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Original Article

Phytochemical Profiling and Antioxidant Potential of Coconut Inflorescence Sap

Raseema Raheema Sharafudeen, Annie Abraham*, Reshma Usha Soman
Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, INDIA.

ABSTRACT

Background: The coconut palm is so much more than just a tree; because all of this is essential with medicinal benefits. Coconut inflorescence sap (CIS) is the sweet, oyster-white colored, non-fermented juice tapped from the immature coconut spadix. Coconut products are widely used in Indian folk medicine for their effects on hemorrhaging, bronchitis, antimicrobial, radical scavenging, analgesics, anti-inflammation, anthelmintics, and immunotherapies. Many studies reported the fantastic health benefit of the coconut palm tree. The coconut inflorescence has cytoprotective and antihyperglycemic properties [Renjith, R.S. *et al.*, 2013]. But not many systematic studies have been conducted on Coconut Inflorescence Sap. The objective of the present study is to analyze the nutritional components, detect the mineral composition, perform preliminary phytochemical screening, and find out biologically active compounds using FTIR Spectrum analysis and LCMS analysis. **Materials and Methods:** CIS's nutritional components and mineral content analysis were done following the standard procedures. The phytochemical screening, FTIR Spectrum analysis, and LCMS analysis are also done to confirm the health benefits of CIS. **Results:** CIS exhibited a significant amount of micro and macronutrients. The Phytochemical analysis also showed the presence of flavonoids, alkaloids, terpenes, steroids, phenols, and glycosides. The LCMS Spectrum analysis of CIS shows the presence of biologically significant compounds, namely 4-HydroxyCoumarin, P-Coumaric acid, Mellein, Leucopelargonidin, Coumarin, 3 Caffeoylquinic acid, Ferulic acid 4-O-Glucosidase and also three essential aminoacids L-Phenylalanine, L-isoleucine and D-Tryptophan. **Conclusion:** The nutritional composition, active phytochemical constituents, and polyphenolic compounds of CIS showed that CIS has a significant beneficial effect in upgrading the antioxidant status. CIS is a natural drink with effective antioxidant potential. Concerning the nutrient composition, mineral and amino acid composition, and scavenging assays, it would be possible to exploit this natural drink in clinical therapy.

Keywords: CIS, Micronutrients, Phytochemical Analysis, Antioxidants, FTIR, LCMS.

Correspondence

Dr. Annie Abraham
Professor, Department of Biochemistry, University of Kerala, Kariavattom-695581, Thiruvananthapuram, INDIA.

Email id: annieab2013@gmail.com

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INTRODUCTION

Coconut Inflorescence Sap is a natural non-alcoholic beverage collected from coconut palms with a rich source of nutrients and antioxidants. Coconut palm is so much more than just a tree; because all part of this incredibly medicinal plant possesses medical benefits.[1-3] Many authors reported that coconut water contains a growth factor that stimulates different bacterial strains and *in vitro* culture of plants.[4-5] Various components such as the variety of coconut, location, and time of collection have a role in determining the chemical constituents of CIS.[6] To explore the potential role of CIS as a natural protective agent against disease, a detailed phytochemical and nutritional analysis is essential. Vegetables, fruits, and medicinal herbs are more effective and less toxic than synthetic antioxidants.[7-8] Habitual consumption of nutritious foods is beneficial as preventive medicine for various diseases. Antioxidants have a vital role in protecting the body against various diseases. Freshly collected CIS is oyster colored with pH Seven and has a pleasant odor. CIS is an unfermented coconut inflorescence sap (NFCIS) from coconut flowers and is not an alcoholic beverage. Renjith *et al.* investigated the coconut inflorescence has cytoprotective and antihyperglycemic properties.[9-10] The significant difference between CIS and toddy is based on fermentation and processing. If CIS is kept at room temperature for a few hours, it becomes an alcoholic drink, toddy. CIS is a natural source of vitamins, namely Vitamin C, Vitamin A and B complex. CIS has a shallow glycemic index and is thus a safe drink even for diabetic persons.[11] A detailed study is essential to analyze CIS's chemical composition and antioxidant potential.

MATERIALS AND METHODS

Collection and Characterization of CIS

Fresh Coconut Inflorescence Sap (CIS) was obtained from the unopened inflorescence of *Cocos nucifera* in an aseptic condition. Healthy Palms with immature blossomed spadix were selected, and the palm crown was sterilized. The spadix was chopped with a sterile knife in its middle. After 15 to 20 days of beating, a coconut sap chiller is inserted in the center of the spadix two times a day, once in the morning and the other in the evening. The flow of CIS is prolonged, so highly prone to fermentation. Raw CIS is collected in a sterile bottle, filtered through microfilters, pasteurized at 75-85°C for 10-15 min with constant agitation, and stored under cold conditions for further studies. The taxonomy of the collected specimen was confirmed at the Department of Botany, University of Kerala, and a voucher specimen with accession no. KUBH 111111 has been deposited in the herbarium of the Department. The fresh CIS was obtained from Kaipuzha Coconut plantation, Mynagappally, Kollam, Kerala.

Nutritional Analysis

The nutritional analysis was analyzed based on the A.O.A.C. lnternational 21st Edition 2019 and quantified the total fat, total ash, total protein, total energy, and total carbohydrates present in 100ml of the sample.

Mineral Content Analysis

The minerals detection was done using an inductively coupled mass spectrometry system (Thermo Scientific ICAP Qc).

Qualitative Phytochemical Analysis

The naturally occurring active secondary metabolites in plants constitute phytochemicals. (The word Phyto in Greek indicates "plant"), and are responsible for color and biological properties. The phytochemical means those chemicals that may have biological significance but are not established as essential nutrients. The CIS was analyzed for the presence of Alkaloids, Phenols, Flavonoids, Terpenes, Steroids, and Glycosides.

Antioxidant Assays

DPPH Radical Scavenging Assay

DPPH radical scavenging activity was analyzed following the procedure of Blois, 1958.[12] Ascorbic acid was used as a standard and the IC_{50} of the extracts was compared with the standard. The lower IC_{50} value indicates higher free radical scavenging activity.

Superoxide Radical Scavenging Assay

O2- scavenging activity of the CIS was analyzed by Fontana *et al.,* 2001. Phosphate buffer devoid of the CIS served as control. IC_{50} of the extracts was compared with quercetin.

Hydrogen Peroxide Radical Scavenging Activity

The H_2O_2 scavenging ability of the CIS was determined by the method of Ruch *et al.*, 1989.[13] Phosphate buffer devoid of H_2O_2 was used as blank. Quercetin was used as the standard and the IC_{50} of the extract was compared with the standard.

FTIR Spectrum of CIS

Fourier transform infrared spectroscopy (FTIR) is the method used to identify the chemical bond or functional group. CIS was loaded in the FTIR spectrometer (Perkin-Elmer LS-5S-Luminescence Spectrometer). The measurement was taken at ATRA mode at room temperature. (Range from 400 cm^{-1}– 4000 cm^{-1}), and changes in the spectral pattern were analyzed and noted.

Determination of Phenolic Compounds of CIS using LCMS Analysis

LCMS analysis was performed using SHIMADZU LC MS-8045. To identify the active components, the particulate matters are to be separated by centrifugation for 20 min at 4000rpm, followed by sterilization using a filter of 0.22 µm. The filtrate thus obtained was dried by lyophilization. The final powdered material (1 mg) was dissolved in 1 ml of ultrapure water and prepared in a stock solution (1 mg ml^{-1}) to detect the active constituents.

RESULTS

Nutritional Composition of CIS

The nutritional composition of CIS is depicted in Table 1 and it showed that CIS possesses an excellent macronutrient composition with a total energy of 57.4Kcal /100ml.

Table 1: Nutritional composition of CIS.

Sl. No.	Component	Quantity/100ml
1	Total Energy	57.4KCal
2	Total Fat	0.2g
3	Total Protein	0.5g
4	Total Carbohydrates	13.4g
5	Total PH	6.5-7.5
6	Total ash	0.3g

Table 2: Mineral Content Analysis of CIS by ICPMS.

Minerals	Quantity/ 100 ml
K	100mg
Na	15.2mg
Mg	6.0 mg
Al	0.35mg
Ca	1.8mg
Fe	0.36mg
Zn	0.19mg
Ni	0.11mg
Cr	0.03mg
Ba	0.02mg
Li	0.01mg
V	0.01mg
Co	0.01mg
Cu	0.39mg
Mn	0.01mg
Be	ND
Cd	ND
In	ND

Mineral Composition of CIS

CIS showed the presence of micronutrients Iron and Zinc in significant quantity and also showed the presence of other essential minerals as shown in Table 2.

Preliminary Phytochemical Analysis

The phytochemical characteristics of *Coconut Inflorescence Sap* (CIS) were summarized in Table 3. The results showed the presence of bioactive molecules such as flavonoids, alkaloids, glycosides, phenols, and tannins were present in CIS.

Antioxidant Assays

Antioxidant potential of CIS was determined by DPPH assay, Hydroxyl radical scavenging assay and Superoxide radical scavenging assay. Figure 1 shows the DPPH radical scavenging activity of CIS and the standard. The IC_{50} of CIS was 2.92 µg/mL, and ascorbic acid (standard) was 4.35 µg/mL

Figure 2 shows that CIS's hydroxyl radical scavenging activity was dose-dependent. The IC_{50} of CIS was 35.29 µg/ ml, and GA was 14.69 µg/ ml, indicates that the scavenging activity of CIS was adequate and comparable to that of the standard GA

Figure 3 represents the abilities of the CIS and the standard quercetin to scavenge superoxide radicals from the reaction medium. As depicted

Table 3: **Preliminary phytochemical analysis of CIS.**

Phytochemical constituents	Tests	Observation
Alkaloids	Mayer's test	+
	Hager's test	+
Flavonoids	Alkaline reagent test	+
	Lead acetate test	+
Glycosides	Keller kelliyani test	+
	Baljet's test	+
Tannins	Gelatin test	-
	KOH test	-
Steroids	Salkowiski's test	+
	Acetic acid test	+
Phenols	Ferric chloride test	+
Saponins	Foam test	-
Diterpenes	Copper acetate test	+

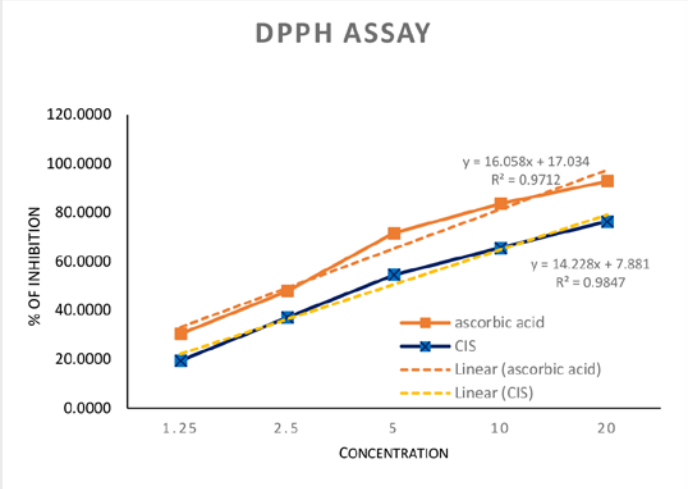


Figure 1: Comparative DPPH activity of CIS.

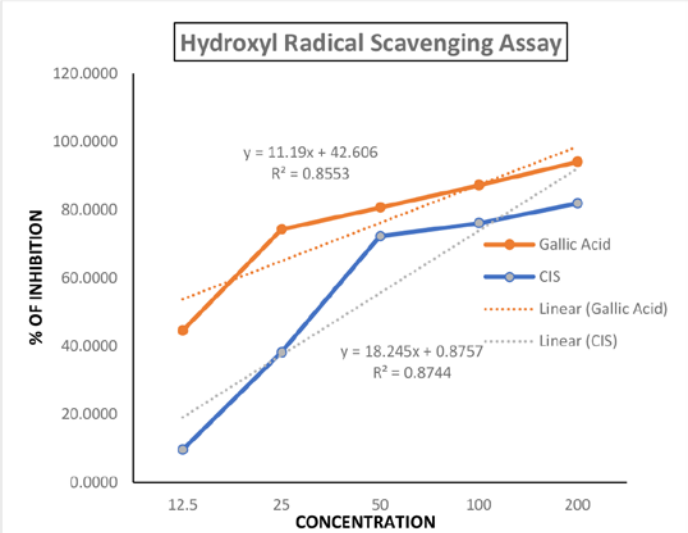


Figure 2: Hydroxyl Radical Scavenging Assay of CIS.

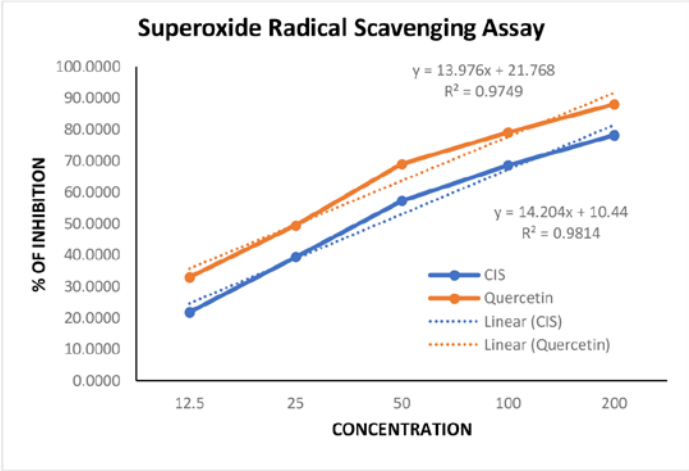
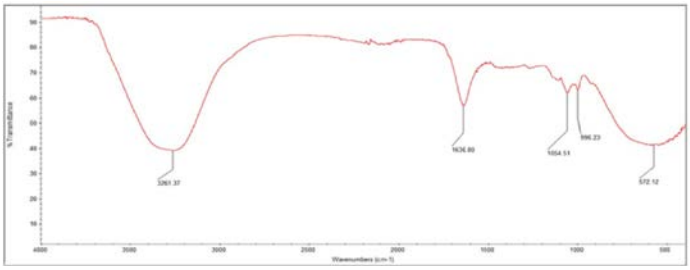


Figure 3: Superoxide Radical Scavenging Assay of CIS.

Table 4: **List of functional groups in FTIR Spectrum of CIS.**

FTIR Peak Values	Functional group
3261	X-H stretch (X is C, O, or N)
1636	C=C stretch(alkene)
1054	C-N stretch(aliphatic Amines)
996	C-O(ethers)
572	C-Br(alkyl halides)



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in Figure 3, the IC_{50} values of CIS and quercetin on scavenging superoxide anions were 45.785 μg/mL and 32.20 μg/mL, respectively.

FTIR Spectrum Analysis of CIS

The FTIR Spectrum of CIS is depicted in Figure 4, and the prominent functional group present in CIS is illustrated in Table 4.

Characterization of Phenolic Compounds of CIS using LCMS Analysis

The LCMS Spectrum analysis of CIS in Figure 5 showed the presence of biologically significant compounds, as depicted in Table 5.

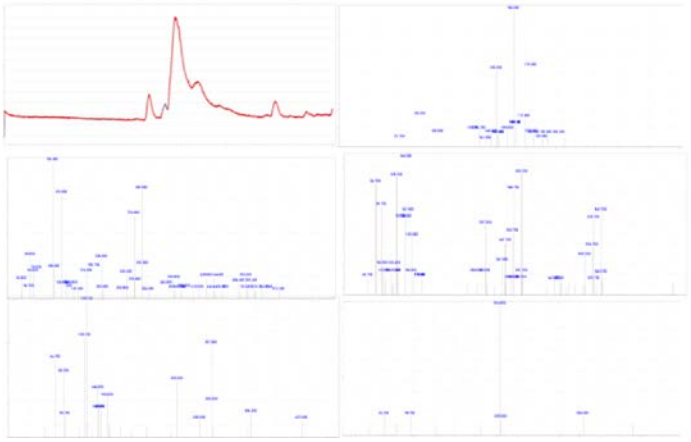

Figure 5: Characterisation of Phenolic compounds of CIS using LCMS analysis.

Table 5: Characterization of Phenolic Compounds of CIS using LCMS Analysis.

Component Name	Observed mass m/z	Absolute intensity	Relative Intensity
4-Hydroxy Coumarin	164.85	7709234	100
P-Coumaric acid	165.90	1237359	16.56
Mellein	178.85	4327772	56.14
Leucopelargonidin	290.55	4923286	76.08
Coumarin	146.80	14229472	100
3 Caffeoyl quinic acid	364.75	10359060	72.80
Ferulic acid 4-O-Glucosidase	380.75	12079800	84.89
L-Phenylalanine	165.85	3919564	30.41
L-Isoleucine	129.75	12590519	100
D-Tryptophan	204.90	6404787	100

DISCUSSION

Phytochemicals are secondary metabolites obtained from plants having a protective role in preventing diseases. Preliminary phytochemical screening of CIS indicates the presence of significant phytochemicals such as flavonoids, alkaloids, phenolics, terpenes, steroids, and glycosides. The antioxidant assays also indicate that CIS possesses high antioxidant potential comparable to standard antioxidants. The biochemical analysis of CIS elevates the importance of CIS as a health drink. CIS was subjected to analysis of mineral contents and identified the presence of 15 elements. Magnesium, an essential element, is present in ample quantity in CIS. The presence of Cobalt, a vital trace element, Iron, Potassium, and Sodium in a significantly better portion. The present investigation observed that CIS has significant amounts of alkaloids, phenolic compounds, glycosides, and flavonoids. Antioxidants have the property of scavenging free radicals, offering resistance to oxidative stress and inhibiting lipid peroxidation.[14] Antioxidant activity is expressed in IC_{50}, and a lower IC_{50} value corresponds to a more significant scavenging activity.[15] The beneficial effects of antioxidants are due to their ability to provide hydrogen atoms to DPPH. The scavenging activities of natural antioxidants can prevent the toxic effects of free radicals. The antioxidant activity of CIS was analyzed by DPPH free radical scavenging assays. In the assay, the violet color DPPH solution was reduced to a yellow-colored product, diphenylpicryl hydrazine, by adding the extract concentration-dependent. The method requires a very short period for analysis and is thus extensively used for antioxidant activity assays. The degrees of discoloration of DPPH by its reduction indicated the radical scavenging activity of CIS. This study revealed that the CIS had a similar free radical scavenging activity to ascorbic acid. The IC_{50} value of CIS was 4.35 µg/ml and standard ascorbic acid 2.92 µg/ ml, demonstrating that the inhibitory activity of CIS was higher than the standard. The results obtained in this study indicate that CIS possess radical scavenging activity due to their hydrogen donating or electron transfer or ability. In the present study, CIS showed significant antioxidant activity and inhibition percentage. The present study suggests that CIS contains phytochemical constituents capable of donating hydrogen atoms to free radicals and thus alleviating the potential damage. The ability of CIS and the standard to scavenge superoxide radicals from the reaction mixture are indicated by the decrease in absorbance at 560 nm. The IC_{50} values of CIS and the standard on SO^{2-} scavenging activity were 45.785 μg/mL and 32.20 μg/mL, respectively. At the pathogenesis, biomolecules are damaged directly or indirectly by superoxide radicals due to the formation of peroxyl nitrite, H^2O^2, singlet oxygen, or^{--}OH. Superoxide anions now initiate lipid peroxidation. The PMS-NADH system assayed the superoxide anion radical scavenging activity of CIS. SO^{2-} scavenging activity of CIS was increased significantly with the increase in concentrations. Thus, the increased inhibitory effects of the CIS on radical formation suggest the potential of CIS as an effective antioxidant. The FTIR spectrum shows a broad spectrum 3261cm^{-1} corresponds to X-H stretch (X is C, O, N), indicating the presence of alkanes, hydroxyl group, or imides. The C= C stretch at 1636cm^{-1} is due to the presence of alkenes. The C-N stretch observed at 1054cm^{-1} indicates the presence of aliphatic amines. The peaks 996 and 572 indicate the presence of ethers and alkyl halides. The LCMS Spectrum analysis of CIS shows the presence of biologically significant compounds, namely 4-Hydroxy Coumarin, P-Coumaric acid, Mellein, Leucopelargonidin, Coumarin, 3 Caffeoylquinic acid, Ferulic acid 4-O-Glucosidase and also three essential aminoacids L-Phenylalanine, L-isoleucine and D-Tryptophan. The studies confirmed the presence of biologically active compounds with protective efficacy. Coumarin and its derivative, 4-hydroxycoumarin, p-coumaric acid was reported to have significant roles in alleviating inflammation. Geranylnaringenin is also reported to have potential antioxidant and anti-inflammatory effects. In short, the results of this study scientifically validated the nutritional composition, phytochemical constitution, and antioxidant potential of the natural drink Coconut Inflorescence Sap. The regular consumption of CIS may protect the body from various chronic diseases, especially where oxidative stress plays a key role.

CONCLUSION

The findings of the present study confirm the nutritional importance and health benefits of CIS. The phytochemical analysis showed the presence of flavonoids, alkaloids, terpenes, steroids, phenols, and glycosides. The antioxidant assays, mineral content analysis, FTIR spectrum and LCMS analysis suggest that CIS is an effective nutritional drink to be exploited in clinical therapy. So further *in vivo* and *in vitro* studies are essential to elucidate the biochemical effects of the natural drink in alleviating oxidative stress and inflammation.

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