Research Article

Antimicrobial Potential and Screening of Antimicrobial compounds of *Ruellia tuberose* Using GC-MS

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ABSTRACT

The methanol leaf extracts of *Ruellia tuberosa* showed significant antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis, Proteus mirablis* and antifungal activity against *Aspergillussp, Mucorsp, Penicilliumsp* and *Fusarium sp*. The antibacterial potential of *Ruellia tuberose* methanol extract was tested by using Agar well diffusion method. The (100mg/ml) leaf extract showed maximum inhibition against *Proteus mirablis* (7mm). Further the extract showed maximum zone of inhibition against the fungus of *Aspergillus sp* (8mm). Phytochemical tests were performed and showed that the antibacterial activity of plant *Ruellia tuberosa* leaves was due to the presence of phytochemical compounds like alkaloids, tripenoid, tannins, glycosides, saponins. GC-MS analysis revealed the presence of 27 compounds.

Keywords: *Ruellia tuberosa,* Phytochemical Analysis, Antimicrobial activity, GC-MS analysis.

INTRODUCTION

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to the world health organization, 80% of the world populations rely mainly on traditional therapies which involve the use of plant extracts or their active substances[1]. The microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs[2]. Antibiotics are sometimes associated with side effects[3] whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature[4]. All these data high lights the need for new alternative drug regimens. Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases [5]. The plant extracts have been developed and proposed for use as antimicrobial substances[6]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine[7]. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential[8-10]. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants[11]. In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities have been intensively investigated as a source of medicinal plants [12].Thus, it is anticipated that phytochemicals with adequate bacterial efficacy will be used for the bacterial infections. Since, man has used various part of plants in the treatment and prevention of various ailments [13].The present study was aimed to evaluated the antibacterial potential of methanol extract of *Ruelliatuberosa* against bacterial pathogens and phytochemical analysis of and identifing the compounds using GCMS.

MATERIALS AND METHODS

Collection and Drying of plant materials

Mature leaves of *Ruellia tuberosa* were collected from Coimbatore in 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.

Preparation of plant extract

10 g of powdered leaves were extracted successively with 100 ml 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 [14].

Test microorganisms

Nine pathogenic bacteria, *viz*., *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Klebsiella pneumonia*, *Vibrio cholera* and *Pseudomonas aeruginosa* and Fungi such as *Penicillium, Mucor, Tricoderma, Aspergillus* were used during 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 5 ml 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 (8 mm in diameter) were punched in the agar. Methanol extracts with same concentrations 100 mg/ml. 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 Alkaloids (Meyer's Test)

The extract *Ruellia tuberosa* of was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent[15]. The samples were then observed for the presence of turbidity or yellow precipitation [16].

Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated Sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer [14].

Test for Terpenoid and Steroid

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml 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 [14].

Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml 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 [14].

Test for Reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Triterpenes

300 mg of extract was mixed with 5 ml 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)

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

Test for Tannins

To 0.5 ml 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 [17].

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 5 ml 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.

GC-MS

The compounds were identified by using GC-MS.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of methonol leaf extract of the study species is given in Table 1. The results of the phytochemical screening revealed the phytochemicals such as alkaloids, glycosides, tripenoid, tannins and triterpenes are present in the methanol extract. The other phytochemical compounds flavonoids, saponins are absent. Among the seven phytochemical constituents tested such as, alkaloids, glycosides, tripenoid, flavonoids, tannins, saponins and triterpenes, the five constituents such as, alkaloids, glycosides, tripenoid, tannins and triterpenes 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 mass spectrum was done at the south India textile research association (SITRA), coimbatore. The spectrum of the unknown component was composed with known component stored in SITRA library. The name molecular weight structure of the component of test material was ascertained.

Twenty seven compounds were identified in methanol leaf extract of the study species by GC-MS analysis. The active principle molecular weight, concentration (%), molecular formula arepresented in table 2 and figure 1. The prevailing compounds are Neophytadiene (13.87%), 9,12,15-Octadecatrienoic acid (11.63%), 2-Hexadecene, 3,7,11,15-tetramethyl (8.66%), 2-Furancarboxaldehyde, 5-(hydroxymethyl) (8.29%), 9-Octadecenoic acid (7.81%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (7.66%) and Squalene (4.93%).

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 and four fungal species

(Table 3). The methanol extract of *Ruellia tuberosa* (100mg/ml) showed maximum zone of inhibition (7mm) against the bacteria, *Proteus mirablis* (Table 3). The methanol extract of *Ruellia tuberosa* (100mg/ml) showed maximum zone of inhibition (8mm) against the fungus, *Aspergillus sp* (Table 4).

Table 1: Phytochemical analysis of *Ruellia tuberosa* extract:

S.No	Test	Result
1	Glycoside	+
2	Tripenoid	+
3	Triterpenes	+
4	Tannins	+
5	Saponins	-
6	Alkaloids	+
7	Flavonoids	-

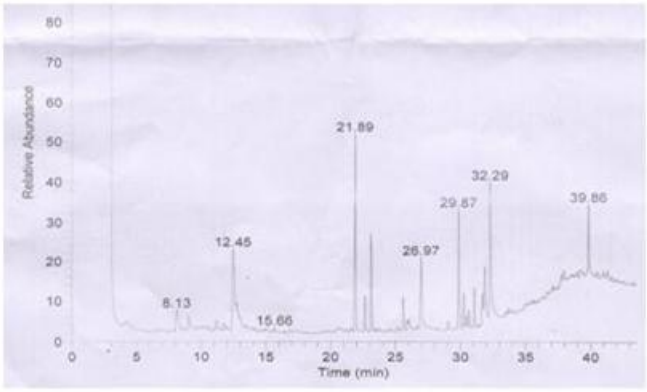

Figure 1: GC-MS chromatogram of methonal extract of *Ruellia tuberosa*:

Table 2: Table Showing Compounds, Molecular weight and Abandance % From GC-MS Analysis:

Compound	Molecular weight	Molecular formula	Abandance %
2-Furancarboxaldehyde (CAS)	96	$C_5H_4O_2$	0.68
1-Amino-2,6-dimethylpiperidine	128	$C_7H_{16}N_2$	2.22
2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	128	$C_6H_8O_3$	1.45
trans-á-methylstyrene-à,á-d(2)	118	$C_9H_8D_2$	1.59
D-(+)-à-Amino-î-caprolactam	128	$C_6H_{12}N_2O$	0.66
2-Furancarboxaldehyde, 5-(hydroxymethyl)	126	$C_6H_6O_3$	8.29
Neophytadiene	278	C_20H_{38}	13.87
1-[[Bis(methylthio)methylene]acetyl]-2-(4-(4-methoxy phenyl)-1,3-butadienyl)cyclopropane	346	$C_{19}H_{22}O_2S_2$	2.48
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	296	$C_{20}H_{40}O$	7.66
Hexadecanoic acid, methyl ester (CAS)	270	$C_{17}H_{34}O_2$	2.39
3-(P-TOLYL)-1,2-BENZOPYRONE	236	$C_{16}H_{12}O_2$	1.72
Hexadecanoic acid (CAS)	256	$C_{16}H_{32}O_2$	7.00
2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	280	$C_{20}H_40$	8.66
Phytol	296	$C_{20}H_{40}O$	2.56
13-Octadecenoic acid, methyl ester, (Z)- (CAS)	296	$C_{19}H_{36}O_2$	1.05
Octadecanoic acid, methyl ester (CAS)	298	$C_{19}H_{38}O_2$	1.25
9,12,15-Octadecatrienoic acid, methyl ester (CAS)	292	$C_{19}H_{32}O_2$	2.63
9-Octadecenoic acid (Z)- (CAS)	282	$C_{18}H_{34}O_2$	7.81
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$	11.63
2,6-Bis[5-cyano-6-(4-bromophenyl)-1,2,4-triazin-3-yl]pYridine	595	$C_{25}H_{11}Br_2N_9$	1.00
2,6-Bis[5-cyano-6-(4-bromophenyl)-1,2,4-triazin-3-yl]pYridine	595	$C_{25}H_{11}Br_2N_9$	0.84
3',4'-Dihydro-Stephasubine	592	$C_{36}H_{36}N_2O_6$	1.40
2,6-bis(Dibromomethyl)-3,5-diphenyl-4H-pyran-4-one	588	$C_{19}H_{12}Br_4O_2$	0.82
1,3,8-trimethyl-4-propyl-5-ethyl-2-(1-hydroxyethyl)-7-m ethoxycarbonylethyl-6,ç-methylenecarbonyl-porphine	594	$C_{36}H_{42}N_4O_4$	0.99
Squalene	410	$C_{30}H_50$	4.93
(+)-6-Acetyl-7-hydroxy-6-[2-(4-methylphenyl)ethyl]-9-phenoxy-1-azabicyclo[6.2.0]dec-4-en-10-one	419	$C_{26}H_{29}NO_4$	0.91
(+)-(P,1R,3S)-5-(4,5-dimethoxy-2-methyl-1-naphthyl)-6 ,8-dimethoxy-1,2,3-trimethyl-1,2,3,4-tetrahydroisoquin oline [(+)-O-Methylancistrocline]	436	$C_{27}H_{34}NO_4$	1.17

Table 3: Antibacterial activity of *Ruellia tuberosa* methanol extract against bacterial pathogens:

Organism	Concentration of extract and zone of inhibition (mm)		
	50 µl	75 µl	100 µl
Escherichia coli	4mm	5mm	6mm
Pseudomonas aeruginosa	4mm	5mm	5mm
Klebsiella pneumonia	3mm	5mm	7mm
Bacillus subtilis	2mm	4mm	6mm
Proteus mirablis	4mm	6mm	7mm

Table 4: Antifungal activity of methanol *Ruellia tuberosa* extract against pathogenic fungi:

Organism	Concentration of extract and zone of inhibition(mm)		
	50 µl	75 µl	100µl
Penicilliumsp	3mm	5mm	7mm
Mucorsp	6mm	6mm	7mm
Tricodermasp	2mm	3mm	4mm
Aspergillussp	5mm	6mm	6mm

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. In the present work methanolic extract of *Ruellia tubrosa* showed higher activity to the majority of organism tested. The result of phytochemicals in the present investigation showed that the plant leaves contain components like tannins, saponins, alkaloids, flavonoids. This study reports the presence of different phytochemicals with biological activity that can be valuable therapeutic index[18,19]. In the present study, shows that the biologically active phytochemicals were present in the methanolic leaf extract of the study plant. The antibacterial properties of these extracts may be due to the presence of above mentioned phytochemicals.

CONCLUSION

The plant extract studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs.

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