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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Determination of bioactive components of *Barleria cuspidata* and *Barleria buxifolia* by Gas Chromatography-Mass Spectrometry analysis

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**ABSTRACT**

*The present study was carried out to identify the phytoconstituents present in the methanol extract of whole plant of Barleria buxifolia Linn and Barleria cuspidata Heyne ex Nees by GC-MS analysis. The plants of Barleria buxifolia and Barleria cuspidata after air dried were subjected to sequential extraction with chloroform and methanol by soxhlet extraction apparatus. Then the methanol extract of both the plants was further subjected to gas chromatography - mass spectrometry analysis to determine different biological active components. From crude methanol extracts of Barleria buxifolia and Barleria cuspidata the qualitative determination of different biological active compounds using gas chromatography - mass spectrometry revealed the presence of different types of high and low molecular weight chemical compound with different quantities, such as flavonoids, tannins, alkaloids, steroids and fatty acids from each plant extract. These chemical compounds are considered biologically and pharmacologically important.The study results will make a way for the isolation of active constituents and to examine their pharmacological aspects.*

**Keywords:** *Barleria buxifolia* and *Barleria cuspidata*, Extraction and GC-MS.

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## INTRODUCTION

Now a day's herbal medicines are gaining more importance because of their safety,easy availability & low cost [1].The medicinal activities of these plants is mainly due to the presence of bioactive phytochemical constituents such as alkaloids, steroids, tannins, proteins and amino acids, flavonoids, saponins, essential oils etc., that produce certain physiological actions [2].Nevertheless, people deter from using herbal medicines because of the difference in the concentration of active constituents in herbal drugs. The various factors that bring about inconsistency in the percentage of active constituents of herbal drugs are genetics, climatic conditions,bacteria& viral infections etc., [3].

Standardization and validation parameters are implemented to ensure that active constituents, moisture content, inorganic impurities or heavy metals, microbial limits, pesticides etc within the prescribed limits. Separation techniques viz high-performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), gas chromatography mass spectroscopy (GC-MS), Liquid chromatography mass spectrometry (LCMS) and capillary electrophoresis provide a new dimension for herbal drug analysis [4]. GC-MS chromatogram provides data regarding the retention time, peak area and mass spectra of phytoconstituents present in plant extract. GC-MS analysis revealed the existence of major phytoconstituents such as esters, fatty acids, terpenes, phenols, sterols etc in several plant extracts [5 & 6].

Genus *Barleria* belongs to the family Acanthaceae. Whole-plant extract *Barleria* contains a number of active compounds such as alkaloids, terpenes, flavonoids, glycosides, lignins, and phenolics, which have shown potent therapeutic activities against several diseases [7, 8, 9 & 10]. Barleria also shows various pharmacological effects such as antimicrobial, anti-helminthics, anti-fertility, antioxidant, anti-diabetic, anti-arthritic, hepatoprotective, diuretic, cytoprotective, anti-diarrheal, analgesic, anti-leukemic, anti-inflammatory, and hypoglycaemic properties without any toxic effects [11 & 12].The current examination

were attempted to standardize the entire plants of *Barleriabuxifolia* and *Barleria cuspidate* by using GC-MS studies.

## MATERIAL AND METHODS

### Plant collection and authentication

Fresh whole plants of *Barleria buxifolia* Linnand *Barleria cuspidata*Heyne ex Nees (Acanthaceae) were pull together from Chittoor districts in the areas of Tirumala Hills and Tirupathi surroundings and authentified by Dr. K. MadavaChetty, Professor, Department of Botany, Sri Venkateswara University, Tirupathi. Andhra Pradesh, India. Voucher specimens (No: BB- 1418 and BC- 1419 respectively) for these plants has been kept in the P. Rami Reddy Memorial College of Pharmacy, Kadapa, Andhra Pradesh, India.

### Plant material

The gathered entire plants of *Barleriabuxifolia* and *Barleria cuspidata* were separately washed with running water, cut into little pieces and shade dried at room temperature to maintain a strategic distance from loss of phytoconstituents of plant. The absolute shade dried materials beat for powder and sieved up to 80 cross sections. By then it was homogenized to fine powder and set aside in air tight compartment for extra considers [13].

### Preparation of plant extracts

Whole plants powder of the *Barleriabuxifolia* and*Barleria cuspidata* were individually extracted successively with two different solvents like chloroform and methanol in a Soxhlet apparatus in batches of 500 gm each. The overabundance solvent was expelled from extract utilizing a rotary vacuum evaporator and later on concentrated on a water bath. The rate yield of the extract was determined.

### Instrumentation

GC-MS investigation of the methanol concentrate of *Barleria buxifolia* and *Barleria cuspidata* was performed utilizing Equipment Scion 436-GC Bruker auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) outfitted with a segment BR-5MS (5% diphenyl/95% dimethyl poly siloxane), 30m x 0.25mm ID x 0.25µm df. For GC-MS recognition, an electron ionization framework was drive in electron impact methodology with ionization energy of 70eV. Helium gas (99.999%) was utilized as carrier gas at a constant stream pace of 1ml/min.

### Extraction of plant material and GC-MS analysis

Around 10 gm of the entire plant powder was added to 100 ml of ethanol. It was incubated for the time being and filtered through filter paper. Sodium sulphate was likewise utilized during filtration to eliminate the sediments and traces of water in the filter paper. The filtrate was concentrated. 2µl of the example arrangement was utilized in GC-MS for examination. The injector temperature was kept up at 280°C, the ion-source temperature was 250°C and the oven temperature was modified for 110°C (isothermal for 3.50min). Mass spectra were taken at 70eV; a scan time period of 0.5 seconds and parts from 45-500Da. The solvent postponement was 0 to 2min and the complete GC-MS running time was 40 to 50min. The relative percentage amount of every segment was determined by contrasting its average peak area to the total areas. Estimation of peak areas and information preparing were carried out by Turbo-Mass OCPTVS-Demo SPL programming.

### Identification of compounds

Interpretation was done by GC-MS using the database from National Institute of Standards and Technology (NIST) library which has more than 82,000 patterns. Comparison of the spectrum of unknown components with the spectrum of the known components stored in the NIST library was carried out. The retention time, name of the compound, molecular formula, molecular weight and peak area of the phytoconstituents are given in Table 1 & 3. The nature of compound, molecular structure and activities of the phytoconstituents are given in Table 2 & 4.

## RESULTS AND DISCUSSION

### GC-MS analysis of B*arleria cuspidata*

GC-MS analysis of the methanol extract of *Barleria cuspidata* revealed the presence of 13 phytocompounds as shown in the Figure 1. The compounds with their retention time, molecularformula, molecular weight, peak area % are presented in Table1 and their structure, nature and activity of the compounds were listed in Table2

The vast majority of the compounds identified by GC-MS analysis of MEBC are known to possess pharmacological property compounds like pentadecanoic acid, eicosanoic acid, 9 oxononanoic acid, heptafluorobutyric acid, N-tridecayl ester, octa decaine 3-ethyl-5-(2-ethyl butyl)- have antioxidant activity and the compounds like sulphurous acid, 2-propyl tetra decyl ester, sulphurous acid, penta decyl 2- propyl ester possess anticancer activity. Aside from the compound referenced above there are the reports of different compounds exhibiting exercises like antimicrobial (carbamic acid, (3,4,4-trimethyl-

1,2-dioxetan-3-yl) methyl ester, 3,7-diacetamido-7H-S-triazolo[5,1-C]-S-triazole, 1,3 dioxolan-2-one,3-methyl-3-(4,8-dime- thylnona-3,7-dienyl) – 4methylene).

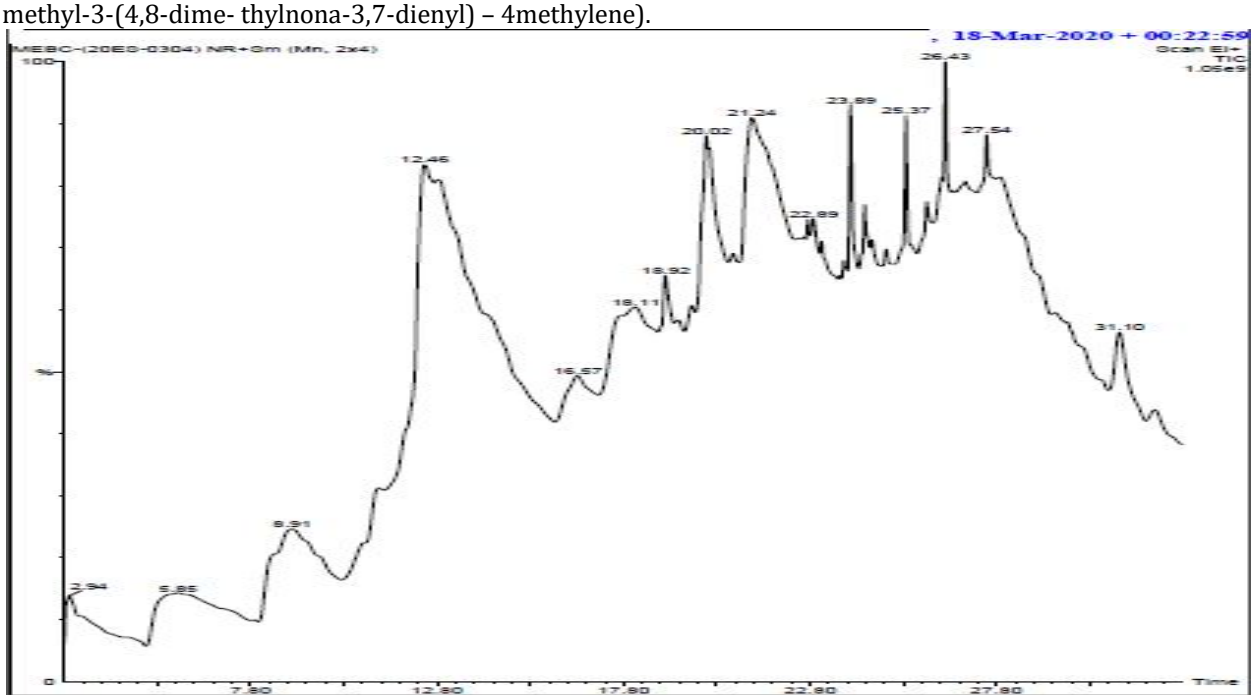
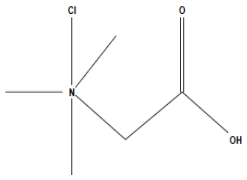
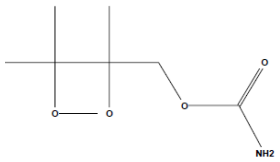
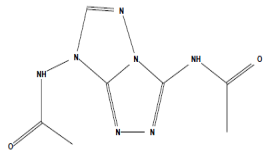
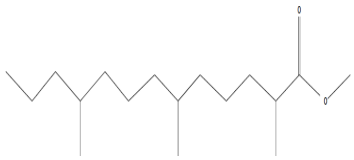
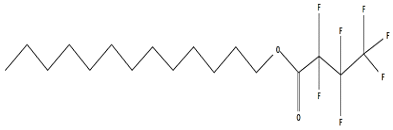
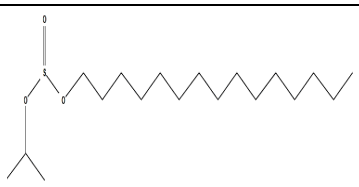


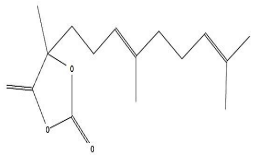
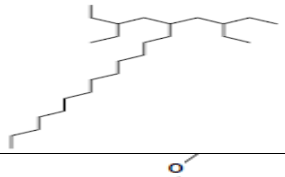
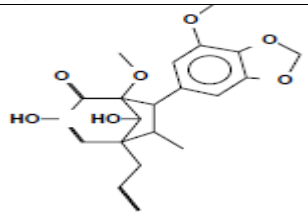
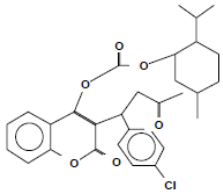

**Figure 1:Chromatogram of *Barleria cuspidata***

**Table 1: Phytoconstituents identified in MEBC**

| S. No. | RT | Name of the compound | Mol.For. | Mol.Wt. | Peak area% |
|---|---|---|---|---|---|
| 1. | 12.517 | Betaine hydrochloride | $C_5H_{12}O_2NCl$ | 153 | 21.283 |
| 2. | 16.504 | Carbamic acid, (3,4,4-trimethyl-1,2-dioxetan-3-yl) methyl ester | $C_7H_{13}O_4N$ | 175 | 2.640 |
| 3. | 18.190 | 3,7-diacetamido-7H-S-Triazolo[5,1-C]-S-Triazole | $C_7H_9O_2N_7$ | 223 | 8.277 |
| 4. | 18.905 | Methyl 2,6,10-trimethyl tridecanoate | $C_{17}H_{34}O_2$ | 270 | 3.462 |
| 5. | 20.731 | Eicosanoic acid | $C_{20}H_{40}O_2$ | 312 | 2.363 |
| 6. | 21.206 | 9 Oxononanoic acid | $C_9H_{16}O_3$ | 172 | 17.719 |
| 7. | 22.751 | Heptafluorobutyric acid, N-tridecayl ester | $C_{17}H_{27}O_2F_7$ | 396 | 1.677 |
| 8. | 22.876 | Bicyclo[3.2.1] oct-3-en-2one, 3,8 dihydroxy 1-methoxy-7[7-methoxy-1,3-benzodioxole-5-yl]-6-methyl-5 | $C_{21}H_{24}O_7$ | 388 | 1.353 |
| 9. | 23.867 | 1,3 dioxolan-2-one,3-methyl-3-(4,8-dimethylnona-3,7-dienyl) – 4methylene | $C_{16}H_{24}O_3$ | 264 | 1.266 |
| 10. | 25.918 | Sulphurous acid, penta decyl 2- propyl ester | $C_{18}H_{38}O_3S$ | 334 | 1.944 |
| 11. | 26.423 | Octa decaine,3-ethyl-5-(2-ethyl butyl)- | $C_{26}H_{54}$ | 366 | 4.196 |
| 12. | 26.963 | 2-isopropyl-5-methyl cyclohexyl 3-[1-(4-chlorophenyl)-3oxobutyl]-coumarin-4-yl-carbonate | $C_{30}H_{33}O_6Cl$ | 524 | 4.277 |
| 13. | 27.538 | Pentadecanoic acid | $C_{15}H_{30}O_2$ | 242 | 16.208 |

**Table 2: Activities of phytoconstituents identified in MEBC**

| S. No. | Name of the compound | Structure | Activity |
|---|---|---|---|
| 1. | Betaine hydrochloride |  | Gastric acidifier [14] |
| 2. | Carbamic acid, (3,4,4-trimethyl-1,2-dioxetan-3-yl) methyl ester |  | Antimicrobial activity [15] |
| 3. | 3,7-diacetamido-7H-S-Triazolo[5,1-C]-S-Triazole |  | Antibacterial, antifungal [16] |
| 4. | Methyl 2,6,10-trimethyl tridecanoate |  | No activity |
| 5. | Pentadecanoic acid |  | Antioxidant activity, anticancer [17] |
| 6. | Eicosanoic acid |  | Antioxidant activity [17] |
| 7. | 9 Oxononanoic acid |  | Antioxidant activities [17] |
| 8. | Heptafluorobutyric acid, N-tridecayl ester |  | Antioxidant activity [18] |
| 9. | Sulphurous acid, penta decyl 2- propyl ester |  | Anticancer [19] |

| | | | |
|---|---|---|---|
| 10. | 1,3 dioxolan-2-one,3-methyl-3-(4,8-dimethylnona-3,7-dienyl) – 4methylene |  | Antibacterial [20] |
| 11. | Octa decaine,3-ethyl-5-(2-ethyl butyl)- |  | Antioxidant [17] |
| 12. | Bicyclo[3.2.1] oct-3-en-2one, 3,8 dihydroxy 1-methoxy-7[7-methoxy-1,3-benzodioxole-5-yl]-6-methyl-5 |  | Diuretic |
| 13. | 2-isopropyl-5-methyl cyclohexyl 3-[1-(4-chlorophenyl)-3oxobutyl]-coumarin-4-yl-carbonate |  | Antioxidant, antimicrobial and anti-inflammatory activity [20] |

**GC-MS analysis of *Barleria buxifolia***

GC-MS analysis of the methanol extract of *Barleria buxifolia* revealed the presence of 6 phytocompounds as shown in the Figure 2. The compounds with their retention time, molecularformula, mol. wt, peak area % are presented in Table3 and their structure, nature and activity of the compounds were listed in Table4.

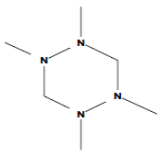
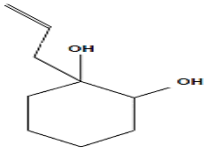
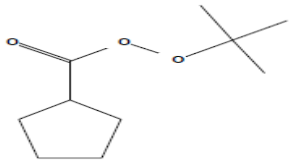
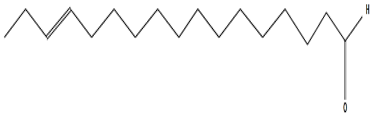
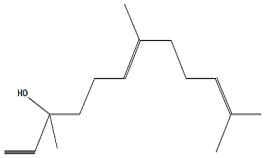
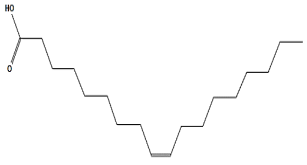
The vast majority of the compounds identified by GC-MS analysis of MEBB are known to possess pharmacological property compounds like 1,2,4,5-tetrazine, hexa hydro-1,2,4,5-tetra methyl, T-butyl cyclopentane peroxy carboxylate, 14-heptadecenal, oleic acid with anticancer activity.


**Figure 2: Chromatogram of *Barleria buxifolia***

**Table 3: Phytoconstituents identified in MEBB**

| S. No. | RT | Name of the compound | Mol.Formula | Mol.Wt. | Peak area% |
|---|---|---|---|---|---|
| 1. | 15.158 | 1,2,4,5-Tetrazine, hexa hydro-1,2,4,5-tetra methyl | $C_6H_{16}N_4$ | 144 | - |
| 2. | 18.715 | 1-allyl-cyclohexane-1,2-diol | $C_7H_{13}O_4N$ | 156 | 2.668 |
| 3. | 21.171 | T-butyl cyclopentane peroxy carboxy- late | $C_7H_9O_2N_7$ | 186 | 3.992 |
| 4. | 21.156 | 14-heptadecenal | $C_{17}H_{34}O_2$ | 252 | 23.012 |
| 5. | 24.172 | 6,10-dodecadien-1-yn-3-ol,3,7,11-trimethyl | $C_{15}H_{30}O_2$ | 220 | 3.433 |
| 6. | 27.363 | Oleic acid | $C_{20}H_{40}O_2$ | 282 | 66.895 |

**Table 4: Activities of phytoconstituents identified in MEBB**

| S. No. | Name of the compound | Structure | Activity |
|---|---|---|---|
| 1. | 1,2,4,5-Tetrazine, hexa hydro-1,2,4,5-tetra methyl |  | Anticancer[21] |
| 2. | 1-allyl-cyclohexane-1,2-diol |  | Analgesic[22] |
| 3. | T-butyl cyclopentane peroxy carboxylate |  | Anticancer[23] |
| 4. | 14-heptadecenal |  | Anticancer<br>Antibacterial<br>Antioxidant |
| 5. | 6,10-dodecadien-1-yn-3-ol,3,7,11-trimethyl |  | --- |
| 6 | Oleic acid |  | Anticancer [24] |

## CONCLUSION

GC-MS profiling of the methanol extract of *Barleria cuspidata*and *Barleria buxifolia* was done to determine their qualitative and quantitative parameters. Based on the results this study clearly indicated the presence of various active principles with several pharmacological activities in MEBC and MEBB. So, this can be helpful for the isolation of phytoconstituents present in and for examine their respective pharmacological studies.

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**CONFLICT OF INTEREST**
The authors declared that there was no conflict of interest in this research.

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