Recent Research in Science and Technology 2011, 3(8): 73-75
ISSN: 2076-5061
www.scholarjournals.org

www.recent-science.com

RRST-Biochemistry

Evaluation of *Gymnema sylvestre* Antimicrobial Activity in Methanol

Bhuvaneswari CH, Kiranmayee Rao, Archana Giri*

Centre for Biotechnology, Institute of Science & Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad – 500 085

Article Info

Article History

Received : 27-05-2011
Revised : 28-07-2011
Accepted : 28-07-2011

*Corresponding Author

Tel : +91-40-23156129
Fax : +91-40-23156129

Email:
archanagirin@yahoo.co.in
b_moram@rediffmail.com

Abstract

G. sylvestre is a medicinal plant known for its sugar destroying property, as an anti-diabetic agent. The major phytoconstituents are the triterpenoid saponins, responsible for the various activities. The antimicrobial activity of this plant has been assessed in methanol as the solvent system for the extraction of active principles. The gram positive and gram negative organisms used in the study, have shown susceptibility towards the extracts, with the root extracts at acidic pH, showing higher activity. *E. coli* and *E. cloacae* were found to be the most sensitive and *Pseudomonas aeruginosa*, the resistant type of microorganisms, based on the results obtained from the zones of inhibition. The broad spectrum activity of the plant can be utilised in the development of new antimicrobial drugs.

Key Words: *G. sylvestre*, Aerial and Root extracts, Antimicrobial activity, Methanol, Antibiotic resistance.

Introduction

The alarming increase in the bacterial resistance towards the existing chemotherapeutics has paved the way for exploration of new alternatives. One of the options – folk medicines could provide a platform for yielding effective compounds with potent activity against microbes. The last three decades have seen the development of several synthetic drugs, but the resistance towards these drugs is also developing at a faster stroke, as the bacteria can acquire and transmit genes responsible for antibiotic resistance [1]. The main advantage of natural agents is that, the crude extracts contain a mixture of compounds like phenols, acids, esters, aldehydes etc., for which it is difficult to develop resistance by bacteria unlike the synthetic antibiotics that contain a single compound [2]. The world health organization has taken an initiative in the development of plant based health care, making it available to maximum population [3]. Developing countries are fast making use of the traditional medicines with already 1400 preparations in use.

Gymnema sylvestre R. Br belonging to the family Asclepiadaceae, is found in various parts of India and Tropical Africa. Its major active principles are triterpene saponins commonly called as the Gymnemic acids. The gymnemic acids can cause potential reduction in the triacylglycerol, cholesterol and VLDL levels. They also possess antimicrobial [4], hepatoprotective [5] and antidiabetic activities [6]. The other important phytoconstituents are flavones, anthroquinones, acontanes, phytin, quercitol, lupeol, β-amyrin related glycosides, alkaloids and stigmasterol [7]. The methanol extracts of *G. sylvestre* were used for evaluating the antimicrobial activity of the aerial and root parts in two pH ranges. The extraction at acidic pH range was much superior in activity towards all the microorganisms in comparison to the neutral range extraction.

Material and Methods

Materials

Plant material was obtained from A G Biotek. Methanol, Amikacin and bacteriological media (Mueller Hinton Agar (MHA) & Mueller Hinton Broth (MHB)) were procured from Himedia.

Extract preparation

The plants were thoroughly washed and separated in different parts like shoots (aerial parts) and roots. They were oven dried at 60°C for 24h to avoid moisture interference and finely ground using a blender. Four different extracts (aerial & root) were obtained at two different pH ranges: acidic pH (5.5) and neutral pH (7.0). The pH was adjusted using 0.1N HCl. The obtained extracts were concentrated using a rotavapour (IKA, Germany).

Microbial cultures

The MTCC cultures – *Bacillus subtilis* MTCC 2391, *Escherichia coli* MTCC 1563 and *Pseudomonas aeruginosa* MTCC 6642 were obtained from IMTECH, Chandigarh. The other test organisms were obtained as clinical isolates from Global hospitals, Hyderabad, India. All the cultures were tested for purity and maintained on Mueller Hinton agar at 4°C. The strains were sub-cultured every fortnight and reactivated in Mueller Hinton broth before the antimicrobial assay.

Antimicrobial assay

The antimicrobial activity of the extracts was assessed using the Agar well diffusion method suggested by Perez et al in 1920 [8]. An inoculum size of 10^6 colony forming units (cfu)/ml of bacteria was used for the pour plate technique. A borer with a diameter of 8mm was used for making the wells in

the MHA plates. Plant extracts at a concentration of 500 µg in the respective solvents were dispensed into each of the wells. Amikacin, a broad spectrum antibiotic was used at a concentration of 50 µg as the positive reference standard. The zones of inhibition around each well were measured after a 24h incubation period at 37°C. The sensitivity of the pathogens towards the extracts was determined by comparing the inhibitory zones around the well. All the assays were performed in triplicate and expressed.

Statistical analysis

Results were expressed as the means ± standard deviations from the obtained triplicate data. The data was compared by least significant difference test using Statistical Analysis System (ver 9.1).

Results and Discussion

Plants synthesize a wide variety of substances as part of defence mechanism against attack by insects, herbivores and microorganisms [9]. The increasing incidence of antibiotic resistance among microbes is paving the way in search of new drugs from different sources. There is a pressing need to discover new compounds differing in structure as well as activity. One such source is the plant biodiversity, as there is a widespread belief that, green medicines are nontoxic, healthier and safe, in comparison to the synthetic drugs. The antimicrobial activity of plant extracts towards the drug resistant bacteria has been studied by many researchers [10].

Table No. 1 Inhibition zones of *G. sylvestre* plant extracts in different solvents systems

Name of the Organism	Diameter of the inhibition zone (mm*)				
	Aerial		Root		Antibiotic
	Acidic(5.5)	Neutral(7.0)	Acidic(5.5)	Neutral(7.0)	
Gram positive					
B. subtilis	15.17±0.30	13.96±0.22	17.93±0.54	16.24±0.25	34.12±0.23
E. faecalis	12.42±0.01	10.83±0.17	13.45±0.26	12.27±0.19	32.09±0.24
S. aureus	13.98±0.01	13.32±0.30	19.01±0.20	16.44±0.24	29.03±0.17
S. epidermis	13.61±0.36	12.70±0.09	17.52±0.01	15.25±0.19	28.45±0.38
Gram negative					
E. aerogene	15.46±0.13	13.97±0.29	18.92±0.27	16.52±0.43	30.63±0.51
E. cloacae	15.25±0.03	13.23±0.29	12.72±0.24	11.70±0.01	29.86±0.47
E. coli	17.64±0.80	15.86±0.45	20.02±0.36	18.88±0.19	31.63±0.89
K. pneumonia	13.19±0.34	11.14±0.25	14.61±0.21	12.05±0.34	26.83±0.42
P. aeruginosa	11.87±0.19	10.88±0.24	13.11±0.56	11.74±0.56	25.92±0.64
S. typhimurium	10.45±0.29	12.64±0.21	18.86±0.24	16.55±0.21	27.74±0.15

*Borer is 8mm

In the present study, methanol has been used for extraction of active principles from the different parts of *G. sylvestre*. The presence of phytoconstituents were analysed separately in aerial and root parts. The efficient extraction of active principles depends on the competence of the organic solvent and the extraction protocol. Clinically important microorganisms were used to assess the activity of extracts. The methanol extracts have shown good activity towards all the pathogens showing its broad spectrum nature. The activity was more prominent at acidic pH (5.5) in comparison to the neutral pH (7.0). Amikacin served as the positive control, while the solvent (methanol) served as negative control. The impact of pH on the secondary metabolite extraction from plant material has been previously established [11, 12, 2].

Among the aerial and root extracts, the root extracts were much superior to the aerial extracts. The inhibition zones of root extracts at acidic pH were in the range of 20.02 ± 0.36 mm – 12.72 ± 0.24 mm (Table 1). Both the gram negative and gram positive organisms were equally susceptible. The highest zone of inhibition was obtained against *E. coli*, which is known to play an important role in nosocomial infections. The efficiency of the extracts can be seen from the zones of

inhibition obtained against potent microbes like *S. aureus* (19.01 ± 0.20mm) and *S. typhimurium* (18.86 ± 0.24mm). The most resistant organism in the present study was found to be *P. aeruginosa* (13.11 ± 0.56mm). The permeability of the compounds and the resistance mechanisms displayed by the microbes could be the reason for the variable zones of inhibition exhibited by the organisms.

Earlier reports of strong antimicrobial activity of *G. sylvestre* towards pathogens like *S. aureus* and *S. typhimurium*, have confirmed the choice of the plant in the present study [13, 14, 15]. Gram negative organisms are known to be resistant to many of the drugs as well as the natural agents, as the outer membrane acts as a selective barrier for the passage of molecules in and out of the cell. However, in the present study, the gram negative bacteria have been successfully inhibited by the extracts of *G. sylvestre* owing to the high content of saponins. The antimicrobial activity of plants attributing to the presence of saponins has been established by Soetan et al., 2006 [16]. Also the sterols viz. stigmasterol present in the plant could essay an important role in the activity due their lipophylic nature that helps them in the outer membrane penetration.

Conclusion

The exploration of secondary metabolites from plant sources seems to be an excellent choice for the development of new age antimicrobials, given the vast biodiversity in the subcontinent. The methanol extracts of *G. sylvestre* have displayed good activity against both the gram positive and gram negative microorganisms displaying its potential in the development of new phytopharmaceuticals. As these drugs are plant based, they can be considered safe for human consumption.

Acknowledgements

The authors are grateful for the financial assistance provided by Council of Scientific and Industrial Research (CSIR) and University Grants Commission (UGC).

References

[1] Gislene, G. F. N., Juliana, L., Paulo, C. F and Giuliana, L. S. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria, Brazilian Journal of Microbiology. 31: 247–256.

[2] Kiranmayee, R., Bhuvaneswari, Ch., Lakshmi, M. N and Archana, G. 2010. Antibacterial activity of *Alpinia galanga* (L) Willd crude extracts, Appl. Biochem. Biotechnol. 162: 871–884.

[3] Goud, P. S. P., Murthy, K. S. R., Pillaiah, T and Babu, G. V. A. K. 2005. Screening for antimicrobial and antifungal activity of some medicinal plants of Nallamala in Andhra Pradesh. India, J Econ Taxon Bot. 29: 704–708.

[4] Venkatesan, G. K and Kannabiran. K. 2008. Antimicrobial activity of saponin fractions of the leaves of *Gymnema sylvestre* and *Eclipta prostrate*, World Journal of Microbiol. Biotechnol. 24: 2737–2740.

[5] Rana, A. C and Avadhoot, Y. 1992. Experimental evaluation of hepatoprotective activity of *Gymnema sylvestre* and *Curcuma zedoaria*, Fitoterapia. 63: 60–62.

[6] Gopi, C and Vatsala, T. M. 2006. *In vitro* studies on effects of plant growth regulators on callus and cell suspension culture biomass yield from *Gymnema sylvestre* R. Br., African Journal of Biotechnol. 5(12): 1215–1219.

[7] Dateo, G. P and Long, L. 1973. Gymnemic acid, the antisaccharine principle of *Gymnema sylvestre*: Studies on the isolation and heterogeneity of gymnemic acid A1, Journal of agricultural and food chemistry. 21(5): 899–903.

[8] Perez, C., Pauli, M and Bazevque, P. 1990. An antibiotic assay by the agar well diffusion method, Acta Biologiae et Medicine Experimentalis. 15:113–115.

[9] Marjorie M. C. 1999. Plant products as antimicrobial agents, Clinical Microbiology Reveiws. 12: 564–582.

[10] Pesewu, G. A., Cutler, R. R and Humber, D. P. 2008. Antimicrobial activity of plants used in traditional medicine of Ghana, with particular reference to MRSA, Journal of Ethnopharmacology. 116: 102–111.

[11] Syed, S. B., Lakshmi, N. M and Naveen, A. 2009. Comparative activity against pathogenic bacteria of the root, stem and leaf of *Raphanus sativus* grown in India, World Journal of Microbiol. Biotechnol. 25: 465–473.

[12] Soma, R., Kiranmayee, R., Bhuvaneswari, C., Archana, G and Lakshmi, N. M. 2010. Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity, World Journal of Microbiol. Biotechnol. 26: 85–91.

[13] Satdive, R. K., Abhilash, P and Fulzele, D. P. 2003. Antimicrobial activity of *Gymnema sylvestre* leaf extract, Fitoterepia. 74: 699–701.

[14] Ramasubramania, R. R and Prameeladevi, B. 2010. Phytochemical and antimicrobial screening of *Gymnema sylvestre, Mentha arventis, Solanum surratense* extracts in dental carries, Journal of Pharmacy Research. 3: 21–23.

[15] Deb, R. S and Kamaljeet, S. 2010. *In Vitro* antibiotic activity of various extracts of *Gymnema sylvestre*, International Journal of Pharma Research & Development. 1–3.

[16] Soetan, K. O., Oyekunle, M. A., Aiyelaagbe, O. O and Fafunso, M. A. 2006. Evaluation of antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench, African Journal of Biotechnology. 5: 2405–2410.