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Screening for Physical, Phytochemical and Antimicrobial Activities of Leaf Extracts of *Sapindus emarginatus* Vahl.

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Abstract: The crude extracts from leaf of *Sapindus emarginatus* Vahl. in different solvent, were subjected to pharmacognostic and fluorescence analysis, phytochemical and antimicrobial screening against selected gram positive, gram negative bacteria and fungus. Methanol and aqueous extracts of leaf were used for phytochemical screening and antimicrobial activity. Phytochemical studies indicated that the leaf contain a broad spectrum of secondary metabolites. Carbohydrates, alkaloids, phenol, tannins, fixed oils & fats, gums & mucilage and flavonoids were predominantly found in both the solvent extracts of leaf followed by steroids, saponins and proteins. Both the extracts showed varying degree of inhibitory potential against all the tested bacteria. The aqueous extract showed the highest activity against *Escherichia coli* (36mm) and *Pseudomonas aeruginosa* (36mm) but methanol extract of leaf had maximum against *Pseudomonas aeruginosa* (29mm). In the case of antifungal activity methanol extract of leaf had higher inhibitory action against *Aspergillus niger*. Aqueous extract of leaf had no inhibitory action against the test fungus.
Key words: phytochemical, methanol extract, aqueous extract, *Sapindus emarginatus*, antimicrobial.

INTRODUCTION

Medicinal plants are gifts of nature used to cure number of human diseases. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the medicinal plants [1]. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes [2]. Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against microbial infections [3].

The increasingly high numbers of bacteria that are developing resistance to classical antibiotics drive much of the current interest on natural antimicrobial molecules in hope that they may provide useful leads into anti-infective drug candidates. Several antimicrobial agents were isolated from plant including secondary metabolites as essential oil and terenoides, amongst which can be cited xanthones, benzophenones, coumarins and flavonoids [4].

The genus *Sapindus* belongs to family Sapindaceae posses tremendous medicinal value. Since past, it is used as emetic, tonic, astringent, anthelmintic, for asthma, colic, diarrhea, cholera, tubercular glands and paralysis of limbs. The fruit is commonly used as a remedy for hair problems and also in preparation of shampoos.

Sapindus emarginatus traditionally, used as anti-inflammatory and antipyretic. The seed is an intoxicant, and the fruit rind has oxytropic action. Its powder is used as nasal insufflations [5]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoural and antimicrobial agents [6].

MATERIALS AND METHODS

Preparation of plant extracts

Fresh Plant leaf of *Sapindus emarginatus* was collected from Kattampoondi Village, Thiruvannamalai district, Tamil Nadu, India; they were identified with the help of Gamble's flora.

Preparation of powder

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sheaved using 80 meshes and stored in an airtight container. These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis [7].

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia [8]. The leaves were dried in shade and the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for phytochemical screening of compounds, antimicrobial and pharmacological activity.

Qualitative phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate *et al.* (1995). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Test organisms

The stored culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Sector 39-4, Chandigarh, India.

The pathogenic fungal strains *Aspergillus niger, Aspergillus flavus, Rhizopus indicus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Antibacterial Studies

Bacterial Media (Muller Hindon Media)

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies.

Antifungal studies

Fungal media (PDA)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork porer.

Well diffusion method

Antibacterial and Antifungal activity of the plant extract was tested using well diffusion method [10]. The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2^{o}C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

RESULTS

Fluorescence analysis and quantitative determination of pharmacognostic characters

The results of Fluorescence analysis of the powder in visible range has been shown in Table1. The results of quantitative determination of pharmacognostic characters of *Sapindus emarginatus* were helpful in evaluating the pharmacognostic value of the medicinal plant. The total ash, acid insoluble ash, acid soluble, alcohol soluble and water soluble ash contents were found to be 36 %, 27 %, 73 %, 52 % and 32 % for leaf extract respectively.

Table 1: Analysis of fluorescence characters of leaf powder of *Sapindus emarginatus* in different chemical reagents

Sl. No	Chemical reagent	Appearance
1	Powder colour	Green
2	5% NaOH	Green
3	10% NaOH	Light green
4	Con. H_2SO_4	Light brown
5	Acetic Acid	Light yellow
6	1N NaOH in H_2O	Light green
7	5% KOH	Light green
8	50% HNO3	Brown
9	5% $FeCl_2$	Light green
10	1N HCl	Light green
11	Con.HNO3	Light yellow
12	1N NaOH in Ethanol	Light green
13	50% H_2SO_4	Light green
14	Con. HCl	Light green

Table 2: Results of phytochemical screening of aqueous leaf extracts of *Sapindus emarginatus*

S. No.	Name of the compounds	Name of the Test	Status of the substances	
			Aqueous extract	Methanol extract
1	Carbohydrates	Fehling's Benedict's	++ ++	+ ++
2	Alkaloids	Mayer's Hager's Wagner's Dragen Dorfff's	++ ++ ++ -	++ ++ + -
3	Steroids	Chloroform + Acetic acid + H_2SO_4	++	+
4	Tannins & Phenols	10% Lead acetate 5% Ferric chloride 1% Gelatin	+ ++ -	++ ++ -
5	Saponins	Foam test	+	+
6	Fixed oils & Fats	Spot test	++	++
7	Gums & Mucilage	Alcoholic precipitation	++	+++
8	Proteins	Biuret test	+	+
9	Flavonoids	NaOH / HCl	++	++
10	Volatile oils	Hydro distillation method	-	-

++++ - High rich amount +++ - Rich amount
++ - Moderate amount + - Minimum amount
- - Absent

Phytochemical evaluation

Phytochemical analysis of the aqueous and methanol extract indicated that the leaf contain a broad spectrum of secondary metabolites. Carbohydrates, alkaloids, phenol, tannins, fixed oils & fats, gums & mucilage and flavonoids were predominantly found in both the solvent extracts of leaf followed by steroids, saponins and proteins and results were presented in Table 2.

Table 3: Inhibition zone of Aqueous and Methanol extracts of *Sapindus emarginatus* against bacterial pathogens

Sl. No.	Name of the organisms	Zone of Inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	*Staphylococcus aureus*	16 ±2.4	18±3.7	22±2.8	-	-	-
2	*Escherichia coli*	12±2.8	26±2.4	36±3.7	-	-	-
3	*Leuconostoc lactis*	10±2.8	14±2.8	24±5.1	16±1.4	18±3.7	23±2.8
4	*Salmonella typhi*	-	-	-	-	-	-
5	*Pseudomonas aeruginosa*	11±1.4	18±2.4	36±2.8	18±1.4	20±4.9	29±3.7
6	*Streptococcus pyogenes*	-	-	-	-	-	-

Table 4: Inhibition zone of Aqueous and Methanol extracts of *Sapindus emarginatus* against fungal pathogens

Sl. No.	Name of the organisms	Zone of Inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	*Aspergillus flavus*	-	-	-	06±1.4	12±2.8	21±2.4
2	*Mucor indicus*	-	-	-	-	-	-
3	*Aspergillus niger*	-	-	-	18±2.8	24±4.9	32±2.8
4	*Rhizopus indicus*	-	-	-	09±1.4	12±2.8	19±4.2

Antimicrobial activity

The present investigation was to evaluate the antimicrobial activity of methanol and aqueous extract of *S. emarginatus* by well diffusion against five bacterial and four fungal species. The specific zone of inhibition against various types of pathogenic bacteria and fungus was shown in Table 3 and 4, respectively.

Methanol and aqueous extract were effective against both bacteria and fungus. The aqueous extract was better than methanol extract against bacteria but in the case of fungal pathogens it is in voce versa. The maximum antibacterial activity of aqueous extract of *S. emarginatus* was found 36 mm at 120 µg against *Escherichia coli* and *Pseudomonas aeruginosa* and minimum 10mm at 30 µg levels against *Leuconostoc lactis* whereas, in methanol extract showed maximum 29mm of inhibition zone establish at 120 µg of extract against *Pseudomonas aeruginos.* There is no activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Streptococcus pyogenes*. The significant antifungal activity of methanol extract was found 32 mm at 120 µg against *Aspergillus niger* and less significant activity was found 6 mm at 30 µg against *Aspergillus flavus* but in the case of aqueous extract didn't showed any activity against the test fungus.

DISCUSSION

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem [11, 12]. The presence of antifungal and antimicrobial substances in the higher plants is well established as they have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine have been used for the treatment of diseases as in done in cases of Unani and Ayervedic system of medicines, a natural blueprint for the development of new drugs. Much of the exploration and utilization of natural product as antimicrobial arise from microbial sources. The present study was conducted to analysis the pharmacognostic, phytochemical, fluorescence characteristics and antibacterial potential of leaf extracts of *Sapindus emarginatus*.

Florescence analysis of powders and crude extracts of different parts of medicinal plants (leaf, stem, root, bark and fruit) gives a clue if powder and extracts are in adulteration, thus can be used as a diagnostic tool for testing the adulteration. Such studies were done previously in *Morinda tinctoria* [13] and *Abutilon indicum* [14].

Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances [15]. In our study the phytochemical screening of extracts of leaf indicate the strength of active principle depends on the use of a suitable solvent besides the type of the plant species to achieve positive results. Hence leaf extracts of *S. emarginatus* is highly recommended for the herbal preparations to the traditional medicinal practioners and for the pharmaceutical industries for the mass scale extractions of the therapeutic agents. In the present investigation, methanol and aqueous extracts of *S. emarginatus* was evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive pathogenic bacteria and fungus. Aqueous and solvent extracts of medicinal plant have shown very high antimicrobial susceptibility against bacterial and fungal strains. Antimicrobial activity of tannins [16] flavonoids [17], saponins [18], terpenoids [19] and alkaloids [20] has been documented. In the present study, phytoconstituents namely flavonoids, alkaloids, steroids and saponins were detected in the extracts which may account for the activities.

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