Research Article

GC-MS Analysis of Phytochemicals, Fatty acids and Antimicrobial Potency of Dry Christmas Lima Beans

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ABSTRACT

Seeds are very important for the plants to reproduce continuously in the existing world. The human life depends partly on the seeds derived from plants either by means of cooked or raw or in the form of active principles in case of pharmacological use. So, it is essential to study the nutrients present in the seeds and also its fatty acid content as it is an important factor in giving antimicrobial activity. The components were assessed by means of analytical technique called GC-MS. The obtained results showed that it contains higher oleic acid and 9-Octadecenoic acid (Z)-methyl ester and found to be effective against fungi *Aspergillus flavus.*

Keywords: Dry lima beans, Fatty acid profile, GC-MS, Phytonutrients, *Phaseolus lunatus*.

INTRODUCTION

The genus *Phaseolus*, family Fabacea has a complex taxonomic and nomenclatural history, which is well illustrated by *Phaseolus lunatus*, the Lima bean. The lima bean plant is named after the city of Lima, Peru, where people have been eating them since 6000 BC. In the U.S., Native Americans grew lima beans before the English settlers landed. Lima beans are also known as butter beans or chad beans. Lima bean is a cool season vegetable requiring dry and cool climate where temperatures are ranging from 16 to 27°C and where annual rainfall is about 900-1500mm. Compared to other legumes, it is a long duration crop and is retained in field for 9 months. Lima bean is an important crop in Maharashtra. Storage proteins represent nearly 50% of the total proteins found in the seeds of flowering plants. Most of the storage proteins found in legumes are globulins. Seed storage proteins are used as markers in the following areas: analysis of genetic diversity within and among populations, plant domestication with respect to genetic resources conservation, breeding, genome associations in polyploid series, and as an instrument in plant breeding.[1] Lima bean is rich in niacin, thiamine and riboflavin.[2] They are said to contain high levels of potassium, phosphorus, calcium and iron.[3,4]However, like other tropical legumes, lima bean seed contains some anti-nutritional factors, which limit its utilization in animal feeding. These include phytins and tannins,[5,6] hydrogen cyanide and trypsin inhibitors.[7] Hence, considering the importance of seeds it was decided to analyze the chemical constituents by gas chromatography and mass spectrophotometry and also its antimicrobial potency.

MATERIALS AND METHODS

Sample used for the study

The dry lima beans (*Phaseolus lunatus* seeds) were purchased from Nilgiris shop located at sarada college road, Salem, Tamil Nadu, India. The beans purchased were cleaned, freed from debris and then grounded to obtain powder. The powdered beans were transferred in to an air tight plastic container and used for analysis.

Extraction process

10gm of powdered beans were extracted with 30ml ethanol overnight and filtered. The extract was concentrated to 1ml by bubbling nitrogen in to the solution. 2µl of the ethanolic extract was employed for GC-MS analysis for the identification of phytochemical compounds.[8] Further, the given samples were extracted with hexane and methylated, analyzed through GC-MS for the identification of the fatty acid profile.

GC-MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-5MS fused capillary column (30 x 0.25mm x 0.25µm df) composed of 5% Diphenyl / 95% Dimethyl poly siloxane. Helium (99.999%) was used as carrier gas at a constant flow of 1ml per min and injection volume of 2µl was adopted (split ratio of 10:1) The injected sample was detected by Turbo mass gold detector (Perkin Elmer) with the aid of Turbomass 5.2 software. During the 36 minute GC extraction process, the oven temperature was programmed from of 110°C with an increase of 10°C/min up to 200°C, then 5°C/min up to 280°C (9 minutes hold). The injector temperature was set at 250°C (mass analyzer). Other parameters involved in the operation of Clarus 500MS, was also standardized (Inlet line temperature: 200°C; Source temperature: 200°C). Mass spectra were taken at 70eV and fragments from 45-450 Da. The MS detection was completed in 36 minutes. The detection employed the NIST (National Institute of Standards and Technology) - Year 2005 library.

Antimicrobial Assay

The antimicrobial activity was assessed by the method of Kirby-Bauer disk diffusion method.[9] Results of the zone of inhibition was observed and measured after 24hr period of incubation time.

RESULTS AND DISCUSSION

Gas chromatography and mass spectrometry is an effective combination for chemical analysis. The results of various phytochemicals observed are depicted in Table 1 and the chromatogram is shown in Figure 1. Similarly, the fatty acid profile is depicted in Table 2 and Figure 2, While antimicrobial activity in Table 3.

Table 1: Bioactive Components identified in dry Christmas lima beans [GC MS study]

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	8.21	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	5.30
2	12.62	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.62
3	12.76	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.02
4	13.97	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	0.89
5	14.93	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	8.45
6	16.01	Oleic Acid	$C_{18}H_{34}O_2$	282	57.92
7	19.62	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268	2.64
8	20.85	3',8,8'-Trimethoxy-3-pip eridyl-2,2'-binaphthalene-1,1',4,4'-tetrone	$C_{28}H_{25}NO_7$	487	4.12
9	22.04	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	0.99
10	24.30	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356	1.98
11	30.14	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	$C_{27}H_{44}O_3$	416	3.22
12	32.77	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	2.92
13	33.82	Digitoxin	$C_{41}H_{64}O_{13}$	764	1.93

Table 2: Fatty acids identified in dry Christmas lima beans [GC MS study]

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	9.64	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	186	0.79
2	10.36	Tetradecanoic acid, ethyl ester	$C_{16}H_{32}O_2$	256	0.17
3	10.69	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	0.25
4	11.51	7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268	0.95
5	11.82	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	9.14
6	12.47	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	2.10
7	12.83	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284	0.54
8	13.99	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	39.74
9	14.18	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	7.25
10	14.66	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	9.14
11	14.92	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.88
12	15.34	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$	312	0.30
13	16.37	11-Eicosenoic acid, methyl ester	$C_{21}H_{40}O_2$	324	3.99
14	16.68	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	1.87
15	17.17	E-11-Hexadecenoic acid, ethyl ester	$C_{18}H_{34}O_2$	282	0.44
16	18.33	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	0.62
17	19.05	13-Docosenoic acid, methyl ester	$C_{23}H_{44}O_2$	352	1.72
18	19.56	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	13.01
19	22.28	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382	7.09

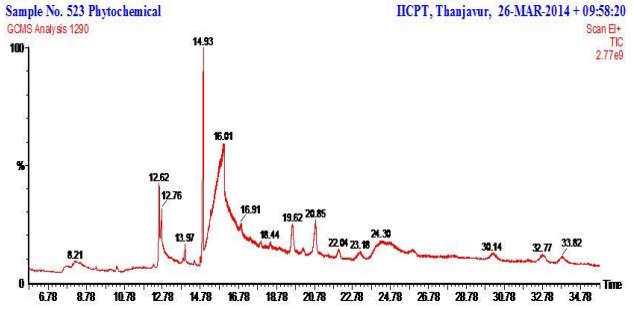

Figure 1: Chromatogram of phytochemicals present in dry christmas lima beans

Phytochemicals

Totally 13 phytochemicals were identified in dry lima bean seeds (Table 1, Figure 1). The compound 1 identified was á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-, $C_{12}H_{22}O_{11}$, showing peak ratio percent of 5.30. Compound 2 identified was Hexadecanoic acid ethyl ester, $C_{18}H_{36}O_2$, with peak ratio of 2.62%. Compound 3 identified were n-Hexadecanoic acid $C_{16}H_{32}O_2$, showing peak ratio of 7.02%. Compound 4 identified was 9-Octadecenoic acid (Z)-methyl ester, $C_{19}H_{36}O_2$, with peak ratio of 0.89%. Compound 5 identified was 9,12-Octadecadienoic acid (Z,Z)-,$C_{18}H_{32}O_2$ showing peak ratio of 8.45%. Compound 6 identified was oleic acid, $C_{18}H_{34}O_2$, with peak ratio of 57.92%. Compound 7 identified was 10-Methyl –E-11-tridecen-1-ol-propionate, $C_{17}H_{32}O_2$ showing peak ratio of 2.64%. Compound 8 identified was 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone $C_{28}H_{25}NO_7$, with peak ratio of 4.12%. Compound 9 identified were 2-Myristynoyl pantetheine, $C_{25}H_{44}N_2O_5S$, showing peak area percent as 0.99%. Compound 10 identified was 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, $C_{21}H_{40}O_4$, with peak area percent as 1.98%. Compound 11 identified was 9, 10- Secocholesta-5, 7, 10 (19)-triene-3, 24, 25-triol,(3a,5Z,7E)-, $C_{27}H_{44}O_3$, showing peak ratio of 3.22%. Compound 12 identified was Ethyl iso-allocholate, $C_{26}H_{44}O_5$, with peak area of 2.92%. Compound 13 identified was Digitoxin, $C_{41}H_{64}O_{13}$, showing peak area percent as 1.93. From our results, it is known that oleic acid is present in higher concentration and the remaining compounds in moderate amount only. The retention time starts at 8.21 and ends at 33.82. The molecular weights of the compounds range from 256 to 764. (Table 1, Figure 1)

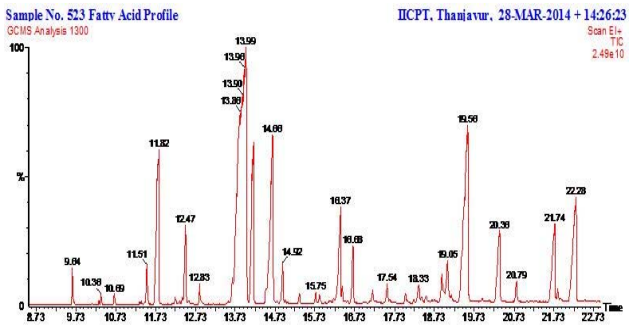

Figure 2: Chromatogram showing fatty acid profile of dry christmas lima beans.

Fatty acid profile

Totally 19 compounds were identified using GC-MS (Table 2, Figure 2). Compound 1 identified was Decanoic acid, methyl ester, $C_{11}H_{22}O_2$, having peak ratio of 0.79%, Compound 2 identified was Tetradecanoic acid, ethyl ester, $C_{16}H_{32}O_2$ having peak ratio of 0.17%, Compound 3 identified was Pentadecanoic acid, methyl ester, $C_{16}H_{32}O_2$ showing peak ratio of 0.25%, Compound 4 identified was 7- Hexadecanoic acid, methyl ester (Z)-, $C_{17}H_{32}O_2$, with peak ratio of 0.95%. Compound 5 identified was Hexadecanoic acid, methyl ester, $C_{17}H_{34}O_2$ showing peak ratio of 9.14%. Compound 6 identified was Hexadecanoic acid, ethyl ester $C_{18}H_{36}O_2$ with peak ratio of 2.10%. Compound 7 identified was Heptadecanoic acid methyl ester $C_{18}H_{36}O_2$ showing peak ratio of 0.54%. Compound 8 identified was 9- Octadecanoic acid (Z)- methyl ester, $C_{19}H_{36}O_2$, with peak ratio of 39.74%. Compound 9 identified was Octadecanoic acid, methyl ester, $C_{19}H_{38}O_2$, showing peak ratio of 7.25%. Compound 10 identified was 9,12-Octadecadienoic acid (Z,Z)- $C_{18}H_{32}O_2$ with peak ratio of 9.14%. Compound 11 identified was Octadecanoic acid, ethyl ester, $C_{20}H_{40}O_2$ showing peak ratio of 0.88%. Compound 12 identified was Nonadecanoic acid, methyl ester, $C_{20}H_{40}O_2$ with peak ratio of 0.30%. Compound 13 identified was 11-Eicosenoic acid, methyl ester, $C_{21}H_{40}O_2$ showing peak ratio of 3.99%. Compound 14 identified was Eicosanoic acid, methyl ester $C_{21}H_{42}O_2$ with peak ratio of 1.87%. Compound 15 identified was E-11-Hexadecenoic acid, ethyl ester, $C_{18}H_{34}O_2$ showing peak ratio of 0.44%. Compound 16 identified was 5, 8, 11, 14-Eicosatetraenoic acid, methyl ester (Z,Z,Z,Z),$C_{21}H_{34}O_2$ with peak ratio of 0.62%. Compound 17 identified was 13-Docosenoic acid methyl ester $C_{23}H_{44}O_2$ showing peak ratio of 1.72%. Compound 18 identified was Docosanoic acid, methyl ester, $C_{23}H_{46}O_2$ with peak ratio of 13.01%. Compound 19 identified was Tetracosanoic acid methyl ester, $C_{25}H_{50}O_2$, showing peak ratio of 7.09%. The retention time observed was 9.64 to 22.28. C_{11} to C_{25} carbon compounds were observed in the study. Among the 19 compounds identified, the following compounds such as 9-Octadecanoic acid (Z) – methyl ester, Docosanoic acid, methyl ester, Hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z)-, Octadecanoic acid, methyl ester, Tetracosanoic acid methyl ester, Octadecanoic acid methyl ester, 11-Eicosenoic acid, methyl ester, Eicosanoic acid, methyl ester, 13-Docosenoic acid methyl ester are prominent. All the other compounds are present in trace amount. (Table 2, Figure 2)

Antimicrobial activity

Table 3: Antimicrobial activity of dry Christmas lima beans

Microorganisms	Zone of Inhibition (mm)	
	50 µg	100 µg
Aspergillus niger	11	13
Aspergillus flavus	14	25
Escherichia coli	--	--
Staphylococcus aureus	13	14

Antimicrobial activity of dry lima bean seeds was tested against *Aspergillus niger, Aspergillus flavus*. Among the fungi tested, *Aspergillus flavus* is found to have good activity at 100µg concentration. While, it was not active against *E.coli* but found to be active against *Staphylococcus aureus*. (Table 3)

CONCLUSION

Every living organism in this planet depends ultimately on plants and its products like fruits, seeds, leaf for the food we eat and for the oxygen we breathe. Hence, it is essential to study the phyto nutrients present in edible dry lima bean seeds. According to our obtained results, it is evident that dry lima bean seeds composed of huge amount of oleic acid and 9-Octadecenoic acid (Z)-, methyl ester and also other phytochemicals in moderate amount, which impart antimicrobial potential.

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