ORIGINAL ARTICLE

Antibacterial evaluation of *Sapindus emarginatus* Vahl leaf in *in-vitro* conditions

Yogeshkumar Vaghasiya, Rathish Nair, Sumitra Chanda
Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot -360 005, Gujarat, India

The emergence of antibiotic resistant bacterial strains is a growing problem and is an important concern for patients, physicians, healthcare managers and policymakers as it results in poorer health and economic outcomes. This has led to an urgent global call for new antimicrobial drugs, particularly from natural resources. In the present study, antibacterial evaluation of aqueous, 1, 4-dioxan, methanol and acetone extracts of *Sapindus emarginatus* Vahl leaf was studied against 6 medically important bacterial strains. Antibacterial assay was performed by agar well diffusion method. Gentamicin (5mg/10ml) was used as standard antibiotic for comparison. Dioxan extract exhibited potential antibacterial activity

Key words: Agar well diffusion method, antibacterial evaluation, *Sapindus emarginatus*

INTRODUCTION

Plants contain numerous biologically active compounds many of which have been shown to have antimicrobial properties.[1] Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials is the need of the hour. They have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.[2] Also there is an alarming incidence of antibiotic resistance in bacteria of medical importance; there is a constant need for new and effective therapeutic agents.[3] The effect of plant extracts on bacteria has been studied by a very large number of workers in different parts of the world.[4] *Sapindus emarginatus* Vahl. belongs to the family Sapindaceae. Seeds of *S. emarginatus* contains oil which is traditionally used as anti inflammatory. It is used to purify the blood. In the present work, *S. emarginatus* leaf is subjected to Soxhlet extraction in different solvents and the extract thus obtained is used for antibacterial and phytochemical analysis.

Plant Material

Fresh leaves of *S. emarginatus* were collected randomly from the semi-arid region of Rajkot, Gujarat, India. The taxonomic identification of *S. emarginatus* (PSN-131) was determined by Dr. P. S. Nagar, Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaves of *S. emarginatus* were washed under tap water, air dried, homogenized to fine powder and stored in air tight bottles.

Extraction

The dried leaf powder was extracted by Soxhlet extraction method.[5] Four different solvents (Aqueous, 1,4-dioxan, methanol and acetone) were used for the extraction. The solvents were evaporated from the extracts and dried extracts were collected, the extractive yield was calculated and all the extracts were stored at 4°C.

Bacterial Strains

The bacterial strains used were obtained from National Chemical Laboratory (NCL), Pune. The studied bacterial strains were *Pseudomonas testosteroni* NCIM5098, *Staphylococcus epidermidis* ATCC12228, *Proteus morganii* NCIM2040, *Bacillus subtilis* ATCC6633, *Micrococcus flavus* ATCC10240 and *Klebsiella pneumoniae* NCIM2719. The bacteria were grown in the nutrient broth and maintained on nutrient agar slants at 4°C.

Antibacterial Assay

The antibacterial activity of solvent extracts was done by agar ditch diffusion method.[6,7] The media along with the test bacterial culture was poured into the Petri plate (Hi-media). The test strain (0.2 ml) was inoculated into the media (inoculum size 10^8 cells/ml) when the temperature reached 40-42°C. Care was taken to see proper homogenization occurred. A well was prepared in the plates with a cup-borer (0.85 cm) and 0.1ml of the extract (10mg/ml in dimethylsulphoxide) was pipetted directly into the well. Each bacterial strain controls were maintained using pure dimethylsulphoxide instead of the extract. The plates were incubated for 24h at 37°C. The control zones were subtracted from the test zones

Address for correspondence: Dr. Sumitra Chanda, Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot - 360 005, Gujarat, India. E-mail: svchanda@gmail.com
Received: 12-03-2008; **Accepted**: 26-06-2008; **DOI:** 10.4103/0973-8258.54913

and the resulting zone diameter is shown in the Table 1. The experiment was performed three times to minimize the error and the mean values are presented. The extracts were compared with standard antibiotic gentamicin at 5mg/10ml concentration.

RESULT AND DISCUSSION

Initially *S. emarginatus* leaf was extracted both in aqueous and methanol and its antibacterial activity against *Ps. testosteroni, K. pneumoniae*, *M. flavus, P. morganii*, *B. subtilis* and *S. epidermidis* were studied.[8] The previous results showed that methanolic extract exhibited considerably more activity than the aqueous extract and based on these results, *S. emarginatus* leaf was extracted in three different solvents (1,4-dioxan, methanol, and acetone) and the crystalline powder thus obtained was dissolved in DMSO and its antibacterial activity was studied by agar ditch method.

The antibacterial study of *S. emarginatus* in different solvents against *Ps. testosteroni* is shown in Table 1. The maximum inhibitory activity was shown by TDi (1,4-dioxan extract) where as minimum activity was shown by TMe (methanol extract), TAc (acetone extract) and TAq (water extract). All the extracts were inactive against *K. pneumoniae* and could not show any inhibitory activity. TDi showed maximum activity against all the Gram positive bacterial strains studied. TAq was inactive against either of the Gram positive bacterial strains. TMe and TAc extracts did not show any activity against *B. subtilis* while they could inhibit *S. epidermidis* to a certain extent. In this study, different solvents proved better for different strains but the solvent TDi proved to be the best for *Ps. testosteroni, S. epidermidis, B. subtilis* and *M. flavus*. Amongst the bacterial strains studied *K. pneumoniae* was the most resistant strain while *M. flavus* was the most susceptible ones. The aqueous extract showed activity only against *Ps. testosteroni* and *P. morganii*, the Gram negative bacterial strains while it did not show any activity against the remaining four strains.

CONCLUSION

From the above results, it can be concluded that all the compounds extracted in five different solvents were able to inhibit the growth of the investigated bacterial strains; the most susceptible bacteria was *M flavus* while most resistant was *K pneumoniae*. Dioxan extract was most potent extract as antibacterial agent compared with other extracts studied. The various phytochemical studies revealed the presence of steroids, glycosides and saponins in the leaf of *S. emarginatus* [Table 2].

Table 1: Antibacterial activity of different extracts of *Sapindus emarginatus* leaf

Bacterial strain	*Zone of inhibition (mm)				
	TDi (9.31%)**	TMe (14.74%)**	TAc (4.06%)**	TAq (7.56%)**	Gentamicin
Ps. testosteroni	10.5	9.5	9.5	9.5	30
S. epidermidis	12.5	10.5	10.5	-	-
P. morganii	15.5	15.5	14.5	15.5	-
B. subtilis	9.5	-	-	-	26.5
M. flavus	19	16.5	15.5	-	33
K. pneumoniae	-	-	-	-	-

Values are mean of triplicates, *Diameter of the well is 8.5 mm, G - Gentamicin (5 mg/10 ml), TDi - 1,4 - Dioxan extract, TMe - Methanol extract, TAe - Acetone extract, TAq - Water extract, ** Percent Yield obtained, - means no activity

Table 2: Preliminary qualitative analysis of the extracts of *S. emarginatus* leaf extracted in different solvents

Plant constituents (Test/Reagent used)	TDi	TMe	TAc	TAq
Alkaloids				
Dragendroff test	-	-	-	-
Wagner's test	-	-	-	-
Mayer's test	-	-	-	-
Carbohydrates	-	-	-	-
Glycosides	+	+	-	+
Steroids	+	+	+	-
Saponins	+	+	+	+
Tannins	-	-	-	-
Phenols	+	+	+	+

TDi - 1,4 - Dioxan extract, TMe - Methanol extract, TAe - Acetone extract, TAq - Water extract, + means present, - means absent

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
2. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J, editor Perspectives on New Crops and New Uses. Alexandria VA: ASHS Press; 1999; p. 457.
3. Bhavnani SM, Ballow CH. New agents for Gram positive bacteria. Curr Opin Microbiol 2000;3:528-34.
4. Mothana RA, Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. J Ethnopharmacol 2005;96:177-81.
5. Vaghasiya Y, Nair R, Baluja S, Chanda S. Antibacterial and preliminary phytochemical analysis of *Eucalyptus citriodora* Hk leaf. Nat Prod Res 2008;22:754-62.
6. Perez C, Paul M, Bazerque P. An antibiotic assay by the agar well diffusion method. Acta Biol Med Exp 1990;15:113-5.
7. Parekh J, Chanda S. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 2007;31:53-8.
8. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol 2005;29:41-7.

Source of Support: Nil, **Conflict of Interest:** None declared.