Antimicrobial Activity of *Ruellia tuberosa* L. (Whole Plant)

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ABSTRACT

The present communication attempts to study the antimicrobial activity of successive extraction using n-hexane, chloroform, ethyl acetate, alcohol and separate aqueous extract from whole plant of ***Ruellia tuberosa*** **L.** against different bacterial and fungal organisms (ATCC, MTCC) using disc diffusion method. The chloroform, ethyl acetate, alcohol and aqueous extracts were active against all the bacteria tested and showed significant antibacterial properties. The aqueous extract exhibited less activity against fungal organisms. Thus it may be informed that ***Ruellia tuberosa*** **L.** extract may be used to treat oral bacterial diseases.

Key words: *Ruellia tuberosa,* Antimicrobial activity, Successive extractions, MIC

INTRODUCTION

Since ancient time, plants have been a veritable source of drugs. Recent research work on herbal medicinal plants is intensified and information on these plants be exchanged. This thought will go a long way in the scientific exploration of medicinal plants for the benefit of man and is likely to decrease the dependence on drugs. Today plant compounds are providing the models for 50% of western drugs.[1] The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment. Some studies have concentrated exclusively on oils on microorganism. While these data are useful, the reports are not directly comparable due to methodological difference such as choice of plant extracts, test microorganism and antimicrobial test methods.[2]

Ruellia tuberosa **L.** is a Minnie root medicinal plant; a tropical plant widely distributed in South East Asia. It belongs to the family of Acanthaceae. It is a tropical perennial growing up to a height of 6.5" with a hairy stem. The simple leaves are opposite and elliptic; the plant flowers after the start of the rainy season. It has thick finger like roots. The big bisexual flowers are violet in colour. The ripe fruits are in a pod with 7-8 seeds each, burst open, when they get wet and the black seeds are hurdled away.[3,4] ***Ruellia tuberosa*** **L.** is medicinally used as an anthelmentic, against joint pains and strained muscles. In folk medicine, it has been used as diuretic, antidiabetic, antipyretic, analgesic, anti-hypersensitive, thirst-quenching, and antidotal agent. It has also recently been incorporated as a component in herbal drink in Taiwan,[5,6] and traditionally used for reducing toxicity, healing urine tract inflammation,[7] However, very few chemical constituents and pharmacological activities have been reported for this species.[5,8,9]

The present communication attempts to study the antimicrobial activity of successive extraction using n-hexane, chloroform, ethyl acetate, alcohol and separate aqueous extract from whole plant of ***Ruellia tuberosa*** **L.** against different bacterial and fungal organisms (ATCC, MTCC) using disc diffusion method. The scope of this study is to analyze antimicrobial compound and to generate data for this plant crude extract on which little information exist.

MATERIALS AND METHODS

Plant materials

The fresh plant materials of ***Ruellia tuberosa*** **L.** (Acanthaceae) were collected in July 2008 from district of Tamilnadu, India, and identified with the help of the Flora of Presidency of Madras[10,11] and a dried specimen (No: 00628) was deposited in museum of CSMDRIA Chennai.

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Extract preparation

The whole plant of ***Ruellia tuberosa* L.** was collected and allowed to dry at room temperature in the laboratory for a period of 2 weeks in sun shade, coarsely powdered and weighed. Then it was soaked with n-hexane for 48 hrs, the extract was filtered, the filtrate was concentrated by distillation over boil water batty and the last traces of solvent were removed under vacuum. Extraction was repeated with n-hexane and the residue was then extracted twice with dichloromethane, ethyl acetate and methanol respectively and successively. Aqueous extract was separately taken by using soxlet apparatus and concentrated under reduced pressure (T < 40 ºC). The yield of n-hexane was 134.34%, for chloroform 16.05%, yield with ethyl acetate 39.85%, yield with alcohol 56.63% and aqueous 83.06% per kg of plant powder. One gram from each successive extract were weighted in dry clean bottle and diluted by using 10% solution of dimethylsulfoxide (DMSO), dimethlyformamide (DMF), to make the concentration of 100 mg/ml. The aqueous extract is diluted by using saline (0.9%). The diluted solution was used for further antimicrobial work.

Microorganism

Both Gram positive and Gram negative bacteria's and fungus (ATCC and MTCC) were used as test organism for this study. They were obtained from the stock cultures of department of microbiology CSMDRIA & from SRM medical college hospital and also from Dr. Jeorge Mosses Lab, Chennai, India. **Gram positive bacteria** such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella sonnei,* and *Protease*. **Gram negative bacteria** such as *Salmonella, Staphylococcus spp, Seratia, Bacillus spp* and *Protease* and fungus like *Saceromyces cervesiae, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Candida albicans,* and *Candida tropicallis*. The organisms were sub cultured on to nutrient agar in order to determine their viability. The identity of each test organism was confirmed by using standard culture, morphological and biochemical techniques as described.[12] Stock cultures were maintained on nutrient agar slants at 4 ºC and then sub-cultured in nutrient broth at 37 ºC prior to each antimicrobial test. Inoculants of the test organisms were standardized by methods.[13] This was done by suspending 5 colonies of a 24 hrs culture in 5 ml of nutrient broth and comparing the turbidity with that of 0.5 Mac farina standards after incubating at 35 ºC for 2 hrs. Then the plant extract fractions were subjected to antimicrobial assay using disc diffusion method.[3,4,14,15]

Anti-microbial assay

Muller Hinton Agar (Hi media) for bacteria and Saber Dextrose Agar (Hi-media) for fungus were prepared according to the manufacturer's instructions. Muller Hinton Agar (MHA) contains Beef-2 g, casein acid hydrolysate 17.5 g, starch 105 g and agar 17 g; pH 7.4 ± 0.2. MHA (38 g) was weighed and dissolved in 1000 ml of distilled water. Sabouraud Dextrose Agar (SDA) was used for cultivation of fungi and particularly pathogenic fungi associated with skin infections. It contains Peptone –10 g, dextrose 40 g and agar 15 g; pH 5.6 ± 0.2. SDA (65 g) was dissolved in 1000 ml of distilled water. The medium was sterilized by autoclaving at 121 ºC for 15 minutes at 15 psi pressure and was used for tests. Sterile molten cool (45 ºC) agar was poured aseptically into sterile petridishes (15 ml each) and the plates were allowed to solidify at room temperature in sterile condition. After gelling and drying, the plates were seeded with appropriate micro organisms by streaking evenly on to the surface of the medium with a sterile cotton swab or pouring the appropriate microorganism on the surface of dry agar plate present in peptone broth. Care was taken for the even distribution of culture all over the plate. The inoculums were allowed to dry for 5 minutes. The discs of 6 mm diameter were prepared from Whatmann filter paper No. 1 and were sterilized in a hot air oven at 160 ºC for 1 hrs. The discs were then impregnated with the extracts and solvent DMSO, Amikacin, Gatifloxacin, Ciprofloxacin, Amphitrosine, discs were used as standard. Each disc contained 5 µg of corresponding standards. Sterile Whattman No 1 filter paper with 100 mg/ml were placed on to the agar with flamed forceps and gently pressed down to ensure contact along with the diluted extract, one appropriate control dry disc also placed at the center. Then the plates were incubated below 37 ºC for 24 hrs to allow perfusion of drugs being tested. The next day the zones of inhibition were measured with a measuring scale. This experiment was carried out in triplicate for their conformation. The results were read by the presence or absence of zone of inhibition. The lowest concentration of the each extract that inhibited the organisms was recorded as the MIC. This experimental procedure was repeated using several dilution of different successive extract until the minimal inhibitory zone was obtained.

RESULT AND DISCUSSION

Table 1 shows that preliminary antimicrobial test for ***Ruellia tuberosa* L.** whole plant successively extracted by n-hexane, chloroform, ethyl acetate and alcohol, and separate aqueous extract by the disc diffusion method. **Table: 2** shows that *Escherichia coli* have no characteristic zone of minimal inhibition for all extracts. It is indicated that *Escherichia coli* does not show any growth inhibition for all the extract at any concentration. Chloroform extract has zone of minimal inhibition of 1.0 cm at (50 µl), ethyl acetate extract has 1.0 cm at (200 µl) and alcohol extract has 0.8 cm at (100 µl) but hexane shows no characteristic zone of minimal inhibition at any concentration for *Pseudomonas aeruginosa.*

Table 1: Preliminary antimicrobial test for different solvent extracts of *Ruellia tuberosa* L. whole plant.

Name of the Micro organism	H	C	E	M	A	Con
Gram positive bacteria						
Escherichia coli ATCC – 73	–	–	–	–	–	Ak
Pseudomonas aeruginosa ATCC – 25583	–	+	+	+	–	Ak
Klebsiella pneumoniae ATCC – 700693	–	+	+	+	+	Ak
Shigella sonnei ATCC – 29508	+	+	+	+	+	Gt
Protease ATCC -9484	–	+	+	–	–	Cf
Gram negative bacteria						
Salmonella ATCC -10749	–	+	–	–	–	Ak
Staphylococcus spp ATCC – 25923	–	+	+	+	–	Ak
Seratia ATCC -14460	–	+	+	+	+	Gt
Bacillus spp ATCC – 6633	+	+	+	+	+	Gt
Fungus						
Saceromyces cervesiae	–	–	–	–	+	A
Aspergillus niger ATCC – 2587	–	–	–	–	–	A
Aspergillus fumigatus MTCC – 2551	–	–	–	–	–	A
Aspergillus flavus MTCC – 1884	–	–	–	–	–	A
Candida albicans	–	–	–	–	–	A
Candida tropicallis	–	–	–	–	–	A

H: n-Hexane, C: Chloroform, E: Ethyl acetate, M: Alcohol, A: Aqueous, Con: Control Ak = Amikacin, Gt = Gatifloxacin, Cf = Ciprofloxacin, A = Amphitrosine, (+) = Positive results, (–) = Negative results.

Table 2: Antibacterial effect of successive extract of n-Hexane, Chloroform, Ethyl acetate, and Alcohol extract of from whole plant of *Ruellia tuberosa* L. for Gram positive bacteria (100 mg/ml)

Name of the Bacterial organism	Zone of minimal inhibition (cm)										
	Successive extracts										
	Control	n-Hexane (µl)	Chloroform (µl)			Ethyl acetate (µl)		Alcohol (µl)			
		100	10	25	50	100	50	200	25	50	100	800
Escherichia coli	2.0	–	–	–	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa	3.0	–	–	–	1.0	–	–	1.0	–	–	0.8	–
Klebsiella pneumoniae	3.0	–	2.2	–	–	–	0.8	–	0.8	–	–	–
Shigella sonnei	3.5	1.2	–	–	–	0.8	0.8	–	–	–	–	1.2
Protease	1.5	–	–	–	–	0.8	–	–	–	–	–	–

It is indicated that the growth of *Pseudomonas aeruginosa* is inhibited at 50 µl for chloroform, 200 µl for ethyl acetate and 100 µl for alcohol extract. More over *Pseudomonas aeruginosa* does not show any growth inhibition for hexane extract at any concentration. Chloroform extract has zone of minimal inhibition of 2.2 cm at (10 µl), ethyl acetate extract has 0.8 cm at (50 µl) and alcohol extract has 0.8 cm at (25 µl) but hexane extract show no characteristic zone of minimal inhibition at any concentration for *Klebsiella pneumoniae*. It is indicated that the growth of *Klebsiella pneumoniae is* inhibited at 10 µl for chloroform, 50 µl for ethyl acetate and 25 µl for alcohol extract. More over *Klebsiella pneumoniae* does not show any growth inhibition for hexane extract at any concentration. Hexane extract has zone of minimal inhibition of 1.2 cm at (100 µl), chloroform extract has 0.8 cm at (100 µl), ethyl acetate extract has 0.8 cm at (50 µl) and alcohol extract has 1.2 cm at (800 µl) concentration for *Shigella sonnei*. It is indicated that the growth of *Shigella sonnei is* inhibited at 100 µl for hexane, 100 µl for chloroform, 50 µl for ethyl acetate and 800 µl for alcohol extract. **Table: 3**

Table 3: Antibacterial effect of Aqueous extract from whole plant of *Ruellia tuberosa* L. for Gram positive bacteria (100 mg/ml)

Name of the Bacterial organism	Zone of minimal inhibition		
	Separate aqueous extracts		
	Control	10 µl	100 µl
Escherichia coli	2.0 cm	–	–
Pseudomonas aeruginosa	3.0 cm	–	–
Klebsiella pneumoniae	3.0 cm	1.3 cm	–
Shigella sonnei	3.5 cm	–	0.5 cm
Protease	1.5 cm	–	–

shows that aqueous extract shows no characteristic zone of minimal inhibition at any concentration for *Escherichia coli, pseudomonas aeruginosa* and *Protease, but* aqueous extract has 1.3 cm at (10 µl) for *Klebsiella pneumoniae* and 0.5 cm at (100 µl) concentration for *Shigella sonnei*. It is indicated that the growth of *Klebsiella pneumoniae* and *Shigella sonnei* are inhibited at 10 µl and 100 µl for aqueous extract. **Table: 4**

Table 4: Antibacterial effect of successive extract of Hexane, Chloroform, Ethyl acetate, and Alcohol extract from whole plant of *Ruellia tuberosa* L. for Gram negative bacteria (100 mg/ml)

Name of the Bacterial organism	Zone of minimal inhibition								
	Successive extracts								
	Control	n-Hexane (µl)		Chloroform (µl)		Ethyl acetate (µl)		Alcohol (µl)	
		25	10	100	1600	100	400	10	50	100
Salmonella	3.0 (cm)	–	0.8	–	–	–	–	–	–	–
Staphylococcus	4.0 (cm)	–	–	1.1	–	–	1.2	–	1.8	–
Seratia	3.2 (cm)	–	–	0.8	–	0.9	–	–	–	0.6
Bacillus	4.0 (cm)	0.9	–	–	0.8	–	1.2	0.8	–	–

shows that chloroform extract has zone of minimal inhibition of 0.8 cm at (10 µl) but hexane, ethyl acetate, and alcohol extract shows no characteristic zone of minimal inhibition at any concentration for *Salmonella*. It is indicated that the growth of *Salmonella* is inhibited at 10 µl for hexane. Chloroform extract has zone of minimal inhibition of 1.1 cm at (100 µl), ethyl acetate extract has 1.2 cm at (400 µl) and alcohol extract has 1.8 cm at (50 µl) but hexane extract shows no characteristic zone minimal inhibition at any concentration for *Staphylococcus spp*. It is indicated that the growth of *Staphylococcus spp* is inhibited at 100 µl for chloroform, 400 µl for ethyl acetate and 50µl for alcohol extract. More over *Staphylococcus spp* does not show any growth inhibition for hexane extract at any concentration. Chloroform extract has zone of minimal inhibition of 0.8 cm at (100 µl), ethyl acetate extract has 0.9 cm at (100 µl) and alcohol extract has 0.6 cm at (100 µl), but hexane and aqueous extract shows no characteristic zone minimal inhibition at any concentration for *Seratia*. It is indicated that the growth of *Seratia* is inhibited at 100 µl for chloroform, 100 µl for ethyl acetate and 100 µl for alcohol extract. More over *Seratia* does not show any growth inhibition for hexane extract at any concentration. Hexane extract has zone of minimal inhibition of 0.9 cm at (25 µl), chloroform extract has 0.8 cm at (1600 µl), ethyl acetate extract has 1.2 cm at (100 µl) and alcohol extract has 0.8 cm at (10 µl) concentration for *Bacillus spp*. It is indicated that the growth of *Bacillus spp* is inhibited at 25 µl for hexane, 1600 µl for chloroform, 100 µl for ethyl acetate and 10 µl for alcohol extract. **Table: 5** shows that aqueous extract shows no characteristic zone minimal inhibition at any concentration for *Salmonella and Staphylococcus spp,* but aqueous extract has 0.6 cm at (100 µl) for *Seratia* and 0.8 cm at (10 µl) concentration for *Bacillus spp*. It is indicated that *Seratia* and *Bacillus* shows resistance to aqueous extract at any concentration. It is indicated that there is no inhibition for growth of *salmonell*a and *staphylococcus spp*, but there is growth inhibition for *Seratia* at 100 µl and *Bacillus spp* at 10 µl for aqueous extract. **Table: 6** Shows that no characteristic zones of minimal inhibition for Hexane, Chloroform, Ethyl acetate, Alcohol extract for *Saceromyces cervesiae, Aspergillus niger. Aspergillus fumigatus, Aspergillus flavus, Candida albicans* and *Candida tropicallis*. It is indicated that the *Saceromyces cervesiae, Aspergillus niger. Aspergillus fumigatus, Aspergillus flavus, Candida albicans* and *Candida tropicallis* does not show any growth inhibition for hexane, Chloroform, Ethyl acetate, and Alcohol extract at any concentration. **Table: 7** Shows that no characteristic zone of minimal inhibition for *Aspergillus niger. Aspergillus fumigatus, Aspergillus flavus, Candida albicans* and *Candida tropicallis* for Aqueous extract but *Saceromyces cervesiae* has zone of minimal inhibition of 0.8 cm at (10 µl). It is indicated that the *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Candida albicans* and *Candida tropicallis* does not show any growth inhibition for aqueous extract at any concentration, but there is growth inhibition for *Saceromyces cervesiae* at 10 µl for aqueous extract. All fractions showed a very good level of broad spectrum antibacterial activity tested at a concentration 500 µgm/ml particularly good activity was observed in the ethyl acetate and alcohol fractions from the whole plant extract. None of the fractions demonstrated antifungal activity expects aqueous extract from the whole plant for *Saceromyces cervesiae*. Though no valuable activity was observed against fungi there results may provide scientific support for some uses of the plant in traditional medicine. Chloroform and hexane extract showed less activity than that of ethyl acetate and alcohol extract at the tested concentration against bacterial strain.

Table 5: Antibacterial effect of separate Aqueous extract from whole plant of *Ruellia tuberosa* L. for Gram negative bacteria (100 mg/ml)

Name of the Bacterial organism	Zone of minimal inhibition		
	Separate aqueous extracts		
	Control (µl)	10 (µl)	100 (µl)
Salmonella	3.0 cm	–	–
Staphylococcus	4.0 cm	–	–
Seratia	3.2 cm	–	0.6 cm
Bacillus	4.0 cm	0.8 cm	–

Table 6: Antifungal effect of successive extract of Hexane, Chloroform, Ethyl acetate, Alcohol extract of from whole plant of *Ruellia tuberosa* L. for fungus (100 mg/ml)

Name of the Fungal organism	Zone of minimal inhibition				
	n-Hexane, Chloroform, Ethyl acetate, Alcohol extract				
	Control (µl)	10 (µl)	25 (µl)	50 (µl)	100 (µl)
Saceromyces cervesiae	1.2 cm	–	–	–	–
Aspergillus niger	1.2 cm	–	–	–	–
Aspergillus fumigatus	2..0 cm	–	–	–	–
Aspergillus flavus	1.1 cm	–	–	–	–
Candida albicans	1.1 cm	–	–	–	–
Candida tropicallis	1.1 cm	–	–	–	–

Table 7: Antifungal effect of separate extract of Aqueous from whole plant of *Ruellia tuberosa* L. for fungus (100 mg/ml)

Name of the Fungal organism	Zone of minimal inhibition				
	Aqueous extract				
	Control (µl)	10 (µl)	25 (µl)	50 (µl)	100 (µl)
Saceromyces cervesiae	1.2 cm	0.8 cm	–	–	–
Aspergillus niger	1.2 cm	–	–	–	–
Aspergillus fumigatus	2..0 cm	–	–	–	–
Aspergillus flavus	1.1 cm	–	–	–	–
Candida albicans	1.1 cm	–	–	–	–
Candida tropicallis	1.1 cm	–	–	–	–

CONCLUSION

In conclusion data presented in this study explain and justify the use of ***Ruellia tuberosa* L.** extract in the treatment of diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, skin disease etc. all the extractives showed a range of activity against all the tested bacteria drastically improved the level of activity for ethyl acetate and alcohol fractions exhibiting in all cases at better levels of activity. Though no activity was observed against fungi, these results may prove scientific support for some uses of the plant in traditional medicine. The present work has shown that the whole plant of ***Ruellia tuberosa* L.** studied is potentially good source of antimicrobial agents and that further investigations are carried to support the view that traditional use in medicine and also assisting primarily health care.

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