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Original Research Article

Effect of Processing Variables on Anthocyanin and Total Polyphenol Extraction from Water Caltrop *(Trapa bispinosa)* Hull

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Abstract:

Anthocyanins are water soluble pigments responsible for the brilliant orange red through deep purple colors in flower and fruits. The effects of different extraction variables (pH, particle size, temperature and time, extraction method) on anthocynin, total polyphenol content and antioxidant of the extracts were studied. The anthocyanin content of fresh and dried (55 ^{0}C for 2 h) water caltrop hull were 68.68 and 44.38 µg/100 g d.b. respectively. Temperature, particle size and pH played a significant role ($p<0.05$) in aqueous extraction of anthonycinin. The optimum aqueous extraction condition were pH of 4.49, particle size of 300 µm, temperature of 67.2 ^{0}C and time of 25.38 min which gave 70.3% anthocynin extraction. There was a significant effect of extraction method (acidified ethanol, acidified ethanol and pH differential methods) and particles size on anthocynin extraction ($p<0.05$). The total polyphenol content in the aqueous extracts from water caltrop hull powder of 300, 690 and 1080 µm were in the range of 55.02-60.31 mg GAE/g dm. The extraction of total polyphenol from 300 µm was significantly different from 690 and 1080 µm particle sizes, while there was no significant difference between from 690 and 1080 µm respectively ($p>0.05$). The particle size had a significant effect ($p<0.05$) on the antioxidant activity of the extract and the values ranged from 173.36 µg/mL to 193.69 µg/mL.

Key words: Water caltrop, anthocyanin, polyphenol, antioxidant activity, sorption isotherm

Introduction

Polyphenols are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens (Pandey and Rizvi, 2009). Plant polyphenols have been implicated for disease resistances (Bravo, 1998). In foods, polyphenoles may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability and their stable radical intermediates prevent the oxidation of various food ingredients, particularly, fatty acids and oil (Maillard et al, 1996). The consumption of diets rich in plant polyphenols offered some protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Graf et al, 2005).

Anthocyanins are the water soluble pigments responsible for the brilliant orange red through deep purple colors in flower and fruits (Wong, 1996). They are polar molecules, thus the most common solvents used for its extraction are aqueous mixtures of ethanol, methanol or acetone (Kahkonan et al, 2001), acidified alcoholic solvents (Philip, 1974) and even water only (Esselen and Sammy, 1975). They contribute to the most important attributes of food, like aesthetic value and quality judgment. Their metal chelating capability, together with radical scavenging property, has enabled phenolic compounds to be considered effective antioxidant derived from natural plants (Lopes et al, 1999), that protect human body from the attack of free radicals, retard the progress of many chronic diseases (Pyror, 1991), and lipid oxidative rancidity in foods. Thermal stability of anthocyanins varies with temperature and pH. It is the most stable and highly colored at low pH values and gradually loses its color as the pH increases. However, if the product being colored contains components capable of acting as co pigments, color may be retained and also, the light stabilized it to certain extent (Bobbio et al, 1992). The presence of oxygen and interaction with other components, like sugar and ascorbic acid, also affect on its stability. reported that the degradation of blackberry's anthocyanins increased with increasing heating temperature from 60 to 90^{0}C for

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different times.

Water caltrop (*Trapa bispinosa*) belongs to the family Trapaceae, is an aquatic nut crop grown as submersed plant community mainly in the tropical and sub-tropical region and has special taste and medical function, Due to high activity of enzymes and phenolics content, the color of water caltrop hulls easily changes from the original pink to dark brown color during transportation and processing. Hence, the aim of this study was to extract anthocyanin pigment from water caltrop hull at different extraction conditions.

Materials & Methods

Fresh water caltrops were collected from Biratnagar, Morang Nepal. The hulls were separated manually, cleaned in pure water, dried at 55±5^{0}C for 12 h, milled and then sieved into 300, 690 and 1180 µm particle size. The fresh and dried hulls were analyzed for anthocynanin and total polyphenol content. The effect of different processing variables pH (3, 5, 7), particle size (300, 690, 1180 µm), extraction temperature (30, 55, 80^{0}C) and time (10, 35, 60 min) on the aqueous extraction of anthocyanin were studied. Response surface methodology (RSM) was used for the experimental design (Table 3) using a three-level; four-factor Central Composite Face centered Design (Mayers et al, 1976). A numerical multi-response optimization was applied to determine the optimum combinations of extraction variables. Similarly the effect of extraction method (acidified ethonal, acidified methonal and pH differential) from particle size of 300, 690, 1080 µm on anthocynanin and total polyphenol extraction, and antioxidant activity in the extracts were studied. The correlation between anthocyanin content and antioxidant activity was also determined.

Determination of chemical parameters:

Moisture content (hot air over method), crude fat (ether extraction method), protein (micro-Kjeldahl), crude fiber, ash content and the pH of the extract (pH meter) were determined as per Ranganna (2010).

Extraction and determination of anthocyanins:

The total anthocyanins in aqueous extract was measured as per Shrivastava and Kumar (2002) with some modification. Briefly, to 3 mL of the extract, 15 mL of 0.1 N HCl was added. Then it was allowed to equilibrate in the dark for 1 h. The absorbance was measured at 510 nm using a spectrophotometer.

The total anthocyanins in acidified ethanol (95% ethanol and 1.5N HCL in ratio 85:15) extract was measured as per Shrivastava and Kumar (2002). One gram of the dried samples was blended with 5 mL of acidified ethanol and diluted to 25 ml. . The mixture was allowed to stand overnight in a refrigerator at 4^{0}C, and optical density was measured at 535 nm using a spectrophotometer.
The anthocyanin content in the extract by acidified methanol ectraction method (methanol: water: HCl, 79:20:1, v/v) was determined as per Mancinelli et al, (1975). Briefly, to 1 g dried samples, 20 mL of acidified methanol was added and allowed to stand at refrigeration for 72 h in the dark . The extracts were then centrifuged for 10 minutes at 5000 rpm and the absorbance was measured at 530 and 657 nm. The absorbance readings at 530 nm (A_{530}) were corrected for scattering using the absorbance readings at 657 nm (A_{657}) using formula as following:

$$A = A_{530} - 1/3\,A_{657}$$

Total anthocyanin in the extract by pH differential method was determined as per Wrolstad and Giusti (2001) with slight modification. One gram of the sample was dissolved in 50 mL buffers of pH 1 and 4.5 seperately and the absorbances were recorded at 510 and 700 nm against a blank cell containing deionized water. The absorbance (A) of the samples were calculated as follows:

$$A = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5}$$

Anthocyanin content (mg/100gm) = (A× MW× DF× 10)/(ε×1)
Where, MW is molecular weight (449.2) of cyaniding-3glucoside; DF is dilution factor (60); ε is molar absorptivity constant (29,600)

Determination of *total polyphenols content:* Total phenols content was determined by using Folin-Ciocalteu reagent (Singleton and Rossi, 1965) and expressed as gallic acid equivalent (GAE) /mg of extract on db, with slight modification Briefly, to 0.5 mL of an aqueous extract sample, 2.5 mL of dilute Folin-Ciocalteu reagent (10 fold, v/v) and 2 mL of sodium carbonate (7.5%) were added The mixture was kept for 5 min at 50 ^{0}C, cooled and the absorbance was measured at 760 nm using digital specotrophotometer.

Determination of antioxidant activity: Antioxidant activity was determined as DPPH free radical scavenging activity or IC_{50} (µg/ mL) as described by Marinova and Batchvarov (2011). To 1.5 mL diluted aqueous extract sample, 1.5ml of 0.06mM DPPH solution was mixed. It was then stored in dark for 30 minutes and absorbance at 517 nm. The percentage of DPPH scavenging was calculated as follows:

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DPPH radical scavenging activity (%) = $[(A_c - A_s)/A_c] \times 100\%$

Where, A_c = Absorbance of control at 517 nm and A_s = Absorbance of aqueous extract

The concentration of sample necessary to decrease the DPPH concentration by 50% was obtained by interpolation from linear regression analysis denoted IC_{50} value (µg/ml).

Statistical analysis: The anthocyanin content as affected by independent variables namely pH of medium, particle size, temperature and time of extraction was modeled by multiple regression analysis and the statistical significance of the terms was examined by analysis of variance (ANOVA) for each response.: The polyphenols and IC_{50} value were determined by linear regression using Excel, Microsoft Office, 2010. For significance analysis, the data were separated using Tukey's LSD range at P=0.05. The statistical analysis was performed using SPSS version 20.

Results and Discussion

Proximate composition of oven dried hull: The proximate composition of the oven dried hull is given in the Table1. The crude protein, crude lipid and carbohydrate obtained were slightly higher but the crude fiber was slightly less than that obtained by in water caltrops . This may be due to the difference in season and location of the plant grown area. found 79-84% total fiber in a Norwegian oat hull. The protein content of oat hull was in the range of 2– 4.9% (Welch and Yong, 1980).

Table 1. Proximate composition of oven dried hull

Composition (db)	g/100 g Sample
Crude protein	7.2±0.9
Crude lipid	15.5±0.5
Crude fiber	68.3±1.2
Ash	1.1±0.3
Moisture	15.2±0.7
Carbohydrate	8.2

Values are means ±standard deviations of three replications

Effect of drying on anthocyanin: The total anthocyanin content of fresh and dried water caltrop hull was 68.68 and 44.38 mg/ 100 g d.b. (i.e after drying anthocyanin content was decreased by 35.3%). This might be due to the degradation of anthocyanin with increasing drying temperature and time (Wang and Xu, 2007). The difference in anthocyanin content of fresh and oven dried grape skin; and fresh and oven dried mulberry was reported to be 34.5% and 41% respectively (De Torres et al, 2010). Phenolic compounds are often found in the external areas of the vacuoles. Thus, if the cellular structure deteriorates during the drying process, the compounds stored outside of the organelles are more sensitive to degradation, which should have been more marked in the case of anthocyanin (Chism and Haard, 1996).

Table 2. Effect of Extraction method and particle size on Anthocyanin extraction

Method	Particle size (µm)	Total anthocyanin (mg/100 g d.b.)
Using Acidified Ethanol	300	38.65 ± 0.42^{b}
	690	37.99 ± 0.31^{a}
	1080	36.85 ± 0.57^{a}
Using Acidified Methanol	300	50.27 ± 1.25^{e}
	690	47.38 ± 0.68^{d}
	1080	43.32 ± 0.79^{c}
pH differential	300	39.76 ± 0.57^{b}
	690	39.47 ± 0.77^{b}
	1080	36.59 ± 0.55^{a}

Values are means ±standard deviations of three replications

Effect of extraction method and particle size: The effect of extraction method and particle size on the anthocyanin extraction were significant but there was no significant difference in the anthocyanin content for 690 and 1080 µm by acidified ethanol extraction and for 300

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and 690 µm by pH differential method. Similarly there was no significant difference in anthocyanin extraction from 1080 µm by acidified ethanol and pH differential method. There was an interaction between particle size and extraction methods on anthocyanin extraction ($p>0.05$) as shown by Table.2.

Effects of variables on aqueous extraction of anthocyanin: The aqueous extraction of anthocyanin at different conditions with respect to total anthocyanin in oven dried water caltrop varied from 27.5% to 67.8% (Table 3; Fig. 1 & 2) The results were in agreement with the research work reported by Fan *et al.*, (2008).

Table 3: Effect of variables on aqueous extraction of anthocyanin

Experimental design for aqueous extraction				% Anthocyanin extraction
Initial pH	Size	Tem (ºC)	Time (Min)	
3	300	30	10	29.7
7	300	30	10	33.8
3	1080	30	10	27.5
7	1080	30	10	30.3
3	300	80	10	61.9
7	300	80	10	66.7
3	1080	80	10	42.4
7	1080	80	10	51.4
3	300	30	60	31.8
7	300	30	60	36.1
3	1080	30	60	29.2
7	1080	30	60	30.9
3	300	80	60	60.9
7	300	80	60	67.8
3	1080	80	60	53.1
7	1080	80	60	54.4
3	690	55	35	42.2
7	690	55	35	50.5
5	300	55	35	58.8
5	1080	55	35	40.4
5	690	30	35	32.5
5	690	80	35	55.1
5	690	55	10	44.5
5	690	55	60	61.7
5	690	55	35	53.2
5	690	55	35	58.6
5	690	55	35	56.8
5	690	55	35	52.1
5	690	55	35	57.7
5	690	55	35	59.3

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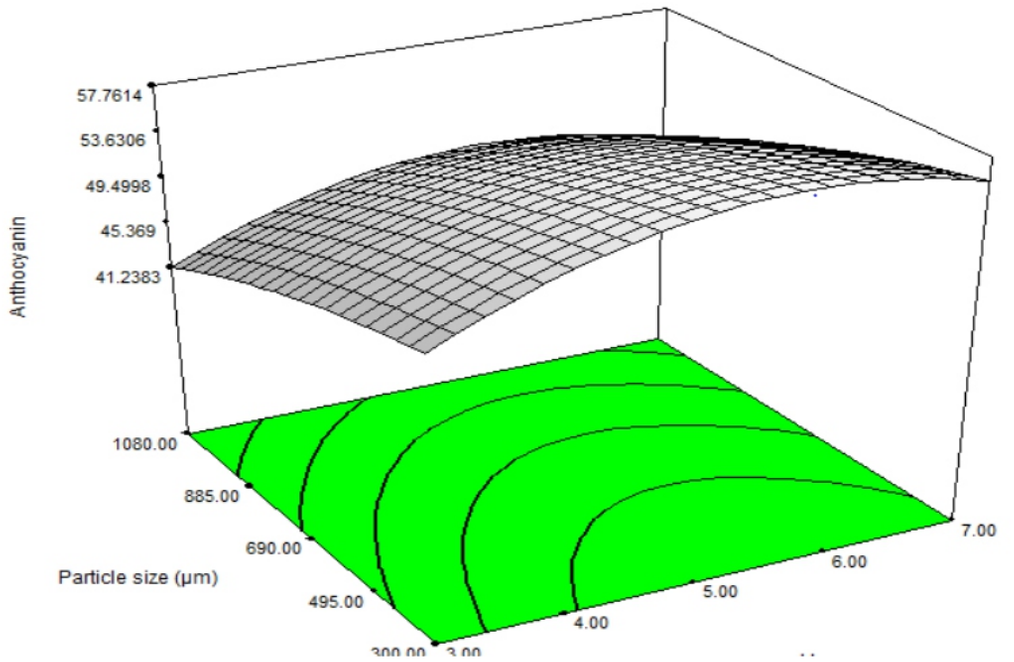

Fig 1. Response surface plot for % anthocyanin extraction as a function of particle size and pH

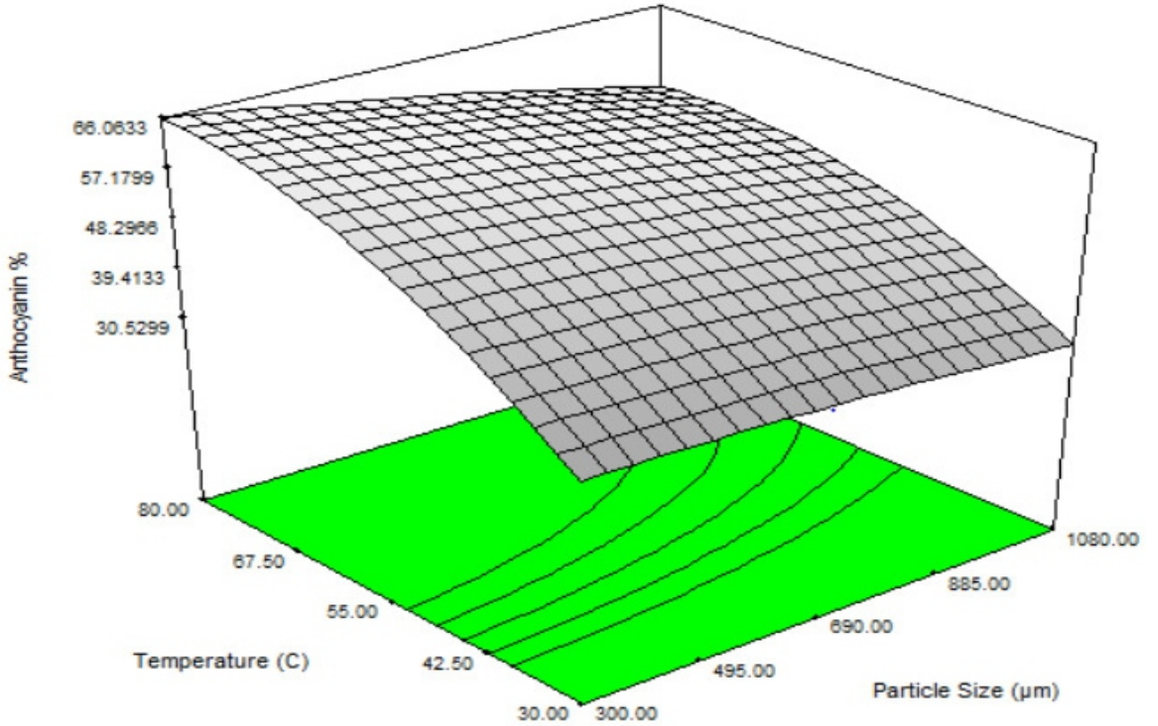

Fig 2. Response surface plot for % anthocyanin extraction as a function of temperature and particle size

The Figure 3 shows that decreasing particle size increase the anthocynin extraction. found higher yields in total phenolics and anthocyanins in the extract with decrease in pomace size of black currant juice residues. Increasing extraction temperation increased the anthocyanin extraction (Fig. 4) Increase in temperature favors the extraction by increasing the solubility of anthocyanins and increasing the diffusion coefficient (). The anthocyanins contain from purple sweet potato was significantly affected by temperatures on acid-ethanol solvent extraction (Fan et al, 2008).

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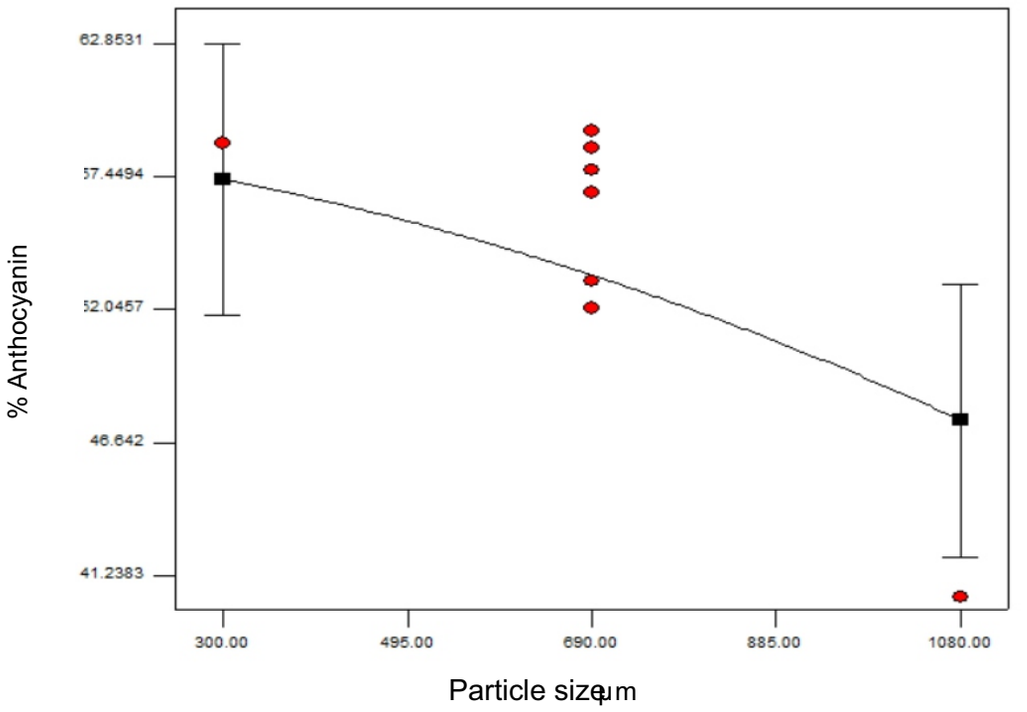

Fig 3. One factor plot for % extraction of anthocyanin as function of Particle size (µm).

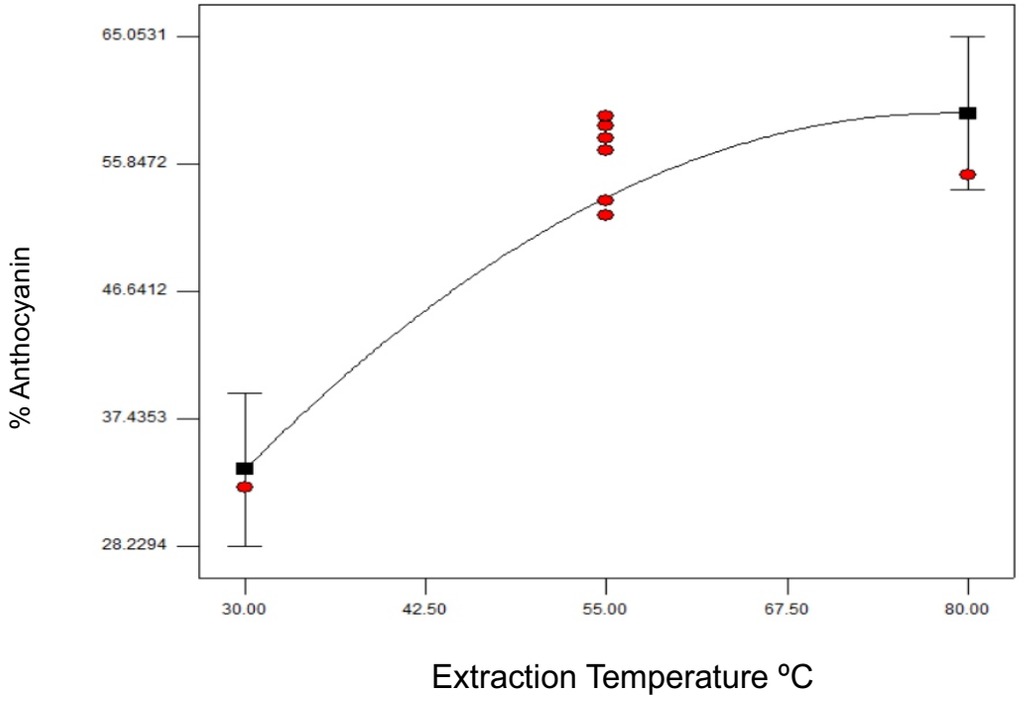

Fig 4. Response surface plot for % anthocyanin extraction as a function of temperature and particle size

Effect of Particle size on Total Polyphenols: The total polyphenol content in the extracts were 55.02, 56.31 and 60.31 mg GAE/g dm for particle sizes of 1080, 690 and 300 µm respectively. The polyphenol contents of 300 µm was significantly higher than those of 690 and 1080 µm, while there was no significant difference in the polyphenol contents between 690 and 1080 µm sizes (Fig 5, $p>0.05$) . The total phenolics in methanolic extracts of water calrop hull ranged from 5.21 to 8.59 g GAE/100 g, . (Jihh-Ying et al, 2007).

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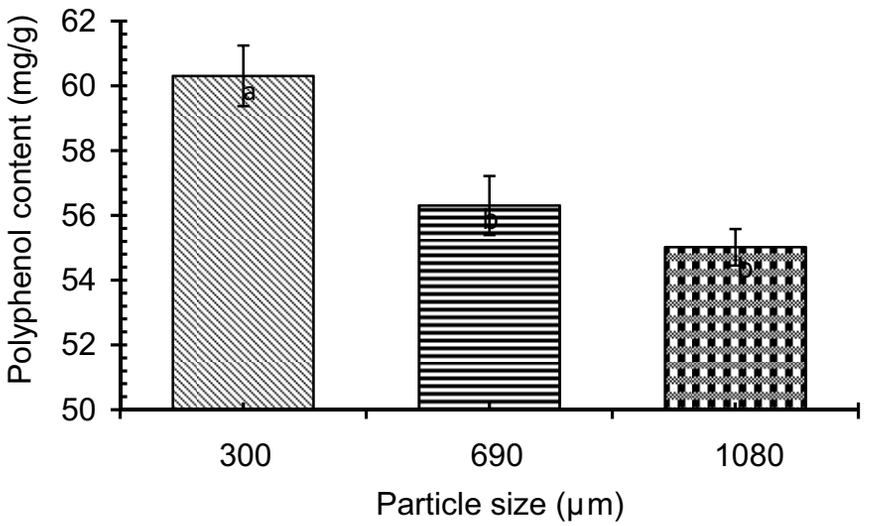

Fig. 5 Total polyphenol content of aqueous extract for different particle size

Effect of particle size on antioxidant activity: Particle 300 µm showed lowest IC50 value (173.36 µg/ mL), followed by 690 µm (187.73 µg/ mL) whereas 1080 µm showed the highest IC50 value (193.69 µg/mL) which were significantly different from each other ($p<0.05$). The results were similar to those obtained in research by , which was reported to be 2 mg/ mL concentrations for scavenging activity of 79.3% for hot air dried water caltrop hulls. Similarly, determined the scavenging ability of DPPH free-radical and the value was 78.3% at the dose of the 250 µg/ mL methanolic extracts for dried water caltrop hulls.

Conclusion

Water caltrop hull contains substantial amount of anthocyanin. Drying temperature affected the anthocyanin content in the water caltrop hull. Percentage extraction of anthocyanin was affected by pH of the extraction medium, particle size of water caltrop hull, extraction temperature and time. Increasing particle size and temperature increased anthocynanin extraction.

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