Annals of Phytomedicine 13(2): 859-870, 2024

DOI: http://dx.doi.org/10.54085/ap.2024.13.2.88

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Analyzing the minerals and phytochemicals composition of *Phaseolus vulgaris* L. using ethanol extract by gas chromatography and mass spectroscopy (GC-MS) analysis

T. Velmurugan, R. Balakumbahan*, K. Kalpana◆, K. Sundharaiya***, M. Gnanasekaran*** and M. Kabilan**

Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam-625 604, Theni, Tamil Nadu, India

**Horticultural Research Station, Tamil Nadu Agricultural University, Thadiyankudisai-624 212, Dindigul, Tamil Nadu, India*

*** Department of Plant Protection, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam-625 604, Theni, Tamil Nadu, India*

**** Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam-625 604, Theni, Tamil Nadu, India*

Article Info

Article history
Received 1 August 2024
Revised 11 September 2024
Accepted 12 September 2024
Published Online 30 December 2024

Keywords
Bioactive compounds
Crude protein
GC-MS
Minerals
Pole bean
Phaseolus vulgaris L.

Abstract

The study employed a randomized complete block design with three replications, and included forty-three genotypes and one standard check (TKDPV(P) 13 from TKDPV(P) 1 to TKDPV(P) 44. The results showed that the genotype TKDPV(P) 8 showed the highest levels of crude protein (25.98%), crude fibre (6.53%), Fe (10.02 ppm), Zn (5.02 ppm), Cu (5.44 ppm), and Mn (2.21 ppm). Major bioactive compounds identified from the genotype TKDPV(P) 8 includes stigmasterol (19.02%), chondrillasterol (19.02%), eicosane (7.40%), phenol (6.29%), ethyl 3-hydroxybenzoate (6.29%), myristoyl chloride (5.79%), 1,3,12-nonadecatriene (3.68%), 15-hydroxypentadecanoic acid (3.63%), isopropyl linoleate (3.46%), n-methyl-1-adamantaneacetamide (2.92%), 9,12,15-octadecatrienoic acid, ethyl ester (Z, Z, Z) (2.32%), pyrazine (2.18%), cyclohexane (1.80%), oxazole (1.79%), phthalic acid (1.70%), phytol (1.69%), 2-diamino-3,4,6-trifluorobenzene (1.37%), benzenemethanol (1.35%), morpholine (1.23%), benzoic acid (0.60%), and pyrocatechol (0.41). Among forty-three genotypes and one standard check, the genotype TKDPV(P) 8 is deemed the highest mineral content, and enhancing phytochemical quality of *P. vulgaris*.

1. Introduction

Pole bean (*Phaseolus vulgaris* L.) a warm season vegetable, is one of the most important legumes cultivated in India (Baruah *et al*., 2022). It is an annual, highly self-pollinated, indeterminate type, grow vertically up to 4 m and are normally trained in pandal system. *Phaseolus* originated in Central and South America, with secondary origins in Peru, and Ecuador. There are three landraces of beans such as Mesoamerican, Durango, and Nueva Granada. It is also called as french bean, fresh bean, garden bean, kidney bean, common bean, snap bean, navy bean, pinto bean, haricot bean, and string bean (Jhanavi *et al*., 2018). Pole bean, often referred to as the "meat of the poor", the "grain of hope" and a "superfood" (Abou Hadid., 2012). It has a higher nutritional index of protein in dried pods (21.1%) as well as in fresh pods (1.7%), and it is a rich source of fibre, protein, and vitamins (Salehi *et al*., 2008). According to Sarakamis *et al*. (2009), it is the third most significant source of calories, and the second most significant source of diet proteins for humans. Key drug classes include immunosuppressants, anticancer, antifungal, antimalarial, antiparasitic, antihyperlipidemic, and antidiabetic medications are all made from natural materials, particularly plants (Nupur, 2021). Consuming beans regularly can lower the risk of cancer, type 2 diabetes, and coronary heart disease. It also contains carminative properties, lowers the risk of chronic and diseases like diabetes, cardiovascular disease, cancer, and obesity (Jhanavi *et al.,* 2018).

Several combinations of elemental and phytochemical compositions affect the plant's medicinal potency (Goel *et al*., 2022). The best and most responsible use of natural resources is made possible by phytochemical screening, which is essential in finding new sources of compounds with medicinal significance that are valuable both industrially, and therapeutically. More and more analysis of the Fabaceae family is required to obtain novel phytochemicals (Bhawana *et al*., 2021). An advanced analytical method called gas chromatography-mass spectroscopy (GC-MS) is used to find compounds in extracts, that are present in trace amounts. It works especially well for identifying bioactive substances such as amino acids, alcohols, long-chain hydrocarbons, esters, nitro compounds, and steroids. Through interpretation and comparison with reference spectra, unknown organic compounds in complex mixtures can be identified (Shunmugapriya *et al*., 2017). The aim of this research is to investigate the phytochemicals found in the ethanolic pod extract of *P. vulgaris* and to use GC-MS analysis to identify and characterize the bioactive compounds.

Corresponding author: Dr. K. Kalpana
Associate Professor (Plant Pathology), Department of Plant Protection, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Periyakulam-625 604, Theni, Tamil Nadu, India
E-mail: kalpssri73@gmail.com
Tel.: +91-9994387816

2. Materials and Methods

2.1 Sample collection

The experimental site was located at Horticultural Research Station (10° 29'80.47 North latitude, 77° 70' 97.27 East longitude), 1100 m above MSL, F block, Thadiyankudisai, Perumparai Post, Dindigul district, Tamil Nadu. The research was carried out using a randomized complete block design (RCBD) with three replications with 43 Pole type bean genotypes and one standard check (TKDPV(P) 13-KKL 1), *viz.,* TKDPV(P) 1 to TKDPV(P) 44 during 2023-2024. The details of Pole bean accessions/genotypes and source of collections are represented in the Table 1.

Table 1: Details of Pole bean accessions/genotypes and source of collection

S. No.	Genotypes/Accessions code	Source of collection
1.	TKDPV(P) 1	Dharwad, Karnataka
2.	TKDPV(P) 2	Gundur-Yercaud, Salem
3.	TKDPV(P) 3	Iyyarpatti, Udhagamandalam
4.	TKDPV(P) 4	Iyyarpatti, Udhagamandalam
5.	TKDPV(P) 5	Iyyarpatti, Udhagamandalam
6.	TKDPV(P) 6	Iyyarpatti, Udhagamandalam
7.	TKDPV(P) 7	Kavunji-Kodaikanal, Dindigul
8.	TKDPV(P) 8	Naidupuram-Kodaikanal, Dindigul
9.	TKDPV(P) 9	Naidupuram-Kodaikanal, Dindigul
10.	TKDPV(P) 10	Pallangi-Kodaikanal, Dindigul
11.	TKDPV(P) 11	Periyur, Dindigul
12.	TKDPV(P) 12	Perumparai, Dindigul
13.	TKDPV(P) 13	(KKL 1)-Kodaikanal, Dindigul
14.	TKDPV(P) 14	Kodaikanal, Dindigul
15.	TKDPV(P) 15	Kodaikanal, Dindigul
16.	TKDPV(P) 16	Ebbanad, Udhagamandalam
17.	TKDPV(P) 17	Ebbanad, Udhagamandalam
18.	TKDPV(P) 18	Kengamudi, Udhagamandalam
19.	TKDPV(P) 19	Kengamudi, Udhagamandalam
20.	TKDPV(P) 20	(EC 25500), ICAR - NBPGR, Shimla
21.	TKDPV(P) 21	(EC 25502), ICAR - NBPGR, Shimla
22.	TKDPV(P) 22	(EC 25516), ICAR - NBPGR, Shimla
23.	TKDPV(P) 23	(EC 26408), ICAR - NBPGR, Shimla
24.	TKDPV(P) 24	(EC 42961), ICAR - NBPGR, Shimla
25.	TKDPV(P) 25	(IC 17887), ICAR - NBPGR, Shimla
26.	TKDPV(P) 26	(IC 39050), ICAR - NBPGR, Shimla
27.	TKDPV(P) 27	(IC 39052), ICAR - NBPGR, Shimla
28.	TKDPV(P) 28	Odane-Kotagiri, Udhagamandalam
29.	TKDPV(P) 29	Odane-Kotagiri, Udhagamandalam
30.	TKDPV(P) 30	Odane-Kotagiri, Udhagamandalam
31.	TKDPV(P) 31	Kadanad, Udhagamandalam
32.	TKDPV(P) 32	Kadanad, Udhagamandalam
33.	TKDPV(P) 33	Oormalai, Udhagamandalam
34.	TKDPV(P) 34	Oormalai, Udhagamandalam
35.	TKDPV(P) 35	Karaipillu, Udhagamandalam

36.	TKDPV(P) 36	Karaipillu, Udhagamandalam
37.	TKDPV(P) 37	Vadakaraiparai-Kodaikanal, Dindigul
38.	TKDPV(P) 38	Dirang-West Kameng, Arunachal Pradesh
39.	TKDPV(P) 39	Dirang-West Kameng, Arunachal Pradesh
40.	TKDPV(P) 40	Dirang-West Kameng, Arunachal Pradesh
41.	TKDPV(P) 41	Namshu-West Kameng, Arunachal Pradesh
42.	TKDPV(P) 42	Namshu-West Kameng, Arunachal Pradesh
43.	TKDPV(P) 43	Namshu-West Kameng, Arunachal Pradesh
44.	TKDPV(P) 44	Namshu-West Kameng, Arunachal Pradesh

Note: TKDPV(P)–Thadiyankudisai *P. vulgaris* (Pole type)

2.2 Proximate and mineral composition

The proximate composition of Pole bean pods, including crude protein and crude fibre, was assessed three times using the Association of Official Analytical Chemists (AOAC, 1990). The crude protein content was measured by Lowry method (1951) while crude fibre was measured using the Maynard method (1970). Micronutrient content, *viz.,* Fe, Zn, Cu and Mn were assessed through an atomic absorption spectrophotometer method (Jackson, 1973).

2.3 Preparation of sample extract

The beans were thoroughly cleaned with tap water to eliminate any residual particles. Subsequently, they were set out to dry in a protected location for a span of 3 to 5 days, contingent on their moisture content. Once dry, the pods were reduced to a fine powder using a blender. 30 ml of ethanol was used to extract 10 g of powdered beans overnight and the mixture was then filtered by Whatman filter paper No.42. By adding bubbling nitrogen to the mixture, the extract was concentrated to 1 ml. To identify the phytochemical compounds, GC-MS analysis was performed using 2 µl of the ethanolic extract.

Figure 1: TKDPV(P) 8 Pole bean.

Figure 2: TKDPV(P) 8 pod powder.

2.4 Gas-chromatography and mass-spectrometry (GC-MS) analysis

GC-MS plays an essential role in chemotaxonomic studies and phytochemical components evaluation regarding pharmacologically active compounds of plants (Srivani *et al*., 2023). To begin the extraction process, ethyl alcohol was added to a sealed flask containing the necessary amount of powdered bean pods. After letting the mixture infuse for a full day, a vacuum distillation device was used to filter and dry the mixture. The GC-MS system was then used to analyse the residue that was left over. Utilizing an Elite-I fused RMS 6 silica capillary column made of dimethyl polysiloxane, a gas chromatography and mass spectrometry (GC-MS) and the Thermo GC Ultra Clarus 550 system was utilized to conduct the analysis. The process of detection involved the use of an electron ionization apparatus with an ionizing energy of 60 eV.

A single microliter of the sample (1 µl) was injected at a split ratio of 12:1 and helium (99.9 per cent) were utilized as the carrier gas, flowing at a constant 2 ml daily. 240°C and 230°C were set as the new temperatures for the injector and ion source. Set to start at 90°C, the oven would rise by 5°C per min to 240°C before holding

at that temperature for three min. For pieces whose diameters ranged from 50 to 650 Da, mass spectra were acquired at a scan interval of 0.5 sec. Utilizing turbo mass software, the mass spectra and chromatograms were examined (Dutta *et al*., 2020).

2.5 Identification of bioactive compounds

Using retention values for over 95,000 compounds, the National Institute of Standards and Technology (NIST) database (https://www.nist.gov/srd/nist-standard-reference-database1a-v14) was used to analyse and interpret the mass spectra obtained from the GC-MS. In order to match unknown components with known substances, spectra from the NIST and Wiley libraries were reviewed. Through the use of this method, the test materials identity, composition and molecular weight were all possible.

2.6 Biological activity of identified substances

Using PASS (Prediction of Activity Spectra for Biologically Active Substances), we were able to predict the biological effects of the compounds based on their structural formulas. As per the online database PASS, this procedure comprised projecting a variety of pharmacological impacts, possible toxicities and potential mechanisms of action connected to the substances (Filimonov *et al.,* 2014).

3. Results

3.1 Proximate analysis

3.1.1 Crude protein (%)

The observations recorded on the crude protein content of Pole bean genotypes are depicted in Table 2, with the mean values ranging from 16.80 per cent to 25.98 per cent. Among the 43 genotypes and one standard check, TKDPV(P) 8 displayed the highest crude protein content of 25.98 per cent, followed by TKDPV(P) 3 with 25.56 per cent. Whereas, TKDPV(P) 44 exhibited the lowest crude protein concentration of 16.80 per cent.

3.1.2 Crude fibre (%)

The data pertaining to changes in crude fibre content in Pole bean genotypes are presented in Table 2, with the mean values ranging from 3.02 per cent to 6.53 per cent. Among the 43 genotypes and one standard check, TKDPV(P) 8 exhibited the highest crude fibre content of 6.53 per cent, followed by TKDPV(P) 3 with 6.49 per cent. Alternatively, TKDPV(P) 44 displayed the lowest crude fibre content of 3.02 per cent.

3.2 Mineral analysis

3.2.1 Iron content (ppm)

Observations on the iron content are presented in Table 2. The mean value of iron content varied between 4.09 to 10.02 ppm among different genotypes. In terms of genotypes, TKDPV(P) 8 noted the highest iron content (10.02 ppm), followed by TKDPV(P) 12 with 9.97 ppm, while the lowest iron content was recorded in TKDPV(P) 4 with the value of 4.09 ppm.

3.2.2 Zinc content (ppm)

The observations recorded on the zinc content of pole bean genotypes are depicted in Table 2, with the mean values ranging from 2.18 ppm to 5.02 ppm. Among the 43 genotypes and one standard check, TKDPV(P) 8 displayed the highest zinc content of 5.02 ppm, followed by TKDPV(P) 12 with 5.00 ppm. Whereas, TKDPV(P) 41 exhibited the lowest zinc concentration of 2.18 ppm.

3.2.3 Copper content (ppm)

The data pertaining to changes in copper content in pole bean genotypes are presented in Table 2, with the mean values ranging from 3.03 ppm to 5.44 ppm. Among the 43 genotypes and one standard check, TKDPV(P) 8 exhibited the highest copper content of 5.44 ppm, followed by TKDPV(P) 21 with 5.29 ppm. Alternatively, TKDPV(P) 7 displayed the lowest copper concentration of 3.03 ppm.

3.2.4 Manganese content (ppm)

Observations on the manganese content are presented in Table 2. The mean value of manganese content varied between 0.93 to 2.21 ppm among different genotypes. In terms of genotypes, TKDPV(P) 8 noted the highest manganese content of 2.21 ppm, followed by TKDPV(P) 1 with 2.16 ppm, while the lowest manganese content was recorded in TKDPV(P) 35 with the value of 0.93 ppm.

Table 2: Effect of different genotypes on crude protein (%), crude fibre (%), iron (ppm), zinc (ppm), copper (ppm) and manganese (ppm)

S. No.	Genotypes/ Accessions code	Crude protein (%)	Crude fibre (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
1.	TKDPV(P) 1	21.03	5.85	6.27	3.05	3.60	2.16
2.	TKDPV(P) 2	19.72	6.25	5.58	4.58	3.57	1.19
3.	TKDPV(P) 3	25.56	6.49	9.91	4.91	5.07	0.98
4.	TKDPV(P) 4	17.36	6.15	4.09	3.96	4.14	1.24
5.	TKDPV(P) 5	18.52	5.27	7.13	3.72	4.89	1.37
6.	TKDPV(P) 6	16.99	5.58	5.28	3.15	4.64	1.33
7.	TKDPV(P) 7	21.30	4.26	6.71	3.90	3.03	1.47
8.	TKDPV(P) 8	25.98	6.53	10.02	5.02	5.44	2.21
9.	TKDPV(P) 9	19.25	4.98	8.01	4.25	3.35	1.13
10.	TKDPV(P) 10	23.57	6.35	9.90	4.89	5.04	1.96
11.	TKDPV(P) 11	20.91	6.38	9.94	4.98	5.25	2.09

12.	TKDPV(P) 12	22.14	6.32	9.97	5.00	4.98	1.82
13.	TKDPV(P) 13 (KKL 1)	19.37	5.21	7.87	4.10	4.51	1.57
14.	TKDPV(P) 14	20.91	4.81	7.95	4.08	4.67	0.95
15.	TKDPV(P) 15	23.11	3.59	8.83	4.75	3.40	1.68
16.	TKDPV(P) 16	18.27	3.87	6.13	3.91	3.38	1.53
17.	TKDPV(P) 17	20.59	4.61	6.86	3.96	3.86	1.61
18.	TKDPV(P) 18	22.84	4.25	4.92	3.10	4.51	1.04
19.	TKDPV(P) 19	17.65	4.97	7.49	3.82	3.28	1.08
20.	TKDPV(P) 20	20.46	5.93	5.41	3.26	4.01	1.29
21.	TKDPV(P) 21	24.02	6.29	8.99	4.84	5.29	2.02
22.	TKDPV(P) 22	20.15	5.27	6.33	4.13	5.22	1.58
23.	TKDPV(P) 23	22.36	4.68	7.18	4.54	4.95	1.78
24.	TKDPV(P) 24	19.67	4.38	4.21	3.59	3.97	1.65
25.	TKDPV(P) 25	18.92	4.81	5.83	3.93	3.24	1.48
26.	TKDPV(P) 26	22.45	5.43	8.01	4.62	4.58	1.39
27.	TKDPV(P) 27	17.63	5.76	6.08	4.16	4.19	1.25
28.	TKDPV(P) 28	17.84	4.28	8.51	4.71	4.68	1.91
29.	TKDPV(P) 29	21.09	4.35	6.13	4.00	4.07	1.83
30.	TKDPV(P) 30	20.81	3.95	6.49	4.08	4.33	1.05
31.	TKDPV(P) 31	18.37	4.60	6.06	3.92	4.59	1.90
32.	TKDPV(P) 32	23.62	5.20	6.27	4.28	4.22	1.87
33.	TKDPV(P) 33	19.82	4.89	5.57	3.87	4.57	1.24
34.	TKDPV(P) 34	21.37	3.74	4.38	2.77	4.14	1.00
35.	TKDPV(P) 35	17.58	4.51	6.19	4.18	4.72	0.93
36.	TKDPV(P) 36	20.53	4.73	5.38	4.99	3.48	1.21
37.	TKDPV(P) 37	16.81	3.11	6.97	3.85	3.61	1.53
38.	TKDPV(P) 38	22.38	3.12	7.81	3.01	3.85	1.69
39.	TKDPV(P) 39	20.12	3.71	5.94	3.86	3.66	1.82
40.	TKDPV(P) 40	18.73	3.75	4.61	2.99	3.27	1.36
41.	TKDPV(P) 41	16.84	3.36	5.19	2.18	3.79	1.49
42.	TKDPV(P) 42	17.24	3.25	6.08	2.28	3.11	1.74
43.	TKDPV(P) 43	17.59	3.09	4.27	2.63	3.58	1.16
44.	TKDPV(P) 44	16.80	3.02	5.46	2.89	3.65	1.59
	SE (d)	0.41	0.10	0.15	0.08	0.09	0.03
	CD (0.05)	0.83**	0.21**	0.31**	0.17*	0.18*	0.06*

3.3 Metabolite profiling using GC-MS analysis

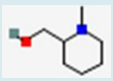
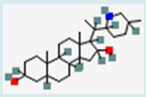
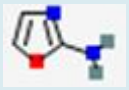
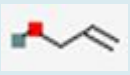
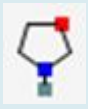
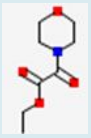
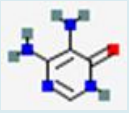
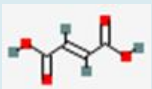
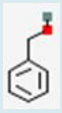
GC-MS analysis was used to determine which bioactive substances were present in the Pole bean**.** Among the genotypes, TKDPV(P) 8 has recorded high yield coupled with proximate and mineral composition. So, TKDPV(P) 8 has been taken for GC-MS analysis to identify and characterize the bioactive compounds. The components of the Pole bean from TKDPV(P) 8 genotype shown by the chromatogram and mass spectrum (Figures 3 to 7). The identified bioactive compounds, along with their peak area (%), retention time (min) and molecular weight (g/mol) are presented in the Table 3. Notably, totally 136 compounds found in Pole bean from TKDPV(P) 8, the significant compounds such as stigmasterol (19.02%), chondrillasterol (19.02%), eicosane (7.40%), phenol (6.29%), ethyl 3-hydroxybenzoate (6.29%), myristoyl chloride (5.79%), 1,3,12-nonadecatriene (3.68%), 15-hydroxypentadecanoic acid (3.63%), isopropyl linoleate (3.46%), n-methyl-1-adamantaneacetamide (2.92%), 9,12,15-octadecatrienoic acid, ethyl ester (Z,Z,Z) (2.32%), pyrazine (2.18%), cyclohexane (1.80%), oxazole (1.79%), phthalic acid (1.70%), phytol (1.69%), 2-diamino-3,4,6-trifluorobenzene (1.37%), benzenemethanol (1.35%), morpholine (1.23%), benzoic acid (0.60%), and pyrocatechol (0.41).

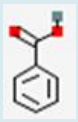
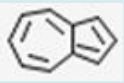
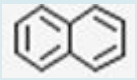
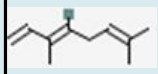
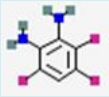
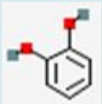
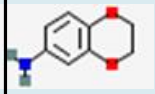
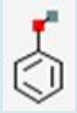
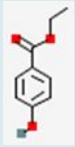
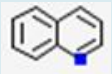
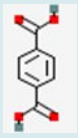
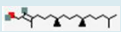
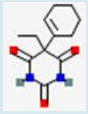
Table 3: Different bioactive compounds present in the pods of Pole bean genotype TKDPV(P) 8 with their IUPAC name, molecular weight, peak area and retention time

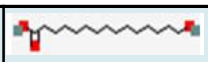
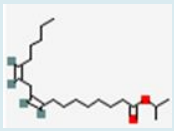
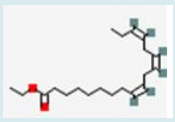
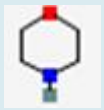
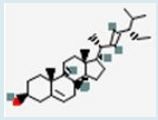
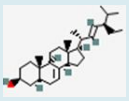
S.No.	Compound name	IUPAC name	Molecular weight (g/mol)	Peak area (%)	Retention time (min)
1.	1-Methyl-2-piperidinemethanol	(1-methylpiperidin-2-yl) methanol	129.199	0.77	4.198
2.	Tetrahydrosolasodine	10,13-dimethyl-17-[1-(5-methylpi-peridin-2-yl) ethyl]-2,3,4,5,6,7,8, 9,11,12,14,15,16,17-tetradecahydro-1 H-cyclopenta[a]phenanth-rene-3,16-diol	417.7	0.77	4.198
3.	2-Amino-oxazole	1,3-oxazol-2-amine	84.08	0.61	4.509
4.	2-Propen-1-ol	prop-2-en-1-ol	58.08	2.82	4.865
5.	Oxazolidine	1,3-oxazolidine	73.09	0.39	5.331
6.	monomorpholide	ethyl 2-morpholin-4-yl-2-oxoacetate	187.19	0.39	5.331
7.	Benzonitrile	benzonitrile	103.12	1.08	5.531
8.	4,5-Diamino-6-hydroxypyrimidine	4,5-diamino-1H-pyrimidin-6-one	126.12	0.77	5.720
9.	Pyrazine	pyrazine	80.09	2.18	5.909
10.	Fumaric acid	(E)-but-2-enedioic acid	116.07	0.61	6.042
11.	Benzenemethanol	phenylmethanol	108.14	1.35	6.642
12.	benzoic acid	benzoic acid	122.12	0.60	6.831
13.	Azulene	azulene	128.169	0.59	7.087
14.	Naphthalene	naphthalene	128.169	0.59	7.087
15.	Cyclohexane	cyclohexane	84.16	1.80	8.520
16.	Ocimene	(3E)-3,7-dimethylocta-1,3,6-triene	136.23	1.80	8.520
17.	2-Diamino-3,4,6-trifluorobenzene	3,4,6-trifluorobenzene-1,2-diamine	162.11	1.37	8.642
18.	Pyrocatechol	benzene-1,2-diol	110.11	0.41	9.431
19.	1,4 Benzodioxan-6-amine	2,3-dihydro-1,4-benzodioxin-6-amine	151.16	0.41	9.642
20.	Phenol	phenol	94.11	0.46	9.875
21.	Ethylparaben	ethyl 4-hydroxybenzoate	166.17	6.29	10.053
22.	Quinoline	quinoline	129.16		
23.	Phthalic acid	terephthalic acid	166.13	1.70	13.241
24.	Phytol	(E,7R,11R)-3,7,11,15-tetramethyl hexadec-2-en-1-ol	296.5	1.69	14.197
25.	Cyclobarbital	5-(cyclohexen-1-yl)-5-ethyl-1,3-diazinane-2,4,6-trione	236.27	0.35	14.363
26.	15-Hydroxypentadecanoic acid	15-hydroxypentadecanoic acid	258.399	3.63	15.308
27.	Isopropyl linoleate	propan-2-yl (9Z,12Z)-octadeca-9,12-dienoate	322.5	3.46	16.285
28.	9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z)	ethyl (9Z,12Z,15Z)-octadeca-9,12,15 -trienoate	306.5	2.32	16.330
29.	Morpholine	morpholine	87.12	1.23	16.374
30.	Stannane	stannane	122.74	0.59	16.608
31.	Oxazole	1,3-oxazole	69.06	1.79	18.152
32.	Eicosane	icosane	282.	7.40	20.329
33.	Stigmasterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R, 5S)-5-ethyl-6-methylhept-3-en-2-yl] -10,13-dimethyl-2,3,4,7,8,9,11,12,14,15, 16,17-dodecahydro-1H-cyclopenta[a] phenanthren-3-ol	412.7	19.02	22.307
34.	Chondrillasterol	(3S,5S,9R,10S,13R,14R,17R)-17-[(E,2R,5R)-5-ethyl-6-methylhept-3-en -2-yl]-10,13-dimethyl-2,3,4,5, 6,9,11,12,14,15,16,17-dodecahydro-1 H-cyclopenta[a]phenanthren-3-ol	412.7	19.02	22.307

Among the various genotypes, TKDPV(P) 8 yielded the highest minerals and richest phytochemical composition. Therefore, from the best-performing genotypes were collected for GC-MS analysis. The GC-MS chromatogram, shown in Figure 3, illustrates the diverse phytochemical compounds found in Pole beans under the genotype TKDPV(P) 8. Table 4 provides a comprehensive list of these bioactive compounds, detailing their molecular formula, molecular structure, and pharmacological activities.

Table 4: Pole bean pod compounds and their pharmaceutical property with molecular formula and molecular structure identified by GC-MS

S. No.	Compound name	Molecular formula	Molecular structure	Pharmaceutical property
1.	1-Methyl-2-piperidinemethanol	$C_7H_{15}NO$		Neuroactive, antidepressant and anesthetic property
2.	Tetrahydrosolasodine	$C_{27}H_{47}NO_2$		Anticancer, diuretic, antifungal, antispermatogenetic, cardiotonic, antiandrogenic, antipyretic, immuno-modulatory and various effects on central nervous system
3.	2-Amino-oxazole	$C_3H_4N_2O$		Antimicrobial property
4.	2-Propen-1-ol	C_3H_6O		Antimicrobial property
5.	Oxazolidine	C_3H_7NO		Antibacterial property
6.	Monomorpholide	$C_8H_{13}NO_4$		Antifungal and anticancer property
7.	Benzonitrile	C_7H_5N		Anti-inflammatory and antimicrobial activity
8.	4,5-Diamino-6-hydroxypyrimidine	$C_4H_6N_4O$		Anticancer activity, antiviral and antimicrobial property
9.	Pyrazine	$C_4H_4N_2$		Anticancer, anti-inflammatory and antidiabetic property
10.	Fumaric acid	$C_4H_4O_4$		Treating psoriasis and multiple sclerosis
11.	Benzenemethanol	C_7H_8O		Antiseptic property

12.	Benzoic acid	$C_7H_6O_2$		Antibacterial property
13.	Azulene	$C_{10}H_8$		Anti-inflammatory, antibacterial and antihyperglycemic property
14.	Naphthalene	$C_{10}H_8$		Anticancer, antimicrobial, antiviral, antitubercular, antidiabetic, anti-neurodegenerative and antipsychotic property
15.	Cyclohexane	C_6H_{12}		Anti-pain and antifungal property
16.	Ocimene	$C_{10}H_{16}$		Antimicrobial , anti-inflammatory and antioxidant property
17.	2-Diamino-3,4,6-trifluorobenzene	$C_6H_5F_3N_2$		Anti-inflammatory and anticancer property
18.	Pyrocatechol	$C_6H_6O_2$		Antioxidant and antimicrobial property
19.	1,4 Benzodioxan-6-amine	$C_8H_9NO_2$		Antidepressant potential and neuroprotective effect
20.	Phenol	C_6H_6O		Antiseptic and disinfectant
21.	Ethylparaben	$C_9H_{10}O_3$		Antimicrobial property
22.	Quinoline	C_9H_7N		Anti-inflammatory, anticancer and antibacterial property
23.	Phthalic acid	$C_8H_6O_4$		Anti-inflammatory, anticancer and antibacterial property
24.	Phytol	$C_{20}H_{40}O$		Antioxidant, anti-inflammatory, antimicrobial and neurological property
25.	Cyclobarbital	$C_{12}H_{16}N_2O_3$		Anticonvulsant and anxiolytic effect

26.	15-Hydroxypentadecanoic acid	$C_{15}H_{30}O_3$		Anti-inflammatory, antioxidant property, neuroprotective effect
27.	Isopropyl linoleate	$C_{21}H_{38}O_2$		Anti-aging property
28.	9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z)	$C_{20}H_{34}O_2$		Anti-inflammatory and neurological benefits
29.	Morpholine	C_4H_9NO		Antimicrobial and anti-inflammatory property
30.	Stannane	H_4Sn		Anticancer, antimicrobial and antiparasitic activity
31.	Oxazole	C_3H_3NO		Antimicrobial, anticancer, anti-inflammatory, antihypertensive and antiviral property
32.	Eicosane	$C_{20}H_{42}$		Anti-inflammatory and antioxidant property
33.	Stigmasterol	$C_{29}H_{48}O$		Anticancer and antioxidant property
34.	Chondrillasterol	$C_{29}H_{48}O$		Anti-inflammatory, anticancer activity and antimicrobial property

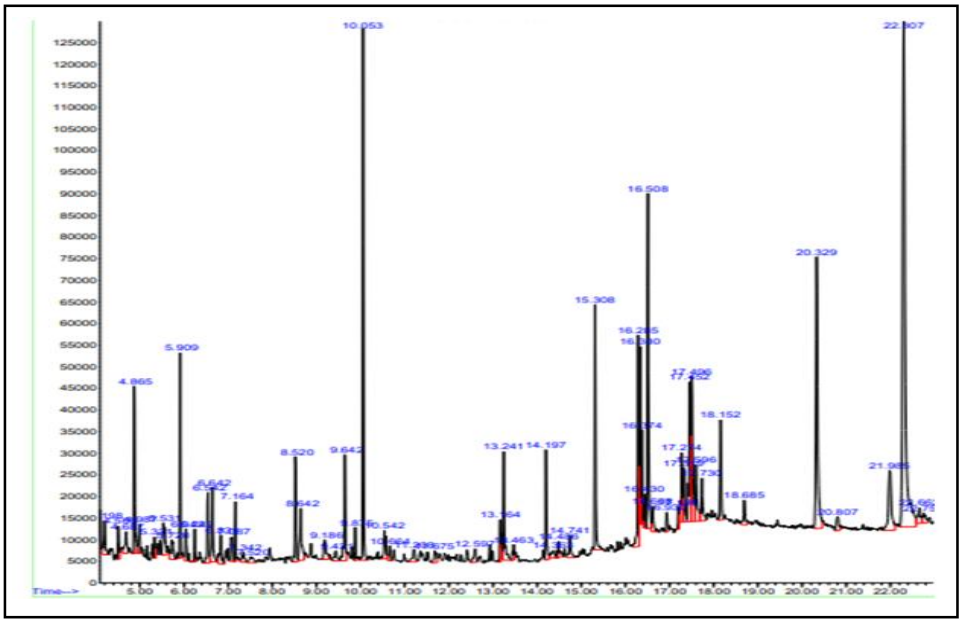


Figure 3: Chromatogram of ethanolic extract of Pole bean pods.

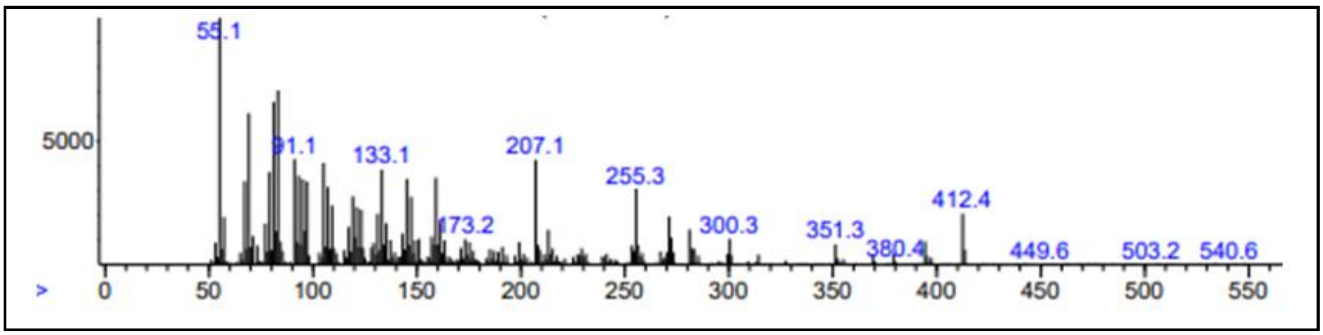


Figure 4: Mass spectrum of stigmasterol (19.02%).

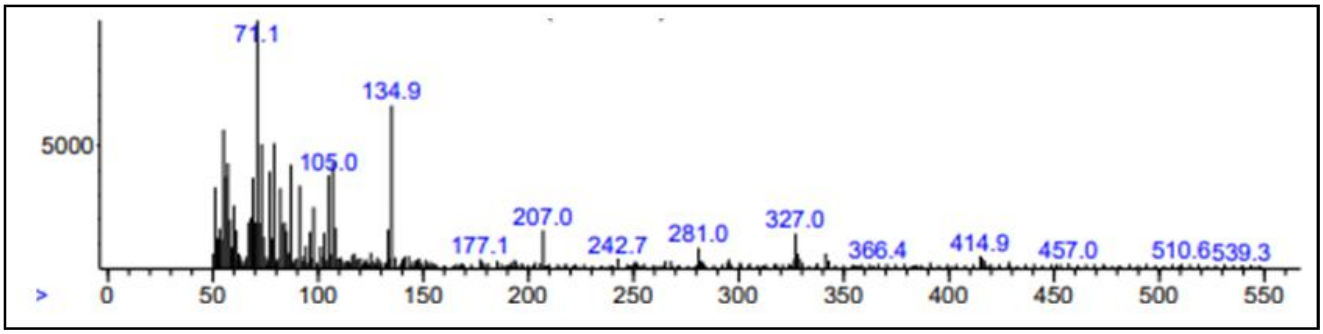


Figure 5: Mass spectrum of phenol (6.29%).

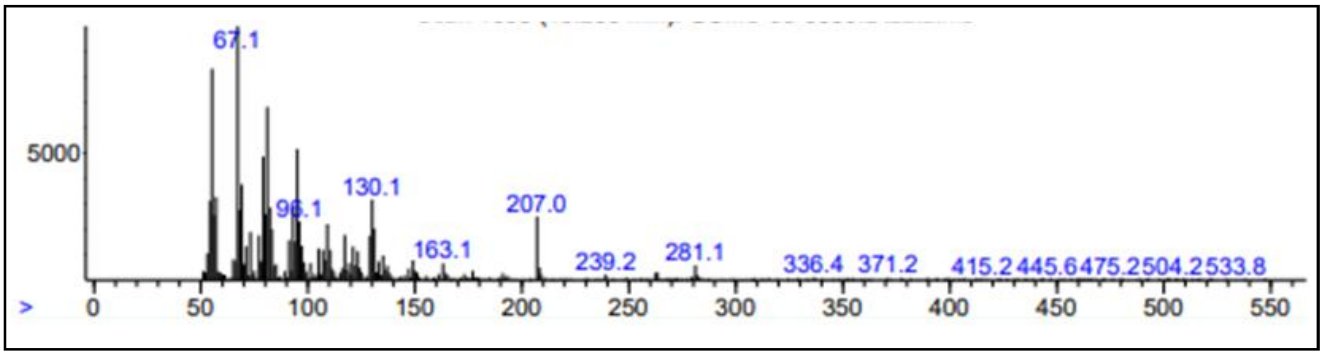


Figure 6: Mass spectrum of isopropyl linoleate (3.46%).

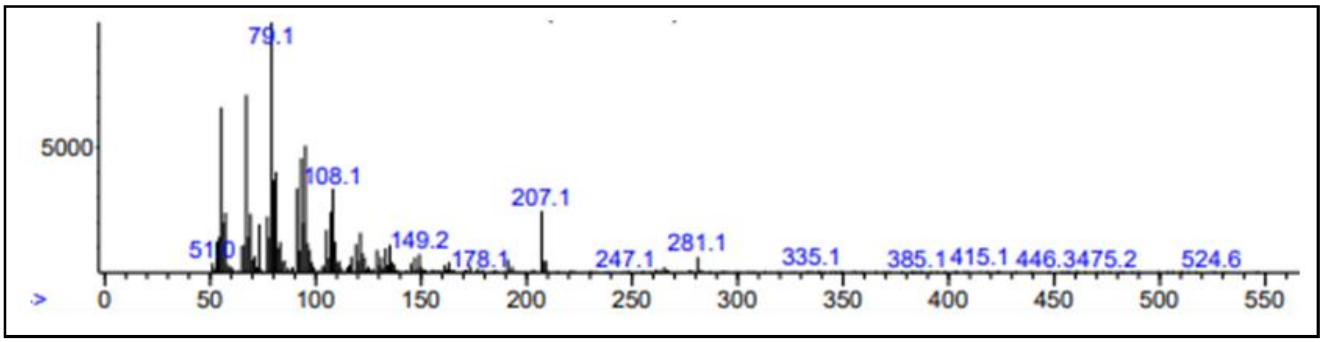


Figure 7: Mass spectrum of 9,12,15-octadecatrienoic acid, ethyl ester (Z,Z,Z) (2.32%).

4. Discussion

Common beans are a great source of antioxidants, which can include a wide range of phenolic acids and various flavonoids like anthocyanins, flavonols, proanthocyanidins, tannins, and glycosides (Hayat *et al*., 2014). The natural synthesis and maintenance of bodily tissues, hormones, enzymes, and other chemicals necessary for proper operation depend on dietary proteins. When compared to the crude protein reported by Ketema *et al*. (2019), which ranged from 18.62% to 25.98% respectively, the crude protein content of those forty-four Pole bean genotypes was relatively similar, ranging from 16.80 per cent to 25.98 per cent. Despite having little nutritional value, fibre is composed of a number of substances that are good for the

body's other systems as well as the digestive system. It also lowers blood cholesterol, which lowers the chance of heart disease (Soujanya *et al*., 2021). When compared to the crude fibre reported by Mekonen *et al*. (2012), which ranged from 4.86 g/100 g to 7.01 g/100 g, respectively, the crude fibre content of those forty-four Pole bean genotypes was relatively low, ranging from 3.02 per cent to 6.53 per cent.

Different physiological and cellular processes in the body depends on minerals. Some health problems are frequently linked to deficiencies in minerals like iron, zinc, copper, and manganese (Katoch *et al*., 2023). Worldwide, micronutrient deficiencies are common, impacting nearly all age groups and leading to grave health consequences. Pregnant women and children under five years old are especially deficient in iron, zinc, copper, and manganese. The human body recycling process, mineral absorption, food intake and dietary habits are controlled by these vital nutrients. Serious illnesses and disorders can result from mineral deficiencies, which are either directly or indirectly linked to physiological problems like insufficient oxygen delivery to tissues, weakness, impaired cognitive function, lower productivity, and heightened susceptibility to infections (Jindal *et al*., 2023). For vital biological processes like oxygen transport and cellular respiration, iron is a necessary nutrient. A healthy adult body has 3 g to 5 g of iron, of which 70 per cent is used by red blood cells to make haemoglobin. According to diet, dietary intake varies depending on the type of diet; mixed diets are thought to absorb 14 per cent to 18 per cent of iron, while vegetarian diets absorb 5 per cent to 12 per cent of iron (Charlebois *et al*., 2023). Insufficient iron intake results in anaemia, cephalalgia, exhaustion, heart problem, restless legs syndrome, pregnancy complications, and delayed child development. Zinc is an essential metal for the proper functioning of many enzyme systems. A growing body of research suggests that zinc is essential for the healthy growth of bone tissue and the preservation of homeostasis. Zinc is not only a part of bone tissue but also plays a role in mineralization, bone turnover, and synthesis of the collagen matrix (Molenda *et al*., 2023). Lack of zinc, especially in children can cause weakness, slow growth, loss of appetite, and even postponed sexual development (Monika *et al*., 2023). Copper helps to maintain the immune and nervous systems, brain development, and activates genes. Other essential bodily functions like iron absorption, reactive oxygen species detoxification, and energy metabolism can all be impacted by a copper deficiency. Manganese helps the body form bones, connective tissue, sex hormones, and blood clotting factors. It also plays a vital role in carbohydrate and fat metabolism, blood sugar regulation, and calcium absorption. Manganese deficiency might cause poor growth in children, bone demineralization, skin rashes, decreased serum cholesterol, hair depigmentation, and increased alkaline phosphatase activity in men; and increased premenstrual pain in women. Among the various genotypes, the highest iron, zinc and manganese content was recorded in the genotype TKDPV(P) 8. Similar pattern of results were also reported by Gouveia *et al*. (2014). The copper content ranging from 3.03 ppm to 5.44 ppm. A similar finding was mentioned by Quamruzzaman *et al*. (2022) in yard long bean.

5. Conclusion

The study concludes that the genotype TKDPV(P) 8 is the best performed genotype among forty-three genotypes and one standard check (TKDPV(P) 13-KKL 1) and the genotype TKDPV(P) 8 will be used as parent material for further breeding programmes. The hypothesis that *P. vulgaris* may offer substantial pharmacological and therapeutic advantages for people is further supported by the GC-MS analysis. More than 136 compounds were found in the ethanolic extract of Pole bean pods, indicating their potential pharmacological use. The compounds found in this study using GC-MS have a broad range of biological activities, which makes them extremely valuable for the pharmaceutical and biochemical industries. These activities include antiageing, antioxidant, antimicrobial, antifungal, anti-inflammatory, and anticancer effects. This analysis emphasizes the importance of the crop and the variety of active compounds found in Pole bean pods. Because of this, Pole beans are becoming an increasingly important raw material for pharmaceutical industries and researchers to use in the development and production of new drugs.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abou Hadid, A.F. (2012). Prediction and adaptation of dry bean yield under climate change conditions. Research Journal of Agriculture and Biological Sciences, **8(2)**:147-153.

AOAC (1990). Official methods of analysis. Association of Official Analytical Chemists: Washington, DC. Chemistry, **146**:299-307.

Baruah, S.; Dihingia, S.; Sharma, J.; Gogoi, S.; Sarmah, A.; Khound, A. and Pathak, P. (2022). Performance evaluation of French bean (*Phaseolus vulgaris* L.) varieties Arka Komal and Arka Sukomal in different agro-climatic zones of Assam. The Pharma Innovation Journal, **11(7)**: 2664-2667.

Bhawana, S.; Shiv Charan, S. and Afroz, A. (2021). Phytochemical screening and GC-MS analysis of *Tamarindus indica* L. (Angiosperms: Fabaceae). Ann. Phytomed, **10(1)**:215-221. http://dx.doi.org/ 10.21276/ ap.2021.10.1.23

Charlebois, E. and Pantopoulos, K. (2023). Nutritional aspects of iron in health and disease. Nutrients, **15(11)**:2441.

Dutta, A.; Hingmire, S. and Banerjee, K. (2020). Multiresidue analysis of pesticides in moringa pods by GC-MS/MS and LC-MS/MS. Journal of AOAC International, **103(6):**1486-1497.

Filimonov, D.A.; Lagunin, A. A.; Gloriozova, T.A.; Rudik, A. V.; Druzhilovskii, D.S.; Pogodin, P.V. and Poroikov, V.V. (2014). Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chemistry of Heterocyclic Compounds, **50:**444-457.

Goel, N.; Kumari, S.; Singh, S.; Sangwan, V.; Bhardwaj, K.K.; Moond, M.; Panghal, M. and Rani, I. (2022). Evaluation and comparison of the leaves and stem of *Argemone mexicana* L. in various solvents for total phenolics, total flavonoids and antioxidant activity. Ann. Phytomed, **11(2):**494-499.

Gouveia, C.S.; Freitas, G.; Brito, J.H.D.; Slaski, J.J. and Carvalho, M.A. (2014). Nutritional and mineral variability in 52 accessions of common bean varieties (*Phaseolus vulgaris* L.) from Madeira Island. Agricultural Sciences, **5(04):**317-329.

Hayat, I.; Ahmad, A.; Masud, T.; Ahmed, A. and Bashir, S. (2014). Nutritional and health perspectives of beans (*Phaseolus vulgaris* L.): An overview. Crit. Rev. Food. Sci, **54(5)**:580-592. doi: 10.1080/10408398.2011. 596639

Jackson, M.L. (1973). Soil chemical analysis, pentice hall of India Pvt. Ltd., New Delhi, India, **498**:151-154.

Jhanavi, D.R.; Patil, H.B., Justin, P.; Hadimani, R.H.; Mulla, S.W.R. and Sarvamangala, C. (2018). Genetic variability, heritability and genetic advance studies in french bean (*Phaseolus vulgaris* L.) genotypes. Indian Journal of Agricultural Research, **52(2)**:162-166.

Jindal, A.; Patil, N.; Bains, A.; Sridhar, K.; Stephen Inbaraj, B.; Tripathi, M. and Sharma, M. (2023). Recent trends in cereal-and legume-based protein-mineral complexes: Formulation methods, toxicity, and food applications. Foods, **12(21)**:3898.

Katoch, R.; Sanadya, S.K.; Pathania, K. and Chaudhary, H.K. (2023). Nutritional and nutraceutical potential of rice bean (*Vigna umbellata*): A legume with hidden potential. Frontiers in Nutrition, **10**:1126544.

Ketema, D.A.; Gebeyehu, H.R. and Gebreyes, B.G. (2019). Evaluation of proximate, mineral and anti-nutritional composition of improved and released common bean varieties in Ethiopia. Int. J. Novel. Res. Life. Sci, **6(6)**:13-27.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem, **193(1)**:265-275.

Maynard, A.J. (1970). Methods in food analysis. Academic Press, NewYork, **pp**:176.

Mekonen, D. and Admasu, S. (2012). Canning quality evaluation of common bean (*Phaseolus vulgaris* L.) varieties grown in the central rift valley of Ethiopia. East African Journal of Sciences, **6**:65-78.

Molenda, M. and Kolmas, J. (2023). The role of zinc in bone tissue health and regeneration: A review. Biological Trace Element Research, **201(12)**:5640-5651.

Monika, M.; Sushila, S.; Ritu, D.; Rajita, B.; Pinki, M.; Sachin, K. and Rajni K. S. (2023). Proximate and mineral analysis of *Trigonella foenum-graecum* (Fenugreek) seeds and leaves of variety HM444. Ann. Phytomed., **12(1)**:546-552. http://dx.doi.org/10.54085/ap.2023.12.1.10.

Nupur, M. (2021). Herbs that heal: Natures pharmacy. Ann. Phytomed, **10(1)**:6-22. http://dx.doi.org/10.21276/ ap.2021.10.1.2

Quamruzzaman, A.K.M.; Islam, F.; Akter, L.; Khatun, A.; Mallick, S.R.; Gaber, A.; Laing, A.; Brestic, M. and Hossain, A. (2022). Evaluation of the quality of yard-long bean (*Vigna unguiculata* sub sp. *sesquipedalis* L.) cultivars to meet the nutritional security of increasing population. Agronomy, **12(9)**:2195.

Salehi, M.; Tajik, M. and Ebadi, A. (2008). The study of interrelationship between different traits in common bean using multivariate analysis. American Eurasian J. Agri. Environ. Sci., **3**:806-9

Sarikamis, G.; Yasar, F.; Bakir, M.; Kazan, K. and Ergul, A. (2009). Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from eastern Turkey. Gen. Mol. Res., **8**:880-887.

Shunmugapriya, K.; Vennila, P.; Thirukkumar, S. and Ilamaran, M. (2017). Identification of bioactive components in *Moringa oleifera* fruit by GC-MS. Journal of Pharmacognosy and Phytochemistry, **6(3)**:748-751.

Soujanya, B.; Kiran Kumar, A.; Bhagwan, A.; Sreedhar, M.; Vanisri, S. and Saidaiah, P. (2021). Estimation of crude fiber content in different cultivars of mango (*Mangifera indica L.*) grown in Telangana State, India. Ann. Phytomed, **10(1)**:319-324. http://dx.doi.org/10.21276/ ap.2021.10.1.35

Srivani, A. and Krishna Mohan, G. (2023). GC-MS analysis and isolation of few bioactive phytoconstituents from *Ixora parviflora* Lam., Ann. Phytomed, **12(1)**:783-794. http://dx.doi.org/10.54085/ap.2023.12.1.77.

Citation **T. Velmurugan, R. Balakumbahan, K. Kalpana, K. Sundharaiya, M. Gnanasekaran and M. Kabilan (2024). Analyzing the minerals and phytochemicals composition of *Phaseolus vulgaris* L. using ethanol extract by gas chromatography and mass spectroscopy (GC-MS) analysis. Ann. Phytomed., 13(2):859-870. http://dx.doi.org/10.54085/ap.2024.13.2.88.**