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Evaluation of antioxidant activity and GC-MS analysis of bioactive compounds present in leaf extract of *Youngia japonica* (L.)DC. from Chandigarh

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Abstract: **The present study reports the phytochemical investigation as well as nutraceutical aspect of leaves of *Youngia japonica* from Chandigarh. *Youngia japonica*, commonly known as Oriental false hawksbeard, is a weed species of flowering plant belonging to family Asteraceae. *In vitro* antioxidant analysis of plant extract through three different methods viz. DPPH, H_2O_2 and Phosphomolybdenum assay shows significant results as compared to the ascorbic acid which is taken as positive control. Results indicate that plant has good potential of phenolic (82.60±0.0071 at 250µg/ml conc) and flavonoid (41.64±0.0612 at 250µg/ml conc) compounds as well it can be regarded as natural source of antioxidants. Gas chromatography–mass spectrometry (GC-MS) analysis of the methanol extract showed a total of 10 components out of which 9,12,15Octadecatrienoic acid,2,3bis[(trimethylsilyl) oxy]propyl ester, (Z,Z,Z) (RT=58.34%), psi.,.psi. Carotene, 3, 4 didehydro1,1', 2,2'tetrahydro1' hydroxyl1methoxy (RT=23.20%) and hexadecanoic acid, methyl ester (RT=3.64%) were the major constituents identified in the methanol extracts, respectively. Methanol extract showed the presence of several bioactive components and offers reference data for additional research of its active components.**

Keywords: **Phytochemical, antioxidant, bioactive, GC-MS, methanol.**

1. INTRODUCTION

Plants play a substantial role in the prevention and treatment of ailments and can even avert and reduce the adverse effects of conventional treatments. They can be a source of chemical constituents of pharmacological and biological significance. History reveals that plants are important for screening of new lead compounds and will continuously be sources of successful drugs. An important aspect in the investigation of plant is the identification of the biologically active compounds present, leading to further pharmacological and biological studies.

Youngia japonica, commonly known as Oriental false hawksbeard, is annual common weed species of flowering plant belonging to family asteraceae growing along the roadsides, garden, and waste area. Generally it is native to eastern Asia, presently it is found as a weed nearly worldwide in the Himalayas, from Kumaon to Bhutan, India, Ceylon, Malaysia, China, Indo-China, Japan, Philippines at altitudes of 230-2900 m. It is an annual herb with bright yellow flowers. Flower-heads are many, 4-7 mm across in corymb like panicles. Involucre bracts are 6-8, narrowly oblong, green and erect. Florets are yellow, 10-20. It blooms in late spring and early summer. Plants are variable in height depending on growing conditions which range from 15 cm to 150 cm. Basal leaves are rosette, toothed lobes, terminal lobe largest and pinnately divided. Its fruits are dispersed by wind. Stem leaves reduced above into bracts and few in no. Seed pods are brown, 1.5-2.5 mm long, fusiform, tip narrowed; pappus white, 3-3.5 mm long. Plant is used as febrifuge and antitussive as well as used in treatment of snakebite and boils. Plant contains sesquiterpines that exhibit antitumor activities (Yulan *et al*., 2014). The young leaves and stalks were consumed as a wild vegetable when there was a famine in the ancient time. The plant leaf is edible and is used nowadays as wild health food for salads in North America.

The use of natural products for health, especially of plants origin is increasing day by day. Plants play an important role in the health services around the globe. Around three quarters of the world's population depends upon plants and their extracts for health care. According to World Health Organization 80% of the world population still depends on plant based traditional medications for primary health care management (Bodeker *et al*., 2005). In recent years, much attention has been paid towards plant based antioxidants and their association with health aids. Consumption of antioxidant constituents are reported to have protection against oxidative damage induced degenerative and pathological processes including ageing and cancer. Free radical-induced oxidative damage is involved in the pathogenesis of many chronic and degenerative diseases, such as cardiovascular disease, cancer, diabetes *etc. (*Ramarathnam N. *et al*., 2005). Detrimental effects which originate from the imbalance in the antioxidant pro-oxidant balance can be barred by the consumption of antioxidant substances.

Antioxidant compounds in food plays an important role as a health protecting factor (Uttara *et al*., 2009). Antioxidant properties have been studied in several plant species for the development of natural antioxidant formulations in the areas of food, medicine and cosmetics. Recently, researchers have tried to separate nontoxic antioxidants from edible plants to avoid autoxidation and lipid peroxidation with an initiative to replace synthetic antioxidants. Plant extracts containing high amounts of bioactive compounds especially antioxidants and have the prospective of being used in food, agriculture, nutraceuticals, cosmetics and pharmaceutical products.

In the past few years, GC-MS has been proven as a key technological metabolic profiling in plant species. The present exploration, of this work was to analyse the natural bioactive products and their phytochemical constituents. It was aimed to investigate the phyto-components through GC-MS study and antioxidant properties along with total phenolic contents and flavonoid content so that this plant could be used as a safe alternative as antioxidant.

2. METHODS

2.1 Plant material

Plant collection and identification: The young leaves of *Youngia japonica* was collected from Chandigarh during the month of January 2015. Identification of species was done by comparing with authenticated herbarium specimens, Botany department, Panjab University Chandigarh (PAN) and a voucher specimen number PAN- 21625 was deposited in the department for future references and later confirmed with the help of diagnostic keys and morphological descriptions given in various floras.

2.2 Preparation of Plant Extracts: Plant extract was prepared by taking fresh young leaf portion. Fresh plant material was collected, dried under shade conditions and crushed to fine powder with the help of pestle and mortar. About 25 gm of dried powder was taken and 250 ml of methanol was poured and kept on shaker for 24 hrs. After 24hrs it was kept open under normal room conditions so that solvent could evaporate and concentrated extract obtained after that was used for further experiments.

Total Phenolic content (Ainsworth 2007)

Total Phenolic content of plant extract was determined by using Folin Ciocalteu reagent.

Total Flavonoid Content (Chang *et al*., 2002)

Total flavonoid content of plant extract was determined by using $AlCl_3$ colorimetric assay.

Study of antioxidant activity:

Antioxidant activity of the plant extract was done by DPPH free radical scavenging activity, H_2O_2 activity and Phosphomolybdenum assay.

1. DPPH free radical scavenging activity (Harini *et al.*, 2012)

The free radical scavenging capacity of each methanolic plant extract was determined by using DPPH. Ascorbic acid was used as standard and DPPH was taken as positive control. Methanol served as blank for the experiment.

The % inhibition was calculated by the formula: % inhibition = $\frac{Control-test}{Control} \times 100$

The results are expressed as mean % antioxidant activity.

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2. Scavenging of hydrogen peroxide (Nabavi *et al*., 2009)

The ability of the extract to scavenge the hydrogen peroxide was determined by the standard method. Methanol was taken as blank and ascorbic acid served as standard. The % of hydrogen peroxide scavenged by the plant extract and the standard was calculated by the formula: % scavenged $[H_2O_2] = [(A_O - A_1)/A_O] \times 100$

Where A_O was the absorbance of the control and A_1 was the absorbance of the test.

3. Phosphomolybdenum assay (Prieto *et al*., 1999)

The total antioxidant capacity of the methanol extract of leaves was evaluated by the phosphomolybdenum reduction assay method. The % inhibition of the plant extract and standard was calculated by the formula:

% inhibition $= [(A_O - A_1)/A_O] \times 100$

Where A_O was the absorbance of the control and A_1 was the absorbance of the test.

GC-MS analysis (Gas Chromatography- Mass Spectroscopy)

The GC-MS analysis of the leaf sample was performed with Thermo Trace 1300GC coupled with Thermo TSQ 800 Triple Quadrupole Mass Spectrometer for Gas Chromatography - thermo trace 1300 Gas Chromatography and for Mass Spectrometer - thermo tsq 8000 fitted with an TG 5MS capillary column (30m X 0.25mm, 0.25µm). The oven temperature was programmed from 60° C to 250° C and a hold for 10 mins. Helium was used a carrier gas. The injector temperature was 250° C with injection size 1.0µL. The interface and MS transfer line temp were maintained at 230 ˚C and 280˚C respectively. Data handling was done using software XCalibur 2.2SP1 with Foundation 2.0SP1.

Identification of compounds

Mass spectrum of GC-MS was interpreted using the mass spectral database of National Institute of Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in NIST library. The name, molecular weight and retention time of the components were determined.

3. RESULTS AND DISCUSSION

Total Phenolic and Flavonoid content:

Phenolic and flavonoid compounds are widely distributed in plants and are associated with antioxidant activity and play important role in stabilizing lipid peroxidation. Total Phenolic content of extract is expressed in terms of gram gallic acid equivalent and calculated using standard curve equation. In *Youngia Japonica* it has been observed that at concentration of 250 µg/ml phenolic content is 82.62±0.0467 whereas flavonoid content was 41.64±1.415. Flavonoid content is expressed in terms of mg of quercetin/g of extract and calculated using standard curve equation. Further it has been observed that as the concentration of the extract is increased there is increase in phenolic and flavonoid content which attributes towards various activities of the plant such as antibacterial, antidiabetic, antioxidant and its usage as food. The results of the present study at various concentrations are shown in Table 1 and Fig. 1 and 2 given below.

Table 1: Total Phenolic and Flavonoid content

Concentration	Total Phenolic content (mg GAE equivalent/g)	Total flavonoid content (mg quercetin equivalent/g)
50	20.18±1.405*	13.15±0.142*
100	34.29±0.825*	20.83±0.105*
150	49.64±1.094*	29.04±0.353*
200	65.67±0.618*	36.44±0.471*
250	82.62±0.467*	41.64±1.415*

All values were calculated in triplicates *(Values represent mean ± SEM of three replicates)

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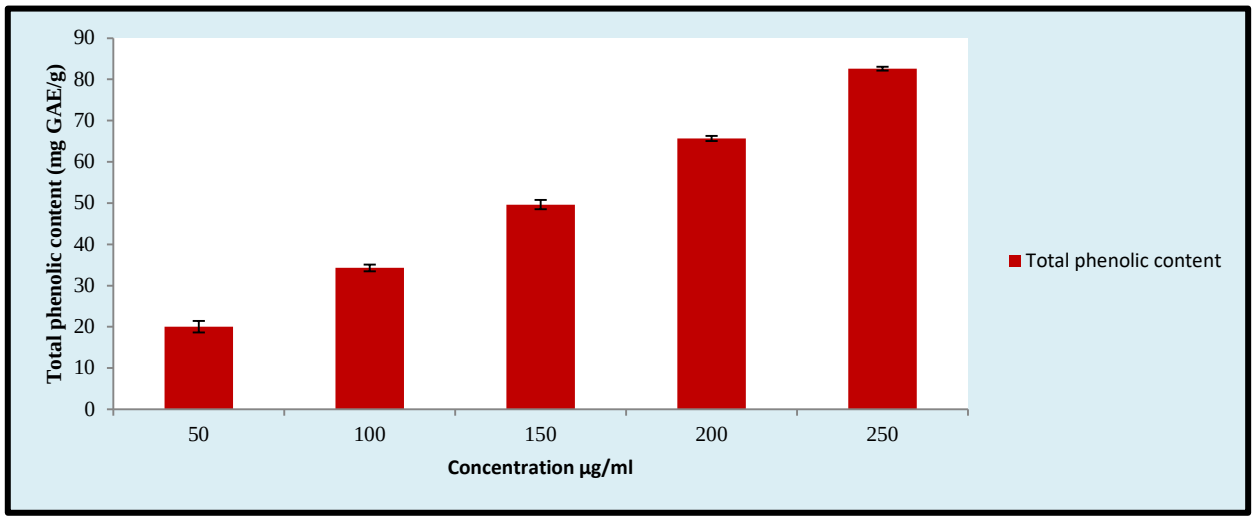

Figure 1: Represents total phenolic content of methanolic extract of *Youngia japonica*

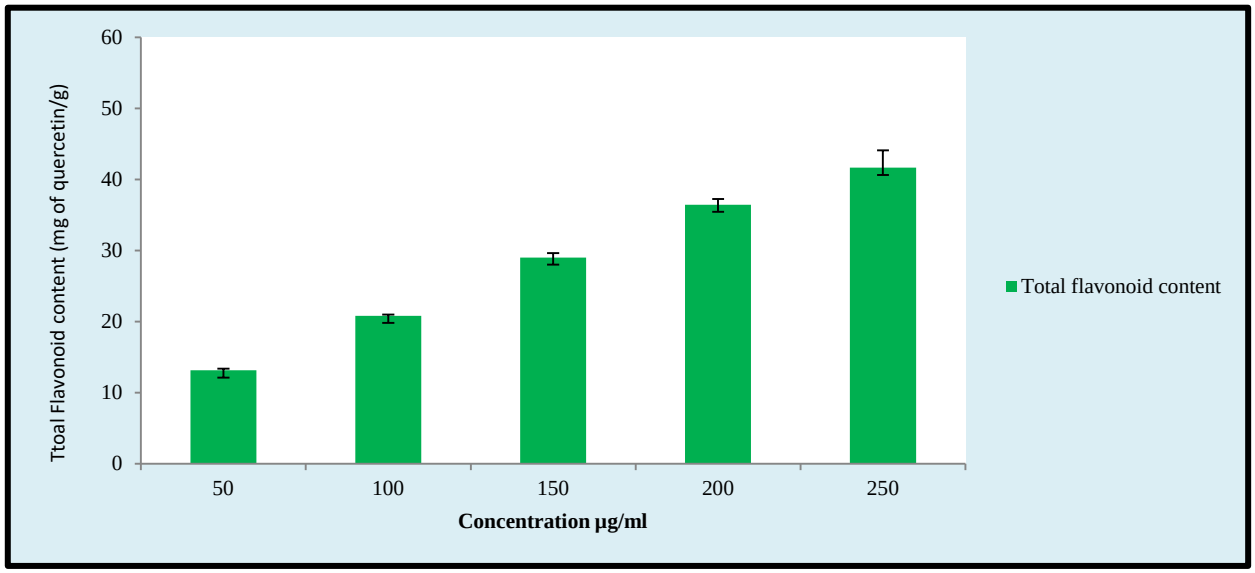

Figure 2: Represents total flavonoid content of methanolic extract of *Youngia japonica.*

DPPH Free Radical Scavenging Activity:

The ability of methanolic plant extract of *Youngia japonica* to scavenge DPPH free radical was calculated by percentage inhibition, where inhibition was found to be 51.97±0.026% at concentration 250µg/ml, whereas at same concentration it was found 43.54±0.0014% for ascorbic acid which was used as a positive control. The results of the present study at various concentrations are shown in Table 2 and Fig. 3 given below.

Table 2: % inhibition of DPPH by methanolic extract of *Youngia japonica* and standard (ascorbic acid)

Concentration µg/ml	% inhibition (plant extract)	% inhibition (Standard)
50	39.08±0.077*	20.96±0.0144*
100	41.19±0.049*	27.95±0.0144*
150	44.64±0.029*	34.94±0.0072*
200	50.42±0.026*	40.32±0.0024*
250	51.97±0.026*	43.54±0.0014*

*All values were calculated in triplicates *(Values represent mean ± SEM of three replicates)

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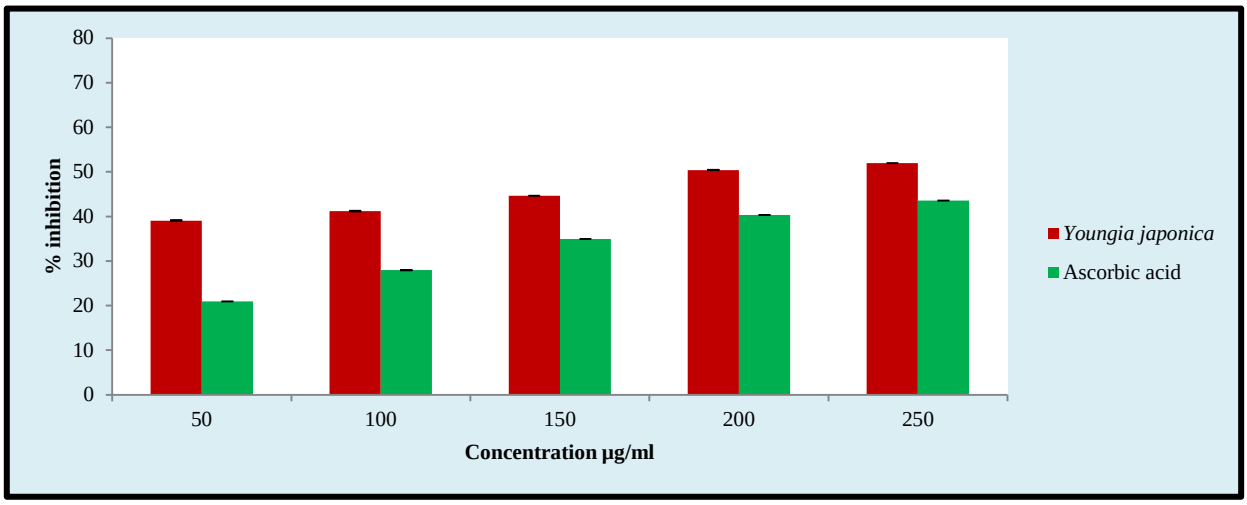

Figure 3: Difference in % Inhibition of DPPH by methanolic extract of *Youngia japonica* and ascorbic acid at various concentrations

H_2O_2 Scavenging activity

The scavenging ability of methanol extract was calculated as percentage inhibition. The % inhibition values are given in Fig 3. At 250µg/ml it was found to be 66.5±0.012% for *Youngia japonica* and 61.9±0.032% for ascorbic acid which was used as a positive control. These results suggest that % inhibition of *Youngia japonica* was found to be higher than standard. It can be attributed that methanolic leaf extract can protect the living system from oxidative stress by eliminating H_2O_2 as it can react with Fe^{2+} ions and Cu^{2+} ions to form hydroxyl radicals. The results of the present study at various concentrations are shown in Table 3 and Fig. 4 given below.

Table 3: % inhibition of H_2O_2 Scavenging ability by methanolic extract of *Youngia japonica* and standard (ascorbic acid)

Concentration µg/ml	% inhibition (plant extract)	% inhibition (Standard)
50	18.2±0.087*	18.6±0.100*
100	34.77±0.016*	28.3±0.020*
150	51.49±0.054*	46.15±0.149*
200	63.8±0.105*	61.4±0.018*
250	66.5±0.012*	61.9±0.032*

*All values were calculated in triplicates *(Values represent mean ± SEM of three replicates)

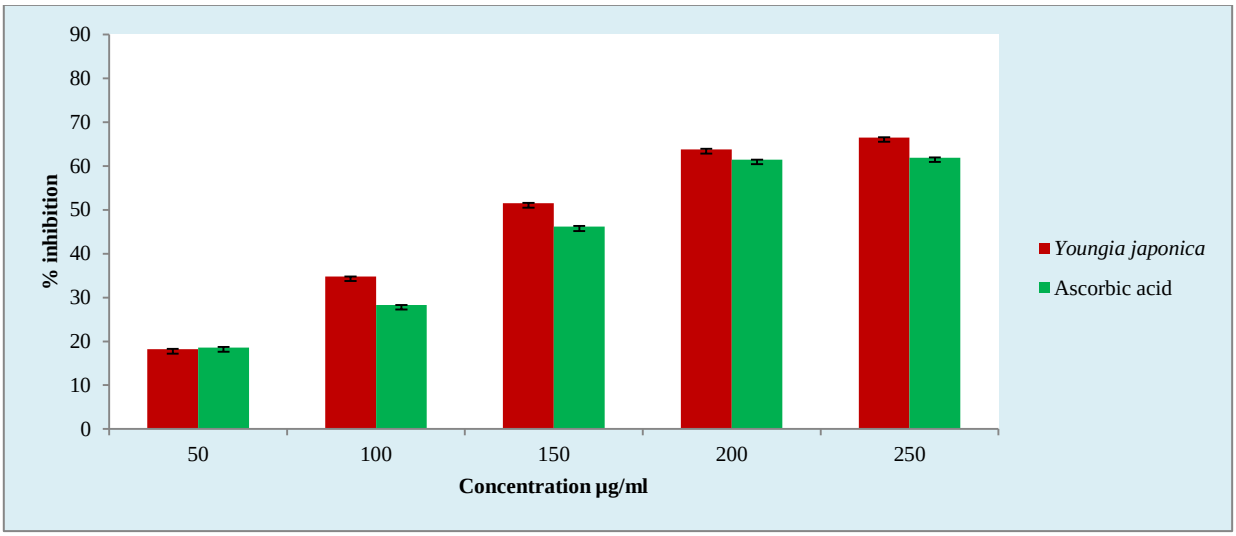

Figure 4: Difference in scavenging % of H_2O_2 by methanolic extract of *Youngia japonica* and ascorbic acid at various concentrations

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Phosphomolybdenum assay:

The Phosphomolybdenum reduction assay was based on the reduction of Mo(VI) to Mo(V) in presence of antioxidant compound and subsequent formation of a green phosphate/Mo(V) complex at acidic pH and at higher temperature. The Phosphomolybdenum reduction assay increases with increase in concentration of methanol extract of leaves of *Youngia japonica* as shown in Figure 4. At concentration of 250µg/ml it was found to be 93.62±0.016% for *Youngia japonica* and 78.62±0.015% for ascorbic acid which was used as a standard. The results of the present study at various concentrations are shown in Table 4 and Fig. 5 given below.

Table 4: % inhibition of Phosphomolybdenum by methanolic extract of *Youngia japonica* and standard (ascorbic acid)

Concentration µg/ml	% inhibition (plant extract)	% inhibition (Standard)
50	39.83±0.018*	47.66±0.039*
100	52.25±0.017*	54.43±0.027*
150	74.03±0.015*	66.04±0.049*
200	80.48±0.011*	73.14±0.053*
250	93.62±0.016*	78.62±0.015*

*All values were calculated in triplicates *(Values represent mean ± SEM of three replicates)

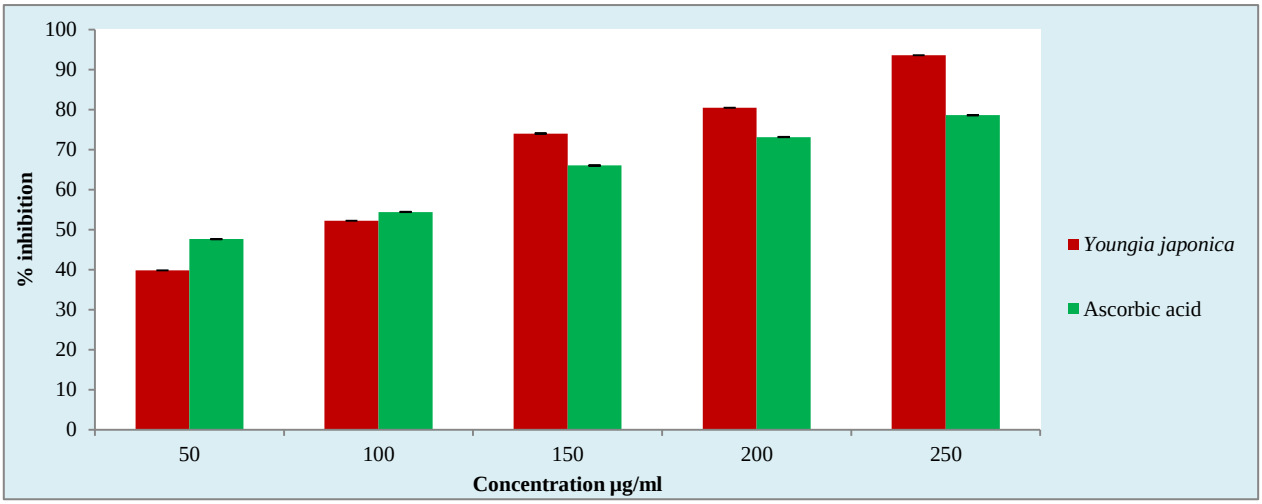

Figure 5: Difference in % Inhibition of Phosphomolybdenum by methanolic extract of *Youngia japonica* and ascorbic acid at various concentrations.

GC-MS study:

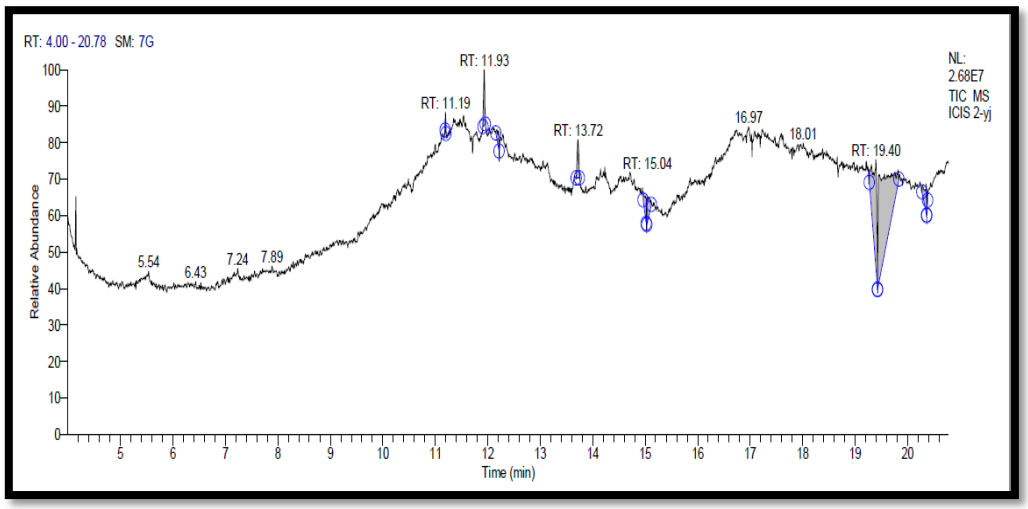

Figure 6: GC-MS spectra of methanolic leaf extract of *Youngia japonica.*

Table 5: GC-MS analysis of phytochemical compounds in the methanolic leaf extract of *Youngia japonica*.

RT	Compound name	Molecular formula	Peak Area	Area %
11.19	(5á)Pregnane3,20adiol,14a,18a[4methyl3oxo(1oxa4azabutane1,4d iyl)], diacetate (borreledin)	C28H43NO6	943128.40	0.55
11.93	Hexadecanoic acid, methyl ester	C17H34O2	6263142.10	3.64
12.20	psi.,.psi.Carotene,3,4didehydro1,1',2,2'tetrahydro1' hydroxy1methoxy	C41H60O2	2608637.10	1.52
13.72	9Octadecenoicacid(Z),2,3dihydroxypropyl ester	C21H40O4	5877825.56	3.42
15.01	Glycine,N[(3a,5a,7a,12a)24oxo3,7,12tris[(Trimethyl silyl)oxy]cholan24yl]methyl ester	C36H69NO6Si3	4054832.01	2.36
15.04	17(1,5Dimethylhexyl)10,13dimethyl3styrylh exadecahydrocyclopenta[a] phenanthren2one	C35H52O	5586454.41	3.25
19.40	psi.,.psi.Carotene,1,1',2,2'tetrahydro1,1' dimethoxy	C42H64O2	39927241.94	23.20
19.45	9,12,15Octadecatrienoic acid, 2,3bis [(trimethylsilyl)oxy] propyl ester, (Z,Z,Z)	C27H52O4Si2	100377488.13	58.34
20.35	Glycine,N[(3a,5a,7a,12a)24oxo3,7,12tris[(trimethyls ilyl)oxy]cholan24yl], methyl ester	C36H69NO6Si3	5639139.22	3.28
20.38	Glycine,N[(3a,5a,7a,12a)24oxo3,7,12tris[(trimethyls ilyl)oxy]cholan24yl], methyl ester	C27H52O4Si2	788717.08	0.46

In the present study we characterized the chemical profile of *Youngia japonica* using GC-MS analysis. The chromatogram showed the relative concentration of various compounds getting eluted as a function of retention time. Peak heights indicate the relative concentrations of the compounds present in the plant extract. The mass spectrometric analyses of the compound eluted at different times to identify the nature and structure of the compounds. Compound elution give rise to presence of peaks at different M/Z ratios. These mass spectra are fingerprint of that compound which can be identified from the NIST data library. Various compounds present in methanol leaf extract were (5a)Pregnane3,20adiol,14a,18a[4methyl3oxo(1oxa4azabutane1,4d iyl)], diacetate, Hexadecanoic acid, methyl ester, psi.,.psi.Carotene,3,4didehydro 1,1',2,2'tetrahydro1' hydroxy1methoxy, 9 Octadecenoic acid(Z),2,3 dihydroxy propyl ester, Glycine,N [(3a,5a, 7a,12a)24oxo3,7,12 tris [(Trimethylsilyl) oxy] cholan24yl]methylester,17 (1,5Dimethylhexyl) 10,13dimethyl3 styrylhexadecahydrocyclopenta[a]phenanthren2one,psi.,.psi.Carotene,1,1',2,2'tetrahydro1,1' dimethoxy, 9,12,15 Octadecatrienoic acid, 2,3bis [(trimethylsilyl)oxy] propyl ester, (Z,Z,Z). Among these (5a) Pregnane3, 20adiol, 14a, 18a[4methyl3oxo (1oxa4azabutane1,4d iyl)], diacetate exhibits antimalarial and antibacterial activity, Glycine, N[(3a,5a,7a,12a)24oxo3,7,12tris[(Trimethylsilyl)oxy]cholan24yl]methyl ester has antibacterial activity as reported by (Ganesh and Vennila, 2011) in *Acanthus ilicifolius*, Hexadecanoic acid, methyl ester has Antibacterial and antifungal activity (Abubakar, M.N and Majinda, R.R.T 2016), 9-octadecenoic acid, (Z)- 2,3-dihydroxypropyl ester (monoolein), is known for its surfactant and emulsifying properties, and is regarded as a 'magic lipid' (Ganem-Quintanar *et al*., 2000) due to its varied application in pharmaceuticals, agriculture, cosmetics, food and protein crystallisation. More notably is its use in drug delivery applications as a drug delivery enhancer, protein crystallization (Kulkarni *et al*., 2011).

4. CONCLUSION

Searching plant based sources may bring new natural products into food, cosmetic and pharmaceutical industry. An *in-vitro* antioxidant study provides scientific evidence to prove the traditional claims to the Asteraceae member, *Youngia japonica*. On the basis of the results obtained in the present study, it was concluded that the methanolic leaf extracts of this plant species possess significant antioxidant activity. Presence of enormous amount of phenol and flavonoid compounds may account for this information. So these findings of present study suggest that this plant have a potential source of natural antioxidant. Further studies are necessary for the isolation and characterization of antioxidant compounds, and also *in vivo* studies are desired for understanding their mechanism of action as oxidative scavengers. Presence of some of the active constituents in the plant extract provides the scientific evidences for the antimicrobial, antioxidant and antidiabetic properties of the plant. In addition to this the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant and paves the way for several treatment regimens based on the extract.

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