

Chemical Composition and Antimicrobial Activity of Essential Oil from *Eupatorium triplinerve* Vahl. Aerial Parts

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ABSTRACT

Eupatorium triplinerve Vahl belongs to the Asteraceae family, popularly known as Ayapana .It is a perennial shrub native to Amazon rainforests of South America. Its leaves are used through infusions, decoctions, baths, and tea. It is largely used in Brazilian folk medicine as sedative, febrifuge, stimulant, tonic and anti-inflammatory. The essential oil from aerial parts of *Eupatorium triplinerve* was analysis by gas chromatography mass spectroscopy (GC-MS). Thirty compounds representing 98.24 % of the oil were identified. The major compounds were 2-tert- butyl – 1, 4- methoxybenzene (74.3 %) and b – Selinene (8.6 %). The antibacterial activity of the essential oil was evaluated against ten bacteria (including Gram positive and Gram negative) and six phytopathogenic fungi. The oil exhibited moderated antibacterial and strong antifungal activity against all the test pathogens. The highest zone inhibition was recorded against *Salmella typhae* (21mm) followed by *Shigella sonnei* (18mm) at a concentration of 20µl/ disc on the other standard *Ampicillin* 20mg/ disc showed on average of 14-34mm diameter of zone inhibition against the test organism. The lowest antifungal activities were recorded against *Macrophomina phaseoline*, and *Botryodiplodia theobromae*. The highest *Alternaria alternate* (12000ppm).Further pharmacological and toxicology studies will be required to establish the therapeutic uses of this oil.

KEYWORD: *Eupatorium triplinerve*, Antimicrobial activity, Essential oil, GC-MS, 2-tert- butyl-1, 4-methoxybenzene.

INTRODUCTION

Eupatorium triplinerve Vahl. Commonly called Ayapana (Syn. *Ayapana triplinerve* and *Eupatorium ayapana*) is an ornamental erect perennial herb with aromatic leaves is under the family Asteraceae. Most of the people in rural and urban areas of the world were depended on the medicinal plants for the treatment of infectious diseases. The Ayurvedic and Unani systems of medicines are widely used by the people of Indian subcontinent. Among the different plant derivetives, secondary metabolites proved to be the most important group of compounds that showed wide range of antibacterial and antifungal activity (**Ahmed et al.2002, Rahman et al.**

1998). *Eupatorium triplinerve* (Compositae) is a slender herb with narrow lanceolate leaves and large number of pedicelled flower-heads at the top of the branch, cultivated in Chittagong and Chittagong Hill Tracts. Extract of the plant is used as antiseptic, and in the treatment of various ulcers and hemorrhages (Ghani 1998). This paper reports the result of antimicrobial activity of *E. triplinerve* on folk usages by traditional practitioners in Tribal areas of Bangladesh. Ayapana is a rich source of naturally occurring coumarin chemicals and is used in herbal medicine as anti-tumor remedy. It was reported that a coumarin chemical named hernarin (7-methoxycoumarin) was toxic to cancer cells including multi drug resistant cancer cells (Canales *et al.*, 2005; Yadava and Saini, 1980) and leukemic cells (Garg and Nigam, 1970). Garg and Nigam (1970) reported the presence of thymohydroquinone dimethylether, coumarin, borneol and bornyl acetate along with seven monoterpenoids in the leaf oil. Later, (Garg and Nakhare, 1993) analyzed the flower oil of *E. triplinerve* and found thymohydroquinone dimethylene (50.3%), followed by borneol (5.81%) and isoeugenol (4%) as the major constituent, along with other minor constituents. The essential oil of the flowers has yielded much better antimicrobial results than the plant itself. Ayapana flowers essential oil possess strong antifungal activity against 10 strains of fungi in vitro (Sharma and Singh, 1979). It also possesses antibacterial (against *staph*, *cholera*, *pneumonia* and *shigella*) as well as antiparasitic (*Ascaris*) and anthelminthic (*Taenia*) actions (Garg and Nakhare, 1993). In an early animal study, the flower essential oil injected into mice was reported to have CNS depressant, analgesic and sedative effects (as well as an in vitro antibacterial effect) (Kokate and Verma, 1971). Ghani (2003) reported the presence of coumarin, ayapanin and ayapin having pronounced haemostatic properties. There is no report on the essential oil of *E. triplinerve* of local origin and its antimicrobial properties. So, the aim of the present study was to investigate the chemical composition and antimicrobial activity of the essential oil of *E. triplinerve* aerial parts growing in Bangladesh in order to find out new natural antimicrobial agents.

MATERIALS AND METHODS

Collection of Plant material The fresh leaves of the plant *Eupatorium triplinerve* Vahl were collected from State Forest Research Institute Vandal, Kanchipuram District, and TamilNadu. It was identified and authenticated in P.G Department of Plant Biology & Plant Biotechnology, Presidency College Chennai, and TamilNadu.

Extraction of essential oil: The experiment was conducted when the plants were healthy and well-grown. For extracting of essential oil aerial parts (5 Kg) were subjected to hydro distillation using a modified Clevenger apparatus (Clevenger, 1928) for 4 hours. The oil sample was stored at freeze in air-tight containers after drying them over anhydrous sodium sulfate for GC-MS analysis.

Test organisms: The essential oil obtained from *E. triplinerve* aerial parts was tested for its antibacterial activity against ten pathogenic bacteria, viz., *Bacillus subtilis*, *B. megaterium*, *Staphylococcus aureus*, *B. cereus*, *Escherichia coli*, *Pseudomonas sp.*, *Shigella dysenteriae*, *S. sonnei*, *Salmonella typhi*, and *Vibrio cholerae*, and six phytopathogenic fungi viz., *Alternaria alternata* (Fr.) Kedissler, *Botryodiplodia theobromaepat.*, *Colletotrichum corchori* Ikata (Yoshida), *Curvularia lunata* (Wakker) Boedijin, *Fusarium equiseti* (Corda) Sacc. and *Macrophomina phaseolina* (Maubl) Ashby.

Antibacterial activity: The in vitro antibacterial activity of the oil was determined by disc diffusion method (Bauer and Kirby, 1966). Nutrient agar medium was used for culture of bacteria. Each experiment was repeated thrice. All the results were compared with the standard antibacterial antibiotic Ampicillin (20 µg/disc).

Antifungal activity: The in vitro antifungal activity of the essential oil of *E. triplinerve* was determined by poisoned food technique (Grover and Moore, 1962). Potato dextrose agar medium was used for culture of fungi. Each experiment was repeated thrice. Linear mycelia growth of fungus was measured after 3-5 days of incubation. The percentage inhibition of radial

Determination of minimum inhibitory concentration (MIC) against bacteria and minimum bacterial concentration (MBC)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil against ten test bacterial isolates were determined by micro and macro dilution broth technique (Jones et al., 1985) using Muller Hinton medium. The MIC and MBC experiments were carried out using 100-25000 ppm concentrations of the essential oil.

Determination of minimum inhibitory concentration (MIC) against fungus and minimum fungicidal concentration (MFC)

The MIC and MFC of essential oil against six test fungi were determined by micro and macro-dilution broth technique (Jones et al., 1985) using Sabouraud medium. The MIC and MFC experiments were carried out using 100 to 12,000 ppm concentrations of the essential oil.

GC-MS analysis The essential oil from aerial parts of *E. triplinerve* was analyzed by GC-MS electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu) coupled to a GC-MS QP 5050A Mass Spectrometer (Shimadzu); fused silica capillary column (30m x 0.25mm; 0.25 mm film thickness), coated with DB-5 (J&W); column temperature 100 oC (2 min) to 250oC at the rate of 3oC/min; carrier gas, helium at constant pressure of 90Kpa. Acquisition parameters full scan; scan range 40-350 amu.

Identification of the constituents Constituent's identification was done by comparing the NIST library data of the peaks with those reported in literature. Percentage composition was computed from GC peak areas on DB-5 column without applying correction factors.

RESULTS AND DISCUSSION

Essential oil from the aerial parts of *E. triplinerve* was analyzed by GC-MS. The oil yield was 1.10%. Thirty compounds (Table 1) were identified by GC-MS analysis with 2-tert-butyl-1,4-methoxybenzene (74.27%) and b-selinene (8.59%) as major. Other notable compounds are b-pinene (1.51%), b-pinene (2.19%), a-phellandrene (2.2%), thymolmethyl ether (2.03%), b-selinene (1.28%) and 6-isopropenyl-4, 8adimethyl-1, 2, 3, 5, 6, 7, 8, 8a-octahydronaphthalene-2-ol (2.63%). Gupta et al. (2004) reported selina-4(15), 7(11)-dien-8-one (36.6%), b-caryophyllene (14.7%) and d-elemene (5.9%) as major constituents in the leaf oil of Indian *E. triplinerve* which are totally different from our sample. Results showed that the aerial parts oils of *E. triplinerve* was a complex mixture of numerous compounds; many of which were present in trace amounts. This confirms that the variations in the oil from local origin is not due to geographic divergence and ecological conditions, it may be due to different chemotype.

The results of the in vitro antibacterial activity of the essential oil (2, 5, 10, 15 and 20µl/disc) against the ten bacterial strains (including Gram-positive and Gram-negative) are summarized in (Table 2.). It was observed that the essential oil was moderately effective against the test bacterial strains even at the lowest concentration (2µl/ disc) of the essential oil tested. 10-25mm diameter of zone of inhibition was recorded in 20µl /disc concentration. The highest zone inhibition was recorded against *Salmella typhi* (21mm) followed by *Shigella sonnei* (18mm) at a concentration of 20µl/ disc (Table) on the other standard *Ampicillin* 20mg/ disc showed an average of 14- 34mm diameter of zone inhibition against the test organism.

The MIC value of the essential oil varied between 17000- 21000ppm against the test bacteria (Table 3). The oil showed the lowest MIC (17000ppm) against *Shigella typhae* followed by 18000ppm against *Shidella sonnei*, *Shigella dysenterae* and *Pseudomonas* and highest (21000ppm) against *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Vibro cholera* and *E.coli*. The oil exhibited the lowest MBC value (22000ppm) against *Shigella sonnei*, *Shigella dysenterae*, *Pseudomonas* and *Salmonella typhae*. The in vitro antifungal activity of essential oil against six phytopathogenic fungi was recorded and presented in (Table 4). The oil exhibited strong pathogen tested. In most of the cases, the essential oil exhibited better antifungal activity than the standard antibiotic, Nystain (Table 4). The MIC value of the essential oil was found of very between 6000-9000ppm against the test fungi (Table). The lowest MIC value (6000ppm) was recorded against *M .phaseoline* and *C. corchori*. MFC value of the essential oil were found to very between 8000- 12000ppm (Table 5). The oil exhibited the lowest MFC value (8000ppm) against *M phaseoline* and *B. thebromae*. Similar antibacterial and antifungal activity of essential oil of other plant have also been reported. Medicinal as well as aromatic plant is an important source of potentially useful new compound for the discovery and development of chemotherapeutic against. *E.triplinerve* is a medicinal plant and also an important source of essential oil which contains 2- tert butyl-1, 4 methoxybenzene as major constituent (74.3 %) followed by b- selinene (8.6%) is more antifungal compared to antibacterial agent. Further pharmacological and toxicological studies will be required to establish the therapeutic uses of this oil.

Table: 1 Chemical constituent of the essential oil from *Eupatorium triplinerve* aerial parts

PEAK NO:	NAME OF THE CHEMICAL CONSITUENTS	PERCENTAGE %
1	a- Thujene	0.03
2	a- Pinene	1.53
3	Sabinene	0.03
4	b- Pinene	2.19
5	c- Myrcene	0.11
6	a- phellandrane	2.20
7	O- cymene	0.58
8	D- Limonene	0.67
9	Terpinolene	0.06
10	Sabinenehydrate	0.07
11	Mthyl chavicol	0.35
12	Thymol methyl ether	2.03
13	Terpinyl acetate	0.06
14	Anethole	0.06
15	(+) Myrtenyl acetate	0.04

16	Isolongifolene , 9,10- dehydro	0.05
17	Camphene	0.05
18	3- Oxabicyclo (4,2,0) oct – ene, ento- 8- methyl- exo- 8- (2-propenyl)	0.25
19	Cis – myrtanal acetate	0.07
20	b- elemene	0.57
21	a- gurjunene	0.91
22	2- tert- butyl- b- 1,4- metho oxy benzene	74.27
23	a- caryophylliene	0.40
24	3-4, dimethoxy – b- nitrostyrene	0.17
25	a- Selinene	1.28
26	b- Selinene	8.59
27	Patchoulene	0.11
28	Caryophyllene oxide	0.35
29	6- isopropencyl 1-4, 8a dimethyl 1- 2,3,5,6,7,8,8a, octa hydro naphthalene- 2-01	2.63
30	Ayapanin	0.16

Table : 2 Antibacterial activity essential oil from *Eupatorium triplinerve* aerial parts

S. No	Bacterium	2ml	5ml	10ml	15ml	20ml	Ampicillin 20mg/disc
	Gram-Positive Organism						
1	Staphylococcus aureus	11	12	13	16	16	20
2	Bacillus megaterium	10	11	11	12	13	21
3	Bacillus cereus	11	12	14	15	17	21
4	Bacillus subtilis	9	11	13	15	16	25
	Gram-negative organism						
1	Shigella dysenteriae	12	13	14	15	17	34
2	Shigella sonnei	12	14	15	16	18	30
3	Vibro cholerae	10	11	12	13	14	25
4	Escherichia coli	8	8	9	9	10	14
5	Salmonella typhi	13	15	16	18	21	29
6	Pseudomonas species	11	12	13	14	15	19

Table : 3 MIC and MBC of the essential oil from aerial parts from *Eupatorium triplinerve* against ten bacterial strains

S.No	Bacteria	MIC(ppm)	MBC(ppm)
1	Vibria cholerae	21000	24000
2	E.coli	21000	24000
3	Pseudomonas	18000	22000
4	Salmonella typhae	17000	22000
5	Shigella dysenterae	18000	22000
6	Shigella sonnei	18000	22000
7	Staphylococcus aureus	21000	23000
8	Bacillus cereus	21000	23000
9	Bacillus subtilis	21000	23000
10	Bacillus megaterium	21000	23000

MIC – Minimum inhibitory concentration

MBC – Minimum bacterial concentration

Table: 4 MIC and MFC essential oil from aerial parts of *Eupatorium triplinerve* against six fungal pathogens

S.No	Fungus	MIC (ppm)	MFC(ppm)
1	Macrophomina phaseoline	6000	8000
2	Colletotrichum corchori	6000	9000
3	Botryodiplodia	7000	8000
4	Curuularia	8000	10000
5	Fusarium equiseti	8000	10000
6	Alternaria alternate	9000	12000

MIC – Minimum inhibitory concentration

MFC – Minimum fungicidal concentration

Table: 5 Antifungal activity of essential oil from *Eupatorium triplinerve* aerial

S.No	Fungus	100(ppm)	250(ppm)	500(ppm)	750(ppm)	Nystetin (ppm) 1000(ppm)	100(ppm)
1	Fusarium equiseti	60	67	72	75	85	52
2	Curuularia lunate	59	64	70	73	82	68
3	Macrophomina phaseolina	80	84	85	87	89	70
4	Alternaria alternate	70	73	76	77	82	53
5	Colletotrichum corchori	71	72	75	78	81	40
6	Botryodiplodia theobromae	79	81	84	85	88	69

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