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Research Article

COMPARATIVE PHYTOCHEMICAL ANALYSIS AND ANTIDIABETIC ACTIVITIES OF TENDER AND RIPENED COCONUT FRUIT PARTS

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ABSTRACT

Objectives: Diabetes mellitus is a chronic metabolic disorder, which leads to a decrease in quality of life and even death. There are various treatments for diabetes available in the market, but they have several side effects. In the search for natural antidiabetic agents or medicinal plants, coconut (*Cocos nucifera L.*) is a suitable choice due to its lesser adverse effects. This study investigates and compares the phytochemical profile and antidiabetic activities of tender and ripened coconut parts (e.g., husk, water, and milk).

Methods: Different quantitative and qualitative tests were conducted to identify various bioactive compounds, such as alkaloids, flavonoids, phenolics, tannins, etc., and antidiabetic potential was evaluated through α-amylase and α-glucosidase enzyme inhibition assays using *in vitro* models to evaluate glucose-lowering potential.

Results: Both quantitative and qualitative assays showed differences in bioactive substances in tender and ripened coconut fruit parts. Antidiabetic activity showed that both ripened and tender coconut husks had significant inhibition, which is comparable to the standard drug acarbose, showing strong antidiabetic potential. The ripened coconut husk showed a half maximal inhibitory concentration value of 821.45 µg/mL for α-amylase and 1172.82 µg/mL for α-glucosidase, while tender coconut husk showed 1079.11 µg/mL for α-amylase and 1196.76 µg/mL for α-glucosidase ($p<0.05$ compared to control). Both the ripened and tender coconut milk showed low to minimal antidiabetic activity, while coconut water demonstrated moderate activity.

Conclusion: Both ripened and tender coconut husks demonstrated strong antidiabetic potential. Coconut water showed moderate activity, while coconut milk was the least effective.

Keywords: Diabetes mellitus, Antidiabetic activity, *Cocos nucifera*, α-amylase inhibition, α-glucosidase inhibition.

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INTRODUCTION

Diabetes is a long-term metabolic condition characterized by persistent hyperglycemia. This condition occurs because of a malfunction in insulin production or resistance to its action [1,2]. It affects the quality of life and increases complications (e.g., delayed wound healing, cardiovascular disease), and can also lead to disability and death [3]. There are several conventional methods to manage diabetes, such as insulin injections (for type 1 diabetes) and various hypoglycemic agents (for type 2 diabetes), including metformin, thiazolidinediones, etc. These medicines are effective in controlling diabetes but have side effects like gastrointestinal disturbances, hypoglycemia, hepatotoxicity, weight gain, and even increased cardiovascular risk [4-7]. Along with these side effects, they are also costly, making them less affordable for low-income groups. Hence, the demand for a natural, safe, and cost-effective plant-based alternative is increasing day by day, which can meet all the criteria and be available worldwide.

Coconut is a versatile tree also known for its medicinal properties [8]. It has the scientific name *Cocos nucifera L.*, and is a tall, unbranched tree classified under the family *Arecaceae* [9]. Coconut has different synonyms like copra, coco, coconut kernel, coconut meat, buko, etc., and vernacular names like–in Bengali: narkel, dab; in Hindi: Nariyal, shreephal; in Tamil: tenkay; in Telugu: Kobbari; in Kannada: Haralu; in Marathi: Naral; in Punjabi: Khopra; in Thai: Ma-phrao on; in Indonesian: Degan, etc.

From fruit to root, every part of this plant has medicinal use. Comparison between different fruit parts was conducted in both stages (ripened and tender) because biochemical composition changes with time and maturity [10]. Tender coconuts are rich in electrolytes and sugars, whereas ripened coconuts contain high quantities of saturated fats and phenolic compounds. Phytochemical analysis provides details about all the components available at different maturity stages, which are responsible for antidiabetic activity. Hence, in this study, the antidiabetic potential is compared between different coconut fruit parts in tender and ripened stages.

METHODS

Chemicals, reagents, and equipment

All the chemicals, reagents, and enzymes were obtained from a reputable supplier and used according to the manufacturer's instructions. The fruits of tender and ripened coconut were collected from the local market of Sodepur, North 24 Parganas, India. The plant specimen was authenticated by the Botanical Survey of India (CNH), Howrah. A voucher specimen was deposited under the number GNIPST/2025/06.α-Amylase (from *porcine pancreas*) and α-glucosidase (from yeast) were purchased from Sisco Research Laboratories, India. All other chemicals and reagents used were of analytical grade. An analytical balance (Citizen, India), ultraviolet (UV)-visible spectrophotometer (Systronics 2201, India), centrifuge (Remi R-8C, India), and Incubator (Tempo Instruments, India) were used during procedures.

Preparation of coconut samples

The outer surfaces of both fruits were washed. The ripened and tender coconut husks were removed. Then they were dried under shade conditions for 2–3 days. After that, husk powder was made using a mixer grinder to obtain coarse powder. The ripened and tender coconut meats were removed with a knife and then a smooth paste

was made using distilled water and strained to obtain milk. Ripened and tender coconut water was directly collected from the ripened and tender coconut fruit.

Preparation of extracts

Twenty-five grams ripened coconut husk and 25 g of tender coconut husk powder were subjected to decoction with 250 mL of distilled water in two separate beakers at 90°C for 60 min [11]. Both the husk extracts were separately filtered in a suction pump. Liquid of both extracts were evaporated with the help of a hot water bath to make a dry extract for the purpose of storage.

Phytochemical screening

A total of six extracts were examined for the presence of various phytochemicals using established qualitative methods [12,13].

Determination of total phenolic, flavonoid, and alkaloid content

The total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) of the extracts were estimated using standard spectrophotometric procedures [14,15].

Determination of antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

To prepare the DPPH solution, 4 mg of DPPH were mixed with 100 mL of methanol in a small glass beaker and thoroughly mixed until a deep violet color appeared. As DPPH is particularly light-sensitive, it was then wrapped with aluminum foil.

- Standard solution preparation: 10 mg of ascorbic acid was accurately weighed and mixed with 10 mL of methanol (conc. 1 mg/mL).
- Test solution preparation: 1 mL of each solution (plant extract solution or standard solution) was mixed with 1 mL of recently prepared DPPH solution. For approximately 30 min, the mixture was kept at room temperature. DPPH and methanol (ratio 1:1) were used as the blank.
- Spectrophotometric measurement: The absorbance of the final solutions was recorded at 517 nm with the help of a UV-Visible spectrophotometer [16]. The scavenging activity was calculated using:

$$\text{Scavenging activity (\%)} = [[(\text{Absorbance}^{\text{Control}} - \text{Absorbance}^{\text{Sample}}) \times 100] / \text{Absorbance}^{\text{Control}}]$$

Determination of antidiabetic activity using the α-amylase inhibition assay

Dinitrosalicylic (DNS) solution preparation: 1 g of DNS was dissolved in 20 mL of NaOH. Thirty grams of sodium potassium tartrate was added and the volume was made up to 100 mL with distilled water.

- Starch solution preparation: 1 g of soluble starch was diluted into 100 mL of phosphate buffer (pH 6.9). A 1% starch solution was prepared.
- Extract solution preparation: Extract solutions of 5 different concentrations were taken (100 µg/mL, 500 µg/mL, 1000 µg/mL, 1500 µg/mL, 2000 µg/mL).
- Reaction setup: 0.5 mL phosphate buffer (pH 6.9), 0.5 mL α-amylase (1 U/mL), and 0.5 mL of the test sample at different concentrations were mixed together. Incubated at 37°C for 10 min. And 0.5 mL of 1% starch solution was added and incubated for another 10 min. Then the DNS solution was added to each and heated in a water bath (90°C–100°C) for 5 min. Finally, cool to room temperature and dilute with distilled water.
- For standard: Acarbose was used (same concentrations as the extract solution) and carry out the same process.
- For positive control: Similar to the extract solution, but without the extract and without the inhibitor.
- Spectrophotometric measurement: The absorbance of the final solutions was recorded at 540 nm with the help of UV-Visible spectrophotometer [17,18].

$$\text{\% Inhibition} = [[(\text{Absorbance}^{\text{Control}} - \text{Absorbance}^{\text{Sample}}) \times 100] / \text{Absorbance}^{\text{Control}}]$$

Determination of antidiabetic activity using the α-glucosidase inhibition assay

p-nitrophenyl-α-D-glucopyranoside (pNPG) solution preparation: 7.51 mg of pNPG was dissolved in 10 mL of phosphate buffer (pH 6.9) to prepare a 5 mM pNPG solution.

Sodium carbonate (Na_2CO_3) solution: 1.06 g of Na_2CO_3 was dissolved in 100 mL of distilled water to prepare a 0.1 M Na_2CO_3 solution.

Extract solution preparation: Extract solutions of 5 different concentrations were taken (100 µg/mL, 500 µg/mL, 1000 µg/mL, 1500 µg/mL, 2000 µg/mL).

Reaction setup: 0.5 mL phosphate buffer (pH 6.9), 0.5 mL α-glucosidase (1 U/mL), and 0.5 mL of the test sample at different concentrations were mixed together. Incubated at 37°C for 10 min. Moreover, 0.5 mL of pNPG solution was added and incubated for another 20 min. Then 2 mL Na_2CO_3 solution was added to each, and lastly cooled to room temperature.

For standard: Acarbose was used (same concentrations as the extract solution) and carry out the same process.

For positive control: Similar to the extract solution, but without the extract and without the inhibitor.

Spectrophotometric measurement: The absorbance of the final solutions was recorded at 405 nm with the help of UV-Visible spectrophotometer [19,20].

$$\text{\% Inhibition} = [\{(\text{Absorbance}^{\text{Control}} - \text{Absorbance}^{\text{Sample}}) \times 100\} / \text{Absorbance}^{\text{Control}}]$$

Statistical analysis

Statistical analysis was performed using Microsoft Excel and GraphPad Prism. All experiments were conducted in triplicates, and results are expressed as mean ± standard deviation. Half maximal inhibitory concentration (IC_{50}) values were calculated using linear regression analysis based on dose-response data. One-way analysis of variance was considered for assessing statistical significance, with $p<0.05$ as the threshold.

RESULTS

Phytochemical screening

The result of phytochemical screening revealed the presence of alkaloids, tannins, phenols, and terpenoids in both ripened and tender coconut husk extracts. Ripened and tender coconut milk and water showed the presence of carbohydrates and amino acids. Ripened and tender coconut husk and milk showed the presence of glycosides (Table 1).

TPC, TFC, TAC

Results showed that among all parts, ripened coconut husk exhibited the highest TAC, while tender coconut husk showed the highest flavonoid content. Ripened coconut milk showed the highest phenolic content (Table 2).

Antioxidant activity using DPPH

Results of the DPPH assay for standard and different coconut fruit parts are given in Table 3. It showed that tender and ripened coconut husk had significantly high antioxidant activities.

Antidiabetic activity using the α-amylase and α-glucosidase inhibition assays

Tables 4 and 5 showed the % inhibition. Among all the parts, ripened and tender coconut husk exhibited the highest inhibition for both assays. Table 6 shows a comparison of the IC_{50} value between different coconut fruit parts. Fig. 1 represents a comparison of IC_{50}.

DISCUSSION

For this research, coconut fruit parts were analyzed, which include ripened coconut husk, tender coconut husk, ripened coconut milk,

Table 1. Comparison of phytochemical screening between different parts of the coconut fruit

S. No.	Test for	Test	Ripened coconut husk	Tender coconut husk	Ripened coconut milk	Tender coconut milk	Ripened coconut water	Tender coconut water
1.	Alkaloids	Wagner's test	+	+	-	-	-	-
		Hager's test	+	+	-	-	-	-
2.	Amino acids	Ninhydrin test	-	-	++	++	++	+++
3.	Carbohydrates	Molisch's test	-	-	++	++	++	++
		Fehling's test	-	-	+	+	+++	+++
4.	Flavonoids	Shinoda test	+	+	+	+	-	-
5.	Glycosides	Froth formation test	++	++	+	+	-	-
6.	Tannins	Ferric chloride test	+++	+++	-	-	-	-
7.	Terpenoids	Salkowski test	+	+	-	-	-	-
8.	Phenols	Ferric chloride test	+	+	-	-	-	-
9.	Starch	Iodine test	-	-	-	-	-	-

"+++" : Strong presence; "++" : Moderate presence; "+" : Weak presence; "-" : Absent

Table 2: Comparison of total phenolic, total flavonoid, and total alkaloid content between different parts of coconut fruit

S. No.	Sample	TPC (mg GAE/g)	TFC (mg QE/g)	TAC (mg AE/g)
1.	Ripened coconut husk	3.52±0.14	11.72±0.31	12.44±0.27
2.	Tender coconut husk	2.02±0.18	15.42±0.67	5.04±0.56
3.	Ripened coconut milk	6.76±0.23	9.54±0.59	3.65±0.25
4.	Tender coconut milk	3.37±0.16	8.42±0.44	4.46±0.14
5.	Ripened coconut water	1.15±0.13	2.20±0.38	2.02±0.62
6.	Tender coconut water	1.98±0.21	1.43±0.17	3.71±0.11

Values are expressed as mean±SD (n=3). TPC: Total phenolic content, TFC: Total flavonoid content, TAC: Total alkaloid content. GAE: Gallic acid equivalent, QE: Quercetin equivalent, AE: Atropine Equivalent

Table 3: Results of DPPH assay for standard and different coconut fruit parts

S. No.	Sample	% RSA
1.	Ascorbic acid	60.58±0.11
2.	Ripened coconut husk	49.34±0.46
3.	Tender coconut husk	56.13±0.52
4.	Ripened coconut milk	36.18±0.33
5.	Tender coconut milk	47.12±0.25
6.	Ripened coconut water	16.05±0.84
7.	Tender coconut water	20.14±0.52

Values are mean±SD (n=3). DPPH: 2,2-diphenyl-1-picrylhydrazyl, RSA: Radical scavenging activity

tender coconut milk, ripened coconut water, and tender coconut water. Phytochemical tests were conducted. The results showed ripened and tender coconut husks indicated the presence of tannins in very high quantity, while glycosides were present in moderate, and alkaloids, terpenoids, flavonoids, and phenols were present in low quantity. Ripened and tender coconut milk contained moderate amounts of amino acids and carbohydrates, while low quantities of glycosides and flavonoids [21]. Ripened and tender coconut water showed the presence of carbohydrates and amino acids in very high quantity.

Determination of TPC was conducted, which showed among these six, ripened coconut milk contained the highest amount, which was 6.76 mg gallic acid equivalent (GAE)/g, followed by ripened coconut husk 3.52 mg GAE/g. Then TPC for tender coconut milk was 3.37 mg GAE/g, for tender coconut husk was 2.02 mg GAE/g, for tender coconut water was 1.98 mg GAE/g, and for ripened coconut water was 1.15 mg GAE/g.

When TFC was determined, it showed tender coconut husk demonstrated the highest amount, which was 15.42 mg QE/g, followed by ripened coconut husk 11.72 mg QE/g, ripened coconut milk

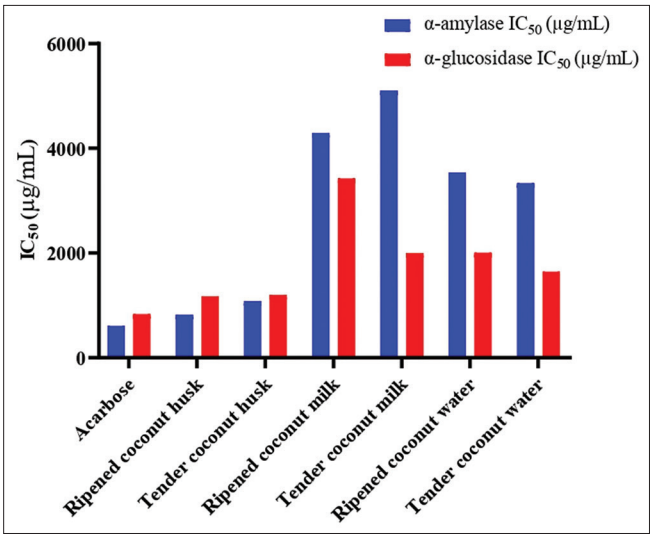

Fig. 1: Comparison of half-maximal inhibitory concentration values for α-amylase and α-glucosidase inhibition assays among coconut fruit parts. Acarbose was used as the standard. IC_{50}: Half maximal inhibitory concentration

9.54 mg QE/g, tender coconut milk 8.42 mg QE/g, ripened coconut water 2.20 mg QE/g, and tender coconut water 1.98 mg QE/g.

For TAC, the highest amount was found in ripened coconut husk 12.44 mg AE/g, then tender coconut husk 5.04 mg AE/g, tender coconut milk 4.46 mg AE/g, ripened coconut milk 3.65 mg AE/g, tender coconut water 3.71 mg AE/g, and ripened coconut water 2.02 mg AE/g. To check for antioxidant activity, the DPPH assay was performed. DPPH assay showed that tender coconut husk had the maximum radical scavenging activity, which was 56.13% [16], then ripened coconut husk 49.34%, tender coconut milk 47.12%, ripened coconut milk 36.18%, tender coconut water 20.14%, and lastly ripened coconut water 16.05%.

To check for antidiabetic activity, α-amylase and α-glucosidase assays were done. α-amylase inhibition assay showed tender coconut milk had the highest IC_{50} value, which was 5102.05 µg/mL, followed by ripened coconut milk 4291.00 µg/mL, ripened coconut water 3538.76 µg/mL, tender coconut water 3334.38 µg/mL, tender coconut husk 1079.77 µg/mL, ripened coconut husk 821.45 µg/mL, and IC_{50} value for standard acarbose was 604.50 µg/mL. Higher the IC_{50}, the lower the antidiabetic potential. So, by analyzing these IC_{50} values, antidiabetic potential was determined. Standard acarbose had the highest antidiabetic potential. Among the coconut fruit parts, ripened coconut husk and tender coconut husk showed maximum antidiabetic activity [16], while tender coconut water and ripened coconut water showed moderate antidiabetic activity, and ripened coconut milk and tender coconut milk showed low to no antidiabetic activity.

Table 4: % Inhibition for α-amylase inhibition assay for standard and different coconut fruit parts

S. No.	Concentration (µg/mL)	% Inhibition						
		Standard acarbose solution	Ripened coconut husk	Tender coconut husk	Ripened coconut milk	Tender coconut milk	Ripened coconut water	Tender coconut water
1.	100	27.15±0.71	26.22±0.28	22.27±0.52	6.73±0.38	10.10±0.49	12.93±0.24	11.12±0.57
2.	500	53.23±0.43	47.84±0.92	40.15±0.63	12.67±0.29	14.28±0.71	19.86±0.66	17.25±0.14
3.	1000	67.77±0.11	57.45±0.26	53.39±0.58	19.45±0.75	19.56±0.56	23.11±0.82	27.39±0.87
4.	1500	75.57±0.27	67.64±0.33	62.31±0.65	22.93±0.62	21.72±0.37	27.53±0.36	29.90±0.71
5.	2000	86.55±0.28	74.82±0.41	65.38±0.17	25.39±0.37	25.23±0.48	34.44±0.11	32.30±0.65

Values are expressed as mean±SD (n=3)

Table 5: % Inhibition for α-glucosidase inhibition assay for standard and different coconut fruit parts

S. No.	Concentration (µg/mL)	% Inhibition						
		Standard acarbose solution	Ripened coconut husk	Tender coconut husk	Ripened coconut milk	Tender coconut milk	Ripened coconut water	Tender coconut water
1.	100	17.12±0.43	14.32±0.53	23.15±0.66	2.13±0.13	8.44±0.72	8.11±0.84	10.11±0.42
2.	500	40.97±0.15	27.67±0.58	35.31±0.26	4.76±0.42	25.83±0.47	18.40±0.47	26.89±0.69
3.	1000	55.00±0.72	50.46±0.43	47.33±0.58	17.02±0.94	29.39±0.63	21.07±0.39	36.22±0.74
4.	1500	85.59±0.64	61.15±1.02	63.15±0.92	22.20±0.14	41.80±0.48	46.18±0.84	50.54±0.88
5.	2000	87.58±0.72	72.79±0.58	69.94±0.51	28.33±0.65	48.34±0.92	47.22±0.45	54.15±0.57

Values are expressed as mean±SD (n=3)

Table 6: Comparison of IC_{50} value for α-amylase and α-glucosidase inhibition assay

S. No.	Sample	IC_{50} value for α-amylase (µg/mL)	IC_{50} value for α-glucosidase (µg/mL)
1.	Acarbose	604.50	830.46
2.	Ripened coconut husk	821.45	1172.82
3.	Tender coconut husk	1079.77	1196.76
4.	Ripened coconut milk	4291.00	3423.34
5.	Tender coconut milk	5102.05	1999.70
6.	Ripened coconut water	3538.76	2006.29
7.	Tender coconut water	3334.38	1644.19

IC_{50}: Half maximal inhibitory concentration. IC_{50} values were calculated from mean % inhibition data of triplicate experiments, without error margins

α-glucosidase inhibition assay showed ripened coconut milk had the highest IC_{50} value, which was 3423.34 µg/mL, followed by ripened coconut water 2006.29 µg/mL, tender coconut milk 1999.70 µg/mL, ripened coconut water 1644.19 µg/mL, tender coconut husk 1196.76 µg/mL, and ripened coconut husk 1172.82 µg/mL. IC_{50} value for acarbose standard was 830.46 µg/mL. Standard acarbose had the highest antidiabetic potential. Among the coconut fruit parts, tender coconut husk and ripened coconut husk showed maximum antidiabetic activity, while tender coconut water, ripened coconut water, and tender coconut milk showed moderate antidiabetic activity, and ripened coconut milk showed low to no antidiabetic activity.

CONCLUSION

This study was a comparison of the phytochemical and antidiabetic activity of six different parts (ripened and tender coconut husk, milk, and water) of *C. nucifera L.* Antidiabetic activity through α-amylase and α-glucosidase inhibition assays showed that both ripened and tender coconut husks had significant inhibition, which is comparable to the standard drug acarbose, showing strong antidiabetic potential. Both the ripened and tender coconut milk showed low to minimal antidiabetic activity, while coconut water demonstrated moderate activity.

From this study, we can conclude that when six parts of the coconut fruit were compared, ripened and tender coconut husks demonstrated the most promising phytochemical and antidiabetic properties, and they can serve as cost-effective, natural candidates for further study in diabetes management.

AUTHORS' CONTRIBUTION

The first author conducted the research, performed the experiments, analyzed the data, and prepared the manuscript. The corresponding author conceptualized the work. The co-authors provided academic guidance, supervision, and reviewed the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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