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Review

Pharmacognostical and Preliminary Phytochemical Screening of *Annona muricata* Linn

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Abstract

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The present research was focused in drawing the pharmacopoeial monographs along with preliminary phytochemical analysis of Annona muricata Linn (leaf). Pharmacognostical studies and phytochemical analysis was carried out by using standard procedures. Pharmacognostical parameters such as macroscopy, microscopy, quantitative microscopy, powder analysis, behaviour of powder with various reagent. Phytochemical screening of the hydroalcoholic extract was performed to investigate the presence of secondary metabolites. Leaves of Annona muricata, simple entire margin, equal base acuminate apex, length - 6.48-12.68cm, breadth 2.08- 4.4cm, alternate phyllotaxy and reticulate venation. Transverse section of leaf showed upper epidermis with thin cuticle, followed by ground cells nad mid rib, that consists of vascular bundle, parenchymatous cells and collenchyma. Quantitative microscopy parameters such as vein islet number, vein termination number, stomatal number, stomatal index and epidermal cells. Powder microscopical analysis showed the presence of uniseriate multicellular covering trichomes, pitted vessels, bundle of fibres and calcium oxalate crystals. Behaviour of powder with various reagent characterised the colour change under visible light and UV of short and long wavelength. Preliminary Phytochemical screening detect the presence of flavonoids, alkaloids, tannin, reducing sugar and phenolic compounds. The results have established a clear identification, authentication pharmacognostical frame work for the leaves of Anona muricata. The results of this study reported that hydroalcoholic extract revealed the presence of alkaloids, flavonoids and phenols.

Keywords: Annona muricata, Pharmacognostical, Phytochemical analysis

INTRODUCTION

Annona muricata (Graviola) belongs to Annonaceae, is a widely distributed in the tropical regions of Central and Soth America, Western Africa, Central and Eastern Africa and south east Asia [1, 2]. In West Indies

traditional practice, decoction from leaves is used to treat hypertension, asthma, difficulty during child birth and diarrhoea. In Togo traditional system of medicine leaves are used to treat malaria [3]. According to West and Peruvian Andes, Brazilian Amazon folklore medicine, leaf preparations are used as sedative, to relieve rheumatism, neuralgia and arthritis to treat liver problems, and as anti- spasmodic effect [4, 7]. In Malaysia customary medicine leaves are used for treating high pressure [5,6]. In the Eastern Andes and Jamaica system of medicine the juice of graviola was used to control diarrhoea, as muscle relaxant and to lower the intestinal acidity [8]. Annona muricata leaves are employed to treat hypertension, diabetes [9,10,11] and cancer [12-17]. For decades, in African countries traditionally leaf, bark, root, and fruits preparations are used as anti inflammatory, to treat diabetes, headaches, rheumatism and insomnia [18,19]. The literature survey of this plant indicated the presence of essential oils, alkaloids, terpenoids, flavonoids, acetogenein, leaves contain muricapentocin [20], annonacin A [21], giganttetrocin [22], solanine [23] annocatachin B [24,25] gallic acid, epicatechine, catechin [26], annoinnol, annoionside, vomifolol, roseoside [27].

Phytochemical review reported the presence of annonaine, quercetin, kaempferol, annonamine, annomuricatin A, annomuricatin C, annonacin, annocatacin. It includes minerals, sodium, potassium, copper, calcium, iron and magnesium. GC-MS analyis of the plant showed the presence of hydrofurans and epoxides [28,29]. It is effective against Herpes infection[30], exhibited anti-ulcer[31], anti-plasmodial [32] anti-protozoal [33], anti-diabetic [34], hypotensive [35], anti-microbial [36], effective against breast and colon cancer [36-39], anti-proliferative [40,41], anti oxidant [42]. Anti-viral effective against dengue type 2 virus and corono virus [43-45], anti-noceptive [46], anti-inflammatory[47], anti-hyperglycemic [48], effective against pancreatic cancer [49,50]. The literature survey revealed that the pharmacognostical parameters for the leaf have not been studied so far

It is crucial to derive the pharmacognostical monographs for this medicinally potent *Annona muricata*. Therefore, it is immediate and essential to investigate the pharamacognostical studies for the leaf of *Annona muricata*

Methods

Collection and authentication

Annona muricata leaves were collected from areas in local garden, Batlagundu, Tamil Nadu, India during September 2023 and was authenticated by Dr Stephen, Department of Botany, The American college, Madurai, Tamil Nadu, India. The herbarium was preserved and kept in the dept for further reference.

Macroscopical studies of Annona muricata

Leaves were studied separately for its morphological characters of the leaves were studied and was presented in figure 1 and table 1.

Microscopical studies of Annona muricata

Hand free thin sections were taken, was stained with routine methods and was observed under microscope. Transverse section and its characters were picture was presented in figure 2.

Quantitative microscopy

Quantitative microscopical parameters such as vein islet ,vein termination number, palisade ratio, epidermal cells, stomata type, number and index are determined as per Ayurvedic pharmacopoeia resulted in table 2.

Preparation of powder

The leaves were collected, air -dried, coarsely powdered and was screened for the powder microscopy, physio chemical analysis and its behaviour with chemical reagents.

Determination of Physico-chemical parameters

The powder is subjected for physico chemical evaluation parameters such as loss on drying, extractive value with different solvents such as petroleum ether, chloroform, ethyl acetate, benzene, ethanol and water were used in increasing order of polarity, Different ash values are determined as per the standard procedure [51-53] and its results was presented in table 3.

Preparation of hydroalcohol extract of Annona muricata (HAEAM).

About 5 gram of powdered *Annona muricata* was taken and macerated with 70% hydroalcohol, extracted until the complete exhaustion of the material. The extract was concentrated and was found to be 2.25 % w/w and was stored in air tight container for further research purpose.

Determination of Phytochemical screening of HAEAM

HAEAM was subjected to preliminary phytochemical analysis as per procedure described in Harbourne [54].

RESULTS

Macroscopy of leaves

It is observed that leaves are green when fresh, pale green when dried, with characterstic taste and odour. Its length varies from 6.48-12.68cm, breadth 2.08- 4.4cm, margin entire, equal base, acuminate apex, with alternate phyllotaxy and reticulate venation.





Fig 1: ventral view dorsal view of Annona muricate

Table 1: Macroscopy of Annona muricata leaves.

S.no	Particulars	Results
1	Colour	Dark Green
2	Odour	Characteristic
3	Taste	Characteristic
5	Length	6.48-12.68 cm
5	Breadth	2.08-4.4 cm
6	Margin	Entire
7	Base	Equal
8	Apex	Acuminate
9	Phyllotaxy	Alternate
10	Petiole	Short
11	Venation	Reticulate

Microscopy of Annona muricata leaves

Transverse section of Annona muricata leaves was displayed in figure 2 and its structures are observed.

Epidermis

Presence of upper and lower epidermis covered with thin cuticle. It consists of epidermal cells consisted of small polygonal paranchymatous cells.

Mesophyll

Below the upper epidermis a single layer of parenchymatous cells is seen. The mesophyll is clearly differentiated into palisade and spongy parenchyma, spongy parenchyma region is present just below the palisade layer and extends up to the lower epidermis.

Mid rib

The midrib region consists of vascular bundle, parenchymatous cells and collenchyma. Both parenchymatous and collenchymatous cells are present below the large vascular bundle.

Vascular bundles

The vascular bundle is ovoid in shape and consists of xylem vessels (lignified) and phloem fibres (non-lignified). xylem vessels are seen towards the outer side that surrounds the phloem. The phloem consisted of sieve tubes, companion cells and phloem parenchyma.

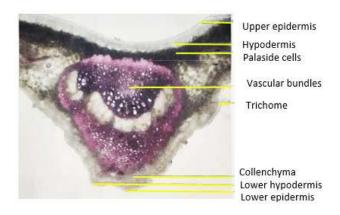


Fig 2: Transverse section of Annona muricata leaf

Table 2: Determination of phyto constants constants of Annona muircata

S.no	Phyto- constants	Results (no of cells /mm ²)
1.	Vein islet number	5
2.	Vein termination number	3
3.	Stomatal number (lower epidermis)	47
4.	Stomatal index (lower epidermis)	25
5	Epidermal cells	208
6	Paliasde ratio	11

Table 3: Determination of physio- chemical parameters of Annona muricate

S.no	Parameters	Results % w/w (Mean ± SEM)
1.	Foreign matter	NIL
2.	Loss on drying	2.691±0.5851
3.	Total solids	97.36±0.58
4.	Total ash	0.09 ± 0.00577
5	Water insoluble ash	0.0330 ± 0.008
6	Acid insoluble ash	0.0475 ± 0.00577
7	Petroleum ether extractive	5.66 ± 0.240
8	Clororform extractive	9.8 ± 0.1
9	Ethanol extractive	16.3 ± 3.07
10	Aqueous extractive	29.5 ± 0.579
11.	Bitterness value	NIL
12	Foaming index	NIL
13	Presence of inorganic elements and	chlorides, nitrates, sulphates, iron
-	metals	-

Behaviour of Annona muricata leaf powder with various reagents

Powder was treated with various chemical reagents and its behaviour colour or fluorescence was observed under visible, UV of (254nm) and (366 nm). The observation may be used as diagnostic identification for this *Annona muricata* leaf powder.

Table 4: Behaviour of Annona muricata leaf powder with various reagents

S.no	Powder +reagent	Visible light	UV light (254nm)	UV light (366nm)
1.	Powder+Conc.Hydrochloric acid	Green	Brown	Black
2.	Powder+Conc.Sulphuric acid	Dull brown	Brown	Black
3.	Powder+Conc.Nitric acid	Dark brown	Black	Black
4.	Powder+Acetic acd	Yellowish brown	Black	golden yellow
5.	Powder+20/Sodium hydroxide	Brown	Dark brown	Black
6.	Powder+Hydrochloric acid + water	Green	Green	Black

7.	Powder+Sulphuric acid+water	Brown	Black	Black
8.	Powder+Nitric acid+water	Orange	Greenish Yellow	Yellow
9.	Powder+20/Sodium hydroxide	Brown	Dark brown	Black

Determination of phytochemical analysis of Haema.

Hydroalcohol extract of *Annona muricata* was screened and the presence of alkaloid, reducing sugar, phenolic compounds, tannins and flavonoids were identified.

Table 5: Determination of phytochemical analysis of Annona muricata.

Test for secondary metabolites	Observation
Flavonoid	+
Saponin	-
Tannin	+
Steroid	-
Alkaloid	+
Triterpenoid	-
Anthraquinone	-
Reducing sugar	+
Cardiac glycosides	-
Phenolic compounds	+
Gum and mucilage	-
	Saponin Tannin Steroid Alkaloid Triterpenoid Anthraquinone Reducing sugar Cardiac glycosides Phenolic compounds

(+ ve) present, (- ve) absent

DISCUSSIONS

It is observed that *Annona muricata* is widely used in various traditional system of medicine in many countries reserves only the knowledge. In oral the scientific approach transforms the traditional information into scientific data base which helps further for other researchers to continue the study of this plant. This transformation may aid to investigate and isolate the phytoconstituents present in *Annona muricata*.

CONCLUSION

This is the first Pharmacognostical report of this medicinally valuable *Annnona muricata* in India. The derived findings help to draw the pharmacopoieal paramters of this plant. Pharmacognostical specifications were determined for the Indian origin of *Annona* includes macroscopy, microscopy, quantitative microscopy and behaviour of powders with various reagents. Physio- chemical and phytochemical reports enhance the usage of this plant medicinally in routine practice. Traditional healers of remote village in Kerela, India is still in practice, using the leaf preparations to treat cancer which can help to restore the information in a scientific manner. The observation may used as diagnostic identification for this *Annona muricata* leaf powder. Total ash reported the presence of chlorides, nitrates, sulpates and iron, upon consumption it can help to reuptake the elements in systemic circulation. Therefore herbal formulations can be developed.

Abbreviation

HAEAM - Hydroalcoholic extract of Annona muricata.

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