Phytoconstituents Screening, TLC, And GC-MS Analysis Of Barleria Cristata Linn. Leaves Methanolic Extract

Harini V1, Kumar P.R2*, Thirumal M3

1, 2*, 3*Department of Pharmacognosy, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu Dt., Tamil Nadu -603 203

*Corresponding Author: Dr Kumar P.R

Abstract
Barleria cristata Linn is an ornamental plant that grows on the sides of roads in India, China, and other countries. This plant belongs to the family Acanthaceae. In India, it is used as herbal medicine and is called Kala Bansa. It has an ancient legacy of application in folk medicine, particularly for the treatment of tuberculosis and snake bites. The purpose of the present study was a preliminary phytochemical analysis, Thin-Layer Chromatography (TLC) analysis and Gas chromatography-mass spectroscopy (GCMS) analysis of the methanol extract of Barleria cristata Linn leaves. The preliminary phytochemical screening finding revealed compounds like Alkaloids, Glycosides, Flavonoids, Carbohydrates, Proteins, and amino acids, and Reducing sugars. Thin-layer chromatography (TLC) of the methanol extract was executed for 5 essential phytoconstituents Alkaloids, Flavonoids, Saponins, Glycosides, and Triterpenoids. The leaf extract of this plant was analyzed using GCMS. This report revealed the 15 compounds. The bioactive chemicals were found by comparing the retention time and peak area with the literature. Barleria cristata possess various medicinal properties, so more research must be done on its traditional claims.

Keywords: Barleria cristata Linn., Phytochemicals, TLC Profiling, Retention factor, and GC-MS.

INTRODUCTION
Humans have looked to nature for cures for various illnesses. Similar to how animals develop their therapeutic abilities, plants also started out on an innate level, since the beginning of time. (1) Numerous active components found in medicinal plants are known to exist in large quantities and may one day be used to create new therapeutics. (2,3) People have probably been interested in medicinal plants from prehistoric times. It is widely acknowledged that the study of medicinal plants is among the earliest medical specialties developed in China, Greece, Egypt, and India (4). According to investigations, plants provide a source of potent chemotherapeutic agents that are non-phytotoxic, more systemic, and rapidly biodegradable. (5) More than 1000 species were employed in India’s traditional medical systems, including Ayurveda, Siddha, and Unani, which have endured for more than four centuries predominantly by using plant-based medicines (6). According to books from antiquity, several plants are used as medicines, including the Rigveda (4500–1600 B.C.) and Athravana Veda. Over 700 different herbs were mentioned in the Charaka Samhita and Sushruta Samhita, the two works on Ayurvedic medicine. (7) Medicinal plants have been extensively used to counteract allopathic health effects and provide a lasting cure for a variety of ailments. (8,9) Barleria cristata Linn (Family: Acanthaceae), often known as Kala Bansa. (10) It is a shrub that is extensively distributed in the subtropical Himalayas, Sikkim, Khasi Hills, central, and southern India. (11) As a traditional herbal medicine, the plant's parts were used to treat conditions like asthma, bronchitis, and skin conditions. It also acts as a diuretic and blood purifier. (12) B. cristata is extremely important in several ethnomedical systems for the treatment of a wide range of ailments, including respiratory conditions and inflammatory disorders. (13)

In this study, phytochemicals in plants will be identified using qualitative phytochemical screening. Thin-layer chromatography, and GC-MS. Then, the relationship between structure and function and bioactivity will be figured out. This will create a new road map for drug discovery by molecular docking. Due to the existence of certain bioactive chemicals, the component with the greatest percentage of peak area in the extract might be used for molecular modeling. Furthermore, our aim is to propose a novel approach for a plant-based drug that will be docked and modeled in order to treat disorders like metabolic and others.
Figure 1: Photograph of Barleria cristata Linn.

MATERIAL AND METHODS

Chemicals
The investigation was conducted using analytical-grade solvents and reagents.

Collection and identification of Plant
Fresh Barleria cristata leaves were collected from Chengalpattu, Tamil Nadu. The leaves were recognized and verified by Prof. P Jayaraman, Ph.D., PARC, Tambaram, Chennai.

Preparation of plant material
The leaves were cleaned with running water to get rid of adherent dust and other foreign objects, and then they were allowed to air dry at room temperature in the shade. The dried sample was individually homogenized to produce fine, coarse powder, which was then kept at room temperature in an airtight container for future research.

Preparation of crude extracts
The powdered plant material was extracted using a Soxhlet continuous extraction. The sample was defatted with the Petroleum ether for 48-72 hr, then separately with methanol solvent for 72 hrs. The extract was filtered and concentrated in a water bath. The methanolic extract was used for the study.

Preliminary phytochemical screening
The extracts obtained were subjected to a qualitative test for the identification of various plant phytochemicals like Proteins and Amino acids, Carbohydrates, Alkaloids, Flavonoids, Glycosides, and Triterpenoids were tested with the standard methods.

Thin layer chromatography
The TLC plates were manufactured using Silica gel "G," which required 35 gm of silica gel to be weighed, suspended in 60 ml of distilled water for two minutes, and then applied to the plate. The plate was then allowed to air dry until the layer's transparency vanished. The plates were drained for 30 minutes in a Hot air oven set to 110°C before being stored in a dry environment and utilized as supposed. Using the appropriate solvent to dilute the crude methanol extract, samples were applied in quantities of 1 to 10 µl, typically 2 cm above the bottom of a TLC plate.

Development of the chromatogram
After the sample was loaded, the plates were put in the glass chamber until the solvent was full. Then, the mobile phase was routed through the adsorbent phase until it attained 3/4 of the way through the plate. TLC was performed for Alkaloids, Flavonoids, Triterpenoids, Saponins, and Glycosides. Once the phytoconstituents were separated, certain reagents were injected and left to dry. The coloured dots that formed on the stationary phase were noted, identified, and measured for distance using a specific reagent.(21) The chromatographic behaviour of sample solutes is frequently described by the term Retention factor (Rf). It was determined using the following formulas:

\[ Rf \text{ value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}} \]

Gas chromatography-mass spectrometry (GC-MS)
The Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane, 30 m, 0.25 mm ID, 250 m df) packed fused silica column utilised in the analysis was used in the Clarus 680 GC. The components were parted using Helium as the carrier gas at a constant flow of 1 ml/min. During chromatographic run, the injector temperature was set to 260°C. A 1 µL aliquot of the extract was put into the apparatus, and the following conditions were met in the oven: After reaching 60 °Celsius for two minutes, 300 °Celsius was reached at a rate of 10 °Celsius per minute, and 300 °Celsius was maintained for six minutes.
Conditions of the mass detector: the transfer line temp was 240°C; the ion source temperature was also 240°C; the ionisation mode electron impact was set at 70 eV; the scan time was 0.2 seconds; and the scan interval was 0.1 seconds. The fragments range in size from 40 to 600 Da. The compound spectrum was compared to a database of known component spectrum maintained in the GC-MS NIST (2008) library.

**Determination of Phytoconstituents**
The identification of the constituents in the Methanol (*B. cristata*) extract that were extracted using different methods was based on a direct comparison of the retention times and mass spectral data with the Nist (2008) library that was used to compare the retention values attached to the GC-MS instrument and the results. Following the determination of the names, molecular weights, and structures of the test extract's constituents, the relative percentage composition of each component was determined by comparing the average peak area of that component to the overall area of the analysis and the they were tabulated.

**RESULTS**
Qualitative Phytochemical screening of Methanol extract of *Barleria cristata*
The phytoconstituents are the major important compounds which are responsible for the revealed phytoconstituents such as Proteins and Amino acids, Carbohydrates, Reducing sugars, Alkaloids, Flavonoids, Glycosides, Triterpenoids, Saponins, and Steroids (Table 1).

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Constituents</th>
<th>Confirmatory Test</th>
<th>Methanol Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Proteins and amino acids</td>
<td>Biuret test</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrate</td>
<td>Molisch’s test</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Reducing Sugars</td>
<td>Benedict test</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoids</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Glycosides</td>
<td>Bontrager’s test</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Triterpenoids</td>
<td>Salkowski’s test</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Saponin</td>
<td>Foam test</td>
<td>+</td>
</tr>
</tbody>
</table>

Thin layer chromatography of *Barleria cristata*
TLC profiling further confirmed the presence of Alkaloids, Flavonoids, Saponin, Glycosides, and Triterpenoids. Methanol was found the best suited solvent for extraction purpose. A robust mobile phase with the right amount has been reported for methanol extract. The Rf value was calculated and reported. The methanol extract of *Barleria cristata* Linn showed the presence of Alkaloid (Rf Value 0.42), Flavonoids (Rf Value 0.65), Saponin (Rf Value 0.33), Glycosides (Rf Value 0.54), and Terpenoids (Rf Value 0.17). (Table: 2)

<table>
<thead>
<tr>
<th>Sl.no.</th>
<th>Phytoconstituents</th>
<th>Solvent system</th>
<th>Spraying reagent</th>
<th>RF value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Benzene: Ethanol (9:1)</td>
<td>Dragendorff’s reagent</td>
<td>0.42</td>
</tr>
<tr>
<td>2.</td>
<td>Flavonoids</td>
<td>Ethyl acetate: Glacial acetic acid: Formic acid: water (7:1:0.5:1.5)</td>
<td>1% ethanolic aluminium chloride reagent</td>
<td>0.65</td>
</tr>
<tr>
<td>3.</td>
<td>Saponin</td>
<td>Methanol: Water (8:2)</td>
<td>Anisaldehyde sulphuric acid reagent</td>
<td>0.33</td>
</tr>
<tr>
<td>4.</td>
<td>Glycosides</td>
<td>Petroleum ether: Ethyl acetate (5:5)</td>
<td>Dragendorff’s reagent</td>
<td>0.54</td>
</tr>
<tr>
<td>5.</td>
<td>Triterpenoids</td>
<td>Ethyl acetate: Glacial acetic acid: Formic acid (3:2:5)</td>
<td></td>
<td>0.17</td>
</tr>
</tbody>
</table>

Gas Chromatography-Mass Spectroscopy profiling of Methanolic extract of *Barleria cristata*
The GC-MS examination of the methanolic extract of *Barleria cristata* leaves revealed a total of 15 compounds, each of which displayed distinct phytochemical properties. The graph is presented in Figure 2, whereas chemical constituents with the Retention time (RT), Molecular formula, Molecular weight, and Concentration were presented in Table 3. The major components present in leaves of *Barleria cristata* are Decanal, 4-Azido-Heptane, 2-Hydroxymethyl-9-[Beta-D-Ribofuranosyl]Hypoxanthine, 11-Tridecen-1-ol, Methyl Ester 13,16-Octadecadienoic Acid, N-[4-Bromo-N-Butyl]-2-Piperidinone, N-Methyl-N-Nitroso-1-Octanamine, 2-Methyl-6-Methylene-Octa-1,7-Dien-3-OL, 4-Acetamido-1-Hexanol, 1-(2-Propenyl)pentane, 4-Undecanone, (R, S)-2-Propyl-5-oxohexanal, 10-Methyl-4-undecanone, 1-Tetradecanamine, and N-Methyl-1-octadecanamine.

The presence of several bioactive metabolites in *B. cristata* leaves supports their use by traditional healers for a wide range of ailments. Accordingly, isolating specific phytochemical components and applying them to biological action will produce an abundance of results.
Figure 2: GCMS chromatogram of Methanol extract of *Barleria cristata* Linn.

Table 3: The components revealed from the *Barleria cristata* Linn.

<table>
<thead>
<tr>
<th>S. NO</th>
<th>RT (min)</th>
<th>Compound Name</th>
<th>Structure of compound</th>
<th>MW (g/mol)</th>
<th>Molecular Formula</th>
<th>CAS Number</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>17.634</td>
<td>Decanal</td>
<td><img src="image" alt="Decanal structure" /></td>
<td>156.2</td>
<td>C_{10}H_{20}O</td>
<td>112-31-2</td>
<td>9.96</td>
</tr>
<tr>
<td>2.</td>
<td>17.694</td>
<td>4-Azido-Heptane</td>
<td><img src="image" alt="4-Azido-Heptane structure" /></td>
<td>163.1</td>
<td>C_{7}H_{15}N_{3}O_{2}</td>
<td>27126-22-3</td>
<td>5.34</td>
</tr>
<tr>
<td>3.</td>
<td>19.020</td>
<td>2-Hydroxymethyl-9-[Beta-D-Ribo Furanosyl]Hypoxanthine</td>
<td><img src="image" alt="2-Hydroxymethyl-9-[Beta-D-Ribo Furanosyl]Hypoxanthine structure" /></td>
<td>298.2</td>
<td>C_{11}H_{16}N_{4}O_{6}</td>
<td>185377-94-0</td>
<td>16.49</td>
</tr>
<tr>
<td>4.</td>
<td>20.235</td>
<td>11-Tridecen-1-Ol</td>
<td><img src="image" alt="11-Tridecen-1-Ol structure" /></td>
<td>198.34</td>
<td>C_{11}H_{23}O</td>
<td>900130-96-8</td>
<td>7.68</td>
</tr>
<tr>
<td>5.</td>
<td>20.300</td>
<td>Methyl Ester 13,16-Octadecadienoic Acid</td>
<td><img src="image" alt="Methyl Ester 13,16-Octadecadienoic Acid structure" /></td>
<td>294</td>
<td>C_{19}H_{34}O_{2}</td>
<td>56846-99-2</td>
<td>57.22</td>
</tr>
<tr>
<td>6.</td>
<td>20.410</td>
<td>N-(4-Bromo-N-Butyl)-2-Piperidinone</td>
<td><img src="image" alt="N-(4-Bromo-N-Butyl)-2-Piperidinone structure" /></td>
<td>233</td>
<td>C_{8}H_{16}ONBr</td>
<td>195194-80-0</td>
<td>100.00</td>
</tr>
<tr>
<td>7.</td>
<td>20.546</td>
<td>N-Methyl-N-Nitroso-1- Octanamine</td>
<td><img src="image" alt="N-Methyl-N-Nitroso-1-Octanamine structure" /></td>
<td>172</td>
<td>C_{10}H_{16}ON_{2}</td>
<td>34423-54-6</td>
<td>7.28</td>
</tr>
<tr>
<td>8.</td>
<td>20.756</td>
<td>2-Methyl-6-Methylene-Octa-1,7-Dien-3-Ol</td>
<td><img src="image" alt="2-Methyl-6-Methylene-Octa-1,7-Dien-3-Ol structure" /></td>
<td>152</td>
<td>C_{16}H_{10}O</td>
<td>22459-10-5</td>
<td>25.24</td>
</tr>
<tr>
<td>9.</td>
<td>21.981</td>
<td>4-Acetamido-1-Hexanol</td>
<td><img src="image" alt="4-Acetamido-1-Hexanol structure" /></td>
<td>159</td>
<td>C_{6}H_{12}O_{2}N</td>
<td>900213-28-5</td>
<td>7.17</td>
</tr>
<tr>
<td>10.</td>
<td>23.492</td>
<td>1-(2-Propenylxylo)-Pentane</td>
<td><img src="image" alt="1-(2-Propenylxylo)-Pentane structure" /></td>
<td>128</td>
<td>C_{9}H_{16}O</td>
<td>23186-70-1</td>
<td>7.89</td>
</tr>
</tbody>
</table>
DISCUSSION

The use of complementary and alternative medicine, particularly with medicinal herbs and plants, is one of the greatest treatments for a variety of disorders. An initial qualitative phytochemical examination of *B. cristata* L leafy sections identified the presence of Alkaloids, Flavonoids, Glycosides, Saponins, Steroids, and Triterpenoids. Many biological and therapeutic activities have been identified for these secondary metabolites. The extraction yield estimated for methanol. The leafy portion of *B. cristata* extract showed that methanolic extract had a greater percentage yield. It may be leading to the high polarity of methanol, which is capable of attract a wide range of plant components. TLC profiling of methanolic extract gives, the result which suggest towards the existence of high content of phytoconstituents. Many phytoconstituents accord various Rf value in various solvent system. The difference in Rf value may give the ideas to identify the polarity and for selecting the solvent system for separation of compounds. The conglomeration of solvents with different polarity in the various proportion can be used for partition of compounds from extract. In qualitative analysis, the use of GC-MS allows for the acquisition of more precise information. The compounds with the highest peak is N-[4-Bromo-N-Butyl]-2-Piperidinone (100.00) was reported with high bactericidal inhibitory which can able to treat bladder spams, shrinkage, and ulcer inflammation. Decanal (9,96) were reported as it is used as perfumes. 11-Tridecan-1-Oil (7.68) were reported, it has antibacterial activity. 4-Undecanone (7.09) were reported with anti-bacterial agent in pharmaceutical preparations. Along with the analysis of mass spectra, the isolated chemicals were identified by contrasting their peak areas and retention times. To confirm their possible benefits, therefore, more research needs to be done to isolate, describe, and evaluate the biological effects of these identified compounds. Traditional healers employ *B. cristata* leaves to treat a wide range of illnesses because the leaves contain multiple bioactive compounds. Therefore, the separation of certain phytoconstituents and exposure to biological activity will gives the abundant result.

CONCLUSION

The methanol extract of *Barleria cristata* contained Triterpenoids, Alkaloids, and Flavonoids, according to the Quantitative phytochemical study. It is evident from the findings of the phytochemical research that *Barleria cristata* produces a large number of secondary metabolites with therapeutic significance. The existence of a broad collection of phytochemicals is confirmed by TLC profiling of plant extract in several solvent systems. Methanol was found to be the most effective solvent for extraction purposes. According to the results of the ethnobotanical survey, the plant exhibited promise as a possible source for the creation of medications to treat a wide variety of ailments. Thus, TLC increased the presence of medicinally significant phytochemicals such as alkaloids, flavonoids, and terpenoids. GC-MS method is the direct analytical approach for identification of phytocompounds with only few grams of plant extract. The biological activity of several of these chemicals highlights the significance of our study. Therefore, from this study it can be concluded the methanolic extract of *Barleria cristata* Linn. has the ability to facilitate as a different source of therapeutic medications, because it contains phytoconstituents and other types of bioactive components which is described.

ACKNOWLEDGEMENT

The authors are obliged to Sophisticated Instrumentation Facility (SIF), VIT University, Vellore.
CONFLICT OF INTEREST
The authors report no conflicts of interest in this work.

ETHICAL APPROVALS
This study does not involve experiment on animals or human subjects.

FUNDING
There is no funding to report.

DATA AVAILABILITY
All data generated and analyzed are included in this research article.

REFERENCES