World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Phytochemical screening and estimation of total phenolics and total flavonoid content of Cocos nucifera endocarp

Nidhi Tyagi*, Vikas Hooda and Sachin Malkani

School of Pharmacy, ITM University, Gwalior, India

Received: 01-05-2015 / Revised: 23-06-2015 / Accepted: 27-06-2015

ABSTRACT

The *Cocos nucifera* (coconut) is the plant of Indian origin and being used since long time for therapeutic as well as domestic purpose. The aim of present study was to investigate the phyto-constituents present in the aqueous and ethanolic extract of *cocus nuifera* endocarp and to estimate the total phenolic and total flavonoid contents. The extracts were prepared and analysed separately for the present phytoconstituents. Total Phenolics contents were estimated and were found 17.2 mg (CNAE) and 21.4 mg (CNEE) gallic acid equivalent) in ethanolic and aqueous extract of Cocus nucifera endocarp respectively, while the estimated total present flavonoid contents were 23.71 mg (CNAE) and 37.57 mg (CNEE) rutin equivalent in respective extract.

Keywords: *Cocos nucifera*, phytoconstituents, phenols, flavonoid

INTRODUCTION

Cocos nucifera L. (Family Arecaceae) commonly known as coconut, is considered as an important fruit crop in the tropical countries. The coconut, *Cocos nucifera* L., has been described as "the tree of life" or the tree of plenty and nature's greatest gift to man. *Cocos nucifera* is a large palm, growing up to 30 meters (98 ft) tall, with pinnate leaves 4–6 meters (13–20 ft) long and pinnate 60–90 cm long; old leaves break away cleanly, leaving the trunk smooth (***Grimwood, 1975***). The fruit is a fibrous drupe. It consists of a thin hard skin (exocarp), a thicker layer of fibrous mesocarp (husk), the hard endocarp (shell), the white endosperm (kernel) and a large cavity filled with liquid (water). The seed comprises the dark brown shell and kernel. The surrounding husk, which is brown and dry at maturity, always remains intact (***Edward Chan & Craig R. E., 2006***). Unlike some other plants, the palm tree has neither tap root nor root hairs; but has a fibrous root system. Leaves pinnate, feather shaped, 4-7m long and 1-1.5 m wide at the broadest part. Leaf stalks 1-2 cm in length and thorn less (***Cobley L.S & Steele W.M, 1976***). Leaves are among the largest of any plant (up to 20 ft), pinnately compound with 200 or more leaflets, and borne in a spiral arrangement at the apex of the trunk (***Orwa et al, 2009***). The coconut plants have a number of uses. It is being used since longer time as a source of timber, food, fermented and unfermented drink, alcohol, vinegar, thatching materials, splints, strips and fibers for making baskets, mats, rope, hats, brushes, brooms and other articles like utensils for household use (such as cups, bowls, spoons). At the same time it is also used for cooking, illumination, for making soap, substitutes for butter and lard, ointments and oil cake for feeding domestic animals and for fertilizers. The palm is ornamental and is frequently planted for decorative (***Sivakumar et al, 2011***). Along with these it has several beneficial health effects like antitumor, antihelmintic, antidotal, antiseptic, aphrodisiac, astringent, bactericidal, depurative, diuretic, refrigerant, stomachic, styptic, suppurative, vermifuge, antioxidant, antihypertensive and vasorelaxant (**Singla et al, 2011**).

MATERIALS AND METHOD

Collection and Authentification of plant material: Cocos nucifera fruits were collected from local market of Rohtak (Haryana, India) and endocarp was separated manually. Before the processing of experimental work the plant part was authentified by Mr. Vinod Sharma, Herbarium Head, Department of Botany, University of Rajasthan, Jaipur. A voucher specimen (viz. no.RUBL 21099) of the plant material was preserved in the Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India and one set

Corresponding Author: Nidhi Tyagi, School of Pharmacy, ITM University, Gwalior, India

was preserved in our laboratory for future reference.

Chemicals and Reagents: Folin-Ciocalteu's phenol reagent, Gallic acid, Rutin, $AlCl_3$, Ethanol, Conc Sulphuric acid, Hydrochloric acid, α-naphthol, Picric acid, Potassium iodide, Ethyl acetate, Sodium Nitroprusside, Glacial acetic acid, Ninhydrin, Sodium nitrate, Sodium hydroxide, Chloroform, Sodium chloride, Sodium lauryl sulphate, Pyridine, Ferric chloride, Calcium chloride, Copper sulphate, Sodium carbonate, Ammonia and Sodium nitroprusside were procured from the standard companies.

Preparation of extracts

Preparation of CNAE (*Cocos nucifera* aqueous extract): The endocarp of Cocos nucifera fruit were air dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve and extracted in a soxhlet extractor with water for 72 hrs. The extract was decanted, filtered with Whatman No. 1. Filter paper and concentrated at reduced pressure below 40ºC through rota vapor to obtain dry CNAE (4% w/w). This CNAE was taken up for further testing.

Preparation of CNEE (*Cocos nucifera* ethanolic extract): The endocarp of Cocos nucifera fruit were air dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40-mesh sieve and extracted in a soxhlet extractor with ethanol (95%) for 72 hrs. The extract was decanted, filtered with Whatman No. 1. Filter paper and concentrated at reduced pressure below 40ºC through rota vapor to obtain dry CNEE (2.5% w/w). This CNEE was taken up for further testing.

Phytochemical Screening: The present phytoconstituents were analysed using freshly prepared reagents. All the used glasswares were thoroughly cleaned before the experimental works. Both the extracts (aqueous and ethanolic were analysed for the presence of carbohydrates, alkaloids, glycosides, phenols, flavonoids, tannins, fats, fixed oils, etc. (**Khandelwal K.R., 2002), (Ansari S.H., 2006).**

Estimation of total phenolic content: The total phenolic content of the extract was estimated according to the method described by Singleton and Rossi (**Singleton & Rossi, 1965**). From the stock solution (1 mg/ml) of the CNAE and CNEE, suitable quantity was taken into a 25 ml volumetric flask and mixed with 10 ml of water and 1.5 ml of Folin Ciocalteu's reagent. After 5 min, 4 ml of 20% (w/v) sodium carbonate solution was added and volume was made up to 25 ml with double distilled water. The absorbance was recorded at 765 nm, after 30 min. The total phenolic content is expressed as milligrams of gallic acid equivalent (GAE) to per gram of dry CNE extract.

Estimation of total flavonoid content: The total flavonoid content was determined with aluminium chloride (AlCl3) according to the known method of Zhishen (**Zhishen et al, 1999**) using Rutin as a standard. The plant extract (0.1 ml) was added to 0.3 ml distilled water followed by 0.03 ml NaNO2 (5%) and incubated for 5 min at 25°C. Later 0.03 ml AlCl3 (10%) was added and further after 5 min, the reaction mixture was treated with 0.2 ml (1mM) NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The total flavonoid content is expressed as milligrams of rutin equivalent (RE) to per gram of dry CNE extract.

Results

Yield of extracts

S.No	*Cocos nucifera* endocarp	% yield
1.	Aqueous extract (CNAE)	4%
2.	Ethanolic extract (CNEE)	2.5%

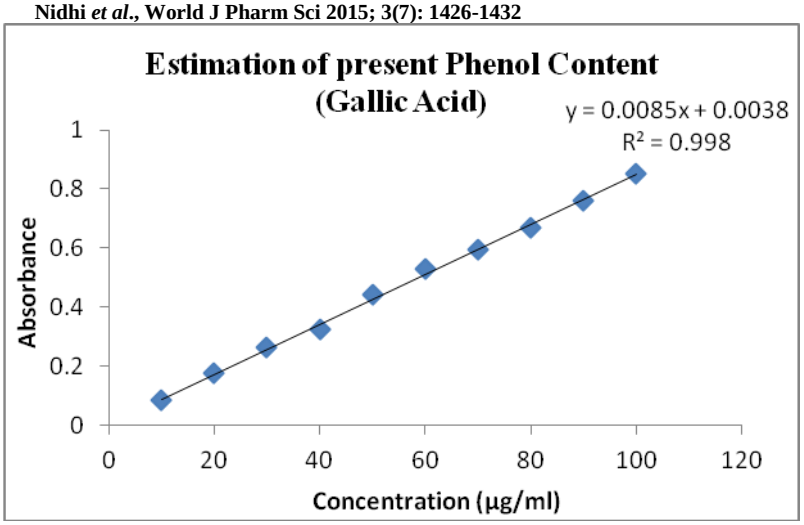
Phytochemical screening

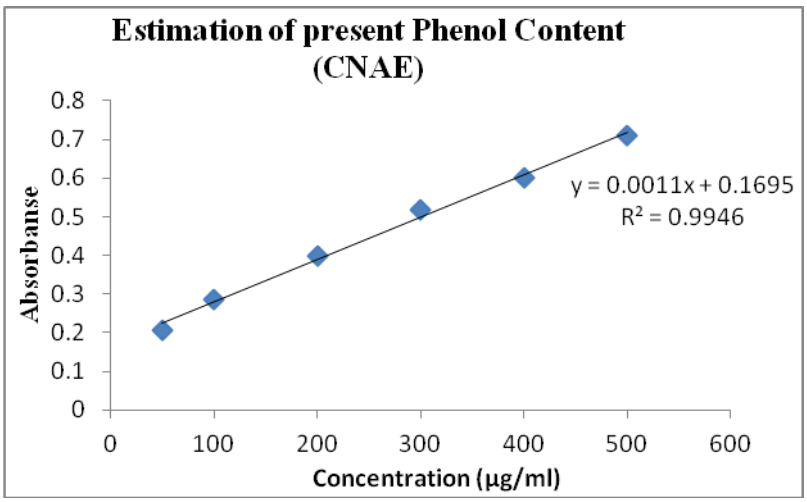
Phytochemicals		Aqueous extract	Ethanolic extract
Alkaloids	General Test	–	–
Carbohydrates (Monosaccharides, Oligosaccharides & Polysaccharides)	General Test	+	+
	Monosaccharides	+	+
	Pentose Sugars	+	+
	Hexose Sugars	+	+
	Non Reducing Polysaccharides	–	–
	Gums	–	–
	Mucilage	–	–
Proteins & Amino acids	Proteins	–	–
	Amino Acids	–	–
Glycosides	General Test		
	Cardiac Glycosides	+	+
	Anthraquinone Glycosides	–	–
	Saponin Glycosides	+	+
	Cyanogenetic Glycosides	–	–
Flavonoids		+	+
Tannis & Phenolic Compounds	General Test	+	+
Steroids		+	+
Volatile Oils		–	–
Fats & Oils		–	–

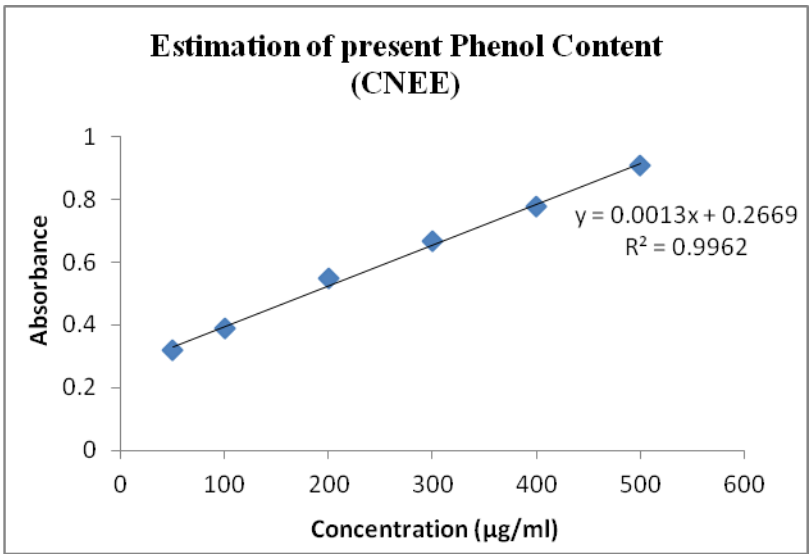
Total phenolic content

Cocos nucifera endocarp contained noticeable amount of phenolic content. CNAE contained 23.71 mg GAE/g and CNEE contained 37.57 mg GAE/g.

Group	Concentration(µg/ml)	Absorbance
STD (Gallic acid)		
	10	0.0827±0.01
	20	0.1759±0.019
	30	0.2617±0.033
	40	0.3231±0.023
	50	0.4430±.0.042
	60	0.531±0.058
	70	0.5964±0.074
	80	0.671±0.088
	90	0.7593±0.077
	100	0.8583±0.053
TEST (CNAE)		
	50	0.2057±0.032
	100	0.2866±0.038
	200	0.3978±0.033
	300	0.5192±0.008
	400	0.6013±0.051
	500	0.7112±0.046
TEST (CNEE)		
	50	0.3195±0.019
	100	0.3896±0.01
	200	0.5487±0.009
	300	0.6687±0.017
	400	0.7771±0.018
	500	0.9089±0.015

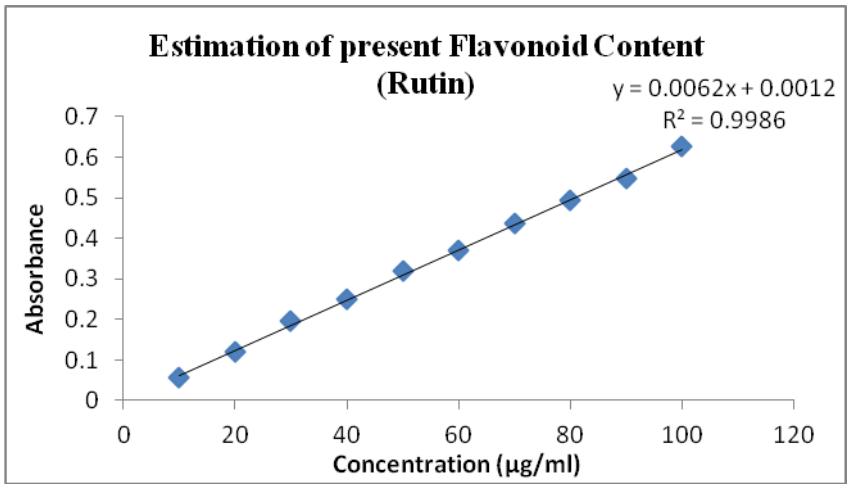

Estimation of present Phenol Content
(Gallic Acid)
y = 0.0085x + 0.0038
R² = 0.998
Absorbance
Concentration (µg/ml)

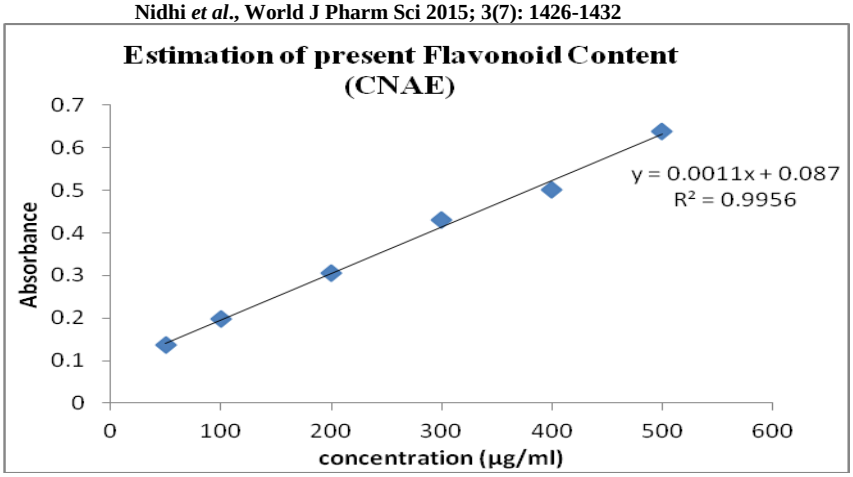

Estimation of present Phenol Content
(CNAE)
y = 0.0011x + 0.1695
R² = 0.9946
Absorbanse
Concentration (µg/ml)

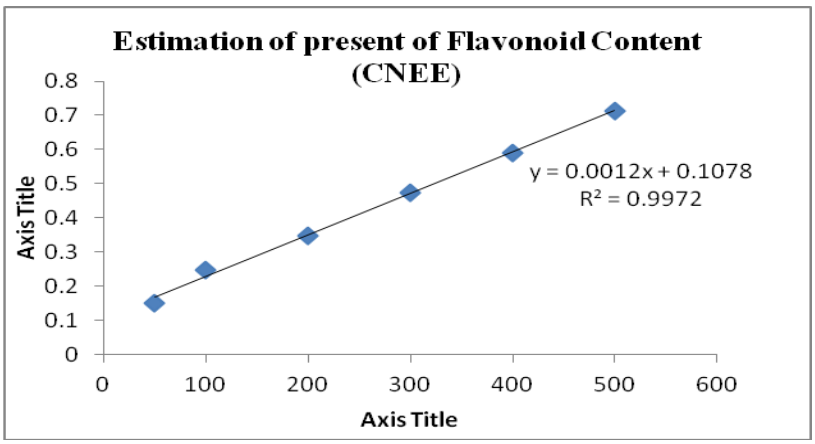

Estimation of present Phenol Content
(CNEE)
y = 0.0013x + 0.2669
R² = 0.9962
Absorbance
Concentration (µg/ml)

Total Flavonoid Content: *Cocos nucifera* endocarp contained noticeable amount of flavonoid content. CNAE contained 17.2 mg RE/g and CNEE contained 21.4 mg RE/g.

Group	Concentration(µg/ml)	Absorbance
STD (Rutin)		
	10	0.0561±0.007
	20	0.1217±0.005
	30	0.1962±0.021
	40	0.2493±0.007
	50	0.3199±.0.02
	60	0.3701±0.01
	70	0.4371±0.007
	80	0.4921±0.011
	90	0.5465±0.014
	100	0.6276±0.021
TEST		
	50	0.1362±0.019
	100	0.1983±0.017
	200	0.3046±0.011
	300	0.4312±0.006
	400	0.5019±0.02
	500	0.6375±0.018
TEST		
	50	0.1518±0.019
	100	0.247±0.011
	200	0.3489±0.016
	300	0.4745±0.012
	400	0.5899±0.012
	500	0.7112±0.019







CONCLUSION

CNAE & CNEE flavonoid and phenolic content are given in table. Cocos nucifera endocarp contained noticeable amount of flavonoid and phenolic content. The amount of flavonoids varied in both the extracts. CNAE contained 17.2 mg RE/g and CNEE contained 21.4 mg RE/g. The amount of phenol also varied in both the extracts. CNAE contained 23.71 mg GAE/g and CNEE contained 37.57 mg GAE/g. The variation can be expected may be due to the presence of different constituents. It was already being reported that flavonoids constitute the active biological principle of most medicinal plants with hypoglycaemic and anti-diabetic properties Howerver the extract should further be subjected to bioactivity-guided drug discovery to isolate the lead compound responsible for anti-diabetic activity and possible mechanism of action.

REFERENCES

1. Grimwood. Coconut palm products: Their processing in developing countries. Food & Agricultural Organizations of United Nations Rome 1975; 18.
2. Edward C, Craig RE. Cocos nucifera (coconut). Species Profiles for Pacific Island Agroforestry 2006; 2.1: 1-25.
3. Cobley LS, Steele WM. An Introduction to the Botany of Tropical Crops. Longman Group Limited 1976.
4. Orwa C et al. Agroforestree Database. a tree reference and selection guide 2009; 4.0: 1-5.
5. Sivakumar MK et al. Preliminary Phytochemical Screening and Anti-Bacterial activity of Cocos nucifera Linn root. Research Journal of Pharmaceutical *B*iological & Chemical Science 2011; 2(4): 469 -476.
6. Singla RK et al. Antioxidant and antimicrobial activities of cocos nucifera linn. (arecaceae) endocarp extracts. Indo Global Journal of Pharmaceutical Science 2011; 1(4): 354-361.
7. Khandelwal KR. Practical Pharmacognosy. Nirali Prakashan 2002; 9: 149-156.
8. Ansari SH. Essential of Pharmacognosy. Birla publication 2005-06; 588-93.
9. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic acid-phosphotungstic acid reagents. American Journal of Enology and Viticulture1965; 16:144-158.
10. Zhishen J et al. Research on antioxidant activity of flavonoids from natural materials. Food Chemistry 1999; 64: 555-559.