IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

ISSN PRINT 2319 1775 Online 2320 7876

 UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

Bio-active Compound Identification in *Phaseolus vulgaris* - A Low-Cost Dietary Supplement through GC-MS

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ABSTRACT

Phaseolus vulgaris, commonly mentioned as beans, is a profoundly hybridized garden vegetable in the Fabaceae family which is most widely used as food are currently being studied for additional physiological effects. The pharmacological significance of eating *Phaseolus vulgaris* has been intensively explored in order to identify several bioactive components responsible for their health benefits. Natural products either as pure compounds or as standardized plant extract, provide unlimited opportunities for new bioactive components primes because of its unmatched availability of chemical diversity. Plant compounds are of interest as a source of safer and effective substitutes than synthetically produced bioactive components. Gas chromatography-mass spectroscopy (GC-MS) is a tool capable of identifying active principles in plant's extractions. Plants are the natural source of bioactive components responsible for the medicinal efficiency due to the presence of derivatives. One hundred and thirty-three compounds were detected in ethanolic extract of *Phaseolus vulgaris* where, 4-O-Methylmannose was identified as the major compound.

Keywords: *Phaseolus vulgaris*, GC-MS, 4-O-Methylmannose

INTRODUCTION

The legume family (Fabaceae or Leguminosae) is one of the largest plant families. Worldwide it comprises almost 23,000 species including chickpeas, lentils, soya beans, peanuts, and many valuable timbers, including the much-prized (Morris, 2003). Legumes are one of the most nutritious foods and when combined with other products are the basis of diet for large part

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

of the world's population, especially in poorer areas where meat, dairy products and fish are economically inaccessible. They are densely packed with proteins, twice the amount found in grain cereals. Legumes are grown agriculturally, primarily for human consumption, for livestock forage and silage, and as soil-enhancing green manure (Albala, 2007). *Phaseolus vulgaris*, also known as the common bean or French bean, is a herbaceous annual plant grown worldwide for its edible dry seeds or unripe fruit. The species *P. vulgaris* was introduced into Europe in the sixteenth century and since then it has become a very important crop in many regions of the world such as Africa (Raja *et al.*, 2020; Longo-Mbenza *et al*., 2013).

The bioactive components in the extracts were identified using GC-MS. Chromatography coupled with mass spectrometry has become a broadly used analytical system in metabolomics. The concluding is a multidisciplinary science that aims to analyse the entire complement of small molecular weight molecules (≤1500 Da) within a biological matrix. The application of metabolomics spans a wide range of fields including medicine, biological and life sciences, nutrition, agriculture, and more recently in food science and technology research (Tugizimana *et al*., 2013; Adebo *et al*., 2017; Adamski, 2020; Feng *et al*., 2020).

MATERIALS AND METHODS

Systematic position

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Fabales
Family	:	Fabaceae
Genus	:	*Phaseolus*
Species	:	*vulgaris*

Sample collection and solvent extraction

The selected *Phaseolus vulgaris* plant was cultivated in the kitchen garden for GC-MS study. The plant was dehydrated under shade condition for one month and pulverized into fine powder and filtered through a mesh. The extraction was made using ethanol solvent.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

Gas chromatography-Mass spectrometry (GC-MS) analysis

GC -MS (QP-ultra-2010, Shimadzu, Japan) analysis was carried out for fatty acid methyl esters using SH-Rxi- 5Sil MS (30m,0.25mm, 0.25 µm Columns (low- polarity phase; Cross bond 1,4 -bis (Dimethylsiloxy) phenylene dimethyl polysiloxane) with electron impact (EI) ionization. Helium was used as a carrier gas at 1.5ml min-1. In GC, injection temperature was maintained at 280ºC.

The oven temperature profile was at initial temperature with 70ºC hold 1 min, increase 5ºC/min up to 255ºC and hold 3min, further increase 5ºC/min up to 300ºC holding time 5 minutes. The total programme time was 54 minutes. The split ratio was 1:10 and the column flow parameter was 1ml/min. In MS, ion source temperature was 230ºC and interface temperature was 280ºC in scan mode with m/z detection from 35-850 Da.

RESULT & DISCUSSION

Table:1 Gas Chromatography-Mass spectrometry (GC-MS) analysis in *Phaseolus vulgaris* using ethanolic extract

Peak	R.Time	Area%	Height%	A/H	MW	Name
1	5.093	0.03	0.11	2.61	274	Glutaconic acid-2TMS
2	5.233	0.10	0.17	5.12	92	Glycerin
3	5.403	0.28	0.38	6.28	144	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on
4	5.544	0.07	0.15	3.98	118	Ethanol, 2-(trimethylsilyl)-
5	5.715	0.05	0.09	4.69	240	Methyl myristoleate
6	5.849	0.05	0.09	4.79	229	Tiglylglycine-TMS
7	6.136	0.06	0.09	5.62	130	Methyl caproate
8	6.392	0.17	0.16	8.62	276	2'-Deoxyribolactone, 2TMS derivative
9	6.771	0.33	0.36	7.72	116	Pentanoic acid, 4-oxo-
10	6.944	0.05	0.07	5.84	276	Methylsuccinic acid-2TMS
11	7.273	0.46	0.57	6.77	148	1-[(Trimethylsilyl)oxy] propan-2ol
12	7.527	0.81	0.62	11.04	126	Cyclopentane, 1-acetyl-1,2epoxy-

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

13	8.067	0.08	0.17	4.03	156	Undecane
14	8.679	0.35	0.39	7.64	160	Dimethylbutylsilyloxyethane
15	8.860	0.16	0.24	5.63	184	2-Furoic acid-TMS
16	8.945	0.26	0.30	7.56	116	Isobutyl acetate
17	9.090	0.05	0.13	3.11	220	Glycolic acid-2TMS
18	9.220	2.51	1.92	11.03	144	4H-Pyran-4-one, 2,3-dihydro3,5-dihydroxy-6
19	9.734	0.10	0.14	5.93	102	Methyl butanoate
20	10.807	0.82	0.63	10.95	232	2-(Hexyloxy)acetic acid, TMS
21	11.285	0.23	0.24	8.24	120	Benzofuran, 2,3-dihydro-
22	11.534	0.85	0.67	10.64	244	Decanoic acid-TMS
23	11.799	1.94	1.23	13.32	134	1,2,3-Propanetriol, 1-acetate
24	12.267	2.72	2.22	10.33	172	Allyl(2-butoxy) dimethylsilane
25	12.585	0.65	0.57	9.77	276	Ureidopropionic acid-2TMS
26	12.823	0.55	0.55	8.50	175	Glyoxylic acid-meto-TMS
27	12.988	0.63	0.64	8.23	172	cyclobutanecarboxylic acid, trimethylsilyl este
28	13.087	0.82	0.62	11.29	195	Nicotinic acid-TMS
29	13.303	0.16	0.28	4.71	194	Niacinamide-TMS
30	13.589	1.46	0.97	12.63	262	Butanedioic acid, 2TMS derivative
31	13.910	0.06	0.10	5.07	276	Ethylmalonic acid-2TMS
32	14.456	0.09	0.14	5.60	216	Octanoic acid-TMS
33	14.685	0.27	0.23	10.05	188	Caproic acid-TMS
34	14.912	0.17	0.22	6.46	190	Propylene Glycol Monoacetate, TMS ether
35	15.202	0.05	0.07	6.76	290	3-Methylglutaric acid-2TMS
36	15.402	1.73	2.46	5.95	188	4-Methylvaleric acid, TMS derivative
37	15.772	0.82	1.19	5.80	232	3,8-Dioxa-2,9-disiladec-5-ene, 2,2,9,9-tetrame
38	15.980	0.08	0.11	6.03	216	Octanoic acid-TMS
39	16.226	0.09	0.14	5.34	189	Acetylglycine-TMS
40	16.343	0.06	0.09	5.93	416	Juniperic acid-2TMS

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

41	16.500	0.10	0.09	9.37	216	Octanoic acid-TMS
42	17.006	5.18	2.26	19.33	130	2-(Isobutoxymethyl)oxirane
43	17.241	0.57	0.79	6.07	188	Caproic acid-TMS
44	17.360	0.27	0.54	4.32	188	Caproic acid-TMS
45	17.460	0.63	0.67	7.98	188	Caproic acid-TMS
46	17.597	0.30	0.37	6.75	386	Thymidine-2TMS
47	18.150	1.18	1.52	6.55	158	2-Penten-1-ol, (Z)-, TMS derivative
48	18.358	2.98	2.01	12.50	160	Neopentyl alcohol, TMS derivative
49	18.907	0.11	0.11	8.70	216	Octanoic acid-TMS
50	19.417	0.38	0.21	15.00	291	N-Acetylserine-2TMS
51	19.781	0.08	0.10	6.39	262	Methylmalonic acid-2TMS
52	19.916	0.12	0.16	6.17	232	2-Butene-1,4-diol, (E)-, 2TMS derivative
53	21.370	15.6	3.15	41.20	194	4-O-Methylmannose
54	22.904	0.11	0.11	8.50	202	Mevalonic lactone-TMS
55	23.127	0.02	0.03	4.51	354	Oleic acid-TMS
56	23.277	0.10	0.10	8.05	354	Oleic acid-TMS
57	24.033	0.03	0.03	6.95	216	Octanoic acid-TMS
58	24.812	0.21	0.32	5.58	350	Methyl cis-13,16-Docosadienate
59	25.827	0.16	0.18	7.71	228	cisZ-11,12-Epoxytetradecan-1-ol
60	26.284	0.12	0.13	7.84	102	Methyl butanoate
61	26.713	0.06	0.09	5.66	324	Methyl cis-11-icosenoate
62	26.888	0.02	0.04	4.21	175	Glyoxylic acid-meto-TMS
63	26.993	0.01	0.03	3.68	376	Arachidonic acid-TMS
64	27.292	0.08	0.14	4.49	311	1-Nonadecanamine, N, N-dimethyl-
65	27.597	0.06	0.13	4.09	270	Pentadecanoic acid, 14-methyl-, methyl ester
66	28.316	7.09	9.25	6.47	256	n-Hexadecanoic acid
67	28.918	0.07	0.12	4.62	200	Methyl undecanoate

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

68	29.503	0.05	0.07	6.10	202	Mevalonic lactone-TMS
69	29.837	0.78	0.50	13.21	328	Palmitic Acid, TMS derivative
70	30.186	0.15	0.17	7.26	102	Methyl butanoate
71	30.763	0.11	0.18	5.04	294	Methyl 11,12-octadecadienoate
72	30.864	0.05	0.10	4.23	292	Methyl linolenate
73	31.085	0.06	0.12	4.37	296	Phytol
74	31.484	3.77	6.09	5.22	280	9,12-Octadecadienoic acid (Z,Z)-
75	31.584	5.28	6.35	7.02	278	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-
76	32.032	1.42	1.48	8.11	284	Octadecanoic acid
77	32.202	0.27	0.30	7.68	411	Metoprolol-2TMS
78	32.612	0.10	0.13	6.21	158	Methyl caprylate
79	32.822	0.50	0.29	14.46	354	Oleic acid-TMS
80	33.479	0.21	0.18	9.78	356	Stearic acid-TMS
81	34.487	0.10	0.16	5.30	327	Dimethylaminoethyl palmitate
82	34.761	0.02	0.04	4.75	203	Acetoacetic acid-meto-TMS
83	35.139	0.02	0.03	3.99	268	Methyl palmitoleate
84	35.844	0.05	0.07	5.62	350	Methyl cis-13,16- Docosadienate
85	35.976	0.05	0.06	6.29	294	Methyl linoleate
86	38.301	0.02	0.05	3.77	175	Dimethylglycine-TMS
87	38.445	0.02	0.04	4.69	175	Dimethylglycine-TMS
88	38.996	0.03	0.06	4.16	175	Dimethylglycine-TMS
89	39.177	0.10	0.13	6.81	244	Decanoic acid-TMS
90	39.468	0.31	0.40	6.58	330	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
91	39.663	0.04	0.07	5.21	416	Juniperic acid-2TMS
92	39.938	0.11	0.15	6.28	350	Methyl cis-13,16-Docosadienate
93	40.307	0.04	0.04	8.80	342	Methyl cis-4,7,10,13,16,19-Docosahexaenoate
94	40.450	0.09	0.14	5.53	248	2-Hydroxyisobutyric acid-2TMS
95	42.297	0.02	0.04	4.83	374	Eicosapentaenoic acid-TMS
96	42.668	0.08	0.12	5.68	352	Linoleic acid-TMS

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

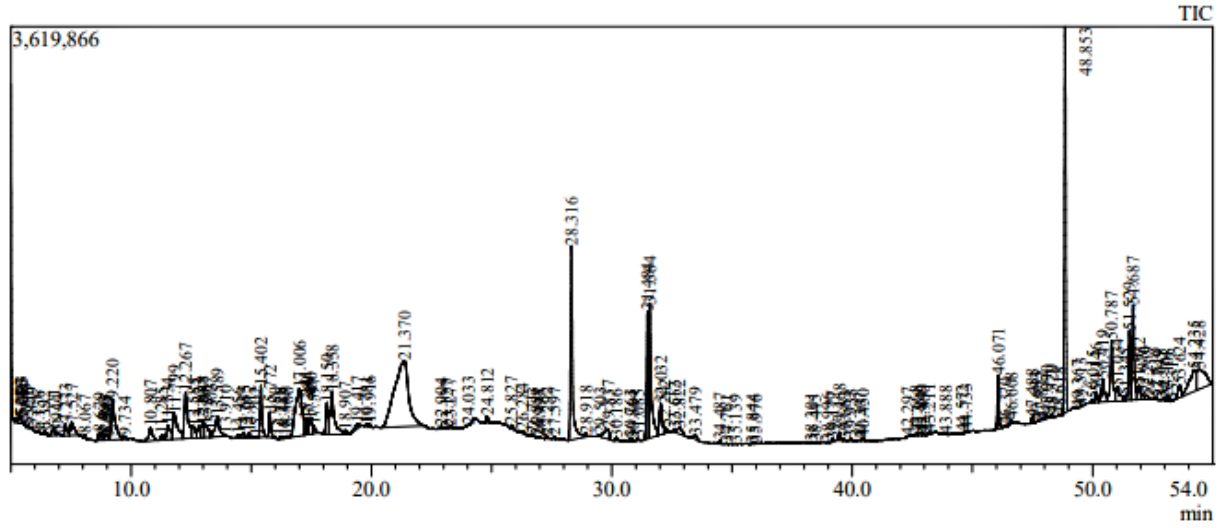
97	42.849	0.15	0.12	10.12	400	Docosahexaenoic acid-TMS
98	42.960	0.05	0.09	4.80	356	Stearic acid-TMS
99	43.211	0.07	0.10	5.79	296	Methyl elaidate
100	43.888	0.03	0.05	5.65	202	Mevalonic lactone-TMS
101	44.572	0.02	0.03	3.74	354	Oleic acid-TMS
102	44.733	0.04	0.07	4.68	354	Oleic acid-TMS
103	46.071	1.51	2.53	5.02	282	Eicosane
104	46.370	0.05	0.04	9.24	296	Methyl elaidate
105	46.608	0.15	0.19	6.33	320	Methyl eicosa-8,11,14-trienoate
106	47.488	0.16	0.29	4.62	184	Nonane, 5-(1-methylpropyl)-
107	47.683	0.01	0.02	4.50	376	Arachidonic acid-TMS
108	47.872	0.05	0.07	6.17	354	Oleic acid-TMS
109	48.090	0.13	0.22	4.85	166	Phenol-TMS
110	48.320	0.09	0.17	4.29	342	Methyl cis-4,7,10,13,16,19-Docosahexaenoate
111	48.618	0.03	0.06	4.20	254	Methyl cis-10-pentadecenoate
112	48.853	10.3	18.41	4.60	618	Tetratetracontane
113	49.367	0.01	0.02	3.49	402	Docosapentaenoic acid-TMS
114	49.513	0.02	0.03	4.89	316	Methyl cis-5,8,11,14,17-Eicosapentaenoate
115	50.015	0.12	0.16	6.30	354	Oleic acid-TMS
116	50.146	0.36	0.52	5.81	338	Tetracosane
117	50.419	1.14	1.10	8.77	414	.beta.-Sitosterol
118	50.787	2.53	2.92	7.30	412	Stigmasterol
119	51.034	0.89	0.68	11.06	458	Cholesterol-TMS
120	51.345	0.17	0.22	6.54	402	Docosapentaenoic acid-TMS
121	51.529	2.11	3.34	5.34	618	Tetratetracontane
122	51.687	3.25	4.54	6.04	414	.beta.-Sitosterol
123	51.932	0.62	0.70	7.42	458	Cholesterol, TMS derivative
124	52.076	0.24	0.26	7.71	296	Methyl elaidate
125	52.269	0.21	0.19	9.40	216	Octanoic acid-TMS
126	52.610	0.07	0.11	5.77	292	Methyl ganma-linolenate

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

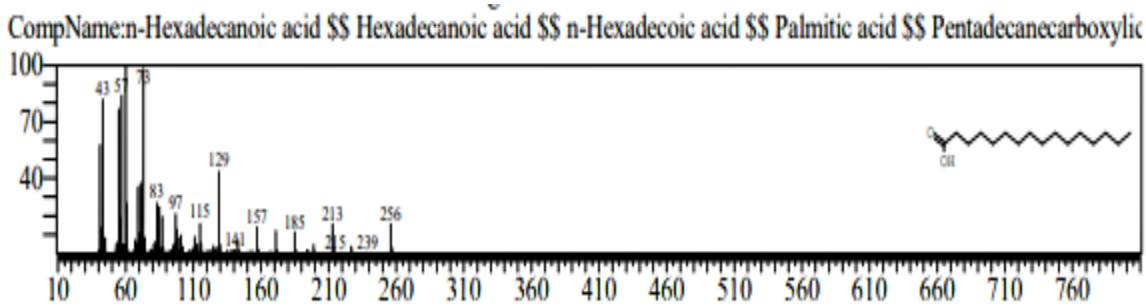
127	52.749	0.01	0.03	3.31	438	Arabinose-4TMS (1)
128	52.961	0.12	0.12	8.13	234	3-Hydroxypropionic acid-2TMS
129	53.108	0.19	0.23	6.78	350	Methyl cis-13,16-Docosadienate
130	53.317	0.01	0.02	4.11	458	Cholesterol-TMS
131	53.624	0.68	0.60	9.69	538	Hentriacontanoic acid, TMS derivative
132	54.235	2.44	1.03	20.02	416	Juniperic acid-2TMS
133	54.428	2.79	0.96	24.67	374	Dodecanedioic acid-2TMS

Figure:1 Gas Chromatography-Mass spectrometry (GC-MS) analysis in *Phaseolus vulgaris* using ethanolic extract



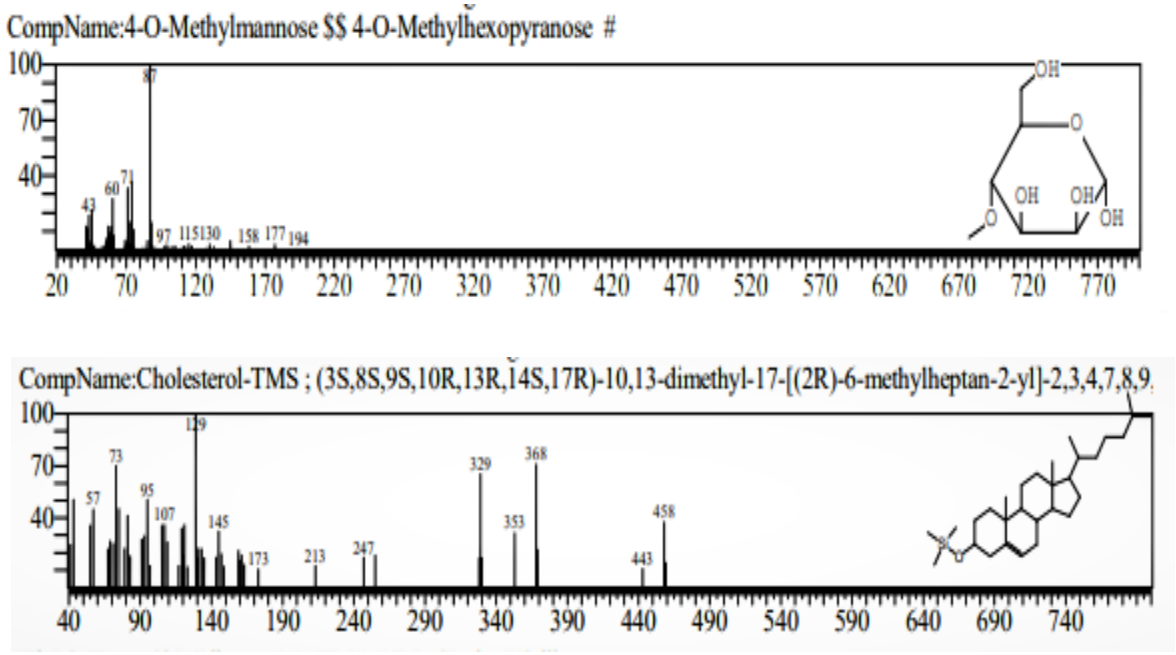
*X-axis - The retention time, *Y-axis - Concentration of sample

*Min - Minutes *TIC – Total Ion Chromatogram



ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022



Bioactive compounds determination by Gas Chromatography – Mass spectrometry (GC-MS) analysis in *Phaseolus vulgaris* using ethanolic extract

The results on GC-MS analysis in ethanolic extract of *Phaseolus vulgaris* with their retention time was showed in Table 1 and Figure 1. One hundred and thirty-three compounds were detected in ethanol extract of *Phaseolus vulgaris.* In retention time 21.370 min, the extraction compound 4-O-Methylmannose occurred with highest peak area of 15.36% which the molecular weight 194. The compound n-Hexadecenoic acid showed the medium peak area of 7.09% at retention time 28.316 min with 256 molecular weights. In retention time 53.317 min, the extraction compound Cholesterol-TMS was observed with lowest peak area 0.01% which has molecular weight 458. *Phaseolus vulgaris* has been investigated as a reservoir of nutritious values, with protein contents ranging between 20-25 percent, complex carbohydrate (50-60 percent), and a good source of vitamins and minerals as reported by (Rahman *et al.,* 2001; Reyes-Moreno *et al*., 1993) and some folacin and fiber contents (Shi *et al*., 2007). Adewole *et al*., 2022 discussed that ethanolic extract includes twelve bioactive compounds whereas 9-octadecenoic acid-methyl ester contributed to have the highest percentage area of 24.985 % which showed dietetic properties in humans. Finally, the discovery of these various beneficial components lends credence to scientific proof that *Phaseolus vulgaris* can be consumed as dietary supplements.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12,Dec 2022

CONCLUSION

Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced bioactive component. Finally, because of its endowered bioactive compounds identified, the *Phaseolus vulgaris* can be a low cost-effective dietary supplement to a large part of the world's population.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, Head of the Department of Botany, and the Management of Scott Christian College (Autonomous), Nagercoil for providing laboratory facilities during the period of this study.

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