Biological Analysis of Cocos Nucifera L Endocarp Extracts

[1]K Lavanya , [2]S Agneswari, [3]S Kala Vetha kumari, [4]K Indirani [5]M Murugan, [6]Abisha B

[1]Department of Biochemistry, PSG College of Arts and Science (Autonomous), *affiliated to* Bharathiar University, Coimbatore, Tamil Nadu, India.

[2]Department of Zoology, Vivekananda College, Agasteeswaram, Kanyakumari, *affiliated to* Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

[3]Department of Botany, Holy Cross College (Autonomous), Nagercoil *affiliated to* Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

[4]Department of Clinical Nutrition and Dietetics, PSG College of Arts and Science (Autonomous), *affiliated to* Bharathiar University, Coimbatore, Tamil Nadu, India.

[5]Department of Biomedical Engineering, Noorul Islam Centre for Higher Education, Kumaracoil, Kanyakumari District, Tamil Nadu, India.

[6]Department of Biotechnology, Noorul Islam College of Arts and Science, Kumaracoil *affiliated to* Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.
email: lavanya@psgcas.ac.in

ABSTRACT
Cocos nucifera. L commonly called as Coconut tree is used for its several beneficial health effects as antitumor, anthelminthic, antidotal, antiseptic, bactericidal activity, etc. Coconut shell or its endocarp is an agricultural waste and is available in very large quantities throughout the tropical countries of the world. Endocarp of C.nucifera. L was supposed to be the hardest part of the fruit but ionically richest source of phenolic and flavonoid compound which are responsible for diverse biological activities beneficial to human health and disease prevention. Therefore the present study was conducted to determine the phytochemical constituents and antioxidant activity of four different extracts of C.nucifera. L endocarp prepared by cold percolation (CNL-01), hot percolation (CNL-02), aqueous extraction (CNL-03) and by dry distillation (CNL-04) methods**.** All the investigated phytochemicals except amino acids, proteins and alkaloids were present in different extracts. Antioxidant activity of extracts using reducing power methods revealed CNL-02 as the better extract.

1. INTRODUCTION

Cocos nucifera Linn commonly known as coconut belonging to *Arecaceae* family is considered as an important fruit tree in the world providing food and used in the world playing a significant role in the economic, cultural and social life of over 80 tropical countries. Currently coconut is mainly an oil crop rich in lauric acid with a variety of others uses in addition to commercial oil production. The most important coconut producing countries in the world are India, SriLanka, Malaysia and Indonesia (Harries, 1995). For thousands of year, coconut products have held a respected and valuable place in Indian folk medicine. It is believed to be antiblenorrhagic, antibronchitis, febrifuga, antigingivitic, immunostimulant, antioxidant, antiparasitic etc. Endocarp of *Cocos nucifera* was supposed to be the hardest part of the fruit but ionically richest source of phenolic and flavonoid compound which are responsible for diverse biological

activities in medicinal plants beneficial to human health and disease prevention. Coconut shell is an agricultural waste and is available in very large quantities throughout the tropical countries of the world as a new source of energy biofuel. Coconut shell is composed mainly of lignin and cellulose having a chemical composition very similar to wood and suitable for phenolic extraction (Pinto *et al.,* 2008). Besides scientific data has been found as evident for antioxidant, antmicrobial, vasorelaxant, antihypertensive and various other activities of coconut endocarp (Singla, 2012). Inhibitory effect of *Cocos nucifera L* endocarp on human oral pathogen was also strongly determined. Hence, the present study is aimed to characterize the coconut shell in order to explore its activity in medicinal world.

2. MATERIALS AND METHODS

Plant material *Cocos nucifera L* endocarp was collected from Vallavilai village in Kanyakumari district, Tamil Nadu (India) and the samples were prepared by four different methods such as cold maceration, soxhlet extraction, dry distillation, aqueous extraction and subjected to phytochemical analysis both qualitatively and quantitatively as per the procedures of Sofowora (1993) followed by antioxidant assay.

2.2 Phytochemical analysis

2.2.1 Test for carbohydrates

The extracts were treated with 2 drops of alcoholic alpha-napthol solution in a test tube and 2 ml concentrated H 2 SO 4 was added carefully along the sides of the test tubes. Formation of red ring at the interphase indicated the presence of carbohydrates.

2.2.2.Test for aminoacids

To each of the solvent extract 0.25% ninhydrin reagent was added and boiled for few minutes for the development of blue colour.

2.2.3 Test for proteins

The extracts were treated with 1 ml of 10% NaOH solution and heated . To this extract a drop of 0.7% $CuSO_4$ solution was added. Formation of purplish violet colour was observed.

2.2.4 Test for Lipids

About 2ml of extract was treated with few drops of Sudan III reagents. The appearance of dark red oil droplet in the upper layer indicated the presence of fatty acids.

2.2.5 Test for alkaloids

The extracts were treated with Mayer's reagent (1.36 gm mercuric chloride and 5 gm of potassium iodide was dissolved in 100 ml distilled water).The formation of yellow cream predicted the presence of alkaloids.

2.2.6 Test for flavonoids

The extracts were treated with few drops of $FeCl_3$ solution. Formation of a blackish red colour indicated the presence of flavonoids.

2.2.7 Test for tannins

To 1 ml of each of the solvent extract, a few drops of $FeCl_3$ solution was added. The appearance of black and a green precipitate indicated the presence of tannins.

2.2.8 Test for phenols

To 1 ml of solvent extract 3 ml of distilled water was added. To this, a few drops of neutral 5% FeCl 3 solution was added. Presence of phenols were confirmed through the formation of a dark green colour.

2.3 Determination of primary and secondary metabolites in *Cocos nucifera.L* endocarp extract

2.3.1 Estimation of carbohydrates

Into a series of test tubes different aliquots (0.2-1ml) of standard glucose solution were pipette out and made up to 1ml with water. A tube containing 1ml of water was taken as blank. A known volume of test solution was taken in another test tube and made up to 1ml with water. To all the tubes 4ml of anthrone reagent was added and mixed well. The test tubes were covered with marbles and kept in a boiling water bath for 10mins and the color formed was read at 620nm.

2.3.2 Estimation of Lipids

About 1ml of the extracts (CNL-03 and CNL-04) was treated with 2ml of chloroform and 1ml of methanol. The extract was treated again with 2ml of chloroform and 1 ml methanol. It was then incubated overnight in dark at room temperature. Again 2ml of chloroform was added and transferred to a separatory funnel. Clear lower layer containing the lipid was transferred to a pre-weighed bottle and dried by a stream of nitrogen. The weight of total lipids were estimated by substracting the initial weight and expressed as mg/g dry weight (Bligh and Dyer, 1959).

2.3.3 Estimation of Total Flavonoid content

Total flavonoids were estimated in the plant extracts using a colorimetric method based on the formation of a complex flavonoid aluminium, having the absorbivity maximum at 430nm. All determinations were made in triplicate and values were calculated from a calibration curve obtained with rutin. Final results were expressed as milligram of rutin equivalent per gram of dried weight (Rajeev *et al.,* 2011).

2.3.4 Determination of Total Tannin content

5ml of the sample was mixed with 50ml of distilled water shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made upto the mark . Then 5ml of the filtrate was pippeted out into a test tube and mixed with 2ml of 0.1N $Fecl_3$ in 0.1N HCL and 0.08M potassium ferrocyanide. The absorbance was measured at 120nm within 10minutes (Van-burden and robinson, 1981).

2.3.5 Determination of Total Phenolic content

Total phenolic content of the all extracts were determined using Folin- Ciocalteu reagent and gallic acid as standard. 3.33 milligrams of the extracts were weighted into 5ml test tube, dissolved in 2ml of DMSO resulting 1.655 mg/ml. Two hundred microlitres (three replicates) were introduced into screw cap test tubes; 1.0 ml of Folin- Ciocalteu reagent and 0.8ml of sodium carbonate (20.25%) were added. The tubes were vortexed and allowed to stand for 2h. The absorption at 750nm was measured (UV Visible spectrophotometer) and the total phenolic content was expressed as milligram of gallic acid equivalents per gram dry materials (Rajeev *et al.,* 2012).

2.4 Antioxidant assay

2.4.1 Reducing power determination

1ml of extracts were mixed with 2.5ml of phosphate buffer (pH 6.6) and 2.5 ml of potassium ferric cyanide (1%w/w) and incubated at 50°C for 20minutes. Trichloro acetic acid (10% 2.5ml) was added to the mixture (2.5ml) were mixed with 2.5ml of distilled water and ferric chloride (0.5ml, 0.1%) and the absorbance was measured at 700nm. Ascorbic acid was used as standard and the results were expressed as percentage (Pandey Manisha *et al.,* 2009).

3. RESULTS

CNL-01, CNL-02, CNL-03 and CNL-04 extracts were successfully extracted from the endocarp of *Cocos nucifera.* The extracts were then subjected to phytochemical analysis and checked for its antioxidant activity.

3.1 Phytochemical screening of *Cocos nucifera.L* endocarp extracts

Preliminary screening of the extracts revealed the presence of carbohydrates in all the extracts except CNL-04, while only CNL-03 and CNL-04 possessed lipid. Proteins and aminoacids were absent in all the four extracts (Table 1). The extracts which responded positively for the preliminary screening were then subjected to quantitative estimatio

Table 1: Qualitative analysis of primary metabolites in *Cocos nucifera L* endocarp extracts

Sample	Carbohydrates	Amino acid	Protein	Lipid
CNL-01	+	-	-	-
CNL-02	+	-	-	-
CNL-03	+	-	-	+
CNL-04	-	-	-	+

3.2 Quantitative estimation of primary metabolites

The amount of total carbohydrates ranged 0.95 to 1.82 mg of the extracts. Among all the three extracts CNL-03 extract contained the highest amount of carbohydrate (1.82mg/g) followed by CNL-02 (1.74mg/g) and the lowest quantity was observed with CNL-01(0.95). The total lipid content of CNL-03 and CNL-04 was found to be 1.3mg/g and 1.6mg/g respectively (Table 2).

Table 2: Quantitative estimation of primary metabolites

Phytochemicals	CNM-01 mg/g	CNM-02 mg/g	CNM-03 mg/g	CNM-04 mg/g
Carbohydrate	0.95	1.74	1.82	-
Lipid	-	-	1.3	1.6

3.3 Preliminary phytochemical screening of secondary metabolites in *Cocos nucifera L* endocarp extracts

Phytochemical screening was done to assess the presence of secondary metabolites such as total flavanoids, total phenolics and total tannin. The result revealed the presence of all the compounds in all the extracts except alkaloids (Table 3). These extracts were then subjected to quantitative estimation using standard procedures.

Table 3: Preliminary phytochemical screening of secondary metabolites in *Cocos nucifera* endocarp extracts

S.no	Compounds	CNL-01	CNL-02	CNL-03	CNL-04
1.	Alkaloids	-	-	-	-
2.	Flavonoids	+	+	+	+

3.	Tannin	+	+	+	+
4.	Phenols	+	+	+	+

3.4 Determination of secondary metabolites in *Cocos nucifera.L* endocarp extracts

The amount of all the analyzed secondary metabolites in all the extracts varied widely. The amount of total flavanoid ranged from 36.32 mg/g to 296.32 mg/g of the extract. Among all the four extracts, CNL-01 contained the highest amount of flavonoids (296.32 m/g) followed by CNL-03(238.32mg/g) and CNL-02(206.00mg/g) while lowest quantity was observed with CNL-04 (36.32mg/g). Total phenolic content of CNL-01, CNL-02, CNL-03 and CNL-04 were found to be 128.32mg/g, 130.20mg/g, 106.50mg/g and 106.00mg/g respectively, while CNL-04 reported the least quantity. Total tannin content was found highest with CNL-02(1.20mg/g) and lowest with CNL-04 (0.20mg/g). On the otherside CNL-01, CNL-03 extract were found to possess 0.80 and 0.32mg/g of tannin content. Thus, the total metabolite content of all the extracts differed from one another. Among all the extracts, CNL-02 possessed major amount of total phenolics and tannin content while flavanoid was found higher with CNL-01 (Table 4).

Table 4: Determination of secondary metabolites in *Cocos nucifera* endocarp extracts

S.no	Sample	Total phenols (mg/g)	Total flavanoids (mg/g)	Total tannin (mg/g)
1	CNL-01	128.32	296.32	0.8
2	CNL-02	130.20	206.00	1.20
3	CNL-03	106.50	238.82	0.32
4	CNL-04	106.00	36.32	0.20

3.5 Antioxidant assay of *Cocos nucifera* L endocarp extracts

Antioxid**a**nt activity of *Cocos nucifera L* endocarp extracts were determined by reducing power method. All the extracts exhibited significant activity from 08.10 mg/g to 26.50mg/g. The highest activity was exhibited by CNL-02 (26.50mg/g), followed by CNL-01(26.00mg/g). The least activity was observed with CNL-04(08.10mg/g) (Table 5).

Table 5: Antioxidant assay of *Cocos nucifera* endocarp extracts

SI.no	Sample	Results (mg/g)
1	Standard (ascorbic acid)-(50µg/gm)	45.20
2	CNL-01	26.00
3	CNL-02	26.50
4	CNL-03	12.62
5	CNL-04	08.10

4 DISCUSSION

The present study conducted using coconut endocarp extracts revealed the presence of most of the phytochemicals analysed including important secondary metabolites such as phenols, flavonoids, tannins etc in

varying amounts in four different extracts of coconut used which are concerned with important therapeutic activities. Such variation in the quantity among the extracts might be attributed towards the difference in extracts, methodology or might be due to the presence of other compounds. Singla *et al.* (2011) analysed the phytochemical constituents, antioxidant activity and antimicrobial activity of *Cocos nucifera L* endocarp extracts. Their phytochemical analysis of endocarp extracts revealed the presence of all the analysed constituents. Quantitative estimation of phytochemical such as total phenolics, total flavonoids, total tannins content revealed the amounts as 145.77mg/g, 208.8mg/g and 6.02mg/g respectively. Among the secondary metabolized analysed, the highest phytochemical content was represented by flavonoids (208.8mg/g) followed by phenols (145.77 mg/g) and tannins. Antioxidant activity of the extracts was assayed using DPPH radical scavenging method, which showed significant antioxidant activity. These above results of Singla *et.al* correlates with the findings of present work. In the present work also, *Cocos nucifera L* endocarp extracts possessed significant amounts of different phytochemicals as well as free radical scaveiging activity which also may be attributed to the presence of total phenolics and other constituents. Therefore, it is suggested to make use of coconut endocarps for beneficial medicinal purpose.

CONCLUSION

The findings of the present study clearly reveal the efficiency of *Cocos nucifera L* endocarp extracts. The overall results provide promising baseline information for the potential use of the crude extracts from *Cocos nucifera L* endocarp extracts to scavenge the free radicals there by to overcome many diseased condition caused by cell damage. The endocarp of *Cocos nucifera. L,* are discarded as waste and it is considered as one of the major agro wastes in most of the countries. Therefore, this study will definitely open up a scope for future utilization of these agro waste for therapeutic purposes.

ACKNOWLEDGEMENT

We the authors jointly acknowledge and PSG College of Arts and Science,Coimbatore and Kamini Biotech, Thuckalay, Kanyakumari District, Tamil Nadu for providing necessary facilities to carry out the research work.

References

1. Bligh, EG, WJ Dyer 1959, ‘ A rapid method of total lipid extraction of purification’, can J, Med SCi, vol. 37, pg. 911-917
2. Evans, WC, Trease, 1997, ‘Harcourt brace and company, Asia Pvt. Ltd, Singapore’, Pharmacognosy, 14th edition.
3. Harries, H.C, 1995, ‘ *Cocos nucifera L* in evolution of crop plants’, J. Simmonds, N.W; Longman: London and NewYork, NMCE, Report on copra. National Multinational Commodity Exchange of India Limited.
4. Pandey manisha, Verma Rohit, Sarat SA, 2009, ‘Antioxidant activity of sida veroniactolia,’ Research J Pharmocognosy and Phytochemistry, vol. 2, issue.1, pg. 79-81.
5. Rajeev Singh, Pawan Kumar Verma, Gagandeep Singh, 2012, ‘Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*’, J Intercult Ethnopharmacol, vol. 1, issue.2, pg.101-104
6. Singla Rk, Jeyalekshmi S, And Yadav,V 2012, ‘Traditional systems of medicine, Now forever Webmed central pharmaceutical Science, vol.3, issue. 4.
7. Sofowora, A 1993, ‘Phytochemical Screening of Medicinal Plants and

Traditional Medicine in Africa Edition', Spectrum Books Ltd, Nigeria, pg.150-156

8. Van-Burden TP, Robison WC 1981, ' Formation of complexes between protein and Tannin acid', J. Agric Food chem. Vol. 1, pg. 17.
9. Yasuma, A and Ichikawa, 1953, 'Ninhydrin-schiff and alloxan- Schiff staining- A new histochemical staining method for proteins', J.Lab clin Med, vol. 41, pg. 296-299.