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Research Article

IN VITRO ANTIBACTERIAL, ANTIFUNGAL & CYTOTOXIC ACTIVITY OF *SCOPARIA DULCIS* L

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ABSTRACT

In vitro antibacterial and antifungal activities of ethanolic extracts of S*coparia dulcis* L. (EESD) whole herb were evaluated in the present study by disc diffusion method using 12 human pathogenic bacterial strains & 3 fungal strains respectively. The activity was measured by determining zone of inhibition and minimum inhibitory concentration. The zone of inhibition values were compared with the standard kanamycin (30 μg/disc) and nystatin (20 μg/disc) for antibacterial & antifungal activity respectively and the minimum inhibitory concentration values were compared with control. The zone of inhibition observed was between 6 to13 mm. The minimum Inhibitory concentration (MIC) was carried out by serial dilution against *Staphylococcus aureus* & *Salmonella paratyphi* and found 256 μg/ml in both cases. The cytotoxic activity of EESD was evaluated by brine shrimp lethality bioassay method & the LC_{50} value found to be 40.39 μg/ml. Vincristine sulphate was used as the positive control in this case. It is concluded that EESD exhibited moderate antibacterial, antifungal & significant cytotoxic activity and thus would be a safer antibiotic and also an anticancer agent.

Keywords: *Scoparia dulcis*, Brine shrimp, Disc diffusion, Antibacterial activity, Antifungal activity, Cytotoxic activity.

INTRODUCTION

Medicinal plants are natural resources yielding valuable herbal products which are often used in the treatment of various ailments[1]. For this purpose the use of plant extracts in traditional medicine has been going on from ancient time[2]. Herbalism and folk medicine, both ancient and modern, have been the source of much useful therapy[3-5]. During the last twenty years renewed interest has emerged to help developing safer antimicrobial drugs from the natural sources[4-8], presumably due to the increasing development of drug resistance to human pathogenic organisms, as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections[9-11].

Scoparia dulcis L, commonly known as sweet broom weed is a perennial herb widely distributed in tropical and subtropical regions[12]. In these regions, fresh or dried *S. dulcis* plants have been traditionally used as remedies for stomach troubles, hypertension[13], diabetes, bronchitis[14] and as analgesic and antipyretic agents[15]. In view of its high reputation and wide acceptance in ethnomedicine, this plant has attracted not only wide publicity but also intensified research efforts by researchers[16-17]. More recently, a number of the speculated medicinal values of *S. dulcis* have been validated by scientific research. These include hypoglycemic activity[12,18-22], antitumour promoting activity[23], antiviral activity[24], hyperlipidemic activity[25], antioxidant and analgesic activity[12, 26]. A significant analgesic activity was also demonstrated along with the antihyperalgesic activity for *S. dulcis* decoction[27]. Later it was investigated the antibacterial and antifungal activity of *S. dulcis*[28] by another author. Pure diterpenes extracted from *S. dulcis* was reported to show cytotoxicity towards six human stomach cancer cell lines[29]. Phytochemical screening of the herb revealed that it is rich in flavonoids and terpenes and the pharmacological actions of *S. dulcis* are believed to be due to the presence of these phytochemicals[12, 24, 26, 30-33]. The main chemicals include scopadulcic acids A and B, scopadiol, scopadulciol, scopadulin, scoparic acids A-C and betulinic acid[34]. *S. dulcis also* contains coumarins, phenols, saponins, tannins, aminoacids, alkaloids, carbohydrates, glycosides[12, 26, 35]. Some of these compounds are likely to be active against certain bacteria, this may account for the traditional use as medicinal plants.

In our laboratory we have previously investigated the antioxidant, antidiabetic and analgesic effects of EESD *in vitro* as well as *in vivo*[12, 26]. The present study was undertaken to further analyze the same extract for *in vitro* antibacterial, antifungal and cytotoxic activity.

MATERIALS & METHODS

Drugs & chemicals

Ethanol and DMSO (dimethyl sulfooxide) were purchased from Merck, Germany. Kanamycin and nystatin were collected from Square Pharmaceuticals Ltd., Bangladesh. Vinchristin sulphate was purchased from Cipla Ltd., Goa, India.

Collection and identification of plants

For the present study the investigated plant *Scoparia dulcis* L. was collected from Manikgong, Bangladesh in July 2008 and was identified initially by Dr. M.A. Razzaque Shah, Tissue Culture Specialist, BRAC Plant Biotechnology Laboratory, Bangladesh and later the expert of Bangladesh National Herbarium, Mirpur, Dhaka recognized the plant & deposited the Voucher specimen no: 32766. The collected plant parts (whole herb) were separated from undesirable materials or plant parts. They were dried for one week. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of extract

The aerial parts of plant were collected, sun dried for seven days and ground. The dried powder of *Scoparia dulcis* L. (180 gm) was soaked in 500 ml of 95% ethanol for 7 days in cold condition with occasional shaking and stirring. The whole mixture was successively filtered through a piece of clean, white cotton material and No. 1 Whatman filter paper. The filtrate (ethanol extract) obtained was evaporated using rotary evaporator. It rendered a gummy reddish black concentrate which was dissolved in ethanol at different concentrations to carry out the following experiments.

Antibacterial screening

In vitro antibacterial screening of the crude extracts was carried out by the disc diffusion method[36-38]. Disc diffusion method is equally suited to screening of antibiotics or the products of plant evaluation[39] and is highly effective for rapidly growing microorganisms and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition. Generally the more susceptible the organism, the bigger is the zone of inhibition. In this method the compounds are applied to the agar medium by using paper discs[40-41]. The method is essentially a qualitative or semi quantitative test which allows classification of

microorganisms as susceptible, intermediate or resistance to the test materials as well as bacteriostatic or bactericidal activity of a compound[42].

The antibacterial activity was determined against four pathogenic Gram- positive (*Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Sarcina lutea)* and eight Gram- negative *(Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Vibrio mimicus, Escherichia coli, Shigella dysenteriae, Shigella boydii* and *Pseudomonas aeruginosa*) bacteria. The extracts were dissolved separately in ethanol and applied to sterile discs at a concentration of 500 μg/disc and carefully dried to evaporate the residual solvent. Here, Kanamycin 30 μg/disc (Oxoid Ltd., England) was used as the standard. These plates were then kept at low temperature (4ºC) for 24 hours to allow maximum diffusion of the test materials and kanamycin. The plates were then incubated at 37ºC for 24 hours to allow maximum growth of the organisms. The test material having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the discs. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm.

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of the test sample or drug at which it shows the highest activity against microorganisms. The extracts that showed high efficacy against microorganisms were subjected to minimum inhibitory concentration (MIC) assay by serial two fold dilution method (2:2)[43-46]. The determination of the MIC involves a semi quantitative test procedure, which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. The method displays tubes of growth broth containing a test level of preservative, into which an inoculum of microbes was added. The end result of the test was the minimum concentration of antimicrobial (test materials) which gave a clear solution, i.e., no visual growth[47-48].

The serial dilution assay determines the anti-microbial activity of a pure compound by providing the MIC value of the compound for specific susceptible test organisms, and indicates important consideration in the further development of bioactive compounds. In this method twelve autoclavable test tubes were used and each of the test tubes containing sterile nutrient broth medium in a dilution (2-512 μg/ml) having 10 μl of the organism (10^7 cells/ml) mixed well. The test sample in various concentrations were applied to the nutrient medium (1ml) in each test tube and incubated at 37.5⁰C for 24 hours. No inhibition was observed in the tube containing sample lower than the above concentrations. Three control test tubes were used to perform control test using nutrient C_M (Medium), C_S (Medium+Sample) and another C_1 (Medium + Inoculum), where bacterial growth was observed in C_1 only, other two were clear. MIC was interpreted as the lowest concentration of the sample, which showed clear fluid without development of turbidity.

Antifungal screening

Antifungal activity of EESD was determined at a concentration of 500 μg/disc by the disc diffusion method[36-38]. Nystatin 20 μg/disc was used as the standard.

Collection of test organisms

The bacterial species used in the present study were *Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus* and *Sarcina lutea, Salmonella paratyphi, Salmonella typhi, Vibrio parahemolyticus, Vibrio mimicus, Escherichia coli, Shigella dysenteriae, Shigella boydii* and *Pseudomonas aeruginosa*. These were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), Dhaka University and International Center for Diarrheal Disease and Research, Bangladesh (ICDDRB) Dhaka,Bangladesh. Tested fungi *Aspergillus niger, Saccharromyces cerevaceae,* and *Candida albicans* were collected from the Microbiology research laboratory, Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

Cytotoxic activity

Brine shrimp lethality bioassay

Cytotoxic activity of the plant extracts was determined by brine shrimp lethality bioassay[38, 49-50] method. It is a recent development in the assay procedure of bioactive compounds, which indicates cytotoxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds[51]. The brine shrimp assay was proposed by Michael *et al.*[52] and later developed by Vanhaecke *et al.*[53] and Sleet and Brendel[54]. It is based on the ability to kill laboratory-cultured brine shrimp (*Artemia* nauplii). The assay is considered as a useful tool for preliminary assessment of toxicity[55] and it has been used for the detection of fungal toxins[56], plant extract toxicity[57], heavy metals[58], cyanobacterial toxins[59], pesticides[60] and cytotoxicity testing of dental materials[61].

Bioactive compounds are often toxic to shrimp larvae (*Artemia salina*); therefore, Brine Shrimp Lethality Assay is in use to monitor different chemicals *in vivo* lethality to shrimp larvae[55-56, 62-63]. In the present study, the lethality test was carried out using brine shrimp nauplii eggs (*Artemia salina* Leach). The eggs of Brine Shrimp was collected from local pet shops and hatched in a tank at a temperature around 37⁰C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). With the help of a pasteur pipette nauplii were exposed to different concentrations of the extracts.

Preparation of test groups

For the experiment, 20 mg of extract were dissolved in 1ml of DMSO and adjusted up to 20 ml by 3.8% NaCl. Then the solutions of varying concentrations (500, 200, 100, 50, 20, 10, 5, 1 μg/ml) were obtained by serial dilution technique. Vincristine Sulphate (25, 10, 5, 1, 0.5, 0.25, 0.125, 0.06 μg/ml) and DMSO (50, 125 μg/ml) were used as positive and negative control respectively. Each test tube contained about 5 ml of sea water with 10 shrimp napulli.

Counting of nauplii

The test tubes were kept at room temperature for about 24 hours and then percent of mortality of napulli was counted with the help of a magnifying glass. The rate of mortality of nauplii was found to be increased in concentration of each of the samples. The median lethal concentration (LC_{50}) and 95% confidence intervals were determined using the probit analysis method described by Finney[64], as the measure of toxicity of the plant extract.

RESULTS

Antibacterial screening

EESD showed moderate antibacterial activity with the average zone of inhibition of 7-13 mm at 500 μg/disc by disc diffusion method (Table 1). Among the tested bacteria, the growth of *Salmonella paratyphi* & *Shigella dysenteriae* (13 mm) was strongly inhibited.

Minimum inhibitory concentration determination

The minimum inhibitory concentration (MIC) of EESD was determined against *Staphylococcus aureus* & *Salmonella paratyphi* and found to be 256 μg/ml in both cases.

Antifungal screening

EESD showed mild to moderate antifungal activity against a number of tested fungi (Table 2). Among the tested fungi, *Saccharromyces cerevacaee showed* highest zone of inhibition (10 mm).

Cytotoxic activity

EESD showed cytotoxic activity against brine shrimp & the LC_{50} value found 40.39μg/ml (Table 4) compared to vincristine sulphate 0.91 μg/ml (Table 3).

Table 1: ***In vitro*** **antibacterial activity of EESD and standard kanamycin discs**

Test microorganisms	Diameter of zone of inhibition (mm)	
	EESD	Kanamycin
Bacteria		
Gram positive		
Staphylococus aereus	10	30
Bacillus megaterium	10	32
Bacillus subtilis	7	30
Sarcina lutea	8	25
Gram negative		
Salmonella paratyphi	13	28
Escherichia coli	7	32
Shigella dysenteriae	13	32
Vibrio minicus	7	28
Vibrio parahemolyticus	7	33
Shigella boydii	11	25
Psedomonas aeruginosa	9	28

Table 2: ***In vitro*** **antifungal activity of EESD and standard Nystatin discs**

Test microorganisms	Diameter of zone of inhibition (mm)	
	EESD	Nystatin
Fungi		
Aspergillus niger	6	25
Saccharromyces cerevaceae	10	20
Candida albicans	9	25

Table 3: Effect of Vincristine sulphate (positive control) on shrimp nauplii

Conc. (C) µg/ml	$Log_{10}C$	No. of naupli taken	No. of naupli dead	% mortality	Corrected % mortality	Probit	LC_{50} µg/ml	95% confidence interval
00	-	-	1	10	-	-		
0.06	-1.221	10	1	10	0.0	0		
0.125	-0.903	10	2	20	11.1	3.77		
0.25	-0.602	10	3	30	22.2	4.23		
0.5	-0.301	10	4	40	33.3	4.56	0.91	0.46-1.79
1	0	10	5	50	44.4	4.85		
5	0.698	10	9	90	88.9	6.23		
10	1	10	10	100	100.0	8.09		

Table 4: Effect of EESD on shrimp naupli

Conc. (C) µg/ml	$Log_{10}C$	No. of naupli taken	No. of naupli dead	% mortality	Corrected % mortality	Probit	LC_{50} µg/ml	95% confidence interval
00	-	-	1	10	-	-		
1	0	10	1	10	0.0	0		
5	0.698	10	2	20	11.1	3.77		
10	1	10	2	20	11.1	3.77		
20	1.301	10	3	30	22.2	4.23	40.39	22.26-73.27
50	1.698	10	6	60	55.6	5.15		
100	2	10	8	80	77.8	5.77		
200	2.301	10	9	90	88.9	6.23		
500	2.698	10	10	100	100.0	8.09		

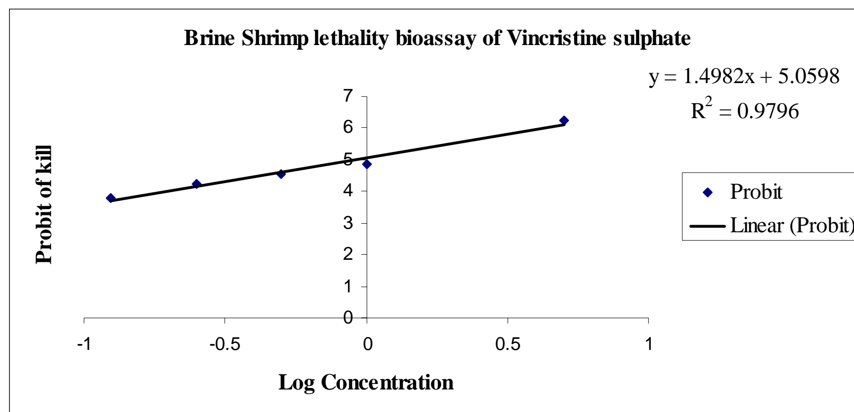

Fig. 1: Plot of adjusted probits and predicted regression line of Vincristine sulphate

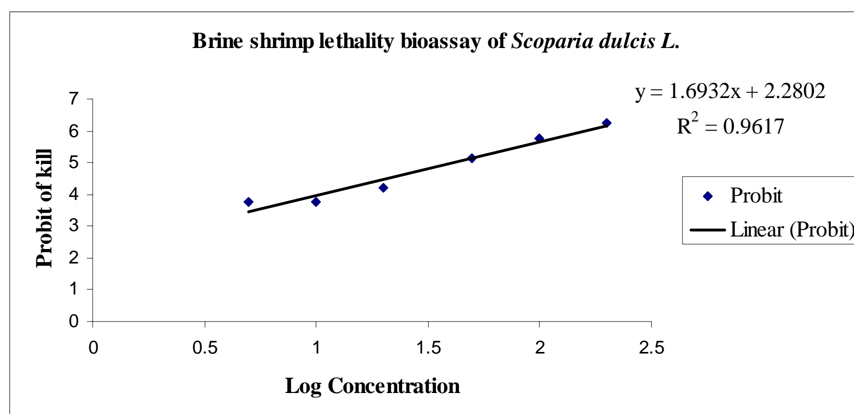

Fig. 2: Plot of adjusted probits and predicted regression line of *Scoparia dulcis* L.

DISCUSSION

The present study revealed that the EESD has antimicrobial and cytotoxic effects and may have potential bioactive principles. Antimicrobial activity was conducted against a wide range of human pathogenic microorganisms including not only Gram-positive and Gram-negative bacteria, but also fungi. Antimicrobial activity of the compounds may be of four types: (1) they hamper cell wall synthesis; (2) they inhibit microbial protein and nucleic acid synthesis; (3) they disrupt microbial membrane structure and function; and (4) they block metabolic pathways through inhibition of key enzymes[28]. In the present study the ethanolic extract of the whole plant of *Scoparia dulcis* L. showed average zone of inhibition ranged from 7-13 mm. A large zone of inhibition was observed 13 mm against *Salmonella paratyphi and Shigella dysenteriae.* It showed moderate activity against *Staphylococus aureus (10mm), Salmonella typhi (10mm)* and *Shigella boydii (11mm).* In antifungal screening, the compound showed mild zones of inhibition against three tested fungi. The earlier reports of antibacterial and antifungal activities[28] support the findings of present studies. From the results obtained, it appears that the antibacterial action of the extracts is more pronounced on Gram-negative than on Gram-positive (*S. aureus*) bacteria in most cases or is even equal. These findings do not correlate with the observations of previous screenings of medicinal plants for antimicrobial activity, where most of the active plant extracts showed activity against Gram-positive strains only[7, 65-66]. However demonstration of antimicrobial activity against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds[67-68].

The cytotoxicity bioassay against *Artemia salina* is a simple and inexpensive method to test cytotoxicity, to biodirect fractionation of natural products and as a predictor of antitumor and pesticidal activity[69]. It indicates also antiviral, antiplasmodial, antifilarial, antimalarial activities[55]. The method allows the use of smaller quantity of the extracts and permits larger number of samples and dilutions within shorter time than using the original test vials[70]. In the present study the crude extracts of *Scoparia dulcis* L. showed prominent result in brine shrimp cytotoxicity assay. The LC_{50} value was 40.39 µg/ml. The inhibitory effect of the extract might be due to the toxic compounds present in the active fraction that possess ovicidal and larvicidal properties. The metabolites either affected the embryonic development or slay the eggs[71]. So the cytotoxic effects of the plant extracts enunciate that it can be selected for further cell line assay because there is a correlation between cytotoxicity and activity against the brine shrimp nauplii using extracts[71-72].

Previous phytochemical screening of ethanolic extracts of *S. dulcis* revealed the presence of flavonoid, alkaloid, tannin, carbohydrate, glycoside[12, 26]. These chemical constituents may be responsible for the antimicrobial activity. These observations may be attributed to two reasons; firstly, the nature of biologically active components (Alkoloids, flavonoids, carbohydrates, tannins etc.) which could be enhanced in presence of ethanol. Secondly, the stronger extraction capacity of ethanol could have produced greater active constituents responsible for anti-microbial activity[73]. The isolated terpenoids with a cyclic structure containing one or more functional groups may also be responsible for antimicrobial activity[28]. Flavonoids[74]

and other compounds like terpens, steroids and glycosides[75] isolated from other plants were found to be responsible for the effective antimicrobial activity. So antimicrobial activity in the plant extracts depends not only on the presence of phenolic compounds but also on the presence of various secondary metabolites[5, 76]. Many of these secondary metabolites present in the various medicinal plants are also well known for their cytotoxic property[71]. Among them sesquiterpenes[77], halogenated monoterpenes[78] and bromophenols[79] isolated from other plant extracts were found to have strong cytotoxic property against several cell lines.

The comparison between cytotoxicity and antimicrobial activities shows that cytotoxicity assay can not predict antibacterial action[69, 80]. This may be due to the difference of the prokaryotic cell structure of a bacterial cell and eukaryotic cell of the brine shrimp. Therefore, although the brine shrimp assay is a simple and reasonable for predicting a vast array of activities, an antimicrobial bioassay (e.g. MIC) should be included also in order to fully understand the potential activity of a natural compound (s) or a crude extract [80].

CONCLUSION

Based on these results it can be concluded that the crude extracts have the potential as antimicrobial compounds against microorganisms and may be utilized in the treatment of infectious diseases caused by resistant organisms. They can also be a source of the development of novel anticancer drug leads. The present study therefore offers a scientific basis for traditional use of EESD. Further evaluation of the antibacterial and antifungal properties of the plant extracts against a more extensive panel of microbial agents is reasonable. The mechanism of selective cytotoxicity is also needed on further studies. Likewise detailed chemical studies for the purification and identification of the bioactive components followed by pharmacological investigations and toxicological assessment are still required to examine the mechanisms of action of these agents. In conclusion, both brine shrimp and antimicrobial bioassays should be used together to entirely identify the promising activity of plant crude extracts or compounds derived from them.

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