

Structural Elucidation of Bioactive Constituents Isolated from *Areca catechu* L.

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ABSTRACT

Background: *Areca catechu* is a significant tropical palm, primarily cultivated for its nut, which holds traditional, medicinal, and socio-cultural importance across Asia. **Objectives:** This current study shows the isolation and structural elucidation of bioactive constituents from plant species *Areca catechu* L. (betel palm) nuts belonging to the family of Arecaceae. **Materials and Methods:** Unripe fruits of *Areca catechu* were authenticated and evaluated for macro-morphological, microscopic, physicochemical, and phytochemical characteristics as per WHO standards guidelines. Preliminary screening confirmed the presence of secondary metabolites as tannins, phenols, steroids, alkaloids, and flavonoids. Ethanol extraction of dried *Areca catechu* by using cold maceration method was followed by solvent fractionation (n-hexane, toluene, 1,2-dichloromethane, chloroform, ethyl acetate). TLC profiling guided the column chromatographic separation using toluene: methanol: glacial acetic acid (15:7.5:2.5), elucidating three isolates (B-3, B-4, B-7). **Results:** Spectroscopic analyses give UV maximum absorption at wavelength 254-273 nm (B-3, B-4) and 260 nm (B-7). FTIR spectroscopy identified different functional groups as hydroxyl, carbonyl, amino, aromatic, and alkyl groups. The nuclear magnetic resonance spectroscopy (1H-NMR) showing aromatic and alkyl proton signals. Mass spectrometry provided molecular ion peaks and fragmentation patterns, facilitating probable structure proposals. **Conclusion:** These results establish a detailed phytochemical and structural profile of *Areca catechu* constituents, supporting its traditional uses and laying the groundwork for further pharmacological and therapeutic investigations.

Keywords: *Areca catechu*, Column Chromatography, FTIR, Mass Spectrometry, NMR, Phytoconstituents.

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INTRODUCTION

Plant secondary metabolites are compounds of medicinal utility. Natural products were studied for secondary metabolites, their isolation in pure form along with investigation of their structure, formation, use, and purpose in the organism. Plants produce a variety of secondary metabolites to protect themselves from infections and predators and to aid in reproduction by acting as intraspecific and interspecific attractants. They might also operate as toxins to provide competitor species a competitive edge. Natural products are classified as acetogenins as well as propanogenins, terpenoids, derivatives of amino acids, and aromatic compounds.

They protect the plant against herbivores and are attractants. Phenolic compounds like Tannins, lignans, flavonoids serve as

defences against herbivores and pathogens. Plant cell wall gets strengthened because of the presence of lignin. Flavonoid pigments are important attractants for pollinators and seed dispersers. Alleopathic activity of some phenols may adversely influence the growth of neighbouring plants. Adaptogenic nature of plants developed defences against herbivory and microbial attack and produced other natural products to foster competitiveness. The capacity to make and safely store such ecologically beneficial metabolites has become broadly established throughout the plant as a result of the more progeny produced by the more competitive, better-defended plants. The natural product study led to the detection of a variety of valuable drugs for the treatment of various diseases and contributed to the development of separation science and technology, spectroscopic methods of structure elucidation and synthetic methodologies that now make up the basics of analytical organic chemistry (Rizvi and Saleh, 2017).

Areca catechu L. [betel palm] is one of the valuable plants used from ancient times. This plant is known for its fruits which are obtained from medium-sized palm trees. In low altitude, this plant has a life span of 60-100 years. The Pacific islands found two different



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varieties red (ugam) and white (changnga) are available in the Pacific Islands. The white variety is collected immature and soft while the red variety is preferred fully mature and hard. Betel nuts are often sold in village stores and munched by combining it with betel vine leaf (*Piper betle*), tobacco, lime, or other ingredients (Staples and Bevacqua, 2006). Different others Common Names: Angiro, Adakka, Areca nut palm, Adike, Areca, Areca-nut, Areca palm, Betel palm, Arequier, Arec cachou, Betelnuss palme, Buei, Bu, Bua, Indian palm, Jambe, Kamuhu, Pan, Pinang palm, Poc, Pu, Puak, Puga, Puguu, Puwak, Supari, Vua Buai, Bunga (Rashid *et al.*, 2015; Ahuja and Ahuja, 2011; Lim, 2011).

Synonyms: *Areca cathecu*, *Areca faufel*, *Areca hortensis*, *Areca himalayana*, *Areca nigra*.

Family Name: *Arecaceae*.

Plant Appearance.

Distinctive feature

Betel nuts are identified by their red-orange ripe fruits and slender, single-stemmed trunk with leaf scars. The Manila palm (*Veitchia merrillii*), which has dense clusters of vivid red fruits, is frequently mistaken for it.

Leaves are narrow, oval-shape lanceolate, Even-pinnate, compound leaves and spirally arranged at the top of the stem (Ansari *et al.*, 2011; Keys, 2011).

Flowers are triangular, cone, 0.5-0.8 in. long, creamy white. Flowers are located in every terminal branch beneath the leaves. Each terminal branch is made up of both female and male flowers. The female flowers, which are larger, are situated at the tip of the base, while the smaller male flowers grow on the lower section up to the branch tip. Plant begins to flower after 4-6 years, year-round.

Drupe types fruits having size of 2-4 x 1.2-2 in., green and turns yellow to orange or red when ripe. One seeded edible fruits.

Betal nut is unripe and ripe fruit obtain from the species of *Areca catechu* L. [betel palm], from the *Arecaceae* family (George *et al.*, 2006).

Chemical constituent

Arecoline, Arecaidine, Guvacine and Guvacoline. Seed contains 50-60% sugars, 15% lipid [glyceride of lauric, myristic and oleic acid] 15% condensed tannins polyphenolic and alkaloids as arecoline, arecaidine, guvacine and guvacoline (Wang *et al.*, 2010; Hong *et al.*, 2015; Pathak and Mathur, 1954; Rathod *et al.*, 2015).

Taxonomical Classification

- **Kingdom:** Plantae [Plants].
- **Subkingdom:** *Tracheobionta* [Vascular plants].

- **Order:** *Arecales*.
- **Class:** *Liliopsida* [Monocotyledons].
- **Subclass:** *Arecidae*.
- **Genus:** *Areca* L. [areca palm].
- **Species:** *Catechu*.

Traditional uses

- In Ayurvedic Medicine to treat fever, headaches and rheumatism.
- In Southern region of India it is used treat migraine headache.
- Used to treatment for urinary problems.
- Used to treat malaria and dysentery.
- Eliminate tapeworms as well as intestinal parasites.
- It provides- relief from hunger, exhaustion, abdominal pains, leprosy, leucoderma, anemia and obesity (Wang *et al.*, 2024).

The adverse effects of Areca Nut [AN] chewing habit on oral health have been well known since no review reporting the harmful effects of AN chewing on systemic health is yet available (Javed *et al.*, 2010).

MATERIALS AND METHODS

Plant Material

Areca catechu were collected from medicinal plant garden of Marathwada Mitra Mandal's College of Pharmacy Pune. The Identification and authentication was confirmed by Department of Pharmacognosy Marathwada Mitra Mandal's College of Pharmacy Pune.

Macromorphological and microscopical Evaluation

Macromorphological and Microscopical characterization of *Areca catechu* was done as per standard WHO guidelines (Patel *et al.*, 2019; Yusriah *et al.*, 2016; Trease and Evans, 2009).

Physicochemical and Phytochemical Evaluation

The physicochemical parameters, including foreign organic materials, loss on drying, ash values, and extractive values, were determined as per WHO guidelines (Indian Pharmacopoeia, Vol-II, 1996; Patel *et al.*, 2019).

Preliminary Phytochemical Screening of Crude Drugs

Chemical tests were performed for the qualitative estimation of the Phytoconstituents as per standard procedures (Khandelwal K., 2010; Sapaat *et al.*, 2022).

Extraction and Fractionation

1000 g of dried *Areca catechu* was extracted with 500 mL of Ethanol by cold maceration technique keeping it at room temperature till solvent becomes Colorless. Then filter the extract using whatman filter paper. Solvent is removed by using Vacuum evaporation by maintaining temperature between 30-40°C (Sayuti *et al.*, 2023). A dark Brown residue obtained having characteristic odour. Further remaining solvent is evaporated to dryness by using vacuum oven. The fractionation of ethanolic extract was carried out with solvents viz. n-Hexane, toluene, 1,2 dichloromethane, chloroform, ethyl acetate (Chavan and Singhal, 2013).

Chromatography of Extract

The composition of the crude extracts was checked by using TLC. Optimization of Chromatographic system was performed.

Isolation and Characterization of Phytoconstituents

Preparation of the Column

Glass column was packed by wet filling. The slurry of adsorbent [Silica gel for column chromatography, 60-120 mesh] was prepared by mixing the adsorbent in the suitable solvent and used as stationary phase. It was then poured into glass column [600 cm x 4.5 cm] [having glass wool at its bottom] and allowed to settle. The air entrapped was removed by stirring with glass rod. A small amount of sand was kept a top the column to provide the latter a flat base. Excess of solvent was run off until the level of mobile phase reach to one cm just above the top of the sand layer.

Preparation of sample and loading

Extract of *Areca catechu* were impregnated with silica gel and loaded onto silica gel column to separate possible phytoconstituents. The column was eluted with Toluene and mixture of Toluene: Methanol: Glacial Acetic Acid with increasing

polarity. Alteration in the composition of the eluting solvent was achieved by adding the second solvent of more polarity gradually to a reservoir of the first column. Mobile phase was passes with constant flow rate [25 mL/min]. At uniform interval, 9 elutes were collected and progress of separation was monitored by Thin layer chromatography using various solvent system (Guo *et al.*, 2024; Khandelwal, 2006).

Characterization of Isolated Phytoconstituents

Preliminary Investigation of Isolated Phytoconstituents was performed followed by analysis using spectroscopic methods. 0.1mg of Phytoconstituents individually dissolved in 10 mL Ethanol. This solution was scanned between 200 nm to 600 nm in a double beam UV spectrophotometer [Shimadzu 1800]. For IR absorption spectrum for Phytoconstituents was recorded by FTIR, where drug sample was placed on IR compartment and scanned between wave number 4000⁻¹ to 600 cm⁻¹ using a Shimadzu Model 8400 FTIR with ATR. For Isolated phytoconstituents from Ethanolic extract of *Areca catechu* Small amount of Phytoconstituents was dissolved in CDCl₃ and NMR spectra interpreted by Varian Mercury 500 MHz. The study has been carried out in CIF, SPPU PUNE-411007. For Isolated phytoconstituents from Ethanolic extract of *Areca catechu* Isolated compounds were scanned between 50m/z to 1200m/z by using Electrospray ionization technique. The study has been carried out in CIF, SPPU PUNE-411007 (Sumalatha and Ishwara, 2019; Yen *et al.*, 2020; Letha *et al.*, 2020).

RESULTS

Morphology and Microscopic Characterization

Morphological characters of unripe fruits of *Areca catechu* shows light brown color with characteristic odour, having round shape, rough texture having Length and width of 1.7 cm.

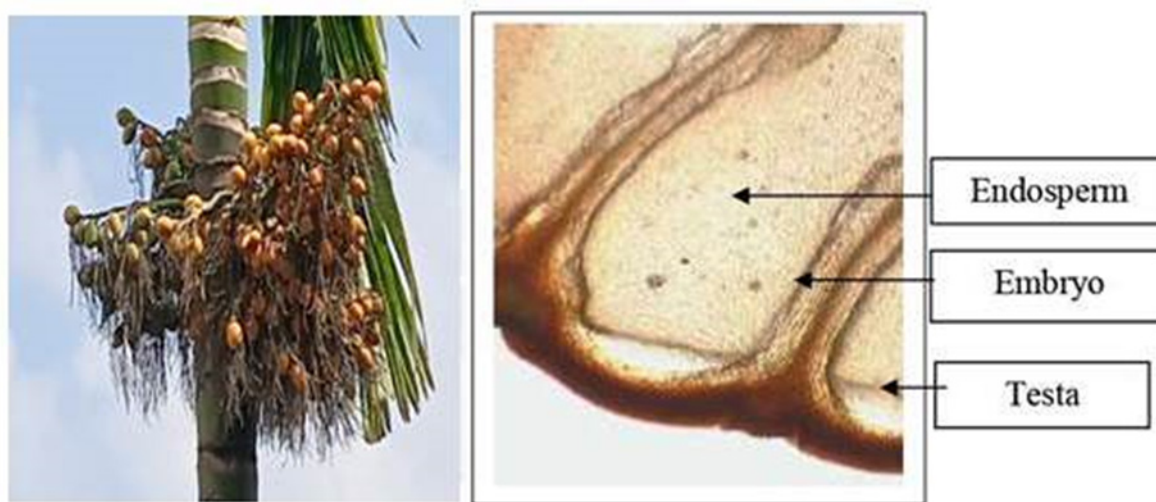


Figure 1: Morphology and Microscopical characterization of *Areca catechu* nut. Location: Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune.

Table 1: Physicochemical Parameters.

Sl. No.	Parameters (%w/w)	Observation
1.	Foreign organic matter	1.3±0.05
2.	Moisture content (LOD)	11.08±0.33
Ash Values		
3.	Total ash	6.05±0.004
4.	Acid insoluble ash	1.02±0.015
5.	Water soluble ash	2.05±0.025
Extractive values		
6.	Water soluble extractive value	24.1±0.033
7.	Alcohol soluble extractive value	03±0.055

Table 2: Fractionation: Fractionation of Ethanolic Extract of *Areca catechu* from n-Hexane-Ethyl Acetate.

Sl. No.	Parameter	Observation				
		n-Hexane	Toluene	1,2-DCM	Chloroform	EA
1.	Colour	Colourless	Colourless	Light brown	Colourless	Colourless
2.	% yield	0.3%	0.5%	0.4%	0.5%	0.3%

Table 3: Preliminary Investigation of Isolated Compound.

Sl. No.	Parameters	Observation		
		B-3	B-4	B-7
1.	Nature	Liquid	Liquid	Liquid
2.	Colour	Brown	Brown	Colourless
3.	% Yield	0.8%	0.8%	0.6%

B-3 - Elute No 3rd, B-4 - Elute No 4th, B-7 - Elute No 7th.

The transverse section of *Areca catechu* Fruit shows the presence of Endosperm, Sclerenchyma, Endosperm, Testa and Parenchyma (Figure 1).

Physicochemical and Phytochemical Evaluation

Preliminary Phytochemical Screening of Unripe *Areca catechu* Powder

Preliminary Phytochemical Screening of Powder of unripe Areca catechu

Preliminary Phytochemical Screening of Powder of unripe *Areca catechu* shows presence of Tannins, Phenol, Flavonoid, Steroids and Alkaloids.

Extraction and Fractionation

Preliminary investigation of ethanolic extract of Areca catechu

Ethanolic extract of *Areca catechu* was found to be Brown color solid with characteristic odour.

Fractionation of Extract

Characterization of Fraction and Extract

Isolation of Phytoconstituents by Column Chromatography

Isolation of Phytoconstituents was done by Column Chromatographic technique using Mobile Phase Toluene: Methanol: Glacial Acetic Acid (15:7.5:2.5). Total 09 Elutes are collected out of 3,4,7 Elutes are further identified.

Preliminary Investigation of Isolated Compound

Spectroscopic Evaluation of Isolated Compound

Determination of UV Spectrum (λ_{max})

For Isolated Compound from Ethanolic Extract of *Areca catechu* Maximum Absorption of Isolated Compound B-3 have been found to be 254 nm. for (B-4) 258 nm and for (B-7) 260 nm.

Determination of Infra-Red Absorption Spectrum

The FTIR analysis revealed the presence of various functional groups in three isolated bioactive (B-3, B-4, B-7) of *Areca catechu* (Figure 2).

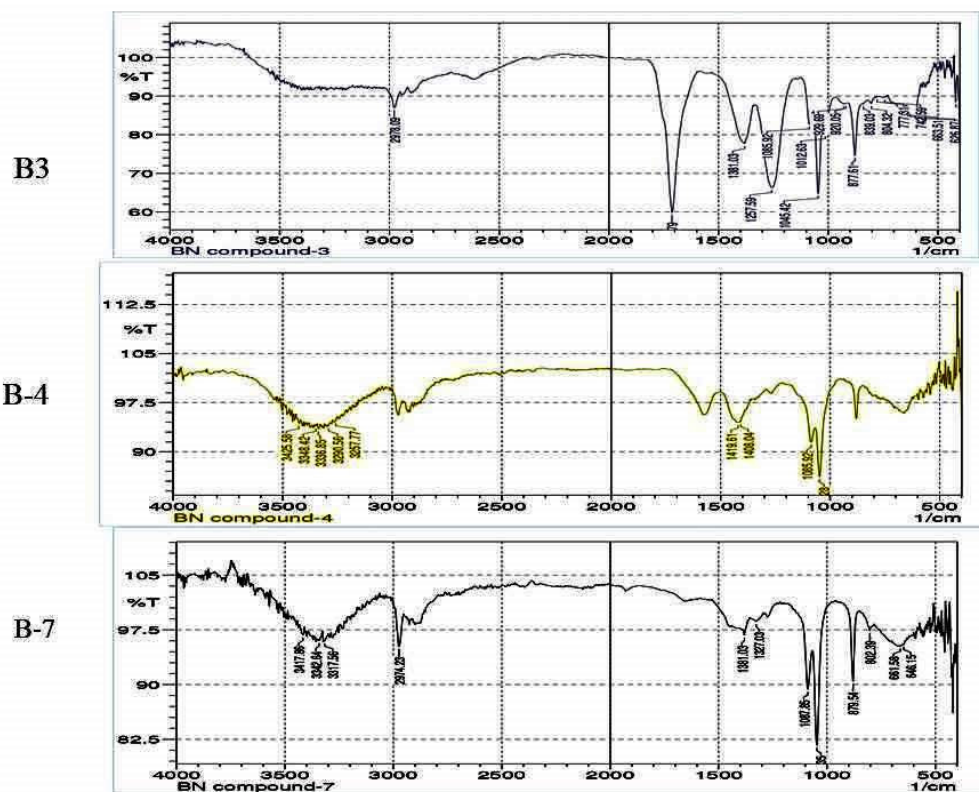


Figure 2: IR Spectra of Isolated Compounds (B-3), (B-4) and (B-7) from extract of *Areca catechu* nut.

Table 4: IR Spectra Values of Major Peaks (B-3).

Sl. No	(B-3)		B-4		B-7	
	Wave Number Observed (cm ⁻¹)	Group Identified	Wave Number Observed (cm ⁻¹)	Group Identified	Wave Number Observed (cm ⁻¹)	Group Identified
1.	2978	-OH Stretch	3428	N-H Stretch	3417	N-H Stretch
2.	1381	-CH Bend	1419	C-H Bend	3317	OH Stretch
3.	1712	C=O	1408	C-C in ring	2974	C-H Stretch
4.	1257	C-N Stretch	1085	C-O Stretch	1381	C-C Aromatic ring
5.	1082	C-O Stretch	1049	C-N Stretch	1327	C-N Stretch

Determination of NMR Spectrum

¹H-NMR spectroscopy was performed for isolated bioactive (B-3, B-4, B-7) from *Areca catechu* to identify the adulterants in herbal (Figure 3).

Determination of MS Spectra

Mass spectroscopy helps us to know the molecular mass of a compound. Compound B3 showed following peaks: 525 (Base Peak), 349,437,481,569, 613. Compound B4 showed following peaks: 365 (Base Peak), 274,425,621,753,945. Compound B7 showed following peaks: 393 (Base Peak), 312,551,659,704,763 (Figure 4).

Structural Elucidation of Isolated Compound

Probable Structure for Isolated Compound B-3, B-4 and B-7 (Figure 5)

DISCUSSION

Identification of low quality and adulteration with many undesirable elements, or improper processing of the crude drug during collection, drying, storage, etc., are crucial components of raw material evaluation. Organoleptic Characteristics reveals that unripe *Areca catechu* have light brown colour with characteristic odour. Microscopic evaluation shows the presence of endosperm, embryo and testa (Aurelien *et al.*, 2011). For checking the purity and quality of raw material physicochemical parameters play a

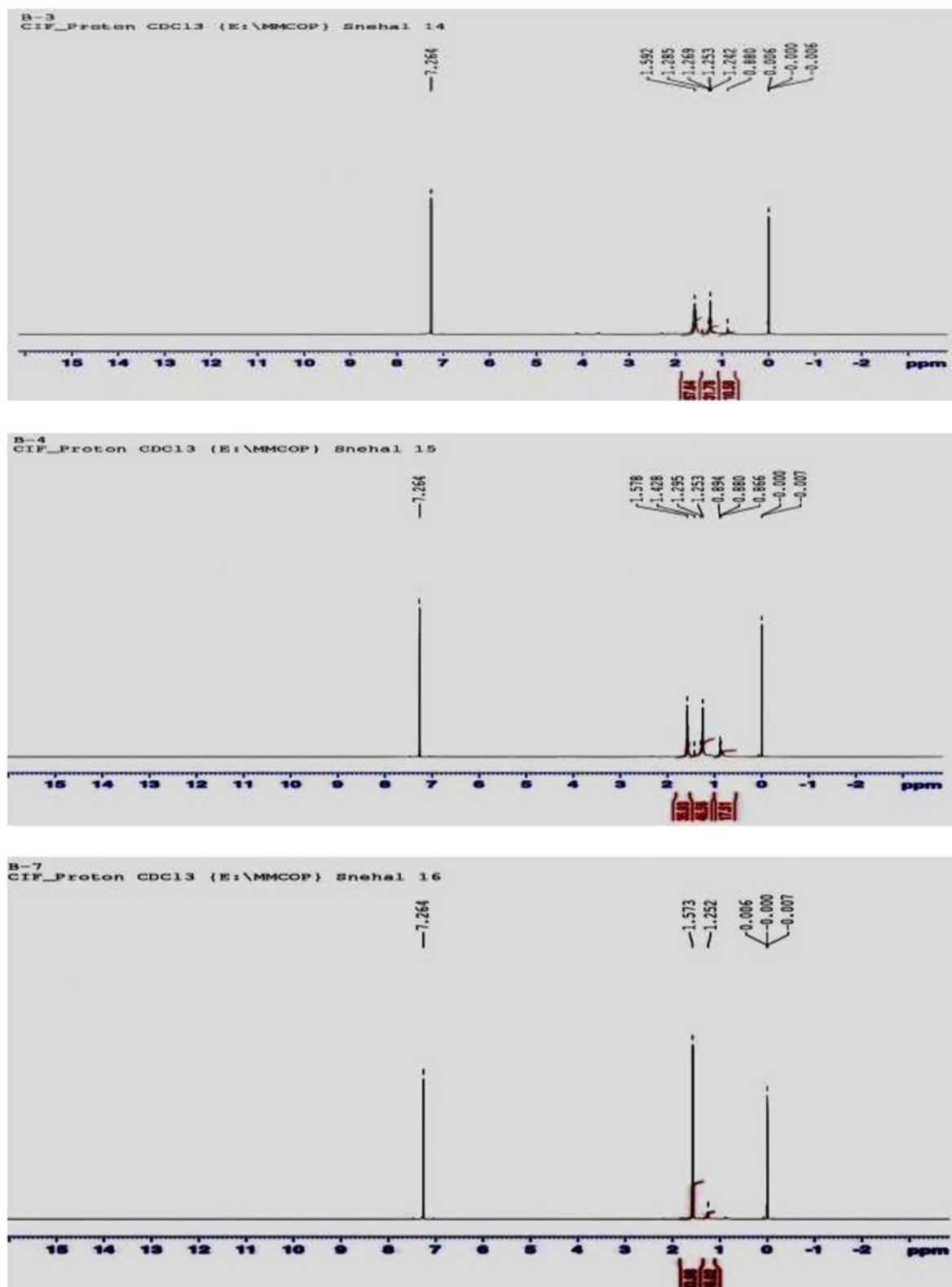


Figure 3: NMR Spectra of Isolated Compounds (B-3), (B-4) and (B-7) from extract of *Areca catechu* nut.

crucial role unwanted parts of drugs, adulteration, possess a character that will hamper physicochemical constants. The result of physicochemical parameters is within the limit (Table 1). Result of foreign organic matter present in crude *Areca catechu* is very

less. Not only the ultimate dryness is important, equally important is rate at which the moisture is removed thus the determination of moisture contain also provides the method of preparation of drug the results shows that *Areca catechu* is properly dried, which lies

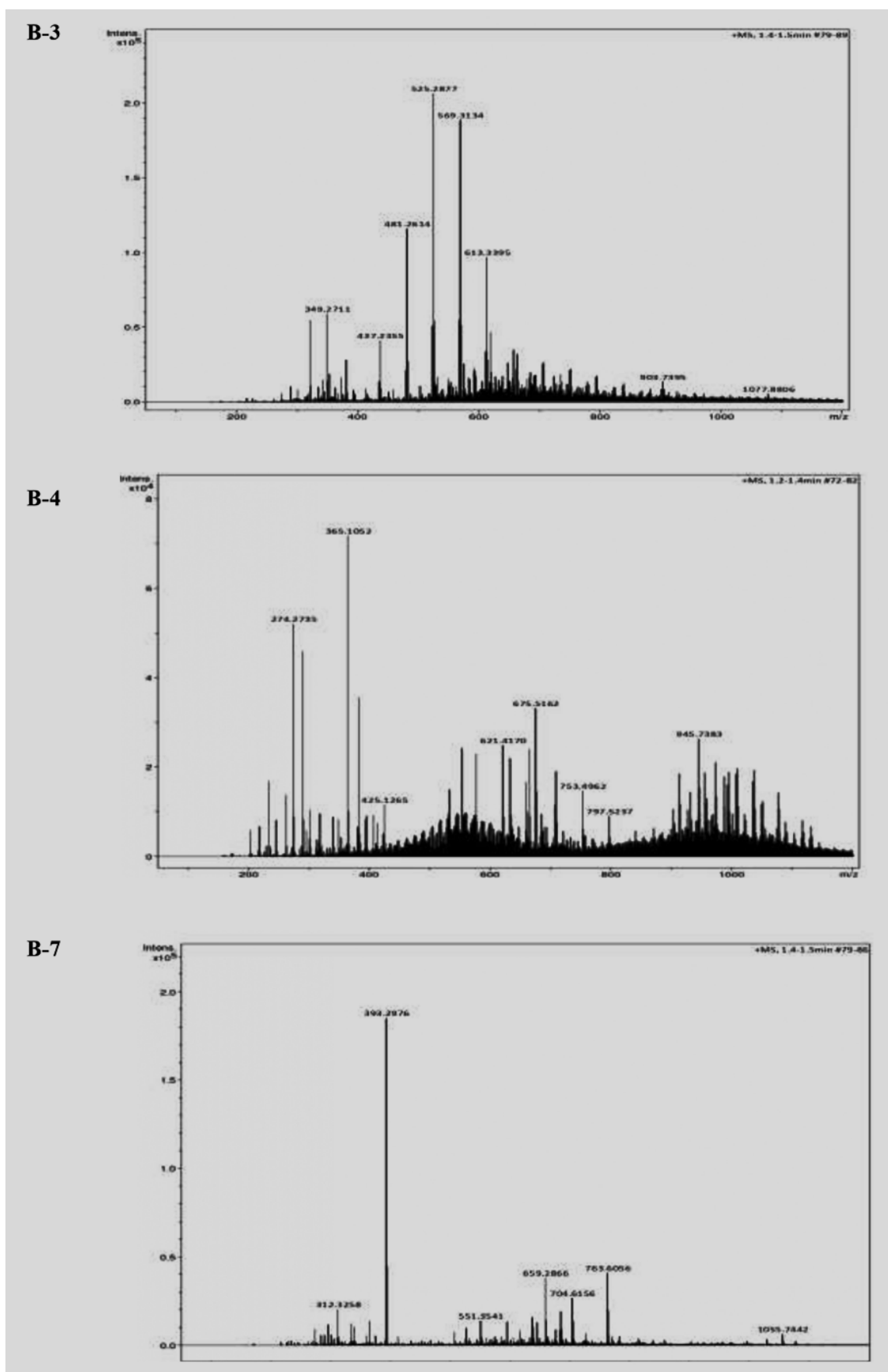
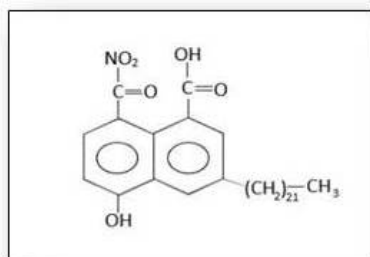
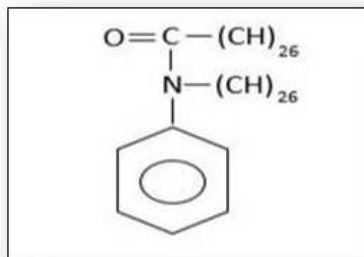
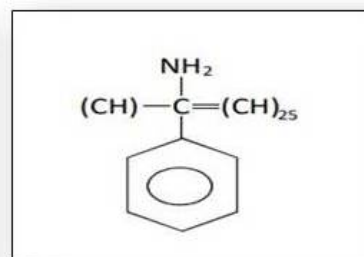


Figure 4: MS Spectra of Isolated Compounds (B-3), (B-4) and (B-7) from extract of *Areca catechu* nut.

Table 5: NMR Data of Isolated Compound (B-3).

Sl. No.	B-3		B-4		B-7	
	Peak Observed	Inference	Peak Observed	Inference	Peak Observed	Inference
1.	7.264	Aromatic Proton (s)	7.264	Aromatic Proton (s)	7.264	Aromatic Proton (s)
2.	1.592	Alkyl Proton (s)	1.578	Alkyl Proton (s)	1.873	Alkyl Proton (s)
3.	1.258	Alkyl Proton (s)	1.428	Alkyl Proton (s)	1.252	Alkyl Proton (s)
			1.295	Alkyl Proton (s)		

**B-3****B-4****B-7****Figure 5:** Probable Structure for Isolated Compound B-3, B-4 and B-7.

within the limit (Evans W., 2009). Ash values of the *Areca catechu* lies within the limits which signify its quality and purity and gives idea about the total inorganic content in crude drug (The Ayurvedic Pharmacopoeia of India., 1999). The Phytochemical investigation is probably responsible for activity. The preliminary phytochemical investigations of crude drug of *Areca catechu* were performed which shows the presence of Tannins, Phenols, Flavonoids, steroids, Alkaloids (Table 1) (Khandelwal, 2006). Extractions are a method to separate a preferred substances and phytoconstituents from raw material. Extraction of *Areca catechu* is carried out by using maceration technique by using ethanol as solvent. Ethanolic extract of *Areca catechu* was dark brown in colour with characteristic odour. Fractionation is process to separate the phytoconstituents from extracts via increasing polarity. In present study fractionation was carried with five different solvents with increasing polarity (Table 2). Thin layer chromatography is generally used for separation and identification of phytoconstituents from extracts. Ethanolic extract of *Areca catechu* subjected to TLC and satisfactory resolution was obtained in the solvent was found to be Toluene: Methanol: Glacial Acetic Acid [0.6:0.3:0.1]. The column chromatography is well known tool for separation and isolation of phytoconstituents. It provides wide range separated phytoconstituents helps in screening unknown fractions/constituents. In present study mobile phase used for column chromatography is Toluene: Methanol: Glacial Acetic Acid [15:7.5:2.5]. 9 elutes were collected based on literature review 3 elutes were selected for further study (Table 3). For the structure elucidation purpose Spectroscopic and Chromatographic technique were implemented. UV Visible Spectroscopy is mostly used for identification of saturation and

unsaturation in structure. FTIR was subjected for identification of functional groups present in compound. NMR is mainly used for no. of proton present in compound which helps in structure elucidation. Mass is elemental analysis used for determine masses of particles and of molecules elucidation of chemical structure of compound. Isolated Phytoconstituents exhibited maximum absorption at λ_{max} at 254 and 273. FTIR was used for the identification of functional groups present in isolated fractions. IR spectrum of isolated compound B-3 shows OH [stretch] at 2974, CH [Bending] at 1381, C-O [stretch] at 1082, C-N (stretch) at 1257, B-4 Compound Shows N-H [Stretch] at 3428, C-O [Stretch] at 1085, C-N (stretch) at 1049, C-H (Bending) at 1419, and B-7 Compound Shows OH [stretch] at 3317, C-H [Stretch] at 2974, N-H (stretch) at 3417, C-N [stretch] at 1327 (Table 4). Mass Spectra data used for the identification of major and minor fragments, which helps to establish the structure of compounds, by giving exact molecules elucidation of chemical structure of compound. NMR of Isolated Compound B-3 shows the Chemical Shift Between [0.5-3] which is probably for various groups (Table 5) (Jarald and Jarald, 2007; Rangari, 2005).

CONCLUSION

The present study highlights the knowledge of the identity and quality of *Areca catechu* nuts. Physico-chemical and Chemical analysis of the nuts provides the quality and purity of the nuts. *Areccca catechu* nuts were extracted with ethanol by cold maceration technique. From extract Phytoconstituents were isolated with Column Chromatography. The isolated compounds were characterized with various Spectroscopic and

Chromatographic techniques. The present study was useful for further pharmacological and therapeutic assessment.

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ABBREVIATIONS

WHO: World Health Organisation; **FTIR:** Fourier-Transform Infrared Spectroscopy; **UV:** Ultraviolet/Visible Spectroscopy; **NMR:** Nuclear Magnetic Resonance; **LCMS:** Liquid Chromatography-Mass Spectrometry; **SPPU:** Savitribai Phule Pune University; **TLC:** Thin Layer Chromatography.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Areca catechu L. [betel palm] is one of the valuable plants used from ancient time. This plant is known for its fruits which are obtained from medium sized palm tree. The chemical constituents present in *Areca catechu* fruits are Arecoline, Arecaidine, Guvacine and Guvacoline. As per Ayurveda this plant is used to treat fever, headaches and rheumatism. In other parts of India the plant has been used to treat migraine headache, urinary problems, leprosy, leucoderma, anemia, obesity, malaria and dysentery. It is used to get relief from exhaustion and hunger. Research on its bioactive components can further expand its role in medicine.

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