Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 2 (1): 39-42, 2008
ISSN 1819-3455

Antimicrobial Activity of Saponin Fraction from the Roots of *Hemidesmus indicus*

Venkatesan Gopiesh Khanna and Krishnan Kannabiran
School of Biotechnology, Chemical and Biomedical Engineering,
VIT University, Vellore-632 014, Tamil Nadu, India

Abstract: The antimicrobial activity of saponin fraction from the roots of *Hemidesmus indicus* was evaluated against pathogenic bacteria and fungi in an *in vitro* condition by agar diffusion assay. Pure saponin extract exhibited remarkable antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*. The present study suggests that the saponin fraction possesses significant antibacterial activity. It can be concluded from this study, saponin may be a phytochemical of choice to develop as a potential antimicrobics against pathogenic microorganisms.

Key words: *Hemidesmus indicus*, saponin, antimicrobial activity, phytochemicals

INTRODUCTION

In the last few years, a number of studies have been conducted in different countries to prove plant has antimicrobial activity. Most of the studies reported were using crude extracts (Reddy *et al*., 2001; ErdoÛrul, 2002; Atefl and ErdoÛrul, 2003). But studies with purified plant compounds are very scanty. The objective of this study was to evaluate the potential of pure saponin fractions from the roots of *H. indicus* on selected bacterial and fungal strains.

Roots of *H. indicus* was widely used as a tonic in Ayurveda, Unani and folklore medicines. It acts as demulcent, diaphoretic, diuretic and used for the treatment of fever, leprosy, rheumatism. It possesses antisnake venom property (Alam *et al*., 1996) and potent anti-inflammatory activity (Alam and Gomes, 1998). Roots have been shown to possess anti-microbial properties (Satyavati *et al*., 1987). Ethanolic extract of root was reported to be antihepatotoxic (Prabakan *et al*., 2000) and antienterobacterial activity (Das and Devaraj, 2006). Several pregnane steroids have been isolated from the aerial parts of the plant (Prakash *et al*., 1991).

The fragrant roots of *H. indicus* were also used as ingredient in herbal tea (Indian native medicine) preparations. Some of the volatile compounds obtained by steam distillation from *H. indicus* roots contained 2-hydroxy-4-methoxybenzaldehyde and ledol, as the major constituents. The GC-MS analysis of the residual oil showed the presence of over 40 minor constituents. Among them, nerolidol, borneol, linalyl acetate, dihydrocarvyl acetate, salicylaldehyde, isocaryophyllene, α-terpinyl acetate and 1, 8-cineol were reported to be important aromatic and bio-active principles (Nagarajan *et al*., 2001).

Hemidesmus indicus (Asclepiadaceae), roots was collected in December 2006 from Javadi Hills, Eastern Ghats of Tamil Nadu, India. A voucher specimen of the roots *H. indicus* was prepared and deposited in the VIT University. The roots were washed with distilled water, shade dried, powdered and stored in an air-tight container separately for further use.

Corresponding Author: Dr. Krishnan Kannabiran, School of Biotechnology, Chemical and Biomedical Engineering, VIT University, Katpadi-Ranipet Road, Vellore-632014, Tamil Nadu, India
Tel: +91-0416-2202473 Fax: +91-0416-2243092/2240411

The powdered sample was defatted by petroleum ether 3×1 h at 40°C. After filtering the petroleum ether, the sample was extracted with methanol 3×1 h with mild heating. The combined methanol extract was concentrated and methanol extract of sample was obtained. In order to get the crude saponins extract the sample was dissolved in methanol and acetone was added (1:5 v/v) to precipitate the saponins (Yan *et al*., 1996). The precipitate was dried under vacuum, turning to a whitish amorphous powder named as Crude Saponin Extract (CSE). To get the Pure Saponin Fraction (PSF), certain amount of CSE was fractionated by applying to Merck silica gel-60 (230-400 mesh) column chromatography and eluted successfully with chloroform-methanol-water (70:30:10) (Favel *et al*., 2005). Five fractions were collected and the solvents were evaporated under reduced temperature; fraction 1 was chosen based on the detection of the total saponin concentration. The total saponins concentration of each fraction was measured by spectrophotometer method with some modification (Baccou *et al*., 1977; Umetsu *et al*., 2000). Standard procedures were followed to identify the chemical constituents in the aqueous extract of the roots of *H. indicus* (Sofowara, 1953; Trease and Evans, 1989; Evans, 1997; Harborne, 1973).

Bacterial strains such as *Staphylococcus aureus* (ATCC 700699), *Escherichia coli* (ATCC 11105), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 10273), *Salmonella typhi* (ATCC 700931) and *Proteus mirablis* (ATCC 19181) were used as test organisms. Exactly 0.2 mL of overnight cultures of each organism was inoculated into 5 mL of sterile nutrient broth and incubated for 3-5 h to standardize the culture to 10^6 cfu mL^{-1}. Mueller Hinton Agar solid media was used for culturing of bacteria. Agar diffusion assay was carried out to check the antimicrobial activity. The plates were incubated at 37°C for 24 h during which activity was evidenced by the presence of a zone of inhibition surrounding the well.

Aspergillus fumigatus, *Aspergillus flavus* and *Aspergillus niger* strains were clinical isolates obtained from Christian Medical College, Vellore, Tamil Nadu, India. The fungal strains were maintained in Sabouraud Dextrose Broth at 4°C. Antifungal activity was tested by well diffusion method. Each fungal culture inoculum was applied on plate and evenly spread on Sabouraud Dextrose agar using a sterile swab. At the end of the 48 h period, inhibition zones, formed in the medium were measured in millimeters (mm). All experiments were done in three replicates.

It is interesting to note that the pure saponin fraction from the roots of *H. indicus* showed significant antifungal activity when compared to the standard fungicide Amphotericin -B (Table 1). The extent of inhibition was greater against fungal pathogens than bacteria. The zone of inhibition by the pure saponin fraction (10 mg mL^{-1}) from the roots of *H. indicus* was compatible with that of Chloramphenicol (25 mg mL^{-1}). Among the bacterial pathogens tested *S. typhi* (14 mm) was more susceptible to inhibition followed by *S. aureus* (12 mm), *K. pneumoniae* (12 mm), *P. aeruginosa* (8 mm) and *E. coli* (8 mm).

Table 1: Antimicrobial activity of saponin fraction of roots of *H. indicus*

	Zone of inhibition (mm) 10 (mg mL^{-1})	
Organisms	Standard antibiotic[a]	10 (mg mL^{-1})
Pseudomonas aeruginosa	12	8
Escherichia coli	22	8
Salmonella typhi	20	14
Klebsiella pneumoniae	8	12
Proteus mirablis	18	-
Staphylococcus aureus	18	12
	Standard antibiotic[b]	20 (mg mL^{-1})
Aspergillus fumigatus	-	20
Aspergillus flavus	-	22
Aspergillus niger	8	18

a: Chloramphenicol, b: Amphotericin-B

Table 2: Screening of phytochemicals of roots of *H. indicus*

Phytochemicals	*H. indicus*
Tannins	+
Saponins	+
Flavonoids	+
Steroids	+
Terpenoids	+
Cardiac glycosides	+
Carbohydrates	+
Proteins	+
Phytosterols	-
Phenolic compounds	+
Volatile oils	+
Cardenolides	+

+: Presence of the phytochemical; - : Absence of the phytochemical

The significant outcome of this study is that the inhibition of microbial growth by the saponin fraction which was significantly higher with increase of saponin concentration. The inhibition was more pronounced when compared to standard antibiotic tested. Antidiarrhoeal effects of methanolic root extract of *H. indicus* was already been reported (Das *et al*., 2003). Further, the methanol and petroleum ether extracts of *H. indicus* roots have been shown to be very active against 15 human pathogenic microorganisms (Chakradhar *et al*., 2005).

Phytochemical screening of aqueous extract from roots of *H. indicus* showed the presence of Tannins, saponins, flavonoids, terpenoids, cardiac glycosides, phenolic compounds, carbohydrates, proteins, cardenolides and volatile oils (Table 2). Antibacterial activity of tannins and saponins isolated from plant species are well documented (Chung *et al*., 1998; Mandal *et al*., 2005). The results of this study suggest that plant saponins can be used as therapeutic agent to control common microbial diseases.

ACKNOWLEDGMENT

The authors wish to thank the management of VIT University for providing the necessary facilities to carryout this study.

REFERENCES

Alam, M.I., B. Auddy and A. Gomes, 1996. Viper venom neutralization by Indian medicinal plant (*Hemidesmus indicus* and *Pluchea indica*) root extracts. Phytother. Res., 10: 58-61.

Alam, M.I. and A. Gomes, 1998. Viper venom-induced inflammation and inhibition of free radical formation by pure compound (2-hydroxy-4-methoxy-benzoic acid) isolated and purified from anantamul (*Hemidesmus indicus* R. Br) root extract. Toxicon, 36: 207-215.

Atefl, D.A. and O.T. ErdoÛrul, 2003. Antimicrobial activities of various medicinal and commercial plant extracts. Turk. J. Biol., 27: 157-162.

Baccou, J.C., F. Lambert and Y. Sauvaire, 1977. Spectrophotometric method for the determination of total steroidal sapogenin. Analyst, 102: 458-465.

Chakradhar, T., P.J.N. Prasad and T. Pullaiah, 2005. *In vitro* antimicrobial activity of *Hemidesmus indicus* root extracts. J. Trop. Med. Plants, 6: 187-191.

Chung, K.T., T.Y. Wong, C.I. Wei, Y.W. Hung and Y. Lin, 1998. Tannins and human health: A review. Crit. Rev. Food Sci. Nutr., 38: 421-464.

Das, S., R. Prakash and S.N. Devaraj, 2003. Antidiarrhoeal effects of methanolic root extract of *Hemidesmus indicus* (Indian sarsaparilla). An *in vitro* and *in vivo* study. Indian J. Exp. Biol., 41: 363-366.

Das, S. and N. Devaraj, 2006. Antienterobacterial activity of *Hemidesmus indicus* R. Br. root extract. Phytother. Res., 20: 416-421.

ErdoÛrul, O.T., 2002. Antibacterial activities of some plant extracts used in folk medicine. Pharmaceuti. Biol., 40: 269-273.

Evans, W.C., 1997. An Index of Medicinal Plants. A Text Book of Pharmacognosy. 14th Edn., 7: 12-14.

Favel, A., E. Kemertelidze, M. Benidze, K. Fallague and P. Regli, 2005. Antifungal activity of steroidal glycosides from *Yucca gloriosa* L. Phytother. Res., 19: 158-161.

Harborne, J.B., 1973. Phytochemical Methods, London. Chapman and Hall, Ltd., pp: 49-188.

Mandal, P., S.P. Sinha Babu and N.C. Mandal, 2005. Antimicrobial activity of saponins from *Acacia auriculiformis*. Fitoterpia, 76: 462-465.

Nagarajan, L. J.M. Rao and K.N. Gurudutt, 2001. Chemical composition of the volatiles of *Hemidesmus indicus* R. Br. Phytother. Res., 16: 212-214.

Prabakan, M., R. Anandan and T. Devaki, 2000. Protective effect of *H. indicus* against rifampicin and isoniazid induced hepatotoxicity in rats. Fitoterapia, 71: 55-59.

Prakash, K., A. Sethi, D. Deepak, A. Khare and M.P. Khare, 1991. Two pregnane glycosides from *Hemidesmus indicus*. Phytochemistry, 30: 297-299.

Reddy, P.S., K. Jamil and P. Madhusudhan, 2001. Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*. Pharmaceut. Biol., 39: 236-238.

Satyavati, G.V., A.K. Gupta and N. Tandon, 1987. Medicinal Plants of India, New Delhi, 2: 16-17.

Sofowara, A., 1953. Medical Plants and Tropical Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp: 289.

Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. 11th Edn., Brailliar Tridel Can. Macmillan Publishers.

Umetsu, Y., K. Hirata, K. Saito and I. Kudo, 2000. Spectrophotometric determination of saponins in Yucca extract used as food additive. J. AOAC. Int., 836: 1451-1454.

Yan, W., K. Ohtani, R. Kasai and K. Yamasaki, 1996. Steroidal saponin from fruits of *Tribulus terrestris*. Phytochemistry, 42: 1417-1422.