Studies on the Antibacterial and Antifungal Activity of Natural Dye (Guava Leaves) in Aqueous Medium

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Abstract

This work aims to prepare and evaluate a natural dye named Guava and antibacterial and antifungal substance. The antibacterial properties of extracted dye were evaluated towards Gram- positive and Gram-negative bacteria. As well as antifungal properties of extracted dye were evaluated towards selected fungus. Results showed that the extracted dyes have higher antibacterial activity and extracted dyes have no antifungal activity. They are considered as eco-friendly, nontoxic medicinal properties fitting into similar trends of repurposing, up cycling which is very important for environment balance. Guava extracted dye are eco-friendly to the human skin.

Keywords *Natural dye, Guavatree leaves, Antibacterial activity, Antifungal activity.*

Introduction

Nowadays natural dyes have received a great interest by scientists all over the world. Research works in dyeing using natural dyes for textile are done, applied and compared to the synthetic ones. Natural dyes are biodegradable, and eco- friendly in nature with a variety of shades. Natural dyes are obtained from different sources like plants, insects, animals and minerals. Multifunctionalization of fabrics is of great interest, beside the environmental benefits of using natural dyes. A number of researches are now available for multifunctional properties of natural dyes such as antimicrobial.[1]Nature provides a wealth of plants which will yield their colour for the purpose of dyeing, many natural dyes have been used since antiquity. In the early 21st century, the market for natural dyes in the fashion industry is experiencing a resurge. Western consumers have become more concerned about the health and environmental impact of synthetic dyes in manufacturing and there is a growing demand for products that use natural dyes. Completely capturing the market with natural dyed fabric is an urgent need to maintain a safe environment.[2]

Textile materials and clothing are known to be susceptible to microbial attack, as these provide large surface area and absorb moisture required for microbial growth.[3] Natural fiber have protein and cellulose, etc, which provide basic requirements such as moister, oxygen, nutrients and temperature for microbial growth and multiplication.[4] This necessitates the development of clothing that could provide a desired antimicrobial effect.[5]

Natural products have been used in traditional medicine through out of the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants or compound in treating disease has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on earth to treat various infections, although only 1% have gained recognition by modern scientists.[6]

Nowadays, fortunately, there is an increasing awareness among the people toward the use of natural dyes as substitute for synthetic dyes. Due to its non-toxic property, low pollution, and less side effects, natural dyes are used more often in food products as well as other important regular uses. Above all, they are environment-friendly and after use, can be recycled.[7]In developing countries like India people use folk medicine for the treatment of common infections. These plants are ingested as decoctions, teas and juice preparations to treat various infections. It is necessary to evaluate, in a scientific base, the potential

use of folk medicine for treatment of infectious diseases produced by common pathogens. Medicinal plants or natural product might represent an alternative treatment in non-serve cases of infectious diseases.[8]

They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant. Owing to their popular use as remedies for many infectious diseases searches for plants containing antimicrobial substance are frequent. According to World Health Organization medicinal plants would be the best source for obtaining variety of drugs.[9]These evidences contribute to support and quantify the importance of screening natural products. Although known for a long time for dyeing as well as medicinal properties, the structures and protective properties of natural dyes have been recognized only in the recent past. Many of the plants used for dyeing extraction are classified as medicinal and some of these have recently been shown to possess remarkable antimicrobial activity.[10]

The aim of the present study was to investigate the antibacterial and antifungal properties of guava leaves extracted natural dye.

Guava is a tropical tree which is cultivated in many parts of world. Its edible fruits are included in super fruits and are very rich in vitamin C. Its leaves, fruits and roots are used in treatment of cough, fever, diarrhea, constipation, bad breath, gum problems and other health problems. In India it is cultivated throughout the country. Guava is a small tropical evergreen shrub or shade tree. It easily propeagates by seeds and cuttings. Since guava fruit is rich in vitamin c, it protects body against bacterial and viral infections by improving body immunity.[11] Guava leaves aid in weight loss by preventing the complex starches from being converted into sugars. Guava leaf tea can effectively lower blood glucose in diabetics by reducing the alpha-glycosidase enzyme activity. The guava leaves are a great liver tonic. Guava leaves are herbal remedy for diarrhea and dysentery. Guava leaf tea aids in digestion by stimulating digestive enzyme production. The powerful antibacterial agents kill bacteria in the lining of the gut and stop proliferation of toxic enzymes by bacteria. Guava leaf tea is effective in treating bronchitis by opening up the lungs. Guava leaves can even be made into a natural paste at home for brushing the teeth. Guava leaves are considered a natural remedy for dengue fever. Guava leaves can be beneficial in case of prostate cancer. Guava leaves have great healing properties. Guava leaves effective for acne and black spot.[12]

The study this envisaged to investigate the antibacterial and antifungal activity of natural dye extracted from guava leaves.

Materials and Methods

2.1. Materials:-

2.1.1. Source:- The leaves of tree(Guava) were collected from Tanshipur village, Haridwar district.

Figure-1. Guava tree leaves

2.2. Experimental Methods:-

2.2.1. Dye Extraction:-The collected leaves were properly cleaned under running tap water and then allowed for air drying in shade. The air dried leaves were chopped into small pieces and coarsely ground using electric grinder. Aqueous medium was prepared in 300 ml of water without using chemicals and maintained the pH 6 separately 30gm of dyestuff was added and the dye was extracted for 90 min. at 90 æ%C the solution was filtered. The extract was directly used without any dilution.

2.2.2. Test solutions of natural dye:- Test solution of a series of concentrations viz. 20, 50, 100, 250, 400 mg/ml were prepared by dissolving natural dye obtained from different leaves in aqueous medium. All test solution were kept in refrigerator at 4æ%C for future use.

2.2.3. Antibacterial screening test:-

2.2.3.1. Bacterial strains: -Antibacterial activity of natural dye obtained from leaves. The natural dye was tested against gram positive bacteria, Staphylococcus aureus and gram negative bacteria, Escherichia Coli. The pure bacterial cultures were maintained on nutrient agent medium and each bacterial culture was further maintained by sub culturing on the same medium and was stored at 4æ%C before use in experiments. The bacterial strains obtained from IIT, Roorkee were used for evaluating antibacterial activity.

2.2.3.2. Preparation of bacterial inoculums: -Stock culture was maintained at 4æ%C on slopes of nutrient agar active culture for experiments was prepared by transferring bacteria in nutrient broth and that inoculated without agitation for 24 hrs. at 37æ%C. The bacteria spore suspension was adjusted to give a final concentration of 10×10^{10} PBS ml.

2.2.3.3. Preparation of media: -The medium was prepared by dissolving Muller Hinton Agar Medium (Himedia) in distilled water. The dissolved medium was auto calved at 15 Ibs pressure at 121æ%C for

15 minutes. The autoclaves medium was mixed well and poured into 100 mm petriplates (25ml plates) while still molten.

2.2.3.4. Assessment of antibacterial activity of natural dye: -The antibacterial activity of natural dye was tested against several bacterial isolated using agar well diffusion method. The culture plates were inoculated with 0.1 ml of standardized inoculums (1.0×10^{10} PBS ml) of each bacterium and spread with sterile swabs wells of 0.9 mm diameter were punched into MHA petriplates containing the bacterial inoculums with sterile core borer. The wells were filled with test solutions of natural dye. Commercially available antibiotics Ampicillin and Streptomycin discs (1.0 mg/ disc each) were used. The plates thus prepared were left at room temperature for 15 minutes allowing the diffusion of the extract into the medium. After incubation for 24 hrs at 37æ%C, the plates were observed. Antibacterial activity was observed by an inhibition zone surrounding the well containing the natural dye. The zone of inhibition was measured and expressed in millimeters. Each experiment was repeated and diameters of inhibition zones were calculated. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism.[13]

2.2.4. Antifungal screening test:-

2.2.4.1. Fungal strains: -The in vitro antifungal activities of the natural dye obtained from selected leaves. The natural dye was assessed against standard strains of two fungi namely Chrysosporium fungus and Fusarium Oxysporium fungus. The pathogenesis grown in pure culture were maintained in potato dextrose agar (PDA). Culture slants at 4æ%C and used as stock culture throughout the study. The fungal strains obtained from IIT, Roorkee were used for evaluated antibacterial activity.

2.2.4.2. Preparation of fungal inoculums: -For the antifungal assay, cultivated slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of 1×10^{5} Cfu/ml.

2.2.4.3. Preparation of media:-The medium was prepared by dissolving PDA media (Himedia) in distilled water and autoclaving at 121æ%C for 15 min. 20 ml of sterile PDA media was poured in sterilized pertridishes (9cm diameter) and allowed to solidify which were used for the antifungal assay.

2.2.4.4. Antifungal activity assay: -Antifungal activity of natural dye from different selected leaves and barks determined, using agar-well diffusion method. Spore suspensions (0.2 ml) were applied on the surface of the presterilized and autoclaved PDA pertridishes and spread by using a sterile glass spreader. Wells of 0.6 mm diameter

hole were made in the center of each of the PDA petriplates with help of sterilized cork border. The wells were filled with test solutions of natural dye. All the petriplates including treatment and controls were allowed to diffuse at room temperature for 2 hrs and then incubated at room temperature for 72 hrs. after incubation, the antifungal activity of extracts was expressed in terms of diameter of zone of inhibition.[14]

Result and Discussion

In the present study, the inhibitory effect of natural dye extractedfrom selected leaves. The antibacterial and antifungal activity of natural dye was determined using agar well diffusion method and quantitatively assessed on the basis of inhibition zone.

Textile dyeing industry at present uses excessive amount of synthetic dyes to meet the required coloration o global consumption of textiles due to cheaper prices, wider ranges of bright shades, and considerably improved fastness properties in comparison to natural dyes.[15] The application o such dyes causes serious health hazards and influences negatively the eco-balance of nature.[16] In the present study the antibacterial efficacy of selected leaves natural dye was evaluated according to their zone of inhibition against various bacteria and fungus.

3.1. Evaluation of antibacterial activity of natural dye

In this study, two different bacterial pathogens were used to screen the possible antimicrobial activity of dye extract. Dye extract exhibited antibacterial activity against all tested microorganisms.

Antibacterial activities of dye extract from Guava leaves

Table 1- Antibacterial test against Escherichia coli

SR.NO.	Natural dye Concentration(mg/ml)	Zone of inhibition (mm) Escherichia coli
1.	100	4
2.	200	6
3.	250	6
4.	400	9

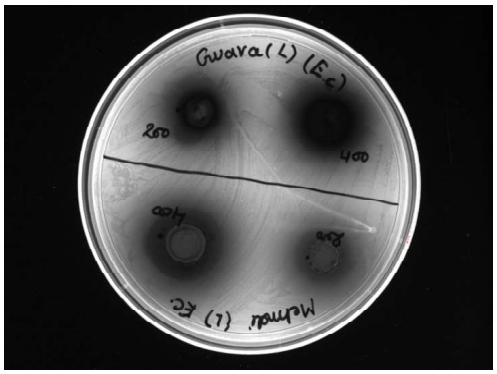

Figure-2. Zone of inhibition (E. Coli) of guava leaves extract

It is evident from table 1 that test solution of the natural dye at 200, 400 ml concentration of showed highest antibacterial activity against other concentration. . Treatments of the natural dye at 100, 200

ml concentration show minimum activity against tested bacteria.

Table 2- Antibacterial test against Staphylococcus aureous

SR.NO.	Natural dye Concentration(mg/ml)	Zone of inhibition (mm) Staphylococcus aureous
1.	100	0
2.	200	0
3.	250	4
4.	400	7

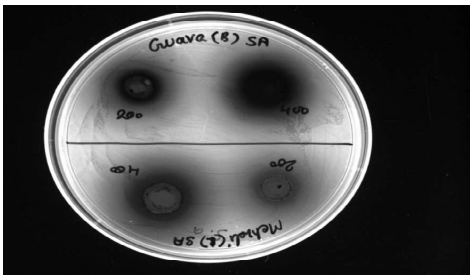

Figure-3. Zone of inhibition of (Staphylococcus aureous) guava leaves extract

It is evident from table 2 that test solution of the natural dye at 250, 400 ml concentration of showed highest antibacterial activity against other concentration. Treatments of the natural dye at 100, 200 ml concentration show no activity against tested bacteria.

3.2 Evaluation of antifungal activity of natural dye

The antifungal efficiency of different selected barks and leaves natural dye was evaluated according to their zone of inhibition against altogether two pathogenic fungi. The result as summarized in table that the natural dye exhibit antifungal activity against all the test fungi studied at all the experimented concentrations.

Antifungal activities of dye extract from Guava leaves

Table 3- Antifungal test against Chrysosporium fungus

SR.NO.	Natural dye Concentration(mg/ml)	Zone of inhibition (mm) Chrysosporium fungus
1.	20	Some activity
2.	50	Some activity

It is evident from data presented in table 3 that the natural dyes from Guava leaves test solution at different concentration show some antifungal activity against all tested fungus. If natural dye extract medium is change, antifungal activity definitely show.

Figure-4. Zone of inhibition of (Chrysosporium fungus) guava leaves extract

Table 4- Antifungal test against Fusarium Oxysporium

SR.NO.	Natural dye Concentration(mg/ml)	Zone of inhibition (mm) Fusarium Oxysporium fungus
1.	20	Some activity
2.	50	Some activity

It is evident from data presented in table 4 that the natural dyes from Guava leaves test solution at different concentration show some antifungal activity against all tested fungus. If natural dye extract medium is change, antifungal activity definitely shows.

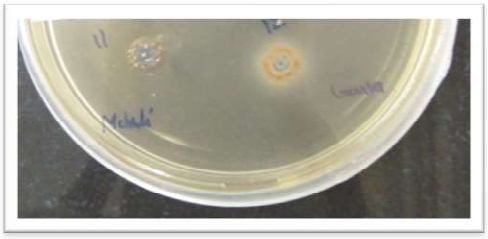

Figure-5. Zone of inhibition of (Fusarium Oxysporium) guava leaves extract

The extract of B. pilosa is used in folk medicine as an anti-helminthes and protozoacide agent; it also has antiseptic properties. It contains flavanoids.[17]Henna was used even before 2500 BC, while saffron is mentioned in the Bible.[18]In Egypt, mummies have been found wrapped in dyed cloth. Primitive men used plant dyestuff for colouring animal skin and to their own skin during religious festivals as well as during wars. Solider believed that the colour would give them magical powers, protect them from evil spirits and help them to achieve victory in war.[19]

The yellow pigment curcumin of Turmeric is reported to have antiviral, antifungal antibacterial effects and strong antiseptic potency.[20]In another study, Singh et al. have studied on antimicrobial activity of different natural dye source such as Acacia catechu, Kerria lacca, Quercus infectoria, Rubia cordifolia and Rumex maritimus and tested the antimicrobial efficncy of samples against some common pathogens. Finally they found different antimicrobial efficiencies against the tested pathogens.[10]

This study looks into the antibacterial and antifungal activity of guava leaves extract against two bacteria gram negative bacteria (E.Coli) and gram positive bacteria (S. Aureus) and two funguses the Chrysosporium Fungus and Fusarium Oxysporium Fungus. Results of antibacterial activity of Guava leaves natural dye had greater antibacterial activity toward the gram negative bacteria (E.Coli) and gram positive bacteria (S. Aureus) and results of antifungal activity of guava leaves natural dye had some antifungal activity toward the Chrysosporium Fungus and Fusarium Oxysporium Fungus. If natural dye extract

medium is change, antifungal activity definitely shows.

Conclusion

At present worldwide there is a growing trend towards the usage of natural colours in various industries due to the environment hazards caused by the excessive use of synthetic dyes. The usage of vast amountof synthetic dye cause pollution disturbs the ecological balance and causes health hazards to human beings.[22] Because of this Germania government was the first to ban the manufacturing of synthetic dyes. Netherlands, India and some other countries also followed the ban.[23] At present, research is focused to find out alternative dyes for clothing and it should be eco-friendly, readily available, cost effective.

The usage of synthetic dyes was done commercially for attractive colours but it is hazardous to skin and environment. Usage of natural dyes obtained from Guava leaves in textile has good use for antibacterial purpose and may help in keeping the skin healthy by preventing from allergy reduce the risk o skin cancer. The dye obtained is biodegradable and non- toxic. The natural dye obtained from Guava leaves also has medicinal properties. Natural dye from Guava is evaluated in present study and showed better result in antibacterial activity. Because of its antibacterial property, its usage in dyeing medical cloth.

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