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Original Research Article

Preliminary Phytochemical and Physiochemical Profile of *Annona Squamosa* Leaf

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Abstract: The macroscopical, microscopic, and early phytochemical studies on Annona squamosa Linn's leaf are the subject of the current communication. The herb Annona squamosa Linn has stimulant, antispasmodic, sudorific, anthelmintic, and insecticidal qualities, among other medicinal uses. All of the criteria were investigated in accordance with WHO and Pharmacopoeial standards. The quantitative evaluation of flavonoids included analysis that showed the leaf's potential.

Keywords: Annona squamosa, phytochemical analysis, physiochemical analysis & TFC.

INTRODUCTION

The family Annonaceae includes Annona squamosa Linn, also called Sitaphala, Custard Apple, and other names. Throughout India, Annona squamosa Linn. Is commonly grown as an ornamental plant and a deciduous tree. The qualities of leaves have been described as stimulating, antispasmodic, sudorific, anthelmintic, and insecticidal. According to reports, crushed leaves are put on the nostrils during fits and hysteria. In order to cause suppuration, the poultice of the leaves is applied as a cataplasm over boils and ulcers. Additionally, it reduces pain and edoema. [1]. In tissue culture, it has been discovered that the ethanolic extract of the leaves exhibits activity against the epidermoid carcinoma of the human nasopharynx, Walker carcinoma 256, sarcoma 180, and L-1210 lymphoid leukaemia. Micrococcus pyogenes var. aureus has demonstrated antibiotic action when exposed to extracts containing acid, ether, and acetate buffer. The water- soluble component of the alcoholic leaf extract was found to have oxytocic activity on the uterus of rats and to exert spasmogenic effects on the ileum of guinea pigs, as well as stimulate the isolated heart and calm the isolated duodenum of rabbits. The pharmacological activity of the active principle is very similar to that of adrenaline [2]. Herbal medicine is a success of widely accepted medicinal variety. The medicinal plants almost always play a vital role and serve as the foundation of traditional medicine. Setting up standards for quality, safety, and efficacy is a crucial first step in ensuring the safe use of these medications [3]. These facts are taken into account as attempts are made to develop pharmacognostic standards for plant leaves. A huge, straggling, evergreen shrub or small tree with a height of 7 metres that was introduced to India and is now grown in a number of locations up to 900 metres above sea level is called Annona squamosa Linn. Carpels are numerous, lozenge-shaped, on a central torus, fused into an irregularly globose or heart-shaped, tubercled, yellowish green syncarpium, 5-10 cm in diameter; seeds are oblong, deep brownish black, with arils shining; the bark is thin and grey; the

leaves are oblong lanceolate or elliptic, pellucid-dotted, and peculiarly scented, ranging in size from

The current study examines the leaf of Annona squamosa Linn for its macroscopical, phytochemical, and early phytochemical characteristics.

EXPERIMENTAL WORK

Collection of Plant Material

The plant material was gathered in the Shivpuri region between September and October of 2021. (M.P).

Procurement and Authentication of Crude Drugs

The leaves were gathered and verified. The raw medication was then broken up into small pieces for extraction and extractive values and allowed to dry in the air.

Evaluation Parameters

Pharmacognostic Examination

Macroscopic Examination: [5]

Color

Samples of unprocessed crude drugs were tested in dappled daylight. It is also possible to employ an artificial light source with daylight-like wavelengths. It was noted what colour the samples were.

Surface Characteristic, Texture and Fracture Characteristics

To determine surface, texture, and fracture properties, materials were touched.

Odor

A little amount of the sample was placed on the palm of the hand, and the air was inhaled slowly and repeatedly over the substance.

Taste

The taste of a little amount of medication powder was noted as it was maintained over the tongue.

Microscopic Examination: (Kokate, C.K, 2003 & Khandelwal K.R. 2003)

Microscopical analyses of medicinal plants are crucial for both the accurate identification of the plants as well as the research of adulterants.

Evaluation of Physicochemical Parameters

Physical Evaluation: [6]

Determination of Foreign Matter

Microscopical analyses of medicinal plants are crucial for both the accurate identification of the plants as well as the research of adulterants.

Determination of Solvent Extractive Value: [7]

Determination of Water Soluble Extractive Value

In a closed flask, 5 g of powdered crude drug was macerated with 100 ml of water for 2 hours. After that, the mixture was occasionally shook for 6 hours and kept firmly for 18 hours. Following filtration, 25ml of the filtrate was evaporated to dryness in a shallow dish with a flat bottom, dried at 105°C, and weighed. The percentage of extractives that are water soluble was estimated using the drug's air drying value.

Determination of Alcohol Soluble Extractive Value

For extracting different compounds like tannins, alkaloids, resins, etc., alcohol is the best solvent. For the purpose of determining the alcohol soluble extractive, ethyl alcohol (95 percent v/v) was utilised. 5 gm of powdered medication was macerated with 100 ml of ethanol, maintained in a flask with the lid closed for 24 hours, sometimes shaken for 6 hours, then firmly held for 18 hours before filtering. The filtrate was evaporated to dryness in a shallow dish with a flat bottom, dried at 105°C, and weighed. Calculating the percentage of ethanol soluble extractives was done using the air dried medication as a reference.

Determination of Moisture Content

It's important to gauge and manage the crude medications' moisture content. In order to stop the degradation of crude pharmaceuticals due to chemical changes or microbiological contamination, the moisture content should be kept to a minimum.

Procedure

5gm of correctly weighed, coarse-powdered medication samples were stored in IR moisture balance. In comparison to an air-dried sample of the crude medication, the weight loss was expressed as a percentage (percent) of moisture.

Determination of Ash Value

The inorganic salts that adhere to the medicine, either naturally occurring or intentionally added as a type of adulteration, are simply represented by the ash content that is left after cremation. Frequently, different mineral ingredients like sand, soil, calcium oxalate, chalk powder, or other medications with differing inorganic compositions are added with the crude drugs. Ash value was developed to assess the purity of unprocessed medicines. In general, total ash value, acid-insoluble ash value, or both are determined. Phosphates, silicates, silica, adherent dirt, and sand are typically included in this.

Determination of Total Ash

Weighing 2 mg of the air dried crude drug sample, placing it in a tared silica dish, and burning it at a temperature no higher than 450°C until it was carbon-free allowed us to calculate the total amount of ash.

Determination of Acid Insoluble Ash

The ash produced by the previous procedure was heated for 5 minutes with 25ml of 2M HCI, and the insoluble material was then collected, washed with hot water, ignited, cooled in a dessicator, and weighed on ash-free filter paper. With reference to the sample that had been air dried, the percentage of acid-insoluble ash was calculated.

Determination of Water Soluble Ash

The ash was boiled in 25ml of water for 5 minutes, and the insoluble material was then collected on ash-free filter paper, heated in hot water for 15 minutes, and ignited while keeping the temperature below 450°C. The water soluble ash was calculated by subtracting the weight of the insoluble material from the weight of the ash. With reference to the sample that had been air dried, the percentage of water soluble ash was calculated.

Preparation of Extracts

Soxhelation was used to extract the powdered plant material (200gm) using redistilled, analytical grade petroleum ether (40-600C), chloroform, ethanol, methanol, and water.

Qualitative Phytochemical Analysis: [8]

The resulting extracts were subjected to a number of qualitative tests to determine whether or not common phytopharmaceuticals were present.

Determination of Total Flavonoids Content

Using the aluminium chloride colorimetric method, the amount of total flavonoids was calculated as the quercetin equivalent. AlCl3 (2 percent w/v) in methanol was combined with plant extract (10 mg/ml) in the appropriate solvent (stock solution), and the solution was then diluted to 25 ml with a methanolic solution of acetic acid (0.5 percent v/v) (Probe solution PS). 1ml of SS was diluted to 25ml with a methanolic acetic acid solution (contrast solution CS). After 30 minutes, the absorbance of PS and SS was assessed at 420 nm. The outcomes were presented as a percentage of the content of all flavonoids [9].

%TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\ cm}$ x wt. of extract in gm

RESULT AND DISCUSSION

Fruit trees, including Annona squamosa L. (Annonaceae), have been utilized for a variety of purposes for a very long time. A. squamosa, an evergreen plant, is most frequently found in tropical or subtropical regions. Ice cream, sweets, and beverages are typically made with the srikaya fruit, which comes from an A. squamosa plant. A. squamosa plant parts have been linked to a variety of traditional medical purposes, including tonic, apophlegmatisant, cool medicine, abortient, and cardiac sedative. Numerous scientific studies on A. squamosa have revealed that it has anticancer, antioxidant, antidiabetic, antihypertensive, hepatoprotective, antiparasitic, antimalarial, insecticidal, microbicidal, and molluscicidal properties.

As the main source of active plant components, leaves were considered for the inquiry. According to Table 1, the outcomes of the morphological and macroscopic evaluation of leaves are satisfactory in terms of both quality and raw material quality. Many physiochemical variables, such as those in Table 2 and Graph 1, can be used as a reliable indicator of adulteration.

The resulting extracts were also put through a number of qualitative tests to determine whether or not common phytopharmaceuticals were present. The results in table 3 demonstrate whether or not numerous common phytochemicals, including alkaloids, carbohydrates and glycosides, proteins, terpenoids, volatile oils, tannin, and others, are present or absent. According to preliminary phytochemical experiments, the chloroform, methanolic, and aqueous extracts of A. squamosa leaf contained flavonoids. Additionally, the amount of total flavonoids in various leaf extracts was measured using the aluminium chloride colorimetric method, which revealed that the methanolic leaf extract had the greatest level of flavonoids at 2.6 percent TFC (Table 4 and graph 2).

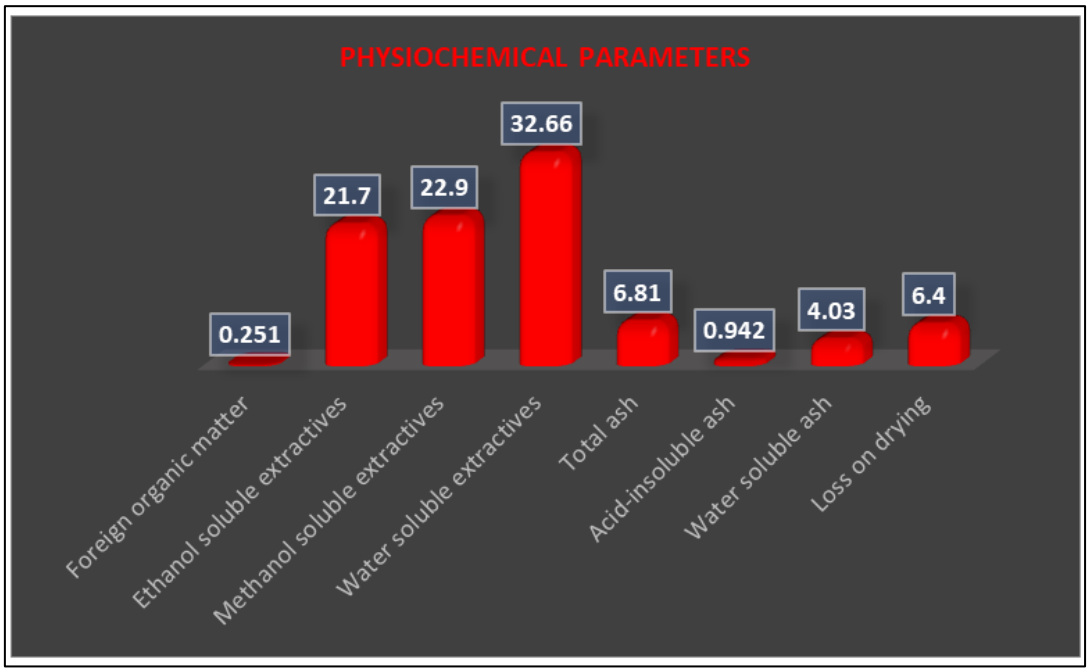
Table 1: Macroscopic and Microscopic Description

Macroscopic Characteristics		Microscopic Characteristics			
Charac-teristics	*A. squamosa* leaf	T.S of Lamina of leaf		T.S of midrib of leaf	
Size	Length: 10-15 cm, Width: 3-6 cm	Charac-teristics	*A. squamosa* leaf	Characteristics	*A. squamosa* leaf
Shape	Acute, oblong-lanceolate or elliptic, pellucid-dotted	Epidermal cells	Single layer	Epidermis	Single layer on the both surface

Macroscopic Characteristics		Microscopic Characteristics			
Color	Upper surface - deep green, Lower surface – pale green	Parenchyma	Parenchyma (spongy) of 3 to 5 layers	Collenchymas	Present followed by thin walled, round or oval parenchymatous cells
Odor	Bitter	Stomata	anomocytic	vascular bundle	consisting of xylem and phloem, present in centre
Taste	Bitter				

Table 2: Physicochemical Parameters

S. No.	Parameter	% Content
		A. squamosa
1.	Foreign organic matter	0.251
2.	Ethanol soluble extractives	21.7
3.	Methanol soluble extractives	22.9
4.	Water soluble extractives	32.66
5.	Total ash	6.81
6.	Acid-insoluble ash	0.942
7.	Water soluble ash	4.03
8.	Loss on drying	6.4



Graph 1: Physicochemical Parameters of *A.squamosa*

Table 3: Phytochemical Screening of *A. squamosa* leaves

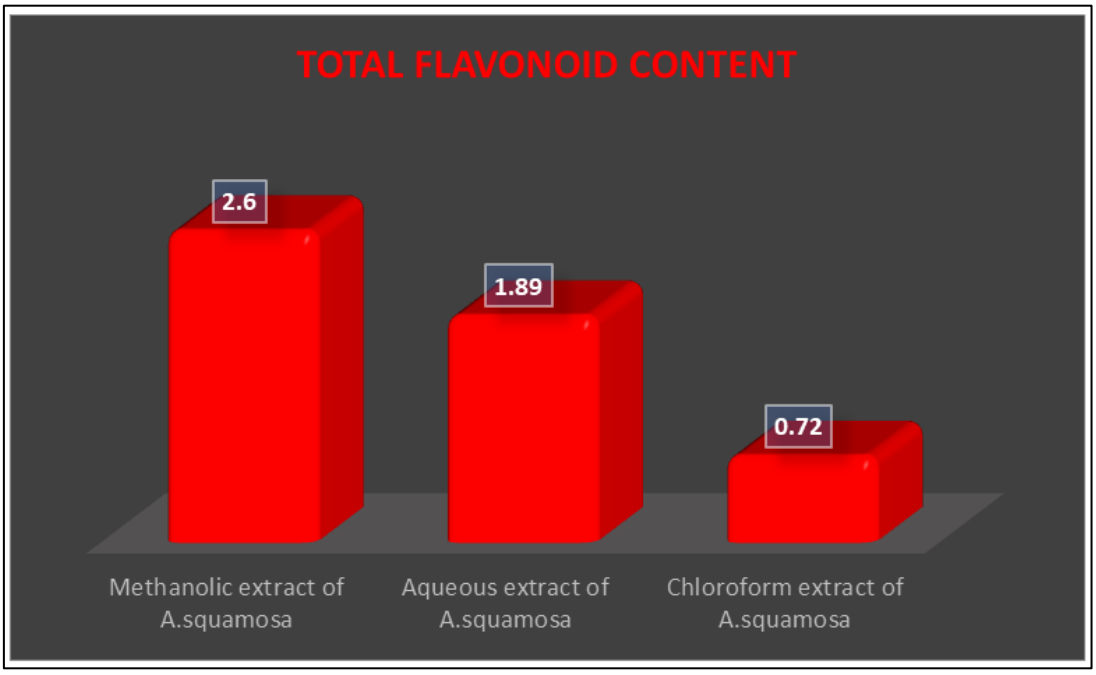
Test	Pet.ether	Chloroform	Ethanolic	Methanolic	Aqueous
a) Carbohydrate					
Molish	(+)ve	(-)ve	(-)ve	(-)ve	(-)ve
Benedict	(+)ve	(-)ve	(-)ve	(+)ve	(-)ve
Starch	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Hexose sugar	(+)ve	(+)ve	(-)ve	(+)ve	(-)ve
b) Tannin					
$FeCl_3$	(+)ve	(+)ve	(-)ve	(+)ve	(+)ve
c) Protein					
Biuret	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Xanthoprotein	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
d) Amino acid					
Ninhydrin	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

Test	Pet.ether	Chloroform	Ethanolic	Methanolic	Aqueous
e) Alkaloids					
Dragnodroff	(-)ve	(+)ve	(+)ve	(+)ve	(-)ve
Mayer	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
f) Steroid					
Salkowski Libermann – Bucher	(+)ve (-)ve	(+)ve (+)ve	(+)ve (+)ve	(+)ve (+)ve	(+)ve (+)ve
g) Flavonoids					
Shinoda	(-)ve	(+)ve	(-)ve	(+)ve	(+)ve
NaOH	(-)ve	(+)ve	(-)ve	(+)ve	(-)ve
Lead acetate	(-)ve	(+)ve	(+)ve	(+)ve	(+)ve
h) Coumarin	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
i)Glycosides					
Baljet	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Legal	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Killer-Killani	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

(+)ve = Present (-)ve = Absent

Table 4: Total Flavonoid Content

S. No	Sample	%TFC
1.	Methanolic extract of *A.squamosa*	2.6
2.	Aqueous extract of *A.squamosa*	1.89
2.	Chloroform extract of *A.squamosa*	0.72



Graph 2: TFC of different extract of *A.squamosa*

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