

Medicinal and Aromatic Plant Science and Biotechnology

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Scope and target readership: *Medicinal and Aromatic Plant Science and Biotechnology* will provide a complete analysis and understanding of all aspects of medicinal and aromatic plant science and biotechnology.

Medicinal and Aromatic Plant Science and Biotechnology primarily wishes to examine:

- 1) Cultural practices (greenhouse growth, hydroponics, aeroponics, organic farming);
- 2) *In vitro* propagation (micropropagation, somatic embryogenesis, tissue culture, bioreactor system production);
- 3) Mycorrhizal symbioses (and effects on plant physiology, productivity, reproduction and disease resistance);
- 4) Novel techniques for analysis (genetic, biochemical, biophysical);
- 5) Physiology, genetics, molecular biology, structural botany (integrated, pure and applied);
- 6) Pathology;
- 7) Production of secondary metabolites, organic and inorganic biochemistry, and phytochemistry; pharmacological properties;
- 8) Storage of valuable genetic material (cold-storage or cryopreservation).

For publication in *Medicinal and Aromatic Plant Science and Biotechnology* the research must provide a highly significant new contribution to our understanding of medicinal and aromatic plants (from any climatic or geographic origin) and must generally be supported by a combination of either: physiological, biochemical, genetic or molecular analyses. All areas of study are welcome and the experimental approaches used can be wide-ranging. Results that simply provide a description without an integrated multi-disciplinary approach might not be considered, as might descriptive or overly-localized studies and reports on conventional propagation without a wide impact. Manuscripts pertaining to breeding, post-harvest technology, nutritional aspects or influences on human health of medicinal and aromatic plants will also be considered.

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Cover photos: Left plate: Stages of *Plumbago rosea* micropropagation (Reddy *et al.*, pp 85-89). Top, center plate: Some indigenous orchids of Bangladesh with therapeutic uses (Hossain, pp 100-106). Bottom, center plate: Direct organogenesis and rooting of *in vitro* propagated shoots of *Andrographis paniculata* (Roy *et al.*, pp 94-96). Top right: *Linum grandiflorum* Desf. leaves (Mohammed *et al.*, pp 37-41). Center right: *Sclerocarya birrea* tree; Bottom right: bark of *Bauhinia rufescens* (Usman *et al.*, pp 110-116). Chemical structures: Quercetin-3-*O*-(3"-*O*-galloyl)- α -D-apiofuranoside (lower right) and quercetin-3-*O*-galactopyranoside (upper left), two phenolic compounds from *Dichrostachys cinerea* L. (Abou Zeid *et al.*, pp 42-49).

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SPECIAL ISSUE: Proceedings of 4th Conference on Research and Development of Pharmaceutical Industries (Current Challenges)

Nagwa M. Ammar, Lamia T. Abou El-Kassem, Nabil H. El-Sayed (Egypt), Lalita M. Calabria, Tom J. Mabry (USA) Flavonoid Constituents and Antimicrobial Activity of Date (*Phoenix dactylifera* L.) Seeds Growing in Egypt (pp 1-5)

ABSTRACT

Original Research Paper: The methanolic extract of date palm (*Phoenix dactylifera* L.) seeds cultivated in El-Dakhla Oases, Egypt, was investigated for their phenolics and antimicrobial activity. Seven flavonoids were isolated and identified as isoquercetrin (1), luteolin 7-O- β -D-neohesperopyranoside 3'-O-methylether (2), luteolin 7-O- β -D-neohesperopyranoside (3), acacetin 7-O- β -D-neohesperopyranoside (4), apigenin 7-O- α -D-apiofuranoside (5), apigenin 7-O- α -D-apiofuranosyl-(1 \rightarrow 2)-O- β -D-glucopyranoside (6) and genistein 8-C- β -D-glucopyranoside (7). The structures were determined mainly by spectroscopic methods (UV, ¹H-, ¹³C-NMR, 2D-NMR). Results of an antimicrobial test showed that the methanolic extract of the *P. dactylifera* seeds moderately inhibited the growth of Gram-positive and Gram-negative bacteria.

Nagwa E. Awad, Hemaia M. Motawe, Mohamed A. Selim, Azza A. Matloub (Egypt) Antitumorigenic Polysaccharides Isolated from the Brown Algae, *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe (pp 6-11)

ABSTRACT

Original Research Paper: Mannitol, other carbohydrates of low-molecular weight, fucose-containing polysaccharides, laminarin and alginic acid were isolated from Egyptian brown algae *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe. Fucose-containing polysaccharides were isolated with cold and hot water; glucuronic acid was the major hydrolysate in both extracts isolated from *Padina pavonia* (L.) Gaill. while glucuronic and galacturonic acids were the major hydrolysate in cold extract polysaccharides of *H. clathratus*; fucose was the major hydrolysate in hot extract polysaccharides. The hot extracts of both were achieved by fractional precipitation with ethanol. The hot water extract of both algae and their fractions exhibited antitumour properties against HepG2 human cell line *in vitro*.

Nagwa E. Awad, Hemaia M. Motawe, Mohamed A. Selim, Azza A. Matloub (Egypt) Volatile Constituents of the Brown Algae *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe and their Antimicrobial Activity (pp 12-15)

ABSTRACT

Short Communication: GC/MS analysis of the volatile oils of *Padina pavonia* (L.) Gaill. and *Hydroclathrus clathratus* (C. Agardh) Howe led to identify 54 volatile constituents in the former, or 75.64% of total constituents and 39 in the latter, or 92.67% of total constituents. The volatile constituents of both brown algae consisted mainly of esters (25.06 and 42.25%), hydrocarbons (20.38 and 12.63%) and fatty acids (6.59 and 11.68%). The antimicrobial activity of the volatile fractions of these algae was tested on 12 microorganisms (6 bacteria, 2 yeasts and 3 fungi). The volatile fraction of *P. pavonia* exhibited obvious antimicrobial activity against *Bacillus cereus* compared with amoxycillin as the reference drug while the volatile fraction of *H. clathratus* showed pronounced antimicrobial activity against *Saccharomyces cerevisiae* compared with canestin.

Salma Ahmed El-Sawi, Mohamed Elsayed Ibrahim, Amal M. Ali (Egypt) *In Vitro* Cytotoxic, Antioxidant and Antimicrobial Activities of Essential Oil of Leaves of *Laurus nobilis* L. Grown in Egypt and its Chemical Composition (pp 16-23)

ABSTRACT

Original Research Paper: In this study, the essential oil (EO) of leaves of *Laurus nobilis* L. (family Lauraceae) was evaluated as a new source of EO under Egyptian conditions. The EO was extracted by hydrodistillation yielding 0.5-0.8% (v/w). The EO was analyzed by GC/MS. Fifty three compounds were identified accounting for 96% of the total EO constituents. The fresh EO contains approximately 50.38% 1,8-cineole. Other major oxygenated monoterpenes were α -terpenyl acetate (19.97 %) and 4-terpineol (6.48%). α -Terpinene, γ -eudesmole, α -terpineole, and 3-carene were identified in concentrations above 1%. Minor qualitative and quantitative differences were reported in the constituents of the leaf EO after cold storage (4°C) for one year. The *in vitro* cytotoxicity of the EO on five human cancer cell lines (Hela, U-251, HepG2, MCF7 and H460) was examined. The EO was found to be very active against all five cell lines tested. The EO was also found to possess antioxidant activity as

demonstrated by the 1-diphenyl-2 picrylhydrazyl (DPPH) radical method. The antimicrobial activities of the EO were tested using the inverted Petri-plate method. The EO showed prominent antimicrobial activities at a very low concentration (100 µl).

Rasmia Ali Hassan, Amira Abdelazim Habib, Azza Amin Ezz El-Din (Egypt) Effect of Nitrogen and Potassium Fertilization on Growth, Yield and Alkaloidal Content of Periwinkle (*Catharanthus roseus* G. Don) (pp 24-26)

ABSTRACT

Short Communication: Two field experiments were conducted at the farm of Saft El-Laban, Giza, Egypt, during two successive seasons (2005 and 2006) to study the effect of nitrogen (N) and potassium (K) fertilization on growth, yield and alkaloidal content of periwinkle (*Catharanthus roseus* G. Don). Periwinkle seedlings were transplanted from the nursery to a permanent field in April in both seasons. The trials included three doses of N (50, 100 and 150 Kg/fed (4200 m²). as ammonium nitrate) and three doses of K (25, 50 and 75 Kg/fed as potassium sulfate) and their combinations. Plant height (cm), number of branches per plant, fresh and dry weights of plant, as well as the herb yield were determined. These applications produce a significant increase in all the different characters under investigation. The high level of both N and K resulted in the highest values of all parameters. The yield of total *C. roseus* alkaloids under different fertilization treatments was determined with TLC densitometry analysis, where the highest percentage were recorded in plants fertilized with 150 kg/fed of N and 25 kg/fed of K. Special attention had been paid in this paper to the indole alkaloids, specifically vincristine and vinblastine.

Nabawya Ibrahim, Ahmed Shalaby, Radwan Farag, Gamal Elbaroty, Emad Hassan (Egypt) Chemical Composition and Biological Evaluation of *Vitex agnus-castus* L. (pp 27-31)

ABSTRACT

Original Research Paper: The chemical composition of the essential oil (EO) of chaste tree (*Vitex agnus-castus*) leaves, Verbenaceae, was identified by GC/MS analysis. The oil contained hydrocarbons (62.77%) and oxygenated compounds (36.38%) as the main chemical groups. Mono-, sesqui- and diterpenoid compounds represented 47.37, 49.12 and 2.69%, respectively. T-caryophyllene (18.76%) was the major constituent, followed by 1,8-cineole (17.38), sabinene (15.38%) and germacrene B (13.72%). The unsaponifiable matter (USM) content of chaste tree fruits was 65%. The most predominant hydrocarbons identified by GLC were: tricosane (9.99%), heptadecane (7.76%), dotriacontane (7.74%), eicosane (7.45%), hexadecane (7.28%), β -sitosterol (5.56%), campesterol (1.01), and stigmasterol (1.32%). GLC of fatty acid methyl esters revealed that oleic acid (26.11%) and linoleic acid (24.76%) were the major unsaturated fatty acids, while palmitic acid (21.01%) was the major saturated fatty acid. Crude proteins of chaste tree fruits amounted to 6.65%. The protein hydrolysate was analyzed with an amino acid analyzer. Seventeen amino acids were detected; the major essential ones were leucine (7.85%), phenylalanine (6.09%) and threonine (5.83%); the non-essential ones were glutamic acid (15.95%), aspartic acid (14.61%) serine (11.99%), glycine (7.38%) and alanine (6.62%). The mucilage hydrolysates, analyzed by HPLC, were characterized by the presence of a high concentration of galacturonic acid and rhamnose. The antimicrobial activity of the EO was performed and the minimum inhibitory concentration (MIC) determined. The growth of both Gram-positive and -negative bacteria yeast and fungi were inhibited at a 1:50 (v/v) dilution; the growth of *Candida albicans* (yeast) and *Aspergillus niger* (fungus) were completely inhibited at 19 mg/ml. Also, the ethanolic extract (70%) of fruits showed antioxidant and antidiabetic activities.

Amal A. Mohamed (Egypt) Effect of Low Dose Gamma Irradiation on some Phytochemicals and Scavenger Ability of *in Vitro* Culantro (*Eryngium foetidum* L.) Plantlets (pp 32-36)

ABSTRACT

Original Research Paper: This research work aimed to enhance the concentration of some bioactive compounds in culantro (*Eryngium foetidum* L.) plantlets through gamma irradiation (0.0, 10.0, 20.0 and 40.0 Gy range) treatments. Gamma irradiation at 40.0 Gy significantly stimulated total phenolic, flavonoids, tannin and saponin contents. The effect of irradiation on antioxidant properties of culantro extracts was investigated by radical-scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals and the content of thiobarbituric acid-reactive substances (TBARS). Some significant changes were observed in thiobarbituric acid-reactive substances between non-irradiated and irradiated samples. Irradiation at 40 Gy affected the DPPH radical-scavenging activity significantly. Total soluble sugars increased from 2.68 g/100 g dw at 0.0 Gy to 6.08 g/100 g dw at 10.0 Gy. Gamma radiation enhanced the level of ascorbic acid accumulation compared to control (non-irradiated) plantlets. Total chlorophyll decreased significantly under all irradiation treatments. These results suggested that both low γ -irradiation doses and tissue culture techniques could be used to produce culantro plantlets with high quantities of certain metabolites.

Magdy M. D. Mohammed (Denmark/ Egypt), Lars P. Christensen (Denmark), Nabaweya A. Ibrahim, Nagwa E. Awad, Ibrahim F. Zeid (Egypt), Erik B. Pedersen, Kenneth B. Jensen (Denmark), Paolo L. Colla (Italy) Anti-HIV-1 Activities of the Extracts from the Medicinal Plant *Linum grandiflorum* Desf. (pp 37-41)

ABSTRACT

Original Research Paper: As part of our screening of anti-AIDS agents from natural sources e.g. *Ixora undulata*, *Paulownia tomentosa*, *Fortunella margarita*, *Aegle marmelos* and *Erythrina abyssinica*, the different organic and aqueous extracts of *Linum grandiflorum* leaves and seeds were evaluated *in vitro* by the microculture tetrazolium (MTT) assay. The activity of the tested extracts against multiplication of HIV-1 wild type III_B, N119, A17, and EFV^R in acutely infected cells was based on inhibition of virus-induced cytopathicity in MT-4 cells. Results revealed that both the MeOH and the CHCl₃ extracts of *L. grandiflorum* have significant inhibitory effects against HIV-1 induced infection with MT-4 cells. The MeOH extract of the leaves is more potent than other extracts against MT-4 cell cultures infected with the wild type HIV-1, strain III_B with an ED₅₀ of 46 ± 6 μM, while the CHCl₃ extract of the seeds is more potent than other extracts against MT-4 cell cultures infected with the double mutation K103N+Y181C with an ED₅₀ of 57 ± 4 μM.

Aisha Hussein Abou Zeid, Mohamed Saeed Hifnawy, Reda Sayed Mohammed (Egypt) Phenolic Compounds and Biological Activities of *Dichrostachys cinerea* L. (pp 42-49)

ABSTRACT

Original Research Paper: Two new flavonoidal compounds in addition to eight known ones, as well as two phenolic acids (1→12) were isolated for the first time from the methanol successive extract of the aerial parts of *Dichrostachys cinerea* by fractionation on polyamide column chromatography and further purification on a Sephadex column. They were identified by studying their chromatographic, hydrolytic and spectral characters (UV, ¹H-NMR, ¹³C-NMR, ESI). These compounds were identified as: (1) 4, 3', 4'- trihydroxyauron-6-O-galactopyranoside, (2) quercetin-3-O-*apiosyl*-3''O-gallate, (3) myricetin-3-O-rhamnopyranoside, (4) myricetin-3-O-glucopyranoside (5) quercetin-3-O- α -L-rhamnopyranosyl (1'''→6'')-O- β -D-glucopyranoside, (6) quercetin-3-O-rhamnopyranoside, (7) quercetin-3-O-glucopyranoside, (8) quercetin-3-O-galactopyranoside, (9) apigenin, (10) kaempferol, (11) isovanillic acid, and (12) vanillic acid. The LD₅₀ of the total ethanol extract was 6.9 g/kg body weight. The anti-ulcer and anti-inflammatory activities of the total ethanol extract and successive extracts (petroleum ether, chloroform, ethyl acetate, and methanol) of the aerial parts of *D. cinerea* were studied. The total ethanol extract (200 mg/kg body weight) and the chloroform successive extract (100 mg/kg body weight) were found to exhibit the highest anti-ulcer activity (71.5% protection), and anti-inflammatory activity (89.83% potency), respectively. The antioxidant activity measured by DPPH using electron spin resonance spectroscopy of some of the isolated compounds was evaluated. Compounds 3 and 4 having a myricetin nucleus were found to exhibit the highest scavenging activity (95.70 and 94.60%, respectively) at 1 mg/ml as compared with ascorbic acid.

Samy Mostafa Mohamed, Elsayed Abou El-fotowh Omer (Egypt) Seasonal Variation in the Volatile Oil of *Myoporum laetum* Leaves (pp 50-51)

ABSTRACT

Short Communication: *Myoporum laetum* (Myoporaceae), an evergreen shrub, is cultivated in Egypt as an ornamental plant and flowers from May to June. In order to study the changes of the essential oil (EO) percentage and its main components due to seasonal variation, four samples were taken from the same tree in February, May, August and November. EO percent was determined separately in each sample by hydrodistillation. The EO percentage was 0.12, 0.18, 0.23 and 0.13%, respectively. GC/MS analysis of the resulting EO revealed the presence of 27 compounds, the major one being elemicin, a sesquiterpene, which ranged from 16.6 to 50%. Ngaione, the second major compound, a furanoid sesquiterpene ketone, ranged from 26 to 44.7% (v/w) of the EO. There was a clear effect by season on EO production and its chemical composition. The major constituents of the EOs were sesquiterpenes. The oxygenated compounds were more than non-oxygenated ones in all samples.

Khalid A. Khalid, Sahar M. Zaghloul, Abd-Elazim A. Yassen (Egypt) Responses of Thyme (*Thymus vulgaris* L.) to Magnesium Applications (pp 52-57)

ABSTRACT

Original Research Paper: The effects of soil and foliar applications of Mg^{2+} ($MgSO_4$) on *Thymus vulgaris* L. plants were investigated in sandy soil during two successive seasons, 2007 and 2008. 20 g L^{-1} $MgSO_4$ was most effective, resulting in a significant increase in vegetative growth characters, essential oil (EO) content, main components of EO, photosynthesis pigments, total sugars, protein and most nutrient contents.

Jafer Adinee, Khosro Piri, Omid Karami (Iran) Essential Oil Composition of Lemon Balm (*Melissa officinalis* L.) Leaves Grown in Hamadan Province, Iran (pp 58-60)

ABSTRACT

Short Communication: This paper focuses on the analysis of the chemical composition of lemon balm essential oil (EO) cultured in Hamedan province, Iran. The EO of leaves (0.32% yield, w/v) was obtained by steam distillation with a Clevenger apparatus and analyzed by capillary GC and GC/MS. 18 substances were identified. The main components of the EO were geraniol (44.23%), citronellol (23.3%), β -caryophyllene (5.66%), citronellal (4.74%), spathulenol (3.4%), geranyl acetate (3.3%) and γ -muroloene (2.13%).

Abiodun Falodun (Nigeria), Rizwana Siraj, Muhammad Irfan Qadir, Sheraz Ahmad Khan Tanoli, Muhammad Iqbal Choudhary (Pakistan) Chemical Composition and Insecticidal Activity of Volatile Oil of *Khaya grandifoliola* (pp 61-63)

ABSTRACT

Short Communication: *Khaya grandifoliola* has been used in traditional medicine against infections, rheumatic and parasitic diseases. The essential oil (EO) from the stem bark of *K. grandifoliola* was extracted with *n*-hexane, analysed by GC and GC-MS. The insecticidal activity of the EO was evaluated against two insects, *Rhyzopertha dominica* and *Tribolium castaneum* using an established standard procedure. The chemical composition of the EO obtained through GC-MS revealed 24 components. The major components were α -pinene (10.56%), limonene (1.25%), β -carophyllene (3.87%), β -pinene (7.80%), α -phellandrene (7.45%), and citronellol (5.10%). The EO exhibited significant insecticidal activity against *Rhyzopertha dominica* and *Tribolium castaneum* (85 and 80%, respectively).

M. Naeem, M. Masroor A. Khan, Moinuddin, Manzer H. Siddiqui, M. Nasir Khan (India) Role of Calcium in Ameliorating Photosynthetic Capacity, Nitrogen Fixation, Enzyme Activities, Nutraceuticals and Crop Productivity of Hyacinth Bean (*Lablab purpureus* L.) under Calcium-Deficient Soil (pp 64-73)

ABSTRACT

Original Research Paper: Plant biological yield appears to be comparatively low in calcium (Ca)-deficient soils of Aligarh, Western Uttar Pradesh, India. Here, Ca deficiency poses a serious yield and quality limitation for several crops, including various legumes. Hyacinth bean (*Lablab purpureus* L.) is a good source of vegetable protein in the human diet. Its seeds and pods contain as much as 20-28% protein. It contains tyrosinase enzyme, which has potential use in the treatment of hypertension. Because of the importance for hyacinth bean as a bio-functional medicinal legume, an experiment was designed to determine whether Ca application through soil could enhance hyacinth bean production, nitrogen fixation, photosynthesis, enzymatic activities, nutraceuticals and quality attributes for this legume. The plants were grown in pots containing soil and supplied with five levels of calcium, viz. 0, 40, 80, 120 and 160 mg Ca kg^{-1} soil applied as calcium chloride ($CaCl_2$). The performance of the crop was assessed in terms of various growth, physiological, biochemical, yield and quality attributes at 60, 90, 120 and 150 days after sowing (DAS). Ca application proved to be significantly effective on most of the parameters studied. Of the five levels, Ca at 120 mg kg^{-1} soil showed the best results, significantly stimulating most of the attributes studied at 60, 90, 120 and 150 DAS. In fact, this level of Ca increased seed yield, seed protein content and tyrosinase activity by 30.3, 16.6 and 20.3%, respectively, compared to control plants. This need for Ca by hyacinth bean should be included as a fertilizer recommendation for this region.

Parale Anuradha Prakash, Nikam Tukaram Dayaram (India) Influence of Auxins, Cytokinins and Biotic Elicitors on Accumulation of Memory Enhancer Compound Bacoside-A in Tissue Culture of *Bacopa monniera* (L.) Pennell. (pp 74-81)

ABSTRACT

Original Research Paper: The effects of the auxins [indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA) and

2,4-dichlorophenoxyacetic acid (2,4-D)], cytokinins, [6-benzyladenine (BA), Kinetin (Kin) and thidiazuron (TDZ)] and biotic elicitors were evaluated individually and in combinations, on callus proliferation, shoot growth and accumulation of bacoside-A through liquid and semi-solid nutrient media. Maximum shoot proliferation (19.4 ± 0.91 shoots/explant) was observed in liquid MS medium containing $5 \mu\text{M}$ BA, which was 1.3 times higher than semi-solid MS medium fortified with same concentration of BA. The callus and shoots showed sustained growth and accumulation of bacoside-A even after two years of initiation of subcultures. The nature of nutrient media did not influence the accumulation of bacoside-A in shoot biomass. Except for *Saccharomyces cerevisiae*-derived elicitors that showed about a 20% increase in shoot biomass production, the growth of callus as well as shoots was inhibited after the addition of other fungal elicitors in MS medium. Among the shoot and callus cultures exposed to *Penicillium notatum*, *Rhizopus stolonifer*, *Coriolus versicolor*, *Mucor* sp. and *S. cerevisiae*-derived elicitors, incorporation of 750 mg/l *S. cerevisiae* or *Mucor* sp. showed about 3.2- and 1.7-fold higher production of bacoside-A in callus and shoot biomass, respectively. This protocol would be helpful in reducing pressure on natural populations of *Bacopa monniera* (L.) Pennell. harvested indiscriminately for bacoside-A.

Abdol Rahman Rahimi, Kambiz Mashayekhi, Sheno Amini, Elias Soltani (Iran) Effect of Mineral vs. Biofertilizer on the Growth, Yield and Essential Oil Content of Coriander (*Coriandrum sativum* L.) (pp 82-84)

ABSTRACT

Short Communication: In order to study the influence of nitrogen and biofertilizer on the growth, yield, and essential oil content of coriander (*Coriandrum sativum* L.), an experiment was conducted in 2007 at the experimental field of Gorgan University, Iran. Treatments were control (T1), biofertilizer (*Azotobacter* + *Azospirillum*) (T2), biofertilizer + 37.5 kg N (T3), and 75 kg N without inoculation (T4). Application of T4 and T3 significantly increased plant height, number of branches/plant, total dry weights, fruit yield, essential oil (EO) percentage, EO yield/plant, content of linalool in EO and linalool yield compared with control. The highest values were always obtained by T3 for all traits, followed by T4, but there were no significant differences in most cases. The lowest values were obtained in the control.

Kalpana N. Reddy, A. Manoj Kumar, N. B. Shanmugam (India) Simple and Rapid Regeneration of *Plumbago rosea* using BAP for Direct and Indirect Plant Regeneration from Leaf and Nodal Explants (pp 85-89)

ABSTRACT

Original Research Paper: A rapid and highly effective method for plant micropropagation from leaf bits and vegetative shoot buds was established for the medicinal plant *Plumbago rosea* Linn. The number of multiple shoots per explant ranged from 8-10 when cultured on Murashige and Skoog (MS) basal medium supplemented with 0.5 mg l^{-1} indole-3-butyric acid (IBA) and 6 mg l^{-1} 6-benzylamino purine (BAP). Nodal explants initially produced basal callus followed by 10 ± 0.71 multiple shoots. Recently matured leaves i.e., third leaves from the top, when cultured on MS with 0.25 mg l^{-1} BAP, 0.25 mg l^{-1} kinetin and 0.5 mg l^{-1} ascorbic acid and maintained in the dark, produced dark green calli which in later stages produced shoot buds in a 16-h photoperiod regime. About 12 ± 1.22 multiple shoots also developed directly within 15 days from the leaf margin as well as from the wounds when maintained at a 16-h photoperiod regime. More than 85% of the matured leaf explants regenerated shoot buds, while very young or fully mature leaves did not respond. Excised shootlets cultured on half-strength MS with 0.2 mg l^{-1} IBA rooted within 45 days. Successful transfer of the rooted shots to pots was accomplished with 100% survival.

Mohammad J. Saharkhiz, Askar Ghani, Mohammad Hassanzadeh-Khayyat (Iran) Changes in Essential Oil Content and Composition of Clary Sage (*Salvia sclarea*) Aerial Parts during Different Phenological Stages (pp 90-93)

ABSTRACT

Short Communication: The variations in quantity and quality of essential oils (EOs) of *Salvia sclarea* L. (Lamiaceae) were examined at different phenological stages (i.e. rosette, stem initiation, full flowering, and fruit set) of the life cycle of species. The EOs of air-dried samples were obtained by hydrodistillation. The yields of EOs (w/w %) were 0.23, 0.15, 1.36, and 1.35% at rosette, stem initiation, full flowering, and fruit set stages, respectively. The EOs were analyzed by GC and GC-MS. A total of 17, 27, 19, and 51 components were identified and quantified at the above mentioned stages, respectively. The proportions of linalool and linalyl acetate, as major oil constituents were highest at the full flowering stage. However, the main group of compounds at the rosette stage was the monoterpene esters (53.36%) and at stem initiation the sesquiterpenes (57.7%). Moreover, monoterpene alcohols were the predominant compounds at full flowering (43.7%) and at fruit set (34.88%) stages.

Soma Roy, Archana Giri, Chodiseti Bhubaneswari, M. Lakshmi Narasu, Charu C. Giri (India) High Frequency Plantlet Regeneration via Direct Organogenesis in *Andrographis paniculata* (pp 94-96)

ABSTRACT

Research Note: An efficient protocol was standardized for high frequency plant regeneration via organogenesis from stem base explants of *Andrographis paniculata*. Seeds of *A. paniculata* were germinated on MS basal media. Shoot multiplication occurred on MS media supplemented with various concentrations of cytokinins viz. benzyladenine (BA), kinetin (Kn), thiodiazuron (TDZ) and zeatin (Zn). However, high frequency direct organogenesis generated maximum number of adventitious shoots ($62.0 \pm 4.2/\text{explant}$) on MS media supplemented with 2.0 mg/l Zn. Histological observations revealed the organogenic path of regeneration. Shoot elongation from proliferated shoots occurred on MS media supplemented with BA (2.0 mg/l). Shoots rooted after they were dipped in indole-3-butyric acid (1000 mg/l) and upon its further transfer to MS basal media.

Adewale Adewuyi, Rotimi Ayodele Oderinde, Mopelola A. Omotoso (Nigeria) Comparative Study of the Antibacterial and Cytotoxicity of the Essential Oils from the Leaves, Stem Bark and Roots of *Blighia unijugata* Baker (Sapindaceae) (pp 97-99)

ABSTRACT

Research Note: The antimicrobial activity of the essential oils (EOs) from *Blighia unijugata* Baker (Sapindaceae) was studied. EO was extracted from the leaves (BUL), stem bark (BUB) and roots (BUR) by hydrodistillation. These were all colorless and soluble in water except for BUB. The EOs were active against *Proteus mirabilis*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas mallei*. BUB EO had the highest inhibitory zone (40 mm) against *E. coli*. BUB was also active against all the tested organisms except for *P. fluorescens*. All the EOs exhibited significant inhibitory activity against the pathogenic microorganisms. They all showed potent cytotoxicity with an LC_{50} of 85.20 $\mu\text{g/ml}$ (BUL), 70.50 $\mu\text{g/ml}$ (BUR) and 155.10 $\mu\text{g/ml}$ (BUB), which suggests an ethnomedicinal application of these EOs.

Mohammad Musharof Hossain (Bangladesh) Traditional Therapeutic Uses of Some Indigenous Orchids of Bangladesh (pp 100-106)

ABSTRACT

Research Note: The traditional therapeutic uses of some indigenous orchids of Bangladesh are described in this paper. Terrestrial (11) and epiphytic (18) orchids, 29 in total, are used by Bangladeshi rural and tribal people for the treatment of nearly 45 different diseases and ailments. Roots, tubers, pseudobulbs, stems, leaves and even whole plants are used. Some herbal preparations have miraculous curative properties. Unfortunately, these preparations have not typically been subjected to the precise scientific clarification and standardization which are consequently required for clinical implementations. Some of the orchids are endangered due to over-exploitation and habitat destruction. Conservation strategies for orchids and further pharmacological studies on traditional medicines are suggested.

Shalini Kakarla, Deepak Ganjewala (India) Antimicrobial Activity of Essential Oils of Four Lemongrass (*Cymbopogon flexuosus* Steud) Varieties (pp 107-109)

ABSTRACT

Research Note: Antimicrobial activity of the essential oils (EOs) of four lemongrass (*Cymbopogon flexuosus*) varieties 'Krishna', 'Cauveri', 'Nima' and 'Cheerharit' and major EO constituents, viz. citral, geraniol, and geranyl acetate were evaluated. EOs from 30 d-old tillers of these varieties were extracted. Antimicrobial screening of the EO and major constituents was performed by the agar well diffusion method. All the EOs screened displayed strong antibacterial than antifungal activity against the microorganisms used. EOs from 'Krishna' and 'Cauveri' had exceptionally strong inhibitory effects against *Bacillus subtilis*. Among all the bacteria, *Staphylococcus aureus* was highly susceptible to all four EOs. Citral displayed remarkable antimicrobial activity against bacteria and fungi. Geraniol was also effective against fungi *Aspergillus flavus* and *A. fumigatus* while geranyl acetate had reasonable activity against *S. aureus*.

Hamidu Usman, Fanna Inna Abdulrahman, Haruna Abdu Kaita, Irfan Zaheer Khan (Nigeria) Comparative Phytochemical and Antimicrobial Evaluation of Stem Bark Extracts of *Bauhinia rufescens* Lam. (Caesalpinioideae-Leguminosae) and *Sclerocarya birrea* (A. Rich.) Hochst (Anacardiaceae) (pp 110-116)

ABSTRACT

Research Note: The methanolic extracts of plants *Bauhinia rufescens* (BRME) and *Sclerocarya birrea* (SBME) were screened phytochemically and investigated *in vitro* against five Gram⁻, five Gram⁺ bacterial isolates and three fungal species using hole-in-plate agar diffusion technique. The extractive value of BRME was 17.90% w/w (dark brown) and SBME was 15.37% w/w (reddish brown). The secondary metabolites present in both extracts were cardenolides, cardiac glycosides, flavonoids, saponins, resins, tannins and phlobatannins; anthraquinones were only present in BRME. The diameters of inhibition zones exhibited by the BRME extract against Gram⁺ and Gram⁻ organisms ranged from 11.00-28.17 and 10.67-27.17 mm, respectively. SBME had values ranging from 13.00-26.50 and 11.67-26.00 mm, respectively against Gram⁺ and Gram⁻ species. The overall susceptibility data revealed that BRME was more susceptible to Gram⁻ organisms, although inhibition was in particular cases insignificant ($P>0.05$) against *Pseudomonas aeruginosa*, *Klebsiella* spp., *Proteus vulgaris*, *Bacillus subtilis*, *Streptococcus pneumoniae* (5 mg/hole). The ranges of MIC and MBC data obtained from BRME against the tested organisms were 0.78-6.25 and 1.56-12.5 mg/ml, respectively while for SBME these values were 1.56-12.5 and 1.56-25 mg/ml, respectively. BRME was more susceptible to Gram⁻ organisms since an MIC/MBC value of 1.56 mg/ml was noted for *Salmonella typhi* and the MBC value for *E. coli*. SBME had an MIC/MBC value of 3.13 mg/ml for *Corynaebacterium* spp. and *Staphylococcus aureus* and the same MBC value for *Streptococcus pneumoniae*. There were no antifungal activities on BRME but little activities were expressed by SBME. Finally, both plant extracts showed very good activity against the pathogenic strains tested and hence, could be a yardstick for their traditional use.

H. P. Shilpashree, V. Ravishankar Rai (India) Fatty Acid Composition and Antimicrobial Activity of Leaf and Flower Extracts of *Hypericum mysorense* (pp 117-119)

ABSTRACT

Research Note: *Hypericum mysorense* is an important medicinal source for screening bioactive compounds. The chemical composition of the essential oil (EO) obtained from leaf and flower extracts of *H. mysorense* was analyzed by GC-MS. Linoleic acid was abundant in both samples. The EOs of both extracts showed antimicrobial activity against several microorganisms at 60-80 µg/ml.