

Pharmacological evaluation of the aqueous extract of the leaves of *Averrhoa carambola*

*Aklima Akter, Md. Mominur Rahman, and Fahadul Islam

Department of Pharmacy, Faculty of Allied Health Sciences,
Daffodil International University, Dhaka-1207.

Abstract: The aim of the study was to screen different chemical groups in aqueous extract of the leaves of *Averrhoa carambola* and evaluate the pharmacological activities. The plant was reported earlier to contain anti-inflammatory and hypoglycemic properties. The present study was conducted in vivo to evaluate the analgesic and anti-diarrheal activity of the aqueous extracts of leaves. Phytochemical screening of the sample indicated the presence of carbohydrates, tannins, and alkaloids. When tested for its analgesic effects on acetic acid-induced writhing in mice, the extract produced significant analgesia at doses of 250 and 500 mg/kg body weight and percentage (%) of inhibition was 32.8% and 36.9% respectively which was compared with the standard diclofenac at a dose of 10 mg/kg body weight. In the evaluation for its anti-diarrheal effects on castor oil induced defecation in mice, the extract produces anti-diarrheal effects at dose 250 mg/kg body weight and percentage (%) of activity was 62.5% compared to the standard loperamide at a dose of 5 mg/kg body weight. The overall result obtained from the study suggests the analgesic and anti-diarrheal properties of leaves of the plant.

Keywords: *Averrhoa carambola*, leave extract, analgesic, acetic acid, anti-diarrheal, castor oil.

Introduction

Averrhoa carambola L., commonly known as star fruit or carambola, is a tree originally from Asia which has become acclimatized in many tropical countries, including Brazil. It is a small tree or shrub that grows 5–12 meters tall, with rose to red-purple flowers. The flowers are small and bell-shaped, with five petals that have whitish edges¹. The ripe fruit of *Averrhoa carambola* L. (commonly known as “Kamranga”) is considered as digestible, tonic strengthening, for bleeding piles and causing biliousness. The dried fruit is also used in fever; it is cooling and possesses antiscorbutic properties². This tree is used to treat headaches, vomiting, coughing and hangovers³. The topical anti-inflammatory activity of the ethanolic extract and two isolated flavonoids from the leaves of *A. carambola* were evaluated on a classic model of skin inflammation—croton oil-induced mouse ear edema⁴. The leaves and fruits of *A. carambola* have been used in folk medicine as an appetite stimulant, a diuretic, an antidiarrheal, and a febrifugal agent, as well as in the treatment of eczemas. Also, decoction of the leaves has been used in diabetes treatment. Previous investigations on *A. carambola* have revealed the presence of steroids and triterpenes, cyanidin glycosides, O-glycosyl flavonoids, and C-glycosyl flavones. The species *A. carambola* presents hypoglycemic, hypocholesterolemic, antimicrobial, antioxidant, and anti-inflammatory effects^{1,5-6}. The carambola juice is usually used for making confectionary, juice concentrate, and refreshing drinks. After the juice extraction process, thousands of tons of carambola pomace are produced and discarded as feeds. Carambola was reported to be rich in dietary fiber, especially insoluble dietary fiber. Some authors have pointed out that agricultural by products from fruits, cereals, and vegetables can be a potential source of fibers and functional compounds⁷.

*Corresponding Author: Ms. Aklima Akter, Lecturer, Department of Pharmacy, Daffodil International University (DIU), Dhaka-1207, Bangladesh. Email: aklima.ph@diu.edu.bd

Uremic patients with severe intoxication manifested after ingesting star fruit juice showed important evidence of cardiovascular system involvement. This was manifested by bradycardia, cardiorespiratory arrest, tachycardia, hemodynamic instability, and arterial hypotension. In the cardiovascular system, the effects of the Averrhoa carambola aqueous extract made from leaves promoted a reduction of guinea pig atrial contractility and automaticity, indicating L-type Ca^{2+} channel blockade and electrophysiological changes in the normal guinea pig heart⁸⁻⁹. In Ayurveda, the ripe fruit is considered as digestive, tonic and causes biliousness. The dried fruit is also used in fever; diabetes, it is cooling and possesses antiscorbutic properties. It is considered as one of the best Indian cooling medicines¹⁰⁻¹². An alcoholic extract of the stems of A. carambola has been shown to exhibit selective activity against brain tumor cells while that of the leaves was effective against liver carcinoma cells¹³. In the present study, we evaluated the analgesic and antidiarrheal effects of the Averrhoa carambola aqueous extract of leaves and both are significant.

Material and Methods

A. Collection of plant material:

The experimental plant of Averrhoa carambola was collected from Feni, Bangladesh in May 2018 and the genus as well as the family was identified from National Herbarium, Bangladesh (accession number is 45747) and sample specimens have been kept over there.

B. Extraction:

200 grams of dry powder was taken in a 1000 ml beaker and soaked with about 500 ml of water. Then the beaker was sealed using aluminium foil paper and kept in phytochemical laboratory room, Department of Pharmacy, Daffodil International University, accompanied by occasional stirring. After 2 days, the moisture was filtered through cotton followed by filter paper. The filtrate was then taken in a rotary evaporator to concentrate the extracts. The semi-solid residue was then taken in a 50 ml beaker. The weight of the extract was found 10g. The extract was stored in freeze for further investigation.

C. Animal:

Swiss albino mice (male) were collected from Jahangir Nagar University, Dhaka, Bangladesh. The mice weighed between 25-30 grams. The mice were acclimatized for 7 days prior to experiment. The study was conducted following approval by the Institutional Animal Ethical Committee of Daffodil International University, Dhaka, Bangladesh.

D. Drugs and Chemicals:

Acetic acid, diclofenac, distilled water, needle, syringe, electronic balance, hand gloves, face masks, boxes, α -naphthol, sulfuric acid, hydrochloric acid, sodium hydroxide (pellet), ferric chloride, lead acetate, potassium iodide, mercuric iodide, bismuth nitrate, cupric sulfate, sodium citrate, anhydrous sodium carbonate were used.

E. Phytochemical screening

The collected plant extracts of Averrhoa carambola was subjected to qualitative phytochemical screening for identification of various classes of active chemical constituents including alkaloids, glycosides, steroids, gums and tannins¹⁴.

F. Experimental animal

The animals were kept in a stainless steel cage under controlled temperature ($24 \pm 2^\circ\text{C}$), in a 12 hours light-dark cycle. ICDDRDB formulated rodent food and pure water was given to the mice since these animals very sensitive to environmental changes.

G. Analgesic test activity:

The analgesia is characterized by the writhing of the mice and it is induced by injecting 1% acetic acid. The analgesic activity is evaluated by visual observation of reduction in frequency and number of writhing after administration of the test sample of crude extract. The total count of writhing is compared with the standard group. Analgesic agents decrease the writhing count¹⁵.

H. Anti-diarrheal test activity

Time taken before the first defecation is the 'Latent period'. The total count stool and latent period of test group are compared with positive control group. Antidiarrheal agent increase latent period and decrease total stool count¹⁶⁻¹⁷.

Results and Discussions

A. Phytochemical group presence test

Major classes of therapeutically important compounds like carbohydrate, tannins, and alkaloids were found in the plant leaves after performing different chemical test for identification of the compounds. The phytochemicals present in the plant leaves are outlined in the following table:

Table 1: Different chemical group test results

Different chemical group test results	
Test for carbohydrate	
Molisch's test	Positive
Test for tannin	
Ferric Chloride test	Positive
Potassium Dichromate test	Negative
Lead Acetate test	Negative
Test for alkaloid	
Mayer's test	Positive
Dragendroff's test	Positive
Test for glycosides	Negative
Test For steroids	Negative

B. Analgesic activity:

The extract was also subjected to Swiss albino mice to study the analgesic activities against acetic acid induced writhing and analgesic actions were found with oral doses of 250 and 500 mg/kg which is shown in the following table:

Table 2: Result obtained from evaluation of analgesic action

Group	Treatment	Dose	Writhes counted	% of Inhibition
Control	Normal saline	-	24.3	-
Standard	Diclofenac	10	15.0	38.27
Test group 1	Leaves extract	250	16.33	32.75
Test group 2	Leaves extract	500	15.33	36.89

C. Anti-diarrheal activity:

In case of castor oil-induced diarrheal test, the AEL showed a potent anti-diarrheal effect in the mice. The leaves extract (AEL) showed anti-diarrheal effect, in the mice upon administration of extract at doses 250 mg/kg compared to the standard group at the dose of 5 mg/kg. The above discussion indicates that Averrhoa carambola has anti-diarrheal activities which prove the traditional use of this plant.

Table 3: Result obtained from evaluation of anti-diarrheal action

Code No.	Mice no	1 st hour	2 nd hour	3 rd hour	4 th hour	Average	TotalAverage with SEM	Std. Deviation	% Reduction Diarrhea
CTL	1	1	1	1	2	1.25	4±0.083	0.144	-
	2	1	0	3	2	1.5			
	3	1	2	0	2	1.25			
STD	0	0	0	0	0	0	0	0	100
	0	0	0	0	0	0			
	0	0	0	0	0	0			
AEL	1	0	0	2	0	0.5	1.5±0	0.25	62.5
	2	0	0	1	0	0.25			
	3	0	1	0	2	0.75			

with SD

Nociception is commonly referred to as acute pain, i.e., an extreme noxious stimulus that places an extreme and painful feeling at risk. The excitement of unique receptors, non-ciceptors, or their numerous fibers causes pain. All these extraordinary cells respond to a wide variety of physical stimuli (heat, cold, pressure) or chemical stimuli¹⁸. To achieve as complete a representation as possible of the analgesic properties of a drug using behavioral nonciceptive measures, tests that vary in terms of stimuli content, strength, and period must be applied. The research findings suggest that the extract has a mild dose-dependent analgesic impact on the different pain models used. The effects of the extract on these pain models shows that it could be performing centrally¹⁹. Acetic acid prompted writhing in visceral

pain imposed by mice attracts a great deal of attention from analgesic drug screening²⁰. In general, tannin and flavonoids anti-diarrheal activity has been recognized for intestinal motility inhibition, antimicrobial action, antisecretory and anti-secretory effects. In addition, antinociceptive, anti-inflammatory and antidiarrheal effects are believed to be caused by the astringent properties of tannins²¹. Castor oil is widely used in mice to test for GIT motility. The principal portion of the castor oil is ricinoleic acid triglycerides, which are decomposed into glycerol and ricinoleic acid by lipases in the small intestine. Ricinoleic acid production is facilitated by the release of prostaglandins arising from the effects of inflammation and agitation, resulting in increased peristalsis²². In addition, it induces fluid and electrolyte secretion in the tiny intestine that accelerates the passage of the bowel. The extract's antidiarrheal action against experimentally induced castor oil diarrhea may be attributed to permeability of anti-electrolyte action. The plant's leaves substantial antispasmodic effects may be due to its flavonoids and composition of anthraquinones, since these phytomolecules have an antispasmodic influence²³.

Conclusion

The phytochemical screening confirmed the presence of several organic compounds. Pharmacological studies for the corresponding effects of the compounds may lead to the discovery of medicinally active lead compound. Acetic acid-induced writhing test confirmed significant analgesic activity compared to standard diclofenac by reducing the writhing count. From this test, the peripheral analgesic action of the plant had been confirmed. Castor oil induced defecation confirmed significant anti-diarrheal activity compared to standard imotil by reducing defecation. From this test, the anti-diarrheal action of the plant had been confirmed. The results of the current studies indicate that further isolation and purification of the crude extract may lead to the discovery of lead compounds with potential activity.

Acknowledgments

We are thankful to Head, Department of Pharmacy, Daffodil International University for all sorts of supports throughout the study.

Conflict of interest: None.

Funding: None.

References

1. Moresco HH, Queiroz GS, Pizzolatti MG, Brighente I. Chemical constituents and evaluation of the toxic and antioxidant activities of *Averrhoa carambola* leaves. *Revista Brasileira de Farmacognosia*. 2012;22(2):319-24.
2. Singh R. Chemotaxonomy of Medicinal Plants: Possibilities and Limitations. In *Natural Products and Drug Discovery 2018* (pp. 119-136).
3. Carolino RO, Belebani RO, Pizzo AB, Del Vecchio F, Garcia-Cairasco N, Moyses-Neto M, dos Santos WF, Coutinho-Netto J. Convulsant activity and neurochemical alterations induced by a fraction obtained from fruit *Averrhoa carambola* (Oxalidaceae: Geraniales). *Neurochemistry international*. 2005;46(7):523-31.
4. Cabrini DA, Moresco HH, Imazu P, Silva CD, Pietrovski EF, Mendes DA, Prudente AD, Pizzolatti MG, Brighente IM, Otuki MF. Analysis of the potential topical anti-inflammatory activity of *Averrhoa carambola* L. in mice. *Evidence-Based Complementary and Alternative Medicine*. 2011;2011.
5. Dasgupta P, Chakraborty P, Bala NN. *Averrhoa carambola*: an updated review. *International Journal of Pharma Research & Review*. 2013;2(7):54-63.
6. Corrêa MP. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. Imprensa Nacional, ISBN, Rio de Janeiro, Brazil. 1984;1:74

7. Chau CF, Chen CH, Lin CY. Insoluble fiber-rich fractions derived from *Averrhoa carambola*: hypoglycemic effects determined by *in vitro* methods. *LWT-Food Science and Technology*. 2004;37(3):331-5.
8. Soncini R, Santiago MB, Orlandi L, Moraes GO, Peloso AL, dos Santos MH, Alves-da-Silva G, Paffaro Jr VA, Bento AC, Giusti-Paiva A. Hypotensive effect of aqueous extract of *Averrhoa carambola* L.(Oxalidaceae) in rats: An *in vivo* and *in vitro* approach. *Journal of ethnopharmacology*. 2011;133(2):353-7
9. Neto MM, da Costa JA, Garcia-Cairasco N, Netto JC, Nakagawa B, Dantas M. Intoxication by star fruit (*Averrhoa carambola*) in 32 uraemic patients: treatment and outcome. *Nephrology Dialysis Transplantation*. 2003;18(1):120-5.
10. Das BN, Ahmed M. Analgesic activity of the fruit extract of *Averrhoa carambola*. *Int J Life Sci Biotech Pharm Res*. 2012;1(3).
11. Parrotta JA. *Healing plants of peninsular India*. CABI publishing; 2001.
12. Shahreen S, Banik J, Hafiz A, Rahman S, Zaman AT, Shoyeb A, Chowdhury MH, Rahmatullah M. Antihyperglycemic activities of leaves of three edible fruit plants (*Averrhoa carambola*, *Ficushispida* and *Syzygiumsamarangense*) of Bangladesh. *African Journal of Traditional, Complementary and Alternative Medicines*. 2012;9(2):287-91.
13. Neto MM, Robl F, Netto JC. Intoxication by star fruit (*Averrhoa carambola*) in six dialysis patients?(Preliminary report). *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association-European Renal Association*. 1998;13(3):570-2.
14. Islam F, Azad AK, Faysal M, Islam S, Sugandha NJ, Saha S, Isah SI. PHYTOCHEMICAL INVESTIGATION AND COMPARATIVE ANTHELMINTIC ACTIVITY OF BETWEEN METHANOL AND ACETONE EXTRACT OF LIMONIA ACIDISSIMA L (FRUIT PEEL).
15. Islam F, Azad AK, Faysal M, Azad MA, Islam S, Al Amin M, Sultana N, Dola FY, Rahman MM, Begh MZ. A Comparative Study of Analgesic, Antidiarrhoeal and Antimicrobial Activities of Methanol and Acetone Extracts of Fruits Peels of *Limoniaacidissima*L.(Rutaceae). *Journal of Drug Delivery and Therapeutics*.2020 Feb 15;10(1-s):62-5.
16. Azad AK, Islam F, Begh ZA, Amin A, Alam SM, Sugandha NJ, Shekh N, Islam A. Analgesic, Antidiarrheal and Antimicrobial Activities of *Rhynchochumellipticum* (Gesneriaceae) Stem and Leaf Extracts. *Journal of Pharmaceutical Sciences and Research*. 2020;12(1):147-50.
17. Akter A, Begh MZ, Islam F, Afroz T, Hossain MS, Faysal M, Rahman MM. Phytochemical Screening and Evaluation of Thrombolytic, Analgesic and Antidiarrhoeal Activity of the Leaves of *Cucumis sativus* Linn.(Cucurbitaceae) of Methanolic Extracts. *Journal of Pharmaceutical Sciences and Research*. 2020 Mar 1;12(3):448-51.
18. RAO AS, LATHA P, DHAKATE O, GUNDA S, SRI M. Anti-inflammatory and Analgesic Activity of *Cicer Arietinum*. L. Plant Extracts in Rats. *International Journal of Pharmaceutical Research*. 2014 Oct;6(4):75.
19. Hossain A, Hossain A, Mannan SJ. Evaluation of antioxidant and analgesic activities of three medicinal plants. *Pharmacognosy Research*. 2019 Jul 1;11(3):248.
20. Faiza RT, Mahmud ME, Islam M, Faisal MS, Islam MA, Asad AB, Hossain MS. Determination of thrombolytic, antioxidant and analgesic activity of methanolic extracts of *Rudbeckiahirta*. *Discovery Phytomedicine*. 2019;6(2):77-82.
21. Ali K, Ashraf A, Biswas NN. Analgesic, anti-inflammatory and anti-diarrheal activities of ethanolic leaf extract of *Typhoniumtrilobatum* L. Schott. *Asian Pacific journal of tropical biomedicine*. 2012 Sep 1;2(9):722-6.
22. Ullah S, Ibrar M, Muhammad N, Ali J, Mustafa S, Farooq U, Khan A. Antinociceptive and Anti-diarrheal Activities of *Debregeasiasalicifolia*. *Journal of the Chemical Society of Pakistan*. 2014 Dec 31;36(6).
23. Shibata S, Harada M, Budidarmo W. Studies on the constituents of Japanese and Chinese crude drugs. Parr III: Antispasmodic action of flavonoids and anthraquinones. *YakugakuZasshi*. 1960;80:620-4.