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Phytochemical Profiling of *Phaseolus vulgaris* Linn. Using HR-LCMS: Identification of Bioactive Compounds with Diverse Therapeutic Potential

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Abstract

High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) was utilized to conduct a phytochemical analysis of the methanol extract of a *Phaseolus vulgaris* Linn., aiming to identify bioactive compounds with potential therapeutic uses. The study was performed on an Agilent G6550A system employing optimized conditions and a gradient solvent system consisting of acetonitrile and 0.1% formic acid. Data acquisition was performed in both positive and negative ion modes, spanning a mass range of 126–1200 m/z. The analysis yielded 19 bioactive compounds in positive ion mode and 15 in negative ion mode. These substances exhibit various pharmacological activities, including anticancer, anti-inflammatory, antidiabetic, antibacterial, and antimicrobial effects. These findings serve as a foundation for future investigations aimed at isolating and evaluating the biological activities of these compounds, particularly with respect to cancer, inflammation, diabetes, and microbial infections.

Keywords: *Phaseolus vulgaris* Linn., Leaves, Methanol extract, HR-LCMS

Introduction

Phaseolus vulgaris Linn., commonly referred to as the common bean, is a ubiquitous legume that has drawn considerable interest due to its nutritional content and potential therapeutic applications. (Padmavathi et al., 2021) Historically, various plant components, including seeds, leaves, and seed coats, have been utilized for their medicinal qualities. The methanol extract of *Phaseolus vulgaris* Linn. has emerged as a focal point in pharmaceutical research, owing to its abundance of bioactive constituents. Recent pharmacological studies have underscored the significance of identifying and quantifying bioactive molecules to elucidate their medicinal potential. High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LC-MS) represents a cutting-edge analytical technique that enables comprehensive characterization of complex plant extracts. This method facilitates the identification of diverse metabolites in the methanol extract of *Phaseolus vulgaris* L., offering valuable insights into its chemical composition and the potential for discovering novel therapeutic agents. This research aimed to employ HR-LCMS for analyzing methanol extracts derived from *Phaseolus vulgaris* Linn. leaves. The primary objective was to identify key bioactive compounds that might explain the plant's medicinal properties. The findings of this investigation could provide a scientific basis for the therapeutic use of *Phaseolus vulgaris* leaves, potentially facilitating their integration into modern pharmacological applications.

Materials and Methods

Chemicals

All the chemicals required for extraction are obtained from analytical grade from sigma Aldrich.

Authentication of plant material

The plant is collected from Dapoli region from Maharashtra. It is authenticated from Xavier college, Mumbai. The herbarium submitted matches with Blatter herbarium D.P.2275 of D.P. Panthaki.

Extraction by maceration

The collected leaves were cleaned with water and allowed to dry naturally in protected areas. Once fully dehydrated, the leaves were pulverized into fine powder using a mechanical grinding device. A 250 ml conical flask received 5 g of powdered plant material, followed by the introduction of 50 ml of methanol. The standard ratio of the plant powder to solvent was maintained at 1:10. To avoid contamination and ensure complete immersion of the plant material in the solvent, the flask was sealed with a filter paper. The mixture was subjected to intermittent agitation on a shaker for approximately 72 h. Subsequently, the plant material was filtered and the resulting liquid was concentrated using a rotary evaporation process. The final yield of the crude methanol extract from the plant amounted to approximately 3.11 grams.(Cacique et al., 2020)

HR-LCMS method

Phytochemical profiling of the crude methanol extract obtained through maceration extraction was conducted using high-resolution liquid chromatography-mass spectrometry (HR-LCMS). The analysis employed an Agilent G6550A model with 0.01% mass resolution. The acquisition parameters were set to an MS minimum range of 126 (m/z) and a maximum range of 1200 Da (m/z) with a scanning rate of one spectrum per second. The gas chromatography conditions included a flow rate of 13 psi/min at 250 °C with a stop time of 35 min. Chromatographic separation was achieved using a gradient solvent system comprising solvent A (100% acetonitrile) and solvent B (0.1% formic acid).**(Noumi et al., 2020)**

Results

HR-LCMS for Phytochemical Analysis

High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) was employed to conduct phytochemical analysis on the plant's crude methanol extract. An Agilent G6550A model was utilized under optimized conditions, employing a gradient solvent system of pure acetonitrile (solvent A) and 0.1% formic acid (solvent B). The mass range was set between 126 and 1200 m/z, with a scanning rate of one spectrum per second. The analysis revealed 19 bioactive compounds in positive ion mode and 15 in negative ion mode.

Discussion

Positive Ion Mode Analysis

The positive ion mode identified 19 bioactive compounds, demonstrating its effectiveness in detecting substances that form stable cations. This mode is particularly adept at identifying metabolites such as alkaloids, glycosides, and flavonoids. Many of these compounds are likely to exhibit a range of therapeutic effects, including anticancer, anti-inflammatory, antidiabetic, and antibacterial properties. For instance, certain alkaloids and flavonoids typically detected in this mode have been extensively documented for their anticancer and anti-inflammatory activities. These substances have been shown to influence crucial signaling pathways involved in cell proliferation, apoptosis, and inflammation, making them potential candidates for cancer treatment and inflammatory disorders.

Negative Ion Mode Analysis

In the negative ion mode, 15 bioactive compounds were detected. This mode excels at identifying acidic compounds, including phenolic acids, flavonoid aglycones, and organic acids, which are prevalent in plants. These substances are renowned for their potent antioxidant, antimicrobial, antidiabetic, and anticancer properties.

Comparative Evaluation of Ion Modes

The positive and negative ion modes provided complementary data regarding the plant's chemical composition. While the positive mode was more effective in identifying a broader range of compounds, including alkaloids and glycosides associated with anticancer and anti-inflammatory activities, the negative mode excelled at detecting more acidic substances like phenolic acids and flavonoid aglycones, known for their antioxidant, antibacterial, antidiabetic, and anticancer properties. The identification of these bioactive compounds in both modes suggests that the plant possesses a wide spectrum of pharmacological effects, particularly in addressing chronic diseases such as cancer, diabetes, and inflammation. The combined information from both modes strengthens the hypothesis that plants contain a complex array of bioactive molecules with diverse therapeutic potential

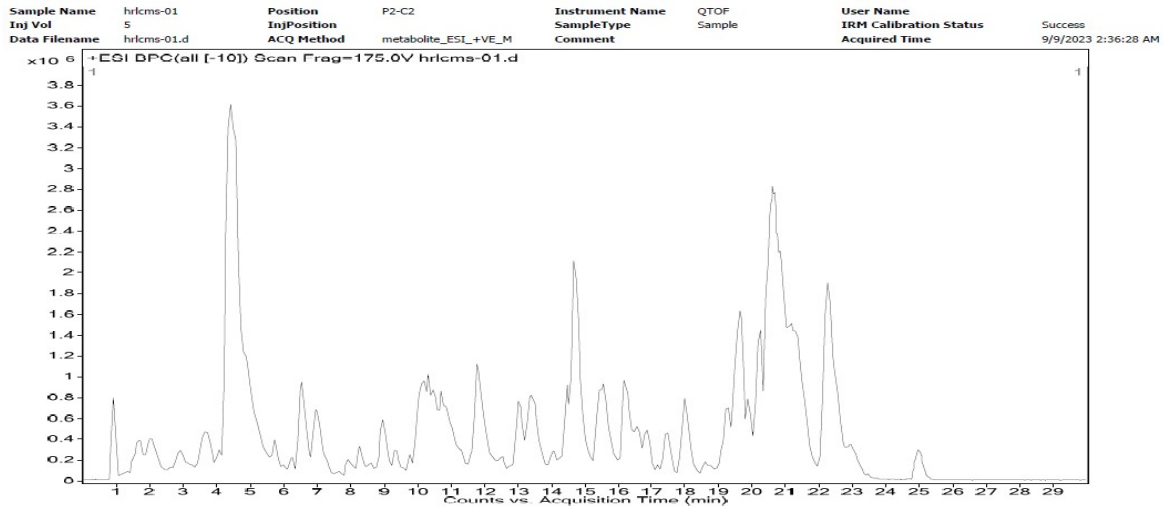

Fig.1: Positive mode of HR-LCMSS chromatogram of crude methanol extract of *Phaseolus vulgaris* Linn leaves

Table 1: Compounds obtained from positive mode of ESI

Sr.No	Name	Retention time	Mass	Formula	DBDiff(ppm)	Reported bioactivity	References
1	Anthranilicacid	1.555	137.0468	$C_7H_7NO_2$	6.64	Antimicrobial, Anticancer Anti-inflammatory,Anti-viral, anti-insecticidal	(Marinova & Hristov, 2023)
2	N-(1-Deoxy-1-fructosyl)isoleucine	1.636	293.1448	$C_{12}H_{23}NO_7$	9.03	Not Found	
3	L-isoleucyl-L-proline	1.647	228.1452	$C_{11}H_{20}N2O_3$	9.78	Normotensive	(Bernard et al., 2005)
4	Pirbuterol	1.692	240.1452	$C_{12}H_{20}N_2O_3$	9.2	Anti-asthma	(Cells, 2006)
5	N-(1-Deoxy-1-fructosyl)phenylalanine	2.074	327.1289	$C_{15}H_{21}NO_7$	8.95	Antibacterial	(Bernardo-bermejo et al., 2021)
6	LarixinicAcid	2.917	126.0308	$C_6H_6O_3$	7.07	Antioxidant, Anticancer	(Ramadan et al., 2022)
7	Chromone	3.665	146.0358	$C_9H_6O_2$	6.67	Antimicrobial,Anti-viral,Anticancer, Anti-oxidant	(Khadem & Marles, 2012)

8	Glucocaffeic acid	3.67	342.0923	$C_{15}H_{18}O_9$	8.01	Antioxidant, Anticancer, Anti-inflammatory, Antidiabetic	(Monteiro Espíndola et al., 2019)
9	N(alpha)-Benzyloxycarbonyl-L-leucine	3.671	265.1292	$C_{14}H_{19}NO_4$	8.47	Skin and bones	(Zheng et al., 2021)
10	Isobutyl N-methylanthranilate	4.089	207.1241	$C_{12}H_{17}NO_2$	8.67	Anticancer	(Zheng et al., 2021)
11	2,4-Dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one	4.091	241.056	$C_{10}H_{11}NO_6$	10.88	Not reported yet	-------
13	Ruspolinone	4.48	249.1343	$C_{14}H_{19}NO_3$	8.93	Organocatalyst and building blocks in organic synthesis, Antioxidant and anticancer	(Eze et al., 2019)
14	Quercetin	4.733	302.0399	$C_{15}H_{10}O_7$	8.98	Antioxidant, cytotoxic active	(Anand David et al., 2016)
15	Metalaxyl	4.85	279.1447	$C_{15}H_{21}NO_4$	8.47	Antioxidant and Fungiside	(De Sousa et al., 2017)
16	Maritimetin	5.203	286.0451	$C_{15}H_{10}O_6$	9.07	Antiviral, antibacterial, antifungal, anti-inflammatory, antitumor, antimalarial, and antioxidant:	(Jin, 2010)
17	N-[(Ethoxycarbonyl) methyl)-p-menthane-3-carboxamide	5.372	269.1966	$C_{15}H_{27}NO_3$	9.36	Flavouring and fragrance agent	(Aids, 2012)
18	N-Carboxyacetyl-D-phenylalanine	5.924	251.0766	$C_{12}H_{13}NO_5$	10.97	Not reported	------
19	Fabianine	6.556	219.1606	$C_{14}H_{21}NO$	7.88	Diuretic	(O.E.Edward, 1961)

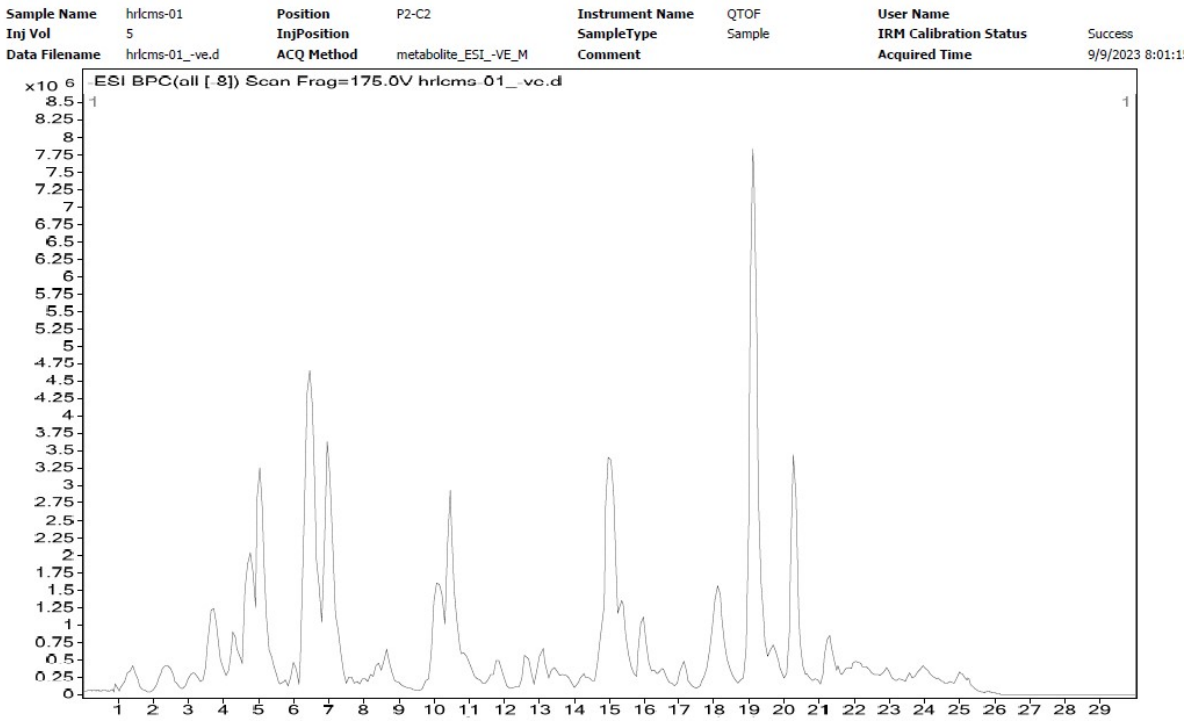

Fig.2: Negative ESI mode of HR-LCMS chromatogram of crude methanol extract of *Phaseolus vulgaris* Linn leaves

Table 2: bioactive compounds obtained from negative mode of HR-LCMS of methanol extract of *Phaseolus vulgaris* Linn

Sr. No.	Name of Compound	Retention time	Molecular weight	Molecular formula	DB (ppm)	Reported bioactivity	Reported Bioactivity
1	Melibiose	1.338	342.116	$C_{12}H_{22}O_{11}$	0.7	Anticancer	(Lin & Tzi, 2008)
2	L-Arabinose	1.4	150.0521	$C_5H_{10}O_5$	4.92	Anti-Glycemic and insulinemic	(Pol et al., 2022)
3	L-Malicacid	1.498	134.0207	$C_4H_6O_5$	6.51	Antibacterial	(Wei et al., 2021)
4	D-Lombricine	2.393	270.0727	$C_6H_{15}N_4O_6$	0.64	Antibacterial	(Yan, 2022)
5	Caffeicacid3-glucoside	3.108	342.094	$C_{15}H_{18}O_9$	3.06	Antioxidant	(Cizmarova et al., 2020)
6	Hamamelose	3.748	196.0573	$C_6H_{12}O_7$	5.19	Anti-inflammatory, Antioxidant	(Cheesman et al., 2023)
7	3,5-dihydroxybenzoicacid	3.93	154.0257	$C_7H_6O_4$	6.18	Inhibits lipolysis in wild-type mouse adipocytes.	(Liu et al., 2012)

8	2,6-dihydroxybenzoicacid	3.95	154.0258	$C_7H_6O_4$	5.56	Oxidation	(Liu et al., 2012)
9	Monomethyl phthalate	4.859	180.0417	$C_9H_8O_4$	3.11	Thyroid cancer	(Huang et al., 2021)
10	4-Hydroxycinnamicacid	6.01	164.0469	$C_9H_8O_3$	2.87	Antioxidant, Antimicrobial, Anticancer	(Kim et al., 2016)
11	Manghaslin	6.021	756.2112	$C_{33}H_{40}O_{20}$	0.11	Antioxidant and Anti-inflammatory	(Teh et al., n.d.)
12	N-Acetyl-D-tryptophan	6.397	246.0997	$C_{13}H_{14}N_2O_3$	3.18	sclerosis and rheumatoid arthritis	(Hogan et al., 2017)
13	Benzoicacid	6.674	122.0362	$C_7H_6O_2$	4.48	Antimicrobial	(Georgousaki et al., 2020)
14	D-Pinitol	6.903	194.0783	C7H14O6	3.67	Antidiabetic, Anticancer	(Ramadan et al., 2022)
15	Azelaicacid	7.404	188.1044	$C_9H_{16}O_4$	2.46	Anti-inflammatory	(Spaggiari et al., 2023)

Conclusion

The application of High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) to analyze the methanol extract of the plant under study yielded significant results. In positive ion mode, 19 bioactive compounds were identified, while 15 were detected in negative ion mode. These findings showcase HR-LCMS's capability in elucidating and characterizing bioactive metabolites within plant extracts. The higher compound count in positive mode suggests its particular effectiveness for detecting certain bioactive molecule types, such as alkaloids, flavonoids, or other positively charged entities. Conversely, the negative mode contributed to identifying additional bioactive compounds, potentially representing distinct chemical classes with unique polarity profiles, like phenolics or organic acids. The extensive range of bioactive compounds detected across both ion modes points to the plant's intricate chemical composition, indicating potential for a wide array of biological activities. These compounds may offer various pharmacological and therapeutic possibilities.

Further investigation, including biological assays, is essential to evaluate the specific bioactivities of these compounds, such as their antimicrobial, anti-inflammatory, and antioxidant properties, as well as to explore their mechanisms of action. These results underscore the importance of HR-LCMS in comprehensive phytochemical profiling and bioactive compound identification within plant extracts. This approach provides a valuable foundation for future research aimed at uncovering novel natural products with potential therapeutic applications.

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Conflict of interest statement

Authors declares no conflict of interest.

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