Antimicrobial activity and phytochemical analysis of *Rivea ornate*

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Abstract

Rivea ornate is an important medicinal plant in the family Convolvulceae. Extracts of young leaves, matured leaves, stem and roots of *Rivea ornata* were obtained by extraction method using hexane and methanol. Antifungal, antibacterial assay, antibiotic resistance and preliminary phytochemical analysis were carried out. Antibacterial activity was determined against *Pseudomonas* sp., *Proteus* sp., *Enterococcus* sp., *Staphylococcus* sp. and *E, coli* and antifungal activity was determined against *Mucor* sp., *Rhizopus* sp. and *Aspergillus* sp. by using the standard agar well diffusion method. Antibiotic resistance of tested bacteria was determined by the disc method against bacitracin, gentamycin, amoxycilin and streptomycin. The standard Mancozeb had no effect on *Rhizopus* sp. Only methanol extract of young leaf showed the higher degree of antifungal activity against *Mucor sp*. and *Aspergillus* sp. Methanol extract of stem inhibited the growth of *Rhizopus* sp. predominantly.

Hexane and methanol extracts of stem showed activity only against *Enterococcus* sp. and *E. coli* respectively. Methanol extract of root only exhibited activity against *Enterococcus* sp. Methanol extract of young leaf had the predominant antibacterial activity among the tested different extracts against the bacterial species. This study also revealed that *E.coli* and *Pseudomonas* sp. had the multi resistance ability for antibiotics. Phytochemical analysis of methanolic extract of aerial parts revealed that the presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides. The antimicrobial activity of Methanolic extracts of young leaves, and matured leaves of *Rivea ornata* is due to the presence of bioactive compounds.

Keywords: *Rivea ornate*, antibacterial, antifungal, Phytochemical analysis

Introduction

Rivea ornata belonging to the family Convolvulaceae is distributed throughout southern part of India[1]. They are erect shrubs or scandent from a woody rootstock. Leaves are orbicular to reniform. The inflorescence 3-10 flowers. Fruits are subglobose, glossy brown and glabrous. Seeds are brown in colour and are embedded in crumbly crust[11]. In Tamil, it is known as '*Machuttai*', in Sinhala called as "*Dumbutu*" and in folklore, it is well known as '*Baravat*' and '*Phaang*'. The leaves are given after parturition. In folklore, it is used topically in hemorrhagic diseases and piles[1]. *R. ornata* Cleanses the blood and strengthens all the organs of the body. It can be used for rheumatism and diseases such as white disease, body cooling, cooling to the eyes and good for hair growth. It contains iron and should be included in meals[2]. The aerial parts possess anti-inflammatory activity[3]. It was cooked with garlic and consumed as a green leafy vegetable to increase haemoglobin level[4]. Since there is no reported work on antimicrobial activity, the present study was carried out to analyse the bioactive compounds qualitatively and to determine the antimicrobial activity of the extracts of Matured leaves, young leaves, stem and root of *Rivea ornate* (Figure 1).

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Fig.1: Plant of *Rivea ornate*

Materials and Methods

Preparation of plant extracts

The healthy plant parts were collected from Navaly area in Jaffna, Sri Lanka. The plant material was taxonomically identified and authenticated by the support from the taxonomist of Department of Botany, University of Jaffna, Sri Lanka. They were dried in shade. Completely dried of Matured leaves, young leaves, stem and root were ground into fine powder using an electric blender. The powder was used to get hexane and methanol extractions as described below.

Preparation of Hexane extract

The n-hexane extracts were prepared by soaking 40 g of dried plant parts powder with 200ml n-hexane in a clean, dry-sealed bottle. Then it was time to time manually shacked well for 48 hours and filtered through Whatman filter paper No 1 in a Buchner funnel. The remaining residue was re-extracted two more times. All three n-hexane extracts were combined and the solvent was removed by using Buchi rotary evaporator under reduced pressure. After that the crude extract was then transferred into the glass vial (table 1) and kept in oven at 40 0C to remove the solvent completely.

Preparation of Methanolic extract

The air-dried remaining residue from above was further extracted with 200ml methanol by shaking for 48 hours as above and it was filtered through Whatman filter paper No. 1 in a Buchner funnel. The remaining residue was re-extracted two times more and the corresponding filtrates were pooled. The pooled filtrate of methanol was concentrated on a Buchi rotary evaporator at 40 0C under reduced pressure. The crude extract was then transferred into the glass vial (table 1) and kept in oven at 40^0C to remove the solvent completely.

Table 1: Weight of dried crude extracts

Plant part	Hexane extract (g)	Methanol extract (g)
Young leaf	1.1	1.6
Matured leaf	0.9	1.3
Stem	0.8	1.0
Root	0.8	0.9

Phytochemical Analysis

Different types of phytochemical constituents of the *Rivea ornata* young and matured leaves extracts were determined by using standard procedures[5, 6, 12]. The colour intensities of each extract and/or the appearance of solids in those extracts during the identification reactions revealed a semi-quantitative evaluation of the presence of various kinds of secondary metabolites. Standard procedures were used to determine Tannins, saponins, flavonoids, steroids, cardiac glycosides, alkaloids and coumarins.

Collection of Microbial cultures

The fungi *Aspergillus* sp., *Mucor* sp., *Rhizopus* sp. and the bacteria *E. coli*, *Enterococcus* sp., *Pseudo-monas aeruginosa*, *Proteus* sp., *Staphylococcus aureus* were obtained from the culture collections of the Department of Botany, University of Jaffna, Sri Lanka. Fungal cultures were maintained in Potato Dextrose Agar (PDA) medium whereas bacterial cultures were maintained in Nutrient Agar (NA)medium.

Determination of antibacterial activity

Antibacterial activity of *Rivea ornata* extracts of stem, root, young leaves and matured leaves was determined using agar well diffusion method. Young

bacterial suspension of *Pseudomonas aeruginosa* was standardized with 0.5M Mc Farland standard. 0.1 mL of a particular bacterial suspension was spread on the entire surface of the Mueller Hinton agar plate uniformly with the help of a sterile spreader. 9 mm diameter wells were made by using a sterile Corkborer. Then 150 µL of test solutions (500ppm), standard (0.01M Streptomycin), controls (hexane, methanol) were eluted into each well separately with the help of a sterile micropipette. All the plates were incubated at 37°C for 24 hours and the zone of inhibition around the well was measured. Each experiment was repeated thrice and the mean value was obtained. The above procedure was repeated for *E.coli* , *Enterococcus* sp., *Proteus* sp. and *Staphylococcus aureus* bacterial suspensions.[7,9]

Determination of antifungal activity

Fungal spore suspension [x* 10^5 number of spores/mL] of 0.1 mL was spread separately on the entire surface of the PDA plate with the help of a sterile spreader. 9 mm diameter wells were made by using a sterile Corkborer. Then 150 µL of test solutions (500ppm) of stem, root, young leaves and matured leaves extracts, standard (20mg/100mL Mancozeb- Dithane M-45), control (hexane, methanol) were eluted into each well separately with the help of a sterile micropipette. All the plates were incubated at room temperature for 4-5 days and the zone of inhibition around the well was measured after 72 hours. Each of the experiment was repeated thrice and the mean value was obtained. The above procedure was repeated for each of the fungus.[8,10]

Determination of antibiotic resistance of tested bacteria

Multiple antibiotic resistance against Bacitracin, Gentamycin, Amoxicillin and Streptomycin of tested bacteria was determined by the disc method on Mueller Hinton agar medium. Zone of inhibition was determined after 24 hours of incubation.

Results

The qualitative tests for the presence of phytochemicals revealed that the methanolic extracts of the aerial parts of the *Rivea ornata* presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides, whereas flavonoids and steroids were not detected (Table 2).

Table 2: Phytochemical constituents of methanolic extract of aerial parts of *Rivea ornate*

Tests for Phytoconstituents	Methanol extract of young leaves	Methanol extract of Matured leaves
Test for alkaloids. (Mayer's reagent)	+ve	+ve
Test for flavonoids. (lead acetate test)	-ve	-ve
Test for saponins. (Froth test)	+ve	+ve
Test for Cardiac Glycosides (Test with FeCl3)	+ve	+ve
Test for Tannins. (Reaction with fecl3)	+ve	+ve
Test for coumarins. (EtOH/ KOH)	+ve	+ve
Test for steroids (AC_2O + $C.H_2SO_4$)	-ve	-ve

-ve- absent, +ve - present

Bacterial growth was observed after 24 hours of incubation period. Antibacterial assay revealed that the standard streptomycin exhibits the highest antibacterial activity rather than the concentration of 500 ppm test solutions of stem, root, young leaves and matured leaves extracts and hexane and methanol as control. Antibacterial activity was high against all bacteria except *E. coli, Proteus* but *Pseudomonas* sp., *Staphylococcus* sp., and *Enterococcus sp*. were predominantly inhibited by test solutions compared with controls (Table 3).

Table 3: Antibacterial activity of different test samples

Name of the bacteria	Average Zone of inhibition in mm After 24 hours of incubation										
	Stem		Root		Young Leaves		Matured Leaves		Control		Standard
	Hex	Meo	Hex	Meo	Hex	Meo	Hex	Meo	hex	Meo	
Pseudomonas sp.	-	9.75± 0.95	-	-	-	13.25± 0.21	-	10.4± 0.8	10.3± 0.15	9.75± 0.95	21.25± 0.06
Proteus sp.	-	-	-	-	-	-	-	-	-	-	17.75± 0.75
Enterococcus sp.	10.75 ±0.06	-	-	10.25 ±0.5	-	10.25± 0.5	-	10.5± 0.92	-	-	-
Staphylococcus sp.	-	12.5± 0.30	-	10.25 ±0.5		12.25± 0.06	10.25± 0.5	13.5± 0.79	10.5± 0.3	12.5± 0.08	19.75± 0.40
E-coli	9.75± 0.96	10.5± 0.57	-	-	-	11.00± 0.81	-	-	11±0.82	-	-

The growth of all fungi was observed after 72 hours of the incubation period. It was observed that the diameter of the zone of inhibition decreased with an increasing incubation period. The standard mancozeb exhibited the highest antifungal activity rather than the concentration of 500 ppm test solutions and controls. Antifungal activity was exhibited against all fungi. But *Aspergillus* sp. was predominantly inhibited by test solutions compared with controls. The degree of inhibition depends on the type of fungi, spore concentration, types of antifungal compounds and their concentrations (Table 4).

Table 4: Antifungal activity of different test samples

Average zone of inhibition (mm) after 72 hours of incubation			
Test solution	***Mucor sp.***	***Rhizopus sp.***	***Aspergillus sp.***
Stem (hexane)	_	_	_
Stem (methanol)	12.5	18.5	12.5
Root (hexane)	_	_	_
Root (methanol)	12.8	12.5	12.5
Y.leaf (hexane)	_	_	_
Y.leaf (methanol)	16	16	19.5
M.leaf (hexane)	_	_	_
M.leaf (methanol)	12.25	12.25	18.5
Hexane (control)	_	_	_
Methanol (control)	_	_	_
Mancozab (standard)	18.5	_	15.5

Y. leaf-young leaf: M. leaf-matured leaf

Table 5: Antibiotic resistance of different bacteria

Average zone of inhibition in mm after 24 hours of incubation				
	Antibiotics			
Bacteria	**Bacitracin**	**Gentamycin**	**Amoxicillin**	**Streptomycin**
Enterococcus sp.	12	10.5	15.5	_
Pseudomonas sp.	_	22.5	_	11.5
E. coli.	_	18.5	_	_
Staphylococcus sp.	14.5	23.5	13.5	14.5

Discussion

The present study is to find out the degree of antifungal activity of different extracts of *Rivea ornata* against the tested fungi *Aspergillus* sp., *Mucor* sp. and *Rhizopus* sp. Using fungal conidia or spores as inoculum is more preferred than the fungal hyphae. Because the hyphal form cannot be accurately counted, diluted or transferred because of the adherence of hyphae to the surface and the macroscopic, interconnected nature of the hyphal mat. Due to this reason spores/ conidial suspension was used as the inoculum and these can be easily counted, diluted and transferred. The antifungal activity was detected by the inhibition in the growth of fungal hyphae.

In this study, a synthetic fungicide Dithane M-45 was used as a standard in order to assess the degree of effectiveness of the crude extracts by comparison. The trade name of this fungicide is Mancozeb. This fungicide was used at its recommended dosage (2g/l). Most commonly this can be used to control the diseases like downy mildews, rust diseases, anthracnose and leaf blights in the field crops except rice plants.

The growth of all fungi was observed after 72 hours of the incubation period. The standard mancozeb exhibited the highest antifungal activity except had no effect on *Rhizopus* sp. Methanol, Hexane and all stem, root and leaf hexane extracts did not show any effect on the growth of all tested fungi. Methanol extract of young leaf showed a higher degree of antifungal activity against *Mucor sp*. And *Aspergillus* sp. whereas methanol extract of stem inhibits the growth of *Rhizopus* sp. predominantly.

In the preparation of bacterial suspensions, the turbidity of each suspension was compared with the McFarland standard. Generally, McFarland Standards are used to standardize the approximate number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard is a chemical solution of barium chloride and sulfuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulfate. In this study concentration of 0.5 mol L^{-1} McFarland standard was used to compare the turbidity.

In this study an antibiotic Streptomycin was used as a standard in order to assess the degree of effectiveness of the crude extracts by comparison. Streptomycin is an aminoglycoside antibiotic that is produced by the soil actinomycete *Streptomyces griseus*. Normally, it performs by binding to the 30S (Svedberg unit) ribosomal subunit of susceptible organisms and disrupting the initiation and elongation steps in protein synthesis. A concentration of 500 ppm streptomycin solution was used in this study.

The degree of antibacterial activity varies not only with bacterial species but also with the tested samples. The standard streptomycin exhibited the highest degree of antibacterial activity. Hexane and methanol extracts of stem showed antibacterial activity only against *Enterococcus* sp. and *E.coli*

respectively when compared with the control. Methnol extract of root only exhibited activity against *Enterococcus* sp. Young and mature leaf extracts of methanol had activity against four and three bacterial species respectively out of five bacterial species that were tested. Methanol extract of young leaf had the predominant antibacterial activity compared to the tested different extracts against the bacterial species. The degree of antimicrobial activity varies with the species, type of extracts, types of bioactive compounds, growth media and incubation conditions. The phytochemical analysis of the methanolic extract of aerial parts revealed the presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides, whereas flavonoids and steroids were not detected[7,8].

Staphylococcus sp. was sensitive to all tested antibiotics. *Enterococcus* sp. showed resistance against only streptomycin. *Pseudomonas* sp. exhibited resistance against bacitracin and amoxicillin. But *E. coli* was only sensitive to gentamycin. This study revealed that *E. coli* and *Pseudomonas* sp. had the multi-resistance ability for the tested antibiotics[7, 9].

Conclusion

The phytochemical analysis of methanolic extract of aerial parts revealed that the presence of alkaloids, saponins, tannins, coumarins and Cardiac Glycosides. Bioactive compounds play a major role in antimicrobial activities. The standard mancozeb had no effect on *Rhizopus* sp. Only the methanol extract of the young leaf showed the higher degree of antifungal activity against *Mucor sp*. and *Aspergillus* sp. whereas the methanol extract of the stem inhibits the growth of *Rhizopus* sp. predominantly. Hexane and methanol extracts of stem showed antibacterial activity only against *Enterococcus* sp. and *E.coli* respectively. Methanol extract of root only exhibited activity against *Enterococcus* sp. Methanol extract of the young leaf had the predominant antibacterial activity among the tested different extracts against the bacterial species. The degree of antimicrobial activity varied with the species, type of extracts, groups of phytochemicals and amount of bioactive compounds, growth media and condition of incubation.

Reference

1. Chandu B.R., Kanala K., Hwisa N.T., Katakam P., Khagga M. (2013), Bioequivalence and pharmacokinetic study of febuxostat in human plasma by using LC-MS/MS with liquid-liquid extraction method, Springerplus, 2, 194 - 204.
2. Patel T.K, Barvaliya M.J, Sharma D., Tripathi C. (2013), A systematic review of the drug-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Indian population, *Indian journal of dermatology, venerology and leprology:* 79 (3), 389 – 98.
3. Sharma V.J., Patel P. M. (2013), Evaluation of anti-inflammatory activity of plant *Rivea ornate* (2013), *Journal of drug delivery & therapeutics:* 3 (1), 50 – 60.
4. Soujanya K., Kumari A., B Jyothsna E.B., Kavitha K.V. (2021), Traditional Knowledge on Uncultivated Green leafy vegetables (UCGLVS) used in Nalgonda district of Telangana, *International Journal of Bio-resource and stress management*:12 (4) 278 – 288.
5. Trease G.E, Evans W.C (1989), A text book of pharmacognosy, 12th Edition, Bailliere Tinall Ltd, London, 333 -337.
6. Edeoga1 H.O, Okwu D.E, Mbaebie B.O, (2005), Phytochemical constituents of some Nigerian medicinal plants, *African Journal of Biotechnology*: 4 (7), 685-688
7. Venkata S.P., Mohan C., Murali, Jame A., de Silva T., Akondi R.B., Sravani R. (2012). Screening the Antimicrobial and Antioxidant potential of *Ventilago denticulate*, *Scolopia crenata* and *Rivea hypocrateriformis* from Maredumilli forest, India. *Medicinal and Aromatic Plant Science and Biotechnology:* 6(i): 58- 62.

8. Saboo S., Tapadiya G. G., Khadabadi S.S. (2014). Antimicrobial and Phytochemical Analysis of *Rivea hypocrateriformis*. *Microbiology Journal:* 4:22-26.
9. Valle D. L., Cabrera E.C., Puzon J.J.M., Rivera W.L. (.2016) Antimicrobial activities of Methanol, Ethanol and Supercritical CO2 extracts of Philippine *Piper betel* L. on clinical isolates of gram positive and gram-negative bacteria with transferable multiple drug resistance. PLOS One 11(1): e0146349.
10. Balouiri M., Sadiki M., Ibnsouda S.K. (2016). Methods for In Vitro evaluation of antimicrobial activity: *A Review. Journal of Pharmaceutical Analysis*: 6(2):71 – 79.
11. Steples G. (2010). Convolvulaceae.FI Thailand,10(3):330-468.
12. Parekh J., Chanda S.V. (2007) *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish Journal of Biology*: 31:53 – 58.