Asian Journal of Chemistry; Vol. 36, No. 1 (2024), 223-228

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2024.28159

Effects of Drying Techniques and Different Solvents on Phytochemical Composition and Antioxidant Activity of *Carica papaya* Seed Extracts: Implications for Industrial Use and Medicinal Benefits

AYOMADEWA MERCY OLATUNYA

Department of Chemistry, Ekiti State University, P.M.B. 5363, Ado, Ekiti, Nigeria

Corresponding author: Tel: +234 8160850627; E-mail: ayomadewa.olatunya@eksu.edu.ng

Received: 10 June 2023; *Accepted*: 10 December 2023; *Published online*: 31 December 2023; AJC-21507

Plant has always been of immense health benefits to humanity because, plant contains different parts that have been found to have various medicinal and industrial values. Therefore, plants play a crucial role in obtaining their most effective extracts for medical and industrial applications. The primary components of these preparations involve the extraction and drying methods. *Carica papaya* is a plant that is known to have different parts with numerous and unexplored medicinal and industrial benefits. Thus, this study investigated the effects of drying techniques and different extracting solvents on the phytochemical composition and antioxidant activity of *Carica papaya* seeds extract using standard analytical procedures. The results showed that drying technique has significant effect ($p < 0.05$) on the phytochemical composition of the *C. papaya* seeds with air-drying at room temperature having the highest amount of total phenolic compounds, flavonoids, saponin content and antioxidant activity when compared to other techniques. Hexane extracted the highest amount of total phenolic compounds (121.08 mg/GAE/100 g). Diethyl ether extracted the highest amount of total flavonoid and saponin content while polar solvents (aqueous and ethanol) extracts have the highest amount of antioxidant activity. This study showed that air drying at room temperature is a good method of preservation of the phytochemicals present in medicinal plants and that stereochemistry and structural diversity of compounds determine the efficiency of their extraction in any solvent. Furthermore, the highlighted phytochemicals and antioxidant activity of *C. papaya* seed extracts could be of immense benefits in various industries and for medicinal purposes.

Keywords: Drying techniques, Phytochemicals, Aqueous extract, Antioxidant activity.

INTRODUCTION

One of the most widely available fruits, the *Carica papaya* is cultivated in a variety of regions across the globe and is also one of the most widely consumed fruits [1]. It is currently grown in more than sixty nations of the world with about 13,894,705 million tonnes of the fruit produced in the year 2020. The colour of the fruit changes from green to yellow-orange or red depending on its stage of ripening [2]. Each matured and ripe pawpaw fruit possess numerous seed when cut. Generally, the seed constitutes about 22% of processing waste thus they are generally regarded as waste. Even though, they are generally regarded as waste, they have been found to have some traditional uses. In Nigeria, it is used to make an indigenous food called *dadawa* - a fermented food [3], which has antibactericidal activities against several illnesses and protect the body from oxidative stress [4,5]. As secondary metabolites, phytochemicals play an important role in plant defense mechanisms. However, these phytochemicals have been found to be responsible for some of the medicinal properties found in plants. They play a key role in preventing occurrence of some human diseases like cancer, neurodegenerative diseases, diabetes and obesity [6]. These compounds are structurally different and are heterogenous in nature, their structure sometimes, may vary from simple to complex compounds thus their solubility depend on their chemical structure, nature and polarity of solvent. These factors have been found to have different effects on the extraction and quantification of some polyphenolic compounds and phytochemicals obtained from plants [7]. It has also been found that the method of preparation of medicinal plants is important in determining the quality and quantity of the bioactive compounds obtained from them. Some studies have been carried

out on the phytochemical composition of *Carica papaya* plant extract [8-10] but only few studies have worked on the phytochemical composition of *Carica papaya* seeds [5,11]. Fewer researchers also investigated the effects of drying techniques and solvents used for extraction on the constituents of the phytochemicals and antioxidant activity of the seeds [12].

Therefore, the aim of this research work is to investigate the effect of drying techniques and different extracting solvents on the quantitative analysis of the phytochemical constitution and antioxidant activity of *Carica papaya* seeds. This could shed more lights on the various contents of *Carica papaya* and the potential benefits of its seeds for various industries and medicinal use rather than being considered as waste considering the fact that *Carica papaya* fruits contain considerable amount of seeds and can be grown in many parts of the world.

EXPERIMENTAL

Ripened *Carica papaya* fruits were collected from the tree plantation site in Ado Ekiti metropolis. The fruits were peeled and the seeds were also removed. The seeds were rinsed several times under running water to remove any fruit pulp. Thereafter, it was rinsed with distilled water several times. The seeds were divided into two parts, one part was air dried under shade at room temperature for 5 days while the other part was dried under intense sun for 5 days. After drying to a constant weight, the seeds were ground separately to fine powdered and kept in airtight container till further analysis. All the chemicals used for this study were of analytical grade and purchased from Sigma-Aldrich (United Kingdom).

Extraction of *C. papaya* seeds: The powdered sample (5 g) was extracted with 150 mL of hexane for 24 h at room temperature using a mechanical shaker. After extraction, it was filtered through a filter paper to remove insoluble residues. The solvent was evaporated under vacuum using rotary evaporator at 30 ºC and the extracts were stored at 4 ºC for further analysis.

Determination of effect of different solvents and drying technique on seed extract: To determine the effect of different solvent on the seed extract, the same procedure was carried out as described above with three different solvents which are diethyl ether, ethanol and aqueous solution. This was also done for the sun dried and air dried samples separately to determine the effect of drying techniques on the sample.

Determination of phytochemical and antinutritional composition of *C. papaya* seed extract

Total flavonoid content: The total flavonoid content of the extract was determined using a colourimeter assay [13] with slight modification. The extract (0.5mL) was mixed to 0.3 mL of 5% $NaNO_3$ and shaked well. After 5 min, 0.6 mL of 10% $AlCl_3$ was added and after 6 min, 2 mL of 1 M NaOH was added to the mixture followed by the addition of 2.1 mL of distilled water. Absorbance was read at 510 nm against the reagent blank and the total flavonoid content was expressed as the rutin equivalents (mg RE/100 g) of the dry weight of sample.

Total phenolic content: The total phenolic content of the extract was determined by the method of Otang *et al.* [14]. The extract (0.5 mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent which was previously diluted with water (1:9 v/v). After 5 min, 2 mL of 7.5% sodium carbonate was added, then the tubes were vortexed for 5 s and finally the reaction mixture was subsequently incubated in the dark at 25 ºC for 40 min. The absorbance was measured at 700 nm using UV vis spectrophotometer, garlic acid was used as standard and TPC (total phenolic content) was expressed as mg gallic acid equivalents per 100 g of sample.

Determination of total saponin content: The method of Benyong *et al*. [15] was used for the determination of the total saponin content with slight modifications. The extract (2 µL) was added to 100 mL of isobutyl alcohol or (butan-2-ol) and then the mixture was vigorously shaken using a mechanical shaker for 30 min, filtered and the residue was reextracted again. The combined solution was concentrated under reduced pressure to 30 mL and then transferred to 250 mL separatory funnel and extracted with petroleum ether. The etheral layer was discarded and the purification process was repeated. Now, 50 mL of *n*-butanol was added and the extraction process was continued till a clean colourless solution was obtained. A 1 mL of colourless solution was taken into 50 mL volumetric flask, 2 mL of 5% $FeCl_3$ solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min and the absorbance was taken against the blank at 540 nm. The standard saponins solutions in the range of 0 to 5 ppm were prepared and the absorbance were also taken to obtain the gradient plotted curve.

Determination of total alkaloid content: The total alkaloid content was determined using the method of Obadoni & Ochuko [16]. A 5 g of the sample was added to 200 mL of 10% acetic acid in ethanol and allowed to stand for 4-5 min. The solution was filtered and then concentrated on a water bath to one quarter of the original volume followed by the addition of conc. NH_4OH dropwise to the extract until the precipitation was completed. Once the solution settled, the precipitate was collected, washed with dilute NH_4OH and filtered.The residue which contained alkaloid was dried and then weighed.

Determination of antinutrient composition: The oxalate composition of *C. papaya* seed extract was determined using the method of Day & Underwood [17]; the phytate content was determined using Wheeler & Ferrel [18] and the total cyanide was determined by the method of AOAC [19].

Antioxidant activity

Free radical scavenging ability using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay: The antioxidant activity of the *C. papaya* seed extract was determined using DPPH free radical scavenging assay described by Gyamfi *et al.* [20] with slight modifications. The extract was serially diluted to concentrations of 0.1-0.3 mg/mL. Each dilution (1 mL) was mixed with freshly prepared DPPH solution (1 mL) in methanol and incubated in dark for 30 min. After which the absorbance of the mixture was measured at 517 nm. The percentage DPPH scavenging activity was thereafter calculated using the following equation:

$$\text{Inhibition } (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

Determination of ferric reducing activity: The reducing activity of the extract was determined by the method of Pulido *et al.* [21]. The ferric reducing antioxidant power (FRAP) reagent was prepared in sodium phosphate buffer (pH 6.6). Then, 0.25 mL of extract was added to 0.75 mL of reagent and the mixture was incubated at 50 ºC for 20 min and finally centrifuged at 2000 rpm for 10 min. Aliquots of the sample was taken and the absorbance was read at 700 nm. The standard curve was prepared with ascorbic acid and the results were expressed as mg ascorbic acid equivalent per gram of the sample.

Statistical analysis: All the values were expressed as means of triplicate analysis. Analysis of variance using Tukey's test, correlation paired 't'-test and Bonferroni post tests were performed using GraphPad Prism program version 5 for windows. Differences among the means were also determined for significance at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical and antinutritional composition of the *C. papaya* seed using different solvents for extraction: The results of the phytochemical and antinutritional composition of the *C. papaya* seed is presented in Table-1. Hexane and diethyl ether extracts have high amount of total phenolic compounds and total saponin content. For the antinutrients, diethyl ether has higher amount of phytate content while there was no significant difference in the amount of phytate extracted by other solvents.

The choice of a solvent is known to be very important when carrying out extraction, because different solvents have different polarities and this affect the type of material to be extracted. In this study, hexane has the highest amount of total phenolic compound extraction while diethyl ether has the highest amount of flavonoid and saponins as shown in Table-1. This observation may be due to the fact that phenolic compounds are composed of different compounds with different polarities and thus, the type of compound with the highest concentration will determine the solubility of the total phenolic compound present in the substance. Phenolic compounds with higher polarity will be extracted by solvents with higher polarity and *vice versa*. So also, is flavonoids; flavonoids with less polarity like flavonones, aglycones, flavonols, methylated flavones will be extracted more by less polar solvents [22]. This observation agrees with the report of Gaikwad & Kshirsagar *et al*. [10] on the extraction of phytochemicals from *C. papaya* flower using different solvents and also with the report of Nawaz *et al.* [23] on the extraction of phytochemicals from beans seeds. Saponins are slightly polar and will be more compatible with solvents, which are less polar than water.

Phytochemicals are composed of numerous compounds with different polarity thus, it can be concluded that the extraction of phytochemicals in different samples is influenced by the solubility of each compound in different extracting solvents and also, the polarity of the extracting solvent. Furthermore, the solubility of these compounds depend on stereochemistry and the intermolecular forces occurring between the molecules of the compound and the solvents [7]. This work has confirmed that due to the various physical and chemical diversity observed in natural products, it is not feasible to give a general extraction conditions but rather the stereochemistry and structural diversity of the separate compounds should be taken into consideration during solvent extraction process [24].

The observed high quantity of phytochemicals in these samples confirmed that pawpaw seeds are rich sources of these phytochemicals which have been confirmed to have beneficial health effects on human like acting as antioxidant, anti-aging, anti-inflammatory and antiproliferative agents [25,26]. This has also confirmed the potentials of the seeds for use in traditional and folk medicine and this could also been an eye opener to the potential use of the *C. papaya* seeds in modern day industries. The results of the total phenolic content (TPC) of the *C. papaya* seeds in this present work is higher when compared to the report of the TPC of *C. papaya* seeds by Pokhrel & Karki [27]. The differences in the results may be due to differences in the extraction process and geographical location.

The results of the antinutrients showed that only phytate has significantly high amount in diethyl ether extract thus showing that the antinutritive content of *C. papaya* seed can generally be extracted by any of the different solvents used in this study. Antinutritional components were detected in low concentration in the seed extract thus confirming the safety of the seeds. Phytate, cyanide and oxalate are the antinutritional components that have been found to chelate and form complex with dietary minerals thereby reducing their bioavailability in the body system. However, these antinutrients becomes significantly dangerous at a level higher than 50 mg/day [28-30]. The concentration detected in this sample is far lower than the toxicity level thus confirming that *C. papaya* seeds are safe for consumption. The result presented in this work is comparable to the report of Oyeleke *et al.* [11] on the antinutritional composition of *C. papaya* seeds.

Antioxidant activity of *C. papaya* seeds using different solvents for extraction: The result of the DPPH radical scavenging ability of the seed extract using different solvent is

TABLE-1
PHYTOCHEMICAL AND ANTINUTRITIONAL COMPOSITION OF *C. papaya* SEED USING DIFFERENT SOLVENTS FOR EXTRACTION

Solvent used	TFC (mg RE/100 g)	TPC (mg GAE/100 g)	TSC (mg/100 g)	TA (mg/100 g)	Oxalate (mg/100 g)	Phytate (mg/100 g)	Cyanide (mg/100 g)
Hexane	7.27^{b}	121.08^{e}	57.82^{a}	2.30^{c}	0.180^{k}	4.12^{m}	2.29^{o}
Diethyl ether	18.27^{a}	90.88^{f}	58.91^{a}	4.15^{d}	0.63^{h}	12.36^{l}	2.95^{o}
Ethanol	6.53^{c}	42.09^{g}	53.96^{b}	4.96^{d}	0.42^{h}	6.32^{m}	3.14^{op}
Aqueous	5.65^{d}	35.49^{h}	40.54^{c}	3.89^{d}	0.56^{h}	5.05^{m}	3.32^{p}

TFC = Total flavonoid content; TPC = Total phenolic compound; TSC = Total saponin content; TA = Total alkanol; values represent mean of triplicate analysis; Values with different letter down the column are significantly different at $p < 0.05$.

presented in Fig. 1. Aqueous extract has the highest percentage of DPPH radical scavenging ability when compared to other extracts. This same trend was also observed for the reducing abilities of the extract (Table-2). Aqueous extract had the highest radical scavenging ability and the highest reducing ability than the other solvents. This showed that polar solvents are more suitable for extraction of antioxidant components from plant materials. This result agrees with the reports of Nawaz *et al.* [23] on the antioxidant activity of *Phaseolus vulgaris* seeds.

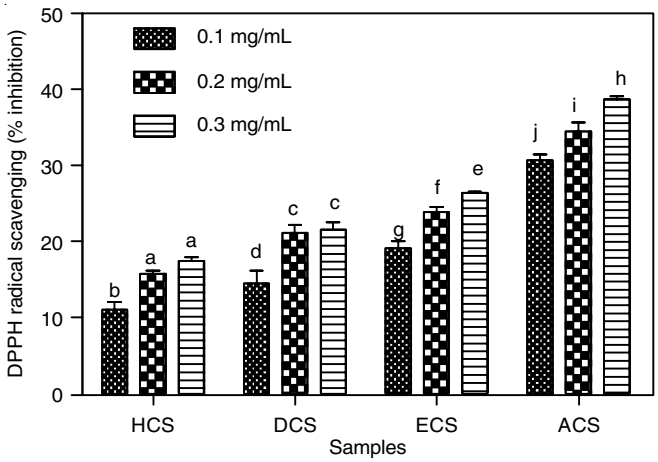

Fig. 1. DPPH radical scavenging ability of *C. papaya* seed samples in different extracting solvent. HCS = hexane extract *C. papaya* seed; DCS = diethyl ether extract *C. papaya* seed; ECS = ethanol extract *C. papaya* seed; ACS = Aqueous extract *C. papaya* seed. Values are expressed as mean of triplicate analysis. Bars with different letters indicate significant difference at $p < 0.05$

The good antioxidant ability observed for *C. papaya* seed extract showed that the seeds will be good for use in food industries as sources of natural antioxidants which have been advocated for because of their several health benefits and little or no adverse effects. The antioxidant potential of natural components is based on their redox properties that facilitate their activity as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers [10]. The result of antioxidant activity of *C. papaya* seeds in this study is in agreement with the reports of other authors on the antioxidant activity of paw-paw seed [31,32], which shows that the seed extract has high radical scavenging ability and good reducing ability which could be linked to the high content of phytochemicals present in the seed.

Effect of drying technique on the phytochemicals and antinutritional composition of the different extracts: The result of the effect of drying technique on the phytochemical and the antinutritional composition of the different extracts is presented in Table-3. There was a significant reduction in the amount of all the phytochemical parameters investigated when the two drying techniques were compared (air drying at room temperature and sun drying) except for total alkanols and saponin contents of some solvents while for the antinutritional factor there was no statistical difference in the composition of the antinutrients with regards to drying technique except for phytate and oxalate in hexane extract.

The significantly high amount of phytochemicals detected in the air dried samples at room temperature as compared to the sun dried samples showed that exposure to high temperature could cause loss of some vital phytochemicals in biological components. This observation is in agreement with the report of Irondi *et al.* [12] on the effect of different drying method on *C. papaya* seeds. Oxidation of bioactive compounds at high temperature could account for the reduction in the amount of total phenol and other phytochemicals present in the investigated samples. Thus, air drying at room temperature will help to preserve the phytochemicals present in the *Carica papaya* seeds therefore helping the manufacturing, health and complimentary health industries in their method of preservation.

Effect of drying techniques on the antioxidant activity of the different extracts: The result of the effect of drying technique on the antioxidant activity of the different extract is shown in Fig. 2. The result showed that air dried samples at room temperature has significantly higher DPPH radical scav-

TABLE-2
FERRIC REDUCING ABILITY OF *C. papaya* SEED USING DIFFERENT SOLVENTS FOR EXTRACTION

Solvents	Hexane	Diethyl ether	Ethanol	Aqueous
mg Ascorbic acid equivalent/g	6.55^{d}	9.84^{c}	13.02^{b}	16.06^{a}

Values with different letter along the row are significantly different at $p < 0.05$.

TABLE-3
EFFECT OF DRYING TECHNIQUE ON THE PHYTOCHEMICAL AND ANTINUTRITIONAL COMPOSITION OF *C. papaya* SEED EXTRACTS

	ASH	SSH	ASD	SSD	ASE	SSE	ASA	SSA
Total flavonoid content (mg RE/100 g)	7.269^{e}	5.04^{e}	18.27^{a}	15.65^{b}	6.53^{d}	4.25^{f}	5.65^{g}	3.95^{h}
Total phenolic compound (mg GAE/100 g)	121.08^{a}	99.01^{b}	90.88^{c}	81.00^{d}	42.09^{e}	35.58^{f}	35.49^{g}	33.10^{h}
Total saponin content (mg/100 g)	57.82^{b}	48.55^{e}	58.91^{a}	57.95^{a}	53.96^{d}	42.74^{f}	40.54^{g}	40.02^{g}
Total alkanol (mg/100 g)	2.30^{h}	2.14^{h}	4.15^{h}	4.07^{h}	4.96^{h}	3.33^{h}	3.89^{h}	3.76^{h}
Oxalate (mg/100 g)	0.18^{s}	0.14^{r}	0.63^{q}	0.61^{q}	0.42^{q}	0.31^{q}	0.56^{q}	0.51^{q}
Phytate (mg/100 g)	4.12^{v}	2.96^{x}	12.36^{t}	10.37^{t}	6.32^{v}	4.14^{v}	5.05^{v}	4.14^{v}
Cyanide (mg/100 g)	2.29^{d}	2.16^{d}	2.95^{c}	3.07^{c}	3.14^{b}	2.04^{d}	3.32^{a}	2.94^{a}

ASH = Air-dried at room temperature seed in hexane; SSH = Sun dried seed in hexane; ASD = Air dried at room temperature seed in diethyl ether; SSD = Sun dried seed in diethyl ether; ASE = Air dried at room temperature seed in ethanol; SSE = Sun dried seed in ethanol; ASA = Air dried at room temperature seed in aqueous; SSA = Sun dried seed in aqueous; values represent mean of triplicate analysis. Values with different letter along the row are significantly different at $p < 0.05$.

TABLE-4
FERRIC REDUCING ABILITY OF AIR DRIED AND SUN DRIED *C. papaya* SEED EXTRACTS

	Hexane*	Diethyl ether*	Ethanol*	Aqueous*
Air dried seed at room temperature	6.55[a]	9.84[c]	13.02[e]	16.06[f]
Sun dried seed	5.01[b]	7.83[b]	4.86[d]	11.75[g]

*Ferric reducing ability in mg ascorbic acid equivalent/g; Values with different letter down the column are significantly different at $p < 0.05$.

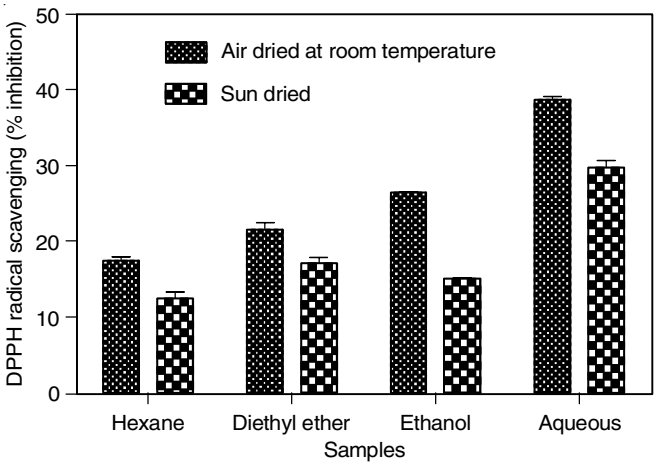

Fig. 2. Effect of drying technique on the antioxidant activity of the *C. papaya* seed extract using DPPH radical at concentration of 0.3 mg/mL

enging ability when compared to the sun dried samples. The same trend was also observed for the reducing ability of the extracts (Table-4). The significantly higher antioxidant activities of air dried samples also confirmed that air drying at room temperature helps to preserve the loss of some bioactive components which also have antioxidant power.

Conclusion

This study has shown that drying has significant effect on the quantity of phytochemicals obtained from natural plants. Air dried samples at room temperature has higher phytochemical and better antioxidant activity compared to the sundried samples. In addition, the composition of the phytochemicals present in the samples determines the type of solvent that gives better extraction of the phytochemicals while the antioxidant activities were higher in more polar solvent. The high amount of phytochemicals, high free radical scavenging abilities and reducing abilities of the *Carica papaya* seeds showed that the seeds are rich in total phenolic compounds and some other phytochemicals. These showed that they could be of great importance in prevention of some deadly diseases in humans. The antinutritional components also showed that the samples are safe for consumption. Thus making *C. papaya* seeds to be of great importance in many industries because of the various beneficial and medicinal benefits of its phytochemicals. Due to its strong antioxidant properties, *C. papaya* seed could be recommended as a substitute for synthetic antioxidants, as it is both safe and readily accessible. In order to optimize the numerous benefits of *C. papaya* seeds, close attention must be paid to the choice of solvent used for extraction especially in resource limited settings where *C. papaya* seeds can serve as a major raw material for various domestic and industrial uses.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. Food and Agriculture Organization (FAO), FAOSTAT; Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 22 August 2023).
2. O.R. Alara, N.H. Abdurahman and J.A. Alara, *Adv. Tradit. Med.*, **22**, 17 (2022); https://doi.org/10.1007/s13596-020-00481-3
3. N.S. Kumar and P. Sreeja Devi, *J. Pharmacogn. Phytochem.*, **6**, 424 (2017).
4. J.K. Peter, Y. Kumar, P. Pandey and H. Masih, *IOSR J. Pharm. Biol. Sci.*, **9**, 29 (2014); https://doi.org/10.9790/3008-09272937
5. Masfufatun, N.P.W. Yani and N. Putri, *J. Phys. Conf. Ser.*, **1277**, 012018 (2018); https://doi.org/10.1088/1742-6596/1277/1/012018
6. A. Vassallo, M.F. Armentano, R. Miglionico, C. Caddeo, C. Chirollo, M.J. Gualtieri, A. Ostuni, F. Bisaccia, I. Faraone and L. Milella, *Pharmaceutics*, **12**, 553 (2020); https://doi.org/10.3390/pharmaceutics12060553
7. D.-H. Truong, D.H. Nguyen, N.T.A. Ta, A.V. Bui, T.H. Do and H.C. Nguyen, *J. Food Qual.*, **2019**, 1 (2019); https://doi.org/10.1155/2019/8178294
8. K. Saravanasingh, M. Ramamurthy and P. Parthiban, *Int. J. Adv. Res. Biol. Sci.*, **3**, 329 (2016).
9. A.A. Gaye, O.I.K. Cisse, B. Ndiaye, N.C. Ayessou, M. Cisse and C.M. Diop, *Food Nutr. Sci.*, **10**, 276 (2019); https://doi.org/10.4236/fns.2019.103021
10. S.S. Gaikwad and S.J. Kshirsagar, *Beni. Suef Univ. J. Basic Appl. Sci.*, **9**, 1 (2020); https://doi.org/10.1186/s43088-019-0027-7
11. G.O. Oyeleke, A.D. Isola, M.A. Salam and F.D. Ajao, *J. Environ. Sci. Toxicol. Food Technol.*, **4**, 8 (2013).
12. A.E. Irondi, K.K. Anokam and U.S. Ndidi, *Int. J. Biosci.*, **3**, 154 (2013); https://doi.org/10.12692/ijb/3.11.154-163
13. J. Bao, Y. Cai, M. Sun, G. Wang and H. Corke, *J. Agric. Food Chem.*, **53**, 2327 (2005); https://doi.org/10.1021/jf048312z
14. W.M. Otang, D.S. Grierson and R.N. Ndip, *BMC Complement. Altern. Med.*, **12**, 43 (2012); https://doi.org/10.1186/1472-6882-12-43
15. H. Benyong, C. Ying, R. Ying and C. Chaoyin, *Indian J. Biotechnol.*, **10**, 10400 (2014).
16. B.O. Obadoni and P.O. Ochuko, *Glob. J. Pure Appl. Sci.*, **8**, 203 (2002); https://doi.org/10.4314/gjpas.v8i2.16033
17. R. Day and A. Underwood, Qualitative analysisin, 5th Ed., 701 (Prentice-Hall Publication, 1986). https://doi.org/10.1021/ed059pA105.1
18. E.L. Wheeler and R.E. Ferrel, *Cereal Chem.*, **48**, 312 (1971).
19. AOAC, Official Methods of Analysisin, Association of Official Analytical Chemists INC., Virginia, USA, edn. 15 (1990).
20. M.A. Gyamfi, M. Yonamine and Y. Aniya, *Gen. Pharmacol.*, **32**, 661 (1999); https://doi.org/10.1016/S0306-3623(98)00238-9
21. R. Pulido, L. Bravo and F. Saura-Calixto, *J. Agric. Food Chem.*, **48**, 3396 (2000); https://doi.org/10.1021/jf9913458

22. J.O. Chaves, M.C. de Souza, L.C. da Silva, D. Lachos-Perez, P.C. Torres-Mayanga, A. Machado, T. Forster-Carneiro, M. Vázquez-Espinosa, A.V. González-de-Peredo, G.F. Barbero and M.A. Rostagno, *Front Chem.*, **8**, 507887 (2020); https://doi.org/10.3389/fchem.2020.507887
23. H. Nawaz, M.A. Shad, N. Rehman, H. Andaleeb and N. Ullah, *Braz. J. Pharm. Sci.*, **56**, e17129 (2020); https://doi.org/10.1590/s2175-97902019000417129
24. A.R. Abubakar and M. Haque, *J. Pharm. Bioallied Sci.*, **12**, 1 (2020); https://doi.org/10.4103/jpbs.JPBS_175_19
25. F.S. El-Safy, R.H. Salem and M.E. Abd El-Ghany, *World J. Dairy Food Sci.*, **7**, 59 (2012).
26. D. Lin, M. Xiao, J. Zhao, Z. Li, B. Xing, X. Li, M. Kong, L. Li, Q. Zhang, Y. Liu, H. Chen, W. Qin, H. Wu and S. Chen, *Molecules*, **21**, 1374 (2016); https://doi.org/10.3390/molecules21101374
27. S. Pokhrel and P. Karki, *Nepal J. Sci. Technol.*, **20**, 126 (2021); https://doi.org/10.3126/njst.v20i1.43362
28. G.I. Onwuka, Food Analysis and Instrumentation: Theory and Practice. Naphthali Print, Lagos, pp. 140–146 (2005).
29. K. Fredlund, M. Isaksson, L. Rossander-Hulthén, A. Almgren and A.-S. Sandberg, *J. Trace Elem. Med. Biol.*, **20**, 49 (2006); https://doi.org/10.1016/j.jtemb.2006.01.003
30. R. Siener, D.J. Bade, A. Hesse and B. Hoppe, *J. Transl. Med.*, **11**, 306 (2013); https://doi.org/10.1186/1479-5876-11-306
31. E. Panzarini, M. Dwikat, S. Mariano, C. Vergallo and L. Dini, *Evid. Based Complement. Alternat. Med.*, **2014**, 1 (2014); https://doi.org/10.1155/2014/281508
32. S. Salla, R. Sunkara, S. Ogutu, L.T. Walker and M. Verghese, *LWT-Food Sci. Technol.*, **66**, 293 (2016); https://doi.org/10.1016/j.lwt.2015.09.008