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Preliminary Cytotoxicity Screening of Some Medicinal Plants of Bangladesh

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ABSTRACT: The cytotoxic activity of the methanolic extracts of 35 plant species, including 28 traditionally used plants of Bangladesh was evaluated by the brine shrimp lethality bioassay technique. Among these, 19 plant extracts exhibited significant toxicity to brine shrimps with LC_{50} less than 10 µg/ml.

Key words: Cytotoxic activity, Brine shrimp lethality bioassay.

INTRODUCTION

Plants are the natural reservoir of many antimicrobial and anticancer agents. Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for the treatment of various diseases. However, scientific studies have been conducted only to a limited extent with few medicinal plants.[1-4] In this investigation, 35 locally used plants were selected and tested to justify their existing bioactivities by the brine shrimp lethality bioassay.[5] The method utilizes *in vivo* lethality in a simple zoological organism brine shrimp nauplii as a convenient monitor for screening cytotoxicity of the plant extracts which can be furhter correlated with its anticancer potentiality and other bioactivities.

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MATERIALS AND METHODS

Plant collection. The plants selected for the study (Table 1) were collected from Dhaka, Chittagong and Khulna districts of Bangladesh during January - April 2004 and identified at the Department of Botany, University of Dhaka where voucher specimens for these collections are maintained.

Extraction. The air-dried and powdered plant materials were separately extracted with methanol for 5 days at room temperature with occasional shaking and stirring. The extracts were then filtered off through a cotton plug and finally with Whatman no.1 filter papers. The volume of the filtrate was reduced using a Buchii rotary evaporator at low temperature and pressure.

Bioassay. Brine shrimp lethality bioassay[5] technique was applied for the determination of cytotoxic property of the plant extractives. Vincristine sulphate and DMSO were used as positive and negative control, respectively.

Table 1. LC_{50} of methanol extracts of some medicinal plants of Bangladesh

Plant (Family)	Uses in traditional medicine	RMC	RPPS	LC_{50} (µg/ml)
Aglaia roxburghiana (Meliaceae)	Dysentry, leucoderma, leprosy, fever, thirst, tumors, vomiting.[6]	24, 25-epoxy-29-norcycloartan-3-ol, 29-norcyclorart-23-ene-3, 25-diol , 24,25-epoxy-29-nor-24-cycloarten-3β-ol, roxburghiline, hydroxyroxburghiline, aglaroxin-A, roxhurghiadiol A.[10]	Terpenoids, alkaloids	11.66 ± 1.10
Amoora cucullata (Meliaceae)	Inflammation.[6]	No information found.	Steroids	5.16 ± 1.13
Amoora rohituka (Meliaceae)	Cancer, tumours, spleen and liver disease, rheumatism.[7]	6b,7b-epoxyguai-4-en-3-one, 6b,7b-epoxy-4b,5-dihydroxyguaiane,[11] stigmasta-5,24(28)-dien-3β-O-β-D-glucopyranosyl-O-α-L-rhamnopyranoside,[12] 7-keto-octadec-cis-I 1-enoic acid.[13]	Terpenoids	5.95 ± 0.94
Brunfelsia americana (Solanaceae)	No information found.	No information found.	Steroids	7.43 ± 1.17
Brunfelsia latifolia (Solanaceae)	No information found.	No information found.	Steroids	6.82 ± 0.47
Buchanania lanzan (Anacardiaceae)	fever, thirst, diarrhea, itch.[8]	Myricetin 3′-rhamnoside-3-galactoside.[14]	flavonoid	63.42 ± 1.34
Chukrasia tabularis (Meliaceae)	As an astringent and antidiarrhoeic.[6]	tabulalides A–E,[15] tabularin, [16] scopoletin, melianone, [17] chukrasin A-E.[10]	Terpenoids, coumarins	15.16 ± 1.27
Combretum coccineum (Combretaceae)	No information found.	A hydroxylated mansumbinen-28-oic acid.[18]	Terpenoids	5.95 ± 0.37
Erioglossum edule (Sapindaceae)	Fever, whooping cough.[6]	No information found.	Steroids	158.74 ± 1.24
Ficus indica (Moraceae)	Relieve toothache, rheumatism, lumbago, inflammations, diarrhoea, dysentry, vomitting, biliousness.[7]	Bengalenoside, leucoanthocyanidins, leucoanthocyanin glycoside, betasitusterol glycoside, mesoinositol, friedelin, beta-sitosterol, qurecetin-3-galactoside and rutin, tiglic acid ester of gamma-tarxerol, cyanidin rhamnoglycoside, ficusin and bergaptin.[7]	Steroids, terpenoids, phenolics	17.67 ± 1.17
Garuga pinnata (Burseraceae)	Asthma, opacity of conjucntiva.[7]	Garuganins I-VI, eupha-7, 24-diene-1, 3- diol, eupha-7, 24-diene-3, 11, 16-triol.[10]	Terpenoids	37.72 ± 3.10
Indigofera tinctoria (Papilionaceae)	Epilepsy, nervous disorder, bronchitis, sores, old ulcers.[7]	Galactomannan [19], indirubin.[20]	Steroids, terpenoids	1.86 ± 1.14
Lannea coromandelica (Anacardiaceae)	Leprous and obstinate ulcers, toothache, mouth sores, impotency.[9]	(2*R*,3*S*)-(+)-3′,5-dihydroxy-4′,7-dimethoxydihydroflavonol, (2*R*,3*R*)-(+)-4′,5,7-trimethoxydihydroflavonol, (2R,3R)-(+)-4′,7-di-O-methyldihydroquercetin, (2R,3R)-(+)-4′,7-di-O-methyldihydrokaempferol and (2R,3R)-(+)-4′-O-methyldihydroquercetin [21], Quercetin-3-arabinoside, ellagic acid, β-sitosterol, physcion, physcion anthranol B, leucocyanidin.[22]	Phenolics	53.59 ± 1.33
Nephelium litchi (Sapindaceae) Syn: *Litchi chinensis*	Neurological disorders, smallpox, throat infection.[7]	Folic acid , L - ascorbic acid, cyanidin-3-glucoside, cyanidin-3-rutinoside, malvidin-3-acetylglucoside, α-[methylene cyclopropyl] glycine.[7]	Steroids, terpenoids	143.98 ± 1.64
Nephelium longana (Sapindaceae) Syn: *Euphoria longan*	Anthelmintic, fever.[6]	Saponin,[6] 2-amino-4-methylhex-5-ynoic acid, 2-amino-4-hydroxymethylhex-5-ynoic acid, 2-amino-4-hydroxyhept-6-ynoic acid.[23]	Steroids, terpenoids, amino acids.	2.21 ± 0.66
Passiflora coccinea (Passifloraceae)	No information found.	Passicoccin.[24]	Cyanogenic glycoside	131.95 ± 1.33

(Contd.)

Table 1 (Contd.)

Plant (Family)	Uses in traditional medicine	RMC	RPPS	LC_{50} (µg/ml)
Petunia meleagris (Solanaceae)	No information found.	No information found.	Steroids	53.59 ± 2.31
Petunia phoenica (Solanaceae)	No information found.	No information found.	Steroids	30.31 ± 1.28
Petunia punctata (Solanaceae)	No information found.	No information found.	Steroids	4.90 ± 1.64
Petunia violaceae	No information found.	No information found.	Steroids	41.14 ± 1.67
Phyllanthus reticulatus (Euphorbiaceae)	Spongy and bleeding gums, as astringent, diuretic.[6]	Friedelin, sitosterol, friedelan-38-02 glochidonol, 2la-hydroxyfriedelan3-one, 21a-hydroxyfriedel-4(23)-en-3-one, betulinic acid.[25]	Terpenoids, steroids	3.72 ± 1.98
Pongamia glabra (Leguminosae)	Bleeding piles, fistulous sores, bronchitis, gonorrhoea, whooping cough, as tonic.[9]	Karanjin , ovalitenone, pongachromene, lanceolatin, betulinic acid, caffeic esters, pongapin, glabrachromene, desmethoxykanugin, (-)-isoglabrachromene, kanugin, glabra-ii, fisetin tetramethyl ether, 5-methoxy-3',4'-methylenedioxy-2",2"-do(7,8-6'',5") flavone, [26] glabone.[27] pongagallone-a, pongagallone-b, [28] isopongachromene, pongamol, kanjone, pongaglabrone, [29]	Flavonoids	8.54 ± 1.31
		glabrachalcone, [30] isopongaglabol and 6-methoxyisopongaglabol, 5-methoxyfurano(8,74",5")flavone, 5-methoxy-3',4'-methylenedioxyfurano(8,7-4",5")flavone, ovalichromene B, cycloart-23-ene-3p,25-diol, friedelin, and β-sitosterol-β-D-glucoside,[31] pongaglabol, aurantiamide acetate [32] pongaglabrone.[33]		
Pterospermum suberifolium (Sterculiaceae)	Smallpox, hemicrania.[6]	3,4-Di-O-methylrhamnose	Phenolics	2.48 ± 0.88
		2,3,4,6-Tetra-O-methylglucose		
		2,3,6-Tri-O-methylglucose		
		2,3,4,6-Tetra-O-methylgalactose		
		2,3,6-Tri-O-methylgalactose, 4,6-Di-O-methylgalactose 3,6-Di-O-methylgalactose.[34]		
Quisqualis indica (Combretaceae)	Diarrhea, fever, rickets in children, boils, ulcers, helminthiasis.[7]	Quisqualic acid,[35] quisqualin A.[10]	Amino acids, tannins	4.42 ± 1.57
Semecarpus anacardium (Anacardiaceae)	Scrofulous, venereal and leprous infections, nervous debility.[6]	Anacardic acid, semicarpol, bhilawanol, monolefin I, diolefin II, bhilawanol-A, bhilawanol-B, amentoflavone tetrahydroamentoflavone, tetrahydrobustaflavone, jeediflavanone, semecarpuflavanone,galluflavanone anacarduflavone, anacardoside,[36] semecarpetin, nallaflavanone, [37] jeediflavanone, [38] semecarpuflavanone, [39] galluflavanone,[40] O-trimethyl biflavanone A_1, O-trimethyl biflavanone A_2, O-Tetramethyl biflavanone A_1, O-hexamethyl bichalcone A,		
		O-dimethyl biflavonone B, O-heptamethyl bichalcone B_1, O-hexamethyl bichalcone B_2, O-tetramethyl biflavanone C.[41]	Phenolics	35.36 ± 0.94
Shorea robusta (Dipterocarpaceae)	Ulcers, wounds, gonorrhea, leprosy, helminthiasis.[8]	9,10-dihydroxystearic acid, 3,25-epoxy-1,2,3,11-tetrahydroxy-12-ursen-28-oic acid,[7] 28-nor-12-ursen-3-ol, shorea phenol, 2,3,23-trihydroxy-11-methoxy-12-ursen-28-oic acid.[10]	Terpenoids	3.50 ± 1.38

(Contd.)

Table 1 (Contd.)

Plant (Family)	Uses in traditional medicine	RMC	RPPS	LC_{50} (µg/ml)
Solamum indicum (Solanaceae)	Astringent, carminative, cardiac tonic, aphrodisiac.[6]	Isoanguivine, protodioscin, solasonine and solamargine, indioside A-E.[42]	Steroids	4.42 ± 0.67
Solanum ferox (Solanaceae)	Coughs, asthma, fever, vomiting, sore throat, gonorrhea.[6]	Carpesterol, solanocarpine.[6]	Steroids, alkaloids	12.92 ± 1.49
Spondias mangifera (Anacardiaceae)	Antiscorbutic, astringent, dysentry, diarrhoea, vomitting, gonorrhoea and leuchoohoea.[7]	3, 16-dihydroxy-12-oleanen-28-oic acid -3-O-[β-D-galactopyranosyl-(1-->5) xylopyranoside].[10]	Terpenoids	4.42 ± 0.99
Swintonnia floribunda (Anacardiaceae)	No information found.	No information found.	Steroids	32.66 ± 1.68
Terminalia bellirica (Combretaceae)	Hepatitis, breathing problem, coughs, eye disease, constipation purgative.[7]	Cardenolide,[43] 2-dotriacontanol, bellericagenin B, bellericaside B, termilignan , bellericagenin A, bellericaside A, thannilignan, 9-tritriacontanone,[10] sitosterol, gallic aad, ellagic, acid, ethyl gallate, galloyl glucose, mannitol, glucose, galactose, fructose		
		and rhamnose, bellerlc acid, bellericoside, arjungenin, arjunglycoside.[44]	Terpenoids, phenolics, steroids,	3.62 ± 1.35
Terminalia chebula (Combretacacae)	Indigestion, constipation jaundice, piles, painful menstruation, asthma, colic, as diuretic and cardiotonic.[6]	Terflavin A, chebulagic acid, chebulinic acid, corilagin, 2α-hydroxymicromeric acid, luteolic acid, 12-oleanene-2,3,19,23,28-pentol, terchebin, terchebulin, terflavin D, terfalvin B, 1,3,6-trigalloyl glucose. [10]	Phenolics	8.85 ± 0.38
Trachyspermum ammi (Umbelliferae)	Diarrhea, colic, flatulence, indigestion, cholera, dyspepsia.[6]	An essential oil containing thymol.[6]	Terpenoids	4.95 ± 1.61
Xylocarpus granatum (Meliaceae)	Dysentery, diarrhea, and other abdominal problems.[7]	xyloccensins O-P, xyloccensins Q-V, [45] Xyloccensin L, [46] xyloccensin K, [47] xyloccensin I-J.[48]	Terpeonoids	6.81 ± 0.22
Zizyphus mauritiana (Rhamnaceae)	Diarrhea, fever, delirium, gout, wounds and ulcers.[7]	Mauritine A-H, frangufoline, amphibine B, amphibine D-F,[49] zizogenin, [50] laccaic acid D.[10]	Alkaloids	22.75 ± 0.34

RMC, repoted major constituents; RPPS, results of preliminary phytochemical screenings; LC_{50}, 50% of lethal concentration; LC_{50} were calculated as mean ± SD (n=3).

4 mg of each of the extractives was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml were obtained by serial dilution technique. Then the solutions were added to the premarked 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 (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

Statistical analysis. The experiment was conducted in triplicate and the LC_{50} were calculated as mean ± SD (n=3).

RESULTS AND DISCUSSION

In the present study, extracts of 35 local plants used in Bangladesh were evaluated by the brine shrimp lethality bioassay using the procedure

designed by Meyer *et al*.[5] The LC_{50} values of the brine shrimp assay obtained for extracts of these medicinal plants are listed in Table 1.

The methanolic extract of *Indigofera tinctoria* showed the highest activity with LC_{50} 1.86 µg/ml. The extracts of *Nephelium longan*, *Pterospermum suberifolium*, *Shorea robusta*, *Terminalia bellerica* and *Phyllanthus reticulatus* exhibited strong brine shrimp lethality with LC_{50} values of 2.21, 2.48, 3.50, 3.62 and 3.72 µg/ml, respectively. In addition, *Solanum indicum, Quisqualis indica, Spondias mangifera, Petunia punctata, Trachyspermum ammi, Amoora cucullata, Amoora rohituka* and *Combretum coccineum* have also shown significant brine shrimp lethality and the LC_{50} values were found to be lower than 6.00 (Table 1). On the otherhand, *Xylocarpus grantum, Brunfelsia latifolia*, *Brunfelsia americana, Pongamia glabra* and *Terminalia chebula* showed moderate cytotoxicity with LC_{50} less than 10.00 µg/ml.

It is observed that 19 plants extracts were highly lethal to brine shrimp nauplii out of the 35 plants used in the study. This indicates that these plants contain potential bioactive compounds, which if properly and extensively studied, could provide many chemically interesting and biologically active drug candidates, including some with potential antitumor and antiproliferative properties. A thorough chemical study is required to isolate the molecules that are responsible for the activities.

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