OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2009 **9(2)**, 174-181
DOI 10.3742/OPEM.2009.9.2.174

Antibacterial potential of the extracts derived from leaves and *in vitro* raised calli of medicinal plants *Pterocarpus marsupium* Roxb., *Clitoria ternatea* L., and *Sanseveiria cylindrica* Bojer ex Hook

M Shahid[1,*], A Shahzad[2] and M Anis[2]

[1]Section of Antimicrobial Agents & Drug Resistance Researches, Department of Microbiology, Jawaharlal Nehru Medical College & Hospital, India; [2]Plant Biotechnology laboratory, Department of Botany, Aligarh Muslim University, Aligarh – 202002, U.P., India

Received for publication June 18, 2007; accepted February 12, 2009

SUMMARY

Pterocarpus marsupium, *Clitoria ternatea*, and *Sanseveiria cylindrica* are some of the important and endangered medicinal plant species of India. Despite of medicinal properties, antibacterial potential of the plants have not yet been explored. The present study was designed to optimize the *in vitro* technique for micropropagation and to screen the extracts from leaves and *in vitro* raised calli for antibacterial properties. Excised leaf-explants from the parent plants were surface sterilized and cultivated on Murashige & Skoog's (MS) medium containing N^6-benzyladenine (BA) in concentrations of 1, 2, 5, and 10 μM. Optimal growth of calli was noticed at a concentration of 5 μM, therefore the extracts from calli grown at this concentration were further studied for antibacterial activity. Both alcoholic and aqueous extracts from leaves of respective plants, and their *in vitro* raised calli were tested for antibacterial activity by agar well diffusion method against a range of Gram-positive and Gram-negative bacteria. Aqueous extracts showed antibacterial activity against limited number of bacterial species; notably the extracts of *C. ternatea* which showed antibacterial activity against *Streptococcus pyogenes*, *Bacillus subtilis* and *Bacillus cereus*. Alcoholic extracts of all three plants showed antibacterial activity against a wider range of bacteria. Among the Gram-positive bacteria, extracts from *C. ternatea* showed strong antibacterial activity against *Bacillus* spp., whereas the extracts of *S. cylindrica* showed good antibacterial potential for *Staphylococcus aureus*, *S. epidermidis* and *S. pyogenes*. The extracts from all three plants showed antibacterial activity against Gram-negative bacteria, including, *Salmonella* spp. and *Shigella dysenteriae*; organisms causing enteric fever and dysentery. In most of the cases, the extracts from respective calli showed comparable, and in some cases better, result in comparison to the extracts from parent leaves. To the best of our knowledge this is the first preliminary report on antibacterial potential, especially through calli extracts, of these plants; and *in vitro* cultivation of the explants may be used to obtain phytotherapeutic compounds.

Key words: *Pterocarpus marsupium*; *Clitoria ternatea*; *Sanseveiria cylindrica*; Antibacterial activity; *In vitro*; Callus

*Correspondence: M Shahid, Section of Antimicrobial Agents & Drug Resistance Researches, Department of Microbiology, Jawaharlal Nehru Medical College & Hospital, India. Tel: +915712720382; Fax: +915712721776; E-mail: shahidsahar@yahoo.co.in

INTRODUCTION

Pterocarpus marsupium Roxb. (Fabaceae), commonly known as Indian Kino, is a deciduous and multi-purpose leguminous tree which is found in the Deccan Peninsula, Central-India and some parts of North-India. It is an important forest tree that yields timber, ranking next to the Teak and Rose wood in peninsular India. The natural propagation of this tree is through seeds but the percentage of germination is very low in natural conditions (10 - 20%). Because of the low germ-inability and survival rate of the seedlings, the native natural stand of this tree is disappearing fast, and is now listed as an endangered tree legume. In Indian traditional medicine, an aqueous infusion of the wood is known to used in diabetes and milk stored in the vessels made of the wood is reputed to have anti-diabetic qualities (Anonymous, 1988). The tree yields gum, which is a powerful astringent, used for diarrhea, dysentery, leucorrhoea, hemorrhages and toothache (Anuradha and Pullaiah, 1999; Chand and Singh, 2004).

Clitoria ternatea L. (Fabaceae), commonly known as butterfly pea, is distributed in tropical Asia, the Philippine Islands, and Madagascar (Anonymous, 1988). It is an attractive perennial climber with conspicuous blue or white flowers. It is highly palatable forage legume and generally is preferred by livestock over other legumes. Butterfly pea is also used as a cover crop or green manure. The roots are bitter, laxative, intellect promoting, alexetiric, diuretic, anthelmintic, depurative, aphrodisiac and tonic. The root is used in treatment of various diseases such as indigestion, constipation, arthritis and eye infections (Morris, 1999). Leaves are useful in hepatopathy, eruptions and antidotes to animal stings. The seeds are laxative and safe for colic, dropsy, enlargement of abdominal viscera and swollen joint.

Sanseveiria cylindrica Bojer ex Hook (Agavaceae), commonly known as Indian bowstring's hemp, is a stem-less herb arising from a creeping underground rhizome. The plant is native to Tropical Africa however; it grows as an escape from cultivation in the eastern coast of India, and from West Bengal to Tamil Nadu, in south-India. It has cylindrical leaves (20 - 60 cm × 1.3 - 2.5 cm), which are faint-green with black streaks. The plant has long been the source of a fiber used for bowstring in India. The plant has also been reported to contain some important medicinal compounds (Anis and Shahzad, 2005).

Despite the medicinal importance of these plants, the antibacterial activities of the plants have not yet been explored. As the plants have been reported as endangered plants in India, an attempt was made to optimize the *in vitro* callus induction from the leaves and then to find out the antibacterial activity of the extracts from their respective calli as well as from the parent leaf-explants.

MATERIALS AND METHODS

Plant part used

The fresh leaves of *P. marsupium, C. ternatea*, and *S. cylindrica* were collected from Tropical Forest Research Institute, Jabalpur, India; Department of Botany Aligarh Muslim University, Aligarh India; and Botanical Garden of the A.M.U. Aligarh respectively. The plants were identified and deposited in the herbarium of survey of Medicinal Plant Unit of Central Council for Research in Unani Medicine at Aligarh, Uttar Pradesh, India.

In vitro culture of explants for callus induction

Collected explants were subjected to *in vitro* cultivation for callus induction. The explants were washed in running tap water for 30 min followed by 1% Bovestin (a broad spectrum fungicide) for 30 min. The explants were further washed in 5% (v/v) teepol for 10 min., rinsed in sterile DDW and dipped in 70% ethanol for 30 s. The source tissue were surface sterilized with 0.1% (w/v) $HgCl_2$ (Bhojwani and Razdan, 1996) for seven min and thoroughly washed several times in sterile DDW to

remove the traces of mercury ion. The sterilized explants were aseptically inoculated in MS medium (Murashige and Skoog, 1962) containing BA in concentrations of 1, 2, 5, and 10 μM. All the MS-media tubes were agarified with 1% Agar (HiMedia Lab Ltd, India) and incubated at 25 ± 2°C with a relative humidity of 55 ± 5% and exposed for 16 h photocycle of 2,500 Lux intensity. Fifty replicates for each plant explant were prepared and the experiments were repeated thrice.

Plants extracts tested for antibacterial activity

Both aqueous and alcoholic extracts were tested for antibacterial activity. The extracts were derived according to the method of Singh and Singh (2000) with some modifications as described below.

Aqueous extracts

Fresh plant leaves (15 g) and their respective calli were taken, thoroughly washed in sterile DDW, surface sterilized in 70% ethanol (v/v) for 30 s, and then washed thrice in sterile DDW at an interval of 5 min. The sterilized materials were grounded with a sterile pestle and mortal in 150 ml sterile distilled water. The homogenized tissue was centrifuged at 7,000 rpm for 15 min. and the supernatant was taken as the aqueous extract.

Alcoholic extracts

To prepare alcoholic extracts, the fresh plant materials (15 g) were homogenized in 30 ml of 95% ethanol and centrifuged as above. The supernatant was kept in a hot water bath at 60 °C to evaporate the organic solution. The extract was dissolved again in 95% ethanol to achieve the desired concentrations. The extracts were immediately used for experimentation and they were filter sterilized before use.

Bacterial strains used

Standard and clinical bacterial strains (stocked in the department of Microbiology, Jawaharlal Nehru Medical College & Hospital, Aligarh) of various Gram-positive and Gram–negative bacteria were used for detection of antibacterial activity of the extracts. The bacterial strains used were *Staphylococcus aureus* (ATCC 25923), *S. epidermidis*, *Streptococcus pyogenes*, *S. viridans*, *Enterococcus faecalis*, *Bacillus subtilis*, *B. cereus*, *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae*, *Proteus vulgaris*, *P. mirabilis*, *Salmonella typhi*, *S. typhimurium*, and *Shigella dysenteriae type 1*.

Antibacterial susceptibility testing

Mueller Hinton Agar (M 173, HiMedia, India) was used for antibacterial susceptibility testing. For fastidious organisms such as *streptococci*, the agar supplemented with 5% sterile, defibrinated blood was used. Agar well diffusion method as described by Vanden- Berghe and Vlietinck (1991) and Akinpelu (2001) was used in the present study with some modifications as adopted in our previous studies (Shahid *et al.*, 2007). Briefly, stock bacterial solutions were thawed and immediately suspended in Mueller Hinton broth. The turbidity of the bacterial suspensions was adjusted corresponding to 0.5 McFarland Barium sulphate tube. Two sets of Mueller Hinton Agar plates (one for aqueous extracts and the other for alcoholic extracts) were lawn cultured with the respective bacterial suspensions. Wells (7 wells/plate) in a diameter of 5 mm were made in each plate with the help of a sterile borer. A volume of 50 μl of the plant extracts (alcoholic extracts in a concentration of 10 mg/ml) were poured in the wells. Sterile distilled water and ethanol was used to serve as blank controls in the respective plates. The plates were kept upright to 5 - 10 min until the solution diffused into the medium and then incubated aerobically at 37 °C overnight. The cultures of *streptococci* were incubated in an atmosphere enriched with 10% CO_2. Each experiment was performed in triplicate.

Statistical analysis

The Tukey multiple analysis test was used to compare the antibacterial effect of the different extracts (SPSS Software, Chicago, Ill.). *P* values of $\leq$ 0.05 were considered statistically significant.

RESULTS

In vitro callus induction

The cut end of the leaf explants started callusing after 10 days on MS + BA (5 μM), and within 4 weeks, 3 - 5 g compact callus mass was obtained from leaves (Fig. 1A-C). On lowering concentrations of BA to 1 and 2 μM, callusing response was drastically reduced with a very slow growth. However, on higher concentration (10 μM), the growth of the callus was moderate (Table 1). As optimal response was noted on MS + BA (5 μM), the calli from the same medium was used for evaluation of the antibacterial effect.

Antibacterial activity

The detailed results of the antibacterial activities of the aqueous and alcoholic extracts of the explants and their respective calli, against the Gram-positive and Gram-negative bacteria are shown in Tables 2 and 3. Of the aqueous extracts tested, only the extract of *C. ternatea* showed antibacterial activity against limited Gram-positive bacterial species; notably the extracts from leaves and leaves-derived calli against *Bacillus subtilis* and *B. cereus*. Moderate activity against *S. pyogenes* was noticed in extract derived from leaves of *C. ternatea*. Aqueous extracts from the calli of *C. ternatea* showed better antibacterial activity against *Bacillus* spp. as compared to that of extracts derived from respective leaf explants (Table 2). None of the other aqueous extracts showed any antibacterial activity against any of the bacteria tested.

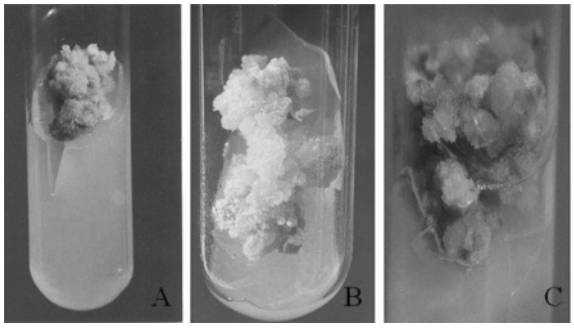

Fig. 1. Calli induced through leaf-explants of *Pterocarpus marsupium* (A), *Clitoria ternatea* (B), and *Sanseveiria cylindrica* (C) on MS + BA (5 μM) medium.

Alcoholic extracts of all the three plants showed antibacterial activity against a wider range of bacteria. Among the Gram-positive bacteria, extracts from *C. ternatea* showed strong antibacterial activity against *Bacillus* spp. The extracts derived from calli of *C. ternatea* showed antibacterial activity against *Streptococcus viridans* and *Enterococcus faecalis* whereas the extracts from parent plant do not (Table 2). The extracts of *S. cylindrica* showed good antibacterial potential for *Staphylococcus aureus*, *S. epidermidis* and *S. pyogenes*. The extracts derived from *P. marsupium* showed remarkable activity against *Bacillus subtilis*, *B. cereus* and *Enterococcus faecalis* (Table 2). The extracts from all three plants showed antibacterial activity against Gram-negative bacteria, including, *Salmonella* spp. and *Shigella dysenteriae*, organisms causing enteric fever and dysentery. The best activity against *Salmonella* and *Shigella* spp. was found in extracts derived from *P. marsupium* (Table 3).

Table 1. Response of BA for callus induction through leaf-explants of *Pterocarpus marsupium*, *Clitoria ternatea*, and *Sanseveiria cylindrica* on MS medium

BAP	*P. marsupium*		*C. ternatea*		*S. cylindrica*	
	% Response	Fresh Wt ± S.D. (g)	% Response	Fresh Wt ± S.D. (g)	% Response	Fresh Wt ± S.D. (g)
1 μM	40 - 60	1.979 ± 2.63	35 - 40	1.570 ± 2.16	30 - 40	1.873 ± 4.34
2 μM	45 - 55	2.769 ± 2.43	35 - 40	1.970 ± 2.86	30 - 40	1.873 ± 4.34
5 μM	70 - 90	4.890 ± 4.27	65 - 70	2.782 ± 4.03	45 - 55	3.040 ± 3.88
10 μM	40 - 60	2.997 ± 2.54	45 - 55	1.970 ± 2.66	30 - 40	1.435 ± 3.25

Data taken after 4 weeks of incubation, ten replicates were taken for each treatment

Table 2. Activity of the aqueous and ethanol extracts of leaf- explants and their *in vitro* raised calli of respective plants against pathogenic Gram-positive bacteria

Extract	Sa	Se	Sp	Sv	Ef	Bs	Bc
	Zone of inhibition (mm) ± S.E.						
P. marsupium							
Leaf							
Aqueous							
Ethanol	14.50 ± 0.28^{d}	12.16 ± 0.16^{d}	14.00 ± 0.00^{c}	15.50 ± 0.00^{b}	14.23 ± 0.12^{b}	14.33 ± 0.16^{d}	14.50 ± 0.00^{d}
Leaf callus							
Aqueous							
Ethanol	12.20 ± 0.11^{g}	12.23 ± 0.14^{d}	12.23 ± 0.14^{d}	10.16 ± 0.16^{c}	12.00 ± 0.00^{c}	16.33 ± 0.16^{c}	16.33 ± 0.16^{c}
C. ternatea							
Leaf							
Aqueous	-	-	8.33 ± 0.33^{e}	-	-	12.16 ± 0.16^{e}	10.00 ± 0.00^{e}
Ethanol	15.16 ± 0.16^{c}	14.30 ± 0.17^{c}	14.26 ± 0.14^{c}	10.33 ± 0.17^{c}	9.50 ± 0.28^{e}	27.33 ± 0.16^{a}	32.16 ± 0.16^{a}
Leaf callus							
Aqueous	-	-	-	-	-	16.00 ± 0.00^{c}	16.00 ± 0.00^{c}
Ethanol	14.00 ± 0.00^{e}	12.00 ± 0.00^{d}	14.33 ± 0.16^{c}	18.33 ± 0.33^{a}	18.33 ± 0.33^{a}	22.26 ± 0.26^{b}	20.00 ± 0.00^{b}
S. cylindrica							
Leaf							
Aqueous	-	-	-	-	-	-	-
Ethanol	20.33 ± 0.33^{a}	18.16 ± 0.16^{a}	17.33 ± 0.33^{a}	16.20 ± 0.20^{b}	11.26 ± 0.26^{c}	6.13 ± 0.13^{f}	6.26 ± 0.13^{f}
Leaf callus							
Aqueous							
Ethanol	19.30 ± 0.15^{b}	16.33 ± 0.17^{b}	16.10 ± 0.10^{b}	16.10 ± 0.10^{b}	11.33 ± 0.16^{c}	6.00 ± 0.00^{f}	6.00 ± 0.00^{f}
Ethanol	13.16 ± 0.16^{e}	12.00 ± 0.00^{d}	14.13 ± 0.13^{c}	16.00 ± 0.00^{b}	11.00 ± 0.00^{d}	6.00 ± 0.00^{f}	6.00 ± 0.00^{f}
DDW	5.00 ± 0.00^{f}	5.00 ± 0.00^{e}	5.00 ± 0.00^{f}	5.00 ± 0.00^{d}	5.00 ± 0.00^{f}	5.00 ± 0.00^{g}	5.00 ± 0.00^{g}
CAM	16.00 ± 0.00^{c}	12.16 ± 0.16^{d}	12.16 ± 0.16^{d}	18.00 ± 0.00^{a}	9.16 ± 0.16^{e}	16.40 ± 0.11^{c}	16.40 ± 0.11^{c}

Diameter of zone of inhibition is a mean of triplicates ± S.E.. Differences were assessed statistically using one-way ANOVA followed by Tukey's test $P \leq 0.05$. The mean represented by same letter is not significantly different within the column. CAM= chloramphenicol (30 ug) disc, DDW= Double Distilled Water. Sa: *Staphylococcus aureus* (ATCC 25923); Se: *S. epidermidis*; Sp: *Streptococcus pyogenes*; Sv: *S. viridians*; Ef: *Enterococcus faecalis*; Bs: *Bacillus subtilis*; Bc: *B. cereus*.

DISCUSSION

The present study explored that in most of the cases the extracts from respective calli showed comparable, and in some cases better, results in comparison to the extracts from parent leaves. In various other studies, on different plant species, the same fact has been reported where they found ethanolic extracts to be more effective than the aqueous extract in inhibiting the bacterial growth (Sener, 1994; Nkere and Iroegbu, 2005). To the best of our knowledge, this is the first report analyzing the antibacterial property of these extracts against a wider range of bacteria, especially through their *in vitro* raised calli, and therefore our results could not be compared with other reports in greater details.

P. marsupium produces numerous secondary metabolites, including flavonoids, and following subclasses have been identified: chalcones, dihydrochalcones and related compounds (e.g. 4,2′,4′-trihydroxychalcone, pterosupin, propterol),

Table 3. Activity of the aqueous and ethanol extracts of leaf- explants and their *in vitro* raised calli of respective plants against pathogenic Gram-negative bacteria

Extract	Ec	Klb	Pv	Pm	Sty	Stym	Shd-1
	Zone of inhibition (mm) ± S.E.						
P. marsupium							
Leaf							
Aqueous	-	-	-	-	-	-	-
Ethanol	16.00 ± 0.00^{c}	16.16 ± 0.16^{b}	16.50 ± 0.00^{c}	14.13 ± 0.13^{c}	15.13 ± 0.10^{b}	16.10 ± 0.10^{a}	17.17 ± 0.17^{a}
Leaf callus							
Aqueous	-	-	-	-	-	-	-
Ethanol	18.13 ± 0.13^{b}	18.00 ± 0.00^{a}	16.26 ± 0.26^{c}	16.00 ± 0.00^{b}	17.06 ± 0.10^{b}	18.13 ± 0.10^{b}	18.83 ± 0.17^{b}
C. ternatea							
Leaf							
Aqueous	-	-	-	-	-	-	-
Ethanol	18.33 ± 0.16^{b}	18.33 ± 0.16^{a}	19.00 ± 0.00^{a}	21.20 ± 0.20^{a}	16.27 ± 0.17^{a}	16.06 ± 0.10^{c}	18.10 ± 0.10^{c}
Leaf callus							
Aqueous	-	-	-	-	-	-	-
Ethanol	15.33 ± 0.33^{d}	16.20 ± 0.20^{b}	14.13 ± 0.13^{d}	13.16 ± 0.16^{d}	14.27 ± 0.18^{a}	14.10 ± 0.10^{c}	13.06 ± 0.10^{c}
S. cylindrica							
Leaf							
Aqueous	-	-	-	-	-	-	-
Ethanol	16.16 ± 0.16^{c}	14.33 ± 0.16^{c}	18.20 ± 0.20^{b}	14.00 ± 0.00^{c}	14.10 ± 0.10^{c}	14.03 ± 0.10^{c}	17.00 ± 0.00^{c}
Leaf callus							
Aqueous	-	-	-	-	-	-	-
Ethanol	19.13 ± 0.13^{a}	16.20 ± 0.20^{b}	16.00 ± 0.00^{c}	16.40 ± 0.23^{b}	12.13 ± 0.10^{c}	12.10 ± 0.10^{c}	18.03 ± 0.17^{c}
Ethanol	11.00 ± 0.00^{e}	11.33 ± 0.16^{d}	10.10 ± 0.10^{e}	11.00 ± 0.00^{e}	11.00 ± 0.00^{c}	12.00 ± 0.17^{b}	12.00 ± 0.00^{c}
DDW	5.00 ± 0.00^{f}	5.00 ± 0.00^{e}	5.00 ± 0.00^{f}	5.00 ± 0.00^{f}	5.00 ± 0.00^{e}	5.00 ± 0.00^{d}	5.00 ± 0.00^{d}
CAM	16.00 ± 0.00^{c}	00.00 ± 0.00^{f}	16.00 ± 0.00^{c}	16.00 ± 0.00^{b}	18.00 ± 0.00^{e}	19.00 ± 0.00^{e}	22.00 ± 0.00^{e}

Diameter of zone of inhibition is a mean of triplicates ± SE. Differences were assessed statistically using one way ANOVA followed by Tukey's test $P \leq 0.05$. The mean represented by same letter is not significantly different within the column. See Table 2 for the description of abbreviations. Ec: *Escherichia coli* (ATCC 25922); Klb: *Klebsiella Pneumoniae*; Pv: *Proteus vulgaris*; Pm: *P. mirabilis*; Sty: *Salmonella typhi*; Stym: *S. typhimurium*; Shd-1: *Shigella dysenteriae type 1;* BA: N^{6}-benzyladenine

flavones (e.g. 7,4′-dihydroxyflavone, liquiritigenin, isoliquiritigenin), isoflavones (e.g. 7-hydroxy-5,4′-dimethoxy-8-methylisoflavone-7-rhamnoside), aurones (e.g. marsupin, 6,4′-dihydroxy-7-methylaurone-6-*O*-rhamnoside, 4,6,3′,4′-tetrahydroxyaurone-6-*O*-rhamnoside), and (-)-Epicatechin (Dev, 2006). Several flavonoids of *P. marsupium* have shown antibacterial activity, and of which "propterol" have been reported to possess most potent antibacterial activity (Dev, 2006). A protein designated "finotin" has also been isolated from *C. ternatea* that showed some antimicrobial activity (Kelemu *et al.*, 2004). *C. ternatea* also produces a wider range of secondary metabolites including triterpenoids, flavonol glycosides, anthocyanins and steroids (Mukherjee, 2008). However, during our search of extensive literature, we could not get any study describing in detail the active constituents possessing antibacterial property from the calli of these plants. We presume that possibly the compounds discussed above, to occur in native plants, could also be responsible for antimicrobial activity of the extracts from their respective calli. We are planning further researches in this area and suggest that looking for specific

active compounds in these calli would prove rewarding. Moreover, studies looking for interaction between these extracts and animal cells, especially observing the cytotoxicity, are also elementary; whatever literature present is of decades earlier. For example, Bhakuni *et al.* reported in year 1971 that ethanol (50%) extracts of *P. marsupium* had LD_{50} > 1,000 mg/kg in mice. Researches looking for the cytotoxicity of these extracts, especially from calli, will also provide new rooms for this rewarding area.

Based on this preliminary study, it is thus suggested that the alcoholic extracts of these plants, including those from their respective calli, may be used in phytotherapy as an antibacterial agent. As the extracts from the calli gave good results, *in vitro* cultivation of the explants may be used to obtain phytotherapeutic compounds, especially, at places where the plants cannot be grown because of the adverse atmospheric conditions. Through *in vitro* cultivation it would also be possible to preserve and conserve these important endangered plant species. Moreover, the bioactive compounds responsible for the antibacterial effects could further be enhanced in the culture conditions by nutritional and hormonal manipulations in the cultivation medium.

ACKNOWLEDGEMENTS

M Shahid and A Shahzad are grateful to Department of Science & Technology, Ministry of Science & Technology, Government of India for awarding "Young Scientist Project Awards" under sanction numbers FT/SR-L-111/2006 and SR/FT/L-23/2006, respectively. The authors also wish to thank A Arif and Uzma Zafar for technical assistance, M Mansoor for photographic, and Ikramuddin for statistical assistance.

REFERENCES

Akinpelu DA. (2001) Antimicrobial activity of *Anacardium occidentale* bark. *Fitoterapia* **72**, 286-287.

Anis M, Shahzad A. (2005) Micropropagation of *Sansevieria cylindrica* Bojer ex Hook through leaf disc culture. *Propag. Ornament. Plant.* **5**, 119-123.

Anonymous. (1988) The wealth of India: a dictionary of Indian raw materials and industrial products, Vol. II. New Delhi: Publication and Information Directorate, Council of Scientific and Industrial Research Publications (CSIR).

Anuradha M, Pullaiah T. (1999) In vitro seed culture and induction of enhanced axillary branching in *Pterocarpus santalinus* and *Pterocarpus marsupium* : a method for rapid multiplication. *Phytomorph.* **49**, 157-163.

Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Gupta B, Srimal RC. (1971) Screening of Indian plants for biological activity. III. *Indian J. Exp. Biol.* **9**, 91-102.

Bhojwani SS, Razdan MK. (1996) Plant Tissue Culture: Theory and Practice, pp. 31-32, Elsevier, Amsterdam.

Chand S, Singh AK. (2004) In vitro shoot regeneration from cotyledonary node explants of a multipurpose leguminous tree, *Pterocarpus marsupium* Roxb. *In vitro Cell. Div. Biol. Plant.* **40**, 167-170.

Dev S. (2006) A Selection of Prime Ayurveduc Plant Drugs-Ancient Modern Concordance, pp. 359-362, Anamaya Publishers, N. Delhi.

Kelemu S, Cardona C, Segura G. (2004) Antimicrobial and insecticidal protein isolated from seeds of *C. ternatea* (L.): a tropical forage legume. *Plant Physiol. Biochem.* **42**, 867-873.

Morris JB. (1999) Legume genetic resources with novel 'value added' industrial and pharmaceutical use. In: Perspectives on new crops and new uses, edited by Janick J, pp. 196-201, Alexandria, VA: ASHS Press.

Mukherjee PK, Kumar V, Kumar NS, Heinrich M. (2008) The Ayurvedic medicine *Clitoria ternatea*-From traditional use to scientific assessment. *J. Ethnopharmacol.* **120**, 291-301.

Murashige T, Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Planta* **15**, 473-497.

Nkere CK, Iroegbu CU. (2005) Antibacterial screening of the root, seed and stembark extracts of *Picralima nitida. African J. Biothech.* **4**, 522-526.

Sener B. (1994) Recent results in the search of bioactive compounds from Turkish medicinal plants. *Pure Appl. Chem.* **66**, 2295-2298.

Shahid M, Shahzad A, Malik A, Anis M. (2007) Antibacterial activity of aerial parts as well as *in vitro* raised calli of the medicinal plant *Saraca asoca* (Roxb.) de Wilde. *Canadian J. Microbiol.* **53**, 75-81.

Singh I, Singh VP. (2000) Antifungal properties of aqueous and organic extracts of seed plants against *Aspergillus flavus* and *A. niger*. *Phytomorph.* **50**, 151-157.

Vanden-Berghe DA, Vlietinck AJ. (1991) Screening methods for antibacterial and antiviral agents from higher plants. In: Methods in Plant Biochemistry, edited by Dey PM, Harborne JB, and Kohostettmann, pp.47-70, Academic Press, London.