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BIOACTIVITY OF THE BARK OF *MACARANGA INDICA* WIGHT IC (EUPHORBIACEAE)

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ABSTRACT

The ethanol extract of *Macaranga indica* Wight Ic, the tralatitiously used medicative plant belonging to the family Euphorbiaceae has been investigated for the presence of its secondary metabolites and evaluation of biological activities of the crude extractive for the brine shrimp lethality bioassay and antioxidant activity for the first time. In chemical group tests, the result showed that the ethanol extract of *Macaranga indica* contains tannin, flavonoid, saponin, gum, steroid and alkaloid groups. In brine shrimp lethality bioassay after 18 hrs, the LC_{50} of crude extract and standard vincristine sulphate were 95.17 and 95.15 µg/ml respectively, after 24hrs, the values were 21.71 and 2.75 µg/ml respectively. In free radical scavenging assay, IC_{50} value of ethanol extract was 0.79 µg/ml in comparison to the standard trolox 0.40 µg/ml. From the study, it was evident that, the ethanol extract of *M. indica* showed moderate cytotoxicity and very strong antioxidant activity.

Keywords: *Macaranga indica*, Euphorbiaceae, Cytotoxic, Brine shrimp, Antioxidant, DPPH.

INTRODUCTION

Macaranga indica Wight Ic (Family: Euphorbiaceae), synonym- *M. flexuosa* Wight, *M. adenantha* Gagnepain, *Tanarius indicus* (Wight) Kuntze, *Trewia hernandifolia* Roth, is

locally known as Burna, Malata; Nuibothi bang in Marma. It is a large evergreen tree with soft wood, branchlets stout, glaucous, marked with leaf scars. Fruits are very small [1]. Leaves are orbicular ovate, acuminate, entire, broadly peltate, large, long petiole. Flowers are dioecious, in axillary panicles and light yellow in color. It is found in the hilly range of Bangladesh [2]. Besides, the plant is found in India, Srilanka, Bhutan, Mayanmar and China [3]. The red gum of the plant is used to clean wounds[2] and applied to sores [1]. Different parts of the plant are used quite frequently in various traditional medicines. The plant is used in the treatment of anaemia, paralysis and tumour [3]. It is also used for treatment of venereal sores[1], cuts, wounds, stomach-ache [4]. Sometimes, a gum exuded from the cut branches, petiole bases, young shoots and fruits of the plant are applied externally to get relief from venereal sores. The other parts of the plant are also used in different ways to treat various ailments[4]. Another species, *M. peltala* is also locally used for enlarged spleen and to get relief from venereal sores[5] and reported to have antioxidant, antimicrobial and cytotoxicity activity[6]. No activity of *M. indica* is yet been reported. Antioxidant properties of other species; *M. tanarius*[7], *Macaranga barteri* [8] along with *M. peltala*[6] has been reported. Compounds isolated from *M. peltala* have very potent antimicrobial activity[9].

MATERIALS AND METHODS

Collection and identification of plant material

Barks of *Macaranga indica* were collected from National Botanical Garden, Mirpur, Dhaka, Bangladesh (Coordinate-23°49'6"N 90°20'50"E) in March 2013. The samples of the plant were mounted on herbarium sheet and the species was taxonomically confirmed (Accession no.- DACB-37929) by Sarder Nasir Uddin, Principle Scientific Officer, Bangladesh National Herbarium (BNH), Mirpur, Dhaka. The voucher specimen of the plants has been deposited and preserved in BNH library for further collection and reference.

Preparation of ethanol extract

The collected barks were separated from undesirable materials. They were dried in open air under shade for two weeks. The shade dried plants part ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 204 gm powdered bark was taken in a clean, flat-bottomed glass container and soaked in 1.5 L of ethanol. The container with it contain was sealed and kept for a period of 15 days accompanying occasional shaking and stirring (Khatun et al., 2014).

The whole mixture then underwent a coarse filtration by a piece of clean, white, cotton material. It was then filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate was concentrated under air and dried. It rendered a19 g concentrate (9.31% yields) greenish brown color and was designated as crude ethanol extract. Extraction was conceded following the standard method[10].

Chemicals and reagents

Standard chromogenic reagents used for chemical group test were of reagent grade and purchased from Sigma-Aldrich Co. LLC, Missouri, United States. Vincristine sulphate, used as a standard drug in the cytotoxic assay was collected from the Techno Drugs Limited, Bangladesh. Ethanol supplied by Laboratory Patterson Scientific, U.K. was used as solvent. Dimethyl sulfoxide (DMSO), ≥99.9% purchased Sigma-Aldrich, India was used as solvent to dissolve the extracts. Sodium Chloride Crystal GR from Merck Ltd., Mumbai, India was used to prepare sea water in brine shrimp lethality bioassay. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was from Nacalai Tesque Inc., Kyoto, Japan and standard trolox was from Merk KGaA, Dermstadt, Germany.

Instruments and equipment

Electronic balance (serial no.- 1508, OHAUS, Germany) was used for all weight measurement in this study. Vortex mixer (VM-2000, 220 V, Digisystem Laboratory Instruments Inc. Taiwan) was used to mix the extract with the solvent. Double beam Analykjena UV/Visible spectrophotometer (model- Shimadzu, UV-1800, Japan) was used to take the absorbance of the extract and standard in free radical scavenging assay. Glass made hatching tank, air pump and cover lamp to grow shrimp were purchased locally. Pipettes, Micro-pipette, test tubes and other glass apparatus used were of laboratory standard and procured from authorized dealer.

Brine Shrimp

Brine shrimp (*Artemia salina*) eggs were purchased from Carolina Biological Supply Company, Burlington, NC, USA. Nauplii were obtained by hatching the eggs in artificial sea-water (3.8% NaCl solution) for 24 hrs.

Assessment of secondary metabolites

The ethanol extract of *Macaranga indica* was tested for its different chemical groups of plant secondary metabolites as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and

tannins using standard protocol[11]. 10% (w/v) solution of the extract in ethanol was used for each of the above test.

Test for cytotoxic activity

The cytotoxicity assay was performed on brine shrimp nauplii using the modified method of Mayer *et al.* [12] determining the 50% lethal dose (LC_{50}) of the extract and standard Vincristine sulphate. Sample was prepared by suspending 50 mg of plant extract in 5ml of artificial sea water containing DMSO to have concentration of 10 µg/µl. From this solution 0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 µl were transferred to each 10 ml vial and using artificial sea water volume was adjusted to 10 ml water to give concentrations of compound of 0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 µg/ml respectively. Brine shrimp nauplii were grown in these solutions and observed their mortality after 18 and 24 hrs. The resulting data were transformed to probit analysis software (LdP Line software, USA)[13, 14] for the determination of LC_{50} values of the extract and standard. Artificial sea-water medium containing DMSO used for the analysis was employed as negative control.

Test for antioxidant activity

The antioxidant activities of plant extract and the standard antioxidant, trolox were assessed on the basis of free radical scavenging effect of the stable DPPH free radical[15, 16]. Stock solution (10 mg/ml) of the ethanol extract of *M. indica* was prepared in respective solvent systems from which serial dilutions were carried out to obtain concentrations of 0.1, 0.5, 1, 5, 10, 50 µg/ml. In this assay, an equal amount of sample solution was added to an equal amount of 0.1 mM ethanolic DPPH solution, vortexed and allowed to stand at the dark place at 25°C for 30 min for the reaction to occur. After 30 min of incubation period, the absorbance was read against a blank at 517 nm with Analykjena UV/Visible spectrophotometer. The radical scavenging activity was expressed as the percentage of inhibition (I%) and calculated as per the equation:

$$I(\%) = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound with all reagents. IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from the plot of inhibition (%) against extract concentration. All the tests were carried out in triplicate and average of the absorptions was noted. Ascorbic acid was used as positive control standard for this study.

Results

Assessment of secondary metabolites

Different chemical tests on the ethanol extract of *Macaranga indica* showed the presence of steroids, alkaloids, tannins, gums, flavonoids and saponins as the major plant secondary metabolites (Table 1).

Table 1: Results of phytochemical screening of *Macaranga indica* extracts.

Test for phytochemical group	Reagent	Results of the extract of *Macaranga indica*
Reducing sugar	Fehling's test	-
	Benedict's test	-
Alkaloid	Hager's test	+
	Wagner's test	+
	Dragendorff's test	+
Steroid	Salkowski's test	+
Tannin	Ferric chloride test	+
	Lead acetate test	+
	Potassium dichromate test	+
Gum	Molisch's test	+
Flavonoid	Shinoda test	+
	Alkaline reagent test	+
Saponin	Frothing test	+

+: Positive result; - : Negative result

Cytotoxic activity

Table 2 shows the cytotoxic effect of the ethanol extract *Macaranga indica* using brine shrimp lethality bioassay. In the test, the extract and the standard showed LC_{50} at 95.17 and 95.15 µg/ml after 18 hours and after 24 hours 21.71 and 2.75 µg/ml respectively. Control group did not show any mortality. An approximate linear correlation was observed in a dose and time dependant manner when concentrations versus percentages of mortality were plotted on graph paper.

Table 2: Brine shrimp lethality bioassay of ethanol extract of *Macaranga indica* Wight

Conc. (µg/ml)	After 18 hours				After 24 hours			
	Macaranga indica		Vincristine sulphate		*Macaranga indica*		Vincristine sulphate	
	% mortality	LC_{50} (µg/ml)	% mortality	LC_{50} (µg/ml)	% mortality	LC_{50} (µg/ml)	% mortality	LC_{50} (µg/ml)
1	40	95.17	30	95.15	50	21.71	50	2.75
5	50		40		60		60	
10	60		50		60		70	
50	60		70		70		80	
100	70		80		80		80	
500	80		80		90		90	

Antioxidant activity

In free radical scavenging assay, IC_{50} values of ethanol extract of *Macaranga indica* and standard trolox were 0.79 and 0.40 µg/ml. This plant exhibited significant antioxidant activity (Table 3).

Table 3: Free radical scavenging activity of the ethanol extract of *Macaranga indica* Wight at 517 nm

Concentration (µg/ml)	*Macaranga indica* Wight		Standard Trolox	
	% Inhibition	IC_{50} (µg/ml)	% Inhibition	IC_{50} (µg/ml)
1	12.87	0.79	21.17	0.40
5	30.34		27.21	
10	62.67		59.42	
50	77.75		81.88	
100	86.78		82.35	
500	90.91		93.5	

DISCUSSION

Cancer, malignant tumour or neoplasm is a broad term for a large group of diseases that can affect any part of the body. 8.2 million people worldwide died from cancer in 2012 and 60% of world's total new annual cases occur in Africa, Asia and Central and South America[17]. From the beginning of history, natural products have afforded a wealthy source of compounds that have found many applications in the fields of medicine, pharmacy and biology. Within the sphere of cancer, a number of significant new commercialized drugs have been obtained from natural sources, by structural alteration of natural compounds, or by the synthesis of new compounds, designed following a natural compound as model. The search for improved cytotoxic agents continues to be a key line in the finding of modern anticancer drugs. The enormous structural diversity of natural compounds and their bioactivity potentials have meant that several products separated from plants, marine flora and microorganisms can serve as "lead" compounds for development of their therapeutic potential by molecular modification. Furthermore, semisynthesis processes of new compounds, obtained by molecular modification of the functional groups of lead compounds, are able to produce structural analogues with superior pharmacological activity and with lesser side effects[18]. Even today, over 60% of the 140 plus agents presently available in Western medicine can trace their provenance to a natural-product source[19].

To evaluate the traditional exercise in tumour, the extract was underwent brine shrimp lethality bioassay using *Artemia salina* comparing with standard anti-cancer drug vincristine

sulphate. Among the methods to assay antitumour and cytotoxic activities namely brine shrimp lethality assay, cytotoxicity against cultured cells[20], Sulphorodamine B (SRB) assay[21], MTT assay[22], *Agrobacterium tumefaciens* induced potato disc assay[23] etc., brine shrimp lethality bioassay is an easy, non-expensive and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity[24]. In the present study the extract showed LC_{50} at a very low concentration with very quick response indicating that the extract is significantly potent; specially after 18 hours, the extract and standards showed almost same toxicity. Further investigation is required to find the responsible compound(s) for the cytotoxic activity observed for *M. indica*.

Definite antioxidant supplements may lessen adverse reactions and toxicities. Noteworthy reductions in toxicity may alleviate dose-limiting toxicities so that more patients are capable to complete prescribed chemotherapy regimens and thus, in turn, get better potential for success in terms of tumor response and survival[25]. Cell oxidation can lead to the onset and development of a wide range of diseases including Alzheimer and Parkinson, the pathologies caused by diabetes, rheumatoid arthritis, neurodegeneration in motor neuron diseases, and cancer. Reactive species (RS) of various types are powerful oxidizing agents, capable of damaging DNA and other biomolecules. Increased formation of RS can promote the development of malignancy, 'normal' rates of RS generation may account for the increased risk of cancer development. Oxidants and free radicals are inevitably produced during the majority of physiological and metabolic processes and the human body has defensive antioxidant mechanisms; these mechanisms vary according to cell and tissue type and may act antagonistically or synergistically. They include natural enzymes like Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx), as well as antioxidants such as vitamins, carotenoids, polyphenols, and other natural antioxidants, which have attracted great interest in recent years.

So, cancer and reactive species are responsible for lots of health complication and the pace is increasing in a surprisingly higher rate. Searches for new source of cytotoxic and antioxidant compounds are the major concern of the time. As certain groups of plant secondary metabolites like tannin, reducing sugar, alkaloid, flavonoid, gum, saponin and steroidal compounds are responsible for some specific pharmacological actions, the ethanol extract of *M. indica* was tested to determine whether these definite groups were present in the extract.

The study demonstrated the presence of steroids, alkaloids, tannins, gums, flavonoids and saponins as the major secondary metabolites.

There has been a great deal of interest of late in the role of complementary and alternative drugs for the treatment of various acute and chronic diseases. Among the several classes of phytochemicals, interest has focused on the anti-inflammatory and antioxidant properties of the polyphenols that are found in various botanical agents. Plant vegetables and spices used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemopreventive drug discoveries and development[26].

In brine shrimp lethality bioassay, the ethanol extract and the standard showed LC_{50} at 95.17 and 95.15 µg/ml after 18 hours and after 24 hours 21.71 and 2.75 µg/ml respectively. In free radical scavenging assay, IC_{50} values of ethanol extract was 0.79 µg/ml in comparison to the standard trolox 0.40 µg/ml. From the study, it was evident that, the ethanol extract of *M. indica* showed significant cytotoxicity and antioxidant activity.

The plant is applied in sores [1, 4] and used to clean wounds[2]. In this study, we found the plant to have strong antioxidant activity which evidently contributes in wound healing[27, 28]. Some other species of this genus were reported to have antimicrobial property[6, 9, 29].

CONCLUSION

Present study is based on the report of preliminary phytochemical and biological screening of *Macaranga indica* extract to evaluate its conventional benefits in traditional medicine. The obtained results are significant and justify the applications. Advanced studies including LC-MS can be carried out to get a bigger picture of the chemical constituents present in the plant and correlate the bioactivities with their mechanism of action.

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