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Identification of Bioactive Compounds from *Spirulina* by Gas Chromatography Coupled with Mass Spectrophotometer (GC-MS)

Vasanth Ramasamy¹, Velliyur Kanniappan Gopalakrishnan^{1*}

*1. Department of Biochemistry and Bioinformatics, Karpagam University, Coimbatore – 641 021
Tamil Nadu, India*

ABSTRACT

The aim of the present study was to investigate the chemical composition of *Spirulina* subjected to different solvent extraction (acetone, methanol and ethyl acetate) by using soxhlet methodology and the extracts were analysis by Gas chromatography coupled with mass spectrophotometer (GC-MS) using DB-5 capillary column. During GC-MS analysis, it was observed that fatty acid components are present in their extracts. In acetone extract, totally forty compounds were identified and heptacosane (17.25%), hexacosane (17.04%), heneicosane (14.47%), pentacosane (13.22%) and Nonacosane (11.29%) as major components. Thirty five compounds were identified in methanol extract and the major compounds are n-Hexadecanoic acid (19.9%), Cyclononasiloxane octadeca methyl (7.81%) and phytol (6.53%). Thirty components were identified in ethyl acetate extract and dodecane (17.35%), heptadecane (15.31%), sufurous acid butyl heptadecyl ester (11.31%) and n-hexadecanoic acid (4.26%) most abundant in ethyl acetate extract.

Keywords: *Spirulina*, GC-MS, acetone, methanol, ethyl acetate

*Corresponding Author Email: vkgopalakrishnan@gmail.com

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INTRODUCTION

Spirulina is an algae containing a valuable combination of substances, including those practically absent in common food. Energetic value of 10 g of dried *Spirulina* powder – 29 kcal: fat-0.7g , protein - 5.7g, carbohydrate – 2.4g vitamin A – 57 IU, vitamin B1-0.24 mg, B2(riboflavin)-0.38 mg, B3 (nicotinamide)-1.3mg, B6 (pyridoxine) – 0.4mg, B9(folic acid) – 9.4 mg, vitamin C – 1mg, Vitamin – 0.5 mg, Natrium – 104 mg, potassium – 136 mg, calcium – 12 mg, magnesium – 20 mg, iron – 2.8 mg, zinc – 0.2 mg¹.

Spirulina is a source of β -carotene and iron². Research shows that *Spirulina* stimulate the immune system, build both red and white blood cells and assist detoxification³. *Spirulina* enhances the hematopoietic system and increases resistance to hypoxia^{4,5}.

A large number of microalgae and cyanobacterial extracts and extracellular products have been found to have antibacterial and antifungal activity. The volatile compound and crude solvent extract of algae species has ability to inhibit the growth of bacteria^{6,7,8}.

Spirulina has been studied because of its therapeutic properties⁹ and the presence of antioxidant compound such as phenolics^{10,12}. The occurrence of phenolic compound in plant is well documented and these compounds play important role of antioxidant activity in biological system. However the antioxidant properties of algae and cyanobacteria are less well documented, although decreased cholesterol level has been reported in hypercholesterolemic patient fed *Spirulina* and the antioxidant activity of phycobiliproteins extracted from *Spirulina* has also been documented¹³.

The present study was to investigate the *Spirulina* was subjected to different solvent extraction and identifies the individual components in their extract by gas chromatography coupled with mass spectrometer (GC-MS).

MATERIALS AND METHODS:

Sample Collection and Reagents

Dried form of *Spirulina* material was procured from Sanet products limited, kodai Raod, Tamilnadu. Acetone, Methanol and ethyl acetate were purchased from Coimbatore Scientific Suppliers, Coimbatore, Tamil Nadu.

Preparation of various extracts of *Spirulina*

10 g of *Spirulina* was extracted separately with acetone, methanol and ethyl acetate by using a soxhlet apparatus for 24 hours. The resulting extracts of *Spirulina* from these solvents were kept at fridge for further use^{14,15}.

GC-MS analysis

The following GC-MS conditioned maintained throughout the analysis

Column - DB-5 Length 30m, Diameter 0.25mm, Film thickness- 0.25 μ m or equivalent.

Column Flow rate – 1.0 ml/min, Detection – Mass /Abundance (AMU)

Temp. Programme - 70°C - 2min - 25°C/min - 150°C - 3°C/min - 200°C - 8°C/min - 260°C - 1.133min

Injector Temperature -250°C

Injection Mode - Pulsed Split

Split ratio - 1:2

Septum Purge flow - 3ml/min. Gas saver Mode:-20ml/min after 2min.

Aux. Temp - 260°C

MS Conditions

MS Source Temperature - 230°C, MS Quad Temp - 150°C, Solvent delay:- 12min. EMV mode:- Gain factor,

Gain factor: - 5.00 = 1224V, Acq Mode: - SCAN, LIBRARY – NIST

RESULTS AND DISCUSSION:

The present study reveals that the use of organic solvents (such as acetone, methanol, and ethyl acetate) in the preparation of *Spirulina* extraction and identified more compounds by GC-MS. From GC-MS analysis of solvent extracts of *Spirulina*, it was observed that acetone extract showed forty components (Figure 1) and methanol extract showed thirty five compounds (Figure 2) and in ethyl acetate extract, thirty compounds were identified (Figure 3). Some of the compounds were present in all the extracts. Heptacosane (17.25%), Hexacosane (17.04 %) Heneicosane (14.47%), Pentacosane (13.22%) and Nonacosane (11.29 %) were present as major constituents in acetone extract (Table 1). In methanol extract, the most abundant compounds were n-Hexadecanoic acid (19.9%), Cyclononasiloxane octadeca methyl (7.81%) Cyclodecasiloxane (6.57 %) and phytol (6.53%) (Table 2). Dodecane (17.35%), Heptadecene (15.31%), Sufurous acid butyl heptadecyl ester (11.31%) and n-Hexadecanoic acid (4.26%) were major components present in the ethyl acetate extract (Table 3). Compounds such as Octadecene and Heptadecene were found in both algae and plant species shows potent antioxidant, anticancer and antimicrobial activity (Lee *et al.*, 2007 and Mishra *et al.*, 2007). In present study, Octadecene and heptadecane were identified in ethyl acetate extract of *Spirulina*. The

hexadecane, heptadecane, Eicosane, octadecene, phytol and pentadecene were major constituents in the

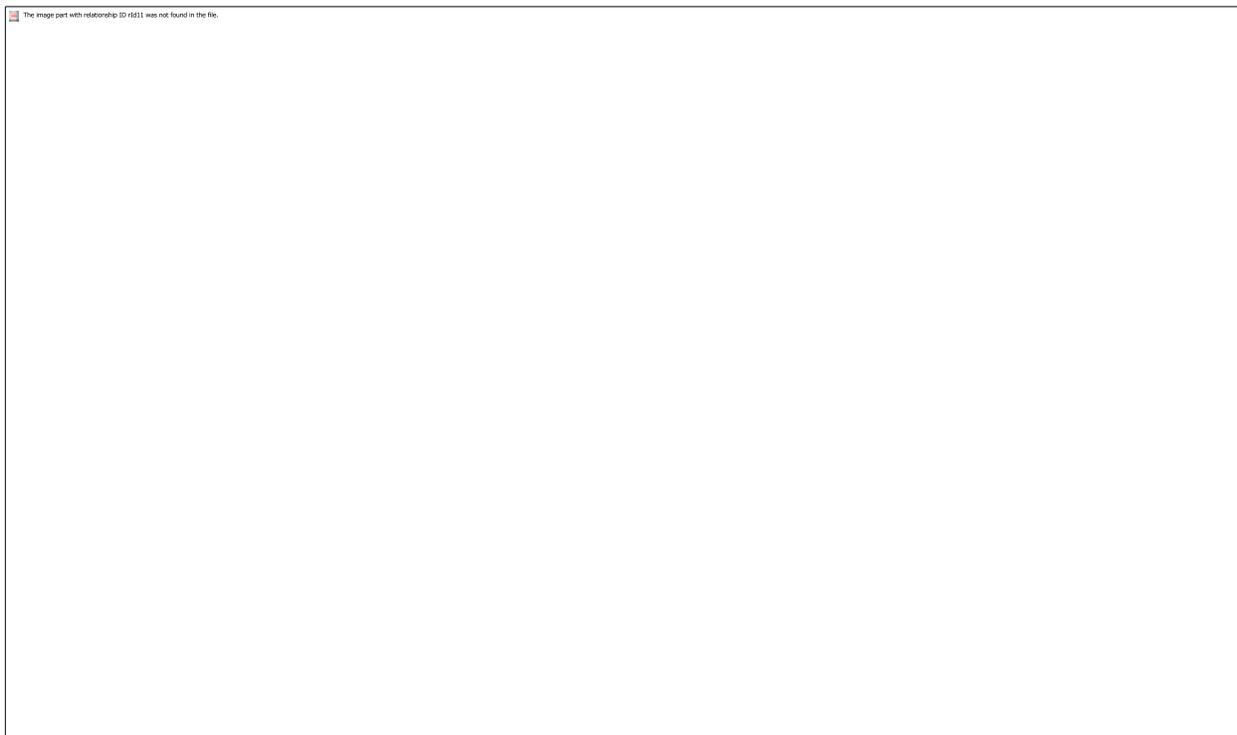


Figure 1: GC-MS chromatogram of acetone extract of *Spirulina*.

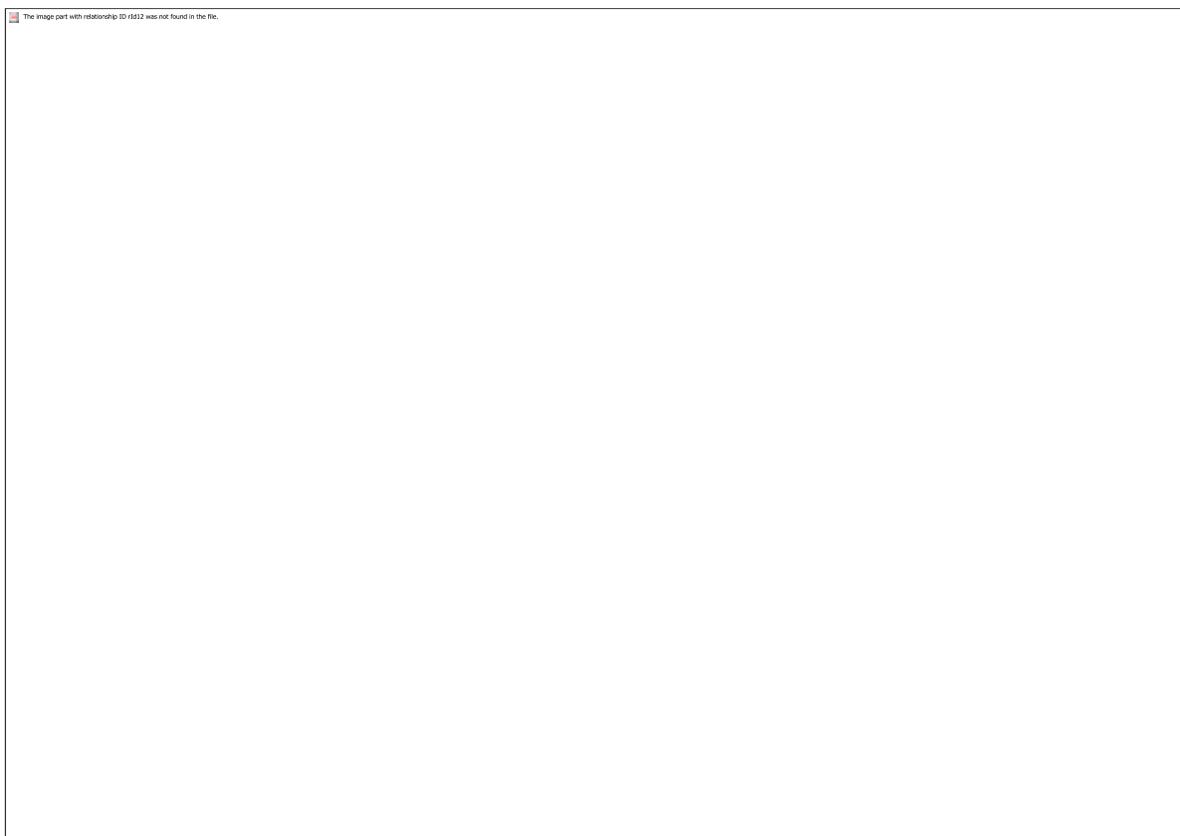
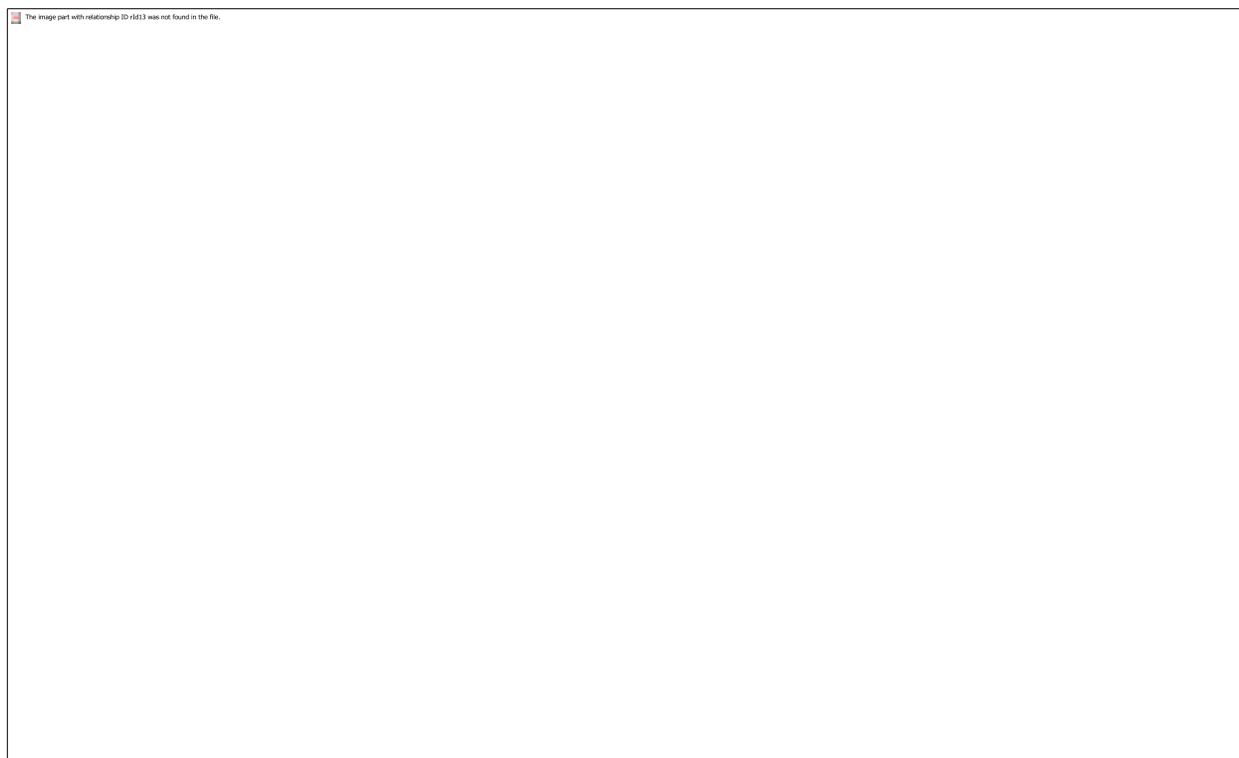


Figure 2: GC-MS chromatogram of methanol extract of *Spirulina*.**Figure 3: GC-MS chromatogram of ethyl acetate extract of *Spirulina*.****Table 1: GC-MS analysis of different compound in acetone extract of *Spirulina***

Peak	RT	Compounds	%
1	7.868	Cycloheptanol, 2-methylene	0.02
2	9.601	Phenol, 2,5-bis(1,1-dimethylethyl)	0.03
3	9.731	Butylated hydroxytoluene	0.2
4	10.978	1-Hexadecene	0.03
5	13.227	Heptadecane	0.38
6	19.863	n-hexadecanic acid	0.04
7	20.801	Carbonic acid ,isobutyl octadecyl ester	0.03
8	23.519	Heneicosane	0.1
9	25.448	Docosane	0.6
10	27.033	Tricosane	2.56
11	28.404	Tetracosane	7.03
12	29.146	Docosane	0.09
13	29.626	Pentacosane	13.22
14	30.362	Pentacosane	0.38
15	30.493	3-methyltricosane	0.09
16	30.914	Hexacosane	17.04
17	31.763	Tetracosane	0.79
18	31.923	Tricosane	0.32
19	32.137	Cyclopentane	0.04
20	32.428	Heptacosane	17.25
21	33.223	Tetracosane	0.03

22	33.354	Tetracosane, 3 - ethyl	0.11
23	33.449	Docosane	0.96
24	33.651	Pentacosane	0.51
25	33.935	9-Eicosene	0.11
26	34.25	Heneicosane	14.47
27	35.235	Pentacosane,5 -methyl	0.09
28	35.413	Hexacosane	0.2
29	35.514	Docosane	0.87
30	35.796	Pentacosane	0.68
31	36.173	9-Hexacosene	0.24
32	36.494	Nonacosane	11.29
33	37.176	Cyclopentane, (4-octyldodecyl)	0.03
34	37.592	Pentadecane	0.05
35	37.746	Hexacosane,9-octyl	0.15
36	37.96	Pentacosane	0.26
37	38.096	Nonacosane	0.86
38	38.417	Undecane,3-methyl	0.63
39	39.963	Cyclopentane, decyl	0.31
40	39.295	Heptacosane	7.93

Table 2: GC-MS analysis of different compound in methanol extract of *Spirulina*

Peak	RT	Compounds	%
1	7.072	4-Chloro-2-trifluoromethyl-6-[4-trifluoromethyl phenyl] pyridine	0.35
2	7.915	3-Pyridinecarboxamide	0.29
3	7.956	Tetradecane	0.28
4	9.292	Cycloheptasiloxane, tetradecamethyl	0.7
5	9.345	3-Buten-2-one,4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	0.47
6	10.224	2(4h)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	0.55
7	11.108	Hexadecane	0.34
8	12.538	Silane	5.51
9	13.221	Heptadecane	6.47
10	14.562	Tetradecanoic acid	0.46
11	16.456	Cyclononasiloxane, octadecamethyl	7.81
12	16.658	2-propenoic acid, undec-10-enyl ester	0.24
13	17.489	Phthalic acid, butyl isohexyl ester	1.82
14	18.96	Pentadecanoic acid, 14 methyl-methyl ester	0.95
15	19.317	9-Hexadecanoic acid	0.43
16	19.317	n-Hexadecanoic acid	19.9
17	20.658	Cyclodecasiloxane	6.57
18	23.044	Sulfuric acid 5,8,11-heptadecatrienyl methyl ester	0.45
19	23.418	9,12 -octadecadienoic acid acid(Z,Z)- methyl ester	0.35
20	23.822	Phytol	6.53
21	23.922	1,3-cyclodode	1.89
22	24.195	9,12 -octadecadienoic acid acid(Z,Z)- methyl ester	2.14
23	24.373	Benzoic acid,2,4 -bis(trimethylsilyl)oxyl-trimethylsilylester	6.04
24	25.05	Hexadecanamide	0.51
25	26.854	6,8-dichloro-2-[4-chlorophenyl]-4-bromoacetylquinoline	4.98

26	28.777	Cyclononasiloxane, octadecamethyl	4.97
27	29.353	1-(3-chloro-4-phenyl)-3-(1,2,3,4-dihydro-naphthalen-1-yl)	0.33
28	29.549	Hydroxy butyric acid ethyl ester	0.23
29	29.692	Hexadecanoic acid, 2,3 -dihydroxypropyl ester	3.23
30	30.511	Hexasiloxane tetradecamethyl	4.9
31	32.178	E-Z-1,3,12-Nonadecatriene	0.9
32	32.624	Hexasiloxane tetradecamethyl	3.54
33	33.561	Tritriacontane	2.25
34	35.401	1,1,1,5,7,7,7-heptamethyl-3-3-bis tri methyl siloxy) tetrasiloxane	2.53
35	39.164	Cyclononasiloxane, octadecamethyl	1.09

Table 3: GC-MS analysis of different compound in ethyl acetate extract of *Spirulina*

Peak RT	Compounds	%
1	6.53 Sufurous acid, hexyl pentadecyl ester	0.9
2	6.69 Heptadecane, 2,6,10,15-tetramethyl	1.07
3	7.96 Tetradecane	3.08
4	9.34 Heptadecane, 2,6,10,15-tetramethyl	1.97
5	9.6 Phenol, 2,5-bis(1,1-dimethylethyl)	3.14
6	10.22 2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4 7a trimethy	1.21
7	11.1 Hexadecane	2.34
8	13.22 Heptadecane	15.31
9	15.63 Octadecane	0.99
10	16.66 1,12-Dodecanediol	2.76
11	17.79 Z-11-Tridecen-1-ol, acetate	0.94
12	18.94 Tridecanoic acid, 4,8,12-trimethyl - methylester	1.18
13	19.85 n-Hexadecanoic acid	4.26
14	23.83 Phytol	1.81
15	24.33 Carbonic acid, isobutyl tetradecyl ester	3.1
16	24.98 Cyclodecanone	0.97
17	26.08 2-Piperidinone, N[4-bromo-n-butyl]	0.97
18	26.71 Tetrapentacontane,1,54-dibromo	0.99
19	27.52 Sulfurous acid, octadecyl 2-propyl ester	4.16
20	28.56 1-Bromo-11-iodoundecane	5.82
21	28.95 Dodecane	17.35
22	30.29 1-Hexacosanol	0.78
23	31.25 Carbonic acid, isobutyl tetradecyl ester	0.76
24	31.78 2-Thiopheneaceticacid, 6-ethyl-3-octyl ester	2.27
25	32.35 Triacontane - 1-bromo	2.55
26	33.27 1-Bromo-11-iodoundecane	1.19
27	33.66 Sufurous acid, butyl heptadecyl ester	11.31
28	35.36 Heptadecanoic acid heptadecyl ester	1.6
29	35.84 2-Heptafluorobutyroxypentadecane	4.07
30	38.14 Silanamine	1.13

methanol and acetone extract of *Spirulina platensis* by GC-MS and these compounds shows antibacterial activity against *Staphylococcus aureus* and *Salmonella typhimurium* (Vinaykumar *et al.*, 2011). Ethyl acetate extract of *Anabaena variabilis*, *Oscillatoria anustissima* and *Anabaena flosaquae* shows potent antimicrobial activity against gram positive, gram negative, yeast and

fungi and GCMS analysis of ethyl extract reveals that heptadecane, octadecene, hexadecanoic acid, docosane (Hanan *et al.*, 2010). In present study, these similar compounds were identified. Costantino *et al* (1993) reported that Phenol, 2,5-bis(1,1-dimethylethyl) has good anti-inflammatory activity in rats and also report in present study. The benzene extract of *Trichodesma amplexicaule Roth*, it contains terpenoids (beta-sitosterol, alpha-amyrin, lupeol, hexacosanoic acid, ceryl alcohol and hexacosane) and bioactivities against selected pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus flavus* and *Penicillium Chrysogenum*. The isolated compound hexacosane was more active against *E. coli* and hexacosanoic acid had greater activity against *A. flavus* (Singh *et al.*, 2003). The oil from aerial part of *anthesis mixta* and *anthesis tomentosa* shows good antibacterial activity against gram-positive bacteria and it contains hexadecanoic acid, nonacosane and hexacosane (Formisano *et al.*, 2012). The hexane extract of *Turkish, Achillea, satureja* which contains mainly hexacosane and heneicosane and extract has exhibit the potent antibacterial activity over a board spectrum against 25 phytopathogenic bacterial strains (Kotan *et al.*, 2010). The chemical composition of *Ceratonia siliqua* which contains nonadecane, heneicosane, heptadecane, hexadecanoic acid, octadecanoic acid and it shows antimicrobial activity against 13 bacteria and 8 fungal strains and it also has cytotoxic effect against two tumoral human cell lines HeLa and MCF-7 (Hsouna *et al.*, 2011).

CONCLUSION:

This study revealed a high level of chemical composition characteristic of fatty acids extracted from *Spirulina* and analysis by GC-MS. From GC MS data, identification of more compounds in their extract and it previously reported that these compounds has antibacterial, antifungal, antioxidant and anticancer activity but further researches should be made to isolation and purification of natural products in their extract.

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