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## Comparative evaluation of antioxidant profiling and quantitative phytochemicals of *Ixeris polycephala* Cass. and *Youngia japonica* (L.) DC. (Asteraceae)

Deepti Rawat and PB Rao

### Abstract

*Ixeris polycephala* and *Youngia japonica* (Oriental hawkbeard) both are herbs and harmless for human consumption. They have a traditional medicinal history in various countries of the world, especially in China. In the present investigation pharmacological potential of methanolic and acetone extract of the both selected plant species was determined by the DPPH inhibition (%), Fe<sup>3+</sup> reducing antioxidant power (FRAP), Phospho-molybdenum assay (PMA, total antioxidant activity), total phenol content (TPC), and total flavonoid content. In the methanolic extract, the DPPH scavenging activity of *Ixeris polycephala* is higher than *Youngia japonica* while in acetone *Youngia japonica* shows higher scavenging activity than *Ixeris polycephala*. While the highest ferrous reducing activity was shown by the acetone solution of *Youngia japonica*. Total antioxidant activity and total phenol content ( $\mu\text{g GAE/ mg}$ ) was shown by the methanolic solution of *Ixeris polycephala* whereas total flavonoid content ( $\mu\text{g QE/ mg}$ ) by acetone extract of *Youngia japonica*.

**Keywords:** ixeris polycephala; youngia japonica; pharmacological; antioxidant; phytochemicals

### 1. Introduction

A plant possesses a variety of bioactive compounds mostly in their bark, leaves, and roots and sometimes in their flowers. There are many bioactive compounds such as alkaloids; flavonoids, tannins, and phenols are well documented to have antioxidant properties [12]. These bioactive compounds have a significant role in curing various precarious human ailments such as cancer, cardiovascular and neurological disorders [14]. As a result, plants have made a monumental place in medical science and pharmaceutical industries. These bioactive compounds are the result of resistance mechanisms through which plants can endure with assorted biotic and abiotic stresses [1].

These compounds are acknowledged for their imperative role in plants for acclimatization to changing environment and defense but not necessary for the sustenance of life processes. *Ixeris polycephala* (Cass.) and *Youngia japonica* both are annual herbs which are reported to have various bioactive compounds included steroids, triterpenes, sesquiterpene, lactones, amino acids, and fatty acids [6]. These herbs belong to family Asteraceae (Daisy family) and harmless to human consumption. *Ixeris polycephala* is extensively cultivated in the domains of the east, southeast and south part of Asia. The leaves of *Ixeris polycephala* are consumed and other parts are used to increase usage and commercial values of beverages and other vegetables by local peoples of China. It also used in the treatment of various ailments such as in inflammation of the appendix, viral disease e.g. measles, contusion, analgesic (pain-relieving) and hemostasis (stop bleeding) [9].

*Youngia japonica* is extensively distributed all over the world especially in temperate regions. It is used by Chinese people as traditional medicine [10]. Various secondary metabolites such as Taraxasteryl acetate, n-docosanol,  $\beta$ -sitosterol and stigmasterol, retinol,  $\beta$ -daucosterol, docosanoic acid, apigenin are reported in *Youngia japonica* which makes it a potent candidate for medicinal properties [16]. It is used to diminish pyrexia, detoxification, and atrophy. It possesses other medicinal properties such as antitumor, antioxidant, antiallergic activity and it is very effective for the treatment of boils and snakebites [10]. Approximately eleven sesquiterpenoids have been reported in this plant [4].

The purpose of the present investigation is to compare pharmacological properties via DPPH scavenging activity, ferrous reducing antioxidant power assay, phospho-molybdenum assay, total flavonoid content and total phenol content of *Ixeris polycephala* and *Youngia japonica* by

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using two different solvents (aqua methanol and aqua acetone) for extraction.

## 2. Material and Methods

### 2.1 Sample collection and authentication

Fresh plant material was collected from G.B.P.U.A. &T., Pantnagar, Uttarakhand, India in March 2019 and properly identified by Dr. D.S. Rawat, Assistant Professor, Deptt. of Biological Sciences, according to the “eflora of Pantnagar”.

### 2.2 Preparation of extract

Fresh harvested mature plant leaves were washed with distilled water, shade dried then powdered with the help of an electric grinder. 10 gm of dried powder of plant sample was soaked (1:10 w/v) in two different solvents aqua methanol and aqua acetone (20:80 each) in order to obtain plant extract for 7 days at temp 27 °C. The extracts were filtered, evaporated in the oven (40 °C) and stored at 4 °C for further experimentation.

### 2.3 Qualitative phytochemical screening

Different phytochemicals such as alkaloids, tannins, phenols, protein, flavonoids, quinines, carbohydrates, saponins, steroids, cardiac glycosides, and terpenoids qualitatively determined by applied methods of Harbone [7] and Sofowora [13].

### Phospho-molybdenum assay

Phospho-molybdenum assay, for quantification of total antioxidant activity (TAA) of plant extracts. The method described by Prieto *et al.* [11] with required adjustments were adopted for the determination of total antioxidant activity. The reaction mixture was prepared with (1 ml) of plant extract with different concentration (50 to 300 µg/ml) with reagent (3 ml) prepared by mixing sulphuric acid (0.6 M), ammonium molybdate (4 mM) and sodium phosphate (28 mM) was performed and the test tubes were incubated at 95 °C for 90 min. The absorbance of resultant products was measured at 695 and ascorbic acid (Vit-C) was used as a standard reference or positive control while extraction solvents as a negative control.

### 2.3.2 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

DPPH• scavenging activity (%) of the plant extract was analyzed according to the method [3] procedure with required alterations. The reaction mixture was prepared with 1 ml of plant extracts (50 to 300 µg/ml concentrations) with 3 ml of DPPH solution (0.004%) and the reaction mixture was incubated for 60 min. The absorbance of the reaction mixture after incubation was measured at 517 nm and decreasing optical density with increasing concentration represents radical inhibition capacity of plant extract/ standard. Scavenging activity was calculated by the following equation.

$$\text{Scavenging activity (\%)} = [1 - (A_t/A_c) * 100]$$

Where,  $A_t$  indicates absorbance of sample and  $A_0$  indicates absorbance of control at 517 nm.

### 2.3.3 Fe<sup>3+</sup> Reducing Antioxidant Power (FRAP) assay

The Fe<sup>3+</sup> reducing power of the plant extract was assayed by using the method [2]. Fresh FRAP reagent was prepared with 300 mM sodium buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl) and 20 mM ferric chloride (FeCl<sub>3</sub>.6H<sub>2</sub>O) solution in 10:1:1 ratio, respectively. The reagent was incubated at 37 °C before using. For reaction, 1 ml of both plant extracts (50-300 µg/ml concentration) was reacted with 3 ml of reagent for 30 min at 37 °C. The absorbance of the blue-colored product was determined by taking absorbance at 593 nm. The value of FRAP was determined by using a standard curve of the Trolox and respective solvents (80 % aqua methanol and 80% aqua acetone) were used as a negative control.

## 2.4 Quantitative phytochemical analysis

### 2.4.1 Total phenolic content (TPC)

Folin-Ciocalteu colorimetric method [15] was used with required modifications. The reaction mixture was prepared with varying plant extracts (50 to 300 µg/ml concentration) was 0.2 ml of Folin-Ciocalteu reagent for 5 min and the reaction was neutralized by the adding 2 ml of saturated 7% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). The reaction mixture was incubated for 60 min. The absorbance of reaction products after incubation was measured at 765 nm and values were expressed in µg gallic acid equivalents (GAE) using a gallic acid standard curve.

### 2.4.2 Total flavonoid content (TFC)

For analysis of flavonoid content in the plant extract, the procedure [5] with convenient modifications was used. Both plant extracts (200 µg/ml concentrations) were reacted with 2% aluminium chloride (1:1 v/v). After 60 min incubation, absorbance at 420 was measured against a methanol control. The total flavonoid was analyzed by using the quercetin equivalents (QE) standard curve.

## 2.5 Statistical analysis

The data (Three replicates and six concentrations) was represented as mean±S.E. Duncan's multiple range test (DMRT) of SPSS 16.0 version was used for the determination of significant difference ( $p < 0.05$ ) of all means.

## 3. Results and Discussion

### 3.1 Extraction Yield and Qualitative Test of Phytochemicals

The extraction of different phytochemical compounds or bioactive compounds from the plants is a very crucial step, which is affected by nature of solvent, extraction method, polarity of various biological compounds of plant sample and other abiotic factors such as temperature, pH and time [19]. In the present investigation, extraction yield was performed by using (20:80 v/v) aqua methanol and aqua acetone. The higher yield was obtained in aqua methanol solvent in both selected plant species which was 23.7% (w/w) and 11.6% (w/w) *I. polycephala* and *Y. japonica* respectively. A similarly higher amount of different qualitative phytochemicals was observed in aqua methanol as compare to aqua acetone extract that may be because of superior extraction capacity, polarity and slightest vaporization nature of methanol (Table 1).

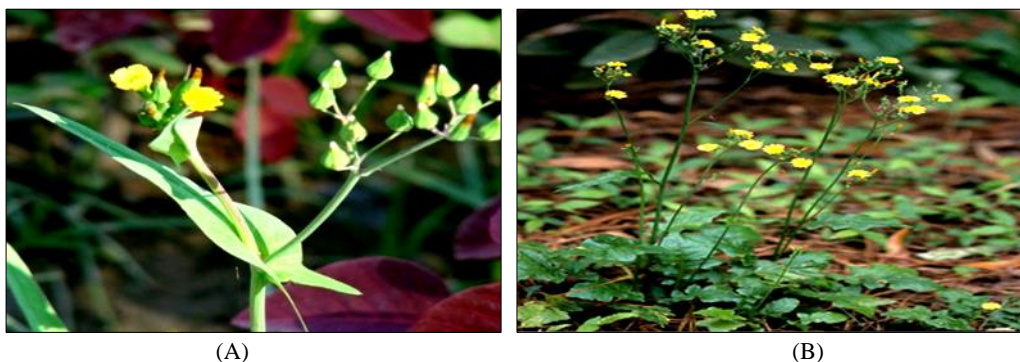


Fig: (A) *Ixeris polycephala* and (B) *Youngia japonica*

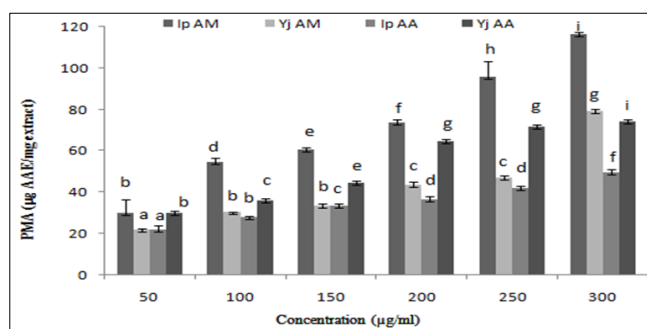
Table 1: Extraction yield and qualitative phytochemical constituents in aqua methanol and aqua acetone of *I. polycephala* and *Y. japonica*

Phytochemicals	Test	Extracts			
		<i>I. polycephala</i> (AM)	<i>I. polycephala</i> (AA)	<i>Y. japonica</i> (AM)	<i>Y. japonica</i> (AA)
Alkaloids	Mayer's	+	+	+	+
Carbohydrate	Molisch's	+++	+++	+++	++
Cardiac Glycosides	Keller-Kiliani	-	-	-	-
Flavonoids	Sulfuric acid	++	+	++	+
Phenols	Ferric chloride	+++	++	+++	++
Proteins	Xanthoproteic	+	+	+	+
Quinines	Hydrochloric acid	++	+	++	-
Saponins	Froth	+	-	+	-
Steroids	Liebermann-Burchard's	+	+	+	+++
Tannins	Lead acetate	++	+	++	+
Terpenoids	Salkowski	++	+	++	+
Yield%		23.7% (w/w)	10.6% (w/w)	11.6% (w/w)	5% (w/w)

(+) Present, (-) absent of respective phytochemicals, AM= Aqua methanol, AA= Aqua acetone

### 3.2 Phospho- molybdenum assay

The method is based on the reduction rate of molybdenum (VI) to molybdenum (V) to form the green complex (phospho-molybdenum) via thermal auto-oxidation [20]. The total antioxidant activity ( $\mu\text{g AAE}/\text{mg extract}$ ) of both plants increases in a dose-dependent manner in both aqua methanol and aqua acetone extract. In aqua methanol extract TAA at 300  $\mu\text{g}/\text{ml}$  of *Ixeris polycephala* ( $116.33 \pm 0.69^j$ ) was comparatively higher as compared to *Youngia japonica* ( $79.33 \pm 0.57^e$ ) while in aqua acetone *Youngia japonica* ( $74.33 \pm 0.57^i$ ) exhibits higher TAA than *Ixeris polycephala* ( $49.67 \pm 0.83^f$ ).



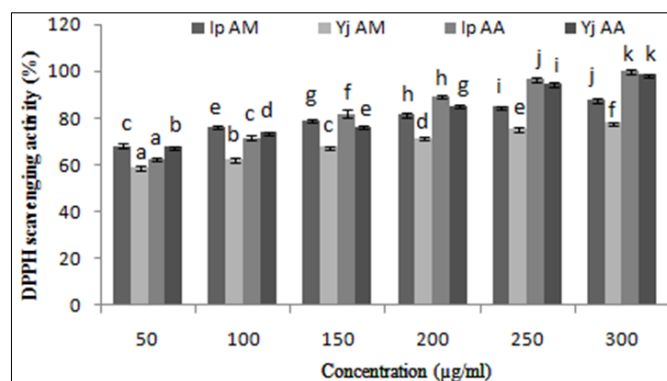
Values with different lowercase letters indicate significant difference ( $p < 0.05$ ).

Fig 1: Phospho-molybdenum assay (TAA,  $\mu\text{g AAE}/\text{mg extract}$ ) in both AM and AA leaf extracts of *Ixeris polycephala* and *Youngia japonica* at varying concentrations.

### 3.3 DPPH inhibition activity (%)

DPPH inhibition activity in both selected plant species increases in a concentration-dependent manner. Both the plant species showed comparatively higher DPPH inhibition

in aqua acetone, in the case of *Ixeris polycephala* ( $99.81 \pm 0.46^k$ ) which was slightly higher as compared to *Youngia japonica* ( $98.77 \pm 0.11^k$ ). While in aqua methanol *Ixeris polycephala* exhibits ( $87.80 \pm 0.32^j$ ) and *Youngia japonica* ( $77.83 \pm 0.30^f$ ), which is again the same as the first one. The DPPH inhibition activity of aerial parts of some members of the family Asteraceae reported [21] ethanolic extract of *Pluchea indica* 16.66  $\mu\text{g}/\text{ml}$ , *Elephantopus scaber* 18.43  $\mu\text{g}/\text{ml}$  and *Eclipta alba* 23.79  $\mu\text{g}/\text{ml}$ , whereas ethyl acetate extract of *Eclipta alba* 24.33  $\mu\text{g}/\text{ml}$ .



Values with different lowercase letters indicate significant difference ( $p < 0.05$ ).

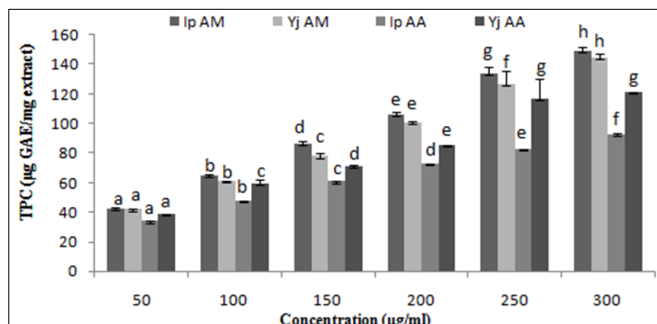
Fig 2: DPPH scavenging activity (%) in both AM and AA leaf extracts of *Ixeris polycephala* and *Youngia japonica* at varying concentrations.

### 3.4 Fe<sup>3+</sup> reducing antioxidant power

The ferrous reducing power of both selected plant extract increases in the concentration supportive pattern in the case of both solvents with significant difference ( $p < 0.05$ ) (Fig.



3). The value of FRAP ( $\mu\text{g TAE}/\text{mg extract}$ ) of *Ixeris polycephala* ( $149.2 \pm 1.25^{\text{h}}$ ) and *Youngia japonica* ( $144.70 \pm 1.44^{\text{h}}$ ) at 300  $\mu\text{g}/\text{ml}$  concentration, which reveals that both plants exhibit more or less similar ferric reducing antioxidant power in aqua methanol extract. However, in aqua acetone, *Youngia japonica* ( $121.04 \pm 0.21^{\text{e}}$ ) exhibits a significantly higher ferrous reducing power as compare to *Ixeris polycephala* ( $92.45 \pm 0.55^{\text{f}}$ ).

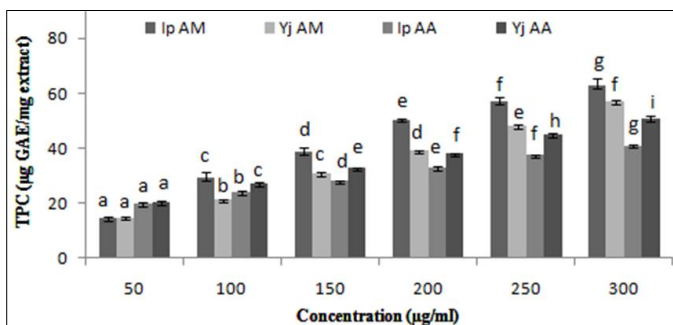


Values with different lowercase letters indicate significant difference ( $p < 0.05$ ).

**Fig 3:** Fe<sup>3+</sup> reducing power (FRAP,  $\mu\text{g TE}/\text{mg extract}$ ) in both AM and AA leaf extracts of *Ixeris polycephala* and *Youngia Japonica* at varying concentrations

### 3.5 Total phenol content

Plant phenols are an extremely diverse group of compounds due to their variability in structure and numerous substitutions for some groups. Plant phenol composition mainly depends upon the plant species, their stage of growth and development, environmental conditions and geographical area. Phenols are the main phytochemicals that have antioxidant activity [22]. The amount of total phenol content ( $\mu\text{g GAE}/\text{mg}$ ) was determined higher in aqua methanol extract of *Ixeris polycephala* ( $63 \pm 1.32^{\text{e}}$ ) as compare to *Youngia japonica* ( $57.23 \pm 0.20^{\text{f}}$ ); whereas higher total phenol content was determined in *Youngia japonica* ( $50.83 \pm 0.42^{\text{i}}$ ) as compare to *Ixeris polycephala* ( $41.03 \pm 0.14^{\text{e}}$ ) at 300  $\mu\text{g}/\text{ml}$  with significant difference at  $p < 0.05$ . The TPC ( $\mu\text{g GAE}/\text{mg}$ ) in both species at different concentrations showed in a dose-dependent manner (Fig. 3). In an investigation of different species of the same family, the TPC in *E. purpurea* (13.34 mg GA per g-1 FW) species and in *H. annuus* (2.65 mg GA per g-1 FW) [22]. In another study, [23] the reported values of TPC (mg GAE/g) 5.6 g/100 g DW for *Artemisia monosperma* in 80% methanol extract.



Values with different lowercase letters indicate significant difference ( $p < 0.05$ ).

**Fig 4:** Total phenol content (TPC,  $\mu\text{g GAE}/\text{mg extract}$ ) in both AM and AA leaf extracts of *Ixeris polycephala* and *Youngia Japonica* at varying concentrations.

### 3.6 Total Flavonoid content

Flavonoids are naturally occurring in the most prevalent and ubiquitous class of polyphenolic compounds and possess various biological activities such as anti-inflammatory, anti-diabetic, anti-rheumatic and anti-carcinogenic. The value of flavonoid content (TFC,  $\mu\text{g QE}/\text{mg extract}$ ) was determined higher in aqua acetone as compare to aqua methanol in both plant species. It may be due to the large size and somewhat non-polar nature of flavonoid that can be dissolved more frequently in a less polar solvent. The total flavonoid content in *Ixeris polycephala* ( $19.76 \pm 0.08$ ) which is higher than *Youngia japonica* ( $10.67 \pm 0.13$ ) in aqua acetone while in aqua methanol the value of total flavonoid in *Ixeris polycephala* ( $11.70 \pm 0.05$ ) which is again higher than *Youngia japonica* ( $6.13 \pm 0.08$ ) at 200  $\mu\text{g}/\text{ml}$  concentration (Table 2) with significant difference at  $p < 0.05$ . According to a study, the total flavonoid value of methanol extract of *Calendula officinalis* L. ( $6.5 \pm 0.004$ ) [24], while in another investigation in nine varieties of ethanolic leaf extract of *Calendula officinalis* it was 6.11–15.74 mg/g [25], a difference can be seen in the value of flavonoids within the family.

**Table 2:** Total flavonoid content (TFC,  $\mu\text{g QE}/\text{mg extract}$ ) in both AM and AA leaf extracts of *Ixeris polycephala* and *Youngia Japonica* (Mean $\pm$ S.E).

S. No.	Plant Species	Extract Concentration (200 $\mu\text{g}/\text{ml}$ )	
		Aqua Methanol	Aqua Acetone
1	<i>I. polycephala</i>	$11.70 \pm 0.05^{\text{c}}$	$19.76 \pm 0.08^{\text{d}}$
2	<i>Y. japonica</i>	$6.13 \pm 0.08^{\text{a}}$	$10.67 \pm 0.13^{\text{b}}$

\*DMRT was applied. Mean values with varying alphabets imply the significant difference ( $p < 0.05$ ).

### 4. Conclusion

The present study reveals that both plant species illustrated the antioxidant potential, but *Ixeris polycephala* showed higher DPPH inhibition activity (%), Phospho-molybdenum assay (PMA or TAA) and Fe<sup>3+</sup> reducing antioxidant power (FRAP) in aqua- methanolic extract while *Youngia japonica* exhibits higher antioxidant activity and quantity of phytochemical in aqua acetone extract. Both plant species are harmless to human consumption and are available in large quantities in nature. The aqua methanol and aqua acetone leaf extracts of both plant species proved itself as a potent antioxidant resource for pharmaceutical industries to the development of new remedies.

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