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Research Article

IN VITRO ANTIBACTERIAL, ANTIFUNGAL AND INSECTICIDAL ACTIVITIES OF ETHANOLIC EXTRACT AND ITS FRACTIONATES OF *SANCHEZIA SPECIOSA* HOOK. F.

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ABSTRACT
The present study was carried out to evaluate the antimicrobial and insecticidal activities of plant *Sanchezia speciosa* Hook. F. extract against pathogenic bacteria and fungi by disc diffusion method and insect by surface film activity test. Fifteen different species of Gram-positive and Gram-negative bacteria, six species of fungi and *Tribolium castaneum* (Herbst) insect were used for this screening. The result revealed that among the three fractions obtained by solvent-solvent partitioning, chloroform fraction was the best extractive for antibacterial and antifungal properties than other two fractions (pet-ether, ethyl acetate). The ranges of zone of inhibition were 8 ± 0.01 to 23 ± 0.02 mm using 500 µg/disc. In addition the minimum inhibitory concentrations (MICs) of different solvent fractions tested were found to be in the range from 16 µg/ml to 128 µg/ml against fifteen pathogenic bacteria depending on isolates and extracting solvent. The insecticidal assay also indicated reasonable activity with 60 %, 40 % and 20 % mortality rate of *Tribolium castaneum* (Herbst) at a dose of 50 mg/ml in 48 hours for chloroform, ethyl acetate and petroleum ether extract respectively. This is the first report of antibacterial, antifungal and insecticidal activities of the ethanolic extract and it's fractionates (Chloroform, ethyl acetate and petroleum ether fraction) of *Sanchezia speciosa* Hook. F.
Keywords: *Sanchezia speciosa* Hook F, *Tribolium castaneum* (Herbst), antibacterial, antifungal, insecticidal activity.

INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses and parasites remain a major threat to public health despite of tremendous progress in human medicine. The impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance[1]. Many of the medicinal plants were screened for various biological and pharmacological activities including antibacterial, antifungal, insecticidal activities[2-4]. Synthetic antimicrobial and insecticidal compounds are widely used but their use is being limited because of their toxic and carcinogenic effects. Therefore, it is of great significance and necessity that research focuses on discovering effective and nontoxic antimicrobial and insecticidal compounds from natural sources, to replace synthetic ones because of their probable carcinogenic activity. *Tribolium castaneum* (Herbst) is considered to be a major pest of stored grains in Bangladesh. It is abundantly found in stored grains of different cereals[5,6]. The large scale use of chemical pesticides in agriculture and public health leads to adverse effects such as development of pesticide resistance, frequent pest out breaks, emergence of new pests pollution and health hazards. In order to search an environmentally safe alternative, scientists considered the pesticides of biological origin (biopesticides) in the place of synthetic insecticides. Throughout history plant products have been successfully exploited as insecticides, insect repellents and insect antifeedants[7]. *Sanchezia speciosa* Hook. F. (Family: Acanthaceae) is a stout erect shrub commonly known as Fire Fingers in English. The plant is native to Ecuador and Peru. It is cultivated for both its attractive orange flowers and green leaves with yellow veins and is very popular as a hedge, screen or border plant. The species is commonly planted as an ornamental plant and found in wet and shady areas and in many Pacific islands, Hawaii, Fiji and New Caledonia[8]. The present study was conducted to search for newer, safer and potent antimicrobial and insecticidal compounds from the plant *Sanchezia speciosa* Hook. F. and it was our first report on above biological activities of this plant. Literature survey only reveals that methanolic extract of plant *Sanchezia speciosa* Hook. F. leaves had antioxidant and anticancer effects[9]. As part of our continuing studies of medicinal plants in Bangladesh the present review is, therefore, an effort to give a detailed account on it's an extensive survey on antimicrobial and insecticidal activities of the ethanolic extract and the different fractions of *Sanchezia speciosa* Hook. F and we, here in, report the results of our preliminary investigation.

MATERIALS AND METHODS

Plant material collection

The plant *Sanchezia speciosa* Hook. F. was collected in huge amount during the month of December 2012 from the village Haibatpur of Natore district of Bangladesh and identified by Dr. AHM Mahbubur Rahman, Associate Professor, Department of Botany, University of Rajshahi, Bangladesh.

Plant materials extraction and fractionation

The collected plant *Sanchezia speciosa* Hook. F. were sun dried and pulverized into coarse powder with the help of grinding machine. The plant powder (500 g) was extracted with ethanol (3 litres) in an air tight container for five days at room temperature with occasional stirring and shaking. The extracts were then filtered first through a fresh cotton plug and finally with a Whatman No.1 filter paper. The filtrate was evaporated to dryness in vacuum by a rotary evaporator at 40-50ºC. The prepared mass was then fractionated by solvent-solvent partitioning with petroleum ether, ethyl acetate and chloroform[10].

Growth media and conditions

Nutrient agar media (Difco laboratories) pH 7.2, Nutrient broth media pH 6.2 and Sabouraud dextrose agar media (Biolife Vole Monza) pH 5.6 were used for antibacterial screening, MIC determination and antifungal activity determination respectively.

Antibacterial screening

The Disc diffusion method[11,12,14] was used to test antibacterial activity against fifteen pathogenic bacteria (Table 1). Disc containing the test materials were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs (kanamycin 30 µg/disc) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at 4ºC for 24 hours to allow maximum diffusion followed by incubation at 37ºC for 24 hours for maximum growth of the organisms. The activity was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Determination of Minimum Inhibitory Concentration (MIC)

Serial tube dilution technique[13,14] was used to determine MIC of each solvent fraction of plant *Sanchezia speciosa* Hook. F. Each extract was dissolved in 2 ml distilled water to obtain stock solution having concentration of 512 µg/ml. 1 ml of the prepared stock solution was transferred to test tube containing 1 ml nutrient broth medium to give concentration of 256 µg/ml and so on. After preparation of suspension of test organisms, 1 drop of suspension (0.02 ml) was added to each broth dilution. After 18 hours incubation at 37ºC, the tubes were then examined for growth. Growth was observed in those tubes where the concentration of extract was below the inhibitory level and the broth was observed turbid. Concurrently, kanamycin and distilled water with 3 drops of Tween 80 was used as positive and negative control respectively.

Antifungal screening

In vitro antifungal activity of crude extracts was carried out on Sabouraud Dextrose agar plate by disc diffusion method[11,12,14] against six pathogenic fungi at a concentration of 500 µg/disc as described in antibacterial screening section. Standard disc of antifungal agent ampicillin (30 µg/disc) was used as positive control.

Insecticidal screening

To conduct surface film activity test each extract (50 mg) was dissolved into 1 ml respective solvent. Then were poured in the petridish and allowed them to dry out. The insects were released in each of the treated petridish. A control experiment applying only the solvent was also set at the same time under same condition[15]. Treated petridishes were placed in a secured place at room temperature. The mortality was observed first after 30 minutes and then after 12 hours, 24 hours, 36 hours and finally after 48 hours of exposure and data were recorded.

RESULTS

The inhibitory effect of the plant *Sanchezia speciosa* Hook. F. against pathogenic bacteria are shown in Table 1. The chloroform extract had interesting highest activity against *Escherichia coli, Salmonella paratyphi* and *Bacillus megaterium* followed by *Shigella flexneri*, *Pseudomonas aeruginosa* and *Shigella shiga*. The moderate zone of inhibition was noted in ethyl acetate fraction against *Shigella sonnei* and *Shigella dysenteriae* whereas petroleum ether extract had least effect. The MIC values of chloroform fraction against these bacteria ranged from 16 to 64 µg/ml where the ethyl acetate fraction had 32 to 128 µg/ml and petroleum ether fraction had 64 to 128 µg/ml (Table 1). The plant *Sanchezia speciosa* Hook. F. extract showed significant antifungal activity against a number of test fungi as indicated by the zone of inhibition (Table 2). Maximum inhibition was obtained in chloroform extract against *Candida albicans* followed by *Rizopus oryzae*, *Aspergillus niger* and *Trycophyton rubrum*. Moderate inhibition was obtained in ethyl acetate extract against *Rizopus oryzae* and *Trycophyton rubrum* whereas petroleum ether fraction had insignificant activity. Chloroform fraction have shown 60 % mortality rate of *Tribolium castaneum* (Herbst) at a dose of 50 mg/ml in 48 hours and ethyl acetate fraction have shown 40 % mortality at the same dose (Table 3). Weak insecticidal activities (20 %) were shown by petroleum ether fraction. Control group showed 0 % mortality.

DISCUSSION

Medicinal plants play an important role for the management of different microbial infections because over medication and long-term side effects of synthetic drugs have assumed alarming range. Bioactivity evaluation is an important part for the development of new drugs from medicinal plants and screening crude extract and various fractions against micro-organisms is usually first step during bioactivity evaluation[16]. Successful fraction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Different solvents extract possess different compounds and some active components can only be extracted by polar compounds, while some by less polar and yet some by non-polar compounds. Considering the fact that the petroleum ether fraction lacks some components and hence least zone of inhibition was seen. In contrast the chloroform fraction showed significant antimicrobial activity which is probably due to the presence of some potential phytochemicals. The insecticidal study indicated that the mortality caused by each solvent fraction was increased with the increasing of exposure time. The exposure time played an important role in influencing susceptibility. Developing countries in Asia and Africa, including Bangladesh have a long history to protect stored grains with locally available herbal substances[17]. Chloroform fraction possesses high insecticidal activity against *Tribolium castaneum* (Herbst) as compared to other two solvent fractions and might be useful as potent insect control agents if used in higher concentration than the tested one. The results therefore established a good support for the use of *Sanchezia speciosa* Hook. F as traditional medicine.

CONCLUSION

To the best of our knowledge there is no earlier report on the antibacterial, antifungal and insecticidal activity of the plant. The results of this study indicate that the plant is significantly antibacterial, antifungal and moderately insecticidal in action. Further investigations are currently on to isolate the natural constituents responsible for the observed activity and these will be reported at a later date.

Table 1: Antibacterial activities and Minimum Inhibitory Concentrations of the plant *Sanchezia speciosa* Hook. F. extract

Bacterial strains tested	Diameter of zone of inhibition (mm) Extract (500 µg/disc) of -			Kanamycin (30 µg/disc)	MIC values (µg/ml)			
	Pet.ether	Ethyl acetate	Chloroform		Pet. Ether	Ethyl acetate	Chloroform	Kanamycn
Gram (+ve)								
Staphylococcus aureus	9 ± 0.03	14 ± 0.5	18 ± 0.01	22 ± 0.31	128	64	32	16
Bacillus subtilis	9 ± 0.12	16 ± 0.11	19 ± 0.22	24 ± 0.01	128	32	16	4
Bacillus megaterium	10 ± 0.04	16 ± 0.11	21 ± 0.03	27 ± 0.01	64	32	16	2
Bacillus cereus	11 ± 0.04	15 ± 0.22	18 ± 0.03	25 ± 0.01	64	64	64	4
Sarcina lutea	8 ± 0.01	14 ± 0.22	18 ± 0.01	26 ± 0.04	128	128	64	16
Gram (-ve)								
Escherichia coli	11 ± 0.01	16 ± 0.02	23 ± 0.02	29 ± 0.12	64	32	16	2
Pseudomonus aeruginosa	9 ± 0.11	15 ± 0.02	20 ± 0.11	27± 0.18	128	64	16	8
Salmonella paratyphii	9 ± 0.21	16 ± 0.04	22 ± 0.25	24 ± 0.03	128	32	16	4
Salmonella typhii	10 ± 0.31	14 ± 0.13	19 ± 0.02	26 ± 0.03	64	64	16	2
Shigella boydii	8 ± 0.21	14 ± 0.44	18 ± 0.01	23 ± 0.01	128	64	32	8
Shigella. sonnei	9 ± 0.20	17 ± 0.11	19 ± 0.01	27 ± 0.11	128	32	16	2
Shigella shiga	10 ± 0.03	14 ± 0.11	20 ± 0.13	26± 0.11	64	128	32	8
Shigella flexneri	9 ± 0.12	15 ± 0.16	21 ± 0.20	25 ± 0.21	128	128	16	4
Shigella dysenteriae	10 ± 0.03	17 ± 0.02	18 ± 0.05	22 ± 0.21	64	32	64	8
Vibrio mimicus	8 ± 0.04	15 ± 0.02	18 ± 0.05	24± 0.11	128	64	64	16

The assay was performed in triplicate and the results are the mean of three values ± Standard Deviation.

Table 2: Antifungal activities of the plant *Sanchezia speciosa* Hook. F extract

Fungal strains tested	Diameter of zone of inhibition (mm) Extract (500 µg/disc) of -			Ampicillin (30 µg/disc)
	Pet. ether	Ethyl acetate	Chloroform	
Candida albicans	10 ± 0.02	10 ± 0.31	18 ± 0.41	25 ± 0.01
Aspergillus niger	8 ± 0.11	11 ± 0.16	16 ± 0.02	23 ± 0.01
Saccharomyces cerevisiae	7 ± 0.11	10 ± 0.42	15 ± 0.02	26 ± 0.27
Aspergillus ustus	7 ± 0.22	9 ± 0.27	14 ± 0.04	21 ± 0.03
Rizopus oryzae	9 ± 0.17	12 ± 0.20	17 ± 0.11	22 ± 0.03
Tricophyton rubrum	8 ± 0.33	12 ± 0.20	16 ± 0.11	24± 0.13

Table 3: Insecticidal activities of the plant *Sanchezia speciosa* Hook. F extract

Extract of plant *Sanchezia speciosa* Hook. f	Amount of extract (mg/ml)	Number of insect used	Number of insect dead					Mortality (%)
			30 minutes	12 h	24 h	36 h	48 h	
Pet. Ether	50	15	---	---	1	2	3	20 %
Ethyl acetate	50	15	---	---	2	5	6	40 %
Chloroform	50	15	---	3	5	7	9	60 %

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