https://doi.org/10.17221/360/2018-CJFS

Chemical, microbial and antioxidant activity of *Cola lepidota* K. Schum fruits

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Citation: Oranusi S., Onibokun A., Afolabi O., Okpalajiaku C., Seweje A., Olopade B., Obafemi Y. (2020): Chemical, microbial and antioxidant activity of *Cola lepidota* K. Schum fruits. Czech J. Food Sci., 38: 11–19.

Abstract: This research was to investigate the chemical composition, antioxidant activity, and microbial profiles of *Cola lepidota* fruits. One hundred grams each of the fruit exocarp, mesocarp and endocarp were blended and analyses were carried out by the following standard methods. Active acidity and vitamin C contents were 5.5 and 6.34 mg 100 g^{-1} in endocarp, 4.5 and 14.39 mg 100 g^{-1} in mesocarp and 6.7 and 10.02 mg 100 g^{-1} for exocarp. Moisture and carbohydrate contents of 12.31 and 68.72% were in the mesocarp while protein and crude fibre contents of 8.13 and 26.18% were in the exocarp and endocarp. Iron (Fe), zinc (Zn) and manganese (Mn) contents were 1.79, 0.27 and 0.57 mg 100 g^{-1} in exocarp while lead (Pb), cadmium (Cd) and chromium (Cr) were absent in the endocarp. Predominant isolates were *Aspergillus niger*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Bacillus*, *Staphylococcus* and *Pseudomonas* species. *C. lepidota* had no antimicrobial effect against the tested organisms. Tannins, flavonoids, terpenoids, phenols, coumarins and anthocyanins were present while alkaloids, quinolones, glycosides, steroids and cardiac glycosides were absent. The fruit was observed to have antioxidant property by hydrogen peroxide scavenging activity. This study presents *C. lepidota* as good for human consumption and can be exploited for animal feed production.

Keywords: *Cola lepidota*; microbial profile; antioxidant activity; chemical composition

Fruits are a vital part of human diet as they provide antioxidants, low-calorie and protective micronutrient-rich diets (Sachdeva et al. 2013). They have been associated with reduced blood cholesterol, prevention of large bowel diseases, reduced incidence of cancer and cardiovascular diseases (Williams et al. 2009; Ene-Obong et al. 2016). Despite these, it has been reported that 77.6% of men and 78.4% of women from 52 mainly low- and middle-income countries consume less than minimally recommended five daily servings of fruits and vegetables (Hall et al. 2009). This may account for the high prevalence of malnutrition, particularly micronutrient deficiencies, and the increasing prevalence of diet related non-communicable diseases in low- and middle-income countries (Hall et al. 2009). The lack of good database and poor knowledge of the nutrient composition and quality of traditional food crops are some of the reasons for low fruit and vegetable consumption in developing countries (Grivetti et al. 2000). Tropical African sub-regions are home to many valuable fruit species whose potentials have not been fully realized. Most of them have not been identified and evaluated for their nutritional and functional properties and therefore are underexploited. One of such plant foods is *Cola lepidota* (Monkey kola). *C. lepidota* belongs to the group of Monkey kola, a member of the family *Malvaceae* and subfamily *Sterculiaceae*. The Monkey kola varieties include red (*C. lateritia*), yellow (*C. lepidota*) and white (*C. parchycarpa*) types (Okudu & Ene-Obong 2015). About forty Cola species have been described in West Africa, however, in Nigeria only about twenty-three species are known and some are used in traditional medicine as a stimulant, to prevent dysentery, headache and to suppress sleep (Essien et al. 2015). The fruit is a good source of crude

https://doi.org/10.17221/360/2018-CJFS

protein, fibre and fat, Ca, Mg, Zn, Cu, β-carotene and niacin, while the pulp is a good source of ash, starch, carbohydrate, K, P and Se contents (Okudu et al. 2015). The pulp (mesocarp) is the most commonly consumed part of this fruit. Microbial contamination of fruits is mostly responsible for yield losses. Contamination by microorganisms may occur through direct contact with soil, dust, water and by handling at harvest or during postharvest processing (Eni et al. 2010). Some spoilage microorganisms are capable of colonizing and creating lesions on healthy, undamaged plant tissue (Miedes & Lorences 2004). The understanding of the microbial profile of *C. lepidota* is important to know the source(s), course(s) and possible mechanisms of microbial spoilage for this fruit. WHO (2007) has shown continuous interest in the use of therapeutic plants for the management of different diseases. Thus, exploring and understanding the phytochemical composition and antioxidant potential of non-conventional plants may encourage utilization of these plants for nutraceutical and pharmaceutical purposes. This study was therefore designed to investigate the chemical composition, antioxidant activity and microbial diversity of the exocarp, mesocarp, and endocarp of *C. lepidota* fruit with the aim of improving its utilization.

MATERIAL AND METHODS

Sample collection

A total of 200 samples of *C. lepidota* fruits were randomly purchased from ten different fruit vendors in Abia (5°25'0.01" N 7°30'0.00" E) and Imo (5°30'0.00" N 7°10'0.01" E) States, Nigeria. The samples were pooled and transported in sterile plastic bags to the Microbiology Laboratory of the Covenant University within 48 h from purchase for analysis.

Sample preparation

The samples were inspected and sorted to remove fruits with physical injury, and to separate fruits with signs of spoilage but with no visible physical injury, from completely healthy fruits. The physically injured fruits were discarded while those with signs of spoilage and the completely healthy fruits were used for this research. The choice of spoilt fruit with no physical injury was to eliminate as much as possible microorganisms possibly introduced via injury material(s) other than organisms able to initiate spoilage. With the aid of a sterile scalpel blade, the exocarp, mesocarp and endocarp of the completely healthy fruits were separated for determination of heterotrophic microbial profiles, chemical compositions and antioxidant activity analysis. a similar separation of the exocarp, mesocarp and endocarp was carried out from the spoilt parts of the fruits to be used for screening of microorganisms associated with the fruit spoilage. The fruit samples were separately evaluated for their microbial profile, however pooled samples were used for another analysis. Duplicate sample portion (100 g), each of the exocarp, mesocarp and endocarp, was measured; one portion was oven dried at 40 °C for 5 days and blended for phytochemical and antioxidant analysis. The other portion was blended and used for the determination of proximate composition and to further confirm the heterotrophic microbial composition.

Microbiological assessment

Isolation of microorganisms. a sample (approximately 1.00 g) of already blended fruit parts was diluted with sterile distilled water (9 × the sample weight). Serial dilutions of 10 : 1 to 10 : 4 were carried out. A homogenate aliquot of the sample (0.1 mL) was inoculated onto already prepared and cooled Nutrient agar (Oxoid, UK), MacConkey agar (Fluka, Germany), Eosin Methylene Blue agar (EMB) (Oxoid, UK) and Potato Dextrose Agar (PDA) (Fluka, Germany) using the streak plate method. Lactose Broth for microbiology (Lab M, UK) in test tubes (9 mL, with inserted Durham tube) was inoculated by samples (1.0 g of homogenate) for the Coliform Bacteria Test (indicated by gas accumulation in the Durham tube due to sugar fermentation and change in the colour of dye in the medium due to change in pH). The Petri dishes and test tubes were incubated at 37 °C for 24 h, but except for PDA plates which were incubated at 25 °C for 7 days.

Identification of microbial isolates. Grown colonies were isolated and purified (repetitive inoculation and cultivation procedures). The bacterial isolates (Gram staining – differentiation of bacteria) were subjected to the following biochemical tests: indole test, methyl red test and Voges-Proskauer test, citrate utilization test, sugar fermentation test, urease test, oxidase test, starch hydrolysis test, gelatine liquefaction test, catalase test, coagulase and motility tests. Further identification of the bacterial isolates was done by use of the API kits (bioMérieux, FRA) and including amino acid decarboxylation (ADH, LDC, ODC), ONPG, H_2S production, tryptophan deaminase (TDA) test, and sugar fermentations (Sorbitol, Rhamnose, Inositol, Mannose, Arabinose, Amygdalin and Melibiose). The fungal and yeast isolates were observed both macroscopi-

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cally (e.g. shape, size, colour, gloss/mat of colonies) and microscopically (e.g. shape, size, spore formation of cells). For mounting and staining of moulds and yeasts was used the lactophenol cotton blue stain principle (Watanabe 2010; Xu et al. 2015). Fungi and yeasts were identified by standard biochemical tests including needle mount, germ tube test, and sugar fermentations and with reference to the standard identification atlas.

Determination of antimicrobial activity of ethanolic crude extracts of C. lepidota fruits

Already blended samples (100 g), each of the exocarp, mesocarp and endocarp, of the *Cola lepidota* were respectively soaked in 95% ethanol (250 mL) for 24 h at a laboratory temperature of 28 ± 2 °C on a shaker (MaxQ400; Thermo Fisher Scientific, USA) at 100 rpm. The extracts were concentrated in a rotary evaporator (RE300B; Stuart, UK) and allowed to dry completely. The weight of extracts was determined and they were stored at 4 °C for further use. The antimicrobial activity of the crude extracts was investigated using the agar well diffusion method as described by Abah et al. (2017). Bacterial suspensions were prepared from fresh cultures of the isolates and standardized to 0.5 scale of the McFarland standard (l.0 × 108 cells mL^{-1}). Mueller Hinton agar was seeded with the suspensions; crude extracts were introduced into uniform wells (of 6.0 mm in diameter) cut on the surface of the seeded agar using a sterile cork-borer. The plates were allowed to stand for 1 h to allow for diffusion of the extracts, and then incubated at 37 °C for 24 hours. Plates were observed for inhibition zones and their diameters were measured in millimetres. Gentamicin sensitivity discs (10 µg) (Rapid Labs Ltd., UK) and dimethyl sulfoxide were used as the positive and negative control, respectively, and each test was carried out in triplicate.

Chemical screening

The active acidity (pH) and proximate composition of the exocarp, mesocarp and endocarp of *C. lepidota* were determined according to standard procedures in the AOAC (Association of Official Analytical Chemists, USA) method (2005: 925.10; 2003.05; 923.03; 960.52; 944.02). Vitamin C content was determined according to the AOAC method (1990: 932.06). Qualitative phytochemical analysis was carried out to test the presence of tannins, flavonoids, terpenoids, alkaloids, saponins, quinones and anthroquinones, steroids, cardiac glycosides, reducing sugars, using standard methods and following the description (Njoku & Obi 2009).

Determination of antioxidant activity of ethanolic crude extracts of *C. lepidota* fruits

Hydrogen peroxide scavenging activity. The method of Ruch et al. (1989) was used, by following the description (Oranusi et al. 2013). Different concentrations (25.000; 12.500; 6.250 and 3.125 mg mL^{-1}) of the extracts were prepared. To each extract (100) was added H_2O_2 (600 µL) in 0.1 M phosphate buffer (pH 7.4). Samples were incubated at room temperature in the dark for 10 minutes. A negative control was set up in parallel with the entire reagent except extract or standard. Absorbance was read at 230 nm against a blank solution containing the phosphate buffer without H_2O_2, using the spectrophotometer model M106 (Spectronic Campsec, UK). The percentage of H_2O_2 scavenging of the extracts and standard compounds was calculated. Total flavonoid content assay to distilled water (490 µL), the extract was added (10 µL) and followed by 5% sodium nitrate (30 µL) and 10% aluminium chloride (30 µL). Samples were incubated at room temperature for 5 min and 1 M NaOH (200 µL) was added, followed by distilled water (240 µL), and vortexed thoroughly. Absorbance was read at 570 nm, using the Genesys, G105 UV-VIS spectrophotometer (Thermo Fisher Scientific, USA). Standard pyrocatechol was used as the control, mean of the triplicate analysis was recorded and results were expressed as milligrams of catechin equivalents per 100 g of sample (Zhishen et al. 1999; Oranusi et al. 2013).

Total phenol content assay. The Folin-Ciocalteu method was used as was described by Alothman et al. (2009). To the extracts (10 µL), distilled water (600 µL) was added and followed by the 10% Folin-Ciocalteu reagent (50 µL). Then 7% Na_2Co_3 was added (150 µL) and vortexed thoroughly for 2 minutes. It was incubated for 8 minutes at room temperature and then distilled water was added (190 µL). Afterwards, it was allowed to stand for 2 h and absorbance was read at 765 nm, using the Genesys, G105 UV-VIS spectrophotometer (Thermo Fisher Scientific, USA). Gallic acid was used as the standard, mean of the triplicate analysis was recorded and results were expressed as milligrams of gallic acid equivalent per 100 g of sample.

RESULTS AND DISCUSSION

In this study, *Bacillus* sp., *Pseudomonas* sp., and *Staphylococcus* sp. were isolated from the *C. lepidota* fruits that were sampled (Table 1). Cells of *Bacillus* sp. create spores and are common environmental contaminants. They are a part of diverse microbial com-

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Table 1. Distribution of microbial isolates in *C. lepidota* samples

SN	Microbial isolate	Number of isolates n = 150	Occurrence (%)	Source		
				exocarp*	mesocarp**	endocarp
1	*A. niger*	110	73.33	+	+	–
2	*S. cerevisiae*	100	66.67	+	+	–
3	*Penicillium* sp.	20	13.33	+	+	–
4	*A. flavus*	70	46.67	+	+	–
5	*Rhizopus* sp.	50	33.33	+	–	–
6	*Alternaria* sp.	50	33.33	+	–	–
7	*Bacillus* sp.	150	100.00	+	+	–
8	*Staphylococcus* sp.	105	70.00	+	–	–
9	*Pseudomonas* sp.	60	40.00	+	–	–

SN – sample number; *found in both healthy and spoilt fruits; **found only in spoilt fruits

munities, for example as normal microflora on plant surfaces (Barth et al. 2009). A few of them are associated with the food spoilage due to their ability to produce extracellular lytic enzymes such as cellulase, hemicellulase, lignocellulase and pectinase (Barth et al. 2009). Most of the reported isolates were present in the exocarp of the fruit, however, a few species isolated in the mesocarp were also present in the exocarp. This establishes the fact that the exocarp of the fruit was the route of entry for the spoilage microorganisms. The high bacterial population of the exocarp could be caused by the use of waste water for irrigation or manure used for fertilization and the unhygienic environment where the fruit was grown. Lam et al. (2015) reported that the use of wastewater excreta in agriculture may put communities at risk especially when contaminated fruits and vegetables are consumed. This may be a pre- and/or post-harvest contamination (Mritunjay & Kumar 2015). Bacteria of the *S. aureus* species are considered to be a common microflora on human skin (Ajayi et al. 2017). Therefore, the fruit may have been contaminated by these bacteria by improper handling techniques at the fruit vendors. Generally, *B. subtilis* and *Serratia* sp. are among the most common fruit spoilage bacteria (Akhtar et al. 2016). *A. niger, A. flavus, Penicillium* sp. and *S. cerevisiae* were the most predominant fungal and yeast species isolated in this study while *A. niger* had the highest occurrence (73.33%). The presence of these microorganisms in the exocarp and mesocarp may have occurred as a result of the fruit exposure to fungal/yeast spores pre-/post-harvest or during fruit storage (Bhat *et al*. 2010). Assessment of the antimicrobial activity of ethanolic extracts of the exocarp, mesocarp and endocarp of *C. lepidota* showed that the extracts at all concentrations had no effect on *B. subtilis, S. aureus* and *P. aeruginosa* (Table 2). The pH of the fruit was acidic ranging from 4.5 in the mesocarp to 6.7 in the exocarp (Table 3). A pH value in the acidic range has been reported to prolong the shelf life of fresh fruit and inhibit the multiplication of micro-

Table 2. Antimicrobial activity of the ethanolic extract of *C. lepidota* fruits

Sample	Tested bacterium	Concentration (mg mL^{-1})				
		undiluted	200 mg mL^{-1}	100 mg mL^{-1}	50 mg mL^{-1}	25 mg mL^{-1}
Endocarp	*Staphylococcus aureus*	R	R	R	R	R
	Pseudomonas aeruginosa	R	R	R	R	R
	Bacillus subtilis	R	R	R	R	R
Mesocarp	*Staphylococcus aureus*	R	R	R	R	R
	Pseudomonas aeruginosa	R	R	R	R	R
	Bacillus subtilis	R	R	R	R	R
Exocarp	*Staphylococcus aureus*	R	R	R	R	R
	Pseudomonas aeruginosa	R	R	R	R	R
	Bacillus subtilis	R	R	R	R	R

R – resistant

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Table 3. Mean pH, vitamin C and percentage proximate composition of *C. lepidota* fruits

Sample	pH	Vitamin C	Moisture	Proteins	Lipids	Crude fibre	Ash	Carbon-hydrate
		(mg 100 mL^{-1})	(%)					
Endocarp	5.5	6.34	9.29	4.20	2.72	26.18	4.42	53.17
Mesocarp	4.5	14.39	12.31	3.60	2.66	8.18	4.52	68.72
Exocarp	6.7	10.02	10.36	8.13	0.93	15.54	1.23	63.80

organisms (Ashie et al. 1996). The mesocarp had the highest amount of vitamin C and other proximate parameters with the exception of protein, lipid and crude fibre (Table 4); however, a higher lead content was observed in the mesocarp (0.3580 mg 100 g^{-1}) when compared to the other two parts. Table 5 presents the phytochemical components of *C. lepidota* with flavonoids and phenols present in all the parts.

Proximate compositions of *C. lepidota* revealed the moisture content of 9.29, 12.31 and 10.63% for the endocarp, mesocarp and exocarp, respectively. The moisture content of *C. lepidota* is relatively lower when compared with other varieties of the fruit (Ethonihu et al. 2013). The lowest moisture content observed in the endocarp corroborates the results of Osabor et al. (2015) where they reported a higher moisture content of 22.00 + 0.12% wet maceration for exocarp and 20.00 + 0.10% for endocarp. The higher moisture content of fruits has been established as a contributory factor to the microbial attack. Okudu et al. (2016) reported a moisture content of less than 10% (exactly 9.29%) for the endocarp, which is in accordance with the result of this study. However, they reported a higher ash content than observed in this study. The high fibre content of the endocarp also agrees with the previous reports (Osabor et al. 2015). The study further presented *C. lepidota* as a rich source of minerals, specifically iron (Fe), and a moderate amount of copper (Cu). The mineral values were many times higher than the values reported for most fruits (Stadlmayr et al. 2012). The vitamin C content observed in this study was 6.34, 14.39, 10.02 mg 100 g^{-1} for the endocarp, mesocarp and exocarp, respectively. This was way higher than the report of Ogbu and Umeokechukwu (2014) where they recorded a vitamin C content of 11.28 mg 100 mg^{-1} of fruit sample. The relatively high vitamin C content of the mesocarp suggests that it may be a suitable source of antioxidants (Bello et al. 2008) and also improve iron absorption (Ene-Obong 2001). Phytochemical and antioxidant screening indicated the presence of tannins and saponins in both the endocarp and exocarp. The mesocarp contains anthocyanins, coumarins and betacyanin, while flavonoid and phenols are present in all the fruit parts (Table 5, Figures 2 and 3). Hydrogen peroxide scavenging activity was observed in all components of the fruit mesocarp, endocarp and exocarp,

Table 4. Mean mineral composition (mg 100g^{-1}) of *C. lepidota* fruits

Sample	Fe	Cu	Zn	Pb	Mn	Cd	Cr
Endocarp	0.0400	0.0070	0.0330	0.0000	0.0120	0.0000	0.0000
Mesocarp	1.4167	0.0698	0.1515	0.3580	0.2013	0.0003	0.0034
Exocarp	1.7930	0.1722	0.2727	0.0151	0.5679	0.0005	0.1343

Fe – Iron; Cu – Copper; Zn – Zinc; Pb – Lead; Mn – Manganese; Cd – Cadmium; Cr – Chromium

Table 5. Phytochemical profiles of the endocarp, mesocarp and exocarp of *C. lepidota* fruits

Sample	Tannins	Saponins	Flavonoids	Alkaloids	Anthocyanin	Quinones	Glycosides	Terpenoids	Phenols	Coumarins	Steroids	Gallic acid	Betacyanin	Cardiac glycosides
Endocarp	+	+	+	–	–	–	–	–	+	–	–	–	–	–
Mesocarp	–	–	+	–	+	–	–	+	+	+	–	–	+	–
Exocarp	+	+	+	–	–	–	–	+	+	–	–	–	–	–

+ positive (present), – negative (absent)

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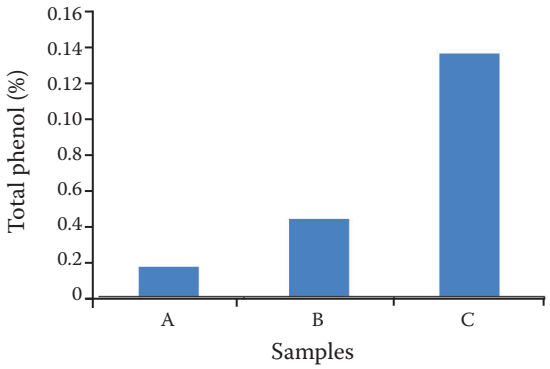

Figure 2. Mean total phenol content (mg gallic acid equivalent g^{-1} extract) in different parts of *C. lepidota* fruits

A – endocarp; B – exocarp; C – mesocarp

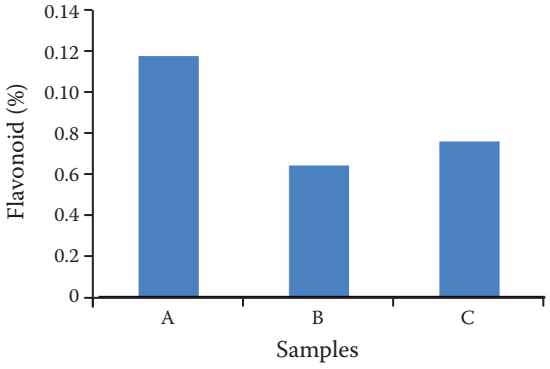

Figure 3. Mean total flavonoid content (mg pyrocatechol equivalent g^{-1} extract) of different parts of *C. lepidota* fruits

A – endocarp; B – exocarp; C – mesocarp

respectively (Figure 1). The fruit exocarp however had the highest distribution of phytochemicals. In all the fruit parts which were analysed, alkaloids, quinolones, glycosides and steroids were absent. Okudu et al. (2016) earlier reported flavonoids, saponins, phenols and tannins, which agrees with our report. However, they recorded and reported the highest quantity for alkaloids, which is in contrast to the report from this study. a high flavonoid content was observed in this study. This corroborates the studies of Ene-Obong et al. (2016), who reported flavonoid and β-carotene as the most abundant phytochemicals in the fruit sample. Again in this study the highest amount of phenol was observed in the mesocarp as against the report of Essien et al. (2015) where the highest phenol content was observed in the seeds (endocarp). Phenolics and flavonoids are known to be strong antioxidants and have anti-microbial, anti-inflammatory, anti-allergic, anti-mutagenic and anti-cancer activity and protect against heart diseases (Hasan et al. 2012; Swamy et al. 2012; Panche et al. 2016). Though the mechanism of the activity by phytochemicals on bacteria is not fully understood, it is inferred that their antibacterial activity is through one of many mechanisms which include blockage of cell wall synthesis, inhibition of protein synthesis, disruption of nucleic acid biosynthesis, or lysis of microbial cells (Khoo et al. 2016). Tannins suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as proteolytic enzymes (Enwa et al. 2014). Saponins exhibit antioxidant and anti-inflammatory activity and are used in the management of hypercholesterolaemia and hyperglycaemia (Oyinlade 2014). The existence of these phytochemicals in *C. lepidota* provides a basis for fur-

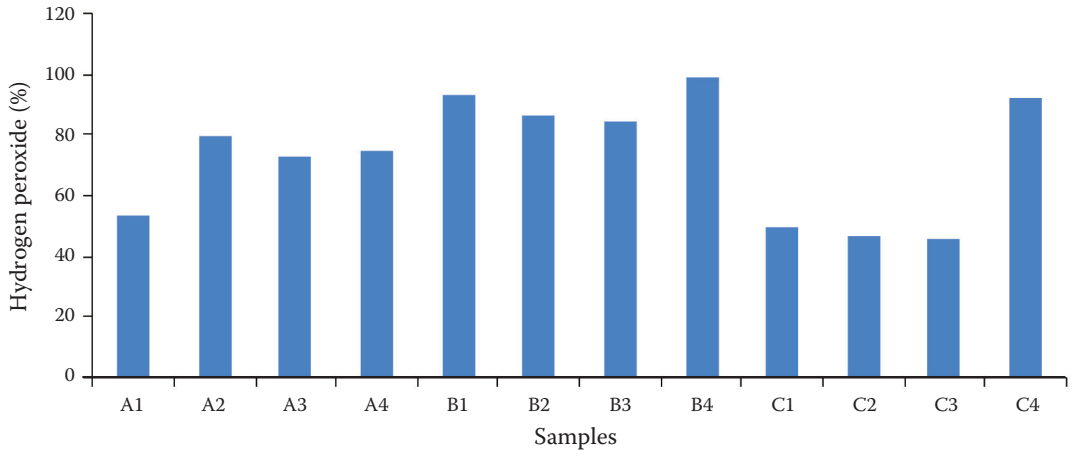

Figure 1. Mean hydrogen peroxide scavenging activity of different parts of *C. lepidota* fruits

A – endocarp; B – exocarp; C – mesocarp; concentrations:1 – 25.000 mg mL^{-1}; 2 – 12.500 mg mL^{-1}; 3 – 6.250 mg mL^{-1}; 4 – 3.125 mg mL^{-1}

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ther development of this fruit into products to be used in disease therapy. However, that *C. lepidota* does not have any antimicrobial activity on the clinical isolates of this work despite the presence of these phytochemicals calls for further investigation.

All the component parts of *C. lepidota* fruits (endocarp, mesocarp and exocarp) presented good antioxidant properties by the hydrogen peroxide scavenging activity. Antioxidants are known to inhibit oxidation and oxidative stress, and remove potentially damaging oxidizing agents in a living organism, thus prevent diseases and serious ill-health in man and animals. Studies have shown a direct correlation between total flavonoid and total phenol contents of plant extracts and free radical scavenging activity (Oranusi et al. 2013; Khandaker et al. 2015; Kefayati et al. 2017; Johari & Khong 2019). Das et al. (2011) and Keser et al. (2012) reported concentration dependent hydrogen peroxide scavenging in tea and *C. monogyna* extracts, respectively. However, Oranusi et al. (2013) observed strong hydrogen peroxide scavenging activity that was not concentration dependent and did not significantly ($P < 0.05$) vary among different spices.

CONCLUSIONS

This study has presented the nutrient and phytochemical compositions, including contaminating microorganisms, of *C. lepidota* fruit. The exocarp, mesocarp and endocarp of *C. lepidota* fruit were compared. The study has shown that the *C. lepidota* fruit is rich in essential nutrients, phytochemicals and in vitamin C, therefore it is suitable for human consumption. The exocarp and endocarp hitherto not consumed by man can be served as a rich nutrient source for animal feed formulation. Because *C. lepidota* is one of the fruits that need to be fully exploited, there is a need for more information on it.

Acknowledgement. Authors acknowledge Covenant University for providing a suitable atmosphere for research and for payment of publication fee.

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Received: December 28, 2018
Accepted: October 25, 2019