The Journal of Phytopharmacology 2024; 13(3):220-229
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2024; 13(3): 220-229
May- June
Received: 19-12-2023
Accepted: 08-05-2024

doi: 10.31254/phyto.2024.13305

Usha Devi
CCRUM-Drug Standardization Research Institute (DSRI), PCIM & H Campus, Ghaziabad, Uttar Pradesh- 201002 India

Shoeb Ahmed Ansari
CCRUM-Drug Standardization Research Institute (DSRI), PCIM & H Campus, Ghaziabad, Uttar Pradesh- 201002 India

Asma Sattar Khan
CCRUM-Drug Standardization Research Institute (DSRI), PCIM & H Campus, Ghaziabad, Uttar Pradesh- 201002 India

Anees Ahmad
CCRUM – Regional Research Institute of Unani Medicine, University of Kashmir, Srinagar, J&K- 190006 India

Mohammed Wasim Ahmed
CCRUM-Drug Standardization Research Institute (DSRI), PCIM & H Campus, Ghaziabad, Uttar Pradesh- 201002 India

Rampratap Meena
Central Council for Research in Unani Medicine (CCRUM), 61-65 Institutional Area, Janakpuri, New Delhi- 110058 India

Correspondence:
Dr. Usha Devi
CCRUM-Drug Standardization Research Institute (DSRI), PCIM & H Campus, Ghaziabad, Uttar Pradesh- 201002 India
Email: usharudhyan19@gmail.com

Pharmacognostic evaluation and development of quality control parameters for Fufal (Areca nut)

Usha Devi, Shoeb Ahmed Ansari, Asma Sattar Khan, Anees Ahmad, Mohammed Wasim Ahmed, Rampratap Meena

ABSTRACT

Seed of *Areca catechu* commonly known as 'Areca nut' or 'Betel nut' belongs to Arecaceae family. It is native to Malaysia and cultivated in subtropical region of South China and India for their economically important seed crop. Areca nut is reddish to light yellowish brown, hard, ovoid, ellipsoidal or globose, internally are mottled with ruminated brownish endosperm tissue alternating with white tissue. In the Indian traditional medicine system Areca nut has been considered one of the important single drug which is therapeutically useful in a number of formulations of Ayurveda, Siddha and Unani preparation. The presence of bioactive phytochemicals in medicinal plants is mainly responsible for various pharmacological properties. It has been widely accepted that medicinal plants are safe and effective treatments for various diseases and ailments. Several pharmacological activities such as antimycotic, antifungal, antiviral, antibacterial, anti-inflammatory, immunomodulatory, hypoglycemic and antioxidant activities are attributed to it. In classical literature of Unani system of medicine, Areca nut is commonly called 'Fufal' and considered astringent, diuretic, emmenogogue, nervine tonic. Local application is useful in acute inflammation, trachoma, sialorrhea, gingivitis and as a tonic for heart. In the current scenario, standardizing herbal materials at a rapid pace has become a major challenge due to the growing demand for herbal medicines. Although herbal drugs are generally effective, and their effectiveness can be impacted by adulteration and lack of standardization. The present study is focused on authentication of the Areca nut by developing different pharmacognostic standards such as macroscopy, microscopy, physicochemical analysis and HPTLC fingerprinting. The majority of the market sample are either contaminated by cancer-causing fungi or adulterated with harmful chemicals, pesticides, and heavy metals that known to cause several health problems on human beings. So, the drug was also evaluated for several quality parameters, like heavy metals, microbial load, aflatoxins, and pesticides. The current study data can serve as reference standards for verifying Areca nut's purity, safety, and effectiveness.

Keywords: HPTLC, Quality control, Phytochemical analysis, Aflatoxins and Pesticides, Areca nut.

INTRODUCTION

The use of herbal drugs is increasing tremendously over the past three decades. The use of substitutes or adulterants as a result of the growing demand of herbal medicines is putting the safety as well as effectiveness of herbal drugs at risk [1]. Herbal drugs are a key factor in pharmaceutical preparation. Consequently, to ensure the authenticity and purity of the crude drug, it would be beneficial to establish scientific standards for herbal medicine [2-6].

Areca catechu L. is a slender, stemmed, perennial palm with about 25 m long trunk. Leaves 8-12 in numbers, light green to green, 1.3-2.7 m long including petiole, fascicles at the top of the stems. The flowers are fragrant, yellow in colour, hermaphroditic, clustered in inflorescences [7,8]. It is an economically important seed crop which cultivated in the Southern India coastal regions, Assam and Bengal during February to March or May to June up to an altitude of 1000 m. The fruits are collected in November to December or March to June and seeds are sun dried for use. The plant is naturally occurred in India, Taiwan, Malaysia and many other Asian countries [9,10].

Areca nut contains two important alkaloids namely arecaidine and arecoline. Besides this, more than 60 compounds i.e. alkaloids, flavonoids, isorhamnetin, chrysoeriol, luteolin, quercetin, catechuins, epicatechuins, arborinol, fernenol, arundoin, palmitic acid, myristic acid, lauric acid, oleic acid, stearic acid, *p*-hydroxybenzoic acid, resveratrol, ferulic acid, vanillic acid etc. have been isolated and identified from this plant [11-14]. It has carries various pharmacological activities include antimycotic, antifungal, antiviral, antibacterial, anti-inflammatory, immuno modulatory, hypoglycemic and antioxidant activities [15-18]. Traditionally Areca nut is used for treatment of multiple disorders such as malaria, arthritis, beriberi, flatulence, diarrhea and dysentery, high blood pressure, inflammation, flatulence [19,20].

In Ayurveda, the plant is stimulant, astringent, diuretic, wound healing, purgative, and digestion-promoting [21].

In the Unani medicinal system, *Areca catechu* is a medicinally significant plant. In Persian, Areca nut is known as Supari and Fuful in Urdu. It is cold and dry in second grade in context of its temperament. In Unani system it is considered astringent (*Qabiz*), diuretic (*Mudirr-i-Bawl*), emmenogogue (*Mudirr-i-Hayd*) and nervine tonic (*Muqawwi-i-A'sab*) and local application is beneficial for the acute inflammation (*Waram al-Haar*). It helps in maintaining the tone of organs. It works as a tonic for heart (*Qalb*) and beneficial in trachoma (*Jarab al-'Ayn*), sialorrhoea (*Kathra al-Lu'ab*) and gingivitis (*Waram al-Litha*). Areca nut is important part in various unani formulation viz. Habb-e-Jiryan, Habb-e-Muqawwi, Majoon hafiz-ul-Janeen, Habb-e-Leemu, Habb-e-Tayyab-ul-Nikhat, Habb-e-Mamool, Qurs-e-Waj-ul-Uzn [22,23]. Keeping view of diverse medicinal uses of Areca nut, the present study was conducted to identify and authenticate the drug through various pharmacognostical parameters.

MATERIAL AND METHODS

Collection and authentication of crude drug

The drug was procured from various places in Delhi and Ghaziabad markets. After cleaning and washing, the samples were dried under the shade at room temperature. The dried crude drug was finely powdered and kept for later use in airtight container. The drug was identified by botanists using pharmacopeial standards [24-26].

Organoleptic evaluation

Various organoleptic characters of the herbal drug were assessed on the basis by color, odor, taste, texture, etc [26-28].

Physicochemical Parameters

The physicochemical parameters of Areca nut included moisture content, ash values (total ash and acid insoluble ash), extractive values (solubility in water, ethanol and hexane), pH values (1% & 10% aqueous solution) were analyzed as per standard methods [29,30].

Sample Preparation for HPTLC analysis

The extraction of two samples, each weighing 2 grams, was done separately using 30 ml of ethanol and chloroform through the sonication process for 30 minutes. The extracts were filtered using Whatman filter paper no. 1 and concentrate them up to 10 ml under vacuum at 50°C. Then, aluminium TLC plate pre-coated with silica gel 60 F 254 (E. Merck) by employing CAMAG Linomat IV automatic sample applicator and 10µl of ethanol extract was applied on it. The plate was developed up to a distance of 9 cm by using 10 ml of the solvent system Toluene: ethyl acetate: formic Acid (9: 1: 0.5) as mobile phase. At room temperature, the plate was air-dried and then examined under ultraviolet light at wavelengths 254 nm and 366 nm. Then the plate was heated at 105°C after being immersed in a 1% vanillin-sulphuric acid reagent until coloured bands emerged. The plate was finally examined under visible light. Furthermore, the chloroform extract was applied to a different TLC plate in the same way and the process was repeated as for the ethanol extract [31, 32,33].

Quality control analysis

The herbal products are used worldwide by a large number of people assuming as effective and safe treatment. This trust of the people impels the scientific world to do equitable quality check on herbal materials. Different quality control parameters, like microbial loads, heavy metals, pesticide residues and aflatoxins were carried out for determination of quality of the drug. The standard method was employed to calculate microbial load [31]. The heavy metal and aflatoxins were detected using Thermo Fisher's Atomic Absorption Spectrophotometer and Thermo Fisher's HPLC [34]. A Triple Quadrupole GC-MS/MS system (Thermo Fisher) was utilized to detect pesticide residues as per standard methods [33, 34].

RESULTS

A) Classification and vernacular name

As per the latest system of classification [35] *Areca catechu* L. syn. *A. faufel* Gaertn, *A. hortensis* Lour, *A. cathechu* Burm.f. is from Arecaceae family. It is commonly known as 'Areca nut' or 'Betel nut' and known by different vernacular names at different geographical regions. These are as following [36].

Urdu – Fufal, Choalia; Assamese – Tamol, Tamul; Andamans – Ahbuddah, Ahpur-ruddah; Bengali – Supari, Gua; English – Betle nut, Areca nut; Gujrati – Sopari; Hindi – Supari; Kannada – Adiks; Konkani – Fufal, Maddi, Supari; Kashmiri – Spari, Supari; Malayalam – Pakku, Adakku; Marathi – Pophal, Supari; Oriya –Pugo, Supari; Persian – Girdchod, Popal, Pupal; Punjabi – Supari, Spari; Sanskrit – Kramuka, Gho; Sinhalese – Puvakka, Pwak; Tamil – Pakku, Kamugu, Pakhumaram; Telugu – Poka, Paka chekka, Pugamu, Vakka.

B) Pharmacognostic study

The correct identification of crude drugs requires standardization. In this study the quantitative determination of some Pharmacognostic parameters viz. botanical identification and authentication through macroscopy and microscopy analysis; physico-chemical parameters viz. total ash and acid insoluble ash value, water and alcohol extractive values, pH, loss in weight on drying; HPTLC finger printing; quality control parameters such as detection of heavy metals, aflatoxins and pesticide residue etc. were performed for setting standards for Areca nut.

Botanical identification and authentication

Macro-morphological study of crude drug is often the first step in the authentication of plant species which completed by analysis of various organoleptic characters such as smell, colour, taste etc; whereas microscopical study brings complementary information of the herbal drug [4].

Macro-morphological characteristics

Areca nut is a very hard, coarsely rounded, slightly conical, externally pale, smooth, reddish to light yellowish brown, 3 to 5 cm long and 2 to 3 cm wide at the base, marked with a network of paler lines; seed hard with ruminated brownish endosperm tissue alternating with white tissue; a semi-circular light brown scar of hilum lies just above the micropyle (Figure 1A).

Microscopic characteristics

A transverse section of seed shows tangentially elongated, several rows cells of seed coat, with more or less thickened inner walls, endosperm tissue abundant and composed of large polygonal whitish cell with irregularly thickened porous walls, occasionally containing aleuronic grains and oil globules; sub-epidermis lignified, consisting of 4 to 5 rows of tangentially elongated sclereids with wide lumen; peg like ruminations of perisperm consisting of radially elongated cells filled with tannin penetrates throughout the endosperm; vascular strands traverse throughout the perisperm (Figure 2).

Organoleptic features

The organoleptic evaluation is crucial for ensuring the authenticity, identity, and purity of herbal drugs. The powder of Areca nut is reddish brown to light brown, astringent and slightly bitter in taste without any odour (Figure 1B).

Powder microscopic analysis

The powder microscopy of the Areca nut showed various diagnostic characters. The microscopic characters of drug consist endosperm tissue with porous walls containing oil globules and aleurone grains; fragment of stone cells of endocarp associated with cells of mesocarp; perisperm filled with tannin; tangentially elongated thick-walled cells of the epidermis containing brown pigment; fragment of epidermal cells; spiral vessel; rhomboidal crystals; transversely cut subepidermal sclereids, isolated or group of subepidermal lignified, pitted stone cells (Figure 3&4).

Physicochemical analysis

The physicochemical analysis of herbal drugs is a vital role in detecting any adulteration or improper handling of drugs. The physicochemical data of Areca nut is depicted in Table 1. The quantitatively assessment data of drug showed that the moisture content was low as the loss in weight on drying at 105ºC occurred between 10.25 – 10.80 %. The total ash value of the drug sample was 2.45 - 2.75% and the acid insoluble ash ranges 0.32 – 0.50 % which is within the limit. The hexane soluble extractive values and water-soluble extractive values of Areca nut were lower ranged between 8.85-9.22% and 14.90 – 15.38 % respectively and followed by the ethanol extractive values which were moderate ranging between 22.10 – 21.65% indicating the polar constituent's extraction. The pH values fall in the range of 5.90 – 6.48 which indicating slightly acidic nature of the aqueous extract.

HPTLC Profile

HPTLC finger printings of Areca nut for chloroform and ethanol extracts developed and were scanned at two wavelengths UV 254 nm, UV 366 nm and under white light after derivatization. The results were consistent as all drug samples had similar colourful bands with replicated Rf values which indicating the consistency of the results (Figure 5&6).

C) Quality Control Parameters

The quality of the herbal ingredients is an essential component to maintain the efficacy of herbal products [3]. The assessment of harmful toxins in medicinal plants is crucial to ensure their safe consumption by humans. The quality control parameters result of microbial load, aflatoxins, pesticide residue and heavy metals analysis are respectively shown in Table 2, 3, 4 & 5.

Heavy metal analysis

The use of medicinal plants has been noted as a potential source of heavy metal toxicity to both humans and animals [37]. It is recommended by the World Health Organization to examine medicinal plants used in herbal remedies for the presence of heavy metals. Even at low concentrations, heavy metal can still have a toxic or poisonous effect on human health and can lead to various deadly diseases. The content of heavy metal in Areca nut was not detected which indicated that the drug was free from heavy metal contamination. The results for heavy metal analysis are shown in Table 2.

Microbial load

Microbial load analysis of crude drug is a tool for determining if the spoilage causing microorganisms are within acceptable levels. The analysis is done to evaluate total fungal count, the total bacterial count like *E. coli*, *Salmonella spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The analysis resulted that the drug is free of any microbial growth and safe for human consumption as shown in Table 3.

Aflatoxins

Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus parasiticus, A flavus.* and *A. nomius*. The results reveal that there are no aflatoxin contents (B1, B2, G1, and G2) in drug samples. The analysis of aflatoxins in the drug has been presented in Table 4.

Pesticide residue

Medicinal plants have been found to possess pesticides, which are primarily applied to crops to protect plants from various pests. Growing agricultural produce without using pesticides is a challenge nowadays. According to WHO guidelines, pesticide residues in herbal drugs must be in permissible limits. There are concerns about this fact and scientific investigation is necessary to assess the health hazards more accurately. The pesticide residues were estimated by analysing the drug through a GC-MS/MS system, and the results confirmed below the quantification limit. The pesticide residues results are given in Table 5.

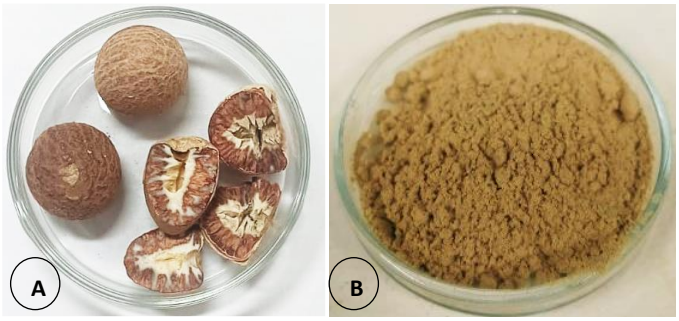
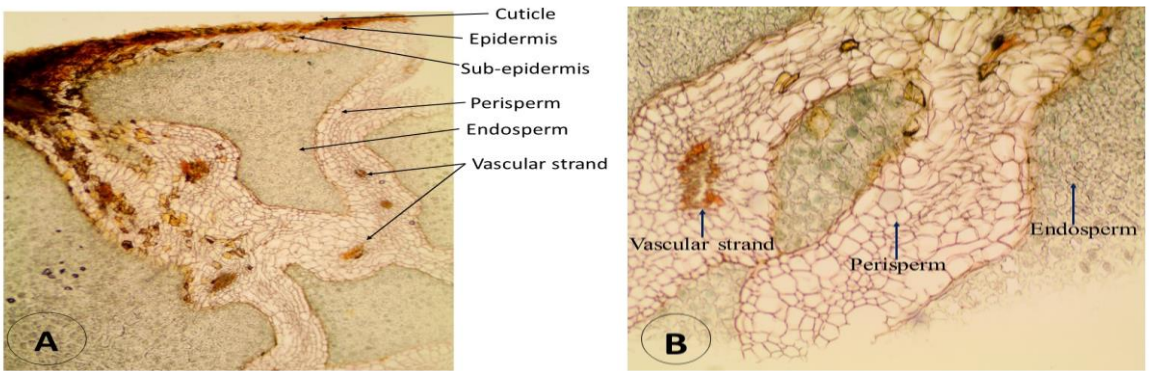


Figure 1: a) Areca nuts (Seeds of Areca catechu); b) Powder of Areca nuts



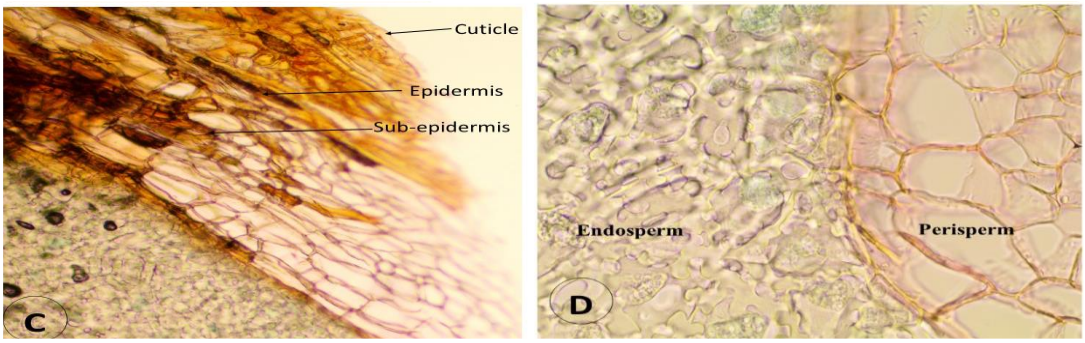


Figure 2: T.S. of Areca nut showed different types of cells. (A) 10x; (B) 20x; (C) 20x; (D) 40x.

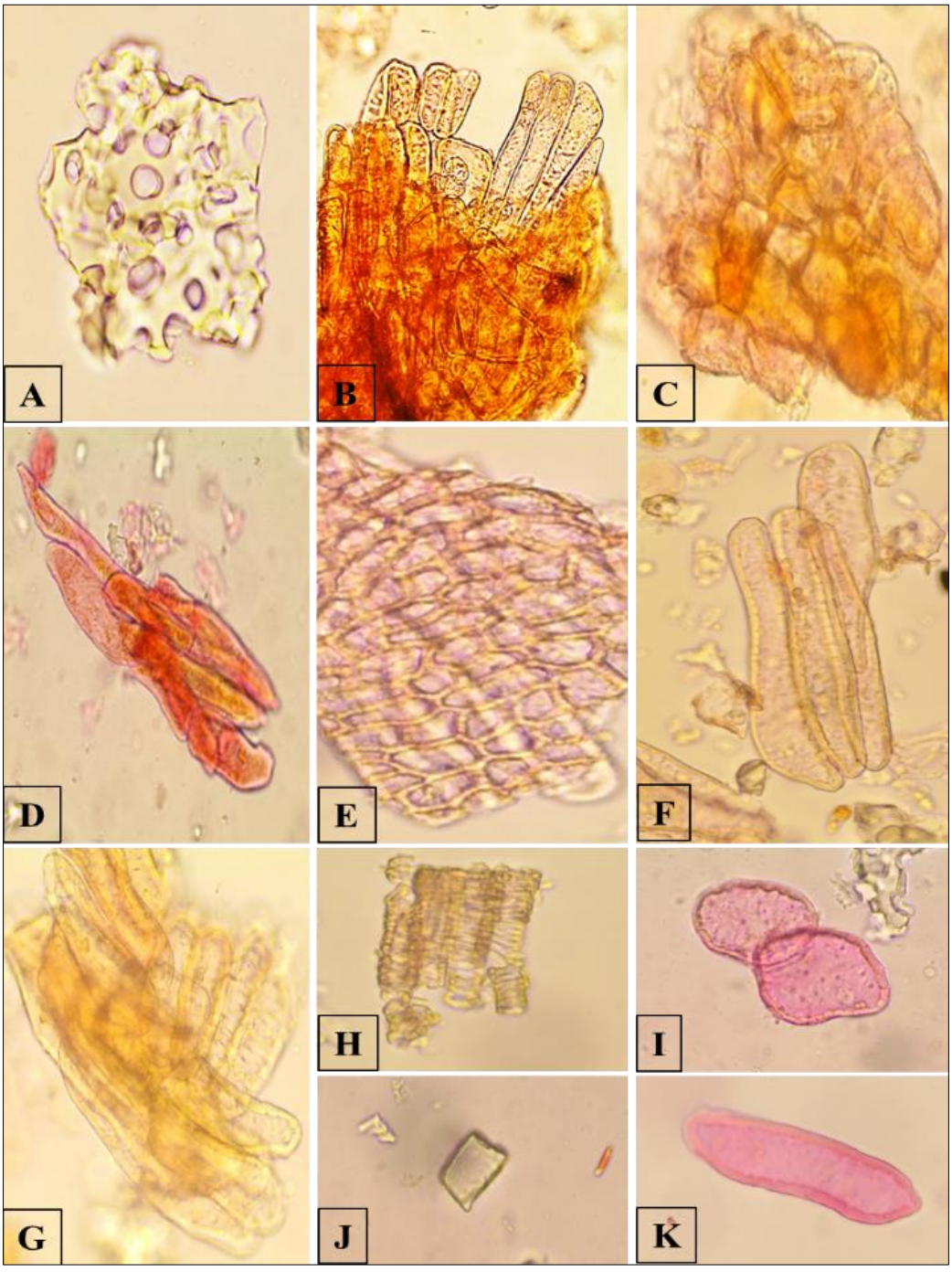


Figure 3: Powder microscopy of Areca nut showed different type of cells. (A) Fragments of endosperm tissue with porous walls containing oil globules and aleurone grains in surface view, 40x; **(B)** Stone cells of endocarp associated with cells of mesocarp, 40x; **(C)** Perisperm filled with tannin in surface view, 20x; **(D)** Tangentially elongated thick-walled cells of the epidermis containing brown pigment, 40x; **(E)** Epidermal cells surface view, 20x; **(F)** Group of pitted stone cells, 40x; **(G)** Group of transversely cut subepidermal cells, 40x; **(H)** Spiral vessel, 40x; **(I)** Stone cell, 40x; **(J)** Rhomboidal crystals, 40x; **(K)** Isolated subepidermal lignified stone cell, 40x

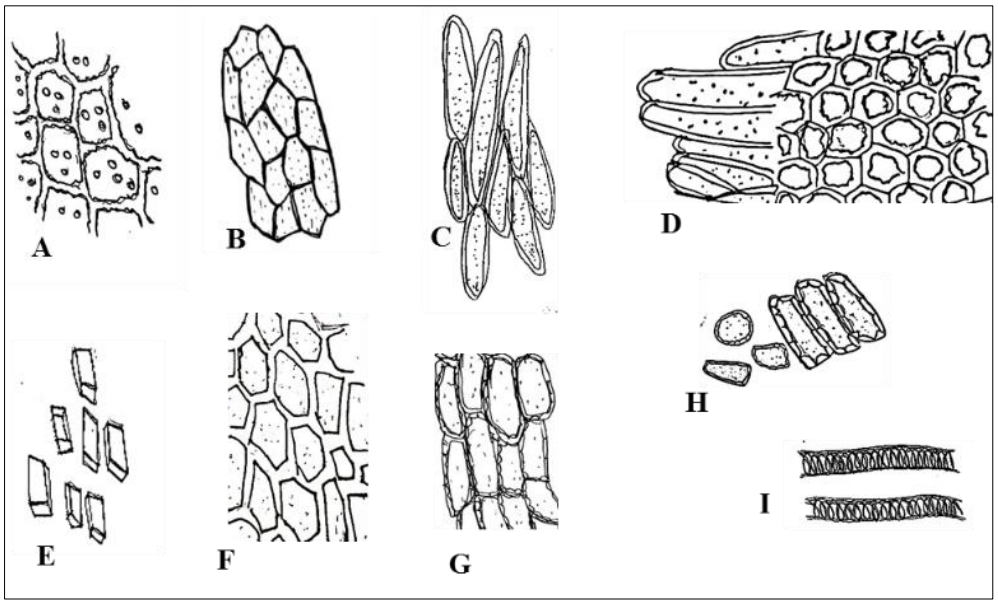


Figure 4: Digramatic representation of different type of cells found in powder microscopy of *A. catechu*. **(A)** Fragments of endosperm tissue with porous walls containing oil globules and aleurone grains (in surface view); **(B)** Perisperm with tannin in surface view; **(C)** Tangentially elongated thick-walled cells of the epidermis containing brown pigment; **(D)** Stone cells of endocarp associated with cells of mesocarp; **(E)** Rhomboidal crystal; **(F)** Epidermal cells surface view; **(G)** Group of transversely cut subepidermal cells; **(H)** Isolated or group of subepidermal lignified, pitted stone cells; **(I)** Spiral vessels.

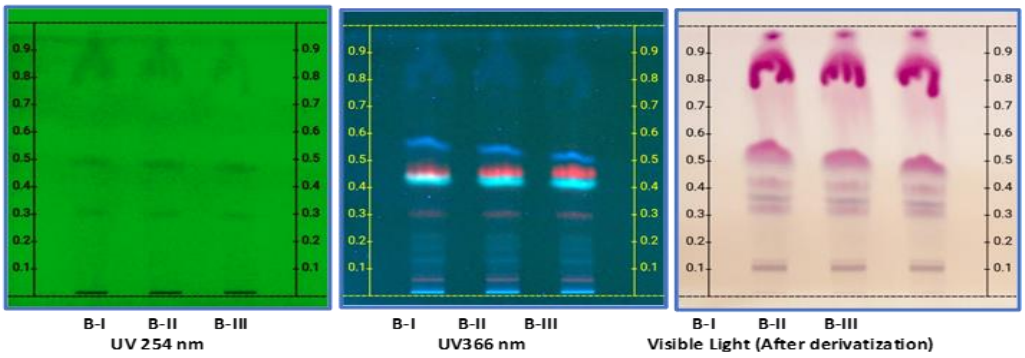


Figure 5: HPTLC of *Chloroform* extracts of Areca nut

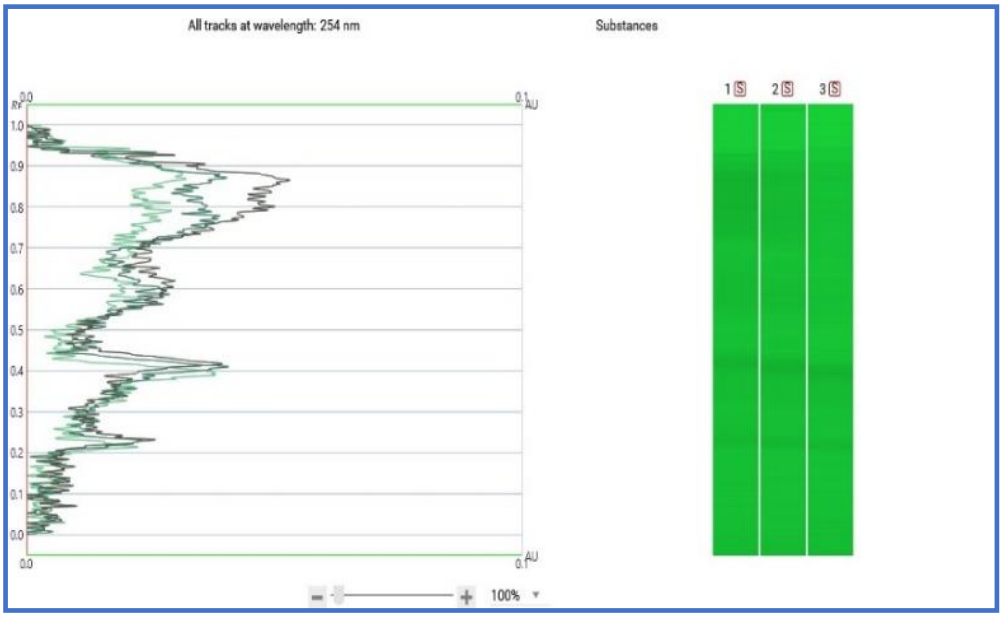


Figure 5a) HPTLC densitometry chromatogram of chloroform extracts (03 batches) at UV 254 nm

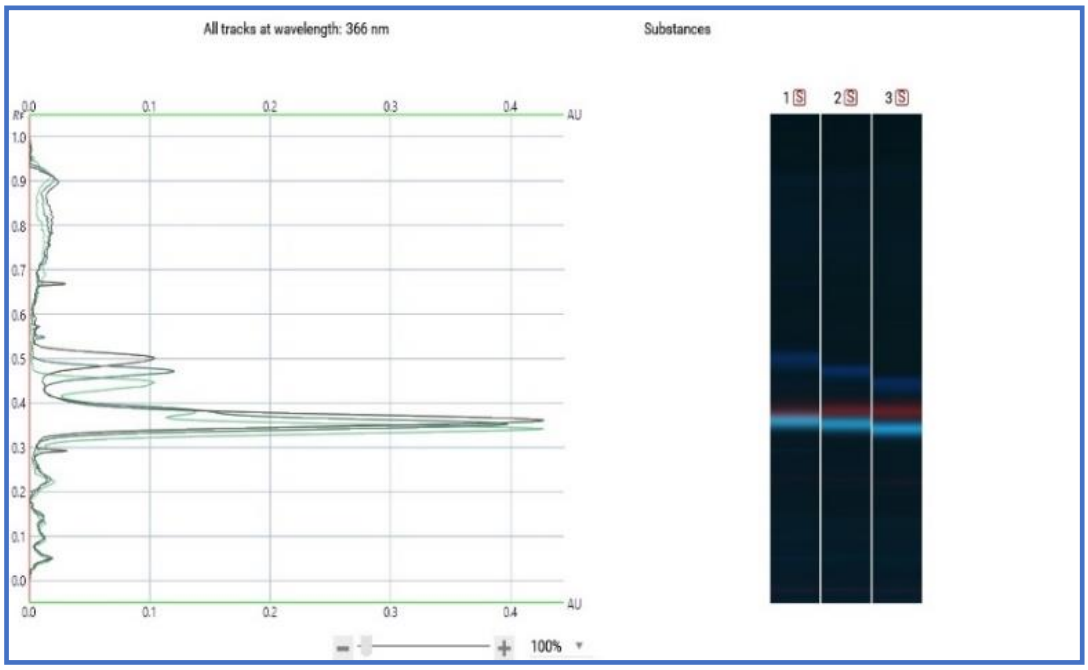


Figure 5b): HPTLC densitometry chromatogram of chloroform extracts (03 batches) at 366nm

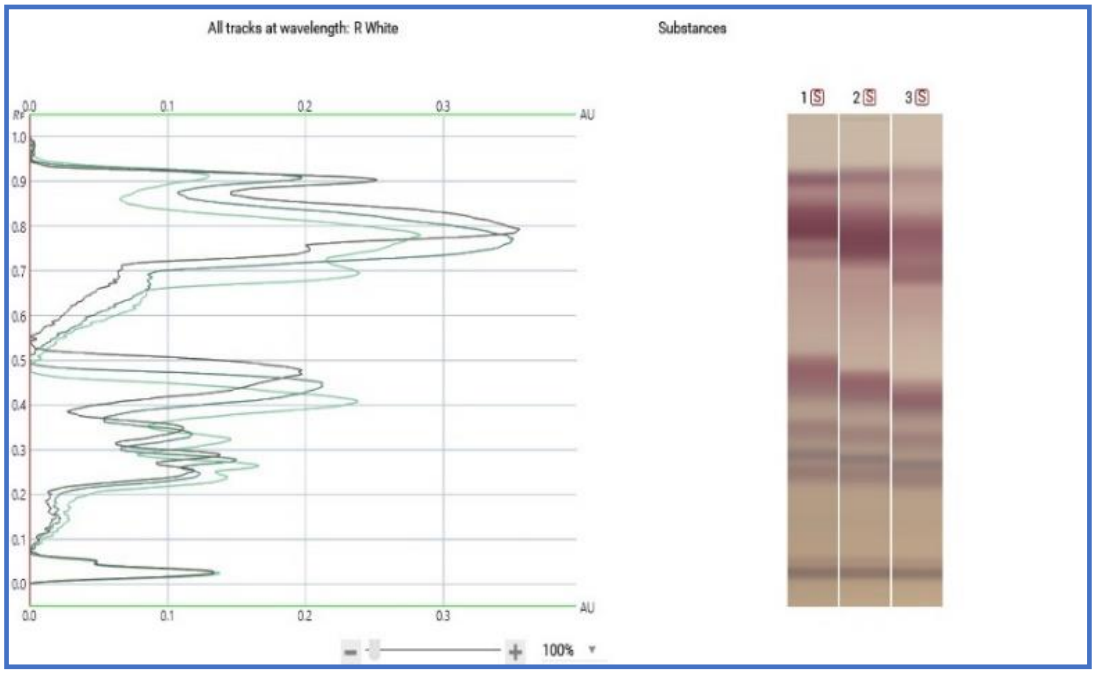


Figure 5c): HPTLC densitometry chromatogram of chloroform extracts (03 batches) under white light after derivatization

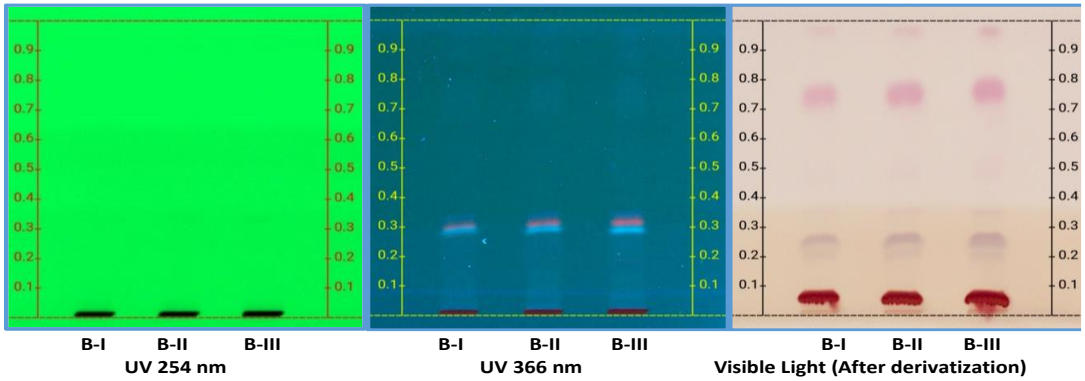


Figure 6: HPTLC of *Ethanol* extracts of Areca nut

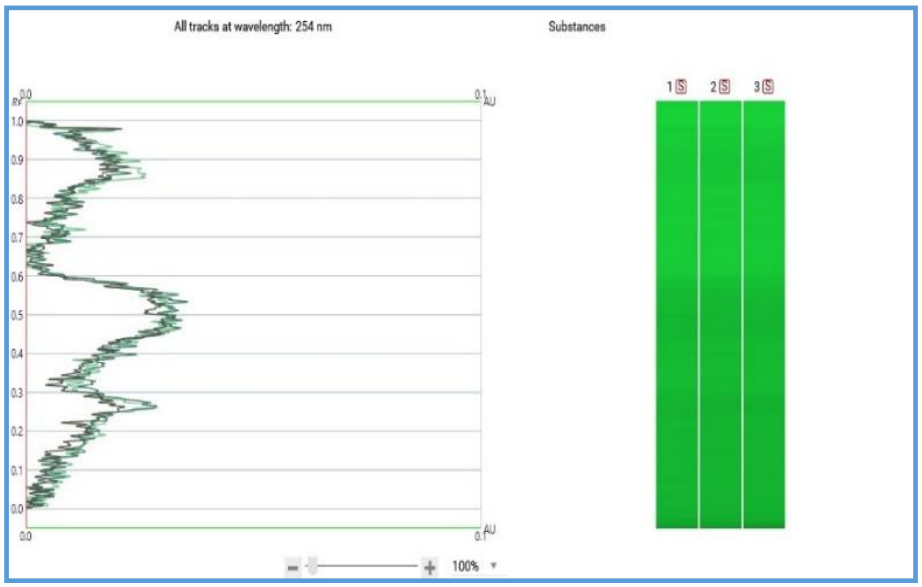


Figure 6a: HPTLC densitometry chromatogram of ethanol extracts (03 batches) at 254 nm

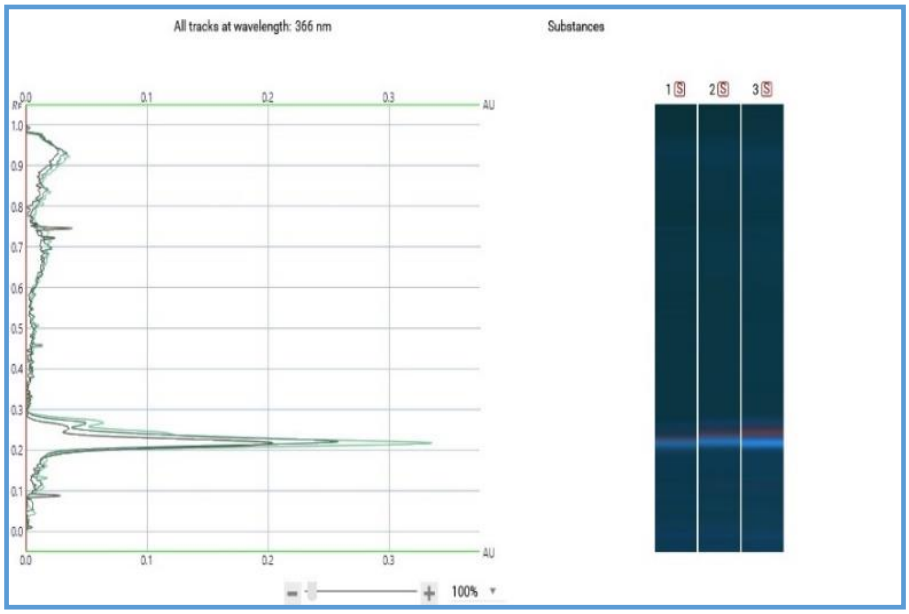


Figure 6b): HPTLC densitometry chromatogram of ethanol extracts (03 batches) at 366 nm

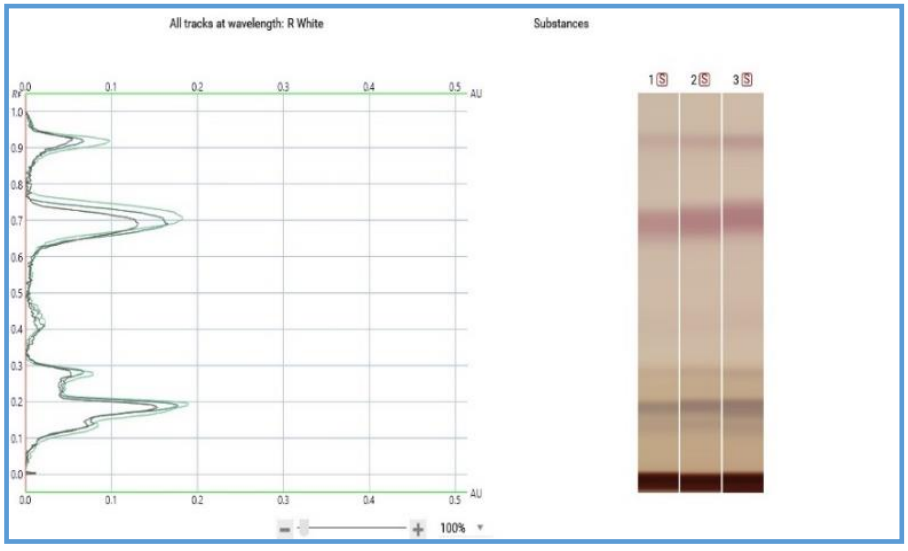


Figure 6c): HPTLC densitometry chromatogram of ethanol extracts of Fufal (03 batches) under white light after derivatization

Table 1: Physico-chemical parameters

S. No.	Parameters	Values
1	Foreign Matter (%)	1.85
2	Loss in weight on drying at 105^0 C (%)	10.25 – 10.80
3	Total Ash (%)	2.45 – 2.75
4	Acid insoluble ash (%)	0.32 – 0.50
5	Ethanol Soluble Extractive (%)	21.10 – 21.65
6	Water Soluble Extractive (%)	14.90 – 15.38
7	Hexane Soluble Extractive (%)	8.85 – 9.22
8	pH 1% Soln.	6.48
	pH 10% Soln.	5.90

Table 2: Heavy Metals Estimation

S. No.	Heavy metals	Result	WHO Limits for internal use
1.	Arsenic	Not Detected	10 ppm
2.	Cadmium	Not Detected	0.3 ppm
3.	Lead	Not Detected	3.0 ppm
4.	Mercury	Not Detected	1.0 ppm

Table 3: Microbial load

S. No.	***Microbial load***		
1	Total aerobic bacterial Count (TABC)	:	1.2×103 CFU/gm
2	Total yeast and molds count (TYMC) CFU/gm	:	9.2×10^2
	Enterobacteriaceae members		
1	*Escherichia coli*	:	ND
2	Salmonella sp.	:	ND
3	Shigella sp.	:	ND
4	Klebsiella sp.	:	ND
	Specific objectionable pathogens		
1	*Pseudomonas aeruginosa*	:	ND
2	*Staphylococcus aureus*	:	ND
3	*Candida albicans*	:	ND
	Aflatoxin producing fungi		
1	*Aspergillus flavus*	:	*ND*
2	*Aspergillus parasiticus*	:	*ND*

Table 4: Aflatoxins analysis.

S. No.	Aflatoxins	
1.	$B_{1:}$	ND
2.	$B_{2:}$	ND
3.	G_1	ND
4.	G2	ND

Table 5: Pesticide Residue Analysis.

S. No.	Pesticide	Result (mg/Kg)	Permissible limit (mg/Kg)
1	Alachlor	BLQ	0.02
2	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	BLQ	0.05
3	Azinophos-methyl	BLQ	1.0
4	Bromopropylate	BLQ	3.0
5	Chlordane (cis, tans and oxychlordane)	BLQ	0.05
6	Chlorfenvinphos	BLQ	0.5
7	Chlorpyrifos	BLQ	0.2
8	Chlorpyrifos-methyl	BLQ	0.1
9	Cypermethrin (and isomers)	BLQ	1.0
10	DDT (all isomers, sum of p,p'-TDE (DDD) expressed as DDT)	BLQ	1.0
11	Deltamethrin	BLQ	0.5
12	Diazion	BLQ	0.5
13	Dichlorvos	BLQ	1.0
14	Dithiocarbamates (as CS2)	BLQ	2.0
15	Endosulphan (sum of isomers & Endosulphan sulphate	BLQ	3.0
16	Endrin	BLQ	0.05
17	Ethion	BLQ	2.0
18	Fenitrothion	BLQ	0.5
19	Fenvalerate	BLQ	1.5
20	Fonofos	BLQ	0.05
21	Heptachlor (sum of Heptachlor & Heptachlor epoxide)	BLQ	0.05
22	Hexachlorobenzene	BLQ	0.1
23	Hexachlorocyclohexane isomer (other than γ)	BLQ	0.3
24	Lindane (γ – Hexachlorocyclohexane)	BLQ	0.6
25	Malathion	BLQ	1.0
26	Methidathion	BLQ	0.2
27	Parathion	BLQ	0.5
28	Parathion methyl	BLQ	02
29	Permethrin	0.14	1.0
30	Phosalone	BLQ	0.1
31	Piperonyl butoxide	BLQ	3.0
32	Pirimiphos methyl	BLQ	4.0
33	Pyrethrins (sum of isomers)	BLQ	3.0
34	Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	BLQ	1.0

***** BLQ – Below limit of quantification

DISCUSSION

The majority of plant-based herbal preparations in Indian traditional medicinal systems like Ayurveda and Unani uses the crude herbal drug in powder form. Since the ultimate goal of the herb drugs are safety and efficacy, the correct identification of the raw material is an essential component to ensure the reproducible quality of the herbal drugs [38]. Herbal products supplied to the market are often in a shredded, rolled, twisted, and distorted state without adequate identification or trade names. The taxonomical identification of herbal drugs based on macro-morphological characters. However**,** unlike macro-morphologically identification, pharmacognostic analysis of

herbal drugs are effective in detecting adulteration in powder [39]. Consequently, macro-morphological findings can be validated and authenticate by pharmacognostic protocols like organoleptic characters, micro-anatomical & powder microscopic analysis, physiochemical analysis, which will help in identifying genuine drugs because these analysis result in specific results for a particular drug [40,41]. All these pharmacognostic parameters are important in compilation of modern monographs.

Authenticating and standardization of crude herbs through anatomical diagnostic tool is the first and fundamental step [42-44]. The microscopic analysis of Areca nut revealed several anatomical features that are peculiar and useful for diagnostic purposes. Transverse section of seed reveals the characteristic features of Areca nut having hard seed coat with ruminated brownish endosperm tissue alternating with white tissue, having a semi-circular light brown scar of hilum lies just above the micropyle [45]. The power microscopic study of nut observed to have endosperm tissue with porous walls containing oil globules and aleurone grains which are of diagnostic importance in microscopic examination of Areca nut.

Physicochemical parameters studied are helpful in setting standards for a crude drug as these parameters are mostly constant for a particular plant [41]. Physicochemical parameters analysis for medicinal plants started with analysis of foreign material present in raw herbal products which helps to determine the adulteration. The foreign matter value in present study is less than 2%; hence Areca nut sample passes the pharmacopoeial limit. Determining the moisture content is important because herbal drugs that include moisture are more likely prone to degradation or deterioration. The percentage of moisture content ranging from 10 - 20% shows an ideal range for minimum bacteria as well as for fungal growth [46]. The moisture content in Areca nut drug sample was low (nearly 11 %) which is apt and unlikely to induce any deterioration during storage. The ash content is another important parameter which helps to measure of inorganic impurities in the herbal drugs i.e the presence or absence of foreign matter such as metallic salts and/ or silica [47,48]. The total ash value and the acid insoluble ash values of Areca nut were found to be relatively low, which suggests that there is a low level of foreign matter contamination. Extractive values of sample were high for ethanol soluble than water and hexane soluble extractives which suggest that the sample were more soluble in ethanol than water and hexane. Similarly, HPTLC analysis of Areca nut may serve as a useful data for the standardization of the drug. The analysis for safety and toxicity evaluation proclaims that the sample of Areca nut was free from any biotic and abiotic contaminants viz., aflatoxins, heavy metals and pesticide.

CONCLUSION

The development of pharmacopoeia standards for Areca nut are based on result of macro-microscopic analysis, physicochemical profiling and HPTLC fingerprinting. Areca nut is an important medicinal plant in various Ayurveda and Unani formulation. Thus, the current study contributes to the authentication of crude drug purchased from the market for the accurate identification of plant ingredients which leads to the ensuring of safety, efficacy and purity of this medicinal plant. As the demand for herbal drugs are growing day by day due to increasing of reliance on herbal products, it is proposed that the present standardization tools for Areca nut will help in sustaining the quality of many herbal preparations.

Acknowledgements

We express our sincere gratitude to the Director of the Central Council for Research in Unani Medicine, New Delhi, for his support and providing necessary facilities to complete the present research work.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

ORCID ID

Usha Devi: https://orcid.org/0000-0001-8243-0044

Shoeb Ahmed Ansari: https://orcid.org/0009-0008-3154-8091

Asma Sattar Khan: https://orcid.org/0000-0002-9825-3617

Anees Ahmad: https://orcid.org/0009-0002-3774-0101

Mohammed Wasim Ahmed: https://orcid.org/0009-0006-4892-9347

Rampratap Meena: https://orcid.org/0000-0003-4131-8069

REFERENCES

1. Chaughule RS, Barve RS. Role of herbal medicines in the treatment of infectious diseases. Vegetos. 2023; 18:1-1.
2. Alam F, Najum us Saqib Q. Pharmacognostic standardization and preliminary phytochemical studies of *Gaultheria trichophylla*. Pharmaceutical biology. 2015; 53(12):1711-8.
3. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products: A review. Future Journal of Pharmaceutical Sciences. 2020; 6:1-9.
4. Muyumba NW, Mutombo SC, Sheridan H, Nachtergael A, Duez P. Quality control of herbal drugs and preparations: The methods of analysis, their relevance and applications. Talanta Open. 2021; 1; 4:100070.
5. Ahmad A, Ansari SA, Khan AS, Devi U, Ahmed MW. Pharmacognostical authentication of heartwood of *Pterocarpus marsupium* Roxb.-An important drug used in traditional systems of medicine. Journal of Pharmacognosy and Phytochemistry. 2023;12(2):138-145.
6. Devi U, Khan AS, Ahmed A, Ansari SA, Ahmed MW, Meena R. Pharmacognostic evaluation and development of quality control parameters for Berg-e-Bed-Sada (leaf of *Salix alba* L.). Journal of Pharmacognosy and Phytochemistry. 2023;12(1):672-678.
7. Staples GW, Bevacqua RF. *Areca catechu* (betel nut palm). Species profiles for Pacific Island agroforestry. 2006;1(13):1-9.
8. Heatubun CD, Dransfield J, Flynn T, Tjitrosoedirdjo SS, Mogea JP, Baker WJ. A monograph of the betel nut palms (*Areca*: Arecaceae) of East Malesia. Botanical Journal of the Linnean Society. 2012;168(2):147-73.
9. Latap NS. Economic Assessment of Betel Nut (*Areca cathecu*) as Component in the Agroforestry (AF) Systems in Ifugao. International Journal of Science and Research. 2015; 4(6):1896:1913.
10. Ansari A, Mahmood T, Bagga P, Ahsan F, Shamim A, Ahmad S, Shariq M, Parveen S. *Areca catechu*: A phytopharmacological legwork. Food Frontiers. 2021;2(2):163-83.
11. Saeed SA, Farnaz S, Simjee RU, Malik A. Triterpenes and Bsitosterol from Piper betle: isolation, antiplatelet and antiinflammatory effects. Biochemical Society Transactions.1993;21(4):462S
12. Zhang X, Mei W, Zeng Y, Liu J. Phenolic constituents from the fruits of *Areca catechu* and their anti-bacterial activities. Journal of Tropical and Subtropical Botany. 2009;17(1):74-6.

13. Yenjit P, Issarakraisila M, Intana W, Chantrapromma K. Fungicidal activity of compounds extracted from the pericarp of *Areca catechu* against Colletotrichum gloeosporioides in vitro and in mango fruit. Postharvest Biology and Technology. 2010; 55(2):129-32.
14. Yang, WQ, Wang HC, Wang, WJ, Wang Y, Zhang XQ, Ye WC. Chemical constituents from the fruits of *Areca catechu*. Journal of Chinese Medical Materials. 2012; 35(3), 400–403.
15. Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, Wu CJ. *Areca catechu* L. (Arecaceae): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. Journal of ethnopharmacology. 2015; 164:340-56.
16. Abbas G, Kashif M, Khan TA, Bhatti HA, Haque S, Naqvi S, Farooq AD. Cytotoxic, embryotoxic, insecticidal and anti-microbial activities of standardized *Areca catechu* nut. Pakistan Journal of Pharmaceutical Sciences. 2018;31(2).
17. Sari LM, Hakim RF, Mubarak Z, Andriyanto A. Analysis of phenolic compounds and immunomodulatory activity of areca nut extract from Aceh, Indonesia, against *Staphylococcus aureus* infection in Sprague-Dawley rats. Veterinary World. 2020;13(1):134.
18. Jerard BA, Damodaran V, Jaisankar I. Promising Unique Accession of Areca nut (*Areca Catechu*). Journal of the Andaman Science Association.2022;27(2):216-21.
19. Chen X, He Y, Deng Y. Chemical composition, pharmacological, and toxicological effects of betel nut. Evidence-Based Complementary and Alternative Medicine. 2021; 18. doi.org/10.1155/2021/1808081.
20. Liu PF, Chang YF. The Controversial Roles of Areca Nut: Medicine or Toxin? International Journal of Molecular Sciences. 2023;24(10):8996.
21. Grover M. Areca catechu L. (Chikni Supari): A Review Based Upon its Ayurvedic and Pharmacological Properties. The Journal of Phytopharmacology. 2021;10(5):338-44.
22. Hakeem HMA. Bustan Ul Mufredat, Idara Kitab-ul-Shifa Dariya Ganj New Delhi. 2002; pp-233.
23. Yusuf Ibn Mohammad Ibn Yusuf Harawi. Reyaz-ul Advia(Urdu Translation) By CCRUM New Delhi, Rakmo Press Pvt Ltd New Delhi 110020. 2022; 121.
24. Johansen, DA. Plant microtechniques. Mc-Graw Hill book company Inc. New York and London. 1940; 13:65-105.
25. Trease GE, Evans WC. Pharmacognosy. Baillière Tindall, London. 1989; 13:5-9.
26. Wallis TE. Textbook of pharmacognosy, 5th ed. CBS Publishers & Distributors Pvt. Ltd., New Delhi. 2005; 578:493-494.
27. Aswatha RH, Kaushik U, Lachake P, Shreedhara CS. Pharmacognosy Research. 2009; 4:224–7.
28. Chandel HS, Pathak AK, Tailang M. Standardization of some herbal antidiabetic drugs in polyherbal formulation. Pharmacognosy Research. 2011; 3(1):49-56. doi: 10.4103/0974-8490.79116.
29. Anonymous. Physicochemical standard of Unani formulations, Part-II. CCRUM, Govt. of India, New Delhi. 1987; 2:268-281.
30. Anonymous. Quality control methods for medicinal plant materials. World Health Organization, Geneva. 1998; 28-33.
31. Wagner H, Bladt S. Plant Drug Analysis- A Thin Layer Chromatography Atlas, springer Verlag, Germany. 1996; 1-3: 293-303.
32. Stahl E. Thin layer chromatography-A Laboratory Handbook. George Allen and Unwin Ltd. London. 1996; pp-900.
33. Anonymous. Official analytical methods of the American Spice Trade Association (ASTA), 4th ed., New Jersey. 1997; 149-152.
34. Anonymous. Official methods of analysis. Horwitz W, Latiner G W (ed.) 18thed. AUAC International, Marryland. 2005; 3: 10-11, 10: 18-23 and 26:17.
35. Angiosperm Phylogeny Group (APG IV). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV– Bot. J. Linn. Soc. 2016; 181: 1-20.
36. Kirtikar KR, Basu BD. Indian medicinal plants. Vols. I – IV (Text) + V-VIII (Plates). Reprinted by Bishen Singh, Mahendra Pal Singh, Dehradun, India, 1935, pp-2793.
37. Dwivedi SK, Dey S. Medicinal herbs: A potential source of toxic metal exposure for man and animals in India. Arch Environ Health. 2002; 57:229–31.
38. Nayak BS, Patel KN. Pharmacognostic studies of the *Jatropha curcas* leaves. Int J PharmTech Res. 2010; 2:140–143.
39. Kumar D, Pravin PV, Zulfikar AB, Jeevan D, Yogesh K, Santosh B. Macroscopical and microscopical evaluation of leaves of *Clerodendrum inerme* Gaertn. International Journal of Biological & Medical Research. 2011; 2(1):404-408.
40. Ajaykumar RS, Rajendra DW. Pharmacognostic study and development of quality parameters of *Hamelia patens* Jacq. Stems. Der Pharmacia Lettre. 2016; 8(8):6-13.
41. World Health Organization (WHO). Quality Assurance of Pharmaceuticals: A Compendium of Guidelines and Related Materials, Good Manufacturing Practices and Inspection. Geneva: WHO, 1996a, 22016;8(8):6-13.
42. Ghani A. Introduction to Pharmacognosy. 1st edition, Ahmadu Bello University Press Ltd., Zaria. c1990; p. 87.
43. Atinga V, Musa TL, Ibrahim HM, Ayeni EA, Namadina MM, UM Jajere, et al. Standardization of stem bark of *Pseudocedrela kotschyi* (Meliaceae). Journal of Pharmacognosy and Phytochemistry. 2021; 10(6):19-23.
44. Devi U, Khan AS, Ansari SA, Ahmed R, Meena RP. Pharmacognostic evaluation and Hptlc fingerprinting of Itrifal Mulayyan-a unani polyherbal formulation. World Journal of Pharmaceutical Research. 2024; 3(6):544-562.
45. Dransfield J. The genus *Areca* (Palmae: Arecoideae) in Borneo. Kew Bull. 1984; 39: 1–22.
46. Sumbul S, Ahmad MA, Asif M, Akhtar M, Saud I. Physicochemical and phytochemical standardization of berries of *Myrtus communis* L. J Pharm Bioallied Sci. 2012; 4(4):322-6. doi: 10.4103/0975-7406.103266. PMID: 23248567; PMCID: PMC3523529.
47. Musa KY, Katsayal AU, Ahmed A, Mohammed Z and Danmalam UH. Pharmacognostic Investigation of the Leaves of *Gisekia pharmacioides*. African Journal of Biotechnology. 2006; 5:956-957.
48. Uba A, Baburo SI. Physico-chemical parameters and heavy metals determination in selected medicinal plants sold in 'Yar Marina market, Sokoto, Nigeria. Chem Mater Res. 2016;8(9):37-41.

HOW TO CITE THIS ARTICLE

Devi U, Ansari SA, Khan AS, Ahmad A, Ahmed MW, Meena R. Pharmacognostic evaluation and development of quality control parameters for Fufal (Areca nut). J Phytopharmacol 2024; 13(3):220-229. doi: 10.31254/phyto.2024.13305