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Phytochemical analysis and antioxidant activity of Jurineadolomiaea Boiss

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ABSTRACT: The herb has been known for its curative properties and has been utilized as antimytotoxic analgesic , antibacterial, antihaemorragic , antihyperglycemic antioxidant , immunomodulatory properties and it is considered as good rejuvenator .

I. INTRODUCTION

Jurinea dolomiaea is also widely used medicinally. Thus, decoction of the stem and root are employed in the treatment of headaches, gonorrhea and as an antidote for poisons.The roots have nutritive, antioxidant, antimicrobial and anti- malaria activities .A wide range 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[1&2]

II. MATERIAL AND METHODS

Collection of Plant material

The plant JurineadolomiaeaBoiss.was collected from the hilly regions of our local area yousmarg Dist. Budgam of Jammu and Kashmir, during the month of August and September 2020. The plant material was thoroughly washed with tap water and kept for drying in shade at room temperature and thoroughly air dried plant material was grinded to powder(40- 60) weighted and stored(Table1).The crude extracts obtained through soxhletapparatus from the plants were subjected to purification process by different chromatographic techniques followed by spectroscopic method[3&4]

Table.1 –Showing percentage weight loss of plant materialsJurineadolomiaeaBoiss

Name of Plant.	Weight of plant material	Weight of plant after drying.	Loss in weight of drying.	Percentage loss in weight.
JurineadolomiaeaBoiss	3000gm	770gm	2260gm	25%

Determination of percentage yield and ash content.

Percentage yield= $\frac{\text{Weight of extract} \times 100}{\text{Weight of powder drug}}$

Determination of Ash Value (Total Ash)

To determine the total ash, place about 10 gm of ground air dried powder, accurately weight in a previously ignited and crucible of silica. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600^{o}C until it is white, indicating the absence of carbon. Cool in a desiccators and weight, and calculated the percentage of ash, (Table 2).

Percentage Ash content

$$= \frac{\text{Weight of ash (g)}}{\text{weight of powddered sample used (g)}} \times 100$$

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Table 2.Showing Ash content of JurineadolomiaeaBoiss.

Name of plant	Wt. of powdered material	After burning in the crucible (ash)	Percentage of ash content
JurineadolomiaeaBoiss	**10 gm**	**0.75 gm**	0.75×100=7.5

Determination of moisture content or Loss on drying (LOD).

An accurately weighed quantity of about 1 to 2 gm of powder was taken in a Petri dish. The powder was distributed evenly. The Petri dish kept open in vacuum oven and the sample was dried at a temperature between 100 to 105^{0}C for 2 h until a constant weight was recorded. Then it was cooled in a desecrator to room temperature, weighed and recorded (Table 3). % Loss on drying was calculated using the following formula.

$$\% \text{ Loss on drying} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \text{ X } 100$$

Table 3: Showing Moisture content (LOD) JurineadolomiaeaBoiss.

Name of plant	Weight of powered material	Loss of weight	Moisture content
JurineadolomiaeaBoiss	2 gm	0.0199 gm.	0.945±0.264

Extraction

The dried plant sample was powdered (coarse) and subjected to Soxhlet using acetone, petreloum ether and methanol respectively. Almost all the chlorophyll and lipid was deposited on the side of the flask and was removed carefully. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. The extracts were stored in refrigerator till any further use. The yield of different extracts was calculated (Table 4)

Table 4: Showing determination of Percentage Yield ofJurineadolomiaeaBoiss.

S. No	Solvent	Wt. of powdered material(gm)	Vol. of solvent (l)	Wt of extract (gm)	Percentage yield %
1	Acetone	650 gm	90cc	146 gm	28.65%
2	Petroleum ether	650 gm	90cc	178 gm	30.40%
3	Methanol	650 gm	90cc	129 gm	43.63%

The percentage yield of extracts was calculated by using following formula:

$$\text{Percntage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder}} \times 100$$

Organoleptic Evaluation of extracts

The three extracts of JurineadolomiaeaBoiss Acetone, petroleum ether and methanol extracts were investigated for their colour, taste and appearance (Table5).

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Table 5. Organoleptic evaluation of extracts of JurineadolomiaeaBoiss**.**

S.No.	Extract	Colour	Taste	Appearance
1	Acetone	Green	Bitter	Sticky
2	Petroleum ether	Dark green	Salty Bitter	Non-Sticky
3	Methanol	Reddish yellow	Bitter	Sticky

Phytochemical Screening of crude extracts of Acetone, petroleum ether and methanol from JurineadolomiaeaBoiss.

This property of selective reactivity of phytochemicals present in extracts forms the basis of chemical tests for identification of compounds. Phytochemical screening is done for analyzing secondary metabolites, which are responsible for curing ailments. Phytochemical screening of the extracts were investigated according to the standard procedures **Test for Carbohydrates**

Molish Test

2 ml of aqueous extract was treated with two drops of alcoholic α-naphthol solution in a test tube and then 1 ml of conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Test for Proteins

Biuret's Test

The extract was treated with 1 ml of 10% sodium hydroxide solution in a test tube and heated. A drop of 0.7% copper sulphate solution was added to the above mixture. The formation of violet or pink colour indicates the presence of proteins.

Test for amino acids

Ninhydrin test

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Test for Saponins

Froth test

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Test for Alkaloids

To the extract, dilute HCl was added, shake it well and filtered. With the filtrate, the following tests were performed.

Mayer's test

To 2-3 ml of filtrate, few drops of Mayer's reagent were added along the sides of tube. Formation of white or creamy precipate indicates the presence of alkaloids.

Wagner's test To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

Test for Terpenoids and Steroids

Salkowski's test

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated H_2SO_4, shaken and allowed to stand. If the lower layer turns red, steroids are present. Presence of golden yellow layer at bottom indicates the presence of Terpenoids.

Test for Flavonoids

Lead acetate test

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Shinoda test

To the extract, 5 ml (95%) 0f ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drops wise addition of concentrated hydrochloric acid. Formation of pink colour indicates presence of flavonoids.

Test for Tannin and Phenolic compounds

Ferric chloride test -Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5 % ferric chloride solution was added. Formation of blue, green or violet colour indicates the presence of phenolic compounds.

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Chromatographic purification

Thin layer chromatography (Harborne)

The methanol crude extract obtained from JurineadolomiaeaBoiss. was subjected to purification process by different chromatographic techniques.

Chromatographic purification of n-butanol and ethyl acetate crude fractions of JurineadolomiaeaBoiss.

S No.	Solvent system	Ratio	Observation
1	Benzene: chloroform	50:50	Moved along with solvent front
2	Chloroform: methanol	50:40	Poor separation
3	Chloroform: methanol	75:25	Poor separation
4	Chloroform: methanol	100:50	Poor separation
5	Chloroform: ethanol	20:01	Separation but tailing
6	Chloroform: methanol: glacialacetic acid	50:40:0.5	Poor separation

Table:6 Summary of Thin Layer Chromatography

Fractions	Solvent system	Detecting reagent	Colour, No. and Rf value
n-butanol	Chloroform:methanol 90:10	Sprayed with vanillin-Sulphuric acid and heated at 100C for 5 minutes	Five violet pink spots with Rf values 0.21,0.32,0.39,0.7 and0.9 were seen
Ethylacetate	n-butanol:aceticacid :water 4:1:5	Anisaldehyde sulphuric acid and heated at 110^0C for 5 minutes	Five spots with Rf values 0.4,0.45,0.46,0.55 and 0.82 were seen

Column Chromatography

Separation of bioactive constituents from n-butanol fraction

n-Butanol fraction was dissolved in a minimum volume of methanol, adsorbed on silica gel (60-120 mesh), dried and applied on the column to separate possible phytoconstituets. Acetone soluble part was eluted gradiently with chloroform, chloroform methanol mixtures 95:5, 90:10, 80:20, 50:50 and methanol. At uniform interval, the eluents (each of five ml) were collected and the progress of separation was monitored by thin layer chromatography (TLC) using solvent system chloroform:methanol (90:10) and iodine vapor as detecting agent (Table 12).

Separation of constituents from ethylacetate fraction

The same procedure was followed for ethyl acetate fraction of JurineadolomiaeaBoiss using chloroform: ethyl acetate (50:50), ethyl acetate: methanol (90:10), ethyl acetate: methanol (50:50)) and methanol. At uniform interval, the eluents (each of five ml) were collected and the progress of separation was monitored by thin layer chromatography (TLC).

Silica gel G 60 F254 TLC plates of E. Merck, layer thickness 0.2mm. The compounds obtained had shown a single spot on TLC plate and the chromatograms were developed in n butanol: acetic acid: water (4:1:5). The plates were sprayed with anisaldehydesulphuric acid and heated for 5 minutes at 110^0C. These compounds were purified by recrystallization from methanol (Table 10).

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Table7: Separation of constituents from n-butanol fraction of methanol extract of JurineadolomiaeaBoiss

S. No.	ElutingSolvent& Composition	Ratio	Fractions Collected	% Yield	Final Fractions
1	$CHCl_3$	100:0	1-6	No Residue after evaporation	----
2	$CHCl_3$:MeOH	95:5	7-10	Very less quantity was isolated	-
3	$CHCl_3$:MeOH	90:10	11-16	Very less quantity was isolated	-
4	$CHCl_3$:MeOH	80:20	17-23	No Residue after evaporationn	-
5	$CHCl_3$:MeOH	50:50	24-40	Fr-I (18.0% w/w, 0.80 gm	A-F-I (R_f = 0.28)
6	MeOH	100	41-47	Very less quantity was isolated	-

Detemination of Antioxidant activity of three crude extracts of JurineadolomiaeaBoiss.

Preperation of Standard Ascorbic acid Solutions

Various solutions of the ascorbic acid were prepared in methanol to obtain different concentrations (50 – 750 µg/ml). 200 µM solution of DPPH in methanol was prepared and 1.5 ml of this solution was added to 1.5 ml of methanolic ascorbic acid solutions prepared in different concentrations (50, 100, 250, 500 and 750 µg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured Spectrophotometrically**(**against methanol (as blank) at 517nm.

B: Preperation of TestSolutions

Various solutions of the Acetone, Petroleum ether, and Methanol extracts were prepared in respective solvents to obtain different concentrations (50, 100, 250, 500 and 750 µg/ml). 200 µM solution of DPPH in methanol was prepared and 1.5 ml of this solution was added to 1.5 ml Acetone, petroleum ether and methanol extract solutions prepared in different concentrations (50, 100, 250, 500 and 750 µg/ml). The reaction mixtures were vortexed thoroughly and left in the dark at room temperature for 30min. The absorbances of the mixtures were measured spectrophotometrically against methanol (as blank) at 517nm.

C: Preperation of Control Solution

For control, 1.5 ml methanol was mixed with 200 µM DPPH solution and incubated for 30 minutes at room temperature in dark. Absorbance of the control was measured spectrophotometrically against methanol (as blank) at 517nm.

D: Estimation of DPPH scavenging activity

A solution of 200 µM DPPH in methanol was prepared as a control and 1.5ml of this solution was added to 1.5ml of standard ascorbic acid and acetone, petroleum ether and methanol extract solutions prepared in different concentrations (50, 100, 250, 500 and 750µg/ml.) The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517nm.

The ability of the ascorbic acid and extracts to scavenge DPPH radical (Antioxidant activity) was calculated by the Equation given below.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Acontrol}} \times 100$$

$$\% \text{ I} = \frac{\text{Ac} - \text{At}}{\text{Ac}} \times 100$$

Reducing power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity **(Oktayet al[7]).** Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates, so they act as primary and secondary antioxidants **(Chandaet al[8]).** The higher the absorbance of the reaction mixture, the higher would be the reducing power.

Protocol for reducing power

Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5ml) and potassium ferricyanide (2.5ml). The mixture was kept at 50°C in water bath for 20 minutes. After cooling, 2.5ml

of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes whenever necessary. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700 nm. Control will be prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power. Reducing power was measured by varying the concentration of the extract and the contact time. Higher value absorbance of the reaction mixture indicated greater reducing power. **(Oyaizu[173], Boonchum, et al[9]).**

Determination of antioxidant activity of three Fractions

DPPH free Radical Scavenging assay

The three fractions i.e., F-I, F-IIand F-III obtained through the course of column chromatography were screened for antioxidant activity by DPPH radical scavenging assay method with the aim of identifying the most potent fraction for the further study of spectral analysis (UV, IR, NMR, and MASS) and of antibacterial activity.

Spectrophotometric Quantification of Total Phenolic content (TPC)

The antioxidant properties of phenolic compounds are well known; they are potent chelators of redox-active metal ions and they can inactivate free radical chain reactions by hindering the conversion of hydroperoxides to reactive oxyradicals **(Sahreenet al).** The amount of total phenolic in Acetone, petroleum ether and methanol extract was determined with the FolinCiocalteureagent **.**Galic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent to (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1 mg/ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test and mixed with 2.5ml of a 10 fold dilute FolinCiocalteu reagent and 2ml 0f 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrophotometrically.

Spectrophotometric Quantification of Total Flavonoid content (TFC)

Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. The amount of Total Flavonoid content in extracts was determined by aluminium chloride assay through Colorimetry. A 0.5ml aliquot of appropriately diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15ml of a 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5ml, then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm. Rutin was used as standard compound for the quantification of total flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin.All samples were analysed in three replications [6&7]

III. RESULT AND DISCUSSION

Chromatographic technique is most versatile option for the required identification tests for the quality control of herbal products. Thin layer chromatography was performed with the aim to confirm the presence of flavanoids present in the methanol crude extract of the plant. In present study a number of developing solvent systems were tried during the study but chloroform: methanol (90:10) and n-butanol: acetic acid: water (4:1:5) were found to be most appropriate solvent systems for ethyl acetate and n-butanol fractions respectively. After developing the TLC plates it was found that n- Butanol crude fraction showed five spots with Rf values of 0.21, 0.32, 0.39, 0.7 and 0.9 and ethyl acetate crude fraction showed five spots with Rf values of 0.4, 0.45 and 0.46, 0.55 and 0.82. These results are in agreement with who gave the same results of the spots characteristic for flavonoids.

During the Column chromatography three compounds were isolated in significant amount which were designated as Fr-I, Fr-II and Fr-III. Fraction first (Fr-I) was from n-butanol soluble part and other two fractions (Fr-II, Fr-III) were from ethyl acetate soluble part Rutin was used as standard compound for the quantification of total flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW).Line of regression (Y=0.0015 + 0.0068) from Rutin was used for estimation of unknown flavonoid content depicts the variation of mean absorbance with different concentrations of Rutin. Different amounts of phenolics were present in Acetone, petroleum ether and methanol extract. and 42 represents the amounts of flavonoids present in Acetone,petroleum ether and methanol extract.

represents the comparison of contents of flavonoids present in Acetone,petroleum ether and methanol extract.[11] The quantitative analysis of TFC in extracts revealed that methanol extract contained highest amount of TFC (107.64mgRE/gm) followed by petroleum ether(86.97mgRE/gm) where as very less amount was found in Acetone (15.86mgRE/gm) [9&10]
Comparison of phenolics and flavonoids of Acetone,petroleum ether and methanol extract was also studied. The mounts of phenolics present in Acetone, petroleum ether and methanol extract were higher than the amounts of flavonoids present in Acetone,petroleum ether and methanol extract. The comparison of phenolics and flavonoids present in the extracts.[12,13,14]

The results of the above mentioned methods have shown methanol extract has shown highest antioxidant activity in both DPPH and Reducing power assays and moreover during Spectrophotometric quantification of total phenolic and flavonoid contents highest amounts of both phenolics and flavonoids were present in methanol extract as compared to ethyl acetate and methanol extract.

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