Asian Journal of Plant Sciences

ISSN 1682-3974

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2019.139.147

Research Article
Drinking Straw from Coconut Leaf: A Study of its Epicuticular Wax Content and Phenol Extrusion Properties

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Abstract
Background and Objectives: Plastics are a ubiquitous part of our daily life but now posing a major threat to marine life, animal and human health. More than 50% of the manufactured plastic including straws are being disposed of after single-use. There is an increasing need to mitigate this trend so that the damage could be brought under control. The aim of this research was to develop a compostable, eco-friendly alternative to plastic straws using the leaves of *Cocos nucifera* L. **Materials and Methods:** The biochemical properties of 6 varieties of *Cocos nucifera* L. leaflets were studied in order to screen the most suitable material for making sustainable straws. Epicuticular wax content was analyzed to choose the best variety for preparation of hydrophobic straws. Total antioxidant activity, total tannin content, phenolic and flavonoid content were assayed to evaluate the potential functionality of the leaflets. The phenol extrusion properties of the material were also checked in acidic and normal beverages. **Results:** Estimation of epicuticular wax and phytochemical analysis in all 6 varieties revealed that all varieties of *Cocos nucifera* L. leaves provide a potent biomaterial for straw preparation. Silicon 732 was found to be a good adhesive agent for straw preparation. Phenol extrusion assays revealed that there is a negligible difference in the release of phytochemicals before and after dipping of straws in the beverages. **Conclusion:** The outcome of this research opens up vistas to carry out further research in a hitherto unexplored area of utilizing the leaf of *Cocos nucifera* in a novel way with far reaching economic and employment implications.

Key words: Biodegradable straws, *Cocos nucifera* L., epicuticular wax, silicon 732

Citation: Jyoti Jeena James, Dylan D. Silva, Saji Varghese, Jobi Xavier and Kuppusamy Alagesan Paari, 2019. Drinking straw from coconut leaf: A study of its epicuticular wax content and phenol extrusion properties. Asian J. Plant Sci., 18: 139-147.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plastic pollution is a major environmental hazard that is responsible for a lot of ecological disasters and needs to be tackled with utmost expediency[1,2]. An estimated 8 million metric tons of plastic end up in our oceans every year posing a huge threat to marine life[3]. With the global plastic demand increasing every year, the rapid production of plastic products has surpassed the production of other man made products[4]. Impacts of plastic pollution are witnessed by a widespread range of organisms comprising microbiota, invertebrates and vertebrates[5]. Microplastics derived from the fragmentation of plastics affect an organism's fitness by causing physical injury and mortality[6]. As plastic waste is one of the prime reasons for land and marine pollution, limiting plastic usage should be at high priority to save the fragile ecosystem[7]. Though paper straws serve the purpose, exploitation of trees for the manufacture of paper straws is a major threat and drawback. However, now having realized the resistance of plastic to degrade and to stay in landfills for hundreds of years, researchers have focused on developing alternatives to replace one-time use plastic straws by using reusable and recyclable materials[8]. With the scientific community focusing on sustainable product development to manage this menace, researchers have developed a number of biodegradable polymers[9]. The biodegradable polymers which are believed to be a substitute for conventional polymers have their origin from both renewable as well as from non-renewable sources[10]. Polyhydroxyalkanoate [PHA], Polyactic acid [PLA] and Polycaprolactone [PCL] have proved to be a potent alternatives to synthetic plastics[11,12]. However, decomposition of biodegradable polymer requires specific circumstances which are not conceivable at natural conditions. This decomposition problem of conventional polymers invites preferences towards natural products. It is a well-known fact that natural products always have a preferred advantage over the synthetic products as they are easy to obtain and the production cost is comparatively less and blends easily to the soil thereby causing no harm to the environment[13].

Cocos nucifera belonging to the genus *Cocos* and family *Arecaceae* are grown predominantly in the Philippines, India, Indonesia and few other South East Asian countries [14]. *Cocos* plants are multipurpose tropical variety that grows well in the littoral soil around the coastal regions of India[15]. Large pinnate leaves of size 5-6 m in length are produced in most of the varieties of *Cocos nucifera*. With an annual production of 12 leaves in each plant, 400 straws can be produced approximately from a single dried compound leaf of *Cocos nucifera* which is very stable and very conducive for commercial usage. The objectives of this research were to (1) Analyze and identify the best leaf variety of *Cocos nucifera* L. for the production of drinking straws, (2) Analyze the epicuticular wax content in each of the leaf varieties to select the best variety for preparation of hydrophobic straws and (3) Analyze phenol extrusion properties in acidic and normal beverages.

MATERIALS AND METHODS

Leaf samples of 6 varieties of *Cocos nucifera* L. were collected from Central Plantation Crops Research Institute [CPCRI] Kasaragod, India and Coconut Development Board, Mandya, India during the months of May-December, 2018. The 6 varieties of *Cocos nucifera* L. used in the study are West Coast Tall [WCT], Ganga Pani, Chowghat Orange Dwarf [COD], Triptur Tall, Fiji and Lakshadweep Ordinary Tall [LCOT]. All plant samples were identified using standard botanical classifications[16].

Estimation of epicuticular wax concentration: Epicuticular wax estimation was done using colorimetric method[17]. Briefly, epicuticular wax from 20 leaf bits of area 3 cm^2 was extracted using 15 mL of chloroform. Midrib was removed from the leaf prior to solvent extraction. The organic chloroform extract was evaporated to dryness and 5 mL of potassium dichromate reagent was added to dissolve the wax and the absorbance was measured at 590 nm in UV-visible spectrophotometer.

Characterization of leaf extracts

Preparation of samples: The dried leaf samples were incubated in a hot air oven at 40°C for 3 h and were ground to a fine powder and were used for phytochemical analysis. About 10 g of the powdered plant sample was extracted with 100 mL of methanol under sonication for 30 min. The slurry was then centrifuged at 8000 rpm for 15 min and the supernatant was placed in a rotary evaporator. Methanol was evaporated at 52°C for 30 min.

Estimation of total phenolic content (TPC): Total phenolic content was measured using Folin-Ciocalteu's assay[18]. About 0.2 mL of the plant leaf extract was mixed with 0.5 mL of the Folin-Ciocalteu's reagent and 2 mL of sodium bicarbonate solution [20% (w/v)] and the absorbance was read at 638 nm. For quantification of the total phenolic content in the plant leaf sample, a standard calibration curve was plotted using catechol.

Total tannin content (TTC): Total Tannin content (TTC) was measured using Folin-Ciocalteu's assay[18]. About 0.2 mL of the extract was mixed with 0.5 mL of the Folin-Ciocalteu's reagent and 2 mL of sodium bicarbonate solution [20% (w/v)] was added to the mixture and incubated for 2 h in a dark environment at room temperature and the absorbance was recorded at 725 nm. For quantification of the TTC content in the sample, a standard calibration curve was plotted using Tannic acid.

Total phenol estimation in different juice samples dipped with the organic straws: The rigidity of the prepared straw was checked by dipping in 25 mL of commercially available juice and soft drink samples (Neera, Carbonated drink and packed fruit juice) of varying acidity. The organic straws were exposed to different beverages for 30 and 60 min at 2 different temperatures (4 and 30°C). After incubation, straws were removed and the total extruded phenol was estimated in straw dipped juice samples using Folin-Ciocalteu's assay[18].

Total flavonoid content (TFC): Total flavonoid content (TFC) was evaluated using a modified Aluminium chloride colorimetric method[19]. Briefly, 0.1 mL of the leaf extract was mixed with 0.1 mL of the Aluminium chloride [10% (w/v)] followed by the addition of 0.1 mL of 1 M Potassium acetate. The obtained solution was maintained for 40 min at room temperature. Absorbance at 415 nm was read for quantification of the TFC content using quercetin as a standard.

Estimation of antioxidant activity: Antioxidant activity of the leaf extract was estimated using DPPH radical scavenging assay[20]. About 30 µL of the plant leaf extract was made up to 3 mL with methanol. About 1 mL of DPPH [0.004% (w/v)] was added under dark conditions and incubated at 30 min and the absorbance was recorded at 513 nm. Ascorbic acid served as a positive control. The antioxidant activity was calculated by using the equation:

$$\text{Radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the blank (methanol with DPPH) and A_1 is the absorbance of the sample (ascorbic acid/plant samples).

Preparation of straws from leaves of *Cocos nucifera*: Leaves from 6 varieties of *Cocos nucifera* were soaked in sterile distilled water for 30 min. Two methods were followed for the preparation of straw. In the first method, the cleaned leaves were boiled for 5 min in water and the boiled leaves were used for straw making using different binding agents (Corn starch, Gelatin mixture, Gum acacia, FDA approved Silicon732). In the second method, instead of boiling, cleaned leaves were subjected to steam for 5-10 min. The treated leaf strips were rolled into straws by applying glue along the margin along its length and rolling it spirally along a cylindrical rod. The process and material is patented with the Department for promotion of Industry and Internal trade, Ministry of commerce and Industry, Government of India, under the title of invention "Biodegradable means for use as drinking straws, stirrer and chopstick and a method thereof."(Patent number: 308356). All experiments were carried out at different time scale to assure the authenticity of the results. Results are indicative of triplicate values expressed as mean±SD.

RESULTS

Estimation of epicuticular wax concentration in *Cocos nucifera* leaves: The highest amount of epicuticular wax was estimated in the leaf of lakshadweep ordinary tall (LCOT) which was 0.194 µg per 60 cm^2. The content of epicuticular wax was observed in the following order: Ganga pani>Triptur tall>West coast tall>Fiji. The least amount of wax was found in Chowghat Orange Dwarf which was 0.090 µg per 60 cm^2 (Fig. 1).

Estimation of total phenolic content in *Cocos nucifera* leaves: The Chowghat Orange Dwarf variety was found to have the highest amount of phenol which was 0.084 g mL^{-1} followed by West coast tall (0.072 g mL^{-1}), Ganga Pani variety (0.052 g mL^{-1}), Lakshadweep ordinary tall had 0.050 g mL^{-1} of phenol and Fiji had 0.048 g mL^{-1} of phenol content (Fig. 2).

Estimation of total tannin content in *Cocos nucifera* leaves: The Tannin content estimated in all 6 different varieties exhibited very minor deviations. West Coast Tall (0.0060 g mL^{-1}) and Ganga Pani (0.0059 g mL^{-1}) had the highest amount of Tannin. Chowghat Orange dwarf and Triptur tall has the same amount of tannin which was found to be 0.0043 g mL^{-1} of Tannin (Fig. 3).

Estimation of total flavonoid content in *Cocos nucifera* leaves: As it is shown in Fig. 4, the flavonoid estimation in different coconut palms depicted Fiji variety is having the highest amount of flavonoid content which was about 9500 µg mL^{-1} followed by West Coast tall which was

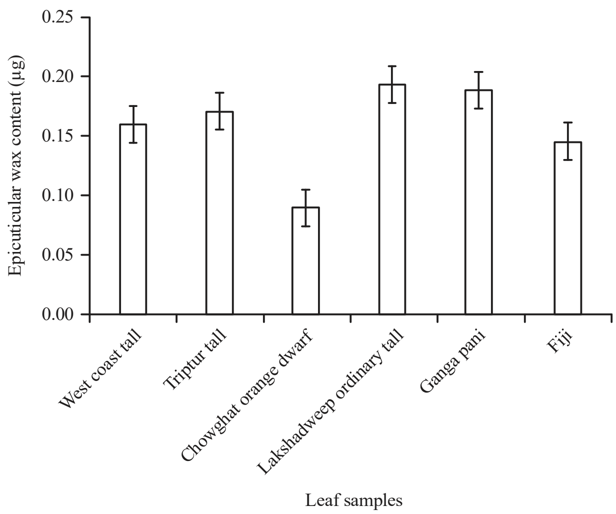

Fig. 1: Estimation of epicuticular wax content of *Cocos nucifera* leaves

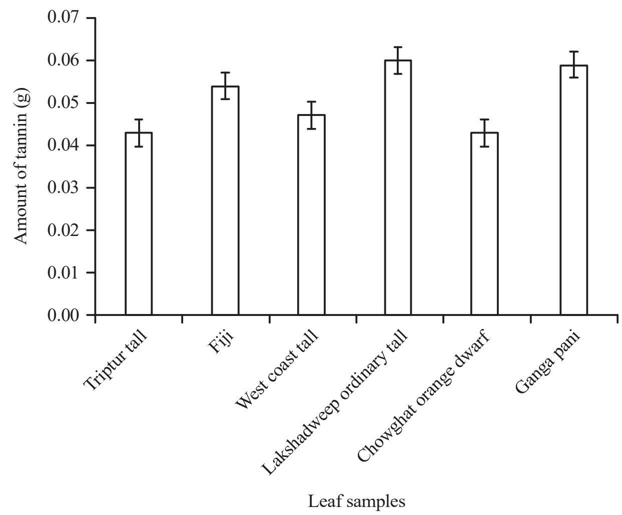

Fig. 3: Estimation of total tannin content of *Cocos nucifera* leaves

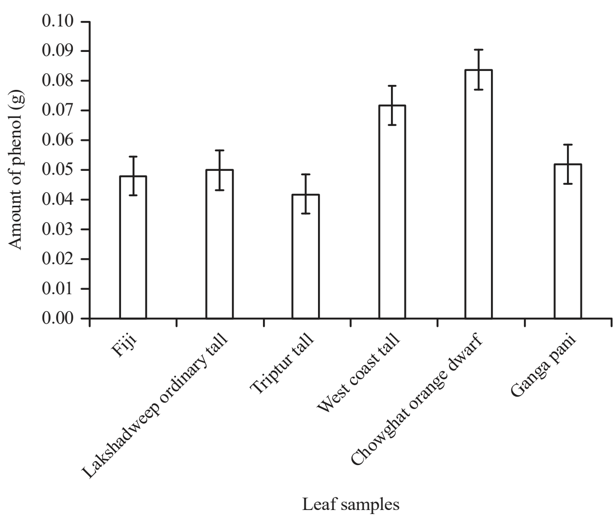

Fig. 2: Estimation of total phenolic content of *Cocos nucifera* leaves

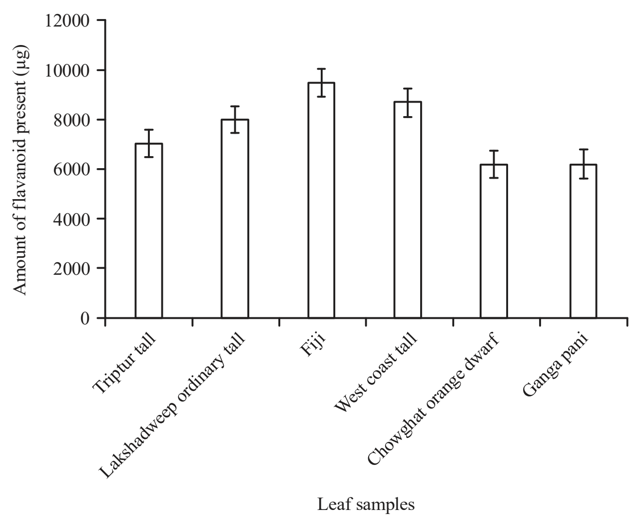

Fig. 4: Estimation of total flavanoid content of *Cocos nucifera* leaves

8700 µg mL^{-1}. Chowghat orange dwarf, variety had the least amount of flavonoid content (6200 µg mL^{-1}) compared to other samples.

Estimation of the antioxidant profile of *Cocos nucifera* leaflets: Higher the percentage of inhibition of diphenylpicrylhydrazyl (DPPH) radical indicates the higher antioxidant activity. The anti-oxidant activity estimated using DPPH assay inferred Chowghat orange dwarf possessing highest anti-oxidant activity (90.6%) compared to West Coast tall (86%) and Fiji (84.2%). Reduced radical sequestering ability was observed in Lakshadweep ordinary tall variety (78.6%) and in Ganga pani (78.5%). The least anti-oxidant activity or radical quenching activity was found in Triptur tall which has been estimated to be 75.4% (Fig. 5).

Analysis of phenol extrusion properties in *Cocos nucifera* straws: The present study also attempted to check the release of phenolic exudates from the prepared *Cocos nucifera* straws. No notable difference in phenolic content was observed at both the incubation temperatures (4 and 30°C) and incubation times (30 and 60 min) when *Cocos nucifera* straws of WCT variety were dipped in neutral juice. A similar trend was observed in all straws made of Fiji, COD, Gangapani

Table 1: Phenol extrusion properties of *Cocos nucifera* straws dipped in neutral juice [Neera]

Varieties	Incubation temperature (°C)	Incubation time (min)	Phenol content before dipping the straws (µg)	Phenol content after dipping the straws (µg)
WCT	4	30	0.191±0.002	0.194±0.003
WCT	4	60	0.191±0.002	0.194±0.002
WCT	30	30	0.191±0.002	0.186±0.004
WCT	30	60	0.191±0.002	0.190±0.001
COD	4	30	0.191±0.002	0.194±0.002
COD	4	60	0.191±0.002	0.194±0.002
COD	30	30	0.191±0.002	0.185±0.004
COD	30	60	0.191±0.002	0.190±0.001
Ganga pani	4	30	0.191±0.002	0.194±0.002
Ganga pani	4	60	0.191±0.002	0.194±0.002
Ganga pani	30	30	0.191±0.002	0.191±0.006*
Ganga pani	30	60	0.191±0.002	0.190±0.001
Triptur tall	4	30	0.191±0.002	0.197±0.002*
Triptur tall	4	60	0.191±0.002	0.195±0.002
Triptur tall	30	30	0.191±0.002	0.191±0.006*
Triptur tall	30	60	0.191±0.002	0.191±0.001
Fiji	4	30	0.191±0.002	0.198±0.002*
Fiji	4	60	0.191±0.002	0.194±0.001
Fiji	30	30	0.191±0.002	0.192±0.006*
Fiji	30	60	0.191±0.002	0.192±0.001
LCOT	4	30	0.191±0.002	0.196±0.001*
LCOT	4	60	0.191±0.002	0.190±0.0007
LCOT	30	30	0.191±0.002	0.196±0.002*
LCOT	30	60	0.191±0.002	0.193±0.001

WCT: West coast tall, LCOT: Lakshadweep ordinary tall, COD: Chowghat orange dwarf, *Significant differences in polyphenol content between the treatment groups, results are indicative of triplicate values expressed as Mean±SD

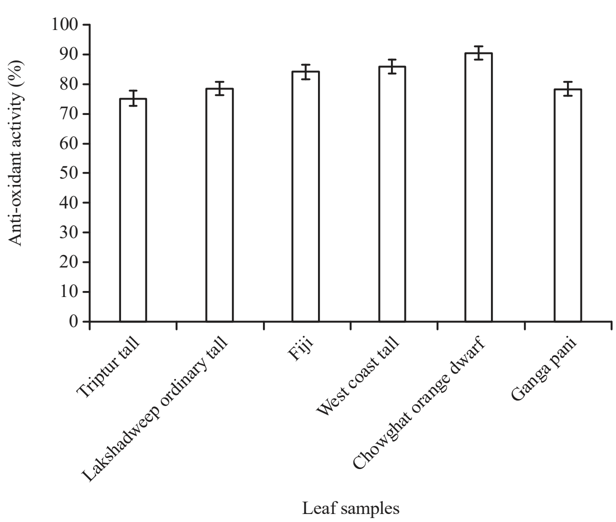

Fig. 5: Estimation of antioxidant activity in *Cocos nucifera* leaves

and LCOT variety. Significant increase in the phenolic content was noted in neera juice dipped with straws made of Triptur tall variety at 4°C. At both the incubation times i.e., 30 and 60 min, the phenolic content raised to 0.197±.002 and 0.195±.002, respectively (Table 1). In contrast to this result, the trend was different when the straws were dipped in a carbonated drink. Marked differences in phenolic content were noted in the samples when treated for longer duration at a higher temperature (Table 2). The difference in the phenolic content before and after dipping *Cocos nucifera* straws in packed fruit drink was very negligible. However, higher temperature caused slightly more extrusion of phenols (Table 3).

DISCUSSION

This study discovered the efficacy of straws made with *Cocos nucifera* leaflets as an alternative to the plastic straw. Steaming of leaves produced better raw material compared to the boiling method. Among the four tested binders (corn starch, gelatin mixture, Gum acacia and FDA approved silicon 732 glue), Silicon 732 glue served as a potent binder. The higher stability of the straws prepared from all leaf varieties was attributed to the concentration of epicuticular wax content[21]. The environmental adaptability of *Cocus nucifera* to saline area is the reason for its higher waxy content. Shiny appearance on the surface of the straw was contributed by the epicuticular wax which is also reported for its capability to act against the biotic and abiotic stress[21]. Epicuticular wax which is a derivative of fatty acids provides a

Table 2: Phenol extrusion properties of *Cocos nucifera* straws dipped in carbonated drink

Varieties	Incubation temperature (°C)	Incubation time (min)	Phenol content before dipping the straws (µg)	Phenol content after dipping the straws (µg)
WCT	4	30	0.0026±0.0002	0.004±0.0003*
WCT	4	60	0.0026±0.0002	0.005±0.001*
WCT	30	30	0.0026±0.0002	0.0080.0009*
WCT	30	60	0.0026±0.0002	0.008±0.0006*
COD	4	30	0.0026±0.0002	0.003±0.0003
COD	4	60	0.0026±0.0002	0.005±0.001*
COD	30	30	0.0026±0.0002	0.007±0.0008*
COD	30	60	0.0026±0.0002	0.007±0.0006*
Ganga pani	4	30	0.0026±0.0002	0.004±0.0001*
Ganga pani	4	60	0.0026±0.0002	0.006±0.001*
Ganga pani	30	30	0.0026±0.0002	0.008±0.0008*
Ganga pani	30	60	0.0026±0.0002	0.008±0.0006*
Triptur tall	4	30	0.0026±0.0002	0.004±0.0001*
Triptur tall	4	60	0.0026±0.0002	0.006±0.0001*
Triptur tall	30	30	0.0026±0.0002	0.009±0.0009*
Triptur tall	30	60	0.0026±0.0002	0.009±0.0003*
Fiji	4	30	0.0026±0.0002	0.005±0.0001*
Fiji	4	60	0.0026±0.0002	0.020±0.03*
Fiji	30	30	0.0026±0.0002	0.009±0.0009*
Fiji	30	60	0.0026±0.0002	0.010±0.0004*
LCOT	4	30	0.0026±0.0002	0.005±0.0001*
LCOT	4	60	0.0026±0.0002	0.007±0.001*
LCOT	30	30	0.0026±0.0002	0.009±0.0008*
LCOT	30	60	0.0026±0.0002	0.010±0.0007*

WCT: West coast tall, LCOT: Lakshadweep ordinary tall, COD: Chowghat orange dwarf, *Significant differences in polyphenol content between the treatment groups, results are indicative of triplicate values expressed as Mean±SD

Table 3: Phenol extrusion properties of *Cocos nucifera* straws dipped in PACKED fruit drink

Varieties	Incubation temperature (°C)	Incubation time (min)	Phenol content before dipping the straws (µg)	Phenol content after dipping the straws (µg)
WCT	4	30	0.176±0.003	0.169±0.002*
WCT	4	60	0.176±0.003	0.170±0.0008*
WCT	30	30	0.176±0.003	0.175±0.001
WCT	30	60	0.176±0.003	0.172±0.006
COD	4	30	0.176±0.003	0.171±0.003
COD	4	60	0.176±0.003	0.200±0.002*
COD	30	30	0.176±0.003	0.176±0.003
COD	30	60	0.176±0.003	0.174±0.012*
Ganga pani	4	30	0.176±0.003	0.158±0.003*
Ganga pani	4	60	0.176±0.003	0.186±0.005*
Ganga pani	30	30	0.176±0.003	0.160±0.004*
Ganga pani	30	60	0.176±0.003	0.172±0.012*
Triptur tall	4	30	0.176±0.003	0.177±0.005*
Triptur tall	4	60	0.176±0.003	0.200±0.010*
Triptur tall	30	30	0.176±0.003	0.174±0.002
Triptur tall	30	60	0.176±0.003	0.182±0.017*
Fiji	4	30	0.176±0.003	0.180±0.005
Fiji	4	60	0.176±0.003	0.203±0.010*
Fiji	30	30	0.176±0.003	0.178±0.002
Fiji	30	60	0.176±0.003	0.248±0.099*
LCOT	4	30	0.176±0.003	0.182±0.005
LCOT	4	60	0.176±0.003	0.208±0.013*
LCOT	30	30	0.176±0.003	0.186±0.006*
LCOT	30	60	0.176±0.003	0.254±0.099*

WCT: West coast tall, LCOT: Lakshadweep ordinary tall, COD: Chowghat orange dwarf, *Significant differences in polyphenol content between the treatment groups, results are indicative of triplicate values expressed as Mean±SD

hydrophobic barrier that plays a crucial role to minimize dust retention and provides absolute protection against bacterial and fungal pathogens[22].

Phenol plays a crucial role in providing resistance to plants against fungal pathogens and insects[23]. The higher amount of phenol in the leaves of *Cocos nucifera* aids the selection of healthy leaves from the 6 varieties for straw preparation. Phenolic compounds present in the leaf extracts contribute to the radical scavenging effect[24]. In addition to radical quenching properties, phenolic compounds are reported to play a crucial role in determining the structure of the cell wall and their antimicrobial properties[25,26]. The tannin content in plants depends upon the nutrient availability in the soil as well as in the plants[25]. This study inferred that the tannin content was higher in tall varieties when compared to dwarf varieties. Though tannin is not considered as the primary antioxidant, it is termed as a secondary antioxidant as it contributes in metal chelation to control oxidation by donating hydrogen atom[27]. In a study carried out by Rebaya *et al.*[28], a significant association was observed amongst DPPH antioxidant activity and tannin concentration in the leaves of *Halimium halimifolium*[28]. Flavonoids are polyphenolic structures present in plants that confer pest and insect resistance[29,30]. Flavonoids in the existence of their substitution units such as flavones, chalcones and isoflavones enhance the antioxidant capacity of the plantlets[31]. Correlation of antioxidant potential with respect to flavonoid content in the *Cocos nucifera* leaflets was not observed in this study but the involvement of phenols in the antioxidant activity of the extracts was observed. Results of our study were similar to the earlier reports that correlate the functional capacity of the flavonoids to antioxidant capacity in vegetables[32]. Presence of reductones in the plant extract contributes to the radical quenching potential that could prevent oxidation in straws thereby extending the shelf life of the products[33]. The correlation between antioxidant activity and phenolic content might also be dependent on the presence of other phytochemicals such as tocopherol and vitamin C.

Phenolic compounds that contribute to the antioxidant activity of the plant extracts exist in the bound form among the polysaccharides in the cell membrane[34]. Stability of these trapped phenolics is disturbed at higher temperatures due to rupturing of the cell membrane and matrix. Changes in the phenolic composition are due to the extent of damage caused to the matrix and also based on the complexity of phenolic structures[35]. Though some studies reported the loss of phenolic content due to extensive heat, few studies have reported the increase in phenolic content in vegetable plants due to higher temperature[36]. *Vitis vinifera* leaves were observed to have reduced phenolic content, at lower temperature[37]. As the usage of straw is limited for a shorter time, phenol extrusion studies were limited to shorter duration (30 and 60 min). Phytochemical concentration before and after dipping the *Cocos nucifera* straws in the beverages provides the evidence to understand the stability of phyto constituents entrapped in the straws.

CONCLUSION

With pollution levels increasing at an alarming rate, biodegradable products are increasingly being used as perfect alternates to plastic and polymer based products. Developing a safe eco-friendly straw from *Cocos nucifera* leaves will benefit the process of conversion of a waste product to a value-added product.

SIGNIFICANCE STATEMENT

The present research focusing on analyzing the epicuticular wax content in different leaf varieties will help in selecting the best leaf variety for the production of straws. The study of phenolic extrusion properties in beverages ensures the safety of the use of straws in beverages. The idea of making eco-friendly straws has the potential to provide a huge opportunity to members of rural communities to augment their income as they can be engaged in the production of these straws. The present research opens up vistas to carry further research in a hitherto unexplored area of utilizing the leaf of *Cocos nucifera* in a novel way with far reaching economic and employment implications.

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