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Phenolic Content and Antioxidant Capacity of Selected Cucurbit Fruits Extracted with Different Solvents

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Abstract

Cucurbits are major economically important species of plants; particularly those with edible fruits having nutritional significance. The present work was to investigate the polyphenolic content and antioxidant capacity of peels and pulps of four cucurbit fruits, namely pumpkin, ash gourd, watermelon and muskmelon. The solvent systems used were methanol, ethanol and acetone at three different concentrations in distilled water (50, 70, and 100%) and 100% distilled water. The extracts were analyzed for their total phenolic content, total flavonoid content and antioxidant activities using Ferric Reducing Antioxidant Power assay (FRAP assay), DPPH free radical-scavenging assay and ABTS radical scavenging capacity. The result showed that the highest extraction was by 50% acetone in case of peels and 50% ethanol in case of pulp. The best solvent was 50% acetone as it gave highest yield as well as showed highest correlation between various assays. The polyphenolic content and the antioxidant activity were high in peels than pulps. The muskmelon fruit extracts (peel and pulp) showed highest antioxidant activity. High polyphenolic content showed significant correlation with high antioxidant activity. The result indicated that these cucurbit fruit are good source of natural antioxidants which can be utilized as an ingredient to functional foods.

Keywords: Cucurbits; Antioxidant activity; Polyphenolic content; Solvent extraction

Introduction

Free radicals, Reactive Oxygen Species (ROS), and Reactive Nitrogen Species (RNS) are implicated in numerous pathological conditions such as inflammation, metabolic disorders, cellular aging, reperfusion damage, atherosclerosis, and carcinogenesis [1,2]. The high levels of ROS and free radicals cause damage to nucleic acids, proteins, and membrane lipids. The antioxidants in diet would terminate attacks by the free radicals and reduce the risks of these diseases [3]. Many plants contain antioxidants viz. vitamin C, vitamin E, carotenoids, polyphenols, phenolic acids, phenolic diterpenes, flavonoids, catechins, procyanidins and anthocyanins. The focus has been shifted to naturally occurring antioxidant. The use of natural antioxidant are considered to be safe rather than synthetic as latter may show carcinogenic potential [4]. Various plant materials with great antioxidant potentials have been identified and are widely used in food, pharmaceuticals, cosmetics and diverse fields related to the utilization of antioxidants. Some plants are commonly cultivated for their culinary purposes but their potential as source of antioxidants is less exploited. Thus, it provides a new approach to develop new sources of antioxidants that can be used in food, neutraceutical and other fields [5].

The proper recovery of phenolic compounds is one of the important tasks. Solvent extraction is frequently used method for the extraction of these compounds from plant material. Different types of solvents are used for the preparation of extracts from the plant materials since solvent system is easy to use and efficient for extraction of different compounds. The physical and chemical property of the sample, type of solvent, extraction time and temperature and sample to solvent ratio effect the extraction yield of the compounds [6]. Solvent polarity and the solubility of the phenolics in the solvent are dependent on each other which affect the recovery of the polyphenols from the plant materials [7]. Polyphenols are mostly extracted from plant matrix by using polar solvents. Basically, the aqueous mixture containing acetone, ethanol, methanol and ethyl acetate are the most suitable solvent for the recovery of polyphenolic compounds [6].

Cucurbits play an important role in human consumption and has higher consumption rate in tropical regions [8]. The fruits are good source of vitamins, minerals and also hold good antioxidant and nutraceutical potential. Watermelon exemplifies one of the most widely cultivated crops in the world, occupying the largest production of all Cucurbits. The watermelon fruit possess high antioxidant potential and free radical scavenging activity in all parts namely peel, pulp and seed [9]. Pumpkin is cultivated worldwide for its nutritional and medicinal importance. Each pumpkin part contains a significant amount of antioxidants, tocopherols, and carotenoids [10]. Muskmelon pulp extracts have shown high antioxidant potential and anti inflammatory activity [11]. Ash gourd is usually renowned for its antioxidant and medicinal property mainly in Asian countries [12].

The literature related to the antioxidant potential of peel and pulp of cucurbit fruits as well as effect of different solvent system on extraction of phenolic compounds in these fruits was scarce. The objective of this study was to determine the effectiveness of different solvent systems i.e., methanol, ethanol, acetone and aqueous mixture of these in different proportions for extraction of polyphenolic compounds from peel and pulp of cucurbits and to investigate the antioxidant potential of the extracts of these fruits in different solvents.

Materials and Methods

Plant materials

Fresh fruit samples were collected at different times from local markets in Allahabad region of Uttar Pradesh, India. Samples included

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pumpkin (*Cucurbita maxima*), ash gourd (*Benincasa hispida*), watermelon (*Citrullus lanatus*) and muskmelon (*Cucumis melo*). All the fruits were free from any physical and microbial damage. Each fruit was identically selected considering the quality traits in terms of shape, size, color, and ripening stage.

Chemicals and reagents

2,4,6-Tri-(2-pyridyl)-s-triazine (TPTZ), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent (FCR), Quercetin were purchased from Sigma- Aldrich Co. (St. Louis USA). Gallic acid (purity >99.0%), trichloroacetic acid (TCA), ferric chloride, sodium carbonate, sodium acetate trihydrate, sodium nitrite, sodium hydroxide, aluminium chloride, acetone, methanol, ethanol were obtained from Merck (Germany). The other chemicals were of analytical grade and the water used was deionized. These chemicals were used as such without undergoing further purification.

Extraction

Fruits were washed with distilled water and separated into different parts i.e., peel and pulp. After that, fruit parts (2.5 g each) were cut into small pieces and blended for 3 min. Then the sample were placed in capped centrifuge tubes and extracted with 10 ml of organic solvent on an orbital shaker (Remi IS 24BL) set at 200 rpm for 2 hrs at room temperature (25 ± 2°C). The samples were again centrifuged using tabletop centrifuge (Remi) for 10 min at 1000 rpm. Next, the samples were filtered through Whatman filter paper No. 1 and the process was repeated twice with each residue obtained after filtration. The filtrate of each extraction was collected simultaneously. The extracts were brought down to dryness using a rotary vacuum evaporator (IKA, RV10) at 50°C. Finally the dried extracts were stored at 4°C to prevent the degradation of compounds. The extraction process was carried out in triplicate, using different fruit samples each time. The solvent system used was methanol, ethanol and acetone at three different concentrations in distilled water (50, 70, and 100%) and 100% distilled water (H_2O).

Total phenolic content (TPC)

The total phenolic content of the samples was determined spectrophotometrically according to the Folin–Ciocalteau method [13]. 0.1 mL of each extract was diluted with deionised water to 4.8 ml, and 0.3 ml Folin - Ciocalteau reagent was added and shaken. After 8 min, 0.9 ml of 20% sodium carbonate was added along with mixing. The solution was incubated at 40°C for 30 min before recording the absorbance at 765 nm in spectrophotometer (Model Evolution 600, Thermoscientific, US). The measurement was compared to a standard curve of Gallic acid solutions (20, 40, 60, 80, 100 mg/L) and results were expressed on fresh weight basis as milligrams of gallic acid equivalents per 100 g (GAE/100 g) samples for the extracts.

Total Flavonoid content (TFC)

The total flavonoid content was measured using aluminum chloride colorimetric assay by Zhishen et al. [14] with slight modification. 1 ml of sample extracts was added to flask containing 4 ml of water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 min, 0.3 ml of 10% $AlCl_3$ and after 6 min, 2 ml of 1 M NaOH was added. The total volume was made up to 10 ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm. A calibration curve was prepared using a standard solution of quercetin (20, 40, 60, 80 and 100 mg/L). The results were expressed on a fresh weight basis as mg quercetin equivalent (QE)/100 g of sample.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the method described by Benzie and Strain [15], Benzie and Szeto [16] with slight modification. Briefly, the FRAP reagent was prepared from sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (40 mM HCl as solvent) and 20 mM iron(III) chloride solution in a volume ratio of 10:1:1, respectively. One hundred microlitres of the diluted sample was added to 3 mL of the FRAP reagent. The absorbance of the mixture was measured at 593 nm after 30 min incubation. The standard curve was prepared using $FeSO_4.7H_2O$ solution (200, 400, 600, 800, 1000 µM), and the results were expressed on fresh weight basis as µM of ferrous equivalent Fe (II) per g of sample.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the extracts were evaluated by 1,1-diphenyl 2-picryl-hydrazil (DPPH) using the method given by Bhat and Karim [17]. An aliquot (100 µL) of fruit extract was mixed with 3.9 ml of 0.1 mM DPPH methanolic solution. The mixture was vortexed thoroughly and kept in the dark for 30 min. The absorbance was measured at 515 nm, against a blank of methanol. The radical scavanging activity was calculated using the ratio: (Acontrol – Asample/Acontrol) × 100, where Acontrol is the absorption of the DPPH solution and A sample is the absorption of the DPPH solution after the addition of the sample. Results were expressed as percentage of inhibition of the DPPH radical.

Antioxidant capacity determined by radical cation ($ABTS^{\cdot+}$)

ABTS assay was carried out according to the method described by Re et al. [18]. ABTS radical cation ($ABTS^{\cdot+}$) stock solution was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate in volume ratio of 1:1 and allowing the mixture to stand in the dark at room temperature for 12–16 hrs before use. The $ABTS^{\cdot+}$ solution was diluted with solvent to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 100 µL of sample or trolox standard to 2 mL of diluted $ABTS^{\cdot+}$ solution, absorbance at 734 nm was measured at exactly 6 min. The calibration curve between absorbance and known solutions of Trolox (200, 400, 600, 800, 1000 µM) was then established. Results were expressed as Trolox equivalent antioxidant capacity (TEAC µmol Trolox/g).

Statistical analysis

All the analysis was carried out in triplicate and values were expressed as means ± standard deviations. Data were analyzed using SPSS version 20 for Window software (IBM corp.) Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences between solvents and samples. Differences were considered significant at $P<0.05$. Correlation analyses between antioxidant activities and polyphenolic content were performed using Pearson's correlation coefficient (r).

Results and Discussion

Extraction yield

Extraction is an important initial step for the recovery and isolation of bioactive compounds from plant samples. The efficiency of extraction as well as yield depends on type of solvents used, solubility

and polarity of the compounds with the solvent, time and temperature of extraction [19]. Sulaiman et al. [20] soaked the sample paste in solvent for 1 hrs and centrifuged it. Musa et al. [21] used 300 rpm for 1 hrs for extraction process which gave better results than maceration. Therefore, the samples were subjected to high speed shaking (200 rpm) for 2 hrs as it have an effect on the morphology of the sample matrix which causes the bioactive compounds to get released more quickly and also increases the extraction process. The extraction yield of peels and pulps in different solvents are shown in Table 1. The extraction yield of the extracts in different solvents ranges from 2.85 ± 0.19 to 10.18 ± 0.66% in a decreasing order of 50% acetone>50% ethanol>50% methanol>70% acetone>70% ethanol>70% methanol>100% etone>100% methanol>100% ethanol>water.

The result showed that the extraction yield of pure acetone was higher than that of pure methanol and pure ethanol. This increase in yield may be due to aprotic nature of acetone as compared to other solvents. It was also found that the extraction yield of the extract with water was somewhat less than that of the extracts of pure solvents. The extraction yield showed increment as the concentration of water in the solvents was increased. This may be due to the extraction of chemical compounds which are soluble in organic solvents and/or water. The phytochemical analysis of the fruits showed the presence of various compounds like tannins, glycosides, terpenoides, carotenoids, phytosterols etc. Cucurbitacins is the most common terpenoides [22]. Tannins are soluble in water or organic solvents like alcohol, acetone while glycosides are soluble in water and insoluble in organic solvents. Terpenoids, carotenoids and phytosterols are soluble in organic solvents and insoluble in water. Therefore the aqueous mixtures of organic solvents gave higher extraction yield than pure solvents and water. Similar results were shown in medicinal plants [23] and rice bran [24].

Polyphenol content

The phenolic compounds in plants are considered to scavenge free radicals and thus it is opinioned that the antioxidant activities shown by plant materials occur due to presence of phenolic compounds [25]. These compounds have the capability to decrease the concentration of free or singlet oxygen, donate hydrogen atom to free radical, decomposition of free radicals to non radical and to prevent removal of hydrogen by breaking chains. Experimental results are similar to those obtained in the phenolic extraction from other fruits [26,27].

Tables 2 and 3 showed the total phenolic content of pulps and peels respectively in different extracting solvents. The TPC of the pulp was highest for muskmelon pulp followed by watermelon, pumpkin and ash gourd in all solvents while in case of peel, it was highest for muskmelon

Solvents	Pumpkin		Ash gourd		Watermelon		Muskmelon	
	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel
Water 100	3.26 ± 0.28^{a}	4.02 ± 0.17^{a}	2.85 ± 0.19^{a}	3.70 ± 0.15^{a}	3.47 ± 0.22^{a}	4.56 ± 0.2^{a}	3.50 ± 0.34^{a}	5.08 ± 0.12^{a}
Acetone: Water								
100:0	4.44 ± 0.30^{c}	6.27 ± 0.12^{c}	3.68 ± 0.25^{c}	5.26 ± 0.20^{c}	4.71 ± 0.20^{c}	5.41 ± 0.13^{b}	4.96 ± 0.11^{c}	5.97 ± 0.11^{b}
70:30	6.05 ± 0.06^{e}	8.42 ± 0.30^{e}	5.57 ± 0.28^{d}	7.32 ± 0.21^{e}	6.09 ± 0.12^{e}	7.48 ± 0.36^{d}	6.43 ± 0.11^{f}	8.79 ± 0.37^{d}
50:50	8.39 ± 0.14^{g}	10.18 ± 0.66^{f}	7.88 ± 0.08^{f}	9.13 ± 0.10^{f}	8.05 ± 0.09^{g}	9.71 ± 0.39^{e}	8.63 ± 0.27^{i}	10.11 ± 0.11^{f}
Methanol: Water								
100:0	3.67 ± 0.19^{b}	5.66 ± 0.31^{b}	3.46 ± 0.32^{bc}	4.93 ± 0.16^{bc}	4.34 ± 0.13^{b}	4.76 ± 0.31^{a}	4.76 ± 0.35^{bc}	5.23 ± 0.23^{a}
70:30	5.43 ± 0.23^{d}	7.37 ± 0.26^{d}	5.29 ± 0.16^{d}	6.61 ± 0.34^{d}	5.64 ± 0.19^{d}	6.56 ± 0.27^{c}	5.92 ± 0.16^{e}	7.27 ± 0.18^{c}
50:50	7.39 ± 0.16^{f}	9.82 ± 0.19^{f}	7.04 ± 0.06^{e}	9.04 ± 0.10^{f}	7.47 ± 0.27^{f}	9.14 ± 0.18^{e}	7.45 ± 0.29^{g}	9.56 ± 0.34^{e}
Ethanol: Water								
100:0	3.50 ± 0.20^{ab}	5.46 ± 0.14^{b}	3.30 ± 0.19^{b}	4.80 ± 0.21^{b}	4.19 ± 0.11^{b}	4.61 ± 0.15^{a}	4.43 ± 0.19^{b}	5.16 ± 0.24^{a}
70:30	5.52 ± 0.23^{d}	7.58 ± 0.29^{d}	5.44 ± 0.22^{d}	6.74 ± 0.26^{d}	5.83 ± 0.24^{de}	6.89 ± 0.18^{c}	6.04 ± 0.12^{ef}	7.40 ± 0.25^{c}
50:50	7.44 ± 0.21^{f}	9.87 ± 0.12^{f}	7.34 ± 0.06^{e}	9.07 ± 0.09^{f}	7.63 ± 0.15^{f}	9.52 ± 0.28^{ef}	7.89 ± 0.21^{h}	9.74 ± 0.25^{ef}

All values are means ± standard deviations of data from three independent experiments
Different superscripts (a, b, c, d…..i) in the same column indicate significant difference (P<0.05)

Table 1: Extraction yield of pulp and peel extracts of cucurbit fruits from different solvent.

Solvents	Pumpkin		Ash gourd		Watermelon		Muskmelon	
	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)
Water 100	13.92 ± 1.49^{a}	3.79 ± 0.51^{a}	11.63 ± 1.00^{a}	2.54 ± 0.28^{a}	18.47 ± 0.94^{a}	4.60 ± 0.28^{a}	22.75 ± 0.95^{a}	5.70 ± 0.28^{a}
Acetone: Water								
100:0	21.59 ± 0.97^{c}	5.30 ± 0.45^{b}	19.33 ± 0.96^{c}	4.49 ± 0.41^{b}	24.41 ± 1.15^{bc}	5.45 ± 0.41^{b}	27.76 ± 1.28^{b}	6.26 ± 0.46^{ab}
70:30	24.86 ± 0.91^{de}	6.80 ± 0.46^{c}	22.63 ± 1.33^{de}	5.70 ± 0.37^{c}	28.94 ± 0.93^{de}	7.24 ± 0.43^{cd}	33.47 ± 0.75^{d}	8.39 ± 0.29^{cd}
50:50	32.42 ± 1.08^{fg}	10.48 ± 0.61^{e}	28.80 ± 0.93^{f}	7.92 ± 0.52^{d}	37.71 ± 1.08^{g}	10.57 ± 0.38^{f}	42.27 ± 1.07^{f}	11.84 ± 0.53^{f}
Methanol: Water								
100:0	19.36 ± 0.99^{b}	4.90 ± 0.43^{b}	17.31 ± 0.81^{b}	3.80 ± 0.35^{b}	22.80 ± 0.93^{b}	5.12 ± 0.21^{b}	27.75 ± 1.26^{b}	6.22 ± 0.36^{ab}
70:30	24.38 ± 1.07^{d}	6.90 ± 0.41^{c}	21.05 ± 0.89^{cd}	5.42 ± 0.46^{c}	27.45 ± 0.95^{d}	6.81 ± 0.31^{c}	31.40 ± 0.73^{c}	7.87 ± 0.30^{c}
50:50	30.69 ± 1.04^{f}	9.84 ± 0.44^{e}	27.72 ± 0.93^{f}	7.62 ± 0.49^{d}	34.56 ± 1.08^{f}	9.68 ± 0.42^{e}	38.50 ± 1.08^{e}	10.78 ± 0.35^{e}
Ethanol: Water								
100:0	21.45 ± 0.90^{b}	5.68 ± 0.42^{b}	20.22 ± 0.95^{c}	4.48 ± 0.31^{b}	25.63 ± 1.10^{c}	5.73 ± 0.40^{b}	30.39 ± 1.13^{c}	6.86 ± 0.41^{b}
70:30	24.38 ± 1.06^{c}	7.73 ± 0.40^{d}	23.41 ± 1.28^{e}	5.82 ± 0.39^{c}	30.14 ± 0.85^{e}	7.56 ± 0.31^{d}	34.50 ± 0.99^{d}	8.62 ± 0.40^{d}
50:50	33.48 ± 1.05^{g}	11.72 ± 0.50^{f}	29.11 ± 0.97^{f}	8.00 ± 0.61^{d}	39.94 ± 0.89^{h}	11.14 ± 0.36^{f}	43.75 ± 1.65^{f}	12.32 ± 0.52^{f}

All values are means ± standard deviations of data from three independent experiments
Different superscripts (a, b, c, d…..h) in the same column indicate significant difference (P<0.05)

Table 2: Total Phenolic content (TPC) and Total flavonoid content (TFC) in pulp of four cucurbits using different solvents.

Solvents	Pumpkin		Ash gourd		Watermelon		Muskmelon	
	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)	TPC (mg GAE/100 g)	TFC (mg QCE/100 g)
Water 100	30.22 ± 1.08^{a}	7.29 ± 0.40^{a}	25.47 ± 1.06	5.60 ± 0.35^{a}	30.45 ± 1.09^{a}	5.75 ± 0.26^{a}	44.22 ± 1.00^{a}	9.04 ± 0.67^{a}
Acetone: Water								
100:0	42.47 ± 0.66^{b}	10.93 ± 0.43^{b}	38.95 ± 0.89^{c}	8.63 ± 0.36^{c}	34.53 ± 1.06^{b}	7.76 ± 0.37^{c}	47.53 ± 1.10^{c}	10.41 ± 0.34^{b}
70:30	52.33 ± 0.81^{d}	14.80 ± 0.40^{d}	46.42 ± 0.95^{d}	11.62 ± 0.38^{d}	43.64 ± 1.21^{d}	10.95 ± 0.30^{f}	55.83 ± 0.58^{e}	13.99 ± 0.35^{c}
50:50	63.30 ± 1.05^{f}	20.21 ± 0.65^{f}	53.53 ± 1.09^{f}	15.18 ± 0.53^{f}	48.63 ± 1.10^{e}	13.65 ± 0.53^{h}	67.45 ± 0.95^{g}	18.93 ± 0.47^{e}
Methanol: Water								
100:0	41.66 ± 1.64^{b}	10.98 ± 0.37^{b}	34.67 ± 1.15^{b}	7.78 ± 0.25^{b}	30.59 ± 1.07^{a}	6.76 ± 0.32^{b}	45.92 ± 0.55^{b}	10.45 ± 0.36^{b}
70:30	50.17 ± 1.04^{c}	13.81 ± 0.23^{c}	44.64 ± 1.03^{d}	11.27 ± 0.45^{d}	35.54 ± 1.05^{bc}	8.94 ± 0.54^{d}	54.29 ± 0.89^{d}	13.62 ± 0.22^{c}
50:50	59.38 ± 1.05^{e}	18.67 ± 0.33^{e}	49.81 ± 1.06^{e}	14.26 ± 0.30^{e}	43.78 ± 1.11^{d}	12.38 ± 0.33^{g}	63.74 ± 0.70^{f}	17.91 ± 0.40^{d}
Ethanol: Water								
100:0	43.42 ± 0.95^{b}	10.79 ± 0.56^{b}	35.48 ± 1.15^{b}	8.03 ± 0.46^{bc}	32.24 ± 1.09^{a}	7.25 ± 0.33^{bc}	46.92 ± 0.45^{bc}	10.72 ± 0.3^{b}
70:30	51.41 ± 1.13^{cd}	14.62 ± 0.29^{d}	45.75 ± 1.17^{d}	11.45 ± 0.33^{d}	37.37 ± 1.11^{c}	10.09 ± 0.18^{de}	54.97 ± 0.84^{de}	13.73 ± 0.43^{c}
50:50	60.90 ± 1.9^{e}	19.48 ± 0.75^{f}	50.67 ± 0.92^{e}	14.35 ± 0.38^{e}	45.35 ± 1.00^{d}	13.07 ± 0.20^{h}	64.67 ± 0.78^{f}	18.16 ± 0.33^{d}

All values are means ± standard deviations of data from three independent experiments
Different superscripts (a, b, c, d…..h) in the same column indicate significant difference (P<0.05)

Table 3: Total Phenolic content (TPC) and Total flavonoid content (TFC) in peel of four cucurbits using different solvents.

followed by pumpkin, ash gourd and watermelon. These result showed that muskmelon fruit have higher polyphenolic content than the other cucurbits. Most of the extracts differed significantly (P<0.05) in their total phenolic content. Furthermore, it was found that 50% aqueous ethanol and 50% aqueous acetone showed no significant difference except in case of watermelon and both occurred as most effective solvent for the extraction of TPC from pulp of each fruit while in case of peels, 50% aqueous acetone was the most effective solvent. The least effective solvent was water as it may be due to insolubility of some complex phenolic compounds in water.

Flavonoids are widely distributed group of phenols which act as effective antioxidants [28]. Table 2 showed that in case of pulp, the TFC content was highest in muskmelon and lowest in ash gourd while the watermelon pulp showed higher in TFC content than pumpkin pulp in some selected solvents (50% acetone, 70% acetone, 100% acetone, 100% methanol, 100% ethanol and water) and in other solvents pumpkin pulp showed higher value than watermelon. In case of peels, except in water extract the highest TFC content was in pumpkin peel followed by muskmelon, watermelon and ash gourd respectively as shown in Table 3. The TFC content showed the similar trend as TPC i.e., for pulp, 50% aqueous ethanol and 50% aqueous acetone showed no significant difference except in case of pumpkin while in case of peel, it was 50% aqueous acetone showed highest values. The results indicated that solvent polarity also effect the flavonoid content extraction.

Effect of solvent system

The solubility of chemical compounds of any sample is influenced by the difference in polarities of the solvents used for extraction. Therefore, it is very important to select an appropriate solvent for determination of TPC, TFC and other antioxidant compounds present in a sample [29]. Extraction of phenolic compounds from plant materials by using different solvents, such as acetone, methanol, ethanol and their aqueous mixture have been reported by various authors [30,31]. From the results shown in Tables 2 and 3, it is evident that the recovery of phenolic compounds was dependent on the type of solvent used and its polarity. Among all the extracts, 50% acetone was found to be the most efficient solvent for extracting phenolic compounds in case of peels whereas 50% ethanol in case of pulps. The recovery of total phenolic compounds was least in pure distilled water. These results may suggest the use of 50% acetone and 50% ethanol for extraction of phenolic compounds in cucurbits. Previous studies showed that the mixture of ethanol and water are usually used for the extraction of phenols from plant materials as it can dissolve wide range of phenolic compounds [17,32,33]. Other than ethanol-water mixture, acetone-water mixture can be used for the higher extraction of polyphenolic compounds from plant materials [20,21,34].

Antioxidant capacity

Fruits and vegetable contain variety of compounds showing antioxidant properties. Different methods have been developed to determine the antioxidant activities of different plant samples [35]. The recovery of the compounds in solvent is totally dependent on the solubility of the antioxidant compounds in solvents used for extraction. Thus, the polarity of solvents can increase the solubility of the antioxidant compounds [17]. In this study, three different methods have been used for the evaluation of the antioxidant capacity of the extracts namely Ferric Reducing Antioxidant Power assay (FRAP assay), DPPH free radical-scavenging assay and ABTS radical scavenging capacity.

Ferric-Reducing Antioxidant Power (FRAP) assay: In FRAP assay, the ferric ion of FRAP reagent is reduced to ferrous at low pH as a result of the activity of antioxidants present in the sample. The reduction of ferric iron produces intense blue color whose absorbance is measured at 593 nm [15]. The muskmelon pulp extracts showed the highest antioxidant activity as FRAP assay (Table 4) and lowest occurred in ash gourd while the watermelon pulp was higher in FRAP value than pumpkin pulp in most of the solvents except water and 100% acetone. The FRAP values of peels given in Table 5 showed that highest activity is in muskmelon extract followed by pumpkin while the ash gourd peel showed higher values than watermelon except in pure organic solvents i.e., ethanol, methanol and acetone. In case of pulps, 50% ethanol was the most effective solvent while 50% acetone had shown the highest antioxidant activity in peels. All the solvents showed significant difference (P<0.05) in their FRAP assay. The peel extracts contain high FRAP value than pulp extracts. This result is similar with other studies showing that the peels of different fruits have more antioxidant activity than pulp [35-37].

DPPH radical scavenging activity: DPPH assay is generally used for the estimation of free radical scavenging activity of the antioxidants. DPPH is stable at room temperature and produces a violet solution in solvent. Antioxidant compounds cause the discoloration of violet color to yellow color indicating the scavenging activity of the added

sample. This reduction results in loss of absorbance measured at 515 nm. The DPPH values of the extracts are presented in Tables 4 and 5 for pulps and peels respectively. The DPPH values for pulp was highest for muskmelon and lowest for ash gourd while the watermelon pulp was higher in DPPH value than pumpkin pulp in most of the solvents except 70% acetone, 70% methanol and 70% ethanol. For peels, highest activity was shown by muskmelon extract followed by pumpkin (except in water extract). The ash gourd peel extracts showed higher value

Solvents	Pumpkin			Ash gourd			Watermelon			Muskmelon		
	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)
Water 100	1.16 ± 0.2^{a}	14.87 ± 0.58^{a}	0.47 ± 0.08^{a}	0.96 ± 0.08^{a}	11.60 ± 1.01^{a}	0.34 ± 0.05^{a}	1.15 ± 0.14^{a}	19.53 ± 1.09^{a}	0.65 ± 0.09^{a}	2.17 ± 0.12^{a}	25.32 ± 0.86^{a}	1.05 ± 0.07^{a}
Acetone: Water												
100:0	1.83 ± 0.10^{bc}	28.68 ± 0.66^{b}	0.84 ± 0.1^{b}	1.13 ± 0.12^{bc}	24.10 ± 0.93^{c}	0.51 ± 0.07^{b}	1.84 ± 0.12^{b}	28.39 ± 1.15^{b}	0.84 ± 0.08^{bc}	3.02 ± 0.10^{c}	33.14 ± 1.22^{b}	1.54 ± 0.10^{b}
70:30	2.36 ± 0.09^{e}	37.67 ± 0.75^{d}	1.19 ± 0.05^{d}	1.26 ± 0.06^{cd}	31.64 ± 1.14^{e}	0.85 ± 0.09^{c}	2.44 ± 0.07^{d}	34.67 ± 1.29^{d}	1.36 ± 0.12^{d}	3.45 ± 0.11^{d}	45.16 ± 1.55^{d}	1.96 ± 0.15^{c}
50:50	2.84 ± 0.14^{g}	49.48 ± 1.10^{g}	1.45 ± 0.09^{e}	2.05 ± 0.10^{f}	41.37 ± 1.20^{h}	1.21 ± 0.13^{d}	3.37 ± 0.12^{f}	51.58 ± 1.08^{g}	2.03 ± 0.09^{f}	4.05 ± 0.13^{f}	57.05 ± 1.58^{e}	2.55 ± 0.12^{d}
Methanol: Water												
100:0	1.56 ± 0.15^{b}	28.31 ± 0.87^{b}	0.77 ± 0.09^{b}	0.99 ± 0.12^{ab}	21.77 ± 1.02^{b}	0.43 ± 0.07^{ab}	2.04 ± 0.07^{c}	29.44 ± 1.06^{b}	0.76 ± 0.06^{ab}	2.77 ± 0.11^{b}	35.30 ± 1.44b^{c}	1.14 ± 0.11^{a}
70:30	2.28 ± 0.14^{de}	38.56 ± 0.65^{de}	1.08 ± 0.12^{cd}	1.24 ± 0.08^{cd}	29.38 ± 1.08^{d}	0.75 ± 0.06^{c}	2.46 ± 0.10^{d}	36.44 ± 1.30^{d}	1.34 ± 0.09^{d}	3.13 ± 0.12^{b}	43.39 ± 1.10^{d}	1.49 ± 0.14b
50:50	2.66 ± 0.18^{fg}	47.64 ± 1.17^{f}	1.36 ± 0.07^{e}	1.77 ± 0.11^{e}	38.51 ± 1.13^{g}	1.12 ± 0.11^{d}	3.31 ± 0.13^{f}	48.52 ± 1.09^{f}	1.82 ± 0.09^{e}	3.84 ± 0.10^{e}	56.52 ± 1.56^{e}	2.41 ± 0.10^{d}
Ethanol: Water												
100:0	2.06 ± 0.13^{cd}	30.33 ± 1.08^{c}	1.04 ± 0.07^{c}	1.34 ± 0.11^{d}	25.52 ± 1.07^{c}	0.79 ± 0.05^{c}	2.17 ± 0.08^{c}	31.73 ± 0.94^{c}	0.96 ± 0.08^{c}	3.15 ± 0.11^{c}	36.53 ± 1.31^{c}	1.57 ± 0.09^{b}
70:30	2.52 ± 0.24^{ef}	39.68 ± 1.06^{e}	1.35 ± 0.08^{e}	1.95 ± 0.06^{f}	34.10 ± 1.14^{f}	1.09 ± 0.08^{d}	2.74 ± 0.10^{e}	39.58 ± 0.88^{e}	1.44 ± 0.1^{d}	3.54 ± 0.14^{d}	45.52 ± 0.94^{d}	1.95 ± 0.08^{c}
50:50	3.23 ± 0.18^{h}	51.16 ± 1.04^{h}	2.04 ± 0.09^{f}	2.26 ± 0.09^{g}	47.11 ± 1.39^{j}	1.54 ± 0.10^{e}	3.45 ± 0.07^{f}	52.53 ± 1.21^{g}	2.24 ± 0.10^{g}	4.21 ± 0.12	63.29 ± 2.46^{f}	2.78 ± 0.14^{e}

All values are means ± standard deviations of data from three independent experiments
Different superscripts (a, b, c, d…..i) in the same column indicate significant difference (P<0.05)

Table 4: Antioxidant activities (obtained from FRAP, DPPH and ABTS assay) in pulp of four cucurbits using different solvents.

Solvents	Pumpkin			Ash gourd			Watermelon			Muskmelon		
	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)	FRAP (µM of Fe(II) per g)	% DPPH	ABTS (µmol Trolox/g)
Water 100	2.14 ± 0.09^{a}	24.23 ± 1.14^{a}	1.25 ± 0.12^{a}	1.77 ± 0.18^{a}	19.55 ± 0.70^{a}	0.99 ± 0.05^{a}	1.18 ± 0.10^{a}	25.28 ± 1.23^{a}	0.86 ± 0.09^{a}	2.92 ± 0.15^{a}	32.52 ± 1.22^{a}	1.15 ± 0.08^{a}
Acetone: Water												
100:0	3.25 ± 0.10^{c}	40.50 ± 1.25^{c}	1.80 ± 0.05^{c}	2.28 ± 0.10^{c}	36.16 ± 0.90^{d}	1.26 ± 0.08^{b}	2.38 ± 0.12^{c}	32.82 ± 1.09^{b}	1.27 ± 0.08^{b}	3.57 ± 0.11^{b}	43.82 ± 1.08^{c}	1.77 ± 0.09^{b}
70:30	4.36 ± 0.07^{f}	49.48 ± 1.08^{e}	2.24 ± 0.13^{ef}	3.33 ± 0.10^{e}	42.58 ± 1.03^{f}	1.74 ± 0.11^{c}	3.13 ± 0.09^{e}	42.67 ± 0.69^{d}	1.66 ± 0.07^{c}	4.70 ± 0.07^{d}	52.54 ± 1.07^{e}	2.44 ± 0.08^{d}
50:50	5.14 ± 0.13^{i}	64.79 ± 1.90^{g}	3.11 ± 0.07^{h}	4.21 ± 0.16^{g}	60.56 ± 1.10^{i}	2.54 ± 0.10^{e}	3.86 ± 0.13^{g}	58.23 ± 0.89^{g}	2.46 ± 0.10^{e}	5.47 ± 0.10^{g}	68.04 ± 1.41^{g}	3.37 ± 0.11^{f}
Methanol: Water												
100:0	3.13 ± 0.09^{bc}	34.57 ± 1.71^{b}	1.34 ± 0.10^{b}	2.03 ± 0.07^{b}	30.25 ± 0.76^{b}	1.06 ± 0.05^{a}	2.13 ± 0.09^{b}	31.31 ± 1.28^{b}	0.96 ± 0.10^{a}	3.38 ± 0.12^{b}	37.99 ± 1.71^{b}	1.63 ± 0.09^{b}
70:30	3.85 ± 0.14^{d}	43.79 ± 1.26^{d}	1.97 ± 0.08^{d}	2.88 ± 0.11^{d}	40.72 ± 1.10^{e}	1.64 ± 0.09^{c}	2.73 ± 0.10^{d}	39.31 ± 1.02^{c}	1.19 ± 0.12^{b}	4.20 ± 0.10^{c}	47.11 ± 1.37^{d}	2.13 ± 0.08^{c}
50:50	4.65 ± 0.15^{g}	59.29 ± 1.05^{f}	2.35 ± 0.15^{f}	3.70 ± 0.07^{f}	54.63 ± 0.87^{g}	2.15 ± 0.05^{d}	3.55 ± 0.13^{f}	51.91 ± 1.55^{e}	2.14 ± 0.10^{d}	5.00 ± 0.11^{e}	62.73 ± 1.28^{f}	2.58 ± 0.10^{d}
Ethanol: Water												
100:0	2.96 ± 0.09^{b}	38.57 ± 1.16^{c}	1.46 ± 0.11^{b}	2.19 ± 0.07^{bc}	32.68 ± 1.20^{c}	1.14 ± 0.08^{ab}	2.26 ± 0.08^{bc}	32.50 ± 0.97^{b}	1.23 ± 0.10^{b}	3.43 ± 0.07^{b}	41.89 ± 1.04^{c}	1.67 ± 0.09^{b}
70:30	4.04 ± 0.11^{e}	47.38 ± 1.11^{e}	2.08 ± 0.09^{de}	3.28 ± 0.06^{e}	42.14 ± 0.95^{ef}	1.66 ± 0.12^{c}	2.77 ± 0.10^{d}	41.63 ± 0.92^{d}	1.55 ± 0.09^{c}	4.38 ± 0.11^{c}	50.80 ± 1.11^{e}	2.16 ± 0.07^{c}
50:50	4.85 ± 0.12^{h}	61.24 ± 1.21^{f}	2.65 ± 0.06^{g}	3.88 ± 0.08^{f}	56.40 ± 1.36^{h}	2.25 ± 0.07^{d}	3.63 ± 0.15^{f}	55.60 ± 1.18^{f}	2.29 ± 0.04^{d}	5.22 ± 0.16^{f}	64.44 ± 1.17^{f}	2.84 ± 0.11^{e}

All valuesare means ± standard deviations of data from three independent experiments
Different superscripts (a,b,c, d….h) in the samecolumn indicate significant difference (P<0.05)

Table 5: Antioxidant activities (obtained from FRAP, DPPH and ABTS assay) in peel of four cucurbits using different solvents.

than watermelon except for extracts in water, 70% acetone and 100% methanol. All the solvents showed significant difference (P<0.05) for their DPPH free radical scavenging activity. The similar trend was obtained as in polyphenols, the extracts in 50% ethanol and 50% acetone showed the highest antioxidant activity in pulp and peel respectively.

Antioxidant capacity determined by radical cation ($ABTS^{\cdot+}$): ABTS assay is based on the reaction of the $ABTS^{\cdot+}$ radical cation generated in the assay with the antioxidant present in the sample. This method takes comparatively less time than the other methods and it is also used to confirm the result obtained with DPPH, as both are similar in their antioxidant mechanism. The result showed that all the fruit pulps and peels exhibit the antioxidant capacity but in different degrees. Table 5 showed that muskmelon peel had the highest activity than pumpkin peel except for water and 100% acetone extracts. After these the ash gourd shows higher value than watermelon peel extracts except in 100% acetone, 100% ethanol and 50% ethanol. In case of pulp extracts, muskmelon pulp showed highest antioxidant activity followed by watermelon. The watermelon extract showed higher antioxidant value than pumpkin in most of the solvent extracts and ash gourd had given the lowest ABTS value. The result showed the similarity with the result of DPPH i.e., the pulp extracts showed highest antioxidant activity in 50% ethanol extract while the peel extracts in 50% acetone.

These results indicated that the TPC, TFC, FRAP, DPPH and ABTS values were susceptible to solvents used for extraction. The pure solvents, acetone 100% and ethanol 100% showed comparatively similar results in extraction efficiency with most of the samples followed by methanol, and water, respectively. Aqueous organic solvent mixture gave the highest values. 50% ethanol and 50% acetone were the best solvents to obtain extracts with higher quantity of polyphenolic content and antioxidant activities. In most of the cases, these two solvents showed significant difference (P<0.05) for all the samples. The value of the antioxidant activities varies in different extracts which might be related to the change in the polarity of different solvents [21]. From the results obtained, it may be suggested that the change in the polarity of organic solvent by addition of water (up to 50%) possibly enhance the extraction of antioxidant compounds. The results also indicated that the polyphenolic content and antioxidant activity of the peels was more than the pulps. The majority of fruit peels exhibit high antioxidant activity than pulp [35]. The antioxidant activity of the fruits might be influenced by its geographical location, types of cultivar, harvest season and storage conditions [38].

Correlation analysis between polyphenolic content and antioxidant activity

Despite of the different fruit, correlation analysis (Table 6) was performed between polyphenolic content and antioxidant activity among all pulp and peel extracts for each solvent. The extracts from 10 different solvents exhibited significant linear correlation (P<0.01) amongst all the parameters tested namely TPC, TFC, FRAP, DPPH and ABTS. The correlation coefficient (r) between TPC and TFC from different solvent extracts, it was shown that TPC and TFC had the similar trend with all the solvent exhibiting high linear correlation coefficient ($r \geq 0.95$). The result also signified a strong correlation between total phenolic content and FRAP assay showing similarity with the correlation found by Benzie and Stezo [16]. In case of correlation between TPC and FRAP, and TFC and FRAP almost a similar trend is observed showing the highest correlation in 50% acetone (r=0.961, 0.938, respectively) and the lowest was observed in 100% ethanol (r=0.792, 0.781, respectively). The correlation between TPC and DPPH, and TFC and DPPH, the highest correlation was in 50% acetone (r=0.959, 0.936, respectively) and lowest in 100% methanol (r=0.796, 0.772, respectively). Between TPC and ABTS, and TFC and ABTS, the highest correlation in 50% acetone (r=0.948) and 70% methanol (r=0.906) respectively, and the lowest was observed in 100% ethanol (r=0.794) and 50% methanol (r=0.761) respectively. These correlations specifies that higher the polyphenolic value, higher the antioxidant activities confirming that polyphenolic compounds are the main components that contribute to the antioxidant activities of these fruits.

Correlation analysis between the antioxidant activities showed significant linear correlation (P< 0.01) in all the solvent extracts. FRAP, DPPH and ABTS follow the same mechanism of single electron transfer (SET) in which it identify the capability of the prospective antioxidant for transferring of single electron for reduction of any compound. Both Fe (III)-TPTZ and $ABTS^{\cdot+}$ have similar redox potential of less than 0.7 V. The conditions of reaction for maintaining the iron solubility differ in both the methods as ABTS assay is done at neutral pH while FRAP assays is carried out at acidic pH 3.6. Thus, the values obtained by both may be comparatively relative [36]. ABTS assay is used to confirm the results obtained by DPPH assay. This shows that all the methods are correlated to each other. The correlation coefficient between FRAP and DPPH, and FRAP and ABTS, the highest value was shown in 50% acetone (r=0.971, 0.977, respectively) and the lowest was observed in water (r=0.813, 0.802, respectively). The correlation between DPPH and ABTS, the highest correlation was in 50% acetone as well as 70% acetone (r=0.963) while lowest occurred in water (r=0.827). Between ABTS and Reducing Power, the highest correlation was in 70% acetone (r=0.950) while lowest occurred in water extract (r=0.827). Among all the solvents used for extraction, 50% acetone showed higher correlation coefficient between most of the assays.

Conclusion

The finding of our study revealed that the extracts of the selected cucurbits have shown the significant antioxidant activity depending upon the type of solvent used for extraction. Acetone was found to be the best solvent followed by ethanol, methanol and water respectively. The mixture of organic solvents and water enhances the efficiency of extraction by making both lipophilic and hydrophilic compounds soluble in the mixture. The 50% aqueous acetone was the most effective solvent while water was found to be the least effective for all extracts. The 50% acetone showed the highest extraction in case of peels while 50% ethanol in case of pulp. The correlation between the polyphenolic content and antioxidants was high for all the extracts. The highest correlation coefficient between various assays was found in 50% acetone indicating it as the best solvent for extraction of polyphenolic compounds in these cucurbits. The phenolic content, flavonoid content, and the antioxidant activity were highest in muskmelon fruit when compared with other three fruits. Thus, the work indicated that these fruits are good source of phytochemicals that can be extracted by using a proper solvent system.

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Correlation coefficient (r)	TFC	FRAP	DPPH	ABTS
100% Water extract (N=24)				
TPC	0.953	0.801	0.926	0.795
TFC	-	0.879	0.921	0.861
FRAP	-	-	0.813	0.802
DPPH	-	-	-	0.827
100% Acetone extract (N=24)				
TPC	0.968	0.822	0.954	0.858
TFC	-	0.815	0.932	0.864
FRAP	-	-	0.910	0.965
DPPH	-	-	-	0.913
70% Acetone extract (N=24)				
TPC	0.984	0.913	0.892	0.885
TFC	-	0.911	0.893	0.878
FRAP	-	-	0.970	0.970
DPPH	-	-	-	0.963
50% Acetone extract (N=24)				
TPC	0.976	0.961	0.959	0.948
TFC	-	0.938	0.932	0.902
FRAP	-	-	0.971	0.977
DPPH	-	-	-	0.963
100% Methanol extract (N=24)				
TPC	0.975	0.871	0.796	0.924
TFC	-	0.864	0.772	0.897
FRAP	-	-	0.947	0.931
DPPH	-	-	-	0.891
70% Methanol extract (N=24)				
TPC	0.986	0.895	0.802	0.914
TFC	-	0.888	0.791	0.906
FRAP	-	-	0.950	0.948
DPPH	-	-	-	0.867
50% Methanol extract (N=24)				
TPC	0.981	0.914	0.882	0.813
TFC	-	0.898	0.862	0.761
FRAP	-	-	0.962	0.927
DPPH	-	-	-	0.929
100% Ethanol extract (N=24)				
TPC	0.977	0.792	0.882	0.794
TFC	-	0.781	0.874	0.765
FRAP	-	-	0.966	0.934
DPPH	-	-	-	0.907
70% Ethanol extract (N=24)				
TPC	0.974	0.908	0.860	0.854
TFC	-	0.876	0.838	0.841
FRAP	-	-	0.958	0.949
DPPH	-	-	-	0.946
50% Ethanol extract (N=24)				
TPC	0.955	0.937	0.834	0.800
TFC	-	0.915	0.779	0.765
FRAP	-	-	0.921	0.919
DPPH	-	-	-	0.922

Correlation is significant at the 0.01 level (2-tailed)

Table 6: Correlations between phenolic contents and antioxidant activities of various solvent extracts.

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