QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *COCOS NUCIFERA* L. INFLORESCENCE

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ABSTRACT

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water. Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body. Plants are the rich source of all the elements essential for human beings. The phytochemical screening of *Cocos nucifera* L. was studied. The aim of the study was to determine the phytochemical constituents of the inflorescence of *Cocos nucifera* L. such as petroleum ether, chloroform and methanol extracts. The qualitative analyses carried out on the inflorescence showed the presence of steroids, terpenoids, phenolics, tannins, saponins, carbohydrates, aminoacids and proteins. The quantitative analysis was carried out using total phenolic and flavanoid content.The methanolic extract showed the high amount total phenolic and flavanoid content. The results obtained showed that *Cocos nucifera* inflorescence extracts have intresting pharmacological active compounds and used in ethnomedicine for treatment of some infections and ailments.

Keywords: phytochemical, *Cocos nucifera,* inflorescence, phenolic, flavanoid.

INTRODUCTION

Phytochemicals are plant or fruit derived chemical compounds that can be used as therapeutic agents. They reduce the risk of cancer due to dietary fibres, polyphenol antioxidants and anti-inflammatory effects[1]. The phytochemicals are produced via secondary metabolism in relatively small amounts[2]. In recent times quite a number of some plants i.e. palms, leaves, stems and roots of some plants have been used due to the presence of phytonutrients in them. Scientifically, research is being undertaken to bring to limelight, the therapeutic properties of the phytochemicals present in these plants and also use them as a yardstick in modern medicinal plant uses[3] .Some groups of phytochemicals, which appear to have significant health potentials, are carotenoids, flavonoids, phytoestrogens, non-digestible carbohydrate i.e. dietary fibre and prebiotics[4] .

Phytochemicals act in numerous ways to assist the human body in combating disease and health problems. They combine with numerous vitamins to boost antioxidants activity of scavenging free radicals before they can cause damage within the body. These phytochemicals boost enzyme activity and increase the benefits of the various protective enzymes consumed with in the diet[2]. The consumption of phytochemicals enhances reduction in the emergence of degenerating diseases following a typical western diet[4]. The aim of this study is to determine the phytochemical constituents of the inflorescence of *Cocos nucifera* L. and possibly relate the constituents to their medicinal and pharmacological uses.

MATERIAL AND METHODS.

Preparation of plant extracts

The fresh inflorescence of *Cocos nucifera* L. was collected thoroughly washed in tap water, shade dried, powdered and was stored in air tight container. The powdered plant material (100 g) was then extracted successively with Petroleum ether, Chloroform and Methanol using a Soxhelet apparatus for about 24hrs each. The extracts were filtered and concentrated under reduced pressure in a rotary vacuum evaporator. The dried extracts were then stored at 0-4°C until further use.

Qualitative analysis of phytoconstituents

The phytochemical analysis of the Petroleum ether, Chloroform and Methanol extracts were carried out with standard protocols[5] [6].

Detection of steroids/ terpenoids

Liebermann Burchard test

Few milligrams of the extracts were dissolved in chloroform and a few drops of acetic anhydride were added, followed by few drops of concentrated H_2SO_4 along the side of the test tube. Blue-green colour indicates the presence of steroids and pink/purple colour indicates terpenoids.

Salkowski's test

Few milligrams of the extracts were dissolved in chloroform and then treated with few drops of concentrated H_2SO_4, shaken and allowed to stand. The development of red colour in the chloroform layer indicates the presence of sterols/steroids.

Detection of phenolics and tannins

Ferric chloride test (general test)

The extract was treated with few drops of neutral ferric chloride solution. The formation of blackish red color indicates the presence of phenolic nucleus.

Gelatin test (for tannins)

To the aqueous solution of the extracts, 2 ml of 2% gelatin solution and 10 % sodium chloride were added. The formation of curdy white precipitate indicates the presence of tannins.

Shinoda test (for flavonoids)

Extracts were dissolved in methanol and a few magnesium metal turnings were added, followed by drop-wise addition of concentrated HCl. The formation of pink color indicates the presence of flavonoids.

Lead acetate test (for flavonoids)

The extracts were treated with a few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Zinc-HCl reduction test (for flavonoids)

To the alcoholic solution of the extracts, a pinch of zinc dust and a few drops of concentrated HCl were added. Formation of deep red colour indicates the presence of flavonoids.

Test for coumarins

Extracts were dissolved in methanol and alcoholic NaOH was added. A yellow colour appeared, which disappeared by adding concentrated HCl drop by drop indicated the presence of coumarins.

Detection of alkaloids

Extracts were dissolved individually in dilute HCl and filtered. The filtrates were used to test the presence of alkaloids.

Mayer's test

Filtrates were treated with Mayer's reagent (potassium mercuric iodide), formation of gelatinous white or cream precipitate indicated the presence of alkaloids.

Wagner's test

Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of brown/reddish brown precipitate indicated the presence of alkaloids.

Dragendroff's test

Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of orange red precipitate indicated the presence of alkaloids.

Detection of saponins

Froth's test

The extracts were diluted with distilled water to 20 ml, shaken in a graduated cylinder for 15 min. Froth formation which lasts for a long time indicated the presence of saponins.

4.2.3.5.Detection of carbohydrates

Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

Molisch's test (general test)

Filtrates were treated with 2 drops of 1% alcoholic α-naphthol solution in a test tube and 2 ml concentrated H_2SO_4 was added carefully along the side of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

Benedict's test (for reducing sugars)

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

Fehling's test (for reducing sugars)

Filtrates were hydrolyzed with dilute HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Detection of proteins and amino acids

Millons test

The extracts were treated with 2 ml of Millon's reagent. The formation of white precipitate, which turns to red upon heating, indicated the presence of proteins.

Ninhydrin test

To the extracts, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated presence of amino acids.

Quantitative analysis of phytoconstituents

Estimation of total phenolic content

Total phenolic content (TPC) of *Cocos nucifera* inflorescence extracts (Petroleum ether, Chloroform and Methanol) were determined according to method described by Lachman *et al.* (2000) [7]. 0.5 ml of each extract, 2.5 ml Folin-Ciocalteu reagent and 2 ml of 7.5% (w/v) sodium carbonate (Na_2CO_3) were mixed. The mixture was incubated at room temperature for 30 min. The absorbance was read using UV-Vis spectrophotometer at 743 nm. The results were expressed as mg GAE (gallic acid equivalents)/g dry extract.

Estimation of total flavonoid content

The total flavonoid content (TFC) of *Cocos nucifera* inflorescence extracts (Petroleum ether, Chloroform and Methanol) were determined according to the aluminum chloride colorimetric method described by Chang *et al.* (2002) [8]. The plant extracts (0.5 ml) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3.6H_2O$), 0.1 ml of 1 M potassium acetate (CH_3CO_2K), and 2.8 ml of distilled water. After incubation at room temperature for 30 min the absorbance of the solution was measured at 415 nm. The results were expressed as mg QE (quercetin equivalents)/g dry extract.

RESULTS

Qualitative analysis of phytochemicals

The result of phytochemical analysis of Petroleum ether, Chloroform and Methanol are as follows (table no.1).

Table 1. : Qualitative Analysis of *Cocos nucifera* inflorescence

Tests	*Cocos nucifera* inflorescence extracts		
	Petroleum ether	Chloroform	Methanol
Detection of steroids and terpenoids			
Libermann Burchard test	+	+	+
Salkowski's test	+	+	+
Detection of phenolics and tannins			
Ferric chloride test	-	+	+
Gelatin test	-	+	+
Shinoda test (for flavonoids)	-	+	+
Lead acetate test (for flavonoids)	-	+	+
Alkaline reagent test (for flavonoids)	-	+	+
Test for coumarins	-	+	+
Detection of alkaloids			
Mayer's test	-	-	-
Wagner's test	-	-	-
Dragendroff's test	-	-	-
Detection of saponins			
Froth test	-	-	+
Detection of carbohydrates			
Molisch's test	-	-	+
Benedict's test	-	-	+
Fehling's test	-	-	+
Detection of amino acids / proteins			
Millons test	-	-	+
Ninhydrin test	-	-	-

Quantitative analysis of phytochemicals

Estimation of total phenolic content

The results of total phenolic content (TPC) of *Cocos nucifera* inflorescence extracts are summarized in table 2.

Table : 2. Estimation of total phenolic content of *Cocos nucifera* inflorescence extracts

Extracts	**Total phenolic content (mg GAE/g dry extract)**
Petroleum ether	0.2453
Chloroform	0.3745
Methanol	0.5176

GAE -Gallic Acid Equivalents.

Estimation of total flavonoid content

The results of the total flavonoid content (TFC) of *Cocos nucifera* inflorescence extracts are summarized in table 3.

Table : 3. Estimation of total flavonoid content of *Cocos nucifera* inflorescence extracts

Extracts	**Total flavonoid content (mg QE /g dry extract).**
Petroleum ether	0.1598
Chloroform	0.8779
Methanol	1.5714

QE -Quercetin Equivalents.

DISCUSSION

The present study showed that the presence of various phytochemicals in cocos nucifera inflorescence such as petroleum ether, chloroform and methanol extracts.The qualitative analyses carried out on the inflorescence showed the presence of steroids, terpenoids, phenolics, tannins, saponins, carbohydrates, aminoacids and proteins. Parker *et al*., 2010 reported that the phytochemical analyses carried out on the milled kernel showed the presence of terpenoids, alkaloids, resins, glyco-sides and steroids. Flavonoids and acidic compounds were not detected. The macronutrient analyses showed the presence of carbohydrate, proteins, reducing sugar, fats and oil. Of the above macronutrients, oil is known to be the major constituent that is necessary for the medicinal uses of coconut, though the phytochemicals: alkaloids, steroids and terpenoids are known to have antioxidantproperties.

The present study showed that the quantitative analysis was carried out using total phenolic and flavanoid content. The methanolic extract showed the high amount total phenolic(0.5176 mg GAE/g dry extract) and flavanoid content (1.5714 mg QE /g dry extract). Chithra *et al*.,2020 investigated that the quantitative analysis of *C. nucifera* inflorescence is rich in polyphenols (222.6 µg GAE/g dry extract) and flavonoids (120.8 µg QE/g dry extract).

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REFERENCES

[1] *Kinderley, D. (2006). Nutrition for life. Lark and Deen Publishers, UK, p 213.*

[2] *Hasler,C. M. (1998). Functional Foods: their role in disease prevention and health promotion. Food Technol., 52: 63-70.*

[3] *Riby, J.E., Xuel, L., Chatterji, U., Bjeldanes, E.L., Firestone, G.L. and Bjeldanes, L. F. (2006). Dept. of Nutrition Sciences and Toxicology, University of California. Berkeley Mol Pharmaco., 69(2): 430-439.*

[4] *Prior, R.I and Cao, G. (2000). Hort Sci., 35: 588-592.*

[5] *Harbone JB. Phytochemical methods. A guide to modern technique of plant Analysis. Chapinan and Hall, London, 1998; 33-185.*

[6] *Trease and Evans WC (2002). Trease and Evans Pharmacognosy, 15th edition. W.B Sauders Company Ltd, London. pp 137-139,230-240.*

[7] *Lachman J., Hamouz K., Čepl, J., Pivec V., Šulc M., Dvořák P. (2000): The effect of selected factors on polyphenol content and antioxidant activity in potato tubers. Chemické listy, 100: 522–527.*

[8] *Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Analaysis, 10: 178-182.*

[9] *Parker Elijah ,Obidoa, Onyechi; Joshua, and Eze, Nkechi J. Phytochemical Analysis of Cocos nucifera L.Journal of Pharmacy Research 2010, 3(2),280-286.*

[10] *Manikantan Ambika Chithra, Thadiyan Parambil Ijinu, Harsha Kharkwal, Rajeev Kumar Sharma, Kainoor Krishnankutty Janardhanan, Palpu Pushpangadan and Varughese George.Cocos nucifera L. inflorescence extract: An effective hepatoprotective agent .Indian Journal of Traditional Knowledge. Vol 19(1), January 2020, pp 128-136 .*