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ORIGINAL RESEARCH ARTICLE

Antimicrobial and Cytotoxicity Studies of the Aerial Part of *Arachis hypogea* Linn.

*Most. Nazma Parvin and Sadia Afreen Chowdhury

Department of Pharmacy, Stamford University Bangladesh
51, Siddeswari Road, Dhaka-1217, Bangladesh.

***Corresponding Author**
Most. Nazma Parvin
Assistant Professor
Department of Pharmacy
Stamford University Bangladesh
Dhaka-1217, Bangladesh
Contact No.: +880 1715 711 659
E-mail: nazma_183@yahoo.com

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ABSTRACT

The petroleum ether, chloroform and methanol soluble extracts of the aerial part of *Arachis hypogea* (Papilionaceae) were screened for their possible antimicrobial activity against thirteen bacteria and three fungi by disc diffusion method and cytotoxic activity by brine shrimp lethality bioassay. The chloroform soluble extract showed moderate antimicrobial activity with a zone of inhibition of 13-15 mm. The petroleum ether soluble extract showed mild and methanol soluble extract showed poor antimicrobial activity against the tested microorganisms. In the brine shrimp lethality bioassay, the most significant cytotoxicity was showed by petroleum ether soluble extract with an LC_{50} of 3.36 µg/ml where vincristine sulphate was used as standard with an LC_{50} of 0.749 µg/ml. The results suggested significant antimicrobial activity of chloroform soluble extract and cytotoxic potential of petroleum ether soluble extract.

Key Words: *Arachis hypogea*, Papilionaceae, Cytotoxicity, Antimicrobial.

INTRODUCTION

Arachis hypogea (Bengali name – Badam, Cheenabadam; English name –Groundnut, Peanut; Family- Papilionaceae) is a creeping herb with oblong leaves, yellow pea-like flowers and oblong, locular underground fruits enclosing round edible seeds, cultivated as a cash crop in sandy areas of Dhaka, Comilla, Mymensingh and Noakhali. The chief constituent of the seeds is a nondrying edible fixed oil, which predominates in monosaturated fats, yields fatty acids, glycerides, carotinoids, tocopherols, triterpenoids, sterols, phosphatides. Cotyledons contain arachins and protease inhibitors. Red skin of Groundnut kernels contains a phenolic glycoside, arachidoside. Kernels contain proteins, carbohydrates and thiamine. Nut meal contains arachin, con-arachin, fat, protein, vitamins and lecithin. The alkaloid arachine isolated from its presscake pellets has been show to be impure choline. Aflatoxin has been detected in the nuts. Seeds are nutritious, aperient and emollient. Seeds and oils are astringent to the bowels. Unripe fruits are used as lactagogue. Peanut skin extracts acts as a haemostatic agent. The mono unsaturated fats in peanut are beneficial for cardiac patient, also preventive of heart attacks (Ghani, 2003). In the present study, we evaluated the antimicrobial and cytotoxic potential the aerial part of *A. hypogea.*

MATERIALS AND METHODS

Plant material

The aerial part of *A. hypogea* was collected from Mymensigh in the month of March 2008. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium, Dhaka, Bangladesh.

Extraction

The powdered aerial part (260 g) of *A. hypogea* was separately extracted to exhaustion in a Soxhlet apparatus at 50°C with petroleum ether then chloroform and finally with methanol. All the extracts were filtered through a cotton plug followed by Whitman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to provide petroleum ether (4.2 g), chloroform (3.00 g) and methanol (3.8 g) extractives.

Antimicrobial assay
The disc diffusion method described by Bauer et al. (1966) was used to test antimicrobial activity against thirteen bacteria and three fungi (Table 1). Solutions of known concentration (mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper disc (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material 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 low temperature (4^0C) for 24 h to allow maximum diffusion. There is a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37^0C for 24 h to allow maximum growth of the organisms. The test materials having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Brine Shrimp lethality bioassay
Brine shrimp lethality bioassay (Meyer et al., 1982; McLaughlin et al., 1998) technique was applied for the determination of general toxic property of the plant extractives. Brine shrimp eggs collected from pet shops were used as the test organism. Seawater was taken in the small tank. Shrimp eggs were added to one side of the tank, and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of a pasteur pipette 10 living shrimps were added to each of the vials containing 5 ml of seawater.

Preparation of positive control group
Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 40 µg/ml from which serial dilutions were made using DMSO to get 20µg/ml, 10µg/ml, 5µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.313µg/ml, 0.15625 µg/ml and 0.078125 µg/ml. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water.

Preparation of negative control group
30 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds.

Preparation of test groups
Stock solutions of plant extract samples were prepared by dissolving the appropriate amount of extracts in calculated volume of dimethyl sulfoxide (DMSO). Samples of different concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781µg/ml) were prepared.10 living nauplii were taken to each of the vial containing different concentrations of test sample with Pasteur pipette.

Counting of nauplii
After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.
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RESULTS AND DISCUSSION

Antimicrobial assay
The petroleum ether, chloroform and methanolic crude extracts of the aerial part of *A. hypogea* (500 µg/disc) were screened for antibacterial and antifungal activity against 13 gram positive and gram negative bacteria (*Bacillus cereus, B. megaterium, B. subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi, S. typhi*, *Shigella*

boydii, *S. dysenteriae, Vibrio mimicus*, *V. parahemolyticus*) and 3 fungi (*Candida albicans*, *Aspergillus niger*, *Sacharomyces cerevacae)* (Table-1) by disc diffusion method.

All the extracts exhibited significant antibacterial and antifungal activity in compared with the standard drug kanamycin (diameter of zone of inhibition 28-36 mm) listed in Table 1. The chloroform soluble extract showed highest antimicrobial activity having the diameter of zone of inhibition of 13-15 mm. The petroleum ether soluble extract also showed mild antimicrobial activity against the entire tested microorganism having the diameter of zone of inhibition of 9-11 mm. On the other hand the methanol soluble extract demonstrated poor antimicrobial activity with the diameter of zone of inhibition 6-8 mm.

Table 1: Antimicrobial activity of the different extracts of *A. hypogea*

Test microorganisms	Diameter of zone of inhibition (mm)			
	PESE	CSE	MSE	Kanamycin
Gram positive bacteria				
Bacillus cereus	10	13	7	32
Bacillus megaterium	11	13	8	35
Bacillus subtilis	9	14	6	31
Staphylococcus aureus	10	13	-	36
Sarcina lutea	9	14	-	35
Gram negative bacteria				
Escherichia coli	11	13	6	28
Pseudomonas aeruginosa	9	15	7	31
Salmonella paratyphi	10	14	-	35
Salmonella typhi	11	13	-	33
Shigella boydii	9	14	8	32
Shigella dysenteriae	10	14	6	34
Vibrio mimicus	9	13	7	35
Vibrio parahemolyticus	10	13	8	36
Fungi				
Candida albicans	9	15	-	28
Aspergillus niger	10	13	8	34
Sacharomyces cerevacae	11	14	-	35

PESE = Petroleum ether soluble extract of the aerial part; CSE = Chloroform soluble extract of the aerial part; MSE = Methanol soluble extract of the aerial part.

Brine Shrimp lethality bioassay

Following the procedure of Mayer et al (1982), the lethality of all the crude extracts to brine shrimp were determined on *A. salina*. Table-2 showed the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate. The LC_{50} obtained from the best-fit line slope were found to be 3.36 µg/ml, 11.07 µg/ml and 15.29 µg/ml for petroleum ether, chloroform and methenol soluble extract of the aerial part of *A. hypogea*, respectively. In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by the petroleum ether soluble extract of the plant showed potent activity whereas the chloroform and methanol soluble extractives showed mild activity.

Table 2: Effects of petroleum ether, chloroform and methanol soluble extracts on brine shrimp nauplii.

Conc. (C) (µg/ml)	Log C	% Mortality			LC_{50} (µg/ml)			Vincristine sulphate			
		PESE	CSE	MSE	PESE	CSE	MSE	Conc (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
400	2.602	100	100	100				40	1.602	100	
200	2.301	90	100	80				20	1.301	100	
100	2	90	80	70				10	1.000	90	
50	1.699	80	70	70				5	0.698	80	
25	1.398	70	60	60	3.36	11.07	15.29	2.5	0.397	70	0.749
12.5	1.097	70	60	50				1.25	0.096	50	
6.25	0.796	60	40	40				0.625	-0.204	40	
3.125	0.495	50	30	20				0.313	-0.505	40	
1.563	0.194	40	20	20				0.156	-0.806	30	
0.781	-0.107	30	10	10				0.078	-1.107	20	

PESE = Petroleum ether soluble extract of the aerial part; CSE = Chloroform soluble extract of the aerial part; MSE = Methanol soluble extract of the aerial part.

This clearly indicates the presence of potent bioactive principles in this crude extract of which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents.

CONCLUSION

From the present study, it can be concluded that different extracts of the aerial part of *A. hypogea* posseses significant antimicrobial and cytotoxic activities. The petroleum ether soluble extract showed potent cytotoxic activity with the LC_{50} value of 3.36µg/ml in comparison with the positive control (vincristine sulphate) which suggests that the plant may contain antitumor or pesticidal compounds (Meyer et al., 1982).The chloroform soluble extract showed highest antimicrobial activity having the diameter of zone of inhibition of 13-15mm. But this study is preliminary type and it would be interesting to carry out further investigations to identify the presence of novel drug candidates which may be responsible for the mechanism of such biological action.

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