

Pharmacognostic, Phytochemical Standards and Antiulcer Activity of Aqueous Extracts of *Annona Squamosa* and *Annona Reticulata* Leaves Onrats

S B Bavage¹, Dr. Birendra Shrivastava², Dr. Ganesh N. Sharma³, Dr. Aamer Quazi⁴

¹Assistant Professor, Department of Pharmacognosy, Latur College of Pharmacy Hasegaon, Maharashtra, India (Corresponding Author)

^{2,3}Jaipur National University, Jaipur. Rajasthan, India

⁴K.T. Patil College of Pharmacy, Osmanabad, Maharashtra, India

Abstract: The present work was undertaken to establish the pharmacognosticphytochemical standards and anti-ulcer activity of aqueous extracts of leaves of *Annona squamosa* Linn. and *Annona reticulata* Linn on wistar rats by pyloric ligation method. These are highly apparent plants in ayurvedic system of medicine for the treatment of various ailments. *A. squamosa* Linn. and *A. reticulata* Linn. are locally known as Sitaphala and Ramphal respectively both belongs to family Annonaceae. The plants are traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever and ulcer. In model the common parameter determined was ulcer-index. aqueous extracts of dosage 175, 350 mg/kg p.o produced significant inhibition of gastric lesions induced by pyloric ligation induced ulcers The extract 175mg/kg & 350mg/kg indicate that the aqueous extracts significantly ($P < 0.001$) decreases the volume of gastric acid secretion, PH, free acidity, total acidity and ulcer index compared to control. This present study indicate that leaves extracts have potential antiulcer activity. This resultmay further suggest that aqueous extracts were found to posses anti ulcerogenic as well as ulcer healing property. No reports are available on the pharmacognostic nature, phytochemical and anti-ulcer activity studies of the both plant leaf. Hence, the present study was carried out to investigate the same. All the parameters were studied according to the WHO & Pharmacopoeial guidelines. This parameters will help for correct identification of these plants for the future references.

Keywords: *Annona squamosa* L., *Annona reticulata* L., Pharmacognostic, WHO , Phytochemical, anti-ulcer

1. Introduction

Annona squamosa Linn and *Annona reticulata* Linn are extensively used as traditional medicine in various culture. The genus name, 'Annona' is from the Latin word 'anon', meaning 'yearly produce', referring to the production of fruits of the various species in this genus.

Annona squamosa Linn also known as Sitaphal, and Sugar apple belongs to the family *Annonaceae*. It is a small ever green tree, cultivated throughout India for its fruits, cultivated in Thailand & originates from the West Indies & south America. It is mainly grown in gardens for its fruits & ornamental value. Its leaves were used as the insecticidal and antispasmodic agents that were used in the treatment of rheumatism and painful spleen. The plant was reported traditionally to possess analgesic, anti-inflammatory, anti-pyretic, anti-ulcer, and antiseptic and abortifacient activities and also as an insecticidal agent. The crushed leaves are reported to be applied to the nostrils in hysteria and fits. The poultice of leaves is used as a cataplasm over boils and ulcers to induce suppuration. It relieves pains and swellings. Moreover Ethnobotanical studies reveals that the leaves are used in ulcers, in scabies, for toothache, wounds, cuts, dysentery, sprains, dandruff, to remove lice, diarrhoea, antidiabetic.¹⁻²



Annona reticulata Linn. also known as Ramphala, Custard-Apple, belongs to the family *Annonaceae*. It is found growing between altitudes of 0 metres (0 ft) to 1,500 metres (4,900 ft) in areas of Central America that have alternating seasons. It is cultivated and naturalized in many tropical countries, and also occurs as feral populations in many parts of the world, including Southeast Asia, Taiwan, India, Bangladesh, Pakistan, Australia, and Africa. The plant is indigenous to the West Indies. In India it is widely cultivated and naturalized as a fruit consuming plant and deciduous tree. Traditionally the plant has been employed for the treatment of epilepsy, dysentery, cardiac problem, parasite and worm infestations, constipation, haemorrhage, bacterial infection, fever, ulcer and as insecticide. Bark is a powerful astringent. Plants are recognized as aromatic as well as source of medicine. The extracts obtained from various plant parts possess medicinal properties and are used as colouring agent, preservative, sweetening agent and as an additive in many medicinal formulations. [1][2]



Peptic ulcer is one of the most widespread common gastrointestinal diseases, is believed to be due to an imbalance between aggressive and protective factors[3]. The gastric mucosa is continuously exposed to potentially injurious agents such as acid, pepsin, bile acids, food ingredients, bacterial products (*Helicobacter pylori*) and drugs [4]. These agents have been implicated in the pathogenesis of gastric ulcer, including enhanced gastric acid and pepsin secretion, inhibition of prostaglandin synthesis and cell proliferation growth, diminished gastric blood flow and gastric motility [5]. Drug treatment of peptic ulcers is targeted at either counteracting aggressive factors (acid, pepsin, active oxidants, platelet aggravating factor “PAF”, leukotrienes, endothelins, bile or exogenous factors including NSAIDs) or stimulating the mucosal defences (mucus, bicarbonate, normal blood flow, prostaglandins (PG), nitric oxide) [6]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are on to find a suitable treatment from natural product sources.

Plant based drugs are one of the most important cultural and traditional parts of the people. Today, most of the world population depends upon plant based drugs for their primary health care needs. World Health Organization (WHO) estimates that 80% of the people living in developing countries almost exclusively use traditional medicines plant. The Ethno-pharmacological approach provides the way for developing new drugs from plants sources, which have historical background. Although modern medicines may be available, due to socio-economical, cultural and historical reasons, these drugs have maintained their importance.[7]

In the present study, we screened pharmacognostic, phytochemical investigation and the leaves extracts of *Annona squamosa* Linn and *Annona reticulata* Linn for antiulcer activity.

2. Materials and Methods

Collection and Authentication of the Plant material

The plants *Annona squamosa* Linn. and *Annona reticulata* Linn were collected from the local area of Latur, in the 1st week of August. It was authenticated by Miss. A. S. Kamble from Department of Botany, Dayanand college of Biotechnology Its herbarium is deposited in the above department. The fresh leaves were used for microscopic study. Collected leaves were shade dried and coarsely powdered. This coarse powder was used for the physico-chemical analysis

Macroscopic evaluation

Macroscopic characters of both the leaves were recorded as per visual observation. The colour, odour, and taste of both the leaves were recorded separately.[8] [9]

Microscopic evaluation

Free hand sections were taken, cleared with chloral hydrate and then add drops of phloroglucinol and hydrochloric acid(1;1). Microphotographs were taken by using binocular microscope attached with camera.[8] [9]

Physico-chemical constants

Physicochemical constants of the leaf such as the total ash, acid insoluble ash, water soluble ash and loss on drying were calculated as per Indian Pharmacopoeia .[10] [11] [12] [13] [14]

Phytochemical analysis

For preliminary phytochemical studies, 60g of powdered material was extracted in soxhlet apparatus with petroleum ether, chloroform and methanol and macerated with chloroform water. Extracts were dried in rotary evaporator and weighed. The presence of various phytoconstituents likes steroids and terpenoids, alkaloids, glycosides, flavonoids, oil, phenol, were detected as per standard procedures given in standard text [16] [17] [18]

Preparation of extract

The collected leaves were shade dried at 21°C over polythene cover and the dried material was crushed to coarse powder with mechanical grinder. The powder was stored in airtight container which was used for extraction. About 70 gm of air dried powdered material was soaked in 1000ml distilled water and heat till solvent separation of extract. Separated filtrate extract is filtered by using muslin cloth and the liquid is centrifuge at 10000 rpm by separating sediment. At the end of the extraction process the marc was taken out and it was dried. After drying, the powdered marc was weighed & again packed. The yield obtained is 15gms.

Experimental Animals

Albino Wistar rats of either sex weighing between 180-250 gm were used. The animals were obtained from animal house and were housed in polypropylene cages. The animals were maintained under standard laboratory conditions (25°C±2°C; 12hr light and dark cycle). The animals were fed with standard diet and water ad libitum. Ethical clearance was obtained from the Institutional Animal Ethical Committee before performing the study on animals was taken for conducting antiulcer activities.

Acute oral toxicity studies: Acute oral toxicity study for aqueous extracts of both leaves was carried out as per OECD guideline 425. [19]

Evaluation of Anti -Ulcer Activity

Pyloric ligation – induced ulcer

Animal Albino Wistar rats are divided in to four groups, each contain 6 animals (mention in above method). Group I- having pyloric ligation and received distilled water orally.

Groups II -received ranitidine (20 mg/kg) as reference drug for ulcer protective study.

Group III - received aqueous extract of *Annona squamosa* leaves in a dose of 175 mg/kg.

Group IV- received aqueous extract of *Annona reticulata* leaves in a dose of 175 mg/kg.

After 45 min of aqueous extracts of both plants leaves and ranitidine treatment, the animals were anesthetized using ether anesthesia, abdomen was opened and pylorus was ligated without obstructing blood supply and abdomen was sutured. At the end of 6h the animals were sacrificed and stomach was isolated after ligation of oesophagus. The gastric juice was collected and volume was measured. Stomach was cut along the greater curvature and ulcer index was evaluated. Total Acidity Total acid output of the gastric juice was estimated by titration of 0.1 ml of gastric juice with 0.01 N sodium hydroxide using phenolphthalein as indicator. Acid output was expressed as mEq/L (Hawk PB and Ostor BL, 1965).[20] [21] [22]

Antiulcer Activity

Ulcer index

The stomach was fixed in 10% formalin and examined with a lens for ulcers.

The ulcer score: Normal colored stomach –0, Spot ulcers-1, Surface ulcer –2, Deep ulcer –3, Perforation –4 (Ganguly AK and Bhatnagar OP, 1973).

The percentage of ulcer inhibition was obtained by following formula:

$$\frac{\text{Control mean ulcer index} - \text{Test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

Effect of extracts on Gastric volume, PH and Acidity

In all treated groups, there was a significant ($p < 0.01$) reduction in the gastric volume when compared to vehicle control. The extracts did not exhibit significant decrease in PH and Acidity. Effect of extracts on Ulcer index

Aqueous extracts of both leaves showed significant ($p < 0.01$) reduction in the ulcer index. However the standard drug Ranitidine produced efficient reduction in ulcer index than that of the test groups.

Statistical analysis

Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using Graph pad PRISM software version 4.03 (2005). 'P' values < 0.05 were considered significant.

Separation of secondary metabolites by TLC

For the thin layer chromatographic studies of secondary metabolites, precoated Silica gel F 254 aluminum plates (20 X 20cm) were used.

TLC study of Flavonoid

The flavonoid spots were separated in aqueous extracts using chloroform and methanol (19:1) and n-Butanol: Glacial acetic acid: water (3:1:1) solvent mixture. The colour and Rf values of these spots were recorded under ultraviolet (UV254nm) light and the spot appeared to be

bright yellow in color under UV with an Rf value 0.59 indicating the presence of quercetin

Total Flavonoid contents

The content of total flavonoids was determined by aluminium chloride colorimetric method. The content of flavonoids was determined as quercetinequivalent. 10 mg/ml of plant extracts in respective solvent (stock solution SS) was mixed with 2 ml AlCl_3 (2% w/v) in methanol and the solution was made up to 25ml with methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). 1ml of SS was made up to 25ml with methanolic solution of acetic acid (contrast solution CS). The absorbance of PS and CS was measured at 420nm after 30 minutes. The result expressed as % of total Flavonoids content²³.

$$\% \text{TFC} = \frac{\text{Absorbance at 420} \times \text{dilution} \times 100}{\text{E}1\%1 \text{ cm} \times \text{wt. of extract in gms}}$$

3. Results and Discussion

Morphology

(A) *A.squamosa* Leaves simple, bright green-coloured above light green below, alternate to spirally arranged on the stem, 10-20 cm long and 4-7 cm wide, petiolate, have a 1.5 cm long petiole, twisted and channeled, stipulate, stipule early withering, measuring about 0.2-0.5 mm, leaf ovate to lanceolate, margin simple, lamina measures about 10x5 cm, lamina base simple, The venation of the leaf is reticulate with a prominent midrib. The leaf is coriaceous and glabrous on both sides (table no.1)

(B) *A.reticulata* Leaves are light green in colour on both the sides, simple, alternate, petiolate, have a 1-1.6 cm long petiole, slightly twisted and channeled, stipulate, stipule early withering, measuring about 0.3-0.6 mm, leaf lanceolate, margin simple, lamina about 15x8 cm, lamina base simple, less pungent and very thin as compared to *A.squamosa*. (table no.1)

Microscopy

T.S. of leaf *A.squamosa*

Transverse section through midrib shows the upper and lower single layered compactly arranged barrel shaped epidermis with thick cuticle and rarely simple trichomes on lower surfaces. Lamina upper 1-2 layered palisade parenchyma and lowers 5-6 layers of spongy parenchyma throughout the lamina lysogenous cavities are very common, prismatic crystals, oil globules and tannin content material spread throughout the lamina and also even in midrib. Through midrib shows vascular bundle radially arranged. Vascular bundle surrounded by pericyclic fibres on both the side, rest of consist parenchyma cells.

T.S. of leaf *A.reticulata*

Transverse section through midrib shows the upper and lower single layered compactly arranged rectangular to barrel shaped epidermis with thick cuticle and multicellular trichomes filled with tannin on lower surfaces. Lamina upper single layered palisade parenchyma and lowers 6-7 layers of spongy parenchyma lysogenous cavities are very common, prismatic crystals, oil globules and tannin content material spread throughout the lamina and also even in midrib. Through midrib shows vascular bundle radially

arranged. Vascular bundle surrounded by pericyclic fibres on both the side, rest of consist parenchyma cells, in center a group of stone cells in observed.

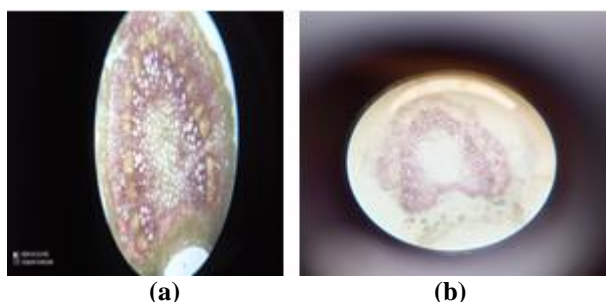


Figure: T. S. of (a) *A. squamosa* and (b) *A. reticulata*

Physico-chemical costants

The Physico-chemical characters of powdered leaves of *Annona squamosa* and *Annona reticulata* such as ash values, extractive values and loss on drying values are given in (table no.2)

Phytochemical Analysis

Represent various phytochemical constituents present in the leaf extracts of *Annona squamosa* and *Annona reticulata*. The phytochemical studies of both the plant extracts conclude that and water extracts of leaf samples had more positive results for glycosides, oils, saponins and flavonoids.(table no.3)

4. Conclusion

From the results it is concluded that both the leaves show some similar and also distinguishing characters in morphology, microscopy and also in micrometric studies. These characters are important in identification of specific species even in powder form. The results of this study may be used as the reference standard in further research undertakings of its kind.

Table 1: Morphology of *A. squamosa* and *A. reticulata* leaf

Characters	<i>A. squamosa</i> leaf	<i>A. reticulata</i> leaf
Size	10-15 cm long and 3-5 cm wide	10-22 cm long, 4-7 cm width
Shape	Alternate, Bilateral, Petiolate, Ovate – lanceolate	Alternate, Bilateral, Petiolate, Oblong-lanceolate,
Venation	Reticulate	Reticulate
Apex	Obtuse	Acute
Base	Asymmetric	Asymmetric

Table 4: The results of anti-ulcer activity against pylorus ligation induced gastriculcer model

Treatment	Dose (mg/kg) p.o	Volume of gastric juice(ml/4h)	PH	Anti -ulcer activity			
				Free Acidity (mEq/L)	Total Acidity (mEq/L)	Ulcer Index	% Inhibition of ulcer
Group-I: Control (Normal saline)	20ml/kg	4.48±0.117	1.74±0.24	25.34±0.08	71.16±0.20	29.6±1.5	-----
Group-II: Standard (ranitidine)	20mg/kg	2.68±0.18	4.86±0.15	10.42 ±0.02	22.24±0.18	11.6±0.8	75.61***
Group-III: Aqueous leaf extract of <i>annona squamosa</i>	175mg/kg	4.28±0.093	3.11±0.14	20.18 ±0.05	51.14 ±0.38	17.88±1.538	33.41*
Group-IV: Aqueous leaf extract <i>annona reticulata</i>	175mg/kg	3.05±0.163	4.36 ±0.16	10.76 ±0.06	30.62 ±0.26	9.16±3.1	71.21**

Plant extracts are some of the most attractive sources of new drugs and have shown promising results in the treatment of gastric ulcers. Several folk medicinal plants and herbs have been used to treat gastrointestinal disorders or gastric ulcer.²⁵

Margin	Simple, Empirical	Simple, Empirical
Colour	Dark Green above Light Green below	Light Green both the surface
Odour	Aromatic	Unpleasant
Taste	Bitter	Bitter, Mucilaginous

Table 2: Physico-chemical constants of *Annona squamosa* L. and *Annona reticulata* L

Phytochemicals	<i>Annona squamosa</i> L	<i>Annona reticulata</i> L
Glycosides	+	+
Phytosterols	+	+
Alkaloids	+	+
Oils	+	-
Saponins	-	-
Phenols	+	+
Flavonoids	+	+

+ = Present; - = Absent

5. Result and Discussion

Phytochemical screening

The preliminary phytochemical screening of the extracts of *Annona squamosa* and *annonna reticulata* showed the presence of steroids and terpenoids, alkaloids, glycosides, flavonoids, oil, phenol.

In the present study, aqueous leaf extracts of both plants were evaluated for its anti-ulcer activity against pylorus ligation induced gastriculcer model.

Results are mean ± S.E.M (n = 6). Statistical comparison was performed by using ANOVA coupled with student 't' test.* P<0.05, ** P<0.01, *** P<0.001 were consider statistically significant when compared to control group. In ligation induced gastric ulcer. Both doses of leaf extracts showed significant reduction in ulcer index, free acidity, total acidity and gastric volume but raised PH of gastric Juice as compared to the control groups. It was showing protection index 33.41% and 71.21% at a dose of 175 mg/kg of both extracts respectively compared with standard drug ranitidine showed 75.61% (Table no.4).

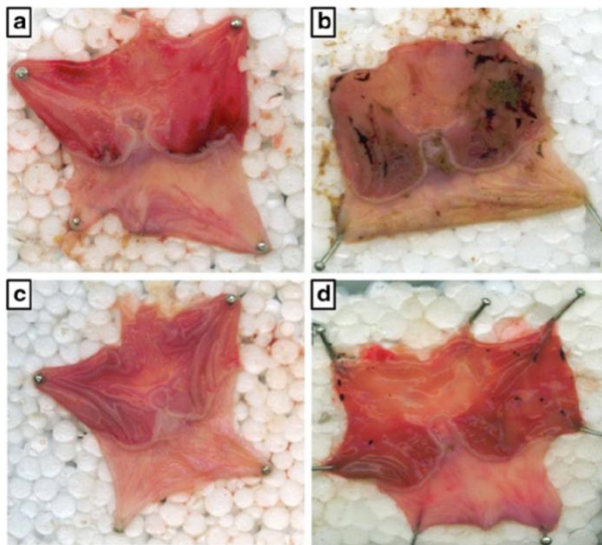


Figure 2

- a) Aqueous leaf extract of *annona squamosa* shows protection of mucosal layer
 b) Control (P.L.) shows severe damage of mucosal layer
 c) Standard (ranitidine) shows protection of mucosal layer
 d) Aqueous leaf extract of *annona reticulata* shows protection of mucosal layer

References

- [1] Sharma O.P. Plant Taxonomy, 2nded., Tata McGraw Hill Education Private Ltd, New Delhi, 2010, 217-220.2.
- [2] Anonymous, Reviews on Indian Medicinal Plants Vol-2, I.C.M.R. New Delhi, 2004, 353-357.
- [3] Alkofahi A, Atta AH., Pharmacological screening of the antiulcerogenic effects of some Jordanian Medicinal Plants in rats, J Ethnopharmacol 1999, 65, 341-5.
- [4] Peskar BM., Maricic N., Role of prostaglandins in gastroprotection, Dig Dis Sci, 1998, 43, S23-9.
- [5] Toma W. Hiruma-Lima CA., Guerrer RO., Souza AR., Preliminary studies of Mammea Americana L (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice, Phytomedicine, 2005, 12, 345-50.
- [6] Borelli F., Izzo AA., The plant kingdom as a source of anti-ulcer remedies, Phytother Res 2000, 14, 581-91.
- [7] Alok Bhardwaj, Rajeev Kumara, Vivek Dabasa, Niyaz Alam. "Evaluation of anti-ulcer activity of citrullus lanatus seed extracts in wistar Albino rats". International Journal of Pharmacy and Pharmaceutical Science, Vol 4, Suppl 5, 2011.
- [8] Kaleem M, Asif M, Ahmad QU, Bano B. Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin induced diabetic rats. Singapore Med J 2006; 47:670-675
- [9] Trease and Evans, Pharmacognosy, 15thedition. W.B. Saunders Company Ltd., 1996.95-99.6.
- [10] Wallis T.E. Text book of Pharmacognosy, 5thedition., CBS Publisher & Distributors, New Delhi, 2002.571-578.
- [11] WHO guidelines for Quality Control Methods for Medicinal Plant Materials. Geneva, 1998:28-30.
- [12] Indian Herbal Pharmacopoeia, A joint publication of regional research laboratory and Indian drug manufacturers association, 1999: 2.
- [13] Indian Pharmacopoeia, Vol II, 4thed, New Delhi: 1996: A-53-54.
- [14] Mukherjee PK. Quality control of herbal drugs-An approach to evaluation of botanicals. 1sted. Business Horizons Pharmaceutical 36 Publisher, New Delhi: 2002: 187-196 and 428-456
- [15] Pollock, J. R. A. and Stevens R. Dictionary of organic compounds. 4th edition, vol. 5, Eyre and Spottish woode, London. 1965
- [16] Wagner H. et al. (1984), Plant Drug Analysis A Thin Layer Chromatography Atlas. Berlin, Germany, Springer-Verlag. 195-211
- [17] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, Nirali Prakashan; 39th edition. Aug. 2007. 284-285.
- [18] Kokate CK. Practical Pharmacognosy. Pune: Vallabh Prakashan; 2003.
- [19] Swati S. Kansara, Manmohan Singhal. "Evaluation of Antiulcer Activity of Moringa Oleifera Seed Extract". JPSBR: Volume 3, Issue 1: Jan Feb 2013 (20-25).
- [20] Pankaj Kushwah, Nandu Kayande, Department of Pharmacognosy, Nimar Institute of Pharmacy, MP. "Antiulcer Activity of Annona Squamosa Seed Extract in Experimental Rats". Pharma Tutor Magazine 174 ISSN: Vol. 2, Issue 6: 2347-7881.
- [21] Shay H, Komarov SA, Fele SS, Meranze D, Gruenstein H, Siple H. A simple method for uniform production of gastric ulceration in rat, Gastroenterol, 1945, 5, 43-61.
- [22] Kulkarni SK. Hand book of experimental pharmacology, Vallabh Prakashan, New Delhi, 1999, pp 148-50.
- [23] Wagner R and Bladt S, Plant Drug Analysis, A Thin Layer Chromatography Atlas, 2nd edition Springer; Berlin, 1996.
- [24] Chen HYL in YC, Hsieh CL. Evaluation of antioxidant activity of aqueous Extract of some selected nutraceutical herbs. Food Chemistry 2007; 104: 1418-20.
- [25] Khare C P Indian medicinal plants, An Illustrated Dictionary, Springer publication.

Author Profile



S B Bavage, Latur college of Pharmacy Hasegaon, Maharashtra, India

Birendra Shrivastava, Jaipur National University, Jaipur. Rajasthan, India.



Ganeshan Sharma, Jaipur National University, Jaipur. Rajasthan, India.



Aamer Quazi, K.T. Patil college of Pharmacy, Osmanabad, Maharashtra, India.