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Antimicrobial activity of traditional formulation

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Abstract

This study was undertaken to investigate the antimicrobial activity of traditional formulation using *phyla nodiflora, Hibiscus rosa sinesis, Phyllanthus emblica, and piper nigrum* plant against pathogens. cold press oil and refined oil extracts of the above plants were prepared and their antimicrobial properties were evaluated by measuring the zone of inhibition using the disk diffusion method. Various fractions showed antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, staphylococcus aureus,* and antifungal activity against *Aspergillus niger* and *Aspergillus flavus.* The results indicated that all of the extracts show antimicrobial properties. The highest potential was observed in formulation 4 (*phyla nodiflora* leaves & cold press oil) 18 mm zone of inhibition. The cold press oil extract shows maximum inhibition against bacteria as compared to fungi. The experiment confirmed the using *phyla nodiflora, Hibiscus rosa sinesis, Phyllanthus emblica, and piper nigrum* oil extracts as natural antimicrobials and suggested the possibility of scalp infectious diseases caused by the test organism. The current investigation shows leaves of *Phyla nodiflora* have potent antimicrobial activity further study is needed to explore its full potential.

Keywords: Traditional formulation; *Hibiscus rosa sinesis; Phyllanthus emblica; Piper nigrum; Phyla nodiflora;* Antimicrobial activity; Cold press oil; Refined oil

1. Introduction

Medicinal plants are the 'backbone' of traditional remedies. Traditional medicine related to the healing of both human and animal diseases with plant-derived preparations is considered precious information for the discovery of new antimicrobials [1]. The importance of medicinal plants as a source of active drugs emerged from the chemical profile that produces a clear physiological action on the biological system. Flavonoids, alkaloids, tannins, and phenolic compounds have been established as the most important bioactive compounds of plants [2]. Medicinal plants are economically important and considered useful. They contain some active substances which are used in the treatment of many human ailments. The plant extracts have been extracted and developed and then used against different microbes [3]. It is well known that even most synthetic drugs have their origin in plant products [4]. Going complex world creates many pathogens that show resistance to many different kinds of drugs. These multidrug-resistant pathogens species create serious troubles and problems in different areas like residential, hospitals, industries, houses, and in open-air communities. Awareness about the importance of medicinal plants is growing in medical communities, government, health care systems, and also in common society in many developing countries. The main source of new drugs and starting products for new drugs comes from medicinal plants [5]. *Phyla nodiflora Linn.* is an essential medicinal plant belonging to the family Verbenaceae. It is scattered in subtropical and tropical regions. Phyla nodiflora is used in colic, asthma, diarrhea, bronchitis, ulcers, gonorrhea fever, knee joint pain, anti-inflammatory and antispasmodic. The phytochemical study of plant shows that it contains flavonoids, sugar, essential oil, sterols, resins, tannins, and non-glucosides bitter substances [6]. In the present study, we have chosen the plant *Phyla nodiflora* used in herbal medicine to determine its antimicrobial property. There are not sufficient scientific studies that confirm the antimicrobial activity of this plant. This study looks into the antimicrobial activity of this plant against some gram-positive and gram-

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2. Material and methods

2.1. Collection of materials

The plant *Phyla nodiflora* was collected from the wetland fields and other irrigated fields in and around Erode, Tamil Nadu, India. The herbarium of these plants was identified and authenticated by Dr. M. U. SHARIEF SCIENTIST 'F' & HEAD OF OFFICE, Southern Regional Centre, Lawley Road, Coimbatore-641003. The remaining ingredients *Hibiscus rosa sinesis, Phyllanthus emblica, and piper nigrum* were collected from in and around Erode, Tamil Nadu, India.

2.1.1. Preparation of formulation

The entire plant of *phyla nodiflora, Hibiscus rosa sinesis, Phyllanthus emblica, and piper nigrum* were collected, shade dried and coarsely powdered using a mechanical grinder. It was then passed through sieve no:10 and the fine powder size was extracted with cold press oil and refined oil by maceration method. The formulation is heated for 15 days and filtered by muslin cloth using vacuum filtration apparatus. The extract was stored in air-tight containers for further studies.

2.2. Microbial strains used

The antibacterial activity of traditional formulation was determined against 2 gram-positive and 2 gram-negative viz., *E. coli, B. subtilus, S. aureus, and P. aeruginosa.* The antifungal activity of traditional formulation was determined against *Aspergillus Niger and A. Flavus.*

2.3. Determination of antimicrobial activity

2.3.1. Determination of anti-bacterial study

Antibacterial assay was used to determine the growth inhibition of bacteria. Bacteria were maintained at ''4 ^{0}C'' on Broth media before use. Nutrient agar medium was prepared and sterilized at ''121 ^{0}C'' for 15 minutes. A total of 25ml of Nutrient agar was poured into sterile Petri dishes and allow setting. Each Petri dish was spread with 0.2ml of different bacterial species (*E. coli, B. subtilus, S. aureus, and P. aeruginosa*). An internal diameter of 6mm and an external diameter of 8mm of the cavity were made by using a sterile borer, Various extracts are poured into the cavity were made into the set agar containing the bacterial culture. The plates were incubated overnight at ''37 ^{0}C''. The result was obtained by measuring the zone diameter.

2.3.2. Determination of anti-fungal activity

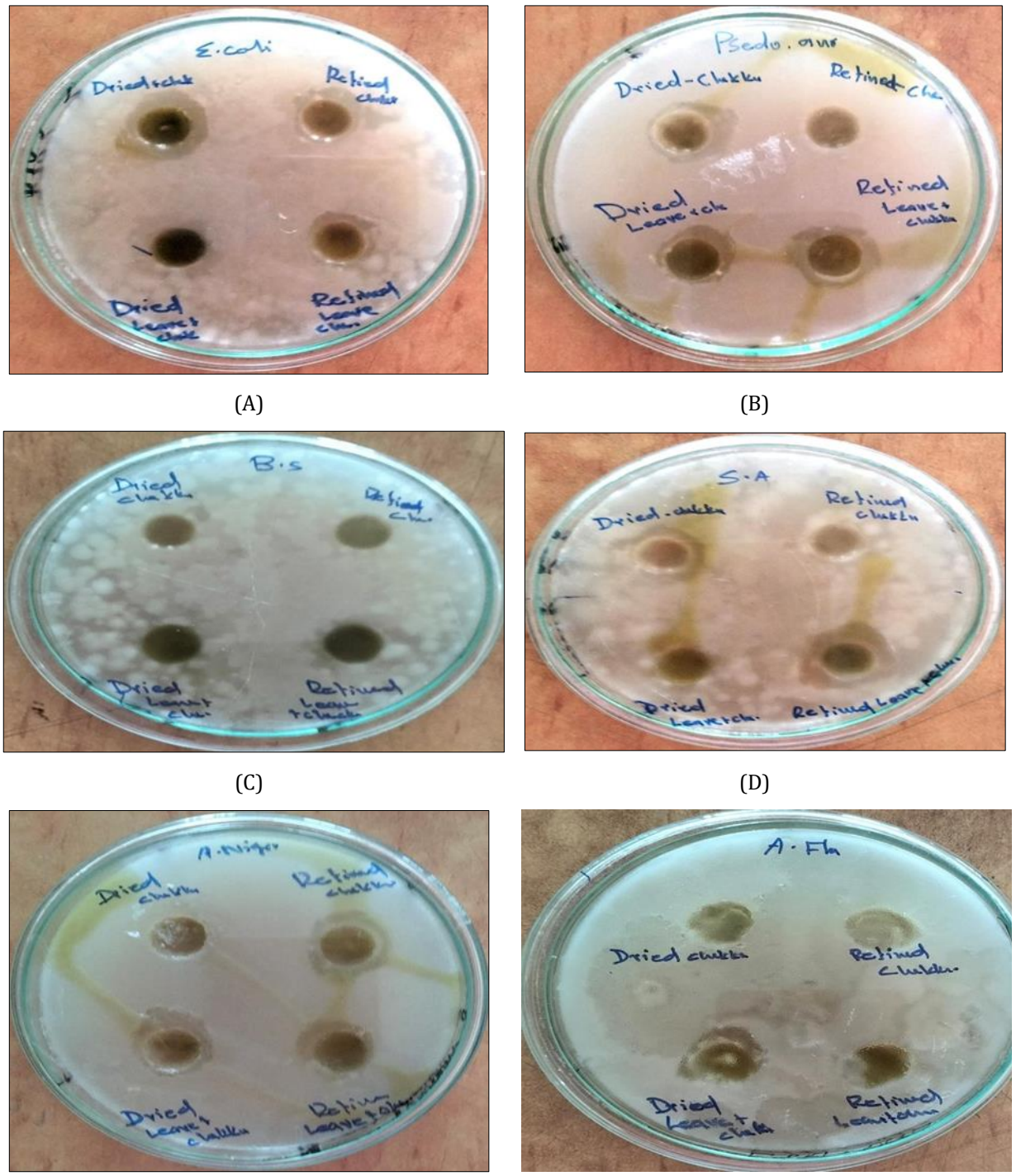
The fungus was maintained at ''4 ^{0}C'' on Broth media before use. Sabouraud dextrose agar medium was prepared and sterilized at ''121 ^{0}C'' for 15 minutes. A total of 25 ml of Sabouraud dextrose agar was poured into sterile Petri dishes and allow setting. Each Petri dish was spread with 0.2ml of different fungi species (*Aspergillus Niger and A. Flavus*). An internal diameter of 6mm and an external diameter of 8mm of the cavity were made by using a sterile borer, Various extracts are poured into the cavity per plate and were made into the set agar containing the fungus culture. The plates were incubated at ''25 ^{0}C'' for 2 days. The result was obtained by measuring the zone diameter.

3. Results

Table 1 Determination of anti-microbial activity

S.No	SAMPLE	Zone of Inhibition (mm)					
		S.aureus	***E.coli***	***B.subtilus***	***P.aeru***	***A.niger***	***A.flavus***
1	F1(Dried &Cold press oil)	15 ± 0.6	18 ± 0.4	12 ± 0.8	14 ± 0.3	10 ± 0.6	10 ± 0.5
2	F2(Dried &Refined oil)	11 ± 0.2	16 ± 0.5	11 ± 0.1	11 ± 0.4	14 ± 0.3	09 ± 0.7
3	F3(Leaves &Refined oil)	12 ± 0.3	13 ± 0.5	15 ± 0.2	15 ± 0.5	16 ± 0.7	13 ± 0.3
4	F4(Leaves &Cold press oil)	18 ± 0.5	14 ± 0.2	15 ± 0.4	17 ± 0.5	16 ± 0.5	14 ± 0.8

The antimicrobial activity of the traditional formulation was studied against two gram-positive & two gram-negative bacteria and two fungal species is summarized in table 1.



(A) (B)

(C) (D)

(E) (F)

Escherichia coli, B- Pseudomonas aeruginosa, C-Bacillus subtilis, D-staphylococcus aureus, E- Aspergillus niger, F- Aspergillus flavus

Figure 1 Zone of inhibition of traditional formulations

4. Discussion

4.1. Antibacterial activity

Antibacterial activity was determined against two gram-positive bacteria and two gram-negative bacteria. The results revealed that the plant extracts showed significant antibacterial activity with varying magnitudes. Among the all formulation, the F4(Leaves & Cold Press oil) and F1(Dried & Cold Press oil) exhibited broad-spectrum activity against

all test bacteria in comparison to other formulations. the F4(Leaves & Cold Press oil) and F1(Dried & Cold Press oil) exhibited high antibacterial activity with a zone of inhibition ranging from 14 to 18 mm and 12 to 18 mm respectively. The F3(Leaves & Refined) formulation parts showed moderate activity of 12 to 16 mm, and the F2(Dried & Refined) formulation exhibited mild activity ranging from 9 to 16 mm. The cold Press oil extract of leaves *(P. nodiflora*) showed the highest inhibition zone against Staphylococcus aureus with an inhibition zone of 18 mm, and theleast activity was observed against *Escherichia coli* (14 mm). The inhibition zone in the cold Press oil extract of the entire dried plant (*P. nodiflora*) was the highest (18 mm) against Escherichia coli and the lowest was observed in *Bacillus subtitles* with a zone measuring 12 mm. The refined oil extract of leaves (*P. nodiflora*) exhibited the highest zone of inhibition of15mm against *Pseudomonas aeruginosa, and Bacillus subtitles* and the least activity was observed against *Staphylococcus aureus* with an inhibition zone measuring 12mm. The inhibition zone in the refined coil extract of the entire dried plant (*P. nodiflora*) was the highest (16 mm) against Escherichia coli and the lowest was observed in *Pseudomonasaeruginosa, Bacillus subtitles, Staphylococcus aureus* with a zone measuring 11 mm.

4.2. Antifungal activity

The fungal strains such as *Aspergillus Niger* and *Aspergillus flavus* used in this study results of antifungal activity of extracts from *P. nodiflora* are summarized in Table 6. Both refined and cold Press oil extract from leaves of *P. nodiflora* exhibited the highest zone of inhibition measuring c. the F1(Dried & Cold Press oil)) formulation parts showed moderate activity of 10 mm and the F2(Dried & Refined) formulation exhibited mild activity ranging from 9 to 14 mm. earlier studies show that the F4(Leaves & Cold Press oil) cold Press oil extract *of P. nodiflora* leaves significantly inhibited the growth of bacteria. A large number ofphytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999). L. citriodora showed the presence of essential oils such as geranial, neral, and limonene (Argyropoulou et al., 2007). The possible reasons for the higher antibacterial activity of F4(Leaves & Cold Press oil) the cold Press oil extract of leaves *(P. nodiflora)* may be due to the stronger extraction capacity of biologically active components such as alkaloids, flavonoids, essential oils, terpenoids, etc. Recently attention has been directed toward extracts and biologically active components isolated from popular plant species.

5. Conclusion

The development of new antimicrobial compounds against different microorganisms is becoming critically important, as infectious scalp diseases are still one of the leading causes of hair and skin disorders in the world. The pharmaceutical industry is searching for new lead compounds with novel chemical structures to overcome the increasing resistance to known antibiotics. Plants can be a useful source of these lead components. The use of plant extract and phytochemicals, both with known antimicrobial properties, can be of great advantage in therapeutic treatments in that way different oil extracts of *L. nodiflora* showed antimicrobial activity against 2 gram-negative, 2 gram-positive, and 2 fungi. Among the all formulation, the F4(Leaves & Cold press oil) showed excellent antimicrobial activity against all test bacteria and fungi in comparison to other formulations.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors have declared no conflict of interest.

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