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Original Article

Quantitative Analysis of Phytochemical Constituents and Antioxidant Efficiency of *Cucumis prophetarum* L.

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ABSTRACT

Background: The current study evaluated the efficiency of different solvent extractions on the yield of total phenolics and flavonoid content as well as antioxidant activity for different plant parts (root, stem, leaves, immature and mature fruits) of *Cucumis prophetarum*. **Materials and Methods:** The antioxidant activity of the extracts was assessed by using the following methods: Ferric reducing antioxidant power assay (FRAP assay), ferrous ion chelating activity, phosphomolybdenum reducing power assay and hydrogen peroxide radical scavenging assay. **Results:** The chemical examination of comparative extractive solvents of different plant parts (root, stem, leaves, immature and mature fruits) showed variations in the amount of active ingredients under investigation. Among the different plant parts analyzed for phenolics and flavonoid content, the aqueous extract of leaf material yielded highest content of phenolics (22.6 mg TAE/g dry weight) and flavonoids (3.15 mg RE/g dry weight) as compared to other plant parts. The aqueous extracts rich in phenolics and flavonoid content also exhibited potent antioxidant activity in all the assays and showed expected significant positive correlation with the phytochemical compounds. **Conclusion:** The study indicated that in phenolics, flavonoids and antioxidant assays, the results were higher for aqueous extraction system than other extragents used. Hence, the aqueous extract represents a source of potential antioxidants that could be used in pharmaceuticals.

Keywords: *Cucumis prophetarum*, Cucurbitaceae, Phenolics, Flavonoids, Free radicals, Correlation.

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INTRODUCTION

Cucurbits are among the largest and most diverse plant families, cultivated worldwide in a variety of environmental conditions. The fruits of cucurbits are very useful in terms of human health, i.e., purification of blood, removal of constipation and good for digestion and give energy. Seeds or fruit parts of some cucurbits are reported to possess purgatives, emetics and anthelmintic properties due to the presence of secondary metabolite cucurbitacins.[1,2] The cucurbitacins are of great interest because of the wide range of biological activities they exhibit in plants and animals. Within Cucurbitaceae, the genus *Cucumis* is considered as the most important one for their medicinal uses. *Cucumber* is one of the most important species of the genus *Cucumis*. Several studies on *Cucumis sativus* have shown multiple biological activities. These include cytotoxicity and exhibiting antibacterial, analgesic and antioxidant properties.[3] They are also rich in flavonoids and polyphenols that exhibit antidiabetic activity.[4] The fruits of *Cucumis trigonus* contain steroid and triterpenoid compounds, cucurbitacin B and proteolytic enzymes and it showed analgesic and anti-inflammatory activities. *C. prophetarum* has also been testified to have various biological activities as abortifacient and antihepatotoxic in the treatment of liver diseases.[5] The previous phytochemical reports have shown the presence of cucurbitacin A and B, sterol and saponins in aerial parts.[6] In spite of all these biological activities, the antioxidant effect of this plant and countless possibilities for investigation still remain in relatively newer areas of its function. This present research is a right step in this direction of searching for novel and more effective therapeutic value of *C. prophetarum*.

MATERIALS AND METHODS

Plant material

Roots, stems, leaves and fruits (Rind, pulp, seeds) of *C. prophetarum* were used as basic materials in this study, were collected from wild habitat around Bhutnal tanda region, Vijayapura, Karnataka. The herbaria for voucher specimen of the *C. prophetarum* was prepared and deposited in the Department of Botany, Karnataka State Akkamahadevi Women's University, Vijayapura (Karnataka), India.

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Reagents and standards

Folin-Ciocalteu reagent, aluminium chloride, ferric chloride, hydrogen peroxide, sodium phosphate, sodium carbonate, sodium acetate, 2,4,6-tripyridyl-s-triazine (TPTZ), ferrozine, ferrous chloride, ammonium molybdate, sulphuric acid, pottassium iodide, sodium thiosulphate (NaS_2O_3), tannic acid, rutin, ascorbic acid, ethanol, acetone and methanol (HPLC grade) were procured from HiMedia Chemical Co. Mumbai, (India). All the solvents used during the study were of AR grade.

Preparation of extract

The extracts of different plant parts of *C. prophetarum* were prepared by using four different solvent systems (aqueous, methanol, ethanol and acetone). The plant parts viz., roots, stems, leaves and fruits were dried under sunlight. Dried plant parts are ground in a blender to fine particles, put in a 5 mL of solvent and shaken vigorously for 5-10 min and left for 24 hr in a shaking machine after which the extract is filtered. Then the extracts obtained were centrifuged at 10,000 rpm for 15 min. The supernatant was collected and the residue was again suspended by adding 5 mL of solvents and centrifuged to complete the extraction. The supernatants pooled and the volume was adjusted to 10 mL by dilution of more distilled water. Same procedure was followed for the preparation of other solvent extracts (methanol, ethanol and acetone). All the extracts were kept at 4°C and for the assays 1% (v/v) extracts (diluted with double distilled water or respective solvents) were used.

Determination of total phenolic content

Total Phenolic Contents (TPC) of the plant extracts were determined using Folin-Ciocalteu method.[7] The reaction mixture was prepared by mixing an aliquot of the extracts (0.125 mL) with Folin-Ciocalteu reagent (0.125 mL) and 1.25 mL of saturated Na_2CO_3 solution. Reaction mixture was thereafter incubated for 90 min at room temperature and the absorbance was measured at 760 nm. The samples were prepared in triplicates for each analysis and the mean value of the absorbance was obtained. A calibration curve was prepared, using a standard solution of tannic acid (10 µg/mL to 100 µg/mL, $r^2 = 0.973$) and the results were expressed in terms of mg Tannic Acid Equivalents (TAE)/ g dry weight (dw) of sample.

Determination of total flavonoid content

Total Flavonoid Content (TFC) of the plant extracts were analyzed according to the spectrophotometric method.[8] The reaction mixture was prepared by adding 1.5 mL of extract to 1.5 mL of 2% ethanolic $AlCl_3$. The samples were incubated for 10 min at room temperature and the absorbance was measured at 420 nm. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained. The same procedure was used for the standard solution of rutin and the calibration curve was prepared (10 µg/mL to 100 µg/mL, $r^2 = 0.986$). The results were expressed on dry weight (dw) basis as mg Rutin Equivalents (RE)/g of sample.

Ferric Reducing Antioxidant Power assay (FRAP assay)

The ability to reduce ferric ions was measured using a method described by Grochowski *et al.*[9] An aliquot (100 µL) of extract was added to 3 mL of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM $FeCl_3$. $6H_2O$ solution) and the reaction mixture was incubated at 37°C for 15 min. After that, the absorbance was measured at 595 nm. A calibration curve was prepared, using an aqueous solution of ascorbic acid (100 µM to 1000 µM, $r^2 = 0.886$). FRAP values were expressed on a dry weight (dw) basis as millimoles of ascorbic acid equivalent per gram of sample.

Ferrous ion chelating activity

The chelating activity of the extracts for ferrous ions Fe2+ was measured according to the method devised by Taroreh *et al.*[10] To 0.5 mL of extract, 1.6 mL of deionized water and 0.05 mL of $FeCl_2$ (2 mM) was added. After 30 sec, 0.1 mL ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe2+-ferrozine complex was measured at 562 nm. The percentage of chelating activity of the extract was determined using the following equation.

$$\text{Chelating activity } \% = A0\text{-}A1/A0 \text{ X } 100$$

Where, A0 is the absorbance of the control and A1 is the absorbance of the sample.

Phosphomolybdenum reducing power assay

The antioxidant activity of the extracts was assessed by the phosphomolybdenum reduction assay according to Siddeeg *et al.*[11] An aliquot of 0.3 mL of sample was mixed with 3 mL of the solution (0.6 M sulphuric acid, 28 mM Sodium phosphate and 4mM ammonium molybdate). The tubes were capped with aluminium foil and incubated at 95°C for 90 min. The tubes were cooled to room temperature and the absorbance of aqueous solution was measured at 695 nm against a blank. For reference, ascorbic acid was used and a calibration curve was prepared (10 µg/mL to 100 µg/mL, $r^2 = 0.984$). The reducing capacity of the extracts was expressed as the ascorbic acid equivalents per gram dry weight (AAE/g dw).

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical scavenging activity was measured by the method described by Keshari *et al.*[12] 0.1 mL of test sample was mixed with 0.3 mL phosphate buffer and of 0.6 mL of 2 mM H_2O_2. The mixed solution was incubated for 10 min and the absorbance was recorded at 230 nm. The percent scavenging activity of the

sample extracts was measured by using the formula of inhibition percentage as applied for ferrous ion chelating activity.

Statistical analysis

Experimental results were statistically analyzed and expressed as mean ± standard deviation. All measurements were replicated three times and the data were subjected to different statistical analysis using MS Excel and GraphPad InStat software.

RESULTS

Total phenolics and flavonoid content

The amount of total phenolics and flavonoids present in the different extracts of *C. prophetarum* were determined and the content of total phenolics is expressed as Tannic Acid Equivalent per gram dry weight (TAE/g dw) and the content of total flavonoids as mg Rutin Equivalent per gram dry weight (RE/g dw). The concentration of total phenolics in the examined extracts ranged from 0.85 to 22.6 mg TAE/g dw (Table 1). Among the four different extracting solvents used, the aqueous extracts of leaf rendered highest phenolic content (22.6 mg TAE/g dw) as compared to methanol extract (20.7 mg TAE/g dw), ethanol extract (19.6 mg TAE/g dw) and acetone extract (16.8 mg TAE/g dw). Similarly, the content of flavonoids in different plant parts of *C. prophetarum* varied from 0.08 mg RE/g to 3.15 mg RE /g dw (Table 1). The leaf extract showed maximum amount of phenolics and flavonoids in comparison to other plant parts viz. root, stem, immature and mature fruit. Thus, the results of TPC and TFC analysis showed that the aqueous extract from the leaves of *C. prophetarum* had maximum phenolics and flavonoids than other extracts. The correlations between TPC and TFC assays were 0.990, 0.815 and 0.943 for root, stem and leaves respectively, which were highly significant at the 0.01 level. On the other hand, the correlations between TPC and TFC were not significant, in the case of immature (0.486) and mature fruits (0.672), at the 0.05 level.

Ferric Reducing Antioxidant Power (FRAP) assay

The ability of the plant extracts to reduce ferric ions was depicted in Table 2. Among the different plant part extracts, aqueous extract of leaf (1773.6 mM AAE/g dw.) exhibited relatively strong ferric ion reducing activities as compared to immature fruit (776.5 mM AAE/g dw.), mature fruit (737.4 mM AAE/g dw.), stem (597.6 mM AAE/g dw.) and root (157.3 mM AAE/g dw.). In this assay, the aqueous extracts again showed relatively high antioxidant activity among all the solvents used for the extraction like phenolics and flavonoid content. Further the highest reducing power in leaves is probably due to the action of hydroxyl group of the phenolic compounds which might act as an electron donor.

Ferrous ion chelating activity

The results of the ferrous ion chelating activity of the different plant part extracts of *C. prophetarum* revealed that the aqueous extracts of leaf exhibited highest chelating activity (89.72%) as compared to root (87.02%), stem (85.31%), immature fruits

Table 1: Total phenolics and flavonoid content in different plant parts of *Cucumis prophetarum* L.

Solvents	Total Phenolics (mg TAE/ g dry weight)					Total Flavonoids (mg RE/g dry weight)				
	Root	Stem	Leaf	Immature fruit	Mature fruit	Root	Stem	Leaf	Immature fruit	Mature fruit
Aqueous	1.47±0.12	12.4±0.12	22.6±0.09	9.37±0.09	9.87±0.096	0.18±0.005	2.20±0.65	3.15±0.85	0.48±0.0024	1.09±0.0024
Methanol	0.85±0.04	9.54±0.09	20.7±0.04	7.89±0.17	8.47±0.173	0.08±0.005	1.91±0.57	2.81±0.56	0.33±0.0043	0.76±0.0028
Ethanol	1.11±0.04	8.78±0.17	19.6±0.17	7.42±0.08	9.00±0.083	0.13±0.000	1.89±0.76	2.68±0.75	0.31±0.0014	1.04±0.0043
Acetone	1.00±0.08	8.75±0.22	16.8±0.09	6.47±0.12	8.75±0.166	0.11±0.001	1.66±0.39	2.47±0.39	0.37±0.0051	0.97±0.0028

Values are expressed as mean ± SD of triplicate measurements. mg TAE/g dry weight: milligram tannic acid equivalent per gram dry weight.mg RE/g dry weight: milligram rutin equivalent per gram dry weight.

Table 2: Antioxidant capacity in different plant parts of *Cucumis prophetarum* L.

Solvents	Ferric reducing antioxidant power (mM AAE/g dry weight)					Ferrous ion chelating Activity (%)				
	Root	Stem	Leaf	Immature fruit	Mature fruit	Root	Stem	Leaf	Immature fruit	Mature fruit
Aqueous	157.3±1.94	597.6±3.37	1773.6±3.89	776.5±33.7	737.4±5.15	87.02	85.31	89.72	83.34	79.38
Methanol	121.3±5.15	525.0±1.94	1760.0±1.94	717.7±5.15	573.1±7.02	81.16	80.89	87.60	77.44	51.05
Ethanol	137.3±3.37	525.0±3.89	1689.8±6.75	692.2±3.89	721.0±9.74	85.28	76.54	84.79	69.36	76.38
Acetone	129.0±1.94	436.0±3.89	1609.2±1.94	664.0±6.00	657.3±3.37	81.23	65.77	82.38	65.50	72.67

Values are expressed as mean ± SD of triplicate measurements.mM AAE /g dry weight: milli molar ascorbic acid equivalent per gram dry weight.

(83.34%) and mature fruits (79.38%) (Table 2). Interestingly, the results revealed that *C. prophetarum* exhibited an effective capacity for iron binding, suggesting that its action as antioxidant may be related to its iron-binding capacity.

Phosphomolybdenum reducing power assay

The extracts of *C. prophetarum* were also assessed for their phosphomolybdenum reducing capacities by the formation of green phosphomolybdenum complex. The phosphomolybdenum reducing power of the extracts is expressed as ascorbic acid equivalents per gram of sample (Table 3). In the ranking of the antioxidant capacity obtained by this method, the aqueous extracts of leaf (14.8 mg AAE/g dw) and immature fruits (14.2 mg AAE/g dw.) of *C. prophetarum* showed higher phosphomolybdenum reduction followed by stem (11.3 mg AAE/g dw), mature fruit (9.19 mg AAE/g dw) and root (4.62 mg AAE/g dw).

Hydrogen peroxide scavenging activity

The analysis of *C. prophetarum* for hydrogen peroxide scavenging activity presented that the extracts of *C. prophetarum* exhibited some extent of hydrogen peroxide scavenging capacity (Table 3). Amongst the various solvent extracts, the aqueous extracts of stem possess stronger scavenging activity (68.57%) as compared to root (65.71%) and leaf (57.14 %) extracts. Whereas aqueous extracts of both immature (37.14%) and mature fruits (37.14%) exhibited a weaker scavenging activity against hydrogen peroxide.

DISCUSSION

Phenolics and flavonoids are ubiquitously found in many plant sources including different vegetables, fruits and medicinal plants. Recently, the role of phenolics and flavonoids in the prevention of free radical mediated diseases has become more important. They possess different antioxidant properties which can be ascribed to a broad range of pharmacological activities.[13] Correlation analysis performed for the phenolics and flavonoid content in the different plant parts indicated that flavonoids are an important phenolic group in representing antioxidant capacity of roots, stem and leaves, where it could be related to other antioxidant compounds in immature and mature fruits. This could be explained by the fact that flavonoids could be related to other antioxidant compounds contained in fruits, such as amino acids and proteins that can also react with Folin-Ciocalteu reagent.

FRAP Assay measures the reducing potential of antioxidant. Antioxidant compound which acts as a reducing agent exerts its effect by donating hydrogen atom to ferric complex and thus break the radical chain reaction.[14] Therefore, the antioxidant potential of different extracts of *C. prophetarum* was estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II). Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress.[15] An important mechanism of antioxidant activity is the ability to chelate/deactivate transition

Table 3: Antioxidant capacity in different plant parts of *Cucumis prophetarum* L.

Solvents	Phosphomolybdenum reducing power (mg AAE /g dry weight)					Hydrogen Peroxide radical scavenging activity (%)				
	Root	Stem	Leaf	Immature fruit	Mature fruit	Root	Stem	Leaf	Immature fruit	Mature fruit
Aqueous	4.62±0.02	11.3±0.04	14.8±0.07	14.2±0.01	9.19±0.01	65.71	68.57	57.14	37.14	35.14
Methanol	3.98±0.031	10.9±0.02	14.2±0.05	11.8±0.01	7.34±0.17	60.00	65.71	54.28	33.28	25.71
Ethanol	4.54±0.012	9.69±0.02	13.1±0.04	13.0±0.26	9.04±0.05	65.71	60.00	51.42	31.57	34.28
Acetone	4.15±0.03	9.27±0.15	11.46±0.02	10.33±0.05	8.45±0.01	62.85	51.42	45.71	29.00	31.42

Values are expressed as mean ± SD of triplicate measurements.mg AAE /g dry weight: milligram ascorbic acid equivalent per gram dry weight.

Table 4: Comparison between phytochemical constituents and different antioxidant assays as represented by correlation coefficient.

Antioxidant activity	Root		Stem		Leaf		Immature fruit		Mature fruit	
	TPC	TFC	TPC	TFC	TPC	TFC	TPC	TFC	TPC	TFC
FRAP	0.997^{ns}	0.990*	0.683*	0.976^{ns}	0.933^{ns}	0.814*	0.992*	0.544^{ns}	0.851*	0.940**
Fe2+ chelation	0.829^{ns}	0.972^{ns}	0.571^{ns}	0.882**	0.962^{ns}	0.945*	0.929*	0.410^{ns}	0.712^{ns}	0.988^{ns}
MoO_2P reduction	0.781^{ns}	0.838^{ns}	0.689^{ns}	0.764*	0.973^{ns}	0.874*	0.776*	0.245^{ns}	0.817^{ns}	0.866^{ns}
H_2O_2 scavenging	0.673^{ns}	0.756^{ns}	0.563^{ns}	0.840**	0.992^{ns}	0.908*	0.997^{ns}	0.468^{ns}	0.648^{ns}	0.991^{ns}

Data were statistically analyzed using Pearson correlation coefficient test.^{ns} Indicates not significant at the level of $p>0.05$,*Indicates a significant difference at the level of $p<0.05$,**Indicates a significant difference at the level of $p<0.01$.

metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton type reactions. Therefore, it is considered important to screen the iron (II) chelating ability of the extracts. All the extracts of *C. prophetarum* demonstrated an extreme level of ability to chelate metal ions. Metal chelating capacity was significant as they reduced the concentration of the catalyzing transition metal in lipid peroxidation.[16] It was reported that chelating agents, which forms bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion.[17]

The phosphomolybdenum method is based on the reduction of molybdenum by the antioxidants and the formation of a green molybdenum (V) complex, which has absorption at 695nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts.[18] This may be explained by the fact that the transfer of electrons/hydrogen from antioxidants depends on the structure of the antioxidants.[19] The Hydrogen peroxide is a highly important reactive oxygen species because of its ability to penetrate biological membranes. However, it may be toxic if converted to hydroxyl radical in the cell.[20] Therefore, removing of H_2O_2 is very important for antioxidant defense in cell or food systems. Our results demonstrated that the extracts of *C. prophetarum* may probably be involved in removing the H_2O_2. H_2O_2-scavenging activity of the extract may be attributed to its phenolic contents as well as other active components such as anthocyanins, tannins and flavonoids which can donate electrons to H_2O_2, thus neutralizing it to water.[21]

From the above results shown it should be noted that the, aqueous extract having the highest amount of TPC and TFC, also exhibited strong antioxidant activities in all the assays. Antioxidant activity assays exhibited considerable antioxidant potential and showed expected significant positive correlation with the phytochemical compounds. The correlation of the phyto compounds with the antioxidant activity showed both positive and negative correlation (Table 4). The positive correlation indicated that phytochemicals are the main factors contributing to the antioxidant properties of *C. prophetarum*. Further the negative correlation between TPC, TFC and antioxidant activity suggested that it could be related to other antioxidant compounds contained in the plant. Thus, there are no universal criteria for presence or absence of antioxidant activity in different plants. However, there are several discrepancies in the correlation. Several studies have investigated the relationship between the antioxidant activity and the content of polyphenol compounds in herbs. Some authors have reported good linear correlation between these two parameters[22-24] whereas others have not observed such correlation.[25,26] Several explanations could be used to account for that. First, it has been reported that polyphenol compounds differ significantly in their antioxidant properties which are determined by several structural features of the polyphenol molecule.[27] Second, the investigated medicinal plant probably contains other substances with antioxidant effect apart from the polyphenols. Moreover, the number of polyphenols does not represent the potential synergism or antagonism between the individual compounds in the samples, which depends on their structure and mutual interactions.

CONCLUSION

The investigation of *C. prophetarum* indicated the presence of phenolics and flavonoids as well as appreciable radical scavenging activity which can be taken as evidence to cure several free radical associated diseases. The study also revealed that the aqueous extract in different plant parts of *C. prophetarum* contains substantial amount of phenolics and flavonoids and it is the extent of phenolics and flavonoids present in this extract being responsible for its marked antioxidant activity as assayed through various models. Thus, aqueous extract of *C. prophetarum* can be used as an accessible source of natural antioxidants with consequent health benefits. Presence of phytochemical constituents may also highlight the importance of this wild plant. Not only could this, but the antioxidant property of this plant together encourages its further analysis to isolate, identify, characterize and elucidate the structure of the bioactive compounds responsible for these properties.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

TPTZ: 2, 4, 6-tripyridyl-s-triazine; **TPC:** Total phenolic content; TAE: tannic acid equivalents; **TFC:** Total flavonoid content; **dw:** dry weight; **RE:** rutin equivalents; **FRAP:** ferric reducing antioxidant power; **AAE:** ascorbic acid equivalents.

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