and proved that the albatross wing provided high lift and good stability. This proves that the application of the albatross wing in UAV industry can reduce high fuel consumption as it has more gliding properties, the albatross wing will have high fuel efficiency and better flight performance for the air vehicles, it will be the green initiative for the future.

KEYWORDS: **Albatross bird, Panel method, Bio-Inspired wing, Lift and Drag.**

1 Introduction

The albatross bird being the largest bird in the earth with high aspect ratio ,the previous studies prove that the albatross wing can produce high lift and less vortices without the use of wingtips by Bachman .T [1], the albatross bird wing can be used in UAV and drone industry to increase the endurance and range with low fuel consumption in some studies[7,8],the other studies also did the analysis of 3D modeled albatross wing and did aerodynamic analysis using 3D panel method in XFLR5 ,using that data in this paper we did stability analysis and aerodynamic analysis of albatross wing modeled in XFLR5 using 3D panel method ,with four different types of airfoils and the selected wing with best airfoil is compared to the traditional tapered wing with two different types of tail configurations. The previous papers regarding the performance of albatross wing which uses dynamic soaring as it gains altitude and speed in strong winds as dynamic soaring does not need sheer wind, the albatross make loops and fly over the sea by Nicholas L M[9].

2. Research methodology

XFLR5 uses a high-order panel method and a fully coupled viscous/inviscid interaction method to evaluate drag, boundary layer transition, and separation. All the airfoils data has been imported from

the website Airfoil Tools, this website has various airfoils with coordinates. The database contains the Selig format DAT files, these DAT files are then imported to XFLR5, the airfoil analysis is performed. In between the airfoils S1223RTL, E-16 and RAF-19.The wing configuration is taken with respect to the albatross bird, and then the wing is designed using wing and plan design in XFLR5, with three airfoils. The methods used for this experiment are taken from the previous

researches, but the static stability method by adding tail to the albatross wing is the key point for this research.

2.1. Wing geometry

The wing is designed in the XFLR5 using Xfoil design and wing and plane design method, this method helps us to design wing ,tail and fuselage with various of options ,the wing geometry used for this experiment is same as the albatross bird ,the wingspan as 3m and chord length as 0.4m and aspect ratio as 15.As shown in Fig 1 it is the cad model designed in XFLR5. The wing dimensions are mentioned in the table 1, the dimensions are taken with respect to the real albatross bird.

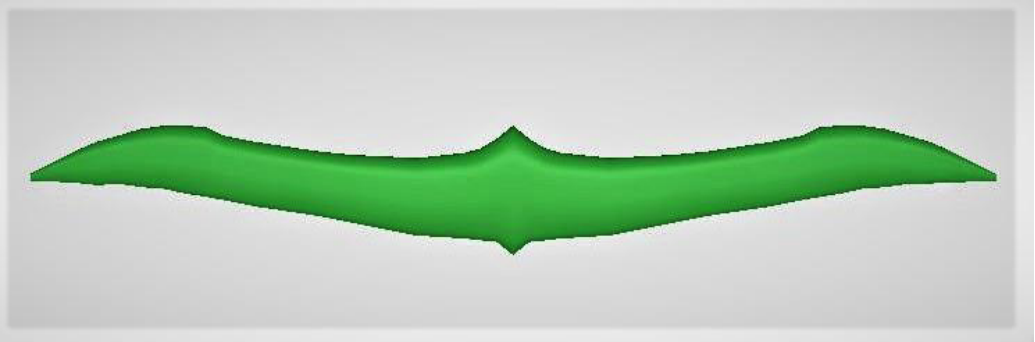


FIG 1:Albatross wing 3D CAD model

Parameters	Dimensions
Wingspan	3m
Wing area	0.598
Wing mass	2kg
Wing loading	3.342kg/m2
Root chord	0.40m
MAC	0.221
Aspect ratio	15.041
Tapper ratio	0.050
Root-Tip sweep	2.100

Table 1: Wing dimensions

2.2. Airfoil selection

The three different airfoils have been taken from the website Airfoil Tools[23] in the Dat format and is imported to XFLR5, the XFOIL design method is used for the three airfoils S1223RTL ,E-16 and RAF-19 as shown in Fig 2,using this method airfoils are analyzed and these airfoils are added to albatross wing designed.

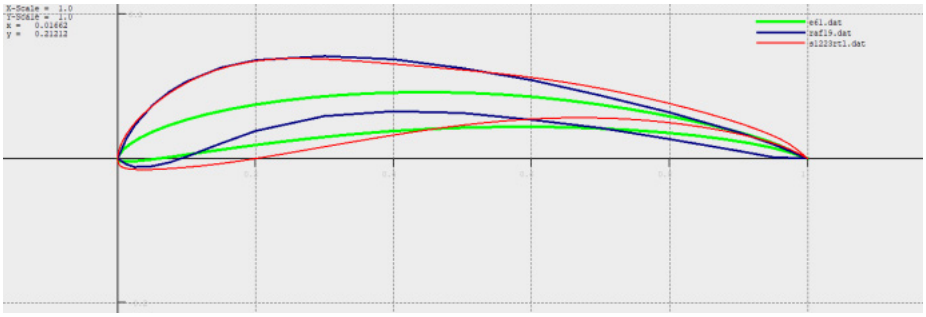


FIG 2 : Airfoils selected

2.3. VLM(Vortex Lattice Method)

VLM is a technique that simulates the aerodynamic behavior of a wing using a lattice of vortex filaments. The foundation of VLM is the superposition principle, which claims that the sum of

each vortex filament flow determines the overall flow around the wing. A wing's lift, drag, pitching moment, and span wise lift distribution may all be determined using VLM by Brooke.M [22]

2.4. Panel method

Using panel methods, the flow equations for a single wing or a collection of wing are solved. The flow over each of the numerous tiny panels that make up the wing surface is modeled as a potential flow. To determine the lift, drag, and other aerodynamic forces and moments acting on the wing, the resulting system of equations is solved as written in these papers [15,16], the panel visualization can be seen in Fig 3.

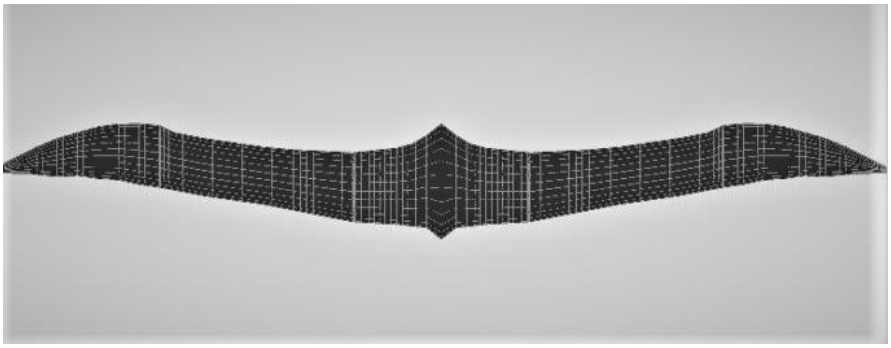


FIG 3 : 3D panel view of albatross wing

3. Theory and calculations

3.1. Airfoil theory

A thin, cambered airfoil's lift and drag characteristics at low angles of attack are predicted using the thin airfoil hypothesis. The theory presupposes that the airfoil is thin enough to allow for two-dimensional treatment of the flow over the top and bottom surfaces of the wing and that the flow is incompressible. The angle of attack and the form of the airfoil are used to determine the lift and drag coefficients by Karz.J [24].

3.2. General formulas

The general formulas used in finding the theoretical values of aerodynamic coefficients i.e. CL and CD, Here are the formulas used in this experiment. The lift and drag are the aerodynamic forces that act on airfoil caused by the pressure differences occur above and below the surface of it referred from [2,3].The moment formula is an important formula for the stability derivative for the aircraft this

formulas are taken as the base for this experiment, these formulas are taken from the books[4,5].

$$\text{LIFT(L)}=\frac{1}{2}*\rho*V^2*S*CL\text{----------}\rightarrow(1)$$

$$\text{DRAG(D)}=\frac{1}{2}*\rho*V^2*S*CD\text{---------->}(2)$$

$$\text{MOMENT(M)}=\frac{1}{2}*\rho*V^2*S*C*(X_{CG}-X_{AC})\text{--------}\rightarrow(3)$$

$$\text{Cm}_{\alpha}=\frac{dm}{d\alpha}*\frac{1}{2}*\rho*v^2*s*c\text{----------}\rightarrow(4)$$

Here V = velocity, λ = air density, S= wing Area, λ= angle of attack , CL= coefficient of lift ,CD= coefficient of drag, XCG= distance from CG, XAC=distance from aerodynamic center, C= chord length

3.3. XFLR5 Calculations

In this experiment the calculations are made using the XFLR5 , the wing and the tail designing are designed and performed aerodynamic and stability analysis, The designed albatross wing with selected airfoil performed an aerodynamic analysis, here the boundary conditions taken are in wing plane analysis the velocity of 17m/s is taken as the average speed of the albatross bird

i.e.500000 Re from Reynolds number the velocity is taken as 17m/s ,the flow taken is non viscous and ring vortex on with the wing mass of 2 kg , the analysis is carried out from -20 to 20 AOA(Angle of attack) the calculations are internally done by XLR5 using panel method and VLM method the aerodynamic coefficients and the CP is recorded at 00 AOA for better streamlines and moment .The tail is added to the albatross wing later on by using the tail volume ratios from Raymer.D.P book, and then the stability analysis is performed using the mix 3d panel/VLM2 method we can provide side slip angle at this stage for the gust loads, and then the analysis is carried out in controlled parameters

4. Results and discussion

The XFLR5 analysis is performed between the three airfoils S1223RTL, E-164 and RAF-19 , the designed albatross wing with these three airfoils , the CP at 00AOA has been performed all the three wing with three airfoils are compared in first part and the best suitable airfoil for better efficiency has been selected , the selected wing is then added a two different types of tail configurations , the results are also taken with respect to CP and compared to the tapered wing with same tail configurations.

4.1. Wing analysis with three different airfoils

The following analysis is carried out using S1223RTL airfoil with albatross wing

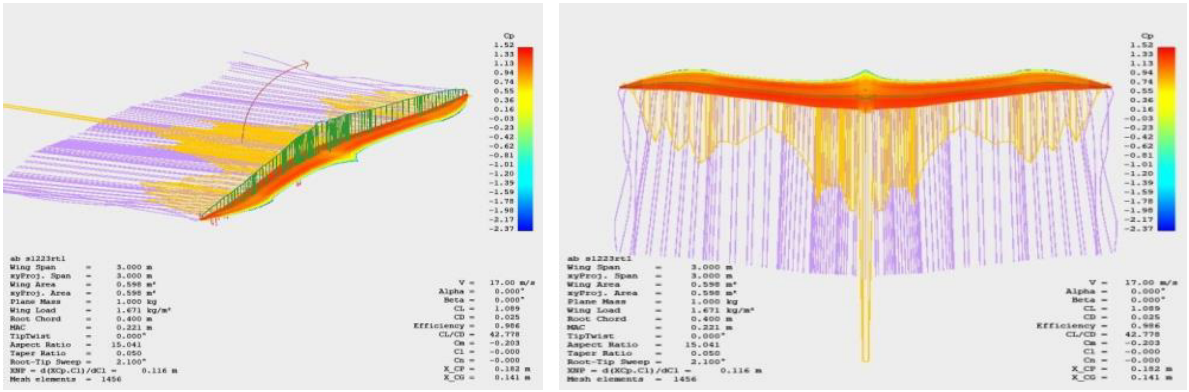


FIG 4: Top and Iso view of albatross wing with S1223RTL

The analysis with E-164 airfoil in albatross wing

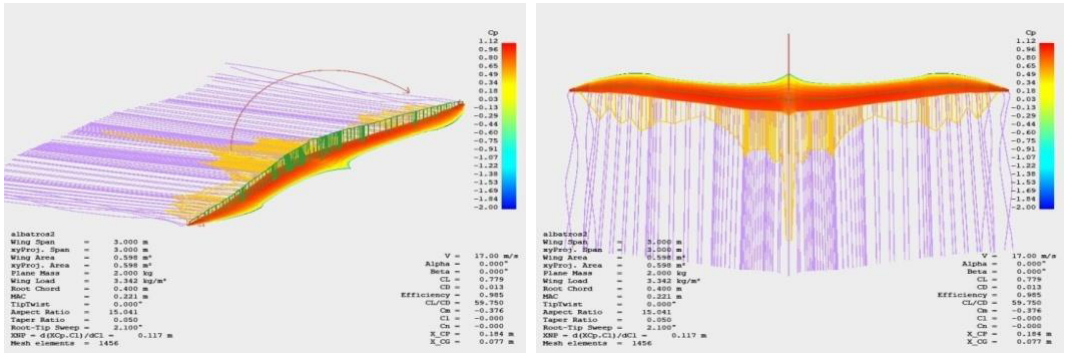


FIG 5: Top and Iso view of albatross wing with E-164

The albatross wing analysis with airfoil RAF-19

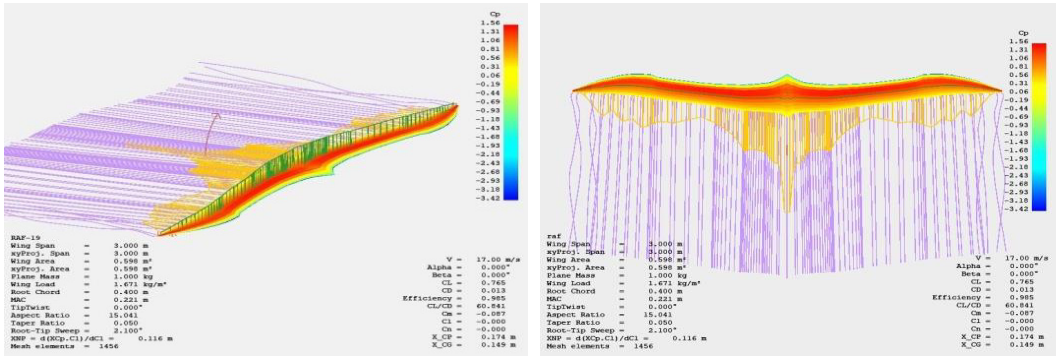
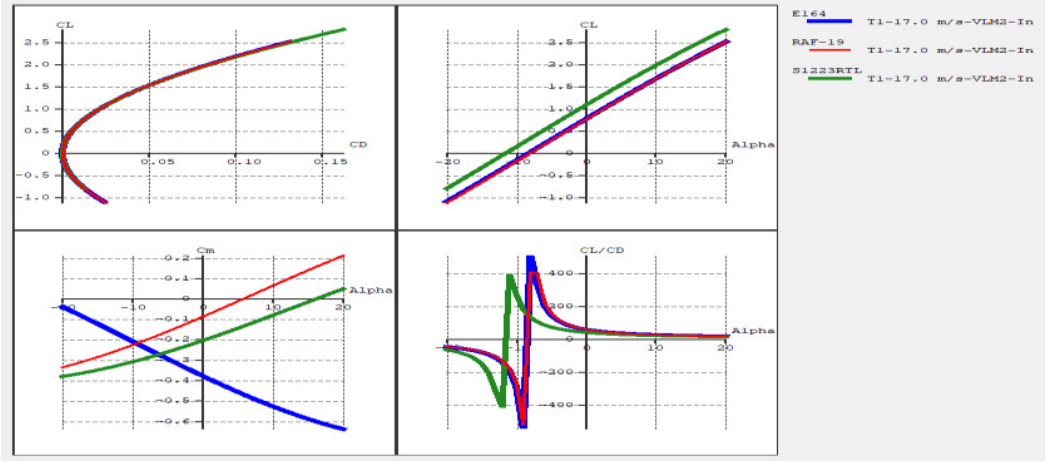


FIG 6: Top and Iso view of albatross wing with RAF-19

By comparing the three wings with different airfoils ,the results indicates that the wing with S1223RT L airfoil shown in Fig 4 ,performed well and produced CL=1.089 ,which is better than the other two airfoils E-16=0.779 Fig 5and RAF-19=0.765 Fig 6, the figure 7 below represents the comparison of these three wings with respect to CL VS ALPHA ,CL VS CD, CM VS ALPHA and CL/CD VS ALPHA. Here by we conclude that the albatross wing with S1223RTL is suitable for this experiment

FIG 7: CL vs. ALPHA ,CL vs. CD, CM vs. ALPHA and CL/CD vs. ALPHA graphs for three wings



4.2. Albatross wing vs Tapered wing

The stability analysis is carried out for the selected albatross wing i.e. wing with S1223RTL airfoil and the tapered wing with same airfoil and same span , the conventional and twin tail are designed and added to the wings, the analysis is carried out between these two wings, Here the longitudinal stability with respect to pitching moment coefficient and angle of attack is observed.

4.2.1 Albatross wing with conventional tail and tapered wing conventional tail

FIG 8 : Albatross wing with conventional tail

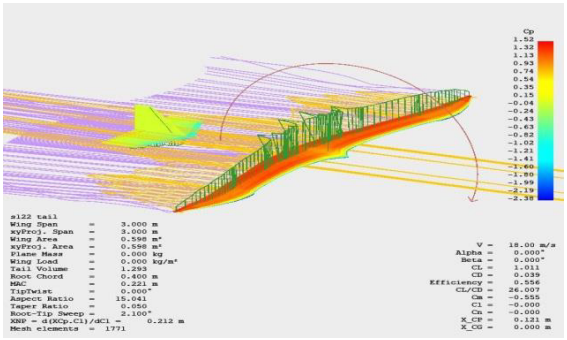
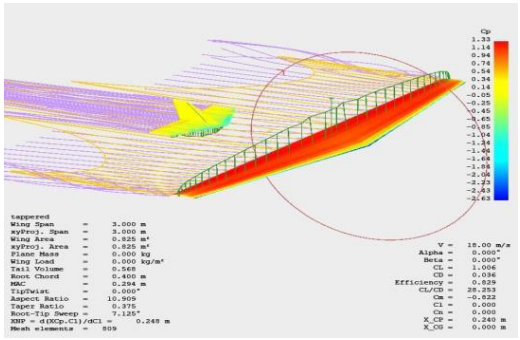


FIG 9:Tapered wing with conventional tail



Here ,from the above comparison the CL of albatross wing is greater than tapered wing and the static stability or pitching moment is better in albatross wing. As we can see in Fig 8 the moment and aerodynamic forces compared to Fig 9 are better and in Fig 8 we can see the high lift.

4.2.2 Albatross wing with twin tail and tapered wing with twin tail

FIG 10: Albatross wing with twin tail

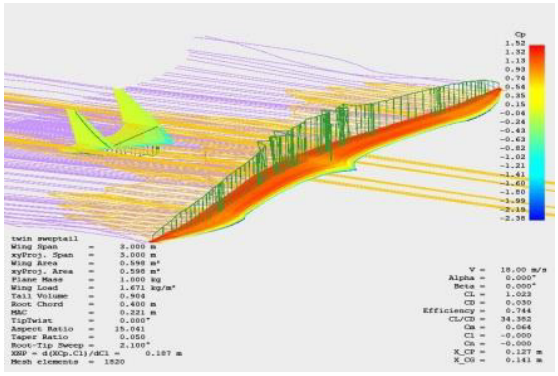


FIG 11:Tapered wing with twin tail

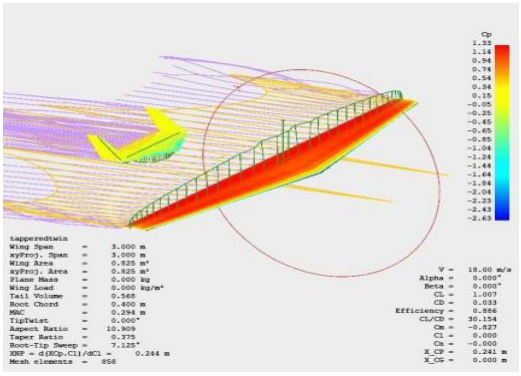


FIG 12: Dynamic analysis of albatross wing with twin tail

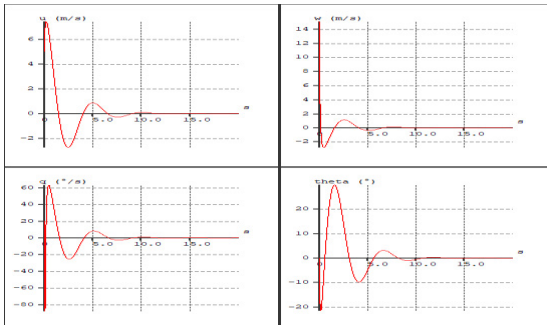
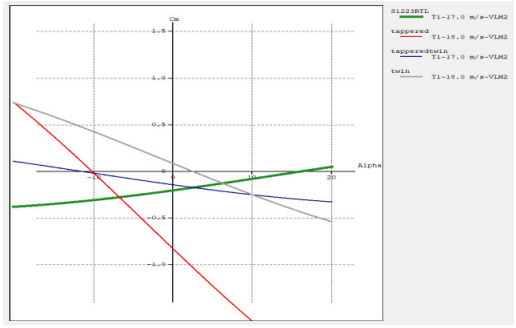


FIG 13:Cm vs alpha graph or four configurations



This figure 13 represents the cm vs alpha graph of albatross and tapered wing with two tail configurations the indications are Green line=albatross (conventional tail), Red=tapered(conventional) , Gray=albatross(twin tail), Blue=tapered(twin).In the figure12 we can see that the albatross aircraft can attain dynamic stability within the time range of 10sec.From the results the longitudinal static stability of albatross wing with twin tail is better than the other three types of wing tail configurations. And the dynamic stability of the albatross twin tail has better results. The bio inspired or corrugated wings provide high stability and high aerodynamic performance by Y D Dwivedi [11,12], the reason

for doing only wing and tail analysis in XFLR5 is the software neglect the flow over the fuselage. So the analysis is carrird out with only wing and tail.

5. Conclusion

The lift coefficient produced by S1223RTL was best when compared to the E-16 and RAF-19,the drag coefficient produced by the airfoil E-16 is least among the airfoils, but S1223RTL is in between E-16 and RAF-19. The aerodynamic performance (CL/CD)of the S1223RTL is not better than other two but the efficiency and CL is greater .The albatross wing is designed with the three airfoils, the wing with S1223RTL airfoil produced high aerodynamic performance ,the analysis is performed at

velocity 17m/s inviscid flow , CL produced by S1223RTL is 1.087 and E-16 is 0.779 and RAF-19 is 0.765. This paper results that the longitudinal static stability of the albatross wing with twin tail is better when compared to the other three wings(albatross with convectional tail and tapered wing with convectional and twin tail).The final results from the study proves that the albatross wing with S1223RTL and twin tail configuration produces high efficiency , better CL/CD ,and static stability of the aircraft is improved than the albatross wing with convectional tail and normal tapered wing with convectional and twin tail.

6. Declarations

6.1. Study limitations

None

6.2. Competing interests

The albatross wing used in the unmanned aerial vehicle (UAV) field can reduce most of the fuel consumption and provides better sustainable aircrafts

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12

A Review of Sustainable Plastic Waste Management

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ABSTRACT

India is currently the 12th highest contributor to the mismanagement of plastic waste, generating 9.4 million tonnes of plastic waste annually. Plastic pollution in the ocean has severely impacted almost all species groups, with negative consequences observed in almost 90% of evaluated species. Even if plastic inputs were to stop today, micro plastic levels in the ocean are projected to double by 2050 and possibly increase 50-fold by 2100 under some scenarios. Moreover, plastic debris entangles marine and freshwater organisms, causing them to suffocate, drown, or starve. In addition, specialists predict that approximately 10% of plastic waste generated will make its way into the ocean. The release of toxic substances is creating a significant danger to the environment, vegetation, and the health of humans and animals. Recycling and landfills are discussed as potential solutions, but the former should only be used if the amount of energy consumed in the recycling process is lower than the energy required producing new materials. A significant sustainability drawback of landfills is that none of the material resources used for plastic production are recovered, resulting in a linear material flow rather than a cyclic one.

KEYWORDS: **Plastic, micro plastic, marine, waste management, recycling, landfill.**

1 Introduction

India has been identified as a significant contributor to plastic waste mismanagement, and its per capita plastic consumption is much lower than that of developed nations. However, India is projected to climb to fifth on the list of highest contributors to plastic waste mismanagement by 2025. The review highlights the impact of plastic pollution on the marine environment, which affects almost all species groups and reduces the productivity of critical marine ecosystems. Plastic waste also poses significant ecological risks in various habitats, including secluded lakes and the deepest parts of the ocean. The review examines conventional technology for plastic waste management, such as recycling and landfilling. While recycling is one approach to reducing plastic waste, it must be applied only when the amount of energy consumed in the recycling process is lower than the energy required producing new materials. Meanwhile, constructing landfills is becoming increasingly challenging due to limited space. The review highlights the importance of exploring alternative methods to manage plastic waste in India to reduce its environmental impact.

2. PLASTIC GENERATION IN INDIA

In 2019, India produced approximately 9.4 million tonnes of plastic waste annually, a mere 3.1% of the total global plastic waste generation of over 380 million tonnes annually. Regarding global plastic

consumption, the packaging and construction sectors consume 42% and 17%, respectively. However, India's packaging and construction sectors consume 35% and 23%, respectively [1]. The total waste generated in India includes 8% of plastic waste [2]. India has been identified as the 12th highest contributor to the mismanagement of plastics waste, despite having a population size similar to that of China. However, by 2025, it is projected to climb to fifth on the list [3]. India's per capita plastic consumption stands at approximately 10 kg per annum, which is significantly lower than the United States, where the annual per capita consumption of plastic is the highest in the world at 110 kg [4].

3. ENVIRONMENTAL IMPACT OF PLASTICS

The annual surge of plastic pollution in the marine environment directly results from the worldwide mass production of polymers and inadequate management of plastic waste. Due to their robustness and weight, plastic materials can travel vast distances and remain in the marine ecosystem for extended periods. As a result of the widespread usage of plastics, contamination has spread to various habitats, including secluded lakes and the deepest parts of the ocean [5]. Plastic pollution has severely impacted the ocean, affecting almost all species groups, with negative consequences observed in almost 90% of evaluated species. This pollution enters the marine food web and significantly reduces the productivity of critical marine ecosystems such as coral reefs and mangroves. Multiple regions, including the Mediterranean and Arctic sea ice, have exceeded plastic pollution thresholds that pose significant ecological risks, and more regions are expected to follow in the future. Even if plastic inputs were to stop today, microplastic levels in the ocean are projected to double by 2050 and possibly increase 50-fold by 2100 under some scenarios [6].

It is nearly impossible to eliminate plastic from the ocean after it has entered it. Additionally, plastic disintegrates in the ocean, with macroplastics becoming microplastics and microplastics transforming into nano plastics [7]. Plastic debris, mainly abandoned fishing gear, entangles marine and freshwater organisms, causing them to suffocate, drown, or starve. Several studies have indicated that aquatic creatures frequently consume and accumulate these novel pollutants in their digestive tracts [8]. In addition, specialists predict that approximately 10% of plastic waste generated will make its way into the ocean [9].

Moreover, according to the World Economic Forum (2016), the ocean's plastic mass will surpass that of fish by 2050. The occurrence of meso, macro, and microplastics (MPs) in aquatic ecosystems is causing concern due to their possible threats to aquatic and human life. Although plastics are inert, MPs with toxic additives and accumulated pollutants can have adverse ecological consequences. Reports indicate that internal tissues can absorb MPs and have toxic effects on critical organs such as the lungs, liver, and brain cells, highlighting their severe health risks [10].

The major routes of releasing plastic-related persistent organic pollutants (POPs) such as flame retardants, dioxins, and furans in India and other developing nations are the open incineration of municipal solid waste and informal plastic waste recycling, including electronic and electrical waste [11]. The release of toxic substances is creating a significant danger to the environment, vegetation, and the health of humans and animals. Polystyrene can harm the Central Nervous System, while the hazardous brominated compounds are carcinogenic and mutagenic. Dioxins, lethal persistent organic pollutants, settle on crops and waterways, eventually making their way into our food and bodies. The most dangerous component of dioxins, 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), called red to as Agent Orange, can cause cancer and neurological damage and disrupt reproductive, thyroid, and respiratory systems. Burning plastic waste can increase the risk of heart disease; aggravate respiratory conditions like asthma and emphysema, cause rashes, nausea, and headaches, and damage the nervous system[12].

4. CONVENTIONAL TECHNOLOGY FOR PLASTIC WASTE MANAGEMENT

4.1. Recycling

Recycling allows the wastes to be reintroduced into the consumption cycle, generally in secondary applications because, in many cases, the recycled products are of lower quality than the virgin ones. Recycling must be applied only when the amount of energy consumed in the recycling process is lower than the energy required producing new materials. Plastics can be recycled using two different approaches: mechanical and feedstock recycling. In the first case, the plastics are recycled as polymers, whereas in the second, plastic wastes are transformed into chemicals or fuels [13].

4.2. Landfill

Although it is a conventional waste management method, constructing landfills is challenging in some countries due to limited space. While a well-managed landfill can minimize immediate environmental harm beyond the impacts of waste collection and transportation, there are potential long-term risks, such as groundwater and soil contamination from certain additives and plastic breakdown, which can lead to persistent organic pollutants.

A significant sustainability drawback of landfills is that none of the material resources used for plastic production are recovered, resulting in a linear material flow rather than a cyclic one [14]

4.3. Incineration

While this process reduces the need for landfilling plastic waste, there are concerns that hazardous materials may be released into the atmosphere. This is especially true for mixed plastic waste containing halogenated additives and PVC, which can release dioxins, furans, and other polychlorinated biphenyls into the environment. The choice of incinerators is crucial, as it is not typically done in a controlled manner that can reduce pollution from off-gases to acceptable levels. Consequently, this method of plastic waste management is often not preferred, as the cost of treating the gases is frequently higher than the energy recovered [15]

With modern incineration technology, solutions are available to address any incineration-related issues without causing harm to the environment and, in many cases, recover the calorific value from the waste being incinerated. Waste incineration plants can use heavily contaminated plastic waste collected from various waste streams for energy recovery. However, this recovery system is generally considered the most expensive among all the other alternatives.

It is important to note that incineration of plastic waste may lead to generating harmful pollutants like dioxins and furans, which is highly undesirable. Therefore, caution must be exercised when considering incineration as an option for plastic waste management to minimize environmental pollution.

5. RECENT TECHNOLOGIES FOR PLASTIC WASTE MANAGEMENT

5.1. Plastic to Road Construction

The use of plastics in the construction of roads provides a novel approach to recycling post-consumer plastics. Plastic roads can be constructed entirely from plastic or a combination of plastic and other materials. In India, flexible roads are being constructed successfully using a specially designed technique involving waste plastics in the road laying process.

Plastic types can be used for road construction, such as Polystyrene (PS) sourced from rigid packaging, cartons, plates, and vending cups. Polypropylene (PP) is obtained from ketchup bottles, yogurt cups, and similar items. Polyethylene (PE) in both high and low densities is acquired from plastic bags,

water bottles, shampoo bottles, Etc. It should be noted that using PolyVinyl Chloride (PVC) sheets or Flux sheets is not recommended.

5.2. Plastic to Alternate Fuel

Co-processing is when materials like plastic waste are used in industrial processes to replace primary fuel and raw materials. This is done in cement, lime, steel production, and power plants. Co-processing plastic waste benefits the cement industry and waste management authorities, as it saves fossil fuel and raw material consumption and reduces the need for other plastic waste disposal methods. Additionally, using this method eliminates the need to invest in other plastic waste practices and helps reduce landfill waste. Plastic waste used for co-processing is called alternative fuel and raw material (AFR).

5.3. Plasma pyrolysis

The plasma pyrolysis method is considered environmentally friendly and is recognized under the Kyoto Protocol as a way to reduce global warming. It operates on a zero-discharge philosophy, and all input materials are recycled back into nature or the market to protect human health and the environment. Plasma technology has the potential to generate electricity and hydrogen from various types of waste without emitting harmful substances like dioxin, furan, and mercury. The EPA recommends that municipalities install plasma facilities to eliminate the need for landfills [16].

6. CONCLUSION

India produces a relatively small amount of plastic waste compared to the global average. However, it is projected to climb to fifth on the list of plastic waste contributors by 2025. Plastic waste has a significant environmental impact, with plastic pollution in the ocean affecting almost all species groups and reducing the productivity of critical marine ecosystems. Plastic disintegrates in the ocean, and macroplastics become microplastics and nanoplastics, posing significant ecological risks. Plastic-related persistent organic pollutants are released through open incineration of municipal solid waste and informal plastic waste recycling in developing nations, creating a significant danger to the environment and health of humans and animals. Conventional technology for plastic waste management includes recycling and landfills. However, recycling should only be applied when the energy consumed in the recycling process is lower than the energy required to produce new materials.

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A Review on Sustainable Composting Methods in Waste Management

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ABSTRACT

Humans induce a lot of waste; Most of them now affect the air we breathe, the water we drink, and land on which we live. According to the United Nations, about 11.2 billion tonnes of solid waste is collected worldwide, nearly all of which comes from humans alone. Shy running of bio waste is a common practice which is not safe and can be replaced with safer eco-friendly system as composting. The world is tending towards the environmental and mortal health. The thing is to reduce the amounts of natural resources consumed, reusing the products taken from nature as much as it's possible, and creating as minimal waste as possible. It's our responsibility to maintain sustainability for the benefit of our terrain as well as future generations. A well-performing sustainable system, should incorporate feedback circles, concentrate on processes, embody severity and divert wastes from the disposal. Composting is efficient in which the conversion of biodegradable products and wastes into stable products with the aid of microorganisms. Although composting is a long-accustomed technology, it has some shortcomings that have reduced its extensive operation and effectiveness. Composting can play a significant part in this sector. This review comprises different global composting styles used in the field of waste operation. And thereby, anatomize the extremity in this sector and should come up with strategies that will manage waste sustainably.

KEYWORDS: **Composting, Sustainable, Environment, Eco-Friendly**

1 Introduction

Indiscriminate dumping of waste is mischievous to mortal health. Piecemeal from being uncomely, it causes air pollution, affects water bodies when ditched into the water, as well as depletes the ozone film when burnt, thereby adding the impact of climate change. Wastes are frequently managed (1, 2) using conventional styles. Wastes are burnt, disposed into abysses, aqueducts, and ditched by the roadsides (3). These practices breed insects and pests, release obnoxious odors, and contribute to global warming (during combustion). (4). When treated anaerobically, biogas and can be used as biofertilizers (5). Composting is a safe system. Composting is an aerobic process where complex degradable accouterments are degraded and converted by microorganisms into organic and inorganic (6). It contains 'humic- such like' composites that separate them from those set up in native soil, coals, and peats. Composting is a means of transubstantiating different degradable wastes into products that can be used safely and beneficially as biofertilizers and soil emendations (7 – 9). The composting process helps to cover underground water from getting defiled compared to the land filling system

of waste disposal, which could pose a pollution trouble to underground water. This is because there's a reduction of the microbes and chemical adulterants during composting. These are the pathogenic microbes present in waste which are dangerous to humans.

SUSTAINABLE COMPOSTING METHODS

1. Ring Compost

It has a capability of four to five weight units of biomass (kitchen waste, theater waste etc.) shall be deposited for corruption of guck. Redundant water in the caddy ends up in decaying rather than foul smell might induce. Chlorinated water or kerosene could not be added which is suitable to have an effect on microorganism growth.

2. Triple bin system

The triple bin system jointly permits you to still compost throughout the time, whereas jointly having compost instantly accessible for formerly you want it within the theater. In a three-caddy system, the primary section of the compost container is for recent scraps. This can be once the composting gets started. Because the food and yard waste begin to intrude down, compost begins to produce. Still if you retain adding new waste on top of the compost that's in development, it takes longer to induce quality compost that you just ought to sow spring seeds, the compost begins to produce, you will be suitable to move the wares to the alternate section, simply move the finished compost to the third caddy for holding till you would like it, without concerning uncomposted waste in your compost.

3. Organic Waste Converter

A waste device could be a machine used for the treatment and employment of solid and liquid garbage material. The system of changing solid waste into compost is incredibly easy, and it saves the setting by turning waste into a solid or appertained to as a helpful-product. This machine performs its medium within the presence of air. The total medium overcomes all the walls which can have an effect on the system in a veritably unhealthy system. The input it takes is perishable waste and thus the affair it produces is a awful substance. The structure of this machine is constructed in a veritably system that it's completely different doors or openings for taking the input and for giving the affair. Composting is the resolution to our waste operation issues Associate in Nursing it's a pressing would like of the hour that must be taken care of incontinently. The application of Organic Waste bias to automatize and ease the system of composting could be a step within the right direction. The before we tend to borrow it, the advanced we're suitable to do for the setting. One should detain mind that the magnitude relation between the carbon and between the gas should be strictly handled formerly waste is being composted. However, the composting can take longer and if there is an inordinate quantum of gas, the system can emit a nasty odor, If there are tons of carbon. Indispensable necessary factors embody the stuffiness content and thus the chemical element content within the pile. All of those factors should be borne in mind once composting. Composting manually is frequently a tough system. One may, thus, suppose about the application of Associate in Nursing Organic waste composter. Before composting, the waste is constantly base up, to cut back the quantum of the waste, and might simply be transported..

4. Berkley Rapid Composting

This is a fast composting system. Then, accouterments compost briskly if the size is between0.5 –1.5 elevations in size. Soft, succulent apkins don't need to be diced in veritably small pieces because they putrefy fleetly. The harder the apkins, the lower they need to be diced to enhance corruption. Once a pile is started, nothing should be added because it takes a certain length of time for the original accouterments to break down, and anything added has to start from the original breakdown stage therefore dragging the corruption time for the whole pile

5. Pot compost

Pot compost is compatible, needs less area, appropriate for tiny families generating up to 2 kg waste per day. It ought to be unbroken far from rain. Make a tiny low hole within the bottom of the pots. Place the [*fr1] metric {capacity unit} capacity plastic vessel below the primary pot. Begin filling the sequestered bio waste (do not place slow degrading things and non-degradable things into the pots) daily into one pot and keep the pot closed. This kind of waste treatment is capable of treating one to a pair of weight units of waste per day solely. Leachate beginning of the pot gets collected within the plastic vessel placed below the pot. Place some salt powder into the plastic vessel to avoid entry of flies into the vessel. The leachate collected will be diluted with water and used as manure within the garden. - Once the primary pot is full to begin victimizing the second pot, by this time the second pot will becomes full of waste, within the initial pot gets revised again into compost. When the initial week of commencement, heap of worms are going to be seen within the pot. Don't try and kill them, they activate the composting method and that they die after three weeks. Throughout time of year unfold the plastic sheets over the pots and place the brick items over the sheet to shield the pots from rains. If the amount of the water within the pot is a lot, add some saw dirt to soak up the water. If an excessive amount of flies are seen round the pot, create an answer of natural resin in copra oil (dissolve a pair of tablets in 25ml of oil) and apply it on a very cheap and high cowl of the pot, by means of the comb. Sprinkling diluted rotten curd or rubbish resolution etc. into the waste can speed up the composting method.

6. Windrow composting

Windrow composting is that the most typical technology enforced in most of the ULBs within the country. Windrows area unit outlined as frequently turned elongated piles, quadrangle in cross section and up to 100 meters or additional long. Windrows composed of MSW area unit sometimes needed to be settled on AN water-repellent surface. Turning the pile provide adequate aeration into the pile and will increase consistency. The windrow dimensions ought to permit conservation of the warmth generated throughout the composting method and permit air to diffuse to the deeper parts of the pile. They will be turned as often as once per week, however additional frequent turning is also necessary, if high proportions of biosolids area unit gift within the feedstock.

7. Vermicomposting

Vermi composting could be a changed and specialized technique of composting, and it's the tip product of the breakdown of organic matter by some species of angleworm. Vermicompost could be a nutrient-made, natural fertilizer and soil conditioner. The angleworm species most ordinarily used are the area unit Eudrilus eugeniae, Eisenia foetida or Lumbricus rubellus. A by-product of vermi composting referred to as vermiwash (which are often collected if there's a faucet at the bottom of the vermicompost tank) conjointly serves an equivalent purpose. Tiny scale vermicomposting is finished in bins of variable sizes and elegance and 3 different kinds of practices like non-continuous, continuous vertical flow and continuous horizontal flow area unit adopted. The ways for big scale vermicomposting area unit windrow and raised-bed or flow through systems. Flows through systems area unit similar temperament to indoor facilities, creating them the well-liked alternative for operations in colder climates. Room wastes except oily and spicy things are area units appropriate for worms. However an excessive amount of room waste ends up in purification before worms will method it and becomes harmful to the worms. Within the usual method vermin composting is practiced now could be labor-intensive and needs some infrastructure. However, at the family level it's found terribly effective. The study allotted by Center for surroundings and Development showed that plastic tumbler and Ferro-cement boxes are often used effectively for vermin composting at family level. The solid waste at family level shall be managed by taking compost pits or by (wherever land is available) establishing vermi composting pit/bins. The vermin composting needs very little care.

The following aspects got to be taken into thought whereas designing a vermi composting program. i) The Vermi composting plant ought to be protected against flies, ants etc., by providing a metal web covering. ii) Extreme wet and dry conditions can damage the worms and care ought to be taken to manage warm temperature by sprinkling water or swinging a wet burlap bag higher than the plant particularly throughout summer season. iii) The Composting plant won't cause any smell, odour, or any unhealthful atmosphere, thus it is often placed within the house presumably in work space or perhaps in a very corner of the room.

8. Black Soldier Fly Composting

It is naturally occurring industrious insects mostly seen in farmyards with a chicken coop. Its larvae feed on rotten food. Place a container outside the yard. Add dry waste in the bottom layer and kitchen waste (both including the meat and dairy products) is regularly in 1:2 proportionality to ensure the heap is moist and purchase the black soldier fly larvae from the farm supply stores., Duration of compost depends upon the number of larvae and quantity of waste. Compost duration will be taken according to the number of larvae and One larvae consumes twice its own body weight

9. Bokashi Bucket

A bokashi bucket system which was developed in Japan is a very ancient form of food waste conversion. It can be kept indoors if needed. Add bio waste including both dairy and meat products into the bucket and sprinkle a layer of bran over the waste and repeat the process until it's full and within one month the residue will be ready to move out. Then remove the residue and cool or dry it under the sunlight for further processing. Twice a week the leftover liquid can be collected from the spigot and can be used as a fertilizer. After ten days, dig up the cured compost and it is ready for harvest

10. Food Recyclers

Most modern version of food waste composting and highly expensive model. Turn on the food recycler machine and add the bio waste including both meat and dairy products. No additives are required for the decomposition of waste. Within 4-6 hours the residue will be ready for further treatment. The collected residue can be transferred to the soil for further composting. The filter used in the food recycler machine should be replaced within 3-4 months.

RESULT AND DISCUSSION

Composting is an important and sustainable way to reduce the amount of waste that ends up in the landfill. Composting allows us to recycle our waste by breaking down into a nutrient rich soil perfect for growing plants and crops. Windrow method have the flexibility to handle changing feed characteristics and peak loads, require relatively simple mechanical equipment, and are simple to operate. Rather than, bokashi bucket and food recyclers require relatively small areas and have the ability to control odors. In the case of Organic waste converters which are an environmentally friendly technology. Its solution is an alternative to traditional methods of organic waste disposal such as landfill dumping and incineration. Vermicomposting creates nutrient-rich compost in an eco friendly nature to reduce waste and it does not release any harmful gases into the environment. Vermicomposting also reduces the amount of solid waste sent to landfills. whereas, in windrow composting which is labour intensive method it is not suitable for small amount of waste and can be difficult to monitor temperature and moisture levels and ensuring proper aeration and oxygenation of the material could be a difficult task. Berkley rapid composting is a fast composting method but if any intermediate materials are added has to start from the initial breakdown stage and thus lengthening the decomposition time for the whole pile. Triple compost bin which is time consuming to build and cannot compost meat and dairy products. Whereas, excess water in the ring compost bin ends up in decaying rather than decomposition and foul smell might generate. Meanwhile in the black solider fly method Meat and dairy will attract other pests which are a risk of pathogens. The byproducts are not technically

compost in the case of bokashi bucket and food recyclers and both requires additional curing period in soil. Regular additives are needed for bokashi bucket and filters are essential for eliminate the odors in food recyclers. Taking too much time for composting and also flies get attracted towards the bin is another issue in the case of pot composting. Composting helps to conserve energy otherwise used to transport and dispose of solid waste. . It is an easy and inexpensive way to recycle organic material such as fruit and vegetable scraps, grass clippings, leaves, and other organic materials. Meanwhile, composting process can emit unpleasant odors if not managed properly. And also it cannot be suitable for some types of waste, such as plastics, metals, and other inorganic materials. And also composting can also attract pests and rodents if not done cautiously.

CONCLUSION

The composting process has been shown to be a largely effective technology for turning organic waste into a usable agrarian product. Numerous dangerous substances, heavy essence from organic waste during the composting process, can be reduced by the composting fashion. It has been successfully demonstrated throughout our country, as well as the world. It's economically competitive with other waste operation styles. With the continued reduction of available tip

space and anticipated high collection and disposal freights demanded to cover the cost of the garbage disposal installations being erected moment, In addition, compost is an environmentally salutary product.(10)While large scale composting operations will be decreasingly important, the most cost-effective way of handling yard, kitchen and theater waste is in our own neighborhoods, avoiding trucking and energy costs.(11) An attempt is made to compare the colorful composting styles for waste operation and after reviewing them it's observed that whatever composting system we borrow, it can be used according to the consumer's time, yard space and plutocrat. Eventually, further exploration should be carried out to discover how to enhance the duration of composting. Though the Berkley system was discovered in the history and is still the fastest composting system, the discovery of faster styles will help to sustain the composting process.

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A Review on Impacts of Urban Heat Islands in Cities and the Importance of Green Buildings

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ABSTRACT

The term "urbanization" refers to the population shift from rural to urban regions, the accompanying decrease in the rural population, and how societies adapt to this change. Global warming and climate change are among the major challenges in the twenty-first century. Rapid development causes numerous environmental issues. As a consequence, urban heat island is a significant issue. It damages our urban ecology and harms public health. Pollutants in the air in metropolitan areas cause more precipitation, which results in the highest temperature. Heat islands raise both total electricity consumption and peak energy demand. Green structures and cool pavements are the most effective ways to decrease urban heat. This encompasses all elements of the ecology of any organism found in urban areas, as well as large-scale concerns of city ecological sustainability. Green building allows us to save energy, reduce waste, and decrease greenhouse gas emissions. Efficient ventilation aids in efficiency, energy saving, and the treatment of health issues. It also enhanced air quality and reduced stress for those who live in those buildings. This review covers various techniques for constructing green buildings. Green buildings contribute to the ecological equilibrium of our cities. Green architecture is also referred to as sustainable or high-performance construction.

KEYWORDS: **Green buildings, Urban Heat Islands, Urban ecology**

1 Introduction

Every human activity creates various adverse effects on our environment. Urban sprawl and the emergence of urban clusters are problems faced by any city. This in turn creates an impact on the micro-scale climate of the area. Rapid urbanization is one of the factors to cause urban problems. Urban heat is a big problem facing many cities. The urban heat island (UHI) is a phenomenon whereby urban regions experience warmer temperatures than their rural, undeveloped surroundings. The UHI is the most obvious atmospheric modification attributable to urbanization, the most studied of climate effects of cities and an iconic phenomenon of urban climate (1). Numerous factors are held accountable for this effect, including anthropogenic heat release, surface cover, climatic conditions, air pollutants, etc. (2). One of the important mitigation measures includes green building. The green building strives to minimize the number of resources consumed in the building's construction, use and operation, as well as curtailing the harm done to the environment through the emission, pollution and waste of its components. Sustainable architecture seeks to minimize the negative environmental impact of buildings by efficiency and moderation in the use of materials, energy, development space and the ecosystem at large.

URBAN HEAT ISLANDS

Urban heat island effect is defined as the presence of significantly higher temperatures in urban areas compared to the temperatures in surrounding rural zones mainly due to human factors (2). Urban heat islands occur many problems in cities. The main causes of urban heat islands include low albedo materials (Albedo is the ratio of the reflected solar energy to the incident solar energy. It depends on the arrangement of surfaces, materials, pavements, coatings, etc (3). If the albedo of the urban surface is low, it will store more solar energy, and the effect will be an increase in urban temperature), paved and impermeable surfaces, lack of vegetation, Buildings contain much thermal mass (they store much heat during the day and are slow to release the heat overnight), Air pollutants – Air pollution is a major issue in urban areas. Exhaust gases from vehicles, industrial pollutants released in the atmosphere, trap solar radiation causing an increase in temperature and climate change (4).

EFFECTS OF URBAN HEAT ISLANDS

Higher energy consumption

The temperature in cities is higher, especially on summer nights. This generates an increase in the demand for energy to power air-conditioning units, which in turn drives up the price of electricity

Elevated emissions of Air pollutants and Greenhouse Gases

Urban heat islands demand more electricity during the summer seasons. It leads to more consumption of fossil fuel power plants to meet much of this demand, which in turn leads to an increase in air pollutants and greenhouse gas emissions.

Impact on health

The high temperatures can affect the health of city dwellers, causing widespread discomfort, respiratory problems, sunstroke, dehydration, tiredness and even increased mortality rates due to heatstroke.

Humidity effects

Although there is little difference in the amount of water that cities and country sides retain in their atmospheres (absolute humidity), the higher urban temperatures effectively lower the relative humidity (since warm air can hold more water than cold air).

Urban haze

The haze of air pollution hanging over many cities can act as a miniature greenhouse layer, preventing outgoing thermal radiation (heat) from escaping urban areas.

However, urban areas are more susceptible to heat because the amount of warmth generated by global climate change is exacerbated by the urban heat island effect. As the climate warms, this means that city dwellers will confront higher temperatures and more intense heat waves in the future. More than half of the world's population now lives in cities, and by 2050, the number of urban people is predicted to reach 70%, implying that the problem of urban heat islands will worsen (5). So, the mitigation of the urban heat island is very important in cities. Green buildings are helpful to reduce urban heat effect in cities. Sustainable architecture is architecture that seeks to minimize the negative environmental impact of buildings by efficiency and moderation in the use of materials, energy, development space and the ecosystem at large.

GREEN BUILDINGS

Green building, also known as sustainable building or green construction, is the concept of designing and building structures and processes that are environmentally responsible and resource-efficient throughout a building's life-cycle, from siting to design, construction, operation, maintenance,

renovation, and deconstruction. Green architecture is one of the options to limit the waste of drinking water for applications where the required quality can be lower (6). In their studies, they reported the applications of three different green architectural solutions and rainwater and treated greywater is used methods like roof wetland, green roof and green wall in three different countries like Tanzania, Italy and India. Bionic building energy efficiency and bionic green architecture are important factors to achieve the sustainable development of buildings (7). They analyzed the applications and typical cases of bionic building energy efficiency and bionic green architecture. With the help of solar energy construction technology improve the indoor thermal environment and leads to low energy consumption for buildings.

The influence of green ecological buildings on energy saving and emission. In their studies, they calculated the total energy consumption of green ecological buildings and energy consumption in the operation stage of green ecological buildings (8). The energy consumption in the operation stage of green ecology is compared with that of ordinary buildings. It revealed the energy consumption of an ordinary building is more than that of green ecological buildings. Many journals work reported the benefits of green buildings. Five major elements of green buildings include Sustainable site design, Water conservation and quality, Energy and environment, Indoor environmental quality and Conservation of materials and resources (9).

BENEFITS OF GREEN BUILDINGS

Energy efficiency

Green building designers make every effort to avoid reliance on non-renewable energy sources such as coal. It installs solar panels to harness solar energy and designs windows to let in as much natural light as possible, reducing the need for artificial lighting.

Protect existing natural spaces

Green buildings tend not to be constructed on environmentally sensitive lands. If they are constructed on or near green spaces, measures are taken to limit the impact on the local ecology.

Preservation of the natural environment

The operation of green buildings helps to conserve their natural habitat. This event encourages residents to develop healthy habits such as walking, exercising, and participating in other physical activities. It also attracts birds and other species, resulting in a diverse environment in the whole region.

Green roofs and vegetation cover

Green roofs present a great method of lessening the impacts of urban heat islands. Green roofing is the practice of planting vegetation on a roof, just like they are planted in a garden. Plants on the roof are excellent insulators during summer and decrease the overall urban heat island effect (10).

Enhanced Health: Eco-Friendly For Life

Living in a sustainable building can literally save your life. According to research, people who live in green constructions benefit from a plethora of health benefits as a result of the eco-friendly materials used in construction. Green buildings, for example, avoid utilizing construction materials that may include dangerous volatile organic compounds (VOCs) or plastic by-products that have been shown to emit poisonous vapours and carcinogens into the environment. These hazardous compounds have been related to respiratory disease, allergies, and other health problems, as well as an elevated risk of cancer in extreme circumstances.

Enhances Indoor Environment Quality

Good indoor environment quality is one that protects the health of the building's occupants, reduces stress and improves their quality of life. Green buildings achieve this through the installation of operable windows that allow in as much sunlight as possible and reduce the use of materials that may emit elements that are dangerous to health.

Reduce Carbon Footprint

As green building consumes less energy during construction as well as in operation. They are more environmentally friendly. Less energy consumption means less carbon footprint on your building.

Sustainable Materials reduce the impact on the environment

Sustainable architects, engineers, and green designers are tapping into existing resources to lower carbon footprints and save natural resources by employing recycled materials and recycling resources (and even repurposing old structures). Green building businesses may produce very efficient structures that can last the test of time by incorporating sustainable tactics into the design process, such as eliminating waste, saving natural resources (such as water and wood), safeguarding our air supply, and limiting energy use.

EXAMPLES OF GREEN BUILDINGS IN INDIA

Rajiv Gandhi International Airport (RGIA), Hyderabad

The sixth busiest airport in India, located in central Hyderabad, has established a precedent for green structures in the country. The airport's structure is meant to use less water and power while conserving natural resources. There is a 273-hectare green belt with numerous vegetation on the airport campus. RGIA has successfully saved almost 3.97 million kWh of energy and reduced its carbon footprint by 3331 tons during the last few years. The GMR airport is the 1st airport in Asia and 2nd globally to have won a LEED silver rating certification

CII- Sohrabji Godrej Green Business Centre, Hyderabad

This architectural marvel has established the best example of passive architectural design in the world. At the time of its opening, the CII-Sohrabji Godrej Green Business Center (GBC) was the first building outside of the United States to be awarded a LEED platinum rating. The building generates no garbage and recycles it entirely inside. The structure is literally constructed entirely of recycled materials. Two air-cooling towers chill the air by up to 8 degrees, a terrace garden covers almost 55% of the roof, and solar cells on the roof produce nearly 20% of the required energy.

Indian Green Building Council

The Indian Green Building Council (IGBC), part of the Confederation of Indian Industry (CII) was formed in the year 2001. The vision of the council is, "To enable sustainable built environment for all and facilitate India to be one of the global leaders in the sustainable built environment by 2025". The council offers a wide array of services which include developing new green building rating programmes, certification services and green building training programmes. The council also organizes the Green Building Congress, its annual flagship event on green buildings (11).

CONCLUSION

The study of ecological processes in urban areas is known as urban ecology. This comprises all aspects of the ecology of any organism found in urban regions, as well as large-scale issues of urban ecological sustainability. Green building contributes to the ecological equilibrium of our cities. Green building is partly responsible for lessening the influence on urban ecosystems. It also enhanced air quality

and reduced stress for folks who live in those buildings. It alleviates heat stress in cities. The initial construction expense is one of the disadvantages of green buildings. Green building materials are not always as widely available as traditional building materials,. Green buildings, on the other hand, are the wealth of the future. Several well-known cities have embraced this method and established councils to determine the reasons and regulations for awarding a "green" stamp to a newly constructed building.

ACKNOWLEDGEMENT

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15

Effect of Vertical location on Canard with three different Location to make Airplane Eco friendly

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ABSTRACT

A canard is a wing configuration in which a small forewing is placed forward of the main wing of a fighter plane. This paper includes the information regarding the aerodynamic analysis of an aircraft with canard configuration at three different vertical position. Canard is used to increase a lift force; it helps in gaining stability and better controls of aircraft and detects the flow and it's changes over the main wing. It also helps in reducing overall drag of the airplane. The flow behaviour of the aircraft changes with respect to the change in position of canard (i.e. high, mid, low position). The effect of canard on the aerodynamic forces has been performed. Airfoil N10 is used for the main wing with air speed of 25 m/s. The NACA 0012 Airfoil was used for the canard configuration. The comparison of canard at different position that is i.e. high, mid and low positions is compared. The result of simulation showed that mid position of the canard performed aerodynamically better than the conventional configuration with lower drag nearly 17% from the traditional configuration. Which is a green concept due to reduction in fuel consumption by 10% which protect the environment.

KEYWORDS: **Canard, Fluid Flow analysis, lift coefficient, Drag coefficient, Fuel consumption.**

1 Introduction

The canard aircrafts are more stable and efficient. The main difficulty observed in the canard setup aeroplane is that the vertical position of the canard is located closer to the nose of the aeroplane. Assume we modify the upward position of the canard setup wing to calculate the difference in lift and drag. This way, we can dazzle with current innovation by utilising the most recent flight control framework. The flow field around the canard wing aircraft was simulated using computational aerodynamics to provide superior aerodynamics characteristics and aerodynamic efficiency. [1]. A canard as a forewing on a fighter wing layout is thought to be capable of increasing manoeuvrability. The employment of canard-delta pairs will have an effect on the plane's performance and aerodynamic properties [2-4]. Delta-configured wings and canards will generate a rolled-up vortex as a lifting force producer on the aircraft. The vortex core generated by the canard and the main wing will interact with each other to sustain lift. As a result, the design of the canard and main wing pairs becomes critical in producing flow interaction patterns [4-6]. The swept-back wing design, which is the fundamental form of the wing and canard of a fighter aircraft, produces a rolled-up vortex, which adds lifting force. The vortex core is a region at the centre of a rolled-up vortex that has very high velocity and low pressure as a lifting force [7, 8]. This vortex core is where the concentration of lift is created on swept-back aircraft. The addition of forewings, such as a canard or Leading-Edge Extension (LEX), creates a powerful vortex

core that leads backward, affecting airflow down the main wing [9,10].According to Yoshimoto, M., and Uchiyama emphasised the benefits of optimised canard surface location for supersonic aircraft in terms of reducing sonic boom and improving lift to drag ratio. The findings indicated that, among the numerous design factors tested, the setting angle was the most sensitive to the two objective functions tested: sonic boom intensity and lift to drag ratio [11-14]. So that reducing drag percentage will be increase and we can save the fuel efficiency in fighter aircraft. Skujins and Cesnik evaluated the influence of the canard on the flow over the elevons control surfaces. The calculated findings revealed that the slipstream behind the trailing edge of a canard had an effect on the elevon's control efficacy [15-17]. Preliminary testing also revealed that the influence of the slipstream on the elevons decreased with increasing distance from the canard [4]. In this research the vertical location of canard effects are studied.

2. Research methodology

The inspiration for the current study stems from prior studies that show the influence of canard structure on air flow around it. The inspiration of this work is the work done by Dr. Dwivedi [5] in which the skin frication drag was study for the canard configuration, this is continuation of his research to study the effects of vertical location of canard with three different location analyse using Computational tools and Static analysis tool.

2.1 Airfoil Selection

Therefore, to overcome this issue, a mid-wing, mid canard configuration was chosen for this study. There are two airfoil geometries ware chosen for this study to create canard and main wing. Fig 2 NACA 0012 airfoil was chosen for the canard plane [5] and Fig 1 N10 airfoil chosen for the main wing of the aircraft [6]. NACA 0012 airfoil is symmetrical airfoil which is more stable, improved the aircraft performance in basic aircraft at low Reynolds number. N10 Airfoil is performed high stability and better aerodynamic Performance of the Aircraft, it is main using for the RC aero modelling community for its providing high lift at delayed stall angle enabling high performance. Preliminary analysis chosen for the work is done by the x-foil analysis in XFLR5 Software.

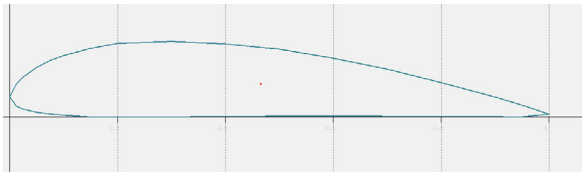

Fig 1: N10 AIRFOIL

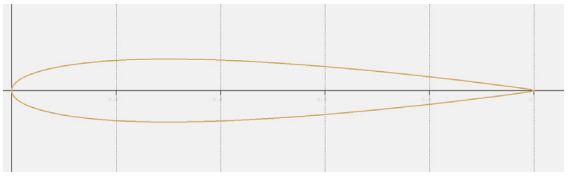

Fig 2: NACA0012 AIRFOIL

2.2 Geometry Modelling

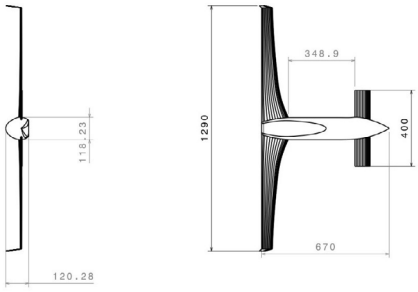

Fig 3: CAD model with Dimensions

The general geometrical specifications from Fig 3 of the wing, canard, and fuselage connecting these two were derived from (5, 16, 17). The wing and canard were built with aspect ratios of 8.15 and 4,

respectively, such that the canard may stall before the wing did. The wingspan for the main wing is 1.27 meter and tip chord of 0.16 meter giving a tapered ratio of 0.33 because a swept back wing produces smaller wingtip vortices than a rectangular wing. The main wing also consists of a swept back angle of 21.49 degrees as a sweep can further increases reduce drag. Here table 2 is the Wing design Parameter (Table 1 & 2).

The canard wing is a Tapered wing section having a canard span is 0.4 meter and chord length of 0.08 meter. The fuselage was also designed having a total length 0.67 meter, width 0.118 meter, and a height 0.12 meter. This study will more focus on the mid canard with mid swept back wing. Here the distance between main to canard wing is 0.34 meter. The main wing located to meter to fuselage reference line and canard was located to meter fuselage reference line. Here table 1 is Canard design Parameter.

CATIA V5 software was used to do 3D CAD modelling of the considered geometry Fig 4. This geometry was then employed in the computational analysis.

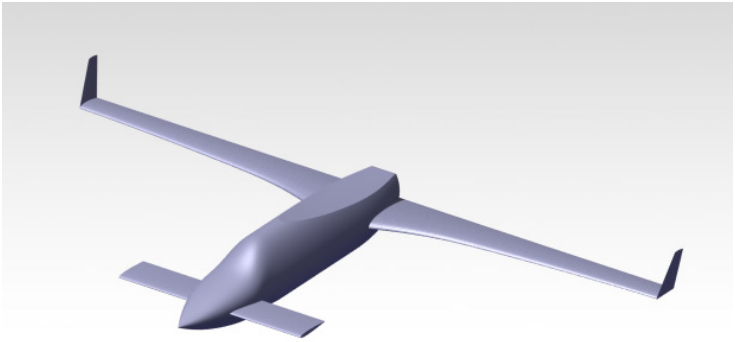

Fig 4: 3D CAD Design model

Canard Parameter	Value
Airfoil used	NACA0012
Canard span	0.4 m
Chord lengt h	0.08 m
Aspect ratio	4.0

Table 1: Canard Design

Wing parameter	Value
Airfoil used	N10
Wingspan	1,27 m
Root chord	0,17 m
Tip chord	0.16 m
Sweep angle	21.49 degree
Taper ratio	0.33
Aspect ratio	8.15

Table 2: Wing Design

2.3 Mesh Generation

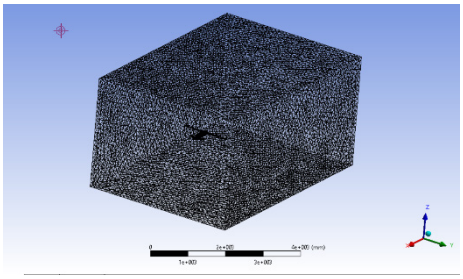

Fig 5: Mesh

Boundary	Type	Value
Inlet	Velocity-inlet	25 ms-1
Outlet	Pressure-outlet	Atmospheric
Walls	No slip	NA

Table 3: Boundary condition

The Mesh independence study from the Ansys CFX proved that the mesh generation from Fig 5 is successful with boundary condition from table 3.

3. Theory and calculation

3.1 XFLR5 Calculation

In this experiment, the XFLR5 is used for calculations, and aerodynamic and stability investigations on the wing-canard and winglet designs are undertaken. The planned sweptback wing with the chosen airfoil was aerodynamically analysed; the wing's boundary conditions were employed. The analysis is carried out from -10 to 10 AOA (Angle of Attack), with calculations made internally by XLR5 utilising the panel methodology and the VLM method. The velocity is assumed to be 25m/s at 00 AOA based on the Reynolds number, and the CP and aerodynamic coefficients are reported.

3.2 General Calculation

The general formulae CL and CD are used to calculate the theoretical values of aerodynamic coefficients equation 1 and 2. The formulae used in this experiment are listed below. Lift and drag are aerodynamic forces experienced by an airfoil as a result of pressure fluctuations above and below its surface. The moment formula, which is the foundation for this experiment and was obtained from literature, is a key formula for the stability derivative of aircraft Equation 3.

$$lif(L) = \frac{1}{2} * \rho * V^2 * S * Cl \quad \text{----------(1)}$$

$$Dra(D) = \frac{1}{2} * \rho * V^2 * S * Cd \quad \text{----------(2)}$$

$$momen(m) = \frac{1}{2} * \rho * V^2 * S * C\,(XCg - XAc) \quad \text{----------(3)}$$

Where ρ = density of the air, V = velocity, Cl = lift coefficient, S = wing surface area, C =chord length.

4. Results and discussion

4.1 Airfoil Analysis

As per the calculation done in the earlier section gave the estimated value of lift and considering this value, so we decided to cambered airfoils over flat bottom airfoil increase lift and induces drag from Fig 6 given below.

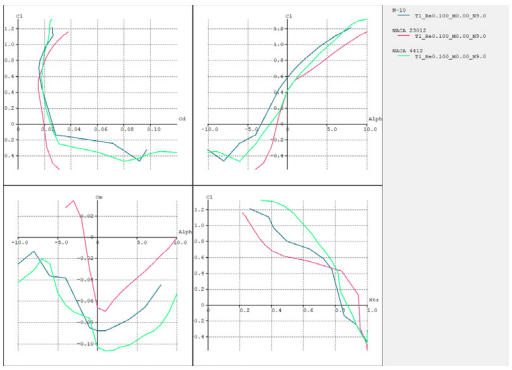

Fig 6: Airfoil Analysis

4.2 Preliminary Aerodynamic Analysis

Aerodynamics study was performed in a non-viscous flow model using the xflr5 programme. A preliminary test on the wings without the fuselage has been carried out. We know that the stall angle is at 13 degrees from using an angle of incidence of 2 degrees in the CFD study of the airfoil. Here Fig 7 is Comparison graph between three different vertical canard position..

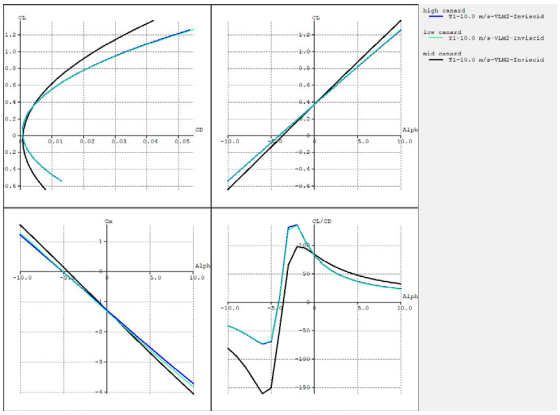

Fig 7: Comparison graph between three different vertical canard position

4.3 Aerodynamics Analysis

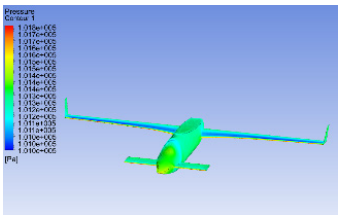

Fig 8: Pressure Contour

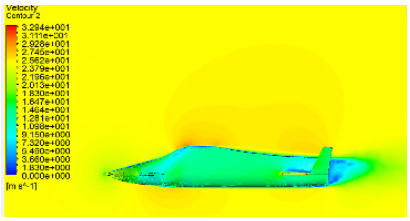

Fig 9: Velocity Contour

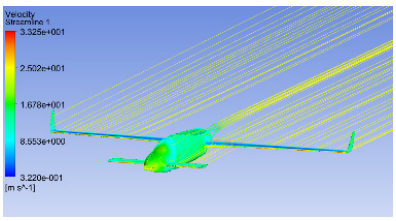

Fig 10: Velocity Streamlines

The aerodynamic analysis carried out an Ansys CFX. The Pressure contours from Fig 8 gives us that lift generation is achieved. From the Fig 9 the Velocity contours are achieved at 25 m/s. From the Fig 10 the Velocity Streamline is shown. From the result of aerodynamic analysis, the aircraft is perfectly streamlined and the pressure and velocity of the aircraft achieved according to the principles.

5. Conclusion

This experiment about the canard aircraft proves that the vertical location of the canard a small wing placed on the aircraft nose, the results and studies from this experimental analysis proved that the mid canard configuration with mid wing increased the lift by 6 % ,the drag is reduced by 17% and the CL/CD increased by the 21% compared to the other configurations , from the XFLR5 software, the range , endurance and the aerodynamic performance is increased compared to the low and high vertical positions of the canard, from the Ansys the pressure, velocity and the streamlines proved that this configuration provides better pressure difference of the aircraft and streamlines are perfect, and the reduction of drag is also achieved from this results can give high aerodynamic efficiency and can save more fuel than normal configurations of the canard , the fuel consumption can be reduced by this aircraft. This is the step to the green initiation towards the future.

6. Declarations

6.1 Acknowledgement

Thanks to Dr. Y D Dwivedi, Professor in Department of Aeronautical Engineering, IARE, Hyderabad, India, and his team for helping in this research work.

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16

Measuring the Chlorophyll Content of Terminalia Catappa l. Based on the their Leaf Ageing

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ABSTRACT

Terminalia catappa L. (Tropical Almond) is a largely grown tree comes under the family Combretaceae. They are largely grown in the subtropical and tropical climate. It is widely planted throughout the tropics for its ornamental purposes and edible nuts. The present study is based on the estimation of chlorophyll content in different aged leaves and to identify their peaks of absorbance in T. catappa. Young leaves, Mature green leaves, Half ripened(orange)leaves and fully ripened(red) leaves were the 4 categories taken for estimate chlorophyll content. Both crude and diluted extract were prepared in 80% acetone. The absorbance was measured in 663, 645, 750 nm of both extracts with the help of UV-VIS (single bean) spectrophotometer. In this study the mature green leaves extract showed maximum absorbance followed by young leaves both in crude and diluted extract. There is not much difference in the chlorophyll content among orange leaves and red leaves. Comparing to the diluted extract crude has the maximum absorbance. UV spectrophotometer (double beam) exhibited the presence of 3 peaks in both young and mature, and a single peak in orange crude extract. In diluted extract 3 peaks present for young leaves 2 peaks for mature leaves and 1 peak for orange leaves. No peaks were observed for red leaves. Highest peaks were observed for mature leaves extract. The total chlorophyll content of a leaf increases with the increase in age of leaves and after a saturation it decrease when comes to ripening.

KEYWORDS: **Absorbance, Chlorophyll, Terminalia catappa, UV-VIS spectrophotometer.**

1 Introduction

Combretaceae family is distributed throughout the tropics, with some extensions to subtropical and warm temperate regions [50]. This family comprises of 20 genera and 500 species comprising herbs, shrubs, trees, and creepers. Terminalia is one of the largest genera of Combretaceae. It is valued as timber tree for African, European, and American markets and some have edible kernels. Terminalia species are native from Africa and are now widely spread out in tropical and sub-tropical regions [37]. Terminalia catappa L. Comes under the family Combretaceae which is native to Southeast Asia T. catappa is growned for ornamental purposes and edible nuts. They are dry-season deciduous; before falling, they turn pinkish- reddish or yellow-brown, due to pigments such as violaxanthin, lutein, and zeaxanthin. The leaf extracts of T. catappa record obvious anti-carcinogenic and antioxidant activities [39] Leaves bark and fruit of the tree Terminalia catappa L. (Combretaceae) have been commonly used as a folk medicine for anti-diarrhoea, antipyretic and haemostatic purposes [36]. The leaves mature during the dry season, changing from green to brown, gold, red, or yellow [40].

Chlorophyll is an important green plant pigment stored in the chloroplast and is used to synthesis food within the help of solar energy. Therefore, the estimation chlorophyll content in plants is very

important that it gives a brief understanding about the plants photosynthetic and physiological activities. Photosynthesis is the most important source of energy for plant growth [41] [38].

Chlorophyll-a and Chlorophyll- b are the 2 most important pigment in the plant photosystem [30]. The plant photosystems contain such many pigments including chlorophyll which absorb light, and the absorbance are used to estimate chlorophyll content in a leaf. The simultaneous increase in the chlorophyll content and the photosynthetic rate from the youngest leaf to the leaf which can be described as "photosynthetically mature" [35]. As we know, chlorophyll is very important macromolecule in plant system which gives an account about the plant energy utilization and development rate. The high amount of chlorophyll in a plant indicates its rate of energy utilization rate in the form of food preparation. Chlorophyll also bears antioxidant properties which can be used in a medicinal drug discovery. So the Present study records the chlorophyll content of different aged leaves (Young, Mature, Half ripened or orange and fully ripened leaves) in T catappa. It is very important to do this kind of study to evaluate the photosynthetic activity changes of leaves of plants

MATERIALS AND METHODS

Material: Terminalia catappa L.

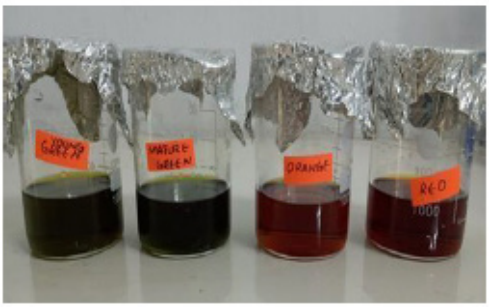
Figure 1: Habitat Sample collected

Collection: Plant materials were collected from S.N.M College, Maliankara campus and identified by Dr. E.C. Baiju.

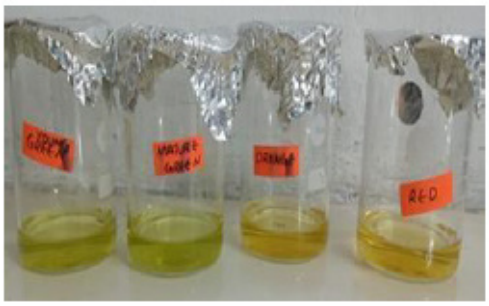
Plant materials were collected as young leaves, mature leaves, half ripened leaves, or orange and fully ripened or red leaves. Disease free clean leaf samples were collected separately, shade dried and powdered with the help of an electronic grinder. Both fresh and dry weight of leaves was recorded. Powder was stored separately in moisture free containers and used for analysis.

Preparation of extract

One gram powder of each leaf type was taken with the help of an electronic weighting balance and transferred to separate beaker. Mix with 20ml of 80% acetone, mix well and rest it for 30minutes. With the help of a filter paper the crude extract was filtered and extracted, and absorbance was recorded. Add 20ml of 80% acetone to the above solution and repeat until the colour of the solution changes to translucent.



Crude extract



Diluted extract

Estimation of Chlorophyll

Both crude and diluted extract in acetone were subjected to measure the absorbance in microprocessor UV-VIS Single bean spectrophotometer in 645,663 and 750nm.The quantitative UV-VIS analyses were also performed in UV-VIS double beam spectrophotometer to observe the peak of absorbance in acetone of acetone in both extracts. The wavelength were recorded, chlorophyll a chlorophyll b and total chlorophyll content of each leaf type in both extract were calculated with the help of formula given below

Equation for the estimation of chlorophyll:

Chlorophyll a (mg/g) = 12.7 (A663) -2.69 (A645) × V/1000 × W

Chlorophyll b (mg/g) = 22.9 (A645) - 4.68 (A663) × V/1000 × W

Total chlorophyll (mg/g) = 20.2 (A645) - 8.02 (A663) V/1000 × W

- A=Absorbance at specific wavelengths
- V=Final volume of chlorophyll extract in 80% acetone
- W=Weight of dried leaves

RESULT AND DISCUSSION

In the present study both crude and diluted extract of T. catappa were used in to evaluate the total chlorophyll content in 4 different leaf types, -young leaves, mature leaves half ripened or orange leaves and fully ripened or red leaves. In both crude and diluted extract, mature leaf indicated the highest absorbance when followed by young leaves. Mature leaves attained an optical density of 0.563, 1.331, and

0.035 at 645, 663 and 750 nm, respectively. The least absorbance were observed in fully ripened or red leave extract in diluted extract, 0.012, 0.011,0.001 at 645,663 and 750 nm respectively. In crude extract, orange leaf extract shows a higher absorbance than red leaves. Chlorophyll content in plants increases from youngest leaf to mature and after attaining this maximum value the chlorophyll content decreases when the leaf starts ripening. An increase in wavelength showed a decrease in absorbance Optical density in UV-VIS spectrophotometer (single beam) analysis of extracts in different leaf types of both crude and diluted extract were showed in table 2.

Sl.no	Type of leave	OD wavelength in nm					
		Crude extract			Diluted extract		
		645 nm	663nm	750nm	645 nm	663nm	750nm
1.	Young leaves	0.563	1.331	0.035	0.046	0.111	0.004
2.	Mature leaves	1.027	2.171	0.093	0.086	0.197	0.015
3.	Orange leaves	0.302	0.292	0.141	0.014	0.015	0.004
4.	Red leaves	0.429	0.379	0.227	0.012	0.011	0.001

Table 2: Absorbance of crude and diluted extract

Estimation of Total Chlorophyll

In young leaves, the chlorophyll-a is 0.307 mg pigment/m3 and Chl. b is 0.131 mg pigment/tissue, respectively in crude extract. The mature green leaves contained Chl. a 0.496 mg pigment/tissue and Chl. b is 0.267 is mg pigment/tissue. Mature leaves contained higher amount of chlorophyll than the young leaves. Similarly, the chlorophyll content in half ripened (orange) leaves were observed as, Chl. is 0.057 mg pigment/tissue and Chl. b is 0.110 mg pigment/tissue which is lower than in fully

ripened leaves (Chl. a-0.073 and Chl. b-0.161). In diluted extract all the values were observed very low. Based on the equation given chlorophyll a chlorophyll b and total chlorophyll content of each leaf types were calculated. Mature leaf extract (both crude and diluted) have the highest total chlorophyll content with 0.763 mg

pigment/tissue followed by young leaves 0.440 mg pigment/tissue. Red leaves in crude extract have higher values (0.234 mg pigment/tissue) than orange leaf extract (0.168 mg pigment/tissue). Diluted extract showed a very low percentage of total chlorophyll content when compared to crude extract (Table3).

Sl.no	Type of leaves	Crude extract			Diluted extract		
		Chl. a	Chl. b	Total Chl	Chl. a	Chl. b	Total Chl
1	Young leaves	0.307	0.132	0.440	0.025	0.001	0.003
2	Mature leaves	0.496	0.267	0.763	0.044	0.020	0.066
3	Orange leaves	0.057	0.110	0.168	0.003	0.005	0.008
4	Red leaves	0.073	0.161	0.234	0.002	0.004	0.006

Table 3: Chlorophyll content of four different leaf types in both crude and diluted extract

UV-VIS spectrophotometer analysis

In UV-VIS spectrophotometer profiling, the peak of absorbance of each leaf type in both crude and diluted extract were evaluated. The crude extract profile showed 3 peaks for young leaves at 783,664,607 nm with an absorbance of 0.151, 0.790, and 0.386. Mature leaves showed 3 peaks at 750,663, 608nm with an absorbance0.190, 1.385, 0.592. Orange or half ripened leaves leaf showed only one peak in 660nm (0.272). In diluted extract young leaves showed peaks at 739,665,608nm0.058, 0.222, 0.106absorbence respectively. Whereas as in mature leaf extract only 2 peaks observed at 666 and608nm with an absorbance observed as0.244, 0.144. Orange leaves or half ripened leaf extract showed peak at 662nm (0.065). Red leaves in both crude and diluted extract have no peaks. The highest peak was observed in crude extract of mature leaf with an absorbance of 1.385 at 663 nm. And a lowest peak was observed in diluted extract of young leaves with an absorbance of 0.058 at 739 nm. The data were details in the below table 4.

Sl.no	Leaf Types	Peak no	Crude extract		Diluted extract	
			Wavelength (nm)	Absorbance	Wavelength (nm)	Absorbance
1.	Young leaves	1	783	0.151	739	0.058
		2	664	0.790	665	0.222
		3	607	0.386	608	0.106
2.	Mature leaves	1	750	0.190	666	0.244
		2	663	1.385	608	0.144
		3	608	0.592	-	-
3.	Orange leaves	1	660	0.272	662	0.065
4.	Red leaves	No peaks				

Table 4: Peak of absorbance in crude and diluted extract of 4 different leaf types.

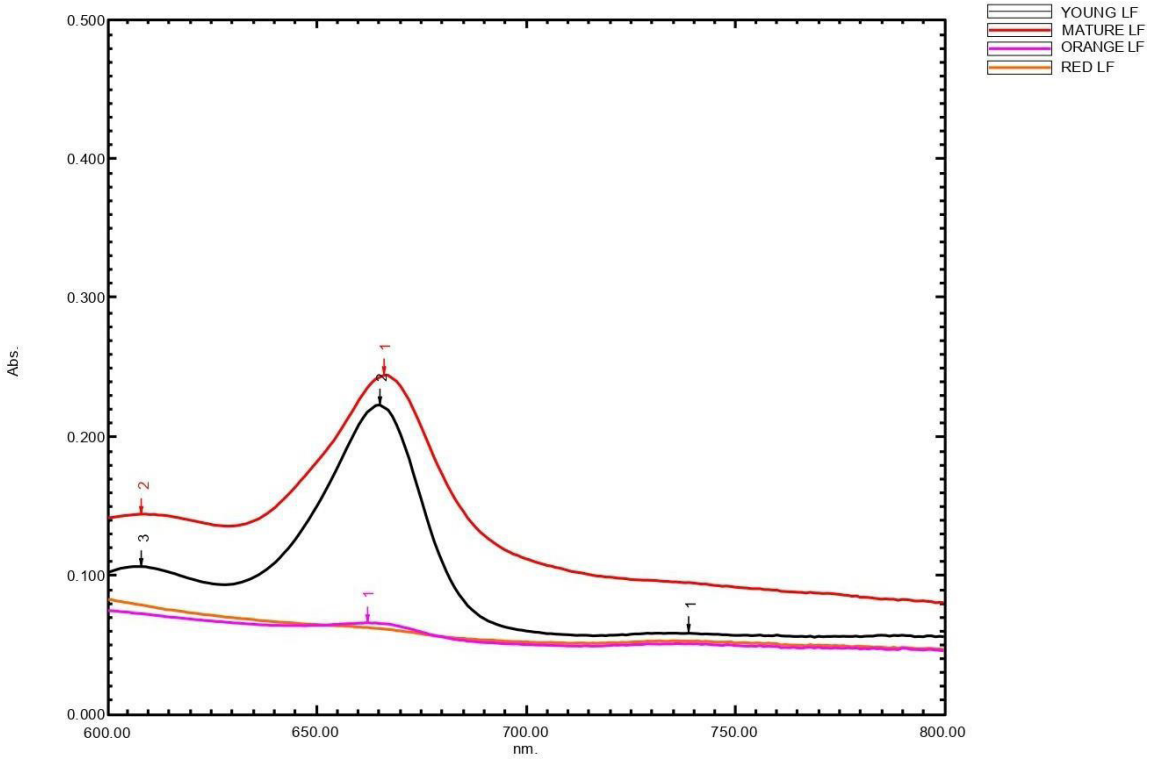


Figure 4: UV-VIS spectra in crude extracts of 4 different leaf type of Terminalia catappa

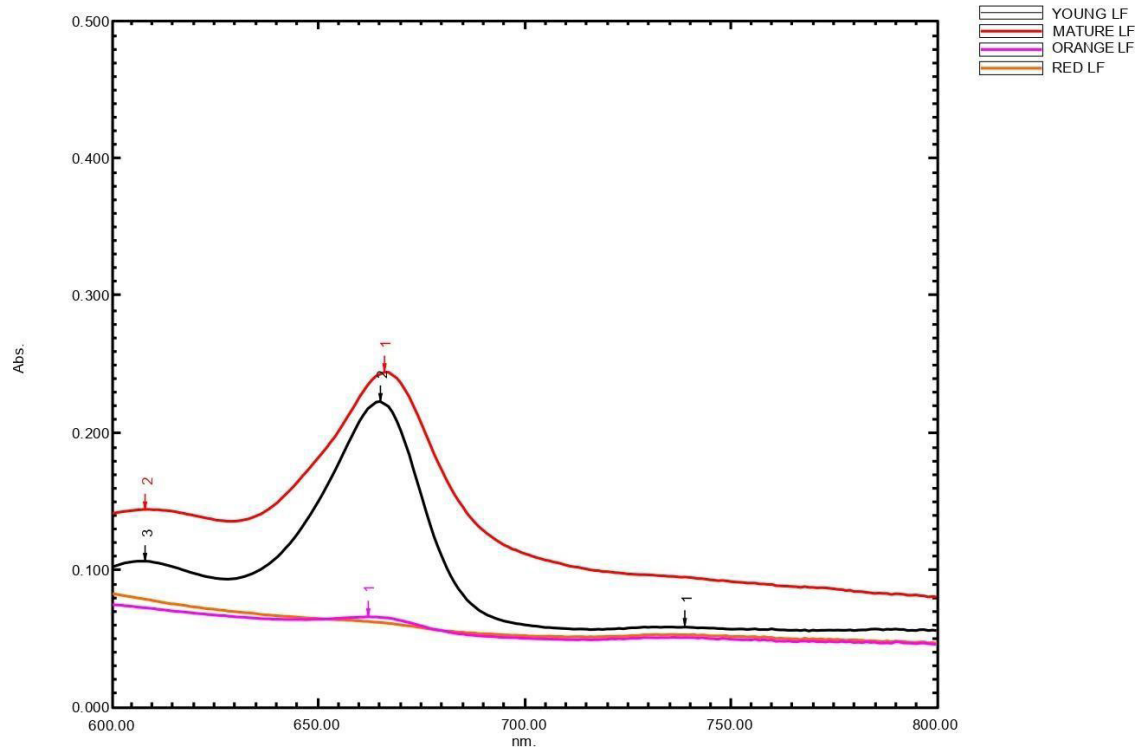


Figure 4: UV-VIS spectra in diluted extract of 4 different leaf type of Terminalia catappa

DISCUSSION

Terminalia catappa L. is a large tree from the family Combretacea. In this present study, we evaluated the amount of chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) in different aged leaves. Both crude and diluted extracts were used for the estimation in 80% acetone and measured at a wavelength of 650,663, and 750 nm. Chlorophyll content is commonly determined by using pestle

and mortar to extract the pigments using an organic solvent such as acetone or dimethyl formamide and absorbance were analyzed at a of 650wavelength [40]. The chlorophyll comprises a group of more than 50 tetrapyrolic pigment with common structural elements and functions [31]. The estimation of chlorophyll content in mature leaves seems to be highest among the rest of leaves. Sestak in 1963 said that Chlorophyll content in plants increases from youngest leaf to the leaf which can be called as "photosynthetically mature" and after attaining this maximum value the chlorophyll content decreases In this present study Chlorophyll a showed greater values than chlorophyll b in both extracts [35]. In a similar study chlorophyll, a and b was found highest in summer season as compared to winter and monsoon in T. catappa [32]. In another study chlorophyll a (0.0087), chlorophyll b (0.0075) and total chlorophyll (0.0163) were estimated [29]. The chlorophyll a is the primary photosynthetic pigment in plants which helps to produce energy in plant [45]. However the chlorophyll a concentration is 2-3 times higher than that of secondary chlorophyll b in plants.

During the study, the mature leaves extract (both crude and dilute) showed maximum chlorophyll content than young and a decrease in chlorophyll content were noticed in ripened leaf extracts. During leaf senescence, chloroplast proteins are gradually degraded as a major source of nitrogen for new growth [18][48][49]. This shows that the chlorophyll content decreases with aging or senescence. There is a simultaneous increase of chlorophyll content and photosynthetic rate from youngest leaf to mature leaf. Total amount of leaf chlorophyll content directly influences the photosynthetic capacity of plants, and this assumption has been verified by a controlled experiment using several plant species [42].

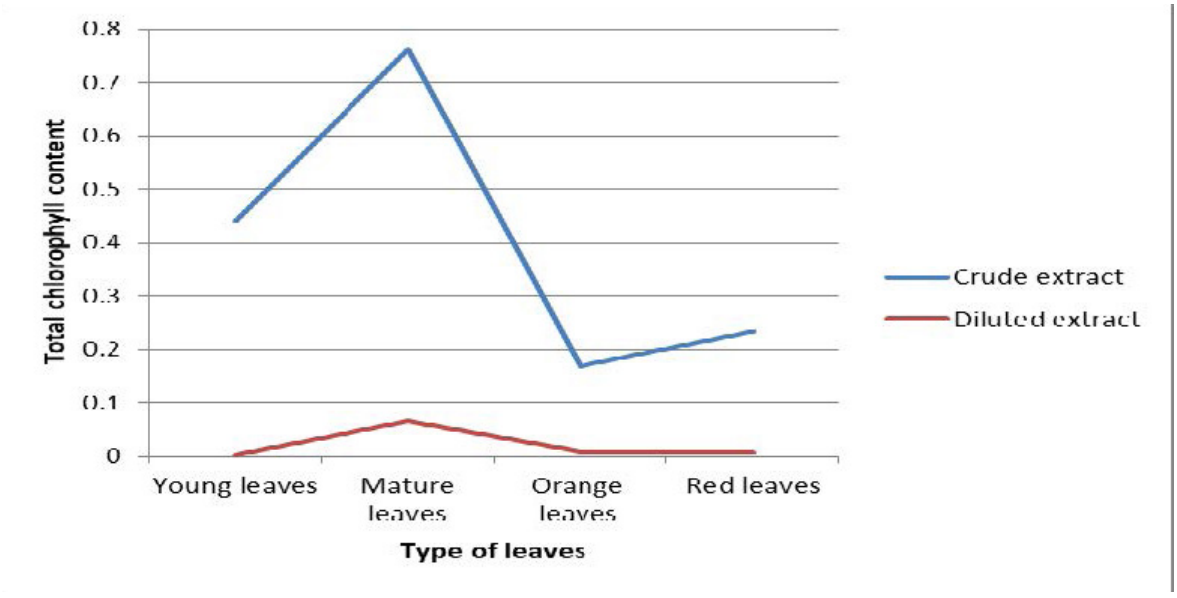


Figure 5: Total chlorophyll content of both crude and diluted extract showing the peak of absorbance, where the crude extracts showing maximum values.

The UV-VIS analysis performed for the identification of absorbance peak in four different aged leaf types of T catappa. The standard analytical methods for chlorophylls are based on spectrophotometry or fluorometery [44]. In UV-VIS spectrophotometer analysis; maximum values were observed in crude extract of mature leaves at 663nm. Evaluation of chlorophyll content using UV-VIS spectrophotometer in T. catappa with a maximum peak value corresponding to chlorophyll is 670nm [34]. In a similar study, Chlorophyll-a, b and total chlorophyll in T. catappa were evaluated at 6am and 11 am. The highest average value of chlorophyll absorbance at 6am was 0,604A and 1,196 A where as in 11am 0,721A and 1,435A in 647 and 663 nm respectively [33].

CONCLUSION

From the present study we can conclude that the maximum absorbance were showed by mature leaf (2.171) both in crude and diluted extract (80% acetone) followed by young leaves (1.331) in 663 nm. The chlorophyll content increases from young leaves, then decreases in half ripened and fully ripened leaf extract. In both crude and diluted extract Chlorophyll a is prominent than chlorophyll b. The total chlorophyll content is more in crude than diluted extract, where mature leaves show a 0.763 mg pigment/m3 which is higher among all the values.

The UV-VIS Spectrophotometer analysis showed the peak of absorbance in all leaf type extract in both crude and diluted extract except in red or fully ripened leaves. Maximum peak of absorbance were showed in mature leaf extract (crude) at 663 nm. Based on the above results we can conclude that the total chlorophyll content in a leaf increases with leaf aging and attains a saturation and then declines when reaches ripening or senescence.

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