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Total Phenolic Content of Organic and Conventionally Grown Gourd Vegetables

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Abstract:*The increasing consumers' demands to acquire healthier fruits and vegetables as well as the urgency in looking to natural compounds with antioxidant activity (AOA) has encouraged a quick expansion of research studies about phenolic in vegetables. Gourd vegetables refer to the fruits of plants in the two Cucurbitaceae genera Lagenaria and Curcubita. There is a diverse source of polyphenols in plant materials.* ***Method:*** *Seven gourd vegetables grown conventionally (CV) were collected from the local market and a set of the same vegetables from certified organic farms (OG). They were analysed for the total phenolic (TP) content by the Folin-Ciocalteu method using Gallic acid as standard. Earlier each vegetable was extracted with ethanol, methanol and water separately for the estimation of TP. The TP content was expressed as µg of GAE/ g of FW.* ***Results:*** *Bitter gourd had the highest amounts of TP in both the conventional (1766.52 µg of GAE/ g of FW)) and organic (1962.0 µg of GAE/ g of FW)) samples. Conventional gourd vegetables had more TP when compared to organic varieties. Among the solvents the extraction of phenolics was the highest in water followed by methanol and ethanol.* ***Conclusion:*** *Gourd vegetables are widely available and can contribute significant amounts of phenolics to the diet.*

Keywords*: Total phenolics; Folin-Ciocalteu method; gourd vegetables; organic farming; ethanol, methanol, water solvents*

I. Introduction

Plants synthesize compounds with biological activity, namely antioxidant, as secondary products, which are mainly phenolic compounds serving in plant defence mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage. Phenolics are secondary plant metabolites ranging from simple structures with one aromatic ring to complex polymers such as tannins and lignins [1]. Such compounds have gained importance in human nutrition as they possess health benefits beyond their nutritional value. Also, their chemical structure and nature vary from simple to highly polymerized substances that include varying proportions of phenolic acids, phenylpropanoids, anthocyanins and tannins, among others [2;3]. Moreover, they might also exist in complex mixtures with carbohydrates, proteins and some quite insoluble high-molecular-weight phenolics [4].

There is a diverse source of polyphenols in plant materials, but both type and amount seem to be highly influenced by their chemical nature, extraction methods, sample particle size, storage time and conditions, as well as by the presence other of interfering substances [5]. Therefore, the phenolic extraction from plant materials is always a mixture of different steps, and many modifications of a particular method are often needed for the removal of unwanted non-phenolic substances such as waxes, fats, terpenes, pigments (chlorophylls and carotenoids). Solid-phase extraction (SPE) techniques, purification and fractionation based on acidity, are commonly used to remove unwanted non-phenolic substances or even other unwanted phenolics [6].

Although the recent advances in the technology provides innovative approaches to obtain enriched polyphenol natural extracts, we must be aware that their extraction efficiency will always be dependent of several factors in which the nature of samples and solvent, pH, temperature, light, length of extraction period, particle size, solvent/sample ratio and liquid-liquid or solid-liquid extraction process [5], among others, are the most critical.

II. Material And Methods

2.1. Vegetable Sampling and Extraction

Vegetable samples were purchased from retail outlets in the Coimbatore area and prepared for analysis during the harvest time. The retail outlets included supermarkets, independent retailers and catering suppliers. One kilogram each was purchased at least from three outlets and was combined into composite samples for analysis. Each composite was made up of three sub-samples, combined on an equal weight basis. Sub-samples included were based on the need to take into account factors including cultivar and region. From this the laboratory sample and test sample were derived for the determination of TP.

The fresh vegetables were sampled in seasons (spring) where the cultivars and geographic origin were known to change between seasons. This process allows a single, robust set of nutrient values to be derived for each vegetable [7]. A voucher specimen was identified by at least three persons viz., the investigator, the vender or farmer and botanist deposited at the Department's Nutrition laboratory for analyses and future reference. The samples were stored in the refrigerator and extracted the same day after removing the skin, stem and seeds.

Figure 1 - The Analysed Gourd Vegetables

Ash Gourd (*Benincasa hispida*) | Bitter Gourd (*Momordica charantia*) | Bottle Gourd (*Lageneria vulgaris*)

Ivy Gourd (*Coccinia cordifolia*) | Pumpkin (*Cucurbita maxima*) | Ridge Gourd (*Luffa acutangula*) | Snake Gourd (*Trichosanthesanguina)*

It is widely accepted that the extraction step is one of the most important stage in isolation of polyphenols, but based in literature, there is no consensus about one single and effective standard extraction method. On the contrary, there are several reported methods with very accurate results, and according to the literature in some cases, the solid-liquid extraction with different types of solvents is more adequate,[8].

In order to extract different phenolic compounds from plants with a high degree of accuracy, three solvents of differing polarities were used [9]. Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute.

Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. However, in our study we used three different solvents for extraction viz., ethanol, methanol and deionised water. The polarity, from least polar to most polar, of a few common solvents is as follows: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water.

The edible portion of the vegetable was cut into pieces and pulverised in a blender (Preeti Mixer, India, 750watts) for one minute. The blended sample (0.3 g) was weighed using an analytical balance (2007 TX/TXB Series, Shimadzu). The sample was then transferred into a Borosil test tube. For each test tube, 6 ml of extracting solvent was added to the sample (solvent ratio of 1:20). The test tube was then placed on a water bath shaker (Precision) of 130 rpm at a room temperature of 30 ºC for 60 min for the extraction. The extracts were collected and filtered using a syringe filter before the determination of total phenolic content [10]. All the extractions were carried out in three replicates. Extracts were stored in brown bottles in the freezer until further analyses.

2.2. Chemicals

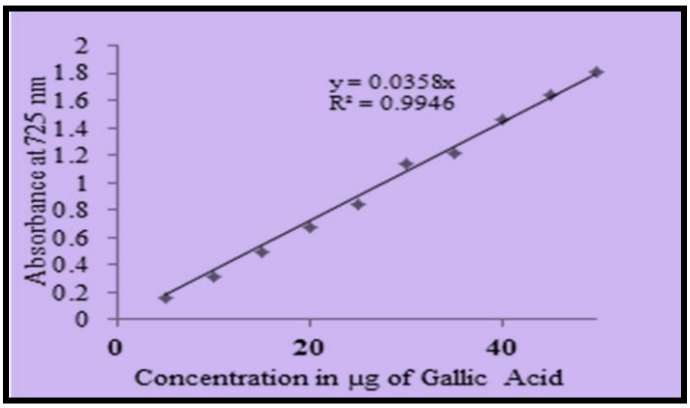
Gallic acid was purchased from Sigma-Aldrich USA. Folin- Ciocalteu reagent was obtained from Merck, Germany. All other chemicals used in the study were of analytical grade. These were supplied by the local agent in the study area.

2.3. Determination of total phenolic content

The total phenolic content of the gourd vegetable extract was determined by using Folin-Ciocalteu reagent following a slightly modified method [11]. Gallic acid was used as a reference standard for plotting calibration curve (Fig. 2). A volume of 0.5 mL of the plant extract (100 µg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v).

Figure 2

Standard Curve of Gallic Acid –Total Phenol Assay



The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for colour development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS Double Beam Spectrophotometer (Systronics, India). The total phenolic contents were determined from the linear equation of a standard curve prepared with Gallic acid. The content of total phenolic (TP) compounds was expressed as µg/g Gallic acid equivalent (GAE) of fresh weight.

III. Results - Total Phenolic Content of Gourd Vegetables

The Gourd Family (Cucurbitaceae) includes hundreds of species bearing coiled, climbing tendrils and some of the most unusual fruits in the world. The total number of species may exceed 700, with at least 100 different genera known as "curcurbits" to gourd lovers. The fruits of this exceedingly diverse family come in an astounding array of shapes and sizes, from tiny to marble-sized "jumbie pumpkins". It is a vegetable of hot climate and snow is very harmful to it. However, it can be grown on any type of soil [12].

The data on TPC of different gourd vegetables in the present study are shown in Table 1**.** The TPC in gourd vegetables varied widely from 29.37 ± 2.84 to 658.04 ± 10.60 µg of GAE/ g FW. Amoung the gourd varieties bitter

gourd and ridge gourd had high TPC while bottle gourd, pumpkin and ivy gourd had medium and snake and ash gourd had low TPC.

The sum of TPC of three extracts in each farming method was between 110.99 to 383.26 µg of GAE/ g FW in OG and CV in the assayed samples such as snake gourd, ivy gourd, ash gourd, OG bottle gourd and CV pumpkin. The TPC was in the range 501-999 µg of GAE/ g FW in OG pumpkin, OG and CV ridge gourd. With the exception, bitter gourd recorded the highest phenolic content (625.30 ± 7.54 and 661.62 ± 0.72 µg of GAE/g FW) in methanolic extracts of both OG and CV vegetable respectively and the sum of values of three the extracts exceeded >1500 µg of GAE/ g FW. It has been reported that ethanolic extracts of Ivorian plants extracted higher concentrations/amount of phenolics compared to acetone, water, and methanol, [13].

The TP of OG gourd vegetables were in the following order: bitter gourd> ridge gourd> pumpkin> ivy gourd> bottle gourd> snake gourd> ash gourd. The order was slightly different in CV gourd vegetables they are bitter gourd> ridge gourd> bottle gourd> snake gourd> pumpkin> ivy gourd> ash gourd.

ANOVA test revealed a high statistically significant ($p<0.05$) result for the difference between three extracts of OG and CV vegetables.

The TPC of OG and CV snake gourd, ivy gourd, ash gourd, pumpkin, bottle gourd ranged from 100- 300 µg GA/ g FW in water extracts (**Table 1**). On the other hand bitter gourd recorded highest TPC in methanolic extracts (> 600 µg).

TABLE 1 - Total Phenolic Content of Gourd Vegetables

S.No	Name of the Gourd Vegetable	(µg of GAE/ g of FW)			
		Ethanol	**Methanol**	**Water**	**Total**
Organic Gourd Vegetables					
1.	Ash gourd (*Benincasa hispida*)	29.37 ±2.84	31.65±1.06	64.44±4.29	125.46^{g}
2.	Bitter gourd (*Momordica charantia*)	567.79±4.18	625.30±7.54	573.43±15.49	1766.52^{a}
3.	Bottle gourd (*Lagenaria vulgaris*)	82.94 ± 8.58	125.03±3.05	124.45±1.35	332.42^{e}
4.	Ivy gourd (*Coccinia cordifolia*)	80.83±6.22	91.49±4.85	210.94±12.11	383.26^{d}
5.	Pumpkin (*Curcubita maxima*)	188.40±5.28	71.20±5.48	255.24±5.26	514.84^{c}
6.	Ridge gourd (*Luffa acutangula*)	115.86±7.19	154.14±2.78	419.78±2.46	689.78^{b}
7.	Snake gourd (*Trichosanthes anguina*)	38.67±9.14	36.74±2.37	101.18±0.69	176.59^{f}
Conventionally grown Gourd Vegetables					
1.	Ash gourd (*Benincasa hispida*)	29.60±1.50	22.28±3.43	59.11±1.58	110.99^{g}
2.	Bitter gourd (*Momordica charantia*)	643.04±1.90	661.62±0.78	658.04±10.66	1962.00^{a}
3.	Bottle gourd (*Lagenaria vulgaris*)	122.23±3.33	232.20±0.40	173.40±10.54	527.83^{c}
4.	Ivy gourd (*Coccinia cordifolia*)	45.24±1.66	68.93±7.31	154.51±7.53	268.68^{f}

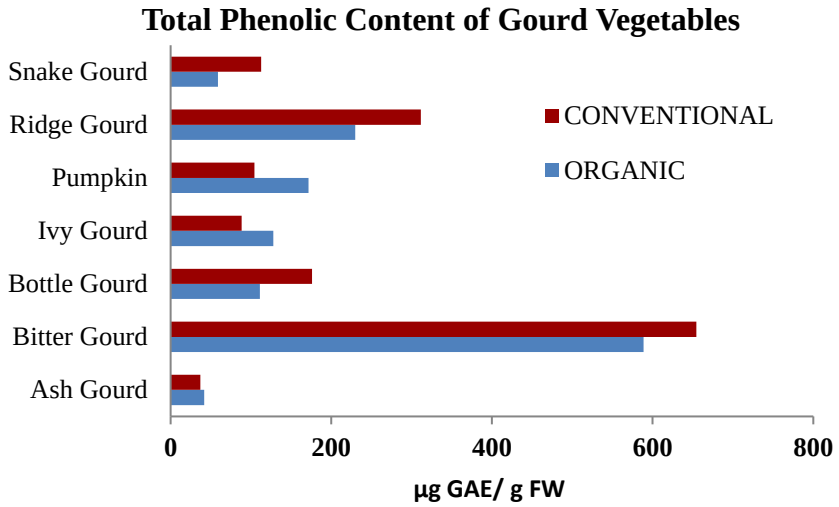
5.	Pumpkin (*Curcubita maxima*)	103.96±7.47	47.94±3.48	160.53±3.46	312.43^{e}
6.	Ridge gourd (*Luffa acutangula*)	165.38±3.07	176.23±0.79	592.66±4.71	934.21^{b}
7.	Snake gourd (*Trichosanthes anguina*)	90.31±5.25	63.72±2.50	183.99±11.37	338.02^{d}

The sum of values of three extracts is arranged in order for all the samples as indicated by the superscript alphabets

From the result, it is clear that the most of the gourd vegetables exhibited high phenolic content in water extracts except bitter and bottle gourd. A statistical significant difference ($p<0.05$) was observed between the similar extracts of one farming method except water extracts of ivy gourd, ash gourd and ethanolic extracts of ash gourd. Thus the result indicates that the recovery of phenolic compounds depended on the solvent used and polarity of phenolics to the solvent from vegetables. The phenolic content of OG ivy gourd and OG pumpkin were high when compared to the CV counterparts and was extremely statistically significant ($p<0.0001$).

Bitter gourd is a good source of phenolic compounds and possesses potent antioxidant property [14; 15]. In another study [16] the TPC of bitter gourd in different ripening stages and the results ranged from 2.8 to 4.2 mg GA/100g FW. The ripening stages were classified based on change in colour expressed as lightness and darkness (L*), redness (+a*) and greenness (-a*) and yellowness (+b*) and blueness (-b*). The increase in L* and b* resulted in moderate TPC with no significant difference in colour. However this result is low with the values of our study which was 56.7 to 66.1 mg GA/ 100g FW.

Figure 3



The above figure was plot using the mean value of three extracts of OG and CV vegetables

In an earlier study [17] the TPC of green and ripe fruit of bitter gourd was 32.4 and 22.4 mg GAE/100 g FW correspondingly. Our result was not consistent with this study results. Others [18] reported the TPC of wild bitter gourd in ethanol and water extracts as 6880 and 5160 mg GAE/ 100g DW. The result of yet another study [19], on TPC of some varieties of bitter gourd flesh varied from 539 to 775 and 640 to 802 mg GAE/ 100 g DW in oven and freeze dried samples respectively.

The determination of total phenolic compound of bitter gourd using subcritical water extraction for two extractions was 769 and 5263 mg/100 g GAE DW. For soxhlet extraction (water as solvent), it was 668 mg/100 g GAE DW and in solvent extraction (methanol as solvent) it was 600 mg GAE/100g DW of sample [14]. The total phenol and flavonoid contents of methanolic extracts of bitter gourd reported in a later study were 10.18 ± 0.501 mg GAE for total phenols and 7.63 ± 1.013 mg QE for total flavonoids [20].

The TPC of 66 vegetables commonly consumed in South Asia and presented TPC in two ways. One in the form of TPC expressed as mg of GAE/g FW and the other was corrected TPC obtained from the difference between TPC and ascorbic acid (AA) content of the vegetables (1 mg AA= 0.872 mg GA). They reported TPC of hairy ash gourd and winter ash gourd to be 0.25 and 0.17 mg GA/ g and FW respectively and the corrected TPC were 0.13 and 0.14 mg GA/ g FW respectively in hydrophilic extracts [21].

Pumpkin is a valuable source of functional components mainly carotenoids, lutein, zeaxanthin, vitamin E, ascorbic acid, phytosterols, selenium and linoleic acid, which acts as antioxidants in human nutrition [22]. In our study the organic pumpkin (514.84 µg of GAE/ g of FW) had more TP when compared to the conventional (312.43 µg of GAE/ g of FW)

The sum of values of three extracts of bitter gourd was 1766.52 and 1962.00 and in ridge gourd was 689.78 and 934.21 in OG and CV vegetables respectively. Considering the solvents methanol and water equally exhibited good phenolic content while ethanol reported the least activity. Gourd vegetables namely bitter gourd, bottle gourd, ridge gourd and snake gourd results favours CV farming while other gourd vegetables supported OG farming.

IV. Conclusion

Gourd vegetables are good sources of TP. In our study Bitter gourd had the highest level of TP. In all the analysed vegetables the conventional variety had greater amounts of phenolics. ANOVA test revealed a high statistically significant ($p<0.05$) result for the difference between three extracts of OG and CV farming methods. There are ample opportunities in this area of research. Data on AO can be posted in food composition tables if the determinations are done as per the established protocol.

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