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Original Article

ANTIOXIDANT, ANTIBACTERIAL AND CYTOTOXIC ACTIVITIES OF VARIOUS EXTRACTS OF *THYSANOLAENA MAXIMA* (ROXB) KUNTZE AVAILABLE IN CHITTAGONG HILL TRACTS OF BANGLADESH

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ABSTRACT

Objective: To evaluate the presence of different phytoconstituents and investigate *in vitro* bioactivities of petroleum ether, chloroform and methanol extracts of *Thysanolaena maxima* available in Bangladesh.

Methods: Phytochemical screening was conducted using the specific standard procedure. Antioxidant activity of the extracts was evaluated using DPPH radical scavenging assay and reducing power assay. Determination of total phenolic and flavonoid contents was also carried out. Antibacterial and cytotoxic activities were investigated using disc diffusion method and brine shrimp lethality bioassay, respectively.

Results: The methanol extract showed highest DPPH radical scavenging activity as well as possessed highest phenolic content (IC_{50} value for DPPH is 36.94±0.62 µg/ml and total phenolic content is 74.39±2.87 in mg/g, GAE) compared to the petroleum ether and chloroform extracts. On the other hand, chloroform extract possessed maximum flavonoid content (81±7.542 in mg/g, QE) and highest reducing power compare to other extracts. All the extracts showed mild to moderate *in vitro* antibacterial activity with a zone of inhibition ranging from 7 mm to 16 mm. In brine shrimp lethality bioassay, the LC_{50} values for petroleum ether, chloroform and methanol extracts were found to be 579.05±78.08 µg/ml, 386.92±80.47 µg/ml and 494.29±104.82 µg/ml, respectively which revealed weak cytotoxic potentials of the extracts compared to the positive control.

Conclusion: The results indicated that *T. maxima* could be a very potent source of natural radical scavenger. Isolation of active compounds from this plant responsible for producing such bioactivities is underway.

Keywords: *Thysanolaena maxima*, DPPH, Total phenolic contents, Total flavonoid contents, Reducing power, Cytotoxicity, Antimicrobial activity

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INTRODUCTION

Plants provide us various nutrition and form an important source of bioactive compounds, which can be used on different physiological condition. Therefore, plants that have medicinal properties are of great importance for the health as well as for their users in different communities. This medicinal property is because of some chemical substances which have the capabilities of producing a definite physiological action on the human body.

The term 'ethnobotany' is defined as the study of human evaluation and manipulation of plant materials and local people's interaction with the surrounding natural vegetation. The tribal people and ethnic races throughout the world have developed their own culture, foods and medicinal practices. Numerous wild and cultivated plants play a very important and vital role among these cultures. The Hill Tracts of Bangladesh includes three districts, viz., Bandarban, Khagrachari and Rangamati, and are located in the south-east corner of the country with Kaptai Watershed area. A large number of tribal populations under 14 major tribes like Chakma, Marma, Murong, Tongchongya, Tripura, Chak, Bhome, Pangkhoa, Khiyang, Rheyang, Rakhain, Lushai, Kuki and Khumi live as forest inhabitants in the remote areas throughout the Hill Tracts where there is no or poor introduction of education and modern medical systems of health care [1]. The majority of them are dependent on the traditional system of treatment which includes various indigenous medicinal plants of those areas.

Thysanolaena maxima (Roxb.) Kuntze (Family-Poaceae) are a perennial grass plant found in hilly regions of Bangladesh, Nepal, northern and eastern parts of India and Bhutan. Various parts of this plant have been used traditionally by various tribal populations of Asia. Two teaspoonfuls of root juice is used twice a day for 2-3 d as anthelmintic by the people of Palpa district in Nepal [2]. Khasi traditional healers of Meghalaya, India used the inflorescence paste of *T. maxima* mixed with a pinch of a slaked lime and applied locally for the treatment of boils. They also use young stem juice of this plant when eyes become red and dirty [3]. The whole plant of *T. maxima* boiled with water which is used as a tonic by the people of the western forest of Thailand [4]. Crushed flowers are taken with water as antiemetics and in the treatment of stomach trouble by Kanda tribal population of Sylhet in Bangladesh. The soft part of young leaves and flower buds are eaten raw to cure flatulence and improve digestion by Dimasa tribes of Assam in India [5]. Chakma community of Chittagong, Bangladesh take pills prepared from the leaves twice daily for the treatment of tuberculosis. The ethanol extract of *T. maxima* showed moderate antimicrobial activities against four bacterial strains and mild antioxidant activities [6].

Literature survey revealed few research works have been performed on this plant to evaluate its medicinal values and active constituents those are responsible for its pharmacological activities. Besides, different solvent systems and conditions have pronounced effect on extracting bioactive molecules which cause variation in their bioactivities [7]. Therefore, taking into consideration the traditional uses of the plant and facilities available for conducting the study, this research work was performed on different solvent extracts of the aerial part of the plant available in Chittagong Hill Tracts of Bangladesh.

MATERIALS AND METHODS

Chemicals and solvents

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was obtained from Sigma-Aldrich co., USA. Folin-Ciocalteu reagent, ascorbic acid, potassium ferricyanide, and sodium carbonate were purchased from Merck, Germany. Trichloroacetic acid and ferric chloride ($FeCl_3$) were

obtained from Fine Chemicals, India. All the other chemicals used, including the solvents, were of analytical grades.

Plant collection and identification

The aerial part of *T. maxima* was collected from Rangamati, Chittagong Hill tracts, Bangladesh on August 20, 2014 and identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen of the plant has been deposited (Accession No.: DACB 42267) in the herbarium for further reference.

Extraction of the plant material

200 gm powdered plant materials were submerged into petroleum ether, chloroform and methanol using 1.0 liter of each solvent in an air-tight flat bottom container for seven days with occasional shaking and stirring. The major portion of the extractable compounds of the plant materials was dissolved in different solvents which were collected and then evaporated with a rotary evaporator (IKA, Germany) at low temperature (40-50 °C) and reduced pressure. The dried crude extracts were stored at 4 °C until used. The yield percentages (w/w) of *T. maxima* in different solvents are shown in table 1.

Table 1: Yield % of *T. maxima* in different solvent extracts

Solvent used	Yield %
Petroleum ether	1.5
Chloroform	2.2
Methanol	3.5

Phytochemical screening

The freshly prepared extracts were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts were performed using the following reagents and chemicals: alkaloids with Wagner's and Hager's reagent, terpenoids with modified Salkowski test, carbohydrates with Molisch's test, tannins with 0.1% ferric chloride, flavonoids with the use of concentrated hydrochloric acid, saponins with ability to produce stable foam and glycosides by using alcohol with few drops of H_2SO_4, followed by neutralization with NaOH solution and boiling with Fehling's solution. These were identified by characteristic color changes using standard procedures [8].

Tests for antioxidant activity

DPPH radical scavenging activity

The free-radical scavenging activity of *T. maxima* extracts was measured by a decrease in the absorbance of a methanol solution of DPPH [9]. A stock solution of DPPH (400 µg/ml) was prepared in methanol, and 100 µl of this stock solution was added to 5 ml of solutions of *T. maxima* extracts of different concentrations (20-100 µg/ml). The solutions were then mixed properly and kept in dark for 20 min and the absorbances were measured at 517 nm by using a spectrophotometer (Shimadzu UV PC-1800). Scavenging activity was expressed as the percentage inhibition calculated using the following formula: $[(A_0-A_1)/A_0]$ x100, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated. Ascorbic acid, a potential antioxidant was used as positive control.

Determination of total phenolic contents

The total phenolic contents of the extracts were determined by using Folin-Ciocalteu reagent [9, 10] and gallic acid (Merck, Germany) as standard. 10% Folin-Ciocalteu reagent was used to oxidize the extracts (250 µg/ml) and gallic acid (50-250 µg/ml) which were neutralized with 700 mM sodium carbonate solution. After 60 min, absorbances were taken at 765 nm. The total phenolic contents of the extracts were determined from a standard curve prepared with gallic acid and expressed as gallic acid equivalents (mg/g, GAE). The estimation of the phenolic compounds was expressed as mean±SD.

Determination of total flavonoid contents

The flavonoid contents were determined using Aluminium chloride colorimetric method, previously described by Kumaran and Karunakaran [11] using quercetin as the reference compound. One millilitre of plant extract in methanol (250 µg/ml) and quercetin (50-250 µg/ml) were mixed with 200 µl of 10% aluminium chloride and 1 M potassium acetate solution followed by addition of 5.6 ml distilled water. The absorption at 415 nm was read after 40 min. The total flavonoid contents were determined from a standard curve prepared with quercetin and expressed as quercetin equivalents (mg/g, QE).

Reducing power

Reducing power of the extracts was determined using potassium ferricyanide where plant extracts showed its reducing power by reducing ferric ion to ferrous ion by forming prussian blue colored complex [12]. 2.0 ml of each extract and standard (ascorbic acid) in different concentrations (25-200 µg/ml) were taken in test tubes. 2.5 ml of potassium ferricyanide $[K_3Fe(CN)_6]$ 1% solution was added into the test tubes followed by incubation for 10 min at 50 °C to complete reaction. 2.5 ml of trichloroacetic acid (10%) was added into the test tubes, and the total mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml supernatant solution was withdrawn from the mixture and mixed with 2.5 ml of distilled water and finally 0.5 ml of ferric chloride (0.1%) solution was added. Then the absorbance of the solution was measured at 700 nm against a blank.

Antibacterial assay

The antibacterial assay was carried out by the disc diffusion method [13] against five Gram-positive and six Gram-negative bacterial strains. 100 µl of suspension of each microorganism containing approximately100-150 CFU/ml was spread over the nutrient agar (Himedia, India). Dried and sterilized filter paper discs (6 mm diameter), impregnated with 400 and 600 µg of different extracts were placed gently in the agar plates. Standard disc (Himedia, India) of Kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as a positive and negative control, respectively. After incubation at 37 °C for 24 h, the antibacterial activity of the extracts was determined by measuring the diameter of the zone of inhibition expressed in mm.

Cytotoxic activity

Brine shrimp lethality bioassay was used [14] for evaluating cytotoxic activity using different concentrations of each extract. The eggs of brine shrimp (*Artemia salina* Leach) were collected and hatched in a tank at a temperature around 37 °C with constant oxygen supply at pH of 8.4. Two days were allowed to hatch and mature the nauplii. A stock solution of the samples was prepared by dissolving required amount of extracts in a specific volume of pure dimethyl sulfoxide (DMSO) (Merck, Germany). 4 ml of seawater was given to each of the vial. Then specific volumes of samples were transferred from the stock solution to the vials to get final sample concentrations of 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml. The Same volume of DMSO (as in the sample vials) was taken as negative control and solutions of different concentrations of Vincristin sulphate were taken as positive control. With the help of a Pasteur pipette, 10 living nauplii were put to each of the vials. After 24 h the vials were observed, and the number of nauplii survived in each vial was counted. After that, the percentage of lethality of brine shrimp nauplii was calculated for each concentration of the extracts and standard.

Statistical analysis

Statistical comparisons were performed using Microsoft Excel, 2007. All experiments were repeated three times and mean values±SD were calculated for expressing the results.

RESULTS

Phytochemical screening

Phytochemical analysis revealed the absence of alkaloids and presence of terpenoids, carbohydrates, tannins, flavonoids, saponins and glycosides in all extracts of *T. maxima* in varying amount (table 2).

Table 2: Results of chemical group tests of the extracts of *T. maxima*

Plant extract	Alkaloids	Terpenoids	Carbohydrates	Tannins	Flavonoids	Saponins	Glycosides
Petroleum ether	-	+	+	+	+	+	+
Chloroform	-	+++	+	+	+++	+	+
Methanol	-	++	+	++	++	++	++

+++: highly present,++: moderately present,+: slightly present,-: absent

Antioxidant activity

DPPH radical scavenging activity

From the analysis of Fig.1, it can be concluded that the scavenging effect of the extracts increases with the concentration. Methanol extract showed the highest radical scavenging activity (IC_{50} 36.94±0.62 µg/ml) whereas petroleum ether extract showed the lowest activity (IC_{50} 94.86±9.20 µg/ml). The IC_{50} values for the extracts and the standard (ascorbic acid) are shown in table 3.

Total phenolic contents

Among the three extracts, the methanol extract showed the highest amount of phenolic compounds (74.39±2.87 mg/g, GAE) followed by the chloroform extract (69.03±3.08 mg/g, GAE) and petroleum ether extract (56.89±13.99 mg/g, GAE) (table 4).

Total flavonoid contents

The highest amount of flavonoid contents was observed in the chloroform extract (81±7.54 mg/g, QE) whereas the petroleum ether extract showed the lowest amount of flavonoid contents (20.66±1.88 mg/g, QE) (table 4).

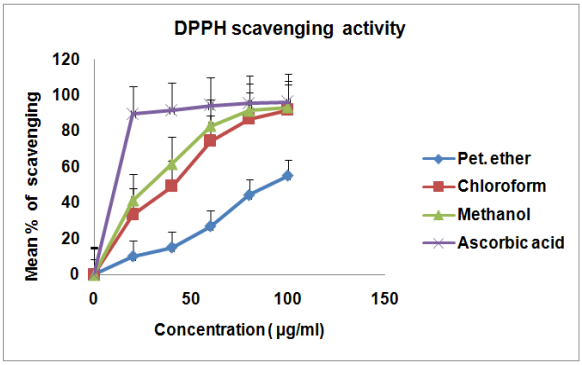

Fig. 1: Mean % of DPPH scavenging activity of different extracts of *T. maxima* and ascorbic acid (n = 3)

Table 3: IC_{50} values of different extracts of *T. maxima* and ascorbic acid

Extract/standard	IC_{50} values (µg/ml)
Petroleum ether	94.86±9.20
Chloroform	43.49±1.07
Methanol	36.94±0.62
Ascorbic acid	10.19±3.43

Values are represented as mean±SD (n = 3)

Table 4: Total phenolic and flavonoid contents of different extracts of *T. maxima*

Extracts	Total phenolic contents (in mg/g, GAE)	Total flavonoid contents (in mg/g, QE)
Petroleum ether	56.89±13.99	20.66±1.88
Chloroform	69.03±3.08	81±7.54
Methanol	74.39±2.87	59.83±4.94

Values are represented as mean±SD (n = 3)

Reducing power

The reducing power of different extracts was found to increase with the concentration. The chloroform extracts showed higher reducing power compared to methanol and petroleum ether extract. Fig. 2 revealed the reductive capabilities of all the plant extracts compared to ascorbic acid which had the highest reductive activity than the extracts.

Antibacterial assay

The chloroform extract displayed zone of inhibition ranging from 8 mm to 16 mm with highest antibacterial activity against *Bacillus subtilis* (15.33±0.29 mm at 600 µg/disc). This extract showed moderate activities against other strains compared to the standard disc Kanamycin (30 µg/disc). The methanol extract showed a zone of inhibition ranging from 8 mm to 11 mm with highest antibacterial activity against *E. coli* (10.33+1.52 mm at 600 µg/disc). Among the three extracts, petroleum ether extract was found to be inactive against all the bacterial strains.

Cytotoxic activity

In brine shrimp lethality bioassay, the lowest LC_{50} value (386.92±80.47 µg/ml) was revealed by the chloroform extract and the highest LC_{50} value (579.05±78.08 µg/ml) was demonstrated by the petroleum ether extract. The extracts showed weak cytotoxic activity compared to the standard Vincristine sulphate (LC_{50} = 1.11±0.45 µg/ml) (table 6 and 7).

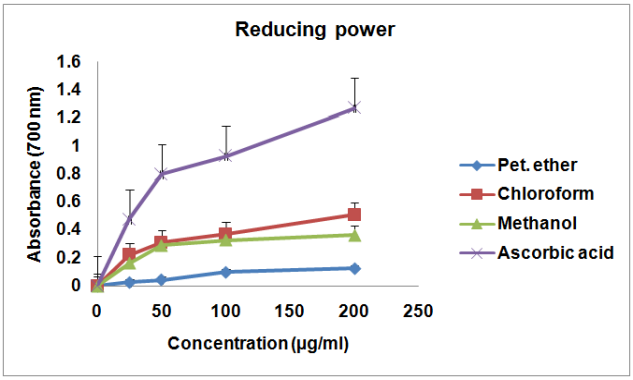

Fig. 2: Mean reducing power of different extracts of *T. maxima* (n = 3)

Table 5: Zone of inhibition of different extracts of *T. maxima* and Kanamycin

Micro-organisms	Petroleum ether		Chloroform		Methanol		Kanamycin
	400 µg/disc	600 µg/disc	400 µg/disc	600 µg/disc	400 µg/disc	600 µg/disc	30 µg/disc
Gram-positive bacteria Zone of inhibition (mm)							
Sarcina lutea	-	-	8±0.5	11.1±0.1	-	-	30.1±0.1
Bacillus megaterium	-	-	10.1±0.1	12±0.5	-	8.66±0.57	30±0.5
Bacillus subtilis ATCC 6059	-	-	11.07±0.06	15.33±0.29	-	-	30.33±0.29
Staphylococccus aureus ATCC25923	-	-	10.1±0.1	13.23±0.25	-	9.66±1.52	34±0.5
Bacillus cereus ATCC 14579	-	-	8.07±0.06	11.33±0.29	-	9±1	30±0.5
Gram-negative bacteria							
Pseudomonas aeruginosa ATCC 27853	-	-	-	8.43±0.15	-	-	28.1±0.1
Salmonella typhi ATCC 13311	-	-	-	-	-	-	17.53±0.58
Escherichia coli ATCC 25922	-	-	12±0.5	13.66±1.527	8.1±0.1	10.33±1.52	30.33±0.29
Vibrio mimicus ATCC 33653	-	-	-	8±0.5	-	-	13.53±0.58
Shigella boydii ATCC13147	-	-	-	8.23±0.25	-	-	23±0.5
Shigella dysenteriae ATCC 26131			8.53±0.58	10.67±0.11	-		24.23±0.25

Values are expressed as mean±SD (n = 3), '–'Indicates no zone of inhibition

Table 6: Mean % mortality of brine shrimp larvae at different concentrations of *T. maxima* extracts and standard

Concentration	Mean % mortality of brine shrimp			
(µg/ml)	Petroleum ether	Chloroform	Methanol	Vincristine sulphate
400	46.66	53.33	56.66	100
200	40	43.33	36.66	100
100	30	36.66	33.33	100
50	26.66	30	26.66	100
25	16.66	20	23.33	93.33
12.5	10	13.33	10	86.66
6.25	3.33	6.66	6.66	76.66
3.125	0	0	0	53.33
1.5625	0	0	0	40

Mean % mortality of brine shrimp larvae after 24 h (n = 3)

Table 7: Cytotoxic activity of various extracts of *T. maxima*

Extracts	LC_{50} values (µg/ml)
Petroleum ether	579.05±78.08
Chloroform	386.92±80.47
Methanol	494.29±104.82
Vincristine sulphate	1.11±0.45

Values are represented as mean±SD (n = 3)

DISCUSSION

Phytochemical tests showed the existence of terpenoids, carbohydrates, tannins, flavonoids and glycosides in all extracts of *T. maxima*. The presence of these phytol compounds can be correlated to the biological activities of *T. maxima* found in this research as chemical constituents of medicinal plants responsible for its therapeutic value.

In recent years, there has been increasing interest in the involvement of reactive oxygen species (ROS) in several pathological incidences because the oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular diseases [15]. As a result, antioxidants with free radical scavenging activities may have great contribution in the prevention and treatment of such diseases. DPPH radical scavenging activity can be used to measure the electron donation ability of natural products [16]. In the present study, Chloroform and methanol extracts showed strong free radical scavenging activity compared to the standard ascorbic acid which found consistent with other literature findings [17].

Phenolic and flavonoid compounds are considered as very important secondary metabolites [18, 19] because of their hydroxyl groups confer scavenging ability. As a result, these compounds scavenges most oxidizing molecules, including singlet oxygen and various free radicals which are responsible for creating several diseases [20]. The methanol extract exhibited the highest total phenolic contents which can be positively correlated with its DPPH free radical scavenging activity.

The reducing ability of a compound generally depends on the presence of reductants [21], which can exert its antioxidant activity by breaking the free radical chain and donating a hydrogen atom [22]. The present study indicated the presence of reductants or antioxidants in all extracts of *T. maxima* which may be responsible for exerting its reducing power. Our results suggested that phenolic compounds and flavonoids may be the major contributors for the antioxidant activity of the extracts.

In the present study, the chloroform and methanol extracts (at different concentrations) exhibited low to moderate antimicrobial activity against various strains of Gram-positive and Gram-negative bacteria whereas petroleum ether extract showed no antibacterial activity. The chloroform extract showed the maximum potential

antibacterial activity against both Gram positive and Gram negative bacterial strains which make it a potential antibacterial candidate.

Various extracts of *T. maxima* produced a concentration-dependent increment in percent mortality of brine shrimp nauplii. All the extracts of *T. maxima* showed weak cytotoxic activity compared to the positive control vincristine sulphate. The results also support the use of *T. maxima* as a daily nontoxic food supplement for highland cattle [17].

This study is suggestive that *T. maxima* can be used as a source of antioxidant and antibacterial agents in the development of new drugs which is supported by its traditional uses. Further work is under progress to identify the bioactive principles and elucidate their mechanisms of action of specific bioactivities.

CONFLICT OF INTERESTS

Declared none

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