Standardization of Majith (Rubia cordifolia Linn.)

Aisha Siddiqui1*, Tajuddin2, KMY Amin2, RH Zuberi2 & Anwar Jamal3

1Department of Ilmul Advia, Faculty of Unani Medicine, Jamia Hamdard (Hamdard University), New Delhi 110 062;
2Department of Ilmul Advia (Unani Pharmacology), AKT College, Aligarh Muslim University, Aligarh 202 002, Uttar Pradesh;
3CCRUM, 61-65 Institutional area, Janakpuri, New Delhi-58
E-mail- ashijamal2003@rediffmail.com

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Standardization and quality control of the herbal drugs including single and compound formulations used in Unani System of Medicine is essential for their acceptance in the international as well as local markets. Thus, there is an urgent need of standardized drugs having consistent quality for reliable beneficial therapeutic purpose. An attempt has been made for phytochemical standardization of Majith (Rubia cordifolia Linn.) (Rubiaceae), a Unani drug having immunomodulatory, antioxidant, antiinflammatory, antipsychotic, anti diarrhoeal, anticonvulsant and antidiabetic properties. Physicochemical parameters, qualitative and quantitative analysis, TLC profile and fluorescence analysis were used for standardization and quality evaluation of Majith. Qualitative analysis of Majith showed the presence of amino acid, proteins, glycosides, flavonoids, phenols, resin, sterol/terpene and tannins except alkaloids.

Keywords: Drug standardization, Unani drug, Majith, Rubia cordifolia, Rubiadin

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Physicochemical standardization is a pre-requisite in quality control of herbal drugs. The efficacy of an herbal drug mainly depends upon its physical and chemical properties. Therefore, the determination of physicochemical characters for the authenticity of the drug is necessary before subjected for pharmacological activity. The qualitative and quantitative analysis of major bioactive chemical components (marker components) of crude drug constitute important and reliable part of quality control protocol as any change in the quality of the drug directly affects the constituents. Study also helps in characterization of constituents or groups of constituents that frequently lead to establish the structure activity relationship and the likely mechanism of action of the drug. Majith (Rubia cordifolia Linn.) (Rubiaceae) is one of the well known drugs being used in Unani System of Medicine since ancient time. It is prickly creeper or climbing perennial herb usually 10 m long, distributed commonly throughout the hilly districts of India, ascending to an altitude of 3,750 m from Northwest Himalaya eastwards and southwards to Ceylon and Malacca and Northeast Asia from Dahuria (East Siberia) to Japan, Java and tropical Africa. It is prescribed in the treatment of many ailments as deobstruent, strong diuretic, detergent, resolvent, detoxifying agent and blood purifier in Unani Medicine. Its immunomodulatory, antioxidant, anti-inflammatory, antipsychotic, anti diarrhoeal, anticonvulsant and antidiabetic activities have been reported. Rubiadin, a major constituent (marker compound) present in Rubia cordifolia Linn. is reported to possess hepatoprotective and antioxidant activity. So, the quantification of rubiadin can be helpful in routine quality control of Majith. Therefore, for the purpose of quality assurance, the physicochemical and phytochemical standardization of Rubia cordifolia Linn. has been undertaken to ensure the potency and purity of the drug.

Methodology

The dried roots of Rubia cordifolia Linn. (Rubiaceae) were purchased from old Delhi market and authenticated at Department of Raw Materials Herbarium and Museum, NISCAIR, New Delhi [Fig. 1(2-3)]. A voucher specimen is also preserved in the Herbal Museum of the Department of Ilmul

*aCorresponding author
Advia, Ajmal Khan Tibbiya College, AMU, Aligarh for further reference. Organoleptic characters of the drug (root powder) were recorded. Phytochemical constituents like alkaloids, tannins, glycosides, resins, terpenes, flavonoids, sugar and proteins were tested\textsuperscript{12}. Quantitative analysis for total ash, acid insoluble ash, water soluble ash, extractive values in alcohol and water, successive soxhlet extractives in petroleum ether, diethyl ether, chloroform, benzene, alcohol and water, loss on drying at 105°C, pH of filtrate of 1% and 10% w/v aqueous solution were carried out\textsuperscript{13}. Determination of total phenolics in \textit{Rubia cordifolia} Linn, isolation and estimation of marker compound rubiadin was also done\textsuperscript{14,15}. For TLC, 2 gm of study drug sample was extracted with 25 ml of methanol on boiling water bath for 25 minutes consecutively and 3 times using fresh portion of 25 ml methanol, filtered and concentrated. TLC was carried out on precoated aluminum plates of silica gel 60 F\textsubscript{254} (E Merck). The mobile phase used was toluene: ethyl acetate: formic acid (85:14:1). The plate was developed over a distance of 6 cm and visualized under visible and UV lights after spraying and \( R_f \) values were calculated. Fluorescence analysis of powdered drug with various chemicals and chemical reagents was also carried out to observe the specific change in colour\textsuperscript{16}.

**Results and discussion**

\textit{Majith} is reddish brown in colour, solid in appearance with scented odour and sweetish taste. The drug sample was subjected to qualitative estimation, phytochemical analysis, TLC profile and fluorescence analysis. Tests like amino acids, proteins, glycosides, flavonoids, phenol, resin, sterol/terpene and tannin were found positive in qualitative analysis of powdered drug [Fig. 1(1)]. Alkaloid was found negative in above analysis. Phytochemical analysis exhibited 7.65% of total ash values; variation was observed in acid insoluble ash, which was 5.11%. Water soluble ash was found 1.09%. pH of 1% and 10% w/v aqueous solution showed 5.81% and 4.39%, respectively. The result obtained from isolates of marker compound rubiadin was found to be 0.12%. Extractives values in alcohol and water were observed to be 29.06% and 54.28%, respectively. Whereas, successive soxhlet extractives in petroleum ether, diethyl ether, chloroform, benzene, alcohol and water were found to have 0.54%, 2.72%, 1.25%, 0.52%, 31.25% and 27.88%, respectively. Total phenolic was 6.46% and bulky density was 1.28. Loss on drying at 105°C was 13.07%. Testing of different mobile phases for the separation of methanolic extract of \textit{Majith} and its constituents by TLC, the desired resolution was achieved using toluene: ethyl acetate: formic acid (85:14:1) (Table 1). Whereas, fluorescence analysis of powdered drug with different reagents under day light, UV light (short and long) were analyzed for the identification of the genuine drug (Table 2).

![TLC profile of Majith (Rubia cordifolia Linn.)](image)

**Table 1—TLC profile of Majith**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent system</th>
<th>Spraying reagent</th>
<th>Visible</th>
<th>Detection / Observations</th>
<th>UV (short)</th>
<th>UV (long)</th>
<th>( R_f ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>toluene: ethyl acetate: formic acid (85:14:1)</td>
<td>Methanolic KOH (5%)</td>
<td>fluorescent green light brown purple</td>
<td>grey</td>
<td>0.34</td>
<td>grey</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bluish purple</td>
<td>0.40</td>
<td>light blue brown black</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Fig. 1—TLC profile of \textit{Majith} (Rubia cordifolia Linn.) (1); Root of \textit{Majith} (lateral view) (2); Root of \textit{Majith} (front view) (3)
Qualitative and physicochemical analysis was carried out to determine the variations in the parameters of adulterated and genuine drugs. Variation was observed in acid insoluble ash which is reported to be not more than 0.8\%\(^{17}\). This difference may be due to variation in the quality of raw material used. The results obtained from isolates of marker compound rubiadin were found to be in encouraging yield, i.e. 0.12\%. Biomarker profiling of maximum number of medicinal plants required to be established to highlight the quality control development based on this new emerging techniques which is being utilized by the people throughout the globe for drug development from natural resources\(^{18}\). The method of sample preparation and the development of a suitable mobile phase are two important steps regarding herbal drug standardization because of the complexity of the chemical composition and affinities of the component towards various solvent. Fluorescence analysis of powdered drugs with different reagents under day light, UV light (short and long) was analyzed for the identification of genuine drugs. UV light is very characteristic and helps in the identification of the drugs from the adulterated ones. This parameter is also utilized in the identification and maintaining the purity of the drug. Thus, it can be concluded that physicochemical parameters, qualitative and quantitative analysis, TLC profile and fluorescence analysis together may be used for quality evaluation and standardization of crude drug to achieve genuine and standard drug for therapeutic purpose.

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**References**