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PHYTOCHEMICAL ANALYSIS OF PHENOLIC COMPOUNDS BY USING LCMS OF *URGINEA INDICA*

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ABSTRACT:

Background: *Urginea indica* is known as a medicinal plant because it contains many chemical compounds with medicinal peroperties. Numerous bioactive components, including alkylresorcinols, bufadienolides, phytosterols, and flavonoids, are present in the plant, particularly in the bulb. The plant possesses antimicrobial, anthelmintic, antidiabetic, anticancer, antioxidant, and wound healing properties, as demonstrated by numerous scientific investigations. This study is carried out to investigate the various chemical constituents that may be present in the plant, including tannins, phenols, alkaloids, flavonoids, glycosides, carbohydrates, resins, quinones, and saponins. **Objective:** In this study, it was aimed to calculate the total phenolic content from ethanolic extact of bulb of *Urginea indica* (UI) and to characterize the phytochemicals present in it with the help of LCMS. **Materials and Methods:** UI collected from Hamirpur himachal Pradesh, and then bulbous parts of UI were air dried. The powdery form of bulb of was extracted with ethanol, and the phytochemical composition of the bulb extracts was analyzed by liquid chromatography with tandem mass spectrometry (LC-MS) method. To determine the total phenolic content the Folin-Ciocalteu method was used. **Results:** The LC-MS analyses identified 12 compounds in the ethanolic extract of UI. Based on the results it had appeared that UI has phenolic content in its extract.

Keywords: *Urginea indica*, bulb, LCMS, phytochemicals, phenolic content.

INTRODUCTION

India is among the most biodiverse nations in the world, as its biomes have demonstrated a great genetic diversity, such as fruitful and flowerful species Protected areas and biodiversity conservation in India [1]. But when it comes to their phytochemicals and therapeutic qualities, some of the local plants are still underutilized. Because of the state's diverse climate brought about by its elevation variation (250–7000 m), Himachal Pradesh also boasts some of India's most diverse flower species [2].

A total of 2,571 species organised into 1038 genera and 180 families of flowering plants are found in the state [3] (Chowdhery, 1999). Of these, 681 species are monocotyledons and 1890 species are dicotyledons, found in 217 genera and 27 families across 821 genera and 153 families. The largest family in the state is Asteraceae, which has 328 species. It is followed in size by Poaceae (321 spp.), Leguminosae (278 spp.), Rosaceae (157 spp.), Scrophulariaceae (138 spp.), Lamiaceae (136 spp.), Cyperaceae (125 spp.), Ranunculaceae (116 spp.), Apiaceae (92 spp.) and Brassicaceae (83 spp.). In terms of genera, Carex (48 spp.) is the largest, followed by Polygonum (37 spp.), Poa (33 spp.), Gentiana (28 spp., Epilobium (26 spp.), Pedicularis (26 spp.), Cotoneaster (25 spp.), Saussurea (25 spp.), Cyperus (23 spp.) and Euphorbia (23 spp.).

Many endemic taxa belonging to different floral families, such as Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Fabaceae, Juncaceae, Papaveraceae, Poaceae, Ranunculaceae, Scrophulariaceae, Violaceae, etc., are found in Himachal Pradesh [4]. *Aconitum heterophyllum, Anemone tetrasepala, Arnebia euchroma, Dactylorhiza hatagirea, Ephedra gerardiana, Ferula jaeschkeana, Hyoscyamus niger, Limosella aquatica, Mahonia jaunsarensis, Nardostachys jatamansi, Picrorrhiza kurroa, Saussurea obvallata, Valeriana jaeschkii,* and *Waldheimia stoliczkae* are a few of the rare flowering plant taxa found in the state. The ten families Asteraceae, Euphorbiaceae, Fabaceae, Acanthaceae, Ceaselpiniaceae, Moraceae, and Solanaceae were found to have the most significant and useful plants across family distribution, followed by Annonaceae, Convolvulaceae, and Liliaceae [5].

The glabrous herb *Urginea indica* is a member of the liliaceae family and has a polytypic genus. In Himachal Pradesh, it is referred to as jungli piyaz locally and as Indian squill or sea onion more generally [6]. Out of all the plant's parts, the bulb has been found to have enormous therapeutic value. It is primarily used to treat a variety of disorders, including edoema, dropsy, asthma, rheumatism, gout, allergies, wound healing, hypertension, dyspepsia, cardiac stimulation, and hypertension [7].

The literature has not gone into enough detail about the phytochemical composition of UI. Consequently, investigations into the phenolic profile are necessary. Thus, the purpose of this study was to examine the phytochemical compound profile, with a particular focus on the phenolic compounds present in the bulb of *Urginea indica*. In order to identify and quantify the phenolic compounds from UI, the high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS) was used.

METHODOLOGY

Plant material

A good number of UI bulbs were collected from Hamirpur, Himachal Pradesh, India. The Department of Botany, Abhilashi Group of Institution Mandi, HP, identified and verified the plant.

The preparation of *Urgenia indica* bulbs ethanolic extracts (UI):

After being collected from the soil, the bulbs of UI were washed and cleaned with paper towel. The bulbs were shade dried. The dried bulbs then powdered by using mortar and pestle. Until it is needed, the powder is kept between 2°C and 4°C. Using cold maceration and 95% ethanol, 500 g of powdered bulbs from each of the examined UIs were extracted until exhaustion. Every time, the combined ethanol extract was dried completely by evaporating it at 40°C and low pressure [8].

Total Phenolic Content Analysis:

Chemicals and reagents

1. Folin ciocalteu reagent -1:10 diluted in DM water
2. Na_2CO_3- 1.0 M
3. Gallic Acid (Prepared in Methanol: Water (50:50 v/v)

All other chemicals and reagents used in the experiments were of analytical grade.

Principle:

The Foiln-Ciocalteu phenol reagent is composed of phosphotungstic acid and hetero-poly phosphomolybdic acid, where tungsten and molybdenum are in 6+ states. When specific reducing agents are used to reduce the material, tungsten blue and molybdenum blue are produced [9].

Procedure:

The phenolic compounds were ascertained using the Folin-Ciocalteu reagent. The test sample dilutions were mixed with 50µl of diluted folin ciocalteu reagent and 40µl of aqueous Na_2CO_3 (1.0 M). After preparing the reaction mixture in line with the reaction mixture setup table and letting it stand for fifteen minutes, absorbance at 760 nm was measured using the double beam JASCO V-630 spectrophotometer. A 50:50 v/v methanol:water mixture was used to prepare gallic acid as a standard curve, with concentrations ranging from 25 µg/mL to 300 µg/ml [10].

Phytochemical analysis by using LC-MS

Principle:

We used LC-MS techniques to perform the phytochemical analyses. Using a dynamic differential migrational process, two or more components can be separated using this technique. The system consists of two phases: one in which the components move in a specific direction continuously and show different mobilities due to variations in their molecular size, adsorption, or partition [11]. Purification takes place in a separation column that has a stationary phase and a mobile phase. A granular substance containing minute porous particles makes up the stationary phase in a separation column. On the other hand, high pressure is used to force a solvent or solvent mixture—referred to as the mobile phase—through the separation column [12]. The sample is injected with a syringe into the mobile phase flow from the pump to the separation column through a valve that has a sample loop connected to it. The sample loop is essentially a tiny capillary or tube made of stainless steel. As a result of their varied degrees of retention from interactions with the stationary phase, the constituent parts of the sample then migrate through the column at different rates [13]. Following their removal from the column, the substances are detected by a suitable detector and signaled to the computer's LCMS software. At the end of this operation or run, the computer's HPLC software generates a chromatogram. The chromatogram makes it possible to identify and measure the different substances.

Procedure

In a vial, 10 mg of the sample were dissolved in 1 ml of methanol, and the vial was then placed into the sample acquisition instrument under the specified instrumental conditions [14,15].

Table 1&2: Setting Up Instruments for Phytochemicals

Time/as per minutes	Solvent System A (Acetonitrile)	Solvent System B (HCOONH4) buffer
0	5	95
25	20	80
40	20	80
55	35	65
65	80	20

Kind of samples	Active compound Extracts
Column type	C18
Wavelength (λ)	280
Flow rate (ml/min)	0.2
Solvent (A)	CH_3CN
Solvent (B)	Ammonium Formate buffe

Details of Equipment

ACQ-TQD#QBB1152 (LC Instrument), SUNFIRE C18, 250 X 2.1, 2.6um (Column), Waters Alliance e2695/HPLC-TQD Mass spectrometer (MS Apparatus)

Table 3: Conditions for HPLC

% Solvent A	0.0 H_2O
% Solvent B	5.0 CAN
% Solvent C	0.0 MeOH
% Solvent D	95.0 5mM AA
Flow rate	1.000(ml/min)
Stop Time	40.0(mins)
Temperature of Column	35.0(°C)
Pressure Min.	0.0(Bar)
Pressure Max.	300.0(Bar)

Table 4: Gradient of HPLC

Time (min)	Flow (ml/min)	%A	%B	%C	%D
1.00	1.000	0.0	5.0	0.0	95.0
10.00	1.000	0.0	30.0	0.0	70.0
16.00	1.000	0.0	60.0	0.0	40.0
24.00	1.000	0.0	80.0	0.0	20.0
32.00	1.000	0.0	80.0	0.0	20.0
35.00	1.000	0.0	5.0	0.0	95.0
40.00	1.000	0.0	5.0	0.0	95.0

Acquisition Mode: Between m/z 150 and 2000, both positive and negative ionization modes of spectrum recording were used.

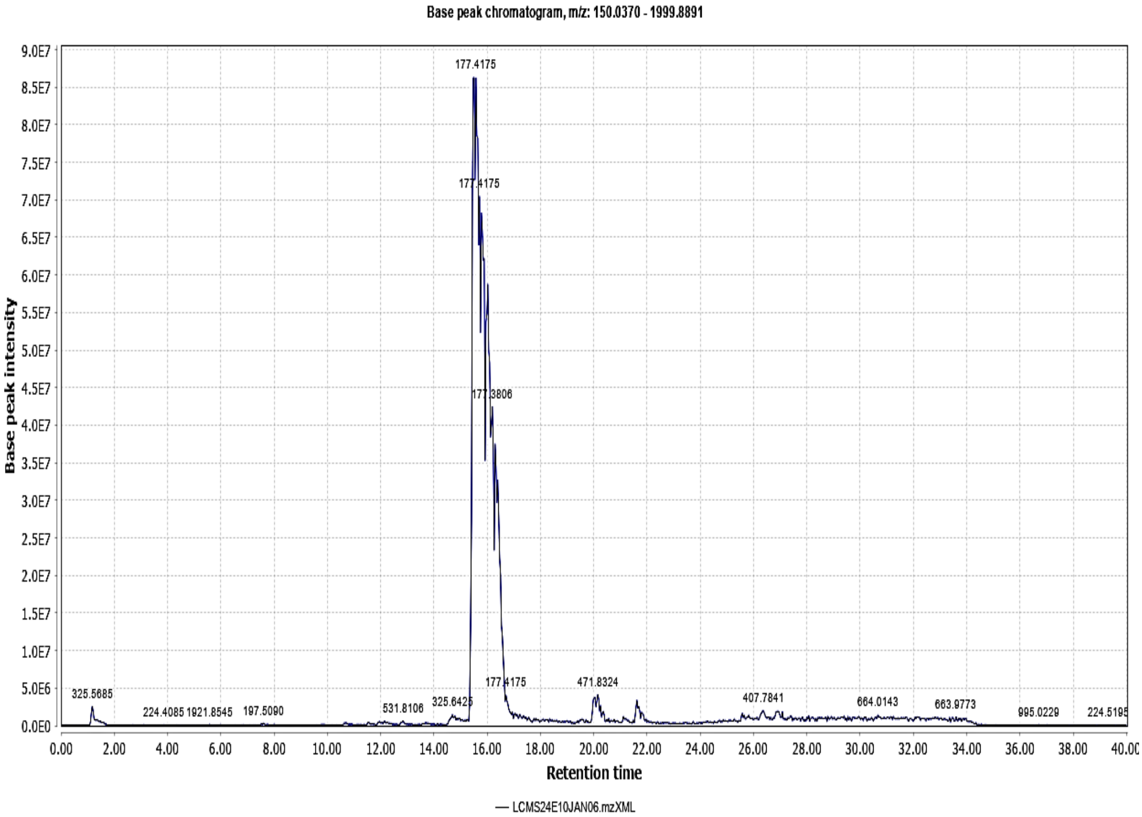
RESULTS

Estimation of total phenolic contents

The hydroxyl groups in phenolic compounds enable them to scavenge, making them potent antioxidants that can break chains. These phenolic substances might have a direct impact on antioxidant activity. The phenolic content of *Urginea indica* was measured in terms of gallic acid equivalents. Based on the results it was found that the ethanolic extract of exhibited 72.2µg/mg phenolic content for UI.

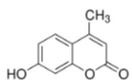
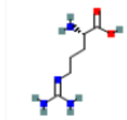
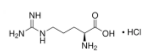
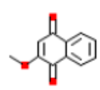
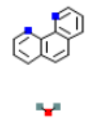
LC-MS analysis of the extracts

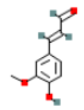
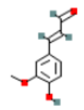
Screening of the extracts by LC-MS/MS enabled the identification of phytochemicals. Ion chromatography was used to identify a total of **12 phytochemical** constituents in the ethanolic extract of UI bulbs. As shown in the table, **seven** of these twelve compounds were identified in the positive mode and **five** in the negative mode.

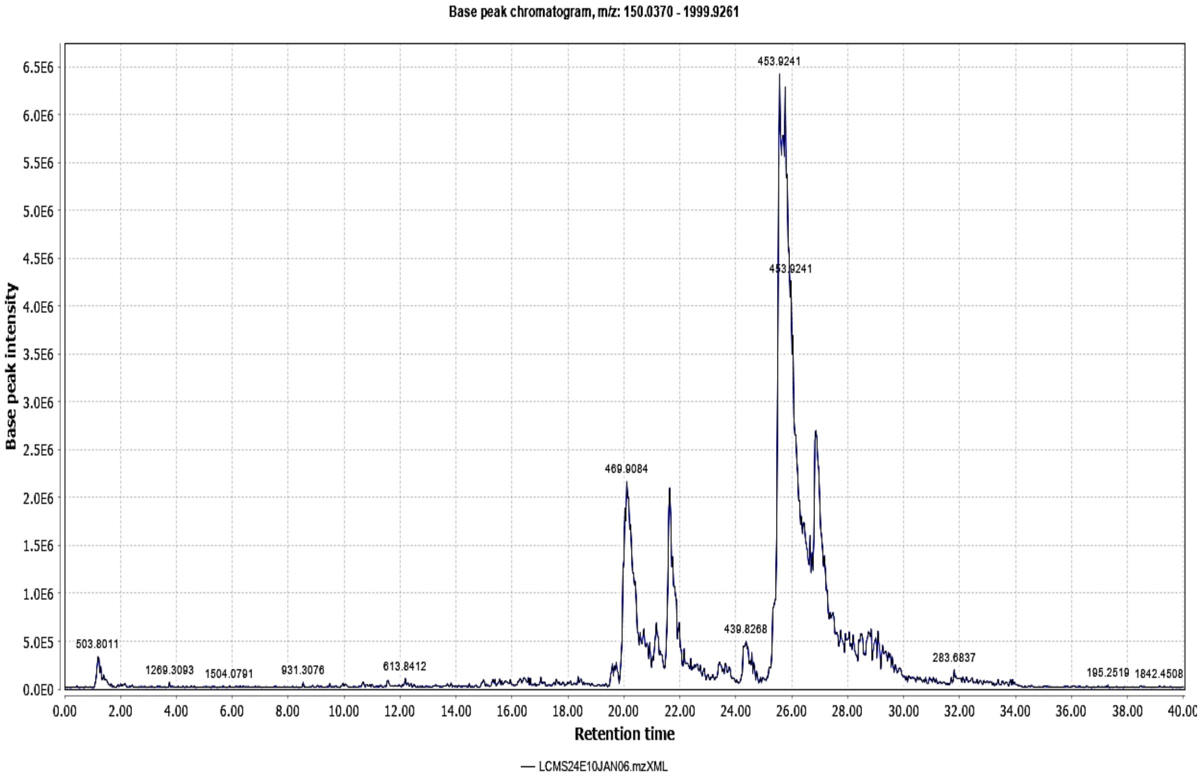


Graph 1: Total Ion Chromatogram (Positive Mode)

Table 5: List of phytochemicals present in UI ethanolic extract (positive mode)

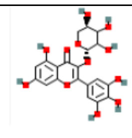
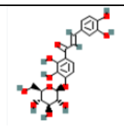
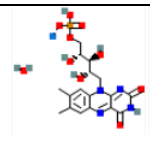
R. Time	Score	Compound Name	Ion	Formula	Exact Mass	Observed Mass	Mass Diff	Chemical Structure
15.58	0.981	7-Hydroxy-4-Methylcoumarin	Positive	$C_{10}H_8O_3$	176.047	177.4175	-1.3705	
15.65	0.986	L(+)-Arginine	Positive	$C_6H_{14}N_4O_2$	174.111	177.4175	-3.3065	
15.72	0.988	L-Arginine Monohydrochloride	Positive	$C_6H_{14}N_4O_2.HCl$	174.111	177.4175	-3.3065	
15.89	0.989	2-Methyl-1,4-Naphthoquinone	Positive	$C_{11}H_8O_2$	172.052	177.4175	-5.3655	
16.02	0.989	1,10-Phenanthroline Monohydrate	Positive	$C_{12}H_8N_2$	180.068	177.3806	2.6874	

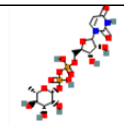
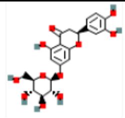
16.19	0.987	4-Hydroxy-3-Methoxycinnamaldehyde	Positive	$C_{10}H_{10}O_3$	178.062	177.3806	0.6814	
16.50	0.99	4-Hydroxy-3-Methoxycinnamaldehyde	Positive	$C_{10}H_{10}O_3$	178.062	177.3806	0.6814	



Graph 2: Total Ion Chromatogram (Negative Mode)

Table 6: List of phytochemicals present in UI ethanolic extract (negative mode)

R. Time	Score	Compound Name	Ion	Formula	Exact Mass	Observed Mass	Mass Diff	Chemical Structure
25.56	0.827	Myricetin-3-Xyloside	Negative	$C_{20}H_{18}O_{12}$	450.079	453.9241	-3.8451	
25.77	0.912	Marein	Negative	$C_{21}H_{22}O_{11}$	450.116	453.8871	-3.7711	
26.04	0.903	Riboflavin-5'-Monophosphate Sodium Salt Hydrate	Negative	$C_{17}H_{21}N_4O_9P$	456.104	453.8871	2.2169	

26.86	0.742	UDP-Beta-L-Rhamnose	Negative	$C_{15}H_{24}N_2O_{16}P_2$	550.06	551.9760	-1.916	
28.84	0.737	Eriodictyol-7-O-Glucoside	Negative	$C_{21}H_{22}O_{11}$	450.116	453.8501	-3.7341	

DISCUSSION

Plants produce very small amounts of low-molecular -weight secondary metabolites called phytochemicals. Carotenoids, phenolic compounds (flavonoids, phytoestrogens, and phenolic acids), phytosterols and phytostanols, tocotrienols, and organosulfur compounds (glucosinolates and allium compounds) are the categories of phytochemicals that have or appear to have a substantial impact on human health. However, a number of studies and reports in recent years have indicated that these phytochemicals have protective effects against a variety of diseases [16]. Major groups such as carotenoids and polyphenols, which comprise phenolic acids, flavonoids, stilbenes, or lignans, can be used to categorise phytochemicals [17].

Urginea indica hold great potential as a natural ingredient for the food and pharmaceutical industries, as they can be used as food supplements, additives, or as a natural antioxidant replacement in functional foods. In addition, the bulb and rhizome contain calcium, iron, and commercial compounds with a range of health-promoting qualities, including quercetin, allose, mindererus spirit, tartaric acid, and paraldehyde. Moreover, it might encourage the creation of new drugs [18]. Phenolic compounds are a family of chemical compounds that have hydroxyl groups directly linked to aromatic hydrocarbon groups. As secondary metabolites, they play a significant function as protective agents. Phenolics have a number of advantageous qualities for people, and their antioxidant qualities are crucial in defining their function as agents of defense against disease processes mediated by free radicals. Nowadays, phenolic compounds' inherent anti-inflammatory, antibacterial, anti-carcinogenic, and antioxidant properties are much sought-after in terms of application and study [19]. The phenolic content were analysed by using the Folin-Ciocalteu method.

A qualitative chemical analysis employing different extraction solvents can be used to determine the chemical composition of a plant product. The primary focus should be on the selection and optimisation of extraction techniques in conjunction with appropriate analytical procedures, taking into account the compound's properties, sources, and solvents utilized [20]. So during this study ethanolic extract of UI leaves was prepared for phytochemical extraction.

Investigating qualitative or quantitative methods for analysing these bioactive chemicals in a variety of plentiful natural sources should be given priority in the research or development of phenolic compounds. This will help to develop methods that are dependable, sensitive, and quick to develop. In the last few years, a wide range of techniques have been investigated or refined. Spectrophotometry is the primary method used to quantify the global assessment of the concentration of phenolic compounds [21]. However, general methodologies enable quantification of this estimation. More precise investigations, however, rely on the detection of particular phenolic classes by sensitive detectors, like mass spectrometry (MS), and their identification using high-performance liquid chromatography (HPLC) or gas chromatography (GC). To achieve improved accuracy in results, an optimised and appropriate method for the extraction and measurement of phenolic chemicals must be developed [22]. Hence, LCMS technique was used to determine the phytochemicals in the extract. As a result of LCMS a total number of 12 compounds were found in the extract.

CONCLUSION

The result of the current study suggests that ethanolic extract of UI Bulbs has phenolic content 72.2μg/mg with respect to gallic acid and it also contains 12 phytochemicals. Many studies supported that phytochemicals have anti-inflammatory and antioxidant qualities that can aid in maintaining a healthy human body. Based on the findings of this study, *Urginea indica* can be regarded as a substitute medicinal plant. Its phytochemicals can be further investigated for their potential use in the production of drugs with anticancer, antidiabetic, anthelmintic, analgesic, antibacterial, antifungal, anti-angiogenic, anti-arthritic, antioxidant, anti-inflammatory, spasmodic, and cardiac stimulant properties.

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CONFLICTS OF INTEREST

All authors declared no conflict of interest.

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