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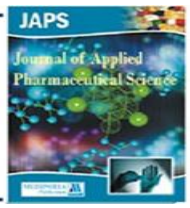

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Phytochemical screening and antibacterial activity of *Gymnema sylvestre* (Retz) R . Br ex. Schultes and *Morinda pubescens* J.E. Smith var. *pubescens*

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ABSTRACT

Two medicinal plants namely *Gymnema sylvestre* and *Morinda pubescens* var. *pubescens* were screened for potential antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The antibacterial activity was determined in petroleum ether, chloroform, acetone, methanol and aqueous extracts using disc diffusion method. The chloroform and methanol extract of leaf of *Gymnema sylvestre* showed highest inhibition against *Escherichia coli* and *Klebsiella pneumoniae* respectively; whereas, acetone extract of *Morinda pubescens* var. *pubescens* leaf exhibited maximum inhibition against *Pseudomonas aeruginosa*. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, tannins, and terpenoids. The results of these studies revealed most valuable information and also support the continued sustainable use of these plants in traditional systems of medicine.

Keywords: *Gymnema sylvestre, Morinda pubescens* var. *pubescens*, antibacterial activity, disc diffusion method.

INTRODUCTION

In recent times, focus on plant research has increased all over the world and immense potential of medicinal plants used in various traditional systems has been highlighted (Dahanukar *et al*., 1999). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Bruneton 1995). In recent years, antibacterial properties of Indian medicinal plants have been increased (Ahmad *et al*., 1998; Ahmad and Beg, 2001; Aqil and Ahmad, 2003; Chendurpandy *et al*., 2011). However, a majority of traditionally used Indian medicinal plants have not yet been systematically screened against various microbial pathogens. In the present study, the antibacterial activity of leaf and stem extracts of *Gymnema sylvestre* (Retz) R. Br ex. Schultes and *Morinda pubescens* var. *pubescens* J.E. Smith have been studied using different bacterial strains by disc diffusion method.

MATERIALS AND METHODS

Collection of Plant Materials

The leaf and stem materials of *Gymnema sylvestre*, leaf and stem bark of *Morinda pubescens* var. *pubescens* were collected from the well grown plants in Grizzled Giant Squirrel Wildlife Sanctuary, Western Ghats, Srivilliputhur, Tamil Nadu. They were shade dried at room temperature for 10-15 days.

Extraction of Plant Material

Various organic solvents were used for the extraction of bioactive compounds. The leaves, stem and stem bark powders (10g) of *Gymnema sylvestre* and *Morinda pubescens* var. *pubescens* were first extracted with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered sample of *Gymnema sylvestre* and *Morinda pubescens* var. *pubescens* were dried and successfully extracted with petroleum ether, chloroform, acetone, methanol and then water in a Soxhlet apparatus.

The extracts obtained were completely evaporated by using vacuum rotary evaporator. The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Brindha *et al*., 1981; Anonymous 1996; Lala 1993*)*. The concentrated extracts were used for antibacterial activity.

Microorganisms

Bacterial strains of *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 109), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 424), *Pseudomonas aeruginosa* (MTCC 443) and *Salmonella typhi* (MTCC 531) were procured from microbial type culture collection, Chandigarh. The bacteria were incubated on a nutrient agar-slant (stationary cultures) for 48h at 37^{o}C followed by inoculation in Muller Hinton Agar (MHA) medium.

Antibacterial Assay

Antibacterial activity was demonstrated using a modified method originally described by Bauer *et al.*, 1966, which is widely used for the antibacterial susceptibility testing (Barry and Thornsberry 1985). A loopful bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20µl) various crude solvent extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram positive and Gram negative bacteria. Respective solvents without plant extracts served as negative control. Standard antibiotics of chloramphenicol and tetracycline were used as reference or positive control.

Plates were incubated at 37^{o}C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs.

RESULTS AND DISCUSSION

The preliminary phytochemical study of the methanol extracts of leaf and stem of *Gymnema sylvestre* revealed the presence of alkaloids, anthraquinones, catechin, coumarin, flavonoids, phenols, steroids, tannins, terpenoids and xanthoprotein (Table1); whereas, leaf and stem bark extracts of *Morinda pubescens* var. *pubescens* exhibited the presence of alkaloids, coumarin, flavonoids, phenols, saponins, steroids, tannins, terpenoids and sugar (Table 2).

The antibacterial activity of the leaf and stem extracts of *Gymnema sylvestre* and leaf and stem bark extracts of *Morinda pubescens* var. *pubescens* are furnished in table 3. All the extracts exhibited different degrees of antibacterial activity. Acetone and methanol extracts of *G. sylvestre* leaf and stem and *M. pubescens* var. *pubescens* leaf and stem bark showed activity against all the six tested pathogens. Petroleum ether extract of *G. sylvestre* leaf showed antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa and S. typhi*; whereas, stem extract did not inhibit *K. pneumoniae, B. subtilis and S. typhi.* Chloroform extract of leaf and stem of *G. sylvestre* showed activity against the entire tested microorganism except *B. subtilis.* Aqueous extract of *G. sylvestre* stem showed minimum activity against *K. pneumoniae* and *S. typhi*. The chloroform and methanol extracts of leaf of *G. sylvestre* showed the highest inhibition zone, observed against *E. coli* and *K. pneumoniae* respectively. Petroleum ether extract of *M. pubescens* var. *pubescens* leaf showed activity against *S. aureus, B. subtilis* and *S. typhi*; whereas, stem bark extract did not inhibit *E. coli.* Chloroform extract of leaf exhibited activity against the entire tested microorganism except *B. subtilis*; whereas, stem bark extract failed to inhibit the growth of *K. pneumoniae* and *E.coli.* Aqueous extract of stem bark showed activity against *K. pneumoniae* and *P. aeruginosa*.

Table 1: Preliminary phytochemical screening of leaf and stem extract of *Gymnema sylvestre.*

Presence/absence of bioactive components	Name of the extract									
	Petroleum ether		Chloroform		Acetone		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	-	-	-	-	-	+	+	-	-
Anthraquinones	-	-	-	-	+	+	+	+	-	-
Catechin	-	-	-	-	+	-	+	+	-	-
Coumarin	-	-	+	+	+	+	+	+	-	-
Flavonoids	-	-	-	-	-	-	+	+	-	-
Phenols	-	-	+	+	+	+	+	+	-	-
Quinones	-	-	-	-	-	-	-	-	-	-
Saponins	+	+	-	-	+	-	-	-	+	-
Steroids	+	+	+	+	-	-	+	+	-	-
Tannins	+	+	-	+	-	+	+	+	-	-
Terpenoids	-	-	-	-	-	-	+	+	-	-
Xanthoprotein	+	+	-	+	+	+	+	+	+	-
Sugar	-	-	+	+	+	+	+	-	-	-

+ Presence - Absence

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Table 2: Preliminary phytochemical screening of leaf and stem bark extract of *Morinda pubescens* var. *pubescens.*

Presence/absence of bioactive components	Name of the extract									
	Petroleum ether		Chloroform		Acetone		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	-	-	-	-	-	+	+	-	-
Anthraquinones	+	-	+	+	+	+	+	-	-	+
Catechin	-	-	-	-	-	-	-	-	-	-
Coumarin	+	-	+	+	-	+	+	+	-	+
Flavonoids	-	-	-	-	-	-	+	+	-	-
Phenols	+	+	+	+	+	+	+	+	+	-
Quinones	-	+	+	+	-	+	-	+	-	-
Saponins	-	-	-	-	-	-	+	+	-	-
Steroids	-	+	+	+	+	+	+	+	-	-
Tannins	-	+	+	+	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+	-	-
Xanthoprotein	-	+	+	+	+	+	+	-	+	-
Sugar	+	+	+	+	-	+	+	+	-	+

+ Presence - Absence

Table 3: Antibacterial activity of *Gymnema sylvestre* and *Morinda pubescens* var. *prubescens.*

Name of the extract	Plant Botanical Name	Plant part & (Antibiotic)	Zone of inhibitor (mm)					
			S. aureus	*K. pneumoniae*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhi*
Petroleum ether	*Gymnema sylvestre*	L	3	0	1	0	2	1
		S	0	2	0	4	3	0
		T	9	8	9	9	9	9
		C	9	9	9	9	9	9
	Morinda pubescens var. *pubescens*	L	3	0	4	2	0	0
		SB	1	2	1	0	1	1
		T	8	9	9	9	9	9
		C	8	9	9	9	9	9
Chloroform	*Gymnema sylvestre*	L	5	4	0	6	5	3
		S	2	4	0	1	3	2
		T	9	9	9	8	9	9
		C	9	9	9	9	9	9
	Morinda pubescens var. *pubescens*	L	2	3	0	2	1	4
		SB	4	0	3	0	5	2
		T	9	9	9	8	9	9
		C	9	9	8	8	9	8
Acetone	*Gymnema sylvestre*	L	2	4	2	2	1	2
		S	2	2	1	2	3	1
		T	8	8	8	9	9	9
		C	9	9	8	9	9	9
	Morinda pubescens var. *pubescens*	L	3	4	3	2	6	1
		SB	4	5	3	3	2	4
		T	9	9	8	8	8	9
		C	9	9	8	9	9	9
Methanol	*Gymnema sylvestre*	L	4	6	2	3	4	2
		S	2	3	2	2	2	1
		T	9	9	8	9	9	9
		C	9	9	9	9	9	9
	Morinda pubescens var. *pubescens*	L	3	3	2	2	4	1
		SB	2	4	1	3	2	2
		T	9	8	8	9	9	9
		C	8	9	9	9	9	8
Water	*Gymnema sylvestre*	L	1	2	1	1	1	2
		S	0	1	4	0	0	2
		T	9	9	8	9	8	9
		C	9	9	9	9	9	9
	Morinda pubescens var. *pubescens*	L	1	2	1	1	2	1
		SB	0	1	0	0	2	0
		T	9	9	8	9	9	8
		C	9	9	9	9	9	9

L- leaf S-Stem SB- Stem bark T- Tetracycline C- Chloramphenicol

Acetone extract of leaf of *M. pubescens* var. *pubescens* exhibited the highest inhibition zone, observed against *P. aeruginosa*. Antibacterial activity was comparable with that of the standard antibiotics, tetracycline and chloramphenicol against the organisms tested. It is concluded that, in the present study, both the plants contain potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments. Although the exact active component of the extracts which showed this effect was not identified, the antibacterial active plant principles such as flavonoids, alkaloids and tannins were observed in the extracts.

REFERENCES

Ahmad I and Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol. 2001; 74: 113-123.

Ahmad I., Method Z and Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 1998; 62: 183-193.

Anonymous. Indian Pharmacopoeia, Government of India. Ministry of Health and family welfare. The controller of publication. Civil lines, Delhi-110054. 1996, vol. I &II.

Aqil F and Ahmad I. Broad spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World J. Microbial & Biotech. 2003: 19: 653-657.

Barry A.L. and Thornsberry C. Susceptibility tests: Disc diffusion test procedures. In: Manual of Clinical Microbiology (Eds). Lennette Balows, EH., W.J. Hauster and H.J. Shadomy. American Society for Microbiology, Washington, DC. 1985; 978-987.

Bauer A.W., Kirby W.M.M., Sherries S.C and Turk M. Antibiotic susceptibility of testing by a standard single disc method. Amer. J. Clinical. Pathol. 1966; 36: 492-496.

Brindha P., Sasikala B. and Purushothaman K.K. Pharmacognostic studies on *Merugan Kizhangu*. Bull.Med.Eth.Bot.Res. 1981; 3: 84-96.

Bruneton J. Pharmacognosy, Phytochemistry, Medicinal plants. France: Lavoisiler Publishing Co. 1995; 265-380.

Chendurpandy P., Mohan V.R and Kalidass C. Screening of ethnomedicinal plants for antimicrobial activity. J. Econ. Taxon.Bot. 2010; 34: 663-669.

Dahanukar S.A., Kulkarni R.A and Rege N.N. Pharmacology of medicinal plants and natural products. Medicinal plants 1999: S 81-S101.

Lala P.K. Lab Manuals of Pharmacognosy. CSI Publishers and distributors, Calcutta, 5th Edition. 1993.