Screening the Antimicrobial and Antioxidant Potential of *Ventilago denticulata*, *Scolopia crenata* and *Rivea hypocrateriformis* from Maredumilli Forest, India

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INTRODUCTION

Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Many infectious diseases are treated with herbal remedies, an age-old tradition. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Ujjwal et al. 2008). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for the treatment and reemerging infectious diseases (Parekh et al. 2007). The fact that microorganisms nowadays tend to develop resistance towards drugs, coupled with the undesirable side effects of certain antibiotics, offers considerable potentials for the development of new effective antimicrobial and antioxidant agents; medicinal plants are a prolific source (Teke et al. 2011).

Historically, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs are reported to be safe and without any side-effects, especially when compared with synthetic drugs (Pandey et al. 2003; Iiaghe et al. 2009).

Various plant extracts possess bacteriostatic and bactericidal effects (Lee 2000) and most of these plants contain many active compounds. The most important of these plant-derived bioactive constituents are alkaloids, tannins, flavonoids and phenolic compounds (Hill 1952). Most of these secondary metabolites, in addition to possessing antimicrobial potential, can also act as potent antioxidants. Antioxidants are closely related to the prevention of degenerative illness such as cardiovascular, neurological diseases, cancer and oxidative stress dysfunctions (Diplock 1995; Black 2000). Therefore, the exploitation of natural antimicrobial agents and antioxidants, especially of plant origin, has increased considerably in recent years.

*Ventilago calyculata* Tul. (syn. *V. denticulata* Willd.), *V. madrasapatana* Roxb., or *V. macrandra silbietiana* belong to the Rhamnaceae. The plants have medicinal qualities, as defined in the Ayurvedic system of medicine (Kirtikar et al. 1933). Plants of the genus *Ventilago*, e.g. *Ventilago leiocarpa* Bunge, have been used in Taiwan for the treatment of cough, rheumatism, and contused wounds (Kan 1980; Hsu 1995). Only six *Ventilago* species have been investigated chemically, i.e., *V. bombaiensis*, *V. calyculata*, *V. gougii*, *V. viminalis*, and *V. vitiensis* (Ali et al. 1994).

*Rivea hypocrateriformis* (Desr.) Choisy (Convolvulaceae) is a climbing shrub found throughout India. Ayurvedic physicians use this plant to prevent fertility in women (Shivalingappa et al. 2001, 2002). This plant has the ability of anti-implantation and is known to completely interrupt early pregnancy and influence the estrous cycle of albino rats (Shivalingappa et al. 2001). Leaves and young shoots are eaten as a vegetable and roots are given after parturition. Cooked leaves of this plant are used as a vegetable curry by the tribes of India (Nataraj et al. 2010). This plant has high vitamin A content (nearly 2.34 retinal equivalents) and has a high retention capacity (75 to 98%) of β-carotene upon pro-

Received: 25 June, 2011. Accepted: 1 September, 2011.

Keywords: methanolic extracts, antimicrobial activity, antioxidant activity, minimum inhibitory concentration, phytochemicals
cessing (Rajyalakshmi et al. 2001, 2003). The ethanolic extract of *R. hypocrateriformis* leaf juice is used to treat rheumatic pain and hair scalp skin diseases (Saikat et al. 2010).

Scoparia crenata (Flacourtiaceae family) leaves are used for treating musco-skeletal pain (Kadaval et al. 2009) although the biological activity of *S. crenata* has not yet been reported. Therefore, our results are the first evidence demonstrating its antimicrobial and antioxidant activities along with its preliminary phytochemical screening.

The present study aims to evaluate the antibacterial potential of the methanolic extracts of the bark and/or roots of these three medicinal plants against bacteria and fungi. The minimum inhibitory concentration (MIC) for antibacterial activity was determined. Other objectives included the search for active phytochemicals present in the methanolic extracts of these plants and also to obtain a preliminary sketch about the chemical nature of the active ingredient(s) associated with their antimicrobial potential. Their antioxidant activity was also evaluated.

**MATERIALS AND METHODS**

**Collection and identification of plant material**

*V. denticulata* bark, *S. crenata* bark and *R. hypocrateriformis* roots were collected from Maredumilli forest located near Rampachadavaram (Mandal), East Godavari (district), Andhra Pradesh (State), South India with the help of a local Ayurvedic doctor. The plants for screening antimicrobial and antioxidant activity were selected based on their use in folk medicine for the treatment of infectious disease-causing agents and were correctly identified at the Department of Botany, Andhra University, Visakhapatnam. Only the most abundantly used part from each plant was selected in this study, based purely on its traditional use by the local tribe.

**Extraction of plant material**

Plant material was brought to the laboratory and washed under running tap water and blotted dry with filter paper and then shade dried on laboratory benches on top of newspaper. After having completely dried, the plant material was then ground into a powder with a hand mill. The powdered plant material (10 g) was placed into a Soxhlet apparatus and was exhaustively extracted using 100 ml of 90% methanol (60-80°C). The crude extracts were concentrated *in vacuo* at 40°C using a rotary evaporator (PBU 6D model; Superfit). The crude extracts were preserved in a freezer at -20°C until use. The successive extracts weights were 29.4, 23.2 and 31.6% (w/w) for *V. denticulata*, *S. crenata* and *R. hypocrateriformis*, respectively.

**Test microorganisms and microbial culture**

Bacterial cultures (*Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella* sp. and *Staphylococcus aureus*) fungal cultures (*Aspergillus niger*, *penicillium* sp. and *Trichoderma viride*) were obtained from the Department of Microbiology, Andhra Medical College, Visakhapatnam. Bacterial strains were cultivated at 37°C and maintained on a nutrient agar (Hi Media) slant at 4°C. Fungal strains were cultivated at 25°C and maintained on potato dextrose agar (PDA) slants at 4°C. Pure cultures were kept on agar slants at 4°C until needed. They were sub-cultured once a month. Each inoculum was standardized using 100 ml of 90% methanol. The bacterial and fungal broth cultures (100 μl each) were inoculated onto nutrient agar and PDA plates (90 mm diameter, Borosil), respectively by the spread plate method. At first, stock solutions (100 μg/ml) of each individual plant extract were prepared separately. Sterile filter paper (Whatman filter paper No. 1) discs 9 mm in diameter were prepared and 10 μl of each extract dilution were impregnated onto the discs and carefully placed at the centre of the previously seeded plates with 0.5 McFarland bacterial and 10 μl CFU/ml fungal cultures with sterile forces. A disc with solvent alone served as the control. Streptomycin (10 μg/ml; Dr. Reddy's Laboratories Ltd.) for bacteria and Albenzadole (10 μg/ml; Ranbaxy Laboratories Ltd.) for fungi were used as the standard antimicrobials for comparison. Bacterial culture plates were then incubated at 37°C for 24 h while fungal cultures were incubated at 25-27°C for 48 h.

**Antimicrobial activity**

The activity of the extracts against the test microorganisms was determined by measuring the zone of inhibition. The minimum inhibitory concentration (MIC) for antibacterial activity was also evaluated.

The antibacterial activity was evaluated by the paper disc diffusion method (Aida et al. 2001). To determine antibacterial activity, each overnight bacterial culture was adjusted to 0.5 using McFarland turbidity standards (Performance Standards for Antimicrobial Susceptibility Testing 2009).

To determine antifungal activity, all fungal isolates were first adjusted to 10^6 colony-forming units (CFU)/ml. The bacterial and fungal broth cultures (100 μl each) were inoculated onto nutrient agar and PDA plates (70 mm diameter, Borosil), respectively by the spread plate method. At first, stock solutions (100 μg/ml) of each individual plant extract were prepared separately. Sterile filter paper (Whatman filter paper No. 1) discs 9 mm in diameter were prepared and 10 μl of each extract dilution were impregnated onto the discs and carefully placed at the centre of the previously seeded plates with 0.5 McFarland bacterial and 10 μl CFU/ml fungal cultures with sterile forces. A disc with solvent alone served as the control. Streptomycin (10 μg/ml; Dr. Reddy's Laboratories Ltd.) for bacteria and Albenzadole (10 μg/ml; Ranbaxy Laboratories Ltd.) for fungi were used as the standard antimicrobials for comparison. Bacterial culture plates were then incubated at 37°C for 24 h while fungal cultures were incubated at 25-27°C for 48 h.

**Preliminary phytochemical screening**

All extracts were analyzed for the presence of alkaloids, saponins, cardiac glycosides (*Trease et al. 1999*), tannins, phenols and flavonoids (*Sofowora 1982*, *Adetuyi et al. 2001*), and steroids (*Odebiyi 1982*).

**In vitro antioxidant study**

The ferric reducing antioxidant power (FRAP; Lim et al. 2007) property of the extract was determined by taking 1 ml of different dilutions of standard solutions of gallic acid (10-100 μg/ml) or methanolic extract that had been adjusted to come under the linearity range (500 μg/ml), placed in 10 ml volumetric flasks and mixed with 2.5 ml of potassium buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min and 25 ml of 10% trichloroacetic acid was added to the mixture to stop the reaction. To the 2.5 ml of the above solution, 2.5 ml of distilled water was added and then 0.5 ml of 0.1% FeCl₃ was added and allowed to stand for 30 min before measuring the absorbance at 593 nm. The absorbance obtained was converted to gallic acid equivalents in mg/g of dry material (GAE/g) using a gallic acid standard curve (*Avani et al. 2010*).

**RESULTS AND DISCUSSION**

**Antimicrobial activity**

The present study was conducted to investigate the antibacterial properties of three selected plants from Maredumilli forest which have not received due scholarly attention, even though they are used in Indian folk medicine. Herbal remedies play a fundamental role in traditional medicine in rural areas of India where they serve as the therapeutic treatment of choice as an antiseptic, anti-inflammatory and in the treatment of infectious diseases, including diarrhea. In the present study, an attempt was made to link traditional herbal
Antimicrobial and antioxidant activity of *V. denticulata*, *S. crenata* and *R. hypocrateriformis*. Venkata et al.

Medicinal knowledge held by the Indian native people with modern scientific laboratory-based assays.

Antimicrobial screening of the methanolic extracts of *V. denticulata*, *S. crenata* and *R. hypocrateriformis* are presented in Table 1 and Figs. 1 and 2. The present study reveals that the extracts of all three plants showed potent antibacterial and antifungal activity against all reference microbial (bacterial and fungal) strains.

**Table 1** Results of antimicrobial activity of methanol crude plant extracts of three different plants against pathogenic bacteria and fungi (*n* = 3).

<table>
<thead>
<tr>
<th>Microorganisms*</th>
<th>Zones of inhibition (mean ± SD) (mm)*</th>
<th>Antibiotics† positive standards</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Venilago denticulata</td>
<td>Scolopia crenata</td>
</tr>
<tr>
<td><strong>Bacterial strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7 ± 1.2</td>
<td>10 ± 1.2</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>7 ± 0.7</td>
<td>9 ± 1.4</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>0</td>
<td>8 ± 0.9</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>7 ± 0.9</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>7 ± 0.6</td>
<td>7 ± 0.9</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>6 ± 0.4</td>
<td>6 ± 0.8</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>6 ± 0.9</td>
<td>9 ± 0.6</td>
</tr>
<tr>
<td><strong>Fungal strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>9 ± 1.3</td>
<td>11 ± 0.4</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>9 ± 0.8</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>7 ± 0.7</td>
<td>7 ± 0.7</td>
</tr>
</tbody>
</table>

*Values are mean of three replicates ± SD.
† Antibiotics used as positive standards: Streptomycin (for bacterial strains); Albendazole (for fungal strains)

**Fig. 1** Bar chart showing results of antibacterial susceptibility of test organisms to methanol extracts of plants. *Venilago denticulata* (A), *Scolopia crenata* (B), *Rivea hypocrateriformis* (C).

**Fig. 2** Bar chart showing results of antifungal susceptibility of test organisms to methanol extracts of plants. *Venilago denticulata* (A), *Scolopia crenata* (B), *Rivea hypocrateriformis* (C).

R. hypocrateriformis exhibited the highest antibacterial activity against all reference strains (8-11 mm), 6 and 9 mm against *Trichoderma viride* and *Penicillium* sp. respectively and no activity against *Aspergillus niger*. Desmethylbergenin hemihydrate is a naturally occurring isocoumarin isolated from this plant (Zammarud *et al*. 2006). The phytochemical screening of *R. hypocrateriformis* tested positive for steroids, alkaloids, glycosides, saponins, fixed oils/fats,
tannins, and phenolic compounds (Shivalingappa et al. 2002).

The next highest activity was observed in S. crenata with (6-10 mm) against bacterial strains, except for S. aureus, and antifungal activity of 7 and 11 mm against A. niger and T. viride, respectively and no activity against Penicillium sp.

V. denticulata showed activity against all bacterial strains (6-7 mm), except for B. cereus, and 7-9 mm activity against all fungal strains. Tambekar et al. (2009) also demonstrated antibacterial properties of V. madraspatana in which the acetone extract showed antibacterial activity against Klebsiella pneumoniae, Enterobacteraerogenes (8 mg/disc) and Shigella flexneri (10 mg/disc). The ethanolic extract was effective against Staphylococcus aureus, Shigella flexneri and Enterobacter aerogenes (10 mg/disc) while the aqueous extract only showed antibacterial effect against Enter. aerogenes. Basu et al. (2005) also observed similar antibacterial activity in the chloroform and ethanol extracts of V. madraspatana, from which emodin and physcion were isolated for the first time from the stem bark. V. denticulata also exhibited therapeutic anti-herpes simplex virus type 1 efficacy in vivo (Vinolmas et al. 2003).

The MIC results of the extracts are shown in Table 2. Among the three plant extracts, R. hypocrateriformis was more potent with least MIC values of 10 and 25 mg/ml against S. aureus, E. coli, B. cereus, B. subtilis and Salmonella sp. whereas S. crenata and V. denticulata had MIC values of 50 and 100 mg/ml against the tested strains.

Preliminary phytochemical analysis of the methanolic extracts of these plants showed the presence of saponins, alkaloids, steroids, cardiac glycosides, tannins and phenolics (Table 3).

**Capacity of the FRAP method**

At low pH, measuring the change in absorption at 593 nm can monitor the reduction of a ferric complex to the ferrous form, which has an intense bluish green color. The change in absorbance is directly related to the combined or "total" reducing power of the electron-donating antioxidants present in the reaction mixture (Avani et al. 2010).

The FRAP method showed the methanolic extracts of V. denticulata bark, S. crenata bark and R. hypocrateriformis roots to have 67.25 ± 0.28, 73.56 ± 1.34 and 47.46 ± 0.72 mg GAE/g of sample, respectively (Table 4). The antioxidant activity of other members of the Rhamnaceae is briefly reviewed. Kaempferol 3-O-β-isorhamnoside (K3O-ir) and rhamnocitrin 3-O-β-isorhamnoside (R3O-ir), isolated from the leaves of Rhamnus alaternus L., inhibited NBT photoreduction and scavenged the free radical ABTS (+). At 150 μg/assay the two compounds showed the most potent inhibitory activity against the superoxide anion by 80.4 and 85.6%, respectively (Bhouri et al. 2011). Free radical scavenging activity of the methanolic extract of Ziziphus rugosa Lam. fruit pericarp exhibited radical scavenging activity with an IC_{50} = 61.88 /g541g/ml by the DPPH method (Kekuda et al. 2010).

The total antioxidant activity of some Australian Flacourtia species was assessed based on scavenging activity of stable ABTS free radicals, among them the leaf extract of Casearia sp. (RB 3051), the mature stem extract of Casearia grayii and the stem extract of Scolopia braunii had the highest antioxidant activity (IC_{50} = 2.9 μg/ml) (Mosadik et al. 2004).

The antioxidant activity of the methanolic extracts of Rivea hypocryptiformis leaf, stem and flower was estimated.

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**Table 2 Minimum inhibitory concentration (MIC) of methanol extracts (n = 3).**

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Test organism</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventilago denticulata</td>
<td>Escherichia coli</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>Rivea hypocrateriformis</td>
<td>Bacillus subtilis</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td>Scolopia crenata</td>
<td>Staphylococcus aureus</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
<tr>
<td><em>Salmonella sp.</em></td>
<td>Pseudomonas aeruginosa</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>*</td>
</tr>
</tbody>
</table>

1 = 10 mg/ml; II = 25 mg/ml; III = 50 mg/ml; IV = 100 mg/ml; + = MIC; - = no growth; + = little growth; ++ = dense growth growth

**Table 3 Results of preliminary phytochemical screening of plant extracts (n = 3).**

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins and phenols</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = presence; - = absence

**Table 4 Results of ferric reducing antioxidant power (FRAP) method on extracts added at 500 μg/ml (n = 3).**

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>mg GAE/g of extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventilago denticulata</td>
<td>67.25 ± 0.28</td>
</tr>
<tr>
<td>Scolopia crenata</td>
<td>73.56 ± 1.34</td>
</tr>
<tr>
<td>Rivea hypocryptiformis</td>
<td>47.46 ± 0.72</td>
</tr>
</tbody>
</table>

GAE = gallic acid equivalents based on standard curve (Fig. 3)
mated using the FRAP reagent (containing 2.5 mL of 20 mmol/L ferric 2,4,6-tripyridyl-S-triazine complex [Fe(III)-(TPTZ)]- solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L FeCl3·6H2O and 25 mL of 0.3 mol/L acetate buffer (pH 3.6) resulted in substantial activity ranging from 484.2 to 1279.5 μmol Fe(II)/g extract (Nataraj et al. 2010).

Another Convolvulaceae member, Evolutus alsinoides L., was also tested for its antioxidant activity in the 2,2'-azino-bis(3-ethylbenothiazoline-6-sulfonic acid) radical cation decolorization assay using ethanolic extracts and water infusion. The results from the ABTS assay showed that the ethanolic extract of E. alsinoides was IC50 = 33.39 μg/ml and the relative antioxidant capacity for the water infusions was IC50 = 172.25 μg/ml (Auddy et al. 2003).

CONCLUSION

Our results favor the use of these plants as potential additives or nutraceuticals to replace synthetic antioxidant compounds. However, further analyses of the antioxidant capacity of the beneficial compounds of these plants are needed to better understand how to implement them in functional medicine. The broad spectrum of antimicrobial activity may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious diseases, chemotherapy and control.

ACKNOWLEDGEMENTS

We are thankful to V. Krishna Rao, CEO, Kovel Foundation, Visakhapatnam for helping in sample collection. The authors thankfully acknowledge the help provided by the Department of Botany, Andhra University, Visakhapatnam for authentication of the plant material and Department of Biotechnology, GIT, GITAM University for providing infrastructure facility and encouragement. The authors are thankful to the management of GITAM University for their kind support.

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Turkish Journal of Biology 31, 53-58

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