

# 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:** Top four photos: Callus formation in *Mirabilis jalapa* in response to 2,4-D and picloram (Ping *et al.*, pp 42-47). Bottom: Effect of 5 mg/L IBA on adventitious root induction from petiole explants of *Centella asiatica* after 8 weeks of culture (Ping *et al.*, pp 36-41).

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**Yong-ping Pei, Jian Chen, Wei-lin Li (China)** Progress in Research and Application of Silymarin (pp 1-8)

**ABSTRACT**

**Mini-Review:** Silymarin (SL), a mixture of flavonolignans mainly including silybin A and B, isosilybin A and B, silidianin, and silychristine from the seeds of *Silybum marianum* L., has been widely used to treat acute and chronic viral hepatitis, toxin/drug-induced hepatitis, cirrhosis, alcoholic fatty liver diseases and other ailments because of its excellent hepatoprotective effect. Silybin is the most active component of SL, which is administered orally in most cases of clinical use. However, its bioavailability is low due to poor water solubility. The present paper reviews the research results on isolation methods, pharmacological activities, action mechanisms, preparation techniques, quality control and market state of SL in the hope that it would be helpful to better understand and use this traditional Chinese medicine.

**El-Saady M. Badawy, Soheir E. El-Sherbeny, Mohamed S. Hussein, Hend M. F. Swaefy, Heba M. Amer (Egypt)** Effect of Organic Manure on Growth, Production and Active Ingredients in Dragonhead (*Dracocephalum moldavica* L.) (pp 9-13)

**ABSTRACT**

**Original Research Paper:** Two field experiments were carried out during two successive seasons to study the response of *Dracocephalum moldavica* L. to different levels of two organic fertilizers (compost and farmyard manure FYM) at 240, 480 and 720 kg N/ha. Compost levels had more pronounced effect on growth characters than those obtained as a result of using farmyard fertilizer. The maximum mean values of growth characters were obtained from fertilization with compost at 240 kg N/ha. Moreover, essential oil percentage and yield were greatly influenced by the application of organic fertilizers. The highest values of essential oil (%) were recorded from applying FYM at 240 kg N/ha in the 1<sup>st</sup> season and compost at 480 or 720 kg N/ha in the second one. Compost at 240 kg N/ha gave the highest mean values of essential oil yield (ml/plant or L/ha). The concentration of N, P and K (%) in different plant tissues showed a remarkable increase with different levels of organic fertilizers. Compost levels were effective on total carbohydrate content (%) compared with FYM levels. The lowest levels of both fertilizers produced the maximum accumulation of total soluble sugars in different organs of plants. Generally photosynthetic pigments were not significantly affected by application of different levels of compost or FYM.

**El-Sherbeny Mohamed Rashad, Mohamed El-Sayed Abo El-Soud (Egypt)** Comparative Study on the Effect of Pre-Sowing Seed Treatments and Ethrel Foliar Application on Growth, Fruiting and Essential Oil Content of *Foeniculum vulgare* Mill. Plants (pp 14-20)

**ABSTRACT**

**Original Research Paper:** In pot experiments, the effects of pre-sowing seed treatments [cooling (2°C/24 h), heating (40°C/30 min) or soaking in 100 mg/l gibberellic acid alone or in combination with a foliar application of ethrel at several concentrations (0, 250, 500 or 1000 mg/l)] on fennel (*Foeniculum vulgare* Mill.) plants were assessed. There was a significant increase in the majority of growth parameters (plant height, number of branches/plant, fresh and dry weight of shoot, and shoot/root ratio) as a result of pre-sowing seeds, especially soaking and heat treatments. When plants were cooled, then heated, then soaked they contained more leaf pigments (chlorophyll *a* and *b*, total chlorophylls and total carotenoids) and total carbohydrate accompanied by a parallel acceleration in flowering and ripening dates. Hardening of fennel through the application of heat resulted in the greatest plant fruit yield and the highest essential oil (EO) yield in both milky and harvest stages. Spraying ethrel at a low concentration (250 mg/l) enhanced growth parameters, leaf pigments and total carbohydrate in most cases; an opposite effect was noticed under the highest concentration used (1000 mg/l), even though all ethrel concentrations accelerated flowering and ripening dates. The highest EO yield was with 500 mg/l ethrel during milky and harvest stages. There was a synergistic effect between pre-sowing seed treatments and the use of low and moderate levels of ethrel in most cases; the combined effect between soaking treatments and ethrel at 250 mg/l resulted in the highest values of most growth parameters during the milky stage while the combined (cooling treatment + 250 mg/l ethrel) treatment resulted in more leaf pigments, total carbohydrate and accelerated flowering and ripening dates. The combination between heat hardening + 500 mg/l ethrel resulted in the highest EO yield during milky and harvest stages. These results demonstrate that heating fennel seeds at 40°C for half an hour could be used effectively for increasing the fruit and EO yield of plants. This increment can be improved by ethrel foliar spray at 50 mg/l.

**El-Sherbeny Mohamed Rashad, Mohamed Slama Ahmed Abd El-Wahed, Aboubaker Ahmed Amin (Egypt)** Effect of  $\beta$ -sitosterol and Gibberellic Acid on Leaf Angle, Growth, Flowering and Biochemical Constituents of Marigold (*Calendula officinalis* L.) (pp 21-27)

#### ABSTRACT

**Original Research Paper:** Marigold (*Calendula officinalis* L.) plant growth and orientation were improved by foliar application of gibberellic acid ( $GA_3$ ; 25, 50 and 100 mg L<sup>-1</sup>) and  $\beta$ -sitosterol (20, 40 and 80 mg L<sup>-1</sup>) singly, or in combination.  $GA_3$  at 25 mg L<sup>-1</sup> more effectively increased the number of branches, fresh and dry weight of shoots and roots/plant while  $GA_3$  at 100 mg L<sup>-1</sup> significantly increased plant height. Leaf angle decreased more with  $GA_3$  than with  $\beta$ -sitosterol application. Both  $GA_3$  (100 mg L<sup>-1</sup>) or  $\beta$ -sitosterol (40 mg L<sup>-1</sup>) significantly induced flowering earliness and number of inflorescences while a low concentration of both substances improved other inflorescence characters. The  $GA_3$  (100 mg L<sup>-1</sup>)  $\times$   $\beta$ -sitosterol (40 mg L<sup>-1</sup>) interaction significantly increased earliness, number and stalk length of marigold plant inflorescences. Pigments (Chlorophyll (Chl) *a* and *b*, and carotenoids) and biochemical constituents (total sugars, indoles and free amino acids) were significantly enhanced by  $GA_3$  (25 mg L<sup>-1</sup>) and/or  $\beta$ -sitosterol (20 or 40 mg L<sup>-1</sup>) as a foliar application. However, the highest concentration of both bioregulators resulted in the highest phenolic content of marigold plant leaves.  $GA_3$  and  $\beta$ -sitosterol were positively correlated to fresh weight of inflorescences and Chl *a* + *b* content. There was a significant negative correlation between all results and phenolic content of marigold plant leaves.

**Hui-lian Xu (Japan), Feifei Qin (Japan/China), Wudeneh Letchamo (USA), André Gosselin (Canada), Shigenori Morita (Japan)** Photosynthetic Responses of Two Angelica Cultivars to Organic Cultures and Supplemental Lighting in Greenhouse (pp 28-31)

#### ABSTRACT

**Short Communication:** Angelica has a multitude of uses in alternative medicines and food processing. In recent years, organic herbs are more popular than ever. In the present study, two cultivars ('Archadie' and 'Munchen') of *Angelica archangelica* L., were cultivated in a greenhouse to investigate the responses of photosynthesis, photosynthetic related factors and dry matter production to organic substrate and supplemental lighting. Peat-based organic substrate was supplied with organic matter-based nutrient solution with sand substrate as control supplied with standard nutrient solution. Light regimes were natural light and 75  $\mu\text{mol m}^{-2} \text{s}^{-1}$  supplemental in addition to natural light. A hyperbolic mathematical model,  $P_N = K P_C i (1 + K i)^{-1} - R_D$ , was used to fit the light photosynthesis curve. Photosynthetic capacity ( $P_C$ ) and the maximum quantum yield ( $Y_Q = K P_C$ ) and dark respiration rate ( $R_D$ ) were analyzed from the model. Under natural lighting, both cultivars produced more total dry mass in organic culture than in sand culture, while under supplemental lighting, total dry mass production of 'Munchen' decreased in organic culture. Within all treatments and cultivars, both photosynthetic capacity ( $P_C$ ) and the maximum quantum yield ( $Y_Q$ ) were not positively proportional to the total dry mass. However, the total dry mass was significantly proportional to the shoot dry mass and the multiplication product of shoot dry mass and  $Y_Q$ , which showed the contribution from the canopy photosynthesis or the leaf area and photosynthetic rate under lower light intensity. With the exception of 'Munchen' in organic culture, total dry mass was increased by supplemental lighting in all other treatments. In conclusion, both organic culture and supplemental lighting are effective in improvement of dry mass production in angelica 'Archadie' but not 'Munchen'.

**Mohammad J. Saharkhiz, F. Ashiri, Mohammad R. Salehi, Jalal Ghaemghami, Sh. Mohammadi (Iran)** Allelopathic Potential of Essential Oils from *Carum copticum* L., *Cuminum cyminum* L., *Rosmarinus officinalis* L. and *Zataria multiflora* Boiss. (pp 32-35)

#### ABSTRACT

**Original Research Paper:** Essential oils (EOs) obtained from dried fruits of *Carum copticum* L. and *Cuminum cyminum* L., and aerial parts of *Rosmarinus officinalis* L. and *Zataria multiflora* Boiss. were evaluated *in vitro* to examine their potential allelopathic effects. The inhibitory effects of EOs at concentrations of 0, 100, 200, 300 and 400  $\mu\text{l. L}^{-1}$  on seed germination and seedling growth of common bermudagrass (*Cynodon dactylon* L.), tall fescue (*Festuca arundinacea* Schreb.) and perennial ryegrass (*Lolium perenne* L.) from the Poaceae family were tested. Germination rate and seedling length were significantly ( $P \leq 0.05$ ) reduced by all EOs, however, the highest effect was observed at 300 and 400  $\mu\text{l. L}^{-1}$ . Moreover, the allelopathic impact of *Z. multiflora* EO on seed germination and seedling growth was most effective (100% germination and growth inhibition) when compared with other tested EOs.

**Anna Pick Kiong Ling, Mee Feng Chin, Sobri Hussein (Malaysia)** Adventitious Root Production of *Centella asiatica* in Response to Plant Growth Regulators and Sucrose Concentrations (pp 36-41)

#### ABSTRACT

**Original Research Paper:** *Centella asiatica* is an important medicinal plant commonly used for wound healing purposes and as a brain tonic. A study was undertaken to investigate the effects of different plant growth regulators at various concentrations on adventitious root induction from the leaf and petiole explants. Full strength Murashige and Skoog (MS) medium supplemented with indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and  $\alpha$ -naphthaleneacetic acid (NAA) at 0, 1, 3, 5, and 7 mg/L were used in this study. All treatments were kept in the dark and data collection was performed after 8 weeks of culture. No adventitious roots formed in the control medium devoid of any plant growth regulators (PGRs). Among the PGRs used, IBA showed the best adventitious root formation for both explants, followed by NAA, and IAA. The highest percentage of explants forming roots, number of roots per explant and longest roots formed from leaf explants was observed in 7 mg/L IBA. Meanwhile, IBA at 5 mg/L showed better rooting efficiency for petiole explants. Petiole explants were better than leaf explant for inducing adventitious roots. The effects of sucrose at 0, 1, 2, 3, 4, 5, 6, and 7% (w/v) on adventitious root induction from petiole explants were also assessed. No adventitious roots formed in MS media without sucrose. Sucrose at 4% and 5 mg/L of IBA showed the highest number of roots per explant and the longest roots.

**Anna Pick Kiong Ling, Kuok-Yid Tang, Jualang Azlan Gansau, Sobri Hussein (Malaysia)** Induction and Maintenance of Callus from Leaf Explants of *Mirabilis jalapa* L. (pp 42-47)

#### ABSTRACT

**Original Research Paper:** *Mirabilis jalapa* L., commonly known as 'four o'clock plant' produces a strong, sweet smelling fragrance after the flowers open at late afternoon. It is a well known ornamental plant as the flowers of different colours can be found simultaneously on the same plant or an individual flower can be splashed with different colours. The colour-changing phenomenon is one of the unique characteristics of *M. jalapa* as it can display flowers with different colour when it matures. Apart from its ornamental value, it has also earned its place in herbal medicine practices around the world. Its array of biological activities continues to support its use worldwide for control of viruses, fungi and yeast. In this study, callus culture was initiated from the leaf explants of *M. jalapa*. The suitable strength of MS (Murashige and Skoog) nutrient media was determined and the effects of different types of auxins [4-amino-3,5,6-trichloro picolinic acid (picloram), 2,4-dichlorophenoxyacetic acid (2,4-D),  $\alpha$ -naphthaleneacetic acid (NAA)] and cytokinin [6-benzyl amino purine (BAP)] at 0.0, 2.5, 5.0, 7.5, 10.0, and 20.0  $\mu$ M were investigated in order to determine the suitable callus induction and maintenance media. The establishment of callus culture was greatly influenced by the strength of MS medium, type as well as the concentration of plant growth regulators (PGRs) used. The best callus induction response was obtained on half strength ( $\frac{1}{2}$ ) MS media supplemented with 20.0  $\mu$ M picloram which produced healthy and friable callus. Meanwhile,  $\frac{1}{2}$  MS supplemented with NAA or BAP as well as PGR-free medium did not produce any callus; rather, explants became necrotic after 3 to 4 weeks of culture. Calli were successfully maintained in  $\frac{1}{2}$  MS supplemented with 10.0  $\mu$ M picloram. Callus in maintenance medium showed a sigmoid growth pattern and reached a maximum growth rate between weeks 1 and 3.

**Salim Khan (Kingdom of Saudi Arabia), Khanda Jabeen Mirza, Md Tayaab, Malik Zainul Abdin (India)** RAPD Profile for Authentication of Medicinal Plant *Glycyrrhiza glabra* Linn. (pp 48-51)

#### ABSTRACT

**Research Note:** Correct identification and quality assurance is indispensable to ensure reproducible medicinal quality of herbal drugs. Authentication is especially useful in the case of those medicinal herbs that are frequently substituted or adulterated with other species or varieties morphologically and phytochemically indistinguishable. In this study, RAPD (Random Amplified Polymorphic DNA) was employed for authentication of *Glycyrrhiza glabra* L. from its adulterant *Abrus precatorius* L. Fifty two decamer oligonucleotide primers were screened in the RAPD analysis for identification of genuine and adulterant samples using the DNA isolated from the dried root of samples. Sixteen primers gave species-specific reproducible unique amplicons, which could clearly distinguish genuine as well as adulterant samples having similar morphology. RAPD could thus, help to serve as a complementary tool for quality control.

**Subramanian Ganesh Manikandan, Cuntheepuram Lakshminarasimhan, Nuruddin Thajuddin, Ramalingam Saravanan (India)** Antibacterial Activity of Epicarp Extract of *Punica granatum* L. against Methicillin-Resistant *Staphylococcus aureus*

(MRSA) (pp 52-54)

#### ABSTRACT

**Short Communication:** The ethanolic extract of *Punica granatum* (Family: Punicaceae) epicarp was studied for its antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). The extract showed intense activity against MRSA. Serial chromatographic purifications by TLC, HPLC and <sup>1</sup>HNMR offered two active compounds which were identified as tannic acid compounds which completely inhibited the growth of MRSA. The MIC value of these components was 25 µg/ml. These tannic acid components found in the epicarp of *P. granatum* might be useful in a phytotherapeutic strategy against MRSA.

**Satyanarjan Mishra, Sabuj Sahoo, Sagar Kumar Mishra, Kedar Kumar Rout, Sukant Kumar Nayak, Nabin Kumar Dhal (India)** Antimicrobial Investigation of Leaves of *Barringtonia acutangula* Linn. (pp 55-58)

#### ABSTRACT

**Original Research Paper:** Methanolic and aqueous extracts of the leaves of *Barringtonia acutangula* Linn. belonging to the family Barringtoniaceae, commonly known as *Hinjal*, were screened for their antibacterial activity against some selected organisms causing urinary and gastrointestinal tract infection, fish-virulent pathogens, human and plant pathogenic fungi. The methanolic extract, which exhibited potent antimicrobial activity, was subjected to high performance thin layer chromatography (HPTLC). Chromatography of the methanolic extract was carried out with an acetone-hexane (4:6) solvent system. HPTLC fingerprinting patterns were developed at three different wave lengths viz. 254, 366 and 650 nm. Antimicrobial activity was carried out against selected bacterial and fungal strains by a disc diffusion assay method at a concentration of 1000 µg/ml and minimum inhibitory concentration values were calculated by a two-fold serial dilution method at a concentration range of 7.8-1000 µg/ml. The yield (%) of methanolic and aqueous extracts was found to be 12.6 and 16.1% (w/w), respectively for the air-dried plant leaves. Antimicrobial screening revealed that the extract showed potent activity against bacterial and fungal strains, namely *Enterococcus faecalis* (22.9 ± 0.23 mm, MIC 31.25 µg/ml), *Aspergillus niger* (27.2 ± 0.15 mm, MIC 62.5 µg/ml) and *Candida albicans* (23.3 ± 0.87 mm, MIC 31.25 µg/ml).

**Karthik Mohan, Jeyachandran Robert (India)** Hepatoprotective Effect of *Stevia rebaudiana* Bertoni Leaf Extract in CCl<sub>4</sub>-Induced Liver Injury in Albino Rats (pp 59-61)

#### ABSTRACT

**Short Communication:** The efficacy of aqueous leaf extract of *Stevia rebaudiana*, a non-calorific natural sweetener was evaluated against carbon tetrachloride (CCl<sub>4</sub>)-induced liver damage in albino rats. Hepatotoxicity was induced by *i.p.* administration of 30% CCl<sub>4</sub> suspended in olive oil (1 ml/kg of body weight). After the treatment regimen animals were sacrificed and liver enzymes AST, ALT, ALP, LDH were assayed in serum along with total protein and antioxidant status was assessed in liver tissues by determining the activities of SOD, CAT, GPx and GSH. The lipid peroxidation levels were also assessed. The observations suggested that pre-treatment of the natural sweetener could possibly protect CCl<sub>4</sub>-induced liver damage and that the exerted effect is connected with its antioxidant and hepatoprotective nature.

**Kumar Manoj, Debasish Mishra, Tapas K. Maity, Snehasish Dutta Gupta (India)** Screening Wound-Healing Potential of Different *Aloe vera* L. Germplasms at the Cellular Level (pp 62-64)

#### ABSTRACT

**Short Communication:** *Aloe vera* L. germplasms were tested for their ability to heal wounds following proliferation and migration of murine L929 fibroblast cells employing MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and an *in vitro* scratch assay. All the germplasms were capable of promoting cell proliferation and migration. A positive correlation was observed in proliferation and migration of cells among the aloe germplasms independent of treatment concentration. Of the tested germplasms, TN exhibited maximum while RJN showed minimum wound-healing potential. This ability of *A. vera* to stimulate fibroblast proliferation and migration might help skin regeneration and repair. The *in vitro* scratch assay employed in the present study for the first time documents its efficiency in evaluating wound-healing potential following fibroblast migration.

**María S. Pérez Gutiérrez, Rosa V. García Rodríguez, Cuauhtémoc Pérez González, Ramses E. Rodríguez, Miguel A. Zavala Sánchez (Mexico)** Anti-inflammatory Properties of *Salvia connivens* (pp 65-67)

#### ABSTRACT

**Short Communication:** The anti-inflammatory properties of chloroform, methanol and aqueous extracts of the leaves of *Salvia connivens* Epling were investigated. It was found that aqueous extract on carraggenan induced edema at dose of 400 mg/kg had no activity. Methanol extract at the same dose inhibited the edema  $64.3 \pm 18\%$  (1 h), however the effect decreased after this time. Chloroform extract showed at this dose an inhibition of  $73.8 \pm 10.6\%$  (4 h), and the effect observed for this extract at dose of 200 mg/kg was  $87.76 \pm 7.6\%$  (5 h). Indomethacin was used as a positive control and inhibited edema by  $73.2 \pm 3.5\%$  at a dose of 8 mg/kg. Tests demonstrated that the chloroform extract inhibited 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced ear edema in mouse by  $50.7 \pm 2.1\%$ ; The chloroform extract inhibited pellet implantation-induced granuloma formation by  $39.34 \pm 5.36\%$  at a dose of 200 mg/kg, a result comparable to that exhibited by naproxen at a dose of 50 mg/kg ( $40.81 \pm 4.78\%$ ). These results suggest that *S. connivens* chloroform extract has anti-inflammatory activity, supporting its folkloric use for treatment of inflammatory diseases.

**Yu Yang, Sheng-Xiong Huang, Hong-Yu Hu, Yi-Min Zhao, Qin-Shi Zhao, Han-Dong Sun (China)** A New Rosane Diterpene from *Lycoris aurea* (pp 68-70)

#### ABSTRACT

**Short Communication:** Plants of the Amaryllidaceae are known to produce structurally unique alkaloids with a wide range of interesting physiological effects. *Lycoris aurea* (Amaryllidaceae), a popular ornamental plant in China, has a wide distribution in the tropics and warm temperature regions. In order to search new bioactive alkaloids from this plant, we investigated the chemical constituents of the bulbs of *L. aurea* collected in Kunming of Yunnan province. A new rosane diterpene, lyrosenonolactone (**1**), has been isolated from the whole plant of *L. aurea*. Its structure was elucidated on the basis of extensive spectroscopic analysis. Additionally, rosolactone (**2**) and seven known alkaloids were also isolated from this plant. To the best of our knowledge, this is the first report of a diterpene from *L. aurea*. Moreover, on the basis of detailed 2D NMR data analysis, the chemical shifts of rosolactone (**2**) reported previously were revised.