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ANTIMICROBIAL ACTIVITY OF STEM BARK EXTRACT OF *MANGIFERA INDICA* AGAINST *STAPHYLOCOCCUS SPECIES* AND FREQUENCY OF USE OF ORGANIC EXTRACTS AS SKINCARE PRODUCTS IN COMMON POPULATION

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ABSTRACT: Antimicrobial activity of stem bark of *Mangifera indica* was determined using the disk diffusion method to determine the susceptibility or resistivity of *Staphylococcus species* to the plant extract. Ethanol extract showed the highest inhibition zone diameter of 34mm, while the aqueous extract showed a zone diameter of 23 mm. The ethanol proved to be a better solvent, and the *Staphylococcus species* was found susceptible to the ethanolic extracts. The stem barks extracts can be used to develop new antimicrobial compounds. Survey results revealed a need to increase awareness to limit the overuse of harmful chemical cosmetics as skin care products and augmentation in the use of organic extract for skincare products among individuals.

INTRODUCTION: *Mangifera indica* (*M. indica*) commonly acknowledged as mango plant, is an evergreen tree with dark green leaves with crown-shaped canopy. Since time immemorial, mango is one the oldest trees that have been cultivated in India and grow well in properly drained loamy soil and in soils rich in organic matter^{1, 2}. In spite of having a wide array of medicinal plants in India, *M. indica* emerged to be a medicinal tree too. It consists of several diseases treating properties³. As the present era is teeming with multi-drug-resistant cases reported concerning different microbes, it is imperative to work out natural medicine to treat diseases. *M. indica* is identified and well-studied medicinal plant. Leaves and stem bark of *M. indica* have been found

to be rich in many useful compounds such as mangiferin, mangiferolic acid, homonangiferin, tannins, flavonoids, steroids, alkaloids, and carbohydrates¹ which have immense antimicrobial properties and might help treat bacterial or other microbial infections and additionally improves skin complexion and tone^{4, 5}. The immediate and most obvious reason for using plant-based medicines is the ease of availability and low price. These plants derived medicines can be easily prepared at home by natural methods and can be used for disease treatment⁶.

Staphylococcus is a Gram-positive coccus that appears as a bunch of grapes when viewed under the microscope. It can be found and isolated from air, soil, and water. It is attributed to the normal microbiota of the human respiratory tract but is also an opportunistic pathogen. It turns into a causal organism of respiratory tract infections, skin infection (most commonly acne) and sometimes causing food poisoning to^{2, 7}. There are many antimicrobial drugs becoming resilient towards the infection caused by *Staphylococcus* species.

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There is a necessity of discovering new antimicrobial compound which is safe and possess no side-effect on the host⁸. This study is aimed to determine the antimicrobial effects of *M. indica* against *Staphylococcus* species and a short survey among 50 individuals to know the use of organic extracts as skincare products.

MATERIALS AND METHODS: For isolation of test organism (*Staphylococcus sp.*) soil sample (garden soil) was collected from the residential areas of Danapur, Bihar. Soil was serially diluted and was plated on the Mannitol salt agar (MSA) plates^{9,10}. The plates were left for incubation at 37 °C for 48 h. On the basis of Grams reaction, appearance of bacterial cell under 100X magnification, colony morphology resembling the characteristics of *Staphylococcus sp.*¹¹, the bacterial colony was selected and used for purification. Pure culture was stored at 4° C to retain its viability.

The bark of *M. indica* was collected from Danapur, Bihar. The area has loamy and dark soil. The collected bark was washed thoroughly with water and then air dried at room temperature for 4-5 days. Upon drying, the plant materials were crushed using mortar and pestle into smaller particles and then the stem bark pieces were grounded into powder and then sieved using a sieve¹². The powdered plant extracts were transferred into airtight containers and was stored in cool and dry place for further use¹³.

Plant active components were extracted from the stem bark extract using the cold extraction method¹⁴. The different solvents used were ethanol and distilled water. 10g of powdered sample was soaked in 100ml solvent in a sterile conical flask. The flask was covered with cotton plug and wrapped with aluminium foil and was shaken vigorously at 3 h intervals for 48 h at room temperature. The crude extract was then filtered using muslin cloth and Whatman no.1 filter paper with the pore size of 125mm. The filtering process was repeated several times until the clear solution was obtained. Antimicrobial activity was performed by standard methods like the disk diffusion method on Mueller Hinton agar media¹⁵. The inoculums were swabbed uniformly over the MHA plates. The inoculum was allowed to dry for 10-15 min with the lid over it.

The discs were impregnated on the surface of the plates using sterile forceps. Sterile paper discs were prepared to have a diameter of 10 mm with 1 ml of each extract at a concentration of 10%. 4 discs were placed on each plate and were placed equidistant from each other to avoid overlapping of inhibition zones¹². The plates were then incubated at 37 °C for 24 h. Control disc was set by soaking the disc in the solvent only. The measurement of zones of inhibition was noted. The procedure was repeated in triplicates, and then the mean diameter was taken. The mean diameter zones were compared to the standard CLSI reference chart¹⁶.

To prove that the above sayings were true in regard to the comments on chemical-based cosmetics, organic skin care products and natural extracts, a survey with the help of google forms with customized questionnaires was conducted in a total population of 50 individuals randomly in Patna (Bihar). The questions from the questionnaire are as follows:

1. No. of individuals prefer chemical-based cosmetics, organic skin care products and natural extracts.
2. Which one is more preferable 'in a long run' among chemical based cosmetics, organic skin care products and natural extracts.
3. Are people around us aware of the harmful chemicals used in their beauty products.

The result of the survey was noted and compared.

RESULTS AND DISCUSSION: The yellow bacterial colony which was yellow in colour, with dry, irregular and wavy margin was purified and regarded as *Staphylococcus sp.* This was further confirmed by Gram-positive reaction with rod-shaped cell and appearance as grape-like clusters under 100X oil immersion microscope. The result showed that the stem bark extracts of *M. indica* possess good antimicrobial activity against *Staphylococcus* species in both solvents. The extracts inhibited the growth of *Staphylococcus*, and the zone of inhibition ranged from 20mm to 34mm. The ethanolic extracts were found to be susceptible to the *Staphylococcus* species.

However, the aqueous extracts were not very much potent because the *Staphylococcus* species were found to be in intermediate. The results were recorded in **Table 1** and **Fig. 1**. The ethanolic

extract proved to be much more effective than the plain aqueous extract. The control showed that the solvents without extracts had minimum or no inhibitory effect on the *Staphylococcus* growth.

TABLE 1: SHOWING THE INFERENCE OF RESISTIVITY AND SUSCEPTIBILITY OF THE STAPHYLOCOCCUS AS COMPARED TO CLSI STANDARDS

Disc used	Zone of resistance (mm)	Zone of intermediate (mm)	Zone of susceptible (mm)	Zone diameter formed (mm)	Inference
Aqueous extract	≤20	21-28	>29	21.6	Intermediate
Ethanolic extract	≤20	21-28	>29	32	Susceptible
Distilled water	≤20	21-28	>29	0.1	Resistant
Ethanol	≤20	21-28	>29	0.1	Resistant

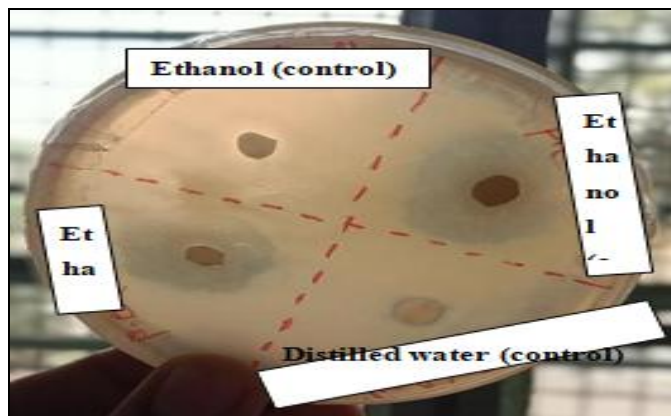


FIG. 1: SHOWING ZONE OF INHIBITION FORMED BY *M. INDICA* STEM BARK EXTRACT (AQUEOUS AND ETHANOLIC) ALONGWITH THE RESPECTIVE CONTROL AGAINST *STAPHYLOCOCCUS SP*

Survey result showed that out of a total of 50 individuals 39 of them preferred to use chemical-based cosmetics, 8 among them preferred to use organic skin care products and only 3 individuals preferred to use natural extracts made at home **Fig. 2**. This is a clear indication that cosmetic industries have already expanded to an extent that people blindly prefer these without knowing its side effects.

From a total of 50 individuals, 6 of them preferred to use chemical-based cosmetics for the rest of their lives, 9 preferred to use organic skin care products, and 35 preferred to use natural homemade extracts for their skin **Fig. 3**. The outcome of the survey depicts that they do not want to fill up their skin with a lot of chemicals that’s why the majority prefer to use natural home remedies for the long run, which has almost no side effects. Natural homemade decoctions or extracts have no or minor side effects on our skin as it is completely natural, and since it has been prepared in this study, we can blindly trust on the results.

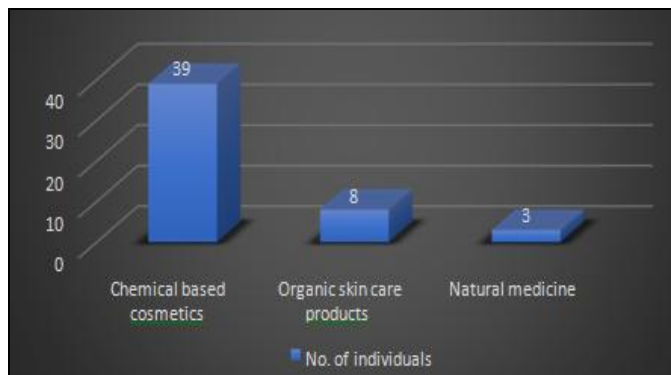


FIG. 2: BAR GRAPH SHOWING NO. OF INDIVIDUALS WHO PREFER CHEMICAL BASED COSMETICS, ORGANIC SKIN CARE PRODUCTS AND NATURAL MEDICINE

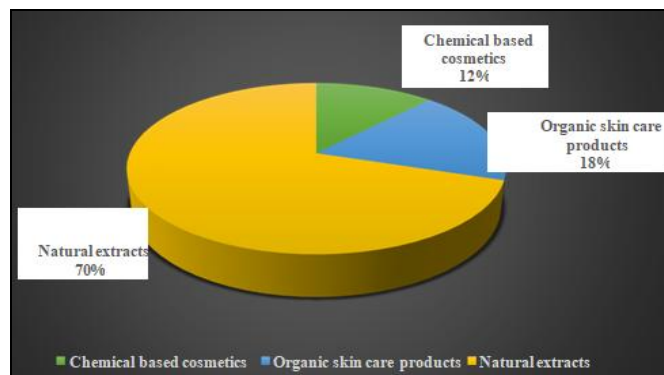


FIG. 3: PIE CHART SHOWING THE PREFERENCE OF INDIVIDUALS FOR USING SKINCARE PRODUCTS FOR A LONG RUN

30 individuals out of a total of 50 were not at all aware of the harmful chemical composition, 12 of them were not sure about the same, whereas only 8 of them were aware the chemical composition and their ill effects **Fig. 4**. The result shows that majority are not aware of the cons of regularly using chemical-based cosmetics. *M. indica* is filled with almost all the properties to improve our skin texture and treat skin-related problems. Stem bark extract of *M. indica* contains active compounds which plays a very important role in improving our

skin health. Stem bark extracts are known to be antiseptic, antioxidant and astringent due to which they are potent in improving skin texture. The *Staphylococcus* bacteria have been found to be resistant to various antibiotics and multiple drugs. The major antibiotics include methicillin, oxycillin, penicillin, ampicillin, amoxicillin, vancomycin, tetracycline, gentamycin etc¹⁷.

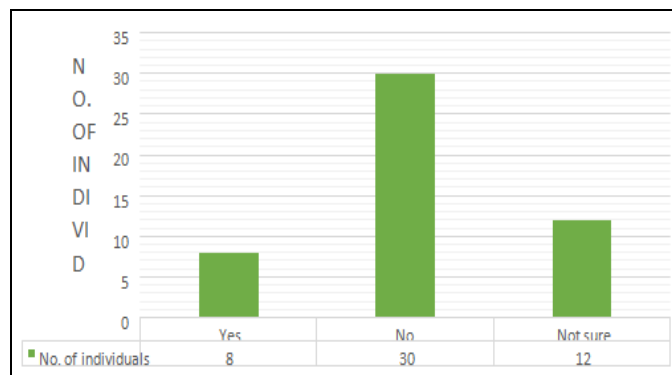


FIG. 4: BAR GRAPH SHOWING THE RATE OF AWARENESS OF PEOPLE REGARDING THE HARMFUL COMPOSITION OF CHEMICAL-BASED COSMETICS

This study found that the ethanolic extract of *M. indica* was more effective against *Staphylococcus* species than the aqueous extract. The aqueous extract could not show good results because water does not act as a good solvent for organic solutes, i.e., *M. indica*. However, the result was on the basis of minimum concentration of the extract, i.e., 10% by weight. The zone of inhibition for the aqueous extract was about 21.6 mm, which was intermediate towards the test organism. So, it can be assumed that upon increasing the concentration of the extract, its efficiency may increase subsequently.

It was reported that the leaves extract of *M. indica* was surprisingly less effective towards *Staphylococcus aureus* and *Escherichia coli*. However, researchers also mentioned that its ethanolic extract was most effective against the test organism. At lower concentrations, *M. indica* acts as a bacteriostatic agent to¹⁸. Joshua and Takudwa, 2013¹ reported the efficiency of *M. indica* stem bark extract against *Staphylococcus aureus*. They concluded their study by showing that the methanolic extract is most effective on the basis of disk diffusion, agar, and broth dilution method. Manzuret al., 2020¹⁹ reported the effectiveness of *M. indica* leaves in reducing biofilms of

Staphylococcus aureus in stainless steel and teet rubbers. The ethanolic extract was found highly efficient in treating *Staphylococcus aureus* biofilms within 5 minutes of contact. The extract was also found to be a potent natural sanitizer too. Earlier work also revealed that the ethanolic solvent of stem bark extract proved more efficient and showed a greater zone of inhibition against *Staphylococcus* species^{20, 21}.

CONCLUSION: Due to old and traditional antibiotics, microorganism is becoming resistant. So, there is a need to find new antimicrobial drugs that will have to be potent and effective against microorganisms, possessing no side effects. The plant extract can serve as a source for the production of antimicrobial compounds, and it would be an efficient way of treating infections.

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