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## Phytochemical analysis of *Bacopa monnieri* (L.) Wettst. and their anti-fungal activities

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Realization of hazardous effects of synthetic fungicides has led to an interest in the usage of biocontrol agents. The present study, therefore, designed to investigate the anti-fungal activities of *Bacopa monnieri* (L.) Wettst. and understand its possible mechanism. To estimate the anti-fungal activity, food poisoning method was applied. Extract of *Bacopa* effectively controlled the growth of chosen fungi as evidenced by the IC<sub>50</sub> value - *Fusarium oxysporum* (31.25 µg/ml), *Sclerotium rolfsii* (6.25 µg/ml), *Alternaria* sp (28.75 µg/ml), *Rhizoctonia solani* (18.75 µg/ml). Phytochemical screening revealed the presence of Tannin, Phlobatannin, Saponin, Steroid, Flavonoid, Cardiac Glycoside, Phenol, Carbohydrate and Alkaloid. However, Anthraquinone and Terpenoid were absent. Quantitative analysis revealed the presence of 12.5 mg, 110 mg and 1.5 mg of tannin, alkaloid and saponin, respectively per gm dry matter of the plant extract, while phenol was estimated to be 24.75 mg GAE/gm methanolic extract. GC-MS analysis showed the presence of 35 compounds in the methanolic extract of whole plant of *Bacopa monnieri*. The *Bacopa monnieri* showed free radicals scavenging activity by DPPH method (94 % inhibition at 1000 µg/ml), CUPRAC assay (0.22622 TE mM at 1000 µg/ml), anthocyanin content (1.436 CGE mg/ml), flavonoid content (29.666 GAE /gm). In conclusion, the plant extracts of *Bacopa monnieri* contains phytochemicals, which shows antifungal activities.

**Keywords:** *Bacopa monnieri* (L.) Wettst., Antioxidant activity, Phytochemicals estimations, GC-MS analysis.

**IPC Int. Cl.<sup>8</sup>:** A61K 36/00, C09K 15/00, C07, C08, C07C-C07K

Plant pathogens are significant to human because they damage plants and its products on which human are dependent for their necessary requirements like food, cloths, furniture, etc. Pathogenic fungi can destroy cellular structure, physiological function and rate of metabolism or pathway can be altered. Now a day, large number of synthetic fungicides are available but they have several side effects like long degradation periods, acute toxicity, decrease soil fertility, accumulation in food chain, non-targeted effects also harm useful microbes as well as several pathogens develop resistance power against them. The residue of synthetic fungicides on plant or plant products also generate free radicals inside the body and damage the cells components, including DNA, proteins and causing several diseases like cancer and other health conditions<sup>1,2</sup>. So that, we have urgent need to develop an alternative of these chemical fungicides from natural resources. Medicinal plants are the one of the most important source of bio-fungicides. A large

number of medicinal plants have been already investigated for their anti-fungal activity as well as antioxidant properties by several researches. Natural compounds are perceived as efficient, safe, cost effective and affordable in comparison with synthetic fungicides and synthetic anti-oxidants that might serve as leads for the development of novel drugs and in food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats<sup>3,4</sup>.

*Bacopa monnieri* (L.) Wettst., also known as *Water Hyssop* (vernacular-*Brahmi*), belongs to the Scrophulariaceae family, is prominently used in *Ayurveda*, a holistic system of medicine that originated in India. *B. monnieri* is a small creeping herb with numerous branches, small fleshy, oblong leaves and purple flowers; it grows in wet and sandy areas and near the streams in tropical regions. Flowers and fruits appear in summer. The stem and leaves of the plants are used for various medicinal purposes. It is used in traditional medicine to treat various nervous disorders, as a brain tonic to enhance memory development, learning, and concentration, and to

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provide relief to patients with anxiety; it is also used in digestive complaints, for skin disorders, and as an antiepileptic, antipyretic, and analgesic<sup>5,6</sup>. Historically, the use of *Bacopa monnieri* (Bm) dates back to approximately 6<sup>th</sup> century AD. Today practitioners of *Ayurveda* recognize it as an adaptogen, a physiological agent that naturally increases the body's resistance to physical and emotional stress.

## Materials and methods

### Collection of plant materials

The plant material of *Bacopa monnieri* (Voucher specimen No. HPS/PJ/101) was collected from the medicinal garden of Ranchi University, Ranchi in 2013. The botanical identity of the plant was confirmed by Professor HP Sharma comparing with the authentic samples at the Herbarium of Taxonomy Department of Ranchi University, Ranchi.

### Extraction of plant material

Whole plant of *B. monnieri* was dried under shade at room temperature and then powdered using a mill. The shade dried plant powder was soaked with methanol (1:10 ratio). The flask was covered with aluminum foil to avoid evaporation and then kept for 48 hrs in 50 rpm shaker incubator at room temperature. After 48 hrs the solution was filtered by using Whatman filter paper No.1 and the filtrate was collected in a beaker. Then, the filtrate was kept in incubator at 37 °C to evaporate the solvent. The prepared extract was then stored at 4 °C for further use.

### Determination of antimicrobial activities

#### Test organism

The antimicrobial activities of plant extract were investigated against *Fusarium oxysporium*, *Alternaria* sp, *Rhizocotonia solani* and *Sclerotium rolfsii*. Pure culture of all the fungal species are maintained in Laboratory of Plant Physiology and Biotechnology, University Department of Botany, Ranchi University, Ranchi- *Fusarium oxysporium* (NCIM 1008); *Alternaria* sp (NCIM 1078); *Rhizocotonia solani* (NCIM 1348) and *Sclerotium rolfsii* (NCIM 1084).

#### Antifungal assay

*In vitro* antifungal activity of plant extracts was tested in Potato Dextrose Agar (PDA) medium. Different concentrations 12.5 µg/ml, 25 µg/ml, 37.5 µg/ml, 50 µg/ml, 62.5 µg/ml of plant extracts were mixed with fungal medium after autoclaving. The

activity was determined to measure the growth of fungal mycelia on control (no extract) and test sample with the help of scale at regular interval.

#### Phytochemical screening

Phytochemical screening was carried out on the aqueous extract of powdered specimens by using standard procedures (Harborne method). Tannin, phlobatannin, terpenoid, saponin, flavonoid, cardiac glycoside, phenol, steroid, alkaloid, anthraquinone and carbohydrate were screened<sup>7</sup>.

#### DPPH Free radical scavenging activity

The total antioxidant activity was measured by the DPPH (2,2-diphenyl-2-picryl hydrazyl) radical scavenging assay method<sup>8,9</sup>. Different concentration of plant extracts (10-1000µg) were taken, final volume made up to 0.5 ml with methanol and incubated with 5ml freshly prepared DPPH solution in dark at room temperature for 30 min. It considers not only the antioxidant concentration but also the reaction time of scavenging reaction to reach the plateau. The antioxidant efficiency is measured at ambient temperature, so that the risk of thermal degradation of the molecules tested is eliminated. Percentage of radicals scavenged was calculated by using following formula:

$$\text{Percentage of radicals scavenged} = \frac{\text{OD}(\text{Control}) - \text{OD}(\text{Sample})}{\text{OD}(\text{Control})} \times 100$$

where, OD- Optical Density

#### CUPRAC

Neocuproine, a methylated phenanthroline derivative which chelates with the copper from cuprous chloride and forms a chromogenic redox reagent bis (neocuproine) copper (II) chloride which is a novel reagent for the CUPRAC antioxidant capacity assay<sup>10</sup>. This assay is based on reduction of Cu (II)–neocuprine complex to highly coloured Cu(I)-neocuprine complex, which is measured at 450 nm. Absorbance is calculated by formula:

$$C = A/\epsilon l$$

where, A is absorbance, C is capacity,  $\epsilon$  is Molar coefficient and l is path length.

1ml each of CuCl<sub>2</sub>, Neocuprine, Ammonium acetate, different concentration of plant extract (10-1000 µg) were mixed together and volume made up to 4.1 ml using methanol, Kept at room temperature for 30 min and absorbance was recorded at 450 nm.

## Phytochemical estimation

### Anthocyanin estimation

The method used estimates total monomeric anthocyanin content, based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5 in two dilution of the sample, one with a buffer of 0.025M KCl (pH1.0) and other 0.4M CH<sub>3</sub>COONa pH (4.5)<sup>11</sup>. Monomeric anthocyanin undergoes a reversible structural transformation as a function of pH (coloured oxonium formed at pH 1.0 and colourless hemiketal form at pH 4.5).

The plant sample was taken in two test tubes and to one KCl was added and in another CH<sub>3</sub>COONa was added and kept for 15 min and absorbance was recorded at 510 nm and 700 nm. Cyanidine3-Glucoside Equivalent was calculated by the formula:

$$C = (A \times MW \times 1000) / (\epsilon \times l)$$

where, A = {(A<sub>520</sub> - A<sub>700</sub>) pH1.0} - {(A<sub>520</sub> - A<sub>700</sub>) pH 4.5}, MW is Molar weight (449.2 gm/l), l is path length in cm and  $\epsilon$  is Molar extinction coefficient of cyanidine (26900/cm/mg).

### Flavonoid estimation

Total Flavonoid was determined by AlCl<sub>3</sub> colorimetric method. Gallic Acid Equivalent was calculated by the formula:

$$y = 0.003x + 0.002$$

where, y is OD value, m is slope value, x is amount if the test component and c is extinction coefficient<sup>10</sup>.

### Estimation of total phenolic content

Total phenolic content in methanolic extract were estimated by Folin-ciocalteau reagent as describe by Banu *et al.*<sup>12</sup>. 1000 µg/ml Gallic acid stock solution was prepared by dissolving gallic acid in methanol. In brief, 1ml of sample was mixed with 5.0 ml of Folin-Ciocalteu reagent. After 5 min 4.0ml of Sodium Carbonate solution was added to the mixture and kept in dark for 30 min at 20 °C and absorbance was recorded at 765 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing 1ml aliquots of 1.0, 2.5, 5.0, 10, 15, 20, 25, 50 & 100 µg/ml of gallic acid solutions.

Total phenolic compound in both the extract was determined by:

$$C = C_1 \times V/m.$$

where, C = Total content of phenolic compounds in mg/gm, in GAE (gallic acid equivalent), C<sub>1</sub> =

The concentration of Gallic acid established from the calibration curve in mg/ml, V = the volume of extract in ml, m = The weight of plant extract in gm.

### Estimation of alkaloid

0.5 gm dried powdered plant sample was mixed with 20 ml, 10 % acetic acid in ethanol, incubated for 4 hrs and filtered. The filtrate was kept on water bath to make it concentrated or to make its volume 1/4<sup>th</sup> the original volume. To this drop by drop concentrated ammonium hydroxide was added to precipitate alkaloid. This solution was left to settle and the precipitate was collected in a filter paper. The precipitate collected was washed with dilute ammonium hydroxide solution and dried in oven at 40 °C, until a constant weight was obtained. Then alkaloid precipitate was calculated in mg/gm of the dried plant material<sup>13</sup>.

### Estimation of tannin

The classic FeCl<sub>3</sub> test was used to identify the presence of tannin. In 0.1gm of dry plant samples 50 ml of water was added, boiled for 30 min and filtered. The filtrate was collected in 500 ml flask and water was added up to the mark. Then to 0.5ml aliquots 1ml 1 % K<sub>3</sub>Fe (CN)<sub>6</sub>, 1ml 1 % FeCl<sub>3</sub> was added and solution volume was made 10ml by adding water. This was left for 5 min and measured spectrophotometrically at 720 nm<sup>14</sup>. The actual tannin concentration was calculated on the basis of the optical absorbance values obtained for the standard solutions calibration curve.

### Estimation of saponin

Two gram of dried powdered plant sample was mixed with 20 ml of 20 % aqueous ethanol and heated over water bath (55 °C) for 4 hrs with continuous stirring. Then the mixture was filtered and residue was re-extracted with another 20 ml 20 % ethanol. This extract was reduced to 4ml over water bath at 90 °C and transferred to 250 ml separatory funnel. To this 10ml of diethyl ether was added and shaken vigorously. Then the aqueous layer was recovered and 6ml n-butanol was added. This extract was washed twice with 10ml of 5 % aqueous sodium chloride and heated on water bath for evaporation. Samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Table 1—Antifungal activities of *Bacopa monnieri* against four plant pathogenic fungi

Concentration of plant extract ( $\mu\text{g/ml}$ )	<i>Fusarium oxysporum</i>		<i>Sclerotium rolfsii</i>		<i>Alternaria</i> sp		<i>Rhizoctonia solani</i>	
	Mean of ZOI	IC <sub>50</sub>	Mean of ZOI	IC <sub>50</sub>	Mean of ZOI	IC <sub>50</sub>	Mean of ZOI	IC <sub>50</sub>
CONTROL	0		0		0		0	
12.5	17.06	31.25 $\mu\text{g/ml}$	70.93	6.25 $\mu\text{g/ml}$	18.13	28.75 $\mu\text{g/ml}$	30.8	18.75 $\mu\text{g/ml}$
25	35.36		94.93		38.7		68.4	
37.5	72.58		96		64.66		92.02	
50	96.14		97		89.22		95.93	
62.5	100		99.1		100		97.65	

IC – Inhibitory Concentration, ZOI- Zone of inhibition

### GC-MS Analysis

The GC-MS analysis of both extracts was performed using GCMS QP-2010 Plus Shimadzu Company instrument equipped with Rtx-5 MS column ( $30 \times 0.25$  mm id, film thickness 0.25  $\mu\text{m}$ ). Initially, oven temperature was maintained at 80 °C for 2 min. and temperature was gradually increased up to 250 °C at 5 min. and 1.0  $\mu\text{l}$  of sample was injected for analysis. Helium was the carrier gas. The flow rate of helium gas was 1.2 ml/min. The sample injector and mass transfer line temperature were set at 250 °C and split ratio is 10 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. Mass spectra were recorded across the range of 40 to 600  $m/z$  for the duration of 50 min. Identification of components was based on comparison of their mass spectra with those of Wiley and NIST libraries.

### Results

Different concentration (12.5  $\mu\text{g/ml}$ , 25  $\mu\text{g/ml}$ , 37.5  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$  and 62.5  $\mu\text{g/ml}$ ) of plant extract were subjected for antifungal activity. Plant extract with concentrations 50  $\mu\text{g/ml}$  and 62.5  $\mu\text{g/ml}$  showed about 100 % zone of inhibition (ZOI). IC<sub>50</sub> value of the plant was recorded as 31.25  $\mu\text{g/ml}$  against *Fusarium oxysprum*. *Sclerotium rolfsii* showed better result exhibiting 90 % zone of inhibition at 25  $\mu\text{g/ml}$  and about 100 % at 50  $\mu\text{g/ml}$  concentration of plant extract; IC<sub>50</sub> value was recorded as 6.25  $\mu\text{g/ml}$  against the *Sclerotium*. At a concentration of 50  $\mu\text{g/ml}$  *Bacopa monneiri* extract exhibited about 89 - 100 % ZOI against *Alternaria* and IC<sub>50</sub> Value was recorded 28.75  $\mu\text{g/ml}$ . Against *Rhizoctonia solani* *Bm* extract at 50  $\mu\text{g/ml}$  concentration showed 95.5 % ZOI and IC<sub>50</sub> Value was recorded as 18.75  $\mu\text{g/ml}$ . The data has been presented in Table 1.

Table 2—Phytochemical screening of the *Bacopa monnieri*

Sl. No.	Phytochemical	Observation	Present/Absent
1	Tannin	Brownish black ppt	Present
2	Phlobetannin	Red precipitate formed	Present
3	Terpenoid	A reddish brown colour formed	Absent
4	Saponin	Frothing not observed	Present
5	Flavonoid	Yellow colour	Present
6	Cardiac glycoside	Ring formed	Present
7	Phenol	Reddish black	Present
8	Steroid	Red colour observed	Present
9	Alkaloid	Turbidity obtained	Present
10	Anthraquinone	Pink colour not observed	Absent
11	Carbohydrate	Black colour was observed	Present

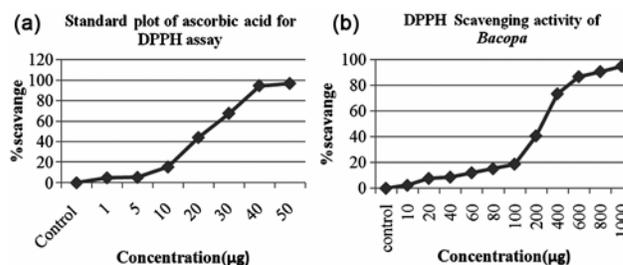


Fig.1A—DPPH scavenging activity of standard ascorbic acid, B- DPPH scavenging activity of *Bm* extract

Phytochemical screening investigation indicated that the dry powder of whole plant contains tannin, phlobotannin, saponin, steroid, flavonoid, cardiac glycoside, phenol, carbohydrate and alkaloid. The Results have been shown in Table 2.

Significant free radical scavenging activity was found to be associated with all concentrations (10  $\mu\text{g}$ -1000  $\mu\text{g}$ ) of *Bm* extract as depicted in Fig. 1.

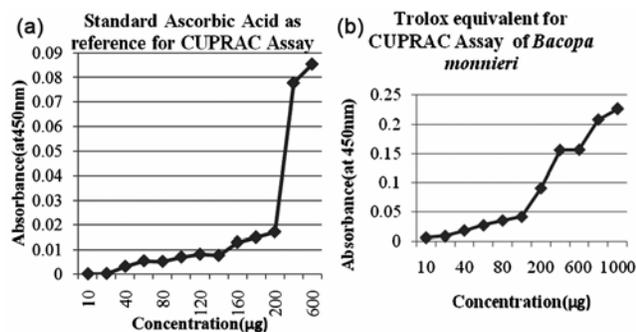


Fig. 2A—CUPRAC assay of standard ascorbic acid, B: CUPRAC assay of *Bm* extract.

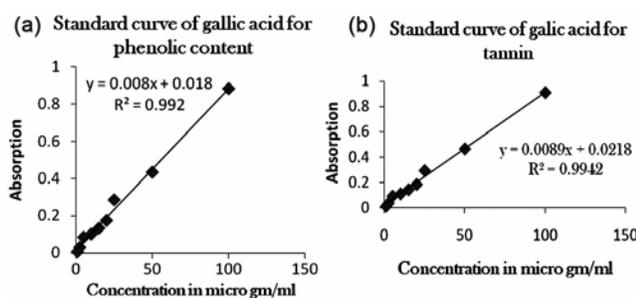


Fig. 3A—Standard gallic acid plot for total phenolic content, B: Standard gallic acid curve for tannin estimation

As revealed by the DPPH method free radical scavenging activity of methanolic extract of *Bacopa monnieri* at 10 µg was about 2 % which increased to 94 % at 1000 µg.

Antioxidant capacity in terms of Trolox equivalent of *Bacopa monnieri* was found to increase with increasing concentration (Fig. 2). At 10 µg of *Bm* extract 0.006 mmol of TE was estimated and it increased to 0.226 mmol of TE at 1000 µg concentration. Quantitative estimation of significant secondary metabolites has been done and standard graph have been presented in Fig. 3. Tannin, alkaloid and saponin per gm dry matter of whole plant were found to be 12.5, 110 and 1.5 mg, respectively. The quantity of total phenols was observed to be 24.75 mg GAE/gm methanolic extract of *Bacopa*. The anthocyanin content of *B. monnieri* was found to be 1.436 CGE (mg/l) by pH differential method as shown in Table 2. Total Flavonoid content was determined by  $\text{AlCl}_3$  colorimetric method and reported as Gallic Acid Equivalent by referring to standard curve ( $y = 0.0039x + 0.0024$ ,  $R^2 = 0.99$ ) represented in Fig. 3. Total flavonoid content at 10 µg was recorded as 6.333 GAE and at 1000 µg it increased to 29.666GAE, as shown in Fig. 4.

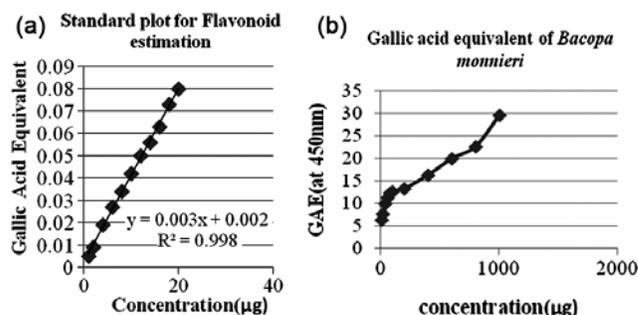


Fig. 4A—Standard gallic acid plot for flavanoid estimation, B: Flavanoid content in *Bm*

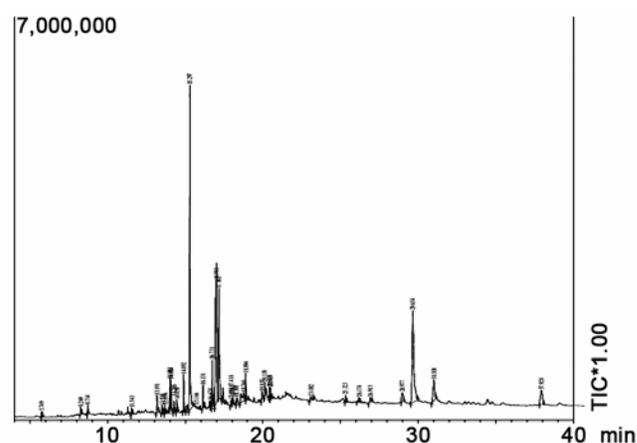


Fig. 5—Chamatogram of GC-MS analysis of *Bacopa monnieri*

After phytochemical screening and estimation the selected plant was subjected for gas chromatography and mass spectroscopy analysis. On analysis of GC-MS chromatogram (Fig. 5), total of 37 compounds were identified in the methanolic extract of *Bacopa monnieri*. The identification of photochemical is based on the Retention Time and quantity is estimated based on peak area. The results have been tabulated in Table 3 showing the Retention Time, Area, Area percent, name of compounds, molecular formula and molecular weight. The structures of major compounds present in *Bacopa* extracts have been shown in Fig. 6.

## Discussion

Previously, *B. monnieri* has been reported to show anticonvulsant, tranquilizing, muscle relaxant, antiseptic, anticancer, and improvement in maize learning activities<sup>15</sup>. It may also serve as the memory boosting alternative source for the development of new neurological agent due to its biological activities. Recent researches have established that it can work as a viable medicine for improving mental health and for

Table 3—GC-MS analysis of *Bacopa monnieri*

Peak	R.Time	Area	Area %	Name of phytochemicals	Mol. Wt.	Mol. formula
1	5.749	205662	0.27	Dodecane	170	C <sub>12</sub> H <sub>26</sub>
2	8.269	331802	0.44	Phenol, 2-methoxy-4-(2-Propenyl)-	164	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>
3	8.714	294251	0.39	Tridecane	184	C <sub>13</sub> H <sub>28</sub>
4	11.543	394558	0.53	3A(1H)-Azulenol,2,3,4,5,8,8A-hexahydro-6,8A-dimethyl-3-(1-M	222	C <sub>15</sub> H <sub>26</sub> O
5	13.191	1127805	1.5	9-Octadecenoic acid (Z)-	282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
6	13.509	524636	0.7	2-Nonenal, 2-Pentyl-	210	C <sub>14</sub> H <sub>26</sub> O
7	13.696	355047	0.47	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	222	C <sub>13</sub> H <sub>18</sub> O <sub>3</sub>
8	14.011	1073495	1.43	2,6,10-Trimethyl,14-ethylene-14-Pentadecne	278	C <sub>20</sub> H <sub>38</sub>
9	14.084	740370	0.99	2-Pentadecanone, 6,10,14-Trimethyl-	268	C <sub>18</sub> H <sub>36</sub> O
10	14.266	618070	0.82	3,7,11,15-tetramethyl-2-Hexadecen-1-ol	296	C <sub>20</sub> H <sub>40</sub> O
11	14.458	497286	0.66	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	296	C <sub>20</sub> H <sub>40</sub> O
12	14.892	1268940	1.69	Hexadecanoic acid, methyl ester	270	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>
13	15.166	575567	0.77	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	292	C <sub>18</sub> H <sub>28</sub> O <sub>3</sub>
14	15.297	17931857	23.91	N-Hexadecanoic acid	256	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
15	16.131	873112	1.16	Octadec-9-enoic acid	282	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>
16	16.628	306770	0.41			
17	16.731	1893715	2.53	Phytol	296	C <sub>20</sub> H <sub>40</sub> O
18	16.981	13927443	18.57	Cis-9-Hexadecenal	238	C <sub>16</sub> H <sub>30</sub> O
19	17.162	5924911	7.9	Octadecanoic acid	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
20	17.418	777619	1.04	Octadecanoic acid, ethyl ester	312	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>
21	17.958	198633	0.26	Cis-10-Nonadecenoic acid	296	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>
22	18.062	253208	0.34	Hahnfett	9999	
23	18.308	174581	0.23	Heneicosane	296	C <sub>21</sub> H <sub>44</sub>
24	18.76	1772162	2.36			
25	18.894	1295754	1.73	Icosanoic acid	312	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>
26	19.937	336854	0.45	Nonacosane	408	C <sub>29</sub> H <sub>60</sub>
27	20.13	731915	0.98	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>
28	20.431	365323	0.49	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	278	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>
29	20.505	460983	0.61	Octadecanoic acid	284	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>
30	23.082	363017	0.48	2-Cyclohexen-1-one, 3-(3-hydroxybutyl)-2,4,4-trimethyl-	210	C <sub>13</sub> H <sub>22</sub> O <sub>2</sub>
31	25.323	390311	0.52	17-(1,5-Dimethyl-hex-2-enyl)-10,13-dimethyl-2,3,4,9,10,11,12,13	382	C <sub>27</sub> H <sub>42</sub> O
32	26.174	352212	0.47	Cholesta-4,6-dien-3-ol, (3-β)-	384	C <sub>27</sub> H <sub>44</sub> O
33	26.915	429707	0.57	Vitamin E	430	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>
34	28.977	1041523	1.39	Ergost-5-en-3-ol, (3-β-,24R)-	400	C <sub>28</sub> H <sub>48</sub> O
35	29.654	12415658	16.55	Stigmasterol	412	C <sub>29</sub> H <sub>48</sub> O
36	31.001	2579540	3.44	Stigmast-5-en-3-ol, (3-β)-	414	C <sub>29</sub> H <sub>50</sub> O
37	37.928	2193847	2.93	2,6,10-trimethyl,14-ethylene-14-Pentadecne	278	C <sub>20</sub> H <sub>38</sub>

the prevention and treatment of age related cognitive decline. Because of the source of various chemical constituents which are used for the treatment of many fatal or life threatening diseases, it helps the body in numerous ways<sup>16</sup>.

Development of resistance by microbes against known antibiotics is a huge concern in medical field, thus searching for new antimicrobial compounds is a never-ending process<sup>17</sup>. It seems that *Bacopa monnieri* is a promising plant in this context.

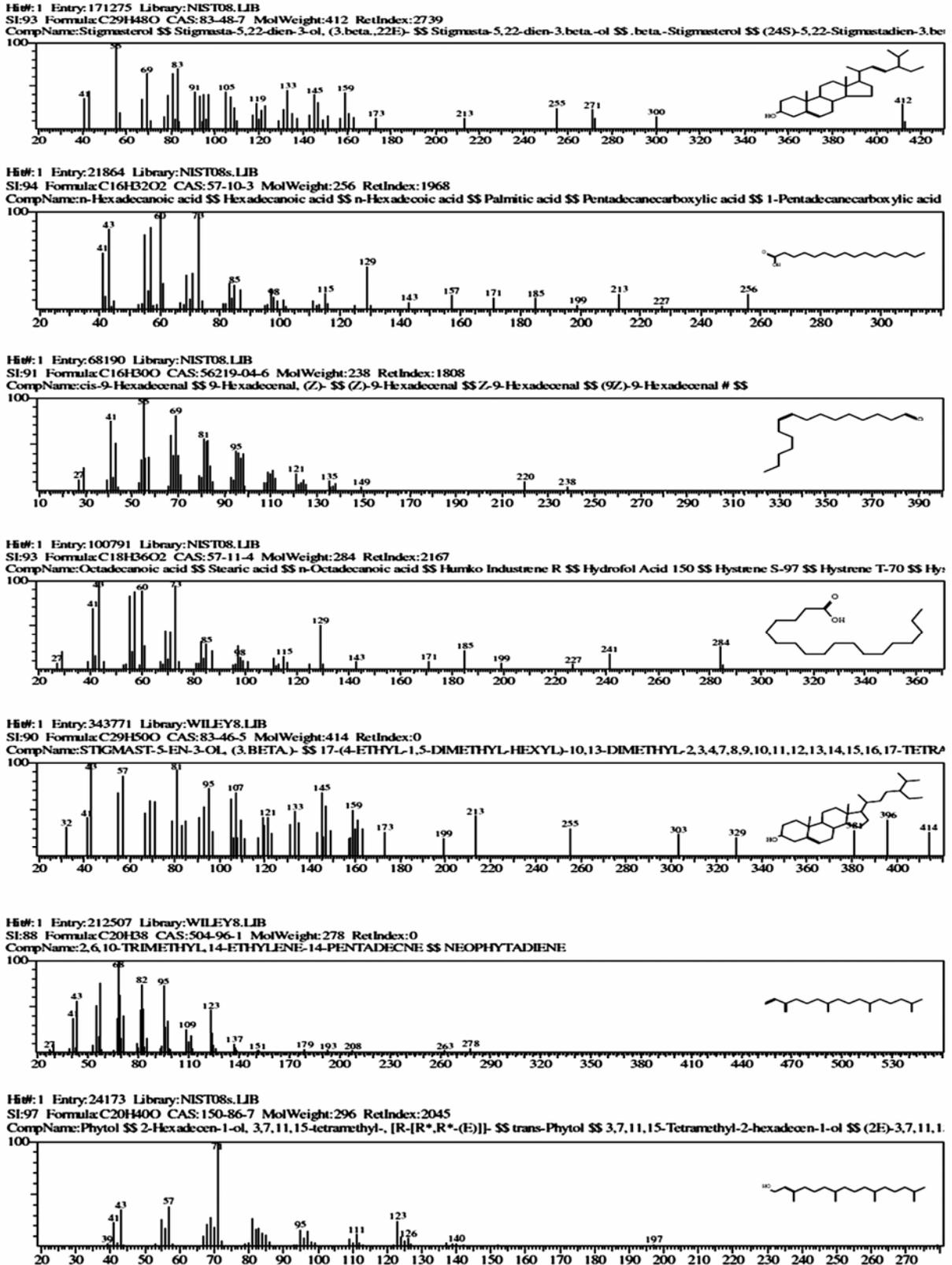


Fig. 6—structure of major components present in GC-MS analysis of *Bacopa monnieri* L.

However, further tests against other strains of bacteria and fungi are needed. In addition, the active ingredient(s) should be isolated and its/their mechanism(s) of action(s) should be enlightened by further studies<sup>18</sup>.

The search for potent plant based antioxidant continues to be of great importance as they serve as effective and easily available remedies against free radical-mediated diseases, prevention of oxidative reactions in foods, protection against DNA damage and carcinogenesis, and possible substances with wide range of pharmacological activities such as anti-inflammatory, anti-bacterial, and anti-fungal properties. Present investigation established *Bacopa monnieri* as a potent source of antioxidant as revealed by DPPH and CUPRAC assay<sup>19,20</sup>.

In present study, the plant extracts contains tannin, phlobatannin, saponin, flavonoid, cardiac glycoside, phenol, steroid, alkaloid, etc., however, it was earlier demonstrated that, the above compounds are good inhibitor to fungal growth<sup>21,22</sup>. GC-MS analysis revealed the presence of compounds like 9-octadecenoic acid (Z)-, phytol, stigmaterol which are known to be potent antifungal compounds<sup>23</sup>. Hence, these results evidently are indicating towards the antifungal potential of the plant.

## Conclusion

Plant extracts of *Bacopa monnieri* possess antifungal and antioxidant activity against *Fusarium oxysporium*, *Alternaria* sp, *Rhizocotonia solani* and *Sclerotium rolfisii* which will be useful in medicinal and pesticide applications. The strong antioxidant and antifungal potential of *B. monnieri* is advantageous to the pesticide industry as a pesticide against plant pathogens. All these activities are due to the composition of the *B. monnieri* that contains many different bioactive compounds. Nowadays, and despite all the efforts deployed by the scientific communities, it has been noticed that the several side effects on environment as well as human health occurs due to the synthetic pesticides. The residues of synthetic pesticides on plant product generate free radicals inside the body, damage cell components and are responsible for several diseases. Hence, by going back to the natural ways of life, and to avoid diseases, especially by avoiding synthetic pesticides, man finds hope in nature through its richness in plants like *B. monnieri*, which can pave the way to a new horizon in prevention of various diseases.

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