Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(2): 207-210
Received: 24-01-2016
Accepted: 26-02-2016

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Antibacterial activity, antioxidant activity and micropropagation of *Gymnema sylvestre* R.Br. a valuable medicinal plant

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Abstract
The present paper reports the antibacterial, antioxidant activity and *In vitro* propagation studies of a threatened plant, *Gymnema sylvestre*. *G. sylvestre* which belongs to the family Asclepiadaceae is a slow growing perennial medicinal woody climber commonly called as "Gudmar". There is a growing demand for leaves of *G. sylvestre* in the pharmaceutical trade due to its use as a remedy for diabetes and also as a tonic of the nerves and as a laxative. Micropropagation of this plant is often difficult and expensive. In the present study the antibacterial activity of extracts of different *G. sylvestre* explants were performed against gram positive and gram negative bacteria by the disk diffusion method. The activities of the compounds were compared with standard strain for antibacterial properties of the imine base and its solvent extract evaluated and presenting in indicate that the compounds are active in exhibiting antibacterial role also carried out. *G. Sylvestre* exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the roots and leaves extract is very much source of natural antioxidant agent. *In vitro* propagation is an alternative method of *in vitro* propagation of the threatened and endangered plant which can aid its conservation. The nodal and intermodal explants were cultured on MS medium containing different concentration and combinations of growth regulators like BAP and IAA. Multiple shoots buds were regenerated successfully from the nodal explants which were efficiently rooted on half strength MS medium supplemented with IBA. The regenerated plantlets were successfully transferred to the glasshouse, acclimatized and transferred to the field.

Keywords: *G. sylvestre*, antibacterial activity, antioxidant activity, DPPH, Micropropagation, nodal and intermodal explants.

Introduction
Plants have been used for the treatment of various diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [1]. Thus over 50% of these modern drugs are of natural products origin and as such play an important role in drug development in the pharmaceutical industry [2]. *Gymnema sylvestre* R.Br. which belongs to the family Asclepiadaceae, is a vulnerable species. It is a slow growing, perennial woody climber of tropical, subtropical regions. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine [3]. *G. sylvestre* plant parts used in the treatment of asthma, inflammations, eye complaints, family planning and snake bite [4]. In addition, it possesses antihypercholesterolemic, hepatoprotective, antimicrobial and sweet suppressing [5]. It also acts as feeding deterrents to caterpillar, prevent dental caries caused by *Streptococcus mutans* [6] and in skin cosmetics [7]. There is a growing demand for leaves and roots of *G. sylvestre* in the pharmaceutical trade due to its use as a remedy for diabetes and also as a tonic of the nerves and as a laxative, as an anti-sweetner and as an anti hypercholesterolemic [8]. It also has stomatic, diuretic and cough suppressant property [9]. Increasing awareness of the side effects of Western drugs have made general public turn towards the herbal medicine, thus the demands for medicinal plants have drastically increased. Due to over exploitation, this plant species has become threatened and is listed in IUCN red data book [10].
G. sylvestre is a slow growing, perennial woody climber [11]. Seeds lose viability in a short period of storage. Conventional propagation methods are hampered due to its poor seed viability, low rate of germination and poor rooting ability of vegetative cuttings [12]. The propagation of plant through seed results in less survivability under natural conditions. Therefore, to fulfill the increasing demand of this potent medicinal plant and population, micropropagation could be an alternative method to aid in its conservation [13, 14].

Therefore, the propagation of this plant species by alternative methods is needed. The active principles which have been identified as glycosides (gymnemic acids) suggest that the topical and selective anaesthetic effect of the plant might result from the competition of the receptor sites between glycosides and the sweet substances [15]. In the present investigation study of *G. sylvestre* has been taken up to carry out antibacterial activity, antioxidant activity and micropropagation through direct organogenesis using nodal and intermodal segments as explants.

Materials and methods

G. sylvestre Plant materials were collected from the Botanical garden, Osmania University, Hyderabad. The collected plant materials were identified by Taxonomist Department of Botany. The plants were subjected to antibacterial activity. Further, a good protocol for *in vitro* propagation was developed to aid in its multiplication and conservation.

Preparation of extracts

Plant samples (leaves) were washed with distilled water and air-dried at room temperature for 7-10 days, then oven-dried at 40 ^{0}C to remove the residual moisture. The dried plant parts were pulverized and stored in air-tight containers at 4 ^{0}C for future use. 50 g of powdered samples of bark, flowers and leaves were extracted with methanol by soxhlation method at 60 to 80 ^{0}C. The three filtrates were separately concentrated in water bath at 40 ^{0}C and evaporated under reduced pressure.

Antibacterial Activity

The disc diffusion method was used to evaluate the antibacterial activity of the synthesized compounds against four bacterial strains viz; *E. coli*, *P. aeruginosa, K. pnemone* and *S. aureus*. Each organism was cultured in nutrient broth at 37 ^{0}C for 24 h. Then 1% broth culture containing approximately 106 colony forming units (CFU/mL) of test strain was added to nutrient agar medium at 45 ^{0}C and poured into sterile petri plates. The medium was allowed to solidify. 5 µL of the test compound (40 mg/mL in DMSO) was poured on 4 mm sterile paper discs and placed on nutrient agar plates. In each plate standard antibacterial drug (ampicillin) and metal complexes were added. The plates were incubated at 37 ^{0}C for 24 h and the antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm) [16].

Radical Scavenging Activity

The percentage of free radical scavenging activity is shown in Fig-1. This assay is based on decrease in absorbance value of DPPH at 517 nm on addition of complex. The experiment involves diluting the working solution of the plant (root and leaves) methanol extracts and the ascorbic acid standard (700, 600, 500, 400, 300 and 200 µg/µL−1) in methanol. DPPH concentration was kept constant (2 mL, 0.004%). To this varying concentration of plant extracts and standard were added. The mixture was shaken vigorously and kept in dark for 30 min at room temperature. Then the absorbance was measured at 517 nm in a spectrophotometer. The whole experiment was carried out using spectroscopic grade methanol solvent at 298 K. The radical scavenging activity has been measured by using the following Eq. 1;

Suppression ratio (%) = $[(A_0-A_i)/A_0)]$ X 100% (1)

Where A_i=the absorbance in the presence of the ligand or its complexes, A_0=the absorbance in the absence of the ligand or its plant extracts.

Micropropagation studies

G. sylvestre plants were subjected to tissue culture and develop a good protocol for micropropagation was developed to aid in its multiplication and conservation. The *in vitro* propagation studies comprised the culture of nodal and nodal explants on defined culture media under standard growth conditions. The nodal and nodal explants were collected from healthy field grown plants. They were washed under running tap water for 15 minutes followed by soaking in 0.1% (v/v) liquid detergent Tween-20 for 5-6 min and then subsequently washed with tap water. The explants were then soaked in 70% ethanol for 4-5 minutes followed by washing with water. Finally the explants were surface sterilized with 0.1% solution of (Hg cl_2) mercuric chloride for 4 to 5 min followed by thorough rinsing in sterile distilled water. A total of thirty explants were inoculated in culture tubes containing MS medium augmented with 2 % sucrose and 0.8% agar and different combinations and concentrations of various plant growth regulators. The experiment was carried out in triplicates. Prior to that, the pH of the medium was adjusted to 5.8, autoclaved at 121^o C for 15 lbs / cm^2 for 15 min and allowed to cool before inoculation. The culture media comprised of the following: MS + BAP (1.0, 1.5, 2.0 and 2.5 mg/l) and MS + BAP (1.0, 1.5, 2.0 and 2.5 mg/l) + IAA (0.5 and 1.0 mg/l). All the inoculated cultures were incubated in sterile growth room under controlled conditions of 22±2 ^{o}C temperature, 75% humidity and 2000 lux illumination of 16 hr / 8 hr L/D cycle. The 1 to 2 cm long regenerated shoots were transferred to root inducing media comprising half MS medium supplemented with IBA (1.0 and 1.5 mg/l). The regenerated plantlets were later transplanted to pots containing a mixture of soil and vermicompost in the ratio of 2:1. The plantlets were gradually acclimatized on the laboratory bench by covering with a plastic bag with holes (to maintain high humidity), which were opened up gradually over a period of one week. The plants in the pots were moved to the glasshouse to a shaded area and gradually acclimatized then transfer to field.

Results and discussion

The antibacterial screening of the *G. sylvestre* leaf extracts were performed against gram positive (*S. aureus*) and gram negative bacteria (*E.coli*, *P. aeruginosa* and *K. pnemone*) by the disk diffusion method. The activities of the compounds were compared with standard Ampicillin for antibacterial activity. The antibacterial properties of the imine base and its solvent extract evaluated and presenting in Fig-1 and Table-1, indicted that the compounds are active in exhibiting antibacterial role like leaf 0.4, 0.2, 0.5 in gram negative bacteria and leaf 0.6 in gram positive bacteria. Study confirms the antibacterial activity of leaf extract of *G. sylvestre* the extract found effective bacterial strain, the activity of leaf extract antibacterial activity higher than in gram negative bacteria, whereas more when compare to in gram positive bacteria. However reported in the present study which agrees with the findings of [17-19].

The model of scavenging the stable DPPH radical is a widely used technique to screen antioxidant properties by spectrophotometer in a very short time period. When the reaction between antioxidant molecule and DPPH radical occurs, it results in decrease in absorbance at 517 nm. This is because the radical is scavenged by antioxidants through donation of hydrogen to form the reduced form (DPPH-H), and this property is also visually noticeable as the color changes from purple to yellow. The more rapidly the absorbance decreases, the more potent is the antioxidant

compound. In the present study the antioxidant activity of R13 and N26 extract was evaluated by scavenging stable DPPH radical (Fig:-2). The DPPH radical scavenging activities were found to be 63.03 % for ascorbic acid, 12.85 % for root and 17. 24% leaf extract, at concentration of the 200 µg/µL-1. Ascorbic acid exhibited higher DPPH scavenging activity than the compound at all concentrations. At the concentration of 700 µg/µL^{-1} scavenging activities were found to be 89.85%, 80.15% and 64.12% for Ascorbic acid, root and leaf extract of respectively. The compounds scavenging activity which is the measure of antioxidant property at the concentration of above compounds at 200 µg/µL^{-1} follows the order: Ascorbic acid > root > leaf extract of while at higher concentration the same order is followed by root and leaf extraction exchanged their position the present study results which agree and similar with the findings of [20, 21].

An efficient micropropagation protocol was developed with a high percentage of shoot regeneration and multiple shoots (fig.1-A to C). The highest response of for production of multiple shoots was recorded with MS + BAP (2.0mg/l) followed by in MS + BAP (1.5 mg/l) (Table-2). The explants proliferated by 6-8 days and shoots regeneration was observed by 10-12 days. Shoots of about 1 to 2 cm with 2-4 nodes were produced by 20-25 days. These were cultured on root induction media containing different concentrations of IBA (1.0, 1.5 mg/l) to induce roots. The higher concentration of IBA (2.5 mg/l) produced better rooting efficiency of (Table-3). The regenerated plants were transferred to the glasshouse for acclimatization (fig.2). Out of a total of 400 explants (pooled from triplicates) inoculated, 320 explants could regenerate shoots and 255 shoots were inoculated on rooting media for root induction out of which 185 shoots could develop roots to enable 150 plants to be transplanted out of which 80 plants survived in pots. In the present study, different concentrations of BAP and BAP with IAA were used to induce regeneration [21]. However, reported the regeneration of *G. sylvestre* through the use of BAP and KN individually and combined with NAA. The present results are agrees well with the above report with supplementation of BAP individually or in combination but a higher frequency of regeneration was obtained with BAP (1.5 mg/l) presently [21] reported the plant regeneration of *G. sylvestre* from nodal explants on MS medium supplemented with BAP (1.0 mg/l) and NAA (2.0 mg/l) whereas, in our present report use of BAP individually produced the highest shoot regeneration frequency without any additional supplementation of NAA. In the present study it was observed that MS + IBA combination produced efficient rooting compared to other reports where they achieved rooting on MS medium supplemented with IAA. This efficient high frequency plant regeneration protocol developed presently can be taken up for large scale micropropagation for its multiplication and conservation.

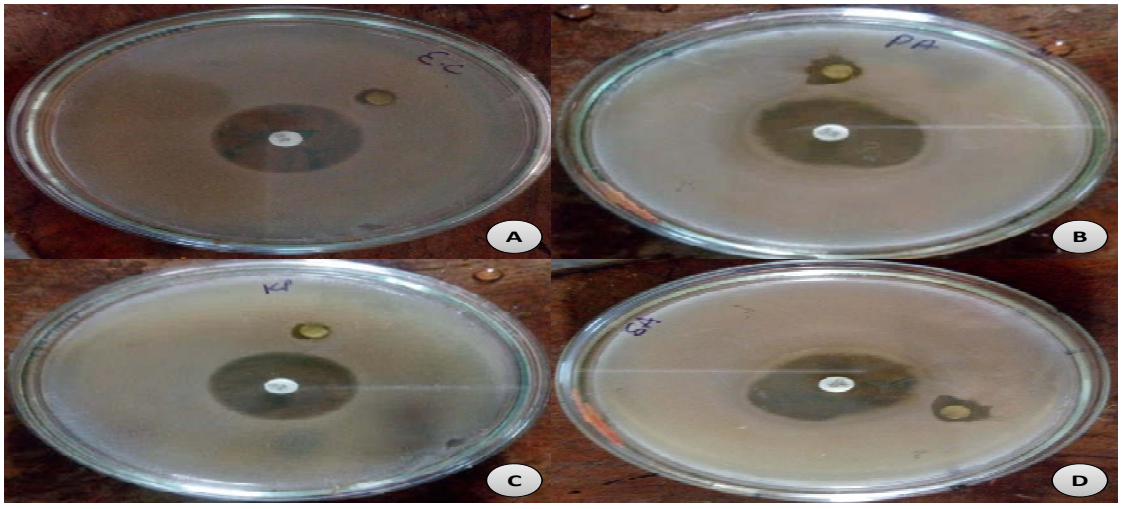

Fig 1: Antibacterial activity of leaf extract of Gymnema sylvestre (A) *E. coli*, (B) *P. aeruginosa* (C) *K. pnemone* (Gram Nagative) and (D) *S. aureus* (Gram Positive) ampicillin as positive control.

Table 1: Minimum inhibition zone (mm) complexes (µg/ml) leaf extract of *G. sylvestre*.

Bacterial inhibition zone (mm) Gram (+)			Bacterial inhibition zone (mm) Gram (-)
E. coli	**P. aeruginosa**	**K. pnemone**	**S. aureus**
0.4	0.2	0.5	0.6

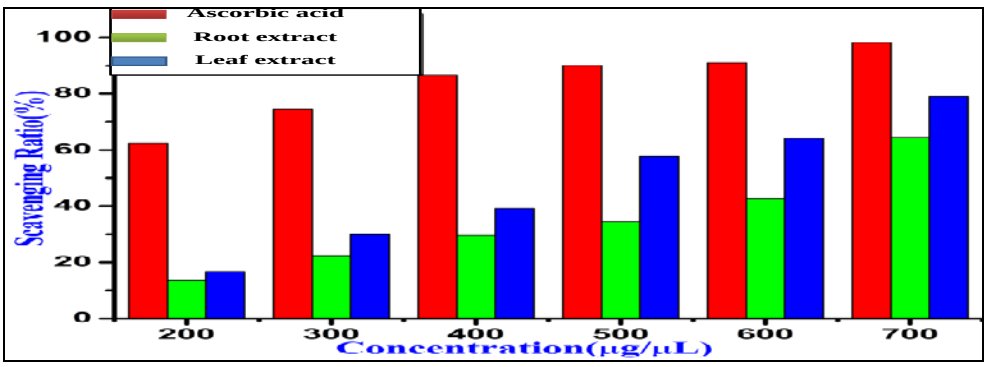

Fig 2: Radical-scavenging activity of the Root and Leaf extract of G. sylvestre on DPPH radicals (%).

Table 2: Efficiency of shoot regeneration and production of multiple shoots from nodal explants of *G. sylvestre*, on different culture media.

Culture medium	No. of explants with shoot induction	Percentage of shoot induction* (Mean±S.E)
MS + BAP (1.0 mg/l)	50	2.78±1.79
MS + BAP (1.5 mg/l)	56	3.34±1.08
MS + BAP (2.0 mg/l)	68	3.60±1.15
MS + BAP (1.0 mg/l) + IAA (0.5 mg/l)	48	1.35±0.25
MS + BAP (1.5 mg/l) + IAA (0.5 mg/l)	35	1.87±0.69
MS + BAP (2.0 mg/l) + IAA (0.5 mg/l)	46	2.15±0.92

Table 3: Percentage of root induction from multiple shoots regenerated from nodal explants of *G. sylvestre*.

Culture medium	No. of shoots with root induction	Percentage of root induction* (Mean±S.E)
MS + IBA (1.0 mg/l)	48	09.00±0.134
MS + IBA (1.5 mg/l)	56	11.78±0.213

Fig 1 A-F: A. *Gymnema sylvestre* plant, B. Shoot regeneration from nodal explants, 10 days after inoculation, C. Multiple shoots, 20 days after inoculation, D. Rooting from regenerated shoot, 20 days after inoculation of shoot, E. Acclimatization of regenerated plantlet, F. Regenerated plant transferred to the field.

Conclusion

It is concluded that *G. sylvestre* antibacterial properties of the imine base and its solvent extract evaluated and presenting in indicted that the compounds are active in exhibiting antibacterial role like leaf 0.4, 0.2, 0.5 in gram negative bacteria and leaf 0.6 in gram positive bacteria. Study confirms the antibacterial activity of leaf extract of *G. sylvestre* the extract found effective bacterial strain, the activity of leaf extract antibacterial activity higher than in gram negative bacteria, whereas more when compare to in gram positive bacteria is a plant with a variety of ethnic medicinal uses. *G. Sylvestre* exhibited potent antioxidant activity by inhibiting DPPH free radicals which indicates the roots and leaves extract is very much of *G. Sylvestre* can be used as an accessible source of natural antioxidant agent.

This is valuable information for preparation of drugs in pharmaceutical industry and stresses the need for more intensive research since they play a great role in healthcare and successful development of *in vitro* propagation protocol of *G. sylvestre.* This protocol provides a successful technique for multiplication and conservation of the valuable medicinal plant which is used in treating various disorders. The protocol developed presently can be taken up in large scale to produce the planting material for development of medicinal plant cultivation programmers.

References

1. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd Edn. Ibadan: John Willey and Sons ltd., 1982, 8-14.
2. Jeyachandran R, Mahesh A. Antimicrobial evaluation of *Kigelia africana* (Lam). Res J Microbiol. 2007; 2(8):645-649.
3. Mitra SK, Gopumadhavan S, Muralidhar TS, Anturlikar SD, Sujatha MB. Effect of D-400 herbomineral preparation on lipid profile glycated haemoglobin and glucose tolerance instreptozotocin induced diabetes in rats. Indian. J Exp. Biol. 1995; 33:798-800.
4. Selvanayagam ZE, Gnanavendhan SG, Chandrasekhran P, Balakrishna K, Rao RB. Plants with antisnake venom activity-a review on pharmacological and clinical studies. Fitoterap 1995; 65:99-111.
5. Kurihara Y. Characteristics of antisweet substances, sweet proteins and sweetness-inducing proteins. Crit. Rev. Food Sci. Nutr 1992; 32:231-252.
6. Hiji Yasutake US. Gymnemic acid for prevention of dental caries. In: Chemical Abst 1990; 113:463-64b.
7. Maeda N, Fukuda M. Skin lightening cosmetics containing *Gymnema* saponins and other ingredients. In: Chemical Abst 1996; 125:1232-89h.
8. Bishayee A, Chatterjee M. Hypolipidaemic and antihyperchloesterolemic effects of oral *Gymnema sylvestre* R. Br. Leaf extract in albino rats fed on a high fat diet. Phytothera. Res 1994; 8:118-120.
9. Kapoor LD. CRC Handbook of Ayurvedic medicinal plants: Boca Raton, Fl, 1990, 200-201.
10. Shailasree S, Ruma K, Kini KR, Niranjana SR, Prakash HS. Potential anti-inflammatory bioactives from medicinal plants of Western Ghats, India, J Pharma. Com. 2012; 2(2):1-2.
11. Shrivastava R, Pratibha S. *In vitro* propagation of multipurpose medicinal plant *Gymnema sylvestre* R.Br. (Gudmar), Ind. J Shodha anusandhan Samachar. 2011; 1:27-30.
12. Komalavalli N, Rao MV. *In vitro* micropropagation of *Gymnema sylvestre*-A multipurpose medicinal plant. Plant Cell, Tissue and Organ Culture. 2000; 61:97-105.
13. Mohan Ch, Manjula P, Kiran Kumar B, Naresh B, Rama Devi B, Prathibha Devi B. *In vitro* propagation of *Clitoria ternatea* L., a valuable medicinal plant. *Trends in Bioscience* 2014; **7**(18):2698-2701.
14. Mohan Ch, Rama Devi B, Manjula P, Prathibha Devi B. Phytochemical investigations and micropropagation of *Tylophora indica* (burm. F.) Merill from nodal explants. The Journal of Indian Botanical Society. 2014; 93(1, 2):42-49.
15. Kumaran A, Karunakaran JR. *In-vitro* antioxidant activities of methanol extracts of five Phyllanthus species from India, LWT-Food Science and Technology 2007; 40(2):344-352.
16. Kishor Naidu G, Chandra Shekhar Naidu K, Sujatha B. *In vitro* Antibacterial Activity and Phytochemical Analysis of Leaves of *Gymnema sylvestre* Retz. R. Br. International Journal of Pharm Tech Research. 2013; 5(3):1315-1320.
17. Murugan M, Mohan VR, Thamodharan V. Phytochemical screening and antibacterial activity of *Gymnema sylvestre* (Retz) R. Br ex. Schultes and *Morinda pubescens* JE. Smith var. pubescens Journal of Applied Pharmaceutical Science. 2012; 02(02):73-76.
18. Mohan Ch, Kistamma S, Vani P, Narshimha Reddy A. Biological Activities of Different Parts of *Saraca asoca* an Endangered Valuable Medicinal Plant, International Journal of Current Microbiology and Applied Science. 2016; 5(3):300-308.
19. Singh Kalyani, Bandita Deo. Phytochemical evaluation and *in vitro* antioxidant activity of *Gymnema sylvestre* R.Br. Journal of Medicinal Plants Studies. 2014; 2(4):19-23.
20. Patel Reena, Patel Yogesh, Prasant Kunjadia, Anju Kunjadia. DPPH free radical scavenging activity of phenolics and flavonoids in some medicinal plants of India Int J Curr Microbiol App Sci. 2015; 4(1):773-780.
21. Mohan Ch, Karnakar A, Sateesh S, Rama Devi B, Manjula P, Sreekanth D *et al*. Phytochemical analysis and *In vitro* propagation of *Gymnema sylvestre* R. Br. a valuable medicinal plant (Published in International Journal of Current Biotechnology. 2014; 2(12):16-21.