

Phytochemical screening and inhibitory effect of *n*-butanol, isopropanol and water extracts leaf extracts of *Sapindus saponaria* Vahl on selected pathogens

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ABSTRACT

The preliminary phytochemical screening of the leaves with n-Butanol, isopropanol and water extracts revealed the presence of alkaloids, saponins, steroids, flavonoids, glycosides, terpenes and phenols. In vitro antimicrobial studies of these leaf extracts were carried out on four medically important microbial strains, including Streptococcus faecalis, Streptococcus aureus, Bacillus subtilis and Bacillus peritolis which were procured from the Microbial Type Culture and Collection, Chandigarh, India, using agar disc diffusion method. The microbial strains were exposed to the following four different concentrations of extracts: 50mg/ml, 100mg/ml, 200mg/ml and 300mg/ml solvent. The results of our antimicrobial assay revealed that the extract showed good inhibitory activity against all the tested pathogens compared with standard antibiotic like streptomycin. The inhibitory activities were found to be dose dependent.

Keywords: Antimicrobial studies, *Sapindus saponaria*, agar disc diffusion method, Pathogens, extracts

INTRODUCTION

The search for selective antimicrobial agents has gained significance in recent era due to the growing cases of microbial resistance to the time honored antibiotics [1-2]. So, this situation fetches the interest of scientist to expand newer broad spectrum antibacterial agents [3]. The higher plants are among the most prominent natural resources. They provide nutrients, fiber, wood and many chemical compounds such as oils, flavonoids, paints and medicinal compounds such as alkaloids [4]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, microbes have the genetic capacity to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [5, 6]. So there is a imperative want to find out new spectrum of antimicrobial agents with minimal side effects, and higher plants are prospective source of novel antimicrobial prototypes [7,8].

Sapindus saponaria (family: Sapindaceae) with alternative Botanical Names *Sapindus thurstonii* and commonly known as Manele, A'e , Hawaiian Soapberry is a known medicinal plant. *Sapindus saponaria* is a tall deciduous tree, growing up to 80 feet in height. It has pale brown bark that falls off in large patches on mature trees. The leaves are made up of 3 to 6 pairs of leaflets. The upper surface of the leaves is shiny green and the lower surface is fuzzy [9]. The present study describes the evaluation and phytochemical screening of antibacterial potential plant species *Sapindus saponaria* against four common human pathogens.

MATERIALS AND METHODS

Plant material:

The matured healthy leaves of plant material *Sapindus saponari* was obtained from the local area in and around Narasaraopet, Guntur district (India). The plant was indentified based on its floral description given in the literature. The plant leaves were air dried under shade and made into fine powder by using hand homogenizer and sieved through sieve no. 40 and the fine powder was used for extraction procedure and other evaluation.

Chemicals:

All the solvents used in this study were purchased from Merck Chemicals, India, of analytical grade.

Preparation of extract:

The leaves were washed in tap water, shade dried for 20 days and made into a fine powder of 40 mesh size using the laboratory mill. Following that, 100g of the powder was filled in the thimble and extracted using 500 ml of distilled *n*-Butanol, isopropanol and water in soxhlet apparatus for 8 – 10 hours. The extract was filtered through Whatman No.1 filter paper to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure. The dried extract was redissolved in *n*-Butanol, isopropanol and water to yield solutions containing 50, 100, 200 and 300mg of leaf extract per ml solvent [10,11]. (Table1).

Preliminary Phytochemical Analysis:

Phytochemical screening of plant extracts was done following the standard procedure by Santaram (1983), Chhabra et al (1984) and Harbone (1998). All the prepared plant leaf extracts were subjected to preliminary phytochemical screening for the presence of alkaloids, quinines, resins, tannins, fixed oils, flavanoids, fats, saponins, phenolic compounds, Proteins and carboxylic acids [12, 13]. The results were shown in Table 2.

Antibacterial Screening:

The four different concentrations of the leaf extracts were tested for antibacterial activity using agar disc diffusion assay according to the method of Bauer *et al.*, 1966 [13]. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37° C for 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37° C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Streptomycin (10-µg/disc) as standards.

a) Micro organisms used:

The screening of the antibacterial activity of crude extracted from the leaf of *Sapindus saponaria* were carried out individually on active cultures of *Streptococcus faecalis*, *Streptococcus aureus*, *Bacillus subtilis* and *Bacillus peritolis*. All the strains were procured from the Microbial Type Culture and collection, Chandigarh, India.

b) Preparation of media:

Muller Hinton Agar (MH, Hi media) was used. The formula (gm/liter) Beef extract 2g, casein acid hydrolysate 17.5g, starch 1.5 g and agar 17g; pH 7.4 ± 0.2. About 38g of MH agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH 7.4 ± 0.2, sterilized by autoclaving at 121 °C for 15 minutes at 15 psi pressure and was used for sensitivity tests [14-16].

RESULTS AND DISCUSSION

The *n*-Butanol, isopropanol and water extracts of *Sapindus saponaria* leaves having extractive value 7.1gm, 6.83 gm and 5.72 gm (Table1) on phytochemical screening showed the presence of alkaloids, saponins, steroids, terpenoids, flavonoids, glycosides and phenols as chemical constituents (Table 2).

Table 1: Extractive value of *n*-Butanol, isopropanol and water extracts of *Sapindus saponaria*

S. No	Type of extract	Extractive Value (in grams)
1	<i>n</i> -Butanol	7.1
2	Isopropanol	6.83
3	Water	5.72

Table 2: Phytochemical screening of leaf extracts of *Sapindus saponaria*

S.No	Plant constituents	<i>n</i> -Butanol extract	Isopropanol extract	Water extract
1	Test for Alkaloids	+	+	+
2	Test for Volatile oils	-	-	-
3	Test for Carboxylic acids	-	-	-
4	Test for Fixed oils	-	-	-
5	Test for Saponins	+	+	+
6	Test for Tannins	+	+	-
7	Test for flavonoids	+	+	+
8	Test for Phenols	+	-	-
9	Test for Carbohydrates	-	-	-
10	Test for Fatty acids	-	-	-
11	Test for Resins	-	-	-
12	Test for Quinones	-	-	-
13	Test for Terpenes	+	+	+
14	Test for Cardinolides	-	-	+
15	Test for Glycosides	+	+	+
16	Test for steroids	+	+	+

+ Indicates the presence of the constituents.

- Indicates the absence of the constituents.

The antimicrobial activities of various extracts like *n*-Butanol, isopropanol and water extracts are evaluated and compared using disc diffusion method. All the test extracts of *Sapindus saponaria* possess significant antimicrobial activity against these common human dental pathogens (Fig 1, 2, 3). Among the three extracts, the water extract showed a higher activity than other extracts (Fig 3). This may be due to the solvent extract containing different constituents having antimicrobial activity. Water was proved as the most effective solvent for extracting broad spectrum of antibacterial compounds from these plants.

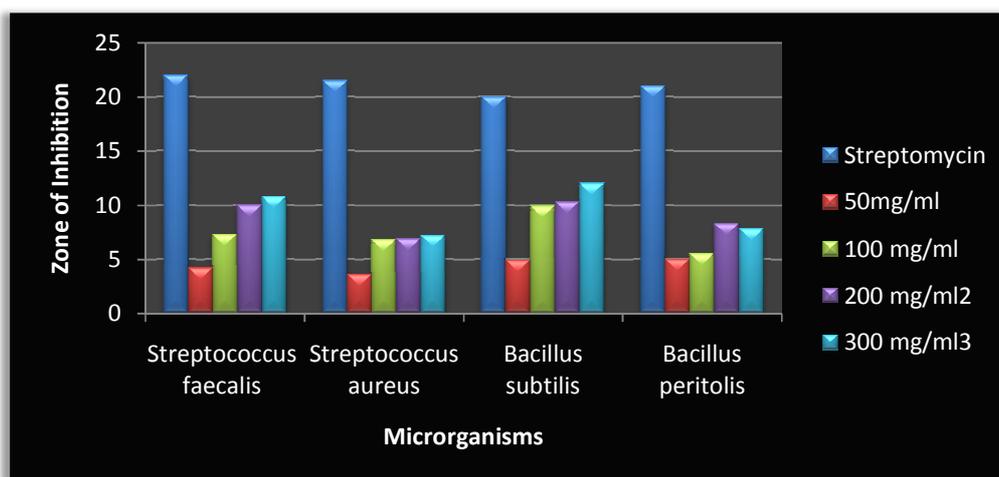
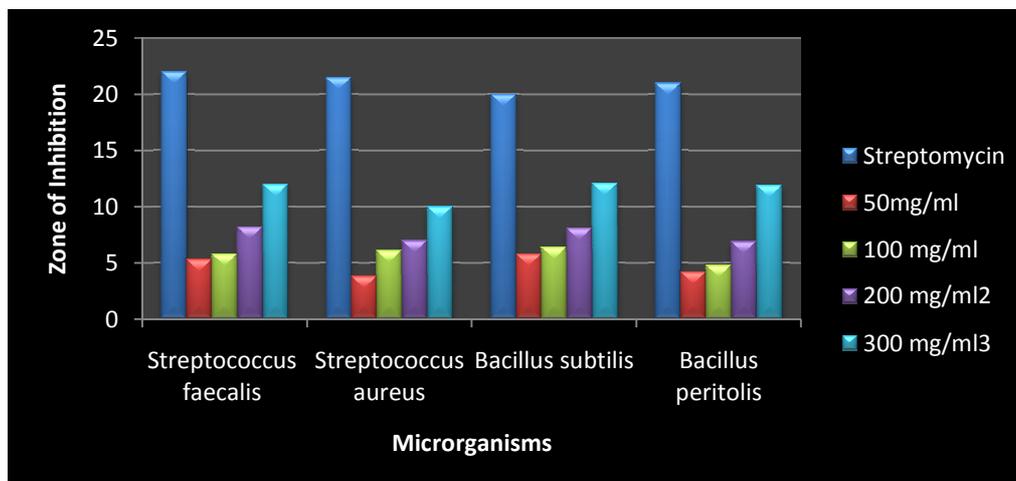
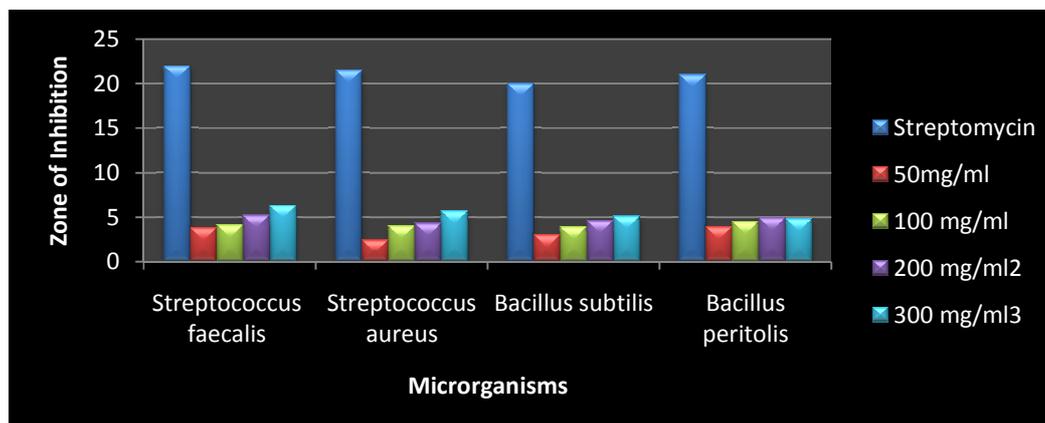
Fig 1: Antibacterial activity of leaf *n*-butanol extracts of *Sapindus saponaria*

Fig 2: Antibacterial activity of leaf Isopropanol extracts of *Sapindus saponaria*Fig 3: Antibacterial activity of leaf water extracts of *Sapindus saponaria*

CONCLUSION

The result of this work suggested that the leaf extracts of *Sapindus saponaria* has potent antibacterial activity against common human pathogens. Therefore the results justify the use of the leaf extract in treating these pathogenic strains and these may serve as leads for the development of new pharmaceuticals that address hither unmet therapeutic needs.

REFERENCES

- [1] Samie A, Obi CL, Bessong PO, Namrita L, *Afr. J. Biotechnol.* **2005**, 4, 1443-1451.
- [2] Francis Xavier T, Arun VR, *J. Plant Sci.* **2007**, 2, 579-582.
- [3] Weisser R, Asscher AW, Winpenny J, *Nature* **1966**, 219, 1365-1366
- [4] Alishahi Norani, F. and S. Mehrabian, **1995**. MS Thesis; Science Faculty. Tehran Tarbiat Moalem. Iran.
- [5] COWAN MM. *Clin Microbiol Rev.* **1999**, 12: 564-582.
- [6] Sofowora A. John Willey and Sons Ltd., Ibadan. **1982**: 8-14.
- [7] Truiti MCT, Sarragiotto MH, Filho BAA, Nakamura CV and Filho BPD. *Mem Inst Oswaldo Cruz.* **2003**, 98(2):283-286.
- [8] Maureer – Grimes B, Macbeth DL, Hallihan B and Delph S. *Int. Journal of pharmacognosy.* **1996**, 34: 243 – 248.
- [9] Wagner, Warren L, Darrel R, Herbst and Sohmer S.H. Honolulu: University of Hawaii Press and Bishop Museum Press. **1990**.

- [10] Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, New Delhi. **2005**.
- [11] Bakshu LMD. Ethnomedicobotanical and Phytochemical evaluation of certain rare, endemic and endangered medicinal plants from Eastern Ghats, Andhra Pradesh, India. **2002**.
- [12] Harbone, JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall London. **1998**.
- [13] Bauer AW, Kirby WMM, Sherris JC, Turck M. *American journal of clinical pathology*. **1966**, 45: 493-496.
- [14] Agnese AM, Perez C, Cabrera JL. *Phytomedicine*. **2001**, 8: 389- 394.
- [15] Acar JF and Goldstein FW. Disc susceptibility test: Antibiotics in laboratory medicine, 4th edition, edited by L. victor, Williams and Wilkins publishers. **1998**.
- [16] National Committee for clinical laboratory standards, performance standards for antibacterial disc susceptibility testing, twelfth information supplement (M100-S12), Wayne, PA: NCCLS, **2002**.