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Phytochemical and Pharmacological Evaluation of Yellow and Green Variety of *Cocus nucifera* Water

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Coconut water widely consumed as a health drink by people around the world. This led to an urge to study various properties of *Cocus nucifera*. We evaluated anti oxidant and phyto chemical constituents of two variety of coconut the yellow and green variety. Phytochemical assays showed the presence of terpinoids, alkaloids, resin, sugars, steroids, glycosides and tannins in both the dwarfs but yellow variety showed more amount of photochemical constituents. In both the variety of coconut water antioxidants were found. In the assay of DPPH, scavenging of nitric oxide assay and scavenging of hydrogen peroxide the presence of antioxidants were proved against the standard ascorbic acid. Results found yellow variety to be more potent antioxidant.
All assays proved yellow dwarf was healthier than green dwarfs with more health benefits and medicinal properties.

Keywords: Antioxidant activity; ascorbic acid; Cocus nucifera; phytochemical.

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1. INTRODUCTION

Cocus nucifera (L) which belongs to the family of Arecaceae and is normally named as “coconut tree”, the utmost naturally wide spread fruit plant on earth. It is popularly known as coconut, coconut of the beach. This plant has originated from south East Asia and islands between Indian and Pacific Ocean. Coconuts are used for their wide variety of uses in food, cosmetics etc [1].

Coconut water is a distinctive, fat-free beverage. It is low in sweets and calories, yet high in essential electrolytes and nutrients. Coconut, named as the "liquid of life," is safe to drink straight from the nut. Coconut water is "dew from the sky," as the Hawaiians say.

Coconut water begins to lose its nutrients and flavours once the coconut is opened. This is due to the naturally occurring compounds contained in coconut water. When peroxidase (POD) and polyphenol oxidase (PPO) come into touch with oxygen, their reactions produce health and flavour problems.

The coconut water is an opalescent fluid found in the shell of the coconut organic product. It is sterile and utilized as a reviving beverage. The coconut is known for its numerous temperances. It is known to be a force to be reckoned with of medical advantages. It assists with treating numerous sicknesses like throat diseases, tapeworms, gonorrhea, stomach related issues, flu, lice, giardia, bronchitis, and numerous different illnesses. It supports the safe framework and has antifungal, antiviral, hostile to parasitic and antibacterial properties.

The coconut can be consumed in various forms, such as raw coconut, coconut oil, coconut milk, coconut margarine, coconut water, and so on. It improves insulin release in the body and increases blood glucose utilisation. Indeed, coconut consumption aids in diabetes management by significantly influencing the chemicals that control glucose in the body [2].

Because of this, the ascent in glucose levels delayed down and this likewise assists with bringing down the glycaemic longings of an individual. The coconut is likewise known to cause fast processing and emphatically affects different side effects that are related with gut and stomach related problems. It is additionally known to assist with the ingestion of supplements and minerals in the body, while likewise giving the quantity of dietary filaments that your body needs. The utilization of coconut, by its rich substance in fiber, eases the weight on the pancreas, adding to the lessening of the diabetes hazard. As of late, the coconut water has become an extremely stylish refreshment [3].

Many studies have shown that the antiviral, antibacterial, anti-inflammatory and antioxidant activities of coconut water may help ease a number of minor to severe health conditions.

This nutrient rich drink has been used to regulate blood pressure, blood sugar, and cholesterol levels, and it has been found to boost energy levels and increase metabolism in human body.

Other conditions that it has been found to be effective in treating include stomach flu, dysentery, indigestion, constipation, intestinal worms, urethra stones, malfunctioning kidneys, dry and itchy skins, age spot and wrinkles [4].

Coconut water has also been reported to aid boost high density in certain recent research.

It is a source of lipoprotein (good) cholesterol. A fantastic natural remedy for keeping one's health in tip-top shape heart and circulatory health Coconut water from a young coconut has Characteristics of oestrogen. It’s simple to combine during World War II; it was used as a source of blood transfusions in an emergency [5].

Lipid peroxidation is an established mechanism of cellular injury, and is used as an indicator of oxidative stress. Polyunsaturated fatty acids peroxides generate malondialdehyde (MDA) and 4-hydroxyalkanals upon decomposition [6]. Superoxide dismutase (SOD) decomposes superoxide anion into hydrogen peroxide and oxygen at very high rates. Superoxide radical is involved in diverse physiological and pathophysiological processes [7]. Lipid profile is a general term that is given to tests for high density lipoprotein, low density lipoprotein, total cholesterol and triglycerides. A shift in the normal level of any of these components of lipid profile is of interest to cases of cardiovascular disorders [8].

This examination plans to assess the phytochemical compounds and pharmacological properties of coconut water.

2. MATERIALS AND METHODS

2.1 Collection

Coconuts were collected from same tree one is yellow dwarf tree and another was green dwarf tree from Mangalore, India.

These varieties were harvested when the fruits begin to mature as the content of *cocus nucifera* water will be more at this time.

Many coconut from both variety were opened and water was removed and filtered and was stored in sterile bottles in -20^0centigrade refrigerator.

These samples were further analyzed for phytochemical and anti- oxidant investigation.

2.2 Preliminary Qualitative Phytochemical Investigation

Coccus nucifera water was subjected to qualitative phytochemical examination for characterizing the active constituents present in it. The tests were carried out by standard methods [6].

2.2.1 Test for alkaloids

A) Dragendorff's test

Coccus nucifera water was taken (2ml), distilled water (5 ml) was added, to which hydrochloric acid (2M) was added for the reaction to occur. 1ml of Dragendroff's reagent was added to the above liquid and the visual observed for orange-red color precipitate formed detects alkaloids are present.

B) Hager's test

Take coconut water about 2 ml in a clean dry test tube later add haters solution a clear observation of yellow precipitate tell alkaloids are present in it.

C) Mayer's test

Take 2 ml of coconut water of both variety in a test tube then add Mayer's reagent drop wise to the test tube. A pale yellow precipitate observed confirms the presence of alkaloids.

D) Wagner's test

Cocus nucifera water (2ml) was taken; 1.5 ml of hydrochloric acid was added, later in the test tube Wagners reagent was put drops wise a buff colored precipitate clearly indicates that alkaloids are present.

2.2.2 Reducing sugar tests

A) Molisch test

Few drops of freshly prepared alcoholic α-naphthol (20%) was added to 2ml of *Cocus nucifera* water, later on the sides of the test tube put 2 ml of concentrated sulphuric acid this is because a layer will be formed bellow the mixture. The presence of carbohydrates was indicated by the formation of violet-red ring . The ring on addition of more amount of alkali will not be seen.

B) Benedict test

Few drops of Benedict's solution was added to the *Cocus nucifera* water and heated for five minutes. A brick red color seen will detect the presence of carbohydrates.

C) Fehling test

Cocus nucifera water (2 ml) was mixed in 10 ml of distilled water and 1ml of Fehling's solution A and B. A brick red color seen tells the presence of reducing sugars.

D) Tollen test

Cocus nucifera water (2 ml) was added to 10 ml of distilled water. To this 1ml of Tollen's reagent was added and heated on a water bath. The formation of black precipitate with silver mirror on the sides of the test tube confirms the presence of reducing sugars.

2.2.3 Test for flavonoids

2.2.3.1 Shinoda test

Cocus nucifera water was added in ethanol (5 ml) and to this dilute HCL about ten drops was added. A small piece of Mg was added and then it was heated. The presence of flavonoids was confirmed by red brown color.

2.2.4 Test for Saponins

2.2.4.1 Emulsion test

In a test tube containing 5ml of *Cocus nucifera* water a drop of sodium bicarbonate solution was added, shaken well and left for 3 min. Formation of honeycomb like froth confirmed the presence of saponins.

2.2.5 Test for tannins

To 2ml of *Cocus nucifera* water added in distilled water, 2ml of lead acetate was added. Formation of white cloudy precipitate confirmed the presence of tannins.

2.2.6 Test for steroids

a) Libermann-Burchard test

About 2ml of the *Cocus nucifera* water was dissolved in acetic anhydride. The solution was heated and cooled, then add along the side of the testtube concentrated sulphuric acid. appearance of green colour confirms steroids are present.

b) Salkowski test

In a test tube take coconut water mixture with chloroform to this solution put concentrated sulphuric acid along the sides of the test tube. In chloroform layer a red color is seen which confirms steroids in the solution.

2.2.7 Test for proteins

a) Biuret's test

Cocus nucifera water (1 ml) was taken, few drops of copper sulphate solution (10% w/v) was added and then heated. A violet red color formed detects proteins in the sample.

b) Millon's test

To 1ml of *Cocus nucifera* water, Millon's reagent (few drops) was added. A white color precipitate which becomes red on heating shows proteins in the sample.

2.2.8 Test for triterpenoids

a) Libermann-Burchard's test

2 ml of the *cocus nucifera* water of the plant material was added in acetic anhydride. The solution was heated and cooled, after which concentrated sulphuric acid (1ml) was added along the sides of the test tube. The presence of triterpenoids was confirmed by the formation of pink color [7].

2.3 Evaluation of Antioxidant Activity of *Cocus nucifera* Water

2.3.1 DPPH radical scavenging activity

The radical scavenging activity of coconut water will be determined by was determined by DPPH free radical assay with some modification. Coconut water or both variety were taken in a test tube 50 µL of coconut water in ethanol were added to 5 mL of 100 µM solution of DPPH.

This mixture was incubated for 30 min after these readings were taken at 517 nm using a UV-VIS spectrophotometer Shimadzu UV-1900. Blank reading was taken by methanol and the percentage inhibition activity was evaluated by the equation given below.

% Inhibtion=[(Ao-A1)/Ao]x100 (1)

Where Ao is the control absorbance and A1 is the coconut water absorbance. Here ascorbic acid was used as a standard. All the readings were performed in triplicates [8].

2.4 Nitric Oxide Radical Inhibition Assay (No°)

The inhibition of NO° can be estimated by the use of Griess Illosvoy reaction [9]. In this investigation, Griess Illosvoy reagent was modified using 0.1% of naphthylethylenediamine dihydrochloride instead of 5% 1-napthylamine. The absorbance of solutions was measured at 540 nm against the corresponding blank solutions using the following formula:

Nitric oxide radical scavenging = Ablank _ Asample × 100 / Ablan

Instrument used is UV-VIS spectrophotometer Shimadzu UV-1900

2.5 Scavenging of Hydrogen Peroxide

A hydrogen peroxide solution was prepared as phosphate buffer solution at pH 7.4. 1 mL of sample and standard was added to methanol

which was mixed with hydrogen peroxide 2 mL (PBS). After 10 minutes the absorbance at 230 nm) was measured using a UV-VIS spectrophotometer Shimadzu UV-1900.

Readings will be done in triplicates and the inhibitory concentration was calculated using equation (i) as represented above) [10-11].

2.5.1 ABTS assay

A solution of ABTS solution (7 mM) with 2.45 mM potassium persulfate (K2S2O8) was prepared. The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 750 nm. Coconut water in a concentration of 1 mg/ml was prepared. An aliquot of 20 µl of solution of each sample was added to 180 µl of ABTS free radical cation solution. The absorbance, monitored for 5 min, was measured spectrophotometrically at 750 nm using a microtitre plate reader. All measurements were performed in triplicate [12].

3. RESULTS AND DISCUSSION

In phytochemical screening of coconut water Alkaloids and reducing sugar were abundant in yellow variety as checked against green variety. Flavanoids were absent in both the species. In the view of saponins tannins and steroids were also seen in higher percentage in yellow variety. Proteins and tri terpenoids were also more in yellow coconut water. Overall the results proved phytochemicals constituents responsible for majority of antioxidant and healing properties were more in yellow variety then in green variety of *Cocus nucifera* t L.

In DPPH, nitric oxide and hydrogen peroxide assay shown in Table 2 and Fig. 1 when IC 50 value were compared of both yellow and green variety as compared to standard ascorbic acid. Yellow variety shows 132, 119, 156 µg/ml as compared ascorbic acid 95. 91, 94 µg/ml which shows comparable results in antioxidant property.

Table 1. Results of phytochemical analysis of yellow and green variety of coconut water

Sl. no	Tests	Observation	Yellow	green
1	Alkaloid			
	a) Dragendroff test	Brick red precipitate Yellow	+++	++
	b) Hager test	precipitate	+++	++
	c) Wagner test	Reddish brown precipitate	+++	++
	d) Mayer test	Milky precipitate	+++	++
2	Reducing sugar			
	a)Molisch test	A brown coloration observed the interface	+++	++
	b)Benedict test	Yellow to bright precipitate Presence	+++	++
	c)Fehling test	of a brick red precipitate Silver	+++	++
	d)Tollen test	precipitate	+++	++
3	Flavonoids			
	a) Shinoda test	No reddish color	---	--
4	Saponins			
	Emulsion test	Emulsion formed	++	+
5	Tannins			
	lead sub acetate test	Presence of cream gelatinous precipitate	+++	++
6	Steroids			
	a)Liebermann-Burchard test	Green blue color	+	+
	b) Salkowski test	Bluish red to purple color	+	+
7	Proteins			
	a)Biuret test	White precipitate Protein present	++	+
	b)Millon's test	White precipitate Protein present	++	+
8	Tri terpenoids			
	a)Liebermann Burchard	Light green to dark green color	+	+

– Absent+ precipitate observed in low concentration, ++ Precipitate observed in moderate concentration+++ Precipitate observed in high concentration

Table 2. Results of antioxidant analysis of coconut water

Name of the compound	Name of the extract and part	IC_{50} values		
		DPPH (µg/ml)	Nitric oxide radical inhibition assay (µg/ml)	Scavenging of hydrogen peroxide
Coconut water	Yellow	132	119	156
	Green	147	125	172
Ascorbic acid		95	91	94

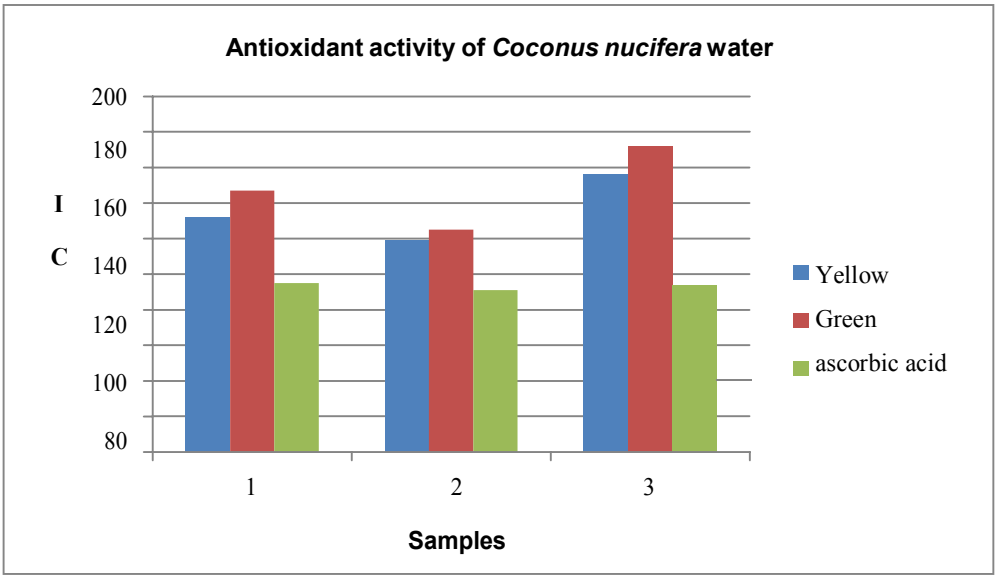

Fig. 1. Antioxidant activity od coconut water

ABTS assay This was done by using the ABTS free radical decolorization assay developed by Re et al. (1999) with some modification. Briefly, the pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7 mM) with 2.45 mM potassium persulfate (K2S2O8). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.7 ± 0.2 units at 750 nm. The plant extracts were separately dissolved in ethanol to yield a concentration of 1 mg/ml. An aliquot of 20 ll of ethanolic test solution of each sample was added to 180 ll of ABTS free radical cation solution. The absorbance, monitored for 5 min, was measured spectrophotometrically at 750 nm using a microtitre plate reader. All measurements were performed in triplicate.

4. CONCLUSION

From the investigation it is inferred that both the coconut assortments uncovered phytochemical constituents yet in yellow coconut showed better outcomes s contrasted with green coconut. Macro nutrients like proteins starches were additionally more in yellow bantam then in green midget. The Pharmacological action of both the organic products had promising enemy of oxidant action yet yellow coconut water showed better movement then, at that point green assortment. The near after effects of the two assortments demonstrated yellow midget a decent cancer prevention agent with bountiful of phytochemical constituents which will be a promising perspective helpful for therapy of different diseases as far as dietary enhancement.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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