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Figure showing Contour plots for a) Gelling capacity b) Viscosity c) Gel strength d) Overlay plot.

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Phytochemical Screening and Liquid Chromatography-Mass Spectrometry Studies of Ethyl Acetate Extract of *Origanum vulgare*

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ABSTRACT

Background: *Origanum vulgare* is a perennial shrub that grows in the mountains of the Mediterranean and Euro/Iran-Siberian regions. **Objectives:** This study was conducted to identify the major constituents of the ethanolic extract of *Origanum vulgare* by Liquid chromatography/mass spectroscopy analysis. **Methods:** The leaves of *Origanum vulgare* were powdered and the powdered sample of plant material was then subjected to extraction by maceration process for 72 h. The extract obtained was filtered through Whatman filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. **Results:** The extract contained monoterpene hydrocarbons and phenolic compounds, the major ones being Chlorogenic acid, p-cymene and phytol. **Conclusion:** Taken together, these results reinforce the importance of this millennial species and open new perspectives

for the research and design of new herbal medicines, obtained from indigenous vegetal material.

Key words: *Origanum vulgare*, Polyphenols, Phytochemicals.

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INTRODUCTION

Medicinal herbs have been the source of a wide range of biologically active compounds for many centuries and they have been used extensively as crude plant extracts or as pure components for treating a variety of disease conditions. Unlike allopathic drugs which are single active compounds that can specifically target one pathway, herbal remedies work in a way that depends on a synergistic approach. A plant contains a multitude of molecules that synergistically act on targeted elements of the cellular complex pathway.¹ When compared to synthetic ones most natural remedies generally have fewer side effects and toxicity. So, nowadays the use of herbal remedies has increased when compared to allopathic drugs.² In the development of potential therapeutic agents, medicinal plants play an important role. The Himalayas, with a very rich repository of medicinal herbs³ may maintain an important position in the production of raw materials either directly for crude drugs or as the bioactive components in the formulation of pharmaceuticals and cosmetics.⁴ *Origanum vulgare* L. (Lamiaceae), native to the Mediterranean region and western Eurasia, is an aromatic perennial herb, 30–50 cm tall, white- or purple-flowered, with the opposite, lanceolate and toothed leaves⁵ (Figure 1). *Origanum vulgare* is recognized throughout the world as a flavoring herb. It is also used in traditional medicine for its health effects, mainly against bronchial disorders^{6,7} as digestive and antiseptic, for treating colds, indigestion and stomach upsets. The aerial parts contain a wide array of medicinally active components, including phenolic glycosides, flavonoids, tannins, sterols and high amounts of terpenoids. Their content can vary due to the presence of different chemo-types in the same species, geographical region of origin and harvest time.⁸⁻¹² These and other factors can result in different pharmacological effects. The current study was conducted to identify the major constituents of the ethyl acetate extract of *Origanum vulgare* by Liquid chromatography-mass spectroscopy analysis.

MATERIALS AND METHODS

The leaves of *Origanum vulgare* (Figure 1) was procured from the market from Srinagar, Jammu and Kashmir, India. This plant was identified and authenticated by Akhtar H. Malik, Curator Centre for Biodiversity and Taxonomy (CBT) Department of Botany, the University of Kashmir under specimen voucher no. 2710 KASH. A sample specimen of collected material was deposited in the herbarium for future references.

Preparation of Extract

The leaves of *Origanum vulgare* were powdered and the powdered sample of plant material was then subjected to extraction by maceration process for 72 h. The extract obtained was filtered through Whatman filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The extract was then dried and transferred into a closed airtight container and stored at -20°C for further use.

Phytochemical screening

The preliminary phytochemical screening was carried out on the ethyl-acetate fraction of *Origanum vulgare* to determine the presence of various phytoconstituents as shown in Table 1.¹³

Liquid Chromatography-Mass Spectroscopy (LC-MS)

The chemical constituents of the ethyl-acetate extract of *Origanum vulgare* were determined using LC-MS. LC-MS analysis was performed using Mariner Bio spectrometry equipped with a binary pump. The HPLC was interfaced with a Q-TOF mass spectrometer fitted with an ESI source. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140°C. HPLC column Phenomenex 5 μ C8, (150 $\times$ 2 mm i.d) was used for the analysis. The solvent was methanol with 0.3% formic acid. Solvents were delivered at a total flow rate of 0.1 mL/min. The solvent was run by isocratic elution. The MS spectra were acquired in

Figure 1: *Origanum vulgare* aerial parts.

Figure 2: LC-MS chromatogram of an ethyl-acetate extract fraction of *Origanum vulgare*.

Table 1: Phytochemical Screening of ethyl-acetate extract of *Origanum vulgare*.

Constituents	Test	Inference	Ethyl-acetate
Carbohydrates	Molish's test	Violet ring	+
	Fehling's test	Brick red ppt	+
	Barfoed's test	Brick red ppt	+
	Selwinoff's test	Pink color	+
Tannins	5%FeCl ₃	Yellow color	-
	Lead acetate	White ppt	-
Saponins	Foam test	Foaming	-
	Froth test	Frothing	-
Flavonoids	Shinoda test	Pink color	+
Phenolics	1%FeCl ₃	Bluish color	+
Anthraquinone Glycosides	Borntrager's test	Pink Color	-
Terpenoids	Salkowski's test	The golden yellow ring at the junction	+
Phytosterols	Libermann's test	Brown ring at the junction	+
Alkaloids	Dragendroff's reagent	Orange ppt	-
	Mayer's reagent	Cream ppt	-
Proteins	Ninhydrin test	Purple color	-
	Biuret test	Blue color	-

Symbol denoted: (+) sign indicates the presence and (-) sign indicates the absence of Phyto-constituents

Table 2: Important compound identified from the ethyl-acetate extract of *Origanum vulgare* by LC-MS.

Peak	Retention time	Name of the compound	[M H] ⁺ , m/z	UV Detection	MS Detection
1	14.23	Quercetin	301	200,268,336	yes
2	15.65	p-cymene	135	201,288	yes
3	13.3	1-hexacosanol	383	201,330	yes
4	11.16	Luteoloin	287	200,308	yes
5	10.57	Chlorogenic acid	355	225,263,296	yes
6	14.07	Phytol	129	200,254	yes

the positive ion mode. The temperature of the drying gas (N₂) was 350°C at a glass flow rate of 6 mL/min and a nebulizing pressure (N₂) of 25 psi. About 0.5 g of sample extract was diluted with methanol and filtered with a 0.22 µm nylon filter before analysis. A 5 µl volume of the ethyl-acetate extract was injected onto the analytical column for analysis. The mass fragmentations were identified by using the spectrum database for organic compounds in SDBS application.^{14,15}

RESULTS

Phytochemical screening

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc. The extracts of *Origanum vulgare* have been evaluated for the presences of alkaloids, flavonoids, glycosides, phenols, saponins, sterols and triterpenoids, tannins, quinines, coumarins, resins, anthraquinones, phlobatannin, catechol, acidic compounds, reducing sugars, carbohydrates, proteins and amino acids, phenols and glycosides. Results of preliminary screening of *Origanum vulgare* extracts are Table 2.

Liquid Chromatography-Mass Spectroscopy(LC-MS)

The Liquid Chromatography-Mass Spectroscopy (LC-MS) of an ethyl-acetate extract fraction of *Origanum vulgare* shown in (Figure 2) showed a molecular peak (base peak) at m/z 301 (Figure 3) is good agreement with the compound having Chemical Formula: C₁₅H₁₀O₇ Quercetin (1). The compound showing molecular peak (base peak) at m/z 135 (Figure 4) is good agreement with compound p-cymene(2)Chemical Formula: C₁₀H₁₄. The fragmentation has shown a peak at m/z 383 (Figure 5) and it showed the presence of compound 1-hexacosanol(3)Chemical Formula: C₂₆H₅₄O. The mass spectrum showed the molecular peak (base peak) at m/z 287 (Figure 6) is good agreement with compound empirical formula Luteoloin (4) Chemical Formula: C₁₅H₁₀O₆. The fragmentation has shown a peak at m/z 355 (Figure 7) and it showed the presence of Chlorogenic acid (5). The compound showed a molecular peak (base peak) atm/z 129 (Figure 8) is exactly in agreement with the compound Phytol (6) Chemical Formula: C₂₀H₄₀O.

DISCUSSION

Plants have provided a source of inspiration in the drug development as plants derived compounds have made a significant contribution towards human health. Plant-derived Phyto-constituents are to be used for the treatment of diseases as is done in the case of the Unani and Ayurvedic system of medicines. Besides, plants are an important source of potentially useful structures for the development of new chemotherapeutic agents and in identifying the active principle responsible for curing various human diseases. In Present study, the chemical constituents of the

Figure 3: Mass and UV spectrum Quercetin at the retention time 21.92.

Figure 4: Mass and UV spectrum at the retention time 15.65.

Figure 5: Mass and UV spectrum at the retention time 13.3.

Figure 6: Mass and UV spectrum at the retention time 11.16.

Figure 7: Mass spectrum at the retention time 10.72.

Figure 8: Mass and UV spectrum at the retention time 7.78.

ethyl-acetate extract were determined using LC-MS. LC-MS analysis was performed using Mariner Bio spectrometry equipped with a binary pump and the mass fragmentations were identified by using the spectrum database for organic compounds in SDBS application. A total of six intense peaks were observed in the LC-MS chromatogram of an ethyl-acetate extract of *Origanum vulgare* which are further characterized by mass and UV spectrum along with retention time. The compounds

identified can be isolated by various chromatographic techniques and can be further evaluated for various biological activities.

CONCLUSION

Origanum vulgare is a flavoring herb of the family Labiatae. The phytoconstituents present in the plant was the potential source for new drug and therapeutic leads. Phytochemical screening showed that the ethyl

acetate extract of *Origanum vulgare* contains compounds either flavonoids or phenolic compounds. The LC-MS analysis has further brought light for the presence of phytosterols and bioflavonoid. The extract contained monoterpene hydrocarbons and phenolic compounds, the major ones being Chlorogenic acid, p-cymene and phytol. Taken together, these results reinforce the importance of this millennial species and open new perspectives for the research and design of new herbal medicines, obtained from indigenous vegetal material.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS

LC-MS: Liquid Chromatography-Mass Spectrometry; **SDBS:** Spectral Data Base, **MS:** Mass Spectrometry; **Q-TOF:** Quadrupole-Time of Flight; **DMSO:** Dimethyl Sulfoxide; **HPLC:** High-Performance Liquid Chromatography.

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