Preliminary phytochemical screening, Antibacterial potential and GC-MS analysis of two medicinal plant extracts

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Abstract: The presence study was aimed to catalyze the primary metabolites and their confirmation by using GC-MS analysis and antibacterial potential of leaf extract of two important medicinal plant viz., *Eucalyptus and Azadirachta indica*. The antibacterial potential of the methanol leaf extract of the studied species was tested against *Escherichia coli, Pseudomonas aeruginosa, Klebsiellap neumoniae, Streptococcus pyogens, Staphylococcus aureus* using by agar well diffusion method. The higher zone of inhibition (16mm) was observed against the bacterium *Pseudomonas aeruginosa* at 100µl concentration of methanol leaf extract. Preliminary phytochemical analysis of studied species shows that presence of phytochemical compounds like steroids, phenolic compounds and flavonoids. GC-MS analysis confirms the occurrence of 20 different compounds in the methanol leaf extract of the both studied species.

Keywords: Methanol extract of *Eucalyptus and Azadirachta indica*, phytochemical analysis, antibacterial activity, GC-MS analysis.

INTRODUCTION

India encouraged scientific investigations of Neem tree as the part of his program to revitalize Indian tradition and also increase commercial interest on Neem (Stix, 1992) and presently some authors believe that no other plant of tree in the world has been so extensively researched or used, in all possible capacities so far, In Africa extracts from Neem leaves have provided various medicinal preparations (Ekanem, 1971). The medicinal properties of the plant *Azadirachta indica* were studied by several workers. They were anti tumour effect (Udeinya *et al*., 2006), antiulcer effect (Fujiwara *et al.*, 1982), antidiabetic effect (Pillai and Santhakumar, 1984). *Eucalyptu*s is native to Australia, and the genus Eucalyptus contains about 600 species. Among these species, *Eucalyptus* is the most widely cultivated in subtropical and Mediterranean regions. Research data has demonstrated that the extracts exhibited various biological effects, such as antibacterial, antihyperglycemic (Gray *et al.*, 1998) and antioxidant (Lee *et al.*, 2001) activities. As eucalyptus is a fast-growing tree, and is a suitable ingredient for paper manufacture, there has been extensive overseas forest plantation of eucalyptus trees. Leaves are a byproduct of tree cutting, and the use of the excess leaves for biomass resources is considered to be an important research subject. The Aborigines (native Australians) have traditionally used eucalyptus leaves to heal wounds and fungal infections. Leaf extracts of *eucalyptus* have been approved as food additives and the extracts are also currently used in cosmetic formulations. Recently, attention has been focused on the medicinal properties of these extracts. The present study was aimed to evaluate the antibacterial potential of methanol leaf extract of *Eucalyptus and Azadirachta indica* against bacterial pathogens and phytochemical analysis of the leaf extract. The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs (Vasantharaj *et al*., 2013).

MATERIALS AND METHODS

Collection and Drying of plant materials

Mature leaves of *Eucalyptus and Azadirachta indica* were collected from different places of Coimbatore, Tamil Nadu. The leaves were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction (Mittal and Subbarao, 2003).

Preparation of plant extract

10g of powdered leaves were extracted successively with 100ml of methanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use (Chessb rough, 2000).

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Test microorganisms

Five pathogenic bacteria, *viz*., *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pyrogens* and *Pseudomonas aeruginosa* were used in the present study and were obtained from MTCC, Chandigarh. The cultures were sub-cultured and maintained on nutrient agar slants and stored at 4°C.

Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5ml of nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards.

Determination of antibacterial activity (Agar well diffusion)

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculums on the surface of the media. Wells (8mm in diameter) were punched in the agar. Methanol extracts with same concentrations of 100mg/ml were used. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

Test for terpenoid and steroid

4mg of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids (Chessbrough, 2000).

Test for flavonoid

4mg of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavonoid (Chessbrough, 2000).

Test for triterpenes

300mg of extract was mixed with 5ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

Test for phenolic compounds (Ferric chloride test)

300mg of extract was diluted in 5ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

Test for tannins

The 0.5ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution wad added. Blue colour was observed for gallic tannins and green black for catecholic tannins (Iyengar 1995).

Table 1: Phytochemical analysis of *Eucalyptus and Azadirachtaindica* methanol extract

S. No	Test	Result
1	Phenolic compounds	+
2	Tripenoid	-
3	Triterpenes	-
4	Tannins	-
5	Saponins	-
6	Steroids	+
7	Flavonoids	+

Test for saponins

2g of the powered sample was boiled in 20 ml of distilled water in a water bath. 10ml of the filterable was mixed with 5ml of distilled water shaken vigorously for a stable persistent broth. The following was mixed with 3 drops of Olive oil and shaken vigorously and then observed for the formation of emulsion.

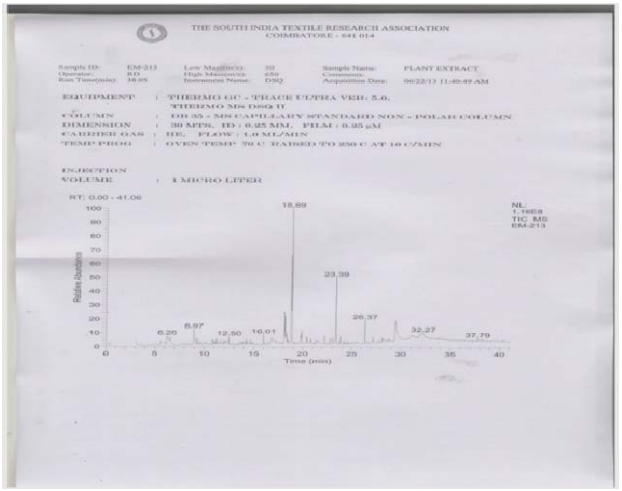

Fig. 1: GC-MS Chromatogram of methanol extract of *Eucalyptus and Azadirachtaindica*

GC-MS

The compounds were identified by using standard procedure and technique of GC-MS (Vasantharaj *et al.*, 2013)

RESULTS

The preliminary phytochemical analysis of methanol leaf extract of the studied species is given in table 1. The results of the phytochemical screening revealed the phytochemicals such as phenolic compounds, steroids and flavonoids are present in the methanol extract. The other

Table 2: The Compounds present, molecular weight and abundance (%) of methanol leaf extract of the study species by using GC-MS analysis

S. No.	Compound	Molecular weight	Molecular formula	Abundance %
1	Serine, acetate (ester)	147	C5H9NO4	0.94
2	l-Phellandrene	136	C10H16	2.45
3	Benzene, 1-methyl-4- (1-methylethyl)-	134	C10H14	2.88
4	ç-Terpinene	136	C10H16	0.80
5	Isopropenyl-3-methoxymethoxy-3-methyl-cyclohexene	196	C12H20O2 1	0.80
6	l-4-Terpineol	154	C10H18O	3.68
7	Linalyl propionate	210	C13H22O2	1.41
8	Terpineol	154	C10H18O	1.41
9	1,3-Oxazinane, 3-(2-bromo-2,2-dinitroethyl)	283	C6H10BrN3O5	0.78
10	6-Acetyl-á-d-mannose	222	C8H14O7	1.03
11	Isonicotinic acid-D1-à-D2	123	C6H2D3NO2	1.03
12	2,3,5-Trimethylanisole	150	C10H14O	1.42
13	Ciscaryophyllence	204	C15H24	1.04
14	á-Selinene (CAS)	204	C15H24	0.74
15	Elemol	222	C15H26O	1.19
16	Xanthosine (CAS)	284	C10H12N4O6	1.74
17	(+,-)-á-Himachalene	204	C15H24	19.14
18	á-Eudesmol (CAS)	222	C15H26O	29.71
19	Sabinene	136	C10H16	2.44
20	d-Mannose	180	C6H12O6	1.92
21	Quinic acid	192	C7H12O6	1.38
22	á-Eudesmol (CAS)	222	C15H26O	1.23
23	Hexadecanoic acid, methyl ester	570	C17H34O2	1.59
24	3-Hydroxycarbofuran	237	C12H15NO4	1.23
25	Rosifoliol	220	C15H24O	19.33

Table 3: Antibacterial activity of *Eucalyptus and Azadirachtaindica* methanol extract against bacterial pathogens

S. No.	Organism	Concentration of extract and zone of inhibition (mm)		
		50µl	75µl	100µl
1	*Escherichia coli*	7	9	13
2	*Pseudomonas aeruginosa*	8	10	14
3	*Klebsiella pneumonia*	10	11	12
4	*Streptococcuspyogens*	7	8	11
5	*Staphylococcus*	9	10	11

phytochemical compounds flavonoids, saponins, triterpenes and terpenoids are absent. Among the seven phytochemical constituents tested such as, alkaloids, glycosides, tripenoid, flavonoids, tannins, saponins and triterpenes, the three constituents such as, phenolic compounds, steroids and flavonoids are present in huge amount. The results indicated the facts that the disparity occurrence of phytochemical compounds in the tested plant extract may be due to extracting efficacy of solvents and solubility nature of the active constituents. Analysis of GC-MS spectrum was done at the South India Textile Research Association (SITRA), Coimbatore. The spectrum of the unknown component was compared with known component stored in SITRA library. The name molecular weight structure of the component of test material was ascertained. Twenty-five compounds were identified in methanol leaf extract of the studied species by GC-MS analysis. The active principle molecular weight, concentrations (%), molecular formula are presented in table 2 and fig. 1. The prevailing compounds are Eudesmol (CAS) (29.71%), Himachalene (19.14%) and Rosifoliol (19.33%). The microbial activity of the leaf extracts of the studied species was assayed *in vitro* by agar well diffusion method against the five bacterial species (table 3). The methanol extract of *Eucalyptus and Azadirachta indica* (100µl) showed maximum zone of inhibition (16mm) against the bacteria, *Pseudomonas aeruginosa* (table 3).

DISCUSSION

There are about 45,000 plant species in India with capacity to produce a large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity (Yiming *et al.,* 2004, Mohato and Chaudhary, 2005). The result of phytochemicals in the present investigation showed that the plant leaves contain components like, steroids, phenolic compounds flavonoids. GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The present study was aimed to evaluate the antibacterial potentiality of methanol extract of *Eucalyptus and Azadirachta indica* against bacterial pathogens and phytochemical analysis of *Eucalyptus* and *Azadirachta indica*. This study reports the presence of different phytochemicals with biological activity that can be valuable therapeutic index (Jignuparekh *et al.,* 2007; Senthilkumar and Reetha, 2009).

CONCLUSION

The antibacterial activity of *Eucalyptus* and *Azadirachta indica* extract showed that it is mainly due to the presence of phytochemical compounds such like phenolic compounds, steroids and flavonoids. The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results. Thus this plant could be utilized as an alternative source of useful anti-microbial drugs.

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