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Antioxidant and Antibacterial Activity of *Jurinea dolomiaea* Boiss Extracts

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ABSTRACT- The aim of this study was to investigate in vitro antioxidant activity and anti-bacterial activity of the petroleum ether, ethyl acetate and methanol extract obtained from the whole part of *Jurinea dolomiaea* Boiss (Asteraceae). Total phenolic and flavonoid contents of these extracts were determined as gallic acid and rutin equivalents, respectively. Total antioxidant activity, reducing power of these extract were evaluated as ascorbic acid and gallic acid equivalents, respectively. ABTS free radical scavenging activity is expressed as trolox equivalent antioxidant capacity (TEAC). The antibacterial activity of the extract was investigated by disc diffusion method. The ethyl acetate and methanol extracts showed moderate activity against *E. coli* and *S. aureus*.

Key words: *Jurinea dolomiaea*; Total phenolic; Total flavonoid; Total antioxidant; Free radical scavenging activity; Antibacterial activity.

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INTRODUCTION

Jurinea dolomiaea Boiss syn *Jurinea macrocephala* Royle is rosette-forming stemless perennial herbs. Leaves flat on the ground, to about 15-45cm long, pinnate to pinnatisect, the leaflets again lobed or toothed, white woolly beneath, mid to grey-green above with a reddish midrib, achenes, ashy grey. Flowerheads purple, 2-3cm across, up to thirty in a central domed cluster in the centre of each rosette, late summer to early autumn, Pakistan to eastern Nepal, on open slopes at 4000m (Naithani BD 1984). Plant is used in Indian traditional system of medicine. A decoction of the root is cordial; it is given in the treatment of colic and puerperal fever & the bruised root is applied as a poultice to eruptions (Chopra RN et al., 1986). The plant is used in Nepal for incense and the juice of the roots is used in the treatment of fevers (Manandhar, 2002).

The limited phytochemical work on *Jurinea* species revealed that their main constituent was the sesquiterpene lactones (Rustaiyan et al., 1981; Todorova and Ognyanov 1984; Zakirov et al., 1975).

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The germacranolides oxygenated at C-14 and C-15are characteristic for this genus (Rustaiyan and Ganji 1988).

The pentacyclic triterpenes were also found in root of *Jurinea albicaulis* (Todorova M and Ognyanov I 1996). *Jurinea* species are reported to be useful as antimicrobial Activity (Kirbag S et al., 2009), antioxidant activity and anticholinesterase Activity (Ozturk H et al., 2011). *Jurinea dolomiaea* have been previously reported antibacterial activity (Dwivedi and Wagay, 2014), and antioxidant activity (Shah N *et al*., 2014).

Materials and Methods

Plant collection and identification:

Fresh plant material of *Jurinea dolomiaea* Boiss were collected in October 2012 from the North-West Himalayas, Uttarakhand, India and identified from Forest Research Institute, Dehradun, India to authentic sample.

Extract preparation:

The whole plant of *J. dolomiaea* was dried at room temperature (25° C). The dried sample was chopped into small parts with a blender. Powder (2 kg) was extracted successively with petroleum ether, ethyl acetate and methanol in soxhlet apparatus for 24 h. The extracts were filtered over filter paper and the organic solvent extracts were concentrated under vacuum using rotary evaporator and the crude extract was obtained, separately.

Determination of total phenolic content:

Total phenols were determined by a Folin-Ciocalteu method (Wolfe G et al., 2003). The measurement was conducted by mixing 2.5 ml of working Folin-Ciocalteu solution, 0.5 ml extracts and 2 ml of saturated

sodium carbonate solution. The absorbance was measured after 30 minutes at 765 nm, along with the blank. The standard gallic acid diagram was prepared by adding gallic acid of different concentration instead of 0.5 ml of sample. The total phenolic content was calculated as phenols equivalent to gallic acid (mg GAE/g).

Determination of total flavonoid content

Total flavonoids in the plant extracts were estimated by using the methods (Patel et al., 2011). The extract (500µL) was diluted appropriately and mixed with 1ml $NaNO_2$ (5%). After standing for 6min, 1ml of 10% $AlCl_3$ and 10ml of NaOH (1M) were added to the mixture. The mixture was adjusted to 25ml with 70% ethanol and allowed to rest for 15min. The absorbance was measured at 510nm, with 70% ethanol as a blank control. Total flavonoid content was estimated by using a calibration curve of rutin and expressed as mg rutin equivalents per g of sample (mgRE/g).

Total antioxidant capacity

Sample (0.3ml) was mixed with 3.0ml reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min under water bath. Absorbance of all the sample mixtures was measured at 695nm (Prieto, Pineda & Aguilar 1999). Total antioxidant capacity was expressed as ascorbic acid equivalent per gram extract (mgAAE/g).

Reducing power

The reducing power of Extracts was determined according to the methods (Oyaizu M 1986). Different concentration of extracts (50-500µg/ml) in 1 ml of extracts in 1ml of alcohol was mixed with 2.5ml phosphate buffer (0.2M, pH6.6) and 2.5ml of 1% potassium ferri-cyanide. The mixture was incubated at 50°C for 20min and 2.5ml of 10% trichloroacetic acid was added. The reaction mixture was then centrifuged for 10min. Further, 2.5ml of the supernatant solution was mixed with 2.5ml of distilled water and 0.5ml of 0.1% $FeCl_3$. The absorbance was measured at 700 nm.

2.7. DPPH free radical scavenging activity:

The free radical scavenging activity of extracts was measured by 1,1-diphenyl-2-picryl-hydrazil ($DPPH^{\cdot}$) using the method (Sheng et al. 2007). A 2ml aliquot of solution was added to 2ml of 2x10-4mol/L ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517nm immediately. The decrease in absorbance was determined at 15 and 30min until the absorbance reached a steady state (after nearly 30 min). The mixture with the addition of standard antioxidants served as a positive control. All the tests were performed in triplicate, and the inhibition rate was calculated according to the formula,

% Inhibition of DPPH free radical = [(A blank – A sample)/ A blank] x 100

ABTS free radical scavenging activity

ABTS free radical was produced by reacting 7mM ABTS aqueous solution with 2.4mM potassium persulfate in the dark for 16h at room temperature (Roberta et al. 1999). Prior to assay, the solution was diluted in ethanol and equilibrated at 30°C to give an absorbance of 0.700±0.02 at 734nm. The stock solution of the sample extracts were diluted such that after introduction of 10µL aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1ml of diluted ABTS solution to 10µL of sample or Trolox standards in ethanol, absorbance was measured exactly 30min after the initial mixing. Appropriate solvent blanks were also run in each assay and the percentage inhibition of the blank absorbance was calculated at 734nm. Triplicate determinations were made at each dilution of the standard and percentage inhibition calculated and plotted as a function of Trolox concentration. The antioxidant activity is expressed as trolox equivalent antioxidant capacity (TEAC).

Anti-bacterial activity

Standard and isolated strains of bacteria used to test antibacterial activities of the extracts are given in (Table 2). Antibacterial activity was evaluated by the disc diffusion method (Ahluwalia et al., 2014) with slight modification against gram-positive and gram-negative bacteria. Bacteria were obtaind from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh (India).

Disc diffusion assay

Nutrient agar medium (20 mL) was poured into the plates to a uniform depth and allowed to solidify. The standard inoculum suspension (106 c.f.u. /ml) was streaked over the surface of the media using a sterile cotton swab to ensure the confluent growth of the organism. Plant extract (10 µL) was diluted with two volumes of 5% dimethyl sulfoxide, impregnated on filter paper discs, and used for the assays. On the surface of the plates, discs were placed with sterile forceps, pressed gently to ensure contact with the inoculated agar surface. Oxacillin (10 µg disc-1) was used as a positive control and hexane as a negative control. The plates were incubated in the dark at 37 oC (24 h) and the inhibition zones calculated. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Total phenolic contents:

Phenolic compounds such as flavonoid, tannins and phenolic diterpenes possess antioxidant activity. Folin-Ciocalteu method of determination of total phenolic content is based on the principle that oxidation of phenol by molybdotungstophosphoric reagent yield a colored product that is estimated by measuring absorbance at 765nm. Gallic acid was used as reference standard and the phenolic contents of the extracts were expressed in mg Gallic acid equivalents per gram of extract (Table 1). The highest amount of phenolic content was found in the ethyl acetate extract (295.36 ± 2.88 mg GAE/g) followed by methanolic extract

(193.3 ± 4.40 mg GAE/g) and the petroleum ether extract (78 ± 1.1547 mg GAE/g) of *J. dolomiaea*.

Total flavonoid contents

Flavonoids possess a wide range of bioactivities including antioxidant activity. The presence of hydroxyl groups in the chemical structure of flavonoids is responsible for their antioxidant activity. The determination of total flavonoid content using aluminum chloride is based on the formation of stable complex between aluminum chloride and keto and hydroxyl groups of flavonoids. The total flavonoid content of the extracts of J. dolomiaea is expressed as rutin equivalents in mg/g extract (Table 1). The high amount of flavonoids in the extract indicated the possible antioxidant potential of the extracts. The methanolic extract (200.18 ± 5.773 mg RE/g) showed the presence of higher flavonoid contents. The high amount of flavonoids in the methanolic extract (200.18 mg/g), petroleum ether extracts (184.78 ± 3.42 mg RE/g) and ethyl acetate extract (162.96 ± 4.3588 mg RE/g) suggested the possible antioxidant potential of the *J. dolomiaea* extracts.

Total antioxidant capacity

Total antioxidant capacity determination by phosphomolybdenum method involves formation of a green phosphate/Mo5+ complex at acidic pH and is measured by absorbance at 695nm. The total antioxidant capacity of the extracts of *J. dolomiaea* is expressed as ascorbic acid equivalent (AAEmg/g extract). The calibration curve of standard ascorbic acid standard solutions was used to determine the total antioxidant capacity of the extracts. The antioxidant capacity of petroleum ether, ethyl acetate and methanolic extracts are 51.3 mg/g, 156.9 mg/g and 119.34 mg/g respectively (Table 1). Ethyl acetate extract showed highest total antioxidant activity.

Reducing power

Reducing power of the extract is determined on the ability to reduce a yellow color Fe3+/ferric cyanide complex to form Fe2+ ferrous complex. The amount of Fe2+ was monitored by measuring the formation of blue color at 700nm. A higher value of absorbance implies higher concentration of Fe+2 complexes and indicates higher reducing power. The methanolic extract demonstrated highest reducing power followed by ethyl acetate extract of *J. dolomiaea* (Fig 1). Ascorbic acid, a synthetic antioxidant demonstrated significant reducing power far better than the extracts and rutin. The results indicate that the methanolic extract of *J. dolomiaea* has a fair ability to act as electron donor and convert free radicals to stable products.

Note: PEJD, EAJD, MEJD and AA are petroleum ether, ethyl acetate, methanolic extracts and ascorbic acid of *Jurinea dolomiaea* respectively.

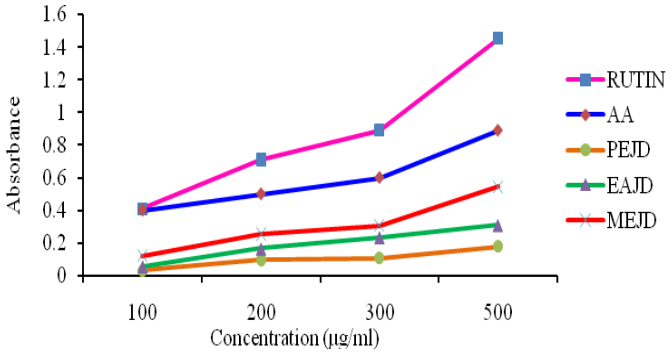

Fig. 1: Reducing power of *Jurinea dolomiaea* extracts

DPPH free radical scavenging ability

The DPPH method is based on the ability of stable free radical 2,2-diphenyl-picrylhydrasyl (DPPH) to react with hydrogen donors, including phenol compounds. DPPH shows an intensive absorption in the visible part of the spectrum and is easily determined spectrophotometrically. The results of the assay demonstrated antioxidant activity of *Jurinea dolomiaea* extracts suggesting that the extracts are capable of donating hydrogen and acting as natural antioxidants. The potential to scavenge DPPH radical was measured by determining IC_{50} value which indicate the concentration required to inhibit 50% of DPPH free radicals. IC_{50} value of the ethyl acetate extract (93.07 µg/ml) was much higher than that of methanolic (102.2 µg/ml) and pet ether extracts (307 µg/ml) of *J. dolomiaea* (Table 1). IC_{50} value of ethyl acetate extract was much higher then methanolic extract and petolium ether extract. It means ethyl acetate extract is powerful antioxidant capability (Fig 2).

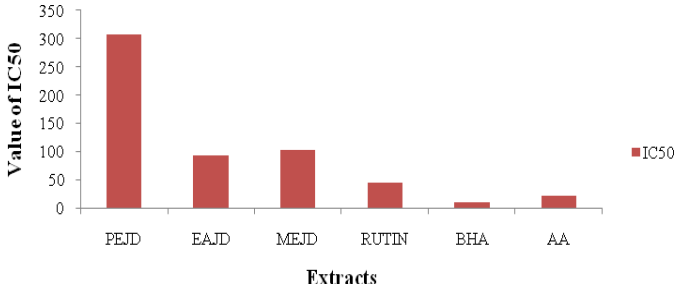

Fig. 2: DPPH free radical scavenging activity (IC50) of *Jurinea dolomiaea* extracts

Note: PEJD, EAJD, MEJD, BHA and AA are petroleum ether, ethyl acetate, methanolic extracts, butylated hydroxy anisole and ascorbic acid of *Jurinea dolomiaea* respectively.

ABTS radical action scavenging assay

An antioxidant is added to preformed ABTS radical cation and after a fixed time period the remaining ABTS is quantified. The activity of the tested sample extracts is expressed as Trolox equivalent antioxidant capacity (TEAC) defined as micromolar Trolox solution having an antioxidant capacity equivalent to 1g extract. Trolox (6-hydroxy-2,5,7,

8-tetramethylchroman-2-carboxylic acid) a water soluble analog of vitamin E is used as standard to represent the antioxidant strength of sample. The extracts exhibited good ABTS radical scavenging ability as all of them were capable of decolorizing the ABTS radical color. The ethyl acetate extracts demonstrated highest Trolox equivalent activity (Table 1) of 11036.62 whereas the methanolic extract (7087.89) and petroleum ether extract exhibited (3129.34).

Table 1: Antioxidant potential of *Jurinea dolomiaea* extracts

EXTRACT/STANDARD	TPC[A] (MG GAE/G)	TFC[B] (MG RE/G)	TAOC[C] (MG AAE/G)	DPPH IC_{50}(µG/ML)	ABTS[D] (TEAC)
PEJD	78 (115.47)	184.78 (3.42)	51.3	307	3129.34
EAJD	295.36(2.88)	162.96 (4.358)	156.9	93.07	11036.62
MEJD	193.3 (4.40)	200.18 (5.773)	119.34	102.2	7087.89
RUTIN	-	-	-	45	-
BHA	-	-	-	10	-
ASCORBIC ACID	-	-	-	21	-

Note: PEJD, EAJD and MEJD are petroleum ether, ethyl acetate and methanolic extracts of *Jurinea dolomiaea* respectively. BHA is butylated hydroxy anisole; [a]total phenolic contents (TPC) are expressed as gallic acid equivalent; [b]total flavonoid contents (TFC) are expressed as rutin equivalent; [c]total antioxidant activity (TAOC) is expressed as ascorbic acid equivalent; [d]TEAC is trolox equivalent antioxidant capacity defined as micromolar trolox solution having antioxidant activity equal to 1g extract; values in parenthesis indicate SD (n=3).

Anti-bacterial activity

Antibacterial Screening of different extracts of *Jurinea dolomiaea* was carried out by disc diffusion method. The zone of inhibition (mm) of the extracts against four pathogenic bacterial strains *E. coli* MTCC-443, *Salmonella typhirium* MTCC-1255, *Klebsiella pneumoniae* MTCC-432 and *Staphylococcus aureus* MTCC-737. The extract was found to be possessing antibacterial activity against some pathogenic bacteria (Table 2). Maximum zone of inhibition was found to be methanol extract (15 mm) against *E. coli*. The petroleum ether extract, ethyl acetate extract and methanol extract do not show activity against *Salmonella typhirium* and *Klebsiella pneumoniae*. Ethyl acetate extracts show antibacterial activity against *E coli* (13 mm) and *Staphylococcus aureus* (10 mm). So from the above study it can be concluded that the methanol and ethyl acetate extract of *Jurinea dolomiaea* possess antibacterial activity against some bacterial strains.

Table 2: Antibacterial activity of *Jurinea dolomiaea* extracts

Samples	*E. coli*	*S. typhirium*	*K. pneumoniae*	*S. aureus*
PEJD	-	-	-	-
EAJD	13 mm	-	-	10 mm
MEJD	15 mm	-	-	12 mm
Oxacillin	23	24	30	25

Note: PEJD, EAJD and MEJD are petroleum ether, ethyl acetate and methanolic extracts of *Jurinea dolomiaea* respectively.

CONCLUSIONS

In conclusion, we found that the ethyl acetate fraction from *Jurinea dolomiaea* had higher total phenolic content, total antioxidant activity, DPPH scavenging activity, and ABTS radical action scavenging activity. The higher total flavonoids contents and anti-bacterial activity observed in methanolic fraction. The petroleum ether extract of *Jurinea dolomiaea* did not show any anti-bacterial activity against *E. coli* MTCC-443, *Salmonella typhirium* MTCC-1255, *Klebsiella pneumoniae* MTCC-432 and *Staphylococcus aureus* MTCC-737. However ethyl acetate and methanolic extract showed moderate activity against *E. coli* and *S. aureus*. The results clearly indicate antioxidant ability of the polar extract of *Jurinea dolomiaea* and potential anti-bacterial activity of its ethyl acetate and methanolic extract. The results of study also indicate the need for further phytochemical investigation of ethyl acetate and methanolic extract of *Jurinea dolomiaea*.

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