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DETERMINATION OF THE THERAPEUTIC COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF *HELIOTROPIUM INDICUM* BY GC/MS

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Abstract

Ethanol extracts of *Heliotropium indicum* was used traditionally in India for the treatment of skin diseases. The present study was investigated for *in vitro* antimicrobial activity against human pathogens namely *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa, Klebsiella pnemonia, Aspergillus níger, Trichoderma viride* and *Candida albicans* using the well diffusion method. The results relevant that the leaf extract possessed the highest inhibitory activity against both bacteria (*Staphylococcus aureus* in 22 mm) and fungi (*Candida albicans* in 24 mm). Among the leaf extracts *H. indicum* possess the highest inhibitory activity then the root extracts. Phytochemical analysis of all the extracts revealed that the antimicrobial activity of the plant material is due to the presence of antimicrobial compounds. The ethanolic extract of the medicinal plant *H. indicum* have been investigated by GC-MS to identify compounds responsible for its therapeutical properties. Two kinds of extracts of plant were investigated. The tincture was prepared by mixing all parts of the plant (roots and leaves) with a 50% alcoholic solution for 30 days. These two extraction methods were compared for extraction of the active therapeutic compounds of *H. indicum*. In parallel, another study was performed to identify the distribution and the concentration of the active compounds in the roots and leaves of this plant. For this purpose we have prepared alcoholic extracts from each part of the plant and we have studied them separately.

Key Words: GC-MS analysis, Antimicrobial activity, *Heliotropium indicum*, phyto-chemical analysis.

Introduction

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 of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. 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 (Forombi, 2003). Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry (Boominathan and Ramamurthy, 2009).

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge in modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. The worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care.

Heliotropium indicum, a very toxic herb, member of the Boraginaeceae family, popularly named Indian heliotrope can be referenced under the symbol HEIN, is a rare medicinal plant which has a very long history of medicinal use, though it is little used by present-day herbalists. It is an aromatic tonic herb that stimulates the antiulcer, reduces inflammation, controls bacterial infections and promotes healing. The flowering herb, with or without the root, is anti-inflammatory (Ramamurthy *et al*., 2010), antiulcer, diaphoretic, emmenagogue, febrifuge, oxytonic and stimulant (Kugelman *et al*., 1976 and Srinivas *et al*., 2000). The plant contains a complex of acids so called "organic acids" which stimulate white blood cell activity and speeds the healing of wounds if it is used in correct concentration. Externally it is used in the treatment of slow-healing cuts, eczema, infected toe and fingernails etc., but internal consumption can cause damage to the kidneys and uterine bleeding.

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In the present work alcoholic extract of *Heliotropium indicum* L root and leaf extracts were investigated for potential antimicrobial activity. To identify and characterized the compounds of therapeutic value extracted from *H. indicum.* The analytical methods chosen are Gas Chromatography/Mass Spectrometry (GC/MS). The methods were applied to characterize the infusion prepared from this plant and to make a comparison between the alcoholic extract of the roots and leaves.

Materials and Methods

Heliotropium indicum L belongs to the family Boraginaeceae was collected from Thanjavur District, Tamil Nadu, India and identified by the special key given Cambell flora. The leaf and root of *H. indicum* were washed with sterile distilled water. After, the leaves were shade dried and powdered by using pestle and mortar. Twenty five gram of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5^{o}C in airtight bottle until further use. All the extracts were subjected to phytochemical analysis and antimicrobial activity assay.

Antimicrobial Assay

The following organisms were employed for this study as test organisms: Bacteria such as *Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa* and *Klebsiella pnemonia.* Fungi such as *Aspergillus níger, Trichoderma viride* and *Candida albicans*. The test microbial pathogen cultures were obtained from the stock cultures maintained in specific agar medium.

Antibacterial and antifungal activity of above mentioned extracts were tested using the agar well method described by Collins and Lyne, (1970). All the above-mentioned bacteria were inoculated into nutrient agar medium and fungi inoculated to potato dextrose agar medium. The wells of 6 mm diameter were punctured in the culture medium using sterile cork borer. Different extracts were administered to fullness in each well. Culture plates were incubated at 37^{o}C for 24 h in bacteria and incubated at 37^{o}C for 4 days in fungi. Bioactivity was determined by measuring diameter of inhibition zones in mm. Solvents used for extraction served as control.

GC-MS Analysis

The leaf and root of *H. indicum* washed with sterile distilled water, and they were shade dried and powdered by using Pestle and Mortar and for the alcoholic extracts (96% alcoholic solution) roots and leaves. The tincture was prepared by mixing all parts of the plant with a 50% alcoholic solution for 30 days. The infusion was also prepared by mixing parts of the plant with hot water for 20 min and the alcoholic extracts by mixing the fresh parts of the plant with a 96% alcoholic solution for 12 days.

The dry fractions (20g) were dissolved in 75ml of alcohol and than soaking for 24 hrs. After soaking, collect a filtrate and evaporate under liquid nitrogen. Then concentrate the filtrate for GC-MS analysis.

For the GC-MS analysis a 30m x 0.25mm I.D x 1.0 µm df fused Elite-1 (100% Dimethyl Poly Siloxane) column; GC Clarus 500 Perkin Elmer gas chromatograph with Mass detector- Turbo mass gold- Perkin Elmer, Software- Turbo mass 5.1. The samples (1µl) were introduced *via* an all – glass injector working in the split mode (10:1), with Helium as the carrier gas.

Oven temperature programme: 110 deg-2min hold, upto 280 deg at the rate of 5 deg /9min hold. Injector temperature: 250 deg C. GC time – 45 mins.

MS Programme: Inlet line temperature: 200^{o} C, Source temperature: 200^{o} C, Electron energy: 70eV, Mass scan: (m/z) 45-450. MS time – 46 mins.

The identification of components was accomplished using computer searches in NIST ver 2.1 library. The identification of the constituents was performed by computer library search, retention indices and visual interpretation of the mass spectra. Compounds were identified by comparing their mass spectrum to those of the database of the GC-MS (NIST 62.lib), literature (McLafferty and Stauffer 1989) and retention indices (Adams 2007).

Results and Discussion

Ethanolic extracts were tested against bacteria and fungi. Among the extracts, the leaf extract of *Heliotropium indicum* were effective against bacteria and fungi. The other root extracts have less inhibitory effect have been noted in bacteria and fungi (Table 1).

The antibacterial activity of crude extract is shown in Table 1. The extracts showed maximum activity against *Staphylococcus aureus, Streptococcus pyogens, Klebsiella pnemonia* and *Pseudomonas aurogonosa*. These data revealed that leaf extracts of *Heliotropium indicum* exhibited significant antimicrobial activity. In testing, inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavanoids (Tsuchiya *et al*., 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya *et al*., 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes (Goren, 1996) etc., are effective antimicrobial substances against a wide range of microorganisms.

The extracts showed maximum activity against *E. coli, Enterobacter aerogenes* and *Alcaligenes faecalis*. These data revealed that extracts of *R. tetraphylla* exhibited significant antibacterial activity (Suresh *et al*., 2008). 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 (Mota *et al.*, 1985). Tannins have important roles such as stable and potent antioxidants (Trease, and Evans, 1983). Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery, thus

exhibiting antimicrobial activity. One of the largest groups of chemical produced by plant is the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications (Raffauf, 1996).

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 (Boominathan and Ramamurthy, 2009). 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. Studies are in progress to further evaluate the mechanisms of action *H. indicum* extracts on some organisms associated with human diseases.

Use of GC/MS enabled identification of the most components in both samples of *Heliotropium indicum* were analyzed by antimicrobial compounds (Table 2). The compounds identified are listed in Tables. In these natural compounds has been a source of medicinal agents for antimicrobial, anti-inflammatory compounds and some essential fatty acids are analyzed in this plant.

The components of the infusion differ from those found in tincture except organic acids derivatives. The concentrations (in %MS) of these derivatives in infusion and tincture are a great deal closed; may be that is why in traditional medicine are used both types of extracts with success.

The differences between the compounds that we have found in the roots, steams and leaves of *Aristolochia clematitis* were studied by GC-FID. This study was performed on the alcoholic extracts of the three parts of the plant. From this study we have concluded that the compounds found in the root and steam are very similar. The aristolochic acid derivatives are present in both extracts, but in the leaves these derivatives are in very low concentration (Podea *et al*., 2001).

In the present study the difference between the compounds that we have found in the roots and leaves of *H. indicum* were studied by GC-MS. This study was performed on the alcoholic extracts of the two parts of the plant. From this study we have concluded that the compounds found in the root and leaves are very dissimilar. The organic acid derivatives are present in both extracts, but in the leaves these derivatives are in very high concentration.

The analytical methods used GC/MS is suitable for medicinal herb organic compounds determination. The sample preparation method is rapid and precise. There is a difference between the compounds extracted from herb by infusion and tincture but the important thing is that the organic acid and fatty acids derivatives are present in both of them. On the other side the study shows that their concentration is higher in the roots and steams. In the leave extracts organic acid derivatives and vitamin F (polyunsaturated fatty acids) are very higher amount present. In conclusion terpenic compounds, fatty acids, phytol, alkaloids and especially organic acid derivatives are responsible for the therapeutic activity of this plant.

Table 1. Antimicrobial efficacy of *Heliotropium indicum*

S. No	Organism	Zone of inhibition in mm	
		Leaf	Root
1	*Staphylococcus aureus*	22	5
2	*Bacillus subtilis*	12	8
3	*Streptococcus pyogens*	24	9
4	*Pseudomonas aurogonosa*	19	8
5	*Klebsiella pnemonia*	20	6
6	*Aspergillus niger*	13	7
7	*Trichoderma viride*	9	5
8	*Candida albicans*	14	8

Table 2. Antimicrobial Components Identified By GC-MS Study

S.No	Antimicrobial Components	Formula	*H. indicum*	
			Leaf	Root
1	Benzene acetaldehyde	C_8H_8O	+	-
2	5H-1-Pyrindine	C_8H_7N	+	+
3	2-Furan carboxaldehyde, 5-(Hydroxymethyl)-	$C_6H_6O_3$	+	+
4	Benzene acetic acid	$C_8H_8O_2$	+	-
5	Dodecanoic acid	$C_{12}H_{24}O_2$	+	+
6	Phenol, 3-Isopropoxy-5-Methyl-	$C_{10}H_{14}O_2$	+	+
7	3`-Acetyllycopsamine	$C_{17}H_{27}NO_6$	+	-
8	Squalene	$C_{30}H_{50}$	+	-
9	Octanoic acid, Ethyl ester	$C_{10}H_{20}O_2$	-	+
10	Benzaldehyde, 3-Hydroxy-4-Methoxy-	$C_8H_8O_3$	-	+
11	Benzaldehyde, 4-Hydroxy-3, 5-Dimethoxy-	$C_9H_{10}O_4$	-	+
12	4-((1E)-3-Hydroxy-1-propenyl)-2-Methoxy Phenol	$C_{10}H_{12}O_3$	-	+
13	Benzaldehyde, 4-Hydroxy-	$C_7H_6O_2$	-	-
14	Butanoic acid, 2-Methyl-	$C_5H_{10}O_2$	-	-
15	Nonanoic acid	$C_9H_{18}O_2$	-	-
16	Benzene acetic acid, 2,5-Dihydroxy-	$C_8H_8O_4$	-	-
17	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$	-	-
18	Phytol	$C_{20}H_{40}O$	+	-
19	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	-	-
20	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$	-	-

+**:** Present; -: Absence

Table 3. Fatty Acids Identified By GC-MS Study

S.No	Name of Fatty Acids	Formula	*H. indicum*	
			Leaf	Root
1	Dodecanoic acid	$C_{12}H_{24}O_2$	+	+
2	Tetradecanoic acid	$C_{14}H_{28}O_2$	+	+
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	+	+
4	Hexadecanoic acid, Ethyl Ester	$C_{18}H_{36}O_2$	+	+
5	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	+	+
6	9,12-Octadecadienoic acid, Ethyl Ester	$C_{20}H_{36}O_2$	+	-
7	9,12,15-Octadecatrienoic acid, Ethyl Ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	+	-
8	Octadecanoic acid, Ethyl Ester	$C_{20}H_{40}O_2$	+	-
9	8,11,14 – Eicosatrienoic acid, (Z, Z, Z) -	$C_{20}H_{34}O_2$	+	-
10	Docosanoic acid, Ethyl Ester	$C_{24}H_{48}O_2$	+	-
11	Hexanoic acid, Ethyl Ester	$C_8H_{16}O_2$	+	+
12	Octanoic acid, Ethyl Ester	$C_{10}H_{20}O_2$	-	+
13	Pentadecanoic acid	$C_{15}H_{30}O_2$	-	+
14	Octadecanoic acid	$C_{18}H_{36}O_2$	-	+
15	Nonanoic acid	$C_9H_{18}O_2$	-	-
16	Undecanoic acid	$C_{11}H_{22}O_2$	-	-

+: Present; -: Absence

Table 4. Antiinflammatory Components Identified BY GC-MS

S. No	Anti-inflammatory Components	Formula	*H. indicum*	
			Leaf	Root
1	Phytol	$C_{20}H_{40}O$	+	-
2	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	+	+
3	9,12-Octadecadienoic acid, Ethyl Ester	$C_{20}H_{36}O_2$	+	-
4	Squalene	$C_{30}H_{50}$	+	-
5	Methyl Salicylate	$C_8H_8O_3$	-	+
6	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$	-	-

+: Present; -: Absence

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