



Antioxidant and antidiarrhoeal activities of methanolic extract of *Artocarpus heterophyllus* seed

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Abstract:

The present study was designed to investigate antidiarrheal and antioxidant activities of the methanolic extract of *Artocarpus heterophyllus* seed (ATS). Total phenolic content, total antioxidant activity, scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical were used to evaluate antioxidant potential of ATS. In DPPH method, ATS showed moderate antioxidant potentiality in a dose dependent manner with the IC₅₀ value of 116.04 µg/ml. The phenolic content of methanol extract of ATS was 437±.006 mg of GAE / gm of dried extract. Total antioxidant capacity of ATS was found to be 170.75±.001 mg/gm equivalent of ascorbic acid. The extract was studied for antidiarrheal property using castor oil in mice. At the doses of 200 and 400 mg/kg body weight, the extract reduced the frequency and severity of diarrhea in test animals throughout the study period. Altogether, these results suggest that the ATS could be used as a potential antidiarrhoeal agent along with its antioxidant potentiality.

Keywords: Antioxidant, Antidiarrhoeal, *Artocarpus heterophyllus*.

Introduction

Nature has been a resource of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Free radicals are the main reason in lipid peroxidation, highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources(1). Free radical oxidative stress caused a wide variety of clinical disorders (2). A serious imbalance between the production of free radicals and the antioxidant defense system is responsible for oxidative stress(3). Antioxidants exert their mode of action by suppressing the formation of reactive oxygen species either by inhibition of enzymes or by chelating trace elements (4).

Diarrhea is one of the main causes of infant death especially in third world country (5). Diarrhea affects the smooth life style due to its huge discomfort, although it is not life threatening for adults (6).

However, twenty percent of total children die from diarrhea before the age of five in developing countries. There are some synthetic drugs available for diarrheal treatment although most of them have side effects like uncomfortable bowel movement, uneasiness etc. A continuous search, therefore, for an alternative treatment is still urged (7).

The jackfruit (*Artocarpus heterophyllus*) belonging to family Moraceae is a commonly known as "Kathal" (8). The various parts of the jack fruit have been used in traditional medicines (9). Different classes of flavonoids are abundant in the jack fruit plant (10). Several reports have cited the antidiabetic effects of jack fruit extracts (JFEs), due to its high proanthocyanidin and flavonoid contents through inhibition of lipid peroxide formation, and via an alpha-amylase inhibitory effect (11)(12)(13)(14)(15).

In addition, it has been reported that JFEs possess anti-inflammatory and antibacterial activity (16), (17). *Artocarpus heterophyllus* possess numerous medicinal properties such as antibacterial, antioxidant, antidiabetic, anti-inflammatory, anti-diuretic, immunomodulatory and have been useful in the treatment of fever, skin diseases, convulsions, constipation, ophthalmic disorders and snake bite (18). Literature reviews indicated that no studies combining the antioxidants as well as antidiarrheal of the seeds of *A. heterophyllus* have so far been undertaken. Taking this in view, the present study aimed to evaluate the antioxidant activity of seeds extracts of *A. heterophyllus* along with their antidiarrheal activities.

MATERIALS AND METHODS

Plant materials

The samples were collected from the Botanical garden of the Jahangirnagar University and verified with Taxonomy Division of the Botany Department of the university.

Preparation of plant extract

The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The dried powder material (1.2 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (310 g).

Antioxidant activity test:

Determination of total phenolics:

Total phenols were determined by determined Folin-Ciocalteu reagent (19). A dilute extract of each plant extract (0.5ml) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (2.5ml, 1: 10 diluted with distilled water) and aqueous Sodium carbonate (2.5 ml). The mixture were allowed to stand for 20 min and the total phenols were determined by spectrophotometer at 760 nm. The standard curve was prepared using 6.25, 12.5, 25, 50, 100, 200 µg/ml solution of gallic acid. Total phenol values are expressed in terms of gallic acid equivalent (mg/g) which is a common standard.

Determination of total antioxidant capacity:

Total antioxidant capacity of different extractives of *A. heterophyllus* was determined by the method of Prieto *et al.*, (20). An aliquot of 0.3 ml of sample solution was combined in a test tube with 3ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The test tube were incubated at 95 °C for 90 minutes. After the sample had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical:

The antioxidant potential of the extracts were determined on the basis of its scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The DPPH assay was carried out as per the procedure outlined by Fresin (21). 0.1 ml of different fraction of extract, at various concentration was added to 3 ml of a 0.004% methanol solution of DPPH and was allowed to stand for 30 min for the reaction occur. Thirty minutes later, the absorbance was measured at 517 nm using spectrophotometer. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation: % inhibition = $[(A_B - A_S) / A_B] \times 100$

Where, A_B is the absorbance of the control reaction (containing all reagents except the test compound and A_S is the absorbance of the test compound.

Ascorbic acid was used as positive control. The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration.

Antidiarrhoeal Activity:

Castor oil induced diarrhea:

This study was conducted by the method described by Shoba and Thomas (22). The animals were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were divided into following four groups containing five mice in each group. Group I: Treated with vehicle (Saline 10 ml kg⁻¹ (p.o.)). Group II and Group III: Treated with 200 and 400 mg kg⁻¹ body weight (p.o.) of ATS, respectively. Group IV: Received loperamide (3mg/kg) body weight (p.o.). Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml castor oil to each mice, 30 min after the above treatments. During an observation period of 4 h, the total number of faecal output and the number of diarrhoeic faeces excreted by the animals were recorded.

Statistical analysis

All values were expressed as the mean ± SEM of three replicate experiments. The analysis were statistically analyzed by applying the Student *t*-test and $p < 0.001$ were considered to be statistically significant.

RESULTS

Acute Toxicity Studies

The acute toxicity studies mainly aim at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. Extracts of ATS were safe

up to a dose of 1000 mg/kg (p.o.) body weight. Behavior of the animals was closely observed for the first 3h then at an interval of every 4h during the next 48h. The extract did not cause mortality in mice during 48h observation. Food and water intake had no significant difference among the group studied.

Total phenolics compound:

Phenolic content of the samples were calculated on the basis of the standard curve for gallic acid as shown in Table 1.1 and in Fig.1.1. The results were expressed as mg of gallic acid equivalent (GAE)/gm of dried extractives. The phenolic content of methanol extract of ATS was $437 \pm .006$ mg of GAE / gm of dried extract.

Determination of total antioxidant activity:

Total antioxidant activity of the samples were calculated on the basis of the standard curve for ascorbic acid as shown in Fig.1.2. Total antioxidant capacity of *A. heterophyllum* seed is expressed as the number of equivalents of ascorbic acid. Total antioxidant capacity of ATS was found to be $170.75 \pm .001$ mg/gm equivalent of ascorbic acid (Table 1.2).

DPPH radical scavenging activity:

The percentage% scavenging of DPPH radical was found to be concentration dependent with the IC_{50} value of $116.04 \mu\text{g/ml}$, while IC_{50} value of standard ascorbic acid was found to be $9.02 \mu\text{g/ml}$ (Table 1.3). The IC_{50} value was obtained from fig 1.3.

Antidiarrhoeal activity in Castor oil-induced diarrhea:

In the castor oil-induced diarrhoeal experiment in mice, the ATS at the dose of 200 and 400mg/kg, reduced the total number of faces as well as the total number of diarrhoeic faces in a dose dependent manner (table 1.4). These results were shown to be statistically significant ($p < 0.001$).

Discussion:

To determine the efficacy of natural antioxidants either as pure compounds or as plant extract, a great number of *in vitro* methods have been developed in which antioxidant compounds act by several mechanisms. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the compounds having antioxidant property and is successfully used to quantify vitamin E in seeds (20).

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (23) and is usually used as a substrate to evaluate the antioxidant activity of a compound (24). Based on the data obtained from this study, DPPH radical scavenging activity of *A. heterophyllum* seed extract ($IC_{50} 116.04 \mu\text{g/ml}$) was comparable with the

standard ($IC_{50} 9.02 \mu\text{g/ml}$). It was revealed that *A. heterophyllum* seed extract did show the proton donating ability and could serve as free radical inhibitor or scavenger. In fact, the radical scavenging capability of phenolic compounds are due to their hydrogen donating ability/number of hydroxyl groups present, which in turn is closely related both to the chemical structure and spatial conformation, that can modify the reactivity of the molecules (25).

Several mechanisms have been previously proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na^+ , K^+ -ATPase activity to reduce normal fluid absorption (26), activation of adenylate cyclase or mucosal cAMP mediated active secretion (27), stimulation of prostaglandin formation (28), platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil (29). However, it is well evident that castor oil produces diarrhea due to its most active component ricinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (30). Since the methanol extract of the seeds of *A. heterophyllum* successfully inhibited the castor oil-induced diarrhea, the extract might have exerted its antidiarrheal action via antisecretory mechanism which was also evident from the reduction of total number of wet faeces in the test groups in the experiment.

CONCLUSION

Based on the results of the present study, we conclude that the methanolic extract of *A. heterophyllum* possesses remarkable antioxidant and antidiarrhoeal activity. However, further studies are indispensable to examine underlying mechanisms of antidiarrhoeal antioxidant effects and to isolate the active compounds responsible for these pharmacological activities.

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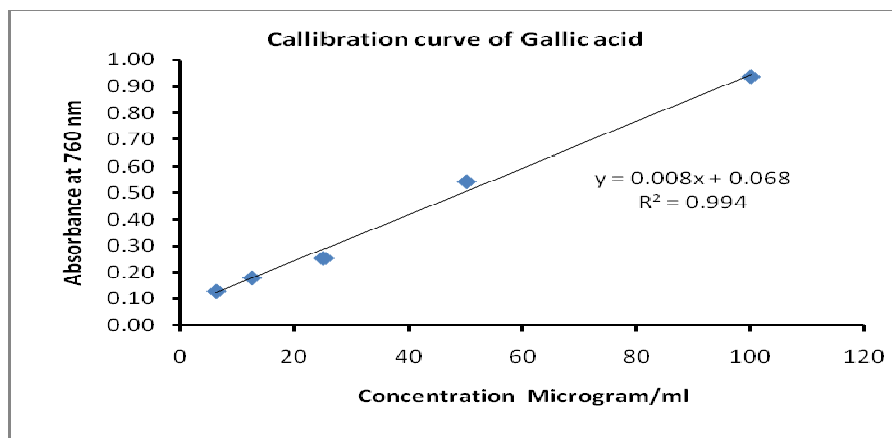


Fig1.1: Standard curve of Gallicacid for the determination of total Phenolics

Table 1.1: Determination of total phenolic content of methanol extract of ATS

Sample	GAE/gm of dried Sample Mean ±STD
Total phenolic content	437±.006

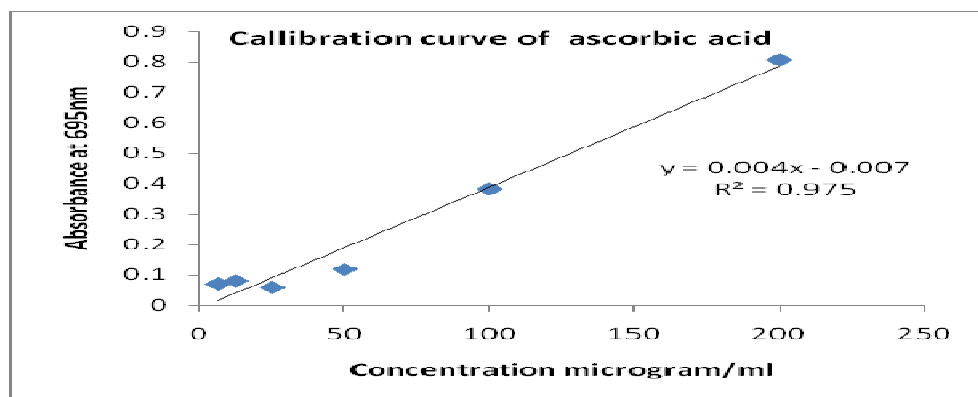


Fig1.2: Standard curve of Ascorbic acid for the determination of total antioxidant activity

Table 1.2: Determination of total antioxidant capacity of methanol extract of Kathal

Sample	GAE/gm of dried Sample Mean ±STD
Total antioxidant capacity	170.75±0.001

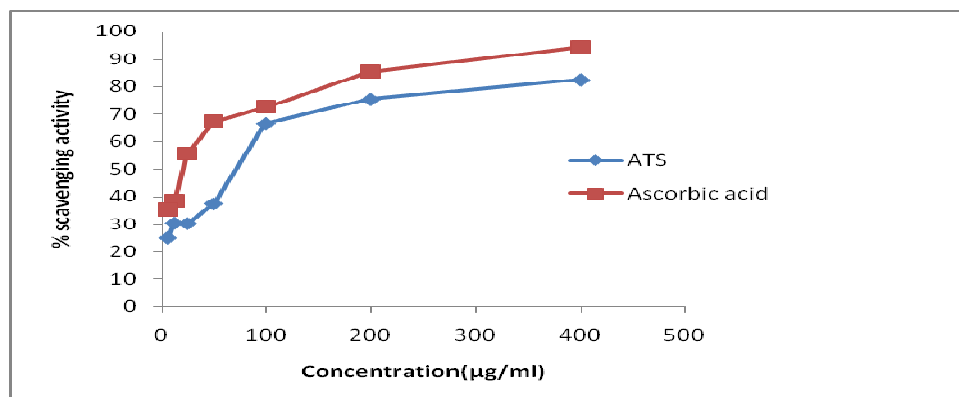


Fig.1.3: DPPH radical scavenging activity of methanolic fractions of ATS

Table 1.3: DPPH radical scavenging activity of methanolic extract of ATS at different concentrations

Con(µg/ml)	% of scavenging Mean±STD		IC ₅₀ (µg/ml) value of Ascorbic acid	IC ₅₀ (µg/ml) value of ATS
	Ascorbic acid	ATS		
6.26	35.12±.45	25.12±.24	9.02	116.04
12.5	38.35±.26	30.43±.36		
25	55.68±.33	30.49±.45		
50	67.24±.65	37.48±.65		
100	72.58±.59	66.64±.71		
200	85.34±.38	75.412±.12		
400	94.27 ±.47	82.45±.36		

Table1.4: Effect of methanolic extracts of ATS on castor oil induced diarrhea in mice

Treatment	Dose(mg/kg)	Total number of faces in 4h	Total number of wet faces in 4h	Percent inhibition of diarrhoeal faces
Control (Saline)		22.6 ± 0.68	18.45± .76	
ATS	200	15.2 ± 1.05*	9.28± .38*	32.74
ATS	400	10.1 ± 0.29*	6.56± .55*	55.30
Loperamide	3	3.8 ± 0.58*	1.5 ± .35*	83.18

Values are presented as mean ± SEM, (n=5); * p<0.001, respectively, compared to control by student's t-test.

References:

- Cheeseman KH, Scater TF. Free Radical in medicine. In British medical bulletin. Churchill, livingstone, London, 2003;9:479-724.
- HalliwellB, Gutteridge JMC. Oxidative stress and antioxidant protection: Some special case. In: Halliwell B, Gutteridge JMC editors. Free Radicals in Biology and Medicine. 3rd edition. Oxford: Clarendon Press; 1999; 530-33.4.
- Nakiboglu M, Urek OR. Kayali AH. Tarhan L. Antioxidant capacities of endemic *Sideritis sipylea* and *Origanum sipyleum* from Turkey. Food chem. 2007; 104:630-635.
- Subhashini N. Thangathirupathi A. Lavanya N. Antioxidant activity of Trigonella foenum graecum using various in vitro and ex vivo models. Int J pharm pharm Sci, 2011; 3:96-102.
- Zavala MA, Perez S, Perez C, Vargal R, Perez RM (1998).Antidiarrhoeal activity of Walthera americana, Commelins cuelestrisand Alternanthera repens.J.Ethnopharmacol.61:41-47.
- Saito T, Mizutani F, Iwanga Y, Morikawa K, Kato H(2002). Laxative and antidirrhoel activity of Polycarbophil in Mice and Rats. Jpn.J.Pharmacol.89:133-141.
- Nester WE, Robbert CE, Nancy NP, Danise GA, Martha TN (1998). Microbiology: A Human Perspective 2nd ed. Mc Graw-Hill CompaniesUSA: 571-573
- Deepika gupta , Sonia Mann, Avijit Sood and Rajinder K. Gupta (2011) Phytochemical, nutritional and antioxidant activity evaluation of seeds of ja ckfruit (artocarpous heterolphyllus lam.) *International Journal of Pharma and Bio Sciences*. P – 336.
- Mukherjee, B. (1993) *Traditional Medicine*. Mohan Primalani for Oxford and IBH Publishing, New Delhi. pp. 45
- Wei, B.L., Weng, J.R., Chiu, P.H., Hung, C.F., Wang, J.P., and Lin, C.N. (2005) Anti-inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *J. Agric. Food Chem.* 53(10), 3867–3871.
- Fernando, M., Wickramasinghe, N., and Thabrew, M. (1991) Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity-onset diabetic mellitus patients. *J.Ethnopharmacol.* 31(3), 277–282.

12. Chandrika, U., Fernando, S., Wickramasinghe, N., and Wedage, W. (2002) Effects of proanthocyanidin and flavonoid fractions from hot water extract of Jack leaves (*Artocarpus heterophyllus*) on the blood glucose levels in healthy male Wistar rats. *Chem. Sri Lanka* 19(2), 10.
13. Chandrika, U., Wedage, W., Wickramasinghe, N., and Fernando, S. (2006) Hypoglycemic action of the flavonoid fraction of *Artocarpus heterophyllus* leaf. *Afr. J. Trad. CAM* 3(2), 42–50
14. Shizuo, T. and Yoshiaki, S. (2006) Inhibitory effect of prenylated flavonoid in *Euchresta japonica* and *Artocarpus heterophyllus* on lipid peroxidation by interaction of hemoglobin and hydrogen peroxide. *Pharm. Biol.* 44(3), 271–273
15. Kotowaroo, M., Mahomoodally, M., Gurib-Fakim, A., and Subratty, H. (2006) Screening of traditional antidiabetic medicinal plants of mauritius for possible α -amylase inhibitory effects *in vitro*. *Phytother. Res.* 20(3), 228–231
16. Wei, B.L., Weng, J.R., Chiu, P.H., Hung, C.F., Wang, J.P., and Lin, C.N. (2005) Anti-inflammatory flavonoids from *Artocarpus heterophyllus* and *Artocarpus communis*. *J. Agric. Food Chem.* 53(10), 3867–3871.
17. Khan, M., Omoloso, A., and Kihara, M. (2003) Antibacterial activity of *Artocarpus heterophyllus*. *Fitoterapia* 74(5),501–505.
18. Prakash O, Kumar R, Mishra A, Gupta R. *Artocarpus heterophyllus* (Jackfruit): an overview. *Phcog Rev.* 2009;6:353–358.
19. Singleton VL, Rossi JA. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acidreagents. *American Journal Enology and Viticulture* 16: 144-158.
20. Prieto P., Pineda M., Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* 1999;269:337–341
21. Feresin, G.E.,A Tapia, G.R. Angel, C. Delporte, N.B. Erazo and G.Schmeda-Hirschman,2002.Free radical scavengers, anti-inflammatory and analgesic activity of *Acaena magellanica*. *J.Pharm.Pharmacol.*;54:835-844.
22. Gaginella TS and Bass P. Laxatives: an update on mechanism of action. *Life Science.* (1978)23:1001-1010.
23. Nakayama T. Suppression of hydroperoxide-induced cytotoxicity by polyphenols. *Cancer Res.* 1994;54(7 Suppl):1991s–1993s.
24. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* 2002;10(3):178–182.
25. Gorelik S, Ligumsky M, Kohen R, Kanner J. A novel function of red wine polyphenols in humans: Prevention of absorption of cytotoxic lipid peroxidation products. *FASEB J.* 2008;22(1):41–46.
26. Nell G, Rummel W. Action mechanism of secretagogue drugs. In: Csaky TZ, editor. *Pharmacology of Intestinal Permeation*. Berlin: Springer-Verlag; 1984. pp. 464–474.
27. Capasso F, Mascolo N, Izzo AA, Gaginella TS. Dissociation of castor oil induced diarrhea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. *Br J Pharmacol.* 1994;113(4):1127–1130.
28. Galvez A, Zarzuelo ME, Crespo MD, Lorente M, Ocete A, Jimenez J. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of active flavonoid constituents. *Planta Med.* 1993;59(4):333–336.
29. Mascolo N, Izzo AA, Gaginella TS, Capasso F. Relationship between nitric oxide and platelet activating factor in castor oil induced mucosal injury in the rat duodenum. *Naunyn Schmiedebergs Arch Pharmacol.* 1996;353(6):680–684.
30. Gaginella TS, Stewart JJ, Olsen WA, Bass P. Action of ricinoleic acid and structurally related fatty acid on the gastrointestinal tract. II. Effect on water and electrolyte absorption *in vitro*. *J Pharmacol Exp Ther.* 1975;195(2):355–356.