International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 17-11-2020; Accepted: 02-12-2020: Published: 11-12-2020
www.botanyjournals.com
Volume 5; Issue 6; 2020; Page No. 579-583

Physicochemical, nutritional analysis and antimicrobial activity of *cocos nucifera* inflorescence sap

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Abstract
Neera, the sweet sap of the coconut palm has a nutritive value, delicious taste and a good flavor. It is obtained by trapping the unopened inflorescence of the coconut palm. It is non alcoholic and is a nutritious drink. Neera obtained from the spadix of coconut palm undergoes natural fermentation due to the innate presence of microorganisms. The fresh neera collected under hygienic condition before sunrise and stored at low temperature is used for analysis. Coconut palm sap mainly contains total sugars, reducing sugar, ethanol, volatile acids, amino acids, vitamins and minerals. In the present study results, fresh and Concentrate neera is sweet, translucent in colour and also has very high nutritional value. It is a very good source of vitamins, minerals and amino acids. Products were formulated and sensory evaluation was done by selected panel members. Concentrated neera is a nutritious drink. It contains a number of minerals and salts, acids like ascorbic acid, nicotinic acid, riboflavin, protein and vitamin C. It has been medically proved that neera is better than mineral water. Neera syrup is used as a health drink in connection with ayurveda and other systems of medicine.

Keywords: coconut palm, concentrate, medicine, Neera, minerals

Introduction
Coconut is highly nutritious and rich in fiber, vitamins, and minerals. It is classified as a "functional food" because it provides many health benefits beyond its nutritional content. The coconut is highly valued as source of food and medicine so it is called "The Tree of Life." Coconut, taxonomically termed as *Cocos nucifera* L., belongs to the family Arecaceae also known as Palmae, and genus Cocos L (Dransfield *et al*., 2008; Nair, 2017) [3, 8]. Coconut is useful in multiple ways to humankind and play a major role in ecological balance and economic importance. Due to its versatile utilization, it is widely acclaimed as 'Kalpaviriksha' or 'Tree of Heaven' (Batugal *et al*., 2005) [1]. It provides both food security and livelihood opportunities to about 20 million people around the globe through cultivation, processing, marketing and trade related activities and thus it exerts profound influence in the rural economy. It is also one of the most important sources of vegetable oil in the world (Uwubanmwen *et al*., 2011) [11].
Cocos nucifera namely coconut palms are one of the oldest flowering trees in the world. For centuries throughout the tropics, cocoti palm wine is the collective name for alcoholic beverages produced by the spontaneous fermentation of the sap of cocoti palm trees. Palm wine is also called as kallu, palm toddy, or simply toddy, is an alcoholic beverage created from the sap of various species of palm tree such as the palmyra, date palms and coconut palms (Rundel Philip, 2002) [9].
This drink is common in various parts of Asia and Africa and goes by various names, such as emu in Nigeria, samba in Democratic Republic of the Congo, kallu in South India etc. Palm wine is an important socio-economic, nutrition and healthy item of many Nigerians especially the low-income rural dwellers.

Neera, the immature coconut inflorescence sap is an important product recently available from Kalpavriksha. A different value-added product prepared from neera is also important with respect to its nutraceutical values. It is a rich source of sugar, minerals, vitamins, fibre in addition to its low glycemic content. It helps in increasing more income generation from coconut palms enhancing employment generation providing natural organic nutrition for all including diabetic patients, resulting in increasing better return from coconut palm as compared to copra (Ghosh et.al., 2018) [4].
However, literature survey reveals that there is no study done on macro and micronutrients of the taxon *Cocos nucifera*. With this aim, an attempt has been made to investigate the main functional components of different nutrients present in the product of this important ethnomedicinal plant.

Materials and methods
Collection of coconut (*Cocos nucifera*) inflorescence sap
Fresh coconut (*Cocos nucifera*) inflorescence sap were collected from the coconut plantation in Kerala. The fresh samples were taken at random after afternoon tapping where one container without preservative and the other one were added limestone powder (approximately 10g/L fresh sap) as a preservative. As soon as the collected fresh sap was brought down approximately 100 ml were filled in a sterilized bottle transported into a cool box to the laboratory and before analysis, the samples were stored in refrigerator at about 4ºC.

Physico: Chemical factors
Physical factors such as Colour, Odour, Taste, pH, Turbidity, Acidity and Alcohol were determined for both

Cocos nucifera fresh sap and *Cocos nucifera* concentrate using standard procedures and were noted.

Nutritional factors

Macro and micronutrients were analysed both for *Cocos nucifera* fresh sap and *Cocos nucifera* concentrate using standard procedures. Macronutrients such as carbohydrate, protein, fat, total free aminoacids, and ash content were analysed. Carbohydrate was analysed by Anthrone method and protein was analysed by Lowrey's method (Lowery *et al.*1951). *Cocos nucifera* contain a wealth of mineral elements. The micronutrients namely phosphorous, calcium, magnesium, potassium, sodium, iron, and iodine were analysed by standard AOAC methods (Raghuramulu *et al.*, 2003). The vitamins such as thiamine, riboflavin, pyridoxine, nicotinic acid, biotin, folic acid, inositol and vitamin C were analysed.

Formulation of the product

Cocos nucifera sap was selected for the preparation of the product. *Cocos nucifera* fresh sap and *Cocos nucifera* concentrate incorporated shake are prepared using standard procedure and the best product was selected.

Table 1: Ingredients for *Cocos nucifera* concentrate incorporated shake

S.No	Ingredients	Diet composition			
		Standard	5%	10%	15%
1.	Vanilla ice cream	100 (ml)	100 (ml)	100 (ml)	100 (ml)
2.	Sugar	50 (gram)	50(gram)	50(gram)	50(gram)
3.	Milk	200 (ml)	200 (ml)	200 (ml)	200 (ml)
4.	*Cocos nucifera* concentrate	-	2.00(ml)	4.00(ml)	8.00(ml)

Preparation of *Cocos nucifera* concentrate incorporated shake

Pour the milk and ice cream, in a blender and partially blend, then add the sugar and flavoring until thoroughly mixed. After mixing add the *Cocos nucifera* concentrate. Then store in refrigerated storage.

Acceptability and Sensory evaluation of *Cocos nucifera* concentrate incorporated shake

The use of standardized recipes ensures that menu items will be consistent in quality each time they are prepared and served. It will also ensure that nutritional values per serving are valid and consistent. The recipes were standardized in terms of the ingredients used, the quantity of each ingredient and the time taken for cooking. Sensory properties, among many other factors influence considerably the quality of food products. Sensory assessment was evaluated on the quality descriptions ie) appearance, taste, color, flavor, texture and over all acceptability using a score card. Score card is a tool which helps in evaluation through direction and degree of judgment using suitable defined scores. Evaluation was carried out for the sample and standard products. They were evaluated by a panel of 20 semi trained panel members from the Department of Nutrition and Dietetics, Muslim Arts College, Thiruvithancode, Kanyakumari District. The panel members did the organoleptic evaluation of these recipes based on five point hedonic scale developed by (Peryam and Pilgrim 1997). The mean score of the organoleptic evaluation was calculated and on the basis of the total obtained scores.

Results

Physical factors

Colour, Odour, Taste, pH and TurbidityColour of the sample *Cocos nucifera* inflorescence sap (fresh (Cn F) and concentrate (Cn C) was measured by using 1.245g of potassium chloroplatinate and 1 g of cobaltous chloride dissolved in distilled water and makes it to 1000 ml. The colour of fresh sap (Cn F) is in white and concentrate (Cn C) is in milky white. In case of odour, fresh sap (Cn F) evolves sweet smell but concentrate (Cn C) gives sour smell with foam which indicates formation of carbon dioxide. Taste of the fresh sap is better than the concentrate (Cn C) taste. *Cocos nucifera* inflorescence concentrate (Cn C) was sour/ tangy due to the acidic level. *Cocos nucifera* inflorescence fresh sap is in sweet taste when it undergoes fermentation taste converts into sour. The pH falls sharply within 3 hours in both sap and concentrate. The fall is from near neutral of pH 6.7 to pH 5.1. In case of concentrate, the pH rate decreases very sharply within 3 hours the pH converts from 4.2 to 3.1. After 12 hours, the pH stabilizes around 2.5 in concentrate. The turbidity of fresh sap was slightly transparent solution, but in the case of concentrate it is different. Turbidity indicating the growth rate of microorganisms (or) fermentation rate was enhanced due to higher fermentation processing.

Chemical factors: Acidity and Alcohol.

The average total amount of acidity in *Cocos nucifera* sap and concentrate was 2.59g/100ml and 4.75g/100ml respectively. There is a sharp rise in titratable acidity of *Cocos nucifera* sap after 4 hours. *Cocos nucifera* sap and concentrate consists of a number of microorganisms. They enhance the rate of alcohol fermentation. Microorganisms convert the sap sugars into alcohol and amino acids. Alcohol percentage majorly depends on temperature and pH. *Cocos nucifera* sap alcohol concentration is 70µl/ml and *Cocos nucifera* concentrate alcohol content is 120µl/ml. Fermentation is a metabolic process that converts sugar to acids, gases and alcohol with the help of microorganisms like yeast and bacteria. The following equation represents the fermentation process in a single step.

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

Table 2: Total alcohol content in fresh (Cn F) and concentrate (Cn C) *Cocos nucifera* inflorescence sap

S. No	Sample	Sample volume in (ml)	Distilled Water in (ml)	O.D values
1	*C. nucifera* sap	0.1	0.9	0.10
2		0.2	0.8	0.21
3		0.3	0.7	0.22
4		0.4	0.6	0.29
5		0.5	0.5	0.11
6	*C. nucifera* Mconcentrate	0.5	0.5	0.16

Table 3: Mean acceptability scores of *Cocos nucifera* sap concentrate incorporated shake

Sl. No	Attributes	Level of incorporation (%)			
		Standard	5%	10%	15%
1	Appearance	4.8	4.3	4.0	3.55
2	Colour	4.2	4.5	4.2	4.1
3	Flavour	4.5	4.3	3.76	3.5
4	Texture	4.8	4.4	4.0	3.6
5	Taste	4.4	4.6	4.3	4.0
	Mean acceptability score	4.54	4.42	4.05	3.72

Fig 1: Collected Samples of *Cocos nucifera* inflorescence sap

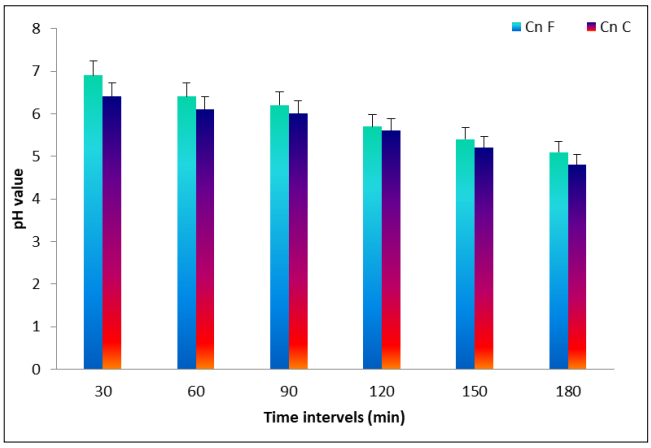

Fig 2: pH difference in-between fresh (Cn F) and concentrate (Cn C) *Cocos nucifera* inflorescence sap

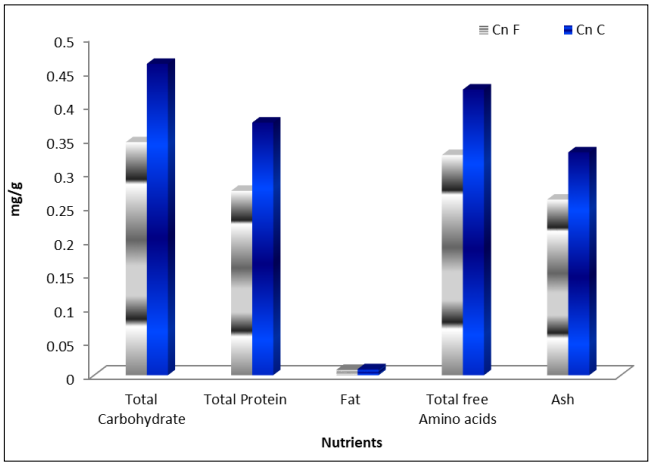

Fig 3: Macronutrient analysis of fresh (Cn F) and concentrate (Cn C) *Cocos nucifera* inflorescence sap

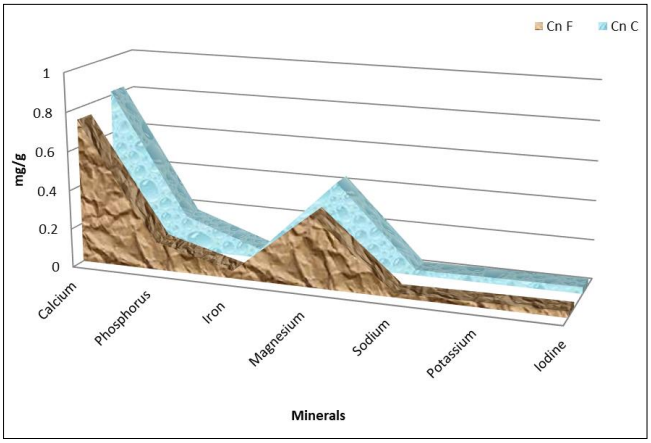

Fig 4: Micronutrient analysis of fresh (Cn F) and concentrate (Cn C) *Cocos nucifera* inflorescence sap

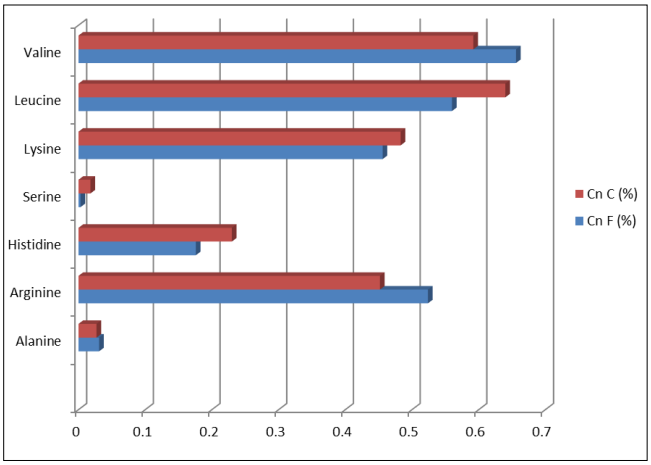

Fig 5: Amino acid profile of *Cocos nucifera* inflorescence sap

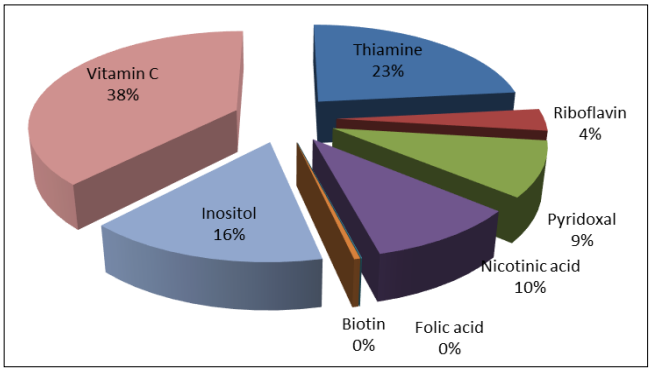

Fig 6: Vitamin profile of fresh (Cn F) Cocos nucifera inflorescence sap

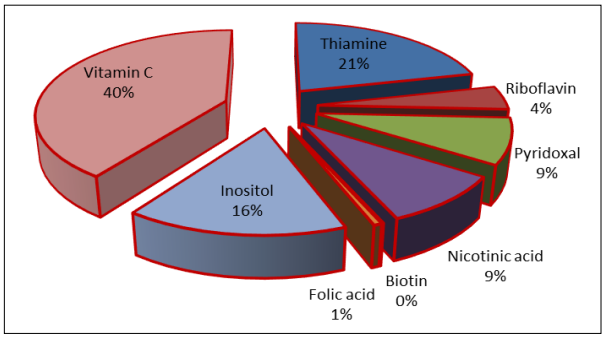

Fig 7: Vitamin profile of concentrate (Cn C) *Cocos nucifera* inflorescence sap

Macronutrient composition

The results of macronutrient composition analysis are shown in Table 4. The total carbohydrate 0.461mg/g and total protein (0.374 mg/g) content were higher in *Cocos nucifera* inflorescence sap concentrate (Cn C). The total carbohydrate 0.345 mg/g and total protein (0.273 mg/g) content were respectively lower in Cn F. The fat content was lower in Cn F (0.008 mg/g) and Cn C (0.009 mg/g). Total free amino acids (0.423 mg/g) and ash (0.33 mg/g) contents were respectively higher in *Cocos nucifera* inflorescence sap concentrate (Cn C). Total free amino acid content was lower in *Cocos nucifera* inflorescence sap fresh (Cn F) (0.326 mg/g) and ash (0.26 mg/g) when compared to others.

Micronutrient composition

The results of micronutrient composition analysis are shown in Table 5. The calcium content was lower in Cn F (0.742 mg/g) and higher in Cn C (0.821 mg/g). Phosphorus level (0.183 mg/g) and iron (0.046 mg/g) contents were respectively higher in *Cocos nucifera* inflorescence sap concentrate (Cn C). The phosphorus (0.145 mg/g) and iron (0.039 mg/g) contents were lower in fresh *Cocos nucifera* inflorescence sap (Cn F). The magnesium 0.44mg/g, sodium 0.028 mg/g and potassium (0.032 mg/g) content were higher in *Cocos nucifera* inflorescence sap concentrate (Cn C). The magnesium 0.37mg/g, sodium 0.021 mg/g and potassium (0.027 mg/g) content were respectively lower in Cn F. Iodine content was lower in *Cocos nucifera* inflorescence sap fresh (Cn F) (0.031 mg/g) and slightly increased in Cn C (0.034 mg/g). The amino acid profile of *C. nucifera* is presented in Table 6. *Cocos nucifera* inflorescence sap fresh (Cn F) recorded lower level Alanine (0.031%), Arginine (0.524%), Histidine (0.176 %), Serine (0.003%), Lysine (0.456 %), Leucine (0.560 %) and Valine (0.656 %). The *Cocos nucifera* inflorescence sap concentrate (Cn C) recorded lower level Alanine (0.027%), Arginine (0.452%), Histidine (0.230 %), Serine (0.018%), Lysine (0.483 %), Leucine (0.640 %) and Valine (0.592 %). All the samples showed significant amino acid profile. Quantification of Vitamins in *C. nucifera* is presented in Table 7. The *Cocos nucifera* inflorescence sap concentrate (Cn C) recorded higher level Thiamine (86 %), Riboflavin (17.9%), Pyridoxal (34.6 %), Nicotinic acid (37.3 %), Biotin (0.26 %), Folic acid (2.34 %) Inositol (62.6 %) and Vitamin C (162.2 %). *Cocos nucifera* inflorescence sap fresh (Cn F) recorded lower level Thiamine (72 %), Riboflavin (11.4%), Pyridoxal (28.4 %), Nicotinic acid (30.6 %), Biotin (0.17 %), Folic acid (1.24 %), Inositol (47.7 %) and Vitamin C (117.5 %). All the samples showed significant vitamin content.

Sensory evaluation

The sensory analysis of the formulated product is given below. The sensory parameters such as appearance, taste, texture, flavor, color and overall acceptability of *Cocos nucifera* concentrate incorporated shake shows the best compared to *Cocos nucifera* fresh incorporated shake.

Mean acceptability scores of *Cocos nucifera* concentrate incorporated shake

The selected *Cocos nucifera* inflorescence sap concentrate were incorporated at different levels of incorporation ranging from 5, 10 and 15 per cent. The recipe prepared were evaluated for acceptability by five point hedonic rating scales. Table 3 shows the mean acceptability scores of *Cocos nucifera* sap concentrate incorporated recipe. Results revealed that the mean total score of 5 – 15 per cent incorporated *Cocos nucifera* sap concentrate shake were found to be possess 4.42, 4.05 and 3.72 percent as the mean scores, when compared to the standard with the mean score of 4.54 respectively.

Overall mean acceptability score of *Cocos nucifera* inflorescence sap concentrate incorporated shake

The overall acceptability of score of *Cocos nucifera* sap concentrate incorporated shake revealed that five per cent incorporation scored higher with respect to the sensory attributes of appearance, color, flavor, texture and taste when compared with 10 – 15 per cent incorporated formulation. *Cocos nucifera* sap concentrate incorporated shake had secured the highest scores and found to be highly acceptable than milk shake.

Discussion

Coconut palm botanically known as *Cocos nucifera* L. belongs to the natural order Arecaceae (Palmoe) an important member of monocotyledons. A sugar containing juice or sap is obtained by tapping the unopened spadix of the coconut palm. The tapping involves the extraction of exuded sap from the inflorescence that yields sweet sap. The tapping methods vary from country to country and within the country. In India and Sri Lanka, the spadix is considered ready for tapping when the mature one is burst or just about to do so. The female flowers within the unopened spathe cause a swelling at the base and its appearance is an indication of the appropriate stage for tapping. The tapping is usually continued for a period of six months and there may be 3 or more spathes on the same tree being tapped at the same time. The maximum yield of coconut sap is usually obtained in the third month after the commencement of tapping. The yield of coconut sap is highly variable. It varies considerably from day to day, season to season, spadix to spadix and tree to tree. The average yield of coconut sap is about 1.5 litres per palm per day. The fresh coconut sap is rich in carbohydrates with sucrose as its main constituent. It is well known that the coconut sap ferments very quickly thus producing alcohol in it. The fermented sap smells obnoxiously thus making it unacceptable as a beverage for consumption. Hence, there is a need to preserve the coconut sap without affecting its nutritional quality. Protein and carbohydrates were moderately present in the sap, while fat and ash in least concentrations. Quantitative estimation of minor components in dried powdered *C.nucifera* revealed the presence of free amino acids, vitamin C, vitamin E, minerals like sodium and potassium. The macronutrient analysis of *C.nucifera* showed the presence of carbohydrates in high concentration and proteins and amino acids in moderate concentration, whereas, fats and oils were absent. *C.nucifera* is a rich source of dietary fibers. Besides this, *C.nucifera* contains free amino acids, minerals like potassium and sodium, vitamins such as ascorbic acid, thiamine, riboflavin, pyridoxine, nicotinic acid, biotin, folic acid, and inositol. The contents of both monosaccharides enhance the final reducing sugars content of the *C.nucifera* sap, because they could probably be the main reducing sugars of *C.nucifera* sap. Their presence in the plant may have resulted from two

biochemical processes. The first source might be the enzymatic hydrolysis of sucrose, during fermentation stages. Indeed, the sap exudates undergoes a spontaneous fermentation by microorganisms occurring from the environment using the inner sucrose. This phenomenon provides glucose and fructose into the sap, which are thereafter also metabolized, leading to alcohol and acid compounds. The second origin of reducing sugars would be a physiological synthesis by the plant. The photosynthesis system and the coconut tree can perform molecules with higher atoms of carbon (C6) from C3 molecules. S This endogenous biological process may have produced other hexoses, like mannose, among the reducing sugars of the coconut sap. The reducing sugars are substrate for enzymatic browning and Maillard reactions which could happen during production of coco syrup and coco sugar from coconut sap. The four coconut cultivars provided reducing sugars contents of their saps equivalent to the sap of the oil palm (Karamoko *et al.*, 2019). The presence of glycerol could be explained by the glycopyruvic fermentation of the reducing sugars (glucose, fructose and mannose) due to yeasts. Simultaneously, this oxidization could lead to other volatile organic compounds, such as acids, aldehydes, ketones and esters, which contribute to the astringency and aroma of the coconut sap (Cordella, 2004).

The differentiation among the physicochemical characters could be originated with the divergence between the four coconut genotypes. The higher contents of total soluble solids and dry matter of the sap collected from coconut farms could be a specificity of the dwarf coconut cultivars. Indeed, a part of the sap may directly result from both hydric and mineral absorption by plant's roots from soil. The other part is synthesized during photosynthesis and water transpiration involved by leaves. So, the sap transports and procures the main nutrients to all the plant organs, including the inflorescences (Konan *et al.*, 2013). In conclusion, the results of the present study indicate that the young inflorescence of coconut possesses significant health benefits. It exerts these beneficial effects mainly due to the presence of various nutritional factors.

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