African Journal of Immunology Research Vol. 2 (3) pp. 108-112, March, 2015. Available online at www.Internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Assessment of some Medicinal plants utilized as a part of customary injury recuperating arrangements for antibacterial property against some pathogenic microorganisms

***Ranbir V. Raman, Moham E. Khan and Anushka Rai**

Department of Clinical Microbiology and Immunology, Faculty of Medical Laboratory Science, University of Hyderabad, Hyderabad, India.

Accepted 26 February, 2015

A total of sixteen extracts of four plants (*Acacia nilotica, Annona squamosa, Azadirachta indica,* and *Ocimum sanctum*) used in traditional formulation in India were investigated for their antibacterial property. Different concentrations (0.5 – 10 mg/ml) of extracts (by the extraction in different organic solvents and water) of plant parts were tested for growth inhibitory activity against infection caused by *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* The aqueous extract of *A. indica* (MIC; 0.07 and 0.5 mg/ml) against *S. aureus* and *P. aeruginosa*; methanolic extracts of *A. nilotica, A. indica,* and *A. squamosa* against *S. aureus, P. aeruginosa* and *E. coli* (MIC; < 0.5 mg/ml); chloroform extracts of *A. indica* (MIC; 0.5 and 0.3 mg/ml) against *S. aureus* and *E. coli*; and petroleum ether extracts of *A. indica* and *O. sanctum* (MIC; 0.5 - 0.79 mg/ml) against *P. aeruginosa* and *E. coli* were found more efficacious. The results revealed that all investigated plants exhibited antibacterial activity against at least one of the screened pathogens. The study also supports the use of above mentioned plants in wound healing formulations.

Key words: Wound, plants extract antibacterial, traditional medicine.

INTRODUCTION

One of the survey conducted by the WHO reports that more than 80% of the world's population still depends upon the traditional medicines for various diseases (Priya et al., 2002; Steenkamp et al., 2004). Forced with the growing resistance of organisms to antibiotics and other drugs, the search for alternatives is urgent (Dharmaratne et al., 1999; Anjaria et al., 2002; Seidal and Taylor, 2004). Herbal Ayurvedic products are used as medicines in form of either extracts or powder; and, they do have growth inhibitory effect against microbial pathogens. Many scientists have validated the biological activities of plants and their chemical constituents and demonstrated that aqueous and alcoholic extracts of several plants elicit antibacterial activity (Colombo and Bosisio, 1996; Ramesh et al., 2002; Fleischer et al., 2003; Karaman et al., 2003; Immanuel et al., 2004). In the present study, 4 plants viz. *Acacia nilotica, Annona squamosa, Azadirachta indica* and *Ocimum sanctum,* based on their use in community people for the cure of various kind of skin ailments were selected for the testing. Plants were evaluated for their antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are most common pathogens causing serious infections (Gnanamani et al., 2003); and *Escherichia coli* which is an opportunistic pathogen at the site of cut wound. *S. aureus* and *P. aeruginosa* are most common pathogens which infect the skin (El-Seed et al., 2002; Jeevan et al., 2004). *S. aureus* express surface proteins that promote attachment to host proteins that form part of the extra cellular matrix on epithelial and endothelial cell surfaces as well as being a component of blood clots (Gnan and Demello, 1999; Baie and Sheikh, 2000). Of the two million nosocomial infections each year, 10% are caused by *P. aeruginosa* (Gnan and Demello, 1999; Baie and Sheikh, 2000).

*Corresponding author. E-mail: rr.vanni@gmail.com

A. nilotica bark is reported to be acrid, hot, alexipharmic, antihelmintic, and used in biliousness, burning sensation leucoderma, and dysentery (Nadkarni, 1908; Chopra et al., 2002). Its pods and bark contain tannin. Samuelsson and coworkers have mentioned the use of *A. nilotica* leaves in dressing of ulcers (Samuelsson et al., 1992). *Annona squamosa* grows wildly throughout India and has antibacterial, pediculocidal, astringent, insecticidal, and vermicidal activity (Anjaria et al., 2002). *A. indica* is known for carminative, expectorant, anthelmintic, and insecticidal properties (Nadkarni, 1908; Anjaria et al., 2002). *O. sanctum* is also found in the literature as antibacterial, mosquito repellent, stimulant, demulcent, disphoretic, antiperiodic, and expectorant; and, it is used in bronchitis, ringworm and flatulence (Anjaria et al., 2002).

The results would be useful for the development of newer cost effective, health and eco-friendly formulation which would help the better efficacy than the existing drugs for skin infections. The promotion of such healing would also be useful in consumer of local biodiversity.

METHODOLOGY

Collection of the plant materials

Bark of *A. nilotica,* leaves of *O. sanctum*, *A. indica,* and *A. squamosa* were collected from their natural habitat (semi arid regions of India) between December 2006 to February 2007, dried under shade, and finally powdered using domestic grinder. The identity of plants was verified by the taxonomist at Botanical Survey of India, Arid Zone Circle, Jodhpur (India). Before the extraction, raw materials were pre-checked for pesticidal contaminations using suitable testing methods that is, US Pharmacopeia methods with Gas Chromatography/Mass Spectrometry. Bacterial strains were procured from MTCC, Institute of Microbial Technology, Chandigarh. All the chemicals and mediums were analytical reagent grade belonging to the E-Merck and Hi Media respectively.

Extraction preparation

Aqueous extract

Individual samples and the mixture of all the samples (20 g) were subjected to boil in 200 m**l** double distilled water in a 500 m**l** flask till the total volume remains half. The water extract was filtered through a 420 m stainless steel filter, cooled and transferred to screw caped glass vials.

Organic solvent extraction

Equal portioned mixture (10 g) of ingredients were extracted with the polar (methanol) intermediate (chloroform) and non-polar (petroleum ether) solvents by cold maceration for 24 h. The extracts were filtered through Whatman filter paper number 1 which was impregnated with same solvent. The organic solvents were concentrated to near dryness under reduced pressure below 40°C using Rotary Evaporator Bath. The amounts of the concentrate organic extract were noted down. The extracts were diluted to 20 mg/ml with dimethylsulfoxide and stored in airtight glass bottles in a refrigerator till further use (Mongelli et al., 1997; Mingarro et al., 2003).

Microorganisms and media

Three bacterial strains Gram positive – *S. aureus* (MTCC 96), gram negative – *P. aeruginosa* (MTCC 741) and *E. coli* (MTCC 443), were selected as test cultures because of their role in primary and secondary wound infections. The cultures were activated on nutrient agar media (HiMedia MM012).

Preparation of inoculums

For bacteria inoculations nutrient agar media (HiMedia MM012) were used and incubated for 24 h (Dykes et al., 2003). The standard curve revealed that 0.5 OD corresponds to 10^7-10^8 CFU/ml density of cultures. Hence, this OD was used as standard for adjusting the culture density. For all the experiments, 0.1 ml cultures of 0.5 OD were inoculated in 10 ml broths giving final cell load of 10^6-10^7 CFU/ml in nutrient broth media (Musumeci et al., 2003; Sohn et al., 2004).

Agar well diffusion assay method

A 0.2 ml volume of the standard inoculum (10^6- 10^7 CFU) of the test bacterial strain was spread on Mueller Hinton Agar (MHA) with a sterile bent glass rod spreader and allowed to dry. Then, 6 mm-diameter wells were bored using cork borer in the MHA. Plant extracts (10, 5, 1, and 0.5 mg/ml concentration) were introduced into each well and allowed to stand for 1 h at room temperature to diffuse the plants extracts in to medium before incubation at 37ºC for 24 h. The inhibition zone diameter (IZD) was measured by antibiotic zone reader to nearest mm (Okoli and Iroegbu, 2004).

Viable cell counting method

Dummy experiment was carried out to check the presence of viable cells in to the broth medium after 24 h treatment. To determine the number of the viable bacteria, 0.1 ml of the suspension mixture from plant extracts and bacteria were used for re- plate on MHA plates. The samples were diluted with 0.85% normal saline solution to an appropriate concentration which gave a countable number of the colonies/plate. Diluted samples (0.1 ml) were spread by sterile bent glass rod on MHA plate and incubated further for 18-24 h. at 37ºC in a biological incubator (Wongkham et al., 2001).

Determination of minimum inhibitory concentration (MIC)

The MIC was determined for the antimicrobially most efficient extracts, using the cylinder agar diffusion method as described by Fyhrquist et al. (2002).

RESULTS

Extract yields of all plants in four different solvents are cited in Table 1. Depending upon the polarity of solvent, extracts yields were found increasing as polarity increased. More yields were found in water extracts of all plants followed by methanol and chloroform. Least yield was found in petroleum ether extracts (Table 1).

Water extract

The effect of water extract of four plants was measured on

Table 1. Solvent extraction and w/w yield in terms of dry plant material.

Scientific name	Plant part	Vernacular name	Solvent and % (w/w) yield			
			Water	Methanol	Chloroform	Petroleum ether
A. nilotica	Bark	Babul	33.288	4.552	1.007	0.002
A. indica	Leaves	Neem	40.813	11.522	3.160	1.620
A. squamosa	Leaves	Sitafal	46.811	12.903	6.075	3.251
O. sanctum	Leaves	Tulsi	29.616	10.079	4.793	2.602

Table 2. Antimicrobial activity of plant extracts against pathogens by agar well diffusion method (n=3).

Plant extracts	Zone of inhibition (mm diameter ± SD)		
	S. aureus	***P. aeruginosa***	***E. coli***
Aqueous extracts			
A. nilotica	4.52± 0.82	0± 0	2.85± 0.85
A. indica	9.52± 1.34	7.08± 0.62	2.42± 0.82
A. squamosa	0.5 ± 0	0± 0	1.07± 0.06
O. sanctum	4.28± 0.18	0.5 ± 0	2.3± 0.7
Methanolic extracts			
A. nilotica	3.7 ± 0.05	6.79 ± 0.4	4.85± 0.76
A. indica	2.92± 1.05	3.02± 0.07	6.27 ± 0.2
A. squamosa	1.78± 0.81	5.42± 0.19	3.18± 0.18
O. sanctum	3.25± 0.04	0± 0	6.17± 2.08
Chloroform extracts			
A. nilotica	3.7 ± 0.06	7.44± 0.16	3.06± 0.24
A. indica	7.5 ± 0.41	4.64± 0.03	8.58± 0.11
A. squamosa	0.69± 0.33	4.7± 0.1	3.43 ± 0.2
O. sanctum	6.08± 0.05	4.67± 0.14	6.25± 0.18
Petroleum ether extracts			
A. nilotica	0.53± 0.06	6.1 ± 0.06	4.07± 0.04
A. indica	2.86± 0.18	9.57± 0.13	4.64± 0.34
A. squamosa	0± 0	5.21± 0.04	3.27± 0.31
O. sanctum	0.53± 0.06	7.35± 0.33	5.5 ± 0.16

on growth kinetics of three test cultures by recording the zone of inhibition and plate count of the cultures. Anti-bacterial activities by agar well diffusion method (Table 2) exhibit that the strong inhibition of growth of *S. aureus* and *P. aeruginosa* was observed in extracts of *A. indica.* Moderate type antibacterial activity was observed in *A. nilotica* against *S. aureus* and *E. coli, A. indica* against *E. coli,* and *O. sanctum* against *S*. *aureus*. The results of the colony count method (Table 3) demonstrate the repro-ducibility of the findings with minor variations. Highest inhibition on the number of colonies in Petri plates was observed in *A. nilotica* against *S. aureus* and *E. coli, A. indica* against *S. aureus* and *P. aeruginosa,* and *O. sanctum* against *S*. *aureus*.

Organic solvent extract

Residues obtained after extraction and evaporation of solvent were dissolved in DMSO (Langfield et al., 2004) because DMSO up to 1% did not influence the growth kinetics of *S. aureus* and other bacteria (Rajani et al., 2002). Hence, 1% DMSO was also tested along with organic solvent extracts for its inhibitory property against *E. coli, S. aureus* and *P. aeruginosa.* Results showed that 1% DMSO did not interfere with the growth kinetics of any of the tested organisms. Table 2 showed that metha-nolic extracts of *A. nilotica* and *A. indica* and chloroform extract of *A. nilotica* and *O. sanctum* exhibited their antibacterial activity against all the screened pathogenic

Table 3. Plate count of bacteria incubated in agar from the broth containing plant extracts for 24 h (n=3).

Plant extracts	Colony counts of test organisms (in 10^6 dilution)		
	S. aureus*	***P. aeruginosa*****	***E. coli*****
Aqueous extracts			
A. nilotica	64	2305	163
A. indica	1033	82	621
A. squamosa	1789	2401	579
O. sanctum	667	2069	523
Methanolic extracts			
A. nilotica	23	76	54
A. indica	410	2	66
A. squamosa	152	56	3
O. sanctum	1612	2208	64
Chloroform extracts			
A. nilotica	743	64	818
A. indica	2	462	64
A. squamosa	196	87	2305
O. sanctum	1323	66	7
Petroleum ether extracts			
A. nilotica	516	54	19
A. indica	62	62	10
A. squamosa	3792	73	19
O. sanctum	466	68	1

*Initial cell load of *Staphylococcus aureus* is 2028×10^6; **Initial cell load of *Escherichia coli* is 2500×10^6; ***Initial cell load of *P. aeruginosa* is 2500×10^6.

pathogenic bacteria. Petroleum ether extract of all plants were found highly inhibitory to *P. aeruginosa* and *E. coli*. Methanolic extract of *A. squamosa* was observed with antibacterial efficiency against *P. aeruginosa* and *E. coli. O. sanctum* was inhibitory against *E. coli*. Chloroform extract of *A. indica* was found effective against *S. aureus* and *E. coli*. *A. squamosa* was found effective against *P. aeruginosa*. Moderate activity was observed in methanol extract of *A. squamosa* and *O. sanctum* against *S. aureus* and chloroform extract of *A. indica* against *P. aeruginosa.* In the rest of the cases either no or very poor activity was reported.

The topical application of these plants at the wound site produced significant wound healing activity which may be due to antibacterial activity of the chemical constituents present in the crude extract. Delays in healing process directly promote the microbial infection. The phyto-chemicals present in different plant parts are responsible for effective antimicrobial activity. Tannins and other polyphenoles inhibit the microbial growth and have the ability to inactivate the microbial adhesine, enzymes, and cells envelop transport proteins (Stern et al., 1996). The results of chloroform extracts of *A. squamosa, A. indica,* and *O. sanctum* against wound pathogens were in agreement with the findings of Thaker and Anjaria (1985).

DISCUSSION

Plants used in Indian folklore system of medicines have been found active against a wide variety of microorganisms. Many biochemical constituents of plants possess excellent antimicrobial activities. Although the report of the studied plants for the treatment of wound infections is available in literature, we found contradictory and equivocal reports on screening of their extracts against pathogens. Similar result of extracts of *A. nilotica* was found as effective growth controller of *E. coli* and *P. aeruginosa* (Bagchi et al., 1999). Methanol extract was also reported inhibitory to *B. subtilis, P. aeruginosa,* and *S. aureus* but not *E. coli* (Deeni and Sadiq, 2002) . Petroleum ether extract was studied for its antibacterial activity by Chariandy et al., (1999) and its high efficacy against *E. coli, P. aeruginosa,* and *S. aureus* was concluded.

Alcoholic extract of *A. indica* was found inhibitory against the *B. subtilis* and *S. aureus* (Ahmad et al., 1998); and, methanol extract was inhibitory against *E. coli, S. aureus* and *P. aeruginosa* (Deeni and Sadiq, 2002). However, aqueous extract was inactive against *E. coli, S. aureus* and *P. aeruginosa* (Srinivasan et al., 2001) . For *O. sanctum,* Ahmad et al. (1998) reported that the aqueous extract was not effective against any bacteria; but the alcoholic extract was highly active against *E. coli, S. aureus* and *P. aeruginosa.*

A number of explanations can be given for the difference in biological activity reports of some common extracts against same or similar microorganism. In this study all plants and plant parts were collected from western region of India. The activity and quantity of phytochemicals presents in extracts can be varying depending upon geographical locations of plant cultivation (Olila et al., 2001). In the findings, there were marked differences in the activities of some extracts in two antimicrobial testing methods. The variation in results during the antimicrobial efficacy in different testing methods of a compound transpires because of effect of medium and supplements (Jones, 1996), temperature and other inoculation conditions (Michel and Blanc, 1994), molecular weight and diffusion rate of compound through medium (Marshall et al., 1999; Olila et al., 2001). The results of present study indicate that plant extracts showing positive microbial activity provide the scientific base to include the traditional practices in modern system of medicines. They may, therefore, provide new leads in the development of new antimicrobial drugs for the therapy of diarrhoea and other infectious diseases caused by *E. coli*, *S. aureus* or *P. aeruginosa.*

Conclusion

The aqueous extract of *A. indica* (MIC; 0.07 and 0.5 mg/ml) against *S. aureus* and *P. aeruginosa,* methanolic extracts of *A. nilotica, A. indica,* and *A. squamosa* against *S. aureus, P. aeruginosa,* and *E. coli* (MIC; < 0.5 mg/ml), chloroform extracts of *A. indica* (MIC; 0.5 and 0.3 mg/ml) against *S. aureus* and *E. coli,* and petroleum ether extracts of *A. indica* and *O. sanctum* (MIC; 0.5 - 0.79 mg/ml) against *P. aeruginosa* and *E. coli* were found efficacious. The obtained results confirm the presence of antibacterial components in all of examined herbs. The results would be helpful in carrying out bioassay- oriented fractionation of the active extracts to isolate best fraction and/or pure compound having antibiotic activities against wound pathogens.

ACKNOWLEDGEMENTS

We are thankful to Dr. Eleumelo S. for his and support and super-vision to carry out this research.

REFERENCES

Ahmad I,Mehmood Z, Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62: 183-193.

Anjaria J,Parabia M, Dwivedi S, (2002).Ethnovet heritage: Indian ethnoveterinary medicine - an overview. Pathik enterprise, Ahmedabad, Preword.pp 1- 612.

Bagchi GD, Singh A, Khanuja SPS, Bansal RP, Singh SC, Kumar S (1999). Wide spectrum antibacterial and antifungal activities in the seeds of some coprophilous plants of north Indian plains. J. Ethnopharmacol. 64: 69-77.

Baie SH, Sheikh KA (2000). The wound healing properties of *Channa striatus* –cetrimide cream-wound contraction and glycosaminoglycan measurement. J. Ethnopharmacol. 73: 15-30.

Chariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BPS(1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. J. Ethnopharmacol. 64(3): 265-270.

Chopra RN, Nayar SL, Chopra IC (2002). Glossary of Indian medicinal plants. National Institute of Science Communication, CSIR, New Delhi, India.

Colombo ML, Bosisio E (1996). Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). Pharmacol. Res. 33(2). 127-134.

Deeni YY, Sadiq NM (2002). Antimicrobial properties and photochemical constituents of leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): An ethnomedicinal plant of Hausaland, Northern Nigeria. J. Ethnopharmacol. 83: 235-240.

Dharmaratne HRW, Wijesinghe WMNM, Thevanasem V (1999). Antimicrobial activities of xanthones from *Calophyllum* species, against methicillin-resistant *Staphylococcus aureus* (MRSA). J. Ethnopharmacol. 66: 339-342.

Dykes GA, Amarowicz R, Pegg RB (2003). Enhancement of nicin antibacterial activity by a bearberry (*Arctostaphylos uvaursi*) leaf extract. Food Microbiol. 20: 211-216.

El-Seed HR, Ohara T, Sta N, Nishiyama S (2002). Antimicrobial deterpenoids from *Eupatorium glutinosum* (Asteraceae). J. Ethnopharmacol. 81: 293-296.

Fyhrquist P, Mwasumbi L, Hæggstrom CA, Vuorela H, Hiltunen R, Vuorela P (2002). Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania. J. Ethnopharmacol. 79: 169-177.

Fleischer TC, Ameade EPK, Sawer IK (2003). Antimicrobial activity of the leaves and flowering tops of *Acanthospermum hispidum.* Fitoterapia, 74: 130-132.

Gnan SO, Demello MT (1999). Inhibition of *Staphylococcus aureus* by aqueous Goiaba extracts. J. Ethnopharmacol., 68: 103-108.

Gnanamani A, Priya KS, Radhakrishnan N, Babu M (2003). Antibacterial activity of two plant extracts on eight burn pathogens. J. Ethnopharmacol. 86: 59-61.

Immanuel G, Vinchybai VC, Sivaram V, Palavesam A, Marian MP (2004). Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquecul, 236: 53-65.

Jeevan Ram A, Bhakshu LM, Raju RRV (2004). In vitro antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. J. Ethnopharmacol. 90. 353-357.

Jones RN (1996). Medium and supplement effects on the antimicrobial activity of quinupristin/dalfopristin tested by agar dilution and e-test methods. Diagn. Microbiol. Infect. Dis., 26(2). 99-102.

Karaman I, Sahin F, Gulluce M, Ogutcu H, Sengul M, Adiguzel A (2003)

Antimicrobial activity of aqueous and methanol extracta of *Juniperus oxycedrus* L. J. Ethnopharmacol. 85: 231-235.
Langfield RD, Scarano FJ, Heitzman ME, Kondo M, Hammond G, Neto C (2004). Use of a modified microplate bioassay method to investigate antibacterial activity in the Peruvian medicinal plant *Peperomia galioides.* J. Ethnopharmacol. 94: 279-281.
Marshall SA, Jones RN, Erwin ME (1999). The Quality Control Study Group. Antimicrobial activity of SCH27899 (Ziracin®), a novel everninomicin derivative, tested against *Streptococcus* spp.: disk diffusion / Etest method evaluations and quality control guidelines. Diagn. Microbiol. Infect. Dis. 33(1). 19-25.
Michel C, Blanc G (1994). Bacterial resistance to antimicrobial agents used in fish farming: A critical evaluation of method and meaning. Ann. Rev. Fish Dis. 4**:** 273-313.
Mingarro DM, Acero N, Llinares F, Pozuelo JM, Mera AG, Vicenten JA, Morales L, Alguacil LF, Peres C (2003). Biological activities from *Catalpa bignonioides* Walt. (Bignoniaceae). J. Ethnopharmacol. 87: 163-167.
Mongelli E, Desmarchelier C, Coussio J, Ciccia G (1997). Biological studies of *Bolax gummifera,* a plant of the Falkland Islands used as a treatment of wounds. J. Ethnopharmacol. 56: 117-121.
Musumeci R, Speciale A, Costanzo R, Annino A, Ragusa S, Rapisarda A, Pappalardo MS, Iauk L (2003). *Berberis aetnensis* C. presl. extracts: antimicrobial properties and interaction with ciprofloxacine. Antimicrob. Agent, 22: 48-53.
Nadkarni AK (1908). Indian materia medica. Bombay Popular Prakashan Pvt. Ltd., Mumbai, India. pp 165-166.
Okoli AS, Iroegbu CU (2004). Evaluation of extracts of *Anthocleista djalonensis, Nauclea latifolia* and *Uvaria afzalii* for activity against bacterial isolates from cases of non-gonococcal urethritis. J. Ethnopharmacol. 92: 135-144.
Olila D, Odyek O, Asibo OJ (2001). Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. Afr. Health Sci. 1(2). 66-72.
Priya KS, Gnanamani A, Radhakrishnan N, Babu M (2002). Healing potential of *Datura alba* on burn wounds in albino rats. J. Ethnopharmacol. 83: 193-199.
Rajani M, Saxena N, Ravishankara MN, Desai N, Padh H (2002). Evaluation of the antimicrobial activity of ammoniacum gum from *Dorema ammoniacum*. Pharm. Biol. 40(7): 534-541.
Ramesh N, Viswanathan MB, Saraswathy A, Balakrishna K, Brindha P, Lakshmanaperumalsamy P (2002). Phytochemical and antimicrobial studies of *Begonia malabarica.* J. Ethnopharmacol. 79: 129-132.
Samuelsson G, Farah MH, Claeson P, Hagos M, Thulin M, Hedberg O, Warfa AM, Hassan AO, Elmi AH, Abdurahman AD, Elmi AS, Abdi YA, Alin MH (1992). Inventory of plants used in traditional medicine in Somalia. III. Plants of the families Lauraceae-Papilionaceae. J. Ethnopharmacol. 37(2). 93-112.
Seidal V, Taylor PW (2004) In vitro activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. Antimicrob. Agent 23: 613-619.
Sohn HY, Son KH, Kwon CS, Kwon GS, Kang SS (2004). Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongoloca* Schneider, *Broussnetia papyrifera* L. Vent, *Sophora flavescens* Ait. and *Echinosophora koreensis* Nakai. Phytomedicine, 11: 666-672.
Srinivasan D, Nathan S, Suresh T, Perumalsamy PL (2001). Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharmacol. 74: 217–220.
Steenkamp V, Mathivha E, Gouws MC, Rensburg CEJ (2004). Studies on antibacterial, antioxidant and fibroblast growth stimulation of wound healing remedies from South Afr. J. Ethnopharmacol. 95: 353–357.
Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996). Phlorotannin-protein interactions. J. Chem. Ecol. 22: 1887–1899.
Thaker AM, Anjaria JV (1985). Antimicrobial and infected wound healing response of some traditional drug. Indian J. Pharmacol. 18: 171-174.
Wongkham S, Laupattarakasaem P, Pienthaweechai K, Areejitranusorn P, Wongkham C, Techanitiswad T (2001). Antimicrobial activity of *Streblus asper* leaf extract. Phytother. Res. 15: 119-121.