ABSTRACT

Background and objective: The present study was design to develop scientific data for identification and quality control of Behrooza.

Methods: GC-MS analysis was done to confirm the Molecular weight, Molecular formula, Molecular structure and chemical composition of Behrooza.

Results: The GC-MS chromatogram of Behrooza revealed the presence of two compounds longicycline with molecular weight and molecular formula and longifolene with molecular weight and molecular formula.

Conclusion: The study was started with an aim to generate the scientific data for identification and quality control of Behrooza.

KEYWORDS: GC-MS; Standardization; Behrooza; Pinus roxburghii.

INTRODUCTION

Natural Resins are Semi-Solid materials, usually a complex mixture of organic compounds called terpenes. These are insolable in water but soluble in certain organic solvents. Resins are often associated with volatile oils (oleo-Resin), with gums (Gum-Resin) or with oil and gum (Oleo-gum Resin).[1] Behrooza is the Unani drug obtained from bark of the Pinus roxurghii Sarg.(Oleo Resin of Pine). Behrooza is used medicinally only after detoxification. The detoxified form is called "Behrooza musaffi" or "Satt Behrooza."[2] In Unani System of Medicine the Behrooza is commonly used for Ehtabas Baul Wa Tams (Anuria and Ammenorrhea), Qurooh Mutaffine (Non-healing ulcer), Khanazeer (Diphtheria), Suzak (Gonorrhea).[3,4] The compound formulations of Behrooza are Marham jadwar, Marham Zangaar, Marham Jalinoos, Marham Rusl, Marham Safeed.[5,6] The Bioactive constituents found in Behrooza are responsible for various properties as revealed by various studies like
Wound healing activity\cite{7}, cytotoxic activity\cite{8}, antibacterial activity\cite{8}, Anti fungal activity\cite{9}, Anti-mosquito activity\cite{10} etc.

MATERIALS AND METHODS

Collection and identification of raw material
Behrooza was procured from the registered crude drug dealer in Kashmir valley. The identity of the drug was confirmed by Pharmacognosist, S. Noorunnisa Begum, Sr. Asst. Prof., Centre for Repository of Medicinal Resources (C-RMR), TransDisciplinary University, (TDU), FRLTH, Bangalore. The sample specimens was deposited in herbarium of NIUM with FRLHT Acc. No. 3875.

Gas Chromatography Mass Spectroscopic Analysis (GC-MS)
GC-MS analysis of Behrooza was carried out by using GC-MS modal thermo scientific TRAYS 1310 and MS TSQ 8000 with a capillary column TG5MS, 30 meter, 0.25 internal diameter. The sample was prepared by dissolving 5mg of powder in 5ml hexane and sonicated for 10 minutes followed by filtration. The filtrate was used as sample for injection under the following conditions: injection volume 1μl with split ratio 1:49, helium as carrier gas at 1.0ml/min constant flow mode, injector temperature 550C, oven temperature was 2400C increasing from 50 ⁰c ramp to 240 ⁰c and detector was full scanned over 50-450 a.m.u range. Identification of compounds was carried out by comparing query mass spectra with NIST 2.2 library reference mass spectra.

RESULTS

GCMS
The GC-MS was done on Behrooza for identification and quantification of components present in the sample. GC analysis of Behrooza was found to contain 2 main peaks one at RT 21.11 with an area percentage of 29.95 and another component at RT 22.03 with area percentage of 70.05 (figure- 1, table- 1). In mass spectroscopy these 2 components were identified by comparing query mass spectra with reference mass spectra in Chemical Abstract Service (CAS) & National Institute of Standard and Technology (NIST) library via spectral matching and the components were detected as Longicycline and Longifolene (figure 2 ad 3). The synonym of Longicycline is 1,2,4-Methenoazulene, decahydro-1,5,5,8atetramethyl-, [1S-(1α,2α,3αβ,4α,8αβ,9R)] with Molecular Formula C_{15}H_{24} and Molecular Weight: 204 Exact Mass: 204.1878 CAS#: 1137-12-8 NIST#: 413540 ID#: 14358 DB: replib. The Molecular Formula of Longifolene: C_{15}H_{24}, Molecular Weight: 204 Exact Mass: 204.1878
CAS#: 475-20-7 NIST#: 62059 ID#: 1406 DB: replib, (figure- 6, table- 9) and the synonyms of Longifolene are found as.

1. 1, 4-Methanoazulene, decahydro-4, 8, 8-trimethyl-9-methylene-, [1S-(1α, 3αβ, 4α, 8αβ)]-

2. 1, 4-Methanoazulene, decahydro-4, 8, 8-trimethyl-9-methylene-, (1S, 3aR, 4S, 8aS)-(++)-

3. (+)-Longifolene

4. D-longifolene

5. Junipen

6. Junipene

7. Kuromatsuen

8. Kuromatsuene

Fig. 1. GC-MS Chromatogram of Oleo Resin of Pine.

RT: 0.00-47.10, No. of Detected peaks-2.
Table 2: Results of GC-MS Chromatogram.

<table>
<thead>
<tr>
<th>I No.</th>
<th>Apex RT</th>
<th>Area</th>
<th>% Area</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.11</td>
<td>8933250.657</td>
<td>29.95</td>
<td>Longicyclene</td>
</tr>
<tr>
<td>2</td>
<td>22.03</td>
<td>20895801.09</td>
<td>70.05</td>
<td>Longifolene</td>
</tr>
</tbody>
</table>

CONCLUSION

The quality and purity of Unani drugs and their widespread acceptance can be improved only if the crude drugs are standardized properly. From the GC-MS analysis and literature survey it can be concluded that Behroza is highly active against microbes, bacteria, resistant fungal infections etc. However further studies like LD$_{50}$ and toxicological report are required to prove its fitness for human consumption. Furthermore the study was started with an aim to generate the scientific data for identification and quality control of Behrooza.

CONFLICT OF INTEREST: Nil.

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REFERENCES