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## Proximate composition, antioxidant, anti-inflammatory and antidiabetic properties of the haustorium from Coconut (*Cocos nucifera* L.) and Palmyra palm (*Borassus flabellifer* L.)



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#### ARTICLE INFO

Article history: Received 23 June 2022 Revised 11 October 2022 Accepted 24 October 2022 Available online 9 November 2022

Keywords: Coconut Palmyra palm Haustorium Proximate composition Antioxidant activity Anti-inflammatory activity

### ABSTRACT

*Objective:* Functional foods play important roles in the management of health; the haustoria from the *Cocos nucifera* and *Borassus flabellifer* are less explored for their nutritional benefits. Therefore, the study evaluated the proximate composition, biological and anti-diabetic effects of the CH and BH.

*Methods:* The total polyphenol content of coconut and palmyra palm haustorium was conducted using the standard Folin-Ciocalteu based assay. The radical scavenging properties of Coconut and Palmyra palm haustorium was analyzed by DPPH radical scavenging assay, Lipid peroxidation inhibition as well as by hydrogen peroxide scavenging assay. Anti-diabetic activity of the coconut and palmyra palm haustorium extracts were analyzed in terms of the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase activities.

*Results:* The results indicated significantly higher amounts of protein, fiber, and fat content in BH. Similarly, higher polyphenol content was present in BH. Substantiating these results, the anti-DPPH radical and anti-hydrogen peroxide radical was higher in BH ( $18.66 \pm 2.09$  and  $30.73 \pm 2.37 \mu g/mL$ ); likewise, the BH had higher lipoygenase inhibition and nitric oxide scavenging potential ( $42.14 \pm 3.45$  and  $72.12 \pm 4.02 \mu g/mL$ ). Both the extracts exhibited anti-diabetic activity ( $58.61 \pm 2.98$  and  $91.17 \pm 3.52 \mu g/mL$ ), however a higher concentration was required for CH compared to the BH.

*Conclusion:* The Palmyra palm and coconut haustorium are highly nutritional in terms of the various components. By virtue of these compounds, the BH and CH may evolve as possible functional foods over time.

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#### 1. Introduction

Functional foods are gaining much attention as a nutritional substitute as well as a pharmaceutical agent. Several underexplored plant products are available with potential biological

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and pharmacological properties. Among these, the *Cocos nucifera* and *Borassus flabellifer* are widely distributed in the Southern parts of Indian Subcontinent. Consumption of the various products from Coconut are common in India and other countries; this include, the fruit, water, milk, toddy and edible oils extracted from their nuts. Coconut is well-studied for the biopharmaceutical and nutritive values; different coconut products such as edible oils, coconut water, kernel milk and proteins are well-known functional foods. The edible oils especially virgin coconut oil is known for its antioxidant (Illam et al., 2017), anti-inflammatory (Famurewa et al., 2017; Famurewa et al., 2020), hypoglycemic, hypolipidemic (Narayanankutty et al., 2018), and anticancer (Narayanankutty et al., 2020), anti-inflammatory (Ratheesh

https://doi.org/10.1016/j.jksus.2022.102404

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et al., 2017), and nephroprotective (Jose et al., 2017) properties. The protein isolated from coconut kernel is reported to have strong hypoglycemic and hypolipidemic (Salil et al., 2014) properties. Coconut milk has been proven to inhibit gastric ulcers in animal models (Ajeigbe et al., 2017). Coconut water is another important component with antimicrobial (Anaya et al., 2020) and antitumor (Mohamad et al., 2019) properties. Compared to coconut, the Borassus flabellifer (Palmyra palm) is less explored; Available reports have elucidated the anti-glycemic properties of flower (Yoshikawa et al., 2007), root (Debnath et al., 2013) and fruit (Duraipandiyan et al., 2020) extracts of palmyra palm. Furthermore, the anti-proliferative potential of a triterpenoid from the seed coats of the plant is also observed against prostate cancer cells (Yarla et al., 2015). Besides, the extract is also having significant inhibitory effects of the microbial growth in vitro (Reshma et al., 2017).

Similar to other products from coconut and Palmvra palm. haustorium is also consumed widely in India. Consuming coconut haustorium has been shown to improve antioxidant potential (Manivannan et al., 2018; Nyayiru Kannaian et al., 2020). In addition, CH haustorium consumption has been shown to protect against fluoride-induced intestinal toxicity in rat intestinal epithelial cells (Job et al., 2021c). Our recent studies indicated that the methanol extract of Palmyra palm protects the cells from different radical-mediated apoptotic cell death via modulating Nrf2 signaling pathway (Job et al., 2021a; Job et al., 2021b; Malayil et al., 2021). However, there is no available literature on the nutritional content of the haustorium of coconut and Palmyra palm. In order to establish the functional food nature of CH and BH, it is necessary to evaluate the nutritional and biological properties of the haustorium. In addition, there needs a comparative study to examine which of this haustorium is more nutritious and health beneficial. Hence, the present study investigated the nutritional content as well as biological properties of the coconut (CH) and Palmyra palm haustorium (BH).

#### 2. Materials and methods

#### 2.1. Chemicals

The chemicals used in the study are of reagent grade; this includes methanol, DPPH, aluminium chloride, Folin-ciocalteu reagent, sodium carbonate, and hydrogen peroxide. The instruments used include UV–Visible spectrophotometer, soxhlet apparatus, vacuum concentrator, centrifuge and.

#### 2.2. Haustoria collection and extract preparation

The coconut haustorium (CH) was collected from the Puthoormadam locality of Calicut district (11.22939° N, 75.87079° E), whereas Palmyra palm haustorium (BH) was obtained from the outskirts of Pallipuram, Palakkad district (10.76932° N, 76.63621° E). The individual haustoria were collected and dried; the extraction was done using Soxhlet apparatus set at 65 °C. All the extracts were dried under vacuum concentration and finally dissolved to 100 mg/mL (w/v) in dimethyl sulfoxide.

#### 2.3. Proximate composition

The nutritional value of the haustorium was determined in terms of the proximate composition; the various parameters explored include the moisture content of the haustorium (AOAC 934.01), total ash content (AOAC 938.08), percentage of crude fat (AOAC 920.58) and crude protein present (AOAC 2001.11), as well as the fiber content (AOAC 985.29). All the analytical methods

were adhering to the protocols described by official methods laid down by the Association of Official Analytical Collaboration (2016). The mineral composition was estimated by atomic absorption spectrometer according to the methods of Anal (2014).

#### 2.4. Phytochemical screening

#### 2.4.1. Estimation of total phenols

The quantitative determination of the total polyphenol content of coconut and palmyra palm haustorium was conducted using the Folin-Ciocalteu-based method (Ahmed and Tavaszi-Sarosi, 2019). The final quantity was expressed in terms of the gallic acid equivalent per gram of the haustorium.

### 2.4.2. LC-MS analysis

The LC-MS analysis protocol was according to the methods described by Illam et al. (2017). Briefly, the Shimadzu LC-MS 2020 system was used for the analysis with the solvent system Methanol: Acetonitrile using the gradient elution method as described earlier.

# 2.5. Radical scavenging properties of coconut and Palmyra palm haustorium

The antioxidant effects of these haustoria was analyzed using the scavenging of DPPH radicals (Liu et al., 2020), hydrogen peroxide (Ahmed and Tavaszi-Sarosi, 2019) and ex vivo lipid peroxidation inhibition (Mahomoodally et al., 2019).

# 2.6. Inhibition of inflammation by coconut and Palmyra palm haustorium

Lipoxygenase inhibition assay was carried out by mixing the coconut and palmyra palm haustorium methanol extract with a reaction system composed of 100 Units/mL lipoxygenase enzyme and 120 nmoles/mL of linoleic acid. Change in the optical density at 234 nm was recorded for 3 min and the inhibition by different concentration of extracts were plotted for the calculation of IC50 values (Ben-Nasr et al., 2015). Scavenging of the nitrate radicals was conducted by mixing the different doses of both extracts with nitric oxide radical generator (sodium nitroprusside) and incubating for 3 h in the dark. At the end of incubation the mixture was mixed with equal volumes of Griess' reagent 1 M sulphanilamide and 0.1 NEDA and the absorbance was measured (Mahomoodally et al., 2019).

#### 2.7. anti-diabetic activities of coconut and Palmyra palm haustorium

Anti-diabetic activity of the coconut and palmyra palm haustorium extracts were analyzed in terms of the alpha-amylase and alpha-glucosidase inhibition as previously described protocols by Patel and Ghane (2021). The  $\alpha$ -Amylase inhibitory activity was determined as the ability to prevent the conversion of starch by the enzyme and the  $\alpha$ -Glucosidase inhibition assay was carried out in terms of 4-Nitrophenyl  $\alpha$ -D-glucopyranoside cleavage inhibition by the methanol extracts.

The percentage inhibition in various in vitro assays was determined by comparing the absorbance as;

$$\% \ Inhibition = \frac{Control - Sample \ (dose)}{Control} \times 100$$

#### 2.8. Statistical analysis

The data of each parameter analyzed were represented in the Mean  $\pm$  SD format of individual experiments carried out. The sig-

nificance of variation was analyzed by one way ANOVA together with Tukey Kramer multiple comparison tests (GraphPad Prism).

#### 3. Results

3.1. Analysis of the proximate composition, fatty acid and mineral contents

The proximate composition of coconut and Palmyra palm haustorium has been given in Table 1. The moisture and protein content as well as soluble fibre is present in higher quantities in coconut haustorium. On contrary, ash content, crude fat, carbohydrate and insoluble fat is high in Palmyra palm haustorium (Table 1).

The fatty acid composition has been listed in Table 2; the total saturated fat content was high in the haustorium of coconut, than the BH. On the other hand, the quantity of medium chain fats was high in the BH (9.82 %), which was 8.63 % in CH. The quantity of lauric acid, whose properties are similar to that of MCFAs, was high in CH than the BH. Unsaturated fatty acids were present in both the haustorium, with a higher level in Palmyra palm.

The minerals present in CH and BH are presented in Table 3. The levels of calcium, potassium and phosphorus were found to be significantly (p < 0.05) high in the Palmyra palm haustorium. The levels of zinc, magnesium and copper were marginally high in Palmyra palm, however with no statistical significance.

# 3.2. In vitro anti-radical, inflammation inhibition and anti-diabetic activity

The IC<sub>50</sub> values of both coconut and Palmyra palm haustorium were calculated and represented in Table 4. The IC<sub>50</sub> value of DPPH radical scavenging activity of BH was 18.66 ± 2.09 µg/mL, whereas the CH had an IC50 value of 27.67 ± 2.34 µg/mL. Similarly, the hydrogen peroxide scavenging (30.73 ± 2.37 µg/mL) and lipid peroxidation inhibition (48.62 ± 3.44 µg/mL) were also high in BH in comparison with CH.

On contrary, the anti-inflammatory properties were found to be almost similar for both the extracts. The IC50 value of LPX inhibition assay in BH treatment was  $42.14 \pm 3.45 \ \mu g/mL$ , which was 54.  $19 \pm 7.32 \ \mu g/mL$  in CH. Similarly, the IC50 value for the scavenging of nitric oxide radical was  $72.12 \pm 4.02 \ \mu g/mL$  in BH and  $87.19 \pm 3$ .  $45 \ \mu g/mL$  in CH.

Anti-diabetic properties of the BH and CH were compared in terms of  $\alpha$ - amylase and  $\alpha$ - glucosidase inhibitory potentials. The anti-diabetic property was also estimated to be higher in BH with respective IC50 values 58.61 ± 2.98 and 91.17 ± 3.52 µg/mL for  $\alpha$ - amylase and  $\alpha$ - glucosidase inhibition assays. Whereas, a significantly lower IC50 value was observed for CH for these assays (Fig. 1).

#### 3.3. Total phenolic content and LC-MS analysis

The total polyphenol content in the haustorium of Palmyra palm was significantly higher than that of the haustorium of *Cocos* 

 Table 1

 Variation in the proximate compositions of coconut and palmyra palm haustorium.

Content	Coconut haustorium	Palmyra haustorium
Moisture content (%)	87.4 ± 2.12	83.9 ± 1.65
Ash content (%)	0.98 ± 0.02	1.25 ± 0.13
Crude fat (%)	1.88 ± 0.11	2.44 ± 0.20
Protein (%)	5.7 ± 0.22	4.68 ± 0.32
Carbohydrate (%)	64.2 ± 3.3	67.96 ± 1.34
Dietary soluble Fibre	5.74 ± 0.44	5.04 ± 0.15
Dietary insoluble Fibre	19.25 ± 3.22	24.1 ± 1.88

#### Table 2

Fatty acid composition of the haustorium obtained from coconut and palmyra palm.

Fatty acid	Coconut haustorium	Palmyra haustorium
Caproic acid (C6:0)	0.28	0.54
Caprylic acid (C8:0)	3.59	4.02
Capric acid (C10:0)	4.76	5.26
Lauric acid (C12:0)	42.79	40.7
Myristic acid (C14:0)	17.4	18.1
Palmitic acid (C16:0)	13.57	12.9
Stearic acid (C18:0)	5.8	5.1
Oleic acid (C18:1)	10.3	12.3
Linoleic acid (C18:2)	1.5	1.08

Table 3
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Mineral and trace element content in Coconut and palmyra palm haustorium.

Minerals	Coconut haustorium	Palmyra haustorium
Sodium	67.9 ± 3.2	79.1 ± 2.3*
Potassium	122.6 ± 4.5	149.6 ± 10.4*
Calcium	39.4 ± 2.8	46.7 ± 1.8*
Zinc	0.9 ± 0.06	$1.2 \pm 0.14$
Magnesium	129.4 ± 4.9	141.5 ± 5.7
Phosphorus	36.2 ± 1.8	48.4 ± 3.0*
Copper	0.18 ± 0.02	0.19 ± 0.03

\* indicate significant difference at p < 0.05.

#### Table 4

The variation in total polyphenol content (expressed as mg GAE/g) and IC50 values of different antioxidant, anti-inflammatory and anti-diabetic assays between the Coconut and Palmyra palm (*Borassus flabellifer*) haustorium extract.

Assay	Coconut haustorium	Palmyra haustorium
Total polyphenol content	179.45 ± 8.77	214.28 ± 8.31*
DPPH scavenging	27.67 ± 2.34	18.66 ± 2.09*
H <sub>2</sub> O <sub>2</sub> scavenging	44.07 ± 1.48	30.73 ± 2.37*
Lipid peroxidation inhibition	83.51 ± 3.34	48.62 ± 3.44*
Lipoxygenase inhibition	54.19 ± 3.32	42.14 ± 3.45*
Nitric oxide scavenging	87.19 ± 3.45	72.12 ± 4.02*
Lipoxygenase inhibition Nitric oxide scavenging	54.19 ± 3.32 87.19 ± 3.45	42.14 ± 3.45* 72.12 ± 4.02*

\* indicate significant difference at p < 0.05.



**Fig. 1.** The anti-diabetic activity of the *Cocos nucifera* (CH) and *Borassus flabellifer* (BH) haustorium.

*nucifera* (p < 0.05) (Table 4). The different polyphenols present in the coconut and Palmyra palm haustorium are listed in Table 5, which indicates more complex polyphenols in BH extract.

### 4. Discussion

Functional foods are gaining attention in recent years, especially with the increasing incidence of various degenerative diseases. The haustorium is a product formed during the

#### Table 5

Composition of the polyphenols in Coconut and Palmyra palm haustorium analyzed by LC-MS.

Retention time (mins)	Coconut haustorium	Palmyra haustorium
2.50	Gallic acid	Gallic acid
4.24	Ferulic acid	Ferulic acid
8.30	4-Hydroxycinnamic	4-Hydroxycinnamic
	acid	acid
14.25	p-coumaric acid	_
16.60	Unidentified	Unidentified
19.14	Quercetin	Quercetin
24.30	_	Myricetin-3-0-
		glucoside
27.22	Quercetin-3-0-	Quercetin-3-0-
	glucoside	glucoside
29.70	_	Unidentified

germination of the nut, where liquid endosperm is converted to a spongy mass of tissue. Studies have indicated that the coconut haustorium contain high protein contents as well as major nutrients (Manivannan et al., 2018). Corroborating with this, our results also indicated the occurrence of essential nutrients in the CH; however, the quantity of insoluble fibre, carbohydrates and crude fat was high in the haustorium obtained from *Borassus flabellifer*, which is an indicator of higher nutritional value for the BH. Together with this, the higher amounts of medium-chain saturated fatty acids and minerals including sodium, potassium and phosphorus reaffirm the advantage of BH over coconut. Medium chain saturated fats are beneficial in preventing various diseases (Roopashree et al., 2021).

The health benefits of haustorium have been analyzed in terms of its antioxidant and anti-inflammatory activities. The Palmyra palm haustorium exhibited significantly higher free radical scavenging activities in vitro and ex vivo models. Though CH extract has been found to have antioxidant potential, the efficacy was significantly lower than that of BH. According to previous studies, the haustorium of coconut (Job et al., 2021c) and palmyra palm (Job et al., 2021a: Job et al., 2021b) has identified the protective effect of these haustoria against pro-oxidants such as fluoride, and alkoxyl radicals. Apart from that, the BH has also been found to inhibit the inflammatory enzyme activities. The lipoxygenase system is actively involved in the inflammatory reaction cascades in the body and activation of the enzyme is observed in various pathological conditions (Chen et al., 2020). Besides, the methanol extract of BH has also been proven to mitigate the LPS-mediated stimulation of macrophages and subsequent cytokine release (Malayil et al., 2021). The increased anti-radical and antiinflammatory capacity are well correlated with the higher polyphenol content observed in the BH. Apart from the quantitative variance, the changes in the composition of polyphenols among these might influence the increased antioxidant properties. The polyphenols identified from the BH and CH including myricetin and quercetin glycosides are well-known antioxidant agents (Devi et al., 2015; Tang et al., 2020). The antioxidant contents are correlated with the functional food status of any dietary component (Serafini and Peluso, 2016). The higher antioxidant potentials of BH may be useful in the prevention of various disease associated with redox imbalance and therefore indicates the possible use as a functional food.

Besides these biological properties, the study observed the inhibitory potential of BH and CH on the enzyme activities associated with Type 2 diabetes mellitus. The  $\alpha$ -Amylase and  $\alpha$  -Glucosidase enzymes are reported to be the targets of anti-diabetic drugs (Alqahtani et al., 2020; Mechchate et al., 2021); hence, by the inhibitory effects on these enzymes, the CH and BH may also have potential anti-diabetic properties. However, further studies using

cell culture and animal models are necessary to determine these particulars.

Overall, the present study indicated a significantly higher proximate content as well as antioxidant activity for *B. flabellifer* haustorium extract. It is also expected that quantitative and compositional variance in the phenolic antioxidants may be responsible for the observed difference in its pharmacological potentials. Hence, the study concludes that haustorium from *B. flabellifer* may be better functional food. Regular consumption of the Palmyra palm haustorium may be beneficial to improve the redox status of the body and therefore prevent various diseases.

#### 5. Conclusion

The study indicated the high nutritional values of *Cocos nucifera* and *Borassus flabellifer* haustorium in terms of the proximate composition, fatty acid content, and traces elemental content. Further, the study also observed the antioxidant and anti-diabetic potential of the two haustoria. Overall, it is possible that the consumption of the haustorium may be beneficial for the body and by virtue of these compounds and their antioxidant properties, the *Borassus flabellifer* and *Cocos nucifera* haustorium may evolve as functional foods.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgement

The authors extend their appreciation to the Researchers supporting project number (RSP-2021/349), King Saud University, Riyadh, Saudi Arabia. Authors also acknowledge Coconut Development Board, Govt. of India for analytical support.

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