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RESEARCH ARTICLE

Evaluation of Antioxidant Capacity and Antimicrobial Properties of Ethnic Bambuseae species and Identification of the Active Components

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ABSTRACT

Bamboo shoots of four different species were evaluated for their antibacterial activity, total phenol content, flavonoid content, antioxidant activity and total phytochemical analysis. The fresh shoots were extracted successively by using various solvents such as petroleum ether, ethyl acetate, methanol and water. The antioxidant and antimicrobial properties were investigated with the extracts. The antioxidant activity of the extracts was evaluated by DPPH assay, FRAP assay, Linoleic acid autooxidation and Hydrogen peroxide radical scavenging activity. The volatile components present in the extracts were analyzed by GCMS-MS technique. A number of compounds with important medicinal properties were detected in the extracts. The antimicrobial activity against gram positive and gram negative bacteria was evaluated by agar well method. All the extracts possessed antimicrobial activity against both *S.aureus , K. pneumonia* bacteria. The antioxidant activity was found to be highest in methanol extracts of all the four bamboo species.The results indicate that these bamboo shoots are rich sources of polyphenols, flavonoids and terpenes with potential antioxidant and antimicrobial activity.

Key words: Antioxidant activity; bamboo shoots; DPPH scavenging assay; FRAP assay; flavonoids.

1. INTRODUCTION

Fruits and vegetables are protective against various types of diseases like cardiovascular diseases and cancer. Reactive oxygen and nitrogen species are known to alter cellular structure and function which induce alterations and cause chronic degenerative diseases also including heart disease and cancer. Antioxidant bioactive compounds are a class of nutrients that can reduce the incidence of these diseases [6]. Bamboo is a perennial fast growing plant which has more than 70 genera and about 1000 species distributed throughout the world. It is recognized as a medicine in ancient Chinese texts and is a well known folk medicine in India. Young bamboo shoots of many different species of bamboo are used in numerous Asian dishes and broths. Bamboo shoots are rich in both amino acids and antioxidants and taste fresh, crisp with aromatic quality, and are delicious[20]. Recently a large number of reports have been published on the medicinal and nutraceutical properties of leaves and shoots of some species of this plant. Different parts of bamboo plant are reported to possess various medicinal properties due to the presence of many important phytochemicals. The phenolic compounds derived from the whole plant extract showed inhibition of P-glycoprotein in adriamycin resistant human breast cancer cells. Bamboo leaves have been used clinically in the treatment of hypertension, arteriosclerosis, cardiovascular disease, and cancer. Bamboo leaves and shoots possess medicinal activities like antidiabetic activity, antifertility effect, antibacterial activity, anti-inflammatory and cancer protective effect[10].

Plants contain assortment of phytochemicals such as phenols, flavonoids, terpenoids and vitamins which possess antioxidant activity [14]. Natural antioxidants derived from plant products have potential health benefits such as reducing aging and possible prevention of cancer and heart diseases. A large number of naturally occurring molecules known for their antioxidative properties are phenolic compounds viz. acid-phenols of

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flavonoids and their esters [11].Moreover, living plants can accumulate heavy metals which are essential for their growth and development from water and soil[8].These metals include Mg, Fe, Mn, Zn, Cu, Co and Ni. The presence of heavy metals is one of the important criteria of the use of plant material in traditional medicines and herbal infusions [7]. In view of the reported medicinal properties of bamboo shoot, the present study was designed to evaluate the antioxidant and antimicrobial property of the shoots of four species of bamboo and to determine the phytochemical constituents and metal ions present in them and also to analyze of volatile compounds by using GCMS-MS.

2.MATERIALS AND METHODS

2.1.Chemicals and plant samples

The shoots of four bamboo species viz. *Bambusa balcooa, Bambusa bambos, Dendrocalamus hamiltoni and Bambusa vulgaris* which are locally known as "Bholukabaah, Kota baah, Keturabaah and Kekubaah" respectively, were collected from different places of Assam, India during spring season (March-May). The *B.balcooa* and *B.bambos* were collected from Kamrup district of Assam and *D.hamiltonii* was collected from Lakhimpur district of Assam. The *B.vulgaris* species was collected from Ribhoi district of Meghalaya. The shoots were cut into small pieces and air dried before extraction. The chemicals and reagents used in the study were of analytical or lab grade and were purchased from Sigma Aldrich and Qualigen, Himedia chemicals, SRL India, Merck India.

2.2. Extraction

About 25 g of dried bamboo shoot was used for extraction using Soxhlet apparatus. Solvents such as methanol, ethyl acetate, n-hexane, acetone and water were used for extraction and the process was continued for 48 hours at 40-60ºC. The extracts were concentrated in a rotary evaporator and stored in refrigerator for further analysis.

The percentage yield of the extract was calculated using the formula

Yield (%) = (weight of solventless extract / weight of initial sample taken) × 100

2.3. Proximate analysis of Bambusa balcooa ,Bambusa bambos, Dendrocalamus hamiltoni, Bambusa vulgaris shoot: The ash and moisture content of the samples of bamboo shoots were analyzed according to method no 44-15A and 08-01respectively as given in AACC (2000)[1].

2.4. Phytochemical Screening of the extract: Initial screening of the five different extracts were carried out to identify the presence or absence of phytochemical constituents [17] such as alkaloid, steroid, tannin terpenoid and saponin. The protein content of bamboo species was estimated by using Bradford method[2]. Alkaloid content was determined as per the method described by Omoruyi*et al*[16].

2.5. Determination of Total Phenolics

Total phenolic content of the extracts was determined spectrophotometrically by using Folin Ciocalteu reagent following the standard procedure. The absorbance was measured at 725 nm after 90 min. Gallic acid was used as a standard[9].

2.6. Determination of Total Flavonoids

The total flavonoid was also determined in a UV Visible spectrophotometer by using aluminium chloride .10 % aluminium chloride and 1 M potassium acetate were mixed with the extract. The absorbance was measured at 415 nm. Quercetin was used as standard [19].

2.7. Trace metal analysis

The metal content in the shoots was determined by using AAS Simadzu Model: AA 7000[12].The dried shoots were converted to ash by heating in a muffle furnace and extracted with nitric acid for the purpose.

2.8. Evaluation of Antioxidant activity

The antioxidant activity of the bamboo shoots was measured by performing the following standard activity tests. Five different extracts of each type of shoot were prepared using solvents of different polarity and the antioxidant activities of the extracts werecompared.

2.8.1. DPPH Scavenging activity: The DPPH scavenging activity of extracts was measured by UV-Visible (Shimadzu UV-1800) spectroscopic method by measuring the absorbance at 517 nm. Ascorbic acid (10-100µg/mL) was taken as the standard [18].

2.8.2. FRAP assay: The Ferric reducing activity was measured taking 0.2 mL of extract(1:20 dilution) mixed with phosphate buffer(pH6.6) and 2.5 mL of potassium ferricyanide. The mixture was incubated at 37℃ for 30 min followed by addition of 2.5 mL of 5%Trichloroacetic Acid(TCA) and 0.1% of ferric chloride. The

absorbance was measured at 700 nm after 20 min. Ascorbic acid (10-100µg/mL) was taken as standard [5].

2.8.3. Hydrogen peroxide radical scavenging activity: 0.2 mL of extract was mixed with 3.4 mL of 0.1 M phosphate buffer (pH 7.4) and 600 µL of 40 mM solution of hydrogen peroxide prepared in same buffer. The absorbance was measured at 230 nm after 40 min. BHA (10-100 µg/mL) was taken as the standard[15].

2.8.4. Linoleic acid autoxidation method: 0.2 mL of extract mixed with linoleic acid 0.13mL, 10 mLof 99.8% ethanol and 10 mL of 0.2 M sodium phosphate buffer of pH 7.4[18]. Then 0.2 mL of ammonium thiocyanate (30%) in water and 0.2 mL of ferric chloride solution (20 mM in 3.5 % HCl) were added to the mixture. After 5 mins, the absorbance was measured at 500 nm.BHT (10-100 µg/mL) was taken as the standard.

All the experiments were repeated for three times and the data obtained were analyzed using statistical method.

The antioxidant activity (%) was calculated using the following equation.

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of the control - Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

2.9. Identification of essential volatile components

The methanol and hexane extracts of all the four species were found to possess more antioxidant activity compared to other extracts.Therefore,GCMS-MS analysis was performed only with the methanol and hexane extracts after separating the fractions by column chromatography.

Column chromatography:

The column chromatography was performed by using 60-120 mesh silica gels and HPLC grade solvents to elute components from crude extracts. The flow rate was maintained at 1ml per min and the fractions collected were tested for antioxidant activity using DPPH assay. The fraction with maximum antioxidant activity was chosen for GCMS-MS analysis.

GCMS-MS analysis:

The GCMS-MS analysis was carried out in a Shimadzu model TQ8030 GCMS-MS instrument employing capillary column measuring 30 mm X 25µm with a film thickness, helium as carrier gas at a flow rate of 1mL/min. The oven temperature was from as 35 to 220 ºC and held for 20 min with incrimate rate 10ºC and the total run time was 43 minutes. The injector temperature was maintained at 250ºC with ion source temperature 230ºC at split ratio 1:20. The mass spectrums of the components were compared with the database of spectrum of NIST library[5].

2.10. Antimicrobial screening

The agar well diffusion method was used to test the antimicrobial activity of the extracts. Two different microorganisms *Staphylococcus aureus and Klebsiella pneumonia* were chosen for the study. These were maintained at nutrient agar media for 24 hours. The nutrient agar media was prepared and poured in sterilized Petri plates. After that the microorganisms were spread over the plates and100 µL of all the extracts were loaded in the well on the agar media. The plates were kept in an incubator for 24 hours at 37^{o}C. The zone of inhibition was measured in mm[10].

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

(**Table 1**) shows the percentage yield (w/w) of extracts obtained from the four *bambuseae* species. In case of all the four bamboo shoots, the higher percentage yield was found for methanol and hexane extracts. In (**Table 2**), the percentage of alkaloid, protein, ash content and moisture present in *bambuseae* species are presented. The preliminary phytochemical studies showed the presence of alkaloids, saponin, steroids, tannins and terpenoids in all the four species (**Table 3**). The ash content was highest in *B.vulgaris* and moisture content was highest in *B.balcooa*.

3.2. Total phenolic and flavonoid content

The phytochemical analysis revealed that the total phenolic content was highest in methanol extracts of all the species. Total phenolic content (TPC) in the extracts ranged from 29.26 to 57.71 mg/g (Fig 1). In plants the TPC is a good index of antioxidant property. The hexane and methanol extracts showed higher phenolic content than other extracts. The TPC was highest in the methanol extract and lowest in the acetone extract of all the four bamboo species (**Fig1a**).

The flavonoid content (TFC) ranged from 25.09 to 53.22 mg/g. TFC was found to be more in the aqueous extract of all the species (**Fig1b**).

3.3. Evaluation of antioxidant activity

The antioxidant activity of *bambuseae* species determined by using 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging capacity assay, ferric reducing ability of plasma (FRAP) assay, hydrogen peroxide scavenging activity assay and linoleic acid peroxidation assay are presented in Fig 2. The results were compared with BHA, BHT, ascorbic acid and quercetin which are efficient antioxidants found in food. The DPPH free radical scavenging activity of all the four bamboo species were found to be in the order ME>AE>HE>ACE>EAE (**Fig 2a**).The order of FRAP was found to be ME>AE>HE>ACE>EAE (**Fig 2b**). In the same way the scavenging activity in hydrogen peroxide assay for all the species were ME>AE>EAE>ACE>HE(**Fig2c**) and for linoleic acid peroxidation assay, the scavenging activity were found to be ME>AE>EAE>ACE>HE(**Fig 2d**). In case of DPPH assay the extracts of aqueous and methanol solvents showed more activity compared to other extracts for all the species. The ferric reducing antioxidant powers of the methanol extract of *B.balcooa*, *B.bambos*, *D.hamiltonii*, *B.vulgaris* species were 44.03, 49.09, 65.71 and 51.3 % respectively (**Fig2**).

3.4. Evaluation of antimicrobial activity

The antimicrobial activities of all the different extracts were shown in (**Table 4**). The level of inhibition was observed with two bacterial species (*S.aureus* and *K.pneumonia*). The fresh extract of *B.vulgaris* showed maximum zone of inhibition. The maximum activity was observed against *S.aureus*,agram positive bacteria. However, very less activity was observed in hexane extract against *K. pneumonia*. The gram positive bacteria (*S.aureus*) were more sensitive than gram negative bacteria (*K.pneumoniae*).Significant inhibition was recorded with methanol and aqueous extracts. It is important to note that the methanol extracts can inhibit the growth in *S.aureus* which is a biogenic amine producer and is major cause of cutaneous infections, furunculosis, impetigo, arthritis, and toxinoses, such as food poisoning, septic shock, scalded skin syndrome and toxic shock syndrome [13].

3.5. Trace metal analysis

The results of estimationof trace metals in the shoots are shown in (**Table 5**). The concentrations of Mg and Ca in the shoots were found to be more compared to other metals. No heavy metal concentration beyond permissible level was found in the shoots. The metals detected in the extracts have well-known biological activity. The concentration of those essential metals has been shown in (Table 5).

3.6. GC-MS analysis

The volatile compounds of bamboo shoots were identified by using GCMS-MS. The identified volatile compounds of *B.balcooa*, *B.bambos*, *D.hamiltonii* and *B.vulgaris* were presented in (**Table 6&7**). The volatile compounds identified in methanolic extracts of bamboo species were methyl stearate, methylparaben, L.proline, adenine, ethylparaben, β.-D-Glucopyranoside, hexadecanoic acid, methyl ester, methylparaben, 9,12-octadecanoic acid; methyl ester, methyl stearate . The identified major compounds in n-Hexane extract of bamboo species were Vanillin, n-Hexadecanoic acid, methyl ester, 2-Propenoic acid, methyl ester, methyl stearate, methylparaben. The results were comparable to finding of El-Ghorab *et al* and Mulyono, N et al[4,10]. The GCMS study also established the presence of some major compounds in the extracts of bamboo species such as methyl esters of myristic acid, ascorbic acid, linoleic acid, stearic acid etc. which are responsible for the medicinal properties of the extracts.

Table 1: Percentage yield of extracts (w/w)

Species	Methanol Extracts	Ethyl acetate extracts	Aqueous extracts	Hexane extracts	Acetone extracts
B.balcooa	32.50	27.52	22.68	10.69	14.96
B.bambos	45.43	19.54	29.9	11.52	15.38
D.hamiltonii	26.48	10.38	28.27	14.91	6.48
D.hamiltonii	19.12	13.12	19.51	13.5	8.54

Table 2: Proximate analysis of all the four Bamboo species

Parameters	*B.balcooa*	*B.bambos*	*D.hamiltonii*	*B.vulgaris*
Moisture %	71.8	78.02	77.61	82.73
Ash %	2.1	2.78	2.06	1.77
Alkaloid %	5.19	7.22	5.32	4.17
Protein %	28.52	33.54	33.73	22.30

Table 3: Screening of phytochemical constituentsof the extracts of Bamboo shoots

Phytochemical constituents	*B.balcooa*	*B.bambos*	*D.hamiltonii*	*B.vulgaris*
Alkaloids	++	+++	++	++
Steriods	+++	++	+++	+
Tannins	++	+	++	++
Terpenoids	++	+	++	++
Saponins	++	++	++	+

+:Presence of phytochemical constituents;- :Absence of phytochemical constituents

Table 4: Zone of Inhibition of Bamboo Species in Diameter (mm)

Staphylococcus aureus					***klebsiella pneumonia***			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Methanol	9	17	16	11	22	28	18	24
Ethyl acetate	11	13	21	12	10	12	17	19
Aqueous	18	22	26	17	17	19	22	20
Acetone	12	10	14	19	5	7	11	16
Hexane	17	8	9	16	No zone	No zone	5	4

(1) *B.balcooa*; (2)*B.bambos*; (3)*D.hamiltonii*;(4)*B.vulgaris*

Table 5: Metals (ppm) present In Bamboo species measured using AAS

Species	Fe	Cu	Mg	Ca	Zn	Mn
B.balcooa	11.15	0.345	180.7	155	131.75	41.09
B.bambos	30.85	1.38	180.3	375.1	176.4	99.9
D.hamiltonii	41.10	0.263	80.5	98.6	4.751	0.878
B.vulgaris	28.95	0.210	75.9	90.12	0.7959	0.925

Table 6: Chemical compounds with its peak area % of the fresh bamboo shoots in methanol extracts identified by GCMS-MS

Compounds name	*B.balcooa*	*B.bambos*	*D.hamiltonii*	*B.vulgaris*
Hexadecanoicacid, methyl ester	-	32.73	8.81	3.81
Methylparaben	27.48	0.65	5.00	-
9,12,15-Octadecatrienoic acid, methyl ester	-	0.28	4.60	-
L-Proline, 5-oxo-, methyl ester	0.15	-	0.65	-
Methyl stearate	0.28	0.26	-	-
Adenine	0.17	-	-	0.60
2-Propenoic acid, 3-(4-hydroxy methyl ester)	-	-	1.43	0.65

Table 7:Chemical compounds with its peak area % of the fresh bamboo shoots in n-hexane extracts identified by GCMS-MS

Compounds name	***B.balcooa***	***B.bambos***	***D.hamiltonii***	***B.vulgaris***
Hexadecanoicacid, methyl ester	51.87	30.17	-	23.72
2-Propenoic acid ;tridecyl ester	0.57	-	18.27	-
7-Tetradecanal	5.56	-	3.19	3.28
Methylparaben	-	1.66	1.07	2.49
Methyl stearate	-	5.41	0.28	9.30
9,12-Octadecadienoic acid (Z,Z)-	7.24	-	-	3.71

Table 8: Phytochemical compounds from GCMS-MS analysis, their nature and their biological activities with molecular weight of methanol and n-hexane extracts of all the four bamboo species

Compounds name	**Molecular Weight**	**Compounds Nature**	**Activity**
Hexadecanoic acid, methyl ester	256	Palmitic acid	Antioxidant, Antiinflammatory
2-Propenoic acid,tridecyl ester	141	Fatty acid ester	Antibacterial
9,12,15-Octadecatrienoic acid, methyl ester	292	Linoleic acid	Antiinflammatory, Cancer preventive
Methylparaben	152.15	Methyl ester	Antibacterial, Anti-fungal
Methyl stearate	298.53	Stearicacid	Antimicrobial
L-Proline, 5-oxo-, methyl ester	115.13	Amino acid	Antioxidant
Adenine	135.5	Nucleobase	Cancer preventive
7-Tetradecanal	253.5	Alkane	Antimicrobial

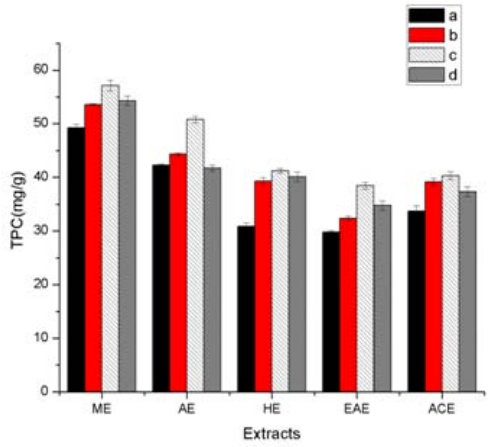

Fig 1a: Comparison of total phenolic contentof (a)*B.balcooa,*(b)*B.bambos,*(c)*D.hamiltonii* and(d)*B.vulgaris* shoots(ME-Methanol extract, AE- Aqueous extract, HE- Hexane extract, EAE-Ethylacetate extract, ACE-Acetone extract)

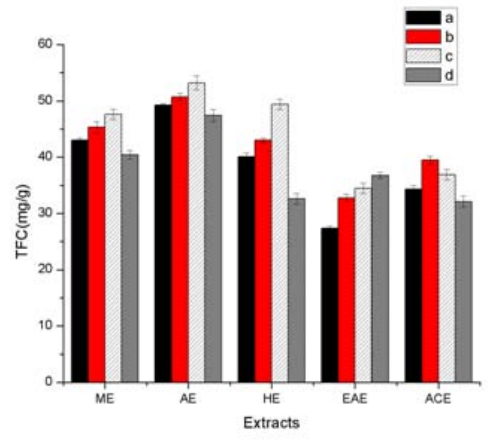

Fig 1b: Comparison of total flavonoid content of (a)*B.balcooa,*(b)*B.bambos,*(c)*D.hamiltonii* and(d)*B.vulgaris* shoots(ME-Methanol extract, AE- Aqueous extract, HE- Hexane extract, EAE-Ethylacetate extract, ACE-Acetone extract)

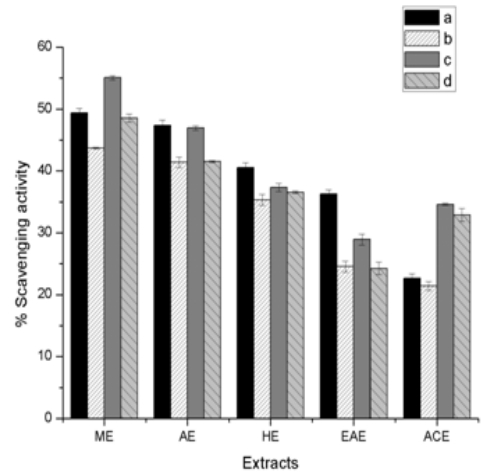

Fig 2a: DPPH free radical scavenging activity of different extracts (ME-Methanol extract, AE- Aqueous extract, HE- Hexane extract, EAE-Ethylacetate extract, ACE-Acetone extract) of *B.balcooa, B.bambos, D.hamiltonii* and *B.vulgaris* shoots

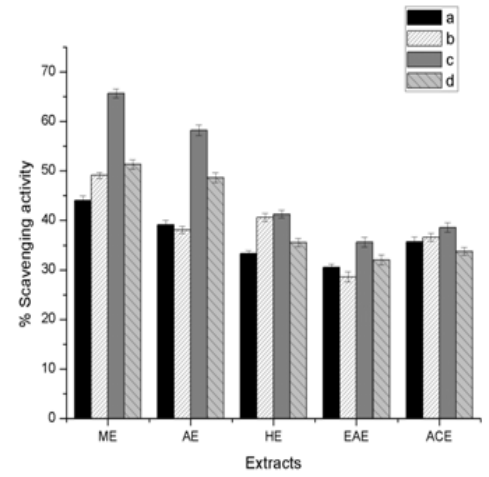

Fig 2b: Ferric reducing ability of plasma assay of different extracts (ME-Methanol extract, AE- Aqueous extract, HE- Hexane extract, EAE-Ethylacetate extract, ACE-Acetone extract) of *B.balcooa, B.bambos, D.hamiltonii* and *B.vulgaris* shoots

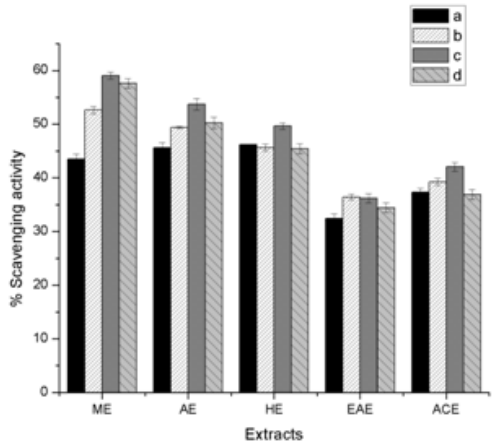

Fig 2c: Hydrogen peroxide scavenging activity assay of different extracts (ME-Methanol extract, AE- Aqueous extract, HE- Hexane extract, EAE-Ethylacetate extract, ACE-Acetone extract) of *B.balcooa, B.bambos, D.hamiltonii* and *B.vulgaris* shoots

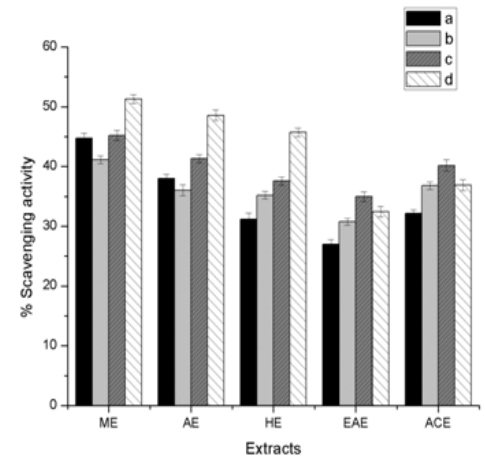

Fig 2d: Linoleic acid peroxidation assay of different extracts (ME-Methanol extract, AE- Aqueous extract, HE- Hexane extract, EAE-Ethylacetate extract, ACE-Acetone extract) of *B.balcooa, B.bambos, D.hamiltonii* and *B.vulgaris* shoots

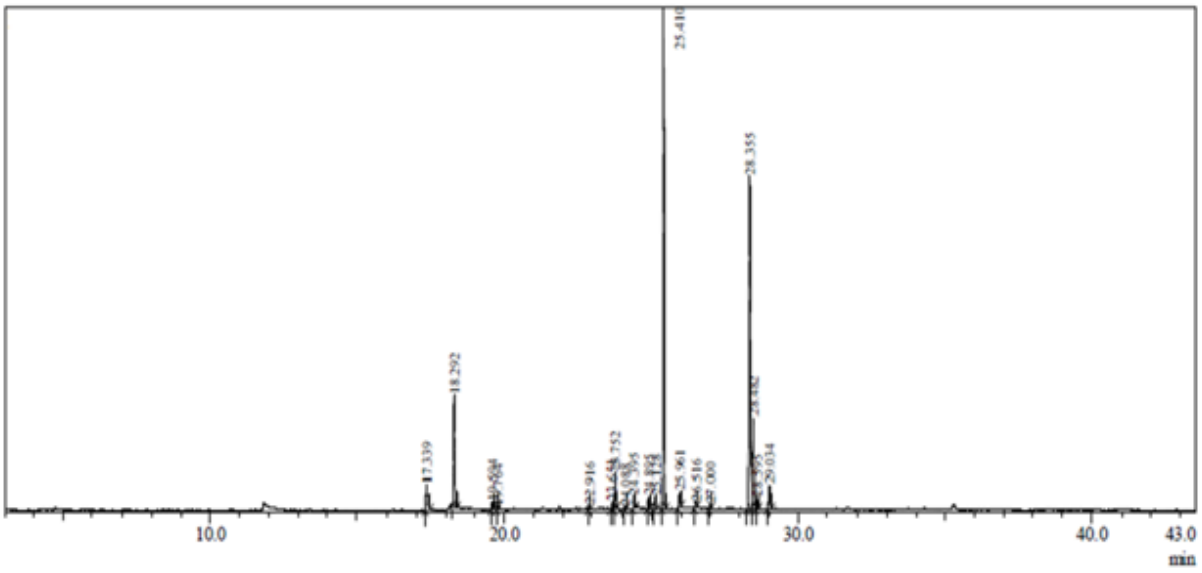

Fig 3a: Chemical composition of fresh bamboo shoots *B.balcooa* in methanol extracts by GCMS-MS.

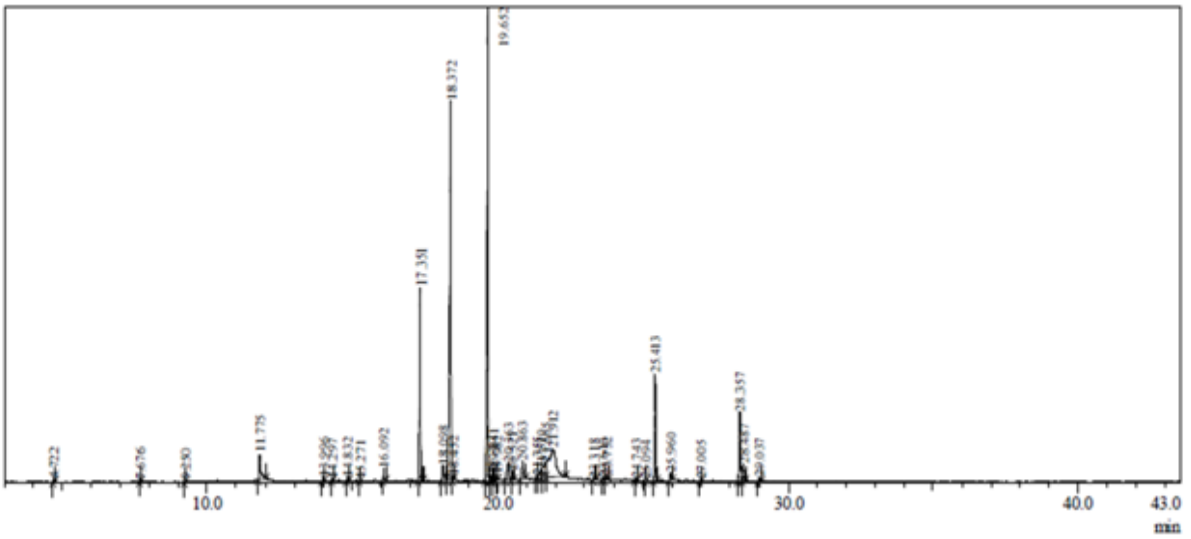

Fig 3b:Chemical composition of fresh bamboo shoots *B. bambos* in methanol extracts by GCMS-MS.

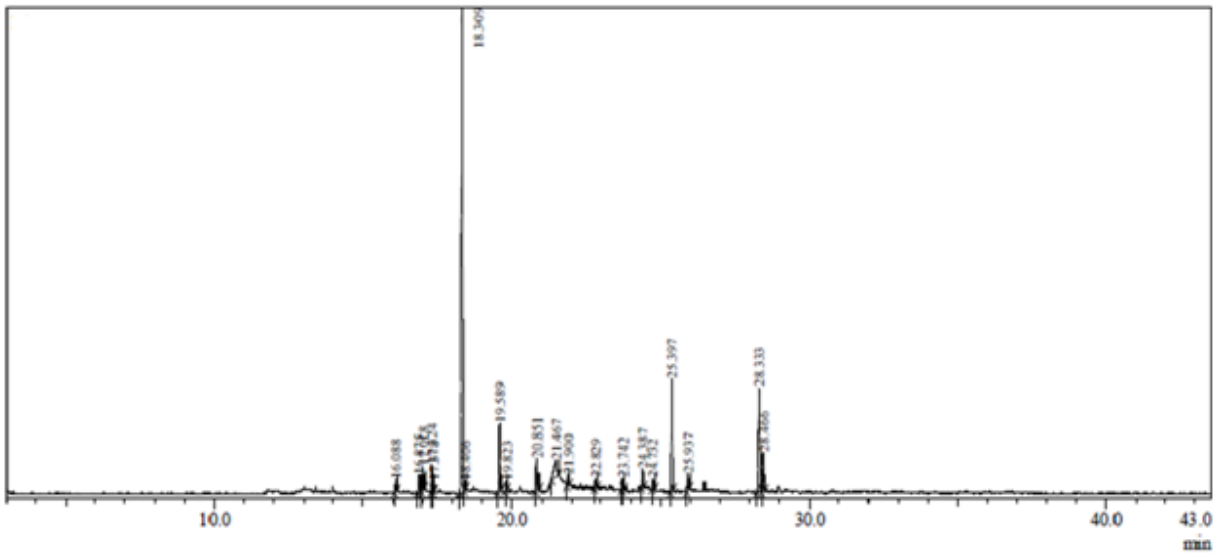

Fig 3c: Chemical composition of fresh bamboo shoots *D. hamiltonii* in methanol extracts by GCMS-MS

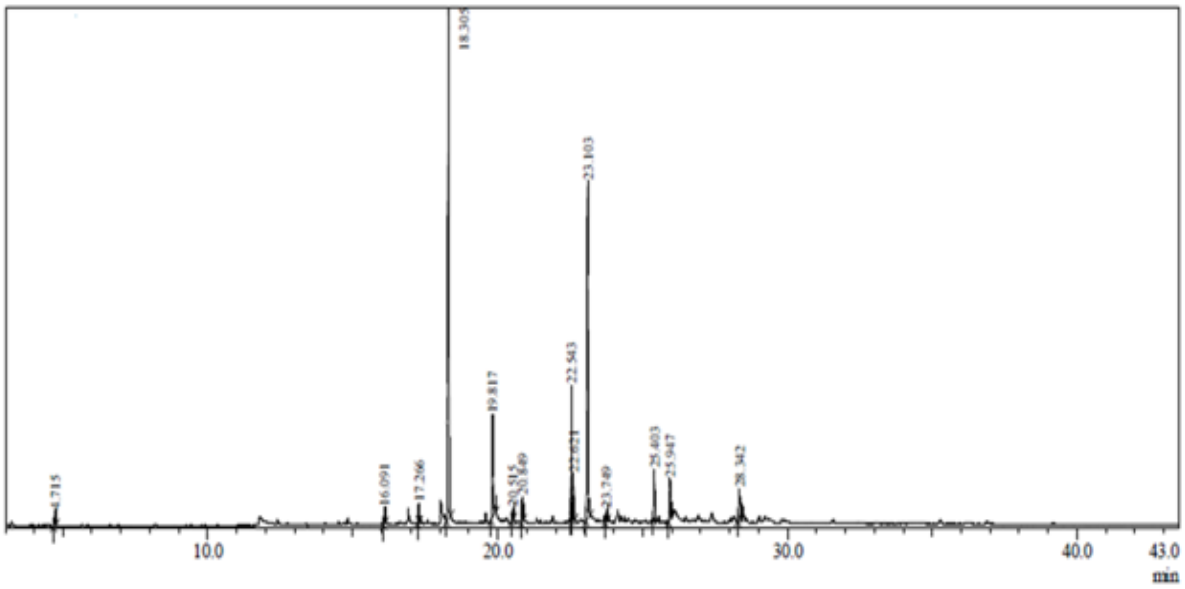

Fig 3d: Chemical composition of fresh bamboo shoots *B.vulgaris* in methanol extracts by GCMS-MS

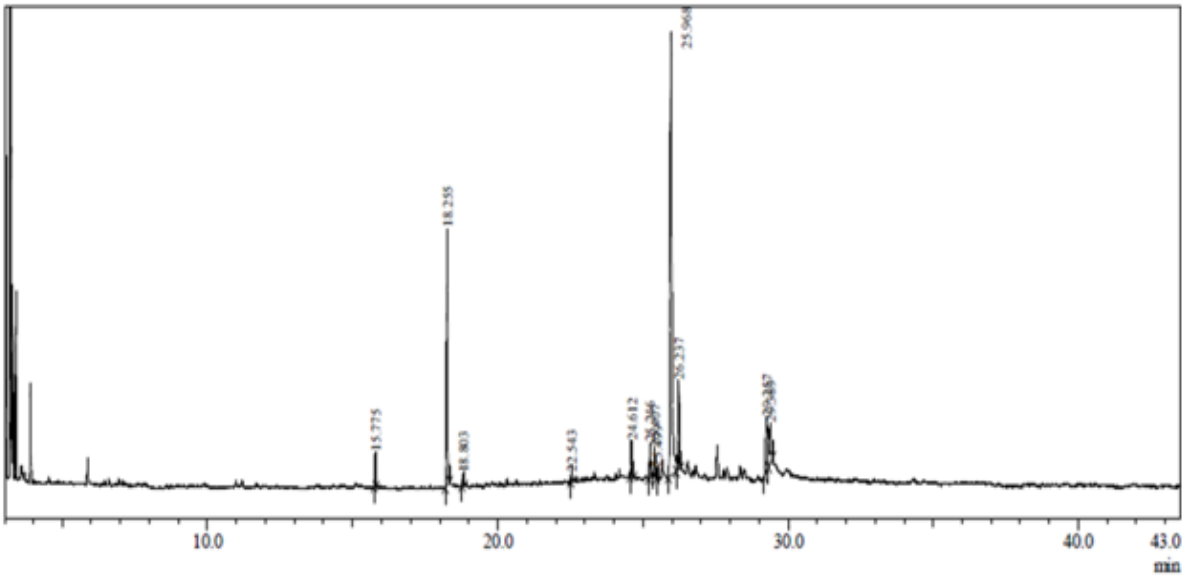

Fig 4a: Chemical composition of fresh bamboo shoots *B.balcooa* in n-hexane extracts by GCMS-MS

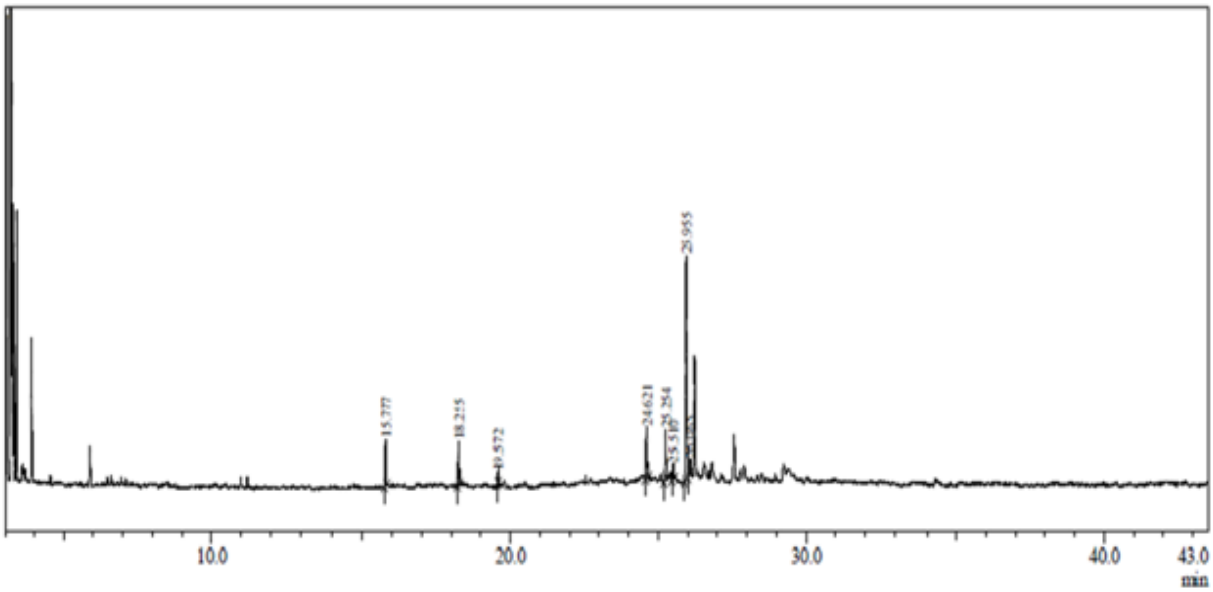

Fig 4b: Chemical composition of fresh bamboo shoots *B. bambos* in n-hexane extracts by GCMS-MS

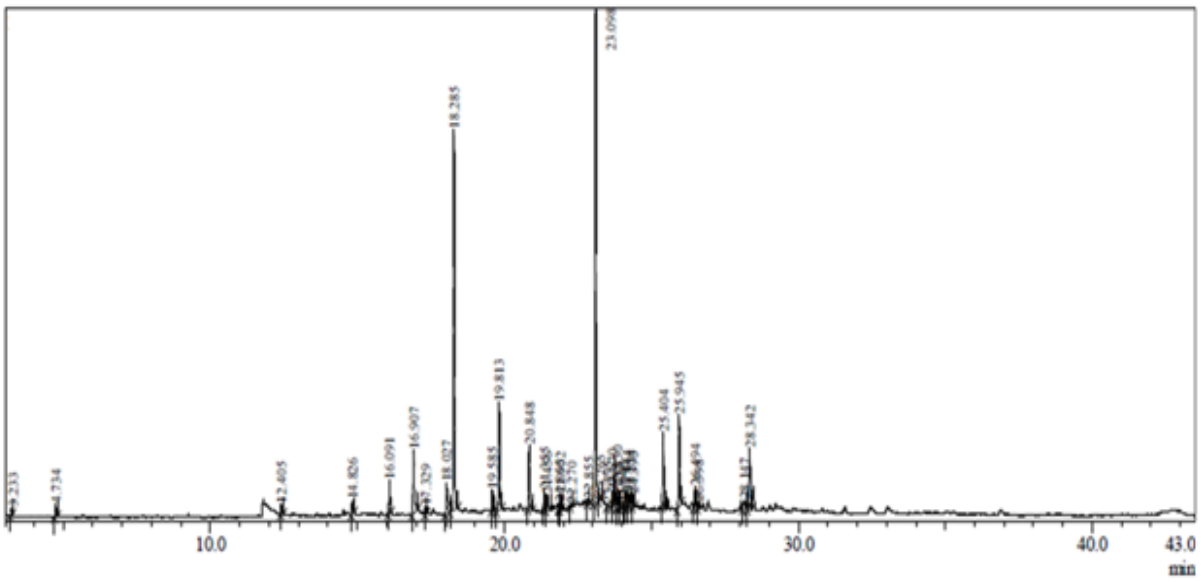

Fig 4c: Chemical composition of fresh bamboo shoots *D. hamiltonii* in n-hexane extracts by GCMS-MS

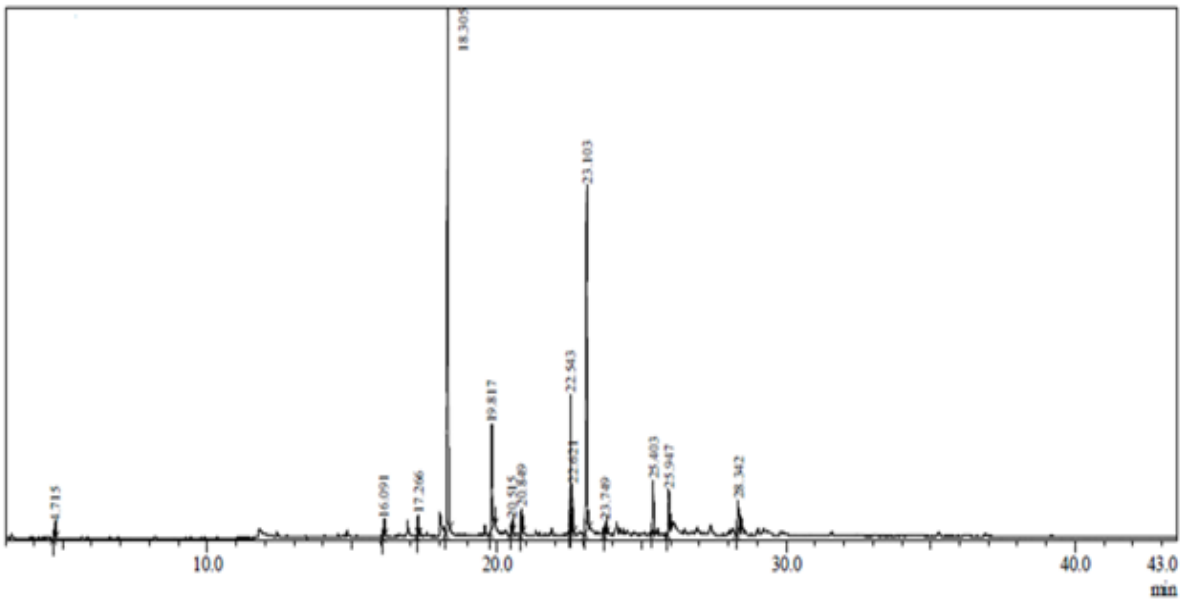

Fig 4d: Chemical composition of fresh bamboo shoots *B.vulgaris* in n-hexane extracts by GCMS-MS

4. CONCLUSION

The volatile compounds of bamboo shoots were identified by GC-MS. The antioxidant compounds like Methyl paraben, methyl stearate, Vanillin, L.proline methyl ester, etc. were identified in the n-hexane and methanol extracts of the shoots. Methylparaben is also known for antifungal effect. L-proline methylester in the extract probably arose from L-proline dehydrogenase enzyme present in the young shoots. The result of the present study showed that the extracts of all the four species of bamboo shootare rich in phenolic compounds and flavonoids which are responsible for the anti-oxidant activity shown by the extracts. The high radical scavenging property of bamboo shootsmay be due to hydroxyl groups present in the phenolic compounds. It is established that phenols are responsible for the antioxidant activity shown by many plants. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. Determination of antimicrobial activity of the species using agar well diffusion assay revealed that bamboo shoots possessed significant antimicrobial properties against both gram positive and gram negative bacteria. Thus the bamboo shoots may have potential as natural antioxidant and antimicrobial agent for use in food preservation. The results obtained may be considered sufficient to initiate further studies for the isolation and identification of the active principles and to evaluate possible synergistic

effect among the components for their antioxidant and antimicrobial activity.

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