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Phytochemical and Pharmacological Evaluation of Leaves of *Averrhoa carambola* Linn. (Family: Oxalidaceae)

Tanzir Hossain[1], Apurba Kumar Barman[1,2], Utpal Kumar Karmakar[1], Bishwajit Bokshi[1], Shrabanti Dev[1], Nripendra Nath Biswas[1]*

[1]Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh.
[2]Institute of Biophysics, University of Chinese Academy of Sciences, Beijing-100101, China.

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Abstract

Phytochemical analysis of the ethanolic leaf extract of *Averrhoa carambola* Linn. indicated the presence of carbohydrate, glycosides, steroid, saponins, flavonoids, gum, tannins and the absence of reducing sugars. On acetic acid induced analgesic test, the plant extract exhibited a significant writhing reflex inhibition by 11.12% and 40.28% ($p<0.01$) at the dose of 250 mg/kg and 500 mg/kg body-weight respectively while the standard drug diclofenac sodium inhibition was found to be 69.45% at a dose of 25 mg/kg body weight. The plant extract showed moderate level of antimicrobial activity against *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Shigella dysenteriae*, *Pseudomonas* spp., *Staphylococcus saprophyticus* when Kanamycine was applied at the dose of 30 µg/disc. On qualitative *in vitro* antioxidant assay (2, 2-diphenyl-1-picryl hydrazyl (DPPH) method), the ethanolic leaf extract was found to have potent antioxidant compounds. In the castor oil-induced diarrhoeal mice, the plant extract significantly reduced the number of stools by 25% and 31.25% ($p<0.01$) at the dose of 250 mg/kg and 500 mg/kg body weight respectively while 60.42% by a standard drug Loperamide at 50 mg/kg body weight. Interestingly, that ethanolic extract exhibited low level of toxicity in the brine shrimp lethality assay. The LC_{50} and LC_{90} of brine shrimp nauplii were found to be 100 µg/ml & 160 µg/ml respectively for the crude plant extract while chloramphenicol displayed 18.62 µg/ml & 134.9 µg/ml respectively. These results suggest that the plant leaf extract possesses significant analagesic, antidiarrheal, antimicrobial and antioxidant effects which support the use of this plant in traditional medicine.

Keywords: *Averrhoa carambola*, analgesic, cytotoxicity, antidiarrhoeal, antimicrobial and antioxidant.

1. Introduction

Averrhoa carambola is a small evergreen tree with a short-trunk or a shrub and belongs to the family Oxalidaceae. It has drooping branches and white wood which turns to reddish. It takes a bushy shape having many branches producing a broad, rounded crown. The soft leaves are compound in nature, medium-green and are spirally arranged around the branches in an alternate fashion (Chakraborty *et al.* **2012**). The pinnate leaves have a single terminal leaflet where each leaf is 15-20 cm long. The leaflets are 3.8-9 cm long and ovate or ovate-oblong in shape. The top sides of the leaves are smooth and the bottomsides are finely hairy and whitish in color. The leaflets are sensitive to light and abrupt shock and thereby tend to fold together at night or tend to close up when shaken. The flowers are lilac or purple-streaked and come up in the axils of leaves at the end of twigs.

Correspondence To: Dr. Nripendra Nath Biswas, Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh. Email: nnathbiswas@gmail.com

The small clusters of flowers are arranged at the ends of the branches or sometimes on the larger stems and trunk. Each cluster is attached to the tree with red stalks (Chakraborty *et al.* 2012). The dimension of each flower is around 6 mm wide, with 5 petals which have recurved ends. The flowers normally takes bell shaped and are produced in loose panicles that are much-branched with pedicellate flowers. The attractive fruits are oblong shaped and in measurement they are longitudinally 5 to 6 angled and 6.35-15 cm long and up to 9 cm wide. The fruits are orange-yellow colored having a thin, waxy skin. The fruits are juicy and yellow inside when ripe and have a crisp texture and looked like a star when cut in cross-section. Usually the fruits are oxalic acidic in odor, which varies from strong to mild depending on the plants. The taste may also vary from very sour to mildly sweetish. Each fruit may contain up to twelve 6-12.5 mm long seeds, and are flat, thin shaped and brown in colou. Some cultivated forms produce seedless fruits (Chakraborty *et al.* 2012).

It is believed that the carambola was originated in Ceylon and the Moluccas but it has been extensively cultivated in Southeast Asia and Malaysia for many centuries. Carambola is widely grown in the provinces of Fukien, Kuangtung and Kuangsi in southern China, Taiwan and India. It is also popular in Philippines and Queensland, Australia and in some parts of the South Pacific islands, particularly in Tahiti, Guam Hawaii and New Caledonia, New Guinea and Netherlands (Morton 1987). The plants contain thiamin, riboflavin, niacin, oxalic acid, ascorbic acid, phytofluene, beta-carotine, beta-cryptoflavin, mutatoxanthin, beta-apo-8-carotene, lutein, cryptoxanthin, cryptochrome (US Department of Agriculture 1992-2016).

The leaves of this plant have been known to be antipruritic, antipyretic and anthelmintic (Avinash *et al.* 2012). It has also been reported to be useful for treating scabies,various types of poisoning, pruritus, intermittent fevers and intestinal warms (Kirtikar and Basu 1984). The fruits are sweet, sour, thermogenic, febrifuge, antipyretic, antiscorbic and tonic. (Bio-india biological 2016). They are also useful in diarrhoea, vomiting, hyperdipsia, haemorrhoids, intermittent fevers, hepatodynia, scabies and various kinds of poisoning and general debility (Sheth 2005, Azeem *et al.* 2010; Payal *et al.* 2012; Vasant *et al.* 2014; Suman *et al.* 2012; Manda *et al.* 2012). From the literature survey we found no scientifically elegant studies supporting traditional uses of this plant has yet been reported. Therefore, to judge the traditional use of this medicinal plant, this study was designed to evaluate the possible analgesic, antidiarrhoeal, antimicrobial, antioxidant and cytotoxic effects of *Averrhoa carambola* Linn. Our results suggest that the plant possesses significant analagesic, antidiarrheal, antimicrobial and antioxidant properties which support the traditional uses of the plant.

2. Materials and methods

2.1 Plant material collection and identification

The leaves of the plant *Averrhoa carambola* Linn. (Family: Oxalidaceae) was collected from Khulna, Bangladesh during the month of July, 2009 at morning and identified by experts of Bangladesh National Herbarium, Mirpur; Dhaka "Accession No: 34467" and a voucher specimen was also deposited there.

2.2 Preparation of the plant extract

Undesirable materials were separated from the plant leaves. The leaves were then washed with water, shade-dried for four weeks and then were 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 150 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its content was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. After the extraction procedure, the whole mixture was filtered by a piece of clean, white cotton material followed by filtration through filter paper. The filtrate (ethanol extract) obtained was evaporated under ceiling fan and in a water-bath until dried completely. It rendered a gummy concentrate (7 gm) of greenish color. The gummy concentrate was designated as crude ethanol extract. The final yield was 2.75%.

2.3 Chemicals and drugs

Glacial acetic acid was purchased from Sigma chemicals, USA. Diclofenac sodium, chloramphenicol and ascorbic acid were collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

2.4 Experimental animal

Young Swiss-albino mice aged 4-5 weeks with average weight ranging from from 20-28 gm were used for the experiment. They were kept in standard environmental condition for one week in the animal house of Pharmacy Discipline, Khulna University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water and maintained at natural day night cycle. All the experiments were conducted on an isolated and noiseless condition.

2.5 Phytochemical screening test

Preliminary phytochemical analysis of the *Averrhoa carambola* L. plant extract was carried out by following standard procedure (Ghani **2003**).

2.6 Evaluation of in vivo analgesic activity by acetic acid induced writhing method

Analgesic effect of the plant extract was investigated by acetic acid induced writhing method (Gawade **2012**). Briefly, experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group- IV consisting of 5 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Test samples, control and Diclofenac-Na were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then, the writhing inducing chemical acetic acid solution (0.7%, 10 ml/kg) was administered intraperitonealy to each of the animals of a group. After an interval of five minutes which was given for absorption of acetic acid, number of squirms (writhing) was counted for 15 minutes.

2.7 In vitro antioxidant activity test

The antioxidant potential of the ethanolic extract was determined on the basis of their scavenging activity of the stable 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical following the method adopted by Sadhu *et al.* **2003**). DPPH is a stable free radical containing an odd electron in its structure and usually used for detection of the radical scavenging activity in chemical analysis. The aliquots of the different concentrations (1-500 µg/ml) of the extract were added to 3 ml of a 0.004% w/v solution of DPPH. After 30 minutes, absorbance of each samples were determined by UV spectrophotometer at 517 nm.

2.8 Determination of cytotoxic activity by brine shrimp lethality bioassay

In vitro lethality bioassay of the ethanolic extract of *Averrhoa carambola* L. was exploited to detect cytotoxic activity following the method described by Meyer *et al.* **1982**. A total of 38 g of sea salt was weighed accurately, dissolved in distilled water to make final volume one liter and then filtered off to get a clear solution. Sea water was taken in the small tank and brine shrimp eggs were added and incubated at 28°C in front of a lamp. The shrimps were allowed for 24 hr to hatch and mature as nauplii (larvae). A solution of 5 µg/µL of the extract was prepared by using dimethyl sulfoxide (DMSO). For this purpose, 24 clean test tubes were taken, 12 of which were for the samples in six concentrations (2 test tubes for each concentration) and 12 for control test. Then 5 ml of sea water was given to each of the test tubes. Then with the help of the micropipette specific volumes (10, 20, 40, 80, 160 and 320 µl) of samples were transferred from the stock solutions to the test tubes to get final sample concentrations of 5, 10, 20, 40, 80, and 160 µg/ml respectively. The concentration of DMSO in these test tubes did not exceed 10 µl/ml. For control, same volumes of DMSO (as in the sample test tubes) were taken in the rest of the 12 test tubes. Finally, with the help of a Pasteur pipette, 10 living shrimps were kept to each of the test tubes. After 24 hr, the test tubes were observed and the number of survived nauplii in each test tube was counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample. Then percent mortality was plotted against log concentration on the graph paper to produce an approximate linear correlation between them graphically.

2.9 Determination of antimicrobial activity by the disc diffusion method

Antimicrobial activity of the ethanolic extract of *Averrhoa carambola* L. was determined by disc diffusion method (Ahmed *et al.* **2003**). Briefly, filter paper discs (6 mm in diameter) were impregnated with the crude extract at the concentration of 250 and 500 µg/disc and then placed onto the agar plates previously inoculated with the test microorganisms. The test microorganisms included *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus agalactiae*, *Shigella sonnei*, *Shigella boydii*, *Streptococcus pyogenes*, *Shigella dysenteriae*, *Pseudomonas* spp., *Staphylococcus saprophyticus*. The Petri dishes were kept at 4 °C for 2 h. The plates were incubated at 37 °C for 16 h to allow the growth of the microorganisms. The diameters of the zones of inhibition were measured in millimeters using a calibrated scale. All the tests were repeated triplicate.

2.10 Antidiarrhoeal activity test

Antidiarrhoeal activity of the ethanolic extract of the fruit of *Averrhoa carambola* Linn. was tested using the model of castor oil induced diarrhoea in mice (Galbez *et al*. **1993**). All the mice were screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the experiment. The test animals were randomly chosen and divided into four groups having 5 mice in each group. Group I was kept as “control” and received 1% tween-80 at the dose of 10 mg/kg body weight; Group II was “positive control” and received standard antimotility drug Loperamide at the dose of 50 mg/kg body weight as oral suspension; and Group III Group IV were “test group” and treated with suspension of leaf extract of *Averrhoa carambola* Linn. at the oral dose of 250 and 500 mg/kg body weight. Control

vehicle and the extract were administered orally 30 min prior oral administration of castor oil at the dose of 0.5 ml. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent papers were counted at each successive hour during the experiment (05 hrs). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed instead of old ones.

2.11 Statistical analysis

The data were analyzed using unpaired t-test as described by Glasnapp *et al.* (**1985**) and expressed as mean ± SEM (Standard Error of the Mean). SPSS (Statistical Package for Social Science) for Windows (Ver. 11) was applied for the analysis of data and $p<0.05$, $p<0.01$, $p<0.001$ was taken as the level of significance. Differences between groups were considered significant at $p < 0.05$, 0.01 and 0.001.

3. Results

3.1 Phytochemical test

Phytochemical analysis of the ethanolic extract of the leaves of Averrhoa carambola Linn. indicated the presence of steroid, glycosides, gum, tannin, alkaloid, saponin and flavonoids.

3.2 Test for analgesic activity

The ethanolic extract of the leaves of *Averrhoa carambola* Linn. at the dose of 250 mg/kg and 500 mg/kg bd wt exhibited significant inhibition of writhing reflex by 11.12% and 40.28% ($p<0.01$) respectively while the standard drug diclofenac inhibition was found to be 69.45% at a dose of 25 mg/kg body weight. Further study is required for more scientific evidence (**Table 1**).

3.3 In vitro antioxidant activity test

The TLC plates were observed under UV detector both in short (256 nm) and long (360 nm) wavelength (images given below) where a lot of colored and fluorescent positive components were observed which indicated the presence of UV positive materials in the plant extract and they were marked. After applying DPPH on the TLC plate, yellow color on purple background was observed which indicated the presence of antioxidant components in the ethanolic extract of *A. carambola* Linn. (**Fig 1**).

3.4 Brine shrimp lethality assay

After 24 hours, the test tubes were observed and the number of survived nauplii in each test tube was counted and the results were noted. From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample. In this bioassay, the crude extract of the dried leaves of *Averrhoa carambola* Linn showed lethality indicating the biological activity of the compound present in the extract. Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase in concentration of the sample and plot of percent mortality versus log concentration on the graph paper produced an approximate linear correlation between them. The plant extract concentrations at which 50% mortality (LC_{50}) and 90% mortality (LC_{90}) of brine shrimp nauplii occurred were obtained by extrapolation and the values were found to be 100 µg/ml and 160 µg/ml respectively while chloramphenicol showed 18.62 µg/ml & 134.9 µg/ml respectively.

3.5 Antidiarrhoeal activity

The plant extract exhibited comparable increase in latent period (25.80% at 500 mg/kg) compared to loperamide (73.56% at 50 mg/kg). It also exhibited moderate inhibition of stool production (30.25% at 500 mg/kg compared to standard drug loperamide 60.42% (**Table 2 and 3**).

3.6 Antimicrobial activity

The plant extract showed moderate level of growth inhibition against *Streptococcus agalactiae*, *Streptococcus pyogens*, *Pseudomonas spp*. and *Staphylococcus saprophyticus* whereas potent inhibition activity against *Shigella dysenteriae* (zone of inhibition 9 mm at 500 mg/kg body weight compared to standard drug kanamycin (zone of inhibition 10 mm at 30 µg/disc) (**Table 4**).

4. Discussion

Medicinal plants have been used for mitigation of lots of ailment as a traditional medicine for more than hundred years which show great character, although it lacks sufficient clinical or scientific data to prove the activity. Natural products are the leading supply of nutrients, vitamins and different phytoconstitutents that attract the researcher or scientists attention to discover novel drugs (Ramawat *et al.* **2009**). Phytoconstitutents like alkaloids, phenols, flavonoids, tannins, glycosides, steroids, volatile oils etc. are synthesized from natural sources like plants, herbs, algae etc. with bearing a prime potential for pharmacological properties that can be utilized as a therapeutic medicine (Akinmoladun *et al.* **2007**, Nasrin *et al.* **2015**, Sharmin *et al.* **2016**). Phytochemical tests of the ethanolic leaf extract of *A. carambola* L. revealed the presence of carbohydrate,

glycosides, steroids, saponins, flavonoids, gum, tannins and the absence of reducing sugars which could be a source of bioactive compounds.

Table 1: Analgesic effects of the ethanoic leaf extract of *Averrhoa carambola* Linn.

Treatment group	Mean of writhing	% Inhibition of writhing
Blank control	14.4±2.00	00.00
Diclofenac Na (25 mg/kg bd wt)	4.4±0.49*	69.45
Extract I: (250 mg/kg bd wt)	12.8±1.23*	11.12
Extract II: (500 mg/kg bd wt)	8.6±0.97*	40.28

Data are represented as Mean±SEM, n=5. *p<0.01, significant compared to control; bd-wt: body weight.

Table 2: Effects of *Averrhoea carambola* Linn. on the latent period of castor oil induced diarrhoea in mice

Treatment group	Mean latent period (hr)	Increase in latent period (%)
Bank control (castor oil (0.7 ml, p.o)+ vehicle, p.o)	0.69±0.00	00.00
Positive control (castor oil (0.7 ml, p.o)+ Loperamide (50 mg/kg bd wt, p.o)	2.61±0.06*	73.56
Extract I (castor oil (0.7 ml, p.o)+ Extract (250 mg/kg bd wt, p.o)	0.88±0.19*	21.59
Extract II (castor oil (0.7 ml, p.o)+ Extract (500 mg/kg bd wt, p.o)	0.93±0.15*	25.80

Data are represented as Mean±SEM, n=5. *p<0.01, significant compared to blank control, p.o.: per oral; bd-wt: body weight.

Table 3: Effects of ethanolic leaf extract of *Averrhoea carambola* Linn. on frequency of defecation in castor oil-induced diarrhoeal mice

Treatment group	Mean no. of stool	Inhibition of defecation (%)
Bank control (castor oil (0.7 ml, p.o)+ vehicle, p.o)	9.6±0.14	00.00
Positive control (castor oil (0.7 ml, p.o)+ Loperamide (50 mg/kg bd wt, p.o)	3.8±0.19**	60.42
Extract I (castor oil (0.7 ml, p.o)+ Extract (250 mg/kg bd wt, p.o)	7.2±0.15*	25.00
Extract II (castor oil (0.7 ml, p.o)+ Extract (500 mg/kg bd wt, p.o)	6.6±0.09**	31.25

Data are represented as Mean±SEM, n=5. *p<0.01, **p<0.001 significant compared to blank control, p.o.: per oral; bd-wt: body weight.

Table 4: *In vitro* antibacterial activity of ethanol extract of *Averrhoa carambola* Linn.

Bacterial strains	Type of Bacterial strains	Diameter of zone of inhibition (mm)			
		Blank	Kanamycin (30 µg/disc)	Extract (250 µg/disc)	Extract (500 µg/disc)
Staphylococcus aureus	Gram(+)	-	24	-	-
Staphylococcus epidermis	Gram(+)	-	25		-
Streptococcus agalactiae	Gram(+)	-	34	8	8
Shigella sonnei	Gram(-)	-	23	-	-
Shigella boydii	Gram(-)	-	30	-	-
Streptococcus pyogenes	Gram(+)	-	25	7	8
Shigella dysenteriae	Gram(-)	-	10	7	9
Pseudomonas spp.	Gram(-)	-	34	10	11
Staphylococcus saprophyticus	Gram(+)	-	33	9	12
Enterococcus coli	Gram(-)	-	30	-	-

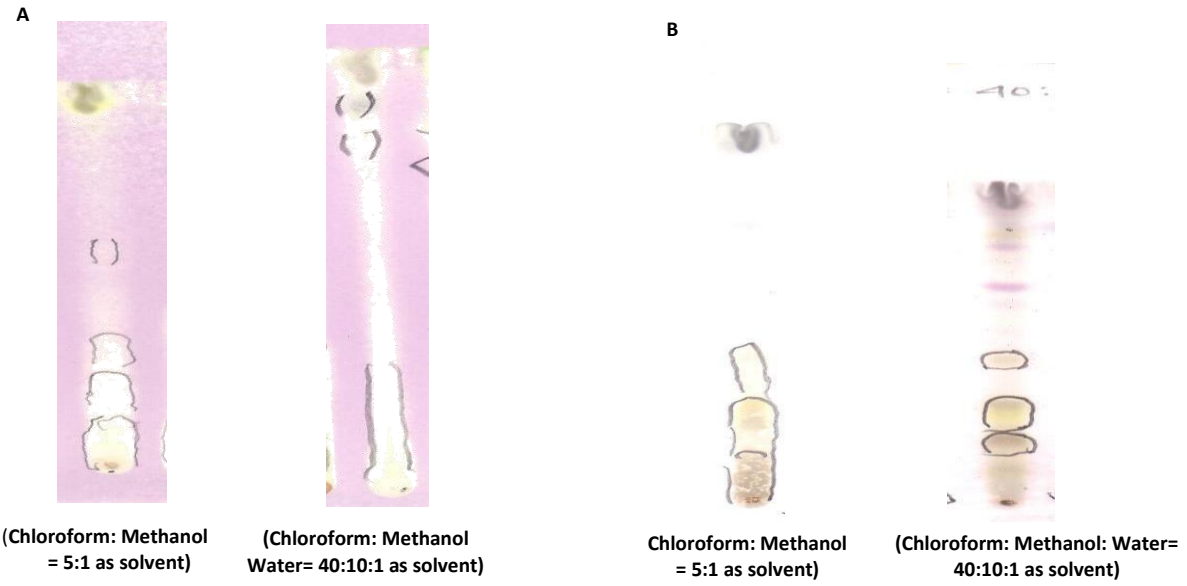

Fig 1 Comparison of TLC plate for *A. carambola* L. with standard (Ascorbic acid) after applying DPPH.

Acetic acid-induced abdominal contraction response called writhing is an effective methods to evaluate the peripherally acting analgesic activity of compounds (Gené *et al.* **1998**, Roberts *et al.* **2001**). The extract reduced the number of abdominal contraction in mice significantly in dose dependent fashion compared to standard drug. Generally, this method has been correlated with prostanoids like high level expression of PGE2 and PGF2α in peripherally (Derardt *et al.* **1980**) as well as lipoxygenase derivatives (Levini *et al.* **1984**, Derardt *et al.* **1980**). The consequences of the acetic acid-induced writhing test, robustly recommend one of the dominant mechanisms of the ethanol extract associated to cyclo-oxygenases and/or lipoxygenases production.

The infectious diseases are of great concern for medicinal chemist to develop newer drugs which can tackle the notorious bacterial activity avoiding their resistance mechanism to antibiotics. The disk diffusion assay is versatile and inexpensive methods and is widely used to determine the antibacterial activity against selected bacterial strains. The ethanolic extract of this plant showed substantial amount of zone of inhibition against all the bacterial strains used. Despite the fact that the disk diffusion method is a simple process, moreover, it has some drawbacks as agar medium is made up of water and it is quite difficult to diffuse non-polar compounds with water which may be responsible for lower zone of inhibition for some bacterial strains (Moreno *et al.* **2006**).

Diarrhoea is a public health concern in developing countries. It is therefore important and useful to identify plants with antidiarrhoeal activity. Although some advanced approaches are available to tackle the diarrhoeal condition in Bangladesh, still local people largely depend on medicinal plants to manage the symptoms of diarrhoea. Diarrhoea is a common symptoms with high incidence of bowel movements as a result of excess release of water contained stools. Castor oil generates diarrhea due to its active metabolite ricinolic acid (Ammon *et al.* **1974**) which is responsible for causing irritation and inflammation of small intestine; consequently, peristaltic progression of intestinal motility and upregulation of secration because of the release of prostaglandins (Zavala *et al.* **1998**). Other mechanisms are also reported apart from castor oil induced diarrhoea including inhibition of intestinal Na^+, K^+-ATPase activity to diminish the normal level of fluid absorption (Nell *et al.* **1984**), up-regulation of adenylate cyclase or mucosal cAMP mediated active secretion (Capasso *et al.* **1994**). In this study, ethanolic leaf extract of *A. carambola* Linn. might have the capability to maintain or increase the reabsorption process of electrolytes or watery components. The extract reduced the number of stools significantly in a dose depended manner by decreasing gastrointestinal motility in mice. It confirmed that the extract could be a potential therapy for diarrhoea treatment.

The brine shrimp lethality bioassay is a simple, quick, inexpensive and convenient technique attempt for evaluating essential pharmacological activity of crude extracts including enzyme inhibition, ion channel interference and cytotoxic activity (Borowitz *et al.* **1992**, Silva *et al.* **2007**). It has been reported that there is a link between brine shrimp lethality test and *in vitro* growth inhibition of human cancer cell line which was recognized by the National Cancer Institute, USA (NCI)

(Anderson *et al.* 1991). In this experiment, both extract and the standard chloramphenicol showed high amount of mortality rate with increase in concentration. The LC_{50} value of this extract is very low which defines the potentiality of this crude extract having biologically active anticancer compounds with possible low toxicity.

Free radicals are responsible for producing different disease stages including inflammation, rheumatoid arthritis, cardiovascular disease, cancer, neurodegenerative ailments (e.g Parkinsonism, Alzheimer's disease) and AIDS (Halliwell B Lancet. 1994). Antioxidant plays an important role in maintaining the disease free condition by blocking or preventing destructive roles of free radicals. The antioxidant activity of plants may rely on presence of phytoconstitutents including polyphenols and flavonoids. Flavonoids and polyphenols are considered as the most powerful secondary metabolite and major bioactive compounds of plants (Surapaneni *et al*. 2009). Flavonoids containing natural products are active against neurodegenerative diseases (Spencer 2010). Present study confirmed that leaf extract of *A. carambola* Linn. contains flavonoids, although we did not measure the total amount of ploy phenols and flavonoid. We found that the plant extract contains potent antioxidant compounds. In our future endeavor, it will be attempted to measure the total polyphenols and flavonoids which may open a new arena for antioxidant drug discovery.

The ethanolic leaf extract of *A. carambola* contains different phytoconstitutents which might act together and their cooperation synergistically increased the observed activities. The present study supports the rational use of this plant as a folklore medicine which could be the source of pharmacologically active compounds.

5. Conclusion

The study clearly indicates that the plant extract possess antioxidant, analgesic, antimicrobial, antidiarrhoeal and cytotoxic constituents. These studies justify the traditional use of this plant in the treatment of headache, diarrhea and wound. However, further study is necessary for elucidating the active principles.

6. Ethical Approval

The study was approved by the ethical review committee, Life Science School, Khulna University, Bangladesh.

7. Conflict of interest statement

We declare that we have no conflict of interest.

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