Isolation And Characterization of Chemical constituents From Leaves of Areca Catechu

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Abstract: The study's goal is to identify, isolate, and characterise bioactive constituents in *Areca catechu* leaves. It's a popular folk remedy.*Areca catechu* L. is a plant in the Arecaceae family that is also known as Areca palm, Betel nut palm, or Betel palm.The best studied species, *Areca catechu* L., contains phytochemicals such as phenolics and alkaloids with biological properties.A survey of the literature revealed that no scientific investigation on the isolation of phytoconstituents from leaves had been done. The plant's leaves were chosen for phytochemical and pharmacological testing as a result of this.The leaves were then pounded to a coarse powder in the shade. Cold maceration extraction was performed on the coarse powder of *Areca catechu* L. leaves using chloroform, methanol, and petroleum ether as solvents.Chemical analysis of Areca catechu L. leaves resulted in the isolation of three compounds: NA1 from chloroform extract, NF1 from methanol extract, and NS1 from petroleum ether extract, with the structures identified as Arecoline, Apigenin, and Stigmasterol using physical, chemical, and spectral characteristics.

Keywords: Arecoline, Apigenin, Stigmasterol, *Areca catechu,* methanol, petroleum ether

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Introduction

Plants have been employed for therapeutic reasons since the dawn of human civilisation. Medicinal plants are linked to several types of traditional medicine, such as Traditional Chinese Medicine, Indian Ayurveda, and Japanese Kampo, but in the rest of the world, they are usually used in conjunction with pharmacological therapy [1]. Herbal product markets have grown significantly over the previous few decades, with diverse end-uses including flavours, colourants, essential oils, sweeteners, antioxidants, and nutraceuticals[2].Herbal medicine, also known as phytotherapy, is the science of treating various illnesses with herbal treatments. It covers all areas of herbal therapy, from plants with strong effects to those with mild effects. People are becoming more aware of the negative consequences of synthetic pharmaceuticals, which has resulted in an increase in the creation of natural items as

treatments for illnesses[3].Traditional medicine, particularly herbal medicine, is often regarded as a key source of healthcare, particularly in rural and remote places [4].This explosion has resulted in highly effective drugs that are widely used in clinics, including many plants natural products and analogues derived from these products, but it has fallen short of delivering effective cures for complex human diseases with complex causes, such as cancer, diabetes, autoimmune disorders, and degenerative diseases [5].Plant bioactive chemicals such as polyphenols, terpenoids, and alkaloids, which have a variety of physiological impacts on the human body, are thought to be responsible for the health benefits [6].

Areca catechu L., often known as Areca palm, Betel nut palm, or Betel palm, belongs to the Arecaceae family of plants. These are commonly farmed and grown throughout the year in India and many other Asian countries [7].Phytochemicals such as phenolics and alkaloids with biological characteristics are found in the best researched species, Areca catechu L. Roots contain the most phenolics, followed by fresh unripe fruits, leaves, spikes, and veins, while alkaloids are found in the following order: roots, fresh unripe fruits, spikes, leaves, and veins. The cardiovascular, pulmonary, neurological, metabolic, gastrointestinal, and reproductive systems have all been said to benefit from this species [8].

The leaves of Areca catechu L. (Arecaceae) are used in folk medicine to cure a variety of diseases. Following a review of the literature, it was discovered that no scientific study had been conducted for the isolation of phytoconstituents and pharmacological activity; in light of this, the plant's leaves were chosen for phytochemical and pharmacological screening. The current research provides the first glimpse into preliminary phytochemical screening.

Materials And Methods

Preparation of Plant material: The leaves of Areca catechu required for the study was collected from in and around Mangalore, Dakshina Kannada (dist), Karnataka; in the month of June and July 2017. The leaves were authenticated by Dr. K. V. Nagalakshamma, Head of the Dept. of Botany, St. Aloysius College, Mangalore. A voucher specimen (No.16PC005) is deposited in NGSM Institute of Pharmaceutical Sciences, Paneer, Deralakatte, Mangalore.

The shade dried leaves were then ground to coarse powder. The coarse powder of leaves of Areca catechu L. were subjected to cold maceration extraction using chloroform, methanol and petroleum ether as the solvents. Cold maceration was done in two parts of the plant powder (100g) for all the solvents, each for 7 days. After the extraction, the macerated powder was filtered using a muslin cloth. The marc obtained was air dried. The filtrate obtained was subjected to steam distillation, to concentrate the extract and the solvent was recollected and was used for further extraction process. A dark green residue was obtained on further concentrating and evaporating the extract on a water bath [9,10,11].

The extract obtained after the cold maceration process was suspended in distilled water (500g in 500ml) and fractioned by using different solvents. The fraction obtained was washed with distilled water and dried over anhydrous sodium sulphate. The solvent was removed by distillation under reduced pressure and then finally it was evaporated to dryness on a water bath. Yields of various fractions obtained were: Chloroform (23 g), Methanol (26 g) and Pet ether (25 g).

Isolation and Purification of compounds:

Chloroform Extract: The chloroform extract (20 g) was taken and partitioned into dilute HCl (0.1 N). The aqueous layer was alkalized with concentrated ammonium hydroxide. It was further partitioned with ether. The ether fraction was then concentrated to give alkaloidal fraction (5.45 g). The alkaloidal fraction (5 g) was taken and was loaded onto the silica gel column (150 g) prepared in chloroform. The column was eluted with 100% chloroform followed by graded mixture of 10%, 25%, 50% and 75% methanol in chloroform. The obtained elution was monitored by TLC (Silica Gel G; MeOH: $CHCl_3$ and visualised using Dragendroff's reagent). Elution with MeOH: CHCl3 (50: 50) showed prominent spots compared to the other fraction. Each time 10 ml eluent was collected and identical eluates (TLC monitored) were combined and kept in a refrigerator. Eluted fractions between 45– 90 resulted in single component. Later these fractions were combined and evaporated to remove the solvent, this was done using a water bath. The resultant residue (1 g) obtained after evaporation was isolated as a pure component. This compound was designated as NA1. It gave orange red precipitate with Dragendroff's test for alkaloids, and a melting point of 160-162°C.

Dragendroff's test for alkaloids: A few crystals of NA1 were dissolved in few ml of distilled water in a test tube. To this 2M Hydrochloric acid was added until the acid reaction occurred. To the above mixture a solution of 1ml of Dragendroff's reagent was added. Formation of orange-red precipitate confirms the presence of alkaloids [11].

Methanol Extract: The residue (20 g) was dissolved in a small volume of methanol (30 ml) and the slurry was loaded onto the silica gel column (150 g) prepared in ethyl acetate. The column was eluted with 100% ethyl acetate followed by graded mixture of 1%, 5% and 10% methanol in ethyl acetate. Elutes of the different fraction were continuously monitored by TLC (Silica Gel G; Ethyl acetate: Methanol and visualised by UV/NH3). The 100% ethyl acetate and 99:1 (ethyl acetate: methanol) elutes showed similar spots. On concentration this was deposited as a yellow coloured crystalline solid. It was recrystallized from methanol obtained as yellow amorphous powder and was designated as compound NF1 (28 mg). It gave brown colour with Shinoda's test for flavonoids and a yellowish colour with NH_3, and melting point was found to be 327°C. The ethyl acetate soluble fraction was chromatographed over silica gel column using a solvent of EtOAc: MeOH: H_2O; 100: 16.5: 13.5 to give compound NF1. Eluates of the different fractions were continuously monitored by TLC and were visualized using UV/NH_3.

Shinoda's test for flavonoids:A few mg of NF1 was dissolved few ml of ethanol in a test tube. To the above solution few drops of dilute hydrochloric acid was added, this was followed by addition of 4-5 pieces of magnesium turnings. Formation of brown colour confirms the presence of flavonoids [12,13].

Petroleum Ether Extract:

The petroleum ether extract (20 g) was dissolved in CHCl3 (20 ml) and adsorbed onto aluminia (20 g). After evaporation of the solvent, it was loaded onto silica gel column (150 g) prepared in petroleum ether (60-80℃). The column was eluted first with petroleum ether (60-80°C), followed by 10%, 25% and 50% of benzene to petroleum ether, later with benzene, tailed by graded mixtures of C_6H_6: $CHCl_3$ and $CHCl_3$: MeOH (95:5, 90:10). Then obtained elution's were monitored on TLC (Silica Gel G; visualization using vanillin in sulphuric acid, heated at 110°C). The eluates from 50% benzene in petroleum ether gave spot on the TLC

plate (C_6H_6: EtOAc, 85:15). Therefore, these eluates were taken, concentrated to yield a compound. This separated compound gave red colour in the Salkowski's test for steroids. The obtained compound was recrystallized from benzene and labelled as NS1 (25 mg).

Salkowski's test for steroids:A few crystals of NS1were shaken with few ml of chloroform. To the chloroform layer, concentrated sulphuric acid was added through the sides of the test tube. Formation of red coloured ring confirms the presence of steroids [14].

Spectroscopic characterization: All the melting points were recorded in a Toshniwal melting point apparatus and were uncorrected. IR spectra of the compounds were recorded using the KBr pellet method on a Bruker Alpha IR Spectrophotometer. ^{1}HNMR spectra of the compound were taken on Bruker AM (400MHz) NMR Spectrometer using $CDCl_3$ as solvent. ^{13}CNMR was recorded on Bruker AM (400 & 1000 MHz) NMR Spectrometer using $CDCl_3$ as solvent. Mass spectra were recorded on AUTOSPEC Spectrophotometer. TLC was carried out on Silica Gel (Merck). Column chromatography was carried out on Silica Gel (Merck 60-120 mesh).

Results and Discussion

Chloroform extract: Compound NA1 showed positive response to Dragendroff's test for alkaloids. It was obtained as a crude residue having melting point 160-162°C. From the IR spectral data, it was found that C-H str. appeared at 2920.67cm^{-1}, C=C str. at 1513.62cm^{-1}, C=O str. at 1709.93cm^{-1} and C-O-C str. at 1033.07cm^{-1}. In ^{1}HNMR data of this compound four aromatic hydrogen atoms appeared in the region δ 6.980-6.998 ppm, three hydrogens of OCH_3 appeared at δ 2.49 ppm and three hydrogens of CH_3 appeared at δ 2.49 ppm. The signal at δ 166.083 ppm accommodated for the C=O, carbon at C-1', the signal at δ 52.580 ppm for C-3', signal at δ 42.279 ppm for C-1, signal at δ 138.032 ppm for C-4, δ 123.204 ppm for C-3, δ 50.472 ppm for C-2, δ 49.449 ppm for C-6 and δ 22.593 ppm for C-5. From the mass spectra the molecular weight of the compound was found to be 155. Its identity as Arecoline was further confirmed by IR, ^{1}HNMR, ^{13}CNMR and Mass spectral characters and by chromatography with an authentic sample of Arecoline.

Methanol extract: Compound NF1 showed positive response to Shinoda's test for flavonoids and a yellow green colour with NH_3. It formed acetate having melting point 113-117°C. From the IR spectral data, it was found that O-H str. appeared at 3300.43cm^{-1}, C=O str. at 1711.39 cm^{-1}, C=C str. at 1604.18 cm^{-1} and C-O-C str. at 1038.25 cm^{-1}. In 1HNMR data of this compound, hydrogen at 3-OH appeared in the region δ 10.80 ppm, and of 4'-OH at δ 10.33 ppm, and of 7- OH at δ 12.94 ppm; H-2' and H-6' appeared at δ 7.92 ppm, H-3' and H-5' at δ 6.92 ppm, H-8 at δ 6.77 ppm, H-6 at δ 6.47 ppm and H-3 at δ 6.18 ppm. The signal at δ 181.6 ppm accommodated for the C=O, the side chain aromatic ring appeared in the range of δ 115.84 - δ 161.03 ppm. The other carbon atoms of the molecules appeared in the range δ 93.83 – δ 164.0 ppm. From the mass spectra the molecular weight of the compound was found to be 270. Its identity as Apigenin was further confirmed by IR, ^{1}HNMR, ^{13}CNMR and Mass spectral characters and by chromatography with an authentic sample of Apigenin[15].

Petroleum ether extract: Compound NS1 showed green colour to Liebermann-Burchard's test and Salkowski's test for steroids. It formed an acetate having melting point 143-145°C. From the IR spectral data, it was found that O-H str. appeared at 3418.96cm^{-1} and C=C str. at

1459.42cm^{-1}. In 1HNMR data of this compound methine protons appeared in the region δ 1.14-2.28 ppm, vinylic proton at δ 5.36 ppm and olefinic protons at δ 5.13 ppm. The signal at δ 141 ppm was accommodated for sp2 (olefinic) carbon at C-5. The signal at δ 120 ppm is to C-6. The carbon at C-3 gave a signal at δ 70 ppm. The next signal δ 56 ppm was accommodated for C-17. Other carbon atoms of the steroidal skeleton side chain appeared in the range of δ 45 - δ 30 ppm. The angular methyl group and the side chain methyl carbons gave signals in the region δ 14.8 – δ 18.4 ppm. From the mass spectra the molecular weight of the compound was found to be 412. Its identity as Stigmasterol was further confirmed by IR, ^{1}HNMR, ^{13}CNMR and Mass spectral characters and by chromatography with an authentic sample.

Conclusion

Chemical investigation of the leaves of Areca catechu L. led to isolation of three compounds namely NA1 (Fig. 1) from chloroform extract, NF1 (Fig. 2) from methanol extract and NS1 (Fig. 3) from petroleum ether extract. These compounds were characterized using IR, ^{1}HNMR, ^{13}CNMR and Mass Spectra data and confirmed as Arecoline, Apigenin and Stigmasterol.

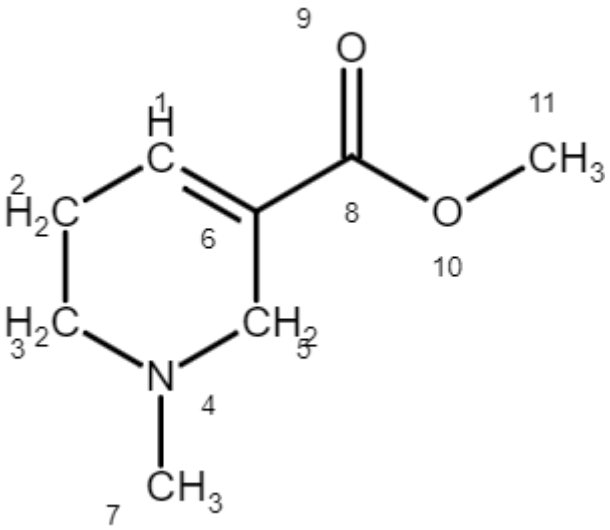

Fig. 1: The Chemical structure of NA1 (Arecoline)

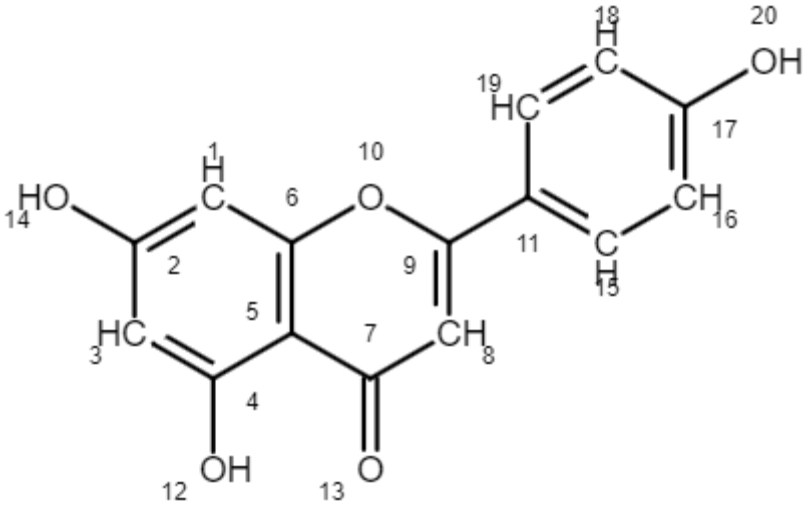

Fig. 2: The Chemical structure of NF1 (Apigenin)

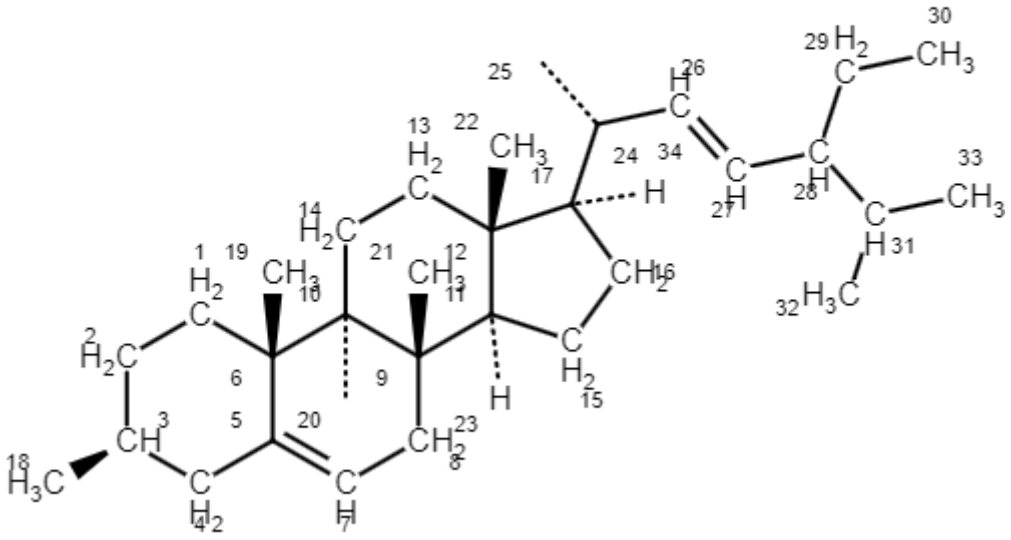

Fig. 3: The Chemical structure of NS1 (Stigmasterol)

Acknowledgement

Authors grateful to NGSM Institute of Pharmaceutical Sciences and Nitte (Deemed to be University) for providing the research facilities and resources to carry out the research work.

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