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Xaverian Research Journal

Peer Refereed Bi-annual
Interdisciplinary Studies and Research

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Contents

Research Articles

Review Article

CHARACTERIZATION OF BETA CAROTENE FROM MANGLICOLOUS YEAST *RHODOTORULA PALUDIGENA* VA 242 FOR APPLICATION IN AQUACULTURE

K. A. Nimsi[1], K. Manjusha[1*], N. Nefla[2], S. N Kutty[3] and V. S. Aneymol[2]

[1] Department of Marine Biosciences, Faculty of Ocean Science and Technology, Kerala University of Fisheries and Ocean Studies, Panangad, Ernakulam, Kerala
[2]Department of Zoology, St. Xavier's College for Women, Aluva, Ernakulam, Kerala
[3] Department of Zoology, NSS College, Nenmara, Palakkad, Kerala
[*]**Corresponding author email id:** manjusha.k@kufos.ac.in or manjushak.kufos@gmail.com

Abstract

Carotenoids have attracted the attention of industries and researchers alike. This biologically valuable molecule can act as a vitamin A precursor and have coloring, antibacterial, and antioxidant properties. They are synthesized by plants, algae, bacteria, and fungi, with plants being a major source. However, the extraction of the pigment from plants is limited by low yields and high production costs. This has resulted in the search for newer promising microbial sources of carotenoids. In this paper, we focused on the characterization of the carotenoid pigment from the yeast *Rhodotorula paludigena* VA 242, an isolate that has already been proven to be a colour-enhancing feed additive. VA 242 gave a good yield of carotene (0.985g/L DCW and 118.25 µg/L) under optimized conditions. The carotenoid extracted from VA 242 was characterized by TLC and FTIR and identified as β-carotene. This β-carotene exhibited strong antibacterial activity against bacterial fish pathogens such as *A. hydrophyla, V. cholerae, V. vulnificus, V. proteolyticus,* and *E. tarda.* It also showed strong antioxidant activity at a concentration of 100µg/ml. The cytotoxicity of the pigmented yeast was assessed in the invertebrate model and a live-feed *Artemia salina* and its proximate composition was determined. The survival rate showed that it was non-toxic and its nutritive value is greater than *Saccharomycetes cervesiae*. The finding of this study has great significance, as this β-carotene from the GRAS strain, *Rhodotorula paludigena* VA 242 can possibly serve as an alternative source of carotenoid suited for use in aquaculture.

Keywords: β-carotene, Antibacterial, Antioxidant, *Rhodotorula,* Yeast

Introduction

The use of synthetic dyes or colour imparting agents has been found to be unsafe for human health and there are only a limited number of such dyes that are permitted to be used. Hence, there is an urgent need for a natural alternative to it. Carotenoids are natural pigments characterized by yellow, orange, red, or purple coloration and exist in a wide variety of plants and microorganisms (Botella-Pavía and Rodríguez-Concepción 2006). These pigments are used as functional food and pharmaceutical supplements and in cosmetic industries as dyes/colorants. Most of the natural pigments are extracted from plants like annatto, beetroot, marigold, grapes, carrot, and paprika (Sankari *et al*., 2018). Despite the availability of a variety of natural carotenoid pigments from plants, they are limited low yields and high production costs. This has currently led to a renewed interest in microbial sources of pigments. As carotenoids are in high demand the world over, a suitable source of these pigments is the need of the hour and mangrove yeasts are one such promising source. Pigmented yeasts are an ideal source as they have the potential to produce large amounts of carotenoids, have a faster growth rate, and are easy to cultivate. Carotenoids like lycopene, β-carotene, astaxanthin, torulene, torularhodin, etc. are produced by yeasts. These carotenoid-producing yeasts are mainly represented by the genera *Rhodotorula* sp., *Rhodosporidium* sp., *Sporobolomyces* sp., and *Xanthophylomyces* sp. (Mannazzu, 2015; Kot *et al*., 2018). Microorganisms that inhabit marine environments have been considered useful natural sources of bioactive compounds including pigments, as they possess unique metabolic and physiological properties (Zhao *et al*., 2019). As there is only limited information on the pigments from yeasts isolated from mangroves and their potential; this study aims to investigate this area. The intended areas of use of these pigments are antimicrobial compounds, antioxidants, and color-enhancing feed additives.

Materials and Methods

(a) The strain used

R. paludigena VA 242 a good pigment-producing yeast isolated as a part of the Kerala State Council for Science Technology and Environment funded project 'Diversity and Biotic Potential of Yeasts from the Mangroves of Central Kerala' was selected for this study.

(b) Optimization of growth and pigment production-One factor at a time method

The factors pH (4-8), temperature (25°C- 33°C), salinity (10-25%), and days of incubation were optimized by one factor at a time method. Yeast Malt (YM) broth was used for optimization. The inoculated broth was incubated for a period of 9 days. Growth and pigment were estimated on the 5^{th}, $7^{th,}$ and 9^{th} day. The flasks were incubated in a shaking incubator with a rotation speed of 160 rpm. The total cell density and pigment production was calculated to determine the optimum condition for growth and pigment production.

(c) Extraction of pigments

For the extraction of pigment, VA 242 was inoculated into YM broth and incubated under the optimized conditions (pH 6, temperature 28ºC, 15% salinity). After 7 days of incubation, the cells were harvested by centrifugation at 8000 rpm at 4ºC for 10 min. Pellets were washed with distilled water and centrifuged. The biomass of VA 242 (dry weight) was quantified by drying at 60°C until a constant mass was obtained. The carotenoids were extracted using the technique described by Lopes *et al.* (2017) with slight modifications. The carotenoids containing supernatant were pooled and analyzed spectrophotometric ally. The total carotenoid concentration was determined (Lopes *et al*., 2017).

Total carotenoids (µg/g of yeast) = Amax × D × V/(E × W)

Amax: the absorbencies of total extract carotenoid at 490 nm

D : sample dilution ratio

V : volume of extraction solvent (mL)

E : extinction coefficient of total carotenoid (0.16)

W : dry weight of yeast (g)

(d) Characterization of pigment from VA 242

- **Thin layer chromatography (TLC)**

To investigate the purity of the extracted pigment thin layer chromatographic separation was carried out using silica gel as the stationary phase and petroleum ether: acetone (80:20.v/v) as the mobile phase and their Rf values were determined. The extracted pigment was identified by comparing the distance traveled by it (R_f value) to the distance traveled by the standard β- carotene (Hi media).

- **Fourier Transform Infra-Red (FTIR) Analysis**

The extracted pigment was dried and partially characterized using a Spectrum recorded in JASCO model 4100 FT-IR spectrometers (CUSAT- STIC).

- **Antimicrobial activity**

The antibacterial activity of the extracted pigment from VA 242 was checked against pathogens such as *Escherichia coli, Pseudomonas* sp., *Bacillus* sp., *Salmonella* sp., *Staphylococcus* sp., *Aeromonas* sp., *Edwardsiella* sp., and *Vibrio* sp. by the disc diffusion method using distilled water as control. After the overnight incubation, the antimicrobial activity was determined by measuring the diameter of the zone of inhibition (ZOI) around the disc (Muthezhilan *et al*., 2014).

- **Antioxidant activity**

The carotenoid content from the acetone extracts was quantified in micrograms, for the antioxidant assays, the pigment was dissolved in water, and

concentrations of 20, 40, 60, 80, and 100 µg/ml were prepared and assayed for antioxidant activity. All experiments were conducted in triplicates. Determination of the antioxidant activity of yeast carotenoid pigment was done using Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (DPPH) assay and ABTS radical assay (Mukherjee *et al*., 2017).

- **Colour stability study**

In order to study the ability of the pigment to retain its overtime colour; 0.5 gm of pigment was added to water agar medium (10 ml) mixed well and poured into an ice tray mould for setting, the color in the medium was visually observed (Muthezhilan *et al*., 2014) over a period of one month. The intensity of the colour retention in the gel was indicative of the stability of the pigment.

- **Cytotoxicity of VA 242**

The experimental setup consisted of round-bottomed glass tanks of 5 L capacity with water (35% salinity), an air pump, and proper lighting, that enabled the hatching process of *Artemia* cysts after 24hrs of incubation. The tanks were set up in such a way that the control and experimental tanks were exposed to the same environmental condition. The hatched nauplii (500 numbers) were transferred to each tank, *Artemia* in the control (C) tank was fed with baker's yeast, and the test (T) was fed with pigmented biomass of *R. paulidigena* (mg/L). The survival rate of hatched nauplii, as well as their colour, was noted every 3rd, 5th, and 7th day of cultivation. The experiments were performed in triplicates.

(e) Proximate composition of VA 242

The selected yeast strain *Rhodotorula paludigena* VA 242 was swab inoculated into Malt Extract broth and incubated at room temperature 28ºC for 72 hrs. The cell suspension was centrifuged at 7000 rpm for 15 minutes in a refrigerated centrifuge (Thermo scientific- ST 8R). It was washed repeatedly with sterile distilled water and centrifuged at 7000 rpm for 15 minutes to remove the

media components. The pellet was then dried at room temperature. The dried yeast biomass was stored at 4ºC until analysis. CNHS analysis of *Rhodotorula paludigena* VA 242 (Test) and baker's yeast (Control) was done with Elemental Vario EL III instrument (STIC-CUSAT).

Results and Discussion

Optimization of VA 242 isolate in YM broth showed maximum growth and pigment production occurred at pH 6, temperature 28ºC, and 15% salinity after 7 days of incubation period at 160 rpm (Figure 1). The total dry cell mass obtained was 0.985 mg/ml after 7 days of incubation. The yield of pigment was 118.25µg/L total carotenoids.

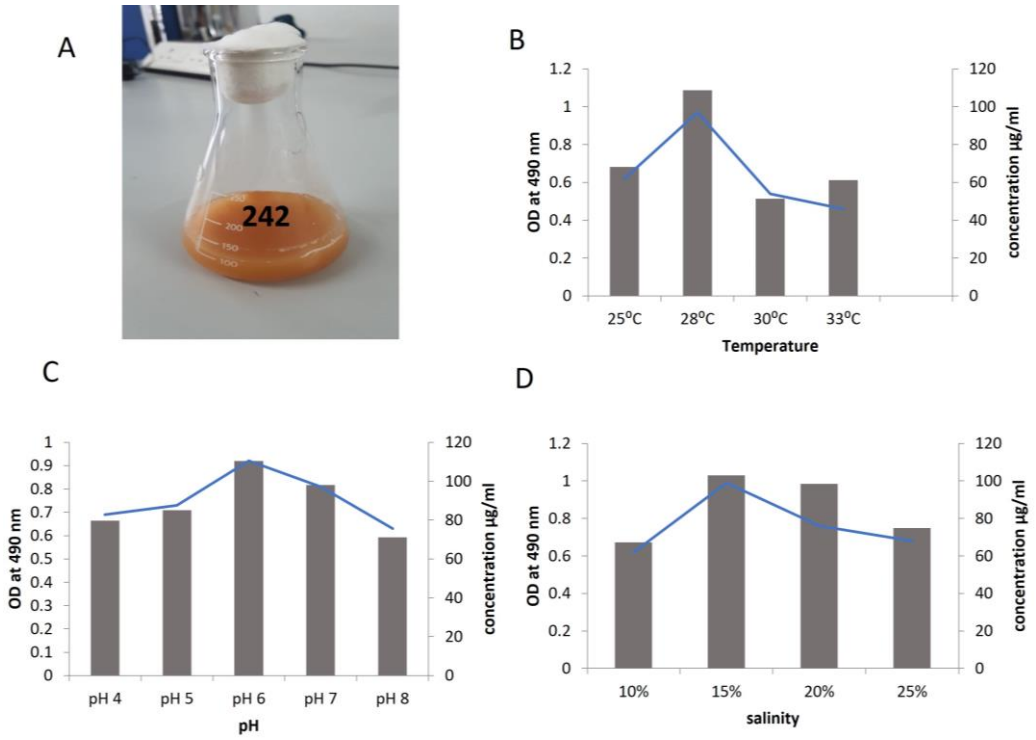

Figure 1. Optimization of growth and pigment production conditions VA 242 A) Production flask B) Optimization of Temperature C) Optimization of pH D) Optimization of Salinity.

The pigment extracted from *Rhodotorula paludigena* VA 242 was effectively separated by the organic solvent Petroleum ether: acetone. The Rf values of a yellow spot in the TLC plate were similar to standard β carotene indicating the identity of the pigment.

The FTIR spectrum of the pigment from VA 242 was recorded in the range of 4000-600 cm^{-1}. The spectrum of the carotenoid extract exhibited transmission peaks at 970, 1365, 1672, and 2931cm^{-1}. The peaks at 1365 and 2931cm^{-1} were due to C-H bending, and the peak at 1672cm^{-1} was attributed to the C--C stretching of the conjugated double bond (Saha *et al*., 2015). Peaks from spectra confirmed the identification of the extracted carotenoid as β-carotene in a trans configuration (Figure 2).

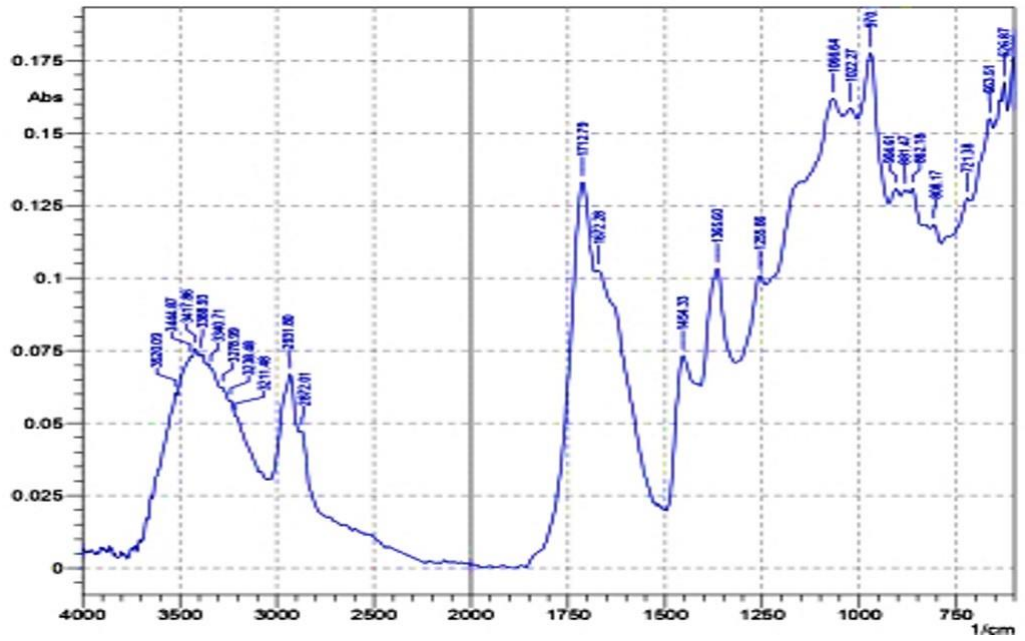

Figure 2. FTIR analysis

The antibacterial activity of the extracted β carotene from *Rhodotorula paludigena* was checked against pathogens such as *Escherichia coli, Pseudomonas* sp., *Bacillus* sp., *Salmonella* sp., *Staphylococcus* sp., *Aeromonas* sp., *Edwardsiella* sp.,

and *Vibrio* sp. by the disc diffusion method using acetone as control (Figure 3). The pigment exhibited activity against all pathogens and it exhibited maximum antibacterial activity against *A.hydrophyla* followed by *V.vulnificus*, and *V.proteolyticus.*

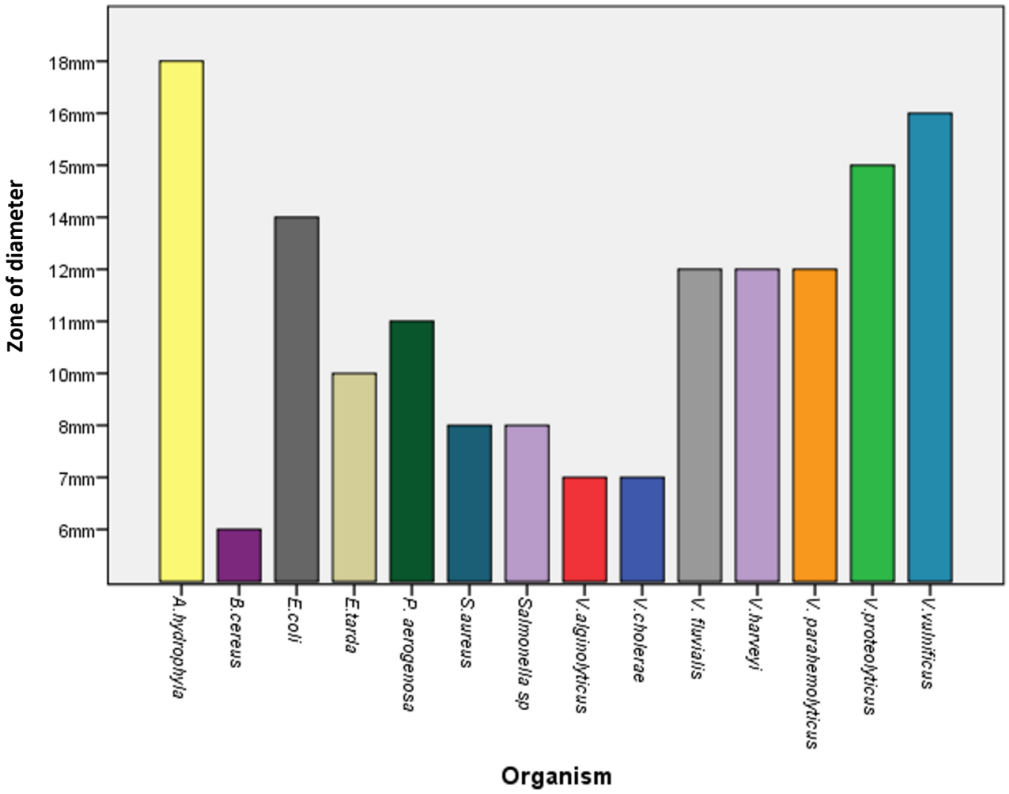

Figure 3. Antibacterial activity of pigment of VA 242

The pigment from *Rhodotorula paludigena* at a concentration of 100µg/ml exhibited 79% and 95% antioxidant activity by DPPH analysis and ABTS assay respectively. The antioxidant activity was found to increase with the increase in pigment concentration. The percentage of inhibition was comparable to the standard ascorbic acid (Figure 4). The results of this study showed that the carotenoid pigment of VA 242 possessed antioxidant activity.

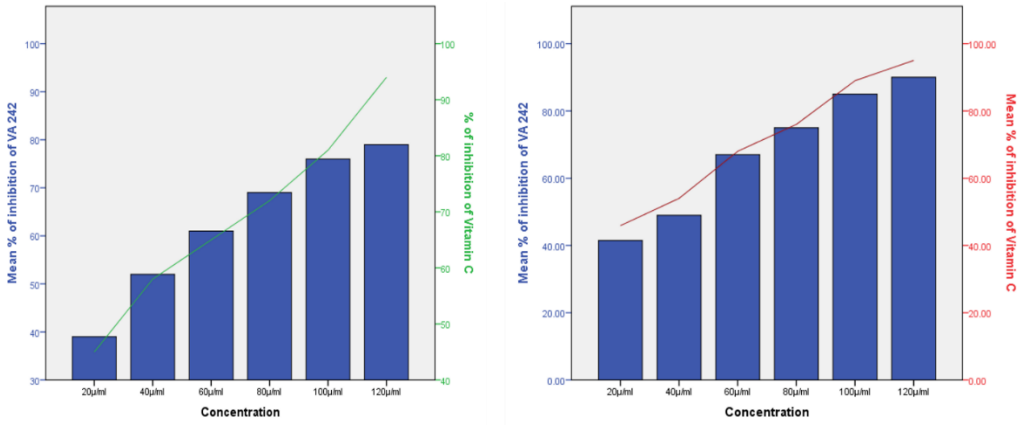

Figure 4. Antioxidant activity of pigment of VA 242

The pigment extracted from the strain *Rhodotorula paludigena* imparted colour to the gel (China grass) into which it was incorporated. The intensity of the colour of the gel was stable even after one month.

The cytotoxicity of the pigment was assessed in the invertebrate model and a live-feed *Artemia salina*. The artemia fed with VA 242 appeared light orange in color unlike the control which appeared colorless (Figure 5).

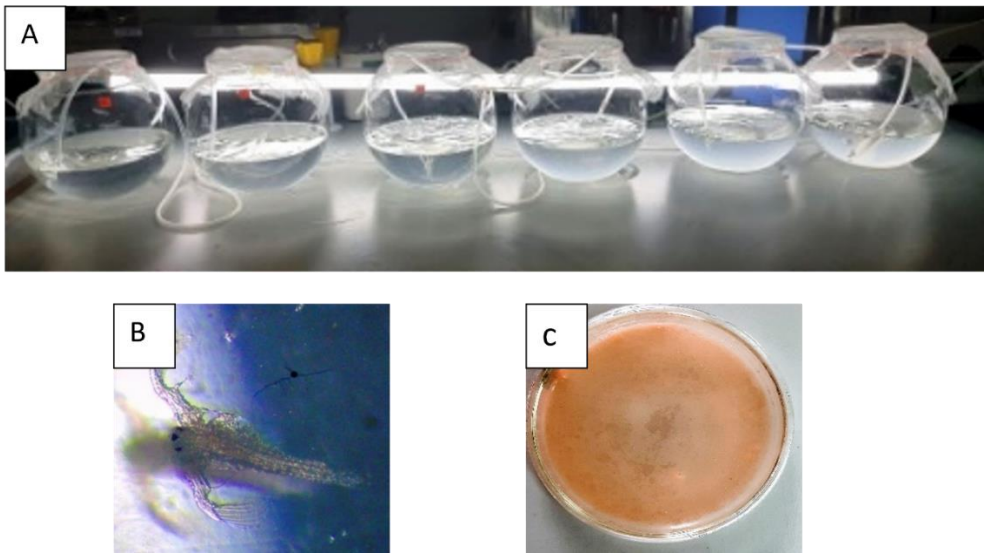

Figure 5 A) Experimental setup for *Artemia* B) Colour enhanced *Artemia* C) Stability test with pigment

The pigment was also seen to influence the survival rate of *Artemia*; with the survival rate being significantly (*P* value <0.05) greater in the test than in the control tank after 7th day of cultivation (Table 1). The proximate composition of the dried biomass of selected yeast *R.paludigena* VA 242 shows slightly more nutritional value than baker's yeast (Table 2). Rekha *et al.* (2022) found *Rhodotorula paludigena* VA 242 to act as a pigment-enhancing feed additive for Koi carp, *Cyprinus carpio*. All these observations indicate the possibility of using VA 242 for the preparation of enriched live feeds for use in aquaculture.

Table 1. Survival rate of *Artemia*

Feed	No. of Nauplii	3rdday	5thday	7thday
VA 242(T)	500	87%	69%	39%
Baker's yeast(C)	500	84%	62%	31%

Table 2. CNHS composition of VA 242

Sample	C%	N%	H%	S%
VA 242	36.32	7.34	7.53	0.35
Baker's yeast	31.5	7.31	5.50	0.12

Conclusion

Terrestrial yeast has held the attention of science and industry for several decades now, but it is only recently that the potential of manglicolous yeast has been recognized. It is clear from this study that *Rhodotorula paludigena* VA 242 is a potent mangrove isolate. Its pigment can be exploited industrially to come up with a natural food colorant after further investigations. There is also the possibility of using VA 242 for the preparation of enriched live feeds and also as a color-enhancing agent in aquaculture. As yeasts are GRAS (Generally Recognised as Safe) they have wide acceptability and great scope in the feed industry.

Acknowledgment

The authors gratefully acknowledge the financial support by The Kerala State Council for Science, Technology and Environment (KSCSTE) for the project entitled 'Diversity and Biotic Potential of Yeasts from the Mangroves of Central Kerala'. (Order No .573/2017/KSCSTE DTD 03/10/2017)

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MEASURING FOLIAR DAMAGE CAUSED BY THE CATERPILLAR OF COMMON CROW (*EUPLOEA CORE)* (LEPIDOPTERA: NYMPHALIDAE) USING BIOLEAF- A PROFESSIONAL MOBILE APPLICATION

Sanika Joseph[1], Saniya Joseph[1] and **Anu Anto[2]***

[1]Department of Microbiology, Sree Narayana Guru Institute of Science and Technology Arts and Science College, North Paravur, Ernakulam, Kerala- 683520
[2]Department of Zoology, St. Xavier's College for Women, Aluva, Ernakulam, Kerala-683101
Corresponding author email id: anuanto@stxaviersaluva.ac.in

Abstract

Herbivory is a fundamental ecological process in which animals consume plant material as a source of nutrients. Understanding the impacts of herbivory is crucial for studying and managing ecosystems, as it influences plant community dynamics, species interactions, and nutrient cycling. In this study, we investigated the foliar damage caused by the larvae of *Euploea core*, in the ornamental plant *Adenium obesum*. Foliar damage is assessed by BioLeaf, a mobile application used for the automatic quantification of leaf damage caused by insect herbivory. In the group with artificial herbivory, 25% herbivory was introduced either inside the leaf or towards the border of the leaf. In the control group, damaged leaves were collected from the host plant and kept with the caterpillars. Overall results show that, leaves that were already damaged by caterpillars while on the plant, exhibited the highest level of herbivory. In this study, the BioLeaf app was able to accurately estimate the amount of herbivory. It could serve as a valuable tool for plant monitoring and early problem detection. Further research is necessary to explore the factors that influence caterpillar preferences and the broader impact of herbivory on plant health. Understanding these factors can help develop more effective pest management strategies and contribute to a better understanding of ecosystem dynamics.

Keywords: *Adenium obesum*, herbivory, BioLeaf

Introduction

Herbivory is the act of animals feeding on plants and it is an important ecological process that has significant effects on primary production, vegetation structure, and composition (Schowalter 2000). Herbivores can eat any part of the plant above or below the soil, affecting habitat and resource conditions for other organisms. The impacts of herbivory can be substantial, affecting the health of habitats, the structure and diversity of plant and soil invertebrate communities, and the productivity of economically important crops. Animals have developed specialized features and feeding strategies to utilize plants as a food source. Herbivory can also have effects on the growth form of plants, such as terminating shoot growth and initiating branching, and affecting shoot-to-root ratios. Changes in the survival, productivity, and growth of individual plant species can affect vegetation structure and community dynamics (Lefcheck and Duffy 2015; Pooter and Nagel 2000; Maron and Crone 2006).

The effects of herbivory on plants are complex and context-dependent, there is evidence to suggest that some plants can benefit from moderate levels of herbivory in terms of growth, reproduction, and survival. However, it is important to note that excessive herbivory can have detrimental effects on plant fitness and ecosystem function (Agarwal 2011; Karban 2011; Stamp 2003; Strauss and Agarwal 1999).

In this study, we investigated the damage caused by the larvae of *Euploea core*, in the ornamental plant *Adenium obesum*. The larvae of the butterfly feed on the leaves and stems of the plant, causing significant damage to the foliage and reducing photosynthetic capacity (Schafleitner *et al*., 2010). Foliar damage is assessed by BioLeaf, a mobile application used for the automatic quantification of leaf damage caused by insect herbivory (Orueta *et al*., 2016).

Methodology

Leaves of *Adenium obesum* were collected (Figure 1). Leaves were divided into three groups: leaves with existing caterpillar attacks (Figure 2a & b), leaves with artificially induced herbivory, and a control group with no injury. The caterpillars used in the experiment were of the *Euploea Core* species (larvae of Common crow butterfly), and the second instar stage caterpillars were used (Figure 6). The caterpillars were left fasting for 24 hours before being exposed to the leaves in the respective groups. In the group with artificial herbivory, 25% herbivory was introduced either inside the leaf (set-3, figure 4 a & b) or towards the border of the leaf (set-4, figure 4a & b), and two sets were replicated. In the control group (set-1, figure 5a & b), damaged leaves were collected from the host plant and kept with the caterpillars. Images of leaves from each group were captured using a Nokia 3.1 mobile camera without flash in a white portable background.

Figure 1. *Adenium obesum* plant

a b.

Figure 2a and b. Leaves with existent attacks from caterpillars

a b

Figure 3a and b. Induced herbivory in the interior of the leaf

a b

Figure 4a and b. Induced herbivory on leaf margins

a b

Figure 5a and b. Control group- Leaves with no injury

Figure 6. Caterpillar of *Euplea core*

Result

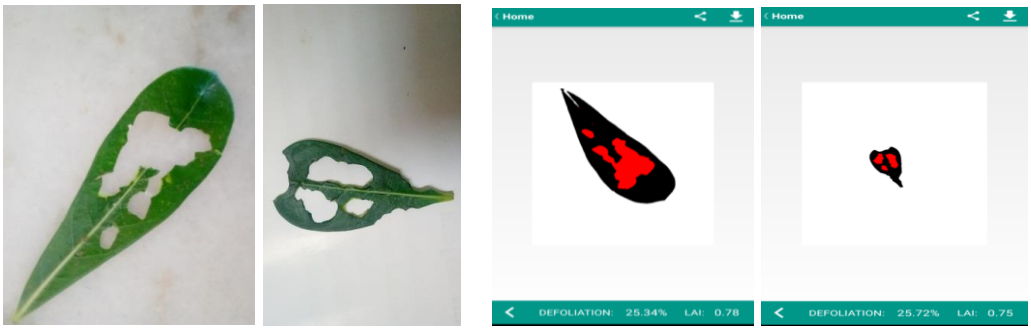
The results of the study show that herbivory is higher in leaves damaged by caterpillars while on the plant (set 1- 44.70%) (figure 7a & b; 8a & b), followed by undamaged leaves. Artificially defoliated leaves are least preferred by caterpillars.

The Bioleaf application determined that the herbivory specifically affected the inner parts of the leaves (set 2a) which were subjected to artificial defoliation in the interior region of leaves. The Bioleaf application calculated that the herbivory accounted for 37.71% of the damaged foliar area on these leaves (figure 9a & b; figure 10a & b).

Herbivory was observed in the leaves with artificial defoliation in the borders (set 2b). The BioLeaf app's automatic estimation determined that the caterpillar accounted for 32.16% of the damaged foliar area in these leaves (figure 11a & b; figure 12 a & b).

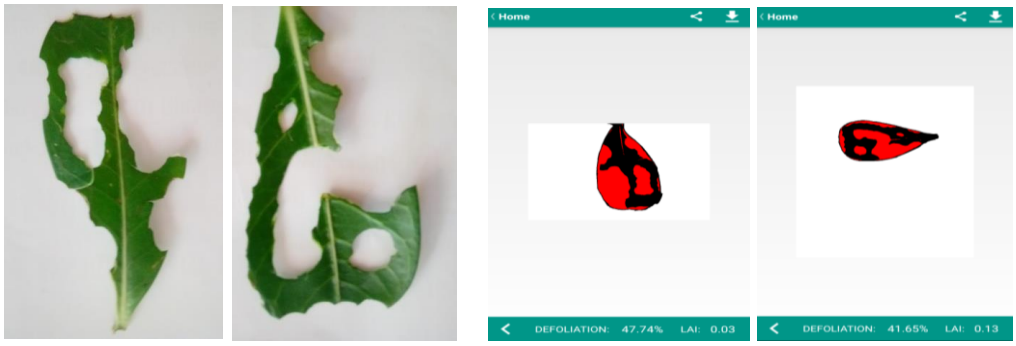
In the leaves without any artificial defoliation (set 3- control), herbivory was 43.99% (figure 13a & b; figure 14a & b).

Overall results show that, leaves that were already damaged by caterpillars while on the plant, exhibited the highest level of herbivory. It suggests that caterpillars preferred to feed on leaves that already had existing damage. Leaves without any artificial defoliation or pre-existing herbivory showed a lower level of herbivory. Leaves that were intentionally defoliated in the interior region, were found to be the least preferred by caterpillars, displaying the lowest level of herbivory. These observations provide insights into the herbivory preferences of caterpillars and highlight the impact of pre-existing damage on their feeding behaviour.



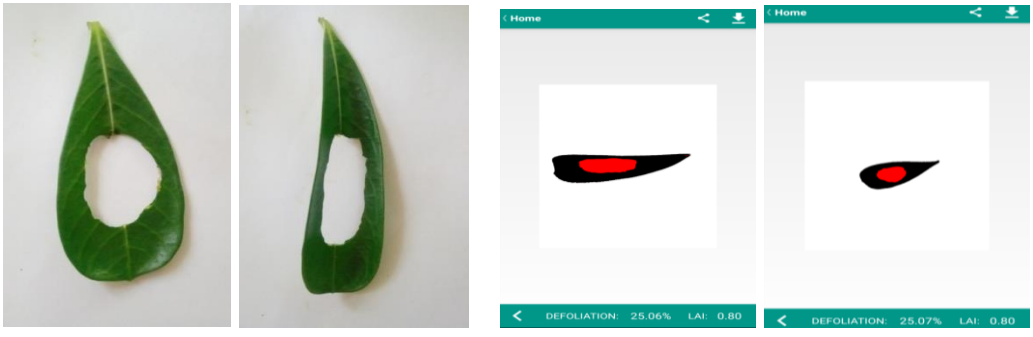
(a) (b)

Figure 7. (a) Leaves with an existent attack by caterpillars while on the plant (before the experiment), (b) estimated herbivory of these leaves using Bioleaf



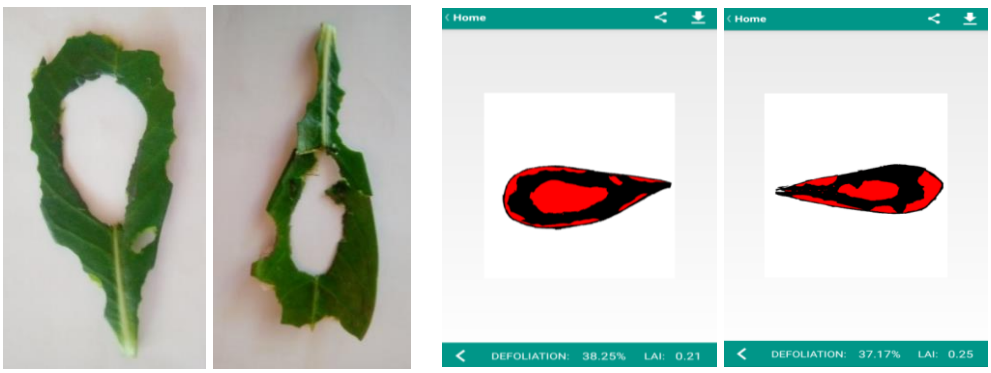
(a) (b)

Figure 8. (a) Set 1 leaves after the experiment (b) estimated herbivory of set 1 leaves using Bioleaf



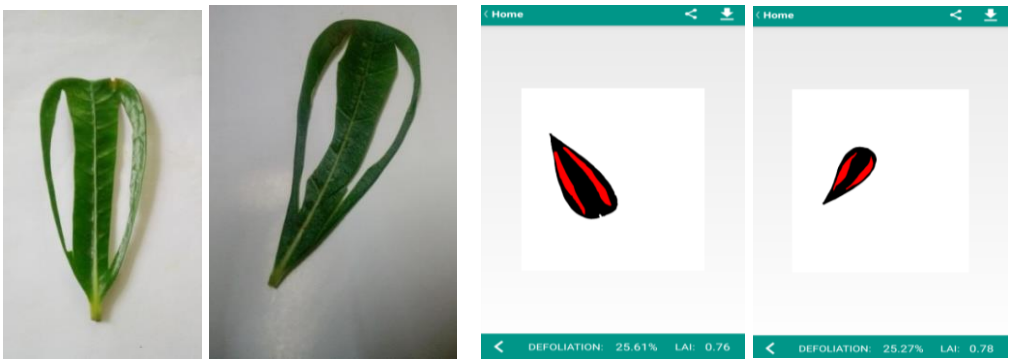
(a) (b)

Figure 9. (a) Set 2 leaves with induced herbivory in the interior of the leaf (before the experiment) (b) estimated herbivory of these leaves using Bioleaf



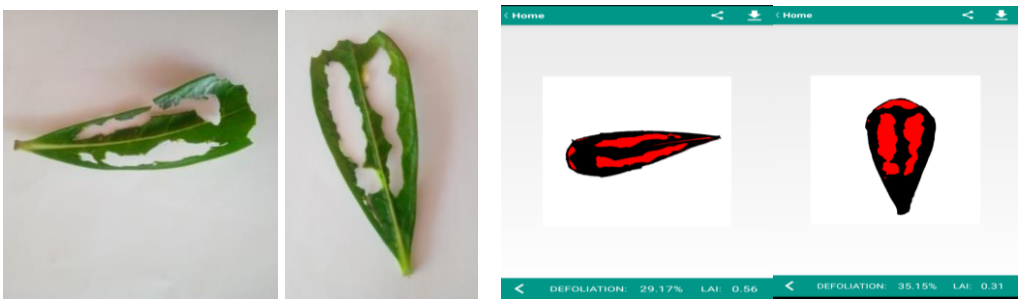
(a) (b)

Figure 10. (a) Set 2 leaves after the experiment (b) estimated herbivory of these leaves using Bioleaf after the experiment



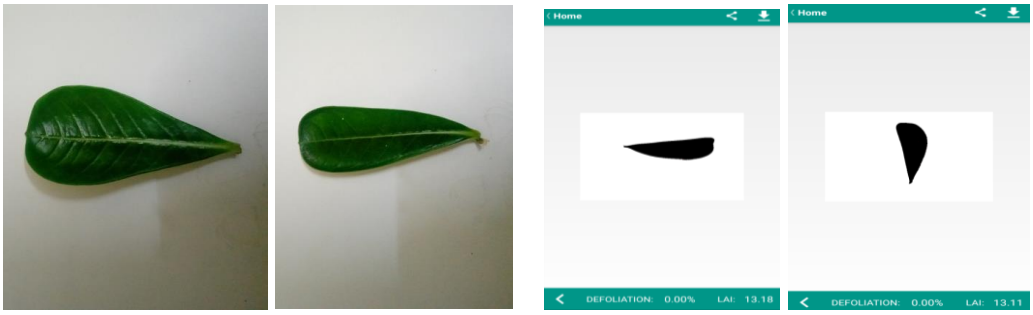
(a) (b)

Figure 11. (a) Set 2 (b) leaves with induced herbivory in the border of the leaf (before the experiment) (b) estimated herbivory of these leaves using Bioleaf



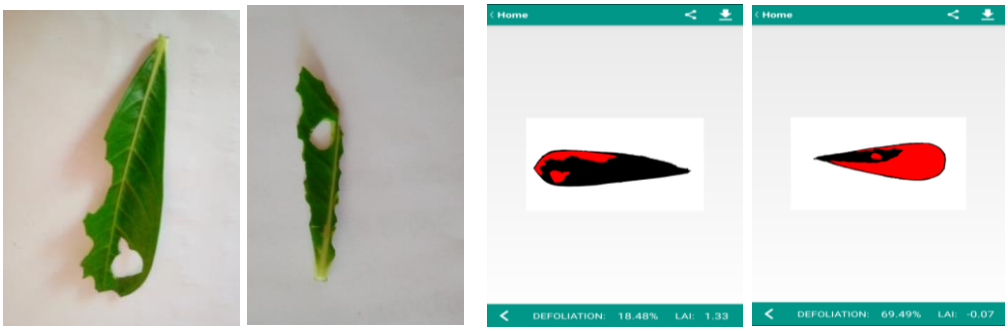
(a) (b)

Figure 12 (a) Set 2(b) leaves after the experiment (b) estimated herbivory of these leaves using Bioleaf after the experiment



(a) (b)

Figure 13 (a) Set 3 (control) leaves with no induced herbivory (before the experiment) (b) estimated herbivory of these leaves using Bioleaf



(a) (b)

Figure 14 (a) Set 3 (control) leaves after the experiment (b) estimated herbivory of these leaves using Bioleaf after the experiment

Discussion

Observations made in this study provide valuable insights into the herbivory preferences and feeding behaviour of caterpillars. It suggests that the presence of pre-existing damage can attract caterpillars and make those leaves more susceptible to further herbivory. This is likely because caterpillars prefer to eat fresh, tender leaves. When they are on the leaf, they can chew through the leaf tissue more easily than when they are eating an artificially defoliated leaf. Additionally, caterpillars may be able to detect the chemicals that are released by damaged leaves, which makes them more attractive (Bezemer and Visser 2008).

The results of this study have implications for the management of insect pests. By understanding the preferences of caterpillars, we can develop more effective strategies for controlling their populations. For example, we could target our efforts on protecting young, tender leaves, or we could use chemicals that mimic the chemicals released by damaged leaves (Gould 1982; Hiker and Mejiden 2007).

In this study, the BioLeaf app was able to accurately estimate the amount of herbivory that had occurred in the inner parts of the leaves. This is a promising finding, as it suggests that the app could be used to monitor the health of plants and identify potential problems early on.

It is important to note that this study was conducted on a small number of leaves, so more research is needed to confirm the accuracy of the BioLeaf app. However, the results of this study are encouraging and suggest that the app could be a valuable tool for plant health monitoring. The app could be used by farmers to monitor their crops for signs of pests or diseases, by gardeners to track the health of their plants, and by researchers to study the effects of herbivory on plant health. Overall, the BioLeaf app is a promising new tool for plant health monitoring. More research is needed to confirm its accuracy, but the results of this study are encouraging (Orueta *et al*., 2016).

Here are some additional thoughts on the results of the study:

- The study was conducted in a controlled environment, so it is not clear how the results would generalize to a natural setting.
- The study only looked at one type of caterpillar, so it is possible that other caterpillars have different preferences.
- The study did not look at the impact of herbivory on the health of the plants. It is possible that even low levels of herbivory can have a negative impact on plant growth and reproduction.

Overall, the results of this study provide valuable insights into the ecology of herbivory. Future research is needed to further explore the factors that influence the preferences of caterpillars and the impact of herbivory on plant health.

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FORMULATION AND EVALUATION OF ANTIMICROBIAL HERBAL SOAP USING *HIBISCUS ROSA SINENSIS* AND *AYAPANA TRIPLINERVIS*

Merin Joyce, Raina Maria Agnes and Asmy Antony K A*

Department of Chemistry, St. Xavier's College for Women, Aluva, Ernakulum, Kerala, India
***Corresponding author email id:** asmyantony@stxaviersaluva.ac.in

Abstract

Bacterial infections are a common occurrence, underscoring the need for antibacterial herbal soaps to maintain healthy and radiant skin. These herbal soaps, derived from plant extracts, are gentle on the skin and do not cause harm. This study aimed to assess the antibacterial properties of ayapana leaf extract (*Ayapana triplinervis*) and hibiscus flower extract (*Hibiscus rosa-sinensis*) by incorporating them into a basic soap formulation. Using oil extracts from hibiscus and ayapana, three distinct types of soaps were created: hibiscus soap, ayapana soap, and a hybrid ayapana-hibiscus soap. Antimicrobial and antibacterial assessments were conducted through disc diffusion and well diffusion methods. All three soaps were tested against *Staphylococcus aureus* using both methods and against *Escherichia coli* using the disc diffusion method. Hibiscus-ayapana soap exhibited a greater zone of inhibition compared to the other soaps. The resulting soap had a pH level of 8, indicating its slightly alkaline nature.

Keywords: Herbal soaps, Disc diffusion method, Well diffusion method, *Staphylococcus aureus, Escherichia coli.*

Introduction

Every day, we incorporate various types of soaps into our hygiene routines. This practice plays a crucial role in halting the spread of diseases instigated by germs. Soap serves as a potent defence mechanism against microbes and germs,

which exist ubiquitously- in the soil, water, air, and even within the human body. Additionally, soap serves the purpose of eliminating dirt, further underlining its indispensability for human well-being (Mukhopadhyay, 2011).

Soaps can be natural or synthetic. Natural soap consists of oil derived from various plants. Such soaps are rich in nutrients and antioxidants. It may also contain antibacterial, anti-inflammatory properties etc. Soap made from the oil extract of herbal plants can be used as an alternative source of antibacterial agents against human pathogens. Herbal soaps do not cause any allergies or skin infections (Devi *et al.*, 2021).

Natural plant products are widely used nowadays because of increasing the burden of diseases (Khristi and Patel *et al.*, 2016). Earlier studies have shown that the Ayapana plant and Hibiscus plant are rich in medicinal properties. Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases (Al-Snafi, 2018).

Hibiscus rosa sinensis is an ornamental plant having wide distribution throughout the world. It has been traditionally used in food, cosmetic, and medicines. Major bioactive constituents include glycosides, terpenoids, saponins, and flavonoids. The plant a has a wide variety of pharmacological applications such as anti-fertility, anti-microbial, anti-inflammatory, anti-diabetic, anti-microbial, and anti-pyretic activities. Toxicological studies indicated that plant extracts are safe to use at higher doses (Khan *et al.*, 2017).

Eupatorium triplinerve Vahl. Commonly called Ayapana (Syn. *Ayapana triplinerve* and *Eupatorium ayapana*) is an ornamental erect perennial herb with aromatic leaves under the family Asteraceae. (Sugumar *et al.*, 2015).

Since ayapana and hibiscus were easily available, the present study was designed to evaluate the antibacterial activity of the leaf extract of ayapana (*Ayapana triplinervis*) and flower extract of hibiscus (*Hibiscus rosa-sinensis*) by incorporating it into basic soap medium.

Materials and methods

Phytochemical Analysis

Preparation of plant extracts

Ethanol extract was obtained by soaking 10g of crushed hibiscus flower and ayapana leaves in 55ml ethanol for 24 hours.

Methods

Phytochemicals	Reagent used	Colour
1. Alkaloids	Dragendroff‟s reagent	an orange or red precipitate
2. Flavonoids	lead acetate	yellow precipitate
3. Glycosides	glacial acetic acid with traces of ferric chloride and conc. H2SO4	blue color
4. Phenol	10%lead acetate	bulky white precipitate
5. Tannins	neutral 5% ferric chloride	dark green color
6. Terpenoids	chloroform and conc.H2SO4	reddish brown color

Antibacterial assay

Preparation of extracts

The aqueous extract was obtained by grinding 14g of Hibiscus flower and Ayapana leaves in 60ml distilled water and the filtrate was taken as an antibacterial aqueous extract.

Well Diffusion Method

Using aseptic techniques, a single pure colony was transferred into 10 ml of nutrient broth and was placed in an incubator at 37°C for overnight incubation. Sterile MHA plates were prepared and the bacterial inoculum was uniformly

swabbed in each plate. A well was made on each plate with the help of a sterilized well borer. Test samples were added to each well over the agar plates. The MHA plates were incubated at 37°C for 24 hours. After incubation, the diameter of inhibitory zones formed around each well was measured in cm and recorded.

Preparation of Soaps

Oil extract of hibiscus flower and ayapana leaves was obtained by boiling 54g of substance with 300g of coconut oil for 20 minutes. The filtrate is taken as an oil extract.

16g of caustic soda was weighed out and dissolved in 45ml distilled water. The temperature of the solution rises and it is kept for cooling for 5hrs. 30g of talcum powder was mixed with 100g of oil extract (hibiscus or ayapana) in a beaker and this mixture was stirred continuously for 30 minutes. The previously prepared solution of caustic soda was added to this mixture and stirred well. This mixture is then transferred into a mould and can be taken out of the mould after one day. Then the soap is kept for setting for seven days.

Hybrid soap is made by taking 50g of ayapana extract and 50g of hibiscus extract.

Soap without extract (Control soap)

About 16g of caustic soda was weighed out and dissolved in 45ml distilled water. The temperature of the solution rises and it is kept for cooling for 5hrs. 30g of talcum powder was mixed with 100g of coconut oil in a beaker and this mixture is stirred continuously for 30 minutes. The previously prepared solution of caustic soda was added to this mixture and stirred well. This mixture is then transferred into a mould and can be taken out of the mould after one day. Then the soap is kept for setting for seven days.

Antibacterial assay of soaps by disc diffusion method

Using aseptic techniques, a single pure colony was transferred into 10 ml of nutrient Broth and was placed in an incubator at 37°C for overnight incubation. Sterile MHA plates were prepared and the bacterial inoculum of Escherichia coli and Staphylococcus aureus were uniformly swabbed in the plate. Test samples of volume 20µl added to the disc and were placed over the agar plates. The plates were incubated at 37oC for 18 hours a period sufficient for the growth. After incubation, the diameter of inhibitory zones formed around each well was measured in cm and recorded.

Disc Diameter = 0.5 cm

Determination of pH of the prepared soap

The soap was mixed with distilled water to form a lather. A pH indicator paper was shown to the lather.

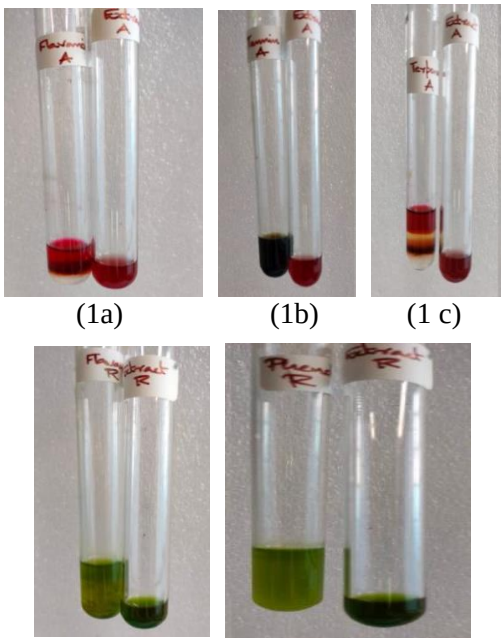
Results and Discussion

Phytochemical analysis of ethanol extracts

Phytochemical analysis of the ethanol extract of *Hibiscus rosa-sinensis* showed the presence of flavonoids, tannins and terpenoids in it. Whereas, ethanol extract of *Ayapana triplinervis* showed the presence of flavonoids and phenols (Table 1, Figure 1).

Table 1. Phytochemical analysis of ethanol extracts of *Hibiscus rosa-sinensis* and *Ayapana triplinervis*

Sl No.	Test	Ethanol extract of *Hibiscus rosa-sinensis*	Ethanol extract of *Ayapana triplinervis*
1	Alkaloid	negative	negative
2	Flavanoid	positive	positive
3	Glycosides	negative	negative
4	Phenols	negative	positive
5	Tannin	positive	negative
6	Terpenoid	positive	negative



(1a) (1b) (1 c)

(2a) (2b)

Figure 1. Photographs of positive results of phytochemical analysis of ethanol extracts of *Hibiscus rosa-sinensis* (Flavanoid- 1a, Tannin- 1b, Terpenoids – 1c) and *Ayapana triplinervis* (Flavanoids- 2a, Phenol- 2b)

Antibacterial assay of aqueous extracts for *Sthaphylococcus aureus* and *Pseudomonas aeruginosa*

Pseudomonas aeruginosa and *Staphylococcus aureus* are the most common bacterial species that cause wound infections. The aqueous extract of both did not show any zone of inhibition against these bacterial species (table 2).

Table 2. Antibacterial assay of aqueous extract of *Hibiscus rosa-sinensis* and *Ayapana triplinervis* in *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Sl.No.	Volume of the sample (µl)	Tetracyclin (C)	Zone of Inhibition
1	25	1.5 cm	No Zone
2	50		No Zone
3	75		No Zone
4	100		No Zone

(2a) (2b)

Figure 2. Antibacterial assay of aqueous extract of *Hibiscus rosa-sinensis* (2a) and *Ayapana triplinervis* (2b) in *Pseudomonas aeruginosa*

(3a) (3b)

Figure 3. Antibacterial assay of aqueous extract of *Hibiscus rosa-sinensis* (3a) and *Ayapana triplinervis* (3b) in *Staphylococcus aureus*

Antibacterial assay of soap for *E.coli* and *S.aureus* using disc diffusion methods

E.coli and *S.aureus* are the most commonly found microbial species in the human body. The prepared soaps were tested against these microbes and the results are shown in table 3.

Table 3. Antibacterial assay of different types of soaps using disc diffusion methods

Sample	***E.coli*- (Width of Zone of Inhibition)**		***S.aureus* (Width of Zone of Inhibition)**	
	Sample	**Tetracycline (Co)**	**Sample**	**Tetracycline (Co)**
Control Soap (C)	No Zone	0.6 cm	1.7 cm	2.6 cm
Hibiscus Soap (H)	No Zone	0.8 cm	1.6 cm	1.5 cm
Ayapana Soap (A)	No Zone	1 cm	0.7 cm	2 cm
Hibiscus- Ayapana Soap (B)	0.9 cm	1.5 cm	1.4 cm	1.8 cm

(a) (b)

Figure 4. Antibacterial assay of control soap (C) using disc diffusion method in (a) *E.coli* (b) *S. aureus*

(a) (b)

Figure 5. Antibacterial assay of Hibiscus soap (H) using disc diffusion method in (a) *E.coli* (b) *S. aureus*

(a) (b)

Figure 6. Antibacterial assay of Ayapana soap using disc diffusion method (a) in (a) *E.coli* (b) *S. aureus*

(a) (b)

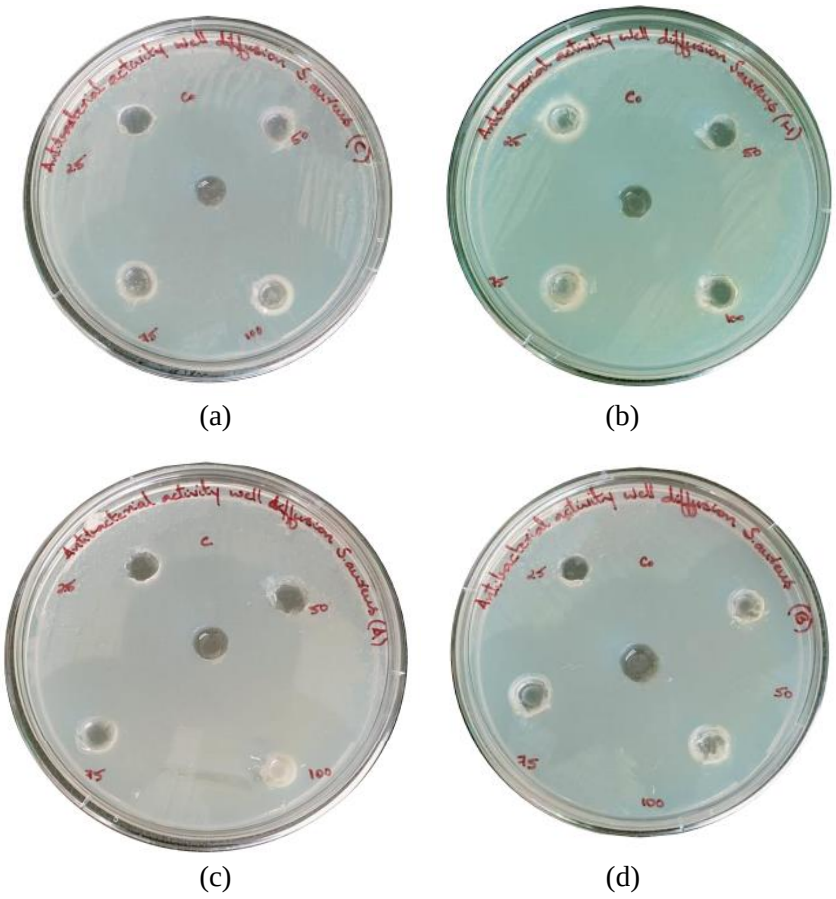
Figure 7. Antibacterial assay of Ayapana soap using disc diffusion method (a) in (a) *E.coli* (b) *S. aureus*

Antibacterial assay of soaps for *Staphylococcus aureus* using well diffusion method

Staphylococcus aureus is a commonly found bacteria in the human body. The prepared soaps were tested against this bacterium and the results are shown in table 4.

Table 4: Antibacterial assay of soaps in *S. aureus* using well diffusion method

Sample	Volume of the sample ((µl)	Tetracycline (C)	Zone of inhibition
Control Soap (C)	25	4.1 cm	No Zone
	50		No Zone
	75		3.7 cm
	100		3.8 cm
Hibiscus Soap (H)	25	4 cm	No Zone
	50		No Zone
	75		No Zone
	100		3.7 cm
Ayapana Soap (A)	25	4 cm	No Zone
	50		No Zone
	75		3.3 cm
	100		3.5 cm
Hibiscus- Ayapana Soap (B)	25	4 cm	No Zone
	50		3.2 cm
	75		3.5 cm
	100		3.7 cm



(a) (b)

(c) (d)

Figure 8: Antibacterial activity of (a) control soap (b) Hibiscus soap (c) Ayapana soap (d) Hibiscus- Ayapana soap in *S.aureus* using well diffusion method

The antibacterial activity of the soaps was assessed against *S.aureus* using varying volumes *(*50 µl, 75µl and 100 µl). The control soap exhibited zone inhibitions of 3.7 cm and 3.8 cm at 75µl and 100 µl, respectively. Hibiscus soap demonstrated a zone inhibition of 3.7 cm at 100 µl. Ayapana soap displayed zone inhibitions of 3.3 cm and 3.5 cm at 75µl and 100 µl, respectively. The combination of hibiscus and ayapana soap exhibited zone inhibitions of 3.2 cm, 3.5 cm, and 3.7 cm at 50 µl, 75µl and 100 µl, respectively, against *S. aureus* (table 4, figure 8).

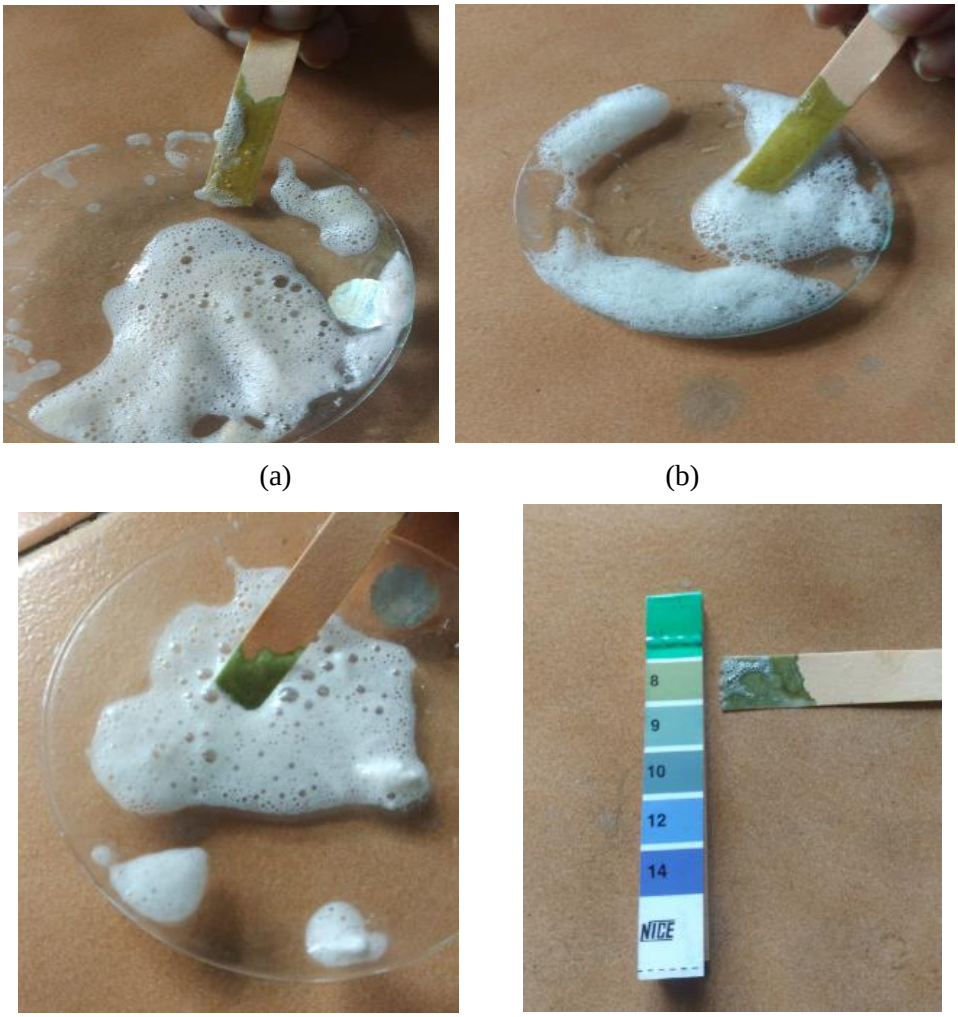
(a) (b)

(c)

Figure 9. Herbal soaps prepared from extracts of (a) *Hibiscus rosa-sinensis* and (b) *Ayapana triplinervis* (c) *Hibiscus rosa-sinensis* and *Ayapana triplinervis*

pH of soaps

The soap was mixed with distilled water to form a lather. A pH indicator paper was shown to the lather. The colour of the pH indicator paper turned light green indicating that the soap has a pH of 8.



(a) (b)

(c) (d)

Figure 10. pH of herbal soaps prepared from extracts of (a) *Hibiscus rosa-sinensis* and (b) *Ayapana triplinervis* (c) *Hibiscus rosa-sinensis* and *Ayapana triplinervis (d)* pH indicator

Conclusion

The ethanol extract of Hibiscus revealed the presence of flavonoids, tannins, and terpenoids, while Ayapana exhibited flavonoids and phenols. However, neither the aqueous extract of Hibiscus nor Ayapana demonstrated antibacterial properties against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

In the antimicrobial assessment of different soaps using the disc diffusion method, the control soap exhibited the highest zone of inhibition against *Staphylococcus aureus* compared to the other three soaps. When comparing Hibiscus soap and Ayapana soap, Hibiscus soap demonstrated a greater zone of inhibition against *Staphylococcus aureus*. Conversely, control soap, Hibiscus soap, and Ayapana soap did not exhibit any zone of inhibition against *Escherichia coli*. Only the hybrid soap derived from the extracts of Hibiscus and Ayapana displayed a zone of inhibition against *Escherichia coli*.

In the antibacterial assay of the prepared soaps using the well diffusion method, the Hibiscus-Ayapana hybrid soap exhibited stronger antibacterial properties against *Staphylococcus aureus*. The pH of the soaps was assessed using pH indicator paper, which turned light green, indicating a pH of 8. This suggests that the soaps have a slightly alkaline nature. Hibiscus-Ayapana soap demonstrated superior antibacterial and antimicrobial properties compared to the other soaps. Consequently, we successfully formulated an antimicrobial and antibacterial herbal soap with a nearly neutral pH, ensuring its safety for daily use on the skin.

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A STUDY ON THE ANTIMICROBIAL ACTIVITY OF NEEM (*AZADIRACHTA INDICA)* AGAINST ORAL PATHOGENS

Remya S. Babu, Nivya Joseph, and Newly Joseph*

Department of Chemistry, St. Xavier's College for Women, Aluva, Ernakulam, Kerala, India

*Corresponding author email id: newlyjoseph@stxaviersaluva.ac.in

Abstract

Plants show antimicrobial activity against different microorganisms because of the presence of phytochemicals in them. Thus, leaves of medicinal plants have the ability to control and cure various periodontal diseases and are effective against various oral pathogens. The study compares the antimicrobial activity of herbal mouthwash prepared from neem against oral microbes like *E.coli, S.aureus*, and *Lactobacillus*. The percentage of inhibition of the mouthwashes in the presence and absence of NaCl and $NaHCO_3$ was estimated by means of the microtitre plate method. The neem-based mouthwash was found to be effective against the microorganisms at different concentrations. The sensitivity towards the microorganisms is found to increase with the concentration. It may be concluded that herbal mouthwashes can be used to replace synthetic mouthwashes.

Keywords: *Azadirachta indica, Lactobacillus, Staphylococcus aureus, Escherichia coli,* Percentage of Inhibition, Optical Density.

Introduction

Medicinal plants, play a vital role in curing diseases due to their antimicrobial and antifungal activity against human pathogens (Banu and Gayathri, 2016). Plants have been used since ancient times to protect and cure teeth-related problems. Mouthwashes prepared from plants are found to cure periodontal diseases and is of less side effects compared to synthetic mouthwashes. Many plants like neem, guava, mango, turmeric, pepper, etc. show appreciable antimicrobial

activity against several oral pathogens. The present study focuses on the antimicrobial activity of *Azadirachita indica* against oral microorganisms.

***Azadirachta indica* (Neem)**

Azadirachta indica commonly known as neem is native of India and found in most of the tropical and subtropical countries. They are of great medicinal importance and are widely distributed all over the world (Hashmat *et al.,* 2012). *Azadirachta indica* is an evergreen tree that has potential medicinal value. The first known use of neem dates back 4500 years, by the Harappa culture in ancient India (Kukreja and Dodwad, 2012). Since time immemorial it has been used by Indians for the treatment of various diseases due to its medicinal properties (Lakshmi *et al.,* 2015).

Each part of the tree possesses some medicinal properties (Hashmat *et al.,* 2012). Various parts like leaf, bark, flower, fruit, twig, gum, seed pulp, oil, and root have been shown *in vivo*, *in vitro* and animal studies to possess analgesic, immunostimulant, hypoglycaemic, anti-inflammatory, antiviral, anticarcinogenic, antifungal, hepatoprotective, antiulcer, abortifacient, anthelminthic, antibacterial, anti-yeast, antimalarial, anti-filarial, antipyretic, antiviral, diuretic, antinematode, antispasmodic, anti-spermatogenic, antifertility, antitumor, hypercholesteraemic, immunomodulator and antioxidant activities (Dhingra and Vandana, 2017). Neem extract also exhibits excellent effects as an insecticide, non-toxic repellent, and pesticide and has also been traditionally used as a skin moisturizer (Lakshmi *et al.*, 2015).

In dentistry, *Azadirachta indica* shows good efficacy in the prevention and treatment of periodontal disorders (Kukreja and Dodwad, 2012). Neem offers a good remedy for curing mouth ulcers, and tooth decay and acts as a pain reliever in toothache problems (Lakshmi *et al.,* 2015). Due to its antiplaque, anticaries, and antibacterial effects, it has been widely used in different parts of the world as an oral hygiene tool (Jalaluddin *et al.,* 2017). The neem solutions are used in

decreasing the inflammation of gums, removing canker, and against dental cavities. Neem shows resistance towards *Streptococcus pyogenes, Lactobacillus bulgaricus, Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli* (Banu and Gayathri, 2016).

The active constituent of neem, Azadirachtin, is a mixture of seven isomeric compounds labelled as azadirachtin A-G and azadirachtin E which is an effective antimicrobial agent (Hashmat *et al.,* 2012). Nimbidin, a major active component isolated from seed kernels of the plant exhibits several biological actions (Lakshmi *et al.,* 2015). From Nimbidin other active constituents like Nimbin, Nimbinin, Nimbidinin, Nimbolide, and Nimbidic acid have been isolated which exert an antibacterial effect against several oral pathogens and reduce bacterial adhesion to the tooth surface (Lakshmi *et al.,* 2015). It also contains gallic acid, gallocatechin, epigallocatechin, and catechin which act as antioxidants by reducing the oxidative burst from neutrophils (Dhingra and Vandana, 2017). Other compounds that exhibit potent antibacterial activity are Salannin, volatile oils, Meliantriol, alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids, and ketones (Lakshmi *et al.,* 2015).

Neem has been extensively used in Ayurveda, Unani, and Homoeopathic medicine and has become a wonder tree of modern medicine. Neem bark is used as an active ingredient in a number of toothpaste and toothpowders for curing periodontal problems and maintaining oral health in a natural way. Regular brushing with Neem containing toothpaste will reduce the deposition of plaque, prevent caries, treat halitosis, and enhance the immune response for overall oral health. Neem twigs are used as oral deodorant, toothache reliever, and for cleaning teeth (Lakshmi *et al.,* 2015). The tender twigs of the tree are used as toothbrushes which keep the body system healthy, and the breath and mouth clean and sweet (Hashmat *et al.,* 2012). Neem leaf is rich in antioxidants and helps to boost the immune response in the gum and tissues of the mouth (Lakshmi

et al., 2015). Leaves of neem demonstrate excellent properties for the treatment of gingivitis and periodontitis (Pandita *et al.,* 2014). Neem oil shows significant antibacterial activity and has been suggested for use in treating dental plaque. Frequent usage of mouthwash containing Neem extract will lessen gingival problems and also treat halitosis (Lakshmi *et al.,* 2015). The neem stick extract possesses excellent bacterial aggregation properties as it inhibits glucan synthesis thereby enabling the denaturation of proteins and ultimately bacteria (Manipal *et al.,* 2016). Thus, neem stick extract can reduce the *Streptococci* to colonize on the surface of the tooth (Banu and Gayathri, 2016). Neem also acts as an anti-inflammatory agent by inhibiting prostaglandin E and 5 HT. "Azadirachtin" which is known to destroy bacterial cell walls is used to explain the antibacterial action. The growth of bacteria occurs by the inhibition and cell death due to the destruction of the cell wall by the change in osmotic pressure (Jalaluddin *et al.,* 2017). However, due to its bitter taste, the overall usage of the neem in various commercial preparations is restricted. But this is compensated by artificial sweeteners and flavours to increase patient compliance and acceptability (Pai *et al.,* 2004).

Materials and Methods

In the present study, natural mouthwash was prepared using leaves of *Azadirachta indica*. The leaves free from insect infestation, infection, or damage were collected from mature plants in the gardens.

Preparation of Plant Extract

The leaves collected were washed 2 or 3 times with fresh water in order to remove any dust or dirt. Each of 10g of these leaves was taken, and ground using mortar, to make fine powder, and the fine powder was packed in air-tight plastic pouches ready for extraction. The powdered plant materials were extracted with 30 ml water and shaken well. It is filtered and kept aside for one hour. The supernatant solution is collected and stored in the refrigerator for use.

Preparation of Mouthwash

15 ml of prepared extracts of *Azadirachta indica* were taken separately in a beaker. To this 0.45g of sodium chloride and 0.3g of sodium bicarbonate were added and made up the volume to 30 ml. The resulting mixtures were poured into labelled bottles and dispensed.

Antimicrobial Assay Using Microtiter Plate Method

a) Preparation of microbial culture

A virulent strain of *Escherichia coli, Lactobacillus, and Staphylococcus aureus* obtained from UniBiosys Biotec Research Labs, an ISO 9001: 2008 certified Laboratory was used in the study. Using aseptic techniques, a pure colony was transferred into a 10 ml nutrient broth/potato dextrose broth capped and placed in an incubator overnight at 37°C. After incubation, using aseptic preparation, the turbidity of suspensions was calculated and adjusted using McFarland standards as a reference.

b) Preparation of the microtiter plate plates

Microtitre plates were prepared under aseptic conditions. A sterile 96-well plate was labelled. 10µl, 25µl, 50µl, 75 µl and 100µl of test material was pipetted into the wells. 100µl of nutrient broth was added to each well. Finally, 100µl of microbial suspension was added to each well. Control dilutions of test material were also kept. The plate was wrapped loosely with cling film to ensure that the organism did not become dehydrated. Each plate had a set of controls: a column with all solutions except the test compound and a column with all solutions except the organism adding 100µl of nutrient broth instead. The plates were incubated at 37^0 C for 24 hours and OD reading was taken (OD_{600}) after sufficient incubation. OD values were taken for both the virgin leaf extracts and the extracts with preservatives ($NaCl + NaHCO_3$).

Optical density was obtained by subtracting the control OD from the sample OD. The % of inhibition was calculated from the following equation

% of inhibition= (Control- Test)/Control *100

The pH of the mouthwashes with and without preservatives (NaCl + $NaHCO_3$) was also measured by means of a pH meter and the obtained readings were compared with the known pH of Listerine.

Results and Discussion

Comparison of pH of *Azadirachta indica* mouthwash with Listerine, a synthetic mouthwash

pH of synthetic mouthwash Listerine is 4.3 and is acidic in nature. Studies show that in a healthy state, saliva has a pH range of 6.7 – 7.4. A lower (more acidic) pH, below the critical value, will favour demineralization of the tooth and remineralization occurs at higher pH levels. Dental erosion, the loss of dental hard tissue due to acid exposure without bacterial involvement can also occur. Dental erosion can be the result of acid from intrinsic sources, such as gastric acids, or extrinsic sources, in particular from the diet and consumption of acidic foods and drinks. (West and Joiner, 2014). On analysis of pH, it has been found that natural *Azadirachta indica* mouthwash, is in the neutral range or in the pH range of saliva. In certain cases, as in the case of modified mouthwash by the addition of preservatives, the pH range is found to increase to 6.5 from 5.58. This increase in pH is due to the alkaline nature of $NaHCO_3$ added as a preservative.

Comparison of percentage inhibition of *Azadirachta indica* against *E.coli*, *S.aureus* and *Lactobacillus* using microtitre plate method at different concentrations

At a concentration of 10 µL, the Neem extract exhibited 37.71%, 34.20%, and 36.83% inhibition against *E. coli*. *S.aureus* and *Lactobacillus* respectively. When the concentration is increased to 25 µL, the percentage of inhibition improved significantly. Further increases in concentration to 50 µL, 75 µL, and

100 µL resulted in higher inhibition percentages, with the highest inhibition observed at 100 µL. (Table 2, Figure 1).

Table 2. Comparison of percentage inhibition of *Azadirachta indica* against *E.coli*, *S.aureus* and *lactobacillus* at different concentrations using microtiter plate method.

Concentration (µl)	*E.coli*			*S.aureus*			*Lactobacillus*		
	OD of control	OD of sample	% of inhibition	OD of control	OD of sample	% of inhibition	OD of control	OD of sample	% of inhibition
10		0.434	37.71		0.579	34.20		0.619	36.83
25		0.169	75.85		0.265	69.88		0.319	67.44
50	0.7	0.124	82.28	0.88	0.165	81.25	0.98	0.201	79.48
75		0.073	89.57		0.11	87.5		0.146	85.10
100		0.064	90.85		0.045	94.88		0.11	88.77

The results indicate that as the concentration of the Neem extract is increased, its antibacterial activity against *E. coli*, *S.aureus* and *Lactobacillus* becomes more potent. This suggests a dose-dependent relationship, where higher concentrations of the extract were more effective at inhibiting microbial growth.

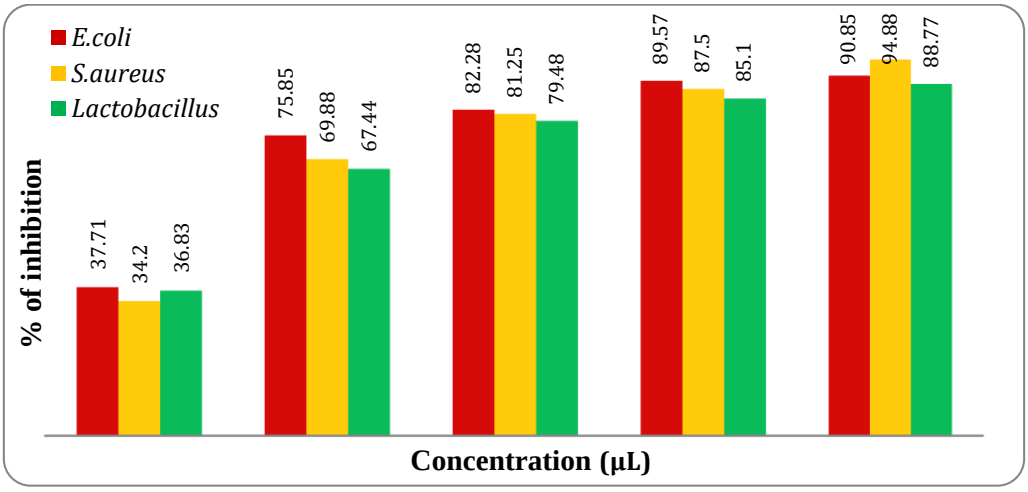

Figure 1. Comparison of percentage inhibition of *Azadirachta indica* against *E.coli*, *S.aureus* and *Lactobacillus* at different concentrations

The results suggest that *Azadirachta indica* (Neem) extract possesses antibacterial properties against *E. coli, S. aureus*, and *Lactobacillus*. The antibacterial activity appears to be concentration-dependent, with higher concentrations of the extract leading to greater inhibition of bacterial growth. This demonstrates the potential of Neem extract as a natural antibacterial agent against these bacterial strains and highlights the importance of concentration optimization for achieving the desired antibacterial effects. Further studies could explore the specific compounds in Neem extract responsible for these inhibitory effects and investigate their mechanisms of action.

Table 3. Comparison of percentage inhibition of *Azadirachta indica* with preservatives (NaCl and $NaHCO_3$) against *E.coli, S.aureus* and *Lactobacillus* at different concentrations

Concentration (µL)	*E.coli*			*S.aureus*			*Lactobacillus*		
	OD of control	OD of sample	% of inhibition	OD of control	OD of sample	% of inhibition	OD of control	OD of sample	% of inhibition
10	0.7	0.624	10.85	0.88	0.749	14.88	0.98	0.755	22.95
25		0.544	22.28		0.608	30.90		0.501	47.9
50		0.392	44		0.557	36.70		0.426	55.4
75		0.329	53		0.533	39.43		0.3	68
100		0.297	57.57		0.479	45.56		0.269	71.1

Table 3 presents the novel results of the antimicrobial assay that evaluates the antibacterial activity of *Azadirachta indica* (Neem) extract in combination with two preservatives, NaCl (Sodium chloride) and NaHCO3 (Sodium bicarbonate), against three different bacterial strains: *E. coli, S. aureus*, and *Lactobacillus,* at various concentrations. The percentage inhibition values indicate the extent to which bacterial growth was inhibited by the combination of Neem extract and the respective preservative at each concentration.

At a concentration of 10 µL, the combination of Neem extract with NaCl and NaHCO3 resulted in 10.85% inhibition only against *E. coli* whereas without preservatives it was 37.71%. As the concentration increased, the inhibition percentages increased as well for samples with preservatives. At the highest concentration (100 µL), the combination with NaCl and NaHCO3 showed a higher percentage of inhibition but less than that for the samples without preservatives.

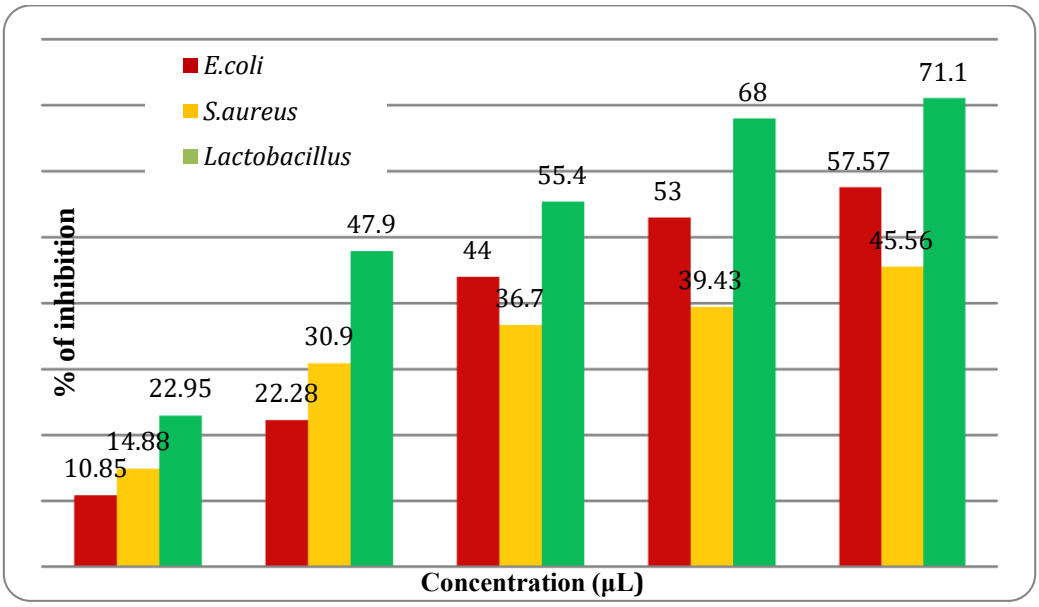

Figure 2. Comparison of percentage inhibition of *Azadirachta indica* with preservatives against *E.coli*, *S.aureus* and *Lactobacillus* at different concentrations

The above results suggest that these combinations have the potential for use as antibacterial agents against the tested bacterial strains but the inhibition potential is less when compared to the samples without preservatives. The decrease in the antibacterial effect of Neem extract in the presence of Sodium Chloride (NaCl) and Sodium bicarbonate (NaHCO3) could be attributed to several factors namely ionic strength, change in pH, chemical interaction of

preservatives with chemical compounds of Neem extract, solubility and precipitation, bacterial response etc. Both NaCl and NaHCO3 are salts that increase the ionic strength of the solution. High ionic strength can interfere with the stability and activity of certain antibacterial compounds present in Neem extract. Some bioactive molecules in Neem extract may be less effective or less stable in the presence of high salt concentrations (Sharma *et al.*, 2022). Sodium bicarbonate (NaHCO3) is a buffer that can alter the pH of the solution. Changes in pH can impact the stability and bioactivity of compounds in Neem extract. Some antibacterial agents may work optimally at specific pH levels, and deviations from this pH range could reduce their effectiveness. They may interact chemically with certain compounds in Neem extract. These interactions can lead to the degradation or modification of bioactive molecules, rendering them less effective against bacteria. Neem extract is a complex mixture of various bioactive compounds, including terpenoids, flavonoids, alkaloids, and others. The presence of salts like NaCl and NaHCO3 can have differential effects on different components within the extract. Some components may be more sensitive to these salts than others, leading to variations in antibacterial activity. The addition of salts can alter the solubility of specific compounds in Neem extract. Some bioactive compounds may precipitate or become less soluble in the presence of salts, reducing their bioavailability and, consequently, their antibacterial effectiveness (Sarkar *et al*., 2021). Some bacteria can adapt to changes in their environment, including changes in salt concentration and pH. They may develop mechanisms to resist antibacterial agents under certain conditions, which could contribute to a reduced antibacterial effect (Reygaert, 2018). It is essential to note that the specific mechanisms underlying the decrease in antibacterial effect may vary depending on the composition of the Neem extract, the concentrations of NaCl and NaHCO3 used, and the target bacteria. Further investigation and research with each parameter are essential to substantiate the mechanism of action in the presence of preservatives.

Conclusion

The results suggest that *Azadirachta indica* (Neem) extract possesses antibacterial properties against *E.coli, S. aureus,* and *Lactobacillus*. The antibacterial activity appears to be concentration-dependent, with higher concentrations of the extract leading to greater inhibition of bacterial growth. This demonstrates the potential of Neem extract as a natural antibacterial agent against these bacterial strains and highlights the importance of concentration optimization for achieving the desired antibacterial effects. Further studies could explore the specific compounds in Neem extract responsible for these inhibitory effects and investigate their mechanisms of action. It can be concluded that mouthwashes prepared from neem are effective against oral pathogens and are safe compared to synthetic mouthwashes.

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Sharma NK, Vishwakarma J, Rai S, Alomar TS, AlMasoud N and Bhattarai A (2022) Green route synthesis and characterization techniques of silver nanoparticles and their biological adeptness. ACS omega. 7(31): 27004-27020.

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CHARACTERISATION OF SILVER NANOPARTICLES GREEN SYNTHESISED USING THE *CARICA PAPAYA* LEAF EXTRACT AND *CINNAMOMUM ZEYLANICUM* BARK EXTRACT

Helna Liston and Newly Joseph*

Department of Chemistry, St. Xavier's College for Women, Aluva, Ernakulam, Kerala, India
*Corresponding author email id: newlyjoseph@stxaviersaluva.ac.in

Abstract

This research work presents the green synthesis and characterization of silver nanoparticles using leaf extracts of *Carica papaya* and *Cinnamomum zeylanicum* bark extracts. This method is sustainable, harmless, and environmentally benign compared to other physical and chemical syntheses. The reducing agents used to synthesize the AgNPs were from the aqueous leaf and bark extracts of papaya and cinnamon. The synthesis of AgNPs was monitored by the color change from yellow to dark brown and UV-visible spectroscopy. The presence of silver ions in nanoparticles was confirmed by X-ray diffraction. The results confirm the fact that the green synthesized AgNPs can be considered for application in pharmaceuticals, owing to their superior anti-inflammatory effect and can be included in the treatment of various inflammatory diseases. In the future, this research can be used to concoct wound healing plasters for diabetic patients since the anti-diabetic property of the plant extracts that act as capping agents have already been studied

Keywords: Silver nanoparticles, green synthesis, antibacterial, anti-inflammatory

Introduction

Silver nanoparticles (AgNPs) have gained significant attention in various fields of medicine, food, health, and industrial processes due to their idiosyncratic properties. Along with these properties, AgNPs also exhibit antibacterial, anticancer, and anti-inflammatory properties and many other therapeutic effects

that make them applicable in medical device coating, optical sensors, and drug delivery. To fulfill the requirement of synthesized silver nanoparticles, different methods are used in its preparation comprising chemical, physical as well as biological methods. Conventional physical and chemical methods were found to be expensive and many hazardous chemicals were needed during those processes. In physical methods, nanoparticles are prepared by evaporation-condensation using a tube furnace at atmospheric pressure (Gurav *et al*., 1994, Magnusson *et al.*,1999, Kruis *et al.*,2000, Schmidt-Ott, 1988). Conventional physical methods for the synthesis of AgNPs include spark discharging and pyrolysis (Tien *et al*., 2008, Pluym *et al*., 1993). Even though physical methods are rapid and less time-consuming radiation is used as a reducing agent. Even though no hazardous chemicals are involved in these processes, the low yield, high energy consumption, solvent contamination, and lack of uniform distribution are the downsides of this method (Shameli *et al*, 2010, Elsupikhe *et al.*, 2015, Tsuji *et al*., 2005). On the other end, chemical methods use water or organic solvents to prepare the silver nanoparticles (Tao *et al*., 2006, Abou El-Nour *et al*., 2010). This process usually embraces three major components namely metal precursors, reducing agents, and stabilizing/capping agents. The major lead of chemical methods is high yield, contrary to physical methods, which have low yield. The above-mentioned methods are extravagant. Additionally, the reducing agents used in the chemical process for the synthesis of AgNPs, such as citrate, borohydride, thio-glycerol, and 2-mercaptoethanol are toxic and hazardous (Wiley *et al*., 2005). Apart from these disadvantages, the manufactured particles are not of the desired purity, as their surfaces were found to be sedimented with chemicals used in the synthesis. Moreover, the use of chemical-reducing agents is harmful to living organisms which makes these silver nanoparticles a non-suitable candidate for biomedical applications. However, in the case of green synthesis, it does not involve any hazardous material. The green approach is one of the best methods for synthesizing stable and environment-friendly silver

nanoparticles, especially for biomedical applications. Apart from this by using the bacterial protein or plant extracts as a reducing agent, we can control the shape, size, and mono-dispersity of the nanoparticles (Gurunathan *et al.,* 2009). The other advantages of biological methods are the availability of a vast array of biological resources, a decreased time requirement, high density, stability, and the ready solubility of prepared nanoparticles in water (Thakkar *et al.,* 2010).

The biological activity of silver nano depends upon the size, shape and particle morphology, coating, agglomeration etc. Therefore, the development of silver nanoparticles with controlled structures that are uniform in size morphology is essential in medical applications. On account of all the above factors, the Green synthetic route of AgNPs using plant extracts is found to be the most cost-effective, simple and environment-friendly approach for the synthesis of AgNPs for biomedical applications.

Hence it is proposed to use the leaves of *Carica papaya* and the bark of *Cinnamomum zeylanicum* for the green synthesis of AgNPs.

Materials and methods

Preparation of *C.papaya* leaf extract

Fresh thoroughly washed *C.papaya* leaves chopped into small pieces were transferred into a conical flask followed by the addition of 200 mL distilled water. The conical flask with chopped leaves was heated at 60℃ for 30 minutes on a hot plate for the effective formation of leaf extract. The leaf extract is then filtered using Whatman no:41 filter paper and kept at 4℃ for further use.

Preparation of *C.zeylanicum* bark extract

C.zeylanicum bark powder was mixed with 200 ml distilled water in a 250 ml conical flask and heated on a hot plate at 60℃ for 30 minutes straight. The extract is then filtered using Whatman no: 41 filter paper and kept at 4℃ for further use.

Preparation of 1mM $AgNO_3$

For the preparation of 1mM AgNO3, 0.169gm of 1mM $AgNO_3$ was made up to the volume in a 100 ml standard flask and mixed thoroughly and the $AgNO_3$ solution was then transferred into an amber-colored bottle to prevent oxidation.

Synthesis of silver nanoparticles *C.papaya* leaf extract

The leaf extract and 1mM $AgNO_3$ solution were mixed in a ratio of 1:4 and heated on a hot plate for 20-30 minutes until the color change was observed. The color change indicates the formation of silver nanoparticles. It was then cooled, centrifuged dispersed in ethanol, and dried in a hot air oven for 5 minutes. Papaya leaves contain bioactive compounds that can act as reducing and stabilizing agents in the synthesis of AgNPs.

Synthesis of silver nanoparticles from *C.zeylanicum* bark extract

The bark extract and 1mM $AgNO_3$ solution were mixed in the ratio 1:4 and heated on a hot plate for 20-30 min until the color change was observed. The color change indicates the formation of silver nanoparticles. It was then cooled, centrifuged dispersed in ethanol, and dried in a hot air oven for 5 minutes.

Characterization of silver nanoparticles formed

- ***UV-visible spectrophotometric analysis of silver nano***

UV-visible spectroscopy is a primary reliable technique for the characterization of nanoparticles which is also used to monitor the synthesis as well as the stability of nanoparticles (Ivanisevic, 2010). UV-visible spectroscopy makes use of the instrument UV-visible spectrometer. Nanoparticles have unique optical properties that are directly dependent on the size, shape, state, and agglomeration of the synthesized nanoparticles (UV/VIS/IR Spectroscopy Analysis of Nanoparticles 2012). In turn, this makes UV-visible spectroscopy a tool for identifying, characterizing, and studying nanoparticles. The characteristic absorption peak of silver nanoparticles was measured by plotting wavelength on the x-axis and absorbance on the y-axis.

- ***Fourier transform infrared (FTIR) analysis***

FTIR is also used to determine the biomolecules present in the plant extract which act as reducing as well as capping agents. The plant extract alone and as well as mixed with $AgNO_3$ was given for FTIR.

- ***X-ray diffraction (XRD)***

XRD is a technique used for the analysis of both molecular and crystal structures for the qualitative identification of various compounds. It makes use of the instrument X-ray diffractometer (Waseda *et al.,* 2011). For XRD, nanoparticles are dispersed in the ethanol coated uniformly on the glass plate and dried in the hot air oven for 5 min and XRD measurements of silver nano formed are measured using an X-ray diffractometer (Vaia, 2002). The average size of the nanoparticles synthesized was calculated using the Debye-Scherrer equation, $d= K\lambda/ \beta\cos(\theta)$ where d is the average crystallite size in nanometers (nm), K is the Scherrer constant, typically with a value between 0.89 and 1, depending on the crystal shape and the diffraction peak used. It is a dimensionless constant. λ is the wavelength of the incident X-ray beam typically in angstroms (Å), β is the full width at half maximum (FWHM) of an hkl X-ray diffraction peak (in radians) at θ value where θ is half of the scattering angle 2θ corresponding to the peak. The working principle of X-ray diffraction is Bragg's law.

Results and Discussion

Formation of silver nanoparticles

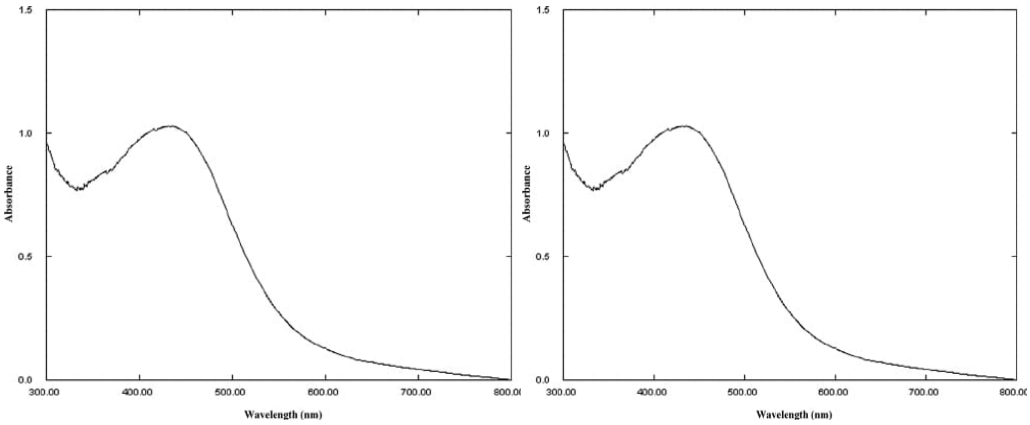
The green synthetic route employed for synthesizing silver nanoparticles from *C. papaya* leaf extract and *C.zeylancium* bark extract is found to be the most simple, cost-effective, and eco-friendly method. The formation of silver nanoparticles can be confirmed by the color change from light yellow to dark brown after the completion of nanoparticle formation (Figure 1).

(a) (b)

Figure 1. Before and after the synthesis of silver nano particles from (a) *C.Papaya* leaf extract and (b) *C. Zeylancium* bark extract.

UV-visible Spectroscopy

The aqueous extract of papaya leaves and cinnamon bark when mixed in an aqueous solution of silver nitrate the reduction of pure Ag(+1) to Ag(0) can be measured by UV visible spectroscopy. When the aqueous extracts are mixed with $AgNO_3$, colour changes from light yellow colour to dark brown colour. AgNPs have unique optical properties which make them strongly interact with specific wavelengths of light. In AgNPs, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band, occurring due to the collective oscillation of electrons of silver nanoparticles in resonance with the light wave.



(a) (b)

Figure 2. UV visible spectra of AgNPs synthesized from (a) *C.papaya* leaf extract (b) *C. Zelyanicum* bark extract

In the above spectrum of wavelength vs. absorbance (Figures 2a & b), a characteristic peak is observed in the range of 400-500nm. which is the characteristic peak of silver nanoparticles. This confirms the formation of stable AgNPs. It can be concluded that AgNPs can be effectively prepared by using leaf and bark extracts of *C.Papaya* and *C.zelyanicum* respectively.

X-ray Diffraction (XRD) Analysis

The XRD analysis was carried out with an X-ray diffractometer, Bruker, D8 Advance model, employing Cu Kα radiation (λ =1.54 A0) and Ni filter operating at 30 kV and 20 mA. The particle size was calculated using the Debye-Sherrer formula, $d = 0.9 \lambda / \beta \cos \theta$ where d is the particle size, λ is the wavelength of the incident x-ray beam, β is the full width at half maximum (FWHM) of an hkl X-ray diffraction peak at θ value where θ is half of the angle 2θ corresponding to the peak.

In the case of AgNPs synthesized using cinnamon bark extract the XRD spectra clearly show the main peaks at (2θ) 38.13 which corresponds to silver nanoparticles. By comparing the jcpds file (file no: 89-37220), the two

unassigned peaks that appeared at 32.25° and 46.21°are weaker than those of silver. This may be due to the bioorganic compound occurring on the surface of AgNPs. The calculated value is in good agreement with the early reported nanoparticle synthesis (Gopinath *et al.,* 2012). The average grain size of silver nanoparticles formed in this bio-reduction process was determined using Debye Scherr's formula, $d = K\lambda / \beta Cos\theta$ and the average value obtained is 20.82nm.

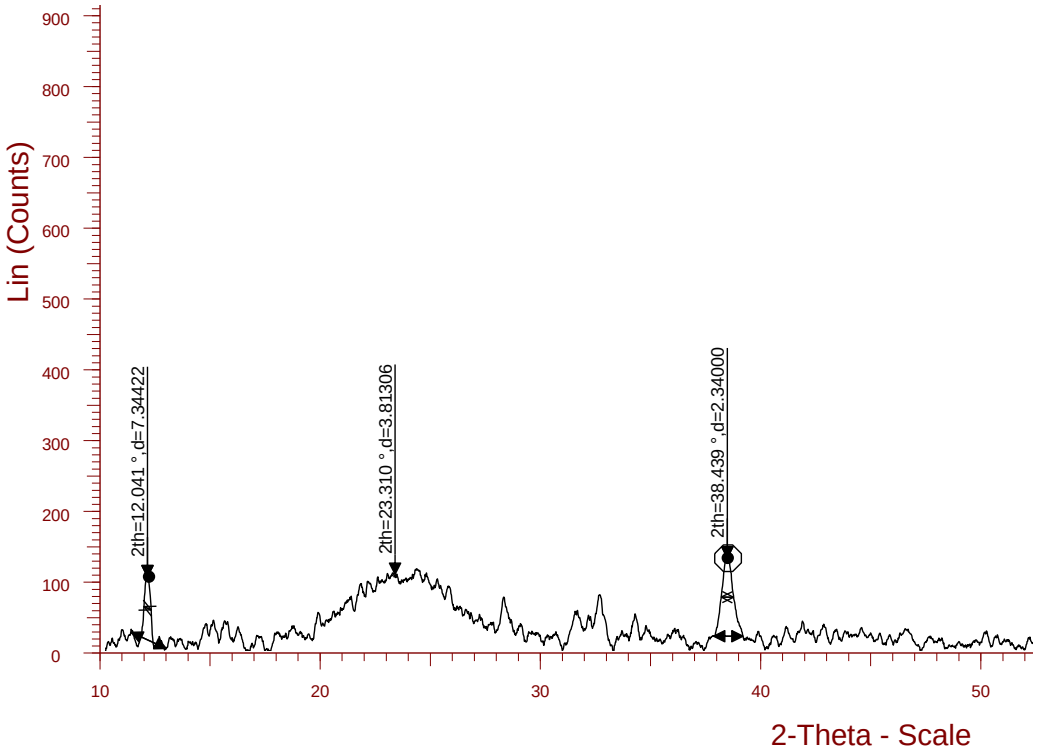


Figure 3. XRD spectra of AgNPs synthesized using cinnamon bark extract

Similarly, in the case of AgNPs synthesized using the papaya leaf extract the pattern clearly shows the main peaks at (2θ) 38.422 which corresponds to silver nanoparticles. By comparing the jcpds file (file no:89-37220), the two unassigned peaks appeared at 32.25° and 46.21°are weaker than those of silver. This may be due to the bio-organic compound occurring on the surface of AgNPs. That is calculated value is in good agreement with the reported. The average grain

size of silver nanoparticles formed in the bioreduction process was determined using Debye Scherr's formula, $d = K\lambda / \beta Cos\theta$ and the average value obtained is 20.98nm.

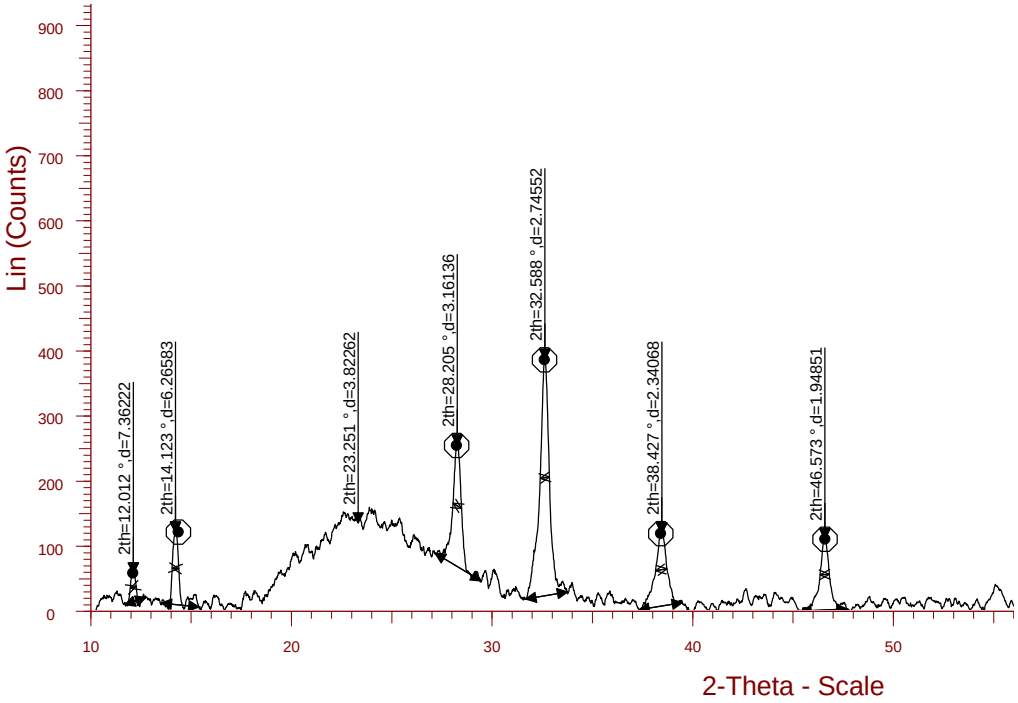


Figure 4. XRD spectra of AgNPs synthesized using papaya leaf extract

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The functional group that might be responsible for the reduction of silver nanoparticles and the groups that act as capping and stabilizing agents can be studied from FTIR analysis. The FTIR analysis of papaya and cinnamon revealed four peaks. The peak corresponding to the range 3416.84,3419.44cm^{-1} might be due to OH stretching. The peak corresponding to 1636.62,1637.43 cm^{-1} might be due to the C=O group which is a characteristic group of flavonoids. The peak at 2069.38 and 2070.56 cm^{-1} may be due to multiple bonding carbon-carbon triple bonds. The peaks at 562.71 cm-1 and 547.46 cm^{-1} might be due to the C-Cl and C-Br bond. All the above details revealed the compound that is present in the

plant extract which acts as a capping and reducing agent may be flavonoids (Veeraputhiran, 2013; Jyoti *et al.*, 2016).

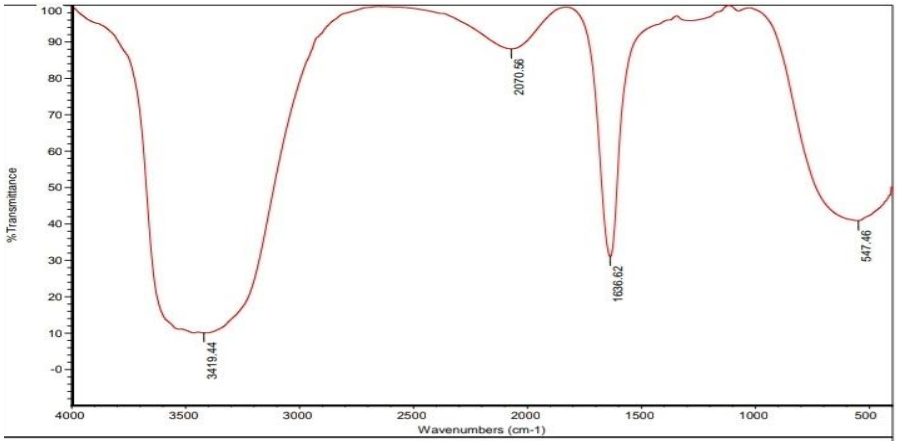


Figure 5. FTIR of AgNPs synthesized from papaya leaf extract

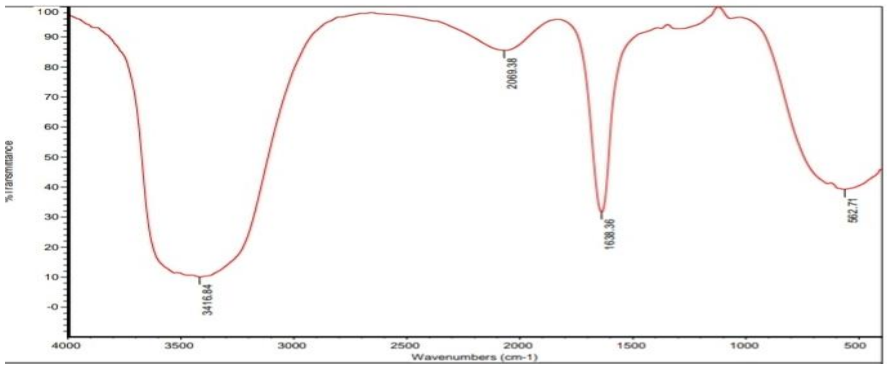


Figure 6. FTIR of AgNPs synthesized from Cinnamon bark extract

Conclusion

The above studies reveal that silver nanoparticles can be effectively synthesized by a green method which does not involve any harmful chemicals. FTIR analysis shows that the flavonoids present in the plant extract act as reducing as well as capping agents in the reduction of silver ions into AgNPs. UV-visible Spectroscopy shows a peak at 400-500nm, which is the characteristic peak of silver, and shows that silver nanoparticles can be effectively synthesized

by the green method analysis gives some characteristic peaks and particle size can be calculated using the Debye Scherrer formula and gives the size of nanoparticles the silver nano synthesized from cinnamon has a size of 20.82nm and from papaya, it has a size of 20.98nm.. This method does not involve any toxic and hazardous material so it can be used in many medicinal applications. Green synthesis can be employed for the instantaneous preparation of silver nano. In the future, this work can be dilated to concoct wound-healing plasters.

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FOLDSCOPE AS A MICROBIOLOGICAL RESEARCH TOOL IN INDIA: A REVIEW

Wafha K.A. and **Baby Divya***

Department of Zoology, St. Xavier's College for Women, Aluva, Ernakulam, Kerala
Corresponding author email id: babydivya@stxaviersaluva.ac.in

Abstract

Microbiology is a field that deals with the organisms visible only through a microscope. Bacteria, fungi, protozoa, algae, and viruses are the major microbial entities. They possess countless leading roles in the environment as well as in the lives of human beings. Hence, for a microbiologist microscope is an essential tool for identification. Foldscope, the paper microscope, a simple and affordable invention in microscopy is considered a part of frugal science. Till date, remarkable microbiological research has been carried out using foldscope ever since its entry to India by supplementing or even replacing the conventional microscopes which is comprehensively reviewed.

Keywords: Foldscope, Bacteria, Fungi, Protozoa, Algae, Application, Microbiology

Introduction

The invention of foldscope: The rationale

Foldscope is an alternative to the simple microscope to visualize objects under 140x magnification. It was developed by Manu Prakash along with his student Jim Cybulski at Prakash Lab, Stanford School of Medicine, Stanford University, USA in the year 2014 with the aim of bringing microscopes to communities worldwide at affordable prices. Prakash Lab established a company, Foldscope Instruments Inc., USA, for the manufacturing of foldscopes in December 2015. The foldscope was designed to cost less than US$1. Until now, 1.8 million foldscope's have been distributed in over 160 countries. In India, the

basic kit is available at a lucrative price only for the reason that its manufacturing material is paper. It is made entirely from a sheet of paper with a series of origami-style folds that can be assembled from simple components; available in a nine-by-twelve-inch envelope. Even so, the kit consists of a lens stage, sample stage, focus ramp, lens (140 X to 2,000X imaging resolution of 2 microns), a light emitting diode, diffuser panel, power LED, etc. similar to other microscopes (Cybulski *et al*., 2014).

Entry of Foldscope to India

Conventional microscopes are expensive and assigned special areas for use. Moreover, frequent maintenance is mandatory at each interval. While compared to the foldscope's benefits, it is satisfactory for field-based researchers and those who handle conventional microscopes. In addition, bright field, darkfield, fluorescence, and lens-array imaging are also feasible with foldscope. Such capabilities make it more comfortable than the conventional microscope. Various research work published in the area of diagnostics, animal health, agriculture, education, sanitation and hygiene, biodiversity, machine learning, etc. with the help of foldscope-based experiments (Dharmendra, 2019; Patil *et al*., 2019; Indu *et al*., 2019; Tambekar *et al*., 2020; Selvakumar and Viswanathan, 2021). In addition, it would be used as a diagnostic tool for detecting many diseases but currently, foldscope is not approved for diagnostics applications in human health. Foldscope was brought to India through the intervention of the Department of Biotechnology (DBT), the Ministry of Science & Technology, and the Government of India. The remarkable features of foldscope are simplicity, portability (lightweight-8 grams), ultra-affordability, durability, and quality similar to conventional research microscopes. In addition, mobile phone cameras can easily be coupled to capture images. It will help to bring the microscope to the field for instant examination and is sturdy to work in all climates. Hence it is known as a part of frugal science: a movement with the aim of inexpensive scientific innovations in the world.

Right away, foldscope has been used in the medical field for the diagnosis of dental plaque and urinary infections, early-stage cancer-causing cells, histopathological analysis, fungal-caused lung infections, biofilm formers, fish infection, food-borne bacterial pathogens, malaria, schistosomiasis, and soil-transmitted helminthiasis. Hence, foldscope helps in rapid analysis to identify the causes. Meanwhile, when compared to conventional microscopes, various disadvantages have been reported such as a lesser magnification capability that is 140 X, a resolution of 2 microns which is not sufficient to observe bacteria, and safety precautions cannot be ensured while handling patient samples (Ganesan *et al*., 2022).

Applications in microbiology

In the field of microbiology, to acquaint visibility of microorganisms sized in the range of 0.2 µm to about 25 µms, foldscope microscopy is a great achievement by promising diagnosis and discoveries. Until now various advanced techniques have been formulated including bright field, dark field, SEM, TEM, Atomic Force Microscopy, etc. Recently scientists are improvising the microscope for deeper imaging of living tissues (Yoon *et al*., 2020). Kaur *et al*. (2020) in their work used foldscope as a primary diagnostic tool for oral and urinary tract infections and its effectiveness in oral health education. In addition, they suggest further improvement in lens and focus to acquire high-quality images. In the study, the effectiveness of the foldscope was analysed by collecting saliva, dental plaque, oral lesions, sputum, and urine samples from hospitals. After processing samples by staining procedures, each sample was observed through the foldscope. In plaque samples, epithelial cells, fungal hyphae, spores, bacteria, and inflammatory cells, and in urine samples, calcium oxalate crystals, pus cells, and epithelial cells were observed. As a result, it was concluded that the foldscope was a great tool to reduce expensive diagnostic procedures. For the detection of thermophilic actinomycetes during the composting process for *Agaricus bisporus*, foldscope was used by Dev and Kaur (2019). Mushroom

compost was prepared with various formulations and its samples were examined randomly on successive days. However, during the study, heavy distribution of actinomycetes was detected from 13-19 days and disappeared on the 25th day. In another work related to water analysis by Dharmendra, (2019) domestic-waste-water sample was observed through foldscope by spot analysis. It was concluded that wastewater contained a large variety of known or unknown microorganisms such as protozoans, rotifers, bacteria, viruses, insects, and helminths. Similarly, Patil *et al*. (2019) used foldscopes to identify the on-site presence of extracellular naringinase-producing bacteria in soil samples collected from citrus agricultural fields.

Mastanamma *et al*. (2019) used foldscopes to create awareness about hygiene and the involvement of microbes in street food. Apart they collected different food samples from streets and subjected them to direct observation using foldscope along with other tests. During their direct observation, many motile and non-motile bacteria were observed including cocci, bacilli and spirillum, and fungal spores. By gram staining along with the traditional bacterial identification method, *Escherichia coli, Salmonella typhi,* and *Vibrio cholerae* were confirmed. Pukhrambam *et al*., 2019 performed screening for the presence of microbes in vegetable and water samples through the foldscope. A large number of gram-positive bacteria with a thick cell wall and a few gram-negative bacteria were observed. An improvised device of fluorescence foldscope was used for the detection of biofilm formation in *Pseudomonas aeruginosa:* an opportunistic pathogen. Biofilm is a mass of anchored microbial cells enclosed by its extracellular polymer. For biofilm detection, the glass slide at a 90° angle was incubated 5 days in Luria Bertani (LB) broth inoculated with *Pseudomonas aeruginosa* and observed through a foldscope (Deshamukhya *et al*., 2020). The application of foldscope in visualizing gram staining results were also supported by Indu *et al*., 2019 during their biofilm-producing bacterial identification procedure and for the confirmation of other bacteria (Jeyakumar *et al.,* 2019; Muthukumaran

and Manikannan, 2019; Mridul, 2019; Mishra *et al*., 2019). The direct view through foldscope without staining process was also done by Buragohain *et al*., (2019).

In the field of protozoology, according to the succession study of Davis and Divya, (2019), studied the relationship between the quality and diversity of leaf litter and protozoan communities was observed through the foldscope. For the study, a pond was selected and the vegetation surrounding the pond was used for the leaf litter microcosms experiment (*Mikania micrantha*, *Mangifera indica*, *Alternanthera reineckii*, *Alternanthera philoxeroides*, *Gliricidia sepium*, and *Bryum sp*.). The study was conducted for six weeks to analyze the abundance and diversity, on average the highest number of protozoa was observed in *Mangifera indica* irrespective of the season. The protozoa observed through the foldscope are *Paramecium, Arcella, Vorticella, Colpodium, Euplotes, Cryptodifflugia, Trinema, Quadralulalla, Centropyxis, Coleps,* and *Heliozoa.* Maya *et al*. (2019) aimed to explore the microflora and microfauna biodiversity in aquatic ecosystems and found that motile protozoa, cyanobacteria, and algae were detected by foldscope.

Malaria has been a major concern all over the world. Even with rapid test kits available, the climate may affect performance. Hence, Gupta *et al.* (2022) used foldscope for malaria detection in their study. However, due to low accuracy, it was concluded that its current magnification and illumination could not be utilized under National Vector Borne Disease Control Programme. The work of Ephraim *et al*. (2015) concluded that foldscope possesses excellent specificity in diagnosing *S. haematobium* and suggests future modifications to improve sensitivity. Urine samples were taken from 50 individuals and processed on the same day and subjected to microscopic analysis. The results showed excellent specificity and limited sensitivity for *S. haematobium* diagnosis.

Tambekar *et al.* (2020) studied foldscope-assisted microscopy for the examination of soil-transmitted helminths. They dealt with parasitic helminth

infection by collecting stool samples from the Wardha district located in Maharashtra. Initially, all the samples were examined under conventional microscopy and later the positive samples were observed through a foldscope to assure their imaging. By analyzing the results, the images of helminth eggs under foldscope were clear and its morphological discrimination was possible. Rajan *et al*. (2019) performed an analysis of endoparasitic infections in elephants using a foldscope. As a result, eggs of *Fasciola* sp., eggs of *Hymenolepis* sp., and larvae of *Strongyles* were observed by foldscope. However, more eggs were observed with conventional microscope than in the foldscope due to the limitation in the volume of the sample. Hence it was concluded that though foldscope had limited sensitivity it had excellent specificity. Ectoparasites, helminth parasites, haemoparasites, fecal and skin scrapings were observed through a conventional microscope by Bal *et al*. (2019) and found that the foldscope could be used as a cheap, effective diagnostic tool to identify parasites. The detection of intestinal parasitic infections using foldscope among school children of South India revealed that about 13.2% were detected positive for intestinal parasitic infestation. Eggs of *Ascaris lumbricoides* (roundworm) and cysts of *Balantidium coli* were commonly found (Kumar *et al*., 2020). Similarly, blood smear analysis for parasitic detection from positive samples, microfilariae and *Babesia bigemina* were observed with clear visibility under the foldscope (Raghavamma, 2019).

Maheswari *et al.* (2018) showed the significance of foldscope for the detection of early blight disease in tomatoes. Early blight is one of the most dominant and serious damaging diseases in potatoes and tomatoes. The primary causative agent is the fungal pathogen *Alternaria solani*. The team collected infected tomato leaves and concluded that this mode of procedure helps them to identify *Alternaria solani* or non-*Alternaria solani* infections. Gurjar and Kanade (2020) analysed phytopathogenic fungi isolated crop plants using morpho-molecular tools such as microscopic evaluation by foldscope along with ITS region sequencing. For that, the infected portions were cut into thin sections to

characterize fungal isolates acquired from various vegetable, fruit, and ornamental plants. Through the study, the aim was to evaluate the foldscope as a preliminary tool for onsite observation and they identified various phytopathogenic fungi and their mode of action. So it was concluded that foldscopes could promote farmers to be aware of plant infection and immediate action to be taken. Selvakumar and Viswanathan, 2021 studied the early detection of airborne Puccinia rust spores using glass slides in the sugarcane field. The uredospores of *P. melanocephala* in the range of 25–39 × 17–28 μm in size and *P. kuehnii* with a size of 33–53 × 21–31 μm were easily observed with the help of foldscope. Vijayakumar and Karthika (2020) assessed soil fungal diversity from agricultural fields using foldscope. For the analysis, the soil sample was serially diluted and inoculated to potato dextrose agar. After the incubation fungus was characterized microscopically under conventional microscope and foldscope. The results showed a similar result of the dominancy of *Trichoderma viridae* and *Aspergillus niger* in both microscopes. Bhattacharyya (2019), observed a total of 6 different fungal species (*Aspergillus sp. Penicillium sp., Curvularia sp., Trichoderma sp., Cladosporium sp.* and *Diasporium sp*.) under foldscope with 140X magnification suggesting the foldscope as a regular monitoring tool for assessment of indoor environment quality. Morphological characteristics of 36 algal genera were observed using a foldscope by Jyotsna *et al*. (2019) during their study from different ponds.

Conclusion

Microscopes are an essential tool in the field of microbiology. The advent of foldscope has facilitated microbiological research, especially in resource-limited settings considering its cost, portability, durability, and quality. Nevertheless, improvisations in the present model with additional options could make foldscopes an indispensable device in microbiological research.

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