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Extraction and Characterization of Novel Polysaccharides from *Annona squamosa* Fruit Peel Using Ultrasound Assisted Extraction

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ABSTRACT

Bioactive compounds were extracted from fruit peel of custard apple (*Annona squamosa*) through ultrasound assisted extraction (UAE). The influence of various green solvents (water, ethanol and ethanol+ water 50% v: v) and temperature (room temperature and 50 ˚C) on global yield (X_0) and chemical composition are studied in detail. Moreover, chemical functional groups in the extracts were identified by Fourier transform infrared spectroscopy (FT- IR). Ethanol + water as a solvent at 50 ˚C was chosen as the best UAE condition for the recovery of cellulose, α- pyranose and acetal from *Annona squamosa* fruit peel. Hydroalcoholic mixture (50 % v: v) shows the best antioxidant activity. The obtained results show that UAE is a simple, cost effective, rapid and environmentally friendly technique to recover the polysaccharides such as cellulose from *Annona squamosa* fruit peel.

INTRODUCTION

Annona squamosa (custard apple) is a small evergreen tree belongs to Annonaceae family, which native to West Indies and South America, and widely cultivated in India, China and tropics areas around the world. Custard apple (CA) fruit globular or heart shaped with many round protuberances with greenish yellow color, ripened aggregate fruit is pendulous on a thickened stalk. This fruit is sweetly aromatic and white-tinged yellow flesh with many dark brown to black seeds dispersed through it [1-4]. CA is an ever-increasing recognition not only by the general public but also, and most remarkably, by the scientific community, due to their health promoting benefits.

CA plant species contain various pharmacological active compounds including glycosides, alkaloids, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, phytosterols and amino acids [3-5]. CA chemical compositions have exhibited wide range of biological activities including antioxidant and anti-inflammatory activity, cardiotonic activity, antimicrobial and insecticidal activity, anti-cancerous activity, antineoplastic activity, cytotoxic activity, genotoxicity and antitumour activity [4-6]. Hence, there is a critical need to develop an efficient and economic method to obtain the bioactive compounds from CA plant.

Extraction is the important step in isolation and purification of biologically active compounds from secondary metabolites, which they are of greater value in cosmetic, nutraceutical and pharmaceutical industries. The use of harmless extraction methods is essential to comply with and sustainable chemical production without negative impact of human health and environment. In recent years, different innovative environmentally friendly techniques have been used for the extraction of bioactive compounds including supercritical fluids extraction [7-9], pressurized liquid extraction [7], microwave assisted extraction [10] and ultrasound assisted extraction (UAE) [11,12]. Among them, UAE has been recognized as efficient extraction technique with strong advantages of high extraction yield, inexpensive, simple operating procedure and low energy input [13-15].

From the literature review, it is found that many studies have reported UAE of bioactive compounds from various plant materials [16-19]. To the best of our knowledge, the extraction of bioactive compounds from CA fruit peel using UAE has not been investigated so for. Nevertheless, biosynthesis of titanium dioxide and silver nanoparticles using CA fruit

peel has been reported [20,21]. The main objective of this study was to obtain extracts containing bioactive compounds from CA fruit peel using UAE. The influence of solvent type (H_2O, C_2H_5OH and H_2O: C_2H_5OH) and temperature was evaluated, and chemical composition and yield of the extract were determined too.

MATERIALS AND METHODS

Materials

Custard apple fruit was purchased in the local market in the region of Dharmapuri, Tamilnadu (India). Fruit peel were separated from fruit and washed thoroughly using double distilled Milli-Q grade water and then dried to constant weight at ambient temperature. Before the extraction, the peel was ground in a Samsung grinder with a sieve of approximately 5 mm. Ethanol analytical gradient were purchased from Changshu Hongsheng fine chemical Co. Ltd, China.

Ultrasound assisted extraction (UAE)

Ultrasound assisted extraction of CA fruit peel was performed in ultrasonic bath (Model Elma S 100 H, Germany). The extraction process was performed on the following operating condition: three types of green solvent such as water, ethanol and water: ethanol; temperature (room temp and 50°C) and an extraction time of 1 h. The experimental conditions are shown in **Table 1**. After the extraction, the extract was centrifuged using Thermo scientific centrifuge (model ST 40 R, USA). Prior to the analysis, the fruit peel extracts stored in Falcon tubes at 4 °C.

Table 1. UAE of bioactive compounds from *Annona squamosa* fruit peel.

run	solvent	Temperature (°C)	Yield (%)
1	H_2O	50	30.41
2	C_2H_5OH	50	13.30
3	H_2O: C_2H_5OH	50	30.26
4	H_2O	rt	29.44
5	C_2H_5OH	rt	10.82
6	H_2O: C_2H_5OH	rt	28.00

Sample-solvent (5gm in 100 mL) Time (60 min) Ultrasonic frequency (37 kHz) rt – room temperature

Extraction yield (%)

The extraction yield (X_0) obtained by UAE of different solvent and different temperature were calculated by the ratio between the extract mass ($M_{extract}$) in dry basis and the mass of sample fed into the extraction cell (M_{Sample}), as stated in Eq 1. To determine $M_{extract}$ 10.0 mL of the extract were dried using Rotavapor after that total weight of the extract could be calculated.

$$X_0 = M_{extract} / M_{sample} \times 100 \quad (1)$$

Fourier transform infrared (FT-IR) spectroscopy analysis

The infrared spectrum of the UAE of custard apple fruit peel in liquid state was recorded on an Agilent Technologies (Cary 660 FTIR, USA) spectrometer. Prior to the analysis, the ATR crystal was cleaned with ethanol and wiped dry before the sample was placed. The spectra were recorded at room temperature over the wave number in the range of 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}.

Antioxidant activity assay with DPPH

The antioxidant activity of the custard apple fruit peel extracts was determined by the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical assay. The DPPH assay is based on the methods described by Brand-Williams [22] and Scherer and Godoy [23]. Ethanolic solutions of CA fruit peel extracts at different concentration were added to 3.9 mL of a 6×10^{-5} mol/L DPPH solution. The absorbance of DPPH was monitored spectrophotometrically at 515 nm at 0 min and 2 min until the reaction reached the steady state. All the tests were performed in duplicate. The concentration of DPPH in the reaction medium was calculated from a calibration curve determined by the equation (2)

$$Abs = 12.709\ C_{DPPH} + 0.002 \quad (2)$$

The percentage of DPPH left behind was calculated by the equation (3)

$$\% \text{ DPPH remaining} = C_{DPPHt} / C_{DPPH0} \times 100 \quad (3)$$

The effective concentration providing 50% inhibition (EC_{50}) was calculated graphically using a non-linear fitting by plotting the sample concentration vs. % DPPH remaining at the steady

state concentration. The AAI (antioxidant activity index), was calculated the DPPH final concentration and the EC_{50} of the tested compounds in the reaction as following equation (4)

AAI = Final concentration of DPPH (µg/mL) / EC_{50} (µg/mL) (4)

The final DPPH concentration was calculated with respect to the concentration of DPPH in the reaction medium. The antioxidant activity is considered to be very strong when AAI ≥ 2.0, strong when AAI is between 1.0 and 2.0, moderate when AAI is between 0.5 and 1.0 and poor when AAI ≤ 0.5 [24].

RESULTS AND DISCUSSION

In the present study, the influence of different green solvents (water, ethanol and ethanol: water) and temperature (room temp & 50 °C) on the global yield (X_0) of the UAE of CA fruit peel have been investigated. The extraction yields of CA fruit peel were shown in Table 1.

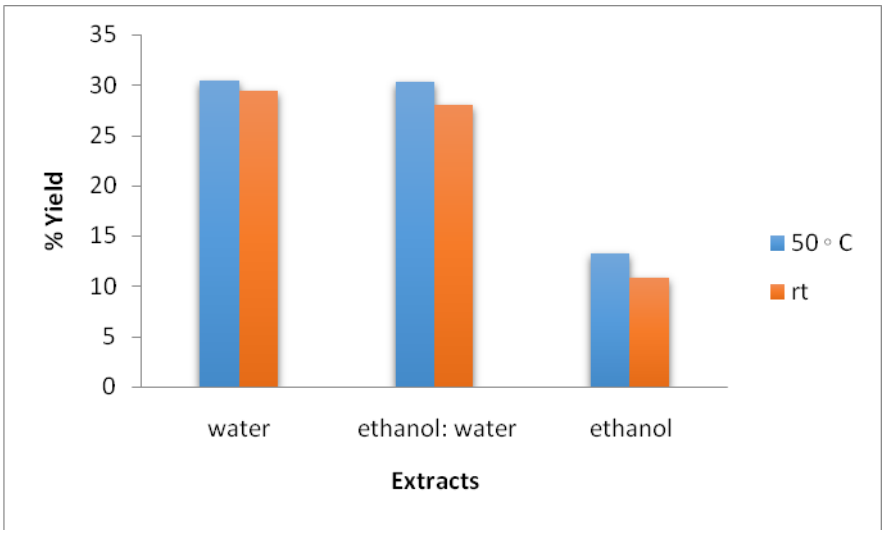
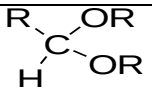
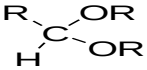
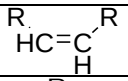
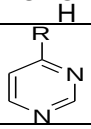
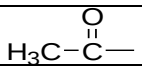
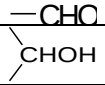
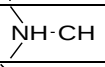
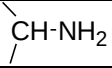
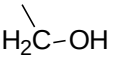
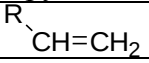


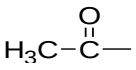
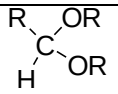
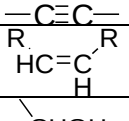
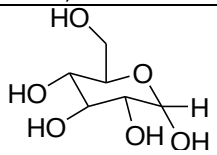
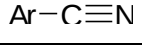
Figure 1. Extraction yield of *Annona squamosa* fruit peel.

From the Fig.1, it can be observed that maximum global yield was obtained with water, water/ethanol as a solvent (30.41% and 30.26% at 50 °C, respectively) which can be attributed to the favorable properties such as high polarity and surface tension. There is no significant difference between water and water/ethanol solvent system. Nevertheless, the use of a binary mixture of an organic solvent (ethanol) and water, which improve the quality of the extract, due to organic solvent enhances the solubility of the analyte and water increases the analyte desorption. [7, 25, 26].

FT-IR spectrums of UAE of CA fruit peel using water, ethanol and water/ethanol were shown in Fig.2. Fourier transform infrared spectroscopy is one of the most powerful qualitative techniques to identify the molecular structures and functional groups [27, 28]. It can be observed in Fig 3a-c, there is no significant difference of the FT-IR spectrum bands absorption of all the extract with increasing temperature. At higher temperature, lower recoveries due to degradation of the active ingredient [29]. Nevertheless, in our case the trend was opposite. The main characteristic vibrations of the UAE of CA fruit peel at 50°C are shown in Table 2. The broad, medium bands observed in the FT-IR spectra of water extract of CA fruit peel at 3314 cm^{-1} was related to the stretching vibration of the O-H bond in cellulose. The small absorption band at 2005 and 2147 cm^{-1} in the spectrum could be associated with acetals group. The stretching vibrations of C=C in alkene group located at 1638 $cm^{-1}$and also the stretching vibrations of C=N bond in 4-substituted pyrimidines are located at 448 cm^{-1}.

Table 2. FT-IR spectral analysis of UAE of *Annona squmosa* fruit peel at 50°C.

Extract	wave number (cm^{-1})	intensity	comment	fragment
Water	3313.87	medium, broad	O−H stretching	cellulose
	2147.08	weak, sharp	-	 acetals
	2004.66	weak, sharp	-	 acetals
	1638.11	medium, sharp	C=C stretching	
	448.31	strong, sharp	C=N stretching	
Ethanol	3314.15	weak, broad	O−H stretching	cellulose
	2971.70	weak, sharp	CH_3- stretching	
	2884.65	weak, sharp	C−H stretching	—CHO
	1381.66	weak, sharp	C-H deformation	
	1328.76	weak, sharp	C-H deformation	
	1085.17	medium, sharp	C-N stretching	
	1044.73	medium, sharp	C-O stretching	
	880.29	weak, sharp	C-H deformation	α pyranose
	631.06	weak, broad	C-H waging	

	431.71	weak, sharp	-	aromatic $-NH_2$ bond
Ethanol: water	3331.00	medium, broad	O−H stretching	cellulose
	2978.56	weak, sharp	CH_3- stretching	
	2218.26	weak, sharp	-	 acetals
	2089.48	weak, sharp	C-C stretching	
	1643.74	medium, sharp	C=C stretching	
	1410.65	weak, broad	C-H deformation	
	873.39	weak, sharp	C-H deformation	 pyranose
	422.77	medium, sharp	aromatic C-CN in plane bending	

From Fig 2, FT-IR spectrum of ethanol extract of CA fruit peel that the broad, weak absorption band around at 3314 cm^{-1} corresponding to O-H stretching vibrations of cellulose, and the peak at 2972 and 2885 cm^{-1} associated with $–CH_3$ stretching vibrations of ketone and C-H stretching vibrations of aldehyde, respectively. The bands at 1381 and 1329 cm^{-1} belong to C-H deformation of an alcohol and secondary amine groups, respectively.

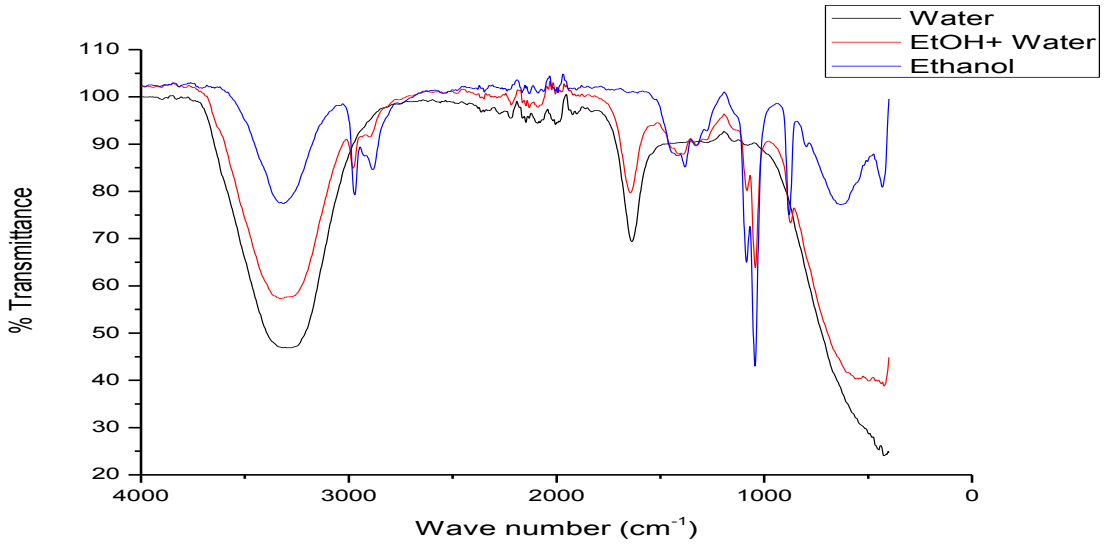


Figure 2. FT-IR diagram of the UAE of *Annona squamosa* fruit peel using different solvents (H_2O, C_2H_5OH, and H_2O:C_2H_5OH) at 50 °C.

The medium, sharp absorption bands at 1085 and 1045 cm^{-1} which refers to C-N stretching vibrations of amine group and C-O stretching vibrations of alcohol groups, respectively. The bands at 880, 631, and 432 cm^{-1} associated with C-H deformation of α- pyranose, C-H waging of alkene and primary aromatic amine group, respectively. These results are shown in Table 2. As can be seen in the Fig. 2 in the ethanol/water extract of the FT-IR spectral characteristic bands associated with vibrations of the bonds are located between 400 and 2500 cm^{-1}. The stretching vibrations of the C-C bonds in alkyne group and C= C bonds in alkene group, C-H deformation vibration in α- pyranose and secondary alcohol group and C-CN in plane bending vibration in aromatic nitrile group were observed. The bands at 3331 and 2979 cm^{-1} associated with O-H stretching vibrations of cellulose and CH_3 stretching ketone groups were identified from Figure 2. The absorption bands of ethanol/water extract of CA fruit peel were almost consistent with that of both the water and ethanol extract of CA fruit peel.

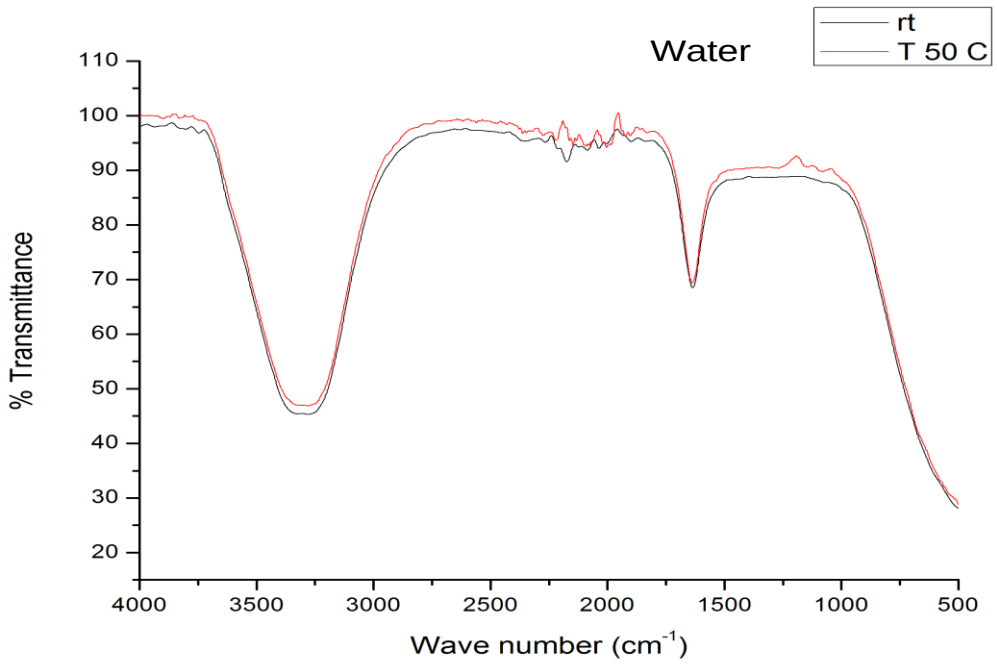


Figure 3a. FT-IR diagram of the UAE of *Annona squamosa* fruit peel using H_2O as a solvent at different temperature (50 °C and room temperature).

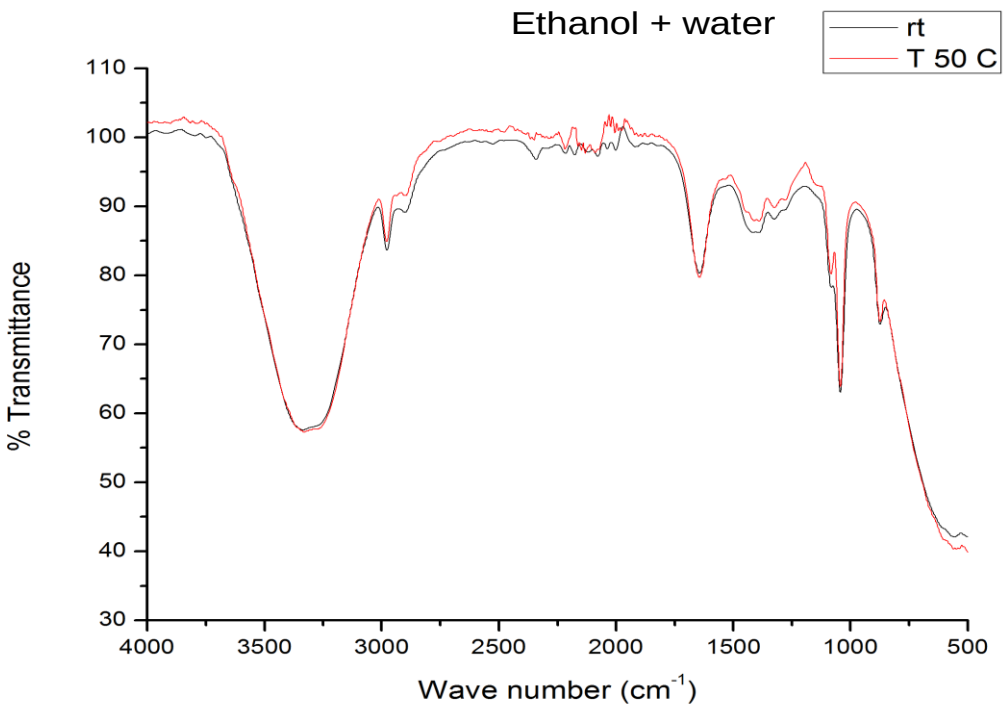


Figure 3b. FT-IR diagram of the UAE of *Annona squamosa* fruit peel using H_2O: C_2H_5OH as a solvent at different temperature (50 ˚C and room temperature).

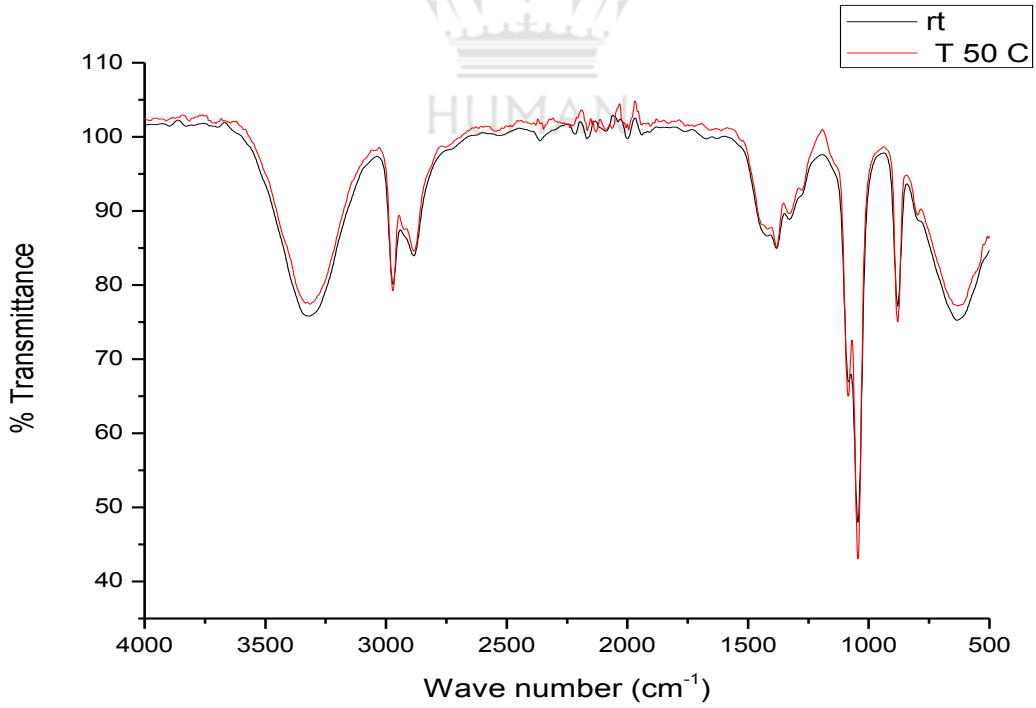


Figure 3c. FT-IR diagram of the UAE of *Annona squamosa* fruit peel using C_2H_5OH as a solvent at different temperature (50 ˚C and room temperature).

Table 3. Antioxidant activity of custard apple fruit peel extracts.

S. NO	Sample	AAI (C_{DPPH}/ EC_{50})
1	Water	1.36
2	Ethanol	0.92
3	Water: ethanol (50 % v:v)	1.84

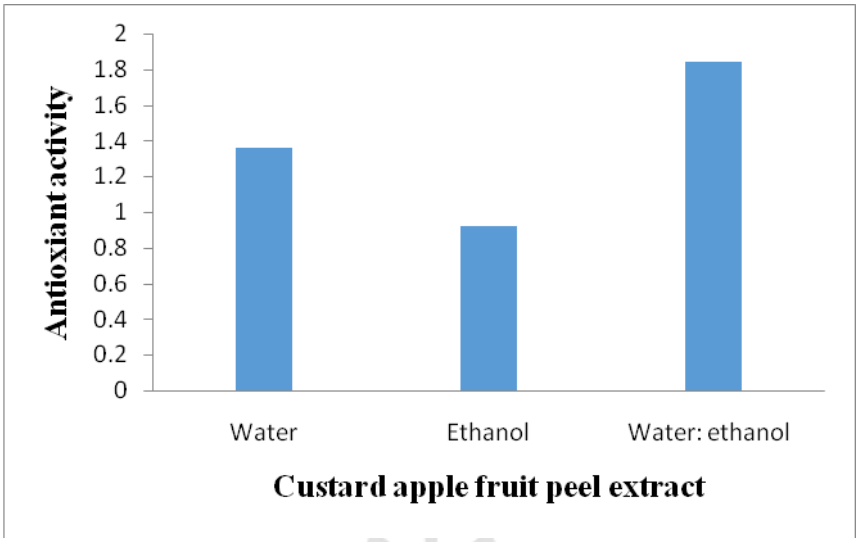


Figure 4. Antioxidant activities of the custard apple fruit peel extract.

Ultrasound assisted extract of custard apple fruit peel extracts were evaluated for their radical scavenging activity with the DPPH assay. The antioxidant activities of the custard apple fruit peel extract are shown in Table 3. As can be seen in Figure 4, the extraction with the water/ethanol shows higher antioxidant activity. This manner has been reported by several authors [7,25,26].

CONCLUSION

According to the above results, it can be concluded that the extraction of bioactive compounds such as cellulose and α- pyranose rich extract recovered from CA fruit peel through ultrasound assisted extraction (UAE), a rapid, simple, cost effective and environmentally clean technique. The solvent type has strong influence on the recovery of bioactive compounds. Binary solvent (ethanol/water) mixture is more efficient to obtain the qualitative and quantitative extracts. Considering the cellulose and α- pyranose content and extraction yield, the best UAE condition was ethanol + water (50% v/v) as a solvent at 50 ˚C.

UAE stands as a promising techniques for the extraction of bioactive compounds from secondary metabolites at moderate temperature.

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REFERENCES

1 Bhakuni DS, Tewari S, Dhar MM. Aporphine alkaloids of *Annona squamosa*. Phytochemistry, 1972; 11: 1819-1822.
2 Baumik PK, Mukherjee B, Juneau JP, Bhacca NS, Mukherjee R. Alkaloids from leaves of *Annona squamosa*. Phytochemistry. 1979; 18: 1584-1586.
3 Jayendra Y, Kumar SS. Two new tetrahydroisoquinoline analogs from Indian medicinal plant *Annona squamosa*. Journal of Pharmacy Research. 2013; 7: 510-515.
4 Jayendra Y, Kumar. New compound 6,7-dimethoxy-2-methylisoquinolinium from Indian medicinal plant *Annona squamosa* L. International Journal of Chemical and analytical research. 2013; 4: 161- 168.
5 Dholvitayakhun A, Trachoo N, Narkkong N, Cushnie TPT. Using scanning and transmission electron microscopy to investigate the antibacterial mechanism of action of the medicinal plant *Annona squamosa* Linn. Journal of Herbal Medicine. 2017; 7: 31-36.
6 Chen Y, Xu S, Chen J, Wang Y, Xu H, Fan N, Li X. Anti-tumor activity of *Annona squamosa* seeds extract containing annonaceous acetogenin compounds. Journal of Ethanopharmacology. 2012; 142: 462-466.
7 Chinnarasu C, Montes A, Fernanez-Ponce MT, Casas L, Mantell C, Pereyra C, Pattabhi S, Martinez de la Ossa EJ. Preparation of polyphenol fine particles potent antioxidants by a supercritical antisolvent process using different extracts of *Olea europaea* leaves, Korean Journal of Chemical Engineering. 2016; 33: 594-602.
8 Chinnarasu C, Montes A, Fernanez-Ponce MT, Casas L, Mantell C, Pereyra C, Martinez de la Ossa EJ, Pattabhi S. Natural antioxidant fine particles recovery from *Eucalyptus globulus* leaves using supercritical carbon dioxide assisted processes, The Journal Supercritical Fluids. 2015; 101: 161-169.
9 Chinnarasu C, Montes A, Fernanez-Ponce MT, Casas L, Mantell C, Pereyra C, Martinez de la Ossa EJ. Precipitation of antioxidant fine particles from *Olea europaea* leaves using supercritical antisolvent process. The Journal Supercritical Fluids. 2015; 97: 125-132.
10 Thirugnanasambandham K, Sivakumar V. Microwave assisted extraction process of betalain from dragon fruit and its antioxidant activities. Journal of the Saudi Society of Agricultural Sciences. 2016; 16: 41-48.
11 Tao Y, Zhang Z, Sun DW. Kinetic modeling of ultrasound-assisted extraction of phenolic compounds from grape marc: Influence of acoustic energy density and temperature. Ultrasonics and Sonochemistry. 2014; 21: 1461-1469.
12 González-Centeno MR, Comas-Serra F, Femenia A, Rosselló C, Simal S. Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (Vitisvinifera L.): Experimental kinetics and modeling. Ultrasonics and Sonochemistry. 2015;22: 506-514.
13 Lavilla I, Bendicho C. Funamentals of ultrasound assisted extraction: Water extraction of bioactive compounds. Elsevier: 2017.
14 Tiwari BK. Ultrasound: a clean, green extraction technology. Trends in Analytical Chemistry. 2015.
15 Chatel G. How Sonochemistry Contributes to Green Chemistry? Ultrasonics and Sonochemistry: 2017.
16 Mercado-Mercado G, Montalvo- González E, González-Aguilar GA, Alvarez-Parrilla E, Sáyago-Ayerdi SG. Ultrasound-assisted extraction of carotenoids from mango (*Mangifera indica* L. 'Ataulfo') by-products on *in vitro* bioaccessibility: 2017.
17 Goltz C, Ávila S, Barbieri JB, Igarashi-Mafra L, Mafra MR. Ultrasound-assisted extraction of phenolic compounds from Macela (*Achyrolcine satureioides*) extracts. Industrial & crop products. 2018; 115: 227-234.

18 Li J, Ai L, Hang F, Ding S, Liu Y. Composition and antioxidant activity of polysaccharides from jujuba by classical and ultrasound extraction. International Journal of Biological Macromolecule. 2014; 63: 150-153.
19 Jang S, Lee AY, Lee AR, Choi G, Kim HK. Optimization of ultrasound-assisted extraction of glycyrrhizic acid from licorice using response surface methodology. Integrative medicine research. 2017; 6: 388-394.
20 Roopan SM, Bharathi A, Prabhakarn A, Rahuman AA, Velayutham K, Rajakumar G, Padmaja RD, Lakshmi M, Madhumitha G. Efficient phytosynthesis and structural characterization of rutile TiO_2 nanoparticles using *Annona squamosa* peel extract. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012; 98: 86-90.
21 Kumara R, Roopan SM, Prabhakarn A, Khannaa VG, Chakroborty S. Agricultural waste *Annona squamosa* peel extract: Biosynthesis of silver nanoparticles. Spectrochimica Acta Part A. 2012; 9: 173- 176.
22 Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technonologies. 1995; 28: 25-30.
23 Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2, 2-diphenyl-1- picrylhydrazyl method. Food Chemistry. 2009; 112: 654-658.
24 Fernandez-Ponce MT, Casas L, Mantell C, Rodriguez M, Martinez de la Ossa EJ. Extraction of antioxidant compounds from different verities of *Mangifera indica* leaves using green technologies. The Journal of Supercritical fluids. 2012; 72: 168-175.
25 Fernandez-Ponce MT, Casas L, Mantell C, Martinez de la Ossa EJ. Use of high pressure techniques to produce *Mangifera indica* L. leaf extracts enriched in potent antioxidant phenolic compounds. Innovative Food Science and Emerging Technology. 2015; 29: 94-106.
26 Otero-Pareja MJ, Casas L, Fernández-Ponce MT, Mantell C, Martínez de la Ossa EJ. Green Extraction of Antioxidants from Different Varieties of Red Grape Pomace. Molecules. 2015; 20: 9686-9702.
27 Coates J. Interpretation of Infrared Spectra, A Practical Approach. Encyclopedia of Analytical Chemistry. 1-23.
28 Gao F, Zhou S, Han L, Yang Z, Liu Z. A novel FT-IR spectroscopic method based on lipid characteristics for qualitative and quantitative analysis of animal-derived feedstuff adulterated with ruminant ingredients. Food Chemistry. 2017; 237: 342-349.
29 Hemwimon S, Pavasant P, Shotipruk A. Ultrasound-assisted extraction of anthraquinones from roots of Morindacitrifolia. Ultrasonics Sonochemistry. 2007; 54: 44 -50.