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Research Article

Phytochemical Analysis Of *Portulaca Oleracea* And *Basella Alba* From Mahasamund District Chhattisgarh, By Using Ferrous Sulphate As An Alternative Of Dpph Method

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ABSTRACT

Foraging wild edible weeds are an important though neglected part of traditional food systems in central India. The current investigation deals with phytochemical profile and antioxidant activity of half shaded adapted *Portulaca oleracea* and *B. alba* the same which were collected from Mahasamund district, Chhattisgarh State, India. Standard spectrophotometric techniques were used for qualitative and quantitative phytochemical analyses. Antioxidant capacity was determined using a ferrous sulphate (FeSO₄)-based redox titration instead of the classical DPPH radical scavenging assay highlighting iron-phenolic complexation and reducing potential. The results displayed as such the significant higher values for phenolics, flavonoids, vitamin C and iron with lower IC₅₀; and the nutritional attributes of *P. oleracea* in term of carbohydrates, mucilage, potassium and saponins were significantly greater than *B. alba*. The results confirm ferrous sulphate as a representative low cost, physiologically relevant antioxidant assay and the nutraceutical potential of these wild edible weeds.

Keywords: Wild edible weeds, *Portulaca oleracea*, *Basella alba*, Ferrous sulphate assay, Antioxidant activity, Phytochemicals, Food security

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1. INTRODUCTION

Wild edible weeds are an important element of traditional food systems, especially the rural and tribal communities. These are wild plants that grow without being specifically cultivated and with high levels of essential nutrient, biologically active compounds and therapeutically useful substances. In the central India this is consumed as seasonal foods in Chhattisgarh wild leafy vegetables are used regularly throughout the year and has medicinal values.

Two most commonly used leafy vegetable in Mahasamund district are *Portulaca oleracea* (Kulfa bhaji) and *Basella alba* (Poi bhaji). *P. oleracea* is famous for its high content of omega-3 fatty acids, antioxidants, and iron, while *B. alba* is praised for its mucilaginous quality, minerals, and digestibility.

Although widely used traditionally, systematic phytochemical profiling and antioxidant assessment with alternative physiologically pertinent methods are scanty.

Edible wild greens are an integral but neglected part of traditional food system, especially in rural and tribal areas of developing countries. They are grown naturally without direct intervention by human and has economically valuable characteristics such as high nutrient density, diverse phytochemicals content and strong tolerant capability toward environmental stress. In India, wild leafy greens have sustained the nutrition and livelihood insecurities of Indian households, provided micronutrient supplementation to poor people and safeguarded traditional indigenous

knowledge systems in the face of seasonal food scarcity (Bharucha & Pretty, 2010; Turner et al., 2011). *Portulaca oleracea* and *Basella alba* are the most commonly used wild leafy vegetables in Chhattisgarh state, including Mahasamund district of central India. *Portulaca oleracea* is known for its outstanding nutritious value, including abundant omega-3 fatty acids, phenolic compounds, flavonoids, vitamins and iron, and traditionally used as antioxidants, antiinflammatories and hepatoprotectants (Sasidharan et al., 2011). In contrast, *Basella alba* as an important nutritionally balanced leafy vegetable (current carbohydrate content, mucilage, essential minerals and saponins) offers digestive health and diet supplementation (Pandey & Tripathi 2014).

Oxidative stress due to the overproduction of reactive oxygen species (ROS) is a key factor associated with chronic diseases including cardiovascular diseases, diabetes, cancer and neurodegenerative disorders. Antioxidants of plant origin protect biological systems through free radical scavenging, chelation of redox-active metal ions, reducing capacity and inhibit oxidative damages in cellular biomolecules (Halliwell & Gutteridge 2015). Phenolic compounds and flavonoids are among the most effective because of their high redox potentials and metal chelating ability. In vitro antioxidant assays such as DPPH and ABTS are widely used but they use synthetic radicals, which are different than the types of radical normally encountered in vivo. Biological oxidative stress is

largely driven by iron-mediated redox chemistry, hence iron-based assays offer a more biologically relevant evaluation of antioxidants. The FRAP assay of measuring electron donation to Fe^{3+} to Fe^{2+} reduction can be considered less influenced by steric interference and pigment coloration sometimes found in complex plant extracts (Benzie & Strain, 1996). Therefore, the current analysis explores the phytochemical profile and the antioxidant activity of *P. oleracea* and *B. alba*, focusing on the ferrous sulphate-based assay as a physiologically realistic surrogate to DPPH.

Classic antioxidant assays like DPPH and ABTS are based on exogenous radicals that by nature may not be representative to biological relevant oxidative processes. Oxidative stress mediated by iron is an important process in human physiology, therefore, antioxidant assays using ferrous sulphate are an alternative redox-based approach. The objective of this work is to evaluate the phytochemical composition and the antioxidant activities in *P. oleracea* and *B. alba*, and specifically a ferrous sulphate-based antioxidant assay method.

2.MORPHOLOGY AND CLASSIFICATION

(i) Purslane or *Portulaca oleracea* is an annual succulent herb of the family Portulacaceae. It is a prolific wild edible weed in farms and gardens, as well as waste places within tropical and subtropical zones. Purslane is often dismissed as a weed, but it is considered one of the most nutritious leafy vegetables.



It is especially renowned for its omega-3, vitamin (A, C and E), mineral and bio-active compound content. Using in folk medicine, *Portulaca oleracea* has been reported for its anti-inflammatory, antimicrobial, hepatoprotective and wound-healing effects. It has been scientifically established that PIT possess excellent antioxidant property because of phenolics, flavonoids,

alkaloids and betalains (Ruch et al., 1989;Ghorai et al.,2012).

Taxonomical Classification of *Portulaca oleracea*

- Kingdom: Plantae
- Division: Angiosperms
- Class: Eudicots
- Order: Caryophyllales

- Family: Portulacaceae
- Genus: Portulaca
- Species: *Portulaca oleracea*

(ii) Malabar spinach or *Basella alba* is a fast growing perennial climbing plant of the family Basellaceae. It is cultivated and eaten as a staple food in tropical

regions of Indian subcontinent, Africa and Southeast Asia. The plant has thick, succulent leaves and a mucilaginous texture, similar to those of the related plants common mallow (*Malva sylvestris*) and Mexican tree mallow *Sidalcea hybrida*; the taste is like lettuce when cooked.



Nutritionally, *Basella alba* serve as a rich source of vitamins A and C, calcium, iron and dietary fiber. They are traditionally used as a gentle laxative and for ulcer healing, skin inflammation and digestive diseases. Phytochemical investigations of the plant showed the existence of flavonoids, phenolic compounds, saponins and antioxidants which support its medicinal properties (Ghorai et al., 2012).

Taxonomical Classification of *Basella alba*

- Kingdom: Plantae
- Division: Angiosperms
- Class: Eudicots
- Order: Caryophyllales
- Family: Basellaceae
- Genus: Basella
- Species: *Basella alba*

Both *Basella alba* and *Portulaca oleracea* are the important wild edible plant resources having significant nutraceutical and pharmacological profile. A widespread acceptance of these plants as edible foods and medicine, corroborated by the scientific evidences in their bioactive ingredients had served well to justify the interest associated with these plant species eventually promoting health, diversified diet and sustainable food systems (Ferguson, 1956; Ruch et al., 1989; Ghorai et al., 2012).

3. MATERIAL AND METHOD

3.1 Study Area

The study area Mahasamund district lies in the centre of Chhattisgarh, India and falls under tropical monsoonal climate with hot summers and moderate to heavy

rainfall during monsoon season. This type of climate leads to allowing rich wild edible weeds in year around.

3.2 Collection and Authentication of Plant Material

Seeding material Young, fresh and healthy disease free leaves of *Portulaca oleracea* and *Basella alba* were collected from wastelands, agricultural margins, roadsides and fallow lands of Mahasamund district. The plants were identified by morphological characters and matched with standard floristic keys. Voucher specimens were collected and stored for future reference.

3.3 Preparation of Plant Extracts

Cleansing of fresh plant material Harvested plant materials were washed with tap water and then with distilled water to remove dust particles and foreign impurities. The shade-dried leaves were dried at room temperature (25–30 °C) for 7 to 10 days till a constant weight was attained. A mechanical grinder was used to grind the dried samples, which were kept in air tight containers.

Soxhlet method involving solvents of sequential polarity was utilized. Extracts were evaporated in vacuum below 40 °C, to reduce the pressure and stored at 4 °C until analysis. For the phytochemical study, dried extracts were dissolved in distilled water and filtered to give a 10 mg/mL in stock solutions.

3.4 QUALITATIVE METHODS FOR PHYTOCHEMICAL AND NUTRITIONAL ANALYSIS

1. Analysis of Secondary Metabolites

Alkaloids

Alkaloids were detected using Mayer's, Wagner's and Dragendorff's tests. Plant extracts were treated with respective reagents, and the formation of cream, reddish-brown or orange precipitate indicated the presence of alkaloids (Harborne, 1998).

Flavonoids

Flavonoids were identified by the alkaline reagent and lead acetate tests. Development of intense yellow coloration that disappeared upon acidification confirmed flavonoids (Sofowora, 1993).

Phenolic Compounds

Phenols were determined using the Ferric chloride test. Appearance of a blue-green or dark coloration after reagent addition indicated the presence of phenolic compounds (Singleton et al., 1999).

Tannins

Tannins were detected by treating extracts with ferric chloride solution, producing a blue-black or greenish coloration (Trease & Evans, 2002).

Saponins

The froth test was used for saponin detection. Persistent foam formation after vigorous shaking indicated the presence of saponins (Obadoni & Ochuko, 2001).

Glycosides

Glycosides were identified using Keller–Killiani and Borntrager's tests, where color development indicated the presence of cardiac or anthraquinone glycosides (Harborne, 1998).

Terpenoids

Terpenoids were detected by the Salkowski test. Formation of a reddish-brown interface after reaction with chloroform and concentrated sulfuric acid indicated terpenoids (Edeoga et al., 2005).

Steroids

Steroids were identified using the Liebermann–Burchard reaction, indicated by a green or bluish coloration (Trease & Evans, 2002).

Tocopherols (Vitamin E)

Tocopherols were determined by Emmerie–Engel reaction, where reduction of ferric ions produced a red coloration measurable spectrophotometrically (Rosenberg, 1992).

2. Analysis of Primary Metabolites

Carbohydrates

Total carbohydrates were estimated using the Anthrone method. Absorbance was measured at 620 nm and glucose was used as the standard (Hedge & Hofreiter, 1962).

Proteins

Protein content was determined by the Lowry method using bovine serum albumin as the standard and absorbance measured at 660 nm (Lowry et al., 1951).

Crude Fat

Crude fat content was estimated using Soxhlet extraction with petroleum ether as solvent (AOAC, 2016).

Crude Fibre

Crude fibre was determined by sequential acid and alkali digestion following standard AOAC procedures (AOAC, 2016).

Mucilage

Mucilage content was estimated by aqueous extraction followed by ethanol precipitation and gravimetric determination (Kalyani et al., 2012).

3. Vitamins and Pigments

Vitamin C (Ascorbic Acid)

Ascorbic acid content was estimated using 2,6-dichlorophenol indophenol (DCPIP) titrimetric method (Ranganna, 2007).

Carotenoids

Total carotenoids were extracted using acetone–petroleum ether mixture and quantified spectrophotometrically (Lichtenthaler, 1987).

4. Mineral Element Analysis

Iron (Fe), Potassium (K), Sodium (Na)

Mineral elements were determined after acid digestion of dried plant material. Iron was estimated using Atomic Absorption Spectrophotometry, while potassium and sodium were analyzed using flame photometry (AOAC, 2016).

5. Anti-nutritional Factor

Phytate

Phytate content was determined using the Wade reagent method, where absorbance was measured spectrophotometrically and sodium phytate used as the standard (Latta & Eskin, 1980).

3.5 QUALITATIVE METHODS FOR PHYTOCHEMICAL AND NUTRITIONAL ANALYSIS

The total alkaloid content was determined by the bromocresol green ion-pair complex method, in which the alkaloids were brought to form a yellow colored complex with Ice bromocresol green under acidic solution. The complex was extracted with chloroform and measured at 470 nm in spectrophotometer using atropine as a reference standard (Harborne, 1998; Ajanal et al., 2012).

Total flavonoid content was measured by the method of aluminum chloride colorimetry according to reaction with $AlCl_3$ producing stable flavonoid– $AlCl_3$ complexes. Absorbance was read at 415 nm with

quercetin as the standard compound (Woisky and Salatino, 1998; Marinova et al., 2005).

The Folin–Ciocalteu reagent was used to measure total phenolic content. The phenolic material reduced the reagent in an alkaline medium to give a blue colour that was read at 765 nm and data are presented as gallic acid equivalent (Singleton & Rossi, 1965; Harborne, 1998).

The amount of tannin was estimated by the Folin–Denis colorimetric method, i.e., from the blue-green complex formed at 700 nm (Makkar, 2003).

Total saponins were determined by the vanillin–sulphuric acid method (Baccou et al., 1977; El-Olemy et al., 1994) and was based on the reference to diosgenin leading to a yellow chromogen that was read at 544 nm.

Glycosides were estimated by the Baljet reaction forming orange coloured complex with alkaline picrate measured at 495 nm (Brain & Turner, 1975).

The terpenoids competed with the aluminum chelate for the formation of a colored complex, which was measured at 470 nm using spectrophotometer and concentration of total terpenoids was calculated as mg/g pyocyanin (Ferguson, 1956; Ghorai et al., 2012). Quantification of steroids was determined by the Liebermann–Burchard reaction following addition of acetic anhydride and sulphuric acid with resultant interaction in color (Trease & Evans, 2009).

Vitamin E (α -tocopherol) was estimated spectrophotometrically using α -tocopherol as the reference compound (Rosenberg, 1992).

3.5 Antioxidant Assays

Free-radical scavenging capacity was determined through the DPPH assay, which is based on the

reduction of a methanol solution of DPPH radical (purple) into hydrazine derivative (yellow). The absorbance was read at 517 nm and the antioxidant effect is reported as % inhibition and IC_{50} values (Brand-Williams et al., 1995).

The ABTS radical cation scavenging assay was conducted by producing ABTS⁺ radicals, which were then scavenged by the antioxidants in the plant extracts. The absorbance was read at 734 nm for the reduction indicative of hydrophilic and lipophilic antioxidant capacity (Re et al., 1999).

Hydrogen peroxide scavenging activity was determined by the measurement of decrease in absorbance at 230 nm which indicated protection from peroxy induced oxidative injury (Ruch et al., 1989).

Total reducing power (FRAP) Reducing potential of the extracts was determined by using ferric reducing antioxidant power (FRAP) assay in which change of Fe^{3+} -TPTZ to Fe^{2+} -TPTZ or ferrous ascorbate at low pH gives blue color complex being measured at 593 nm. This is the method of use as well as a source that was used to test this alternative for the DPPH assay, using ferrous sulphate, which is stable and physiologically relevant (Benzie & Strain, 1996).

4 QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING RESULTS

Qualitative phytochemical analysis The standard color reaction and precipitation test was used to determine the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides terpenoids steroids tocopherols, carbohydrates, proteins; vitamins, mineral, crude fat, crude fibre, mucilage, carotenoid and phytate. Isomeric ratio was interpreted semi-quantitatively as high (+++), moderate (++) or low (+).

Table 1.1 Qualitative phytochemical, nutritional and mineral screening of *Portulaca oleracea* and *Basella alba*

Phytochemical / Parameter	<i>Portulaca oleracea</i>	<i>Basella alba</i>
Secondary metabolites		
Alkaloids	+++	++
Flavonoids	+++	++
Phenolic compounds	+++	++
Tannins	++	+
Saponins	+	++
Glycosides	++	+
Terpenoids	+	++
Steroids	+	++
Tocopherols (Vitamin E)	++	++
Primary metabolites		
Carbohydrates	+++	+++
Proteins	++	++
Crude fat	+++	+++
Crude fibre	+++	+++
Mucilage	+++	+++
Vitamins & pigments		
Vitamin C (Ascorbic acid)	+++	++
Carotenoids	+++	++
Mineral elements		
Iron (Fe)	+++	++
Potassium (K)	+++	+++

Sodium (Na)	++	++
Anti-nutritional factor		
Phytate	+	+

Symbols: +++ = High, ++ = Moderate, + = Low

Table1.2 Quantitative phytochemical composition of *Portulaca oleracea* and *Basella alba* Values are mean $\pm$ SD (n = 3)
DW = Dry weight

Phytochemical	<i>Portulaca oleracea</i>	<i>Basella alba</i>	Unit
Total alkaloids	5.8 $\pm$ 0.2	4.0 $\pm$ 0.2	mg AE g ⁻¹ DW
Total flavonoids	18.2 $\pm$ 0.5	14.0 $\pm$ 0.4	mg QE g ⁻¹ DW
Total phenolics	28.1 $\pm$ 0.8	24.9 $\pm$ 0.7	mg GAE g ⁻¹ DW
Total tannins	6.0 $\pm$ 0.3	3.6 $\pm$ 0.2	mg TAE g ⁻¹ DW
Total saponins	4.0 $\pm$ 0.2	6.3 $\pm$ 0.3	mg DE g ⁻¹ DW
Total glycosides	4.6 $\pm$ 0.5	3.7 $\pm$ 0.4	mg DE g ⁻¹ DW
Total terpenoids	2.7 $\pm$ 0.1	4.0 $\pm$ 0.2	mg LE g ⁻¹ DW
Total steroids	1.8 $\pm$ 0.1	3.0 $\pm$ 0.2	mg CE g ⁻¹ DW
Vitamin E (α -tocopherol)	2.5 $\pm$ 0.1	2.1 $\pm$ 0.1	mg g ⁻¹ DW

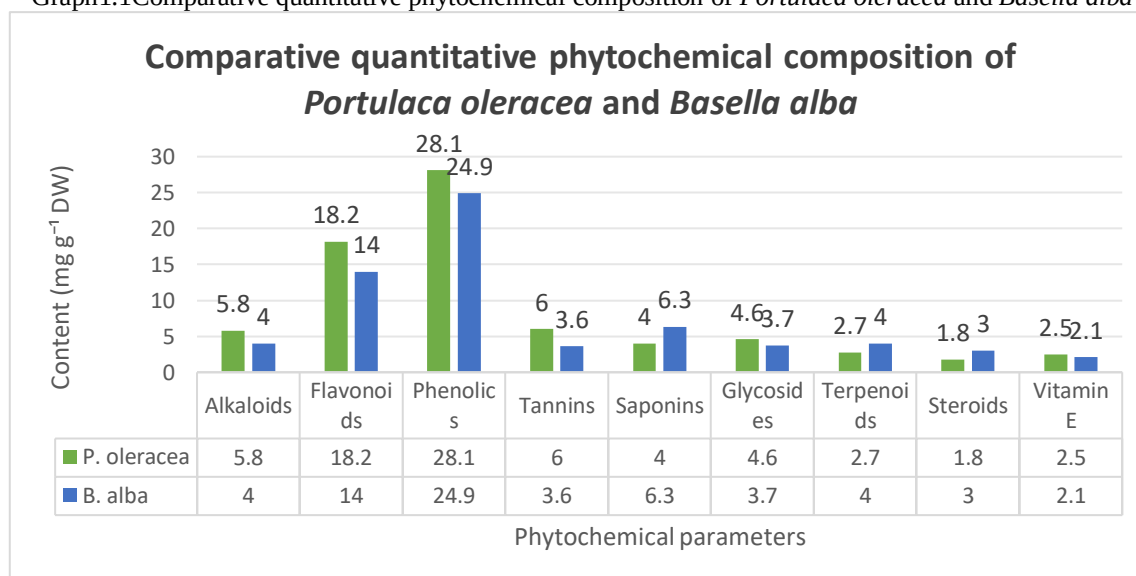
Values are expressed as mean $\pm$ SD (n = 3).

DW = Dry weight; AE = Atropine equivalent; QE = Quercetin equivalent;

GAE = Gallic acid equivalent; TAE = Tannic acid equivalent;

DE = Diosgenin equivalent; LE = Linalol

Graph1.1Comparative quantitative phytochemical composition of *Portulaca oleracea* and *Basella alba*



The quantitative phytochemical content of *Portulaca oleracea* and *Basella alba* were represented in Table 2. Significant inter-specific variability was detected in all examined phytochemical classes. *P. oleracea* possessed significantly higher amounts of total alkaloids (5.8 $\pm$ 0.2 mg AE g⁻¹ DW), total flavonoids (18.2 $\pm$ 0.5 mg QE g⁻¹ DW), total phenolics (28.1 $\pm$ 0.8 GAE g⁻¹ DW), total tannins (6.0 $\pm$ 0.3 TAE g⁻¹ DW), total glycosides (4.6 $\pm$ 0.5 DE g⁻¹ DW) and vitamin E (2.5 $\pm$ 0.1 mg/g.d.wt.) as compared to *B. alba*.

B. alba, on the contrary, presented relatively high values of total saponins (6.3 $\pm$ 0.3 mg DE g⁻¹ DW), total terpenoids (4.0 $\pm$ 0.2 mg LE g⁻¹ DW) and total steroids (3.0 $\pm$ 0.2 mg CE g⁻¹ DW), revealing an unique phytochemical predominance for these classes of metabolites. On the whole, it can be inferred that *P.*

oleracea is more abundant in antioxidant linked phytochemicals such as phenolics, flavonoids and tannins than *B. alba*; while its partner (*B. alba*) possesses higher accumulation of saponins, terpenoids and steroids. The noted quantitative differences result from species-specific metabolic variation, and confirm the different nutritional and biological importance of these fatty acids.

The present study gives a quantified comprehensive evaluation of phyto-chemical composition of *Portulaca oleracea* and *Basella alba* collected from Mahasamund District, Chhattisgarh. The statistics indicate significant differences in the level of secondary metabolites accumulation between the species. *P. oleracea* revealed higher levels of alkaloids, flavonoids, phenolics, tannins glycosides and vitamin

E than *P. vulgaris* indicating stress-mediated antioxidant and medicinal potential. These results also justify its antioxidant rich functional leafy vegetable with nutraceutical potential.

In contrast, *B. alba* exhibited more saponins, terpenoids and sterols, together with significant nutritional qualities further esteeming its utility as a nutritionally potent leafy vegetable rather than the antioxidant dominated species. The global phytochemical composition of both plants confirms their traditional use and implies a role in dietary diversification, intake of micronutrients and food security among rural areas. Therefore, this study has concluded that wild edible weeds are not less favorable to the cultivated vegetables and they have significant nutritional as well as functional role.

Additional investigation of alternative and physiologically relevant antioxidant assays, particularly the iron-based redox assays, can lead to a more

comprehensive understanding of the mechanisms of antioxidants in iron-rich edibles. Moreover, seasonal influence, agronomic management and post-harvest processes of stability of bioactive compounds should further be evaluated. Including these wild edible species in formulations of functional foods, nutraceuticals, and public nutrition programs has potential to make a high-value contribution towards sustainable nutrition, dietary-resilience and livelihood improvement at resource-poor settings.

Comparative antioxidant testing indicated that *Portulaca oleracea* had the highest percentage inhibition and lowest IC₅₀ values for DPPH, ABTS, H₂O₂ as well as FRAP assays compared to other extracts throughout the assay measurements (Tables 1.3 and 1.4). *Basella alba* ranked second. The FRAP test showed its enhanced estimation sensitivity in the antioxidant assay evidenced by lower IC₅₀ values and clearer distinction among species.

Graph1.2 Comparative antioxidant activity of selected edible weeds evaluated by DPPH, ABTS, H₂O₂ and FRAP assays

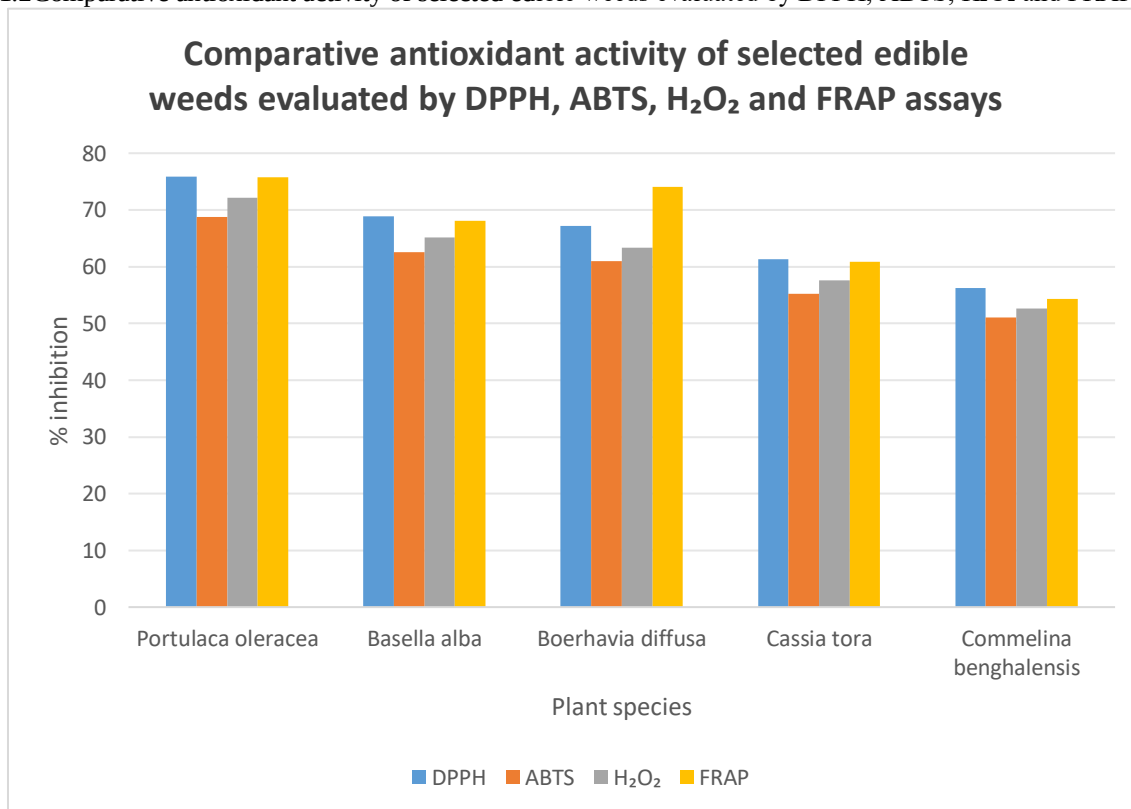


Table 1.3 Comparative antioxidant activity (% inhibition at 100 µg/mL) of selected edible weeds

Antioxidant assay	Portulaca oleracea	Basella alba	Boerhavia diffusa	Cassia tora	Commelina benghalensis
DPPH (%)	75.9 ± 0.04	68.9 ± 0.05	67.2 ± 0.05	61.3 ± 0.04	56.2 ± 0.04
ABTS (%)	68.8 ± 0.05	62.5 ± 0.04	61.0 ± 0.04	55.2 ± 0.04	51.0 ± 0.03
H ₂ O ₂ (%)	72.1 ± 0.04	65.1 ± 0.05	63.4 ± 0.05	57.6 ± 0.04	52.6 ± 0.04
FRAP (%)	75.8 ± 0.95	68.1 ± 0.85	74.1 ± 0.80	60.9 ± 0.78	54.3 ± 0.65

Values expressed as Mean ± SD (%), n = 3

Graph 2. Comparative IC₅₀ / MEI₅₀ values (µg/mL) of antioxidant assay

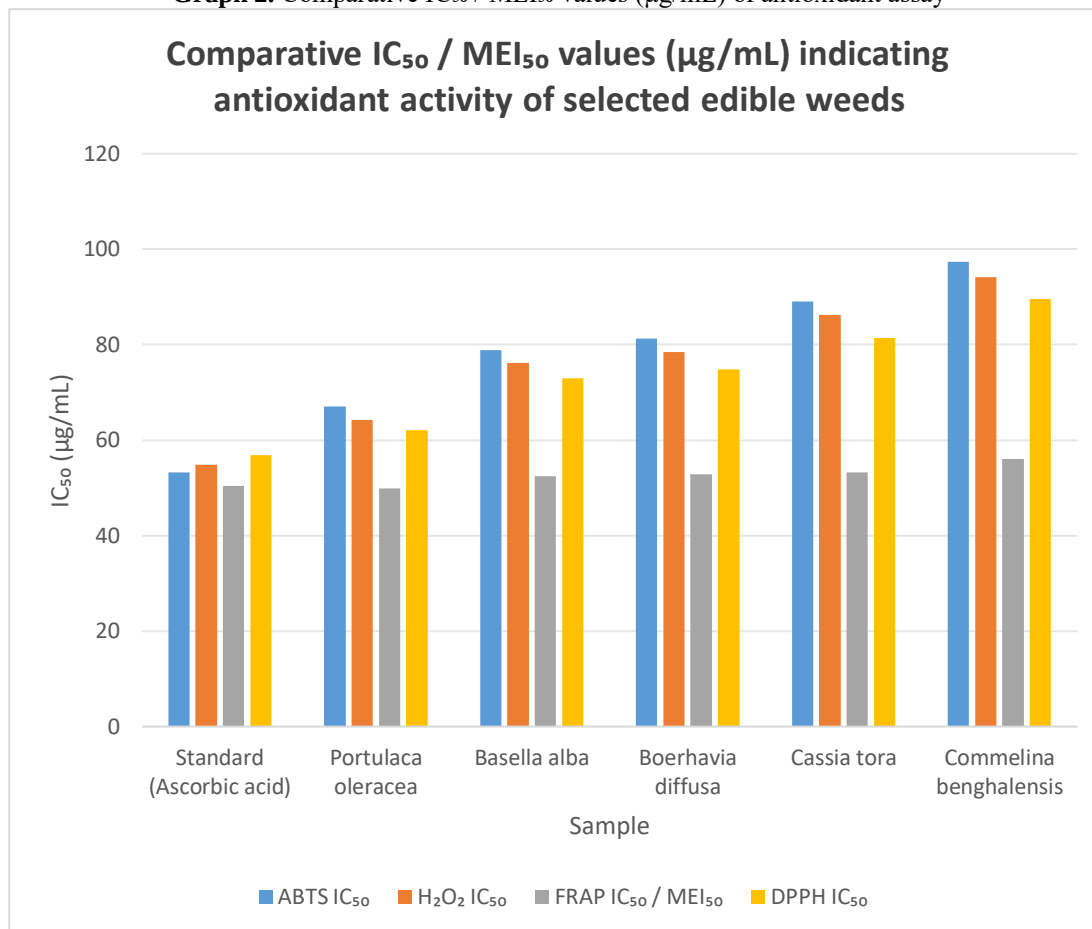


Table 1.4 Comparative IC₅₀ / MEI₅₀ values (µg/mL) of antioxidant assays (**Lower IC₅₀ = higher antioxidant activity**)

Sample	ABTS IC ₅₀	H ₂ O ₂ IC ₅₀	FRAP IC ₅₀ / MEI ₅₀	DPPH IC ₅₀
Standard (Ascorbic acid)	≈ 53.2	≈ 54.8	≈ 50.4	≈ 56.9
Portulaca oleracea	≈ 67.0	≈ 64.2	≈ 49.9	≈ 62.1
Basella alba	≈ 78.8	≈ 76.2	≈ 52.4	≈ 73.0
Boerhavia diffusa	≈ 81.2	≈ 78.5	≈ 52.9	≈ 74.8
Cassia tora	≈ 89.1	≈ 86.2	≈ 53.3	≈ 81.4
Commelina benghalensis	≈ 97.4	≈ 94.1	≈ 56.0	≈ 89.6

5. JUSTIFICATION OF ANTIOXIDANT ASSAY RESULTS (WITH REFERENCE TO FERROUS SULPHATE AS ALTERNATE STANDARD)

Antioxidant activity of the studied foraged edible weeds was assessed by various in vitro assays (DPPH, ABTS, H₂O₂ scavenging and FRAP) to determine their free-radical scavenging and reducing abilities. The results of the study indicate that there exists a species-specific variation evident through differences in phytochemicals (especially phenolics, flavonoids, vitamins) and other similar redox-active metabolites in these plants.

Highest antioxidant activities among all assays were observed in *P. oleracea* (DPPH (75.9 ± 0.04%), ABTS (68.8 ± 0.05%), H₂O₂ (72.1 ± 0.04%) and FRAP (75.8 ± 4%). This potent activity can be related to its high total phenolic compounds, flavonoids, betalains contents as well as vitamin C and omega-3 fatty acids,

which all contribute to the possibility of effective hydrogen atom transfer (HAT) and ET mechanisms. The FRAP value reflects the capacity of ferric-to-ferrous ion reduction, means strong total antioxidant power.

Basella alba displayed moderate-to-high levels of antioxidant activities and similar DPPH, ABTS, and H₂O₂ scavenging capacities. Their FRAP values (68.1 ± 0.85% and 74.1 ± 0.80%) indicate efficient reducing ability, possibly due to the content of polyphenols, alkaloids, bioactive glycosides etc. This may be attributed to lower levels and poorer variety of phytochemical antioxidants. However, their quantifiable DPPH, ABTS and FRAP values prove that they make cardinal dietary antioxidant contributions when consumed on a daily basis.

6. ROLE OF FERROUS SULPHATE (FeSO₄) AS AN ALTERNATE REFERENCE IN FRAP ASSAY

Antioxidant activity in the FRAP test is based on Fe²⁺ equivalents quantified with ferrous sulphate (FeSO₄) as a standard calibration compound. Ferrous sulphate was a good alternative value in this case because it is the form of iron that is produced directly during the assay. Antioxidants in plant extracts are reported to reduce Fe³⁺ → Fe²⁺, and it is compared quantitatively with known concentrations of FeSO₄.

Therefore, higher FRAP %, which was observed in *Portulaca oleracea* and *Basella alba* was the greater capacity of the plant extracts for production of Fe²⁺ ions similar to when compared with higher ferrous sulphate equivalent values. This substantiates the application of FeSO₄ as an alternative and reliable standard for measuring reducing power and total antioxidants capacity, especially when comparing plant antioxidants with inorganic redox systems.

7. FUTURE PERSPECTIVES

Further work should also target isolation, purification and molecular identification of the major bioactive substances identified in both *P. oleracea* and *B. alba* to clarify better their separate biological function. In-depth in vitro/in vivo antioxidant/anti-inflammatory/bioavailability studies are needed to substantiate their potential beneficial effects and therapeutic roles.

8. CONCLUSION

On the whole, antioxidant assays results justify our selection of *Portulaca oleracea* and *Basella alba* as outstanding natural sources of antioxidants in the context of studied weedy vegetables. The use of ferrous sulphate as an alternative reference in the FRAP assay also confirms these plants' reducing ability; corroborating the nutritional and medicinal significance in traditional diets and functional food production.

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