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In vitro Antibacterial Screening and Toxicity Study of Some Different Medicinal Plants

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Abstract: The methanol extract of different parts of seven different medicinal plants, like *Casuarina equisetifolia, Cajanus cajan, Glycosmis pentaphylla, Bixa orellana, Argemone mexicana, Physalis minima* and *Caesalpinia bonduc*, belonging to different families were used for investigation of antibacterial and toxic activities. In antibacterial screening performed by disc diffusion method against some Gram positive and Gram negative bacteria, it was found that the methanol extracts of all the plants samples showed moderate activity against almost all the tested organisms. *C. equisetifolia, C. cajan* and *C. bonduc* plant extracts exhibited comparatively strong activity using 100 µgm/disc. Among these three samples, the MIC value of *C. cajan*, determined by serial dilution technique, was found to be 64 µg/ml, 128µg/ml and 128 µg/ml against *Staphylococcus aureus*, *Bacillus subtilis* and *Shigella sonnei*, respectively. In toxicity study, the methanol extract of *P. minima* and *A. mexicana* showed strong toxic effect using brine shrimp lethality bioassay with LC_{50} values of 36.67 µg/ml and 54.42 µg/ml respectively.

Key words: Casuarina equisetifolia · Cajanus cajan · Glycosmis pentaphylla · Bixa orellana · Argemone mexicana · Physalis minima and *Caesalpinia bonduc* Antibacterial activity · MIC · toxicity

INTRODUCTION

Mankind has been using plants as therapeutic agents for thousands of years and continues to rely on them for health care. According to a WHO estimate, around 80% of the world's 5.86 billion inhabitants depend on traditional medicines for their primary health care, majority of which use plants or their active principles [1]. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast Knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [2]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds [3].

Developing countries like Bangladesh depends on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats and for fire and shade. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries [4,5]. Traditional healers claim that their medicine is cheaper, more effective and impart least side effects as compared to synthetic medicines. In developing countries, low-income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infections [6].

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [7]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries [8]. Although hundreds of plant species have been tested for

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antimicrobial properties, the vast majority have not been adequately evaluated [9].

The *Casuarina equisetifolia* Forst (locally known as Jhau gachh, Hari) belongs to the family Casuarinaceae. Extracts of leaves exhibit anticancer properties (Philippine Journal of Science (PJS), [10]. Bark is astringent and is used in treatment of stomacheache, diarrhea, dysentery and nervous disorders [11]. Seeds are anthelmintic, antispasmodic and antidiabetic [12]. Leaf juice of *Cajanus cajan* (Linn.) Huth (local name Arhar) belonging the family Papilionaceae, is useful in jaundice and disease of the mouth. Aqueous and alcoholic extracts of leaves exhibit anti-hepatotoxic activity in rats [13]. Infusion of leaves of *Glycosmis pentaphylla* Corr. (vernicular name: tooth-brush plant and Family: Rutaceae) is used in fever, liver complaints, cough and jaundice [14].

The seeds of *Bixa orellana* Linn. of which local name is lotkan, shidhur, belongs to the family Bixaceae, are used in fever, apetising agent and stimulant. Extracts of *Argemone mexicana* Linn., locally named as prickly poppy (English), belonging to the family Papaveraceae possesses tonic, anthelmintic, diuretic and hypnotic properties. Latex and extract of plants are used in jaundice, tumors, cancers and eye diseases [15] Bulletin Medico Ethno Botanied Research New Delhi (BMEBR), [16]. Alkaloid of the plant *Physalis minima* Linn. (local name, Tepari or Patka in Bengali) belonging to the family Solanaceae may have potential use for leukemia chemotherapy [17]. Leaves and fruits are tonic, diuretic and purgative and used in gonorrhoea and spleen disorders [18,12]. *Caesalpinia bonduc* (Linn.) Roxb. locally named as Natakaranja in Bengali belonging to Caesalpiniaceae family is used to treat fevers and its roasted seeds are used to treat diabetes [12]. Powder of this plant is an effective in blood dysentery (Journal of Research in Indian Medicine (JRIM), [19].

Considering the vast potentiality of plants as sources for antimicrobial drugs, with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen local flora for antibacterial activity from *Casuarina equisetifolia, Cajanus cajan, Glycosmis pentaphylla, Bixa orellana, Argemone mexicana, Physalis minima* and *Caesalpinia bonduc*. In addition to screening antibacterial activity, methanol extract of different parts of these plant were undergone to determine minimum inhibitory concentration value (MIC). Toxic effects of methanol extracts of these plants were also studied.

MATERIALS AND METHODS

Plant Materials and Preparation of Extracts: Fresh plant materials (Table 1) of *Casuarina equisetifolia, Cajanus cajan, Glycosmis pentaphylla, Bixa orellana, Argemone mexicana, Physalis minima* and *Caesalpinia bonduc* were collected from Botanical Garden of Rajshahi University, Rajshahi, Bangladesh. Their botanical identities were determined and authenticated by Dr. Ashik Mossaddik, Associate Professor, Dept. of Pharmacy, Rajshahi University, Rajshahi-6205, Bangladesh. Some voucher specimen numbers were submitted to the herbarium for future references. The plant materials were washed with water, cut into pieces, sun dried for 5 days and then dried in an oven below 60°C. The dried plant materials were then pulverized into coarse powder in a grinding machine. Ten gm of each (7 samples) plant sample were extracted separately in cold methanol. Solvent from each sample was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract.

Screening of Antibacterial Activity: The methanol leaf and bark extract of *Casuarina equisetifolia, Glycosmis pentaphylla*, and *Caesalpinia bonduc*, the methanol leaf and flower extract of *Argemone Mexicana*, whole plant extract of *Cajanus cajan* and *Physalis minima* and seed extract of *Bixa orellana* were tested for antibacterial activity by disc diffusion method [20]. By reconstituting with methanol, a single conenctration of each extract (100 μg/disc) was prepared. The test microorganisms were inoculated into respective medium by spread plate method with 24h cultured bacteria, grown in nutrient broth media. After solidification the filter paper discs (5 mm in diameter) impregnated with the extracts were placed on test organism-seeded plates. Five Gram positive bacteria like *Staphylococcus aureus, Streptococcus-β-haemolyticus, Bacillus megaterium, Bacillus cerus, Bacillus proteus*

Table 1: The methanolic extract present of the used parts of different plants

Scientific Name	Used Part	Percent Yield
Casuarina equisetifolia	Leaf and bark	15
Cajanus cajan	Whole Plant	11
Glycosmis pentaphylla,	Leaf and bark	14
Bixa orellana	Seed	41
Physalis minima	Whole Plant	12
Argemone mexicana	Leaf and Flower	18
Caesalpinia bonduc	Leaf and bark	12

and four Gram negative bacteria *like Shigella sonnei, Pseudomonus aeruginosa, Klebsillea sp., Shigella siga* were used as test organisms. As negative control, a blank disc impregnated with 10 µl methanol solvent and as positive control Kanamycin (30 µg/disc) were used. The antibacterial assay plates were incubated at 37°C for 24h. The antibacterial activities of the extracts were then determined by measuring the respective zone of inhibition in mm.

Determination of Minimum Inhibitory Concentration (MIC): The Minimum inhibitory concentrations (MICs) of the crude methanol extracts of *Casuariana equisetifolia, Cajanus cajan* and *Argemone mexicana* against *Staphylococcus aureus, Sheigella sonnei, Bacillus subtilis,* were determined by using serial dilution technique [21]. The sample solutions of all the extracts were prepared in dimethyl sulfoxide (DMSO) in such a way that the solutions had a concentration of 1mg/ml. In this technique a large no of sterilized test tubes were used and each of the test tube was filled with 1 ml of sterile nutrient broth media and graded doses of sample solution was added. Then these test tubes were inoculated with said organisms (inoculum contains 10^6 cell/ml) followed by incubated at 37°C for 24 hours to allow growth of the bacteria used. Inhibition of growth observed in that test tube (the solution content was clear) which has lowest or minimum concentration of extract and above of which no growth observed too. This lowest or minimum concentration was considered as minimum inhibitory concentration (MIC). Another three test tubes containing medium, medium plus sample and medium plus inoculum were used as control. Bacterial growth observed only in test tube (solution content was cloudy) containing medium plus inoculum and the other two were clear that means no growth occurred.

Toxicity Bioassay: Brine shrimp eggs, *Artemia salina,* were collected from pet shop (Dhaka, Bangladesh) and hatched for two days to let them be matured as nauplii [22].

The test samples (methanol extracts) were prepared by dissolving it in DMSO (not more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to make concentrations- 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 80µg /ml. Three vials each of which contains 20 µl DMSO diluted to 5 ml were used as negative control.

Then 20 matured alive shrimps were applied to each of all experimental vials containing solution of concentrations-5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml prepared from methnol extracts of all the plants separately and control vial containing only DMSO. After 24 hrs the number of dead nauplii was counted. The data so obtained were presented graphically by plotting log of concentration versus percentage of mortality of nauplii from which LC_{50} for sample was determined by drawing regression line.

RESULTS AND DISCUSSION

The results revealed that the tested methanol extracts of seven plants posses moderated antibacterial activity against *Staphylococcus aureus, spreptococcus-β-haemolyticus, Bacillus megaterium, Bacillus cerus, Bacillus proteus* and *Shigella sonnei, Pseudomonus aeruginosa, Klebsillea sp., Shigell siga* (Table 2). It is clear when tested by disc diffusion method that kanamycin showed positive results against all bacterial strains. The methanols extract of whole plant of *Cajanus cajan showed* significant antibacterial activity (around 25 mm) against *S. aureus, B. megaterium, B. cerus, Shigella sonnei, S. siga* and methanol extract of leaf and bark of *Casuarian equsetifolia* showed good activity against different strains. The methanol leaf and bark extract of *Caesalpinia bonduc* showed less activity (around 15 mm) than *Cajanus cajan* but showed activity against highest numbers of bacteria (6 strains). *B. megaterium, B. cerus* and *S. shiga* are highly sensitive strains against all plants. As standard, kanamycin showed highest zone of inhibition, measured 30 mm. Among the tested plant extracts, *Casuarian equsetifolia* showed largest zone of inhibition against *Bacillus proteus* and *Klebsiella sp.* measured 28 mm. *Cajanus cajan* showed activity against three Gram positive and two Gram negative bacteria and showed strong activity against *Shigella siga*, measured 28 mm. Seed extract of *Bixa orellana* and whole plant extract of *Physalis minima* showed strong activity against *Bacillus cerus* measured at 28 mm. The zone of inhibition of leaf and bark extract of *Glycosmis pentaphylla* was the lowest value (around 10). This plant showed least activity (6 mm) against *B. megaterium* and that was the least value among all the plants tested. Leaf and flower extract of *Argemone mexicana* showed a little bit more activity but not satisfactory, around 20 against three strains.

Results of the Minimum inhibitory concentrations (MICs) of leaf and bark methanol extracts of Casuarina *equisetifolia, Cajanus cajan* and *Argemone mexicana* determined against *Staphylococcus aureus, Bacillus subtilis, Shigella sonnei* were presented in Table 3.

Table 2: *In vitro* antibacterial activity of methanol extracts of the plants studied and standard antibiotics kanamycin in term of zone of inhibition in mm against some bacteria

	Diameter of the zone of inhibition (mm)							
Bacteria	S1 100 µg/di	S2 100 µg/di	S3 100 µg/di	S4 100 µg/di	S5 100 µg/di.	S6 100 µg/di	S7 100 µg/di	K-30 30 µg/di
Gram Positive								
Staphylococcus aureus	--	25	--	--	--	--	18	27
Spreptococcus-β-haemolyticus	--	--	--	--	--	--	--	26
Bacillus megaterium	9	22	6	18	17	10	14	30
Bacillus cerus	--	26	10	28	20	28	--	30
Bacillus proteus	28	--	--	--	--	--	17	30
Gram negative								
Shigella sonnei	11	25	11	--	--	--	14	29
Pseudomonus aeruginosa	--	--	--	--	--	--	--	28
Klebsillea sp	28	--	--	--	--	--	13	26
Shigell siga	10	28	10	22	21	24	15	30

"--'' = No zone of inhibition di = disc

K-30 = Kanamycin std. (30µg/disc), S1 = *Casuarina equisetifolia,* S2 = *Cajanus cajan,* S3 = *Glycosmis pentaphylla,* S4 = *Bixa orellana,* S5 = *Argemone mexicana,*S6 = *Physalis minima,* S7 = *Caesalpinia bonduc*

Table 3: MIC values (µg/ml) of three plant extracts against four pathogenic bacteria

Name of Bacteria	*Casuarina equisetifolia*	*Cajanus cajan*	*Argemone mexima*
Gram positive			
Stapylococcus aureus	64	64	128
Bacillus subtilis	256	128	128
Gram negative			
Shigella sonnei	128	128	128

Table 4: Toxicity study of six different plants extracts against brine shrimp nauplii

Sample	LC_{50}(µg/ml)	Regression equation	R-squared value
Ampicillin trihydrate	12.18	y = 46.507x - 0.5034	0.98
Methanol Ext. of *C. equisetifolia*	137.12	y = 49.834x - 56.501	0.9643
Methanol Ext. of *C. cajan*	93.86	y = 43.185x - 35.181	0.9657
Methanol Ext. of *G. pentaphylla*	76.12	y = 46.507x - 37.503	0.98
Methanol Ext. of *P. minima*	36.67	y = 53.15x - 33.146	0.8767
Methanol Ext. of *C. bonduc*	122.83	y = 58.14x - 71.473	0.9932
Methanol Ext. of *A. mexicana*	54.42	y = 49.834x - 36.501	0.9643

The MICs of *Casuarina equisetifolia* were 64 µg/ml and 128 µg/ml against *S. aureus and S. sonnei,* respectively and 256 µg/ml against *B. subtilis*. Again, the MIC value of sample *Argemone mexicana* against the mentioned organisms were 128 µg/ml. Similarly the MICs of *Cajanus cajan* were 64 µg/ml, 128 µg/ml and 128 µg/ml against the test organism *S. aureus* and *S. sonnei* and *B. subtilis,* respectively. So, it is evident that the extracts under study showed inhibition of bacterial growth even at low concentrations. Among these samples, the MIC is lowest for Casuarina *equisetifolia* and *Cajanus cajan* against both Gram positive *S. aureus* and Gram negative *S. sonnei,* respectively. Thus, Casuarina *equisetifolia* and *Cajanus cajan* are more suitable in aspect of antibacterial activity.

The crude methanol extracts of different parts of six different plants under study (among seven) were tested for toxic activities using brine shrimp lethality bioassay (Fig. 1). The results are presented in Table 4. The median lethal concentration (LC_{50} values) of *Casuarina equisetifolia, Cajanus cajan, Glycosmis pentaphylla, Physalis minima* and *Caesalpinia bonduc,Argemone mexicana* were 137.12, 93.86, 76.12, 36.67, 122.83, 54.42 µg/ml, respectively. Among them, the LC_{50} values of *Physalis minima* and *Argemone mexicana* were the lowest measured 32.20 and 54.42 µg/ml, respectively. With comparison of LC_{50} value (12.18 µg/ml) of positive control ampicillin trihydrate, *Physalis minima and Argemone mexicana* are more toxic than others. So, it can be concluded that the crude

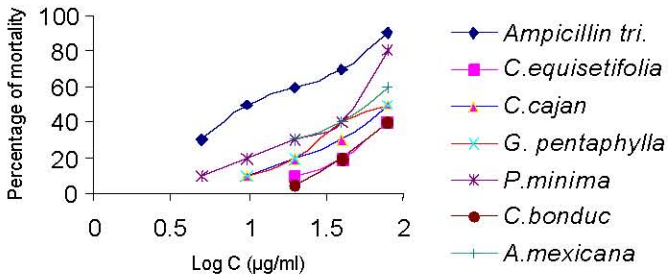

Fig. 1: Log C versus average percentage of mortality of nauplii of different methanol extract of 6 different plants under the present study

methanol extract of whole plant *Physalis minima* and methanol leaf and flower extract of *Argemone mexicana* might be used as larvicide and insecticide.

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