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# Comparative antioxidant profiling and mineral estimation of *Portulaca oleracea* L. and *Portulaca quadrifida* L.

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**Abstract**
The present study aims to assess the antioxidant potential and mineral estimation of the two important conventional medicinal herbs namely *Portulaca oleracea L. and Portulaca quadrifida* L. that are considered to be the richest in having omega-3 and omega-6 fatty acids. In the two crude extracts of aqua-methnol and aqua-acetone, protein denaturation inhibition (%) of *P. oleracea* was almost equal (97.09±0.51) to that of Diclofenac sodium (98.41±0.49) in aqua-methanol. The $IC_{50}$ (µg/ml) of DPPH and ferrous chelation were maximum (37.34 and 54.98, respectively) in aqua-methanol for *P. oleracea*. FRAP (µg TE/mg extract) was maximum in aqua-methanol for both species. The TAA (µg AAE/mg extract) was higher (52.12±0.38) in aqua-methanol extract of *P. oleracea*; while the TAA in aqua-acetone was almost equal in *P. oleracea* (30.60±0.34) and *P. quadrifida* (28.31±0.99). The TPC (µg GAE/mg extract) was highest (80.52±2.69) in *P. oleracea* aqua-methanol extract. The total flavonoid content (µg QE/mg extract) was higher in aqua-methanol extract (7.23±0.09) of *P. oleracea* and aqua-acetone extract (6.58±1.22) of *P. quadrifida*. Both the plant species exhibited mineral content under permissible limit. The present investigation may help in guiding the future pharmacological testing of reference *Portulaca* sp. against inflammation and harmful reactive oxidants related diseases.

**Keywords:** Anti-inflammatory, antioxidants, minerals, phytochemicals, Portulaca sp.

## 1. Introduction
Plants are being routinely practiced by humans as medicines for 60,000 years and since early 1900s, plants (root, bark and leaf) alone are bestowing the world with about 80% medicines. Each and every plant cell nurtures remedial legacy in the form of secondary metabolites (by-products) because of having unique enzyme machinery which requires no cellular-reducing equivalent. These metabolites improve competition with adjacent environmental organisms as well as abiotic stresses by acting as antioxidants, which relieve many inflammation-related problems as they work at molecular level in every cell to suppress inflammation related genes (Kliebenstein, 2004; McChesney *et al.*, 2007; Sharma *et al.*, 2012; Yuan *et al.*, 2016) [27, 32, 46, 55, 46]. *The Portulacaceae members Portulaca oleracea* L. (purslane or Global Panacea) and *Portulaca quadrifida* L. (chickweed) are annual grasses having fleshy stem, succulent leaves and yellow flowers with $C_4$ type of photosynthesis optimization. Both plant species are worldwide distributed in tropical and subtropical areas and categorized as invasive weeds. Pharmacologically, the species help in mitigating several ailments such as inflammations, microbial diseases, different ulcers, cough, arthritis, etc. (PROTA, 2014; Uddin *et al.*, 2014; Durgawale *et al.*, 2018; Rahimi *et al.*, 2019) [39, 50, 11, 40]. Both plant species undoubtedly own the unique trait of soothing different ailments due to their pure wild genetic machinery, which helps to withstand against adverse environmental conditions. Several diseases of liver, stomach, cough, intestine, diarrhea, eczema, arthritis and dysentery are cured by *P. oleracea*, which contains the highest ω-3 fatty acid (α-linolenic acid) than any plant worldwide. Medicinally, *P. quadrifida* has been reported to cure ulcer, asthma, cough, hemorrhoids, urinal infections with antifungal activities against *Candida albicans* and *Aspergillus fumigates* (Saxena and Rao, 2021) [44].
Although, several previous investigations have been done on these plant species, however, their comparative evaluation up to following diverse assays is not accomplished together. In the present study, an attempt is made to evaluate the anti-inflammatory and antioxidant potential of *P. oleracea* and *P. quadrifida* along with mineral elements as these plant species are sometimes misjudged from being effective remedy because of their invasiveness.

## 2. Materials and Methods

### 2.1 Collection and Identification

Fresh and mature leaves were collected from campus (29° 1' 29.07" N and 79° 29' 23.16" E) of Pantnagar, Uttarakhand. Identification and authentication of reference plant species were done with the help of eFloraIndia, eFlora Pantnagar (EfloraIndia, 2016; Eflora Pantnagar, 2015) [12, 13] and Dr. D. S. Rawat, Department of Biological Sciences, College of Basic Sciences and Humanities, G.B.P.U.A.&T., Pantnagar, U.S. Nagar, Uttarakhand, India.

### 2.2 Extraction Procedure

Fresh leaf stock washed (with tap and distilled water), shade dried, mechanically powdered and extracted (1:10 w/v) in aqua-methanol and aqua-acetone (20:80 v/v) for 10 days in orbital shaker (at 150 rpm) at 30 ºC for maximum antioxidant extraction. The filtered extracts in Whatmann No. 1 were centrifuged at 12000 rpm and 4 ºC temperature for 15 min for obtaining clear supernatant. The supernatants were evaporated under water bath at 40 ºC, yielded and stored at 4 ºC temperature for further experimental activities.

### 2.3 Chemicals Required

All the chemicals used namely DPPH (2,2'-diphenyl-1-picrylhydrazyl), diclofenac sodium. Sodium chloride, potassium chloride, sodium hypophosphate, BHT (butylated hydroxytoluene), $Na_2EDTA$ (disodium ethylenediaminetetraacetic acid), ferrozine, TPTZ (2,4,6-tris(2-pyridyl)-1,3,5- triazine), ferrous chloride tetrahydrate, trolox, sodium carbonate, hydrochloric acid, Folin-Ciocalteu reagent, gallic acid, aluminium chloride, quercetin, sulphuric acid, ammonium molybdate, sodium phosphate, ascorbic acid, sodium hydroxide, copper sulfate, sodium potassium tartarate, potassium phosphate buffer, sodium phosphate buffer, BSA (bovine serum albumin), methionine, NBT (nitro blue tetrazolium), riboflavin, pyrogallol, hydrogen peroxide, EDTA (ethylenediaminetetraacetic acid) and nitric acid were of analytical grade belonging to Sigma-Aldrich, Germany; Hi-Media, India; Merck, Darmstadt, Germany; and Ranbaxy, India.

### 2.4 Phytochemical Analysis

Phytochemicals (carbohydrates, proteins, flavonoids, phenols, tannins, alkaloids, quinines, steroids, terpenoids, cardiac glycosides and saponins) were analyzed *via* standardized procedures (Brain and Turner, 1975; Evans, 1996) [8, 15].

### 2.5 Anti-inflammatory Potential by Albumin-Denaturation Assay

Albumin-denaturation analysis was done by using Sakat *et al.* 2010 [42] and Ngoua-Meye-Misso *et al.* 2018 [37] methodologies with minor modifications based decreased protein turbidity (denatured protein) with increasing concentration. The action mixtures of 1 ml Diclofenac sodium (standard drug) or plant extracts (20, 40, 60, 80 and 100 µl), 1.9 ml phosphate buffer saline (8 g NaCl, 0.2 g KCl and 1.44 g $Na_2HPO_4$ in 6.4 pH distilled water) and 0.1 ml fresh hen's egg albumin were incubated (at 37 ºC) for 20 min by slowly increasing the temperature (up to 70 ºC) for 5 min. The absorbance of differently turbid reaction mixtures was measured (at 660 nm) and values were expressed by following formula:

Denaturation inhibition (%) = [1-(At/Ac)*100]

Where, At and Ac are absorbance of sample and control at 660 nm, respectively. Both the aqua-methanol and aqua-acetone solvents (3 ml) were used as blank.

### 2.6 Antioxidant Evaluation

#### 2.6.1 2,2'-diphenyl-1-picrylhydrazyl (DPPH•) Scavenging Assay

The DPPH radical scavenging activity (%) was investigated by using Brand-Williams *et al.* 1995 [9] procedure with slight modifications. About 1 ml BHT (standard) or plant extracts (20, 40, 60, 80 and 100 µl) with 3 ml solution of DPPH (0.004%) was reacted for 60 min. The absorbance (at 517 nm) of resultant yellow product was taken and values were calculated by using following formula:

Scavenging activity (%) = [1-(At/Ac)*100]

Where, At and Ac are absorbance of sample and control at 517 nm, respectively.

The DPPH $IC_{50}$ (50% inhibitory concentration) values were calculated by plotting scavenging effect on ordinate against plant extracts concentration on abscissa.

2.6.2 $Fe^{2+}$ (ferrous) Chelating Activity (FCA)

The chelating activity was performed by Hsu *et al.* 2003 [21] method based on the ability of plant extracts to destabilize $Fe^{2+}$ ion-ferrozine complex. The reaction of 1 ml $Na_2EDTA$ (standard) or plant extracts (20, 40, 60, 80 and 100 µl) with 0.1 ml $FeCl_2.4H_2O$ (2 mM) and 0.2 ml ferrozine (5 mM) was performed for 10 min by making total volume of 5 ml with respective solvents (aqua-methanol and aqua-acetone). The absorbance (at 562 nm) of reduced red color intensity was measured and the values were calculated with following formula:

Chelating activity (%) = [1-(At/Ac)*100]

Where, At and Ac are absorbance of sample and control at 562 nm, respectively.

The FCA $IC_{50}$ (50% inhibitory concentration) values were calculated by plotting chelating activity on ordinate against plant extracts concentration on abscissa.

#### 2.6.3 Ferric ($Fe^{3+}$) Reducing Antioxidant Power (FRAP) Assay

The ferric reducing power of the plant extract was analyzed by using Benzie and Strain, 1996 [5] method with suitable modifications. Freshly prepared FRAP reagent (300 mM sodium buffer, pH 3.6; 10 mM TPTZ in 40 mM HCl; and 20 mM ferric chloride solution in 10:1:1 ratio was incubated (at 37 ºC) before using and reacted with 1 ml plant extracts (20, 40, 60, 80 and 100 µl) for 30 min (at 37 ºC). The absorbance of resultant blue colored product ($Fe^{2+}$ TPTZ complex) was measured at 593 nm and values were expressed as µg trolox equivalents (TE) per mg of extract.

#### 2.6.4 Phosphomolybdenum Assay

The total antioxidant activity (TAA) was analyzed *via* phosphomolybdenum method of Prieto *et al.* 1999 [38], based on reduction of molybdenum (VI) to molybdenum (V). The reaction of 1 ml plant extracts (20, 40, 60, 80 and 100 µl) with

3 ml reagent (0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate) was performed for 90 min in water bath (at 95 ºC) and absorbance of resultant green product was measured at 695 nm. The values were expressed as µg ascorbic acid equivalent (AAE) per mg of extract.

### 2.7 Total Phenolic Content (TPC)
The total phenol content was evaluated by using Folin-Ciocalteu colorometric method of Wolfe *et al.* 2003 [53] with slight modifications. About 0.5 ml plant extracts (20, 40, 60, 80 and 100 µl) were reacted with 0.2 ml Folin-Ciocalteu reagent for 5 min and alkalized with 7% saturated $Na_2CO_3$. The absorbance of blue product was measured (at 765 nm) after 60 min incubation and values were expressed as µg gallic acid equivalents (GAE) per mg of extract.

### 2.8 Total Flavonoid Content (TFC)
The total flavonoid content was analyzed by using aluminium chloride colorometric method of Djeridane *et al.*, 2006 [10] with suitable modifications. The plant extracts (100 µg/ml conc) were reacted with 2% $AlCl_3$ (1:1 v/v) for 60 min and absorbance of yellow product was measured (at 420 nm) and the values were expressed as µg quercetin equivalents (QE) per mg of extract.

### 2.9 Total Protein Content
The total protein of plant samples was quantified by using Lowry *et al.* 1951 [30] procedure based on cupric ions and peptide nitrogen reaction followed by Folins-Ciocalteu phosphomolybdic and phosphotungstic acid reduction into heteromolybdenum (blue coloured product) due to aromatic acids oxidation *via* copper catalysis. The protein was extracted by homogenizing 500 mg fresh leaves in chilled 5 ml potassium phosphate buffer (pH 7.0), centrifuged (15,000 rpm) for 20 min. The reaction of 1 ml plant protein with 4.5 ml reagent A (2% $Na_2CO_3$ in 0.1 N NaOH; and 0.5% $CuSO_4$ in 1% sodium potassium tartarate, 1:1 v/v) for 10 min by further incubation with reagent B (Folin-Ciocalteu in distilled water, 1:1 v/v) for 30 min. The absorbance of resultant blue product was measured at 660 nm and protein values were expressed as µg bovine serum albumin (BSA) equivalent/mg of extract.

### 2.10 Enzymatic Antioxidant Analysis
#### 2.10.1 Superoxide Dismutase (SOD) Activity
The superoxide dismutase activity was evaluated *via* photochemical method proposed by Giannopolitis and Ries, 1977 [19] and values were expressed as unit (U) per mg fresh weight (FW). The enzyme extraction of 1 g fresh leaves was done in 4 ml chilled extraction buffer (100 mM potassium phosphate buffer, pH 7.0 and 0.1 mM EDTA) and centrifuged at 15000 rpm and 4 ºC for 15 min. The 100 µl plant enzyme was admixed with 3 ml reaction mixture (50 mM phosphate buffer, pH 7.8; 0.1 µM EDTA; 13 mM methionine; 75 µM NBT; and 2 µM riboflavin) and illuminated (30 min) along with control (illuminated mixture excluding enzyme) against blank (non-illuminated mixture) and absorbance was read at 560 nm. One unit (1 U) of SOD required for photo-reduction of 50% NBT was calculated by using following formula:

$$Z = [(X-A)/X]*100$$

Where,
Z= photo-reduction % in sample;
X= absorbance of control;
A= absorbance of plant enzyme; and
Z/50= total SOD unit.

#### 2.10.2 Peroxidase (POD) Assay
Peroxidase activity was assayed by using Kar and Mishra 1975 [24] and Reddy *et al.*, 1985 [41] procedures with minor modifications. The fresh enzyme was extracted by homogenizing 1 g mature leaves with chilled 125 µM potassium phosphate buffer (pH 6.8) in ratio of 1:8 w/v and centrifuged at 12000 rpm and 4 ºC for 20 min. About 100 µl plant enzyme was reacted with 3 ml pyrogallol (1 mM) and 0.5 ml $H_2O_2$ (1%) was further added to measure the absorbance change (at 470 nm) against blank (reaction mixture without $H_2O_2$) for 1 min (at 10 sec intervals). The reaction was prevented by adding 1 ml $H_2SO_4$ (5%) and POD values were expressed as nmol/mg of protein.

#### 2.10.3 Catalase (CAT) Activity
The catalase activity was assayed by Kar and Mishra, 1976 [25] methodology with slight modifications. Fresh enzyme was extracted by homogenizing 1 g mature leaves in chilled 100 mM sodium phosphate buffer (6.8 pH) in ratio of 1:8 w/v and centrifuged at 12,000 rpm and 4 ºC for 20 min. Approximately, 0.1 ml plant enzyme was reacted with 2 ml reaction mixture (200 mM potassium phosphate buffer, pH 7.0; 50 mM $H_2O_2$ and 0.1 mM EDTA) and absorbance was read (at 240 nm) at 10 sec intervals for 1 min. The CAT values were expressed as nmol/min/mg of protein.

### 2.11 Atomic Absorption Spectrophotometric (AAS) Analysis for Mineral Estimation
About 40 mg plant powder was reacted with 6 ml mixture of $HClO_4$ and $HNO_3$ (1:5 v/v) in open (for 2 hrs) followed by addition of $HNO_3$ and HCl (1:1 v/v) for complete digestion (for 4 hrs) at 300 ºC on hot plate indicated by resultant colorless liquid. Further, digested and dried plant samples were mixed with 5 ml deionized water for AAS analysis.

### 2.12 Statistical Analysis
The data (in triplicates) were represented as mean±S.D. along with two way-analysis of variance applied with help of STPR to investigate the anti-inflammatory, antioxidant and phytochemical potential of both plant extracts. The significant differences ($p$<0.05) among all means were calculated using Duncan's multiple range test (DMRT) *via* SPSS version 16.0. The Pearson's correlation within varied parameters of both plant species was established by using SPSS version 16.0.

## 3. Results
### 3.1 Phytochemicals and Yield
Aqua-methanol extracts of both plant species exhibited the presence of all phytochemicals along with higher quantity of alkaloids and steroids in *P. oleracea* and steroids in *P. quadrifida*. In aqua-acetone, almost all phytochemicals were present except tannins, steroids and cardiac glycosides in *P. oleracea* and tannins and steroids in *P. quadrifida* (Table 1). However, yield and phytochemicals are much higher in aqua-methanol as compared to aqua-acetone.

**Table 1:** Qualitative analysis of phytochemicals in aqua-methanol and aqua-acetone leaf extracts of *P. oleracea* and *P. quadrifida*.

| Phytochemicals/Yield | Plant Species | | | |
|---|---|---|---|---|
| | *Portulaca oleracea* | | *Portulaca quadrifida* | |
| | Aqua-methanol | Aqua-acetone | Aqua-methanol | Aqua-acetone |
| Carbohydrates | + | + | + | + |
| Proteins | + | + | + | + |
| Falvonoids | + | + | + | + |
| Phenols | + | + | + | + |
| Tannins | + | - | + | - |
| Alkaloids | ++ | + | + | + |
| Quinones | + | + | + | + |
| Steroids | ++ | - | ++ | + |
| Terpenoids | + | + | + | + |
| Cardiac glycosides | + | - | + | - |
| Saponins | + | + | + | + |
| Yield (%) (w/w) | $11.54\pm1.01^{c}$ | $10.48\pm0.86^{b}$ | $10.06\pm0.78^{b}$ | $8.87\pm0.33^{a}$ |

(+) present, (++) more present and (-) absent. Superscripted alphabets indicate significant range differences ($p<0.05$) among yield (%) values.

### 3.2 Albumin-denaturation Inhibition

The albumin-denaturation inhibition (%) in both leaf extracts showed dose dependent relationship with significant differences ($p<0.05$) (Figure 1). The protein (albumin) denaturation inhibition (%) at highest concentration (100 µg/ml) was 97.09±0.51, 85.34±0.67 and 98.41±0.49 in aqua-methanol; while 94.60±0.95, 72.80±1.24 and 97.04±0.91 in aqua-acetone for *P. oleracea*, *P. quadrifida* and diclofenac sodium (standard), respectively. In both aqua-methanol and aqua-acetone extracts, *P. oleracea* exhibited the protein-denaturation inhibition very close to that of standard. The albumin-denaturation inhibition potential (%) assessed by Shettar *et al.*, 2015 [47] in *Ximenia americana* leaf methanol extract (77.51±2.25) is higher; while, by Naz *et al.*, 2017 [36] in *Tribulus terrestris* leaf methanol extract (63.6±1.3) and by Garbi *et al.*, 2017 [18] in *Hibiscus sabdariffa* flower methanol extract (18.62) was lower than present study values.

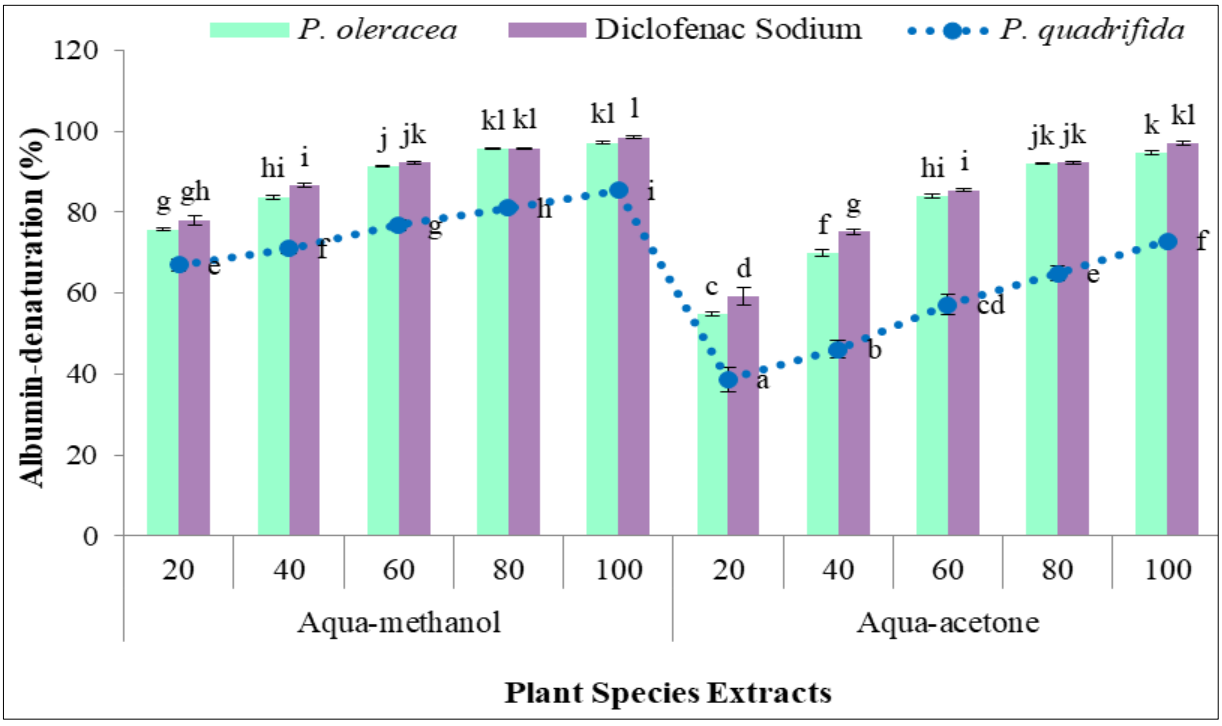


**Fig 1:** Albumin-denaturation inhibition (%) in aqua-methanol and aqua-acetone extracts of *P. oleracea* and *P. quadrifida* at different concentrations. Superscripted alphabets indicate significant range differences ($p<0.05$).

### 3.3 Antioxidant activity

**3.3.1 DPPH Inhibition:** The DPPH inhibition (%) in both extracts increased with increasing concentration in both species (Figure 2) with significant differences ($p<0.05$). The DPPH inhibition (%) at highest concentration (100 µg/ml) was 73.98±4.19 and 70.06±1.37 in aqua-methanol; while 63.48±4.19 and 59.57±1.37 in aqua-acetone for *P. oleracea* and *P. quadrifida*, respectively. The $IC_{50}$ (µg/ml) were 37.34 and 57.11 in aqua-methanol; while 65.50 and 78.98 in aqua-acetone for *P. oleracea* and *P. quadrifida*, respectively. Whereas, for BHT, DPPH inhibition (%) was 96.75±1.00 at similar concentration and $IC_{50}$ (µg/ml) was 1.21. The DPPH inhibition (%) reported by Basma *et al.*, 2011 [4] in *Euphorbia hirta* leaf methanol extract (72.96±0.78); and by Ashafa *et al.*, 2010 [3] in *Felicia muricata* leaf extract (13.81% at 0.1 mg/ml conc.) are lower, while; by Mathappan and Sanjay, 2013 [31] in *Urena lobata* whole plant methanol extract (93.47 at 100 µg/ml conc.) is maximum than the present study values.

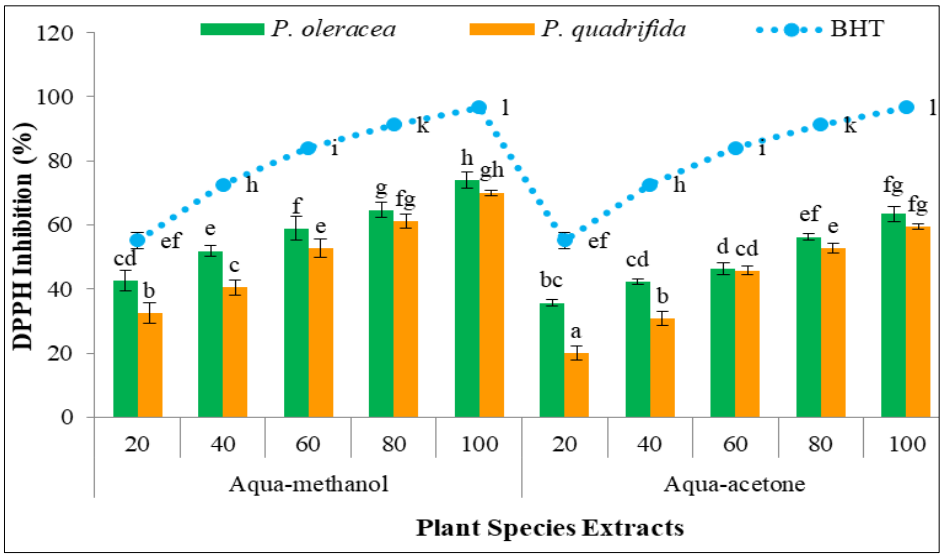


**Fig 2:** DPPH inhibition (%) in aqua-methanol and aqua-acetone extracts of *P. oleracea* and *P. quadrifida* at different concentrations. Superscripted alphabets indicate significant range differences ($p$<0.05).

### 3.3.2 Ferrous Ion Chelation

The $Fe^{2+}$ chelation activity (%) in both aqua-methanol and aqua-acetone plant extracts significantly ($p$<0.05) increased with concentration-dependent manner (Figure 3). The FCA (%) at 100 µg/ml concentration were 70.58±3.44, 66.51±1.53 and 96.36±1.12 in aqua-methanol; while 69.38±4.93, 64.57±0.96 and 96.17±1.18 in aqua-acetone for *P. oleracea*, *P. quadrifida* and $Na_2EDTA$ (standard), respectively. The $IC_{50}$ (µg/ml) were 54.98, 68.15 and 11.6 in aqua-methanol; while, 57.62, 71.35 and 15.92 in aqua-acetone for *P. oleracea*, *P. quadrifida* and standard, respectively. Almost similar FCA has been assessed by Shanmugapriya *et al.*, 2017 [45] in leaf (71.6%), stem (67.8%) and flower (66.8%) extracts of *Gnaphalium polucaulon*; whereas higher by and Emmanuel *et al.*, 2018 [14] in leaf (92.2±10.19%) extract of *Alternanthera brasiliana* regarding present study FCA values.

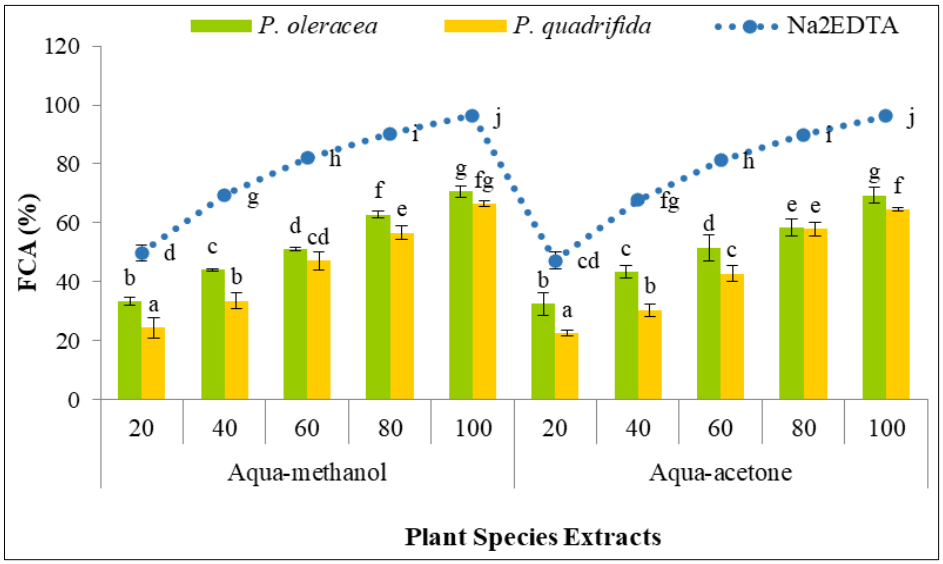


**Fig 3:** Ferrous-ion chelation (%) in aqua-methanol and aqua-acetone extracts of *P. oleracea* and *P. quadrifida* at different concentrations. Superscripted alphabets indicate significant range differences ($p$<0.05).

### 3.3.3 Ferric Reducing Antioxidant Power

The $Fe^{3+}$ reducing antioxidant power values (µg TE/mg extract) in both solvent extracts increased in dose-response manner significantly ($p$<0.05) (Figure 4). The FRAP values (µg TE/mg extract) were 65.59±0.85 and 53.88±6.35 in aqua-methanol; while 13.37±0.15 and 12.11±0.23 in aqua-acetone for *P. oleracea* and *P. quadrifida*, respectively at 100 µg/ml. On comparing present study FRAP values, the lower FRAP values were reported by Uddin *et al.*, 2012 [51] in *Portulaca oleracea* leaf methanol extract (4.3±0.1); Alam *et al.*, 2014 [2] in *Portulaca oleracea* V3 accession (7.39±0.08 mg TE/g DW); Kumar *et al.*, 2014 [29] in *Lantana camara* CPV variety (5.60±0.25); and Jain *et al.*, 2015 [23] in *Abrus precatorius* stem extract (1.77±0.27). However, higher FRAP values were assessed by Ahmed *et al.*, 2013 [1] in hexane (1705.26±9.57), butanol (437.13±3.24), methanol (287.98±5.26) and chloroform (71.98±3.33 µg AAE/mg) extracts of *O. corniculata*; Upadhya *et al.*, 2015 [52] in methanol (164.27 µM TEAC/g) leaf extracts of *Achyranthes aspera*; and Saxena and Rao, 2018 [43] in aqua-methanol (79.67±1.35) and aqua-acetone (67.52±0.95 µg TE/mg) leaf extracts of *Malvastrum coromandelianum*.

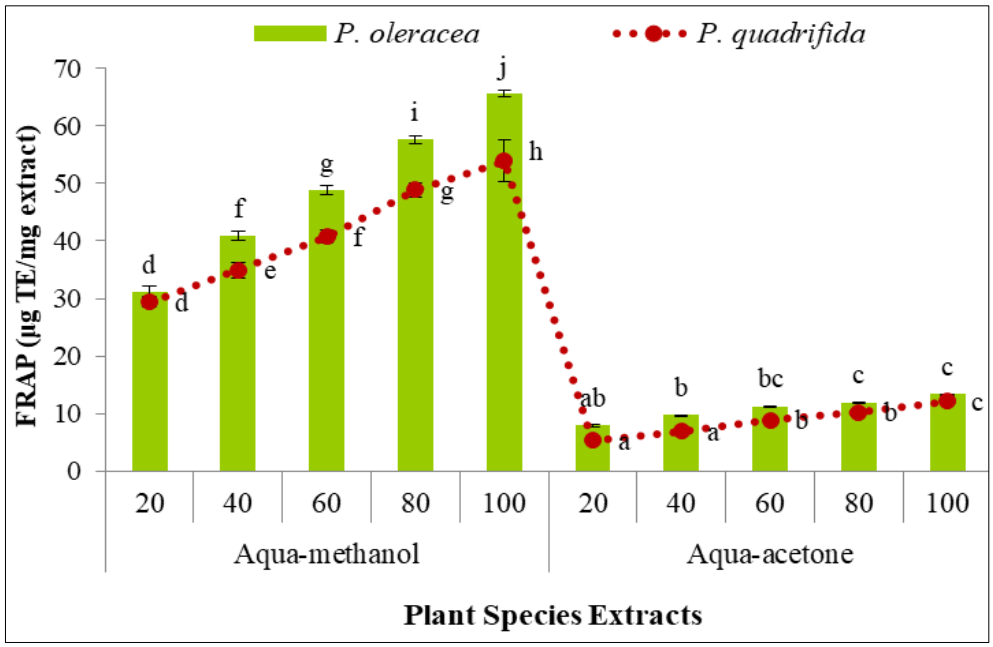


**Fig 4:** Ferric reducing antioxidant power (µg TE/mg extract) in aqua-methanol and aqua-acetone extracts of *P. oleracea* and *P. quadrifida* at different concentrations. Superscripted alphabets indicate significant range differences ($p$<0.05).

### 3.3.4 Total Antioxidant Activity

Both aqua-methanol and aqua-acetone extracts exhibited increased total antioxidant activity (µg AAE/mg extract) in concentration dependent manner significantly ($p$<0.05) (Figure 5). At highest concentration (100 µg/ml), the TAA (µg AAE/mg extract) was 52.12±0.38 and 31.39±1.10 in aqua-methanol; while 30.60±0.34 and 28.31±0.99 in aqua-acetone for *P. oleracea* and *P. quadrifida*, respectively. The TAA values reported by Borah *et al.*, 2012 [7] in acetone (7.44±0.597 mM AAE/g) and methanol (10.449±0.799 mM AAE/g) extracts of *Oxalis corniculata* leaf; by Zeeshan *et al.*, 2012 [56] in methanol extract (> 9 µg AAE/ml) of *Ageratum haustonianum* leaf; and by Krishnaveni *et al.*, 2015 [28] in water extract (3.28±1.32 mg/g) of *Prosopis juliflora* leaf were lower; while by Mozhiyarasi and Anuradha, 2018 [34] in methanol extract (55.2±0.5%) of *Hyptis suaveolens* leaf; by Nasrin, 2013 [35] in methanol extract (333.37±4.22 mg AAE/g) of *Ageratum conyzoides* stem; and Saxena and Rao, 2018 [43] in AM (69.40±1.18) and AA (59.89±0.64) extracts of *Malvastrum coromandelianum* were higher than present study.

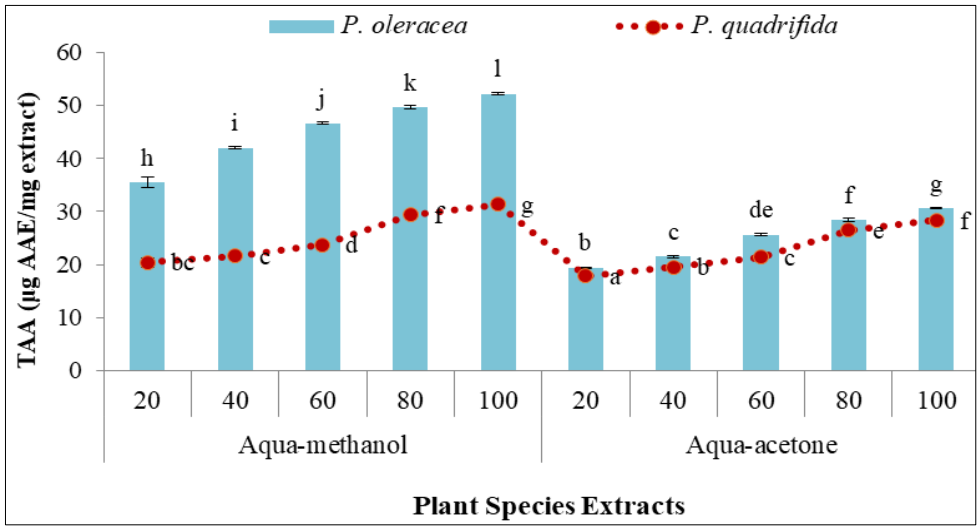


**Fig 5:** Total antioxidant activity (µg AAE/mg extract) in aqua-methanol and aqua-acetone extracts of *P. oleracea* and *P. quadrifida* at different concentrations. Superscripted alphabets indicate significant range differences ($p$<0.05).

### 3.4 Total Phenolic Content

The total phenol content (µg GAE/mg extract) in both solvent extracts exhibited concentrations-based relationship (Figure 6). The TPC values (µg GAE/mg extract) were 80.52±2.69 and 61.98±1.06 in aqua-methanol; while 53.13±1.78 and 40.89±0.70 in aqua-acetone for *P. oleracea* and *P. quadrifida*, respectively at maximum concentration (100 µg/ml) with significant differences ($p$<0.05). The lower TPC values were evaluated by Borah *et al.*, 2011 [6] in *Alternanthera sessilis* leaf (1.529±0.083 mg GAE/g DW); Farhan *et al.*, 2012 [16] in ethanol leaf (2.24±0.031) and stem (1.61±0.043) extracts of *Malva parviflora*; and Goswami *et al.*, 2014 [20] in *Argemone*

*Mexicana* flower (23.5±1.010 mg GAE/g DW), stem (13.5±2.312), fruit (10.5±0.730), leaf (4.5±0.035) and root (4.0±0.426); while, higher TPC ranges by Yakob *et al.*, 2016 [54] in methanol (264.76±0.23 mg GAE/g) extract of *Ludwigia octavalvis* leaves; Shrestha *et al.*, 2015 [48] in methanol (81.047 mg GAE/g) leaf extract of *Lantana camara*; and Sowunmi and Afolayan, 2015 [49] in acetone (126.79±0.55 mg tannic acid equivalent/g extract) leaf extract of *Cleome gynandra* than present study TPC values.

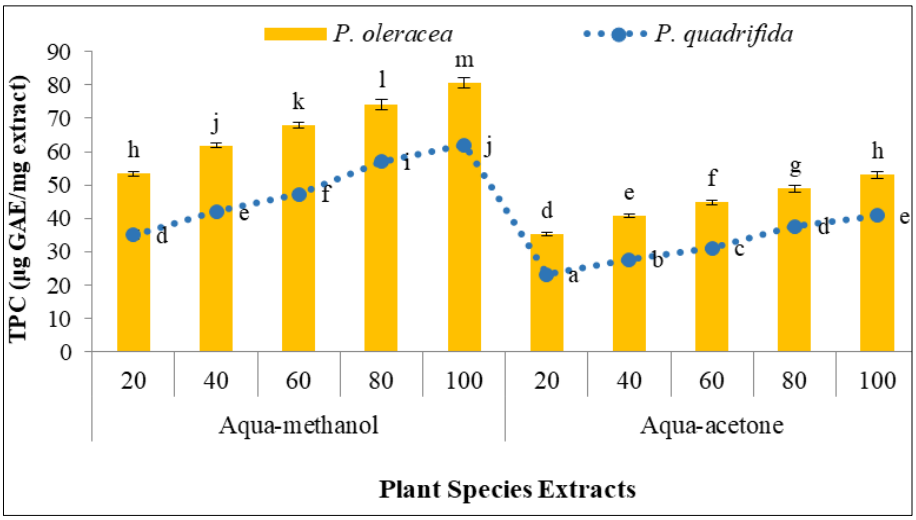


**Fig 6:** Total phenol content (µg GAE/mg extract) in aqua-methanol and aqua-acetone extracts of *P. oleracea* and *P. quadrifida* at different concentrations. Superscripted alphabets indicate significant range differences ($p$<0.05).

### 3.5 Total Flavonoid Content

The total flavonoid content (µg QE/mg extract) in *P. oleracea* was higher in aqua-methanol (7.23±0.09$^{c}$) than aqua-acetone (3.40±1.04$^{a}$); while in *P. quadrifida* was lower in aqua-methanol (5.26±1.17$^{b}$) than aqua-acetone (6.58±1.22$^{c}$) significantly ($p$<0.05). The lower TFC was observed in *Alternanthera sessilis* leaf (0.370±0.011 mg RE/g DW) (Borah *et al.*, 2011) [6] and *Portulaca oleracea* leaf (1.44±0.08 mg RE/g DW) extracts (Alam *et al.*, 2014) [2] than present study TFC ranges. Conversely, the TFC reported by Goswami *et al.*, 2014 [20] in *Argemone Mexicana* methanol flower (34.50±0.221 mg QE/g DW), leaf (32.5±3.28) and fruit (9.375±0.36) extracts were higher.

### 3.6 Total Protein Content

The total protein content (mg/ml fresh wt.) in present study was higher in *P. oleracea* (33.00±1.28$^{b}$) than *P. quadrifida* (20.59±1.71$^{a}$) with significant difference ($p$<0.05). More or less similar protein content was assessed in *Calotropis procera* (25 to 50.80%) (Khanzada *et al.*, 2008) [26]; lower in *Ambrosia maritima* shoots (14.06%) (Hussein *et al.*, 2012) [22]; and higher in *Portulaca oleracea* leaves (44.353 g/100 g DW) (Mohamed and Hussein, 1994) [33].

### 3.7 Enzymatic Antioxidants

The activities of SOD (superoxide dismutase), POD (peroxidase) and CAT (catalase) in present study were examined in support of antioxidant potential at enzyme level (Figure 7). The significant ($p$<0.05) values of SOD (U/mg FW), POD (nmole/mg of protein), and CAT (µmol/min/mg protein) for *P. oleracea* and *P. quadrifida* have been depicted in Figure 7.

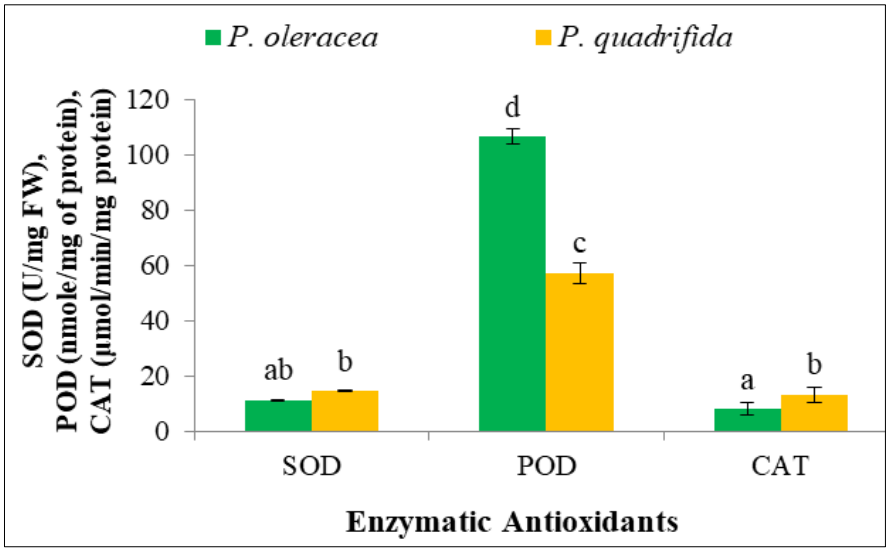


**Fig 7:** Enzymatic antioxidant analysis of freshly extracted leaf enzymes of *P. oleracea* and *P. quadrifida*. Superscripted alphabets indicate significant range differences ($p$<0.05).

### 3.8 Mineral Estimation

The metallic-minerals (manganese, copper and cobalt) in present investigation were analyzed *via* atomic absorption spectrophotometric (AAS) assay for strengthening anti-inflammatory and antioxidant potential of reference plant species. The permissible concentrations for consuming manganese (3 mg), copper (0.9 mg/day) and cobalt (5 to 8 µg) were compared with internationally standardized authorities (FSAI, 2018) [17]. The present study metallic-mineral element values are discussed in Table 2.

**Table 2:** Mineral estimation (µg/mg) *via* atomic absorption spectrophotometric in *Portulaca oleracea* and *Portulaca quadrifida*.

| Plant Species | Metallic minerals (µg/mg) | | |
|---|---|---|---|
| | Manganese | Copper | Cobalt |
| *P. oleracea* | 0.75±0.04$^{d}$ | 0.16±0.04$^{a}$ | 0.18±0.01$^{a}$ |
| *P. quadrifida* | 0.38±0.02$^{c}$ | 0.17±0.02$^{a}$ | 0.24±0.02$^{b}$ |

*Superscripted alphabets indicate significant range differences ($p$<0.05).

### 3.9 Matrix of Correlation

Correlation coefficient in both aqua-methanol and aqua-acetone extracts of *P. oleracea* and *P. quadrifida*, was evaluated to relate different parameters (DPPH, FRAP, FCA, TAA, TPC and anti-inflammatory potential) with each other in concentration-dependent manner. Both plant species in each solvent exhibited significantly ($p$<0.05) positive correlation (Table 3 and 4). Furthermore, a perfect positive correlation (1.000$^{**}$) was established between DPPH *vs* Anti-inflammatory, FCA *vs* Anti-inflammatory and FCA *vs* DPPH in *P. quadrifida* aqua-acetone extracts. This undoubtedly portrays the precise association of all present study parameters with each other.

**Table 3:** Correlation among different parameters in aqua-methanol extracts of *P. oleracea* and *P. quadrifida*.

| | Anti-inflammatory | DDPH | TAA | FCA | FRAP | TPC |
|---|---|---|---|---|---|---|
| ***Portulaca oleracea*** | | | | | | |
| Anti-inflammatory | 1.00 | | | | | |
| DPPH | 0.965$^{**}$ | 1.00 | | | | |
| TAA | 0.996$^{**}$ | 0.982$^{**}$ | 1.00 | | | |
| FCA | 0.967$^{**}$ | 0.993$^{**}$ | 0.980$^{**}$ | 1.00 | | |
| FRAP | 0.972$^{**}$ | 0.997$^{**}$ | 0.985$^{**}$ | 0.999$^{**}$ | 1.00 | |
| TPC | 0.977$^{**}$ | 0.998$^{**}$ | 0.989$^{**}$ | 0.997$^{**}$ | 0.999$^{**}$ | 1.00 |
| ***Portulaca quadrifida*** | | | | | | |
| Anti-inflammatory | 1.00 | | | | | |
| DPPH | 1.000$^{**}$ | 1.00 | | | | |
| TAA | 0.967$^{**}$ | 0.967$^{**}$ | 1.00 | | | |
| FCA | 1.000$^{**}$ | 1.000$^{**}$ | 0.967$^{**}$ | 1.00 | | |
| FRAP | 0.995$^{**}$ | 0.995$^{**}$ | 0.985$^{**}$ | 0.995$^{**}$ | 1.00 | |
| TPC | 0.990$^{**}$ | 0.990$^{**}$ | 0.986$^{**}$ | 0.990$^{**}$ | 0.999$^{**}$ | 1.00 |

**Table 4:** Correlation among different parameters in aqua-acetone extracts of *P. oleracea* and *P. quadrifida*.

| | Anti-inflammatory | DDPH | TAA | FCA | FRAP | TPC |
|---|---|---|---|---|---|---|
| ***Portulaca oleracea*** | | | | | | |
| Anti-inflammatory | 1.00 | | | | | |
| DPPH | 0.937$^{*}$ | 1.00 | | | | |
| TAA | 0.975$^{**}$ | 0.978$^{**}$ | 1.00 | | | |
| FCA | 0.965$^{**}$ | 0.986$^{**}$ | 0.986$^{**}$ | 1.00 | | |
| FRAP | 0.981$^{**}$ | 0.967$^{**}$ | 0.984$^{**}$ | 0.995$^{**}$ | 1.00 | |
| TPC | 0.977$^{**}$ | 0.987$^{**}$ | 0.990$^{**}$ | 0.998$^{**}$ | 0.995$^{**}$ | 1.00 |
| ***Portulaca quadrifida*** | | | | | | |
| Anti-inflammatory | 1.00 | | | | | |
| DPPH | 0.993$^{**}$ | 1.00 | | | | |
| TAA | 0.975$^{**}$ | 0.951$^{*}$ | 1.00 | | | |
| FCA | 0.994$^{**}$ | 0.983$^{**}$ | 0.991$^{**}$ | 1.00 | | |
| FRAP | 0.998$^{**}$ | 0.989$^{**}$ | 0.971$^{**}$ | 0.988$^{**}$ | 1.00 | |
| TPC | 0.990$^{**}$ | 0.977$^{**}$ | 0.993$^{**}$ | 0.996$^{**}$ | 0.988$^{**}$ | 1.00 |

## 4. Discussions

Naturally occurring anti-inflammatory and antioxidative alternates are in high demand due to adverse influences (mainly carcinogenicity) of synthetic medicines like BHT (butylated hydroxyltoluene), BHA (butylated hydroxylanisole), TBHQ (tert-butylhydroquinone), etc. on humans. Therefore, ethno-therapeutic options (natural products) of plant origin have been considered as secured sources in herbal world by lots of scientists.

In present investigation, anti-inflammatory (*via* albumin-denaturation suppression), antioxidant capacity (*via* DPPH, FCA, FRAP, TAA, TPC, TFC and enzymatic antioxidants) and mineral estimation (*via* AAS) were assessed in *P. oleracea* and *P. quadrifida* leaf extracts (aqua-methanol and aqua-acetone). It's the foremost attempt to analyze metallic-minerals like manganese, cobalt and copper in both plant species as supporting agents of anti-inflammation. All the present assay values were compared to the previous study

done on other invasive plant species.

Phytochemicals (carbohydrates, proteins, flavonoids, phenols, tannins, alkaloids, quinines, steroids, terpenoids, cardiac glycosides and saponins) in both species were higher in aqua-methanol than aqua-acetone, may be due to least vaporization and extra polarity of previous solvent.

Maximum albumin-denaturation inhibition in *P. oleracea* than *P. quadrifida* may be due to presence of more peroxidase (polyphenolic and antioxidant capacity enhancer) in former plant species.

The higher DPPH scavenging and metal chelation (FCA) potential of *P. oleracea* than *P. quadrifida* may be the consequence of higher total phenol content (TPC) existing in *P. oleracea* that candidly boosts antioxidants and hinders metal-induces lipid peroxidation in any natural material.

The better metal-reducing power (FRAP) and total antioxidant activity (TAA) was again shown by *P. oleracea* as compared to *P. quadrifida* that may result from maximum antioxidant composition (DPPH, FCA and TPC) and bioactive phytoconstituents inhabiting in former plant species.

Once again maximum total phenols (TPC) were represented by *P. oleracea* than *P. oleracea* as a result of higher anti-inflammation, antioxidants and phytoconstituents. Moreover, the TPC in both plant species was higher in aqua-methanol than aqua-acetone, may be due to extra polarity of methanol to penetrate deep into cellular membrane for exhuming even low molecular weight bio-compounds than acetone.

However, maximum flavonoids (TFC) were exhibited by *P. quadrifida* than *P. oleracea* that may be attributed to extra SOD activity (natural flavonoid booster) of previous plant species. Moreover, enhanced TFC in aqua-acetone than aqua-methanol may be the consequence of less polar nature of acetone, thus dissolving only big molecules (like flavonoids) with high chemical bond resonance.

Peroxidase (POD) activity in *P. oleracea* was maximum may be due to more polyphenols and antioxidative capacity. While, superoxide dismutase (SOD) and catalase (CAT) were maximum in *P. quadrifida* that may because of enhanced TFC and furthermore, SOD and CAT work in synergetic manner to boost each other's potential.

## 5. Conclusions

The present investigation confirmed anti-inflammatory, antioxidative and mineral estimation of *P. oleracea* and *P. quadrifida* leaf extracts in aqua-methanol and aqua-acetone. Between these plant species, *P. oleracea* showed comparatively high anti-inflammatory and antioxidant capacity than *P. quadrifida*. Further, additional phytoconstituents were unveiled in *P. oleracea* than *P. quadrifida*. However, antioxidative enzymes (SOD and CAT) and mineral elements (copper and cobalt) were maximum in *P. quadrifida*. Thus, *P. oleracea* may substitute several synthetic pills of anti-inflammation and antioxidants in pharmaceutical industries. The assays namely DPPH, FRAP and FCA have depicted that *Portulaca* sp. may be an indispensable treatment of pathologies or damages related to free radicals, metal chelation and reduction that ultimately bring severe inflammation. Conclusively, the current study may pave the way for constructing future therapeutic regimens pertaining to *Portulaca* sp. extracts and purifying precious bio-compounds and tracer elements having anti-inflammatory and antioxidant qualities.

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