

Some antinutrient compositions and in vitro antioxidant properties of milled *Carica papaya* (pawpaw) peels and seeds

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ABSTRACT

This study evaluated some antinutrient compositions and antioxidant properties of milled *Carica papaya* peels and seeds, using standard methods. The result revealed the preponderance of phenol in the *Carica papaya* peels (184.72 ± 0.02 mg/100 g) which was higher than the other determined antinutrients in the peels and seeds. This was followed by oxalates in the peels (28.81 ± 0.15 %), phenols in the seeds (28.32 ± 0.02 mg/100 g) and oxalates in the seeds (11.08 ± 0.17 %) while the least of the determined antinutrients was hydrogen cyanide in the peels (0.15 ± 0.20 µg/g). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of the *Carica papaya* peels (48.03 ± 0.69 %) was higher than that of the seeds (43.70 ± 0.78 %) while the chelating ability of the peels (27.83 ± 0.81 %) and the reducing ability of the peels (36.24 ± 0.23 %) were lower than that of the seeds (32.60 ± 1.21 % and 52.33 ± 0.34 %, respectively). The difference between the *Carica papaya* peels and seeds for the determined parameters, apart from that of flavonoids, phytates and saponins, was significant ($p < 0.05$). Thus, the study revealed that the *Carica papaya* peels and seeds contain varied anti-nutrients with varied and sample specific antioxidant properties. Further studies, including the determination of the other antioxidant compounds in and the concerted antioxidant capacity of the *Carica papaya* peels and seeds are warranted and recommended.

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Key words: *Antioxidant, hydrogen cyanide, DPPH, antinutrients, chelating*



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Introduction

Increasing human health challenges could be compounded by the adverse effects of apparently harmless foods and food condiments to overwhelm the existing drugs (Egbuonu and Osakwe, 2011; Egbuonu et al., 2009). The resultant increasing search and use of plant-sourced drugs may deplete food supply and worsen food shortage in especially developing countries including Nigeria. Thus, there is the need to explore the possible food and drug potentials of plant food and fruit wastes to, in addition, reduce the possible environmental waste burden and the attendant public health implications of such solid food wastes (Egbuonu and Osuji, 2016; Egbuonu et al. 2015).

Carica papaya which belongs to the family Caricaceae is found in tropical regions including Africa (Aravind et al., 2013). It, though short-lived, grows to an un-branched height of 3 – 10 m (Krishna et al., 2008) and even up to 20 - 30 m (Vijay et al., 2014). It is known as pawpaw or ‘okwuru ezi’ in Ojoto and neighbouring towns in the south eastern Nigeria (Egbuonu et al., 2016a). Bioactive compounds (for instance esculetin) in fruits may improve markers of health functions in animals (Egbuonu et al., 2012). The pawpaw fruit is rich in many bioactive components, including phytoalexin, dietary minerals and fibre (Aravind et al., 2013; Krishna et al., 2008; Egbuonu et al., 2016) and its attendant high consumption could generate wastes in the form of peels and seeds.

Free radical-mediated oxidation apart from resulting to food quality deterioration (Sarikurkcu, 2011) was implicated in many human diseases and in aging process (Gulcin, 2012; Romeilah, 2016; Lee and Kim, 2000). These underscore the need for, and the use of, antioxidants in food preservation and in human health management to scavenge the free radicals. However, synthetic antioxidants may have adverse effects in animals necessitating and heightening the search for plant sourced natural antioxidants which could also ameliorate the effect of reactive oxygen species (Fazlina et al., 2016). For instance, plant-sourced flavonoids and phenols (which are antinutrients) are potent antioxidants (Sarikurkcu, 2011; Chua et al., 2008). The seeds and peels of watermelon have nutritive and antimicrobial properties (Egbuonu, 2015a,b,c,d). Possible roles of bioactive compounds in improving and managing even metabolic diseases have been suggested (Ezeanyika and Egbuonu, 2011;

Egbuonu and Ezeanyika, 2013). It is interesting to find out if the *C. papaya* peels and seeds contained antinutrients and had antioxidant properties. These warranted this study aimed at determining and comparing some antinutrient compositions and antioxidant properties of *C. papaya* peels and seeds.

Materials and Methods

Chemicals and reagents

The solvent, ethanol and other chemicals used, including those used in the preparation of reagents, were of analytical grade and products of reputable companies, including Sigma Chemical Company, St. Louis, U.S.A. and British Drug House (BDH) Ltd., Poole, England. This study was conducted between May and August, 2015 at the Department of Biochemistry, Michael Okpara University of Agriculture Umudike, Nigeria.

Collection and identification of plant materials

The *C. papaya* fruits were purchased from Ndioru market in Ikwuano Local Government Area, Abia State, Nigeria. The fruit sample was identified by Mr. Obi, a taxonomist in the Central Laboratory of National Research Root Crop Institute Umudike, Nigeria as *Carica papaya* Linn (Agric or oblong shaped variety).

Samples preparation and extraction

The *C. papaya* fruits were thoroughly washed with clean water, sliced longitudinally into four equal parts and peeled, using a home choice European knife. The seeds were picked from the pulp and washed with clean water. The peels were chopped into bits. The samples (peels and seeds) were separately placed on a foil and weighed with a Satorious Digital Weighing Balance, Model BP210S, Germany before and after sun drying for four days to obtain the wet weight and dry weight respectively. The respective dry weight sample was separately milled using Arthur Thomas Laboratory Mill Crypto model, USA, covered separately in a labeled white nylon and kept in the desiccator until used. The respective ethanol extract of the samples (peels and seeds) was separately obtained as described previously (Egbuonu, 2015d).

Determination of antinutrients

The alkaloid content was determined by the alkaline precipitation gravimetric method (Harbone, 1973) while the flavonoid content was determined by gravimetric method (Harbone, 1973). The tannin content was determined by the Folin-Dennis spectrophotometric method (Pearson, 1976) whereas the saponin content was determined by the double solvent extraction gravimetric method (Harbone, 1973). The hydrogen cyanide content was determined by the alkaline picrate colorimetric method (Balayopalan et al., 1988) while the phytate content was determined by colorimetric method (Oberleas, 1978). The phenol content was determined by the method in Association of Official Analytical Chemists, AOAC (2000).

Determination of antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity)

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Bougateg et al. (2009) as modified by Ladda et al. (2015) and described in Egbuonu et al. (2016b).

Determination of reducing activity

The reducing property of the extracts was determined by assessing the ability of the extract to reduce FeCl_3 solution as described by Oyaizu (1986). A 2.5 ml of FeCl_3 was mixed with 2.5 ml of 200 mmol l^{-1} sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 ml of 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of distilled water and 1 ml of 0.1 % ferric chloride. The absorbance was measured at 700 nm. The ferric reducing antioxidant ability was subsequently calculated using ascorbic acid as standard.

Determination of Fe^{2+} chelating ability

The Fe^{2+} chelating ability of the extracts was determined using the method of Minotti and Aust (1987) as slightly modified by Puntel et al. (2005). Freshly prepared 500 $\mu\text{mol l}^{-1}$ FeSO_4 (150 μl) was added to a reaction mixture containing 168 μL of 0.1mol l^{-1} Tris-HCl (pH 7.4), 218 μl of saline and 0.25 μl of the sample extract. The reaction mixture was incubated for 5 min, before the addition of 13 μl of 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The Fe^{2+} chelating ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample), using the relation:

Fe^{2+} chelating ability (%) = [(Absorbance of control – Absorbance of sample)/Absorbance of control] × 100.

Data analysis

The data obtained by triplicate determinations were subjected to analysis of variance (ANOVA) using SPSS 16.0 for Windows. Comparison of difference in means was based on Students t-test. Difference in mean at a p value < 0.05 was regarded as statistically significant. Results were expressed as mean± standard deviation (SD).

Results

The antinutrient result of the study as presented in Table 1 and Figures 1, 2 and 3 revealed the preponderance of phenol in the *C. papaya* peels (184.72± 0.02 mg/100 g) which was higher than the other determined antinutrients in the peels and seeds. This was followed by oxalates in the peels (28.81±0.15 %), phenols in the seeds (28.32±0.02 mg/100 g) and oxalates in the seeds (11.08±0.17 %). The least of the determined antinutrients was hydrogen cyanide in the peels (0.15±0.20 µg/g). The difference between the *C. papaya* peels and seeds for the determined antinutrients, apart from that of flavonoids, phytates and saponins, was significant (p<0.05).

Table 1: Some antinutrient compositions of milled *Carica papaya* peels and seeds

Anti-nutrients	<i>C. papaya</i> Peels	<i>C. papaya</i> Seeds	Difference
Phenols (Mg/100 g)	184.72± 0.02	28.32±0.02	±156.40*
Hydrogen cyanide (µg/g)	0.15±0.20	1.87±0.23	±1.72*
Alkaloids (%)	4.60 ^a ±0.10	1.07±0.06	±3.53*
Oxalates (mg/100g)	28.81±0.15	11.08±0.17	±17.73*

Result = Value ± SD of triplicate determinations. ns = difference is not significant (p > 0.05). * = difference is significant (p < 0.05)

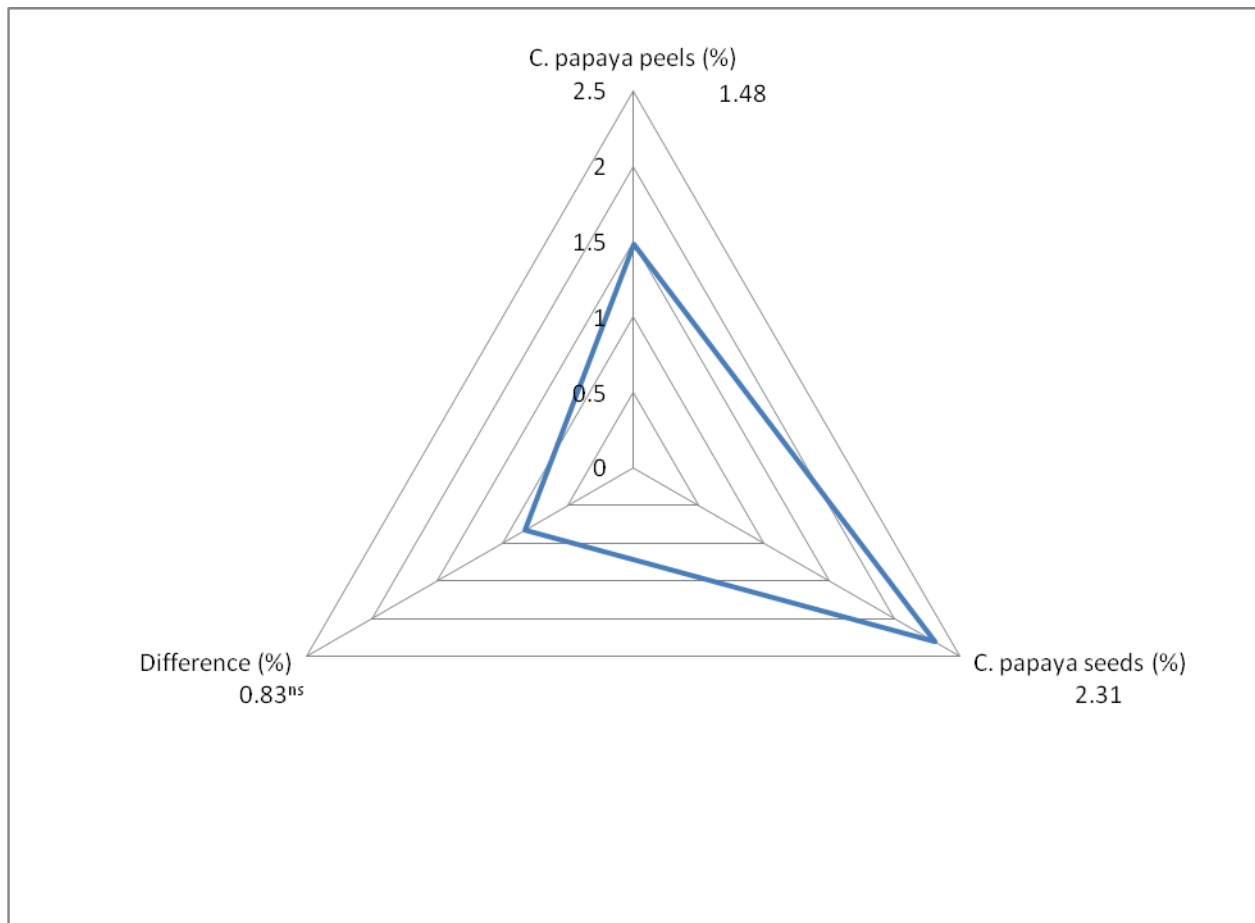


Figure 1: Flavonoids composition (%) of milled *Carica papaya* peels and seeds (^{ns} indicates non significant difference p>0.05)

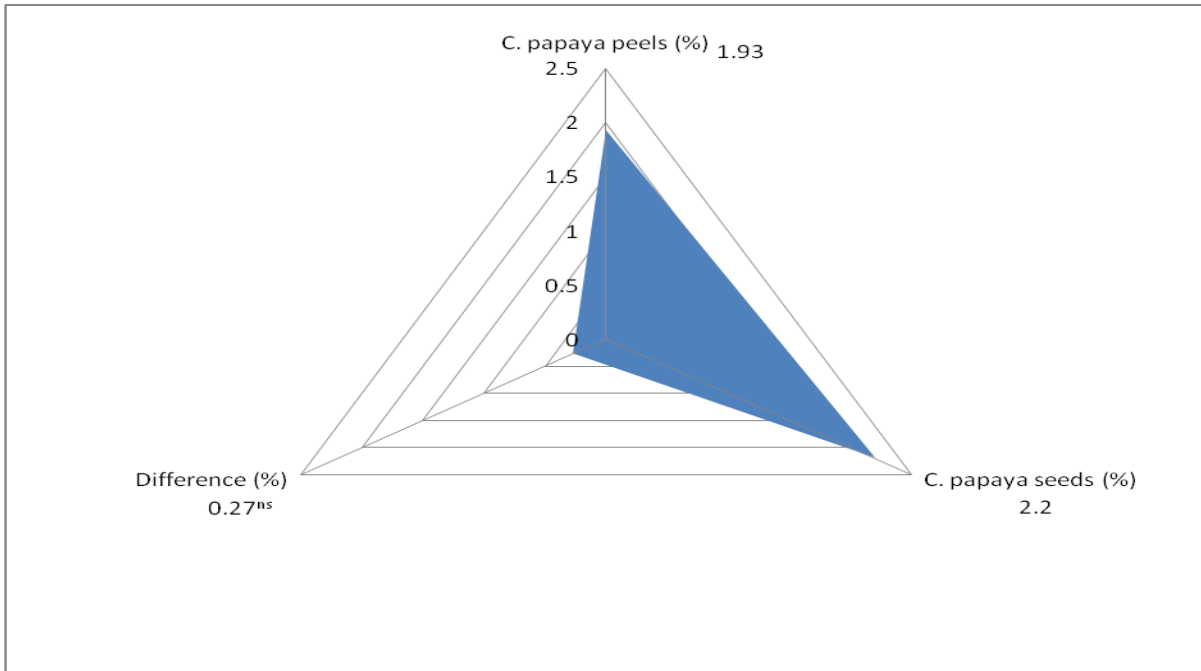


Figure 2: Phytates composition (%) of milled Carica papaya peels and seeds (^{ns} indicates non significant difference $p > 0.05$)

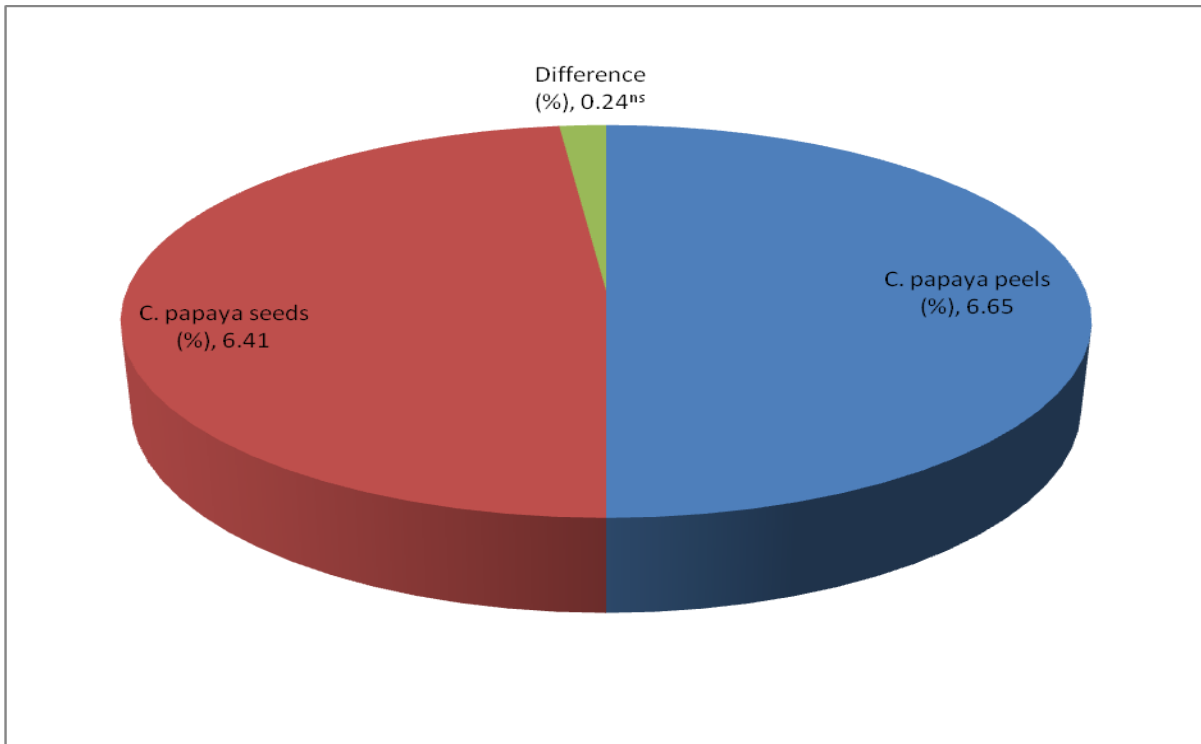


Figure 3: Saponins composition (%) of milled Carica papaya peels and seeds (^{ns} indicates non significant difference $p > 0.05$)

As shown in Table 2, the DPPH radical scavenging ability of the C. papaya peels (48.03 ± 0.69 %) was significantly higher ($p < 0.05$) than that of the seeds (43.70 ± 0.78 %) while the chelating ability of the peels (27.83 ± 0.81 %) and the

reducing ability of the peels (36.24 ± 0.23 %) were significantly lower ($p < 0.05$) than that of the seeds (32.60 ± 1.21 % and 52.33 ± 0.34 %, respectively).

Table 2: DPPH radical scavenging activity (%), Fe^{2+} chelating ability (%) and FeCl_3 reducing ability (%) of milled *Carica papaya* peels and seeds

Sample	DPPH scavenging activity or percentage inhibition (%)	Fe^{2+} chelating ability (%)	FeCl_3 reducing ability (%)
C. papaya peels	48.03 ± 0.69	27.83 ± 0.81	36.24 ± 0.23
C. papaya seeds	43.70 ± 0.78	32.60 ± 1.21	52.33 ± 0.34
Difference	$\pm 4.33^*$	$\pm 4.77^*$	$\pm 16.09^*$

Result = Value \pm SD of triplicate determinations. ns = difference is not significant ($p > 0.05$). * = difference is significant ($p < 0.05$)

Discussion

The preponderance of phenols in the *C. papaya* peels (184.72 ± 0.02 mg/100 g) which was higher than the other determined antinutrients in the peels and seeds could be indicating higher inherent antioxidant properties in the peels (Pourmorad et al., 2006). This is apparently supported by the higher free radical scavenging activity of the *C. papaya* peels recorded in the present study. Phenols are bioactive antioxidants owing to the multiple hydroxyl groups in their structures hence enhanced hydrogen and electron donating abilities (Sarikurkcu, 2011; Boskou, 2006). Oxalate in excess by exceeding the solubility limit results in the formation of calcium oxalate kidney stones (Brzezicha-Cirocka et al., 2016; Holmes and Kennedy, 2000). However, the dietary contribution to excess oxalate was reportedly low (Holmes and Kennedy, 2000). Oxalate content in either sample (Table 1) was low compared to the range of mean values (42 to 469 mg/100 g) from two methods obtained for various types of nuts (Weiwen and Liebman, 2005). Hence the contribution of the respective sample to the formation calcium kidney stones when consumed may not be significant. The saponins content in either the peels or the seeds was quite low compared to the median lethal dose, LD_{50} for saponins (Diwan et al., 2000), hence could be expected to elicit health benefits when consumed by humans (Muhammad et al., 2012). The relatively low hydrogen cyanide in the peels (0.15 ± 0.20 $\mu\text{g/g}$) and even in the seeds (1.87 ± 0.23 $\mu\text{g/g}$) suggests that the samples may not be susceptible to deterioration and may have low hydrogen cyanide-related toxicity following ingestion in animals (Egbonu et al., 2016b). The hydrogen cyanide content in either the peels and in the seeds was lower than the range (40.80 to 42.82 mg/100 g) reported for varieties of groundnut seeds (Shad et al., 2009) and *Citrus sinensis* peels and seeds (Egbonu et al., 2016b).

The DPPH radical scavenging ability of the *C. papaya* peels (48.03 ± 0.69 %) was higher than that of the seeds (43.70 ± 0.78 %) indicating higher free radical-related antioxidant property of the *C. papaya* peels due possibly to the higher phenol content of the peels reported in this study. Generally, iron through Fe^{2+} reacts with hydrogen peroxide (H_2O_2) to produce hydroxyl radicals via the Fenton reaction. However, polyphenols could protect important organs from hydroxyl radicals-mediated oxidative damage by preventing iron (hence Fe^{2+}) overload through Fe^{2+} chelating ability (Obboh et al., 2014). The Fe^{2+} chelating ability for the *C. papaya* peels compares with, whereas that for the *C. papaya* seeds was higher than that, reported for African mistletoes obtained from kolanut and breadfruit (Obboh et al., 2014). The lower chelating (27.83 ± 0.81 %) ability of the peels compared to that of the seeds (32.60 ± 1.21 %) indicates higher chelating-related antioxidant properties of the *C. papaya* seeds. This could imply that the *C. papaya* seeds, more than the peels, could better suppress the formation of reactive oxygen species. Reducing ability is directly proportional to the antioxidant activity (Obboh et al., 2014). Thus, the lower reducing (36.24 ± 0.23 %) ability of the peels compared to that of the seeds (52.33 ± 0.34 %) indicates higher reduction-related antioxidant properties of the *C. papaya* seeds. This could imply that the *C. papaya* seeds, more than the peels, could have higher capacity to react with free radicals to stabilize and terminate radical chain reactions. Overall, the antioxidant results suggest sample specific antioxidant properties of *C. papaya* peels and seeds. This implies that the *C. papaya* peels could achieve higher antioxidant property than the seeds only via scavenging ability whereas the *C. papaya* seeds could achieve higher antioxidant property than the peels through a combination of chelating and reducing abilities. Total antioxidant capacity results from the interplay of antioxidant compounds aside polyphenols (Halvorsen et al., 2006; Lotito and Frei, 2006). Furthermore, the present study did not determine the concerted antioxidant capacity of the *C. papaya* peels and seeds. Thus, further studies, including in animals, are warranted to determine the other antioxidant compounds in, and the concerted antioxidant capacity of, the *C. papaya* peels and seeds.

Conclusion

The study revealed that the *C. papaya* peels and seeds contain varied anti-nutrients with varied and sample specific antioxidant properties. Further studies, including the determination of the other antioxidant compounds in and the concerted antioxidant capacity of, the *C. papaya* peels and seeds are warranted and recommended. Such studies could ensure that the

present findings to be harnessed to minimize the waste status and attendant contribution to environmental burden of *C. papaya* peels and seeds.

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