

New Drug Development strategies with special reference to the endocarp of multifaceted agriculture crop – *Cocos nucifera* Linn.

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Abstract

Coconut shell botanically represents the hard endocarp from the fruit of *Cocos nucifera* Linn. It is the usually discarded part of the plant with folklore claim in metabolic disorders. Being an inexpensive and easily available natural product with ethnomedicinal significance, it seems highly relevant to scientifically validate the pharmaceutical implications of the drug. Till date, the new drug development strategies based on quality and efficacy parameters for the crude drug sample of Coconut shell has not been developed. The present study was intended to develop and scientifically validate the quality, purity, safety, potency and efficacy parameters of Coconut shell and thereby exploring the new drug development strategies for the drug. Materials and methods: The study focused on the screening of Coconut shell based on macroscopic, microscopic, organoleptic, histo-chemical and HPTLC studies. Results: Characteristic presence of thin walled fibres, fibrosclereids, lignins and tannins were observed in the microscopic analysis of Coconut shell. HPTLC studies also exemplified the detailed bioactive chemical profile of Coconut shell. Conclusion: The study outcome suggests Coconut shell as a novel reliable source of bioactive phytoconstituents with broad prospective in new drug development process.

Keywords: *Cocos nucifera*, Ethnomedicine, New drug development, Powder microscopy, Quality control, Ayurveda.

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1. Introduction

Plants and plant based products are globally recognized sources of bioactive compounds [1]. Herbal drugs and their wide application in primary healthcare system is increasing tremendously in world wide. [2]. Nowadays, in the New drug development strategies, the therapeutic agents derived from natural products were given prime focus [3]. Nevertheless, natural products also encounters challenges in this regard, particularly pertaining to the characterization and optimization of chemical compounds [4].

Coconut palm is honored as one among the most versatile trees of the entire world and virtually every part of the coconut palm can be used by humans in some manner and has significant economic value [5]. Coconut shell botanically represents the hard endocarp from the fruit of *Cocos nucifera* Linn [6]. It is the usually discarded part of the plant with folklore claim in metabolic disorders. Being an inexpensive and easily available plant based product with ethnomedicinal significance, comprehensive studies on the medicinal prospects of Coconut shell receives ample contemplation nowadays. However, research updates on the new drug development strategies based on quality, purity, safety and efficacy parameters of Coconut shell is not available till date. The present research work was carried out to develop and scientifically validate the quality and efficacy parameters of Coconut shell in the perspective of new drug development process. The present study is focused on the screening of dried ripe Coconut shell based on macroscopic, microscopic, organoleptic and HPTLC studies.

2. Materials and Methods

Collection and authentication of test drug

Ripe coconuts (*Cocos nucifera* Linn.) were procured from a single plot at Calicut during the month of April- May. The drug was botanically identified and authenticated (Acc. No. DG/21-22/353) in the Pharmacognosy laboratory, Banaras Hindu University. The fruits were further broken and dried well in proper sunlight. After proper drying and removal of the white kernel, the coconut shells (endocarp) were collected as taken as the crude drug for this study.

Preparation of test drug powder

The rough external surfaces of Coconut shells were scraped well to remove the outer fibers. This ripe dried Coconut shell was then manually broke in to small pieces, crushed and pulverized to obtain the coarse powder of test drug. The powdered sample was further subjected to microscopic analysis, histochemical studies, analysis of physicochemical and phytochemical parameters.

1. Macroscopic characterization of test drug

The crude drug sample of Coconut shell was subjected to detailed macroscopic characterization and morphological characters including size, shape, texture, fracture and nature of striations present on both surfaces were documented.

2. Organoleptic characterization of test drug

Organoleptic evaluation refers to evaluation of individual drug based on its characteristic colour, taste, odour, texture etc. The organoleptic characters of Coconut shell was assessed based on sensory observations as per standard methods [7].

3. Powder microscopy of test drug

One pinch of powdered crude drug sample of the test drug was taken in a microscopic slide. Then it was warmed with addition of few drops of chloral hydrate and mounted in glycerin. After mounting, Zeiss AXIO trinocular microscope was used for viewing the slides. The characteristic features were noted and photographed under bright field light using the attached Zeiss Axio Cam camera. Scale-bars are used to indicate the magnifications of figures [8].

The presence of cellulose cell walls, aleurone grains, calcium carbonate, fats, lignified cell walls, inulin, mucilage, cuticular cell walls, starch, tannins, oils and resins in the test drug were analyzed by staining of powdered test drug as per standard guidelines [9]. The test drug powder was stained using Iodine solution in order to identify the presence of starch grains; phloroglucinol and concentrated hydrochloric acid (HCl) for the identification of lignified cells and 5% ferric chloride (FeCl_3) for detection of tannins.

4.HPTLC characterization of test drug

Methanol extraction of Coconut shell was done and 20 µl of the extract was applied on a pre-coated silica gel 60 F254 on aluminium plates (10x10 cm) to a distinct band width of about 8 mm. The Linomat 5 Thin Layer Chromatography applicator was used for test drug application on TLC plates. The solvent system containing Toluene:Ethyl acetate: Formic acid: Methanol (7:5:1:0.5) was used for developing the plates in CAMAG Twin trough chamber (10 x 10 cm). The visualization of these developed plates were done under 254nm and 366 nm. The plates were then derivatized using Anisaldehyde reagent and further densitometry scanning was done under 254, 366 and 540 nm. CAMAG Linomat 5, CAMAG Reprostar 3 and CAMAG Thin Layer Chromatography Scanner 3 were the HPTLC instrumentations employed in this study. HPTLC photo documentation, densitometry scan and colour of distinct spots along with corresponding R_f values of the test sample were recorded as per standard procedures [10].

Observation and results:

1. Macroscopic analysis

Coconut shell which represents the endocarp forms the hard inner layer that surrounds the seed. The shape of Coconut shell is ovoid and about 0.8 – 1.2 cm thick, three angled, outer surface brown, somewhat rough due to shallow reticulated striations, transversely broken; with a large central cavity. The fracture is short and the shell is striated in length by three protruding sutures on the outer surface.

2. Organoleptic evaluation

The powder of Coconut shell is dark brown colour in appearance with no characteristic smell. The drug is predominantly of astringent taste and possess fibrous texture.

3. Powder microscopy

By microscopic analysis, the fine characteristic features were identified and documented as the distinct parameters representing the crude drug sample. Thin walled fibers were abundantly seen in the test drug. Sclerids and fibrosclerids were also present. Spiral vessels, pitted parenchyma cells and Starch grains were also identified in the crude drug sample.

The appearance of characteristic red colour by the addition of Phloroglucinol + Conc. HCl to the powdered sample indicated the presence of lignins in the test drug. Addition of Ferric chloride (5%) has given peculiar dark blue colour corresponding to the tannins present in Coconut shell. Appearance of blue colour by the addition of Iodine indicated the starch grains present in the test drug.

4. HPTLC characterization of the sample

The solvent system containing Toluene: Ethyl acetate: Formic acid: Methanol in the ratio of 7:5:1:0.5; v/v resulted in remarkable separation of the bioactive compounds. The R_f values and areas of different peaks obtained in the HPTLC characterization of Coconut shell at 254 and 366 nm were mentioned in the table 1. Densitometry scan at UV 254 nm in HPTLC of Coconut shell revealed 8 peaks in the methanol extract (Figure 1). The R_f were 0.29, 0.35, 0.40, 0.50, 0.73 for the major peaks with a maximum area of 3682.9 AU obtained for the 6th peak (Table 1). At UV 366 nm, the drug exhibited 7 distinct peaks. The R_f were 0.28, 0.35 and 0.55 for the major peaks (Figure 2). The maximum area of 5939.2 AU is observed for the highest peak with the R value of 0.55.

Discussion

Coconut palm is recognized as one of the most versatile trees and virtually every part of the coconut palm can be used by humans in some manner and has got significant economic value [11]. Being an inexpensive and easily available natural product from our surroundings, comprehensive studies on the new drug development strategies of Coconut shell receives ample contemplation nowadays.

Powder microscopy of Coconut shell indicates the large number of thin walled fibers present in the hard endocarp of fibrous drupe (Coconut fruit). Organoleptic evaluation itself depicts the fibrous texture of test drug. Microscopic study offered a methodological approach that allowed the chemical analysis of cells and tissues of drugs in relation to their structural organization [12]. The lignins present in the dried endocarp of Coconut shell was exemplified in the microscopic staining of test drug. As per previous reports, Coconut shell serves as a natural Lignocellulose material (Lignin -29.4% and cellulose- 26.6%) [13].

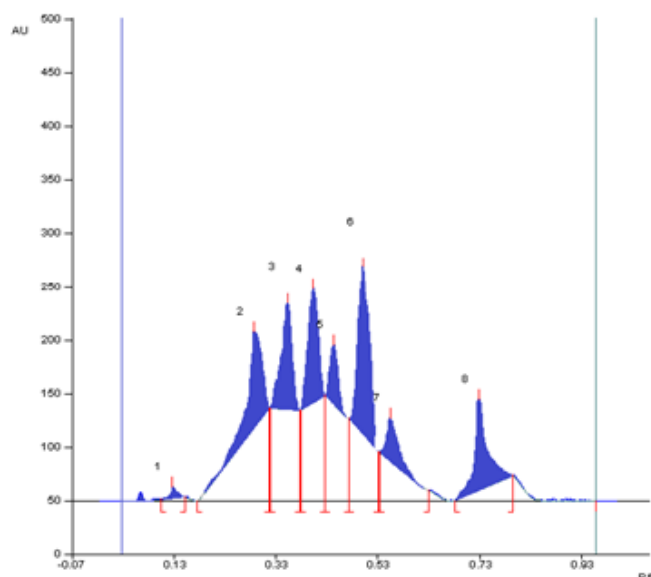


Figure 1. Densitometry scan of test drug at 254 nm

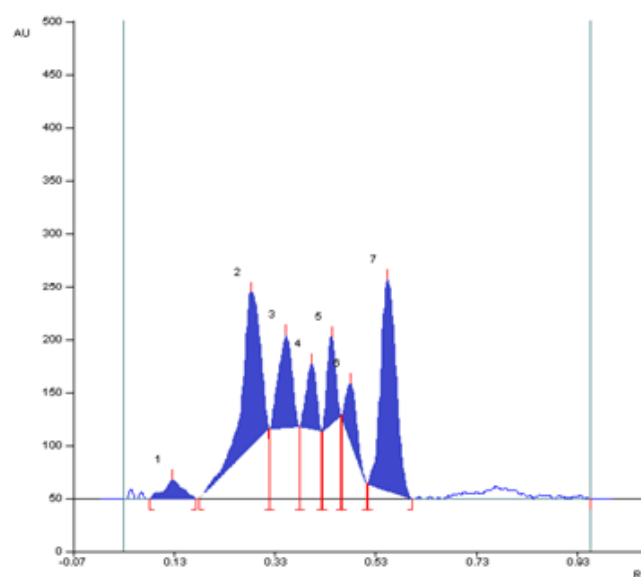


Figure 2. Densitometry scan of test drug at 366nm

Table 3. R_f values and areas in the HPTLC of Coconut shell powder at 254nm and 366nm.

254nm			366nm		
Peak	R _f Value	Area (AU)	Peak	R _f Value	Area (AU)
2	0.29	3425.5 AU	2	0.28	5718.1 AU
3	0.35	2477.1 AU	3	0.35	2185.5 AU
4	0.40	2015.5 AU	7	0.55	5939.2 AU
6	0.50	3682.9 AU			
8	0.73	2875.3 AU			

Lignins are endowed with antioxidant potential and are having diverse pharmacotherapeutic implications in the prevention of lifestyle disorders [14]. The peculiar dark blue colour observed in the microscopic analysis of Coconut shell on addition of Ferric chloride corresponds to the tannins present in the crude drug sample of Coconut endocarp. Organoleptic evaluation suggests the drug to be of predominantly astringent taste that indicates the significant tannin content of Coconut shell. The correlation between tannin content of herbs and astringent intensity was already established by previous studies [15]. Studies have already established the fact that plants endorsed with high tannin content are supposed to have enormous potential as therapeutic agent in wide range of disorders [16]. The presence of tannins in other parts of *Cocos nucifera* including its inflorescence and corresponding therapeutic implications were already reported in previous studies [17].

The HPTLC fingerprint profile served as an important technique employed for elucidating the new drug development strategies of Coconut endocarp. Chromatographic procedures represent the effective analytical tool in new drug development process for the characterization of value-added bio-active compounds from herbal drugs [18]. In this study, the comprehensive bioactive chemical profile and quality standards of Coconut shell were derived by employing the HPTLC fingerprint profiling of the test drug. The eight distinct peaks observed in the densitometry scan at 254 nm indicates the probable presence of value added bioactive chemical constituents in the test drug. The peaks with R_f values of 0.2, 0.3 and 0.4 depicts the phenolic contents present in Coconut shell. The endocarp of test drug may contain some major flavonoid compounds which can be inferred from the peaks with R_f value of 0.50 and 0.55 at 254 and 366nm respectively. Since the maximum area was noted at these specific R_f values at both the wavelengths, the particular flavonoid may be the major chemical constituent of *Cocos nucifera* endocarp that can be further isolated and validated in the

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perspective of new drug development. Various other useful parts of Coconut tree were already reported to be a rich source of flavonoid and phenolic compounds that are responsible for the diverse therapeutic potentials attributed to the respective parts of the plant [19]. All these findings justify the scope of new drug development approach based on Coconut shell and its applications in herbal drug industry.

Conclusion

The present study exemplified Coconut shell as a novel natural source of bioactive constituents like phenols, flavonoids and tannins that can be integrated in the new drug development strategy.

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Conflict of Interest

The authors declare no conflict of interest.

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