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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL STUDIES OF *ANNONA RETICULATA LINN*

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ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. *Annonareticulata Linn* is a multipurpose tree with traditional uses as an antioxidant, antidiabetics, hepatoprotective, cytotoxic activity, genetoxicity, antitumour activity, antilice agent. It is related to contain alkaloids, carbohydrates, fixed oils, tannins & phenolic. To supplement the necessary information for the systematic identification and authentication of this particular species, pharmacognostic standardization, macroscopical, microscopical study of various parts of this plant as per WHO guidelines and phytochemical studies on various crude extracts obtained from the leaf extracts were carried out using three different polar solvents and the results were reported.

Keywords: Annonareticulata, pharmacognostical standardization, physicochemical studies.

INTRODUCTION

According to the WHO survey 80% populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs. Exploration of thechemical constituents of the plants & pharmacological screening may provide us the basis for developing the leads for development of novel agents. In addition, herbs have provided us some of the very important life saving drugs used in the modern medicine. However among the estimated 250,000-400,000 plant species, only 6% have been studied forbiological activity and about 15% have been investigated phytochemically[1,2]. *Annonareticulata Linn* is a small ever green tree is cultivated throughout india for its fruits, differentparts of *Annonareticulata Linn.* are used in folkloric medicine for the treatment of variousdisease[3]. This plant is commonly called custared apple in English &ramaphalam in telugu in india[4]. Annonareticulatalinn.is a shrub or small tree 7 m height & is cultivated throughout India. To ensure reproducible quality of herbal products, authentication of the starting material is essential. According to WHO (1988), the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken.

Annonareticulata Linn, belonging to family*Annonaceae*is commonly found in India &cultivated in Thailand & originates from the WestIndies &south America. It is mainly grown in

gardens for its fruits & ornamental value.It is known as custard apple, sugar apple, sweetapple in english, ramaphalam intelugu in india[5]A root decoction is taken as a febrifuge, whilefragments of the root bark are packed around the gums to relieve toothache. The bark

is very astringent and the decoction is taken as a tonic and also as a remedy for diarrhea and dysentery. The leaf decoction is given as a vermifuge. Crushed leaves or a paste of the flesh may be poulticed on boils, abscesses and ulcers. The unripe dried fruit dried is employed against diarrhea and dysentery. In severe cases, the leaves, bark and green fruits are all boiled together for 5 minutes in a liter of water to make an extremely potent decoction.[6] (Morton, J. 1987. Fruits of Warm Climates.)

Fig. 1: leaf and fruit of Annonareticulate

Collection of Specimens

The leaves of *Annonareticulata*Linn were collected fromthe nearby area of tirumala hills in august 2010 and were authentified by prof. D. Ramakanthraju retirebotanist and a voucher specimen (T.S.N-005, 12/08 /2010)has been deposited in pharmacognosy department Andhrauniversity.

Care was taken to select healthy plants and for normal organs. The leaf, stem, root and stem bark were cut and removed from the plant and fixed in formalin acetic acid solution (Formalin:acetic acid:70% ethyl alcohol in the ratio of 0.5:0.5:9). After 24 h of fixation, the specimens were dehydrated with graded series of tertiary-Butyl alcohol(Sass J E; 1940). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58 - 60°C) until thiobarbituric acid solution attained super saturation. The specimens were then casted out into paraffin blocks.

MATERIALS AND METHODS

Preparation of sections

The paraffin embedded specimens were sectioned with the help of a rotary microtome. 10 - 12 µm thickness of the sections was made. However, dewaxing of the sections was done using customary Procedure[7]. The sections were later stained with toluidine blue[8]. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. For the study of isolated cells leaves were macerated with concentrated nitric acid and potassium chlorate, washed with distilled water and mounted in glycerine.qualitative and quanititave microscopic evaluation was done along with Ultraviolet fluorescence analysis of powdered drug. Collected leaf powder was subjected to preliminary and microscopical examination, physiochemical evaluations which include ash value, acid insoluble and water soluble ash, extractive value (hexane soluble, ethyl acetate solube and ethanol soluble) and moisture contents (loss on drying), swelling index, foaming index were determined[9].

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photomicrographs of different magnifications were taken with electron microscopic unit of quantum model. For normal observation, a bright field microscopy was used and for the study of crystals, starch grains and lignified cells, a polarized light was employed. However, since these structures have birefringent property, under polarized light they tend to appear bright against the dark background[10].

RESULTS AND DISCUSSION

Morphological study

*Annonareticulata*is a small, semi-deciduous tree, 3-7m in height, with a broad, open crown or irregularlyspreading branches, bark light brown with visible leafscars and smoothish to slightly fissured into plates.

Inner bark: light yellow and slightly bitter

Twigs: Become brown with light brown dots.

Leaves: occursingly, 6-17 x 3-6 cm, lanceolate or oblong lanceolate,pale green on both surfaces and glabrate or nearly so[1,2]. Sides sometimes slightly unequal, edges without teeth, inconspicuously hairy, at least when young,minutely dotted on examination with a lens, thin, dullgreen to dark green on top surface, and pale blue-greenand covered with bloom on underside; apex short orlong pointed. Base short pointed or rounded.

Petioles:0.6-1.3 cm long, green, sparsely pubescent.

Flowers:greenish-yellow, fragrant, on slender hairy stalks, Produced singly or in short lateral clusters about 2.5 cmlong, 2-4 flowers but not at the base of the leaves;sepals pointed, hairy, green, about 16 mm long, 3 outerpetals oblong, thick and rounded at the tips, fleshy, 1.6-2.5 cm long, 0.6 cm wide, yellow-green, slightly hairy,inside light yellow and keeled with a purplish orreddish spot at the thin, enlarged base. Inner petals 3minute, ovate, pointed scales.

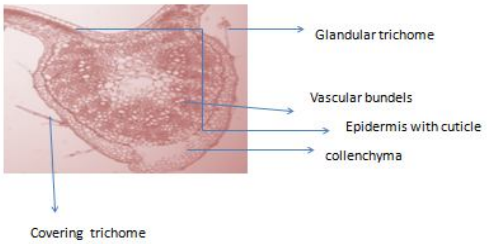
Stamens: very numerous,crowded, white, less than 16 mm long.

Ovary:lightgreen,

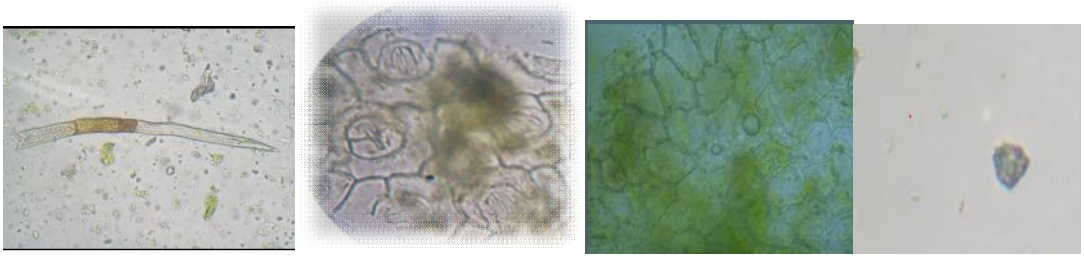
Styles: white, crowded on the raised axis.
Theaggregate fruit formed from the numerous pistils of aflower, which are loosely united, is soft and distinctfrom other species of the genus.
Each pistil forms aseparate tubercle, mostly 1.3-1.9 cm long and 0.6-1.3cm wide. Fruit is round, heart shaped, ovate or conical,5-10 cm in diameter, with many round protuberances;greenish-yellow when ripe, with a white, powderybloom. The pulp is white, edible and sweetly aromatic.
In each carpel is embedded a seed, oblong, shiny andsmooth, blackish or dark brown, 1.3-1.6 cm long,numerous[3-4].(fig. 1)

T.S of leaf: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 radiallyarranged. Vascular bundle surrounded by pericyclic fibres on both the side, rest of consist parenchyma cells, in center a group of stone cells is observed (fig. 2)surface study the upper and lower epidermis of the leaf was peeled off and observed under the microscope, the upper epidermis show only epidermal cells and lysogenous cavity and oil globules where as lower epidermis shows paracytic stomata epidermis cells, lysogenouscavity, oil globules (fig. 2)

T.S of Annona reticulata leaf



Powder microscopy
powder shows paracytic stomata from lower surface, fragment of fibers with narrow lumen, multicellular trichome filled with tannin content from epidermal surface, microrosette crystals of calcium oxalate pitted stone cells with wide lumen, annular vessels from vascular bundle (fig. 3)



Paracytic stomata **multicellular covering trichome with tannin** **epidermal cells with oil glands** **microrossette calcium oxalate crystal**

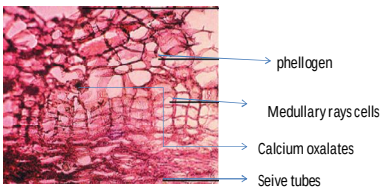
Group of stone cells **annular vessels** **epidermal cells**

Transverse section of stem

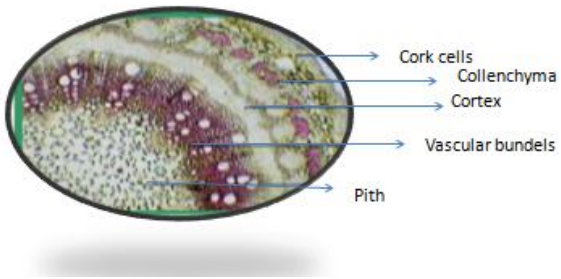
It shows collenchymatous cells below epidermis, followed by pericyclicfibers, xylem, phloem and parenchymatous cells. Xylem is surrounded by starch grains and pith contains lignified stone cells. Starch grains are oval or ellipsoid, turning blue when treated with iodine.

Transverse section of stem bark showed the presence of 7-8 layers of uniformly arranged cork cells followed by cortex cells, it also contains radially dividing parenchyma cells. Also contains wood elements, lignified fibers

T.S of Annona reticulata stem bark

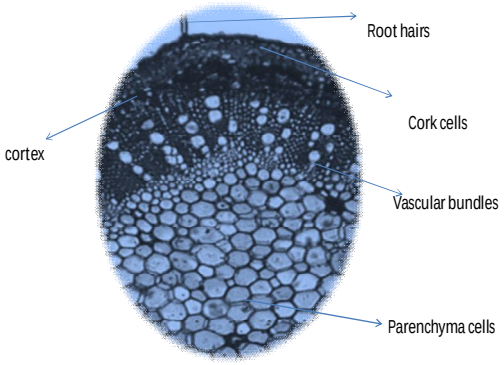


T.S of Annona reticulata stem



Transverse section of rootshows wide cortex made up of a group of stone cells, the phloem shows large sieve tubes interspersed with phloem parenchyma and fibres. The cell types and cell inclusion are detected in the powdered root bark.

T.S of Annona reticulata root



Determination of quantitative microscopic evaluation of Annonareticulata leaf

Stomatal index, stomatal number, vein islet, vein termination number was determined for the peeled of Annonareticulata leaf and the results were given in the Table 1.

Determination of physicochemical parameters of Annonareticulata leaf

Fresh materials of *A. reticulata* leaves, were collected and subjected to various physicochemical parameters such as moisture content were observed and recorded. Ash values are helpful in determining the quality and purity of crude drug, especially in the powder form. Total ash reflects the care taken in its preparation as all traces of organic matters were removed during ash formation and usually consists of carbonates, phosphates and silicates

of sodium, potassium, calcium and magnesium. (Table 2)

Table 1: Quantitative microscopic results

Leaf constants	A.reticulata
Stomal number upper	164-178
Lower	182-196
Stomatal index upper	16.3-19.5
Stomatal index lower	22.5-26.4
Palisade ratio	9-11
Vein islet number	8.5-10.6
Vein termination number	11.3-16.4

Physicochemical properties	A.reticulata Value in %w/w
Total ash	16.5
Acid insoluble ash	16.1
Water soluble ash	7.5
Extractive values hexane extract	5.5
Ethylacetate extract	18.2
Alcohol extract	19.3
L.o.d	5
F.o.m	2.1
Swelling index	6
Foaming index	-
Volatile oil content	0.0

Table 2: Results of physicochemical properties

Table 3: Analysis of leaf powder of Annonareticulatalinn

s.no	Reagents with powder	Daylight	Short wave length	Long wave length
1)	Leaf powder	Green	Light green	Dark green
2)	Powder+water	Dark green	Brown	Brownish red
3)	Powder+ethanol	Drak brown	Light red	Dark red
4)	Powder +dilHCl	Light brown	Light brown	Light brown
5)	Powder + dil H_2So_4	Dark brown	Dark brown	Dark brown
6)	Powder + dil HNo_3	Red	Orange red	Reddish orange
7)	Powder + aq.NaoH	Dark green	Dark brown	Dark green
8)	Powder + alc.NaoH	Dark green	Light Red	Dark Red
9)	Powder + aq.KOH	Light green	Light green	Light green
10)	Powder + alc.KOH	Green	Light brown	Darkbrown

Table 4: Analysis Of Extracts Annonareticulata Linn

s.no	Extract	Nature of extract	Appearance in Day light	Short wave length	Long wave length
1	Hexane extract	Semi solid	Dark green	Light green	Dark red
2	Ethyl acetate extract	Semi solid	Light green	Light green	Dark red
3	Alcohol extract	Semi solid	Greenish brown	Dark brown	Reddish brown

Table 5: results of phytochemical tests of various leaf extracts of Annonareticulatalinn

s.no	Test	Hexane extract	Ethyl acetate extract	Ethanolic extract
1.	Alkaloids	-	+	+
2.	Aminoacids	-	-	-
3.	Carbohydrates	-	+	+
4.	Flavonoids	-	+	-
5.	Mucilage	-	+	+
6.	Proteins	-	+	-
7.	Starch	-	-	-
8.	Steroids&triterpenoids	+	-	-
9.	Glycosides	-	-	+

Preliminary phytochemical studies (Table 5)

The qualitative chemical investigation of all the extractsof selected plant was carried out to check the presence ofvarious phytoconstituents. It revealed the presence of steroids in hexane extract. Ethylacetate extract revealed the presence of alkaloids, carbohydrates, flavonoids, mucilage. Where asethanolic extract revealed the presence of alkaloids, carbohydrates, mucilage, glycosides

CONCLUSIONS

Medicinal plants are valuable natural sources and regarded as potential and safe drugs. They have been playing an important role as natural drugs to alleviate humansufferings by contribution herbal medicines to the primary

health care systems of rural and remote areas where more than 70% of population in India depend on folklore and traditional systems of medicines[11].From the pharmacognostic, and phytochemical investigations, it is quite possible to set the standards of this plant as per the pharmacopoeial guidelines and it will be use full for selecting the proper herb and for the research to carry out.

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