IJPSR (2022), Volume 13, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 March 2022; received in revised form, 24 April 2022; accepted, 25 April 2022; published 01 November 2022

PHARMACOGNOSTICAL STANDARDIZATION AND PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS ON *ANANAS COMOSUS* (L.) MERR.

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Keywords:

Pineapple, Medicinal plants, Phytochemical, Microscopic studies, Standardization, *Ananas comosus*, Pharmacognostic study

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ABSTRACT: Pineapple or 'Ananas,' is a tropical fruit that belongs to the Bromeliaceae family. The stem and fruit of this plant are utilized in conventional and folk medicine for treating various illnesses. In addition to calcium, iron, potassium, pineapples are a rich source of vitamin C, B and A. This study involves determinations various standardization specifications for the plant *Ananas comosus* (L.) Merr such as morphological characteristics, microscopic examinations, physiochemical parameters, and phytochemical screening for significant groups of compounds and other WHO recommended parameters for standardization. A morphology study was done on the fruit and stem portion of the plant. A microscopy study indicated the presence of lignified fibers, ovaries and sepals. The elemental analysis showed the presence of lead, Arsenic, Iron, Zinc, Cadmium, Magnesium, Copper, and Mercury and all the elements are within limits. The phytochemical profile revealed saponins, glycosides, phenols, flavonoids, anthraquinone steroids, tannins, carbohydrates, and terpenoids. The phenolic content was determined by using the Folin-Ciocteau method and found to be highest in ethanolic extract of fruit (40.02 mg GAE/g) and stem (35.83 mg GAE/g), respectively. The flavonoid content was determined using the aluminum chloride method and found to be highest in ethanolic extract of fruit (32.20 mg of qucertein /g) and stem (18.45mg of qucertein /g) portion, respectively. The finding of this study results a useful source of information and give appropriate criteria for recognizing this medicinally active plant for future investigations and applications.

INTRODUCTION: Plants have been the foundation of sophisticated traditional medicine like Chinese, Ayurvedic and Unani. Today, many essential drugs are derived from plant products are used, the conventional system of medicines [1]. In the last three decades, there has been an increase in the utilization of plant supplements and their products. Approximately 80% of the population depends on plant products as a necessary part of primary health care.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(11).4671-79

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(11).4671-79

There is a lot of potential shown by these plants, as they also have good efficacy. The testing of many plant products is still incomplete; hence their use is not fully explored. There is preliminary testing of many plant products; moreover, their use is monitored poorly or even not monitored. As the use of herbal remedies increases, there should be adequate measures by the regulatory authorities to ensure the safety and quality of these products for the health benefits of human beings [2].

Pineapple is an edible fruit in the Bromeliaceae family. It is typically identified as “ananas” or “pineapple.” It is cultivated in Indonesia, Nigeria, India, Costa Rica, Mexico, the Philippines, Colombia, China, and Brazil. It is primarily grown in Goa, Karnataka, Assam, Meghalaya, Tripura,

Mizoram and West Bengal in India. After citrus and banana, pineapple is the 3rd most common fruit globally [3]. India grabs 6th place globally with 1.7 million metric tons of annual production of pineapples [4]. Many pharmacological properties reported in the plant are antimalarial [5], antidiabetic [6], abortifacient [7–9], anticancer [10–16], antioxidant [17–20], antidiarrhoeal [21], antiplatelet aggregation [22, 23], anti-inflammatory [24-27], antidepressant [28, 29], cardioprotective [30–32], debridement [23], diuretic [23], immunomodulatory [33–35]; hypolipidemic [36]. The peel portion of pineapple is used in herbal decoction as a flavoring agent. A proteolytic enzyme is derived from pineapple, shows therapeutic potential in treating various types of cancer and dermatological ailments and acts as an anti-inflammatory agent [37, 38]. Pineapple is used in industry and cosmeceuticals and is used clinically for wound healing [37, 39–42]. Pineapples have a high medicinal value due to diverse bioactive substances; hence pharmacognostical characteristics such as morphological description, microscopical examination, heavy metal presence, and physicochemical parameters must be standardized.

MATERIAL AND METHODS:

Plant Collection and Authentication: The stems and fruits of *Ananas comosus* were procured from the markets of Rohtak, Haryana, and Siliguri in West Bengal, respectively. Dr. Sunita Garg, affiliated to Raw Material and Herbarium and Museum, CSIR-National Institute of Science Communication and Information Resources, New Delhi as chief scientist, authenticated the plant parts in question (fruit and stems) *via* reference no NISCAR / RHMD / Consult / 2018/3305/06 and NICAR/RHMD/ Consult /2020/5665/09. The plant was identified as *Ananas comosus* (L.) Merrill.

Morphological Description: The morphological characteristics of fruit and stem portions include color, odor, shape, size and taste. These characteristics were examined by the naked eye using a dissection microscope.

Microscopic Examination: For the transverse section, the fruit and stem portion of the plant were made smooth by soaking into water. Free-handed sectioning was used for cutting the transverse section of fruit and stem. The transverse section of the plant part was cleared by using chloral-hydrate. Then staining of the T.S. was done with phloroglucinol and HCl mounted in glycerol, followed by a microscopic study. A small amount of powdered stem and fruit took on the slide for powder microscopy. These slides were mounted with phloroglucinol followed by conc. HCl and glycerin. Then observations are made under the light microscope. Various reagents are used to carry out the plant's histochemical studies as per the guidelines of WHO.

Physicochemical Parameters: To find various phytoconstituents, the prepared extract was examined by various chemical tests such as saponins, carbohydrates, phenols, alkaloids, steroids, flavonoids, glycosides and triterpenoids.

Elemental Analysis: The Nitric Perchloric acid digestion method was used for the elemental analysis of samples. The method, as mentioned above, was based on the method given by the Agricultural Association of Chemists (1990). According to the procedure, 10 ml of concentrated nitric acid was used to 1 gram of sample for 30 to 45 minutes. Boiling was stopped on the look of fumes of white color. Again, mixture cooling was carried out, followed by boiling the cool mixture to release fumes. Then, filtration of the cooled solution was carried out by using Whatman Number 42 filter paper specified as 20 & 21. The obtained samples were analyzed using Atomic Absorption Spectroscopy (GBC932 PLUS). The AAS with hollow cathode lamp for arsenic, zinc, cadmium, lead, copper, mercury, magnesium, and iron was used. Calibration of the instrument was done using a solution of zinc, arsenic, cadmium, lead, copper, mercury, magnesium, and iron at wavelengths of 213.9, 193.7, 228.8, 283.5, 324.8, 253.7, 242.1, and 248.3nm, respectively. After that, the calibration curve of reference for sample analysis was prepared. Optimization of the instrument was carried out according to requirements. We have obtained the results in parts per million (ppm) levels.

Extraction: The powdered form of the stem and fruit portion of *A. cosmosus* was defatted using petroleum ether, followed by successive extraction of the above samples with chloroform, ethanol, and water. The triple maceration process was used to

prepare the extracts. Extraction of the powdered drug sample was carried out with one solvent each time, followed by drying below 50 ℃, and then the dried drug was extracted with the successive solvent in the same manner. These obtained extracts were subjected to Rota-evaporator for complete drying, and desiccators were used to store these extracts for further use.

Preliminary Phytochemical Screening: The prepared extract shall be chemically tested to verify the presence of phtyocomponents such as saponins, phenols, steroids, flavonoids and triterpenoids.

Total Phenol Content: Folin-Ciocteau reagent was used to determine phenol content. First, Folin-ciocateu reagent (0.5ml) was added to the ethanolic extract (1.0 ml) and then incubated in the solution for approximately 15 min. In the next step, a saturated sodium carbonate solution was added to the above solution. After that, set the formed solution for 40 min in a dark environment. Finally, solution absorbance was measured by selecting wavelength maxima 725 nm, and for the preparation of the standard plot, gallic acid was used as a reference drug.

Total Flavonoids Content: The aluminum chloride method was used for the determination of total flavonoids content. First, 5% sodium nitrite solution (0.3 ml) and water (4 ml) were added to extract (1.0 ml) and then 10% aluminum chloride (0.3 ml) to the solution.

After 5 min, 1M NaOH (2.0 ml) was added to the solution, followed by dilution to make the volume 10 ml. The obtained solution absorbance was taken by selecting the maximum wavelength of 510 nm. Quercetin was used as a reference drug, and from the standard plot of the drug, flavonoid content is determined.

RESULTS AND DISCUSSION:

Morphological Studies:

Fruit: The shape of the fruit was ovate to cylindrical; it is yellowish to green; its diameter is approximate 16-24 cm long and 13-16 cm long. **Fig. 1** shows fresh and dried fruit portions of pineapple.

Stem: The stem is club-shaped, having a 23-50 cm length; nodes are also present. **Fig. 2** shows the stem portion of the pineapple.

FIG. 1: FRESH AND DRIED PORTION OF PINEAPPLE

FIG. 2: STEM PORTION OF PINEAPPLE

Microscopic Examination:

Qualitative Microscopy: The microscopy of the fruit portion of the pineapple resulted in the presence of ovaries and sepals.

It also showed the presence of a peduncle. The presence of lignified fibers can also be seen in the fruit mesocarp and the transverse section of the fruit is shown in **Fig. 3.**

Histochemical Detection: Histochemical investigation of fruit and stem showed various cell contents presented in **Table 1** and **Fig. 4.**

TABLE 1: HISTOCHEMICAL INVESTIGATION IN *ANANAS COMOSUS* FRUIT AND STEM PORTION

Test	Result	
	Fruit	**Stem**
Cellulose cell wall	**P**	**P**
Aleurone grains	**P**	A
Lignified cell wall	**P**	**P**
Fats	**P**	**P**
Calcium oxalate	A	**P**
Tannins	**P**	**P**
Calcium carbonate	A	**P**
Starch	**P**	**P**

*a= absent, p=present

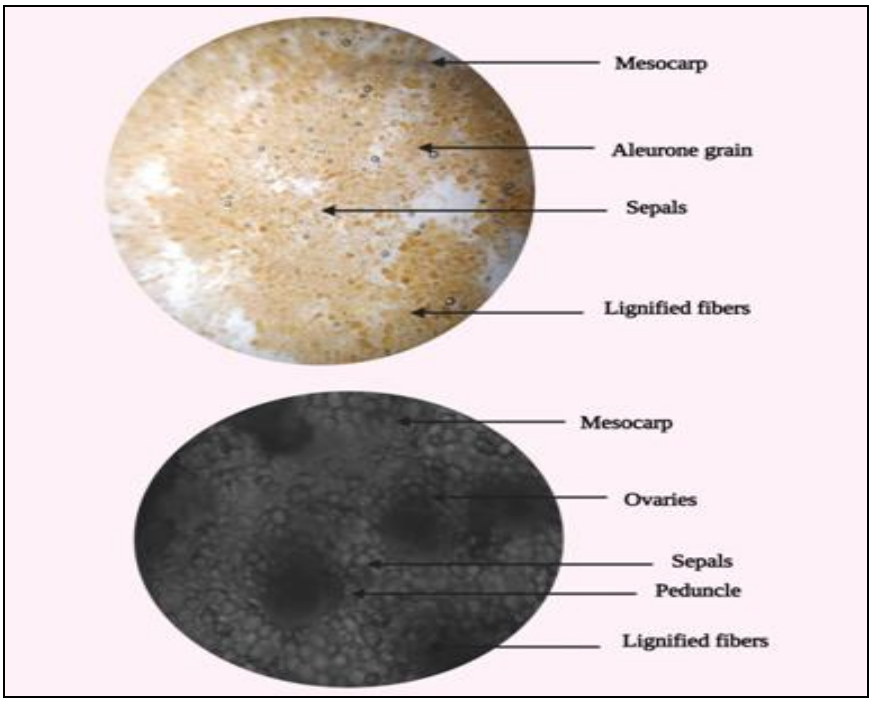

FIG. 3: TRANSVERSE SECTION OF FRUIT

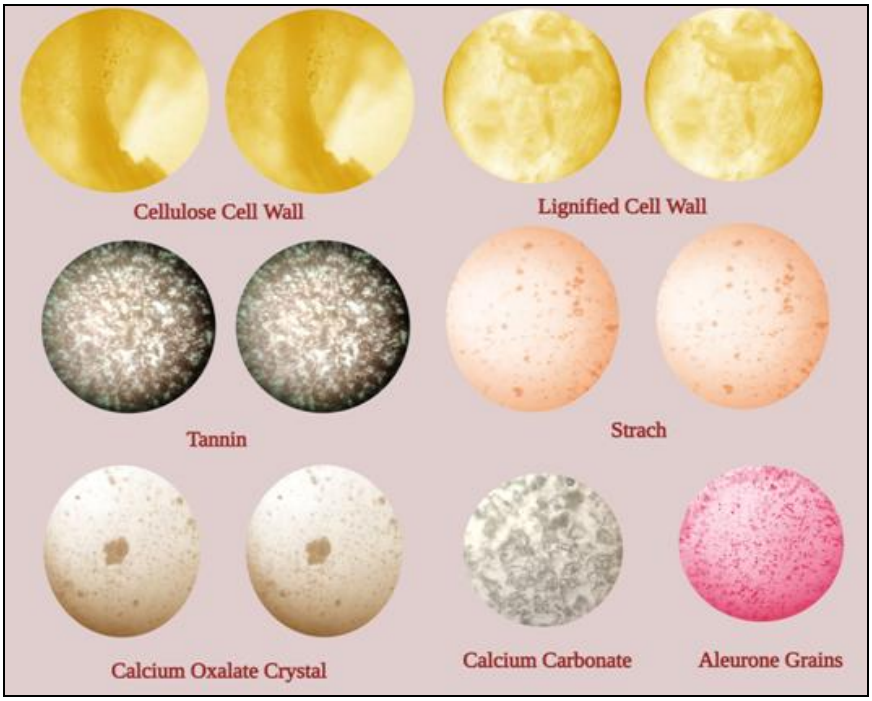

FIG. 4: HISTOCHEMICAL INVESTIGATION IN *ANANAS COMOSUS*

Physicochemical Analysis: The results obtained from the physiochemical analysis for ash values, foaming index, moisture content, extractive values and swelling index are presented in **Table 2.**

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF *ANANAS COMOSUS* FRUIT AND STEM PORTION

S. no.	Parameter	Value	
		Fruit	Stem
1	Moisture content	86.15 %	65.10%
2	Swelling index	3.00	1.00
3	Foaming index	Less than 100	Less than 100
4	**Extractive values**		
	Petroleum ether	0.241%	0.320%
	Chloroform	0.532%	0.411%
	Ethanol	5.156%	3.452%
	Aqueous	3.171%	2.928%
5	**Ash values**		
	Total ash value	3.2% w/w	4.6%w/w
	Acid insoluble ash	1.5% w/w	2.8%w/w
	Sulphated ash	1.8% w/w	1.5% w/w
	Water soluble ash	2.4% w/w	1.9% w/w

Elemental Analysis: The elemental contents of lead, Arsenic, Iron, Zinc, Cadmium, Magnesium, Copper and Mercury were analyzed from the powdered fruit and stem were within limits. The results obtained from the analysis are enumerated in **Table 3.**

TABLE 3: ELEMENTAL ANALYSIS OF *ANANAS COMOSUS* STEM AND FRUIT PORTION

Metal	Concentration (ppm)	
	Fruit	Stem
Lead	0.21	0.10
Arsenic	0.00	0.00
Copper	0.0002	0.001
Cadmium	0.008	0.006
Mercury	0.0444	0.005
Magnesium	55.40	4.08
Zinc	0.00486	0.0023
Iron	0.18	0.14

Preliminary Phytochemical Screening: The analysis of the phytoconstituents of extracts of the stem portion of *A. comosus* was done to identify bioactive compounds according to standards procedures, and the findings are presented in **Table 4.**

TABLE 4: *ANANAS COMOSUS* STEM EXTRACTS SCREENING RESULTS

Phytoconstituents	Test	Inference			
		Petroleum ether	Chloroform	Ethanol	Aqueous
Carbohydrates	Molish test	A	A	P	P
	Barfoed test	A	A	P	A
	Benedict test	A	A	P	P
	Hexose sugar	A	A	A	A
	Fehling test	A	A	P	A
Alkaloids	Hager test	A	A	A	A
	Wagner test	A	A	A	A
	Dragondroff test	A	A	A	A
	Meyer test	A	A	A	A
Anthraquinone glycosides	Modified Borntrager test	A	P	P	A
Coumarin glycosides	Fluorescence	A	A	A	A
Saponins	Foam test	A	P	P	P
Flavonoids	Shinoda	P	P	P	A
	Vanillin HCl	P	P	P	A
	Ammonia	P	P	P	A
Phenols	Ferric chloride	A	P	P	P
	Lead acetate	A	P	P	P
Steroids and terpenoids	Salkovaski	P	P	P	A
	Libermann Burchard test	P	P	P	A

*a= absent, p=present

In the same way, analysis of the fruit portion of *A. comosus* showed the absence or presence of saponins, phenols, anthraquinone glycosides, flavonoids, tannins, terpenoids, steroids and carbohydrates in prepared extracts and the results are given in **Table 5.**

TABLE 5: *ANANAS COMOSUS* FRUIT EXTRACTS SCREENING RESULTS

Phytoconstituents	Test	Inference			
		Petroleum ether	Chloroform	Ethanol	Aqueous
Carbohydrates	Molish test	A	A	P	P
	Benedict test	A	A	P	P
	Fehling test	A	A	P	A
	Pentose sugar	A	A	P	A
	Barfoed test	A	A	P	A
	Hexose sugar	A	A	A	A
Alkaloids	Hager test	A	A	A	A
	Meyer test	A	A	A	A
	Dragondroff test	A	A	A	A
	Wagner test	A	A	A	A
Anthraquinone glycosides	Modified Borntrager's	A	A	P	A
Coumarin glycosides	Fluorescence test	A	A	A	A
Saponins	Foam test	A	A	P	P
Flavonoids	Vanillin HCl	P	P	P	A
	Ammonia	P	P	P	A
	Shinoda	P	P	P	A
Phenols	Ferric chloride test	A	P	P	P
	Lead acetate	A	P	P	P
Steroids and terpenoids	Salkovaski	P	P	P	A
	Libermann Burchard's test	P	P	P	A

*a= absent, p=present

Total Phenolic Content: Phenolic content in prepared extract was determined by Folin-ciocateu. The standard curve's regression equation had a regression co-efficient (R2) of 0.9986. Value of phenolic contents diverse from 2.50 to 40.02 mg of gallic acid per g weight in fruit portion and 1.81 to 35.83.mg gallic acid per g weight in the stem portion of the plant. Phenolic content in the fruit and stem portion of pineapple is shown in **Table 6** below.

TABLE 6: TOTAL PHENOLIC CONTENT OF PINEAPPLE EXTRACTS

Extract	Phenolic (mg of gallic acid equivalents/g) (Fruit)	Phenolic (mg of gallic acid equivalents/g) (Stem)
Petroleum ether extract	Absent	Absent
Chloroform extract	Absent	Absent
Ethanol extract	40.02±2.00	35.83±2.13
Aqueous extract	2.50±0.15	1.81±0.10

Values are mean± S.D.

Total Flavonoid Content: The aluminum chloride method was used for the determination of flavonoid content. The standard curve's regression equation had a regression co-efficient (R^2) of 0.9904. The value of flavonoid content varied from 2.30 mg to 32.20 mg of qucertein/gram weight in the fruit portion and 5.56 to 18.45 mg of qucertein/g weight in the stem portion of the plant. Flavonoid content in the fruit and stem portion of pineapple is shown in **Table 7** below.

TABLE 7: TOTAL FLAVONOID CONTENT IN STEM AND FRUIT PORTION OF PINEAPPLE

Extract	Total flavonoid (mg of qucertein /g) (Fruit)	Total flavonoid (mg of qucertein /g) (Stem)
Petroleum ether extract	Absent	Absent
Chloroform extract	2.32±0.12	Absent
Ethanol extract	32.20±3.46	18.45±1.15
Aqueous extract	16.12±1.02	5.56±0.06

Values are Mean± S.D.

DISCUSSION: In the present study, quality control parameters for standardization of the *A. comosus* stem and fruit were done prior to their biological evaluation. The physiochemical parameters were within the range. Triple maceration was performed for the stem and fruit

portion of *A. comosus* and the extractive value was found to be higher in the case of ethanol. Heavy metals like arsenic, lead and mercury was within both plant portions limits.

Preliminary phytochemical analysis of *A. comosus* showed the presence of carbohydrates, flavonoids, phenol, and triterpenoids in different fractions of extract. Total phenolic content was found to be higher in ethanol extract. The higher concentration of total flavonoids content was determined in ethanol, extract of the stem, and fruit portion of the plant.

CONCLUSION: A pharmacognostical study and preliminary phytochemical tests were carried out to determine quality control considerations for *A. comosus*. The results of these studies will serve as diagnostic tool for standardizing the *A. comosus*.

ACKNOWLEDGEMENT: Declared none.

CONFLICTS OF INTEREST: None.

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How to cite this article:
Kumar V, Garg V and Dureja H: Pharmacognostical standardization and preliminary phytochemical investigations on *Ananas comosus* (L.) Merr. Int J Pharm Sci & Res 2022; 13(11): 4671-79. doi: 10.13040/IJPSR.0975-8232.13(11).4671-79.