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Phytochemical Analysis and Antimocrobial Activity of *Heliotropium indicum* L and *Coldenia procumbens* L.

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Ethanolic extracts of leaves and roots of *Heliotropium indicum* and *Coldenia procumbens* included in Boraginaeceae family were screened for phytochemical constituents and antimicrobial activities towards five pathogens i.e. bacteria and three fungi. Among the four extracts, leaf extract of *H.indicum* showed the highest antimicrobial activity when compared with other extracts. Phytochemical analysis of all the extracts revealed that the antimicrobial activity of the plant material is due to the presence of phenolic compounds.

Key words: *Heliotropium indicum, Coldenia procumbens,* Boraginaeceae, Antimicrobial activity, Phytochemicals.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Over 50% of all modern clinical drugs are of natural product origin[1] and natural products play an important role in drug development programs in the pharmaceutical industry[2]. Various medicinal plants have been used for years in daily life to treat disease all over the world. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[3].

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large number of researchers in different parts of the world[4]. Much work has been

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done on ethanomedical plants in India[5]. Interest in a large number of traditional natural products has been increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents[6]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[7].

Heliotropium indicum L. and *Coldenia procumbens* L belongs to the family *Boraginaceae. H. indicum* grows up to 80cm height with a pleasant aroma. Its stems and leaves are covered with a fine hairy layer, and its flowers are small and grow in clusters, which curve in on themselves at the tips. Many medicinal uses have been recorded for the plant, it is reported to possess emollient, vulnerary and diuretic properties. It is used as local application for ulcers, sores, wounds, gum boils, skin affections, stings of insects and rheumatism; it is also used in poultices[8,9]. In developing countries, infectious diseases remain the main cause of the high mortality rates recorded. In modern medical practice, the alarming worldwide incidence of antibiotic resistant causes an increasing need for new compounds. Therefore, the aim of present work is to investigate the potential phytochemical constituents and antimicrobial activity of *H. indicum* L and *C. procumbens* L.

MATERIAL AND METHODS

Collection of plants

H. indicum L and *C. procumbens* L. belongs to the family Boraginaeceae was collected from Thanjavur District, Tamilnadu State, India and identified by the special key given Gamble flora of the presidency of Madras[10]. Voucher sample was prepared and deposited in the Department of Botany and Microbiology, A.V.V.M Sri Pushpam College (Autononomous), Poondi.

Preparation of leaf and root powder

The leaves and roots of *H. indicum* and *C. procumbens* were washed with sterile distilled water. After, the leaves were shade dried and powdered by using Pestle and Mortar.

Preparation of extracts

25g of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 18 hrs[11]. All the extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottle until further use. All the extracts were subjected to phytochemical analysis and antimicribial activity assay.

Phytochemical analysis

A small portion of the dry extract was used for phytochemical screening test[12, 13]. Dragendorffs reagents were used to test for alkaloids, ferric chloride for tannins, while Bebedict's solution was used to test for saponins.

Test organisms

The following organisms were employed for this study as test organisms:

Bacteria

Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa and *Klebsiella pneumonia*

Fungi

Aspergillus Niger,Trichoderma viride and *Candida albicans*

Pure cultures of these organisms were obtained from Sea Horse Hospital, Trichy and A.V.V.M Sri Pushpam College culture collection center, Poondi. Then they were sub cultured and maintained in a laboratory for further use.

Antimicrobial activity[14]

Antimicrobial activity assay was conducted against *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes Pseudomonas aerouginosa, Klebsiella pnemoniae* and three fungus, *Aspergillus niger, Trichoderma viride* and *Candida albicans*. These microbial cultures were obtained from culture collection facility of A.V.V.M. Sri Pushpam College Poondi. Sterile Nutrient Agar (NA) medium (Peptone 5g; Beef extract 3g; NaCl 2g and Agar 15g/litre; pH 7) and Potato Dextrose Agar (PDA) medium (200g Potato extract, Dextrose 20g, Agar 16g/ litre; pH 6) were used as basal media for growing these pathogenic bacteria and fungus respectively. Inoculums of the pathogen for the assay were prepared in liquid media of the respective composition. One ml of the broth inoculum was mixed with medium poured into the Petri plates and allowed for solidification. After solidification 6mm diameter duplicate well was made with the help of a sterile cork borer in the medium. In each

well 100µl of the filtrate was poured. All the plates were incubated at room temperature and the zone of inhibition was recorded. For bacteria, the plates were incubated for 24 hours and fungi 48 hours. Solvents used for extraction served as control.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical analysis of all the extracts revealed that the presence of alkaloids, carbohydrates and glycosides, phytosterols, fixed oils and fats, phenolic compounds and tannins, flavonoids, proteins and aminoacids and absence of saponins, gums and mucilage and volatile oils in ethanolic extracts of *H. indicum* and *C. procumbens* (Table-1). Further phytochemical analysis of ethanolic extract of both plants[15] revealed that the antimicrobial activity is due to the presence of phenolic compounds.

The phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis[16]. This activity was exhibited against test organisms with the two plant extracts.

Table 1. Phytochemical analysis of *H. indicum* and *C. procumbens*

Test For	*Heliotropium indicum* L		*Coldenia procumbens* L	
	Leaf	Root	Leaf	Root
Alkaloids	+	+	-	-
Carbohydrates and Glycosides	+	+	+	+
Phytosterols	+	+	+	+
Fixed oils and Fats	+	+	+	+
Phenolic compounds	+	+	+	+
Compounds and Tannins				
Saponins	-	-	-	-
Flavonoids	+	+	+	+
Proteins and Amino acids	+	+	+	+
Gums and Mucilge	-	-	-	-
Volatile oils	-	-	-	-

+: Present -: Absent

Table 2. Antimicrobial efficacy of *H. indicum* and *C. procumbens*

S. No.	Pathogens	*Heliotropium indicum* L Zone of inhibition in mm		*Coldenia procumbens* L Zone of inhibition in mm	
		Leaf	Root	Leaf	Root
	Bacterial species				
1	*Staphylococcus aureus*	22	5	9	3
2	*Bacillus subtilis*	10	8	5	4
3	*Streptococcus pyogens*	14	9	8	4
4	*Pseudomonas aurogonosa*	11	8	7	5
5	*Klebsiella pnemonia*	15	6	6	4
	Fungal species				
6	*Aspergillus niger*	13	7	7	5
7	*Trichoderma viride*	9	5	3	1
8	*Candida albicans*	24	8	6	2

Antimicrobial assay

Ethanolic extracts were tested against bacteria and fungi. Among the extracts, the leaf extract of *H. indicum* were effective against bacteria and fungi. The other three extracts have less inhibitory effect been noted in bacteria and fungi (Table: 2). Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Medicinally, this is important for the treatment of inflamed or ulcerated tissues[17]. Tannins have important roles such as stable and potent antioxidants[18]. Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery[19], thus exhibiting antimicrobial activity. One of the largest group of chemical produced by plant are the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications.

H.indicum and *C. procumbens* are used for the treatment of inflammation, wound healing, antitumor and antianelgesic, hence different formulations could be prepared for clinical trials. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin.

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