

Research Article

Cytotoxic and Antimicrobial Activity of the Crude Extract of *Abutilon Indicum*

Muhit Md. Abdul *, Apu Apurba Sarker, Islam Md. Saiful, Ahmed Muniruddin

Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy,
University of Dhaka, Dhaka 1000, BANGLADESH

ABSTRACT

The investigation was conducted with crude methanolic extract of leaf of *Abutilon indicum* for its cytotoxic and antimicrobial activity. Antimicrobial activity of the extract was evaluated against various Gram-positive, Gram-negative bacteria and fungi using disk diffusion technique. For cytotoxic activity, brine shrimp lethality bioassay was performed to estimate LC₅₀ values. The average zone of inhibition produced by carbon tetrachloride extract was found 7-10 mm at a concentration of 400µg/disc. The chloroform extract exhibited no antibacterial activity except *Sarcina lutea* (8.4 mm). In brine shrimp lethality bioassay, LC₅₀ obtained from the best-fit line slope were 0.419, 3.01, 5.62, 1.51, and 11.20 µg/ml for positive control (vincristine sulfate), n-hexane, carbon tetrachloride, chloroform and aqueous fraction respectively. The cytotoxicity exhibited by chloroform soluble fraction of methanol extract was promising. The carbon tetrachloride extract showed mild to moderate antimicrobial activity.

KEYWORDS: *Abutilon indicum*, cytotoxic activity, brine shrimp lethality bioassay, antimicrobial activity.

INTRODUCTION

Infectious diseases are caused by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. Diseases can spread, directly or indirectly, from one person to another. Infectious diseases are the second leading cause of death worldwide. About one-fourth of all the medicines we use, come from rainforest plants. However, scientific studies have been conducted only to a limited extent with few medicinal plants [1], [2]. The present study was designed to search for newer, safer and more potent antimicrobials and cytotoxic components which may accomplish our present need. Herbal medicines have received much attention as a source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and for environment. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth [3], [4].

Abutilon indicum (Bengali name: Jhampi, Petari, Indian name: Atibala, family: Malvaceae) is extensively grown in Bangladesh, India, Pakistan, Srilanka [5]. The plant is considered as astringent, antibacterial, anthelmintic, carminative and diuretic. It is used locally for colds, high fever, mumps, tuberculosis, bronchitis, diabetes, carbuncle, hemorrhoids, hernia, diarrhea and various types of worm infections [5]. Previous phytochemical investigation of the plant revealed the presence of

chemical constituents namely luteolin, chrysoeriol, apigenin 7-O-beta rhamnopyranosyl, quercetin, triacontanoic acid, ursenol, methylstigmaterol, glucopyronoside etc. [6]. Bioactivity guided isolation of *Abutilon indicum* yielded eugenol (4-allyl-2-methoxyphenol), which was found to possess significant analgesic activity [7] in acetic acid induced writhing test. In some places, juice from the leaves of the plant is used in combination with the liquid extract of *A. cepa* to treat jaundice.

As a part of our continuing study on chemical and biological characterization of different plants, attempt was made this time to investigate the antimicrobial activity of *A. indicum* against different Gram-positive, Gram-negative bacteria and fungi species [8]. The cytotoxic activity of the plant materials was performed by using brine shrimp lethality bioassay which was proposed by Michael *et al.* [9] and modified by Solis *et al.* [10].

MATERIALS AND METHODS

Collection of plant materials

Plant sample of *Abutilon indicum* (Family: Malvaceae) was collected from Dhaka in August 2007. The plant was identified and a voucher specimen (Accession number DACB 34459) representing this collection has been deposited in the Bangladesh National Herbarium, Dhaka, for further reference.

Extraction of Plant material

After separating the leaves from the plant, it was cut into small pieces and air dried for several days. The plant materials were then ground into coarse powder. The dried and ground plant powder (600g) was extracted with methanol (2.5 liters) in an air tight, clean flat

Corresponding author: Md. Abdul Muhit, Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh.

Tel: 0721-750041-54/4110, Fax: 0721-750064;
email: muhit_3533@yahoo.com

bottomed container for 3 days at room temperature with occasional stirring and shaking. The extract was then filtered first through a fresh cotton plug and finally with a Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45°C) and pressure. The weight of the crude extract was 23.65 gm. Solvent-solvent partitioning was done using the protocol designed by Kupchan [11] and modified version of Wagenen et al. [12]. The crude extract (5 gm) was dissolved in 10% aqueous methanol which was subsequently extracted first with n-hexane, then carbon tetrachloride and finally with chloroform. All the four fractions were evaporated to dryness by using rotary evaporator and kept in air tight containers for further analysis.

Microbial strains

In total 16 microbial strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both Gram positive, Gram-negative bacteria and fungi were taken for the test. The microorganisms were maintained on nutrient agar medium (Merck, Germany).

Antimicrobial activity

Antibacterial activity of the crude extract was investigated against 16 bacterial strains by the paper disk diffusion technique [13] using 100µl of suspension containing 108 CFU/ml of bacteria spread on nutrient agar medium. Sterile 6 mm diameter filter paper discs were impregnated with 400µg of each of the sterile test material (n-hexane, aqueous soluble partitionates of methanol, carbon tetrachloride and chloroform extract) and placed into nutrient agar medium. Kanamycin (30µg/disc) disc were used as positive control to ensure the activity of standard antibiotic against the test organisms. The sample discs and the standard antibiotic discs were placed gently on the previously marked zones in the agar plates pre-inoculated with the test bacteria

and fungi. The plates were then kept in a refrigerator at 4°C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37°C for 24 hours. The antimicrobial potency of the test agents were measured by their activity to prevent the growth of the microorganisms surrounding the discs which gave clear zones of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale [14].

Cytotoxic activity

For cytotoxicity screening, DMSO (Dimethyl sulfoxide) solutions of the four fractions extracted by n-hexane, chloroform, carbon tetrachloride and water from methanol crude extracts were applied to *Artemia salina* in a one day *in vivo* assay. For the experiment, 4 mg of each of the extracts were dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.123, 1.563, 0.781 µg/ml) were obtained by serial dilution technique. The solutions were then added to the pre-marked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. 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 (LC₅₀) of the test samples were obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration.

Statistical analysis

Different statistical techniques such as; Two tailed T test was performed for analyzing the data of antimicrobial activity and regression analysis were carried out for analyzing the data obtained from different samples to

Table 1: Antimicrobial activity of chloroform (CF), n-hexane (HX), aqueous fraction of methanol extract (AQ) and carbon tetrachloride (CT) of *A. indicum* leaves and positive control kanamycin (KM).

Test Bacteria and Fungi	Diameter of Zone of inhibition (mm)				
	CF	HX	AQ	CT	KM
Gram-Positive					
<i>Bacillus cereus</i>	-	-	-	8.0 ± 0.44	30.4 ± 0.10
<i>Bacillus megaterium</i>	-	-	-	8.2 ± 0.55	33.3 ± 1.20
<i>Bacillus subtilis</i>	-	-	-	7.1 ± 0.66	33.0 ± 1.10
<i>Staphylococcus aureus</i>	-	-	-	6.2 ± 0.25	29.6 ± 0.49
<i>Sarcina lutea</i>	8.4 ± 0.49	-	-	10.4 ± 0.15	34.7 ± 0.60
Gram-Negative					
<i>Escherichia coli</i>	-	-	-	9.2 ± 0.72	33.0 ± 0.49
<i>Pseudomonas aeruginosa</i>	-	-	-	6.2 ± 0.15	34.4 ± 0.40
<i>Salmonella paratyphi</i>	-	-	-	8.4 ± 0.49	29.8 ± 0.90
<i>Salmonella typhi</i>	-	-	-	6.9 ± 0.51	35.0 ± 1.00
<i>Shigella boydii</i>	-	-	-	8.4 ± 0.20	35.0 ± 1.00
<i>Shigella dysenteriae</i>	-	-	-	10.7 ± 0.15	34.5 ± 0.50
<i>Vibrio mimicus</i>	-	-	-	8.4 ± 0.46	30.7 ± 0.60
<i>Vibrio parahaemolyticus</i>	-	-	-	7.6 ± 0.15	32.3 ± 0.30
Fungi					
<i>Candida albicans</i>	-	-	-	6.1 ± 0.62	36.0 ± 1.00
<i>Aspergillus niger</i>	-	-	-	8.7 ± 0.20	36.7 ± 1.50
<i>Sachoromyces cerevaceae</i>	-	-	-	7.6 ± 0.21	35.5 ± 0.50

Values are expressed as mean ± SD (n = 3), “ - “ indicates no zone of inhibition

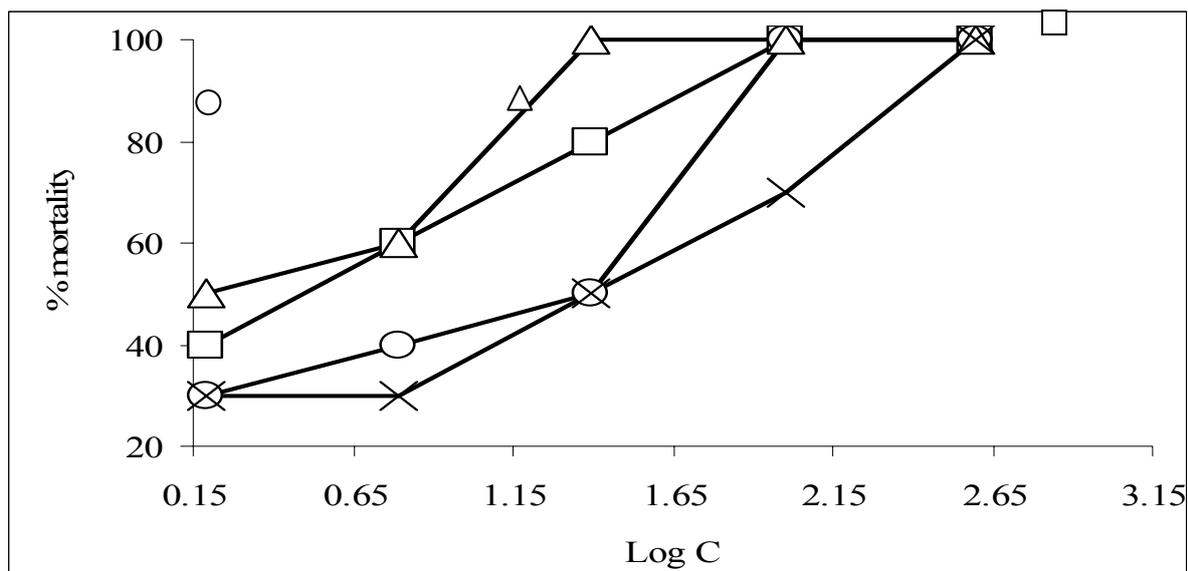


Figure 1: Graphical presentation of log concentration of n-hexane (- -), carbon tetrachloride (- -), chloroform (- -) and aqueous (- X)-soluble partitionates of methanolic extract versus percent shrimp mortality after 24 h of exposure.

study the relationship between cytotoxic activity and vinblastine. Each parameter was measured thrice and the data were taken as mean±SD. Differences at P value of less than 0.05 were considered as statistically significant.

RESULTS

Antimicrobial activity

The antimicrobial effects of methanol extract of *A. indicum* against different test organisms are shown in table 1. The n-hexane and methanol extract exhibited statistically insignificant activity against the tested microorganisms and the chloroform extract exhibited statistically significant antimicrobial activity against only *Sacina lutea* (8.4 mm). On the other hand carbon tetrachloride extract showed statistically significant activity (P value<0.05) against most of the pathogens examined. It showed moderate inhibitory activity against *Bacillus cereus* (8.0 mm), *Bacillus megaterium* (8.2 mm), *Sarcina lutea* (10.4 mm), *Shigella boydii* (8.4 mm), *Escherichia coli* (9.2 mm), *Salmonella paratyphi*

(8.4 mm), *Shigella dysenteriae* (10.7 mm), *Vibrio mimicus* (8.4 mm), and *Aspergillus niger* (8.7 mm).

Cytotoxic activity :

In cytotoxic test activity, % mortality increased gradually with the increase in concentration of the test samples. LC₅₀ values obtained from the best-fit line slope were 0.419, 3.01, 5.62, 1.51, and 11.20 µg/ml for standard, hexane fraction (HF), carbon tetrachloride (CTC), chloroform fractions (CF) and water soluble fractions respectively (Table 2). In comparison to positive control (vincristine sulphate), the cytotoxicity exhibited by chloroform soluble fraction of methanol extract was promising. On the other hand n-hexane, carbon tetrachloride and water soluble fraction demonstrated moderate cytotoxic activity (Table 3) (Figure 1).

DISCUSSION

Antimicrobial activity

In the present study it reveals that *Abutilon indicum* leaf

Table 2: Effect of n-hexane (HX), carbon tetrachloride (CT), chloroform (CF) and aqueous (AQ) soluble partitionate of methanolic extract and positive control vincristine sulphate (VS) on brine shrimp nauplii.

Conc. µg/ml	Log C	% mortality				LC ₅₀ (µg/ml)				Vincristine sulphate (VS)			
		HX	CT	CF	AQ	HX	CT	CF	AQ	Conc. µg/ml	Log C	% mor	LC ₅₀ µg/ml
400	2.60	100	100	100	100					40	1.60	100	
200	2.30	100	100	100	100					20	1.30	100	
100	2.0	100	100	100	70	3.01	5.62	1.51	11.2	10	1.0	90	0.419
50	1.70	80	90	100	70					5	0.69	90	
25	1.40	80	50	100	50					2.5	0.39	80	
12.5	1.09	70	50	70	40					1.25	0.09	70	
6.25	0.79	60	40	60	30					0.625	-0.2	60	
3.125	0.49	50	40	50	40					0.312	-0.5	50	
1.563	0.19	40	30	50	30					0.156	-0.8	30	

Table 3: The result of cytotoxic activity of n-hexane (HX), carbon tetrachloride (CT), chloroform (CF) and aqueous (AQ) soluble partitionate of methanolic extract and positive control vincristine sulphate (VS) on brine shrimp nauplii.

Sample	LC ₅₀ (µg/ml)	Regression equation	R ²
VS	0.419	Y = 30.403x + 61.484	0.9476
HF	3.01	Y = 25.77x + 39.849	0.9622
CT	5.62	Y = 31.61x + 23.564	0.8882
CF	1.51	Y = 25.974x + 44.597	0.8679
AQ	11.20	Y = 28.189x + 20.834	0.8683

extracts demonstrated antimicrobial activity against various worm infections. Only the carbon tetrachloride extract showed mild to moderate antimicrobial activity against most of pathogens examined. The average zones of inhibition produced by carbon tetrachloride extract were found to be 7-10 mm at a concentration of 400 µg/disc. Carbon tetrachloride extract showed maximum significant activity against *Sarcina lutea* and *Shigella dysenteriae*. The inhibitory effect of carbon tetrachloride towards other species was not promising. The previous study reveals that different extracts from the *Abutilon indicum* leaves were not shown statistically significant to inhibit microorganisms [15], [16]. This antimicrobial activity may be due to the greater solubility of the extract in this organic solvent. Previous phytochemical investigations suggested that the presence of flavonoids and triterpenoids compounds in this plant. Flavonoids from the different plants show antimicrobial activity. Thus the work suggested having seven flavonoids [6] to be used in the treatment of infectious diseases.

Cytotoxic activity

From the results of the brine shrimp lethality bioassay it can be well predicted that the crude extracts have considerable cytotoxic potency. Among the four fractions, chloroform extracts showed strongly significant cytotoxicity. The degree of lethality was directly proportional to the concentration of the extracts from the lowest concentration (0.781 µg/ml) to highly significant with the highest concentration (400 µg/ml). Maximum mortalities took place at a concentration of 400 µg/ml, whereas least mortalities were at 0.781 µg/ml concentration. In other words, mortality increased gradually with the increase in concentration of the test samples. Previous phytochemical screening indicated the presence of alkaloids and flavones, which have been shown to possess cytotoxic activity, may be responsible in part for the antitumour effect on Ehrlich ascites carcinoma [17]. The possible mechanism of cytotoxicity of *A. indicum* against brine shrimp nauplii due to poisonous effect on cell mitosis.

CONCLUSION

In general, the mechanisms by which microorganisms survive, the action of antimicrobial agents are poorly understood and remain debatable. On the other hand, the chemical constituents of these extracts may have a

causal role *in vivo* prevention of diseases caused by bacteria, fungi and yeast. Nevertheless, this scientific information can serve as an important platform for the development of further safe and effective natural medicine.

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