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Structural Identification of Chemical Constituents Isolated from Root Extracts of *Jurinea Dolomiaea* Boiss (Astraceae) by Chromatographic and Spectroscopic Methods

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ABSTRACT

Phytochemical screening of the extracts has shown the presence of steroids, flavonoids, saponins, glycosides, tannins, phenolic compounds, fixed oils, and fats in *Jurinea dolomiaea* root extracts. The presence of lupeol has been reported previously by us using high-performance thin-layer chromatography and high-performance liquid chromatography. Present research studies encompasses identification of chemical constituents in *Jurinea dolomiaea* roots of methanol extracts by hyphenated technique such as gas chromatography-mass spectroscopy (MS) which when coupled gives a clear insight of constituents. The components were identified by matching mass spectra with MS libraries. There were 5 different compounds analyzed from *Jurinea dolomiaea* roots. The identified components are (2,4-ditert-butylphenyl)-5-hydroxypentanoate, 2-ethylhexylheptadecyl sulphite, 6-methyltridecane, (9E, 12E)-9,12-Octadecadienoyl chloride, Linoleic acid chloride, linoleoyl chloride, Lup-20 (29)-en-3-ol Lup-20(29)-en-3β-ol Lup-20(29)-en-3α-ol Lupeol, (3α)-isomer Lupeol, (3β,18β,19β)-isomer, in *Jurinea dolomiaea* root extracts.

1. Introduction

Discovery of natural plant-derived drugs from natural botanical herbarium has been the major breakthrough in paving the way for natural product chemistry [1]. Plants are common and important sources in contrast to marine organisms, microorganisms and fungi which are studied to the highest level in order their phytoconstituents [2,3]. But very few research has been done in the field of natural product chemistry, hence it is an indeed a need of an hour to explore these resources in search of new products which have significant pharmacotherapeutic potential as anticancer agents [4-7]. In recent times, the research on plants as medicines has been focusing on isolation and characterization of bioactive constituents and secondary metabolites [8,9].

Triterpenes in plants exhibit good quantity shows structural multiplicity. Terpenoids have common biosynthetic origin based on isoprene units [CH_2=C(CH_3)−CH=CH_2]. Carbon skeletons are built from the union of two or more of C5 units.

Pentacyclic triterpenes such as α- and β-amyrins and derived acids such as oleanolic acid and ursolic acid are found in waxy coatings of leaves and fruits like apple and pear as well as in resins, latex and bark of trees. Lupeol, betulin and betulinic acid of lupane class, oleanane or ursane groups have shown enormous pharmacological potential comprising of antiviral [10-12], anti-inflammatory [13] and antitumor activity. Stigmasterol and its analogues have shown potentiality of acting as chemopreventive agent in cancer by inhibiting the cells in colon and breast cancer [14-22]. During last few decades, infrared spectroscopy has been introduced as a very efficient and nondestructive analytical tool for the reliable identification of triterpenes [23]. Our previous studies [24,25] confirmed the presence of lupeol by high performance thin layer chromatography (HPTLC) and high performance liquid chromatography (HPLC) in *Jurinea dolomiaea* root extracts. Gas-liquid chromatography is an important hyphenated technique which provides an insight to qualitative, as well as quantitative estimation for volatile components such as terpenes. The present work identified and confirmed the structures of the different elucidated constituents by chromatography (TLC, Column, HPLC)-Spectroscopy (UV, IR, NMR, Mass) from the N-butanol, ethylacetate and petroleum ether extracts of *Jurinea dolomiaea* roots.

2. Experimental Methods

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

2.1 Chromatographic Purification

2.1.1 Thin Layer Chromatography

The methanol crude extract obtained from *Jurinea dolomiaea* Boiss was subjected to purification process by different chromatographic techniques. TLC was produced with the aim of identifying the individual substances in a mixture and also testing for purity or for separation of mixtures.

Table 1 Seperation of constituents by TLC of root extract of *Jurinea dolomiaea* boiss

Fractions	Solvent system	Detecting reagent	Colour, No. and R*f* values
n-butanol	Chloroform:methanol 90:10	Sprayed with vanillin Sulphuric acid and heated at 100 °C for 5 minutes.	Seven violet pink spots with R*f* values 0.21,0.32,0.39,0.45,0.7,0.85 and 0.9 were seen
Ethylacetate	n-butanol: aceticacid:water 4:1:5	Anisaldehyde sulphuric acid and heated at 110 °C for 5 minutes	Six spots with R*f* values 0.4,0.45,0.46,0.55,0.67 and 0.82 were seen
Petroleum ether	$CHCl_3$:Pet-ether: water 5:2:3	Anisaldehyde sulphuric acid and heated at 110 °C for 5 minutes	Four spots with R*f* values 0.50, 0.71, 0.86 and 0.98 were seen

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Table 2 Separation of constituents from n-butanol fraction of methanol extract of *Jurinea dolomiaea Boiss*

S.No	Eluting Solvent & 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 evaporation	-
5	$CHCl_3$:MeOH	50:50	24-40	Fr-I (18.0% w/w, 0.80 g)	A-F-I (R_f=0.28)
6	MeOH	100	41-47	Very less quantity was isolated	_

Table 3 Separation of constituents from ethyl acetate fraction of methanol extract of *Jurinea dolomiaea Boiss*

S.No.	Eluting Solvent & Composition	Ratio	Fractions Collected	% Yield	Final Fractions
1	$CHCl_3$	100	1-4	No Residue after evaporation	-
2	$CHCl_3$:Ethyl acetate	50:50	5-20	Fr-II (54% w/w, 2.70 g)	PE-F-I (R_f = 0.55)
3	Ethyl acetate	100	21-26	Very less quantity was isolated	-
4	Ethyl acetate:MeOH	90:10	27-35	Very less quantity was isolated	-
5	Ethyl acetate:MeOH	50:50	36-38	Fr-III (20.4% w/w, 1.02 g)	PE-F-II (R_f = 0.74)
6	MeOH	100	39-41	Less quantity was isolated	-

Table 4 Separation of constituents from petroleum ether fraction of methanol extract of *Jurinea dolomiaea Boiss*

S.No.	Eluting Solvent & Composition	Ratio	Fractions Collected	% Yield	Final Fractions
1	$CHCl_3$	100	1-6	No Residue after evaporation	-
2	$CHCl_3$: Pet.ether	50:50	7-18	Fr-IV (56% w/w, 2.95 g)	PE-F-I (R_f = 0.60)
3	Pet.ether	100	19-28	Very less quantity was isolated	-
4	Pet.ether: MeOH	90:10	29-35	Very less quantity was isolated	-
5	Pet.ether: MeOH	50:50	36-40	Fr-V (22.5% w/w, 2.02 gm)	PE-F-II (R_f = 0.80)
6	MeOH	100	41-45	Less quantity was isolated	-

Table 5 Various components and their fragments in Jurinea dolomiaea root extract

Retention time	Molecular formula	m/z	Synonyms	Figure number
11.80	$C_{19}H_{30}O_3$	307	(2,4-ditert-butylphenyl)-5-hydroxypentanoate	4
25.40	$C_{25}H_{52}O_3S$	432.80	2-ethylhexylheptadecyl sulphite	5
27.84	$C_{14}H_{30}$	199	6-methyltridecane	6
29.15	$C_{18}H_{31}ClO$	299	(9E, 12E)-9,12-Octadecadienoyl chloride, Linoleic acid chloride, Linoleoyl chloride	7
33.13	$C_{30}H_{50}O$	428	Lup-20 (29)-en-3-ol Lup-20(29)-en-3 β-ol Lup-20(29)-en-3α-ol Lupeol,(3α)-isomer Lupeol,(3β,18β,19β)-isomer	8

2.1.2 Column Chromatography

Column chromatography is a method used to purify individual chemical compounds from mixture of compounds. The main advantage of column chromatography is the relative low cost and disposable of stationary phase used in the process. It prevents cross contamination and stationary phase degradation due to recycling. Two methods are generally used to prepare a column, the dry method and the wet method. In column chromatography the stationary phase, a solid adsorbent is placed in a vertical glass column and the mobile phase, a liquid is added to the top which flows down through the column.

2.1.3 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is a chemistry based tool for quantifying and analyzing mixtures of chemical compounds. High Performance Liquid Chromatography (HPLC) is an analytical technique for the separation and determination of organic and inorganic solutes.

2.2 Spectral Analysis

Identification of compounds usually involves a combination of different techniques including UV, IR, NMR and MASS spectrometry. UV spectral studies were carried out (190-110 nm) using V-670 spectrophotometer. Spectral data were collected using the Spectra Manager™ II software. FTIR characterizations were done using JASCO IR spectroscopy, Jasco Inc., Tokyo, Japan. NMR measurements were carried out with a Bruker WM 400 instrument using TMS as internal standard in deuterated benzene, (C_6D_6). GC-MS measurements were carried out on a Hewlett-Packard HP 5890 gas chromatograph equipped with a 25 mm x 0.25 mm polydimethylsiloxane CP-Sil-5-CB (chrompack) capillary column and coupled to a VG Analytical VG 70-250S mass spectrometer with electron impact (70 eV) ionisation. The oven was operated under a linear temperature program from 80 °C to 270 °C at the rate of 10 °C/min. Helium was used as a carrier gas at a flow rate of 0.5 mL/sec. The injector, transfer line and ion source temperatures were 220 °C, 230 °C and 220 °C.

3. Results and Discussion

In the present study, constituents such as lupeol and stigmasterol were eluted in chromatographic and confirmed through spectral studies. The chromatographic and spectral analysis in the recent research studies reveals that the identified compounds belong to the class of triterpenoids has a potential pharmacological role. The identified compounds are probably lipid soluble and found in the plant cell's cytoplasm. The general procedure for their extraction is using light petroleum ether or chloroform since these solvents help to separate the compounds by suitable chromatographic techniques. GC is considered as a vital technique to interpret essential oils and volatile compounds [1]. In the current research study, various compounds were elucidated by TLC (Fig. 1), Column, HPLC (Fig. 2), UV, IR, NMR and MS technique from n-butanol, ethylacetate and petroleum ether extracts of *Jurinea dolomiaea*.

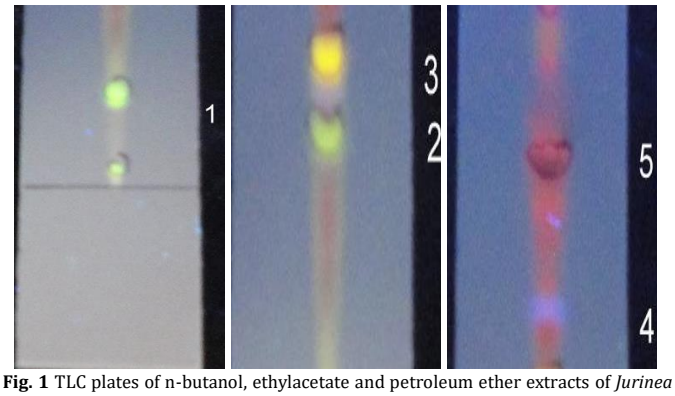

Fig. 1 TLC plates of n-butanol, ethylacetate and petroleum ether extracts of *Jurinea dolomiaea*

Pentanoic acid, 5-hydroxy-2,4-dibutylphenyl esters (Fig. 3) [33.5%] with m/z 307 and fragment ions of 41, 57, 74, 91, 107, 115, 163, 175, 191 is seen most commonly from roots of *Jurinea dolomiaea* (Astraceae). Main component elucidated was sulfurous acid, 2-ethylhexylhepatdecyl sulphate (Fig. 4) with m/z 432.80 and fragment ions of 29, 41, 43, 48, 55, 64, 69, 71, 83, 97, 113, 127, 139, and 155 along with 6-methyltridecane (Fig. 5) as m/z 199 are also seen prominently.

9,12-Octadeca-dienylchloride (Fig. 6) with m/z 299 and fragment ions of 41, 43, 55, 57, 65, 67, 71, 77, 79, 81, 87, 95, 109, 121, 135, 149, 155, 163, 177, 185 is also characterized and Lupeol (Figs. 7 and 8) having m/z 428 with fragment ions of 41, 43, 53, 55, 57, 59, 65, 68, 71, 77, 79, 81, 83, 85, 91, 95, 97, 99, 109, 121, 135, 147, 161, 175, 189, 203, 207, 218, 234, 247, 257, 272, 286, 299, 315, 325, 344, 357, 370, 383, 393, 411. All the components and their fragments are depicted in Table 5.

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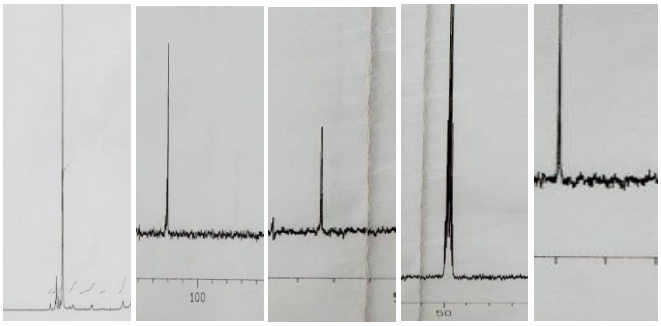


Fig. 2 HPLC of *Jurinea dolomiaea* root extract of Fr.1, Fr.2, Fr.3, Fr.4 and Fr.5

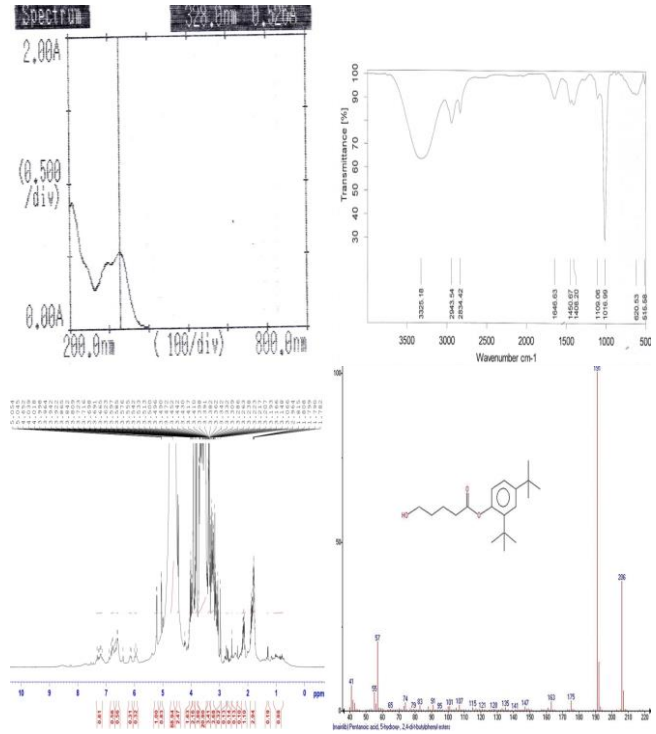


Fig. 3 UV, IR, NMR and Mass spectroscopy of n-butanol (Fr. 1) of methanol root extract of *J. dolomiaea*

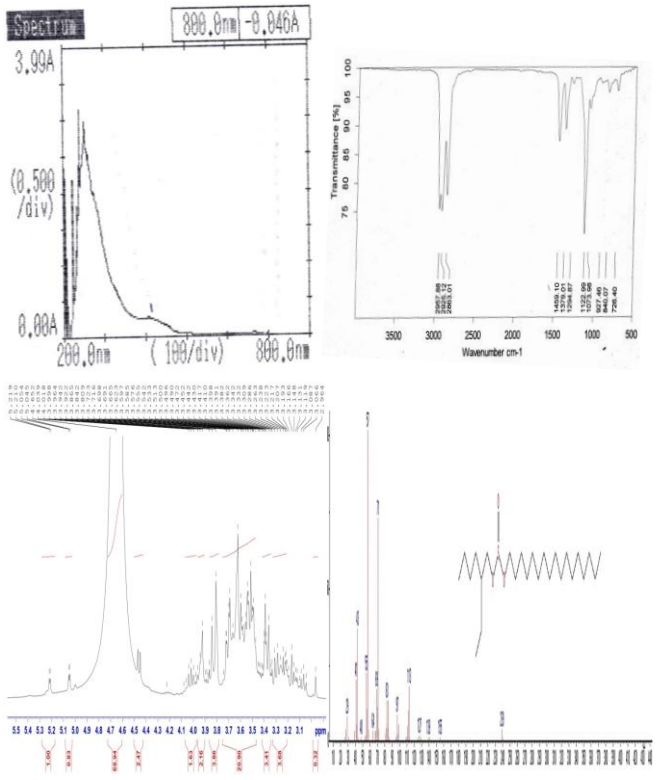


Fig. 4 UV, IR, NMR and Mass spectroscopy of ethyl acetate (Fr.2) of methanol root extract of *J. dolomiaea*

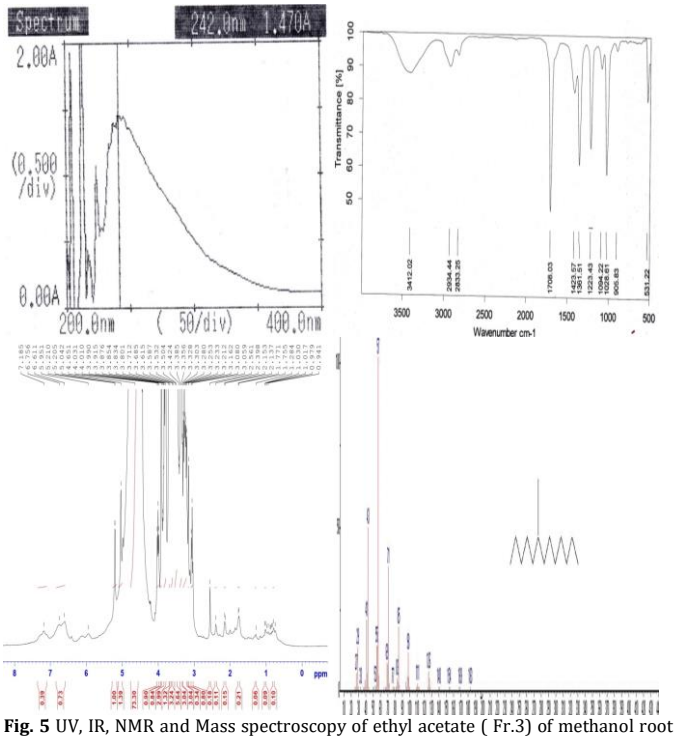
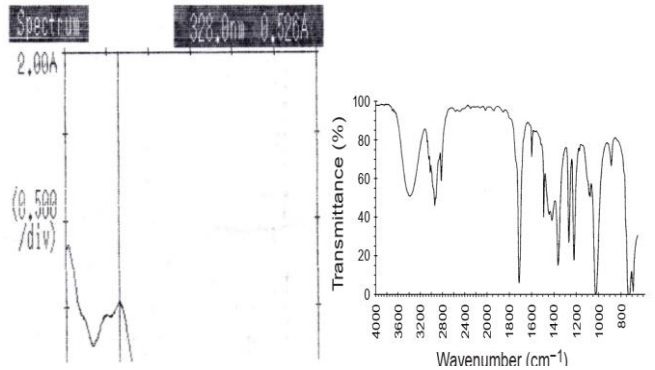


Fig. 5 UV, IR, NMR and Mass spectroscopy of ethyl acetate (Fr.3) of methanol root extract of *J. dolomiaea*



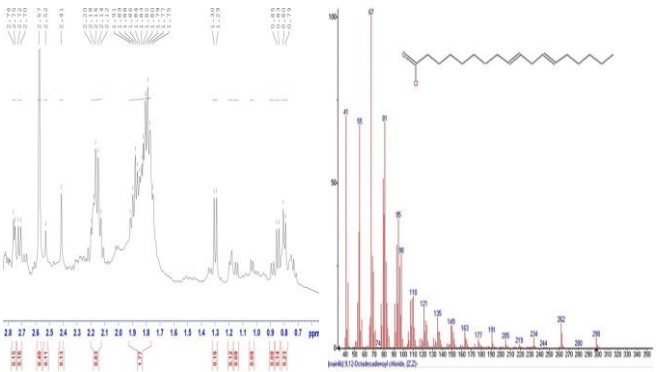


Fig. 6 UV, IR, NMR and Mass spectroscopy of pet ether (Fr.4) of methanol root extract of *J. dolomiaea*

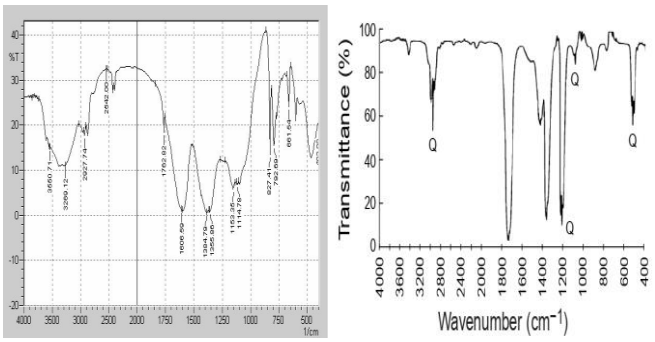


Fig. 7 UV and IR of pet ether (Fr.5) of methanol root extract of *J. dolomiaea*

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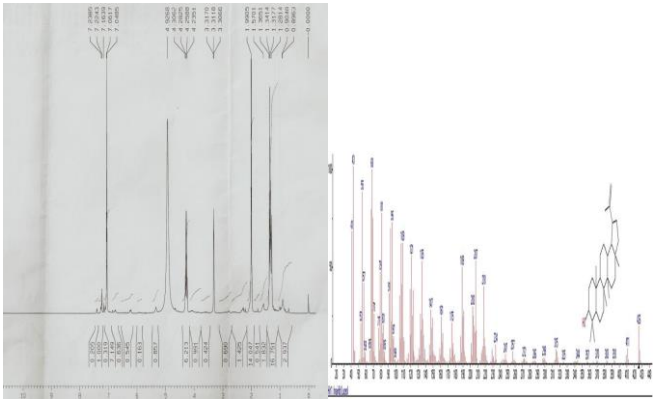

Fig. 8 NMR and Mass spectroscopy of pet ether (Fr.5) of methanol root extract of *J. dolomiaea*

4. Conclusion

On the basis of the results of this study, it is clearly indicated that methanolic extract of root contains number of bioactive components such as pentanoic acid, sulfurous acid , methyltridecane , dienylchloride and Lupeo identified by chromatographic and spectroscopic techniques.

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