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Phytochemical Screening and Antimicrobial Activity of Different Medicinal Plants *Eugenia caryophyllata* (Clove) and *Berbaris Aristata* (Daru Haldi)

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Abstract The antimicrobial activity of extracts was screened against two hyman pathogens : *Escherichia coli* and *Staphylococcus aurreus* employing disc diffusion method. Among four plants tested, *Eugenia caryophyllata* (Clove) was found to be the most effective against *Escherichia coli* and *Staphylococcus aureus.* The largest zone of inhibition (14 mm) was obtained with *Staphylococcus aureus* and (12 mm) with *Escherichia coli. Berbaris aristata* (Daru haldi) was found to partially active against *Staphylococcus aureus* but ineffective against *Escherichia coli*. A qualitative phytochemical analysis was performed for the detection of alkaloids, flavonoids, saponins, steroids and tannins. Ethanolic extracts of *Eugenia caryophyllata* (Clove) was found to contain alkaloids, flavonids, tannins and steroids. *Berbaris aristata* (Daru haldi) extract was also found to contain alkaloids, flavonoids tannins, saponins and steroids. However, alkaloids were present in all tested extracts. Hence, *Eugenia caryophyllata* (Clove) was demonstrated to exhibit maximum antimicrobial activity followed by *Berbaris aristata* (Daru haldi).

Keywords Antimicrobial, Phytochemical, Alkaloids, Flavonoids, Tannins.

Introduction

Medicinal plants are extremely important to health of individuals and communities. Apart from being of great economic value, use of herbal medicine for the treatment of diseases and infections is as old as mankind [1]. According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semisynthesis [2]. Such plants have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical com-

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ponents that are medically active. These non-nutrient plant chemical compounds or bioactive components like alkaloids, tannins, flavonoids and phenolic compounds, are often referred to as phytochemicals or phytocostituents and are responsible for protecting the plant against microbial infections or infestations by pests.

Antimicrobial textiles with improved functionally find a variety of applications such as health and hygiene products, specially the garments worn close to the skin and several medical applications, such as infection control and barrier material . The aim of this work is to carry out a phytochemical analysis of extracts of some natural plants in order to know their antimicrobial activity.

(The authors wish to thank the Punjab Biotechnology Incubator Testing Lab, Mohali).

Materials and Methods

Collection of sample

The medicinal plant parts used for the experiment were bud of *Eugenia caryophyllata* (Clove) and bark of *Berbaris aristata* (Daru haldi). These selective species of plants were identified according to various literature and screened for their antimicrobial properties. These plant parts were selected from different parts of the country . Test organisms were received from Punjab Biotechnology Incubator, Mohali for evaluating antimicrobial activity of different plant extracts.

Preparation of herbal extract through solvent extraction

Seven gram each of the dry plant powder was located in the thimble of Soxlet apparatus. It was fitted with appropriate size round bottom flask with 250 ml absolute ethanol for *Eugenia caryophyllata* (Clove) and *Berbaris aristata* (Daru haldi). The upper part of the flask was fitted with a condenser. Constant heat was provided by heater for recycling the solvent. After complete extraction, the extract in the round flask was transferred into clean and pre weighed universal tubes. These tubes containing the extracts were weighed and the weights were recorded. Percentage yield of extracts was calculated by dividing the initial weight of the raw material by the final weight of the extract in the following manner.

$$\text{Percentage yieald of extract} = \frac{\text{initial weight of raw material}}{\text{final weight of extract}}$$

Phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out for extracts using the standard procedures. For each procedure, details of these have been furnished below.

Test for alkaloids

The presence of alkaloids in the plant extract was tested using Mayer's reagent and Dragendroff's reagent. For this two milliliter of the plant extract was treated with lml of Mayer's reagent. Dull white precipitates indicated the presence of alkaloids. Similarly, two milliliter of the extract was treated with lml of Dragendroff's reagent.Formation of orange or orange red precipitates indicated the presence of alkaloids.

Test for flavonoids

A piece of magnesium ribbon was added to 4mg/ml of each extract. This was followed by drop by drop addition of concentrated hydrochloric acid (HCl). Crimson to magenta color indicated the presence of flavonoids.

Test for saponins

Half a gram (0.5 g) of each extract was placed in a test tube and then 0.5 ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15 mins indicated the presence of saponins.

Test for tannins

Solutions of the extracts were made with distilled

water and addition of 3 drops of 5% ferric chloride solution. A green-black or blue-black color indicated the presence of tannins.

Test for steroids

Salkowski's tests were used to see the presence of steroids in plant extracts. The extract was dissolved in 1 ml of chloroform and equal volume of concentrated sulfuric acid poured by sides of the test tube. The upper layer turning red, the sulfuric acid layer becoming yellow with green fluorescence, represented the steroids and sterol compound in the extract.

Antibacterial activity assay

The antibacterial activity of different plant extracts was determined by the Disc Diffusion Method [3].

Preparation of disc

Sterilized discs (6mm in diameter) 20—25 were soaked in different concentrations 3,5, 7 (gpl) of each plant extract for 24 h at the room temperature. Next day discs were dried at room temperature under sterile conditions.

Preparation of culture broth

The cultures used for the broth wee-Escherichia Coli-ve MTCC 443 and *Staphylococcus Aureus* +ve MTCC 737. Pure culture of *E. coli* and *S. aureus* was inoculated in the nutrient broth and incubated at 37°C for overnight. The turbidity of the above culture was adjusted equivalent to 0.5Mc Farland std. by taking absorbance at 430nm.

Preparation of plates

Tryptone Soya Agar (TSA) plates were prepared and incubated at 37°C for 24 prior to test for sterility check. The plates of *E. coli* and *S. aureus* were prepared using sterile swab in which the sterile swab was dipped in the culture broth (*E.coli* and *S. aureus*), removing excess liquid from the swab and mark the streak three times on TSA plate.

Table 1. Name of plants and their parts used in the experiment.

No.	Scientific name	(Common name)	Plant part used	Family
1	*Eugenia caryophyllata*	clove	Bud	Myrtaceae
2	*Berbaris aristata*	Daru haldi	Bark	Berberidaceae

Placing of disc

Prepared discs were placed on the prepared plates of *E. coli* and *S. aureus*. In each culture plate three discs were placed at 60° to each other. For each dilution, plates were prepared in duplicate. The plates were incubated at refrigerated temperature (4°C) for 4—5h. The plates were shifted to the incubator and incubated at 37°C for 18—30 h. After incubation period the clear zone was measured in mm using a scale. This clear zone represented the zone of inhibition.

Results and Discussion

The interest in medicinal natural plants has been shown all over the world for safe and effective constituents of plant products and in particularly the active principles of medicinal plants.These herbal substances are also renewable sources. Due to the relatively lower incidence of adverse reactions of herbal agents in comparison with synthetic agents, herbal agents can be exploited as an attractive ecofriendly alternative for medicinal and textile applications [4]. In the present study antimicrobial activity of four plant extracts (Table 1) of *Eugenia caryophyllata* and *Berbaris aristata* has been tested. These plants are being known traditionally for their antifungal, antimicrobial, antifungal, antiveinflammatory, antiprotozoal properties.

The antibacterial activity has been attributed to

Table 2. Phytochemical constituents of the plant extracts.

No.	Plant extracts	Alkaloids	Flavonoids	Tannins	saponins	Steroids
1	*Eugenia caryophyllata* (Clove)	+ve	+ve	+ve	+ve	+ve
2	*Berbaris aristata* (Daru haldi)	+ve	+ve	+ve	+ve	+ve

Table 3. Average zone of inhibition (in mm) of the different extracts against test bacteria.

No.	Name of the plant extract	Zone of inhibition	
		Escherichia coli	*Staphylococcus aureus*
1	*Eugenia caryophyllata* (Clove)	12	14
2	*Berbaris aristata* (Daru haldi)	0	12

Table 4. Zone of inhibition and interpretation

No	Zone of inhibition inmm	Interpretation
1	<10	Inactive
2	10–13	Partially active
3	14–19	Active
4	>19	Very active

the presence of some active constituents in the extracts. A qualitative phytochemical screening results presented in Table 2 showed that the plant extracts contain various active compounds including alkaloids, flavonoids, tannins, saponins, and steroids.

In particular, the presence of alkaloids and flavonoids were noted for both the extracts. Flavonoids have been known to exhibit biological activities such as antimicrobials, photo receptors, feeding repellants but most studies focused on the flavonoids' ability as antioxidant [5]. In this study, tannins were also observed to be present in all the plant extracts. This group of active constituents has shown antimicrobial, antidiarrheal and anthelmintic properties [6].

The chemical constituents of plants vary depending on the species, variety and part of the plant, with conditions of growth, and with the age of the plant. The phytochemistry also varies according to the geographical regions, season and time of collection and different climatic conditions [7]. The presence of active constituents, which are not necessarily produced under different environmental conditions, is an expression of the individuality of species [8].

Each class of metabolites has thousands of different compounds that give different phytochemical properties of the plant species [9]. Due to these reasons, plant species found in one place may have different compounds or seceondary metabolites compared with found in other area. However, according to our investigation, ethanolic extracts of *Eugenia caryophyllata* (Clove) were found to contain alkaloids, flavonoids, tannins and steroids. The antagonistic results of these findings may be attributed to different geographical locations and climatic conditions for the growth of the plant. In this study, *Berbaris aristata* extract were found to certain alkaloids, flavanoids tannins and saponins. However, alkaloids were present in all tested extracts.

The results of antimicrobial activity of the herbal plant extracts are presented in Table 3. The gram positive bacteria chosen for the study was *Staphylococcus aureus* and gram negative bacteria was *Escherichia coli*. Among two natural plants the most prominent results were the extract of *Eugenia caryophyllata* (Clove). As shown in Table 2, zone of inhibition of 12 mm and 14 mm resulted with the use of *Eugenia caryophyllata* extract against Escherichia coli and *Staphylococcus aureus*, respectively. It was considered as partially active against and active against. The extract of *Berbaris aristata* (Daru haldi) showed no resistance against *Escherichia coli*. But, the zone of inhibition of 12 mm was found with the use of *Berbaris aristata* extract and it was partially active against *Staphylococcus aureus*. The interpretation of the antibacterial results was based on the standard set by Quinto et al. [10] , which has been shown in Table 4.

Generally, the activity of plant extracts against all the test organisms can be attributed to its secondary metabolite contents. In this study, *Eugenia caryophyllata* (Clove) and *Berbaris aristata* (Daru haldi) contain alkaloids, flavonoids and tannis. Flavonoids are phenolic group of metabolites that are known to be synthesized by plants in response to microbial infection and so are found to be effective antimicrobial agent against different microorganisms. This is due to their ability to complex with bacterial cell walls.

Conclusion

Based on the results obtained, the plants screened for phytochemical constituents and tested for antimicrobial activity seemed to have the potential to act as a source of useful natural medicines and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Finally, the products obtained from these natural plants in any field will be eco-friendly having economic, social and environmental benefits.

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