WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 9, 1160-1171. Research Article ISSN 2277– 7105

SCREENING OF PHYTOCHEMICAL AND ANTIOXIDANT PROFILE OF *COCOS NUCIFERA* FLOWERS IN METHANOL EXTRACT

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Article Received on
31 May 2021,

Revised on 20 June 2021,
Accepted on 11 July 2021

DOI: 10.20959/wjpps20219-21140

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ABSTRACT

Cocos nucifera is mainly used for its nutritive and medicinal values in human life. In drug discovery, origin of plants are developed and it is suitable to reflect natural plants for the management of certain diseases. The aim of this natural medicine is to influence the body system which depends on the chemical composition that it contains. The present study strengthens the pharmacologic knowledge of the photochemical composition of *Cocos nucifera* flowers and antioxidant activity. Phytochemical have the complementary mechanism of exploit in body system such as effect of antioxidant, detoxification of enzyme, resistant system stimulation and variation of endocrine gland. The studies on phytochemical reveals about the confirmation of carbohydrates, glycosides, flavonoids, phenols, tannins, saponins, proteins, amino acid as well as antioxidant potential of *Cocos nucifera* in methanol extract.

KEYWORDS: *Cocos nucifera*, DPPH, FRAP, antioxidant, phytochemicals.

INTRODUCTION

The Coconut tree is of the family Arecaceae and of the *genus Cocos*. Coconut needs high humidity of about 80% intended for its optimal development. Palm of Coconut needs warmer conditions for the effective development more over it is inclined to stony weather condition. Coconut trees are grown-up in about 90 countries with a yield of sixty one million tons annually.

Since propagation of nuts would take a decades, an alterative way could be the micro propagation. A reproducible explants of plumule is based on the embryonic callus multiplication and secondary somatic embrogenesis, where thousands of somatic embryos

can be produced per explants.[1] Majority of natural product antifoulants identified are steroids, terpenoids, carotenoids, phenolics, alkaloids and peptides.[2] It has been isolated from the organisms such as microbes, seaweeds, corals and sponges.[3]

In Indonesia the oil is used for the treatment of wounds.[4-6] The use of coconut plant can act as an alternative treatment was encouraged by the World Health Organization. Fiber of *C.nucifera* is treated for diarrhea in Brazil country.[7] Leaves and roots of young plants are chewed for the treatment of diarrhea and stomach aches.[8,9] For the treatment of renal disorders coconut water is consumed and coconut oil is used for the prevention of hair loss.[10]

The coconut thrives on an alkaline sandy soil that has a fluctuating fresh water table 0.5-1.0m below the surface, which is the typical situation on the strand environment and is highly calcareous and therefore alkaline; micronutrient deficiency may limit growth particularly where rainfall is marginal.

Flavonoid compounds predominantly distributed in vegetables and fruits.[11-13] It was reported that condensed tannins have anti-helminitic effect present in cuticle and oral cavity by binding to its protein.[14] C.nucifera are found in polyphenols, catechins, tannins and flavonoids.[15,17] Coconut water has been used for natural hydration.[18]

The arrangement of 1019 bacterial acyl –ACP TEs plant showed that previously suggested Cys residue is not universally preserved and therefore may not be a catalytic residue.[19] Systematic mutagenesis of this residue to either serine or alanine in three plant acyl-ACP TEs. CvFatB1 and CvFatB2 from cupheaviscosissima and CnFatB2 from Cocos nucifera, resulted in enzymatically active variants, demonstrating that this cys residue (Cys348 in CvFatB2) is not catalytic.

MATERIALS AND METHODS

DPPH, Sodium Nitroprusside, Sulphanalamide, Naphthylethylene diamine dihydrochloride, ferrous ammonium sulphate and other chemicals used were of analytical grade.

COLLECTION OF PLANT MATERIAL

Mature flowers of *Cocos nucifera* were collected mainly in sold town (OT) and around Cuddalore district, Tamil Nadu, India.

Preparation of *Cocos Nucifera* Extracts

Cocos nucifera flowers was to thoroughly cleaned using tap water and shade dried for 2 weeks. Course the powder of flower obtained by crushing the flower in an electronic blender. Twenty gram of powder were taken separately and extracted with 200ml of methanol and aqueous solvent separately in Soxhlet apparatus at 37°C for 24 hours. The extracts were then evaporated under room temperature for 2 days. The obtained dried extracts were stored in an air tight container at 4°C for future studies.

Preliminary Phytochemical Screening

Methanolic extract of *Cocos nucifera* flowers was exposed to screening of phytochemicals.

TEST FOR ALKALOIDS

Dragendroff's test: To 0.5ml of methanolic extract of *Cocos nucifera* flowers, 2ml of HCL and 1ml of Dragendorff's reagent was added. An appearance of orange or red colour precipitate was formed which indicates the presence of alkaloids.

Wagner's Reagent

To 10ml of the extract add 1.5% V/V of HCL, to this add few drops of Wagner's Reagent which shows the presence of yellow or brown colour precipitate confirms the presence of alkaloids.

Mayer's Reagent: To few ml of the extract 0.2ml of dilute hydrochloric acid and 0.1ml of Mayer's reagent were added.

Test For Flavonoids

NaOH Test: To 2-3ml of the extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour which became colourless in addition of few drops of dilute Hcl which indicates the presence of flavonoids.

Test For Carbohydrates

Dissolve few amount of extract in 4ml distilled water which was subjected to filteration. The filterates were used for the analysis of carbohydrates.

Molish's test

The filterate was treated with 2-3drops of 1% alcoholic α-napthol solution and 2.0 ml of conc H_2SO_4 was added along the sides of the test tube. A brown ring was formed at the junction of two liquid layers which indicates the presence of carbohydrates.

Benedict's test: The filterate was treated with Benedict's reagent and gently heated. Presence of reducing sugar was confirmed by Orange red colour precipitate.

Fehling's test: The filterate formed from the extract was hydrolysed with dilute HCl and neutralized with alkali and heated with Fehling's A& B solutions. Presence of reducing sugar was confirmed by the formation of red colour precipitate.

TEST FOR GLYCOSIDES

The extract were hydrolysed with hydrochloric acid for few hours in a water bath and treated with Legal's or Borntrager's test to detect the presence of glycosides.

Legal's test: The hydrolysate was treated with 1.0 ml of pyridine followed by sodium nitroprusside solution and then it was made alkaline with sodium hydroxide solution. Presence of glycosides was confirmed by the appearance of pink to red colour.

Borntrager's test: The hydrolysate was treated with chloroform and the chloroform layer was separated. To this added equal quantity of dilute ammonia solution. Presence of glycosides was confirmed by the pink colouration in the ammonia layer.

TEST FOR SAPONINS

Foam test: The extract was diluted with 20ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. Saponins was confirmed by the presence of foam formation.

TEST FOR TANNINS

$FeCl_3$ test: Few drops of 5% aqueous ferric solution was added to 2ml of extract. Presence of tannins was confirmed by the formation of violet colour.

Lead acetate test

To 5ml of the extract, few drops of 1% lead acetate was added. Formation of yellow colour precipitate indicates the presence of tannins.

TEST FOR PROTEIN AND AMINO ACIDS

Ninhydrin test

To few ml of the extract few drops of Ninhydrin reagent was added and purple colour confirms the presence of amino acid.

Biuret Test: To 1ml of the extract add 5% NaOH solution and 1% copper sulphate which shows the presence of protein and it was confirmed by the appearance of pink colour.

TEST FOR PENOLS

The extract was treated with 1% alcoholic ferric chloride solution or aqueous solution. Phenol is confirmed by the presence of green, purple, blue or black colour.

TEST FOR PHYTOSTEROLS

Salkowski's test: The extract were treated with chloroform and filtered. The filterates were treated with few drops of conc sulphuric acid and shaken well, which is then allowed to stand for few minutes. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burcard's Test: The extract were treated with chloroform and filtered to this few drops of acetic anhydride was added, which is then boiled and cooled. To this Concentrated sulphuric acid was added, a brown ring is formed at the junction indicates the presence of phytosterols.

ESTIMATION OF PROTEIN

Protein estimation was processed according to Lowry's Method.[20]

Antioxidant Scavenging Activity of Methanolic Extract of *Cocos Nucifera Flowers*

DPPH radical scavenging activity

The radical scavenging of methanolic extract of *Cocos nucifera* were determined by the method Mensor.[21] Methanolic extract of *Cocos nucifera* at different concentrations (100-500µg/ml) was diluted with ethanol. 1.0ml of 0.3mM DPPH in ethanol solution was added to the sample at different concentration and allowed to react at room temperature. Ethanol and DPPH were served as blank and ascorbic acid was used as standard.

% inhibition = {Acontrol-Atest/AcontrolX100).

Where,

A(control)=Absorbance of control

A(test)=Absorbance of test sample

Nitric Oxide radical scavenging activity

Nitric oxide scavenging activity was spectrophotometrically measured by the method Govindarajan.[22] To different concentrations of the extract (100-500µg/ml), sodium nitroprusside (5mM) in phosphate buffered saline was mixed and incubated at 25ºC for 30 minutes. Methanol was taken equally and served as a control. After 30 minutes the solution was diluted with 1.5ml of Griess reagent. The absorbance of the chromophore was found during diazotization of nitrate with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was read at 546nm.

$$\% \text{ Inhibition} = (A_{control} - A_{test} / A_{control} \times 100)$$

Hydroxyl radical activity was measured according to the method Halliwell.[23] The reaction mixture consists of 0.8ml of phosphate buffer solution(50mmol/L, pH 7.4, 0.2ml of the sample at (100-500µg/ml) concentrations, 0.2ml of EDTA, 0.2ml of $FeCl_3$ and 0.2ml of 2-deoxyribose. The reaction mixture was kept in a boiling water bath for 37ºC and added 0.2ml of ascorbic acid, 0.2ml of H_2SO_2 respectively. After incubation at 37ºC for 1 hour, 2ml of cold tiobarbituric acid was added to the reaction mixture followed by 2.0ml of Hcl. The mixture was then heated at 100ºC for 15 minutes and cooled. Ascorbic acid was served as a standard solution. The absorbance was read at 532nm spectrophotometrically. The percentage was calculated by

$$\% \text{ inhibition} = \{A_{control} - A_{test} / A_{control} \times 100)$$

Reducing power scavenging activity

The reducing power of *Cocos nucifera* were measured according to Oyaizu method [24]. Various concentration of *Cocos nucifera* (100-500µg/ml) in 1ml of distilled water and mixed with 2.5ml of phosphate buffer (0.2M, pH7.6) and 2.5ml potassium ferricyanide [K_3 Fe (CN) 6] (1%, W/V),and the mixture was incubated at 50ºC for 30 minutes. 2.5ml of TCA (10% W/V) was added to the mixture and centrifuged at 3000 rpm for 10 minutes. 2.5ml of distilled water and 0.5ml $FeCl_3$ (0.1%W/V) was added to it and the absorbance was read at 700nm. Ascorbic acid serves as standard. Higher absorbance of the mixture indicates the greater reducing power.

RESULTS AND DISCUSSION

Table 1: Phytochemical Ascreening of Cocos Nucifera Flowers.

S.No	Phytochemical test	Aqueous extract	Methanol extract
1	Alkaloids	-	-
2	Carbohydrates		
	1.Molisch's test	+	+
	2.Benedicts test	+	+
	3. Fehling's test	+	+
3	Flavonoids	-	+
4	Glycosides	-	+
5	Saponins	-	+
6	Tannins	+	+
7	Protein and Amino Acids	+	+
8	Phenols	+	+
9	Phytosterols	-	-

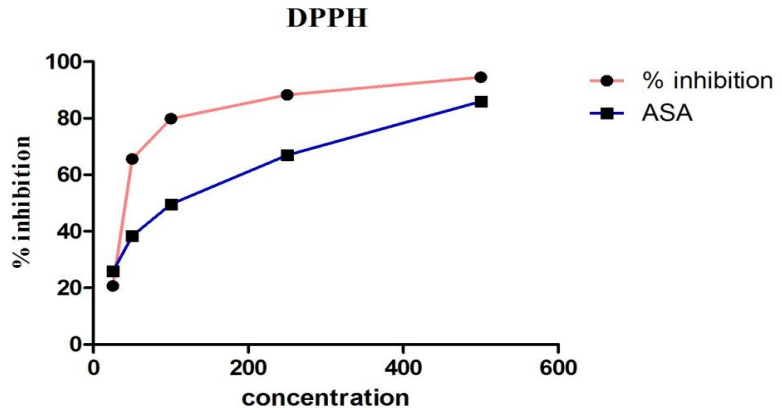

Figure 1: DPPH radical scavenging activity of methanolic extracts of *Cocos nucifera* flowers.

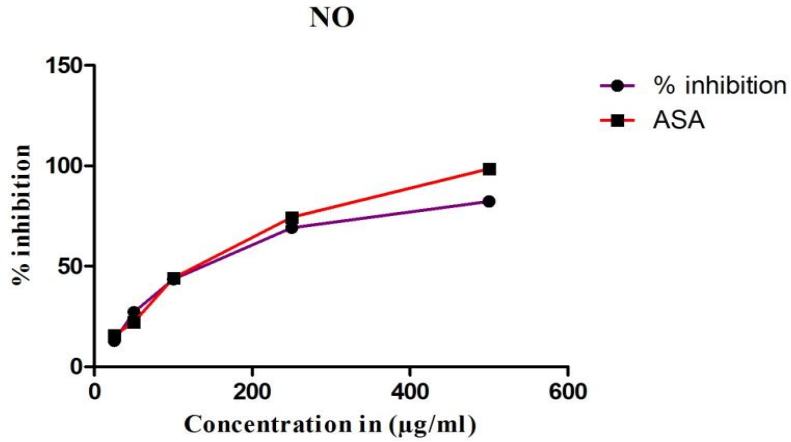

Figure 2: Nitric Oxide scavenging activity of methanolic extract of *Cocos nucifera* flowers.

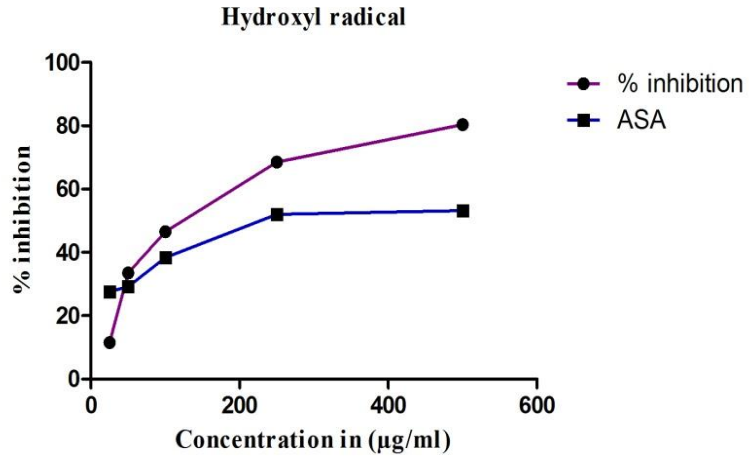

Figure 3: Hydroxyl radical scavenges of methanolic extract of *Cocos nucifera* flowers.

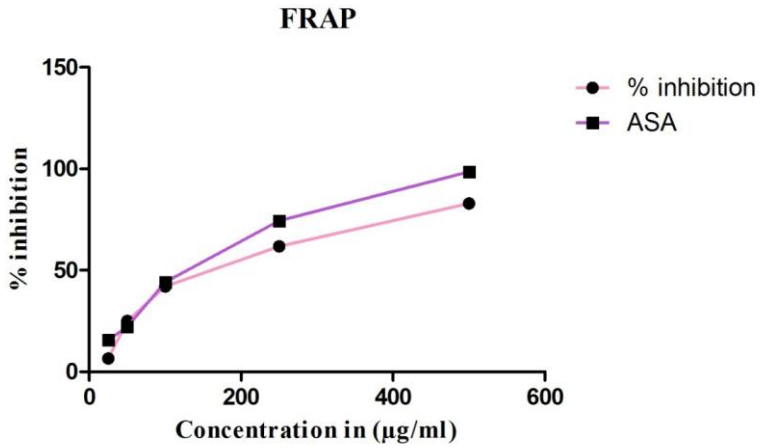

Figure 4: Reducing power of methanolic extract of *Cocos nucifera* flowers.

DISCUSSION

The present study strengthens the pharmacologic knowledge of the phytochemical composition in methanolic extract of *Cocos nucifera* flowers. As new drugs of plant origin are developed, it is appropriate to consider natural plants as a treatment for several diseases. The ability of the natural medicine to influence the body systems depends on the chemical composition and antioxidant scavenging properties against free radicals.

Table 1: reveals the qualitative phytochemical screening of methanolic flower extracts of *C.nucifera* demonstrated the presence of flavonoids, glycosides, tannins, carbohydrates, amino acids and protein etc.

Fig 1 depicts the DPPH radical scavenging activity of *C.nucifera* flower. The inhibitory concentration at 50% of DPPH radical scavenging assay were 37 µg/ml, where as ascorbic acid was found to be 160µg/ml.

Fig 2 illustrates the radical scavenging activity of nitric oxide and it is found to have 143µg/ml whereas ascorbic acid was 1.0µg/ml. This reaction is due to the antioxidant principle of the compound which in turn compete with oxygen to react with nitric oxide.

Herbal extracts possess different phytochemicals with biological activity of reasonable therapeutic index.[25] Alkaloids possess physiological effects when administered to animals and it was further used as drugs.[26,27] They also produce bacterial effects, analgesic, antisapasmodic.[28]

Fig 3 shows the IC_{50} values of hydroxyl radical scavenging activity which was 125µg/ml respectively, whereas ascorbic acid was found to be 160µg/ml. This reaction happens due to the production of cells of superoxide radicals and hydrogen peroxide which then favours the formation of other reactive oxygen and nitrogen species.

Fig 4 shows the IC_{50} value of FRAP assay and found to be 137µg/ml. Methanolic extract showed the good antioxidant properties as the concentration increases.From this result it is evident that *Cocos nucifera* flowers have the potential to develop new drug which might synergistically act as a good therapeutic agent. Further studies have to be carried out to justify the same.

CONCLUSION

Cocos nucifera flowers are considered to be the natural therapy for several diseases and its phytochemical screening were investigated for the phytonutrients present in the *Cocos nucifera* flowers. The phytochemical and antioxidant studies suggest that methanolic extract of *Cocos nucifera* flowers is found to be selective in its action for free radicals where it behaves dependently on dosage. *Cocos nucifera* flowers should also be investigated for various other diseases which include chemical, radiation and viral carcinogenesis models. Thus *Cocos nucifera* flowers were found to be a good source of phytochemicals and radical scavenging activities.

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