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ORIGINAL RESEARCH ARTICLE

Phytochemical Screening of *Coleus aromaticus* and *Leucas aspera* and Their Antibacterial Activity against Enteric Pathogens

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ABSTRACT

In the present study, two important medicinal plants *Coleus aromaticus* and *Leucas aspera* were assessed for phytochemical components and antimicrobial activity. The organic solvents *viz.*, methanol and ethanol were used for the extraction of plant material. The extracts were screened for the presence of phytochemical compounds and also tested antibacterial activity against enteric pathogens such as *Shigella* sp., *Salmonella typhi and Escherichia coli.* The results revealed that the two plant extracts contained tannins, alkaloids and glycosides. The alcoholic leaf extracts (methanol & ethanol) of both of the plants leaf exhibited antibacterial activity against enteric pathogens such as *Shigella* sp., *Salmonella typhi and Escherichia coli.* The aqueous crude extracts of plants leaf did not show any antibacterial activity against the tested enteric pathogens. Hence, concluded that plant leaf extracts showed antibacterial properties due to the presence some phytochemical compounds.

Key words: Phytochemical Screening, antibacterial activity, medicinal plants, solvents.

INTRODUCTION

The value of medicinal plants to the mankind is very well proven. India harbors about 15 percent (3000 – 3500) medicinal plants, out of 20,000 medicinal plants of the world. About 90 percent of these are found growing wild in different climatic regions of the country [1]. It is estimated that 70 to 80% of the people worldwide rely chiefly on traditional health care system and largely on herbal medicines [2-5]. Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. They have been used as remedies and for health care preparations [6]. The tribal and rural people of various parts of India are highly depending on medicinal plant therapy for meeting their health care needs [7-11]. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils [12].

Coleus aromaticus (Benth) is a commonly available medicinal herb in India. The plant chosen for study *Coleus aromaticus* (Benth), belonging to the Family: Lamiaceae is grown as a household herb in Tamilnadu (vernacular Tamil name: ommam or ommavalli).This herb is native to East Indies and is widely cultivated in Africa and almost all tropical countries. It is also popularly known as “Indian Oregano”. It grows to a maximum of 1.5 to 2 meters and has a thick green stem. The leaves of the plant are thick, succulent and juicy. The plant emanates mild, pleasant odour which increases when cut or crushed [13]. The leaves of this plant are traditionally used for the treatment of severe bronchitis, asthma, diarrhea, epilepsy, renal and vesicle calculi and feve [14]. *C.aromaticus* has been reported to exhibit antilithiotic [15], chemopreventive, [16] antiepileptic [17] and antioxidant properties [18].

Leucas aspera (LA), belonging to the family Labiatae, is commonly called as 'chota halkusa'. It grows as a weed on wastelands and roadsides all over India [19]. *Leucas aspera* commonly known as ‘thumbai’ distributed throughout India from the Himalayas down to Ceylon [20]. The plant is used as an insecticide and in traditional medicine for coughs, colds, painful swellings and chronic skin eruptions [19]. Traditionally, the decoction of the whole plant is taken orally for analgesic-

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antipyretic, antirheumatic, anti-inflammatory and antibacterial treatment *etc.* and its paste as an ointment is applied topically to inflamed areas [21]. The aim of this present study is to identify the phytochemical components of *Coleus aromaticus* and *Leucas aspera* and to determine the antimicrobial effects of the plant leaf extracts on *E.coli, Salmonella typhi and Shigella* sp.

Materials and methods

Collection of plant material

Fresh leaves of authenticated *Coleus aromaticus* and *Leucas aspera* free from disease were collected from Cholayil Medicinal plant Conservation Park, Velagapuram, Chennai. The leaves were washed thoroughly 2-3 times with running water and once with distilled water. The leaf parts of both of the plants were blotted and air-dried under shade for seven days. The leaves of *Coleus aromaticus* and *Leucas aspera* were made into powder with the help of mortar and pestle [22].

Preparation of the leaf extracts

The leaf extracts were prepared by the organic solvents *viz.*, ethanol, methanol and distilled water. Ten grams of ground dry leaf samples of the two plants were soaked in 250 ml of 95% ethanol contained in two separate 500 ml capacity conical flasks. The flasks were plugged with cotton wool, wrapped in aluminium foil, shaken vigorously and allowed to stand in the refrigerator for 24 hours.

The crude leaf extracts were obtained and stored in refrigerator in reagent bottles [23]. Simultaneously each extract was tested for growth/contamination by plating them on nutrient agar at 37ºC for 24 hours. No growth was observed visually and the extract was subsequently used to assay for antimicrobial activity using the agar well diffusion method.

Phytochemical screening of Extracts

The standard methods by [24] were used to test for the presence of Phytochemical in the test samples.

Determination of tannins

Extracts of each plant sample (0.5 g) was separately stirred with 10 ml of distilled water and then filtered. To the filtrate was added two drops of 5% Iron (III) Chloride ($FeCl_3$) reagent. Blue – black or blue – green coloration or precipitate was taken as an indication of the presence of tannins.

Determination of alkaloids

Extracts of each plant sample (0.5 g) was separately stirred with 1% hydrochloric acid (HCl) on a steam bath. The solution obtained was filtered and then 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extracts.

Determination of glycosides

Coarsely powered plant material (1 g) was introduced into two different beakers. To one of the beakers was added sulphuric acid (5 ml) while water (5 ml) was added to the other beaker. The two beakers were heated for 3 minutes and the contents were filtered and then poured into labelled test tubes. The filtrates were made alkaline with sodium hydroxide (0.5 ml) and allowed to stand for three minutes. The presence of reddish brown precipitate in the filtrate was taken as positive for glycosides.

Microorganisms used

Clinical isolates of enteric pathogens *viz., E. coli, Salmonella typhi and Shigella* sp. were obtained from Sri Narayani Hospital & Research center. The enteric pathogens were cultured in nutrient broth for 24 hours at 37 °C and the fresh inoculums were used for the test against the crude leaf extracts of plant samples.

Agar well diffusion method

The modified antibacterial test was performed using the agar well diffusion method [25]. The microorganisms were inoculated on Mueller Hinton agar (MHA) and spread uniformly using a sterile spreader. Wells of 5 mm in diameter were made on MHA using a sterile well puncher. The cut agar blocks were carefully removed by the use of sterilized forceps. The crude plant leaf extracts were added to the well and controls were maintained without adding crude leaf extracts. The plates were allowed to stand for one hour at room temperature for diffusion of the substances before the growth of the microorganism commenced. The plates were incubated at 37ºC for 24 hours and then the zone of inhibition was recorded.

RESULTS

The present study carried out on the plant samples revealed the presence of medicinally active constituents. The Phytochemical analysis of the plants assayed in this study revealed that *Coleus aromaticus* and *Leucas aspera* contained tannins, alkaloids and Glycosides (**Table 1**).

The tested leaf crude extracts of plant shows potential antibacterial activity against the enteric pathogens. Methanol leaf crude extracts of *Coleus*

aromaticus showed significant activity against *E.coli, Salmonella typhi and Shigella* sp. by agar well diffusion method.

The highest antibacterial activity of 15 mm againsty *Shigella* sp.and followed by 14 mm against *Salmonella typhi and least recorded in E.coli* measured around 13 mm. Ethanol leaf extract of *Coleus aromaticus* showed similar activity of 11 mm against all the tested pathogens. No activity was recorded for aqueous extract (**Table 2**).

Methanol crude extract of *Leucas aspera* showed antimicrobial activity of 12 mm against *E. coli* and *Salmonella typhi*. Highest antimicrobial activity of 13 mm was recorded against *Shigella* sp. Ethanol crude extract of *Leucas aspera* showed highest antibacterial activity of 15 mm against *Shigella* sp. followed by *Salmonella typhi* (14 mm) and *E. coli* (11 mm). Aqueous crude extract of *Leucas aspera* exhibited the antibacterial activity of 11 mm against *Shigella* sp (**Table 3**).

Table 1: Phytochemical analysis of crude extracts of plant leaves

S.No	Chemical Constituents	*Coleus aromaticus*	*Leucas aspera*
1	Tannins	+	++
2	Alkaloids	±	++
3	Glycosides	+	++

Note: + = Positive; ++ = Strongly positive; ± = Trace

Table 2: Antimicrobial activity of *Coleus aromaticus* leaf crude extracts against enteric pathogens

Test organisms	Ethanol extract	Methanol extract	Aqueous extract
	Zone of Inhibition (mm)		
Shigella sp.	11	15	-
Salmonella typhi	11	14	-
Escherichia coli	11	13	-

Note: - = no activity

Table 3: Antimicrobial activity of *Leucas aspera* against enteric pathogens

Test organisms	Ethanol extract	Methanol extract	Aqueous extract
	Zone of Inhibition (mm)		
Shigella sp.	15	13	11
Salmonella typhi	14	12	-
Escherichia coli	11	12	-

Note: "-" sign indicates no activity

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents [26]. The Phytochemical screening and quantitative estimation of the crude yields of chemical constituents of the plants showed that the leaves and stems were rich in alkaloids, flavonoids, tannins and saponins. They were known to show medicinal activity as well as exhibiting physiological activity [27]. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them [28]. Classes of alkaloids are among the major powerful poisons known [29]. Apart from being poisonous, some alkaloids have also been proved to be useful in correcting renal disorders [30].

The alkaloids of *Coleus aromaticus* and *Leucas aspera* may be a poison that can be tried on lower or higher organism. Glycosides are also found to posses antimicrobial activity. The secondary metabolites identified in the plant materials used in this study could be responsible for antimicrobial activity exhibited by these plants. In the present study, the methanol crude extract of *Coleus aromaticus* leaf showed the highest antimicrobial activity against *Shigella* sp., *Salmonella typhi and E.coli.* For instance, methanol crude extracts of *A.ferore and W.somnifera* were exhibited inhibitory activity against all the strains of *Neisseria gonorrhoea* [31]. The ethanol crude extract of *Leucas aspera* leaf was exhibited the highest antimicrobial activity against *Shigella* sp., *Salmonella typhi and E.coli.*

In the present study, the aqueous crude extract of both plants leaf did not have antimicrobial activity against the tested pathogen (except aqueous extract of *L.aspera* against *Shigella* sp.). On the contrary [32] observed the aqueous extract of *Leucas aspera* along with the eight plants against eleven human pathogenic bacteria showed least antibacterial activity. The plants studied here can be used as a potential source of useful drugs for the treatments of the various enteric pathogens.

CONCLUSION

The alcoholic crude extracts of *Coleus aromaticus* and *Leucas aspera* leaf were exhibited antibacterial activity against *Shigella* sp., *Salmonella typhi and E.coli.* Possible antimicrobial substances contained in the extracts included tannins, alkaloids and glycosides. It therefore suggests that constituents of the plant extracts could serve as a source of drugs useful in the chemotherapy of enteric diseases.

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