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Isolation, Characterisation, and Structural Clarification of Areca catechu Isolates (Areca nut Plant)

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Abstract:

Background: The *Areca catechu*, normally known as the Supari Fruit, is a remarkable Fruit with a rich history of medicinal and healing properties. This evergreen Fruit , native to the Indian subcontinent, is renowned globally for its adaptability and ability to address various health issues.

Objective: This bioanalytical study aims to identify and structurally elucidate the major phytoconstituents present in the Ethanolic extract of the ***Areca catechu.***

Method: In this phyto-analysis, the phytoconstituents from the *Areca catechu* plant were isolated using column chromatography, and their structural elucidation was accomplished using spectral analysis.**Result and Conclusion:**
Two major isolate, **Arecoline** and **Quercetin** were identified and structurally elucidated from *Areca catechu* by spectral analysis (I.R., NMR, and mass spectroscopy).
Key words: Chemical constituents, biological activity, and Areca catechu L.

1. Overview

A tropical crop, Areca catechu L. is a member of the Arecaceae family, which has 2600 species and 181 genera [1]. It is widely dispersed, and Asia's South and Southeast, comprising Bangladesh, India, China, the Philippines, Malaysia, and the country of Myanmar, and, has a long history of consuming its fruit, areca nut [2]. Worldwide, there are already about Six hundred

million chewers of areca nut [3], and the figure is continually rising. Millions of farmers in the planting region of A. catechu rely on it as a cash crop and source of income due to its significant economic benefits. As of 2016, China's Hainan Province has planted 0.38 million acres of A. catechu, and the

The value of the production exceeded forty billion US dollars [4]. Typically, areca fruit has two primary components: the kernel and the husk [5]. Although areca nuts can be eaten in a variety of ways, they are utilised either singly or in combination for culinary or medicinal purposes. Generally speaking, areca nuts can be eaten on their own or wrapped within Piper betel leaves together with tobacco, spices, sweeteners, slaked lime, and other ingredients. They can also be processed into products that are sold commercially [6, 7]. Usually, the cleaned areca husks travel throughout a number of stages of processing, such as soaking, drying, adding bitterness, and enzymatic hydrolysis, during which their typical Their rough Their woody nature puts them at risk for mechanical harm during the act of chewing and their hardness can exceed 105/g [8, 9]. The International Agency for Cancer Research (IARC) has categorised areca nut and betel quid as category 1 carcinogens due to the possibility of gastro-oesophageal and mouth cancers [7], which has had a significant effect regarding the areca nut itself business. Actually, according to the Compendium of Materia Medica [11],

The nut of Are is one of several four primary cures used in the South. [10]. The Chinese Pharmacopoeia has listed areca nut since 1953 [12]. Numerous prescriptions for the medical benefits of various areca nut preparations, such as decoction bits, charred semen arecae, and the pericarpium's arecae [13]. Numerous studies have reported their beneficial benefits on beriberi oedema, tenesmus, malaria, diarrhoea, stomach discomfort, and indigestion [14]. Furthermore, areca nut has been shown to cure glaucoma, premature ejaculation, periodontitis, and trouble urinating [15]. Moreover, areca nut in combination with other medicinal materials has significant effects as well. One such example is the well-known The compound medication Da Yuan Yin can be utilised to treat a range of illnesses. related to inflammation [16], among which areca nut can resolve masses, eliminate mucus, as well as moisture and moisture-related bad [17]. These imply that areca nuts contain a variety of bioactive ingredients that are worth using. several pharmacological properties, including antioxidant, anti-inflammatory, anti-depressant, anti-tumor, and hypoglycemic actions [18–20]. In order to offer an analytical framework for the use of areca nut in medications and functional foods, as well as theoretical direction for the industry's improvement., we summarised the study's progress on the pharmaceutical characteristics and bioactive ingredients of areca nut in this review and presented complete information exploring the numerous health benefits of the bioactive ingredients in areca nut.

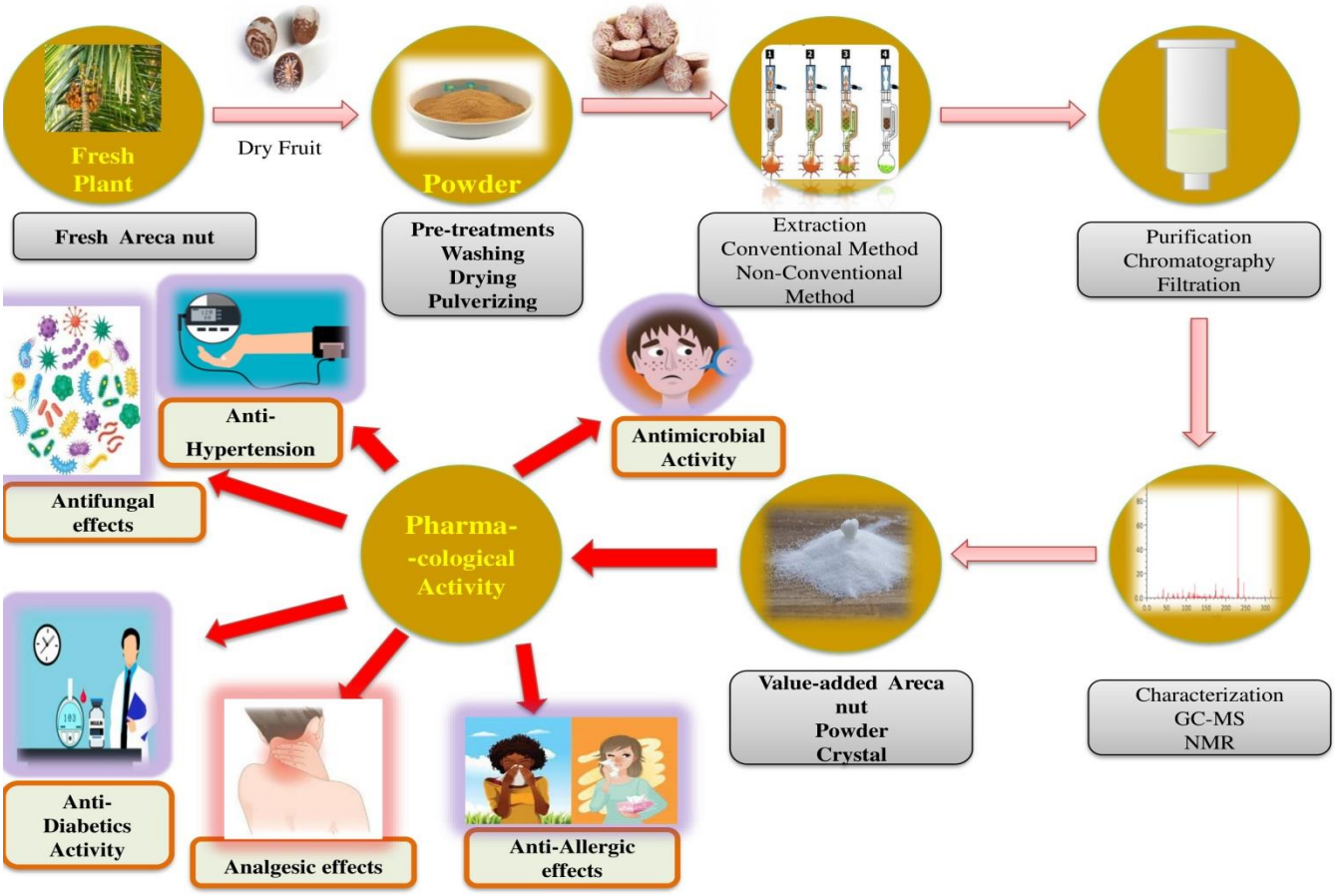
(A)Plant of Areca catechu

(B) Fruit of Areca catechu

(C) Fruits of Areca catechu

(D) Fruits of Areca catechu

(E) Fruits Powder of Areca catechu



2. MATERIALS AND METHODS:

Sample collection: *Areca catechu* Fruit are collected from the source of the **Raebareli Market UP 229001 India**. The *Areca catechu* Fruit are collected, washed with Dist—water, dried in shade, Crushed form a Powder and further stored for extraction and bio-analytical study for Research.

Extraction and isolation of *Areca catechu* Fruit phytoconstituents: After successive extraction in Two different solvents viz. Ethanol (70-78^{0}c), Chloroform ($CHCl_{3)}$ solution, preliminary phytochemical screenings indicate the presence of various constituents like alkaloids, tannins, flavonoids, steroids, glycosides, saponins, phytosterols etc. In TLC Study it was noticed maximum 4 spots obtain in Ethanolic extract of *Areca catechu* Fruit Ethanolic extract of *Areca catechu* extracts were subjected to column chromatography. Column chromatography was used to collect five eluted fractions (10-14) and (25-29) using different proportion of Mobile phase N-Hexane (C_6H_{14}): Alcohol methyl (CH_3OH). Two Pure isolates were obtained by column chromatography through a TLC study. Analyzing the fractions of chemicals by column chromatography has always been done using thin-layer chromatograph. Bioactive compounds have been separated using C chromatography technique and thin-layer chromatography (TLC) using various analytical instruments.

Identification and structural elucidation of organic Compound by Spectrometry:

The two pure isolates M-1 and M-2, obtained by column chromatography, undergoes various spectroscopic approaches, mass spectroscopy, 1H NMR, which is 13C NMR, and FTIR for the identification of isolated compounds. In organic chemistry, infrared (I.R.) spectroscopy is helpful because it makes it possible to distinguish between various functional groups. This is because every functional group has certain bonds that consistently appear in the exact locations across the infrared spectrum. the application of Fourier transform inf is used to identify functional groups (FTIR)spectroscopy. These include vibration bands such as N-H, R-OH, C-H, R-C O. C = C, C = N C = N, and COOH. Atoms and molecules can have their physical and chemical properties ascertained using NMR spectra analysis. Based on the phenomena of nuclear magnetic resonance, it provides extensive details regarding molecules' kinetics, structure, reaction state, and chemical environment. A compound's weight spectrum usually comprises of many signals, the peak at the greatest m/z (molecular ion) value representing the amount of mass of the complete structure.

3. RESULTS AND DISCUSSION:

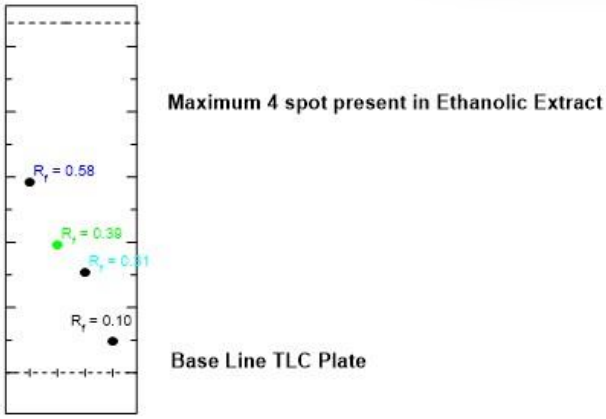


Figure 2: Graphical presentation of TLC of Ethanolic extract of *Areca catechu* Fruit

Figure 3: Isolation of *Areca catechu* Fruit by Column chromatography

3.1: Identification of M-1 Isolate:

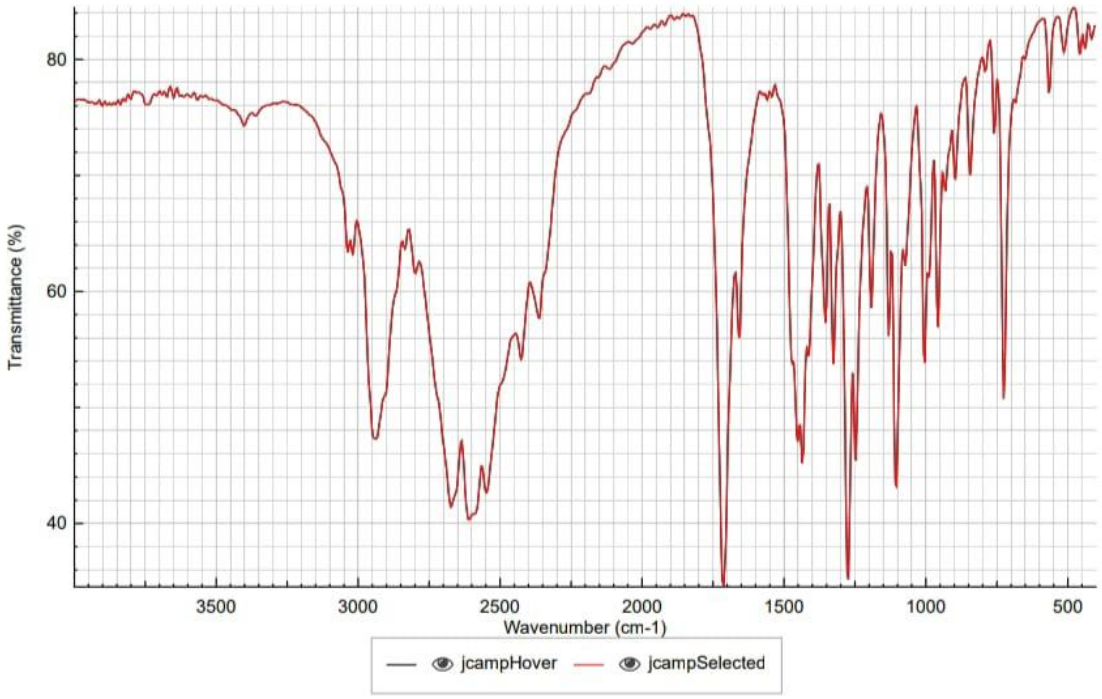


Figure.4: I.R. of M-1

IR of M-1: Compound M-1's FT-IR spectrum shows several significant peaks at different ranges: 3649.69 cm-1 strong, O-H stretching inter-molecular bonded alcohol present; 3271 cm-1 stretching vibration(v) of O-H in the presence of alcohol and phenols; 2983 cm-1 (Chelate H-Bridge, CH stretching vibration presence of alkenes); 1829.11 cm-1 strong C=O stretching and saturated five-member ring carboxylic acid confirmed; 1608.56 cm-1, 1098.61 C=C Alkenes bent C=C trisubstituted stretching alkenes, with a C-C stretching vibration of 1424 cm-1 and a beta keto aldehyde as an enol of 1651 cm-1. Aromaticity, strong (C=C) bending alkene conformation, and 730.88 (cm-1) bending alkene

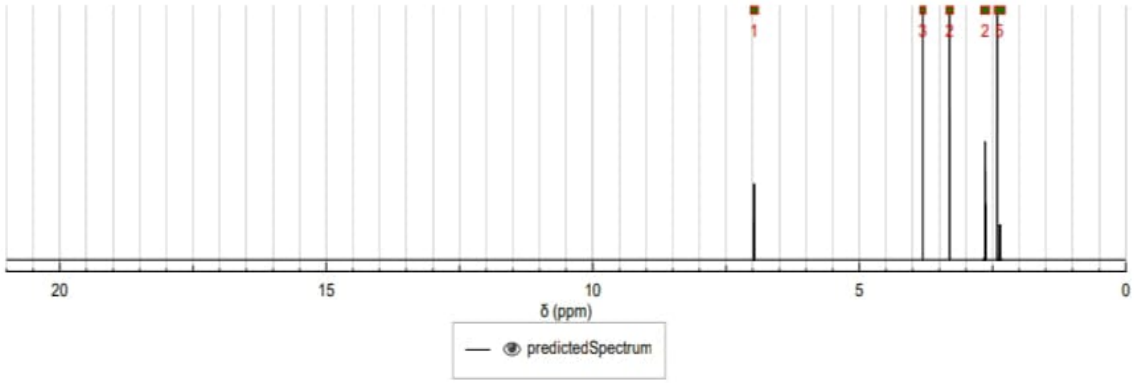


Figure.5 :^{1}H NMR of M-1

1H NMR of M-1: ^{1}H NMR: δ 2.27-2.46 (5H, 2.36 (dddd, *J* = 14.1, 8.1, 6.9, 2.9 Hz), 2.41 (s)), 2.64 (2H, ddd, *J* = 12.2, 6.9, 2.9 Hz), 3.30 (2H, d, *J* = 13.2 Hz), 3.81 (3H, s), 6.97 (1H, dd, *J* = 8.3, 7.8 Hz).

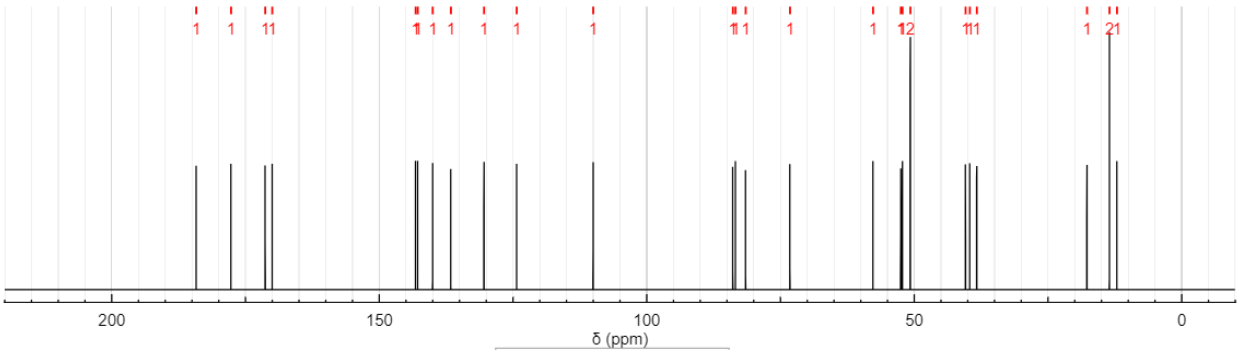


Figure.6:13 C NMR of M-1

^{13}C NMR of M-1: ^{13}C NMR: 25.7 (1C, s), 45.9 (1C, s), 52.2 (1C, s), 52.5 (1C, s), 54.0 (1C, s), 127.7 (1C, s), 138.0 (1C, s), 166.6 (1C, s).

Hydrogen	Chemical Shift(ppm)
12	3.76
13	3.76
14	3.76
15	7.10
16	2.69
17	2.69
18	3.25
19	3.25
20	2.83
21	2.83
22	2.83
23	4.16
24	4.16

Figure.7: Chemical shift of ^{1}H NMR and ^{13}C NMR of M-1

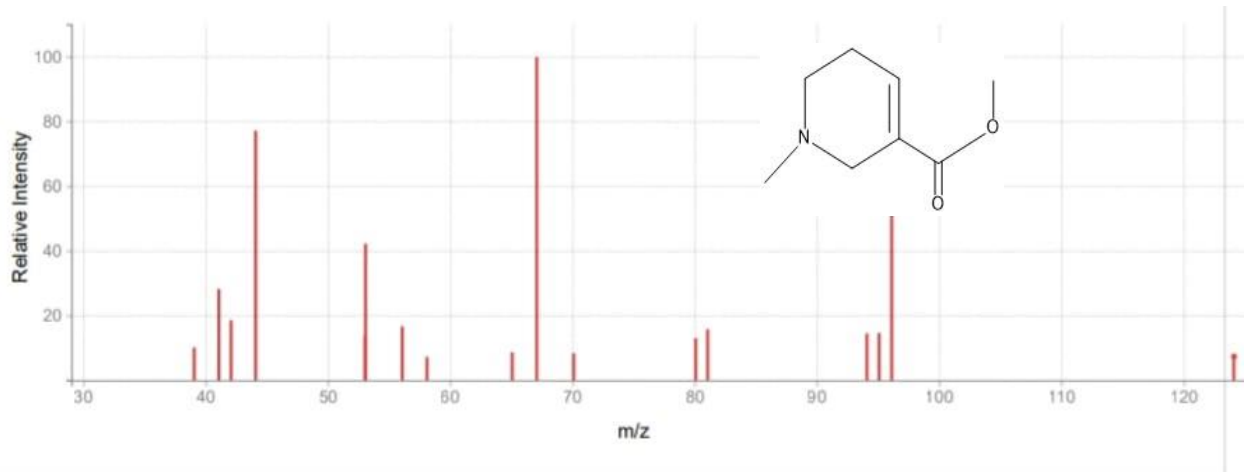


Figure 8: **Mass spectrum of M-1**

Mass spectrum of M-1: Chemical Formula: **$C_8H_{13}NO_2$**, Exact Mass of compound **155.09**

Molecular Weight: 155.19, m/z: 155.09463 (100.0%), 156.09798 (8.7%) and elemental analysis provide C, 61.91; H, 8.44; N, 9.03; O, 20.62.

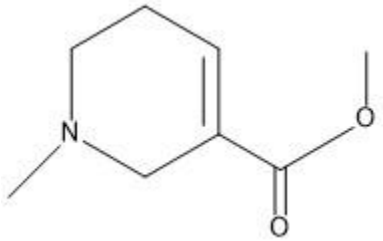


Figure.9: Structure of M-1, methyl 1-carboxylate-1-methyl-1,2,5,6-tetrahydropyridine One methyl 1.

3.2: Identification of M-2 Isolate:

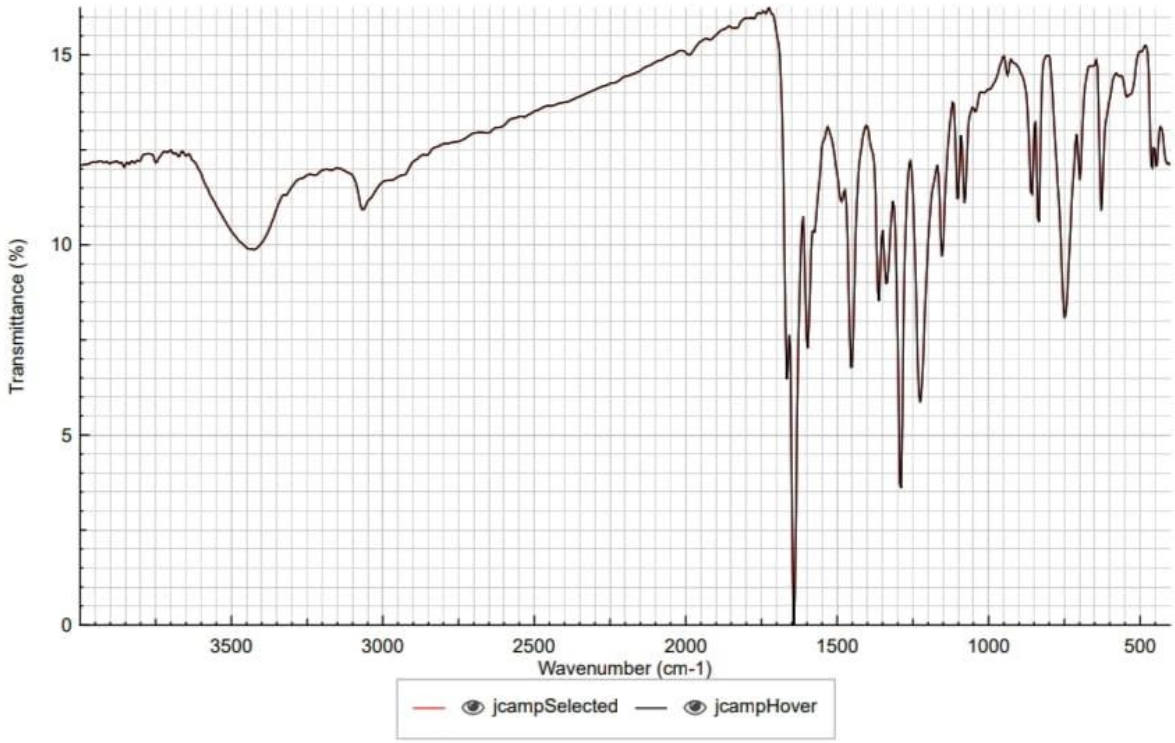


Figure.10.IR of M-2

IR of M-2: 1828.92 cm-1 (w intensity C-H bending aromatic compound), 2306.48 cm-1 (s intensity O=C=O stretching), and 3649.77 cm-1 (v intensity free O-H) 1747.90 cm-1 (stretching with an intensity of C=O) and 1655.51 cm-1 (wrestling with an intensity of C=C)m intensity C=C stretching cyclic alkene: 1605.10 cm-1; s intensity carboxylate ions: 1312.38 cm-1'; s intensity carbonyl group: 1241.57 cm-1; s intensity C-O stretching ester: 1160 cm-1 841.61 cm-1 (w intensity isolated aromatic C-H), 931.11 cm-1 (m intensity C=C bending alkene vinylidene).

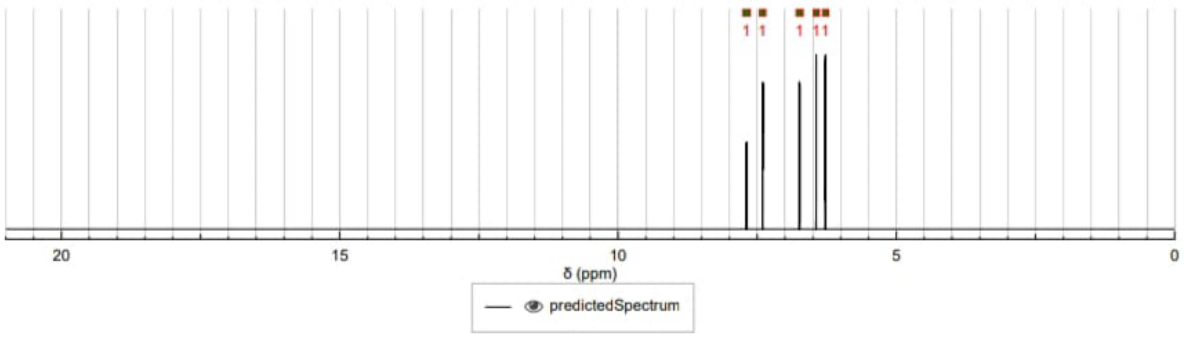


Figure.11.^{1}H NMR of M-2

^{1}H NMR of M-2:

^{1}H NMR: δ 6.27 (1H, d, *J* = 1.9 Hz), 6.44 (1H, d, *J* = 1.9 Hz), 6.74 (1H, dd, *J* = 8.4, 0.5 Hz), 7.40 (1H, dd, *J* = 1.8, 0.5 Hz), 7.69 (1H, dd, *J* = 8.4, 1.8 Hz).

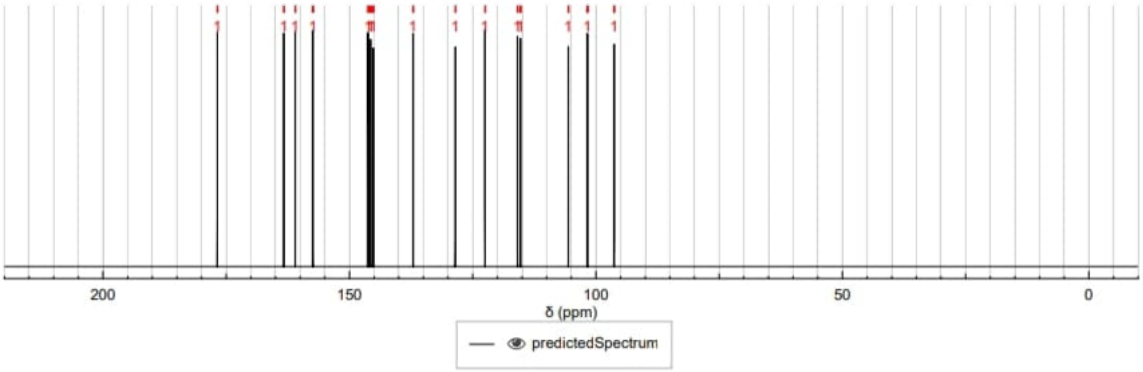


Figure.12.^{13}C NMR of M-2

^{13}C NMR of M-2:

^{13}C NMR: δ 96.3 (1C, s), 101.7 (1C, s), 105.6 (1C, s), 115.3 (1C, s), 115.9 (1C, s), 122.5 (1C, s), 128.5 (1C, s), 137.1 (1C, s), 145.2 (1C, s), 145.7 (1C, s), 146.2 (1C, s), 157.4 (1C, s), 161.0 (1C, s), 163.3 (1C, s), 176.8 (1C, s).

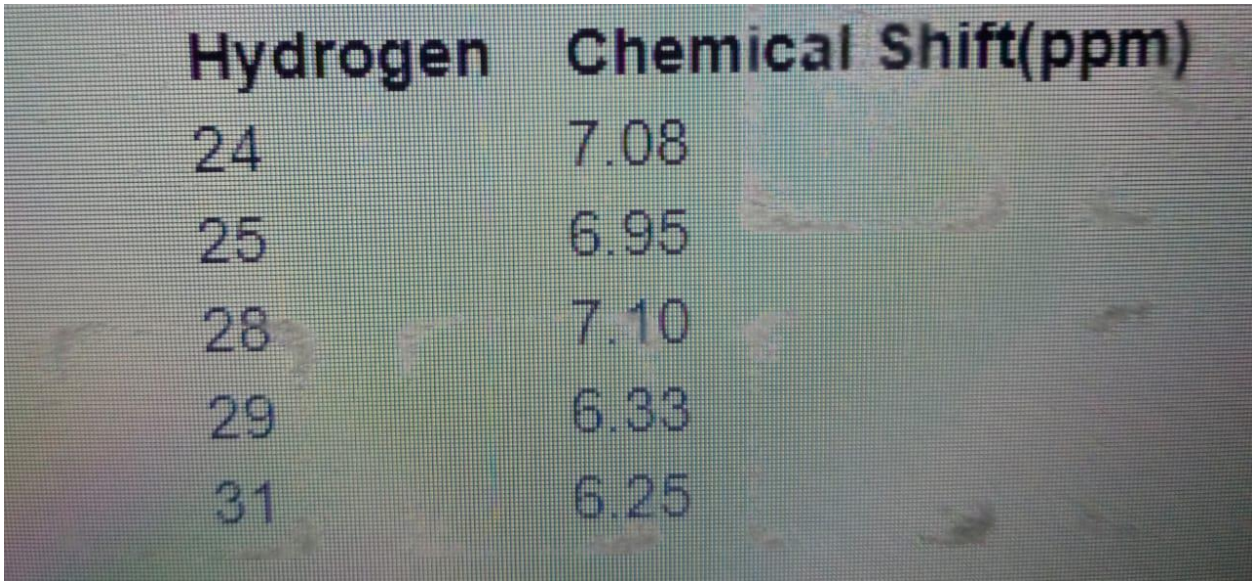


Figure.13: Chemical shift of 1H NMR and 13C NMR of M-2

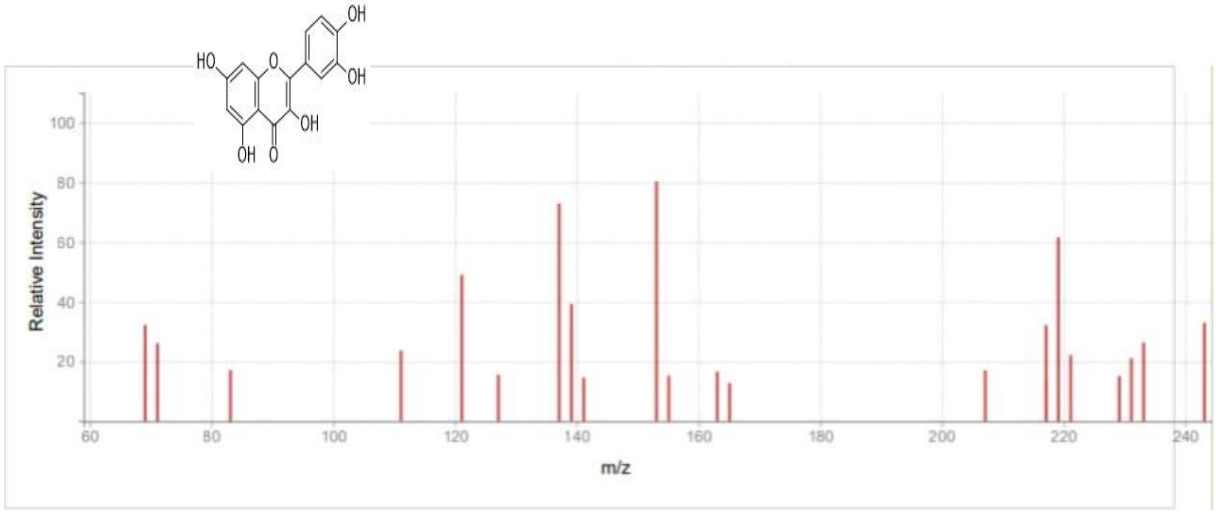


Figure 14: Mass spectrum of M-2

Mass spectrum of M-2: Chemical Formula of M-2 is **$C_{15}H_{10}O_7$** , Exact Mass: 302.04, Molecular Weight: 302.23, m/z: 302.04265 (100.0%), 303.04601 (16.2%), 304.04690 (1.4%), 304.04936 (1.2%) **,** Elemental Analysis of M-2 give C, 59.61; H, 3.34; O, 37.05.

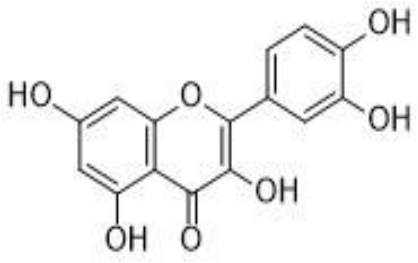


Figure.15 : Structure of M-2, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one

Discussion: From the above, spectroscopic analysis of two isolates collected from *Areca catechu* by column chromatography gives special structural characterization by interpretation of the spectrum. C-1 compound FTIR spectrum conformed that C-H bending aromatic compound at 1828.92 cm-1, O=C=O stretching at 2306.48 cm-1, free O-H at 3649.77 cm-1, C=O at 1747.90 cm-1, C=C at 1655.51 cm-1, C=C stretching at cyclic alkene: 1605.10 cm-1, carboxylate ions at 1312.38 cm-1', carbonyl group at1241.57 cm-1, C-O stretching at 1160 cm-1, isolated aromatic C-H at 841.61 cm-1, C=C bending alkene vinylidene at 931.11 c 1H NMR of M-1 gives chemical shift of 3-furan at δ 7.42, cyclopentene at δ 3.54, 1 alpha -C*R from methane at δ 1.22, cyclopentene at δ 2.44, cyclohexane at δ 1.20, methyl at δ 3.57, methylene at δ 2.27, 1-ethylene at δ 6.73. 13 C NMR of M-1 gives 1-carboxyl at δ 177, 3-furan at δ 143, cyclohexane δ

86.27, δ 70.15, aliphatic compound at δ 55.48 δ41, 1-ethylene at δ 126, 1-carbonyl at δ202. Mass spectrum of M-1 shown Molecular Weight: 155.19, m/z: 155.09463 (100.0%), 156.09798 (8.7%) Similarly FTIR of M-2 compound showed that C-H bending aromatic compound at 1828.92 cm-1, O=C=O stretching at 2306.48 cm-1, free O-H at 3649.77 cm-1, C=O at 1747.90 cm-1, C=C stretching cyclic alkene: 1605.10 cm-1, intensity carbonyl group at 1241.57 cm-1, C=C bending alkene vinylidene, 931.11 cm-1.1H NMR of M-2 shown 3-furan at δ 7.42, Cyclopentene at δ 3.55, Cyclohexane at δ 4.37, Methyl at δ 3.72, methylene at δ 2.63, Ar-H at δ 6.74. 13C NMR of M-2 shown 3-furan at δ 146.27, R-CH_3 at δ 24.43, R-C=OH at δ 177, aliphatic compound at δ 88.3, cyclohexane at δ 70.28 . Mass spectroscopy of the M-2 compound showed that Exact Mass: 302.04, Molecular Weight: 302.23, m/z: 302.04265 (100.0%), 303.04601 (16.2%), 304.04690 (1.4%), 304.04936 (1.2%). From the above spectroscopic analysis, we found that the Exact Mass of M-2 is 302.04, Molecular Weight: is302.23, m/z: 302.04265 (100.0%), 303.04601 (16.2%), 304.04690 (1.4%), 304.04936 (1.2%). From the above spectroscopic interpretation, we found that the chemical name of M-1 is [methyl 1-carboxylate, methyl 1-methyl-1,2,5,6-tetrahydropyridine] common name Arecoline and chemical name of M-2 is [2,5-trihydroxy-3,5,7-dihydroxyphenyl)-4H-chromen-4-one] shortened form Quercetin.

4. SUMMARY AND CONCLUSION:

Areca catechu is a traditional medicinal Fruit in India used in Ayurveda for various medicinal use like antibacterial, antifungal, immunomodulatory, Antifungal effects ,Analgesic effects,Anti-allergic Activity , Antimicrobial, Antihypertenssion type medicinal activities from ancient type. The medicinal activities of *Areca catechu* are due to the multiple phytoconstituents present in this plant. This low cost bio-analytical method used for this spectroscopic analysis of this medicinal Fruit , is a very effective technique for isolating and characterizing phytoconstituents present in this medicinal Fruit. From the results we have drawn the following conclusions that the M-1 isolate is **Arecoline** and M-2 isolate name is **Quercetin** are two very important phytoconstituents responsible for medicinal activates of *Areca catechu.* The methods described in this paper would benefit from further development of bio-analysis of *Areca catechu.*

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