Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 1182-1187
Received: 15-09-2017
Accepted: 17-10-2017

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A study on antioxidant and antibacterial activities of the fruit and seed extracts of two different cultivars of *Momordica charantia* Linn

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Abstract
The seed and fruit extracts of two different cultivars, a white and a green cultivar, were analysed to study the antioxidant, antimicrobial activities and the phytochemical compounds. Oxygen is an important element in the body and the excess amount causes free radical activity in the human body. The antioxidant activities of the different cultivars, both seed and fruit extracts were studied using total phenolic content by Folin-Ciocalteu method, total flavonoid content by the method adopted by Moussa *et al.* and by using FRAP, Thiobarbituric acid and phosphomolebdenum assays. It was found that seeds of the both cultivars exhibit higher absorbance values than the fruits and was understood to be also a provider of antioxidants for our body after consumption. The antibacterial test was conducted using *E. coli* strain for the seed and fruit extracts of both the cultivars. It was found that the zone of inhibition of the extracts were not as convincing as the zone of inhibition of the standard used as Almox 500g. So it was understood that the fruit and the seed do not contain much antibacterial properties. The phytochemical screening was carried out for the seed and fruit extracts of both samples. Little variance in the results obtained for the different cultivars might be the result of the cultivation in soils of different composition and nutrient content.

Keywords: Momordica charantia, phytochemical, antioxidant, assay

1. Introduction
*Momordica Charanti*a or Bitter Melon, also known as balsam pear or Karela belonging tothe family Cucurbitaceae, is a Tropical vegetable, and a common food in Indian cuisine and has been used extensively in folk medicine as a remedy for diabetes. The Latin name *Momordica* means "to bite" (referring to the jagged edges of the leaf, which appear as if they have been bitten). In Ayurveda, the fruit is considered as tonic, stomachic, stimulant, emetic, antibilous, laxative and alterative. Bitter melon has been used in various Asian traditional medicine systems for a long time. Like most bitter-tasting foods, bitter melon stimulates digestion. While this can be helpful in people with sluggish digestion, dyspepsia, and constipation, it can sometimes make heartburn and ulcers worse. The fact that bitter melon is also a demulcent and at least mild inflammation modulator, however, that it rarely does have these negative effects, based on clinical experience and traditional reports. [1] The present study is conducted so as to analyze the antioxidant capacities, antibacterial properties and the phytochemical screening of the seed and the fruit extracts of two different cultivars of *Momordica charantia*. The antioxidant capacity test is conducted using the estimation of total phenolic content, total flavonoid content and total antioxidant content. It is also further analyzed using antioxidant capacity assays like FRAP assay, thiobarbituric assay, etc. The antibacterial property test is conducted to test the antibacterial properties of seed and fruit extracts of the two different cultivars of *Momordica charantia* using bacterial strains of E.coli and Almox 500g as a standard.

Materials and Methods
Extraction of the plant materials and phytochemical analysis: The plant materials are washed with water and the leaves are separated, then are dried in shade and powdered by a mechanical grinder and stored in room temperature. The powdered plant materials were extracted with methanol. The extraction is carried out using soxhlet apparatus of the samples were prepared by 1g of the dried powder in 10ml. The extracts were filtered by using filter paper. The extract is then used for the further phytochemical analysis for steroids, alkaloids, flavonoids, saponins, coumarins etc using standard methods.

Antibacterial analysis: Sterile test tubes containing sterilized agar medium is taken in the petridishes and made ready for streaking with the inoculum containing the known sample of micro-organisms. The secondary metabolite extract of the living plant tissue was prepared. Some discs of filter paper of convenient size were cut and soaked in secondary metabolite placed in a sterile vessel. Then the secondary metabolite discs were appressed on the top of the medium so that it was fixed in the solidified agar. Then the measurement of zone of inhibition were made from the center of the disc to the circular circumference of the zone of bacterial growth after few days of incubation.

Antioxidant capacity

Determination of total phenolic content: The total phenolic content (TPC) of the cultivars was determined by the Folin-Ciocalteu method [23]. To 100 µL of the plant extract, 500 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added and incubated for 6 min at room temperature. The final volume of the solution was made up to 3 mL after addition of 1.25 mL of 7% sodium carbonate. The mixture was incubated for 90 min, followed by measuring the absorbance at 760 nm using UV Visible spectrophotometer (Cyberlab, USA). The total phenolic content was expressed as mg TAE (Tannic acid equivalents) per g of the dry weight of the plant, using a standard plot of Tannic acid.

Determination of total flavonoid content: The total flavonoid content (TFC) of the plant was determined by the method adopted by Moussa *et al*. Two hundred microlitre of the plant extract was taken in a test tube and the solvent was allowed to evaporate. The residue was mixed and shaken well with 5 mL of 0.1 M Aluminium chloride. Upon incubation of the solution for forty minutes at room temperature, the absorbance value was measured at 415 nm. A standard plot of Quercetin at varying concentrations was used to evaluate the total flavonoid content, expressed as µg QE (Quercetin Equivalent) per gram dry weight of the plant material.

Determination of total antioxidants: Phospho-molybdenum method was employed for the estimation of total antioxidant activity. A reagent solution of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was used for the experiment. The plant extract (0.5 mL) was mixed with 4.5 mL of the reagent solution and maintained in a boiling water bath at 950 C for 90 min. The absorbance value was measured at 695 nm upon cooling the solution at room temperature. The total content of antioxidants in the plant was expressed as mg TAE (Tannic acid equivalent) per g of the dry weight of the plant material.

Thiobarbituric acid assay: Two milliliters each of 20% trichloroacetic acid and 0.67% Thiobarbituric acid were mixed with 1 mL of 2.51% linoleic acid and 1 mL of plant extract. The solution was maintained in boiling water bath for 10 min. Upon cooling, the solution was centrifuged at 3000 rpm. The supernatant was passed through UV- visible spectrophotometer at 532 nm to measure the absorbance. The percentage inhibition of the plant against the secondary products of lipid peroxidation was evaluated with reference to the standard solution of butylated hydroxyl toluene (BHT).

Ferric Reducing Antioxidant Power (FRAP) Assay: A mixture of plant extract (1 mL), phosphate buffer - 2.5 mL (of 0.2 M, pH 7) and 1% potassium ferricyanide (2.5 mL) was incubated at 500 C for 30 min. To the solution, 2.5 mL of 10% Trichloroacetic acid was added, mixed and centrifuged for 10 min at 6500 rpm. Distilled water of 2.5 mL and 0.5 mL of 0.1% FeCl3 was added to 2.5 mL of the supernatant. The absorbance of the solution was measured at 700 nm. The reducing ability of the plant was evaluated in terms of percentage by relating the absorbance value of the plant and the standard, FeSO4. [2]

Detection of oils and fats

Spot test: The small quantity of extract is passed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Saponification Test: A few drops of 0.5 N alcoholic potassium hydroxide solution are added to a small quantity of extract along with a drop of phenolphthalein. The mixture is heated on water bath for 2 hr. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil or fats.

Results

Phytochemical Screening

Table 1: Preliminary phytochemical screening of the two varieties (green and white) of Momordica charantia L (medium ripened fruit and its leaves)

Tests		Green fruit	Green seed	White fruit	White seed
1. Steroids		+	+	+	+
2. Alkaloids	a. Wagner's test	+	+	-	+
	b. Mayer's test	+	+	+	+
	c. Dragendroff's test	+	-	+	+
3. Flavonoids	a. Lead acetate test	+	+	+	+
	b. NaOH test		+	+	+
4. Saponins		+	-	+	-
5. Amino acids		+	+	+	+
6. Coumarins		+	+	+	+
7. Cardiac glycosides		+	+	+	+
8. Anthroquinones		+	-	-	+
9. Tanins		+	+	+	+
10. Anthocyanins		+	+	+	+

Antioxidant Activities

Estimation of total phenolic content

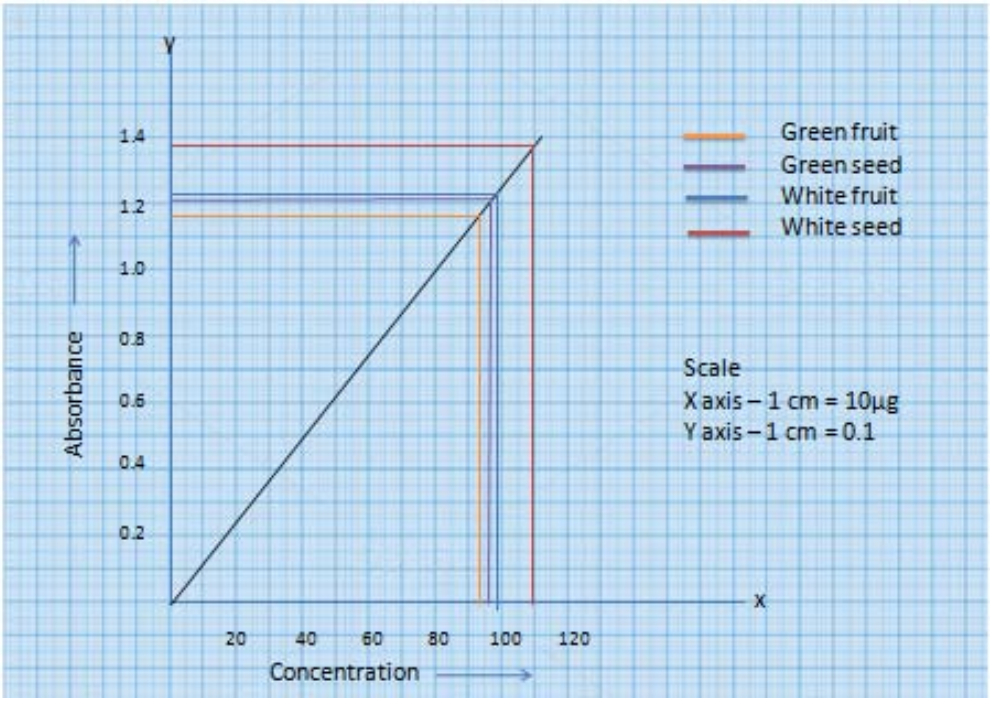

Fig 1: Estimation of total Phenolic Content

Estimation of total antioxidant content

Table 2: Total antioxidant content

Test	Green fruit	Green seed	White fruit	White seed
Total Antioxidant content	1.26	1.39	1.50	1.68

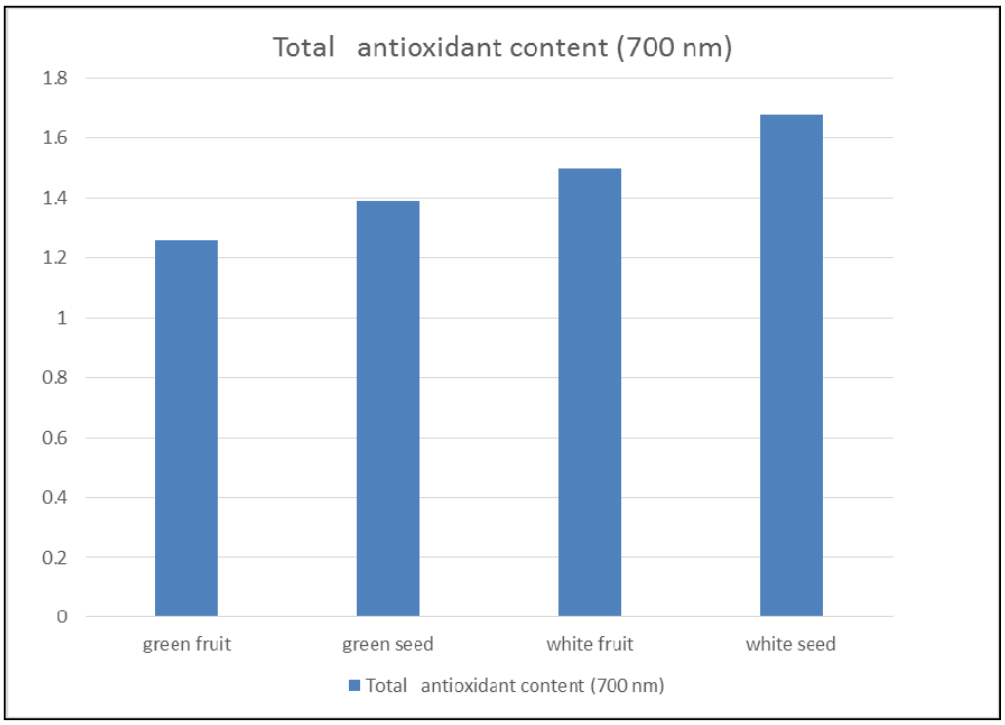

Fig 2: Estimation of total antioxidant content

Thiobarbituric assay

Assays	Green fruit	Green seed	White fruit	White seed
% inhibition	27.778 %	94.444 %	52.778 %	75 %

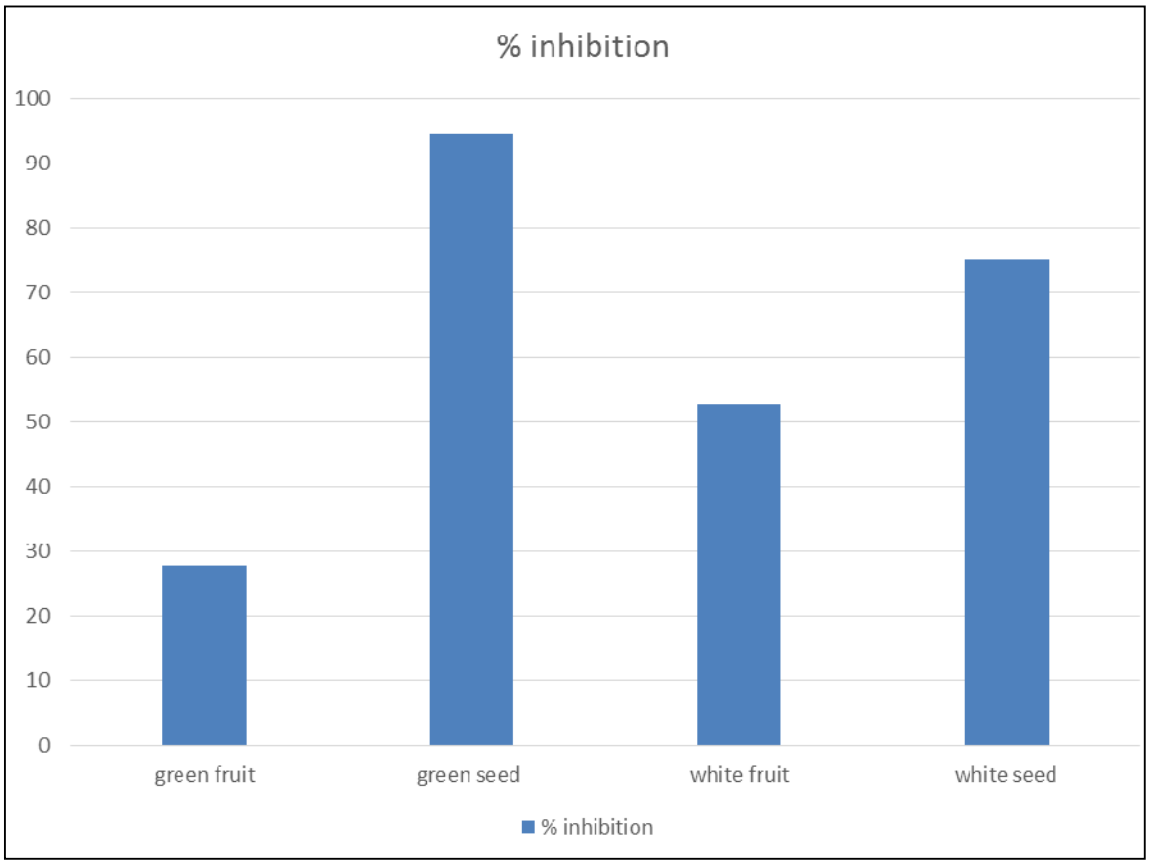

Fig 3: % of Inhibition based on Thiobarbituric assay

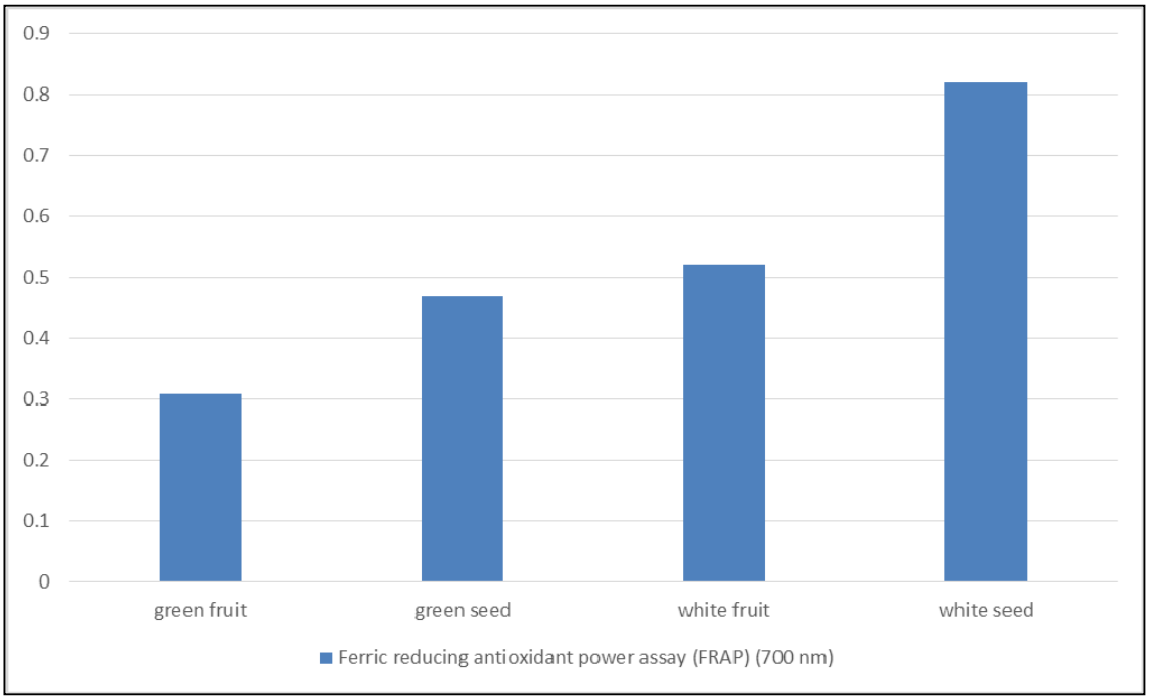

Fig 3: Ferric reducing antioxidant power assay (FRAP) (700 nm

Antibacterial properties

The standard which was used for this experiment was Almox 500mg which is Amoxicillin. The standard had amoxicillin in it so it was able to inhibit the bacterial growth to a large extent. The exact measurement was 40mm.The methanol control didn't have much activity to begin with as it had very less to no amounts of antibacterial properties it. So the zone of inhibition for methanol was 12mm.The zone of inhibitions obtained for the extracts are respectively green fruit, 17mm; green seed 15mm; white fruit, 18mm; white seed, 15mm.The zone of inhibition obtained for the white fruit extract had the biggest diameter and the green seed and white seed share the value of the least diameter.

Fig 4: antibacterial analysis, zone of inhibition of standard

Detection of fixed oils and fats

The tests for the detection of oils and fats were conducted. Spot test and saponification test was done to confirm the amount of oil and fats in the samples. Except for the green seed in spot test, all the other samples gave positive results. Saponification test was answered by all the extracts.

Tests	Green fruit	Green seed	White fruit	White seed
1. Spot test	+	-	++	+
2. Saponification test	+	+	++	++

Discussion

Groups of secondary plant metabolites, antioxidant phenolics, and flavonoids are commonly found in various fruits, vegetables and herbs and they have been shown to provide a fruitful defence against oxidative stress from oxidizing agents and free radicals. Polyphenols, such as phenolic derivatives and flavonoids are also known for their ability to prevent fatty acids from oxidative decay, and provide an additional value to plants used as food ingredients. In the present study, it was observed that the seeds had more phenolic contents compared to the pulp. The results are in agreement with the studies of Udai *et al*, which found that gallic acid, a polyphenol had maximum concentration in seeds followed by the pulp. Ferulic acid and caffeic acid were also comparatively higher in quantity in seeds when compared to the quantity in the pulp. Horax *et al* reported gallic acid as the main phenolic compound besides gastrisic, catechin, chlorogenic and epicortechin. Gallic acid presence and the others indicates the high medicinal value of the fruit and seed taking the cumulative therapeutic properties in account, the entire plant has medicinal values which have already been described in ancient Indian medicine (Ayurveda) where fruits in particular have been recommended for diabetic patients [3].

The studies also revealed that white cultivar had more phenol and phenolic acids as compared to the green cultivar. Phenolic compound included tannins, flavonoid, phenolic acid and other compounds. Phenolic acid had the lower antioxidant capacity than flavonoid. Flavonoid would give higher antioxidant capacity which had OH in ortho C 3, 4, OH in C3, oxo function in C4, double bond at C2 and C3 [4]. It was observed that the seeds have comparatively lesser amounts of flavonoids as compared to the flavonoid contents in the pulp. This may be due to the fact that phenols and phenolic acids are more concentrated in the seed which leaves very less room for the Flavin compounds thus reducing their amount in the seeds. It was also observed that the white seed extract has more amount of flavonoids as compared to that of the green seed extract but the white fruit extract has lesser amount of flavonoids as compared to the green fruit extract. This may be due to the fact that more amount of polyphenols, phenols, phenolic acids like gallic acids are accumulated more in the seed and the fruit of the white cultivar. This proves that the white cultivar has more amounts of phenolic secondary metabolites as compared to the green cultivar and the green cultivar has more amounts of flavonoid and Flavin compounds as compared to the white cultivar.

The highest antioxidant content was seen in the white seed and the lowest was seen in the green fruit. Considering the phenolic content test, the green fruit had the least amount of phenolic content and the white seed had almost twice the amount of phenolic content of the green fruit. Yet, the green fruit had the highest amount of flavonoid content amongst the four extracts. But it had the least antioxidant content. This might be due to the fact that the less amount of phenolic compound it had must have resulted in its low antioxidant capacity even if it had a higher amount of flavonoid content. There are two kinds of flavonoids present in the cultivars, gylcosides and glycones. Glycosides have lower antioxidant capacity as compared to glycones.[5]

Taking into account the amount of phenolic acids, the amount of flavonoid content and the amount of antioxidant capacity, in the white cultivar as compared to the green cultivar, it can be suggested that the white cultivar will prove more beneficial for consumption as it has more amounts of phenols and phenolic acids and less amounts of flavonoids which can be harmful as they can induce flavonoid toxicity, including their pro-oxidant activity, mitochondrial toxicity (potential apoptosis-inducing properties), and interactions with drug-metabolizing enzymes [16] and the white cultivars can be used as a good antioxidant having the optimum amount of required properties. It can also act as an immune-suppressor without causing any cytotoxic effect.

Conclusion

The total phenolic, flavonoid and antioxidant tests done on the two cultivars of *Momordica charantia* revealed the differences between the two cultivars. The white cultivar had considerably more amount of polyphenols as compared to the green cultivar. The seed extract of the white cultivar had the highest amount of phenolic content amongst the four extract. So the white cultivar had the highest amount of gallic acid and the maximum amount of gallic acid was concentrated in the seed of the white fruit. Significant differences were observed in the flavonoid content which is due to the deviations in the content of glycosides. The green fruit had the highest amount of Flavin content which contributes towards its high antioxidant content. There are marginal differences in the antioxidant content for the two cultivars. His is due to the variation in the phenolic and Flavin content for both the cultivars.

Acknowledgement

The authors are grateful to the faculty and supporting staff of Christ University and Bharathiar University for their support and encouragement.

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