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Original Article

MICROBICIDAL AND CYTOTOXIC ACTIONS OF METHANOLIC CRUDE EXTRACTS OF *A. MULTIFLORA.*-ROXB

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ABSTRACT

Objective: The study presented here was carried out for the evaluation of microbicidal and cytotoxic potential of methanolic extract of leaves of *Ammannia multiflora.* The microbicidal activity was tested against 16 bacteria. The cytotoxic activity was tested against brine shrimp species *Artemia salina.*

Methods: The microbicidal effect of the plant extracts was tested *in vitro* by the disc diffusion method with 16 standard bacterial strains. *Artemia salina*; a brine shrimp species; was used to assess cytotoxic activity.

Results: Among the subjected 16 different bacteria; in case of *S. boydii;* the zone of inhibition was higher (22.0 mm) at 500 µg/ml concentration. Whereas in the case of *E. foecalis*, the zone of inhibition was higher (8.0 mm) at 250 µg/ml concentration. In S. pyogenes; zone of inhibition was higher at 500 µg/ml concentration (13.0 mm). The growth of *S. saprophyticus* (9 mm), *S. agalactiae* (7 mm) *and S. sonnei* (7 mm) was also moderately inhibited. The extract exhibited effectiveness against 7 different species of bacteria out of 16. Cytotoxic effect was determined in lethality bioassay of brine shrimp species *Artemia salina*. The plant extracts exhibited significant cytotoxic property which was reflected in LC_{50} and LC_{90} values of 20.42µg/ml and 229.09 µg/ml respectively. Cytotoxic property was found as dose dependent manner.

Conclusion: These results suggest that, *Ammania multiflora* can effectively be used for its significant microbicidal action. It is also effective as cytotoxic agent and thus, could justify its use in traditional medicine.

Keywords: Microbicidal, *Ammannia multiflora*, Inhibition and Cytotoxic.

INTRODUCTION

Nature has blessed us with various herbs which are enriched with nutrient as well as medicinal values. For centuries, the purpose of food as well as medicine is served by the use of herbs. Various herbs were in focus of research interest that possess anti-tumor, immune-stimulating or hypolipidemic properties that may be of use in adjuncts in helping the risk of cancer or cardiovascular disease[1].

Herbal medicine is now in great demand in the developing world for primary health care not because they are inexpensive but because of their better compatibility and acceptability with human body and minimal side effects [2].

The World Health Organization (WHO) estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care which has to compound derive from medicinal herbs [3]. So, investigation should be carried out for better understanding of their properties, safety and efficiency [4]. In the last decades, a remarkable number of researches have been carried out in different countries to prove such efficiency [5-6]. Many plants are used traditionally for their antimicrobial properties, which results from the compounds synthesized in the secondary metabolism of that plant.

Ammannia multiflora (Bengali name-Acid Plant) is an erect glabrous reddish herb belonging to the Lythraceae family [7-8]. It is well distributed in the southern low land and at aisle of paddy field. It grows up to a height of 60 cm, it posses opposite branches and lower opposite leaves, sometimes upper alternate, oblong or narrow-elliptic, rounded or subcordate, usually obtuse or subacute; flowers reddish in dense axillary clusters, apetalous or with minute petals; fruits depressed globose capsules covered by calyx tube up to the middle.

The plants of this genus are known to be useful in burning sensation, anorexia, colic, dyspepsia, flatulence, strangury, renal and vesical calculi, seminal weakness, herpetic eruptions, rheumatism and intermittent fevers [1].

Previous phytochemical studies with other Ammannia genus have led to the isolation and identification of two new terpenic compounds named as ambacinol and ambacinin [2].

More over the following compounds have also been isolated-(i) sitosterol-3-O-glucopyranoside, (ii) Quercctin-3-rutinoside (Rutin), (iii) Kaempferol-3-O-glucopyranoside and (iv) Quercetin-3-O-L-rhamnoside (Quercitrin) [9].

MATERIALS AND METHODS

Collection of Plant materials

The plant under investigation, *A. multiflora,* was collected from low land of southern part of Khulna district. Identification of plant was performed by*Bangladesh National Herbarium, Ministry of Environment and Forest, Dhaka, Bangladesh*. A voucher specimen was preserved there which represented the collection. The identification number is-34429.

Preparation of extracts for microbicidal activity

The leaves of the plant were cut into small pieces, cleaned, dried and pulverized. A quantity of the powdered leaves (200 gm) of *A. multiflora* was soaked in 500 ml methanol, filtered and concentrated using a vacuum rotary evaporator [10, 11].

Test microorganisms

The bacterial strains used for the experiment were collected as pure cultures from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). Gram positive bacterial strains include: *Staphylococcus epidermidis, Streptococcus agalactiae Staphylococcus saprophyticus, Staphylococcus aureus*, *Strepto-coccus pyogenes and Enterococcus faecalis.* Following Gram negative bacteria used, which include: *Shigella dysenteriae, Escherichia coli, Salmonella typhi, Salmonella paratyphi, Shigella sonnei, Vibrio cholera, Shigella boydii, Pseudomonas spp., Shigella flexneri, Proteus spp.*

Microbicidal assay by disc diffusion method

Microbicidal activity of the extracts was determined by the disc diffusion method [12]. Measured amount of the test samples was dissolved in definite volumes of solvent (chloroform) and applied to sterile discs at a concentration of 250 µg/disc and 500 µg/disc respectively and carefully dried, to evaporate the

residual solvent. At the end of pre-determined incubation time, the nutrient agar plates were observed, diameter of zone of inhibition measured and tabulated below (table 1) for various bacterial strains used. In this investigation, Kanamycin (30 µg/disc) standard disc was used as a reference standard. Blank discs were used as control.

Table 1: Microbicidal activity of the test samples of *Ammannia multiflora* extract (500 µg/disc and 250µg/disc) and Kanamycin (30 µg/disc)

S. No.	Test microorganisms	Diameter of zone of inhibition (mm)			
		Blank (control)	Kanamycin (30 µg/disc)	Mel (250 µg/ml)	Mel (500 µg/ml)
	Gram positive bacteria				
01	*Staphylococcus aureus*	–	16.0	–	–
02	*Staphylococcus epidermidis*	–	25.5	4.5	10.5
03	*Staphylococcus saprophyticus*	–	27.0	4.0	9.0
04	*Streptococcus pyogenes*	–	23.0	6.5	13.0
05	*Streptococcus agalactiae*	–	34.5	3.0	7.0
06	*Enterococcus faecalis*	–	16.5	8.0	12.0
	Gram negative bacteria				
07	*Escherichia coli*	–	5.0	–	–
08	*Shigella dysenteriae*	–	19.5	–	–
09	*Shigella sonnei*	–	22.0	2.5	7.0
10	*Salmonella typhi*	–	–	–	–
11	*Salmonella paratyphi*	–	–	–	–
12	*Vibrio cholerae*	–	4.0	–	–
13	*Shigella boydii*	–	22.5	11.0	22.0
14	*Shigella flexneri*	–	22.0	–	–
15	*Pseudomonas spp.*	–	–	–	–
16	*Proteus spp.*	–	–	–	–

MEL: Methanolic Extract of Leaves, "–" indicates no activity

Preparation of plant material for cytotoxic screening

The leaves of the plant were cut into small pieces, cleaned, dried and pulverized. A quantity of the powdered leaves (200 gm) of *A. multiflora* was soaked in 500 ml methanol, filtered and concentrated using a vacuum evaporation method. DMSO (Di-methyl sulfoxide) was used to dissolve the concentrated leaf extract and used for cytotoxic screening.

Cytotoxic screening

For cytotoxicity screening, DMSO solutions of the plant extracts were applied against *Artemia Salina* for 24 hours in a in-vivo simplified assay [13, 14]. For the experiment, 4 mg of the plant extract was dissolved in DMSO and solutions of different concentration level were prepared by serial dilution, e. g. 320, 160, 80, 40, 20, 10 and 5 µg/ml were obtained.

Then each of this test solution was added to test tubes containing 10 shrimps in 5 ml of simulated brine water. After 24 hrs, the median lethal concentration LC50 of the test samples was obtained by a plot of percentage the shrimps killed against the logarithm of the sample concentration.

RESULTS

The methanolic extract of the leaf strongly inhibited the growth of *S. pyogenes* (13.0 mm), *E. faecalis* (12.0 mm) at 500 µg/disc and *S. boydii* (22.0 mm). The growth of *S. saprophyticus (9 mm), S. agalactiae (7 mm) and S. sonnei (7 mm)* was also moderately inhibited. In case of brine shrimp lethality bioassay, the crude methanolic extract of the leaf was studied and the LC_{50} values were found 20.42 µg/ml. The standard Chloramphenicol showed the LC50 value 8.91µg/ml.

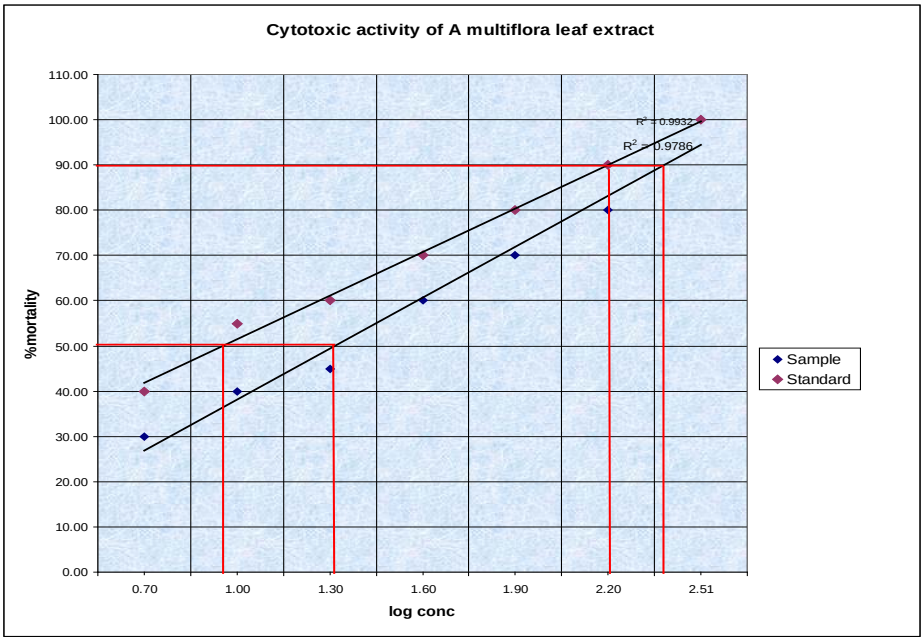

Table 2: Cytotoxic activity of the test sample of *A. multiflora* extract and chloramphenicol

Extracts/Standard	(%) Lethality of brine shrimp at different concentration(µg/ml)							LC_{50} (µg/ml)	LC_{90} (µg/ml)
	5	10	20	40	80	160	320		
Extract of A multiflora	30	40	45	60	70	80	100	20.42	229.09
Chloramphenicol (Standard)	40	55	60	70	80	90	100	8.91	162.18

DISCUSSION

Ammannia multiflora leaf is considered to be a potent source of bioactive compounds. The plant leaf possess many naturally occurring bioactive compound which exhibit strong microbicidal effect and thus can be served as traditional drugs. In the present study, 16 different bacterial strains were used for investigation and out of them, the plant leaf extracts exhibited microbicidal activity against 7 strains of bacteria.

The crude methanolic extract of plant leaves was also produce strong cytotoxic effect. When tested with brine shrimp lethality bioassay method, the plant extracts exhibited LC_{50} value at 20.42 µg/ml concentration. The standard Chloramphenicol showed the LC_{50} value 8.91µg/ml.

There are only few articles which were published focusing pharmacological activity of the genus *Ammannia.* In the present investigation, we confirmed the microbicidal activity of *A. multiflora.* Against seven different species of bacteria. From the obtained result in the case of *A. multiflora* could serve as a potential source of plant derived natural products with microbicidal activity to be used against microbes.

From the experiment, it is clearly evident that the crude methanolic extract of the leaf exhibited significant level of cytotoxicity and microbicidal activity. Hence, *A. multiflora* substantiate the folk uses of this plant in various diseases.

CONFLICT OF INTERESTS

Declared None

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