ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 1 Issue 5

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

Pharmacognostical and Phytochemical Studies on the Leaf and Stem Bark of *Annona reticulata* Linn.

Kamaruz Zaman[1], Kalyani Pathak[1*]

1. Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India. [E-mail: kalyakster@gmail.com, Mob: +91-9707630543]

The present work was undertaken to establish the pharmacognostic and phytochemical standards of Leaf and Stem bark of *Annona reticulata* L. It is a highly apparent plant in ayurvedic system of medicine for the treatment of various ailments. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer . No reports are available on the pharmacognostic nature and phytochemical studies of the leaf and stem bark , 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 this plant for the future references

Keyword: *Annona reticulata* L., Pharmacognostic, WHO , Phytochemical.

1. Introduction

Annona reticulata Linn. also known as Sitaphala , Sarifa, Custard- Apple, belongs to the family Annonaceae. *Annona reticulata* Linn. is widely cultivated throughout India as an fruit consuming plant & deciduous tree. It is a native of South America and West Indies, also cultivated in Bangladesh and Pakistan.

Annona reticulata L. is a highly apparent plant in ayurvedic system of medicine for the treatment of various ailments. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has antifertility, antitumour and abortifacient properties. Ethanolic extracts of leaves and stem are reported to have an anticancerous activity. The aqueous leaf extract has also been reported to ameliorate hyperthyroidism, which is often considered as a causative factor of DM[1]. Ripe fruit is sweet, cooling, good tonic and sedative. It enriches the blood, increases muscular strength, lessens burning sensation, tendency to biliousness and vomiting. Leaf can be used for destroying lice[2]. Previously reported phytochemical constituents from the plant are anonaine, roemerine, norcorydiene, corydine, norisocorydine, Carvone, linalool, samoquasine A, squamocin-I, squamocin-B, squamocenin, motrilin, Kaurenoic acid, phenolic and nonphenolic alkaloids , two crystalline alkaloids – muricine, muricinine, (2, 4-cis and trans)-squamolinone, (2, 4-cis and trans)-9-oxoasimicinone, bullacin B[3,4] etc.

The present investigation deals with the qualitative and quantitative pharmacognostical evaluation of the leaf and stem bark of Annona *reticulata* L.

2. Materials and methods

2.1 Collection and Authentication of the Plant material

The leaves and stems bark of *Annona reticulata* L. were collected from the plant in the month of July 2011 from Sivasagar, Assam. The plant was authenticated by Dr. B.K. Sinha (HOO), Botanical Survey of India, Shillong.

2.1.2 Pharmacognostical studies:

a) Morphology

Morphological studies such as shape, size, apex, surface, colour, margin, venation, taste and odour of leaves were carried out.

b) Microscopy

Microscopical studies were carried out using Nicon Labphot-2 instrument (Japan). Care was taken to select healthy plants and for normal organs. The required samples were cut and removed from the plant and washed with water. After proper washing, the specimens were cleaned with graded series of tertiary-butyl alcohol[5]. In the next step thin sections were cut transversely. Removal of oily/waxy matter from the sections was performed by customary procedure[6]. The sections were stained with saffranin as per the method published by O'Brien[7]. The dye rendered pink colour to the cellulose walls, wherever necessary sections were also stained with safranin and KI (for starch).For studying the stomatal morphology and venation pattern paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 10% potassium hydroxide were prepared[8]. Glycerine mounted temporary preparations were made for macerated/cleared materials.

As a part of quantitative microscopy, stomatal number, stomatal index, vein islet number and vein termination number were studied by taking paradermal sections as well as clearing of leaf with 5% sodium hydroxide and epidermal peeling off .

c) 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 based upon standard procedures[9].

Phytochemical analysis

For preliminary phytochemical studies, 60 g of powdered material was extracted in soxhlet apparatus with petroleum ether, chloroform, methanol and water. Extracts were dried in rotary evaporator and weighed. The presence of various phytoconstituents like steroids and triterpenoids, alkaloids, glycosides, flavonoids were detected as per standard procedures given in standard text[10,11].

3. Result and Discussion

Macroscopically the leaf was found to be leaves oblong-lanceolate, pellucid-dotted, peculiarly scented light green in colour whereas stem bark was found to be acute, yellowish brown to brown in colour with an unpleasant scent [Table 1]. The T.S. of lamina of leaf showed the presence of single layered epidermal cells, mesophyll differentiated into palisade tissues and spongy parenchyma of 3 to 5 layers, stomata anomocytic, present on lower surfaces only [Figure 2]. T.S. of midrib showed single layer epidermis on both surfaces, collenchymatous cell, followed by thin walled, round or oval parenchymatous cells. Xylem is composed of radially arranged vessels, xylem fibres and xylem parenchyma. Vessels are arranged vertically, lignified, having annular, spiral, scalariform thickenings. The T.S. of stem bark showed the presence of single layered cork cell beneath which cortex is present. Lower portion of T.S. Showed the presence of several layers of vascular tissues i.e. xylem and phloem [Figure 3]. Medullary rays are also present with the vascular tissues [Figure 3]. Powder microscopy of leaf shows the presence of spiral vessel, epidermal cell, trichome with leaf attachment, parenchyma cells. Whereas powder microscopy of stem bark shows the presence of polygonal epidermal cells, trichome, vascular bundles, calcium oxalate crystals etc. The values of the physical constant like ash values, extractive values, loss on drying were determined [Table 3]. Preliminary qualitative phytochemical screening of the Leaf extracts revealed the presence of

carbohydrate, fats and oils, terpenoids, flavonoids, amino acids, tannins and phenolic compounds, alkaloids, glycosides and steroids [Table 5] .Methanolic extract of leaf contains maximum number of active constituents. Whereas extracts of Stem bark revealed the presence of alkaloids, fats and oils, steroids, lignin, tannins and phenolic compounds and triterpenes [Table 4]. Here also methanolic extract shows the presence of maximum number of active constituents.

In case of Leaf of *Annona reticulata* L**.**, methanol extract showed more clear and maximum number of spots in three different solvent systems than other extracts of leaf. Whereas, methanol extract of stem bark extract also showed maximum number of spots in TLC plate. It can be concluded that Methanolic extract of both leaf and stem bark contain maximum number of active constituents than other solvent extracts. All the three solvent systems are efficient in separation of phyto-constituents.

Table 1: Macroscopic Features of Leaves and Stem bark of *Annona reticulata* L.:

Characteristics	Leaf	Stem Bark
Shape	Oblong-lanceolate, entire, pellucid-punctate	Acute
Colour	Light green	Yellowish brown to brown
Odour	Unpleasant	Unpleasant
Taste	Mucilaginous, bitter	Mucilaginous, bitter

Table2: Values of the leaf constants of *Annona reticulata* L.:

Characteristics	Values (Average value of 3 replicates ± SD)
Stomatal Number	17.25 ± 0.83
Stomatal Index	14.17 ± 0.22
Vein Islet Number	7.00 ± 0.71
Vein Termination Number	9.25 ± 0.43
Palisad Ratio	4.70 ± 0.28

Table 3. : Values of the physical constants of *Annona reticulata* L.:

Parameters	Leaf (Average value of 3 replicates ± SD)	Stem bark (Average value of 3 replicates ± SD)
Total Ash	15.10±0.16	6.62 ± 0.38
Acid insoluble ash	0.89±0.06	0.503±0.05
Water soluble ash	4.45 ± 0.07	4.41±0.15
Water soluble extractive	3.07± 0.09	4.21±0.05
Alcohol soluble extractive	7.61±0.42	4.32± 1.24
Loss on drying	7.52±0.17	6.27±0.02

Table 4: Preliminary phytochemical tests of *Annona reticulata* L. Stem bark:

Plant Constituents	Petroleum Ether	Acetone	Chloroform	Methanol	Water
Alkaloids	-	+	+	+	-
Amino acids	-	-	-	-	-
Carbohydrates	-	-	-	-	-
Fats & Oils	+	-	-	-	-
Flavonoids	-	-	-	-	-
Glycosides	-	-	-	-	-
Gums	-	-	-	-	-
Lignin	+	-	+	-	-
Mucilage	-	-	-	-	-
Proteins	-	-	-	-	-
Steroid	+	+	+	-	-
Saponins	-	-	-	-	-
Tannins & Phenolic compounds	-	-	-	+	+
Triterpene	+	-	+	-	-

Table 5: Preliminary phytochemical tests of *Annona reticulata* L. Leaf :

Plant Constituents	Petroleum Ether	Chloroform	Methanol	water
Alkaloids	-	+	+	-
Amino acids	-	+	+	+
Carbohydrates	-	+	+	+
Fats & Oils	+	-	-	-
Flavonoids	-	+	+	_
Glycosides	-	+	+	+
Gums	-	-	-	-
Lignin	-	-	-	-
Mucilage	-	-	-	-
Proteins	-	+	+	+
Steroid	+	+	+	_
Saponin	-	-	-	+
Tannins & Phenolic compounds	-	-	+	+
Triterpene	+	-	-	-

Table 6: TLC profile of *Annona reticulata* L. Leaf extracts:

S. No.	Chromatography Solvent	Leaf Extracts	Number of Spots	R_f Values	Visualizing Agents
i.	Petroleum Ether : Ethyl Acetate : Glacial Acetic Acid (4 : 1 : 1)	Petroleum Ether	2	0.63,0.90	Iodine
		Chloroform	2	0.59,0.76,	Iodine
		Methanol	3	0.47,0.64,0.78	Iodine
ii.	Toluene: Ethyl acetate: Formic acid (9:0.5:0.5)	Petroleum Ether	1	0.44	Iodine
		Chloroform	1	0.21	Iodine
		Methanol	2	0.44,0.20	Iodine
iii.	Petroleum Ether : Ethyl Acetate : Glacial Acetic	Petroleum Ether	3	0.57, 0.69, 0.77	Iodine
		Chloroform	2	0.59, 0.82	Iodine

	Acid (2.5 : 1.5 : 1.0)	Methanol	3	0.58, 0.69, 0.87	Iodine

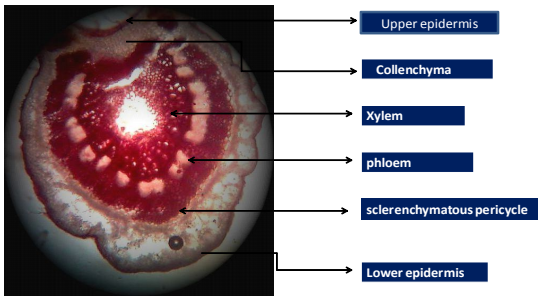

Fig 2: Anomocytic stomata of leaf of *Annona reticulata* L.

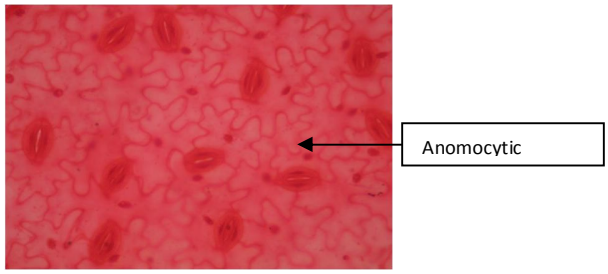

Fig 1: T.S. of Leaf of *Annona reticulata* L.

Table 6.4 . TLC profile of *Annona reticulata* L. Stem bark extracts:

S. No.	Chromatography Solvent	Stem Bark Extracts	Number of Spots	R_f Values	Visualizing Agents
i.	Petroleum Ether : Ethyl Acetate : Glacial Acetic Acid (4 : 1 : 1)	Petroleum Ether	1	0.58	Iodine
		Chloroform	1	0.72	Iodine
		Methanol	2	0.42,0.64	Iodine
		Acetone	1	0.63	Iodine
ii.	Toluene:Ethyl acetate:Formic acid (9:0.5:0.5)	Petroleum Ether	1	0.44	Iodine
		Chloroform	1	0.21	Iodine
		Methanol	2	0.53,0.77	Iodine
		Acetone	1	0.51	Iodine
iii.	Petroleum Ether : Ethyl Acetate : Glacial Acetic Acid (2.5 : 1.5 : 1.0)	Petroleum Ether	2	0.57, 0.63	Iodine
		Chloroform	2	0.59, 0.86	Iodine
		Methanol	2	0.44, 0.69,	Iodine
		Acetone	1	0.35	Iodine

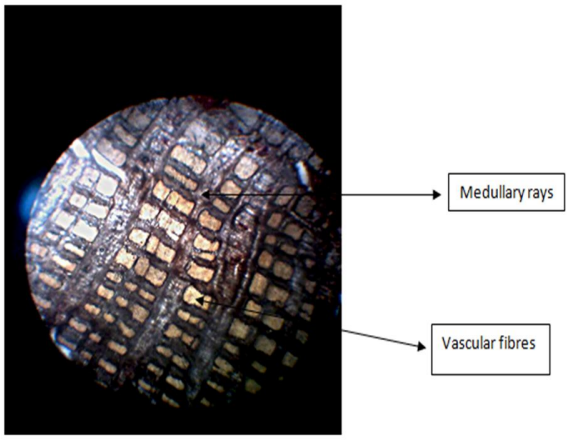

Fig 3 : T.S. of stem bark of *Annona reticulata* L.

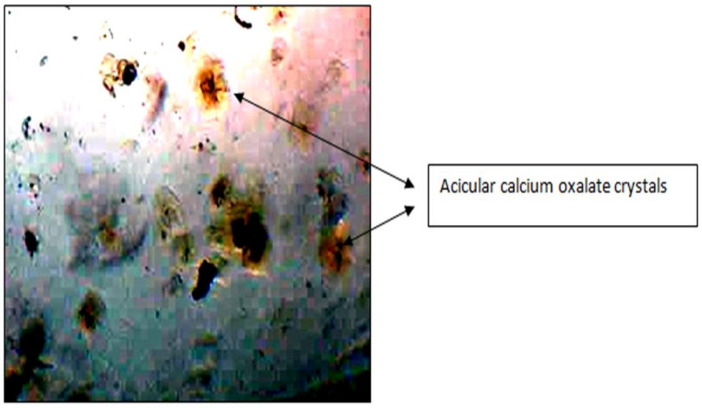

Fig 4: Powder Microscopy of Stem Bark of *Annona reticulata* L.

Fig 5: Powder microscopy –
Leaf multicellular trichome with blunt tip

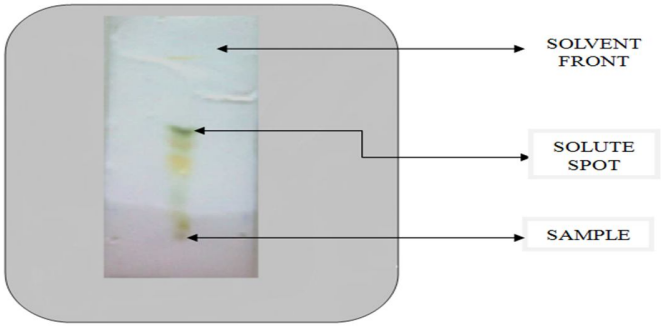

Fig. 6.1: Picture of TLC plate – Methanol extract of Leaf of *Annona reticulata* L. in Petroleum Ether : Ethyl Acetate : Glacial Acetic Acid(4 : 1 : 1)

4. Conclusion

The Plant *Annona reticulata* L. has been studied to give detailed reports on pharmacognostical and preliminary phytochemical studies made on it. These parameters were determined which gives valuable information. This will help for correct identification of this plant for the future references.

The extracts of both leaf and stem bark were subjected to preliminary phytochemical tests and the results indicated the presences of carbohydrate, fats and oils, terpenoids, flavonoids, amino acids, tannins and phenolic compounds, alkaloids, glycosides and steroids in leaf extracts, whereas extracts of Stem bark revealed the presence of alkaloids, fats and oils, steroids, lignin, tannins and phenolic compounds and triterpenes. The maximum number of phytoconstituents was found in the methanolic extract of the leaves using preliminary phytochemical study i.e. phytochemical tests and TLC. The extractive values indicate that plant materials (leaf and bark) contain phytochemicals with better solubility in alcohol than water. The results from the ash value, acid insoluble ash and water soluble ash values suggested that the leaf contains demonstrable quantity of inorganic salts and calcium oxalate.

This could also play an important role in the establishing data for preparation of monograph of the plant.

5. Reference

1. 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.
2. Morton, J., Sugar apple. *Fruits Warm Climate*, 1987; 69–72.
3. Chopra R.N. ; Nayar S.L. ; Chopra I.C. , Glossary of Indian Medicinal Plants, 6th reprinted edition, Publications and Information Directorate, CSIR, New Delhi, 2002, 20.
4. Li, X. H., Hui, Y. H. and Rupprecht, J. K.. Bullatatacin, bullatacinone, and squamone: a new acetogenin from the bark of *Annona squamosa*. *J. Nat. Prod.*, 1990, 53:81–86.
5. Kokate C.T. Purohit A.P. ,Gokhale S.B., Practical pharmacogosy. 4th edition, Vallabh Prakasan, Delhi, 2002
6. Johansen D.A. , Plant Microtechnique. McGraw Hill Book Co., New York,1940
7. O'Brien, T.P., Feder, N., McCull, M.E. . Polychromatic Staining of Plant cell walls by Saffranin. Protoplasma, 1964, 59: 364-373
8. Basu K. , Plant Anatomy. John Wiley and Sons, New York,1964 .767.
9. World Health Organisation, Quality Control Methods for Medicinal Plant Material, Geneva , A.T.T.B.S. Publishers ,New Delhi, 1998 , 28-33,.
10. Pollock, J.R.A. and Stevense, R. . Dictionary of organic compounds., 4th edition, vol. 5, Eyre and Spottish woode, London.1965
11. Wagner H. *et al*. (1984), Plant Drug Analysis—A Thin Layer Chromatography Atlas. Berlin, Germany, Springer-Verlag. 195-211.