*Section A-Research paper*

# Extraction, Isolation and HPTLC fingerprint profiling of bioactive chemical constituents from the Agro-waste of Multifaceted Palm crop in India: An approach of sustainable harvesting and Agro-waste repurposing in Pharmaceutics.

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## ABSTRACT

**Introduction:** Coconut shell botanically represents the hard endocarp from the fruit of *Cocos nucifera* Linn. of Arecaceae or Palm family. It is the usually discarded part of the multifaceted palm crop and constitute a major part of the agriculture waste in India. Limited literature references suggest the internal use of Coconut shell.However, the inclusive pharmaceutical application of Coconut shell with respect to the pertinent advantage of sustainable harvesting and repurposing of agro-waste from the plant source has not yet unveiled in depth. The present research work is focused on extraction, isolation and identification of bioactive chemical constituents of Coconut (Cocos nucifera *Linn*.) shell in order to explore its anticipated therapeutic implications. **Materials andmethods:** The comprehensive phytochemical and HPTLC fingerprint profiling of hot aqueous, cold aqueous and hot ethanol extracts of dried ripe endocarp of Cocos nucifera *Linn.* were done based on the standard protocol for analysis of

extracts described in Ayurvedic Pharmacopoeia of India. **Results:** In the present study, the comprehensive bioactive chemical profile of the test drug were developed and scientifically validated. The standards for identity, purity, efficacy and other qualitative indices of various extract of Coconut shell were derived. The HPTLC fingerprint profile depicts the inclusive chemical profile of hot aqueous, cold aqueous and hot ethanol extracts of Coconut shell. **Conclusion:**The phytochemical standards of various extracts of Coconut shell exemplified in the present study endorses the test drug as a novel reliable source of diverse bioactive phytoconstituents with broad therapeutic potential and immense applications in pharmaceutics. The study outcome can be integrated in further researches on Coconut shell exploring its further strategies in New Drug Development process.

**KEYWORDS:** Cocos nucifera, Coconut shell, Extraction, High Performance Thin Layer Chromatography, Pharmaceutics.

**INTRODUCTION**

Medicinal plants serves as globally valuable sources of herbal products with immense therapeutic prospective. In developing countries around 80 % of people are dependent on herbal drugs for their primary healthcare, and in developed countries almost 25 % of approved medicines are derived from various plant species[1]. The increasing demand for herbal drugs and plant derived products throughout the world led to the alarming rate of extinction of many valuable medicinal plant resources. The subsequent issues of decreased availability, increased cost, adulteration and quality degrading of genuine medicinal plants persists as a serious challenge to herbal drug industry[2]. Therefore new approach of sustainable harvesting of plant sources and exploring the least exploited plant sources for their therapeutic implications need to be unveiled in depth[3].

The coconut tree (*Cocos nucifera*) is a member of the palm tree family (Arecaceae) and the only living species of the genus *Cocos*[4]. It is commonly found in tropical countries such as Indonesia, India, Philippines and many more. States of Kerala, Tamil Nadu, Karnataka,

Puducherry, Andhra Pradesh, Goa, Maharashtra, Odisha, West Bengal, Gujarat and the islands of Lakshadweep and Andaman and Nicobar islands represents the areas of broad-scale coconut cultivation in India[5].

Coconut palm is considered as one of the most versatile trees in the world and virtually every part of the coconut palm can be used by humans in some manner and has significant economic value.

Coconut shell botanically represents the hard endocarp from the fruit of *Cocos nucifera* Linn[6]. It is the usually discarded part of the plant and constitute a major part of the agriculture waste in India[7]. Few applications of Coconut shell as a source of charcoaland raw material for manufacturing handicrafts, potting medium, musical instruments etc are getting attention nowadays [8].In Ayurvedic literatures, we can trace only limited references suggesting the internal use of Coconut shell in strangury and halitosis[9]. However, the inclusive pharmaceutical application of Coconut shell with respect to the pertinent advantage of sustainable harvesting and repurposing of agro-waste from the plant source has not yet unveiled in depth. In this regard, a scientific approach based on screening and validation of phytochemical standards of Cocos nucifera *Linn.* endocarp should be implemented as per standard guidelines. Common criteria for drug evaluation include the screening of quality and therapeutic value of the bulk drug and pharmaceutical product, identification studies, purity, content, uniformity and biological availability. HPTLC studies are among the key identity tests towards developing herbal drug monographs and are used for the identification of bioactive phytoconstituents, identification and determination of impurities, and quantitative determination of therapeutically active compounds of herbal drugs[10]. The present research work is focused on deriving the High Performance Thin Layer Chromatography fingerprint and comprehensive phytochemical profile of various extracts of Coconut (Cocos nucifera *Linn*.) shell in order to explore its anticipated therapeutic implications.

## MATERIALS AND METHODS

### Collection and authentication of test drug

Ripe fruits of Coconut (Cocos *nucifera* Linn.) were procured from Kerala and the drug authentication(Acc. No. DG/21-22/353) was done in the Pharmacognosy laboratory, Centre or Medicinal Plant Research, Kottakkal, Kerala. After proper drying and removal of the white kernel, the coconut shells (endocarp) were collected and taken as the test drug for this study.

### Extraction of *Cocos nucifera* Linn. endocarp

The outer surface of Coconut shells were scraped well to remove the husk fibers. The dried ripe coconut shells were crushed and then subjected to pulverization.The powdered sample of dried ripe Coconut shell was taken in two separate extractors. About 3 times of distilled water and ethanol was added separately for the hot aqueous and alcoholic extraction of the test drug respectively. It was heated at a temperature between 80-85°C for 3 hours followed by filtering

the extract. The filtrate was then concentrated to a syrupy consistency and the solvent was removed by evaporation on a water bath followed by drying under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80°C till the moisture is below 5 per cent [11]. The cold aqueous extract of the test drug was also prepared by following standard methods of extraction[12].

**Isolation and identification of phytochemical constituents in Coconut shell**

The hot aqueous, cold aqueous and hot ethanol extracts of *Cocos nucifera* Linn. endocarp were separately subjected to qualitative analysis for detecting the presence of active principles like alkaloids, steroids, flavonoids, phenols,tannins, saponins and anthraquinones following standard methods of analysis[13].

**HPTLC fingerprint profile of *Cocos nucifera* Linn. endocarp**

*Preparation of test solution*: Approximately 2g powder of coconut shell was refluxed with 50 ml of distilled water for a period of 15min. The solution was further filtered and the extract was concentrated to 25 ml. The same procedure was followed by refluxing with 50 ml of ethanol for preparing the alcoholic test solution.

*Procedure:* High Performance Thin Layer Chromatography of aqueous and ethanol extracts of Coconut shell was performed as per standard technique[14]. Pre-coated silica gel 60 $F_{254}$HPTLCplates (10x10 cm) (Merck,1.05554.0007) were used. Toluene: Ethyl acetate: Formic acid: Ethanol (7:5:1:0.5) was used as the mobile phase. 20 µl of hot aqueous, cold aqueous and ethanol extracts of Coconut shell was applied separately on the HPTLCplates (10x10 cm) to a band width of 10 mm using CAMAG-Linomat 5 automated spray on band applicator equipped with a 100 µL syringe and operated with following settings: band length 10 mm, application rate 10 sec/ µL, distance between 4 mm, distance from the plate side edge1.5 cm and distance from the bottom of the plate 2 cm. The plates were developed usingCAMAG 10 x 10 cm Twin trough chamber. The developed plates were dried in an oven for 5 minutes at 60°C for complete removal of mobile phase. The plates were scanned at 254nm and 366 nm.CAMAG TLC Scanner 3 and CAMAG Reprostar 3 were the HPTLC instrumentations employed in this study. The scanner operating parameters were: (Mode: absorption / reflection; Slit dimension; 5 x 0.1 mm; scanning rate: 20 mm/s and monochromatic band width: 20 nm at an optimized wavelength 254, 366 nm and in visible range).HPTLC photo documentation, densitometry scan, colour of the spots and the corresponding *Rf* values of the test samples were recorded as per standard procedures[14].The peak numbers with its corresponding Rf, height, area, peak display and peak densitogram were identified.

**OBSERVATION AND RESULTS**

**Isolation and identification of phytochemical constituents in Coconut shell.**

Qualitative analysis was carried out to determine the organic compounds present in the active aqueous and alcoholic fractions of dried ripe Coconut shell.The presence of phenols, alkaloids, glycosides, tannins, steroids and Triterpenoids were detected in both the aqueous (hot and cold) as well as ethanol extract of *Cocos nucifera* Linn endocarp. The phytoconstituents like

Flavonoids, saponins, Phlobatannins and Quinones that are present in the aqueous extract were not detected in the ethanol extract of test drug.

**Table 1. Phytochemical constituents in Aqueous and Ethanol extracts of Coconut shell.**

| Qualitative analysis | Aqueous extract (Cold) | Aqueous extract (Hot) | Ethanol extract (Hot) |
|---|---|---|---|
| Test for Alkaloids | ++ | ++ | ++ |
| Test for Flavonoids | ++ | ++ | -- |
| Test for Phenols | ++ | ++ | ++ |
| Test for Steroids | ++ | ++ | ++ |
| Test for Tannins | ++ | ++ | ++ |
| Test for Glycosides | ++ | ++ | ++ |
| Test for Saponins | ++ | ++ | -- |
| Test for Anthraquinones | -- | -- | -- |
| Test for Proteins | -- | -- | -- |
| Test for Triterpenoids | ++ | ++ | ++ |
| Phlobatannins | ++ | ++ | -- |
| Quinones | ++ | ++ | -- |

**(** ++ → Present ; -- → Absent)

**High Performance Thin Layer Chromatography**

In the HPTLC analysis, the solvent system containing Toluene: Ethyl acetate: Formic acid: Ethanol in the ratio of 7:5:1:0.5; v/v resulted in remarkable separation of bioactive compounds present in various extracts of Cocos nucifera Linn. endocarp. HPTLC photo documentation, densitometry scan and Rfvalues of the drug sample were well depicted in this study. The overview graphs depicting the densitometry scan of various extracts at UV 254 nm and 366 nm were represented in Figure 1 and Figure 5 respectively.

In the densitometry scan at UV 254 nm, aqueous extract (cold) of Coconut shell exhibited 12 peaks (Figure 2.) suggesting twelve distinct bioactive constituents present in that active fraction of the test drug. The major peak (Area= 15498.7) for cold aqueous extract at 254 nm corresponds to the Rf value of 0.49. The peaks and corresponding Rf values derived in the densitometry scan at 254 nm for the hot aqueous extract of test drug appears almost same as that of the cold aqueous extract. Only the peak corresponding to the Rf value of 0.85 visualized in the previous graph is not detected in the overview graph for hot aqueous extract of test drug. Also the Rf values of 0.76 and 0.65 were distinctly noted for cold and hot aqueous extracts respectively (corresponding to the $9^{th}$ peak).

The three peaks of higher Rf values (0.76, 0.82, 0.85) observed in the first sample is not visualized in the densitometry scan of ethanol extract of test drug at 254 nm. Even though both the cold and hot aqueous extracts depicted the peak of Rf value 0.43, it was absent in the ethanol extract at 254 nm. All the remaining 8 peaks in the overview graph of ethanol extract somehow matches with that of the aqueous extract of test drug.The Rf values and areas of different peaks

obtained in the HPTLC fingerprint profile of various extracts of Coconut shell at 254nm and 366 nm were illustrated in Table 2 to Table 7.

All the eleven peaks corresponding to Rf value between 0.06 to 0.92 are visualized in both cold and hot aqueous extracts of test drug at 366nm. The peak corresponding to the Rf value of 0.42 represents the highest peak for both the samples whereas peak with Rf 0.44 depicts the major peak for ethanol extract. At 366nm, the area of the peaks were predominant for hot aqueous extract followed by cold aqueous and ethanol extracts of the test drug.

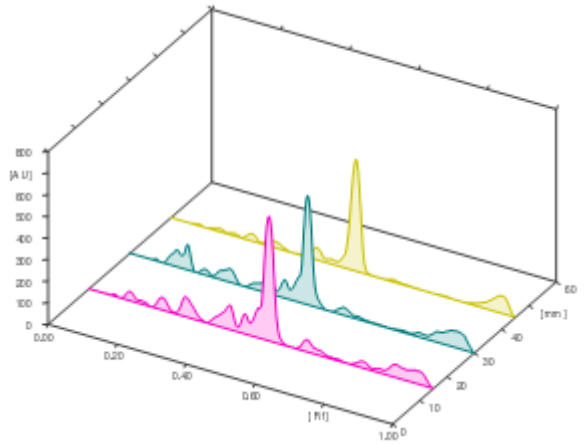

**Figure 1. Overview graph of various extracts of *Cocos nucifera* Linn. endocarp at 254 nm**.

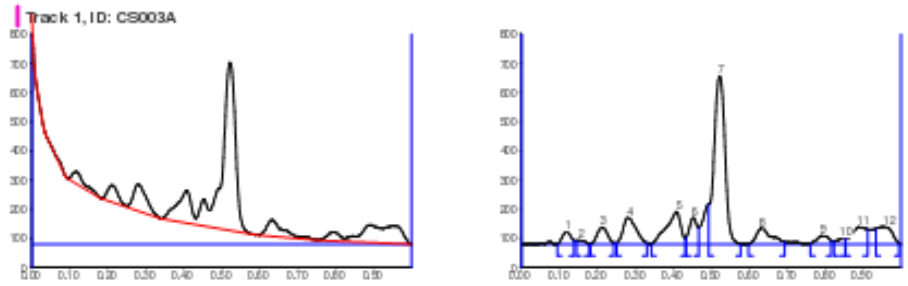


**Figure 2. Overview graph of Aqueous extract (Cold) of *Cocos nucifera* Linn. endocarp at 254 nm.**

**Table 2. Rf values and % areas in the HPTLC of Aqueous extract (Cold) of *Cocos nucifera* Linn. endocarp at 254nm.**

| Peak | Rf | Height | Rf | Height | % | Rf | Height | Area | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.10 | 0.5 | 0.12 | 42.6 | 3.50 | 0.14 | 13.7 | 911.2 | 2.63 |
| 2 | 0.14 | 12.4 | 0.15 | 15.2 | 1.25 | 0.18 | 0.4 | 290.0 | 0.84 |
| 3 | 0.18 | 0.1 | 0.21 | 58.3 | 4.78 | 0.24 | 5.2 | 1483.3 | 4.28 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | 0.25 | 5.0 | 0.28 | 92.6 | 7.59 | 0.33 | 5.8 | 2969.8 | 8.58 |
| 5 | 0.34 | 0.2 | 0.41 | 112.3 | 9.22 | 0.43 | 21.0 | 3945.3 | 11.40 |
| 6 | 0.43 | 22.8 | 0.45 | 92.2 | 7.56 | 0.47 | 57.3 | 1813.1 | 5.24 |
| 7 | 0.49 | 136.6 | 0.52 | 576.5 | 47.30 | 0.58 | 0.5 | 15498.7 | 44.77 |
| 8 | 0.60 | 0.5 | 0.63 | 56.6 | 4.64 | 0.69 | 7.6 | 2069.5 | 5.98 |
| 9 | 0.76 | 2.5 | 0.79 | 31.0 | 2.55 | 0.82 | 13.6 | 882.3 | 2.55 |
| 10 | 0.82 | 8.9 | 0.84 | 19.9 | 1.63 | 0.85 | 17.4 | 377.6 | 1.09 |
| 11 | 0.85 | 18.3 | 0.89 | 60.6 | 4.97 | 0.91 | 52.4 | 2121.9 | 6.13 |
| 12 | 0.93 | 53.9 | 0.96 | 61.0 | 5.00 | 1.00 | 3.7 | 2255.4 | 6.52 |

**Figure 3. Overview graph of Aqueous extract (Hot) of *Cocos nucifera* Linn. endocarp at 254 nm.**

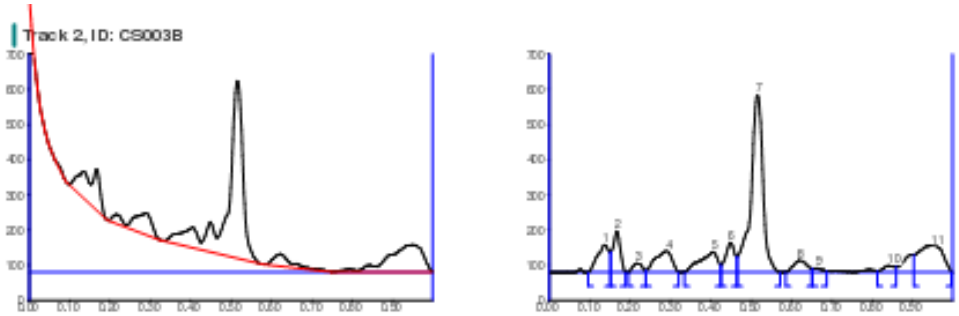


**Table 3. Rf values and % areas in the HPTLC of Aqueous extract (Hot) of *Cocos nucifera* Linn. endocarp at 254nm.**

| Peak | Rf | Height | Rf | Height | % | Rf | Height | Area | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.10 | 0.2 | 0.14 | 77.9 | 7.23 | 0.15 | 58.2 | 2086.2 | 6.65 |
| 2 | 0.15 | 58.4 | 0.17 | 117.5 | 10.91 | 0.19 | 0.2 | 1983.1 | 6.32 |
| 3 | 0.20 | 0.0 | 0.22 | 26.8 | 2.48 | 0.24 | 6.1 | 558.5 | 1.78 |
| 4 | 0.24 | 6.5 | 0.29 | 61.8 | 5.74 | 0.32 | 1.4 | 2332.7 | 7.43 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 0.34 | 1.7 | 0.41 | 58.4 | 5.42 | 0.42 | 21.5 | 2388.4 | 7.61 |
| 6 | 0.43 | 20.8 | 0.45 | 85.3 | 7.92 | 0.47 | 45.0 | 1782.0 | 5.68 |
| 7 | 0.47 | 45.6 | 0.52 | 504.9 | 46.87 | 0.57 | 0.4 | 14043.6 | 44.75 |
| 8 | 0.59 | 0.9 | 0.62 | 35.2 | 3.27 | 0.65 | 10.0 | 1073.9 | 3.42 |
| 9 | 0.65 | 10.3 | 0.66 | 12.5 | 1.16 | 0.69 | 3.7 | 262.6 | 0.84 |
| 10 | 0.81 | 3.9 | 0.84 | 19.9 | 1.84 | 0.86 | 16.3 | 517.9 | 1.65 |
| 11 | 0.90 | 49.5 | 0.95 | 77.0 | 7.15 | 1.00 | 2.6 | 4352.7 | 13.87 |

**Figure 4. Overview graph of Ethanol extract (Hot) of *Cocos nucifera* Linn. endocarp at 254 nm.**

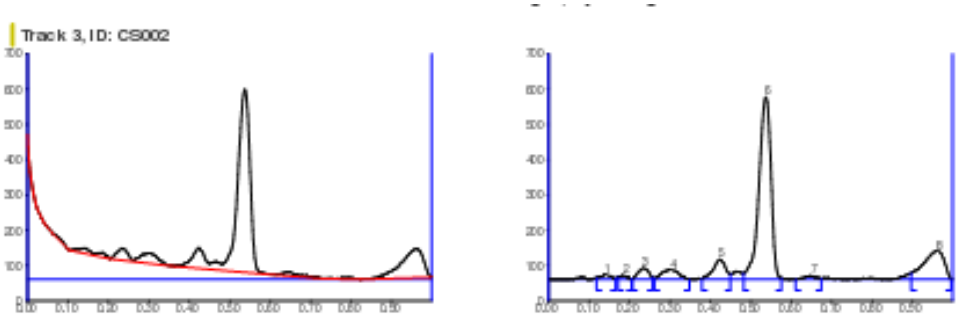


**Table 4. Rf values and % areas in the HPTLC of Ethanol extract (Hot) of *Cocos nucifera* Linn. endocarp at 254nm.**

| Peak | Rf | Height | Rf | Height | % | Rf | Height | Area | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.12 | 5.4 | 0.14 | 15.2 | 2.00 | 0.17 | 3.9 | 372.3 | 1.63 |
| 2 | 0.17 | 7.1 | 0.19 | 13.4 | 1.76 | 0.21 | 1.4 | 226.5 | 0.99 |
| 3 | 0.21 | 2.6 | 0.23 | 34.2 | 4.49 | 0.26 | 6.8 | 831.4 | 3.63 |
| 4 | 0.26 | 4.3 | 0.30 | 29.1 | 3.81 | 0.35 | 0.1 | 1157.8 | 5.06 |
| 5 | 0.38 | 3.3 | 0.42 | 58.1 | 7.62 | 0.45 | 15.8 | 1683.3 | 7.36 |
| 6 | 0.48 | 18.6 | 0.54 | 518.5 | 68.04 | 0.58 | 4.7 | 14360.9 | 62.77 |
| 7 | 0.61 | 1.3 | 0.65 | 11.7 | 1.53 | 0.68 | 3.4 | 326.9 | 1.43 |
| 8 | 0.90 | 19.1 | 0.96 | 81.9 | 10.74 | 1.00 | 2.2 | 3921.0 | 17.14 |

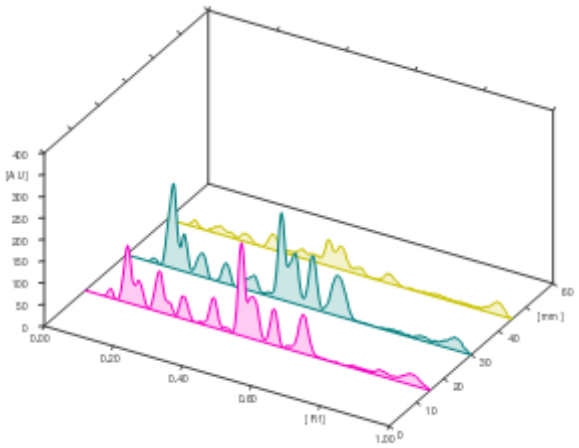

**Figure 5. Overview graph of various extracts of *Cocos nucifera* Linn. endocarp at 366 nm**.

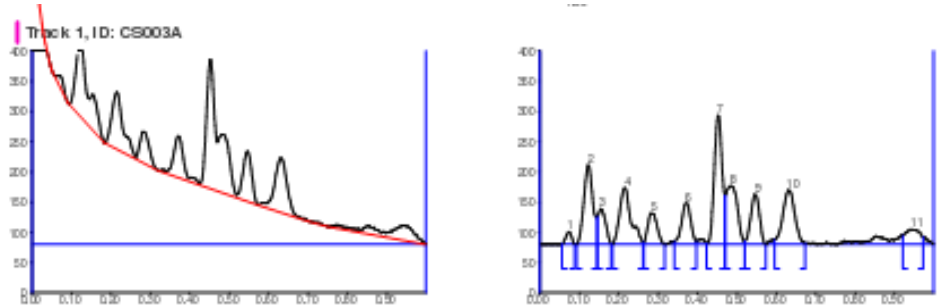


**Figure 6. Overview graph of Aqueous extract (Cold) of *Cocos nucifera* Linn. endocarp at 366 nm.**

**Table 5. Rf values and % areas in the HPTLC of Aqueous extract (Cold) of *Cocos nucifera* Linn. endocarp at 366 nm.**

| Peak | Rf | Height | Rf | Height | % | Rf | Height | Area | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.06 | 1.4 | 0.07 | 21.2 | 2.26 | 0.09 | 0.4 | 287.5 | 1.36 |
| 2 | 0.09 | 0.2 | 0.12 | 131.7 | 14.06 | 0.14 | 45.0 | 2827.5 | 13.35 |
| 3 | 0.15 | 45.9 | 0.16 | 59.3 | 6.32 | 0.18 | 1.2 | 1065.3 | 5.03 |
| 4 | 0.19 | 0.1 | 0.22 | 94.2 | 10.06 | 0.26 | 3.3 | 2536.5 | 11.98 |
| 5 | 0.26 | 3.3 | 0.28 | 51.9 | 5.54 | 0.32 | 0.7 | 1151.2 | 5.44 |
| 6 | 0.34 | 4.7 | 0.37 | 68.8 | 7.34 | 0.40 | 4.8 | 1505.2 | 7.11 |
| 7 | 0.42 | 1.8 | 0.45 | 214.1 | 22.84 | 0.47 | 81.0 | 4037.9 | 19.07 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **8** | 0.47 | 81.3 | 0.49 | 97.4 | 10.39 | 0.52 | 5.0 | 2585.1 | 12.21 |
| **9** | 0.52 | 5.9 | 0.55 | 83.1 | 8.87 | 0.57 | 0.0 | 1718.0 | 8.11 |
| **10** | 0.60 | 8.0 | 0.63 | 90.5 | 9.66 | 0.67 | 2.7 | 2568.3 | 12.13 |
| **11** | 0.92 | 15.0 | 0.95 | 25.0 | 2.67 | 0.97 | 12.4 | 892.5 | 4.21 |

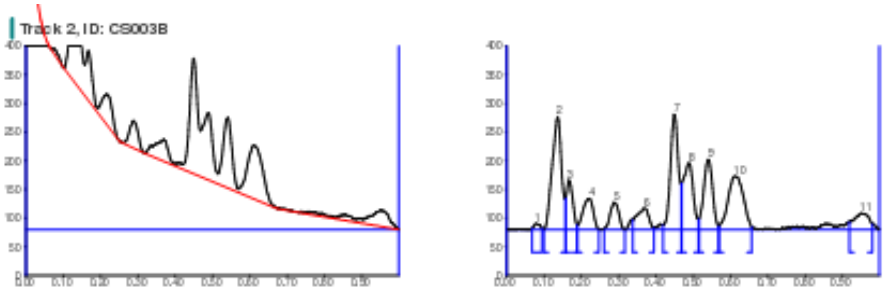


**Figure 7. Overview graph of Aqueous extract (Hot) of *Cocos nucifera* Linn. endocarp at 366 nm.**

**Table 6. Rf values and % areas in the HPTLC of Aqueous extract (Hot) of *Cocos nucifera* Linn. endocarp at 366 nm.**

| Peak | Rf | Height | Rf | Height | % | Rf | Height | Area | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.07 | 2.4 | 0.08 | 11.0 | 1.10 | 0.10 | 2.7 | 161.0 | 0.67 |
| 2 | 0.10 | 0.2 | 0.14 | 196.5 | 19.70 | 0.16 | 53.6 | 4575.4 | 19.16 |
| 3 | 0.16 | 55.0 | 0.17 | 86.6 | 8.68 | 0.19 | 7.3 | 1351.7 | 5.66 |
| 4 | 0.19 | 7.6 | 0.22 | 54.5 | 5.46 | 0.25 | 0.4 | 1395.4 | 5.84 |
| 5 | 0.26 | 0.9 | 0.29 | 47.9 | 4.80 | 0.32 | 0.1 | 1011.2 | 4.23 |
| 6 | 0.34 | 17.4 | 0.37 | 37.9 | 3.80 | 0.40 | 2.9 | 1101.5 | 4.61 |
| 7 | 0.42 | 7.7 | 0.45 | 201.3 | 20.18 | 0.47 | 80.0 | 4045.9 | 16.94 |
| 8 | 0.47 | 81.1 | 0.49 | 116.8 | 11.71 | 0.52 | 17.7 | 2861.2 | 11.98 |
| 9 | 0.52 | 18.4 | 0.54 | 122.6 | 12.29 | 0.57 | 6.1 | 2737.1 | 11.46 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 0.57 | 6.2 | 0.61 | 93.8 | 9.41 | 0.66 | 4.4 | 3573.6 | 14.97 |
| 11 | 0.92 | 13.5 | 0.95 | 28.7 | 2.88 | 0.98 | 10.0 | 1064.7 | 4.46 |

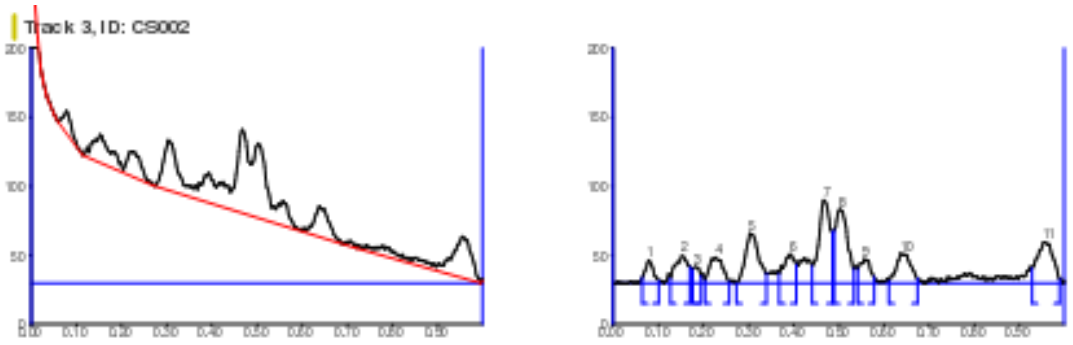


**Figure 8. Overview graph of Ethanol extract (Hot) of *Cocos nucifera* Linn. endocarp at 366 nm.**

**Table 7. Rf values and % areas in the HPTLC of Ethanol extract (Hot) of *Cocos nucifera* Linn. endocarp at 366 nm.**

| Peak | Rf | Height | Rf | Height | % | Rf | Height | Area | % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.07 | 4.5 | 0.08 | 16.8 | 5.40 | 0.10 | 2.0 | 277.3 | 3.59 |
| 2 | 0.13 | 3.8 | 0.15 | 20.3 | 6.52 | 0.17 | 11.9 | 501.4 | 6.50 |
| 3 | 0.18 | 10.1 | 0.18 | 12.6 | 4.04 | 0.20 | 5.5 | 166.7 | 2.16 |
| 4 | 0.20 | 2.2 | 0.23 | 19.0 | 6.10 | 0.26 | 1.1 | 509.5 | 6.60 |
| 5 | 0.27 | 0.1 | 0.30 | 36.4 | 11.69 | 0.34 | 7.1 | 994.1 | 12.88 |
| 6 | 0.37 | 7.0 | 0.39 | 21.4 | 6.87 | 0.41 | 13.5 | 485.4 | 6.29 |
| 7 | 0.44 | 12.4 | 0.47 | 60.5 | 19.47 | 0.49 | 37.3 | 1423.2 | 18.44 |
| 8 | 0.49 | 38.6 | 0.50 | 54.1 | 17.39 | 0.53 | 10.5 | 1345.8 | 17.43 |
| 9 | 0.54 | 10.1 | 0.56 | 18.0 | 5.80 | 0.58 | 4.7 | 383.8 | 4.97 |
| 10 | 0.61 | 1.8 | 0.64 | 22.0 | 7.07 | 0.68 | 3.7 | 648.7 | 8.40 |
| 11 | 0.93 | 10.8 | 0.95 | 30.0 | 9.65 | 0.99 | 3.2 | 983.2 | 12.74 |

**DISCUSSION**

Natural products are important reliable sources for drug development[15]. The inclusive pharmaceutical application of Coconut shell in the perspective of pertinent advantages of sustainable harvesting and repurposing of agro-waste from the plant source *Cocos nucifera* Linn looks highly relevant in this regard. It is very crucial to develop effective and selective methods for extraction, isolation and chemical profiling of bioactive phytoconstituents from natural products before proceeding with intended biological testing. In this regard, deriving the monograph of various extracts of Coconut shell in order to provide qualitative and quantitative standards of authenticity, purity and strength will assure the quality and efficacy parameters for its use in therapeutics[16]. Being the use of novel plant based product, the test drug need to complies with applicable regulations and consequently all relevant aspects of quality of an extract as per API were considered including plant material, collection, solvent used for extraction, extraction procedure and determination of quality and quantity of bioactive constituents in the extract.

By incorporating hot aqueous extraction technique, the isolation and extraction of active ingredients the dried ripe Coconut shells were successfully accomplished in this study. The bioactive chemical profile of various extracts of *Cocos nucifera* Linn endocarp were well established by incorporating the techniques of extraction, isolation, screening and identification of the chemical components by qualitative phytochemical evaluation. Chemical analysis of the test drug reflects the potency of vegetable material in terms of its active principles [17]. The chemical characterization revealed the test drug as a rich source of alkaloids, steroids, phenols, flavonoids and tannins. Previous studies already established the diverse antioxidant, anti-inflammatory, antimicrobial and anti-diabetic activities that are endowed with these bioactive phytoconstituents[18] [19]. Estimation of these active metabolites in the test drug depicts the quality and efficacy standards of Coconut shell. The comprehensive chemical profile also

highlights the broad pharmacotherapeutic implications of Coconut shell. Also the distinct presence of bioactive phytoconstituents like Flavonoids, Saponins, Phlobatannins and Quinones exclusively in the aqueous extract recommends the presence of more water soluble contents of the test drug and thereby direct towards the appropriate dosage forms of Coconut shell in therapeutics.

The HPTLC fingerprint profile of Coconut (*Cocos nucifera* Linn.) shell served as an important parameter to depict the Identity, Purity and Strength of the test drug sample. Chromatographic techniques like HPTLC are most effective analytical tool for the drug standardization and estimation of value-added bio-active compounds from herbal drugs[20]. In this study, the solvent system containing Toluene: Ethyl acetate: Formic acid: Ethanol in the ratio of 7:5:1:0.5; v/v gave remarkable separation and resulted in good resolution of the phytoconstituents present in the test drug. HPTLC photo documentation, densitometric scan and *Rf* values of the drug sample derived by standard procedures indicated several distinct peaks and areas with Rf value ranging from 0.1 to 0.9, revealing the possible presence of diverse phytochemical constituents in the test drug which has broad spectrum detection and broad application in therapeutics. The peaks with Rf values ranging from 0.75 and 0.9 represents some major flavonoid compounds like Quercetin[21]. The presence of phenolic compounds like Catechin can be inferred from the peaks with Rf between 0.3 and 0.4 [22]. Previous studies on *Cocos nucifera* Linn already revealed it as a rich source of flavonoids and phenolic compounds[23]. The occurrence of bioactive phytoconstituents like Catechin and Quercetin in other useful parts of Cocos nucifera were already reported[24].

The difference in the number of peaks, Rf value and corresponding areas of the peaks for three different extracts of the test drug obviously represents the varied concentration of phytoconstituents yielded in specific extracts. The densitometry scan at 254 nm with more number of peaks for aqueous extracts compared to the ethanol extract evidently indicates the abundance of bioactive constituents in the active aqueous portion of the test drug. The maximum peak areas noted in the overview graph of hot aqueous extract in comparison to other two extracts with same number of peaks (11 peaks) represents the presence of more water-soluble and thermostable constituents in the hard endocarp of *Cocos nucifera* Linn. These particular findings of HPTLC analysis were in consistent with the results of qualitative phytochemical analysis of various extracts in which some important chemical constituents identified in the aqueous extract were found absent in the ethanol extract of test drug. .

Using HPTLC fingerprint technique, we were able to derive the chemical profile that depicts the efficacy as well as quality standards of endocarp of *Cocos nucifera* Linn. HPTLC technique provided more accurate and precise data, not only supporting drug discovery and development but also for postmarket surveillance of the test drug.

## CONCLUSION

The qualitative phytochemical characterization and HPTLC fingerprint profile of various extracts of *Cocos nucifera* Linn endocarp depicts its inclusive chemical profile and ascertains the identity, quality, efficacy and potency of the test drug. The bioactive chemical profile of various extracts of Coconut shell embodied in the present study endorses its immense possibilities and inclusive pharmaceutical application of Coconut shell with respect to the pertinent advantage of sustainable harvesting and repurposing of agro-waste from the plant source. The highest content of bioactive secondary metabolites in the hot aqueous extract of the test drug was exemplified in the study. The study outcome can be integrated in further researches on Coconut shell exploring its further strategies of New Drug Development process.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## REFERENCES

1. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014 Jan 10; 4:177. doi: 10.3389/fphar.2013.00177. PMID: 24454289; PMCID: PMC3887317.

2. Sahoo N, Manchikanti P. Herbal drug regulation and commercialization: an Indian industry perspective. J Altern Complement Med. 2013 Dec; 19(12):957-63. doi: 10.1089/acm.2012.0275. Epub 2013 Jul 5. PMID: 23829812; PMCID: PMC3868382.

3. Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med. 2016 Jul 30; 11:37. doi: 10.1186/s13020-016-0108-7. PMID: 27478496; PMCID: PMC4967523.

4. Yong JW, Ge L, Ng YF, Tan SN. The chemical composition and biological properties of coconut (Cocos nucifera L.) water. Molecules. 2009 Dec 9; 14(12):5144-64. doi: 10.3390/molecules14125144. PMID: 20032881; PMCID: PMC6255029.

5. Coconut Development Board; Government of India. (n.d.). "Coconut Cultivation". Archived from the original on December 10, 2015. Retrieved December 8, 2015.

6. Lima EB, Sousa CN, Meneses LN, Ximenes NC, Santos Júnior MA, Vasconcelos GS, Lima NB, Patrocínio MC, Macedo D, Vasconcelos SM. Cocos nucifera (L.) (Arecaceae): A phytochemical and pharmacological review. Braz J Med Biol Res. 2015 Nov; 48(11):953-64. doi: 10.1590/1414-431X20154773. Epub 2015 Aug 18. PMID: 26292222; PMCID: PMC4671521.

7. Ramadhansyah Putra Jaya et al Use of Coconut Shell from Agriculture Waste as Fine Aggregate in Asphaltic Concrete June 2016Journal of Engineering and Applied Sciences 11(12)

8. Somyos Kijkar. "Handbook: Coconut husk as a potting medium". ASEAN-Canada Forest Tree Seed Centre Project 1991, Muak-Lek, Saraburi, Thailand. ISBN 974-361-277-1

9.Gershell LJ, Atkins JH.A brief history of novel drug discovery technologies.Nat Rev Drug Discov. 2003 Apr; 2(4):321-7.

10. Sudberg S, Sudberg EM, Terrazas J, Sudberg S, Patel K, Pineda J, Fine B. Fingerprint analysis and the application of HPTLC to the determination of identity and quality of botanicals, from an industry perspective.J AOAC Int. 2010 Sep-Oct; 93(5):1367-75.

11. 14. Anonymous, 2016. The Ayurvedic Pharmacopoeia of India, Part-I, vol.-IX, first edition, Government of India, Ministry of AYUSH.

12. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 2020 Jan-Mar; 12(1):1-10. doi: 10.4103/jpbs.JPBS_175_19. Epub 2020 Jan 29. PMID: 32801594; PMCID: PMC7398001.

13. Anonymous, 1989. The Ayurvedic Pharmacopoeia of India, Part-I, vol.-I, first English ed., Government of India, Ministry of AYUSH.

14. Eloff JN, Ntloedibe DT, van Brummelen R. A simplified but effective method for the quality control of medicinal plants by planar chromatography. Afr J Tradit Complement Altern Med. 2011; 8(5 Suppl):1-12. doi: 10.4314/ajtcam.v8i5S.11. Epub 2011 Jul 3. PMID: 22754053; PMCID: PMC3252719.

15. Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: An integrated approach. Ayu. 2012 Jan; 33(1):10-9. doi: 10.4103/0974-8520.100295. PMID: 23049178; PMCID: PMC3456845.

16. Simmler C, Graham JG, Chen SN, Pauli GF. Integrated analytical assets aid botanical authenticity and adulteration management. Fitoterapia. 2018 Sep; 129:401-414. doi: 10.1016/j.fitote.2017.11.017. Epub 2017 Nov 22. PMID: 29175549; PMCID: PMC5963993.

17. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Biotechnol Adv. 2015 Dec; 33(8):1582-1614. doi: 10.1016/j.biotechadv.2015.08.001. Epub 2015 Aug 15. PMID: 26281720; PMCID: PMC4748402.

18. Pieczykolan A, Pietrzak W, Gawlik-Dziki U, Nowak R. Antioxidant, Anti-Inflammatory, and Anti-Diabetic Activity of Phenolic Acids Fractions Obtained from *Aerva lanata* (L.) Juss. Molecules. 2021 Jun 8; 26(12):3486. doi: 10.3390/molecules26123486. PMID: 34201147; PMCID: PMC8228310.

19. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D. Flavonoids and Their Anti-Diabetic Effects: Cellular Mechanisms and Effects to Improve Blood Sugar Levels. Biomolecules. 2019 Sep 1; 9(9):430. doi: 10.3390/biom9090430. PMID: 31480505; PMCID: PMC6769509.

20. Attimarad M, Ahmed KK, Aldhubaib BE, Harsha S. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm Methods. 2011 Apr; 2(2):71-5. doi: 10.4103/2229-4708.84436. PMID: 23781433; PMCID: PMC3658041.

21. Kaya B, Menemen Y, Saltan FZ. Flavonoid compounds identified in Alchemilla L. species collected in the north-eastern Black Sea region of Turkey. Afr J Tradit Complement Altern Med. 2012 Apr 2; 9(3):418-25. doi: 10.4314/ajtcam.v9i3.18. PMID: 23983376; PMCID: PMC3746660.

22. Dinakaran SK, Sujiya B, Avasarala H. Profiling and determination of phenolic compounds in Indian marketed hepatoprotective polyherbal formulations and their comparative evaluation. J Ayurveda Integr Med. 2018 Jan-Mar; 9(1):3-12. doi: 10.1016/j.jaim.2016.12.006. Epub 2017 Dec 1. PMID: 29198414; PMCID: PMC5884013.

23. Lima EB, Sousa CN, Meneses LN, Ximenes NC, Santos Júnior MA, Vasconcelos GS, Lima NB, Patrocínio MC, Macedo D, Vasconcelos SM. Cocos nucifera (L.) (Arecaceae): A phytochemical and pharmacological review. Braz J Med Biol Res. 2015 Nov; 48(11):953-64. doi: 10.1590/1414-431X20154773. Epub 2015 Aug 18. PMID: 26292222; PMCID: PMC4671521.

24. Clarice Kirszberg , Daniele Esquenazi, Celuta Sales Alviano, Vivian M Rumjanek. The effect of a catechin-rich extract of *Cocos nucifera* on lymphocytes proliferation. Phytotherapy Res. 2003 Nov; 17(9):1054-8. doi: 10.1002/ptr.1297. PMID: 14595586.