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ORIGINAL ARTICLE **OPEN ACCESS**

Estimation of total phenolic and flavonoid contents and evaluation of antioxidant activity of two plants of family Liliaceae

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ABSTRACT
Plants are known as good sources of natural therapeutic agents. Plants generally contain secondary metabolites like phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids and alkaloids etc. which reveal their specific characteristic properties and attribute to their pharmacological properties. In the present investigation, Chlorophytum and Urginea of family Liliaceae were subjected to estimate total phenolic and flavonoid contents as well as extracts of these plant parts were also evaluated for their antioxidant activity by DPPH assay. Results of the present study indicated the presence of good quantity of total phenols and flavonoid contents in both the selected plants. Both plants also showed good free radical scavenging potential. All experiments were done in triplicates. One way ANOVA analysis showed lack of significant differences in antioxidant activity and total phenolic and flavonoid contents among all the selected plant parts.
Keywords: *Family liliaceae, Total phenols, total flavonoids, antioxidant activity etc.*

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INTRODUCTION

Plants of diverse origins have been utilized for medicinal purposes throughout various nations since ancient times, serving as a valuable reservoir of efficacious and potent pharmaceutical agents. Throughout history, individuals have endeavored to discover pharmaceutical interventions aimed at mitigating discomfort and treating various ailments (1). Plants typically possess secondary metabolites such as phenolics, flavonoids, glycosides, coumarins, saponins, terpenoids, and alkaloids, among others. These compounds are responsible for the distinctive qualities exhibited by plants and contribute to their pharmacological attributes (2). Phytoconstituents possess significant promise for the development of phytomedicines, which are often regarded as being generally safe. In recent years, there has been a growing interest in the natural antioxidants found in plants, owing to their widely recognized nutritional and medicinal benefits. The antioxidant capabilities of plant-derived substances and extracts are regarded as a crucial mechanism behind their therapeutic activity. Ethnopharmacological surveys have provided insights indicating that a significant proportion (80%) of the 122 plant-derived medicines examined may be associated with their traditional medicinal uses (3). Natural antioxidants possess a range of biochemical functionalities, including the ability to hinder the production of reactive oxygen species (ROS), the capacity to directly or indirectly eliminate free radicals, and the ability to modify the intracellular redox potential (4). Antioxidants have been found to exert inhibitory effects on apoptosis due to the initial belief that apoptosis is regulated by oxidative stress (5). Numerous studies have established that a wide range of antioxidant compounds possess notable anticancer or anticarcinogenic characteristics (6, 7). Epigallocatechin-3-gallate (EGCG) included in green tea has been documented to exhibit free radical scavenging properties (8) and to impede the development of carcinogen-induced tumors in the skin, lung, forestomach, and colon of mice (9). Hence, there exists unequivocal evidence of significant interest in the exploration of plant-derived natural antioxidants. The investigation of the bioactivities exhibited by different plant species has become a significant area of study due to the observed discrepancies in the efficacy of plant extracts, which can be attributed to factors such as the choice of extraction solvent, the specific plant component utilized, the age of the plants, and their geographic origin. The overutilization of medicinal plants in the development of pharmaceutical drugs also necessitates an increased supply of plant biomass, a demand that can be addressed by the application of biotechnological techniques such as micropropagation.

Chlorophytum tuberosum Baker is classified within the Liliaceae family and has been traditionally employed in indigenous medicinal practices for its galactogogue and aphrodisiac properties. The product is currently available for purchase in the market, commonly referred to as safed musali. The medicinally valuable components of this plant are its tuberous roots, which are white in color. *Urginea indica*, a significant autochthonous plant of the Liliaceae family, is distributed throughout several regions of India, predominantly inhabiting stony and mountainous terrains. This plant is widely recognized as Indian squill, True squill, or Sea onion, and is commonly referred to as Bon Pollundu. The plant, particularly its bulb, harbors a diverse array of bioactive compounds, including flavonoids, phytosterols, phenols, saponins, alkaloids, proteins, carbohydrates, steroids, and tannins. The bulb and rhizome also possess calcium, iron, and various commercial chemicals, including Bufadienolides, Quercetin, Allose, Mindererus spirit, Tartronic acid, and Paraldehyde, which exhibit diverse health functional qualities. In the present study, along with determination of total phenolic content, total flavonoid content in booth the selected plants, free radical scavenging activity by DPPH assay was also evaluated.

MATERIAL AND METHODS

Sample Collection and Processing:

The two chosen plants were obtained from Jhalawar, Rajasthan, India during separate occasions. All specimens exhibited no signs of microbiological contamination or physical deterioration. The specimens underwent a washing process, following which the various components of the plants were segregated. The specimens were subjected to shade drying at ambient temperature for a duration of 10 days. The dried components were pulverized into a fine powder. The powered samples were maintained in individual airtight containers and were consistently utilized for further phytochemical investigation.

Sample Extraction:

The plant pieces were subjected to a cold percolation process utilizing methanol as a solvent to extract their dry powder. A quantity of 10 grams of the desiccated powder was placed into a conical flask containing 100 milliliters of methanol. The flask was then subjected to agitation on an orbital shaker at a speed of 120 revolutions per minute for a duration of 24 hours. Following a 24-hour period, the extracts underwent filtration using Whatman filter paper no.1 in order to eliminate any remnants of peel particles. Subsequently, the extracts were subjected to evaporation under vacuum conditions.

Determination of Total Phenolic Contents in the Plant Extracts:

TPC (The total phenolic content) was determined by the Folin-Ciocalteau method (10, 11). The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml 7.5% $NaHCO_3$. The mixture was allowed to stand for 15 min at 45° C, and the phenols were determined by the spectrophotometric method. The absorbance was determined at λmax = 765 nm. The samples were prepared in triplicate, and the mean value of absorbance was obtained. Blank was concomitantly prepared, with methanol instead of extract solution. The standard curve was prepared using the standard solution of Gallic acid in methanol in the range 100-1000 mg/L. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight), which is a common reference compound.

Determination of Total Flavonoid Concentrations in the Plant Extracts:

The concentration of TFC (total flavonoid content) was determined using aluminium chloride spectrophotometric method (12) with slight modifications. Plant extracts (0.5 ml) were dissolved with 1.5 ml of methanol, 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water and incubated for half an hour at room temperature. The absorbance of the reaction mixture was measured at 415 nm. All experiments were prepared in triplicate, and the mean value of absorbance was obtained, and values were expressed in mean ± standard deviation. The standard curve was prepared using the standard solution of quercetin in methanol. Total flavonoid content of the extracts was expressed in milligrams of quercetin equivalents per gram dry weight.

Determination of Antioxidant Activity:

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 (13). An aliquot (100µl) of peel 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's scavenging activity was calculated using; $(Ab_{control} - Ab_{sample} / A_{control}) \times 100$ Where, $Ab_{control}$ is the absorption of the DPPH solution and Ab sample is the absorption of the DPPH solution after the addition of the sample. A linear graph of concentration vs percentage inhibition was prepared, and IC_{50} values were calculated. The antioxidant activity of each sample was expressed in terms of IC_{50} (defined as the amount of concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.

Statistical Analysis:
All experimental results were carried out in triplicate and were expressed as the average of three analyses with Standard Deviation. The IC_{50} values were also calculated by linear regression analysis.

RESULTS AND DISCUSSION
Plants possess numerous phytochemicals that serve as valuable reservoirs of natural antioxidants, including phenols, flavonoids, tannins, and phenolic acids (14). Numerous studies have documented that the presence of substantial phenolic and flavonoid compounds in plants serves as an indicative measure of their antioxidant ability. Flavonoids, a category of phenols, are ubiquitously present and exhibit potent antioxidant properties. Phenolics or polyphenols, as plant secondary metabolites, possess significant importance due to their antioxidant properties, which involve chelation of redox-active metal ions, inactivation of lipid free radical chains, and prevention of hydroperoxide conversions into reactive oxyradicals (15). In the present study, total phenolic content and total flavonoid contents were determined in both the selected plants. Results are presented in table 1 and figure 1. Results showed that *Urginea* had higher TPC and TFC (36.48±0.25 and 3.21±0.43 respectively) than *Chlorophytum* (13.07±1.12 and 0.75±0.24 respectively). Result of one-way ANOVA showed presence of significant differences in phenolic and flavonoid contents in both the selected plants. Presence of good amount of phenolic and flavonoid contents showed that all the species are good sources of natural antioxidants as the rich-flavonoid plants could manifest themselves as good sources of antioxidants that would assist in the enhancement of the overall antioxidant capacity of an organism and protection against lipid peroxidation (16). The consideration of polyphenol antioxidant capacity has emerged as a prominent mechanism for inhibiting mutagenesis and cancer initiation. This is attributed to their ability to scavenge reactive oxygen species (ROS), activate antioxidant enzymes, prevent the formation of DNA adducts induced by carcinogens, enhance DNA repair, and reduce oxidative DNA damage overall (17). The DPPH assay is frequently employed as a method to assess the capacity of plant extracts to scavenge free radicals. The current investigation involved the assessment of DPPH free radical scavenging activity in methanolic extracts of the chosen plants. The outcomes are presented in Table 2 and Figure 2. The findings indicated that both of the chosen plant species exhibited a notable capacity for scavenging free radicals.

Table 1: Total phenolic and flavonoid content in the fruit peels of the selected plants

Name of the plant	Total phenolic content (mgGAE/gm.DW)	Total flavonoid content (mgQE/gm.DW)
Chlorophytum	13.07±1.12	0.75±0.24
Urginea	36.48±0.25	3.21±0.43

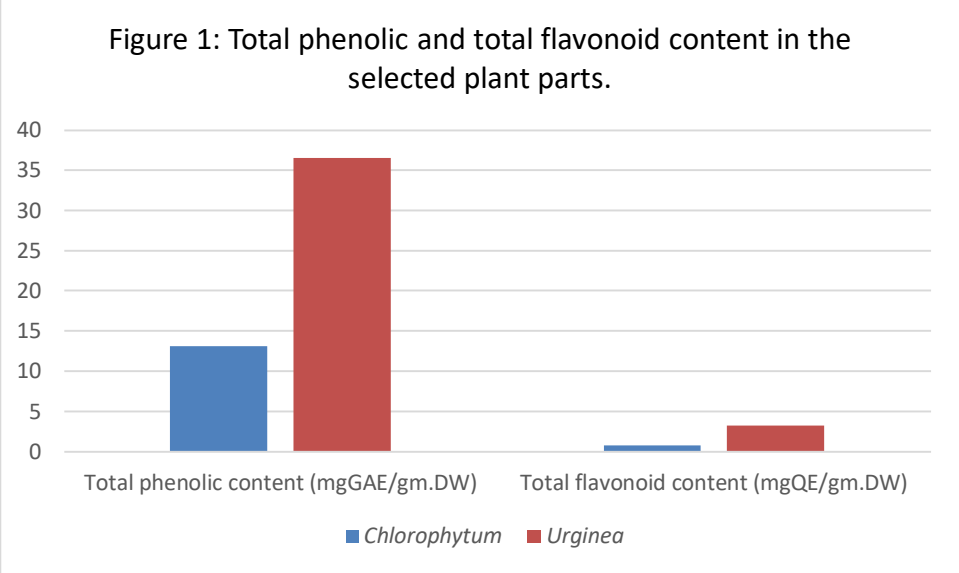
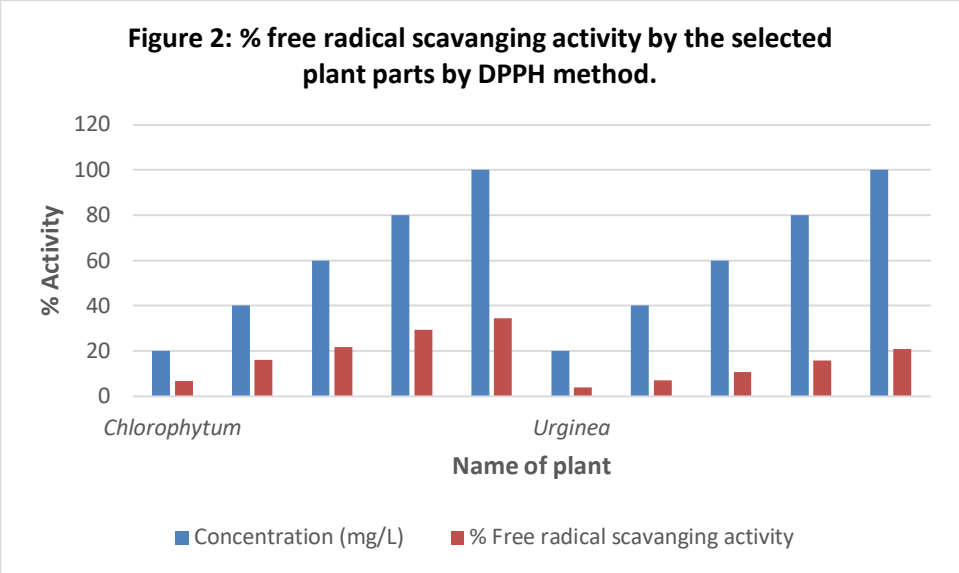


Table 2: Antioxidant potential of methanolic extracts of fruit peels of the selected Plants by DPPH assay.

No.	Name of plant	Concentration (mg/L)	% Free radical scavenging activity	Regression equation	IC_{50} value (mg/L)
1.	***Chlorophytum***	20	6.67±0.65	Y = 0.3447x+0.997	142.16
		40	16.12±1.12		
		60	21.77±1.66		
		80	29.25±0.48		
		100	34.57±2.71		
2.	***Urginea***	20	3.88±0.87	Y = 0.2121x-1.009	240.49
		40	7.12±1.21		
		60	10.65±2.88		
		80	15.65±1.52		
		100	20.82±2.66		



CONCLUSION

The present work shows the presence of high flavonoid and phenolic contents in different parts of both the selected plants which are common edible plant. Results of the study further suggested that these plants are rich source of natural antioxidants and eating these can provide good antioxidants and can protect us from various diseases. The present study may help researchers and scientists to make strategies of developing antioxidant rich varieties of food crops.

Conflict of interest

Authors declare that there is no conflict of interest for this work.

Authors contribution

Author Bahadur Singh Meena conducted the whole study and wrote the manuscript while author Dr Shuchita Jain helped him to designed the study and provided proper guidance for the work.

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