*International Journal of Biomedical and Pharmaceutical Sciences* ©2007 Global Science Books

# Antimicrobial Activity of Extracts from the Leaves of *Clematis gouriana* Roxb.

**H. Raja Naika • V. Krishna***

Phytochemistry and Pharmacology Laboratory, P.G. Department of Studies and Research in Biotechnology and Bioinformatics, School of Biological Sciences, Kuvempu University, Shankaraghatta - 577 451, Karnataka, India

*Corresponding author*: * **krishnabiotech2003@gmail.com**

## ABSTRACT

*Clematis gouriana* (Rananculaceae) is an endemic medicinal plant of Western Ghats, India used in the treatment of dermatopathy, blood diseases, leprosy, wound healing, viral fever, headache, and cardiac disorders. Powdered leaf material of *C. gouriana* was subjected to Soxhlet extraction using three solvents: petroleum ether, chloroform and methanol. The antimicrobial activity of extracts were screened against twenty-seven clinical isolates from different infectious sources belonging to Gram-negative *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, and Gram-positive *Staphylococcus aureus* and five dermatitis fungi: *Trichophyton rubrum*, *T. tonsurans*, *Microsporum gypseum*, *M. audouini*, and *Candida albicans*. The minimal inhibitory concentrations (MIC) of the petroleum ether, chloroform and methanol extracts were determined as 525 µg/µl, 350 µg/µl and 100 µg/µl, respectively. The methanol extract showed a maximum inhibition zone on *S. aureus* (16.56 mm to 24.23 mm), *P. aeruginosa* (12.56 mm to 23.36 mm) and *K. pneumoniae* (14.30 mm to 22.40 mm), and their standard ATCC and MTCC strains by the agar well diffusion method. The antibacterial activity of the petroleum ether and chloroform extracts was not significant against the tested organisms. Among the five dermatitis fungi cultured the maximum zone of inhibition observed in the methanol extract was against the clinical strains of pathogenic fungi *T. rubrum* (13.36 mm) and *C. albicans* (9.96 mm). This study supports the traditional use of *Clematis gouriana* for the treatment of bacterial and fungal infections.

**Keywords:** agar-well diffusion, antimicrobial activity, *Clematis gouriana*, clinical isolates, Ranunculaceae
**Abbreviations:** **ATCC**, American type cell culture; **BHI**, brain-heart infusion agar; **DMSO**, dimethyl sulfoxide; **FDD**, Flora of Davanagere District; **LB**, Luria-Bertani; **MIC**, minimal inhibition concentration; **MTCC**, microbial-type culture collection; **PBS**, phosphate buffer saline

## INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine in India especially in rural areas. Thus, phytotherapy is practiced by a large proportion of the population for the treatment of several physical, physiological, mental and social ailments. To promote the proper use of herbal medicine, it is essential to evaluate the therapeutic properties of the extracts or the isolated constituents in a scientific way (El-Faky *et al*. 1995; Awadh Ali *et al*. 2001). In recent years, infections have increased to a great extent and antibiotic resistance becomes an ever-increasing therapeutic problem (Lis-Balchin and Deans 1996; Maoz and Neeman 1998; Austin *et al*. 1999). The use of higher plants and their extracts may provide a new source of antimicrobial agents with possibly novel mechanisms of action (Hamil *et al*. 2003; Machado *et al*. 2003; Motsei *et al*. 2003; Barbour *et al*. 2004). Due to improper medication and diagnosis many of the pathogenic clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* exhibit multi-drug resistance and are highly disruptive to the intestinal epithelial barriers (Zaborina-Olga *et al*. 2006). Many investigators have evaluated the bioactivity of plant extracts and the isolated constituents against these infectious organisms (Ramzi *et al*. 2005; Wilson *et al*. 2005; Parekh and Sumitra 2006).

*Clematis gouriana* Roxb. (Ranunculaceae) is a woody climber (**Fig. 1**) distributed in Western Ghats, India (Saldanha 1984). In the Indian system of medicine 'Ayurveda' the plant is used to alleviate malarial fever and headache. Root and stem paste is applied externally for psoriosis, itches and skin allergy (Manjunatha *et al*. 2004). The tradi-

**Fig. 1 A twig of *Clematis gouriana* Roxb. showing leaves with flowers.**

Received: 3 March, 2007. Accepted: 16 April, 2007.

Original Research Paper

tional medicine practitioners residing in the vicinity of Bhadra Wild Life Sanctury, India are using the leaf and stem juices for treating infectious old wounds, psoriasis and dermatitis.

The present investigation reports for the first time on the antimicrobial activities of different extracts (petroleum ether, chloroform and methanol) of the leaves of *C. gouriana*. Against the 27 clinical strains of bacteria: *S. aureus*, *P. aeruginosa* and *K. pneumoniae* and five dermatitis fungi: *Trichophyton rubrum*, *T. tonsurans*, *Microsporum gypseum*, *M. audouini*, and *Candida albicans*.

## MATERIALS AND METHODS

### Plant material and extraction

Leaves of *C. gouriana* were collected from the Lakkavalli reserve forest range of the Western Ghats region of Karnataka, India and identified by comparing with the authenticated specimen deposited at the Kuvempu University herbarium (Voucher specimen FDD 80). The leaves were washed in running tap water, shade-dried, powdered mechanically, sieved (Sieve No. 10/44) and stored for 2-3 months in an airtight container. Powdered material was subjected to Soxhlet extracton and exhaustively extracted with petroleum ether (60-80°C), chloroform and methanol for about 48 h in different batches of 250 g each. The resulting extract was filtered, pooled, and concentrated under reduced pressure using a rotary flash evaporator (Büchi, Flawil, Switzerland). The phytochemical tests for the screening of various secondary metabolites of the extracts were evaluated by qualitative tests (Trease 1983).

### Preparation of plant extracts

250 mg of crude extracts of petroleum ether, chloroform and methanol were reconstituted with dimethyl sulphoxide (DMSO). The standard antibacterial drug ciprofloxacin (BioChemika, ≥98.0% (HPLC) (Fluka) and antifungal drug fluconozole (Janssen-Cilag Pharmaceuticals, Bangalore, India) were also tested at 50 µg/100 µl of each.

### Microorganisms and media

The bacterial pathogens used for antibacterial consists of 27 clinical strains of three of the most common bacterial pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and their corresponding ATCC (*Pseudomonas aeruginosa* ATCC-20852; *Staphylococcus aureus* ATCC 29737) and MTCC (*Klebsiella pneumoniae* MTCC-618) strains. The five dermatitis fungi tested are *Trichophyton rubrum*, *T. tonsurans*, *Microsporum gypseum*, *M. audouini*, and *Candida albicans*. The different pathogenic microorganisms and their serotype were isolated from infected patients suffering from different infectious diseases as shown in **Table 1**. The samples were collected from District Health Centre, Gulbarga with the help of an authorized physician and identified at the Department of Microbiology, University of Gulbarga, India and with the help of the National Chemical Laboratory, Pune, India. All the bacterial microorganisms were maintained at 30°C in brain heart infusion (BHI) containing 17% (v/v) glycerol. Before testing, the suspensions were transferred to Luria-Bertani (LB) broth and cultured overnight at 37°C. Inocula were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland standards. Dilutions of this suspension in 0.1% peptone (w/v) solution in sterile water were inoculated on LB agar, to check the viability of the preparations. In case of fungal stocks, cultures were stored on BHI (Merck, India) culture media (pH 6.5).

**Table 1** Profile of the clinical strains used for antimicrobial activity.

| Clinical strains | Clinical condition | Source |
|---|---|---|
| ***Pseudomonas aeruginosa*** | | |
| *Pa-1* | Bronchitis | Wounds |
| *Pa-2* | Otitis media | Pus |
| *Pa-3* | Burns | Sputum |
| *Pa-4* and *Pa-5* | Upper UTI | Stool |
| *Pa-6* | Food poisoning | Hospital effluent |
| *Pa-7* | Cross infection in UTI | Hospital effluent |
| *Pa-8* | Septicemia | Old wounds |
| *Pa-9* | Unknown | Ear swab |
| ***Klebsiella pneumoniae*** | | |
| *Kp-1* | Pneumonia | Mucus |
| *Kp-2* | Gram negative | Follicullitis Stipules |
| *Kp-3* | Burns | Pus |
| *Kp-4* | UTI | Urine |
| *Kp-5* | Septicemia | Sputum |
| *Kp-6* | Cross infections in UTI | Urine |
| *Kp-7* | Abscess in immunodeficiency | Wounds |
| *Kp-8* | Upper UTI | Urine |
| *Kp-9* | Unknown | Hospital effluent |
| ***Staphylococcus aureus*** | | |
| *Sa-1* | Abscess in immunodeficiency | Wounds |
| *Sa-2* | Burns | Pus |
| *Sa-3* | Septicemia | Old wounds |
| *Sa-4* | Food poisoning | Pus |
| *Sa-5* | Burns | Stool |
| *Sa-6* and *Sa-7* | Unknown | Hospital effluent |
| *Sa-8* | Abscess in immunodeficiency | Sputum |
| *Sa-9* | Otitis media | Ear swab |
| **Fungal strains** | | |
| *T. rubrum* | Cutaneous mycoses | skin |
| *T. tonsurans* | Scaring of the scalp | Scalp ringworm |
| *M. gypseum* | Ringworm infections | skin |
| *M. audouini* | Cutaneous mycoses | Skin and hairs |
| *C. albicans* | Opportunistic mycoses candidosis | lungs |

### Antimicrobial assay

Antimicrobial activity was tested by the agar-well diffusion method (Mukherjee *et al*. 1995) and was used to assess the antimicrobial activity of the test samples. Sterilized LB agar (tryptone 10 $gl^{-1}$, yeast extract 5 $gl^{-1}$, sodium chloride 10 $gl^{-1}$, agar-agar 15 $gl^{-1}$, pH 7.2) medium was poured into sterilized Petri dishes (90 mm diameter). LB broth containing 100 µl of 24 h-incubated cultures of the respective clinical isolates and the ATCC and MTCC strains were spread separately on the agar medium. Wells were created using a sterilized cork borer under aseptic conditions. In order to identify the antifungal activity of total extracts against fungal pathogens an agar diffusion assay was performed in BHI culture media (pH 6.5). Fungal cells were obtained by centrifugation at 1500 × *g*, 4°C for 15 min and diluted in PBS, pH 7.2. The final concentration of each strain was $10^6$ cells/ml. Cultures were grown for 3 days at 37°C. One hundred µl of fungal spores were spread on BHI agar plates and wells were made using a sterilized cork borer and 50 µl of test compounds were loaded into each well. The plates were refrigerated for 2 h in order to stop fungal growth and facilitate diffusion of the substances. The reference antibacterial agent ciprofloxacin and antifungal agent fluconozole were loaded in the corresponding wells. As a control the wells were loaded with the same volume of sterile distilled water. Plates were then incubated at 37°C for 48 h. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm.

The minimal inhibitory concentrations (MIC) of the crude extracts were determined by micro dilution techniques in LB broth, according to National Committee for Clinical Laboratory Standard, USA guidelines (NCCLS 2000). The bacterial inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth micro dilution procedure. The micro titer plates were incubated at 37°C and MIC was determined after 24 h of incubation.

### Statistical analysis

The results of these experiments were carried out in triplicate and the mean diameter of the inhibition zone was recorded. The data were evaluated by one-way ANOVA followed by Tukey's pairwise Comparison Test.

## RESULTS AND DISCUSSION

The Soxhlet extraction of 500 g of leaf powder yielded 4.25 g of petroleum ether, 3.90 g of chloroform and 16.50 g of methanol extract respectively. The qualitative chemical tests of the petroleum ether extract showed positive for sterols and quinones. The chloroform extract showed positive test for saponins and methanol extract indicated the presence of alkaloids, triterpenoids and saponins as shown in the **Table 2**.

The MIC of the crude petroleum ether, chloroform and methanol extracts were determined to be 525 µg/µl, 350 µg/µl and 100 µg/µl respectively. The zone of inhibition of the microbial colonies is depicted in **Tables 3-6**. The methanol extract showed the maximum zone of inhibition against all the clinical strains and their serotype of bacterial pathogens i.e., *P. aeruginosa* (12.56 to 23.36 mm), *S. aureus* (16.56 to 24.23 mm) and *K. pneumoniae* (14.30 to 22.40 mm). Among the five dermatitis fungi cultured for antifungal assay, the zone of inhibition of the colony was found to be maximum on *T. rubrum* (13.36 mm), and *C. albicans* (9.96 mm) and negative on *M. gypseum*, *T. tonsurans*, and *M. audouini*. The bactericidal activities of the pet ether and chloroform extracts were not significant.

The results obtained in this study indicated that the methanol extract exhibited significant antimicrobial activity against Gram-positive *S. aureus* and Gram-negative *P. aeruginosa* while in *K. pneumoniae* bactericidal activity was moderate. The biocontrol potency of the methanol extract is comparable with that of the standard antibiotics, ciproflaxin and fluconozole. Generally the Gram-positive bacteria *S. aureus* should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt 1971). In contrast, the Gram-negative bacteria *P. aeruginosa* and *K. pneumoniae* possess an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents (Betoni *et al*. 2006). So the maximum inhibitory activity was observed in the Gram-positive bacterium *S. aureus*. In the case of Gram-negative *P. aeruginosa* and *K. pneumoniae* the zone of inhibitory activity was less significant in petroleum ether and chloroform when compared to methanolic extract because of the multilayered phospholipidic membrane carrying the structural lipopolysaccharide components (Nikaido and Vaara 1985). In spite of these barriers the methanolic extract is more effective in controlling the growth of pathogenic strains to a considerable extent. The highest activity of the methanol was compared to that of petroleum ether and chloroform extracts. Only the methanol extract is solely responsible for antibacterial and antifungal activity and can be used as a broad-spectrum antimicrobial agent.

Plants and plant products have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity (Cowan MM 1999; Nita *et al*. 2002; Ates and Erdogrul 2003; Velickovic *et al*. 2003) Infectious diseases remain the leading cause of death Worldwide and infections due to antibiotic resistant microorganisms have become more widespread in recent years. Resistance rates among key pathogens continue to grow at an alarming rate in distinct geographic regions worldwide (Bell *et al*. 1998; Pfaller *et al*. 1998; Schmitz *et al*. 1999) and the search for novel antimicrobial agents to combat such pathogens have become crucial for avoiding

**Table 2** Phytochemical studies of the leaf extracts of *Clematis gouriana*.

| Tests | Petroleum ether extract | Chloroform extract | Methanol extract |
|---|---|---|---|
| Alkaloids | – | – | + |
| Sterols | + | – | – |
| Flavonoids | – | – | – |
| Glycosides | – | – | – |
| Triterpenoids | – | – | + |
| Tannins | – | – | – |
| Quinones | + | – | – |
| Saponins | – | + | + |
| Carbohydrates | – | – | + |
| Proteins | – | – | – |

+: present; –: absent

**Table 3** Antibacterial activity of the extracts from the leaves of *Clematis gouriana* against clinical strains of *Pseudomonas aeruginosa.*

| Clinical strains* | Diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Petroleum ether | Chloroform | Methanol | Ciproflaxin |
| *Ps-1* | 3.36 ± 0.15 | 3.40 ± 0.17 | 15.30 ± 0.26 | 23.30 ± 0.15 |
| *Ps-2* | 4.23 ± 0.25 | 4.23 ± 0.25 | 12.56 ± 0.20 | 20.50 ± 0.29 |
| *Ps-3* | - | 3.36 ± 0.15 | 18.23 ± 0.25 | 22.23 ± 0.15 |
| *Ps-4* | 2.43 ± 0.40 | - | 21.43 ± 0.40 | 20.20 ± 0.26 |
| *Ps-5* | - | - | 16.26 ± 0.40 | 23.30 ± 0.15 |
| *Ps-6* | 3.33 ± 0.28 | 2.40 ± 0.17 | 23.36 ± 0.15 | 24.33 ± 0.20 |
| *Ps-7* | 4.20 ± 0.20 | 3.36 ± 0.15 | 13.56 ± 0.20 | 21.30 ± 0.15 |
| *Ps-8* | 3.36 ± 0.15 | - | 19.23 ± 0.25 | 23.17 ± 0.17 |
| *Ps-9* | 2.60 ± 0.17 | 3.23 ± 0.25 | 22.30 ± 0.26 | 20.50 ± 0.29 |
| *F*-Value | 162.05 | 345.98 | 576.38 | 52.2 |

*Clinical strains of *Pseudomonas aeruginosa* from different clinical sources.
The values are the mean of three experiments ± SD.
-: No activity

**Table 4** Antibacterial activity of the extracts from the leaves of *Clematis gouriana* against clinical strains of *Klebsiella pneumoniae.*

| Clinical strains* | Diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Petroleum ether | Chloroform | Methanol | Ciproflaxin |
| *Kp-1* | 4.30 ± 0.17 | 3.76 ± 0.25 | 17.23 ± 0.25 | 25.00 ± 0.12 |
| *Kp-2* | 2.63 ± 0.15 | - | 18.33 ± 0.15 | 20.23 ± 0.15 |
| *Kp-3* | 3.40 ± 0.17 | - | 15.36 ± 0.15 | 21.37 ± 0.09 |
| *Kp-4* | - | 1.36 ± 0.15 | 22.40 ± 0.36 | 20.20 ± 0.26 |
| *Kp-5* | - | 3.36 ± 0.15 | 19.36 ± 0.15 | 23.37 ± 0.09 |
| *Kp-6* | 2.23 ± 0.25 | - | 20.33 ± 0.15 | 22.53 ± 0.18 |
| *Kp-7* | 1.40 ± 0.17 | 4.26 ± 0.20 | 21.36 ± 0.15 | 24.37 ± 0.19 |
| *Kp-8* | - | 2.33 ± 0.15 | 13.50 ± 0.30 | 23.43 ± 0.12 |
| *Kp-9* | 1.36 ± 0.15 | - | 14.30 ± 0.26 | 24.43 ± 0.12 |
| F-*Value* | 332.04 | 491.05 | 571.42 | 137.2 |

Clinical strains of *Klebsiella pneumoniae* from different clinical sources.
The values are the mean of three experiments ± SD.
-: No activity

**Table 5** Antibacterial activity of the extracts from the leaves of *Clematis gouriana* against clinical strains of *Staphylococcus aureus.*

| Clinical strains* | Diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Petroleum ether | Chloroform | Methanol | Ciproflaxin |
| *Sa-1* | - | 2.56 ± 0.12 | 19.43 ± 0.40 | 28.33 ± 0.17 |
| *Sa-2* | 1.50 ± 0.30 | 2.36 ± 0.23 | 21.23 ± 0.25 | 26.90 ± 0.21 |
| *Sa-3* | 3.50 ± 0.30 | 2.46 ± 0.35 | 16.56 ± 0.20 | 21.50 ± 0.29 |
| *Sa-4* | - | - | 23.56 ± 0.20 | 24.50 ± 0.29 |
| *Sa-5* | 1.50 ± 0.30 | 1.70 ± 0.17 | 22.50 ± 0.30 | 20.43 ± 0.23 |
| *Sa-6* | - | - | 17.23 ± 0.25 | 27.10 ± 0.21 |
| *Sa-7* | - | - | 21.43 ± 0.40 | 25.50 ± 0.29 |
| *Sa-8* | 1.50 ± 0.30 | 1.36 ± 0.15 | 24.23 ± 0.25 | 23.50 ± 0.29 |
| *Sa-9* | - | - | 17.23 ± 0.25 | 23.83 ± 0.44 |
| F-Value | 104.70 | 134.07 | 296.95 | 88.9 |

*Clinical strains of *Staphylococcus aureus* from different clinical sources.
The values are the mean of three experiments ± SD.
-: No activity

**Table 6** Antifungal activity of the extracts from the leaves of *Clematis gouriana* against clinically isolated fungal pathogens.

| Clinical strains* | Diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Petroleum ether | Chloroform | Methanol | Fluconozole |
| *Trichophyton rubrum* | - | - | 13.36 ± 0.15 | 15.43 ± 0.23 |
| *Tricophyton tonsurans* | - | - | - | 16.57 ± 0.12 |
| *Microsporum gypseum* | - | - | - | 21.37 ± 0.19 |
| *Microsporum audouini* | - | - | - | 11.40 ± 0.10 |
| *Candida albicans* | - | - | 9.96 ± 0.37 | 12.23 ± 0.15 |
| *F*-Value | | | 208.08 | 585.7 |

*Clinical strains of *Staphylococcus aureus* from different clinical sources.
The values are the mean of three experiments ± SD.
-: No activity

the threat of post-antibiotic era.

*Clematis gouriana* is an endemic plant of the Western Ghats treating infectious old wounds and psoriasis. The healing property is due its antimicrobial property against the pathogenic strains of bacteria: *Stapylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The natives are using the leaf extract for dermatitis. During the present investigation showed that it has a pronounced antifungal activity against *Trichophyton rubrum*, *T. tonsurans*, *Microsporum gypseum*, *M. audouini*, and *Candida albicans*. The antifungal activity of the related species *Clematis hirsuta* have been screened against dermatitis fungi *Candida albicans*, *Trichophyton rubrum*, *Epidermophyton floccosum*, and *Microsporum canis* (Cos *et al*. 2002). The significant activity of the methanolic extract may be due to the presence of triterpenoids. The earlier investigators reported the antimicrobial properties of the triterpenoids containing extracts showed significant antimicrobial activity (Sanogo *et al*. 1998; Braca *et al*. 2000; Jain *et al*. 2001; Karterere *et al*. 2003; Jain *et al*. 2004; de Leon *et al*. 2005; Leite *et al*. 2006). Further investigations are under progress on the isolation and characterization of the bioactive constituents from methanol extract of *C. gouriana*.

## ACKNOWLEDGEMENTS

The authors are grateful to the Registrar, Kuvempu University and help of Prof. G. R. Naik, Chairman, Department of Biotechnology, Gulbarga University, Prof. R. Kelamani Chandrakanth, Chairman, Department of Microbiology, Gulbarga University Karnataka, India and Mr. Kumar Swamy (SRF), Mr. Harish (SRF), Mr. Sharath (SRF) and Mr. Gouthamchandra (JRF).

## REFERENCES

**Ates DA, Erdogrul OT** (2003) Antimicrobial activities of various medicinal and commercial plant extracts. *Turk Journal of Biology* **27**, 157-162

**Austin DJ, Kristinsson KG, Anderson RM** (1999) The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proceedings of the National Academy of Sciences USA* **96**, 1152-1156

**Awadh Ali NA, Juelich WD, Kusnick C, Lindequist U** (2001) Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *Journal of Ethnopharmacology* **74**, 173-179

**Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN** (2004) Screening of Selected indigenous plants of Lebanon for antimicrobial activity. *Journal of Ethnopharmacology* **93**, 1-7

**Bell JM, Paton JC, Turnridge J** (1998) Emergence of vancomycin–resistance enterococci in Australia: phenotypic and genotypic characteristics of isolates. *Journal of Clinical Microbiology* **36**, 2187-2190

**Betoni JEC, Mantovani RP, Barbosa LN, di Stasi LC, Fernandes A Jr.** (2006) Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Memórias do Instituto Oswaldo, Rio de Janeiro* **101**, 387-390

**Braca A, Morelli I, Mendez J, Battinelli L, Braghiroli L, Mazzanti G** (2000) Antimicrobial triterpenoids from *Licania heteromorpha*. *Planta Medica* **66**, 768-769

**Cos P, Hermans N, van Poel B, De Bruyne T, Apers S, Sindambiwe JB, van den Berghe D, Pieters L, Vlietinck AJ** (2002) Complement modulating activity of Rwandan medicinal plants. *Phytomedicine* **9**, 56-61

**Cowan MM** (1999) Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**, 564-582

**de Leon L, Beltran B, Moujir L** (2005) Antimicrobial activities of 6-oxophenolic triterpenoids: mode of action against *Bacillus subtilis*. *Planta Medica* **71**, 313-319

**El-Faky FK, Attif O, Aboul Ela M, Gaanem N** (1995) Antimicrobial evaluation of extracts from some Yemeni plants. *Alexandrian Journal of Pharmaceutical Sciences* **9**, 35-37

**Hamil FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD** (2003) Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays. *Journal of Ethnopharmacology* **84**, 57-78

**Jain SC, Jain R, Singh B** (2004) Antimicrobial principles from *Arnebia hispidissima*. *Pharmaceutical Biology* **41**, 231-233

**Jain SC, Singh B, Jain R** (2001) Antimicrobial activity of triterpenoids from *Heliotropium ellipticum*. *Fitoterapia* **72**, 666-668

**Katerere DR, Gray AI, Nash RJ, Waigh RD** (2003) Antimicrobial activity of *Indigofera suffruticosa*. *Phytochemistry* **63**, 81-88

**Leite SP, Vieira JRC, de Medeiros PL, Leite RMP, de Menezes Lima VL, Xavier HS, de Oliveira Lima E** (2006) Antimicrobial activity of *Indigofera suffruticosa*. *BMC Complementary and Alternative Medicine* **3**, 261-265

**Lis-Balchin M, Deans SG** (1996) Antimicrobial effects of hydrophilic extracts of *Pelargonium* species (Geraniaceae). *Letters in Applied Microbiology* **23**, 205-207

**Machado TB, Leal ICR, Amaral ACF, Santos KRN, Silva MG, Kuster RM** (2002) Antimicrobial ellagitannin of *Punica granatum* fruits. *Journal of the Brazilian Chemical Society* **13**, 606-610

**Manjunatha BK, Krishna V, Pullaiah T** (2004) *Floristic Composition of Davanagere District, Karnataka, India*, Regency Publication, New Delhi, 87 pp

**Maoz M, Neeman I** (1998) Antimicrobial effects of aqueous plant extracts on the fungi *Microsporum canis* and *Trichophyton rubrum* and on three bacterial species. *Letters in Applied Microbiology* **26**, 61-63

**Motsei ML, Lindsey KL, van Staden J, Jaeger AK** (2003) Screening of traditionally used South African plants for antifungal activity against *Candida albicans*. *Journal of Ethnopharmacology* **86**, 235-241

**Mukherjee PK, Balasubramanian R, Saha K, Saha BP, Pal M** (1995) Antibacterial efficiency of *Nelumbo nucifera* (Nymphaeaceae) rhizomes extract. *Indian Drugs* **32**, 274-276

**NCCLS** (2000) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard (5th Edn) NCCLS document M7-A5. NCCLS: Wayne, PA, USA

**Nikaido V** (1985) Molecular basis of bacterial outer membrane permeability. *Microbiology Reviews* **1**, 1-32

**Nita T, Arai T, Takamatsu H** (2002) Antibacterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *Journal of Health Science* **48**, 273-276

**Parekh J, Sumitra C** (2006) *In vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). *African Journal of Biomedical Research* **9**, 89-93

**Pfaller MA, Jones RN, Doern GV, Kugler K** (1998) SENTRY participants group: bacterial pathogens isolated from patients with blood stream infection: frequencies of occurrence and antimicrobial surveillance program (United States and Canada). *Journal of Antimicrobial Chemotherapy* **42**, 1762-1770

**Ramzi AA, Mothana, Lindequist U** (2005) Antimicrobial activity of some medicinal plants of the island Soqotra. *Journal of Ethnopharmacology* **96**, 177-181

**Saldanha CJ, Nicolson DJ** (1984) *Flora of Karnataka, India* (1st Edn), Oxford and IBH Publishing Co., New Delhi, pp 90-92

**Sanogo R, Grisafi G, Germano MP, de Pasquale R, Bisignano G** (1998) Evaluation of Malian traditional medicines: Screening for antimicrobial activity. *Phytotherapy Research* **12**, 154-156

**Scherrer R, Gerhardt P** (1971) Molecular sieving by the *Bacillus megaterium* cell wall and protoplast. *Journal of Bacteriology* **107**, 718-735

**Schmitz FJ, Verhoef J, Fluit AC** (1999) Prevalence of resistance to MLS antibiotics in 20 European SENTRY Surveillance program. *Journal of Antimicrobial Chemotherapy* **43**, 783-792

**Trease GE, Evans WC** (1983) *Pharmacognosy* (12th Edn), Bailliere Tindall Publishing, Eastbourne, pp 539-540

**Velickovic DT, Randjelovi NV, Ristic M** (2003) Chemical constituents and antimicrobial activity of the ethanol extracts obtained from the flower, leaf and stem of *Salvia officinalis* L. *Journal of Serb Chemical Society* **68**, 17-24

**Wilson B, Abraham G, Manju VS, Mathew M, Vimala B, Sundaresan S, Nambisan B** (2005) Antimicrobial activity of *Curcuma zedoaria* and *Curcuma malabarica* tubers. *Journal of Ethnopharmacology* **99**, 147-151

**Zaborina O, Kohler EJ, Wang Y, Bethel C, Shevchenko O, Wu L, Turner RJ, Alverdy CJ** (2006) Identification of multi-drug resistant *Pseudomonas aeruginosa* clinical isolates that are highly disruptive to the intestinal epithelial barrier. *Annals of Clinical Microbiology and Antimicrobials* **5**, 1-4