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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF *EUGENIA CARYOPHYLLATA* (CLOVE) AND *PLUMBAGO ZEYLANICA* (CHITRAK) MEDICINAL PLANTS

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ABSTRACT

The antimicrobial activity of extracts was screened against two human pathogens: Escherichia coli and Staphylococcus aureus employing disc diffusion method. Eugenia caryophyllata (Clove) was found to be the more effective against Escherichia coli and Staphylococcus aureus. The largest zone of inhibition (14 mm) was obtained with Staphylococcus aureus and (12mm) with Escherichia coli. Plumbago zeylanica (Chitrak) were 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, flavonoids, tannins and steroids. Hence, Eugenia caryophyllata (Clove) was demonstrated to exihibit more antimicrobial activity as compared to Plumbago zeylanica (Chitrak).

Keywords: Antimicrobial, phytochemical, alkaloids, flavonoids, tannins.

I. INTRODUCTION

In the present day world most of us are very conscious about our hygiene and cleanliness. Clothing and textile materials are not only the carriers of microorganisms such as pathogenic bacteria, odour generating bacteria and mould fungi, but also good media for the growth of the microorganisms[1]. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 per cent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care. 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 semi synthesis [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 components that are medically active. These non-nutrient plant chemical compounds or bioactive components like alkaloids, tannins, flavo1noids and phenolic compounds, are often referred to as phytochemicals or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pests.

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Antimicrobial textiles with improved functionality 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.

II. METHODOLOGY

Collection of sample

The medicinal plant parts used for the experiment were bud of Eugenia caryophyllata (Clove) and root of Plumbago zeylanica (Chitrak). 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 chloroform for Plumbago zeylanica (Chitrak) . 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 [5]. For this two milliliter of the plant extract was treated with 1ml of Mayer's reagent. Dull white precipitates indicated the presence of alkaloids. Similarly, two milliliter of the extract was treated with 1ml of Dragendroff's reagent. Formation of orange or orange red precipitates indicated the presence of alkaloids.

Test for flavonoids

This was carried out according to the method described by Sofowora [6]. 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 colour indicated the presence of flavonoids.

Test for saponins

This was carried out according to the method of Brain and Turner [7]. Half a gram (0.5g) of each extract was placed in a test tube and then 0.5ml of distilled water was added. The tube was then shaken vigorously. A persistent froth that lasted for at least 15mins indicated the presence of saponins.

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Test for tannins

Method described by Ciulci [8] was used to study the presence of 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 colour indicated the presence of tannins.

Test for steroids

Salkowski's tests were used to see the presence of steroids in plant extracts [5]. The extract was dissolved in 1 ml of chloroform and equal volume of concentrated sulphuric acid poured by sides of the test tube. The upper layer turning red, the sulphuric 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 [9].

Preparation of Disc: Details of these are as under:

Sterilized discs (6mm in diameter) 20-25 were soaked in different concentrations 3,5,7 (gpl) of each plant extract for 24 hrs 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 were- Escherichia Coli -ve MTCC 443 and Staphylococcus Aureus +ve MTCC 737. Pure culture of E.coli & S.aureus was inoculated in the nutrient broth and incubated at 37ºC 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 24hrs prior to test for sterility check. The plates of E.coli & S. aureus were prepared using sterile swab in which the sterile swab was dipped in the culture broth (E.coli & S. aureus), removing excess liquid from the swab and mark the streak three times on TSA plate.

Placing of disc:

Prepared Discs were placed on the prepared plates of E.coli & 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 (4ºC) for 4-5hrs. The plates were shifted to the incubator and incubated at 37°C for 18-30 hrs. After incubation period the clear zone was measured in mm using a scale. This clear zone represented the zone of inhibition.

III. 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 eco-friendly alternative for medicinal and textile applications [10]. In the present study antimicrobial activity of *Eugenia caryophyllata* and *Plumbago zeylanica* plant extracts (Table 1) has been tested. These plants are being known traditionally for their antifungal, antimicrobial, antifungal, anti-veinflammatory, antiprotozoal properties.

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Table 1. Plants and their parts used in the experiment

No	Scientific name (common name)	Plant part used	Family	Properties
1	*Eugenia caryophyllata* (Clove)	Bud	*Myrtaceae*	Treats cold, dental abcesses, gum diease, earache and arthiritis pain, anti fungal, anticonvulsant, anticarcinogenic and antimutagenic activities.
2	*Plumbago zeylanica* (Chitrak)	Root	*Plumbaginaceae*	Used to treat fever or malaria. Antiplasmodial, antimicrobial, anti-veinflammatory, antiperglycemic, hypolipidaemic and antiatherosclerotic.

The antibacterial activity has been attributed to 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 flavonoids was noted in both samples. 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 [11]. In this study, tannins were also observed to be present in all *Eugenia caryophyllata* (Clove) plant extract. This group of active constituents has shown antimicrobial, antidiarrheal and anthelmintic properties [12].

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 phyto-chemistry also varies according to the geographical regions, season and time of collection and different climatic conditions [13]. The presence of active constituents, which are not necessarily produced under different environmental conditions, is an expression of the individuality of species [14].

Each class of metabolites has thousands of different compounds that give different phytochemical properties of the plant species [15]. Due to these reasons, plant species found in one place may have different compounds or secondary metabolites compared with found in other area. For example, in the previous study, alkaloids, flavonoids and steroids were absent in the bud extract of *Eugenia caryophyllata* (Clove) [16]. 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.

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	*Plumbago zeylanica*	+ve	+ve	-ve	+ve	+ve

(Chitrak)

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	*Plumbago zeylanica* (Chitrak)	0	11

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*. The extract of *Eugenia caryophyllata* (Clove) was considered as partially active against *Escherichia Coli* and *active* against *Staphylococcus Aureus.* The extract of Plumbago zeylanica (Chitrak) was inactive against *Escherichia .coli* and partially active against *Staphylococcus Aureus.* The interpretation of the antibacterial results was based on the standard set by Quinto [17], which has been shown in Table 4.

Table 4. Zone of inhibition and interpretation

Zone of inhibition, in mm	Interpretation
<10	Inactive
10-13	Partially active
14-19	Active
>19	Very active

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) contain alkaloids, flavinoids and tannins. 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. Tannins, also a phenolic compound, have the ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins while alkaloids can inhibit growth by intercalating with DNA[18].

IV. 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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