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Comparison of Total Phenol, Flavonoid and Antioxidant Activity of Four Leafy Vegetables

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ABSTRACT:

The study aimed to compare the phenolic content, flavonoid content, and antioxidant activity of four underutilized leafy vegetables: *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst., *Boerhavia diffusa* Linn., *Sauropus androgynus* (L.) Merr. and *Talinum fruticosum* (L.) Juss. Phenolic and flavonoid contents were measured using methanol and water extracts, while antioxidant activity was evaluated using percentage inhibition at different concentrations. The findings provide insights into the phytochemical composition and potential health benefits of these leafy vegetables.

Keywords: Leafy vegetables, Phenolic content, Flavonoid content, Antioxidant activity, Phytochemicals, Health benefits

1. Introduction

The utilization of medicinal plants in healthcare has a rich historical backdrop, tracing its roots to ancient civilizations. Across the globe, extensive research endeavors have been undertaken to substantiate the efficacy of medicinal plants, resulting in the formulation of plant-based medicines. Within the paradigm of Primary Health Care (PHC), which encompasses five fundamental principles, the role of medicinal plants in averting common diseases is being reevaluated. Sofowora *et al*. (2013) underscore the pivotal role of medicinal plants in disease prevention, highlighting their compatibility with existing preventive strategies. This viewpoint resonates with the World Health Organization (WHO)'s advocacy for the exploration of medicinal plants as a primary source of diverse pharmaceuticals. Notably, traditional medicines, derived from medicinal plants, are widely embraced, with approximately 80% of individuals in developed countries incorporating them into their healthcare practices (Yadav & Agarwala, 2011). The adoption of plants for medicinal purposes, often referred to as alternative medicine (AM), has permeated various cultures globally, particularly in Asian and Western societies (Mohammed, 2019). Ancient practices of utilizing plants and herbs for culinary, therapeutic, and preventive purposes have been transmitted through generations, attesting to their enduring significance in human communities. These plants, rich in active compounds produced during secondary metabolism, hold promise for treating infectious diseases and warrant further exploration (Singh, 2015).

Amidst the diverse array of plants, green leafy vegetables stand out for their medicinal properties, offering nutritional benefits and potential disease-preventive effects (Kumar *et al*., 2020). Also, leafy vegetables are the only natural sources of folic acid, which is considerably high in leaves of spinach, asparagus, lettuce, mustard green, colocasia green leaf, and turnip green plants compared to other leafy and nonleafy vegetables (Kumar *et al*., 2020). *Cnidoscolus aconitifolius*, commonly known as Chaya, is a good source of protein, vitamins, calcium, and iron; and is also a rich source of antioxidants; however, raw Chaya leaves are toxic as they contain a glucoside that can release toxic cyanide (Kuti & Konuru, 2004). *Boerhavia diffusa* plant is used as an appetizer, alexiteric, eye tonic, flushing out the renal system, to treat seminal weakness, and blood pressure, among other uses (Chaudhary & Dantu, 2011). Ethnobotanical studies have revealed that *Sauropus androgynus* is traditionally used in various formulations against a wide range of ailments including diabetes, weight loss, diarrhea, cough, and ulcer (Anju et al., 2022; Fikri & Purnama, 2020). *Talinum fruticosum* is used for its diuretic properties, management of gastrointestinal disorders, scabies, and high blood pressure (Manikandan & Gayathri, 2022; Kumar *et al*., 2023).

The nutritive value of greens remains underutilized due to a lack of awareness and promotion of appropriate technologies for their effective utilization (Ames & Gold, 1996). They are inexpensive yet high-quality sources of nutrition especially for low-income and marginalized sectors of the economy (Smith & Eyzaguirre, 2007). Green leafy vegetables have long been reported to contribute to the dietary vitamin and mineral intake of local populations (Nordeide *et al*., 1996; Kamble & Jadhav, 2013). In this study, we focus on four underutilized green leafy vegetables—*Cnidoscolus aconitifolius* (Mill.) I.M. Johnst., *Boerhavia diffusa* Linn., *Sauropus androgynus* (L.) Merr., and *Talinum fruticosum* (L.) Juss. selected for their edible nature and high medicinal properties. By exploring their nutritive values and healing abilities through phytochemical studies, we aim to elucidate their potential contributions to healthcare.

2. Materials and Methods

Four different leafy vegetables, namely *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst., *Boerhavia diffusa* Linn., *Sauropus androgynous* (L.) Merr., and *Talinum fruticosum* (L.) Juss.,

were collected from Thirur, Thrissur, during February. These plant materials were authenticated and deposited at the Department of Botany, Vimala College (Autonomous), Thrissur. The samples were cleaned, shade-dried for two weeks, and then powdered for further analysis.

Preparation of Extract

The dried powdered samples of each leafy vegetable (20 g) were separately extracted with methanol and distilled water (200 mL) using an orbital shaker for 72 hours. The extracts were then filtered using Whatman filter paper, and the filtrates were dried in a water bath at 40°C. The dried extracts were stored in a refrigerator at 2–8°C for subsequent analysis.

Determination of Total phenolic content

The total phenolic content was determined for individual extracts using the Folin–Ciocalteu method (Singleton *et al,* 1999). Briefly, 1 mL of extract (100–500 µg/mL) solution was mixed with 2.5 mL of 10% (w/v) Folin–Ciocalteu reagent. After 5 min, 2.0 mL of Na_2CO_3 (75%) was subsequently added to the mixture and incubated at 50 °C for 10 min with intermittent agitation. Afterwards, the sample was cooled and the absorbance was measured utilizing a UV Spectrophotometer (Shimazu, UV-1800) at 765 nm against a blank without extract. The outcome data were expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

Determination of Flavonoid Contents

The flavonoid contents of individual extracts were measured as per the method of Phuyal *et al*.,2020 An aliquot of 1 mL of extract solution (25–200 µg/mL) or quercetin (25–200 µg/mL) were mixed with 0.2 mL of 10% (w/v) $AlCl_3$ solution in methanol, 0.2 mL (1 M) potassium acetate and 5.6 mL distilled water. The mixture was incubated for 30 min at room temperature followed with the measurement of absorbance at 415 nm against the blank. The outcome data were expressed as mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of dry extract.

DPPH Radical Scavenging Activity

The radical scavenging activity (RSA) of the crude extracts was adopted to measure antioxidant activity using the DPPH method of Nithianantham *et al*., 2011 . Briefly, 2 mL of extract solution (1–100 µg/mL) in methanol was added to 2 mL of DPPH (0.1 mM) solution. The mixtures were kept aside in a dark area for 30 min and absorbance was measured at λmax 517 nm against an equal amount of DPPH and methanol as a blank. The percentage of DPPH• scavenging (RSA %) was estimated using the equation:

% scavenging of DPPH $= [(A0 - A1)/A0] \times 100$,

where A0 = absorbance of the control and A1 = absorbance of the test extracts.

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD).

3. Results and Discussion

Plants have been used for centuries as remedies for human diseases. Herbs and plants utilized by traditional medicine practitioners contain a wide range of substances that can treat both chronic and infectious diseases. The medicinal value of these plants lies in certain chemical substances that produce definite physiological actions in the human body (Khaing & Moe, 2019). The most important bioactive compounds in plants are alkaloids, flavonoids, tannins, and phenolic compounds. The leaves of *Cnidoscolus aconitifolius*, *Boerhaavia diffusa*, *Sauropus androgynus*, and *Talinum fruticosum* were studied for their total phenolic, flavonoid,

and antioxidant properties. All of these are edible leafy vegetables that can be consumed as they are, or useful medicines can be developed from them by understanding their phytochemical and antioxidant properties.

For this study, the solvents used for extraction were methanol and water. The successful determination of biologically active compounds from plant material largely depends on the type of solvent used in the extraction procedure (Abegunde *et al*., 2015). Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiological absorption of the extract, preservative action, and inability to cause the extract to complex or dissociate. Methanol extract was chosen because in most phytochemical studies, the extract preparations were done with methanol solvent, aiding in the easy identification of constituents, and methanol leaf extracts exhibited significant antioxidant activity (Altemimi *et al*., 2017). As another extract preparation, water was chosen as this was done to assess their edible aspect, so the best-suited extract preparation was in water. Water is a universal solvent used to extract plant products (Sharma *et al*., 2020). Traditional healers primarily use water for extraction (Kuri-García *et al*., 2017).

Both methanol and water extracts of all the leafy vegetables showed the presence of phenols and flavonoids. Methanol extract exhibited high phenol and flavonoid content in all four vegetables studied. *Talinum fruticosum* (L.) Juss showed almost the same phenol and flavonoid content in both water and methanol extracts (Table 1). Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as anti-apoptosis, anti-aging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection, and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Yadav & Agarwala, 2011). The presence of phenolic compounds makes it impossible to isolate active enzymes by conventional techniques from many plant tissues (Loomis & Battaile, 1966). Flavonoids are a family of plant secondary metabolites that help in plant-pathogen interactions, pollination, light screening, seed development, and allelopathy. Flavonoids have been suggested to act as antioxidants, protecting plants from oxidative stress (Hernandez *et al*., 2009). Many flavonoids have been shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities (Kumar & Pandey, 2013).

Table 1: Total phenolic and flavonoid content of four leafy vegetables

Sl No	**Extract**	**Total phenolic content (mg/gmGAE)**		**Total Flavonoid content (mg/gm QE)**	
		Methanol	Water	Methanol	Water
1.	*Cnidoscolus aconitifolius* (Mill.) I.M. Johnst.	0.734	0.152	0.506	0.142
2.	*Boerhavia diffusa* Linn.	1.115	0.144	0.646	0.168
3.	*Sauropus androgynus* (L.) Merr	0.796	0.141	0.478	0.152
4.	*Talinum fruticosum* (L.) Juss	0.994	0.884	0.460	0.460

Significant correlations were found between the antioxidant activities and the total phenolic and flavonoid contents, indicating that these phytochemicals are the major contributors to the antioxidant capacities of these plants. *Talinum fruticosum* (L.) Juss exhibited the highest antioxidant activity across all concentrations and extracts, particularly notable in its methanol

extract (Table 2). Even though *Boerhavia* exhibited high phenol and flavonoid content better than other vegetables, it doesn't reflect in its antioxidant potential. *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst. and *Sauropus androgynus* (L.) Merr displayed moderate to high antioxidant activity, with variations across different concentrations and extracts. Antioxidant activity positively correlated with phenolic and flavonoid content, indicating the significant contribution of these phytochemicals to the overall antioxidant potential of the leafy vegetables.

Table 2 DPPH activity in methanol and water extracts of four leafy vegetables

Sl no.	**Extract**	**Concentration (µg)**	**% of inhibition**			
			Cnidoscolus aconitifolius	***Boerhavia diffusa***	***Sauropus androgynus***	***Talinum fruticosum***
1.	Methanol	25	17.55	25.16	29.44	24.97
2.	Methanol	50	39.53	38.95	36.05	48.61
3.	Methanol	75	60.09	60.95	64.31	75.12
4.	Methanol	100	87.84	73.17	82.57	98.74
5.	Water	25	38.67	24.70	20.44	16.59
6.	Water	50	47.32	26.61	42.35	32.12
7.	Water	75	56.17	44.55	68.08	61.80
8.	Water	100	72.64	54.85	79.64	91.48

The presence of potential antioxidant bioactive compounds provides a good source of biologically active drug candidates and underscores its great importance as a therapeutic agent in preventing or curing diseases caused by oxidative stress (Bhardwaj *et al*., 2014). According to the present study, phenolic and flavonoid content was higher in methanol extract than in water extract, and consequently, the antioxidant activity was also higher in the methanol extract. Antioxidants may occur naturally in plants, animals, and microorganisms, or they may be synthesized by chemical means. Naturally occurring antioxidants can be isolated from their source material as pure compounds for possible use in nutraceutical/pharmaceutical applications (Shahidi & Zhong, 2015; Chanda & Dave, 2009).

4. Conclusion

This study provides valuable insights into the phytochemical composition and antioxidant properties of *Cnidoscolus aconitifolius* (Mill.) I.M. Johnst., *Boerhavia diffusa* Linn., S*auropus androgynus* (L.) Merr, and *Talinum fruticosum* (L.) Juss. *Boerhavia diffusa* Linn. and *Talinum fruticosum* (L.) Juss emerged as promising sources of phenolic compounds and flavonoids with potent antioxidant activity. Further research is warranted to explore the therapeutic potential and culinary applications of these leafy vegetables.

AUTHORS' CONTRIBUTIONS

Manju Madhavan designed the experiment and is involved in the interpretation of data. Ammu Girish carried out the experimental work, analysis of data, and draft manuscript preparation. Dalie Dominic A corrected the manuscript and helped in final draft preparation. All authors went through the final manuscript.

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