RESEARCH ARTICLE

Fluorescence and FTIR markers for different taxa of Gymnema drug complex from Maharashtra

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ABSTRACT

Gymnema sylvestre known as Madhunashini / Gudmar and being commercially traded as a remedy of diabetes. The other taxa of this genus are also used as alternative drugs. The drug standards are available for the former species but not for the alternative taxa. In the present study, four taxa of Gymnema complex are evaluated to develop drug identification markers through fluorescence study and FTIR analysis. These markers are successful in distinguishing individual drug samples. The study will help to find out the purity of the samples and also for verification of drugs.

Introduction

India is one of the leading countries with a high number of people with Diabetes mellitus and it is estimated that around 57 million people will be suffering from the disorder by the year 2025 (1). The world estimate is about 415 million, i.e., one in eleven persons (2). The majority of the cases are associated with obesity and lifestyle. The prevalence of the disease is very high in India following China which has about 109 million peoples suffering from this disorder. To treat the people in large number quality resources and products are needed. Therefore, the major research work is being done on diabetes eradication or to control diabetes by using herbal medicine. Several plants are claimed to possess anti-diabetic properties in the traditional system and used by tribal people as well as local inhabitants. Due to side effects of allopathic drugs on kidney functions, stomach tiredness, weight gain, risk of liver disorders, etc. herbal medicines are preferred for control of diabetes throughout the world. Of these Gymnema R. Br. is an important plant genus in terms of therapeutic application and commonly called as Gudmar / Gulmar i.e., sugar killer. It consists of 14 species and two varieties in India (3). In Maharashtra, the genus is represented by five species viz., G. cuspidatum (Thub.) Kuntze; G. latifolium Wall. ex Wight; G. montanum (Roxb.) Hook. f.; G. inodorum (Lour.) Decne. and G. sylvestre (Retz.) R. Br. ex Sm. Out of these G. sylvestre is well known medicinal plant having properties like lowering blood sugar, balancing insulin level and excellent remedy for weight loss. Similarly, very few references are also available for G. latifolium and G. montanum mentioning their use in ethnomedicine for curing the disease such as diabetes, obesity, liver disorders, gastrointestinal ailments, constipation, water retention and snakebite (4-6). However, no scientific work is done on G. cuspidatum and G. inodorum regarding any such activities. Our previous studies (7-8) reported the use of these species as alternative drugs under the name Gudmar against diabetes.

These species are locally used as medicinal plants as per availability of taxa and sold in local as well as national market. Due to anti-diabetic property, Gymnema is being exported in the form of dry extract as well as a crude drug (9). However, the efficacy of the drug is dependent upon the purity of plant material as a drug. Many times misidentification of medicinal plants or adulteration in drug sample leads to severe health problems (10-11) therefore, correct identification of the drug using some common
techniques is essential for therapeutic application. Pharmacognostic standards are available for Gymnema sylvestre only, even though other taxa are used as alternative drugs, are not studied and considered as adulterants. This also leads to non-utilization of alternative drugs. Non-availability of markers for alternative drugs provides a lacuna for illicit trade of the important resources. Hence, a study was undertaken to develop identification markers by using Fluorescence analysis and Fourier Transform Infrared spectrophotometry (FTIR).

Fluorescence is the phenomenon exhibited both under visible and UV light by various chemical constituents present in the plant material. Fluorescence is considered to be a very significant and reliable parameter of pharmacognostic evaluations (12). Recently FTIR (Fourier Transform Infrared spectrophotometry) has been applied to identify herbal drugs or herbal drug analysis. The measurements made by FTIR were accurate and reproducible. By using this technique, chemical constituents and its functional group identification become easy. Therefore, these are taken as one of the evaluation parameters for the present study.

Materials and Methods

Leaf materials of Gymnema sylvestre and allied species which are used as alternative drugs were collected from different localities of Maharashtra. During the present study authors were able to collect the materials of four taxa viz., Gymnema cuspidatum, G. latifolium, G. montanum and G. sylvestre and used in the analysis (Fig. 1). The sample materials were tagged. Specimens (two - three) from each species with the flowers and/or fruits were processed for herbarium specimen preparation and identification. The identity confirmation of these specimens was done with the help of available literature (13-16) and herbarium specimens at different herbaria, with the help of available literature (13-16) and IR spectrum table and chart of Merck (19) were followed.

Results and Discussion

Fluorescence analysis

The activities of leaf drug sample with different chemical reagents and fluorescence characters of Gymnema cuspidatum, G. montanum, G. latifolium and G. sylvestre were observed under visible light, short UV and long UV lights and analyzed characters are tabulated in Table 1.

The results of fluorescence analysis showed that leaf powder of G. montanum, G. latifolium and G. sylvestre treated with alcoholic 1N NaOH emitted fluorescent green colour under short UV light and G. cuspidatum with green colour. Leaf powders of G. cuspidatum and G. sylvestre showed fluorescent green colour in short UV light when treated with Picric acid. Leaf powder of G. cuspidatum when treated with Acetic acid shown a fluorescent green colour under short UV light, while shown only light brown colour under long UV light. Gymnema sylvestre sample, when treated with acetic acid, shown a fluorescent green colour under short as well as long-wavelength UV lights. The test of leaf powder with nitric acid + ammonia given fluorescent green colour under short UV light only by the sample of G. latifolium, while for others this test was negative. The treatment with alcholic NaOH under long UV showed orange colour in the sample of G. sylvestre, while samples of other species showed only light brown colour.

On the basis of fluorescence analysis, it is evident that drugs of different taxa exhibit different fluorescence colour under short UV (λ=254 nm) and long UV (λ=365 nm) wavelengths. It is possible to identify them easily on the basis of the present results.
An identification key is prepared for the powder samples of these taxa using fluorescence characters. While using the key, Table 1 can be referred. Other colours under UV exposure can also be useful as accessory parameters which support the identification e.g., development of orange colour under long UV for a mixture of *G. sylvestre* and NaOH (alchoholic).

**Identification Key based on Fluorescence Analysis**

1. Powder + 1N NaOH (alcoholic) = No Florescent Green colour ........................................... *G. cuspidatum*
2. Powder + 1N NaOH (alcoholic) = Florescent Green colour under short UV ........................................... 2
3. Powder + Nitric acid + Ammonia = Florescent Green colour under short UV .......................... *G. latifolium*

**Fig. 1. Gymnema R. Br. species used in the study. A) G. cuspidatum (Thub.) Kuntze; B) G. latifolium Wall. ex Wight; C) G. montanum (Roxb.) Hook. f. and D) G. sylvestre (Retz.) R. Br. ex Sm.**
Table 1. Comparative fluorescence analysis for Gymnema species

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experiment</th>
<th>Visible / Daylight</th>
<th>UV 254 nm</th>
<th>UV 365 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gc</td>
<td>Gl</td>
<td>Gm</td>
</tr>
<tr>
<td>1</td>
<td>Powder + DW</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>2</td>
<td>Powder + 1N Sodium hydroxide (aqueous)</td>
<td>Green</td>
<td>Yellow</td>
<td>Green</td>
</tr>
<tr>
<td>3</td>
<td>Powder + 1N Sodium hydroxide (alcohol)</td>
<td>Light Green</td>
<td>Light Green</td>
<td>Light Yellow</td>
</tr>
<tr>
<td>5</td>
<td>Powder + 50% Sulphuric acid</td>
<td>Black</td>
<td>Brown</td>
<td>Black</td>
</tr>
<tr>
<td>6</td>
<td>Powder + Nitric acid</td>
<td>Light Yellow</td>
<td>Brown</td>
<td>Light Yellow</td>
</tr>
<tr>
<td>7</td>
<td>Powder + Picric acid</td>
<td>Yellow Dark Yellow</td>
<td>Yellow</td>
<td>Green</td>
</tr>
<tr>
<td>8</td>
<td>Powder + Acetic acid</td>
<td>Light Pale Brown</td>
<td>Light Yellow</td>
<td>Light Green</td>
</tr>
</tbody>
</table>

Where, Gc = G. cuspidatum; Gl = G. latifolium; Gm = G. montanum; Gs = G. sylvestre.

Table 2. Wave number (cm\(^{-1}\)) of dominant peak obtained from absorption spectra

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Appearance</th>
<th>Compound</th>
<th>Peak Value / Frequency range (cm(^{-1}))</th>
<th>Gc</th>
<th>Gl</th>
<th>Gm</th>
<th>Gs</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-H stretching</td>
<td>Strong, broad</td>
<td>Alcohol</td>
<td>3550-3200</td>
<td>3291.79</td>
<td>3351.13</td>
<td>3359.44</td>
<td>3358.97</td>
</tr>
<tr>
<td>C-H stretching</td>
<td>Medium</td>
<td>Alkane</td>
<td>3000-2840</td>
<td>2922.92</td>
<td>2919.74</td>
<td>2851.42</td>
<td>2851.02</td>
</tr>
<tr>
<td>C-H bending</td>
<td>Weak</td>
<td>Aromatic Compound</td>
<td>2000-1650</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C=C stretching</td>
<td>Strong</td>
<td>Alkene</td>
<td>1648-1638</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C=C stretching</td>
<td>Medium</td>
<td>Alkene</td>
<td>1662-1626</td>
<td>-</td>
<td>1635.03</td>
<td>1626.17</td>
<td>-</td>
</tr>
<tr>
<td>C=C stretching</td>
<td>Strong</td>
<td>α, β unsaturated ketone</td>
<td>1620-1610</td>
<td>1616.61</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-O asymmetric stretch</td>
<td>Strong</td>
<td>Nitro compound</td>
<td>1550-1475</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C=C stretching</td>
<td>Medium</td>
<td>Aromatic</td>
<td>1500-1400</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O-H Bending</td>
<td>Medium</td>
<td>Phenol</td>
<td>1390-1310</td>
<td>1317.62</td>
<td>1381.81, 1317.38</td>
<td>1375.51</td>
<td>1373.70</td>
</tr>
<tr>
<td>C-N stretching</td>
<td>Medium</td>
<td>Aliphatic amines</td>
<td>1250-1020</td>
<td>1243.55</td>
<td>1246.72</td>
<td>1244.27</td>
<td>1246.52</td>
</tr>
<tr>
<td>S=O stretching</td>
<td>Strong</td>
<td>Sulfoxide</td>
<td>1070-1030</td>
<td>1036.05</td>
<td>1035.26</td>
<td>1065.24</td>
<td>1035.75</td>
</tr>
<tr>
<td>C-H Bending</td>
<td>Strong</td>
<td>1,2,3- tridistributed</td>
<td>780±20</td>
<td>-</td>
<td>780.68</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-Br stretching</td>
<td>Strong</td>
<td>Halo compound</td>
<td>690-515</td>
<td>618.23</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C-I stretching</td>
<td>Strong</td>
<td>Halo compound</td>
<td>600-500</td>
<td>516.13</td>
<td>518.23</td>
<td>517.89</td>
<td>532.09</td>
</tr>
</tbody>
</table>

Where, Gc = G. cuspidatum; Gl = G. latifolium; Gm = G. montanum; Gs = G. sylvestre.

2. Powder + Nitric acid + Ammonia = No Florescent Green colour .................................................. 3
3. Powder + Picric Acid / Acetic Acid = Florescent Green colour .................................................. G. sylvestre
3. Powder + Picric Acid / Acetic Acid = No Florescent Green colour .............................................. G. montanum

**FTIR Analysis**

FTIR spectrum was used to identify the functional groups of active components based on the peak value in the region of infra-red radiation. FTIR peak values for each species and functional groups are tabulated in Table 2.
FTIR spectrum of leaf crude drug sample *G. cuspidatum* (Fig. 2) show characteristic peaks indicating different compound groups at 3291.79 cm\(^{-1}\) indicating O-H stretching of alcohol; 2922.92 cm\(^{-1}\) C-H stretching of alkanes; 1616.61 cm\(^{-1}\) C=C stretching of α, β unsaturated ketones; 1317.62 cm\(^{-1}\) O-H bending of phenol; 1243.55 cm\(^{-1}\) C-N stretching of amine; 1036.05 cm\(^{-1}\) S=O stretching of sulfoxide; and C-I stretching at 516.13 cm\(^{-1}\).

FTIR spectrum of leaf crude drug of *G. latifolium* (Fig. 3) show major peaks with characteristic compound class at 3351.13 cm\(^{-1}\) for O-H stretching of alcohol; 2919.74 and 2851.43 cm\(^{-1}\) for C-H stretching of alkane; 1635.05 cm\(^{-1}\) for C=C stretching of alkene; 1383.81 and 1317.38 cm\(^{-1}\) for O-H bending of phenol; 1246.72 cm\(^{-1}\) for C-N stretching of amine; 1035.26 cm\(^{-1}\) for S=O stretching of sulfoxide; 780.68 cm\(^{-1}\) for 1,2,3-tridistributed C-H bending; 618.23 cm\(^{-1}\) for C-Br stretching and 518.62 cm\(^{-1}\) for C-I stretching.

The FTIR spectrum of leaves of *G. montanum* (Fig. 4) have characteristic peaks at 3359.44 cm\(^{-1}\) for O-H stretching of alcohol; two peaks of C-H stretching of alkane at 2920.10 and 2851.02 cm\(^{-1}\); at 1626.17 cm\(^{-1}\) C=C stretching of alkene; 1519.50 cm\(^{-1}\) for N-O asymmetric stretching of nitro compound; 1446.87 cm\(^{-1}\) for C-C stretching of aromatic compound; 1375.51 cm\(^{-1}\) for O-H bending of phenol; 1244.27 cm\(^{-1}\) for C-N stretching of amine; 1103.75 cm\(^{-1}\) S=O stretching of sulfoxide; and 532.09 cm\(^{-1}\) for C-I stretching of halo compound.

The FTIR spectra of these four species show close relationships and similar components with each other. There were seven common absorbing peaks for O-H, C-H, O-H phenol, C-N, S=O and C-I compound classes. Each of the species still bears its characters such as different peak shapes, numbers, position and intensity indicating different groups. The C-H corresponding to aromatic compound group is present only in *G. sylvestre*. The peak for C=C of alkene is present in...
all taxa except *G. cuspidatum*. The peak indicating C=C stretching in α, β unsaturated ketone is unique to *G. cuspidatum*. The drug *G. montanum* has specific peak for N-O asymmetric stretch of nitro compound. A characteristic peak for 1,2,3-tridistributed C-H bending is present only in *G. latifolium*.

The results of present study in the form of fluorescence and FTIR markers indicated clear differences among the taxa studied. Thus, considering all the evidences from the present study, the separation of drug samples become easy and any adulteration or blending of substitute / alternative drug could be easily identified with present Fluorescence and FTIR markers. Combined fluorescence markers and FTIR markers for each drug belonging to Gudmar complex are formulated on the basis of the present study. These markers for each drug are given below.

**Gymnema cuspidatum**

Fluorescence markers

i. Powder + NaOH (Alc.) → No fluorescence at any light.

ii. Powder + Picric acid / Acetic acid → Green Fluorescence under 254 nm.

iii. Powder + Distilled Water → Black colour under 254 nm.

FTIR markers

i. Absence of peak for C-H bending for aromatic compound.

ii. Absence of peak for C=C for Alkene.

iii. Presence of C=C strong stretching for α, β unsaturated ketone.

**Gymnema latifolium**

Fluorescence markers

i. Powder + NaOH (alc.) → Green fluorescence under 254 nm.
Acknowledgements

The authors are thankful to Principal of Sharachandra Pawar Pharmacy College, Dumbarwadi for providing some facilities and help in phytochemical analysis and authors of Vishal Junnar Institute of Pharmacy and Research, Ale for providing FTIR analysis. The first author (SSR) is grateful to the Savitribai Phule, Pune University, Pune for financial support. Thanks are due to the Principals of respective institutions of authors for support towards collaborative studies.

Authors’ contributions

Savita S. Rahangdale (SSR) has done the analytical work of this study and presentation of the results and discussion. Sanjaykumar R. Rahangdale (SRR) has done the field work, collection and processing of samples, morphological characterization of the taxa and manuscript editing. The work is done on a mutual benefit basis.

Competing interest statement

Authors do not have any competing interests among them as well as any other persons with respect to this study.

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Conclusion

The results of the present study provide significant outcomes in the form of Fluorescence and FTIR markers for four taxa used under Gudmar complex. These markers are of immense importance for the quality assurance of the crude drug samples because any impurity or use of alternative drugs can be identified. The study also help in use of alternative drugs and assuring purity of drug in the trade. This is a cheaper and faster technique for the verification of the drug samples of Gymnema complex.

Gymnema montanum

Fluorescence markers

i. Powder + NaOH (alc.) → Green fluorescence under 254 nm.
ii. Powder + 50% Sulfuric acid → Dark Brown colour under 254 nm and 365 nm.
iii. Presence of unique C=C stretching peak of aromatic compound.

FTIR markers

i. Absence of weak peak of aromatic compound group.
ii. Presence of N-O asymmetric stretch of nitro compound.
iii. Presence of unique C-C stretching peak of aromatic compound.

Gymnema sylvestre

Fluorescence markers

i. Powder + NaOH (alc.) → Green fluorescence under 254 nm and orange colour under 365 nm.
ii. Powder + Picric acid → Green fluorescence under 254 nm.
iii. Powder + acetic acid → Green fluorescence under 365 nm.
iv. Powder + 50% Sulfuric acid → Light blue colour under 254 nm.

FTIR markers

i. Nitro compounds and unsaturated ketones absent.
ii. Peak for C-I stretching present.
iii. Presence of peak for C-H bending of aromatic compound.
iv. Peak for C-C stretch of aromatic compound absent.

Acknowledgements

The authors are thankful to Principal of Sharachandra Pawar Pharmacy College, Dumbarwadi for providing some facilities and help in phytochemical analysis and authorities of Vishal Junnar Institute of Pharmacy and Research, Ale for providing FTIR analysis. The first author (SSR) is grateful to the Savitribai Phule, Pune University, Pune for financial support. Thanks are due to the Principals of respective institutions of authors for support towards collaborative studies.

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