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Phytochemical Investigation and Antibacterial activity of leaf and stem extracts of *Sapium indicum* Linn

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Abstract

Plants have provided a source of inspiration for novel drugs compounds. In this study, the antibacterial activity was of *Sapim indicum* Linn. collected from mangroves area of Odisha was made against some bacterial pathogens by disc diffusion method. All the four crude extracts (methanol, chloroform, hexane, and petroleum ether) tested, showed significant antimicrobial activity with zone of inhibitions ranging between 9-31 mm. Among all the solvent, methanol extracts of both stem and leaf exhibited the highest inhibition zone against *Salmonella typhi*. Further evaluation of minimal inhibitory concentration (MIC) showed values ranging from 4-5.6 mg/ml. Preliminary phytochemical screening revealed the presence of alkaloids, steroids, triterpenoids, saponins, flavonoids, reducing sugars and protein in almost all extract. The present study of *in vitro* antibacterial assay and preliminary phytochemical analysis may open new vistas for identifying potential bioactive molecules for chemical, pharmacological and herbal industry.

Keywords: Mangrove, Sapium indicum, phytochemical screening, antimicrobial activity

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Introduction

Plants have been used to treat or prevent illness since before recorded history and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments [1]. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experiment. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant [2]. According to world health organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances (phytochemical) that were synthesized by plants as secondary metabolites and serve as the molecules of plant defense against predation by microorganisms, insects and herbivores. These chemicals produce a definite physiologic action on the human body. Furthermore, some of these compounds involve in plant odor, pigmentation and flavor. However, several of these molecules possess medicinal properties [3]. In recent years, antibiotics derived from the plants have been receiving increasing attention, as the synthetic antibiotics have shown ineffectiveness against several pathogenic organisms due to increasing drug resistance. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [4].

Bhitarkanika wildlife sanctuary in the state of Odisha is one of the richest mangrove forests of India exhibiting maximum plant diversity. Several workers have reported the usefulness of mangrove plants in traditional medicines [5-7]. *Sapium indicum* Linn. (Euphorbiaceae), commonly known as Batul (Bengali), is a small, semi-deciduous to evergreen mangrove species having oblong, elliptic-oblong or elliptic, lanceolate leaves, yellowish clustered flowers and woody capsules. Traditionally the leaves of this plant were used against the irritation after fish sting to relieve pain. The milky juice of the plant is applied on the wound area and a rapid recovery of irritation and subsequently pain are perceived. The plant is also used in fish poisoning [8]. The species is credited with some medicinal properties and the leaves are useful for dyeing yarn.

This study is aimed at investigating the antimicrobial properties and the major phytochemical constituents of different solvent extracts (methanol, chloroform, hexane, and petroleum ether) of leaf as well stem of *Sapium indicum* Linn. with a view to assess their pharmacological potential. The study will also expose new frontiers or improve on the current applications of the plant extract.

Materials and Methods

Collection of Plant sample

The plant material such as leaves and stem of *Sapium indicum* were collected from mangrove areas of Khola, Bhitakanika, Odisha and identified through consulting the herbarium at CSIR-Institute of Minerals and Material Technology (RRL-B), Bhubaneswar following "The flora of Orissa", Volume III [9].

Preparation of plant extract

The collected samples were washed with distilled water properly for removing adhered soil and other particles and shade dried for about 7 to 10 days. The dried plant samples material was then pulverized into fine powder and stored. Twenty five gram of fine powder was added to a soxhlet apparatus along with a solvent (chloroform, ethanol, methanol and aqueous) for extraction of chemicals. The extracts were concentrated and dried in a rotary evaporator and then placed in a desiccators and was allowed to dry completely. Once completely dry, the extracts were stored at 4^0 C and further used for antimicrobial assay.

Preliminary Phytochemical Analysis

A qualitative phytochemical test to detect the presence of alkaloid, tannin, saponin, flavonoid, glycoside and phenol were carried out using standard procedures [10-12].

Antimicrobial Activity

Test pathogens

Various bacterial pathogenic strains such as Salmonella typhii, Salmonella typhii (MMBB-13), Salmonella typhii (MBBC-3), Proteobacter augustum, Staphalococcus faecalis and Enterococcus faecalis were collected from Kalinga Institute of Medical Science, Bhubaneswar and maintained in Nutrient agar slant.

Antimicrobial assay

Preliminary screening of the extracts was carried out by disc diffusion method [13]. Freshly grown liquid culture of the test pathogens were seeded over the nutrient agar plates with a sterile swab. Sterile filter paper discs were soaked with different concentration of extracts of individual solvents and were placed on the plates at equidistance. Then the plates were incubated at 37°C for 18-24 hrs. Clearance zone formed around the discs indicates a positive antimicrobial activity and were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

Results and Discussion

World Health Organization (1991) defined a medicinal plant as any plant which, in one or more of its organs, contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [14]. The preliminary phytochemical screenings of the *S. indicum* stem and leaf extracts revealed the presence of various secondary compounds (Table 1).

From the results of preliminary phytochemical screening, it can be concluded that leaf extracts of *sapium indicum* L. showed the presence of tannins, saponins, steroids and triterpenoids in all extracts. And presence of alkaloids in all the extract except in hexane. Similarly the stem extracts revealed the presence of flavonoids, saponins, steroids and triterpenoids in all the extracts. Previous phytochemical studies on *S. indicum* have resulted in the isolation of several phorbol esters [15-19]. Analysis of leaf extracts of *Sapium insigne* (Royale). Benth. ex Hook.fil demonstrated the presence of phytochemicals like phenolics, saponins, alkaloids and glycosides which were found to have *in vitro* antimicrobial properties [20].

Preliminary screening of antimicrobial activity was evaluated by using disc diffusion methods against six human pathogenic bacteria were presented in the Figure 1 and 2. From Figure 1, it can be concluded that the antibacterial activity was found to be maximum against *Salmonella typhii* and *Proteobacter augustum* (22 mm) in the methanol stem extract. In comparison to all the solvent, methanol has shown the maximum activity. As shown Figure 2, the antibacterial activity of leaf extract was maximum in methanol solvent as compared to other three solvent. Methanol extract was found to have maximum activity against *Salmonella typhii (MMBB-13)* (31 mm) whereas minimum activity was found against *S. flexineri* (12.9mm). In chloroform extract, maximum activity was found against *Salmonella typhii (MMBB-13)* (27 mm) whereas minimum activity was found against *Proteobacter augustum* and *Salmonella typhii* (11 mm). From the result it can concluded that the methanolic extract has maximum antimicrobial activity as compared to other solvent extract. These results are in agreement with earlier studies with different plants [21-24]. Methanol extract was found to be more active than other extracts. Hence, it seems that polarity of the extracting solvent greatly influences the antimicrobial property of any extract. No work has been done on this plant previously by any worker. But few workers have reported that the traditional practitioners make use of water preliminary as solvent, but our studies showed that the methanol and chloroform extract of this plant were certainly much better and powerful. This may due to the better solubility of their active components in organic solvents. The leaf extract was found to have more antibacterial activity than stem extracts of *Sapium indicum*. To summarize, since both leaf and stem of the plant have

Table 1- Qualitative phytochemical analysis of *Sapium indicum* L.

TEST	LEAF				STEM			
	M	C	PE	H	M	C	PE	H
Alkaloids	+	+	+	-	+	+	-	-
Steroids & Triterpenoids	+	+	+	+	+	+	+	+
Flavonoids	+	-	-	-	+	+	+	+
Glycosides	+	-	-	-	+	-	-	-
Tanins	+	+	+	+	+	-	-	-
Saponins	+	+	+	+	+	+	+	+
Positive = (+), Negative = (-) M = Methanol, C= Chloroform, PE= Petroleum ether, H = Hexane								

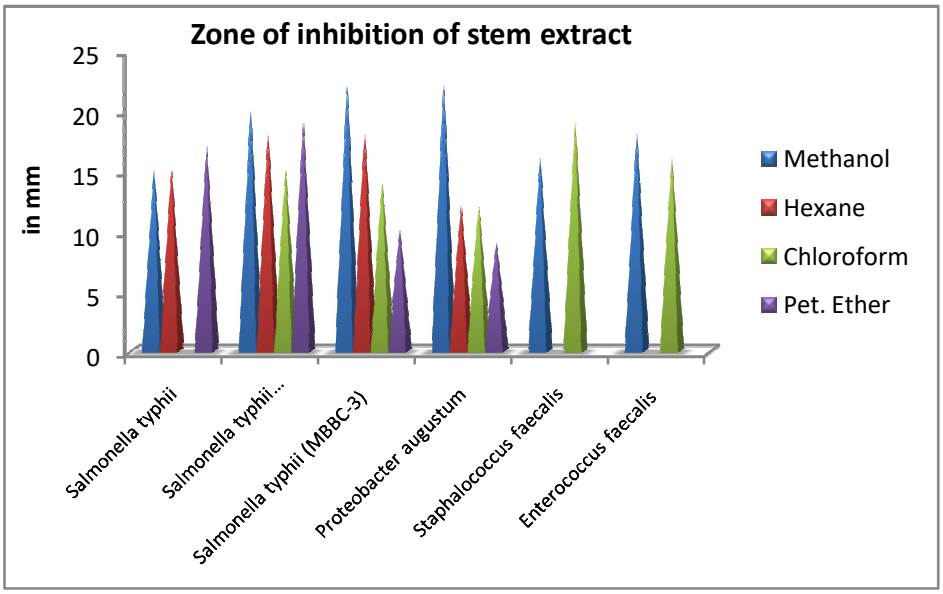

Figure 1- Zone of inhibition of stem extract of *Sapium indicum* in all solvent

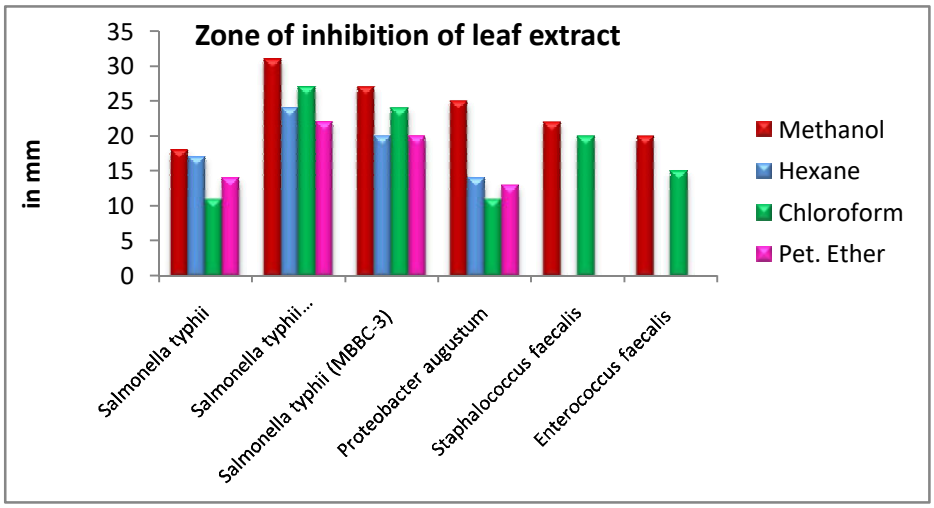

Figure 2- Zone of inhibition of leaf extract of *Sapium indicum* in all solvent.

shown considerable antibacterial properties apart from numerous ethno medicinal uses, efforts should be made for advanced studies on the pharmacological aspects and clinical trials also should be conducted to evaluate the efficacy of the potent drug among various populations.

Conclusion

The results obtained in the present study point to the pharmacological significance of *S. indicum*, a fact that justifies the use of the plant in the treatment of some microbial diseases and infections as claimed by traditional herbalists. The results further suggest that the extracts of this plant contain bioactive elements; which could explain the rationale for the use of the plant in traditional medicine. However, it is recommended that further research be carried out in order to isolate and purify the bioactive constituents using various extraction solvents as isolation of pure compounds increases antimicrobial activity. On the other hand, indiscriminate and non-medical use of antibiotics, plant decoctions and concoctions should be minimized as this could contribute to the emergence of resistant bacterial strains.

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