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Research Article

A COMPARITIVE STUDY OF ANTIOXIDANT ACTIVITY OF BACCOPA MONNIERI (L.) PENNELL USING VARIOUS SOLVENT EXTRACTS AND ITS GC-MS ANALYSIS

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ABSTRACT

Objective: The aim of the present study is to compare *bacopa monnieri* plant species collected from different regions for its antioxidant activity using various solvents and the plant extract with high antioxidant activity was further subjected to phytochemical and GC-MS analysis.

Methods: In the present investigation, the best accession was screened out through antioxidant activity by qualitative and quantitative methods. From the best accession extract, the potent bioactive compound was analyzed by phytochemical and GCMS analysis.

Results: The antioxidant activity of aqueous extract of *bacopa monnieri* collected from Thanjavur was higher when investigated by qualitative and quantitative methods. The GC-MS analysis revealed the presence of twenty three compounds, of the twenty three components identified, major components were Oxepine,2,7 dimethyl- (RT: 14.662), 9-Octadecenamide, (Z)-(RT: 21.096), 2,4-Quinolinediol (RT: 12.207), 3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde(RT: 13.907), Caryophyllene oxide (RT: 13.587), Hexadecanoic acid, methylester (RT: 17.145).

Conclusion: The *Bacopa monnieri* plant collected from Thanjavur was found to have the highest level of antioxidant activity when compared with the plant collected from Dindugal, Chengalpet, Kallakurichi and Trichy. GC-MS and phytochemical analysis of this plant species has established the presence of twenty three bioactive components, further confirming the medicinal use of *Bacopa monnieri.*

Keywords: *Bacopa monnieri*, Antioxidant activity, Phytochemicals, GC-MS analysis

INTRODUCTION

Today bioactive components derived from medicinal plants are being tested for the presence of new drugs with new modes of pharmacological action. Recently, studies are carried out in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases. The most important bioactive constituents of these plants are alkaloids, tannins, flavonoids and phenolic compound [1].

Bacopa monnieri (Scrophulariaceae) has been used to promote memory enhancing activity [2] and intellect, to treat psycho neurological disorders and as a rejuvenator. This plant is also found to possess anticholinesterase activity[3] antioxidant activity[4], Alzheimer disease[5], antidepressant activity[6], antiulcerogenic activity[7], anti-inflammatory activity[8], antibacterial activity[9], anticancer activity [10], antileishmanial properties.

Over the past few years, there has been an exponential growth in study of pharmacological properties of this plant [11-12]. Antioxidant components are micro constituents that inhibit lipid oxidation by inhibiting the initiation or propagation of oxidizing chain reactions, and are also involved in scavenging free radicals. Excess of free radicals has been linked to many diseases, such as cancer problem, heart problem etc. Clinical approaches of antioxidants increased multifold during the recent time for the management and therapeutic implication of neurodegenerative disorders, aging, and chronic degenerative diseases. The objective of the present study is to compare the best accession of *Bacopa monnieri* plant collected from different areas of Tamilnadu and to compare their antioxidant activity; later the best accession plant will be further analysed for phytochemical and GC-MS components.

MATERIALS AND METHODS

Plant collection and extraction

The plants were collected from different areas of TamilNadu such as Dindugal, Thanjavur, Chengalpet, Kallakurichi and Trichy in the month of August 2013.The plant materials were identified and authenticated by Sidha Research Institute, Chennai, TamilNadu, India.

The plant materials were washed with water to remove dust and sand, shade dried at room temperature and powdered. The powdered plant materials were subjected to successive extraction with the solvents ethanol, aqueous, chloroform, acetone and ethyl acetate using soxhlet extractor [13]. The extracts were dried in vacuum pump at 40° C. The dried crude extracts were stored in a refrigerator for further use.

Antioxidant activity

Qualitative and Quantitative analysis was carried out on all the extracts to determine the antioxidant activity.

Qualitative analysis of antioxidant activity of *bacopa monnieri*

50µl of *Bacopa monnieri* plant extract collected from different regions were taken in the micro titer plate. 100µl of 0.1% methanolic DPPH was added to the samples and incubated for 30 minutes in dark condition. The samples were then observed for decoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively [14]. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of *Bacopa monnieri*

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of *Bacopa monnieri* plant extract collected from different regions were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as control [15]. Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectrophotometer (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard such as 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula: % DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance of control)] x 100

Phytochemical Screening

The preliminary phytochemical screening tests were carried out for the best accession plant extract to identify the active phytocompounds such as Tannins, Saponins, Quinones, Terpenoids, Steroids, Flavanoids, Phenol, Alkaloids, Glycosides, Cardiac glycosides, Coumarins, Anthocyanin and Betacyanin by standard methods [16].

GC-MS analysis

For the Identification of bioactive components in extract with greater antioxidant activity, the extract was subjected to GC-MS analysis. GC-MS analysis was carried out on a GC-MS -5975C agilent system comprising an auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument, employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1.51 ml/min and an injection volume of 1µl was employed (split ratio of 10:1) injector temperature 240^{0}C; ion-source temperature 200^{0}C. The oven temperature was programmed from 70^{0}C (isothermal for 2 min), with an increase of 10^{0}C/min, to 300^{0}C/min, ending with a 9 min isothermal at 300^{0}C. Mass spectra were taken at 70eV; with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35 min; Ion source temperature set to 200^{0}C and interface temperature being 240^{0}C.

Identification of Components

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the component of the test materials were identified.

RESULTS

Antioxidant activity

The *Bacopa monnieri* plant species collected from five different regions was extracted using various solvents; its antioxidant activity (qualitative as well as quantitative) was compared. It was found that aqueous extract of *Bacopa monnieri* collected from Thanjavur exhibited maximum antioxidant activity in comparison to other extracts investigated (Table 1& Figure 1). The DPPH was also proved the Thanjavur plant species and the result was confirmed by table 2 and Figure (2-6).

Table 1: Qualitative analysis of antioxidant activity of b*acopa monnieri*. (Note : n=3 triplicate)

S. No.	Extractions	*B. monnieri* (Dindigul)	*B. monnieri* (Thajavur)	*B. monnieri* (Chengalpet)	*B. monnieri* (Kallakurichi)	*B. monnieri* (Trichy)
	BHT (standard)	+++	+++	+++	+++	+++
S1	Ethanol	++	+++	++	++	++
S2	Aqueous	++	+++	++	++	++
S3	Chloroform	-	-	-	-	-
S4	Acetone	+	++	+	+	-
S5	Ethyl acetate	-	-	-	-	-

+++ = very strong positive++ = strong positive

+ = positive - = Negative

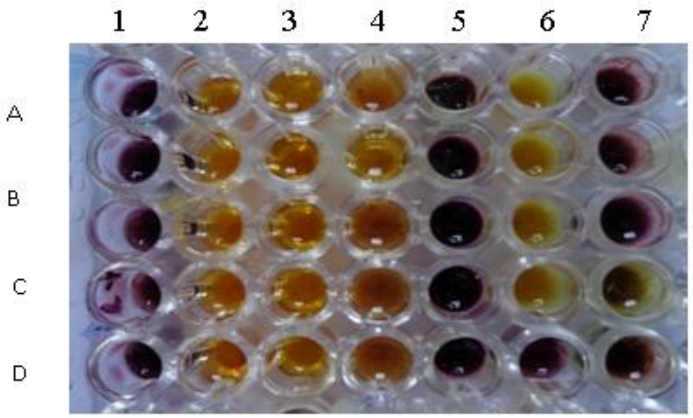

Fig. 1: Shows Qualitative analysis of antioxidant activity of Bacopa monnieri

Table 2: Quantitative analysis of antioxidant activity of *Bacopa monnieri*. (Note n=3 triplicate)

S. No.	Extractions	*B. monnieri* (Dindigul)	*B. monnieri* (Thanjavur)	*B. monnieri* (Chengalpet)	*B. monnieri* (Kallakurichi)	*B. monnieri* (Trichy)
	BHT (standard)	97.2	97.2	97.2	97.2	97.2
S1	Ethanol	84.4	85.7	81.4	78.6	70
S2	Aqueous	87.1	91.4	80	74.3	64.3
S3	Chloroform	42.8	44.2	27.1	32.9	27.1
S4	Acetone	78.5	77.1	62.8	72.8	58.6
S5	Ethyl acetate	41.4	47.1	32.9	24.3	20

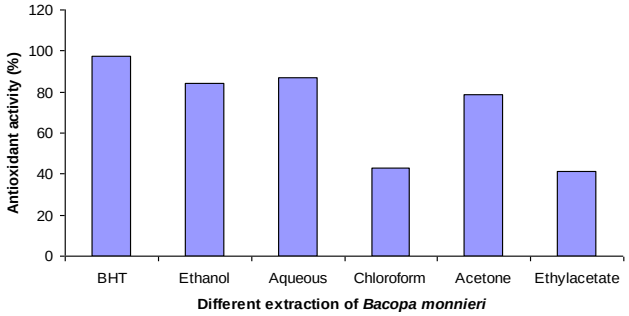

Fig. 2: Quantitative analysis of antioxidant activity from *Bacopa monnieri* (Dindigul)

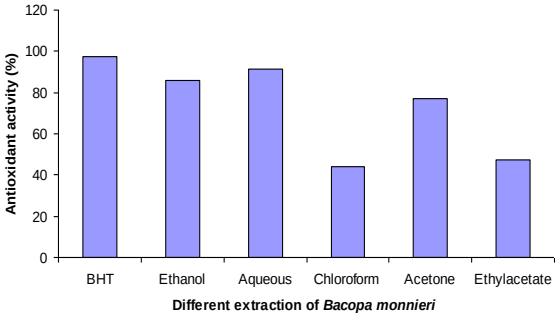

Fig.3: Quantitative analysis of antioxidant activity from Bacopa monnieri (Thanjavur)

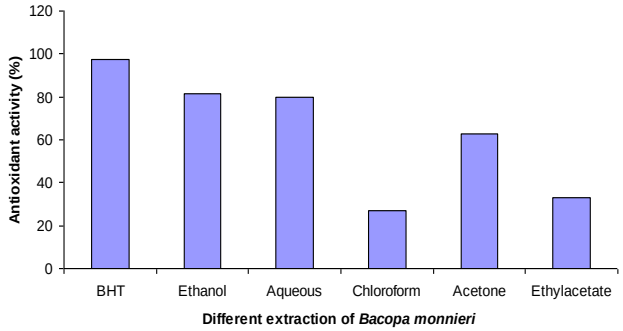

Fig.4: Quantitative analysis of antioxidant activity from *Bacopa* (Chengalpet)

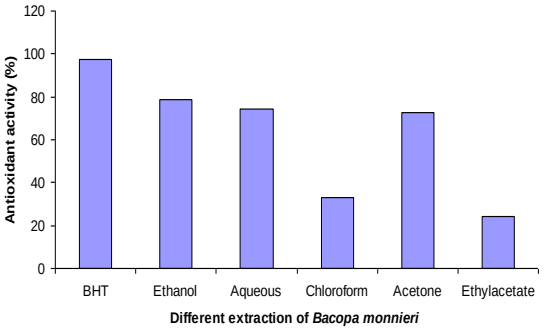

Fig.5: Quantitative analysis of antioxidant activity from *Bacopa* (Kallakurichi)

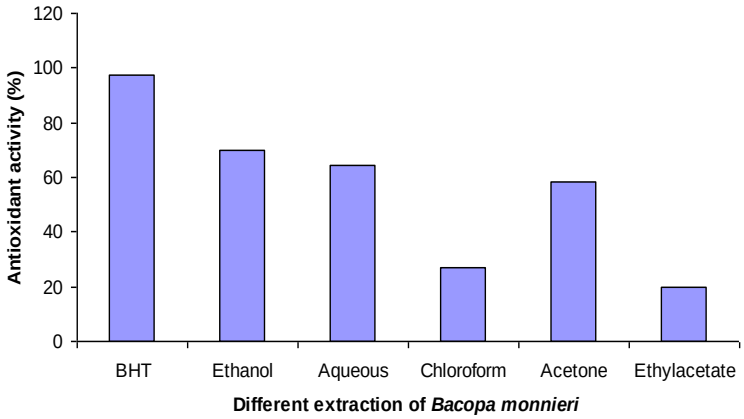

Fig.6: Quantitative analysis of antioxidant activity from *Bacopa* (Trichy)

A1: Control; A2: Standard (BHT), A3: Ethanol; A4: Aqueous; A5: Chloroform; A6:Acetone; A7: Ethyl acetate –Accession I **(Dindigul)**

B1: Control; B2: Standard (BHT), B3: Ethanol; B4: Aqueous; B5: Chloroform; B6:Acetone; B7: Ethyl acetate –Accession II **(Thanjavur)**

C1: Control; C2: Standard (BHT), C3: Ethanol; C4: Aqueous; C5: Chloroform; C6:Acetone; C7: Ethyl acetate –Accession III **(Chengalpet)**

D1: Control; D2: Standard (BHT), D3: Ethanol; D4: Aqueous; D5: Chloroform; D6:Acetone;D7: Ethyl acetate –Accession IV **(Kallakurichi)**

E1: Control; E2: Standard (BHT), E3: Ethanol; E4: Aqueous; E5: Chloroform; E6:Acetone;E7: Ethylacetate –Accession V **(Trichy)**

Table 3: Phytochemical screening of *Bacopa monnieri* (Thanjavur) – Best accessions

Phytochemicals	Plant extract				
	Ethanol	**Aqueous**	chloroform	Acetone	Ethyl acetate
Tannins	++	++	-	+	-
Saponins	+	++	+	++	-
Quinones	-	++	-	+	-
Terpenoids	+	+	+	+	+
Steroids	+	+	+	+	+
Flavonoids	+	++	-	+	-
Phenol	+	++	-	+	+
Alkaloids	+	+	-	+	-
Glycosides	-	-	-	-	-
Cardiac glycosides	+	++	-	-	-
Coumarins	+	+	+	+	-
Antho cyanin	-	-	-	-	-
Beta cyanin	+	+	-	-	-

++ = strong positive+ = positive- = negative

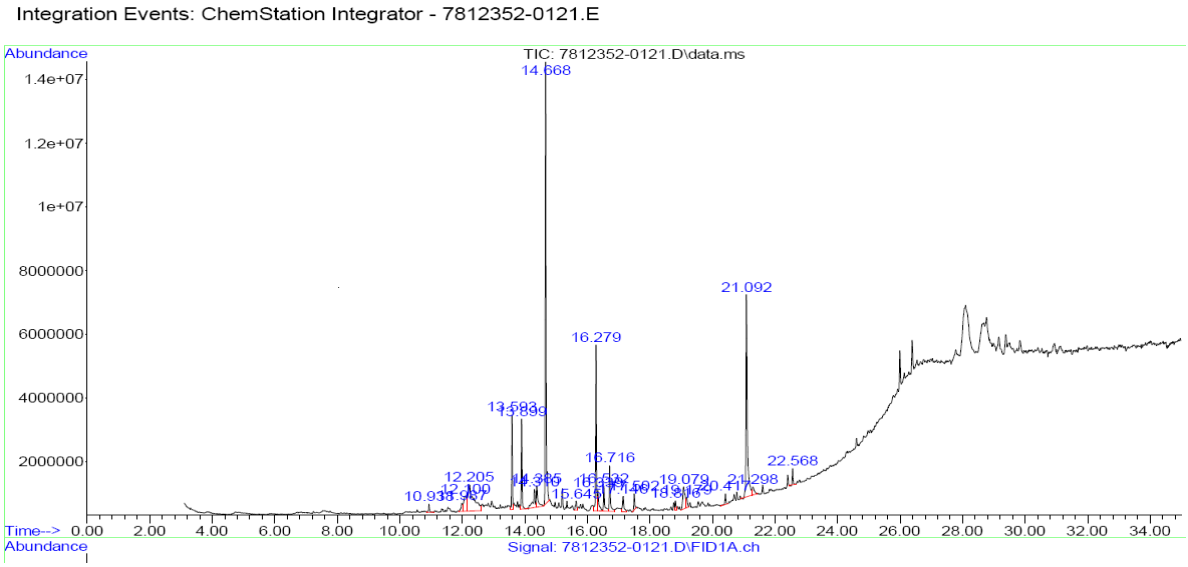

Fig. 7: Show GC-MS Chromatogram of *Bacopa monnieri*

Table 4: Phytocomponents identified in *Bacopa monnieri by GC-MS analysis*

S. No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1	10.944	2 octanol	$C_8H_{18}O$	130	0.60
2	11.990	Dimethoxane	$C_8H_{14}O_4$	174	0.79
3	12.106	Propanal, 2,3-dihydroxy,	$C_3H_6O_3$	90	3.50
4	12.207	2,4-Quinolinediol	$C_9H_7NO_2$	161	10.10
5	13.587	Caryophyllene oxide	$C_{15}H_{24}O$	204	4.70
6	13.907	3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde	$C_{15}H_{24}O$	220	4.93
7	14.313	2-Methyl-1-Phenyl-1-butanol	$C_{11}H_{16}O$	164	2.50
8	14.386	Tricyclo[3.1.0.0(2,4)]hexane, 3,6-diethyl-3,6-dimethyl-, trans-	$C_{12}H_{20}$	164	2.49
9	14.662	Oxepine, 2,7-dimethyl-	$C_8H_{10}O$	122	24.62
10	15.649	Silane, (bromomethyl)-	$C_3H_8BrClSi$	167	0.77
11	16.247	Phytol, acetate	$C_{22}H_{42}O_2$	338	8.78
12	16.332	2-Heptanone, 4-methyl-	$C_8H_{16}O$	128	2.01
13	16.535	Oleyl alcohol, methyl ether	$C_{19}H_{38}O$	282	2.15
14	16.710	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.531	3.15
15	17.145	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.74
16	17.508	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	0.89
17	18.815	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	0.71
18	19.077	Phytol	$C_{20}H_{40}O$	296	3.43
19	19.178	Furazano[3,4-b]pyrazin-5(4H)-one, 6-(1-pyrrolidinyl)-	$C_8H_9N_5O_2$	207	0.91
20	20.413	2-Nanodecanone	$C_{19}H_{33}O$	282	0.69
21	21.096	9-Octadecenenamide, (Z)-	$C_{18}H_{35}NO$	281	18.49
22	21.299	Octadecanamide	$C_{18}H_{37}NO$	283	1.27
23	22.562	Bis(2-ethylhexyl) phthalate	$C_6H_4(C_8H_{17}CO)_2$	390	0.78

Phytochemical Characterization

The phytochemical characterization of *bacopa monnieri* from thanjavur using various solvents is given in table 3. It reveals that terpenoids and steroids were predominately found in all the five extracts, followed by saponin, phenols,coumarins which were found in four extracts and tannins and alkaloids were found in two extracts. The ethylacetate has shown positive result for terpenoids, steroids and phenols.In otherwords, the results confirm the presence of therapeutically potent compounds in the aqueous extract of *bacopa monnieri*.

GC-MS analysis

GC-MS chromatogram of the *Bacopa monnieri* (Fig.2) showed twenty three peaks indicating the presence of twenty three chemical constituents. On comparison of the mass spectra of the constituents with the NIST library the twenty three constituents were characterized and identified. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) in the extract of *Bacopa monnieri* are presented in Table 4. Of the twenty three components identified, major components were Oxepine,2,7 dimethyl- (RT: 14.662), 9-Octadecenamide, (Z)-(RT: 21.096), 2,4-Quinolinediol (RT: 12.207), 3,5-Dimethylcyclohex-1-ene-4-carboxaldehyde(RT: 13.907), Carpohyllene oxide (RT: 13.587), Hexadecanoic acid, methylester (RT: 17.145).

DISCUSSION

The modern day food habits and life style have resulted in many ailments. This in turn has resulted in an increasing interest in the identification of phytochemical compounds from plant species which can be used as medicines without significant side effects. The quantitative and qualitative analysis of various solvent extracts of *bacopa monnieri* plant have established the presence of antioxidant properties. The aim of this study is to provide a comparison of the antioxidant level in the plant species collected from different places and extracted using various solvents. The results indicates that the flavonoids, phenols, tannins, saponins, quinines contents of the aqueous extract of the plant has immense potency which has resulted in the significant antioxidant activity. Further GC-MS analysis of plant species has established the twenty three bioactive components which may be possessing several pharmacological properties. Similar to this study, five major compounds were characterized through GC-MS analysis in *Polygonum chinense* L[17]; seventeen compounds were characterized from methanolic extract of *Cassiaitalica* leaf [18]; seed fourteen compounds were identified from ethanolic extract of *Entada Pursaetha* by GC-MS analysis[19]; fourteen compounds were identified from *Caralluma fimbriata* Wall., through Phytochemical studies and GC-MS analysis [20]

CONCLUSION

This study has revealed that the aqueous extract of *bacopa monnieri* plant species collected from Thanjavur is found to have better level of antioxidant activity as compared to the samples collected from Dindugal, Chengalpet, Kallakurichi and Trichy. Further GC-MS analysis of this sample has established the presence of twenty three bioactive components. The presence of various bioactive compounds justifies the use of the *Bacopa monnieri* for various ailments by traditional practitioners. However, the isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give effective results. So it is recommended as a plant of phytopharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on ecosystem and agriculture products.

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