Available online at *www.ujpronline.com*
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL EVALUATIONS OF METHANOLIC EXTRACTS OF *COCOS NUCIFERA* (L.) LEAVES

Md. Saeedul Alam Nizami, Kazi Mobarak Hossain, Md. Shahidul Islam
Department of Pharmacy, University of science and Technology Chittagong (USTC), Chattogram, Bangladesh.

Article Info:

Article History:
Received: 5 February 2019
Reviewed: 19 March 2019
Accepted: 1 May 2019
Published: 15 May 2019

Cite this article:
Nizami MSA, Hossain KM, Islam MS. Phytochemical screening and *in-vitro* antioxidant and anti-inflammatory potential evaluations of methanolic extracts of *Cocos nucifera* (L.) leaves. Universal Journal of Pharmaceutical Research 2019; 4(2): 24-28.
https://doi.org/10.22270/ujpr.v4i2.253

***Address for Correspondence:**
Md. Shahidul Islam, Department of Pharmacy, University of science and Technology Chittagong (USTC), Chattogram, Bangladesh.
E-mail: *s_i_liton@yahoo.com*

Abstract

Objective: *Cocos nucifera* (L.) (Arecaceae) is commonly called the ''coconut tree'' and is the most naturally widespread fruit plant on Earth. Throughout history, humans have used medicinal plants therapeutically, and minerals, plants, and animals have traditionally been the main sources of drugs. The objective in the present study was to screen the phytochemical profile and pharmacological activities of methanolic extract of coconut leaves.
Methods: To investigate pharmacological activities DPPH scavenging assay and HRBC membrane stabilization methods were performed for antioxidant and anti-inflammatory potential respectively.
Results: The pharmacological studies revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical. The IC_{50} values by DPPH scavenging assay observed for standard and leaves were 97.29 µg/ml and 486.78 µg/ml respectively. Thus, this plant extracts have significant antioxidant effect. It also had moderate anti-inflammatory activity. The IC_{50} values for anti-inflammatory activity by standard and coconut leaves were 21.46 µg/ml and 831.21 µg/ml respectively. These findings suggest that *Cocos nucifera* (L.) may be a possible source for the development of a newanti-inflammatory drug.
Conclusion: The phytochemical analysis of methanolic extract of coconut leaves showed that they contained significant presence of flavonoids, phenols, saponins, terpenoids and triterpenes. Alkaloids, glycosides and tannins are also moderately present. Quantitative evaluations show significant presence of phenols which was more than tannin content.
Keywords: Antioxidant, anti-inflammatory, *Cocos nucifera*, IC50 values, phenols, tannin content.

INTRODUCTION

Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants[1]. Medicines derived from plants are widely famous due to their safety, easy availability and low cost[2]. Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours, fragrances, and medicines[3]. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2 600 BC[4]. Among the substances that were used are oils of *Cedrus* species (cedar) and *Cupressus sempervirens* (cypress), *Glycyrrhiza glabra* (licorice), *Commiphora* species (myrrh) and *Papaver somniferum* (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. In ancient Egypt, bishop's weed (*Ammimajus*) was reported to be used to treat vitiligo, a skin condition characterized by a loss of pigmentation[5,6]. More recently, a drug (methoxypsoralen) has been produced from this plant to treat psoriasis and other skin disorders, as well as T-cell lymphoma[6]. The interest in nature as a source of potential chemotherapeutic agents continues. Natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today.

Table 1: Total phenolic content (TPC) of *C. nucifera* leaves by using Folin and Ciocalteu reagent.

Absorbance	TPC (mg of GAE/g)	Average	TPC (mg of GAE/g)±SEM
0.202	26.306		
0.209	27.435	26.951	26.951 ± 0.33
0.207	27.113		

Higher plants contribute no less than 25% of the total[7]. In the last 40 years, many potent drugs have been derived from flowering plants; including for example *Dioscorea* species (diosgenin), from which all anovulatory contraceptive agents have been derived; reserpine and other antihypertensive and tranquilizing alkaloids from *Rauwolfia* species; pilocarpine to treat glaucoma and 'dry mouth', derived from a group of

Table 2: Total tannin content (TTC) of *C. nucifera* leaves by using Folin Ciocalteu reagent

Absorbance	TTC (mg of TAE/g)	Average	TTC (mg of TAE/g)±SEM
0.364	1.585	1.577	1.577 ± 0.010
0.358	1.557		
0.365	1.590		

South American trees (*Pilocarpus* spp.) in the Citrus family; two powerful anti-cancer agents from the Rosy Periwinkle (*Catharanthus roseus*); laxative agents from *Cassia* sp. and a cardiotonic agent to treat heart failure from *Digitalis* species[8].

Table 3: Test of different metabolites.

Secondary metabolites	Name of the test	Results
Alkaloids	Wagner test	++
Flavonoids	Specific test	+++
Glycosides	General test	++
Phenols	Litmus test	+++
Saponins	Froth test	+++
Tannins	Ferric chloride test	++
Terpenoids	General test	+++
Triterpenes	Salkowski's test	+++

Although discovered through serendipitous laboratory observation, three of the major sources of anti-cancer drugs on the market or completing clinical trials are derived from North American plants used medicinally by native Americans: the papaw (*Asimina* spp); the western yew tree (*Taxus brevifolia*), effective against ovarian cancer and may apple (*Podophyllum peltatum*) used to combat leukaemia, lymphoma lung and testicular cancer[9]. *C. nucifera* (L.) is originally from Southeast Asia (Malaysia, Indonesia, and the Philippines) and the islands between the Indian and Pacific Oceans. From that region, the fruit of the coconut palm is believed to have been brought to India and then to East Africa. After the discovery of the Cape of Good Hope, this plant was introduced into West Africa and, from there, dispersed to the American continent and to other tropical regions of the globe[10]. *C. nucifera* has been called the 'tree of life' or 'tree of heaven' because of its value as provider of so many useful products. This species provides food, water, oil, medicine, fibre, timber, and fuel for many people living on islands in the Pacific Ocean[11].

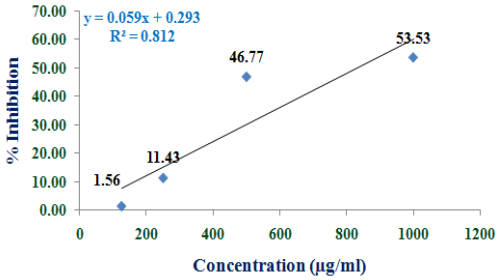

Figure 1: Graphical representation of anti-inflammatory activity of leaves of *C. nucifera*

MATERIALS AND METHODS

In this study, all the chemicals, reagents used here provided from laboratory of Department of Pharmacy, USTC which source from Merck Limited, Mumbai, India and were analytical grade, pure and sorted under optimum storage conditions. Moreover, the drug mixtures and solutions were prepared accurately in standard volumetric flasks about one hour prior to obtain and recording the data.

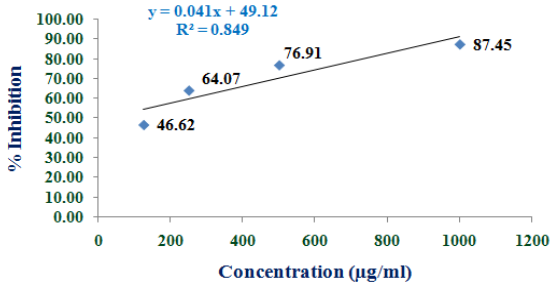

Figure 2: Graphical representation of anti-inflammatory activity of standard.

Total Phenolic Content (TPC)

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu's reagent is used in this ionized phenolic solution, the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu's reagent is yellow and after the oxidation process the solution becomes blue. The intensity of the color change is measured in a spectrophotometer at 760 nm. The absorbance value will reflect the total phenolic content of the compound[12].

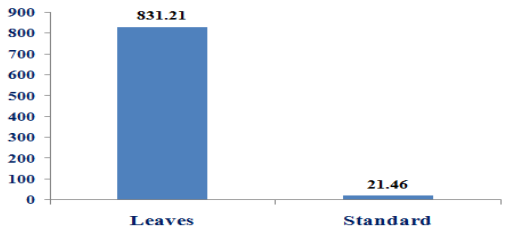

Figure 3: Comparative study based on IC_{50}.

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, following the

method used in previous study[13]. The test sample (0.2 mL) was mixed with 0.6 ml of water and 0.2 mL of Folin-Ciocalteu's phenol reagent (1:1). After 5 min, 1ml of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3 ml with distilled water. The reaction was kept in the dark for 30min and after centrifuging the absorbance of blue color from different samples was measured at 760 nm[14].

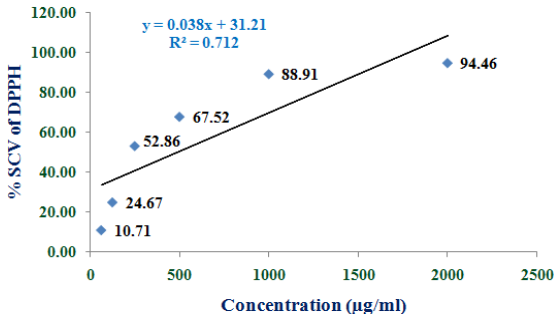

Figure 4: Antioxidant activity of leaves of *C. nucifera* by DPPH SCV assay.

Total Tannin Content (TTC) determination

Fifty micro liters (µl) of tannins extract for each sample was taken in test tube and volume was made to 1.0 ml with distilled water. Then, 0.5 ml Folin Ciocalteu reagent was added and mixed properly. Then 2.5ml 20 per cent sodium carbonate solution was added and mixed it and kept for 40 minutes at room temperature. Optical density was taken at 725 nm in spectrophotometer and concentration was estimated[15]. Tannic acid was used as standard and tannin contents were measured as tannic acid equivalent.

Anti-inflammatory activity

Percent inhibition of protein denaturation was calculated as follows[16]:

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} X100$$

The method of HRBC membrane stabilization was chosen to evaluate anti-inflammatory effect.

Anti oxidant activity

The free radical-scavenging activity of extracts was evaluated with the DPPH assay based on the measurement of the reducing ability of antioxidants toward the DPPH radical[17,18].

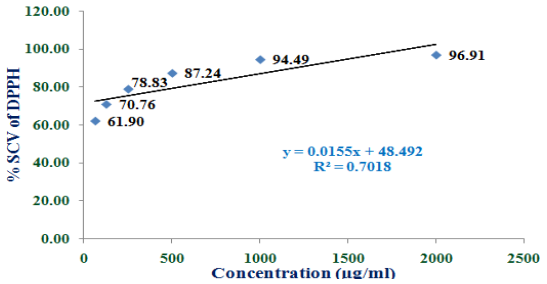

Figure 5: Antioxidant activity of standard by DPPH SCV assay.

RESULTS AND DISCUSSION

The following tests were done to find the presence of the active chemical constituents such as alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and triterpenes is shown in Table 3. Due to the different chemical compositions present in a *C. nucifera* (L.) are obviously responsible for its different therapeutic and pharmacological activities. In this study, the different constituents of the *C. nucifera* (L.) which are found should have some relationship with domestic medicinal applications[1]. It should be mentioned here that the presence of these kinds of chemical constituents, it is expected that the selective plant *C. nucifera* should have anti-inflammatory activity and anti oxidant activity.

Table 4: Spectroscopic determination of anti-inflammatory activity of leaves of *C. nucifera*.

Concentration (µg/ml)	Absorbance	% Inhibition	Average	% Inhibition ± SEM	IC50 (µg/ml)
125	0.443	1.34	1.56	1.56±0.5	
	0.446	0.67			
	0.437	2.67			
250	0.405	9.80	11.43	11.43±0.9	831.21
	0.391	12.92			
	0.397	11.58			
500	0.239	46.77	46.77	46.77±0.5	
	0.235	47.66			
	0.243	45.88			
1000	0.213	52.56	53.53	53.53±0.48	
	0.207	53.90			
	0.206	54.12			

The method of HRBC membrane stabilization was chosen to evaluate anti-inflammatory effect. It is already proved that membrane stabilization of RBC is as effective as healing inflammation in provoking delayed hypersensitivity[9].

From Table 4, it is observed that the degree of membrane stabilization was increased by increase in concentration. That means the drug will give required action at higher concentration. As shown in Figure 2, it is observed that in Comparative % inhibition of protein denaturation, the *Cocosnucifera* leaves under the study of methanolic extract from 1.56±0.5% to 53.53±0.48% has shown moderate inhibition of protein denaturation at any concentration compared to the standard drug Diclofenac Sodium from 79.51±0.46% to 93.47± 0.19%. The antioxidant potential of the methanolic extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl

(DPPH) free radical. So, the free radical-scavenging activity of extracts was evaluated with the DPPH assay based on the measurement of the reducing ability of antioxidants toward the DPPH radical[17,18]. As shown in Comparative % SCV of DPPH, methanolic leaves extract from 62.5 µg/ml to 2000 µg/ml exhibited % SCV ranging from 7.40±0.51 to 94.46±0.39%.

Table 5: Spectroscopic Determination of anti-inflammatory activity of standard compound (Diclofenac-Na).

Concentration (µg/ml)	Absorbance	% Inhibition	Average	% Inhibition ±SEM	IC50 (µg/ml)
125	0.243	45.88	46.62	79.51 ± 0.46	
	0.239	46.77			
	0.237	47.22			
250	0.161	64.14	64.07	85.97 ± 0.25	
	0.159	64.59			21.46
	0.164	63.47			
500	0.101	77.51	76.91	89.31 ± 0.46	
	0.107	76.17			
	0.103	77.06			
1000	0.057	87.31	87.45	93.47 ± 0.19	
	0.053	88.20			
	0.059	86.86			

Table 6: Comparative % inhibition of protein denaturation.

Concentration	Leaves	Standard
125 µg/ml	1.56	46.62
250 µg/ml	11.43	64.07
500 µg/ml	46.77	76.91
1000 µg/ml	53.53	87.45

Table 7: Spectroscopic determination of antioxidant activity of leaves of *C. nucifera.*

Concentration (µg/ml)	Absorbance	% SCV	Average	% SCV±SEM	IC50 (µg/ml)
62.5	0.803	10.38	10.71	7.40±0.51	
	0.806	10.04			
	0.791	11.72			
125	0.679	24.22	24.67	20.20±0.26	
	0.675	24.67			
	0.671	25.11			
250	0.425	52.57	52.86	52.86±0.54	486.78
	0.429	52.12			
	0.413	53.91			
500	0.291	67.52	67.52	67.52±0.26	
	0.287	67.97			
	0.295	67.08			
1000	0.107	88.06	88.91	88.91±0.46	
	0.093	89.62			
	0.098	89.06			
2000	0.049	94.53	94.46	94.46±0.39	
	0.056	93.75			
	0.044	95.09			

Table 8: Spectroscopic determination of antioxidant activity of standard compound (L-Ascorbic Acid).

Concentration (µg/ml)	Absorbance	% SCV	Average	% SCV±SEM	IC50 (µg/ml)
62.5	0.343	61.72	61.90	61.90±0.30	
	0.345	61.50			
	0.336	62.50			
125	0.257	71.32	70.76	70.76±0.36	
	0.268	70.09			
	0.261	70.87			
250	0.195	78.24	78.83	78.83±0.49	97.29
	0.181	79.80			
	0.193	78.46			
500	0.119	86.72	87.24	87.24±0.27	
	0.113	87.39			
	0.111	87.61			

1000	0.047	94.75	94.49	94.49±0.16
	0.052	94.20		
	0.049	94.53		
2000	0.021	97.66	96.91	96.91±0.54
	0.025	97.21		
	0.037	95.87		

CONCLUSIONS

From this research work it was found that qualitative evaluations show significant presence of flavonoids, phenols, saponins, terpenoids and triterpenes. Because each part of *C. nucifera* has different constituents, the pharmacological effects of the plant vary according to the part of the plant evaluated. Alkaloids, glycosides and tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content. There is also moderate anti-inflammatory activity in the methanolic extract of coconut leaves.

ACKNOWLEDGEMENTS

The authors extend their thanks and appreciation to the University of Science and Technology Chittagong (USTC), Chattogram, Bangladesh to provide necessary facilities for this work.

AUTHOR'S CONTRIBUTION

Nizami MSA: writing original draft, conceptualization. **Hossain KM:** Writing, review, supervision. **Islam MS:** writing, review, and editing. Final version of manuscript is approved by all authors.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

REFERENCES

1. Sofowora A. Medicinal plants and traditional in Africa. John willy, New York. 1982; 289-290. *https://doi.org/10.1155/2013/617459*
2. Iwu MM, Duncan AR, Okunji CO. New antimicrobials of plant origin. In: Janick J. ed. Prospective on new crops and new uses. ASHS press, Alexandria, V.A. 1999; 457-462.
3. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem 2005; 77(1):7-24. *https://doi.org/10.1351/pac200577010007*
4. Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of pharmacognosy and phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK. 2004.
5. Rao USM. Phytochemical screening and *in vitro* antioxidant and anti-diabetic potentials of *Persea americana* mill. (lauraceae) fruit extract**.** Universal J Pharm Res. 2018; 3(5): 38-45.*https://doi.org/10.22270/ujpr.v3i5.200*
6. Beissert S, Schwarz T. Role of immune modulation in diseases responsive to phototherapy. Methods 2002; 28 (1):138-144. *https://doi.org/10.1016/S1046-2023(02)00217-7*
7. Farnsworth N, Akerele AO, Bingel AS, Soejarto DD, Guo Z, Bull. WHO 1985; 63:965-981.
8. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat Prod Rep 2000; 17 (3):215-234. *https://doi.org/10.1039/A902202C*
9. Gurib-Fakim A. Medicinal plants: Tradition of yesterday and drugs of tomorrow. Review article. Mol Aspects Med. 2006; 27 (1):1-93. *https://doi.org/10.1016/j.mam.2005.07.008*
10. Kowa TK, Nyunaï N, Tchamgoue AD, Nkoulou Tabi TG. Antihyperglycemic and anti-oxidant potential of ethanol extract of *vitex thyrsiflora* leaves on diabetic rats. Universal J Pharm Res 2018; 3(3): 19-25. *https://doi.org/10.22270/ujpr.v3i3.161*
11. Chan E, Elevitch CR. *Cocos nucifera* (coconut), ver. 2.1. Species profiles for Pacific island agroforestry [ed. by Elevitch CR] Honolulu, Hawaii, USA: Permanent Agriculture Resources (PAR), 2006.
12. Harbertson JF, Spayed S. Measuring phenolics in the winery. Am J Enol Vitic 2006; 54:280-288.
13. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. The American J Enol Viticult 1965; 16:144–158.
14. Maurya S, Singh D. Quantitative analysis of total phenolic content in *Adhatoda vasicanees* extracts. Int J Pharm Tech Res 2010; 2(4):2403-2406.
15. Chioma DE. Anti-inflammatory and anti-oxidant activities of methanol extract of *baphia nitida*. Univ J Pharm Res 2016; 1(2): 42-47. *https://doi.org/10.22270/ujpr.v1i2.R5*
16. Leelaprakash G, Dass SM. *In-vitro* anti-inflammatory activity of methanolic extract of *enicostem maaxillare*. Int J Drug Dev Res 2011; 3(3): 189-196. *https://doi.org/10.3390/biomedicines6040107*
17. Padayatty SJ, Levine M: New insights into the physiology and pharmacology of vitamin C. Canad Med Asoc J 2001; 164(3):353-355.
18. Brand-Williams W, Cuvelier ME, Berset. Use of a free radical method to evaluate antioxidant activity. Leb Wissenchaft Techn 1995; 28:25–30. *https://doi.org/10.1016/S0023-6438(95)80008-5*