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Ethnobotanical, phytochemical and toxicological studies of *Xanthium strumarium* L

Mohammad Rashedul Islam[1], Mohammad Zashim Uddin[2], Mohammad Sharifur Rahman[1], Ershad Tutul[2], Mohammed Zakiur Rahman[3], Md. Abul Hassan[2], M.A. Faiz[4], Moazzem Hossain[5], Maleeha Hussain[6] and Mohammad Abdur Rashid[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000; [2]Department of Botany, University of Dhaka, Dhaka 1000; [3]Center for Biomedical Research, University of Dhaka, Dhaka 1000; [4]Department of Medicine, Sir Salimullah Medical College, Dhaka; [5]Disease Control Unit, Directorate General of Health Services, Dhaka; [6]National Institute of Cancer Research and Hospital, Dhaka, Bangladesh. e-mail: rashid_phdu@yahoo.com

Abstract

The present study describes the ethnobotanical, phytochemical, and toxicological evaluations of *Xanthium strumarium* L. growing in Bangladesh. In toxicity evaluation on rats, the methanol extract of seedlings showed mortality, while both seedling and mature plant extracts raised the serum alanine transaminase and aspartate transaminase values and produced significant abnormalities in the histopathology of liver and kidney of rats. On the other hand, the aqueous soluble fraction of methanol extract of mature plant (LC_{50} = 0.352 µg/mL) and methanol crude extract of seedlings (LC_{50} = 0.656 µg/mL) demonstrated significant toxicity in the brine shrimp lethality bioassay. A total of four compounds were purified and characterized as stigmasterol (**1**), 11-hydroxy-11-carboxy-4-oxo-1(5),2(*Z*)-xanthadien-12,8-olide (**2**), daucosterol (**3**) and lasidiol-10-anisate (**4**). The present study suggests that *X. strumarium* is toxic to animal.

Introduction

The ethnobotanical survey and documentation of traditional knowledge, specifically on the medicinal uses of plants, has provided many important clues for the discovery of new drugs of modern day[1]. Some ethnobotanical survey[2-5] conducted in our country focused mainly on listing medicinal plants, their uses and mode of treatments. However, such works failed to make significant contribution on the ethnobotaninal, phytochemical and toxicological aspects of plants growing in Bangladesh.

Xanthium. strumarium L. (English: Cocklebur, Family: Asteraceae; locally name- ghagra shak) is an annual herb with a short, stout, hairy stem, frequently branched, resulting in somewhat bushy plants from 20-150 cm tall[6]. Although it is known as medicinal plants in Bangladesh previous reports stated that *X. strumarium* is toxic to different mammals such as cattles, horses and swine[7-10]. There was an outbreak in Sylhet, a north eastern district of Bangladesh that claimed more than 30 deaths, who have ages below 12 years. Preliminary investigations have come up with a conclusion that intake of *X. strumarium* with the meals is one of the suspected causes of this outbreak[11]. On this background the present investigation was intended to explore the ethnomedicinal, phytochemical and toxicological aspects of *X. strumarium* available in Bangladesh.

Materials and Methods

Plant materials: Plant samples (mature plant and seedlings) of *X. strumarium* were collected from Sylhet during November 2007 to June 2008. Fertile specimens of *X. strumarium* with flower and fruits have also been collected using herbarium techniques from each study site for the identification of plant up to species level. Voucher specimens have been deposited in Dhaka University Herbarium for future reference.

Ethnobotanical investigation: The ethnobotanical surveys were carried out from November 2007 to June 2008 using semi structured questionnaire[12] in different locations of Bangladesh including, Companiganj, Guainghat, Sylhet, Moulvibazar, Narshindi, Mymensingh, Dhaka, Rajbari, Faridpur, Shariatpur, Comilla, Feni, and Chittagong. Prior to

the use of the questionnaire, conversations with the informants were held with the assistance of local guides to elaborate the objective of the study and to build on trust with the common goal to document and preserve the knowledge on *X. strumarium*. In this case, Participatory Rural Appraisal (PRA) method has been used for data collection[13]. A total of 325 (male-215; female-110) informants were interviewed to record ethnobotanical uses of *X. strumarium*.

Phytochemical investigation: The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument and the spectra were referenced to the residual nondeuterated solvent signal. Column chromatography was run on silica gel (mesh 60-120). Gel permeation chromatography was carried out using lipophilic sephdex LH-20. TLC (20 X 5 cm) and PTLC (20 X 20 cm) were carried out on Merck Si gel 60 PF_{254} on glass plates at a thickness of 0.5 mm. Spots on chromatographic plates were visualised by spraying the developed plates with vanilllin-sulfuric acid followed by heating at 110ºC for 5 min. All solvents used in the study were of analytical grade.

Seedlings and mature plant samples were first sun dried separately for few days followed by oven drying at 40°C to facilitate grinding. The coarse powdered materials (700 g, each) were taken in separate clean, round bottomed flask and soaked in 2.5 liters of methanol for 7 days with occasional shaking and stirring. They were then filtered through a cotton plug followed by whatman No.1 filter paper and the filtrate thus obtained was concentrated at 40°C with a rotary evaporator. A portion (5 g) of the concentrated methanol extract of mature plant was fractionated by the modified Kupchan partitioning method[14] into petroleum ether, carbon tetrachloride, dichloromethane and aqueous soluble fractions and subsequent evaporation of solvents afforded petroleum ether (1.5 g), carbon tetrachloride (1 g), dichloromethane (1.5 g) and aqueous soluble (0.8 g) materials. The methanol extract of seedlings was reserved for toxicity evaluations.

The petroleum ether soluble material was fractionated by gel permeation chromatography and column chromatography. In case of gel permeation chromatography, the column, packed with the slurry of Sephadex LH-20 soaked in a mixture of *n*-hexane-dichloromethane-methanol (2:5:1) was eluted with the same solvent system to give 20 fractions (each 5 mL). Preparative thin layer chromatography of fractions 8-12 using 10% ethyl acetate in toluene as the mobile phase, afforded compound **1** (10 mg). In case of column chromatography separation, the column loaded with the crude extract was eluted with petroleum ether, followed by mixtures of petroleum ether and ethyl acetate, then ethyl acetate and finally with ethyl acetate and methanol mixtures of increasing polarities. The column fractions eluted with 5% ethyl acetate in petroleum ether were subjected to preparative TLC using toluene-ethyl acetate (88:12) as the developing solvent to give compound **2** (5 mg). Similar purification of fractions eluted with 70% ethyl acetate in petroleum ether using chloroform-methanol (90:10) as the developing solvents yielded compound **3** (6 mg). On the other hand, gel permeation chromatography of the carbon tetrachloride soluble partitionate eluted with *n*-hexane-dichloromethane-methanol (2:5:1) provided 25 fractions (each 5 mL). From these, fractions 12-14 yielded compound **4** (6.5 mg) after preparative TLC using toluene-ethyl acetate (80:20) as the mobile phase.

Brine shrimp lethality bioassay: The preliminary cytotoxicity in terms of brine shrimp lethality bioassay was performed following the method described by Meyer et al.[15] All the partitionates (4 mg each), dissolved in DMSO, were subjected to serial dilution technique yielding solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/mL). Ten nauplii (*Artemia salina*) were counted by visual inspection and were taken in test tubes containing 5 mL of simulated sea water. Then samples of different concentrations were added to the pre-marked test tubes through micropipette. The mortality of the brine shrimp nauplii were observed for 24 hours. Here, vincristine sulfate, a known anticancer agent, was used as positive control.

Toxicity evaluation on rats: Twenty Long Evan's rats of either sex of seven weeks old were collected from the Animal Resources Branch of the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B). The rats were kept in properly numbered iron cages (one rat/cage) in a hygienic animal house and were fed with standard pellet diet collected from ICDDR,B. The room was maintained at approximately 25 ± 2^{o}C in a 12-hours light/dark cycle. The rats were maintained in this way for 15 days prior to administration of samples and continued up to the end of the experiment.

Individual weight of the rats was taken and they were divided into five groups (each group comprising of 4 rats) four of which were used as experimentation and the remaining one as control group.

Rats were treated orally with methanol extract of mature plant and seedlings dissolved in saline water for 14 days consecutively. The rats of the control

group were treated with saline water (0.5% body weight/day) only. The rats of experimental groups were given different doses of mature plant and seedling extracts for consecutive 14 days.

The behavior, pattern of diet intake and movement of rats were observed. At the same time, to evaluate the toxic effect of the plant extract on the rat model, liver function tests were performed which included serum alanine transaminase, aspartate transaminase and alkaline phosphatase[16]. In addition, the mortality of the rats was carefully noted throughout the study.

At the end of 14 days, the blood was collected from the rats after sacrificing them and the blood samples were centrifuged (6,000 rpm) and the resultant serum (upper layer) was taken in a stoppered glass vial. The serum samples were stored at -15°C to -20°C temperature. They were brought to room temperature prior to analysis.

Histopathological study: Histopathological investtigation of liver and kidney of all experimental and control rats were performed after sacrificing them at the end of 14 days. The kidneys and livers were removed and observed grossly. These were then fixed in 10% formalin. These tissues were separately sliced in pieces, fixed in 10% formalin for 3 days, processed, stained using hematoxylin, eosin reagent and diphenyl xylene mounting fluid, mounted on glass slides and observed under power microscope.

Data analysis: Data were expressed as mean ± SD. Statistical analysis were done by using the one way ANOVA and $P<0.05$ was considered as significant.

Results

Among the 325 people interviewed, 163 people could recognize *X. strumarium*, while 162 people could not. About the uses of ghagra shak, 85 people mentioned its use as vegetable, 83 as medicine, 18 as fodder, 8 as fire material, 5 in worship and 10 as fun. The survey has also come up with 11 records of poisoning through ingestion of *X. strumarium* that took place a couple of years back particularly in Narshindi, Tejgaon, Bakergonj, Borguna, Barishal, Mehendigonj, Chittagong, Comilla, Patuakhali and Bhola.

It was found that the soft and healthy stem without bark of *X. strumarium* has long been used by the people of Bangladesh as vegetables as well as to treat so many ailments including allergy, joint pain, asthma, diabetes mellitus, jaundices, gastritis (upper abdominal discomfort), urinary disorders and as blood purifier. Fifteen local formulations of *X. strumarium* to treat different diseases were also recorded (Table I).

Table I: Different formulations of *X. strumarium* used by the local people of Bangladesh

Ailments	Part (s) used	Formulations
Asthma	Leaves	Crushed dry leaves put into cigarettes and then a puff of cigarette are taken at least once a day. 60 to 72 cigarettes are required to cure
Urinary disorder	Roots	Roots are cut into pieces, mixed with betel leaf and chewed to cure urinary disease
Impotence	Roots	Root paste is mixed with little water and sugar and is taken to increase libido
Skin disease	Roots	Root paste is mixed with a pinch of table salt and is applied to the affected area twice daily
Burning of foot-sole	Roots	It is an indigenous believe that within a single breath twenty-one plants are to be pulled out and their root paste be applied to the sole of foot to relieve burning
Toothache	Leaves	Young leaves crushed on palm and mixed with a pinch of table salt and put into the root of tooth for a while to relieve the pain.
Allergy	Leaves	The upper side of young leaf should be rubbed on the affected area for a week
Ear infection	Leaves	Extract of young leaves mixed with a pinch of table salt should be poured into ear twice daily
Body or muscle pain	Stems	In case of body or muscle pain, the peeled off stems are cut into pieces and then cooked with small fishes and taken with meal
Jaundice	Roots	Roots mixed with lime are rubbed between the palms of two hands and the yellowish colored hands are washed with water thrice a week
Jaundice	Seeds	4 to 6 thrashed seeds are mixed with one quarter of goat-milk and taken thrice a week to until cure
Diabetic	Stems	Stem is cut into pieces and cooked with small fishes and taken as a vegetable thrice a week to cure
Purification of blood	Stems	Stem is cut into pieces and cooked with small fishes as a vegetable thrice a week to cure
Rheumatism	Stems	Peeled off stem is cut into pieces, cooked with fishes and taken as a vegetable for a week to cure
Gastric disorder	Leaves and stem	Peeled off stem is cut into pieces, cooked with small fishes and taken as vegetable to cure

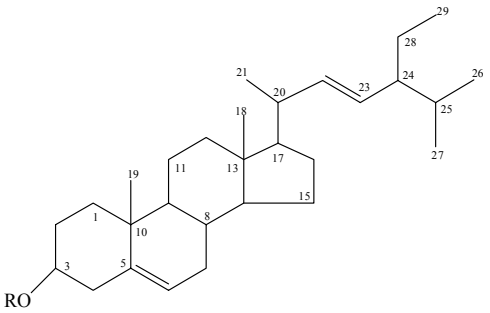
Repeated chromatographic separation and purification of the petroleum ether and carbon tetrachloride soluble partitionates of a methanol extract of the aerial parts of mature *X. strumarium* provided four compounds (**1-4)**, the structures of which were determined by extensive NMR spectral analysis and by comparison with previously reported values as well as co-TLC whenever possible. The ^{1}H NMR spectral data of the isolated compounds are as follows:

Stigmasterol **(1):** Colorless needles; m.p. 160-164ºC (literature[17]-162ºC); ^{1}H NMR spectrum was superimposable to that recorded for an authentic sample.

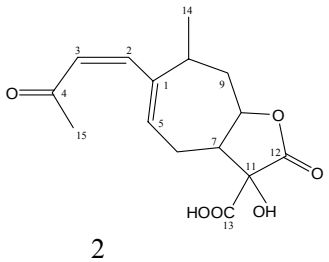
11-hydroxy-11-carboxy-4-oxo-1 (5) , 2 (*Z*) - xantha dien-12,8-olide **(2):** Colorless mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.75 (1H, *d*, *J* = 14.0 Hz, H-2), 6.54 (1H, *dd*, *J* = 9.0, 3.1 Hz, H-5), 6.43 (1H, *d*, *J* = 14.0, H-3), 4.26 (1H, *m*, H-8), 3.04 (H, *m*, H-10), 2.80 (2H, *m*, H_a-6), 2.38 (1H, *t*, *J* = 1.4, H_2-9), 2.35 (3H, *s*, H_3-15), 2.15 (1H, *m*, H_b-6), 2.11 (1H, *m*, H-7), 1.13 (3H, *d*, *J* = 6.2 Hz, H_3-14).

Daucosterol **(3):** White amorphous powder, decomposition point 274-277ºC (literature[21]-273ºC); ^{1}H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD): δ 5.32 (1H, *m*, H-6), 5.01 (1H, *d*, *J* = 7.7 Hz, H-1′), 4.51 (1H, *dd*, *J* = 11.6 , 2.1 Hz, H_b-6′), 4.44 (1H, *dd, J* = 11.6, 2.1Hz, H_a-6′), 4.22 (2H, *m*, H-3′, H-4′), 4.00 (1H, *t, J* = 7.9 Hz, H-2′), 3.93 (1H, *m*, H-5′), 3.85 (1H, *m*, H-3), 0.91 (3H, *d, J* = 6.4 Hz, H_3-21), 0.86 (3H, *s*, H_3-19), 0.82 (3H, *t, J* = 7.3 Hz, H_3-29), 0.80 (3H, *d, J* = 6.8 Hz, H_3-26), 0.78 (3H, *d, J*= 6.9 Hz, H_3-27), 0.58 (3H, *s*, H_3-18).

Lasidiol-10-anisate **(4):** Colorless gum; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (2H, *d*, *J*= 8.6 Hz, H-2′, H-6′), 6.90 (2H, *d, J*= 8.6 Hz, H-3′, H-5′), 5.50 (1H, *s*, H-7), 5.30 (1H, *d*, *J*= 10.9 Hz, H-6), 3.86 (3H, *s*, OCH_3), 1.69 (3H, *s*, H_3-14), 1.06 (3H, *s*, H_3-15), 0.98 (3H, *d*, *J*= 6.5 Hz H_3-13), 0.89 (3H, *d*, *J*= 6.5 Hz, H_3-12), 1.40 (2H, *m*, H_2-3), 1.60 (2H, *m*, H_2-4), 2.10 (1H, *m*, H-2), 1.80 (1H, *m*, H-11).



1: R = H
3: R = $C_6H_{11}O_5$ (glucosyl)



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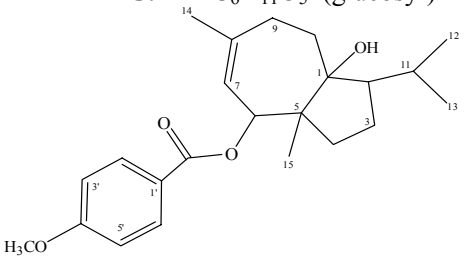

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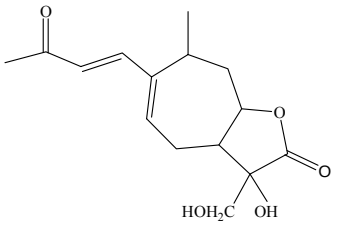

5

The LC_{50} values of methanol extract of seedlings and mature plant and its different partitionates *i.e.* petroleum ether, carbon tetrachloride, dichloro-methane, aqueous soluble fraction of mature plant and vincristine sulphate were found to be 0.656, 1.142, 6.434, 0.775, 1.833, 0.352 and 0.451 µg/mL, respectively (Table II). The aqueous soluble partitionates and methanol extract of seedlings showed highest lethality which was comparable to vincristine sulfate. On the other hand, different other partitionates of the methanol extract of mature plants demonstrated significant toxicity on brine shrimp.

Oral administration of the methanol extract of *X. strumarium* in doses from 0.1 to 0.5% of body weight produced significant changes in behavior, movement and pattern of food intake in experimental rats.

These effects were observed throughout the experimental period. For the control group, the serum alanine transaminase value was found to be

27.33 U/L, whereas it was 33.33, 29.0, 37.0 and 35.0 U/L in rats fed with 0.1% and 0.5% body weight of methanol extract of seedlings and mature plants per day, respectively. The highest increment of alanine transaminase (37.0 U/L) was observed in rats given 0.1% mature plant extract. The aspartate transaminase value in methanol extract was increased whereas alkaline phosphatase level was only increased following administration of methanol extract of mature plant.

Table II: LC_{50} values of extractives of *X. strumarium*

Test samples	LC_{50} (µg/mL)
Vincristine sulphate (standard)	0.451 ± 0.43
Methanol extract of seedlings	0.656 ± 0.52
Methanol extract of mature plant	1.142 ± 0.68
Petroleum ether extract of mature plant	6.434 ± 1.11
Carbon tetrachloride extract of mature plant	0.775 ± 1.35
Dichloromethane extract of mature plant	1.833 ± 0.75
Aqueous extract of mature plant	0.352 ± 1.04

Histopathological study showed infiltration of moderate number of lymphocytes in the stroma and tubules of kidney in the experimental rats. Besides, necroses of tubules were also observed. Ultimately, damage in the kidney was prominent and led to the toxicity. In liver slides of the experimental rats, there was a generalized edema/hypertrophy of the hepatocytes resulting in a marked widespread, sinusoidal congestion. About 60% of the hepatocytes showed cytoplasmic compaction and disintegration, with some apoptotic bodies indicating a degenerative necrotic process on liver cells.

Table III: Effect of methanol extract of mature plant and seedlings of *X. strumarium* on rat model

Drug	Dose (%body weight/day)	No. of rats (deaths)	ALT (U/L)	AST (U/L)	ALP (U/L)
None (saline water)	0.5	4 (0)	27.33 ± 6.85	36.00 ± 4.08	239.67 ± 11.09
Methanol extract of seedlings	0.1	4 (0)	33.33 ± 3.7*	99.33 ± 4.49**	210.5 ± 18.5
Methanol extract of seedlings	0.5	4 (2)	29.0 ± 4.47	107.0 ± 12.96*	229.66 ± 12.76
Methanol extract of mature plant	0.1	4 (1)	37.0 ± 2.73*	95.00 ± 6.04**	265.5 ± 11.5*
Methanol extract of mature plant	0.5	4 (1)	35.0 ± 8.27*	98.50 ± 13.24*	174.33 ± 12.55

Values are given as mean ± SD; Experimental groups were compared with the control; *p<0.05; **p<0.001; ALT: Alanine transaminse; AST: Aspartate transaminase: ALP: Alkaline phosphatase

Throughout the experimental period, a total of four deaths were observed among which two deaths were recorded in case of rats fed with methanol extract of seedlings, indicating the toxic effects of the seedlings in rats. The remaining deaths occurred in rats administered with 0.1% and 0.5% body weight/day of methanol extract of mature plants, respectively which also indicated the toxicity of the mature plant of *X. strumarium.*

Discussion

Bangladeshi believes that the single use of ghagra, as vegetable, is not poisonous. According to them, in most cases seedling is poisonous. Some people mentioned that ghagra shak poisoning shows a number of symptoms in human body including loss of appetite, nausea, vomiting, drowsiness, weakness, ataxia, spasms, lower abdominal pain, diarrhoea, headache, palpitation and unconsciousness. Even a special belief was noted in many locations of Bangladesh that ghagra shak can purify blood, if taken once a year.

Compound **1**, obtained as needle shaped crystals, melted at 160-164°C which was identical to that reported for stigmasterol. Moreover, the ^{1}H NMR spectrum of **1** was superimposable to that acquired for stigmasterol[17]. These features, along with co-TLC with authentic sample confirmed the identity of compound **1** as stigmasterol.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectral data of compound **2** revealed almost similar features to that reported for 11,13-dihydroxy-4-oxo-1(5),2(*E*)-xanthadien-12,8-olide (**5**)[18], except the presence of a carboxyl group instead of a hydroxymethyl (-CH_2OH) group at C-11. In addition, the doublets (J =14.0 Hz) centered at δ 6.75 and 6.43 in compound **2** indicated the presence of a *cis* olefenic double bond between C-2 and C-3, instead of a *trans* double bond present in compound **5**. The spectrum also revealed a methyl doublet (J =6.2 Hz) at δ 1.13 and another downfield methyl singlet at δ 2.35 demonstrating the presence of the characteristic methyl groups in xanthanolide-type carbon skeleton.[18] It also displayed a double doublet (J =9.0, 3.1 Hz) at δ 6.54 suggestive of an olefenic proton, H-5. In addition, multiplets centered at δ 2.15 & 2.80 and a triplet (J =1.4 Hz) centered at δ 2.38 could be attributed to the methylene protons, H_2-6 and H_2-9, respectively. Besides, another multiplet of one proton intensity at δ 2.11 revealed the presence of a methine proton, H-7. The low field value of H-8 at δ 4.26 indicated that it was attached to an oxygenated carbon of the fused

lactone ring. From the above spectral data, the structure of compound **2** was elucidated as 11-hydroxy-11-carboxy-4-oxo-1 (5), 2 (*Z*)-xanthadien-12,8-olide, the identity of which was further substantiated by comparison of its spectral data with reported values[18-20]. This is the first report of this metabolite from *X. strumarium,* although compound **5** has been isolated earlier from some other species of the genus *Xanthium*.

The ^{1}H NMR (400 MHz, $CDCl_3$ + 3 drops of CD_3OD) of compound **3** displayed a multiplet at δ 5.32 indicating the presence of an olefinic proton, H-6 in a steroidal nucleus. The doublet (*J* =7.7 Hz) centered at δ 5.01 suggested the presence of an anomeric proton in a glycoside having a *β*-linkage between the sugar and the aglycone moiety[22]. A double doublets centered at δ 4.51 (*J*=11.6, 2.1 Hz) and 4.44 (*J*=11.6,2.1 Hz) revealed the presence of oxymethylene protons at C-6 in a glucose moiety. On the other hand, the spectrum showed two three proton singlets at δ 0.58 and 0.86 demonstrative of the methyl groups, H_3-18 and H_3-19, respectively.

The three proton doublets at δ 0.91 (*J* =6.4 MHz), 0.80 (*J* =6.8 MHz) and 0.78 (*J* =6.9 MHz) indicated the presence of three methyl groups at C-20 (H_3-21) and C-25 (H_3-26, H_3-27), respectively. The multiplet at δ 3.85 could be assigned to H-3 of the aglycone moiety. On this basis, the identity of compound **3** was established as daucosterol, which was substantiated by comparison of its spectral data with that of reported values[21,22] as well by co-TLC.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **4** showed two doublets (*J* = 8.6 Hz), each of two proton intensity, centered at δ 8.01 and 6.90 indicating the presence of a *para* disubstituted benzene ring. A three proton singlet at δ 3.86 demonstrated the presence of a methoxyl group attached to the *para* position of the aromatic nucleus. A doublet (*J* = 10.9 Hz) centered at δ 5.30 could be assigned to the highly deshielded oxymethine group at C-6 due to its β position to a carboxylic acid group. Another singlet at δ 5.50 revealed the presence of an olefinic proton, H-7. Two singlets of three proton intensity at δ 1.06 and 1.69 suggested the existence of two methyl groups at C-8 and C-5, respectively.

The ^{1}H NMR spectrum also displayed upfield doublets centered at δ 0.89 (*J* =6.5 Hz) and 0.98 (*J* =6.5 Hz), which could be ascribed to the methyl groups at C-11. Four methylene (H_2-3, H_2-4, H_2-9 and H_2-10) and two methine (H-2 and H-11) proton resonances were observed as multiplets between δ 1.40-2.10. By comparing these ^{1}H NMR data of **4** with published values[23] the identity of compound **4** was established as lasidiol-10-anisate.

From the results of the brine shrimp lethality bioassay, it can be concluded that methanol extract of seedlings, mature plant and its different partitionates possess cytotoxic principles. Compared with positive control, the cytotoxicity exhibited by the extractives could be toxic to human and thus, further investigation should be carried out to ascertain its cytotoxicity on human cell.

Alanine transaminase, aspartate transaminase and alkaline phosphatase are enzymes present in hepatocytes. When a cell is damaged, it leaks these enzymes into the blood, where they can be measured[24]. So, the measurement of alanine transaminase and aspartate transaminase values in the experimental rats indicates the toxic response of *X. strumarium* on rat liver.

The overall experimental data gathered from the toxicity evaluation, clearly showed that the plant possesses toxic effects on the liver cell of experimental rats, which might also be toxic to human subjects. This hepatotoxicity can be correlated to the death occurred after administration of extracts of mature plant and seedlings of *X. strumarium* in rats as well as ingestion of this plant among Bangladeshi.

The main toxic compound isolated from *X. strumarium* had been identified as carboxyatractyloside, a kaurene glycoside. Cocklebur seedlings, often consumed by grazing animals, are high in carboxyatractyloside (CAT) until the emergence of their first true leaves after which the compound is present in the whole plant in reduced quantities. Even though the content of CAT in the mature plant is reduced, hay containing mature xanthium plants has caused toxic reactions in animals. When ingested in sufficient quantities by animals, CAT by itself or xanthium that contains CAT, produces hypoglycemia and hepatic damage, the latter possibly due to increased vascular permeability in response to severe hypoglycemia. In addition to CAT, potentially toxic ingredients of xanthium include several sesquiterpene lactones (e.g., guaianolides, germacranolides, elemanolides) that can cause vomiting, weakness, tremors, loss of appetite and convulsions in high doses[7-10].

Although in the present study, four compounds were isolated from *X. strumarium*, possible toxic effects of these compounds could not be ascertained due to scarcity of purified samples.

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Contribution of authors

MRI- Study design, data collection and processing, laboratory work, statistical analysis, NMR interpretation, report writing
MZU- Concept and research question, data collection and processing, report writing
MSR- Lab work, statistical analysis, NMR interpretation
ET- Data collection and processing
MZR- Laboratory work
MAH- Data collection and processing, report writing
MAF- Concept and research question, study design
MH- Concept and research question
MH- Pathological tests
MAR- Concept and research question, study design, statistical analysis, NMR interpretation and report writing

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