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Antioxidant activities and inhibitory effect of *Taraxacum officinale, Cichorium intybus* and *Lectuca sativa* on prooxidant induced lipid peroxidation in mice liver

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ABSTRACT

This study reports the antioxidant and protective properties of three dietary plants, *Taraxacum officinale*, *Cichorium intybus* and *Lectuca sativa* on lipid peroxidation in mice liver. Extracts showed significant ($p = 0.0392$) inhibition against thiobarbituric acid reactive species (TBARS) induced using the pro-oxidants iron (10 μM $FeSO_4$) and sodium nitroprusside (5 μM) in liver homogenates of mice. Free radical scavenging activity was evaluated by the quenching of DPPH radical ($p=0.00236$). Extracts also showed metal chelating activities ($p=0.00143$) and high antioxidant activity in phosphomolybdenum assay ($p=0.00246$). The high content ($p=0.000243$) of phenolics and flavonoids were detected in aqueous extracts of the plants which may be responsible for antioxidant activities. *Taraxacum officinale* comparatively showed higher antioxidant activities followed by *Cichorium intybus* and *Lectuca sativa*. It is concluded that polyphenolic-rich extracts of studied plants are potential sources of natural antioxidants.

Introduction

Free radicals are the molecules or the species having one or more electrons in atomic and also in molecular orbitals. These unpaired electrons are responsible for the reactivity of these species (Padmanabhan et al., 2012). Beneficial effects of free radicals include the defense against infectious agents and cellular signaling process (Valko et al., 2007). Free radicals also contribute to the flow of blood in arteries and fight against infections. These radicals also keep our brain alert and active (Sarma et al., 2010). A harmful effect is the oxidative stress. This is caused when reactive oxygen species or pro-oxidants and antioxidants are not equal. Cellular lipids, proteins and DNA are affected by these species (Valko et al., 2007). Free radicals are responsible for many diseases such as cancer, heart and other diseases. The modification of genetic material will cause the oxidative damage and represents the first step involved in the mutagenesis, carcinogenesis and aging (Gutowski et al., 2013). Oxidative stress is caused by the oxidation of amino acids side chains, oxidation of the peptide backbone of protein and formation of protein-protein cross chains. Protection against the reactive species is better provided by the combination of the different antioxidants than any antioxidant individually (Apak et al., 2013).

Antioxidants are the substances which provide protection from free radical damaging by inhibiting the autoxidation of the free radicals or delaying through different mechanisms, i.e. by scavenging the free radicals and metal chelation (Padmanabhan et al., 2012). Aromatic compounds or phenolic rings are present in the antioxidants. These antioxidants have the ability to donate the hydrogen to the free radicals. Antioxidants act at different stages and neutralize the free radicals. They act at different levels such as prevention level, repairment and interception. Different antioxidants are responsible for the processes including vitamins C and E, thiol compounds, glutathione, flavonoids

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and carotenoids etc. (Devasagayam et al., 2004).
Medicinal properties are associated with the natural products found in the herbs and medicinal plants. Phytochemicals and provitamins that assist in maintaining good health are associated with natural products found in plants (Ivanova et al., 2005). *Taraxacum officinale*, that belongs to family Asteraceae, is herbaceous perennial plant commonly called dandelion. The habitat of the plant are mostly temperate regions on roadsides, in lawns and distributed banks. Traditionally, this plant has been used for poor digestion, water retention and liver diseases including hepatitis (Sohail et al., 2014). The green leaves are used as salad, coffee-like drinks are made from roots, and wine is obtained from flowers. In leaves the concentration of vitamin C and A is high and more iron and calcium is present (Ozcan et al., 2012). *Cichorium intybus*, another plant of the family Asteraceae, commonly known as chicory, is widely distributed in Europe and Asia. The identification of this plant is its auxiliary capitulumns of the lovely blue colour and sometimes of white colour. This plant has tapering, fleshy roots 1-3 cm long angled branches, hispid oblong leaves and its flowers are mostly blue in colour (Zaman et al., 2013). Many medicinally important compounds are present in this plant. This plant is important for the treatment of many diseases such as jaundice, diarrhea and gallstones (Shad et al., 2013). Many other diseases cured by this plant are AIDS, cancer, insomnia and impotence. This plant contains the insulin which is used for the treatment of sugar and for reducing the calories. Investigation of the root extracts of these plants also showed that it has antitumor and anticancer activities. The major phytochemicals present in the roots are alkaloids (Nandagopal and Ranjitha, 2007).
Another plant from the family *Asteraceae* is *Lectuca sativa*, very important because of its medicinal properties. This plant has green leaves. It was found that this plant has many medicinal properties and is used in the treatment of stomach problems, because it increases the digestion, enhances appetite through anti-inflammatory activities due to the presence of triterpenes lactones (Arzu et al., 2009). These plants were chosen as they are frequently used in the diet and also in the herbal medicine as herbal drugs. As the antioxidant activity can vary greatly depending on the pro-oxidant used, we determined the effect of aqueous extracts against hepatotoxic agents such as iron sulphate and sodium nitroprusside. Hence, the objective of this study was to investigate the antioxidant and inhibitory effect of *Taraxacum officinale*, *Cichorium intybus* and *Lectuca sativa* on Fe(II) and sodium nitroprusside lipid per-oxidation in mice liver in vitro.

Materials and methods

Chemicals

Thiobarbituric acid (TBA), malonaldehyde- bis-dimethyl acetal (MDA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, rutin, gallic acid, and phenanthroline were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium nitroprusside (SNP) was obtained from Merck (Darmstadt, Germany) and Iron (II) sulfate from Lahore, Pakistan.

Preparation of plant extracts

The roots and leaves of plants were collected from different areas of district Rawalakot Azad Kashmir from April till June 2015 and identified by the taxonomist at University of Poonch Rawalakot.
The roots and leaves of plants (25 g) were ground and soaked in boiling water (500 ml) for 15 minutes, allowed to cool and filtered using Whatman filter paper No.1. The resulting residue was further extracted twice and finally the whole extract was concentrated in a rotary evaporator (50 °C). Serial dilutions were prepared to obtain the desired concentration of plant for the experiments.

Test animals

All animal procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by University of Poonch, Ethical Council (UPR 10077). BALB/c mice (20 – 25 g) were purchased from National Institute of Health, Islamabad. The animals were kept in separate cages with access to water and food ad libitum, in a room with controlled temperature (22±3 °C) and in 12 h light/dark cycle.

Production of TBARS from animal tissues

Production of TBARS was determined using a modified method (Ohkawa et al., 1997). The mice were anesthetized with chloroform and sacrificed by decapitation. Liver were quickly removed and placed on ice. Tissues (1:10, w/v) were homogenized in cold 100 mM Tris buffer pH 7.4 (1:10 *w/v*) and centrifuged at 1,000 x g for 10 minutes. The resulting homogenates (100 μL) were

incubated with or without 50 µL of freshly prepared oxidant (iron and sodium nitroprusside) and different concentrations of the extracts together with proper volume of deionized water to give a total volume of 300 µl at 37 °C for 1 h. The colour reaction was done by adding 200, 500 and 500 µL of the 8.1% Sodium dodecyl sulphate (SDS), acetic acid (pH 3.4) and 0.6% TBA, respectively. The reaction mixtures, including those of serial dilutions of 0.03 mM standard MDA, were incubated at 97 °C for 1 h. The absorbance of tubes was read after cooling at a wavelength of 532 nm in a spectrophotometer.

DPPH radical scavenging

Scavenging of the stable DPPH radical (ethanolic solution of 0.25 mM) was assayed in vitro (Hatano et al., 1988) and the absorbance was measured at 517 nm. Percent inhibition was calculated from the control. Ascorbic acid was used as a standard in DPPH assay.

Antioxidant potential assay

The total antioxidant potential of the extracts was estimated using the phosphomolybdenum reduction assay (Prieto et al., 1999). The reducing capacity of the extract was expressed as the ascorbic acid equivalents.

Metal chelating activity

The iron chelating ability of the extract was determined using a modified method (Puntel et al., 2005) at 510 nm.

Determination of Phenolics content

The total phenolic content was determined by the method (Singleton et al., 1999). The mean of three readings was taken and the total phenolic content was expressed in milligram of gallic acid equivalents/g extract.

Determination of total flavonoids

The total flavonoid content as quercetin equivalents/g extract was based on the method (Kosalec et al., 2004).

Data analysis

The results were expressed as means ± SD. The data were analyzed by one way ANOVA and different group means were compared by applying Duncan's multiple range test (DMRT); $p<0.05$ was considered significant in all cases. The software package, Statistica was used for statistical analysis.

Table 1. Total Phenolic and flavonoid contents of aqueous extracts of the *Taraxacum officinale, Cichorium intybus* and *Lectuca sativa*

Plants	Phenolic content (mg GAE/g extract)	Flavonoid content (mg GAE/g extract)
TO (leaves)	83.5±4.2^{a}	7.9±0.1^{a}
TO (roots)	77.8±4.5^{b}	6.03±0.2^{b}
CI (leaves)	69.54±3.4^{c}	6.789±0.4^{b}
CI (roots)	60.87±3.0^{d}	5.765±0.9^{c}
LS (roots)	46.98±3.6^{e}	6.5±1.1^{d}
LS (leaves)	39.65±3.0^{f}	4.876±2.0^{e}

Values in table which share different letters are significantly ($p<0.05$) different from each other by DMRT. TO= *Taraxacum officinale*, CI= *Cichorium intybus*, LS= *Lectuca sativa*

Table 2. Percentage DPPH scavenging activity (PS) of the leaves and roots of the *Taraxacum officinale*, *Cichorium intybus* and *Lectuca sativa*

Concentration of extracts (µg/ml)	PS by leaves extract of TO	PS by root extract of TO	PS by leaves extracts of CI	PS by root extract of CI	PS by leaves extract of LS	PS by root extract of LS
25	66±1.2^{a}	46±2.1^{b}	54±2.3^{c}	40±2.1^{d}	55±0.9^{ce}	42±1.1^{f}
50	72±1.3^{a}	49±2^{b}	59±2.1^{c}	49±2.3^{bd}	63±0.8^{e}	49±1.2^{bf}
75	79±1.1^{a}	61±2.3^{b}	68±1.3^{c}	55±2.3^{d}	69±2.1^{de}	58±1.1^{f}
100	83±1.5^{a}	72±2.4^{b}	71±1.2^{c}	60±2.4^{d}	71±2.1^{cd}	67±1.3^{e}
200	86±2.1^{a}	79±2.1^{b}	75±1.9^{c}	68±2.5^{d}	77±2.3^{e}	73±1.3^{f}

PS= Percentage scavenging, TO= *Taraxacum officinale*, CI= *Cichorium intybus*, LS=*Lectuca sativa*. Values in table which share different letters are significantly ($p<0.05$) different from each other by DMRT.

Results and discussion

Phenolic and flavonoid content of extracts

Phenolic compounds are the important class of antioxidants and are termed as free radical terminators. Antioxidant activity of the phenolics is associated with the scavenging of free radicals, hydroxyl radical and single oxygen (Ghaima et al., 2013). The present study revealed that the leaves of the *Taraxacum officinale* have higher phenolic content than the roots (Table 1). A significant difference (p=0.000143) was found between phenolic and flavonoid contents among different plant species. Similar observation was presented in earlier investigations and it was suggested that leaves show high antioxidant activity due to the presence of polyphenols. They play important role in the defense mechanism against endogenous and exogenous free radicals (Shad et al., 2013). The leaves extract of the *Taraxacum officinale* showed the highest phenolic content 26.4 GAE mg/g extracts, while in roots the value was found to be 18 GAE mg/g of extracts (Ozcan et al., 2012). Aqueous extracts of *Taraxacum officinale*, *Cichorium intybus* and *Lectuca sativa* were analyzed for the quantitative determination of the phytoconstituent including phenols and flavonoids (Table 1). The effects of the flavonoids on human health and human nutrition are considerable. All the plants showed the same trend in the flavonoid content as in the phenolic content. The highest amount is found in the aqueous leaves extracts of the *Taraxacum officinale*. The leaves extract of *Lectuca sativa* contained the least quantity of flavonoids. The high content of phenolics and flavonoids in *T. officinale* correspond to the studies of García-Carrasco et al. (2015).

DPPH radical scavenging activity

Free radical scavenging activity by the DPPH method was analyzed for all the extracts in the study. The antioxidant activity of different extracts showed that it also depends upon the concentration (Table 2). There is difference among the antioxidant activities of three plants (p=0.00236). The results clearly indicate that all the extracts have significant antioxidant potential. The strong scavenging activity was shown by the leaves of *Taraxacum officinale* followed by *Cichorium intybus* and *Lectuca sativa*. Total antioxidant activity of the root extracts of the *Taraxacum officinale* is lower than the leaves but higher than other plants extracts. $DPPH^{\bullet}$ is

Table 3. Antioxidant activities of *T. officinale*, *C. Intybus* and *L. Sativa* in mice liver

Concentration µg/ml of extract	TBARS inhibition against iron by root extract of *T. officinale*	TBARS inhibition against SNP by root extract of *T. officinale*	TBARS inhibition against iron by root extract of *C. Intybus*	TBARS inhibition against SNP by root extract of *C. Intybus*	TBARS inhibition against iron by root extract of *L. sativa*	TBARS inhibition against SNP by root extract of *L. sativa*
Basal	246.47±12.0^{a}	249±5.6^{b}	246.47±4.5^{c}	249±4.5^{bd}	246.47±4.6^{ae}	249±3.4^{df}
Control	530.28±10.1^{a}	518±7.1^{b}	530.28±5.1^{ac}	518±5.2^{bd}	530.28±3.4^{ae}	518±3.2^{bf}
25	355±12.3^{a}	478±4.5^{b}	317±6.0^{c}	483±4.6^{d}	292±4.5^{e}	378±4.5^{f}
50	339±1.0^{a}	412±3.5^{b}	299±7.3^{c}	400±5.0^{d}	287±4.7^{e}	345±4.6^{f}
75	362±10.1^{a}	378±4.3^{b}	278±5.0^{c}	378±3.2^{bc}	258±5^{d}	315±8.1^{e}
100	387±11.0^{a}	275±2.3^{b}	256±6.5^{c}	349±4.0^{d}	239±5.4^{e}	288±4.5^{f}
200	393±12.0^{a}	338±5.1^{b}	235±3.4^{c}	316±5.0^{d}	216±4.9^{e}	267±6.1^{f}

SNP= sodium nitroprusside, Values in table which share different letters are significantly (p<0.05) different from each other by DMRT.

Table 4. Metal chelation by aqueous extract of *Taraxacum officinale* (TO), *Cichorium intybus* (CI) and *Lectuca sativa* (LS)

Concentration of extracts (µg/ml)	Percentage chelation by leaves extract (TO)	Percentage chelation by root extract (TO)	Percentage chelation by leaves extract (CI)	Percentage chelation by root extract (CI)	Percentage chelation by leaves extract (LS)	Percen- tage chelation by root extract (LS)
25	51±1.1^{a}	40.9±2.1^{b}	48.5±3.0^{c}	39±1.0^{d}	47±3.1^{e}	39±4.0^{df}
50	57±1.2^{a}	49±1.0^{b}	52±2.0^{c}	42±2.0^{d}	52±3.2^{ce}	46±4.2^{f}
75	63±1.0^{a}	55±2.0^{b}	58±3.0^{c}	46±2.1^{d}	54±2.3^{e}	47±4.3^{f}
100	69±0.9^{a}	60±3.0^{b}	61±4.0^{c}	53±2.3^{d}	58±3.1^{e}	51±4.0^{f}
200	71±3.1^{a}	68±2.0^{b}	64±2.0^{c}	59±2.1^{d}	61±3.4^{e}	55±3.9^{f}

Values in table which share different letters are significantly (p<0.05) different from each other by DMRT.

considered to be a model of stable lipophilic radical. Antioxidants react with DPPH˙ and reduce the number of DPPH free radicals to the number of available hydroxyl groups. The absorbance at 517 nm is proportional to the amount of residual DPPH• and can be noticed as a colour changes from purple to yellow. The results of other determinations carried out by different researchers on the different plants by using the DPPH method. It was also concluded that antioxidant activity has the strong relation with the total phenolic and flavonoids contents (Ozcan et al., 2012). Ethyl acetate extracts of the nettle and the *Taraxacum officinale* showed 76% and 45% scavenging respectively.

This potential is due to the presence of the flavonoids and phenolic compounds (Ghaima et al., 2013). The high DPPH radical scavenging activities of these plant extracts suggest their usage against degenerative diseases. Our results are in line with the studies of Garcia-Carrasco et al. (2015) where the leaves and root extract of *T. officinale* has shown the DPPH activity and total antioxidant activities by FRAP assay.

Lipid peroxidation inhibition by the extracts

Lipid peroxidation in mice liver was induced with iron (10 μM) and sodium nitroprusside (5 μM) and the antioxidant effect of plant extracts was determined. Increases in the formation of TBARS in iron(II) sulphate (10 μM) induced oxidative stress, as compared to the normal, suggest possible damage of tissues with an overload of iron. The iron overload is less frequent, but high contents of tissue iron have been associated with several pathological conditions, including liver, heart diseases, cancer and neurodegenerative disorders (Milman et al., 2001). Sodium nitroprusside is an antihypertensive drug which acts by relaxation of vascular smooth muscle; consequently it dilates peripheral arteries and veins. However, earlier studies have shown that photo degradation of SNP ultimately produces NO, $[(CN)_5\text{-}Fe]^{3+}$ and $[(CN)_4\text{-}Fe]^{2+}$ species (Bates et al., 1990). Nitric oxide is a molecule that is regarded as a universal neuronal messenger in the central nervous system and is involved in pathophysiology of disorders such as Alzheimer's and Parkinson's diseases, stroke, trauma and seizures etc. (Bolanos and Almeida, 1990).

The inhibitory effect of the aqueous extracts of *Taraxacum officinale*, *Lectuca sativa* and *Cichorium intybus* against the lipid peroxidation was analyzed in the present study (Table 3). It is evident from the results that the extracts of the roots of these plants showed lipid peroxidation inhibitory activity. There is significant difference (p=0.0392) in three plant species at different concentrations. To sum up extracts of *Taraxacum officinale* have proved to be stronger and more consistent inhibitor of lipid peroxidation followed by the *Cichorium intybus*. The lowest inhibitory effect is shown by *Lectuca sativa*. Our results are in line with the studies of Garcia-Carrasco et al. (2015) where the leaves and root extract of *T. officinale* have shown their effects on lipid accumulation in adipocytes.

Although the antioxidant activities of *Taraxacium officinale* is reported by several authors (Colle et al., 2012; Ivanov, 2014; García-Carrasco et al., 2015). This study shows the antioxidant and antilipid peroxidative properties of *Taraxacum officinale*, *Cichorium intybus* and *Lectuca sativa* by using different hepatotoxic and neurotoxic agents like iron and sodium nitroprusside using mice liver homogenates. The antioxidant activities of these plants are evaluated for the first time in *In vitro* system against well known prooxidants. The efficacy of the extracts against iron and sodium nitroprusside also suggests that they may be utilized against iron and sodium nitroprusside overload.

Metal chelation by extracts

The plants which are taken in the present study were found to show high iron chelating ability. The chelation ability increased by increasing the concentration of the extracts (Table 4). The significant difference (p=0.00243) was found in the chelating abilities of three plant species. Foods are often contaminated with transition metal ions that may be introduced during processing. Bivalent transition metal ions catalyze the oxidative processes resulting in the formation of hydroxyl radicals, in addition to hydroperoxide decomposition reactions, via the Fenton reaction (Wang and Fordham, 2007). These processes can be delayed by iron chelation and deactivation. The ability of the extract to chelate iron was measured as a percentage of iron chelating. The chelating agent disrupts the complex formation with 1,10-phenathroline and iron leads to a decrease in colour intensity. Metal ions are necessary for normal health physiology. On the other hand, metal ions can cause serious health damage. Transition metals such as iron, zinc and copper make the complexes in the biological systems. During complex formation there is generation of the ROS in the cells leading to metal toxicity. Metal toxicity can be treated by the chelation therapy. In this process the metal ions are chelated and toxic and excess metal ions are removed from the system and reduce the toxic effect.

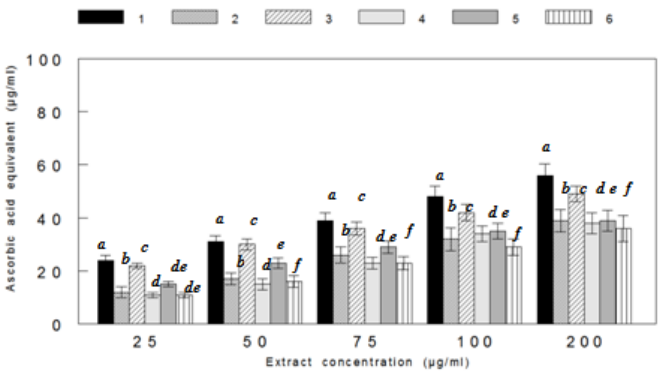

Fig. 1. Total antioxidant activities of plant extracts by phosphomolybdenum assay. 1= total antioxidant activity of leave extract of *Taraxacum officinale*, 2= total antioxidant activity of root extract of *Taraxacum officinale*, 3= Total antioxidant activity of leaves extract of *Cichorium intybus*, 4= Total antioxidant activity of root extract of *Cichorium intybus*, 5= Total antioxidant activity of leaves extract of *Lectuca sativa*, 6= Total antioxidant activity of root extract of *Lectuca sativa*. Values in Figure which share different letters are significantly ($p < 0.05$) different from each other by DMRT.

Oxidative stress caused by ferrous ions lead to many diseases like Alzheimer's syndrome which is a neurological disorder (Ebrahimzadeh et al., 2008). Many types of metal chelators are available for the toxic metals chelation, but selection of the chelator as an ideal chelator is very difficult. Metal chelators should be specific and proper administrated (Flora et al., 2010). Naturally, plants contain the phytochemicals such as phenols and flavonoids, which are responsible for the chelation of the metals and also prevent lipid peroxidation (Khan et al., 2014).

Total antioxidant activity by extracts

Antioxidant activities of the roots and leaves extracts of three medicinal plants *Taraxacum officinale*, *Lectuca sativa* and *Cichorium intybus* was also assessed by phosphomolybdenum reduction method (Fig. 1). The results are shown as ascorbic acid equivalent which was used as standard compound in the assay. A significant difference (p=0.00246) was observed among the antioxidants of plant species. However, the leaves extracts of *Taraxacum officinale* and *Cichorium intybus* have shown almost similar activities. In the phosphomolybdenum assay, which measures the total antioxidant capacity, the extract demonstrated electron-donating capacity showing its ability to act as chain terminators, transforming relative free radical species into more stable non-reactive products (Dorman et al., 2003).

Conclusions

Results of this study demonstrated the high efficacy of crude extracts of *Taraxacum officinale*, *Cichorium intybus* and *Lectuca sativa* for free radical scavenging, inhibition of reactive oxygen species and lipid peroxidation. These effects may be associated with the usage of these plants for medicinal purposes and they can be used as functional food with effectiveness for treatment of degenerative diseases. These plants can be considered as a source of plant antioxidants with a potential use in food, cosmetics, and pharmaceutical fields. However, more detailed in vivo studies are required for evaluation of the antioxidant activities and bio-availability of leaves and root compounds.

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