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RESEARCH ARTICLE

A QUANTITATIVE ESTIMATION OF PHYTOCHEMICAL, ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITY OF CRUDE EXTRACT OF ROOT OF *JURINEA DOLOMIAEA* BOISS

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ABSTRACT

The therapeutic efficacy of Jurinea dolomiaea (root) was selected to analyse the anti-diabetic and anti-oxidant activities of their crude plant extract. The plant samples were extracted from polar solvents viz. n-hexane, ethyl acetate, methanol and water. Further crude extracts were screened for the presence of phytochemicals such as alkaloids, flavonoids, phenols etc. The quantitative analysis of phenols, flavonoids, alkaloids was performed by spectrophotometrically. The evaluation of anti-oxidant activity was determined by DPPH and ABTS methods. The anti-diabetic activity of crude extracts was determined by in vitro α-amylase inhibition method. The methanolic extract was significantly showed DPPH IC50 inhibition at 20 µg/mL, ABTS inhibition IC50 at 30µg/mL and in vitro α-amylase inhibition IC50 at 50µg/mL. It is concluded that methanolic extract has the potential source of anti-oxidant and anti-diabetic activity.

INTRODUCTION

Many medicinal plants have been identified and are used to cure the diseases. Locally many trials were made to identify different parts of the plants for specific illness. Over generations human beings believe the healing power of plants and emphasize miraculous healing mechanisms of the plants (Dhanasekharan *et al*., 2013). The knowledge of medicinal plants was practiced among local communities and information was passed to the people of all over the world. It has been found that plants possess a wide range of biological activity (Shirona, 2014). The medicinal plants are used for their therapeutic or medicinal values and contain a variety of chemical substances. The different parts of the plants are used to prevent, relieve and treat disease (Samuel, 2010). Medicinal plants have a good source of phytochemicals like alkaloids, Flavonoids, phenolics, saponins etc. *Jurinea dolomiaea Boiss.*is a prostrate perennial stem-less herb found in the Himalayas from Pakistan, Kashmir and Kumaon up to East Nepal at altitudes of 3000-4300m. It is found scattered in open slopes, meadows, rock-crevices and glacial moraines. It is a family of over 20,000 species in which the flower is actually a composite consisting of a cluster of many tiny flowers called florets. The local inhabitants of hilly regions extract the roots and the entire plant is destroyed. This has resulted in depletion of the wild population in most areas of its occurance. The plant is relatively a safe and no hazards related to this plant are known till date.

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The herb has been known for its curative properties and has been utilized as antimytotoxic, analgesic, antibacterial, antihaemorragic, anti-hyperglycemic antioxidant, immunemodulatory properties and is considered as good rejuvenator too. A wide range of chemical compounds including alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes and their glycosides have been isolated from this species. Extracts and metabolites from this plant have been known to possess pharmacological properties. The root contain polyacetylene substituted thiophenes.The aerial part is reported to contain a phytosterol, p-amyrin in the n-hexane extract and luteolin-7 glucoside, p-glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone in polar solvent extract. The polypeptides isolated from the plant yield cystine, glutamic acid, phenylalanine, tyrosine and methionine as hydrolysis-nicotine and nicotinic acid are reported. This is a prostrate perennial herb with a central domed cluster of large purple flower heads stretching up to 10cm across. The flower heads are up to 4cm long on a short stalk, are numerous, rosulate in umble like heads, short peduncled, tomentose and often cottony at the base. Outer bracts are lanceolate and hairy; the achenes are curved compressed, 4-5 angled, tubercled and ashy-grey. The root is considered to be stimulant and given in case of fever after the childbirth. Decoction of the roots is given in colic. Bruised roots are applied to eruptions. Aromatic oil from the roots is applied over the affected part in gout and rheumatism. Locally it is known as dhoop or Gugul and its tuberous roots are valued for incense in temples, monasteries, houses and religious ceremonies. The plant roots are boiled in water, which is then cooled and taken orally. The crushed roots are also administered to remit mental depression, excessive thirst, digestive disorders and dyspepsia.

The plant material is also effective in healing eruptions. The aromatic oil extracted from the roots is applied over the affected part. The oil extracted from the plant is added to bathwater as it induces a feeling of freshness.

Experimental Methods

Plant Samples: *Jurinea dolomiaea* Plant was collected from Himalayan region of upper Danchigam in south Kashmir about 38- 45 meters above the sea level. The plants were identified taxonomically and authenticated at the Herbarium, Department of Botany, Kashmir University. Plant roots was washed thoroughly 2 - 3 times with running tap water and then with sterile water followed by shade-dried, powdered and used for extraction.

Plant Extractions: The dried sample plant powder was weighed and packed into a soxhlet apparatus and extracted from low to high polar solvents viz. n-hexane, ethyl acetate, methanol and water. The extract was filtered through Whatman filter paper no-1 and then evaporated to dryness by using rotary evaporator. The final crude extracts were weighed and collected in an air tight container for further use. The weight of the crude extract obtained in each solvent is noted down in Table 1 below.

Table 1. Details of yields of crude extracts

S.No	Compound	Weight in grams	% of extract value
		J.dolomiaea (roots)	
1	Sample	62.90	
2	Hexane	0.40	0.65
3	Ethyl acetate	1.35	2.15
4	Ethanol	12.36	18.25
5	Water	9.42	13.50

Phytochemical screening of the plant Extractions: Phytochemical screening was conducted to the root of *J.dolomiaea* plant for the presence of alkaloids, phenols, saponins, flavonoids, carbohydrates, glycosides, steroids, triterpenoids by using standard phytochemical procedures (4, 5).

Test for Steroids: *Salkowski Test*: Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in colour indicates the presence of steroids.

***Liebermann Burchard's Test*:** Add few drops of acetic anhydride to plant extract and mixed well. 1 ml of concentrated sulphuric acid is also added from the sides of test tube, a reddish brown ring is formed at the junction of two layers indicating the steroids presence.

Tests for Triterpenoids

***Salkowski Test*:** Few drops of concentrated sulphuric acid is added to the extract, shaken and on standing, lower part turns golden yellow colour indicating the presence of triterpenoids.

Lieberman Burchard's Test: Add few drops of acetic anhydride to plant extract and mixed well. 1 ml of concentrated sulphuric acid is also added from the sides of test tube, a red ring indicates the presence of triterpenes.

Test for Saponins

Foam Test: Few amount of extract is shaken with less quantity of water, the foam produced persists for 10 minutes. It confirms the presence of saponins.

Test for Steroidal Saponin

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.
Salkowski Test: Few drops of concentrated sulphuric acid are added to the extract, shake well and on stand for few minutes. The lower layer turns red colour indicates the presence of steroidal saponin.

***Liebermann Burchard's Test*:** Add few drops of acetic anhydride to the extract and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers confirms steroidal saponin.

Tests for Triterpenoidal Saponin

The plant extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids.

Tests for Alkaloids

***Dragendroff's Test*:** The acid layer with few drops of Dragendroff's reagent (Potassium bismuth iodide) gives reddish brown precipitate.

***Hager's Test*:** The acid layer when mixed with few drops of Hager's reagent (Saturated solution of picric acid) gives yellow precipitate.

Test for Carbohydrates

***Molischs's Test*:** Plant extract is treated with Molisch's reagent and conc. sulphuric acid along the sides of the test tube, a reddish violet ring indicates the presence of carbohydrate.

Benedict's test: The plant extract is heating with Benedict's reagent, a brown precipitate indicates the presence of carbohydrate.

Test for Flavonoids

***Ferric Chloride Test*:** Freshly prepared ferric chloride solution is added to the alcoholic solution of extract, blackish green colour indicates the presence of flavonoids.

Test for Glycosides

***Keller-Killiani Test*:** Plant extract and 0.4 ml glacial acetic acid are mixed with ferrous chloride and 0.5 ml of concentrated sulphuric acid. Blue colour of acetic acid layer shows glycosides.

Test for Phenolic Compounds

***Ferric Chloride Test*:** Addition of ferric chloride solution to the plant extract, appearance of blue colour shows hydrolysable tannins and green colour shows condensed tannins.

***Test for Chlorogenic Acid*:** Treat the test solution with liquid ammonia and expose to air, gradually green colour appears shows chlorogenic acid.

Quantitative Estimation of Phytochemicals

Total Phenolic Compounds: The total phenolics content in different solvent extracts was determined with the Folin-Ciocalteu's reagent (FCR). In the procedure, 1 ml of extract was mixed with 0.4 ml FCR (diluted 1:10 v/v). After 5 min 4 ml of sodium carbonate solution was added. The final volume of the tubes were made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenol content of the extract was expressed in milli grams of catechol per gram of dry weight. A total phenolic compound present in crude root extract of *J.dolomiaea* was measured.

Total Flavonoid Content: It was determined by using aluminium chloride and quercetin as a standard. The 1ml plant extract was added to 3 ml distilled water followed by 5 % 0.3ml NaNO2. After 5 min. at 25 °C, 10% 0.3ml AlCl3 was added. After further 5 min. the reaction mixture was treated with 2 ml of 1 M NaOH. Finally, the reaction mixture was diluted to 10 ml water and the absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve (r2 = 0.999) and the results were expressed as mg of quercetin equivalent per gram.

Total Alkaloid content: 5 ml phosphate Buffer (4.7 PH) was added to 1 ml extract and 5 ml BCG (bromo cresol green) solution mixture was shaken with 4 ml chloroform. The extracts were collected in a 20 ml volumetric flask and then diluted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank. Atropine is used as a standard material and compared the assay with Atropine equivalents. The total alkaloid content was calculated from a calibration curve (r2 = 0.998) and the results were expressed as atropine equivalent per gram.

Study of Biological Activities

Measurement of Antioxidant Activity using DPPH Method: The antioxidant activity of crude root extract of *J.dolomiaea* was determined by scavenging activity of 1, 1-dipheny 1, 2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron and is usually utilized for the detection of radical scavenging activity in chemical analysis. 1 ml of each solution of different concentrations (1-500 µg/ml) of the extracts was added to 3 ml of 0.004 % ethanolic DPPH free radical solution. After 30 minutes the absorbance was taken at 517 nm by UV spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid concentration (1-500 µg/ml) (Hatano, 1988). Then the % of antioxidant activity was calculated as below.

$$\% \text{ antioxidant activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Measurement of Antioxidant Activity using ABTS Method: This assay was based on the ability of different substances to scavenge 2, 2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid (ABTS. +) radical cation. The radical cation was prepared by mixing 7 mm ABTS stock solution with 2.45 mm potassium per sulphate and leaving the mixture for 4-16 hrs until the reaction was complete. The ABTS.+ solution was diluted with ethanol to an absorbance of 0.700 ± 0.05 at 734 nm. The photometric assay was conducted on 0.9 ml of ABTS. + solution and 0.1 ml of tested sample and mixed for 45 sec; measurements were taken immediately at 734 nm after 15 min. The anti-oxidant activity of the tested sample was calculated by determining the decrease in absorbance at different concentrations as:

$$E = ((Ac-At)/ Ac) \times 100$$

Where At and Ac are the respective absorbance of tested samples and ABTS. +, was expressed as µmol.

Measurement of Anti-Diabetic Activity: Both 1ml mixture of alpha amylase and plant extract in a test tube was incubated at 37 °C for 10 min. After pre-incubation, 1 ml of 1 % starch solution was added to each tube and incubated at 37 °C for 15 min. The reaction was terminated with 2 ml DNSA reagent, placed in boiling water bath for 5 min, then cooled to room temperature, diluted, and the absorbance was measured at 546 nm (Prabhakar, 2013). The control reaction representing 100 % enzyme activity did not contain any plant extract. The % inhibition of alpha amylase by each plant extract can be calculated as follows.

$$\% \, Inhibition \, of \, \alpha \, amylase = \frac{Enzyme \, activity \, of control - Enzyme \, activity \, of \, extract}{Enzyme \, activity \, of \, control} \times 100$$

RESULTS AND DISCUSSION

Phytochemical Screening: The Phytochemical screening of the *Jurinea dolomiaea* Boiss crude extracts shows the presence of phenolics, steroids, flavonoids and alkaloids, steroids, carbohydrates, saponins. The results are expressed as +ve for the presence and –ve for the absence of phytochemicals as in Table 2. In the Phytochemical screening of *Jurinea dolomiaea* of methanolic extract shows positive presence of phenols, flavonoids and aqueous extract shows positive for steroids, alkaloids, flavonoids, phenols, saponins, steroidal saponins, triterpenoids, triterpenoid saponins.

Total Phenolic, Flavonoid, Alkaloid Contents

***Quantitative Estimation of Phenolics*:** Phenolic compounds are integral part of cell wall structure and capture free radicals they contain hydroxyl groups that will donate hydrogen from their hydroxyl groups to free radicals and form stable phenoxy radicals and neutralize them thus preventing aging process. They are more active due to lower bond dissociation energy of O-H. Higher phenolic content causes higher cytotoxicity. Phenolic antioxidant mechanism includes termination of free radical reaction depending upon their activity with respect to ability to interfere with chain propagation reaction by rapid donation of hydrogen atom to lipid radical (Prabhakar, 2013). Bioactive polyphenols can protect the human body from the oxidative stress (Robards, 1999). A higher phenolic compound shows anticancer activity by exhibit protective action against carcinogens as blocking agents.

The total phenolic contents in different solvent extracts of Jurinea dolomiaea was determined with the Folin Ciocalteus reagent by using UV spectrophotometer and taking catechol as

standard and the total phenolic content was expressed as mg of equivalent of catechol per gram.

Quantitative Estimation of Flavonoids: Flavonoids are a group polyphenolic compounds contain a hydroxyl and carbonyl groups at 3 and 4 position, poly hydroxylation of A&B aromatic rings which influences the radical scavenging, inhibition of hydrolytic and oxidative enzymes (Cook, 1996). The mode of action of Flavonoids include free radical quenching, metal chelating, suppressing the enzymes associated with free radical scavenging, stimulation of internal antioxidant enzyme such as NADPH oxidase, inhibition of low density lipoproteins (LDL), inhibition of hydrolytic and oxidative enzymes (phospholipase A2, cyclooxygenase, lipoxygenase), and anti-inflammatory actions (Kessler, 2003; Banjarnahor, 2013).

They have ability to scavenge the reactive oxygen and chelate free radicals by donating hydrogen atom. Flavonoids also inhibit both cytosolic and membranal tyrosine kinase Integral membrane proteins, such as tyrosine 3-monooxygenase kinase Inhibition of these proteins results in inhibition of uncontrolled cell growth and proliferation can inhibit carcinogenesis. Flavonoids are also potent inhibitors of cell proliferation, angiogenesis, antithromboticity, infectious properties of the viruses (Nijveldt, 2001). Flavonoids are benzo-gamma pyrone derivatives of plant origin, which activate signalling metabolism, insulin sensitising in hepatic cells and adipose tissue leads to exert anti-diabetic activity (Garcia-Diazia, 1983). The total flavonoid content was expressed as mg quercetin equivalent to a gram. The total flavonoids present in different extracts are depicted in Table 4.

Quantitative Estimation of Alkaloid: Alkaloids are biologically active as stimulators, inhibitors and terminators of growth. Alkaloid plays an important role in molecular interactions. Most alkaloids have a strong bitter taste and are very toxic so they are used by plant to protect themselves against herbivores and pests. These compounds affect the central nervous system, reduce appetite and also act as antiparasitic and antimicrobial agents. The total alkaloid content in different solvent extracts of the Jurinea dolomiaea was determined by using phosphate buffer and BCG (bromocresol green) and atropine as a standard. The total alkaloid content was expressed as mg atropine equivalent to a gram. The total amounts of alkaloids present in different extracts are shown as in Table 5.

Anti-Oxidant Activity: The ability of sample was investigated by DPPH assay to scavenge free radicals, which causes damage to natural macromolecules such as nucleic acid, polysaccharides and lipids. Natural antioxidants strengthen the endogenous antioxidant defenses against reactive oxygen species (ROS) and restore the balance. Antioxidants inhibit the formation of free radicals by scavenging species that initiates peroxidation, chelating metal to unable to generate the reactive species by quenching and preventing the formation of peroxide, breaking autooxidation reaction, reducing localized o_2 concentration (Brewer, 2011). The presence of excess oxygen in the human body causes negative effects as it triggers radical chain reactions causes aging and cell destruction. The protective effect is largely attributed to the presence of antioxidant phytochemicals such as phenolics and flavonoids (Badhani, 2016).

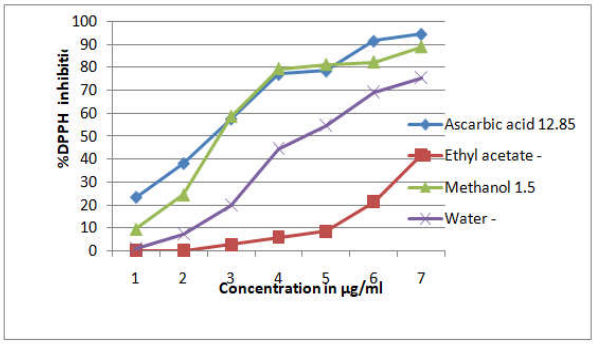

Fig. 1. %DPPH activity of Jurinea dolomiaea

The percentage of DPPH activity of Jurinea dolomiaea crude extracts with that of the standard ascorbic acid was shown in Table 6. The percentage of ABTS inhibition in the Jurinea dolomiaea crude extracts with that of ascorbic acid shown in Table 7. In Jurinea dolomiaea methanol root extract was found to possess an IC50 at 20 µg/ml in DPPH method. The standard Ascorbic acid shows DPPH IC50 at the 20 µg/ml (Fig. 1). In the Jurinea dolomiaea methanol root extract was found to possess IC50 at the lower concentration of 30 µg/ml in ABTS method while standard ascorbic acid shows ABTS IC50 at 20 µg/ml (Fig. 2).

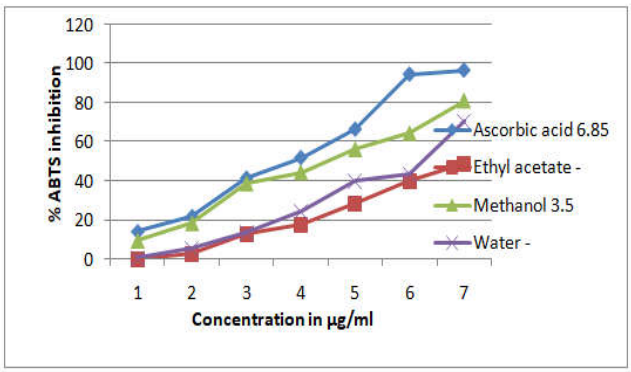

Fig. 2. %ABTS inhibition of Jurinea dolomiaea Boiss

Anti-Diabetic Activity: The anti-diabetic activity of Jurinea dolomiaea and Brassica oleraceae was determined by in vitro α-amylase inhibition method using acarabose as standard. Diabetes is a complex metabolic disorder resulting from insulin dysfunction. In diabetes the oxidative stress causes the reduction in the antioxidant and glycation of protein, inactivation of enzymes and alteration in structural function of collagen basement with age (Baynes, 1991).

Oxidative stress causes various tissue damage in diabetic patients thus antioxidant can stimulate insulin secretion and glucose level will be decreased. The antioxidant protects the pancreatic beta cells against glucose toxicity too. The comparison of α-amylase inhibition of two plant crude extracts with the standard acarabose was presented below as in Table 8. The antidiabetic activity of Jurinea dolomiaea and Brassica oleraceae was determined by in vitro α-amylase inhibition method using acarabose as a standard. In the Jurinea dolomiaea methanolic extract shows α-amylase inhibition IC50 at 50 µg/ml (Fig. 3) and methanolic extract of Brassica oleracea was used as negative control showed α-amylase inhibition IC50 at 50 µg/ml (Fig. 4) while standard drug acarbose shows α-amylase inhibition IC50 at 20 µg/ml.

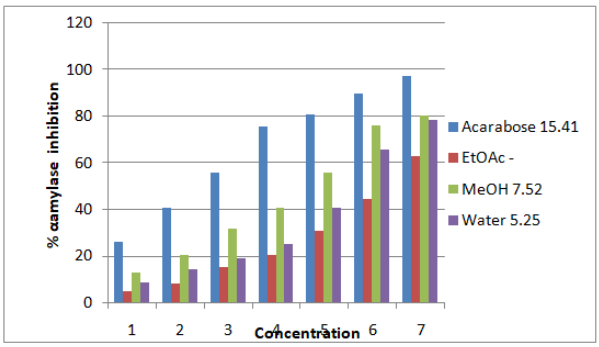

Fig.3.α-amylaseinhibitionofJurineadolomiaeaBoiss

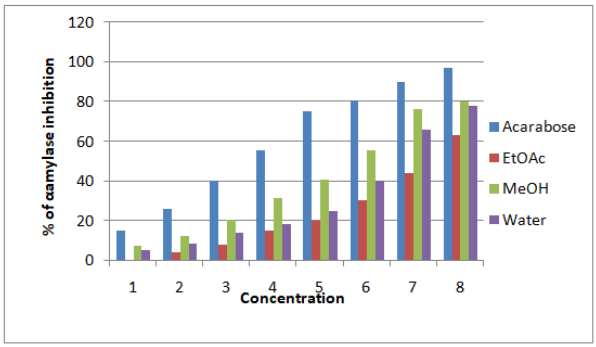

Fig.4.α-amylaseinhibitionofBrassicaoleraceae

Table 2. Phytochemical screening results of Jurinea dolomiaea Boiss.

S.No	ScreeningTests	Jurineadolomiaea (root)		
		Ethylacetateextract	Methanolicextract	Aqueousextract
1	Steroids Salkowski Libermann	-ve -ve	+ve +ve	+ve +ve
2	Triterpenoids Salkowski Liberman	-ve -ve	-ve -ve	+ve +ve
3	SaponinsFoam	-ve	-ve	+ve
4	Steroidalsaponin Salkowski Libermann	-ve -ve	-ve -ve	+ve +ve
5	Triterpenoidsaponin	-ve	-ve	+ve
6	Alkaloids Dragondroffs Picricacid	-ve -ve	+ve +ve	+ve +ve
7	CarbohydratesBenedicts Molisch	-ve -ve	+ve +ve	+ve +ve
8	Flavanoids Ferricchloride (with alcoholicextract)	-ve	+ve	+ve
9	Glycosides	-ve	-ve	+ve
10	Phenols FeCl3 Chlorogenic	 -ve -ve	 +ve +ve	 +ve +ve

Table 3. Results of total amount of phenolics present in different extracts

S.No	Compound	Absorbance λ_{max}	Amount (mg/g)
		Jurinea dolomiaea(root)	Jurinea dolomiaea(root)
1	Hexane	-	-
2	Ethyle acetate	-	-
3	Methanol	0.693	70.80
4	Water	0.235	18.20

Table 4. Results of total content of flavonoids present in different extracts

S.No	Compound	Absorbance λ_{max}	Amount (mg/g)
		Jurinea dolomiaea(root)	Jurinea dolomiaea(root)
1	Hexane	-	-
2	Ethyle acetate	-	-
3	Methanol	0.964	82.65
4	Water	0.634	46.90

Table 5. Results of total amount of alkaloids present in different extracts.

S.No	Compound	Absorbance λ_{max}	Amount (mg/g)
		Jurinea dolomiaea (root)	Jurinea dolomiaea (root)
1	Hexane	-	-
2	Ethyle acetate	-	-
3	Methanol	-	41.25
4	Water	0.318	44.35

Table 6. % DPPH activity of Jurinea dolomiaea Boiss

S.No	Concentration (µg/ml)	Jurinea dolomiaea (root) % of DPPH activity			
		Ascorbic acid	Ethyl acetate	Methanol	Water
1	5	12.85	-	1.50	-
2	10	23.25	-	9.56	1.02
3	15	38.00	-	24.50	7.50
4	20	57.50	2.67	58.75	19.65
5	25	77.02	5.96	79.52	44.50
6	30	78.50	8.50	81.25	54.75
7	50	91.55	21.22	82.40	69.25
8	100	94.60	41.85	89.05	75.50

Table 7. % ABTS inhibition of Jurinea dolomiaea Boiss

S.No	Concentration (µg/ml)	Jurinea dolomiaea (root) % of ABTS activity			
		Ascorbic acid	Ethyl acetate	Methanol	Water
1	5	6.85	-	3.50	-
2	10	14.25	-	9.56	0.76
3	15	22.00	2.80	18.50	5.50
4	20	41.50	12.67	38.75	13.65
5	25	52.02	17.96	44.52	24.50
6	30	66.50	28.50	56.25	39.75
7	50	94.55	40.22	64.40	43.25
8	100	96.60	48.85	81.05	70.50

Table 8. α-amylase inhibition of Jurinea dolomiaea and Brassica oleraceae

S.No	Conc. µg/ml	Standard drug	Jurinea dolomiaea (root) % of inhibition			Brassica oleraceae (root) % of inhibition		
		Acarabose	EtOAc	MeOH	Water	EtOAc	MeOH	Water
1	5	15.41	-	11.90	-	-	7.52	5.25
2	10	25.93	-	23.25	4.30	4.50	12.75	8.55
3	15	40.50	1.70	30.50	10.75	8.10	20.50	14.00
4	20	55.60	10.30	45.25	20.50	15.10	31.55	18.75
5	25	75.40	18.40	55.00	34.80	20.25	40.65	25.25
6	50	80.90	28.20	68.75	45.20	30.50	55.90	40.50
7	100	90.00	38.40	80.50	62.55	44.25	76.20	65.80
8	200	97.10	47.50	90.10	75.20	63.00	80.25	78.25

Conclusion

This study proves the efficacy of methanolic extract of Jurinea dolomiaea had the greatest potential value of antioxidant activity on the basis of response in terms of qualitative and quantitative estimation of phytochemicals followed by DPPH inhibition IC50 at 20 µg/ml. The methanolic extract of Jurinea dolomiaea shows effective antidiabetic activity because it shows α-amylase inhibition IC50 at 25 µg/ml when compared with Brassica oleraceae. The methanolic extract of Jurinea dolomiaea can be better antidiabetic agent as compared to methanolic extract of Brassica oleraceae as negative control. Thus it could be concluded that Jurinea dolomiaea (roots) was possessed both as an anti-oxidant and anti-diabetic agent.

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