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Antimicrobial Activity and GLC Analysis of the Essential Oil of the Leaves of *Eupatorium triplinerve*

R.N. YADAV* and DEEPAK BARSAINYA
Natural Products Laboratory, Department of Chemistry
Dr. Hari Singh Gour University, Sagar-470 003, India

In the present paper, the authors describe the antimicrobial activity and GLC analysis of the essential oil of the leaves of *Eupatorium triplinerve.*

INTRODUCTION

Eupatorium triplinerve [N.O. Compositae][1-4] is known as 'Ayapana' in Hindi. It is distributed in America and has migrated to India. Now it grows in Indian gardens also. It is used as a stimulant diaphoretic. In view of its important medicinal values immediate attention is drawn to study both the phytochemical and antimicrobial nature of the plant.

RESULTS AND DISCUSSION

The leaves of *Eupatorium triplinerve* were found to consist of the following components on GLC analysis:

α-Pinene 4.25%, β-pinene 5.50%, linolyl acetate 7.50%, 1,8-cineol 6.50%, d-limonene 8.25%, α-terpeneol 8.50%, terpinyl acetate 4.00%, myrcene 5.20%, α-borneol 6.25%, nerol 7.50%, camphene 4.50%, citranellal 4.25%, eugenol 16.50%, α-thujene 9.50%.

The essential oil extracted from *Eupatorium triplinerve* was found to be more active against the following bacterial species: *Salmonella newport, Klebsiella pneumoniae, Vibrio cholerae, Staphylococcus aureus.*

In case of fungal species the essential oil was found to be more active against the following: *Penicillium digitatum, Fusarium oxysporum* and *Aspergillus fumigatus.*

Effect of pH and temperature variations on the activities were found to decreases with both temperature and pH.

EXPERIMENTAL

The essential oils were obtained by steam distillation method from leaves of *Eupatorium triplinerve* using Clevenger's apparatus. The oil was separated from water and dried over anhydrous sodium sulphate. The oil was then subjected to GLC and TLC analysis for its various composition.

TLC Analysis[5]

TLC analysis of essential oils was performed over silica gel (1.5 mm). The developing solvents used for the glass plates were benzene, 2,2-dimethyl butane and methyl hexane.

The glass plate was heated in an electric oven at 100°C for 20 min and then separated components were visualised by vanillin/H_2SO_4.

GLC Analysis

GLC was carried out with 26 OV-17 and SE–20. The various parameters for the best results were the following:

Linear Temp. Programming	:	65°–210°C increasing by 2.5°C/m.
Column	:	Glass column, 140 cm long and 0.38 mm in diameter packed with 30% OV. Absorbed on gas chrome detects.
Detector	:	Flame ionization detector
Attenuation	:	4×10^{-4}
Carrier gas	:	N_2
Pressure in carrier gas	:	1.20 kg/cm^{-2}
Injection temp.	:	165°C
Detector temp.	:	240°C
Chart speed	:	1 h
Smaple size	:	0.3–0.5/w of 0.1 solution of the oil in chloroform.

The various components of the essential oil were identified by comparing their retention times at different temperatures with those of pure components and also by co-injection of various authentic samples noted and the increase in the area at the corresponding peaks. Peak areas were determined and employed for the quantitative estimation of various constituents. The results are recorded in Tables 1–6.

TABLE-I
GLC ANALYSIS OF ESSENTIAL OIL FROM THE LEAVES OF *EUPATORIUM TRIPLINERVE*

Peaks S.No.	Corresponding authentic	Temp (°C)	Relative (%)
1.	α-pinene	56	4.25
2.	β-pinene	62	5.50
3.	linolyl acetate	78	7.50
4.	1,8-cineol	90	6.50
5.	*d*-limonene	66	8.25
6.	α-terpeneol	74	8.50
7.	terpinyl acetate	96	4.00
8.	myrcene	102	5.20
9.	α-borneol	84	6.25
10.	nerol	126	7.50
11.	camphene	108	4.50
12.	cirtranellal	152	4.25
13.	eugenol	118	16.50
14.	α-thujene	136	9.50

TABLE-2
ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL FROM THE LEAVE OF *EUPATORIUM TRIPLINERVE*

S. No.	Bacterial species	Diameter of Zone of Inhibition (mm)*				
		Pure oil	1 : 10	1 : 15	1 : 20	1 : 25
1.	*Bacillus anthraces*	2.5	1.5	0	0	0
2.	*Salmonella stanley*	4.5	3.5	1.5	0	0
3.	*Salmonella richmond*	4.0	4.0	3.0	0	0
4.	*Pseudomonas aeruginosa*	2.5	1.5	0	0	0
5.	*Salmonella pullorum*	2.5	1.0	0	0	0
6.	*Klebsiella pneumoniae*	8.5	6.5	5.5	4.5	4.0
7.	*Bacillus subtilis*	1.5	1.0	0	0	0
8.	*Stroptococcus agalactiae*	2.5	1.5	0	0	0
9.	*Vibrio cholerae*	9.5	8.0	7.5	6.5	5.5
10.	*Staphylococcus aureus*	8.5	6.5	5.0	4.5	3.5
11.	*Salmonella newport*	8.5	8.0	6.5	6.5	4.5
12.	*Escherichia coli*	4.5	3.5	2.0	1.5	0
13.	*Proteus vulgaris*	5.5	5.0	3.5	0	0

*The zones of inhibition (mm) were taken as average of four determinations in four different directions and Whatmann 41 (6 mm) were soaked with each sample tested for their activity at a concentration of 6 mg/ml of PBS (w/v).

TABLE-3
ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL FROM THE LEAVES OF *EUPATORIUM TRIPLINERVE*

S. No.	Fungal species	Diameter of zone of inhibition (mm)*				
		Pure oil	1 : 10	1 : 15	1 : 20	1 : 25
1.	*Fusarium oxysporum*	9.5	8.0	6.5	5.5	4.5
2.	*Aspergillus fumigatus*	9.5	7.5	6.5	6.0	5.5
3.	*Aspergillus niger*	4.5	3.0	0	0	0
4.	*Aspergillus flavour*	1.5	1.0	0	0	0
5.	*Penicillium digitatum*	6.5	5.5	5.0	4.5	3.5
6.	*Penicillium notatum*	2.5	1.5	0	0	0
7.	*Rhizipus stolonifera*	2.0	1.5	0	0	0
8.	*Microspermum gypsum*	4.5	3.5	2.0	0	0

*The zones of inhibition (mm) were taken as average of four determinations in four differerent directions and Whatmann 41 (6 mm) were soaked with each sample tested for their activity at a concentration of 6 mg/mL of PBS (w/v).

TABLE-4
MINIMUM INHIBITION CONCENTRATION (MIC) OF ESSENTIAL OIL OF *EUPATORIUM TRIPLINERVE* LEAVES

S. No.	Bacerial species	Minimum inhibition concentration (MIC)		
		Penicillium mg × 10^{-5}/mL	Tetracycline mg × 10^{-2}/mL	Pure oil mg/mL
1.	*Salmonella pullorum*	0	0	1.5
2.	*Escherichia coli*	0.12 × 6.24	0.2	1.0
3.	*Klebsiella pneumoniae*	0	0	8.0
4.	*Bacillus subtilis*	0	0	7.5
5.	*Bacillus anthraces*	0	0	6.0
6.	*Salmonella richmond*	0	0	2.6
7.	*Vibrio cholerae*	0	0	2.5
8.	*Proteus vulgaris*	0·18 × 3.0	0.3	4.5
9.	*Staphylococcus aureus*	0	0	1.0
10.	*Salmonella newport*	0	0	2.0
11.	*Salmonella stanley*	0	0	4.5
12.	*Streptococcus agalactiae*	0	0	4.5
13.	*Pseudomonas aeruginosa*	0	0	2.5

TABLE-5
THE EFFECT OF TEMPERATURE AND pH ON THE INHIBITION OF BACTERIAL GROWTH BY ESSENTIAL OIL FROM THE LEAVES OF *EUPATORIUM TRIPLINERVE*

S. No.	Bacterial species	Temperature (°C)			Diameter of zone of inhibition (mm) (pH)		
		20	50	80	2.0	5.0	8.0
1.	*Salmonella newport*	7.5	6.0	4.5	9.5	7.5	0
2.	*Klebsiella pneumoniae*	8.0	7.0	4.5	9.5	7.5	0
3.	*Vibrio cholerae*	8.0	7.0	5.0	10.5	9.5	0
4.	*Staphylococcus aureus*	7.5	5.5	4.0	8.5	7.5	0

TABLE-6
THE EFFECT OF TEMPERATURE AND pH ON THE INHIBITION OF FUNGAL GROWTH BY ESSENTIAL OIL FROM THE LEAVES OF *EUPATORIUM TRIPLINERVE*

S. No.	Fungal species	Temperature (°C)			Diameter of zone of inhibition (mm) (H)		
		20	50	80	2.0	5.0	8.0
1.	*Penicillium digitatum*	6.0	5.0	4.5	8.0	7.5	0
2.	*Fusarium oxysporum*	7.5	7.0	6.5	7.0	6.5	0
3.	*Aspergillus fumigatus*	6.0	5.5	4.5	8.0	7.5	0

Antimicrobial Activity (antibacterial/antifungal)

The leaves of *Eupatorium triplinerve* were found to contain 1.08% of essential oil. The antibacterial and antifungal activities were tested for pure oil at its various dilutions using ethylene glycol as solvent. The concentration was taken 6 mg/mL of phosphate buffered saline (W/V) against bacterial species [Temp. 20°, 50°, 80°C and pH 2.0, 5.0, 8.0]. Variations in the activity of bacterial species were studied.

The activity was studied by filter paper disc method.[6,7] These were soaked with various samples to be tested and were dried at 50°C. The disc was then placed on 'soft nutrient agar' [2%] petri dishes previously seeded with suspension of each bacterial species. For the fungus, petridishes were placed on 'Saboraud broth' medium [1%]. The zones of inhibition were expressed as an average of maximum dimension in four different directions.

The various bacterial species were first incubated at 40°C for 2 h. The zones of inhibition were recorded at 37°C after 24 h and for fungi at 36°C after 24 h.

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