Antioxidant activity of chloroform extract of *Inula Racemosa* from Kashmir Himalayas

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Abstract

Antioxidant activity of chloroform extract of root parts of *Inula racemosa* was evaluated by measuring the scavenging activity of the extract on stable 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) exhibiting an interesting antioxidant profile. The reducing power was determined using a modification of Zou method. Metal chelating activity of the extract was determined at concentrations of 20, 30, 50 and 100 µg/mL, taking citric acid as standard. The extract displayed significant activity.

Key words: *Inula Racemosa, Chloroform Extract, Antioxidant Activity, Reducing Power, Metal Chelating Activity.*

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INTRODUCTION

Inula racemosa is distributed in temperate alpine Himalayas at an altitude of 1,500- 4,200 from Kashmir to Kumaon, Afghanistan to Central Nepal. It occurs wild among strong alpine scrub vegetation in the cold arid habitat of NW Himalayas between 2,700-3,500 m in the eastern Ladakh (Leh) region of Kashmir. Domesticated forms of this incipient cultigen are cultivated on borders of agricultural fields of wheat, barley and buckwheat both in Kashmir and Lahaul valley of Himachal Pradesh. (Wealth of India -Raw Materials, 1959)

The roots of the plant have a dull brownish skin with yellowish colour inside. They possess a sweet and somewhat camphoraceous odour and have a bitter taste (Chopra RN, et al, 1956). Pushkarmool commercially is a very important medicinal plant of the North western Himalayas. The plant is used in Ayurveda as an expectorant and resolvent in indurations. Considered a 'Rasayana' (rejuvenator, immunomodulator) by Ayurvedic physicians, the drug according to Bhavaprakasha (Bhavaprakasha Bhavaprakash Nighantu, 1961) is bitter pungent in taste. When administered it mitigates vatakapha jwara (fever caused by vata pitta imbalance), sotha (swelling), arachi (anorexia), swasa (breathlessness) and parswasoola (pain in the sides of the chest).

The root is medicinal and considered a specific for cough, dyspnea, asthma, pleurisy, tuberculosis and chest pain especially pre-cordial pain. The aqueous extract of the fresh or dry roots is given orally in rheumatic pains and liver problems. Externally a paste or liniment is used for relieving pain. The root is also used in veterinary medicine as a tonic (Wealth of India-Raw Materials, 2002). The root forms an important ingredient of several polyherbal formulations for heart diseases and inflammatory conditions of spleen and liver (The Ayurvedic pharmacopoeia of India Part 1 Second edition, 1978). Along with *Commiphora mukul*, the drug combination called 'pushkar guggulu' is a popular anti obesity, hypolipidemic indicated in cardiac ailments.

Inula racemosa is used in Chinese medicine for abdominal distension and pain, acute enteritis and bacillary dysentery (Tsarong, T.J, 1994). There are so many medicinal properties reported in literature pertaining to various Inula species. *I. racemosa* contain oxygenated allantolides e.g. 4-(15)-α-epoxy isotelekin and perhydro epoxyalantolide (Goyai R, et al, 1990). It also contains sesquiterpene lactones and lignans. (Tan RX, et al, 1998). *I. britanica* has been reported to contain polyphenolic constituents, exdate flavonoids (So RK, et al, 2002). Pulchellin C, Kaurane glycosides (Yu S, et al,1996) and aromatic esters (Anthonsem, et al,1971). *I. germanica* has been reported to contain sesquiterpenoid lactones (Konovalova OA, et al, 1974). *I. crithmoides* contains methoxylated flavonols, chlorinated thymol derivatives, sesquiterpene lactones (Mohamed AM, et al, 1985). *I. grantiodes* contains triterpenes, sitosteryl glucosides, olefins, flavones, and fatty acids (Burdi DK, et al,1992). I. *caspica* has been reported to possess incapsin, a new sesquiterpene lactone and britanin (Petrisheva; LP,1984) *I. Indica* contains germacranolides (Bhimsen AN, et al, 1981). *I. royleana* contain quinones, abietane triterpenoids, β-caryophyllene, exoxides, diterpenoids (Edwards OE, et al,1962). *I.viscosa* has been reported to contain sesquiterpenoids (Musa H, et al,1998), eucalptol, thymol content, eudesmane acids, oxygenated nerolidol esters, carboxy eudesmadiene (Lauro L, et al,1990).

MATERIALS AND METHODS

Plant Collection

The plant material of *Inula racemosa* was collected in the mid of July 2013 from Indian Institute of Integrative Medicine (IIIM) Srinagar (Kashmir, India) and authenticated by Prof. A.R. Naqshi and Dr. Anzar Khuroo (University of Kashmir) and by comparison of the collected sample with that growing in Department of Plant Taxonomy, University of Kashmir. A voucher specimen (voucher number: IIIM 1125/13) of the plant was deposited in the herbarium of the Institute.

Extraction

The roots of *Inula racemosa* were obtained from the laboratory stock. The roots were dried and powdered. The root material of *Inula racemosa* (200 g) was extracted by soxhlet extraction apparatus using chloroform as the solvent. The soxhlet extraction was carried on for 24 h in two batches and the solvent was distilled off to obtain the crude extract of plant material. The chloroform extract was distilled to yield yellow oil (5 g).

Chemicals

2,2-diphenyl-1-picryl hydrazyl radical, Methanol, Chloroform, α- tocopherol, sodium phosphate buffer, potassium ferricyanide, trichloroacetic acid, ferric chloride, BHT, ferrous chloride, citric acid (Qualigens Fine Chemicals, CDH Chemicals) were used during the study.

Antioxidant Activity

DPPH (1, 1-dihpenyl-2-picrylhydrazyl) Radical Scavenging Activity

The scavenging activity of the 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) radical was measured according to the standard method (Gani A, et al, 2015). The reaction mixture consists of 1 mL of 0.01% methanolic solution of DPPH and 100 µL of sample solution with varying concentration (20-100 µg/mL). The volume was made up to 3 mL by methanol. The solution was incubated for 30 min in dark. The absorbance of sample and α- tocopherol as a standard was read at 517 nm. Percentage inhibition was calculated by using the formula:

$$\% \text{ inhibition} = A_{control} - A_{sample} / A_{control} \times 100 \quad \text{---------- (1)}$$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract.

Reducing Power

The reducing power was determined using a modification of Zou method (Oyetayo VO, et al, 2009). 100 µL extract of varying concentrations (20-100 µg/mL) was separately mixed with 0.2 M sodium phosphate buffer (pH 6.6, 2.5 mL) and 1% (w/v) aqueous potassium ferricyanide (2.5 mL). After incubated the solution for 20 min at 50°C, 10% (w/v) of trichloroacetic acid (2.5 mL) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was diluted with deionized water (2.5 mL) and 0.1% (w/v) ferric chloride (0.5 mL) was added. The absorbance was measured at 700 nm and compared to BHT (standard).

Metal Chelating (Fe^{+2}) Activity

Chelating ability was determined according to the standard method (Dinis TC, et al,1994). The reaction mixture, containing 100 µL of sample with varying concentration (20-100 µg/mL), 0.5 mL ferrous chloride (2 mM), 0.25 mL ferrozine (5 mM) and volume adjusted to 3 mL with methanol, vortexed (mixed using a vortex mixer) and then incubated for 10 min at room temperature. The absorbance of the sample and citric acid standard was measured at 562 nm against the blank.

$$\% \text{ Chelation} = [1 - (A_{sample} / A_{control}) \times 100] \quad \text{---------- (2)}$$

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract.

Results and Discussion

Antioxidant Activity

Free radicals, at high concentration, mediate the damage of cell structures, nucleic acids, lipids and proteins. Examples of reactive oxygen species (ROS) induced DNA damage products involve single or double stranded DNA breaks, purine, pyrimidine or deoxyribose modifications. Such modifications of genetic material may represent the first step in mutagenesis, carcinogenesis and ageing. Free radical mediated DNA damage has been found in various cancer tissues. Thus, it has been hypothesized that free radical scavengers are molecules that could prevent or limit the damage provoked by free radicals (Valko M, et al, 2006).

DPPH, in its radical form, has strong visible absorption and high molar extinction coefficient at 517 nm. On reaction with an antioxidant, the absorbance diminishes (Alkan M, et al,2008).

The anti-oxidative effect of the extract was examined by its radical scavenging effects by measuring changes in absorbance of DPPH radical at 517 nm (Table 1, Fig 1). Results revealed that the DPPH scavenging ability shows the tendency to increase with the increase in concentration. The absorbance of sample and α- tocopherol, as a standard, was read at 517 nm. Percentage inhibition was calculated by using the formula:

% inhibition = $A_{control}$ - A_{sample}/ $A_{control}$ × 100 ---------- (3)

Where, $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract.

Table 1: DPPH scavenging activity (Absorbance)

Concentration (µg/mL)	Extract	α-tocopherol
20	20.91	28.70
30	30.23	38.22
50	46.40	50.29
100	65.21	70.24

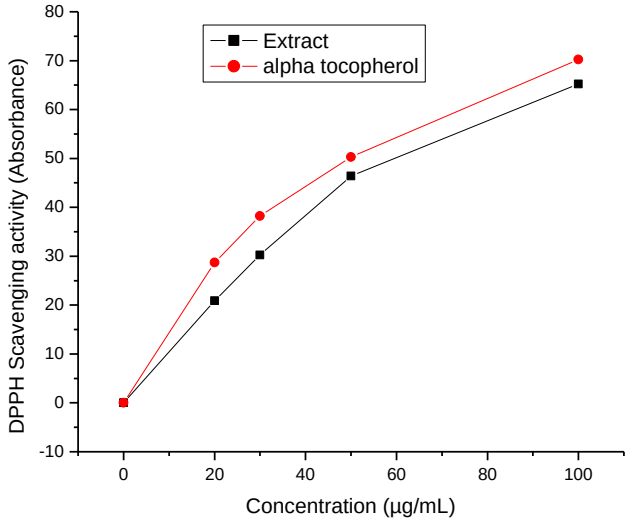

Fig 1 DPPH scavenging activity of the extract at different concentrations

Reducing Power

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe+3) in ferric chloride to ferrous (Fe+2). The reducing capacity of compounds could serve as an indicator of antioxidant properties (Zhao H, et al, 2006) and the increasing absorbance suggests an increase in reducing power.
Compounds with reducing power indicate that they are electron donors and can educe the oxidized intermediates.

The reducing power of the extract, at different concentrations, is presented in Table 2, Fig 2. The absorbance was measured at 700 nm and compared to butylated hydroxytoluene (BHT) as standard. The data revealed that the reducing power of the extract increases with the increase in concentration.

Table 2: Reducing power (Absorbance)

Concentration (µg/mL)	Extract	BHT
20	0.12	0.54
30	0.15	0.66

50	0.35	0.75
100	0.41	0.97

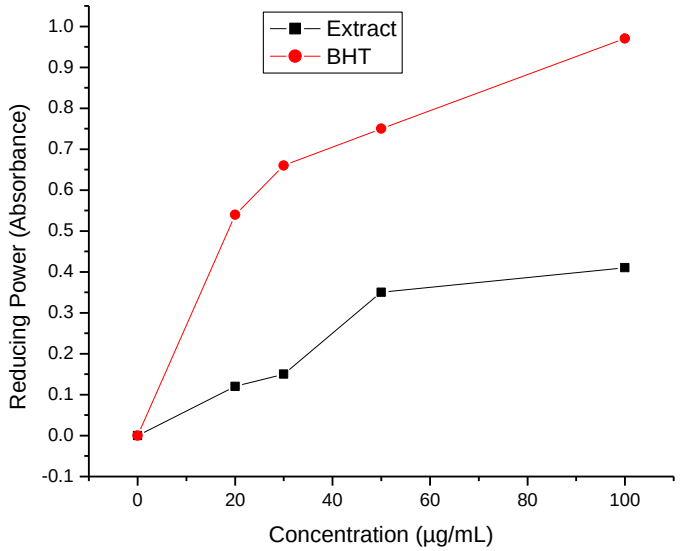

Fig 2 Reducing power of the extract at different concentrations

Metal Chelating Activity

Direct reaction of a substance is not the only mechanism by which the antioxidants may display their activity. Secondary, preventive, or type 2, antioxidants act through numerous possible mechanisms. These antioxidants do not convert free radicals to more stable products, but slow the rate of oxidation by several different mechanisms. One of the most important mechanisms of action of secondary antioxidants is chelation of pro-oxidant metals. Iron and other transition metals (copper, chromium, cobalt, vanadium, cadmium, arsenic, nickel) promote oxidation by acting as catalysts of free radical reactions. These redox-active transition metals transfer single electrons during changes in oxidation states. Chelation of metals by certain compounds decreases their pro-oxidant effect by reducing their redox potentials and stabilizing the oxidized form of the metal. Chelating compounds may also sterically hinder formation of the metal hydroperoxide complex (Reische D W, et al, 2008).

Chelating activity of the extract sample was determined at concentrations of 20, 30, 50 and 100 µg/mL, taking citric acid as standard. The oil displayed significant chelating ability, which appeared to increase significantly with the increase in concentration. The absorbance of sample and citric acid standard was measured at 562 nm against the blank.

% Chelation = $[1- (A_{sample} / A_{control}) \times 100$ ---------- (4)

where $A_{control}$ is the absorbance of the control reaction and A_{sample} the absorbance in the presence of the sample.

Table 3: Metal chelating activity (Absorbance)

Concentration (µg/mL)	Extract	Citric acid
20	0.41	0.57
30	0.50	0.83
50	0.72	0.94
100	0.86	0.93

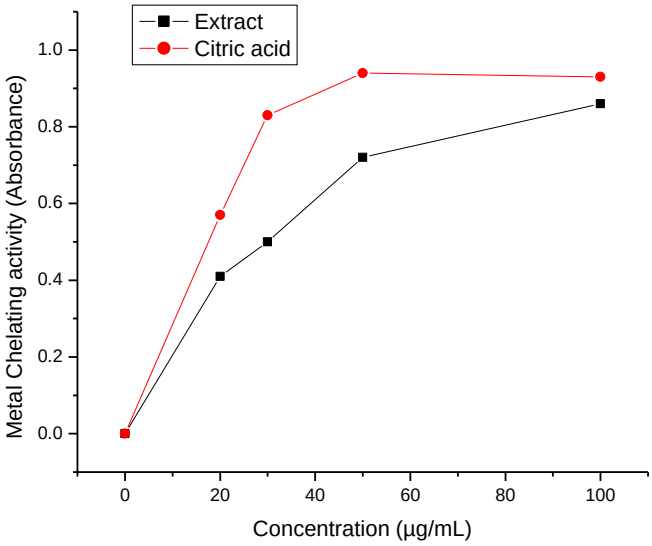

Fig 3 Chelating activity of the extract at different concentrations

Conclusion

The extract displayed significant antioxidant activity at various concentrations on stable 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH). The activity was found to increase with increase in concentration. Same trend was noticed in case of reducing power. Metal chelating activity of the extract was found to be very close to the standard, especially at concentration of 100 µg/mL.

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