

# Evaluation of physicochemical and bacterial contamination of coconut samples based on different time interval and storage condition

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**Abstract**— Coconut water is a well-known natural booster which supplements essential nutrients for a person. Since ancient days coconut water has been reported to be a rich pool of vitamins, phytochemicals, and amino acids etc possessing pharmaceutical importance. The present research has high lightened on comparative study of natural coconut water and synthetic coconut water by emphasizing physicochemical properties and antimicrobial nature. The study was also conducted to compare the previous mentioned parameters at different hours and conditions. Total 5 samples were chosen for this research (Green-Immature without meat, Mature with meat), Brown shelled coconut and 2 commercial products. Based on the results all samples possess protein and calcium but there was diversity in the presence of sulphate, phosphate and acetate, where Green-mature and Mature coconut water has phosphate while sulphate and calcium has same trend as they are only absent in packed coconut water stored in room temperature. Based on the results of quantitative analysis of sample constituents like Phenolic content, ascorbic acid and Carbohydrate that were stored naturally as well as refrigerator conditions, phenolic content was decreased whereas Ascorbic acid and Carbohydrates were slightly increased in both of the condition. To evaluate the antimicrobial nature of samples, total 5 Bacteria were chosen, 4 were Gram Negative (*Pseudomonas sp*, *Bacillus megatirium*, *Bacillus subtilis*, *E.coli*) and 1 Gram Positive (*Staphylococcus aureus*), bases of the results the samples were reported to be negative.

**Index Terms**— Coconut, antimicrobial, Phytochemicals.

## I. INTRODUCTION

Coconut water is the juice of the endosperm produced within the cavity in 2 months (1). According to research, coconut water accounts for 25% of the weight of the fruit, with 95.5% water; 0.7% protein which are functional and structural subunits for immunity, maintenance, cell-signaling in human body and it provides many essential amino acid (2); 4 % carbohydrates (3); 0.1% fat; 0.02% calcium which is an integral part of skeleton and teeth; and required for muscle contraction, cardiac function and nerve impulse transmission (4)(5); 0.01% phosphorous which is an integral part of genome and bones and required for generation of bony tissue and maintenance of acid base balance (6); 0.5% iron; in addition to amino acids (7); 0.3% vitamin C which has a very important function as an antioxidant in association with Vitamin E in scavenging the free radicles from the cells (8)(9); it helps in absorption of iron, promotes healing of wounds, healthy blood vessels and formation of connective tissue; B complex vitamins and mineral salts(10). Sulphate is found in trace amount which can't be synthesized by human body and it is essential for brain and heart function. Consumption of phenols has been linked to the decrease of the risk of diseases associated to oxidative stress and cardiovascular diseases.

Coconut water has many medical therapeutics uses; in some countries coconut water is used as a solution for oral hydration, as part of the daily diet and as a protein supplement when nutritional deficits are intense (11). Some studies suggested that coconut water can be used for intravenous rehydration (12), (13). Other studies suggest that coconut water can be used for electrolyte replacement in a wide range of situations (14)-(16). Coconut water lowers the blood pressure, boosts energy and relax the muscle tension as the result it is the best drink for good athletic performance (17)-(19). Coconut water promotes weight loss by decreasing cholesterol, cleansing and detoxification of gastro-intestinal tract (20).

India is the third largest coconut producing country, after Indonesia and the Philippines, having an area of about 1.78 million hectares under the crop. Annual production is about 7562 million nuts with an average of 5295 nuts/hectare (21). In India, the four south Indian states namely Kerala, Tamil nadu, Karnataka and Andhra Pradesh account for around 90% of the coconut production in the country. In India, different types of coconut have been used for consumption but certain area where natural coconut is not available, packed coconut water is used (21).

In this study the evaluation of physicochemical characteristic is performed on 5 different types of coconut samples i.e. Green coconut, Green-immature coconut, mature coconut and 2 types of coconut water bought from different industries.

## MATERIALS AND METHODOLOGY:

### SAMPLE COLLECTION AND STORAGE:

Different samples were collected and brought to the lab bench for the present study.

**Qualitative analysis:**

Qualitative analysis of different samples were checked for Starch, Protein, ions like Phosphate, Sulphate, Acetate, Nitrate and Calcium (22).

**Starch:** 2-3 drops of Lugol's iodine solution was added to 1 ml of sample. Appearance of Blue- black colour indicates presence of starch.

**Protein:** 2-3 drops of conc. HNO<sub>3</sub> was added to 2ml of sample and observed for appearance of yellow precipitates which indicate the presence of protein.

**Phosphate ion:** 1 ml of conc. HNO<sub>3</sub> and ammonium molybdate were added to 1 ml of sample. Yellow crystalline precipitates indicate presences of phosphate ion.

**Sulphate ion:** Lead acetate powder was added to 1 ml of sample. White precipitates indicate presence of sulphate ion.

**Acetate ion:** 1 ml of conc. H<sub>2</sub>SO<sub>4</sub> was added to 1 ml of sample and the effervescence of vapours was smell to check for vinegar fragrance.

**Nitrate ion:** 1 ml of fresh FeSO<sub>4</sub> and conc. H<sub>2</sub>SO<sub>4</sub> was added drop by drop to 1 ml of sample. Brown ring indicates presence of nitrate ion.

**Calcium ion:** Appearance of white precipitate by adding 1 ml of AgNO<sub>3</sub> to 1 ml of sample indicates the presence of calcium ion.

**Quantitative analysis:**

Quantitative analysis of Total phenolic content, Ascorbic acid and Carbohydrates were performed in this study.

**Determination of the total phenolic content:**

Total phenolic content was estimated by the standard protocol given by Singleton and Rossi (1965). 1 ml of sample was mixed with 4 ml of Folin Ciocalteu reagent and 2ml Alkaline solution followed by incubation at 37°C for 2 h. After incubation absorbency was read at 765nm (23). Amount of phenolic content was calibrated using Standard Gallic acid graph using the following formula.

$$C = C1 * \frac{V}{m}$$

Where C = total phenolic content in mg/g, in GAE (Gallic acid equivalent),

C1 = concentration of Gallic acid established from the calibration curve in mg/ml,

V = volume of extract in ml,

m = the weight of the plant extract in g.

**Determination of ascorbic acid:**

Ascorbic acid extraction was carried using 1:1 ration of sample and 6% meta-phosphoric acid followed by proper mixing and centrifuging for 5 min at 5000 rpm. 0.1 ml supernatant was mixed with 2.3 ml 6% meta-phosphoric acid and 0.8 ml DTCS [DNPH (Di-nitro phenyl hydrazine), Thiourea, Copper sulphate] reagent and incubated at 37°C for 3 h. After incubation 0.4 ml of cold 12 M sulfuric acid was added and absorbance was measured at 520 nm (24). Calibration of ascorbic acid in the sample was done using standard Ascorbic acid graph (20, 80, 165, 250, 415 and 625 µg/ml).

**Determination of carbohydrate:**

Extraction of carbohydrate was carried out by adding 0.1 ml of sample in 5 ml of 2.5 N HCl and boiled for 3 h in water bath followed by neutralizing the solution by adding Sodium carbonate and final volume was made upto 100 ml, which is used as extract. 0.1 ml of extract were mixed with 0.9 ml of distilled water, 4 ml of Anthrone reagent and boiled in water bath for few minutes and absorbance was measured at 630 nm (25). Amount of carbohydrate was calibrated using Glucose standard graph (20-100 mg/ml).

**Antimicrobial activity:**

Antimicrobial activity was analyzed by agar cup plate method. 5 bacterial were chosen for this study (*Pseudomonas sp*, *Bacillus megatirium*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*). 24 h culture was spread on the nutrient agar surface followed by making wells using a sterile cork borer and 20 µl of sample was loaded into wells. Plated were incubated at 37°C for 24h and observed for the appearance of zone of inhibition (26).

**RESULTS****I. Sample collection:**

Total 5 samples were collected for the study. Out of 5 samples, 3 were collected from natural habitat (Green, Green Mature and Mature coconuts), 2 were packet samples (Active cool & Coco sip).

For this study 3 different conditions were chosen sample as soon as they are exposed to open environment [A] and preserved sample in sterile culture vials was labelled as B that has been stored in room temperature and C that has been stored in refrigerator.

**Qualitative Analysis:**

Qualitative analysis of different samples [A, B, C] were checked for Starch, Protein, Phosphate ion, Sulphate ion, Acetate ion, Nitrate ion and Calcium ion. Based on results there was no variation in the chemical constituent of Green coconut water in terms of protein, sulphate and calcium ions, whereas in case of Green-Mature coconut water, change was not observed in terms of protein, sulphate and calcium ions but phosphate was reported after incubation in both B and C. In mature coconut water there was no variation in phosphate, sulphate and calcium while protein was only present in A and C. In Active cool packed coconut water, sulphate and calcium ions were present in sample A and C, while in Coco-sip samples all constituents were positive as shown in Table: 1.

**Table 1.** Qualitative Analysis of all samples

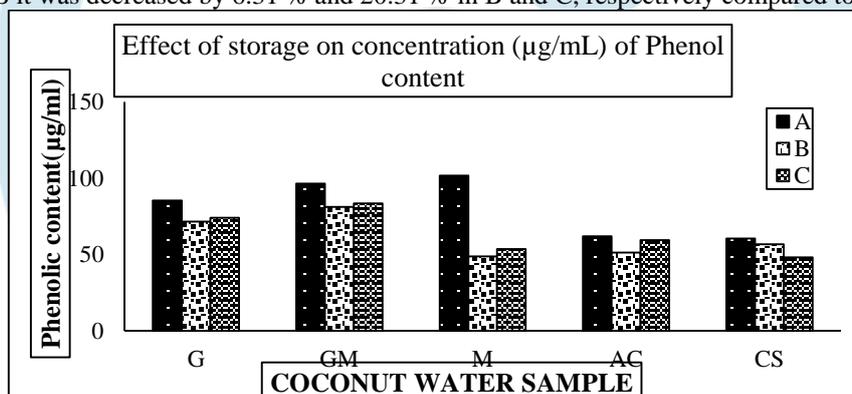
Components	G			GM			M			AC			CS		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Protein	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
PO <sub>4</sub> <sup>3-</sup>	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-
SO <sub>4</sub> <sup>2-</sup>	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
CH <sub>3</sub> COO <sup>-</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NO <sub>3</sub> <sup>-</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ca <sup>2+</sup>	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+

**Quantitative analysis of Total Phenol, Ascorbic acid and total carbohydrates:**

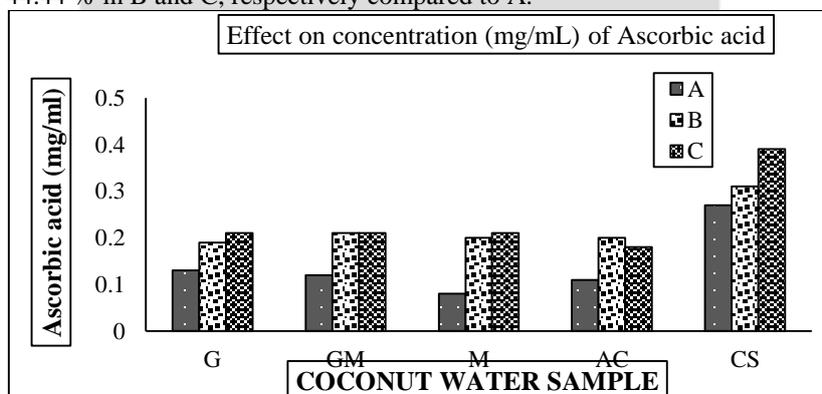
Quantitative analysis of total phenolic content, Vitamin C and total carbohydrates of all samples were done.

**Phenol:**

Based on % of increase and decrease of phenolic content there was a decreased in phenolic content after storage in both cases (B and C). In green sample, phenolic content was decreased by 16.22% in B and 13.29 % in C, in green mature sample by 15.81 % and 13.47 % in B and C, in mature sample by 51.99 % and 47.295 % in sample B and C, in AC it was decreased by 17.14 % and 4.19 % in B and C, in CS it was decreased by 6.31 % and 20.31 % in B and C, respectively compared to A.

**Figure 1.** Effect of storage on concentration (µg/ml) of Phenol content**Ascorbic acid:**

Based on % of increase and decrease of ascorbic acid, all the samples have shown increase in ascorbic acid content in both cases (B and C). In green sample, it was increased by 46.15% in B and 61.53 % in C, in green mature sample by 75 % in B and C, in mature sample by 150 % and 162.5 % in sample B and C, in AC it was increased by 81.81 % and 63.63 % in B and C, in CS it was increased by 14.81 % and 44.44 % in B and C, respectively compared to A.

**Figure 2.** Effect of storage on concentration (mg/ml) of ascorbic acid**Carbohydrates:**

Based on % of increase and decrease of carbohydrates there were variations of increase and decrease of %. In green sample, ascorbic acid was increased by 21.16% in B and 26.37 % in C, in green mature sample by 372.23% and 291.51% in B and C, in mature sample by 338.78 % and 206.31 % in sample B and C, in AC it was increased by 125 % in sample B and decreased by 5.58 % in sample C, in CS it was increased by 71.36 % in sample B and decreased by 11.58 % in sample C compared to A.

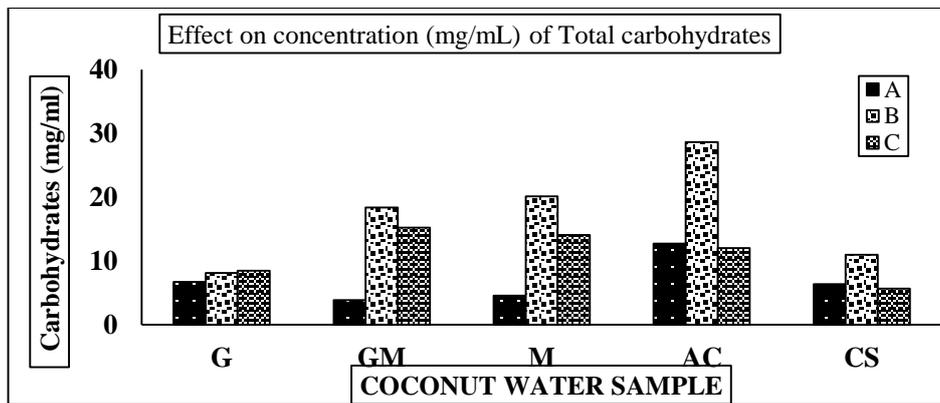


Figure 3. Effect of storage on concentration (mg/ml) of carbohydrates.

**Antimicrobial Activity:**

All samples were reported to be negative in terms of antimicrobial activity.

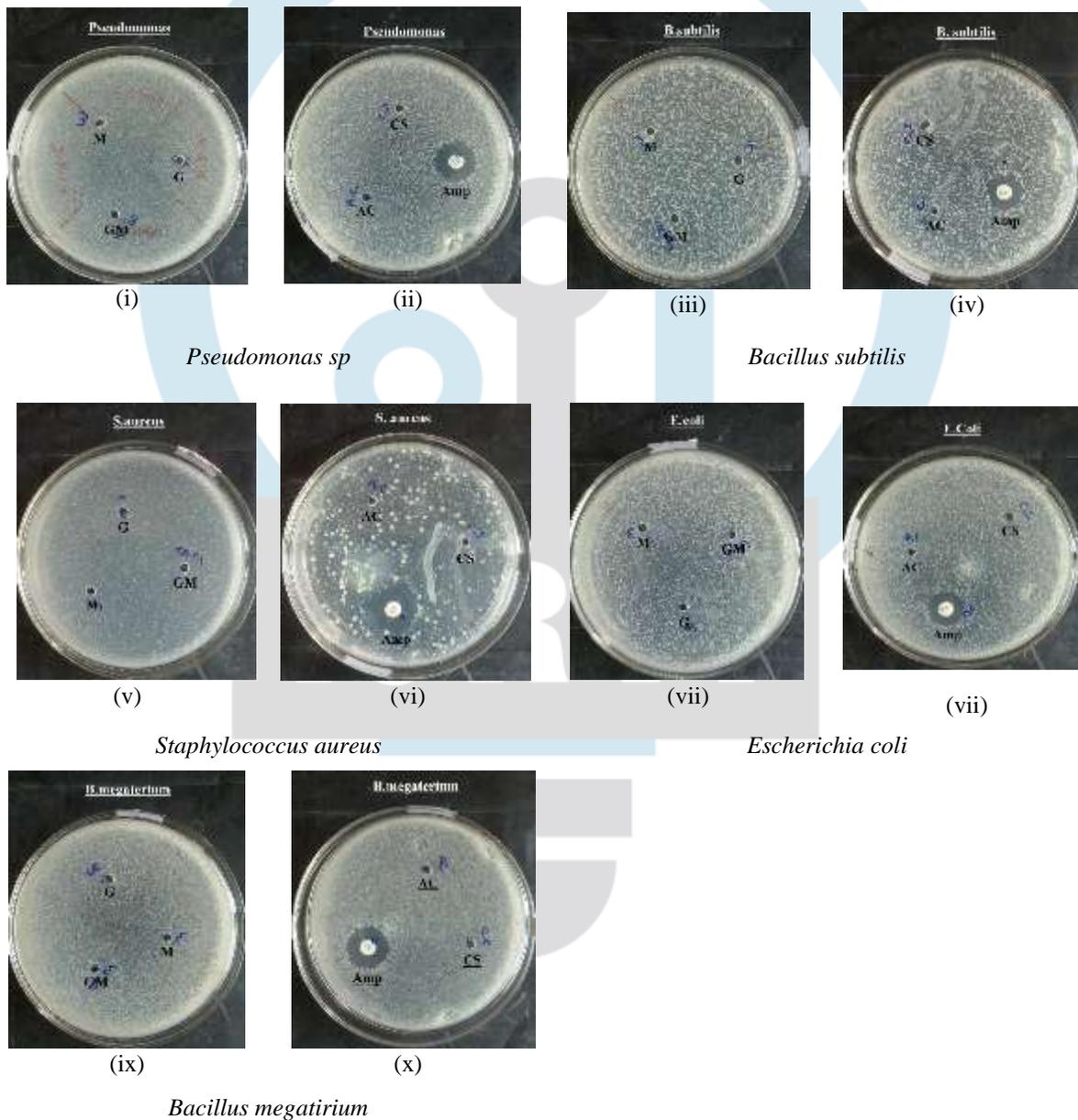


Figure 4. Antimicrobial activity of all samples of coconut water against *Pseudomonas sp*, *Bacillus megaterium*, *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*

**CONCLUSION:**

The present study has focused on two parameters to analyze the qualitative as well as quantitative analysis of various phytochemicals and ions, antimicrobial activity was also evaluated. To perform the objectives total 5 samples were chosen (Green, Green-Mature, Mature, 2 packaged coconut water: Active cool, Coco sip). This is the first time report on comparative study of different coconut water preserved in different conditions such as room temperature and refrigerator. Based on the results, it was revealed that on storage of coconut water had laid to decrease of phenolic contents, and increase of ascorbic acid whereas in terms of carbohydrates it was increasing in all sample stored at room temperature but samples stored in refrigerated condition showed increase in natural sample and decrease in packed sample. The increase and decrease of components have impact on consumers such as decrease in antioxidant ability due to phenol content deterioration (27), increase of diabetes and heart strokes due to increase in carbohydrates (28), on decrease may scavenge the essential nutrients. Based on % of increase and decrease of phenolic content there was a decreased in phenolic content after storage in both cases (B and C). But the highest effect was found to be on mature coconut water sample B, while the least effect was found on Active cool packed coconut water sample C. Green mature and Green coconut water sample C were showing decrease in total phenolic content. Almost in all samples, sample C has more total phenolic content compared with sample B except Active cool packed coconut water. In case of ascorbic acid, highest increase was found in mature coconut water sample C, while the least was found in Coco sip packed coconut water sample B. For Green-mature coconut water sample B and C, increase was found to be same of 75%. In case of total carbohydrates, highest increase was found in Green mature coconut water sample B, while least increase was found in Green coconut water sample B. Whereas in packaged coconut water sample C, concentration of total carbohydrates were decreased. Our study recommends the usage of natural coconut water as fresh as possible without storing in any conditions.

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