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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 *Gudmar* 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

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techniques is essential for therapeutic application. Pharmacognostic standards are available for *G. 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., *G. 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 species was done with the help of available literature (13-16) and herbarium specimens at different herbaria, especially Herbarium of Botanical Survey of India, Western Regional Centre, Pune (BSI). Authenticated specimens are deposited in Herbarium of Department of Botany, Balasaheb Jadhav Arts, Commerce and Science College, Ale. The recent nomenclature is applied as per POWO (17). Leaf materials after collection were cleaned and dried in the shade for two weeks. The materials were ground to a coarse powder by using a household electric blender and passed through sieve no. 25. The fine powder was subjected to analysis for fluorescence and FTIR.

Fluorescence analysis

Some components are prominent and actively show fluorescence under a visible range of light, whereas, characterized fluorescence is not observed in others. These components showed distinguished fluorescence in UV light. Hence, the behaviour of each leaf sample with different chemical reagents was observed under visible light, a short and a long wavelength of ultraviolet light. For the fluorescence observations following procedure was followed.

Crude drug sample (0.5 gm) was taken in each (nine replicates) clean and dry test tubes of 15 ml capacity. In each separate tube, 5 ml of different organic solvents viz., distilled water, glacial acetic acid, 50% sulphuric acid, nitric acid, 1N hydrochloric acid, 5% $FeCl_3$, picric acid, 1N NaOH (aqueous) and 1N alcoholic NaOH were added separately. Then, all the tubes were shaken and they were allowed to stand for about 30 min at room temperature. The solutions obtained were observed under the visible daylight and UV light of short-wavelength (254 nm) and UV light of long-wavelength (365 nm) for their characteristic fluorescent colour.

Fourier Transform Infra-Red (FTIR) spectrometry

The FTIR spectra are recorded by using Perkin-Elmer spectrometer Model 65. Measurement range was 400 cm^{-1} to 4000 cm^{-1} with a resolution of 4 cm^{-1}. Conventional transmission mode using the KBr pellet method was applied. KBr pellet spectra were recorded from the sample (c. 2 mg) which was ground and mixed with dry KBr powder (c. 200 mg). Then the mixture was crushed in a mechanical mould to form a tablet with a diameter of 3 mm. The IR light passes the pellet before it reaches the detector. For the characterization of compound class on the basis of prominent peaks in the spectrum, Spectrometric identification of organic compounds (18) 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 *G. 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 alcoholic 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.

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.

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*

1. Powder + 1N NaOH (alcoholic) = Florescent Green colour under short UV 2

2. Powder + Nitric acid + Ammonia = Florescent Green colour under short UV ..... *G. latifolium*

Table 1. Comparative fluorescence analysis for *Gymnema* species

Sl. No.	Experiment	Visible /Daylight				UV 254 nm				UV 365 nm			
		Gc	Gl	Gm	GS	Gc	Gl	Gm	GS	Gc	Gl	Gm	GS
1	Powder + DW	Green	Green	Green	Green	Black	Dark Green	Black	Green	Dark Green	Black	Dark Green	Light Green
2	Powder + 1N Sodium hydroxide (aqueous)	Green	Yellow Green	Green	Brown Yellow	Dark Green	Pale Green	Dark Green	Light Green	Dark Brown	Black	Dark Brown	Dark Green
3	Powder + 1N Sodium hydroxide (alcohol)	Light Green	Light Green	Light Green	Light Yellow	Green	Fluorescent Green	Fluorescent Green	Fluorescent Green	Light Brown	Light Brown	Light Brown	Orange
4	Powder + 1N Hydrochloric acid	Dark Green	Yellow Green	Dark Green	Brown	Dark Green	Light Green	Dark Green	Light Brown	Brown	Greyish	Brown	Brown
5	Powder + 50% Sulphuric acid	Black	Brown	Black	Brown	Dark Brown	Dark Green	Dark Brown	Light Blue	Dark Black	Black	Dark Brown	Light Green
6	Powder + Nitric acid	Light Yellow	Light Brown	Light Yellow	Reddish-Brown	Light Green	Light Green	Light Green	Light Green	Light Brown	Dark Brown	Light Brown	Light Green
7	Powder + Picric acid	Yellow	Dark Yellow	Yellow	Green	Fluorescent Green	Light Green	Green	Fluorescent Green	Dark Brown	Black	Dark Brown	Green
8	Powder + Acetic acid	Light Brown	Pale Green	Light Brown	Yellow	Fluorescent Green	Light Green	Green	Fluorescent Green	Light Brown	Light Brown	Light Brown	Fluorescent Green
9	Powder+ Ferric Chloride	Light Brown	Light Brown	Light Brown	Light Brown	Dark Green	Light Green	Dark Green	Light Green	Dark Brown	Dark Black	Dark Brown	Green
10	Powder + Nitric acid + Ammonia	Light Brown	Brown	Light Brown	Light Brown	Light Green	Fluorescent Green	Light Green	Light Green	Brown	Light Brown	Brown	Pale Green

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

Functional Group	Appearance	Compound	Peak Value / Frequency range (cm^{-1})	Gc	Gl	Gm	Gs
O-H stretching	Strong, broad	Alcohol	3550-3200	3291.79	3351.13	3359.44	3358.97
C-H stretching	Medium	Alkane	3000-2840	2922.92	2919.74, 2851.43	2920.10, 2851.02	2920.54, 2851.40
C-H bending	Weak	Aromatic Compound	2000-1650	-	-	-	1733.98
C=C stretching	Strong	Alkene	1648-1638	-	-	-	1647.76
C=C stretching	Medium	Alkene	1662-1626	-	1635.03	1626.17	-
C=C stretching	Strong	α, β unsaturated ketone	1620-1610	1616.61	-	-	-
N-O asymmetric stretch	Strong	Nitro compound	1550-1475	-	-	1519.50	-
C-C stretching	Medium	Aromatic	1500-1400	-	-	1446.87	-
O-H Bending	Medium	Phenol	1390-1310	1317.62	1383.81, 1317.38	1375.51	1373.70, 1318.49
C-N stretching	Medium	Aliphatic amines	1250-1020	1243.55	1246.72	1244.27	1246.52
S=O stretching	Strong	Sulfoxide	1070-1030	1036.05	1035.26	1065.24	1035.75
C-H Bending	Strong	1,2,3- tridistributed	780±20	-	780.68	-	-
C-Br stretching	Strong	Halo compound	690-515		618.23		
C-I stretching	Strong	Halo compound	600-500	516.13	518.23	517.89	532.09

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 sulphoxide; 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 sulphoxide; 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; 1065.24 cm^{-1} S=O stretching of sulphoxide; and C-I stretching at 517.89 cm^{-1}.

FTIR spectrum of leaf crude drug of *G. sylvestre* (Fig. 5) indicates different compounds in the form of major peaks at 3358.97 cm^{-1} for O-H stretching of alcohol; 2920.54 cm^{-1} and 2851.40 cm^{-1} for C-H stretching of alkane; 1733.98 cm^{-1} C-H stretching of aromatic compound; 1647.76 cm^{-1} for C=C stretching of alkene; 1373.70 cm^{-1} and 1318.49 cm^{-1} for O-H bending of phenol; 1246.52 cm^{-1} for C-N stretching of amine; 1035.75 cm^{-1} S=O stretching of sulphoxide; 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

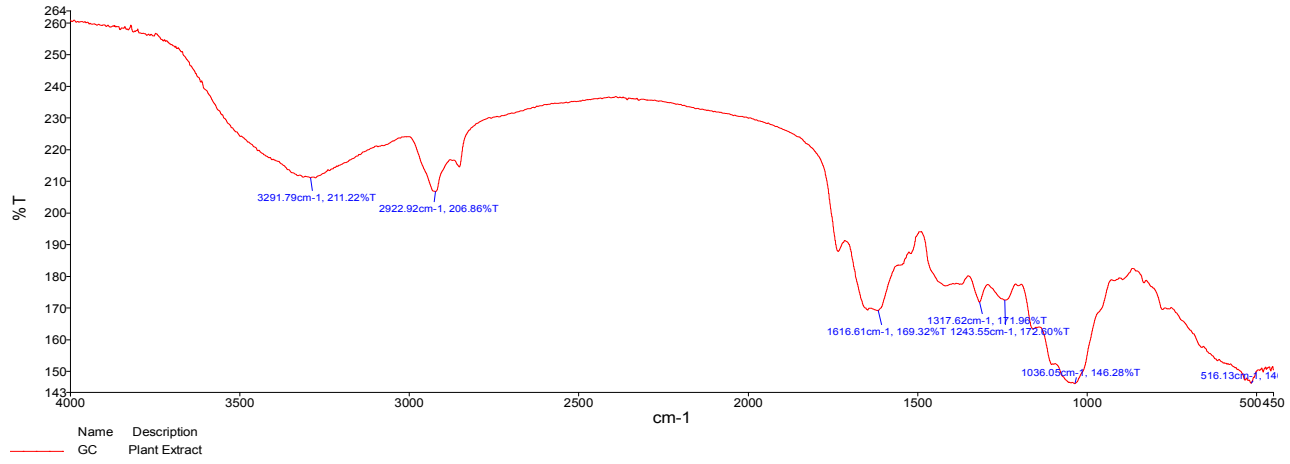

Fig. 2. FTIR spectrum of leaves of *G. cuspidatum*.

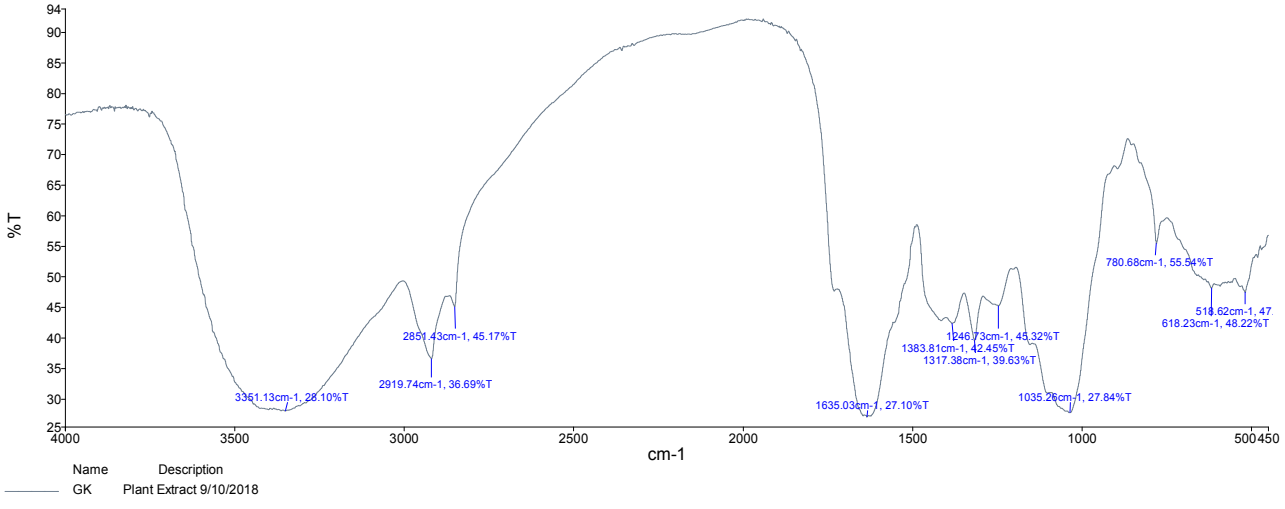

Fig. 3. FTIR spectrum of leaves of *G. latifolium*.

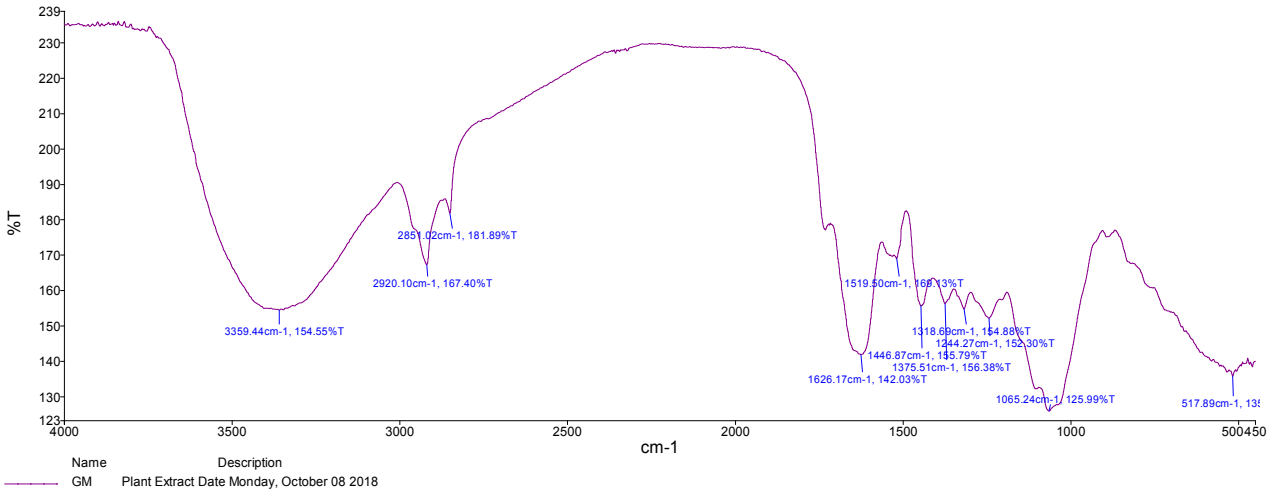

Fig. 4. FTIR spectrum of leaves of *G. montanum.*

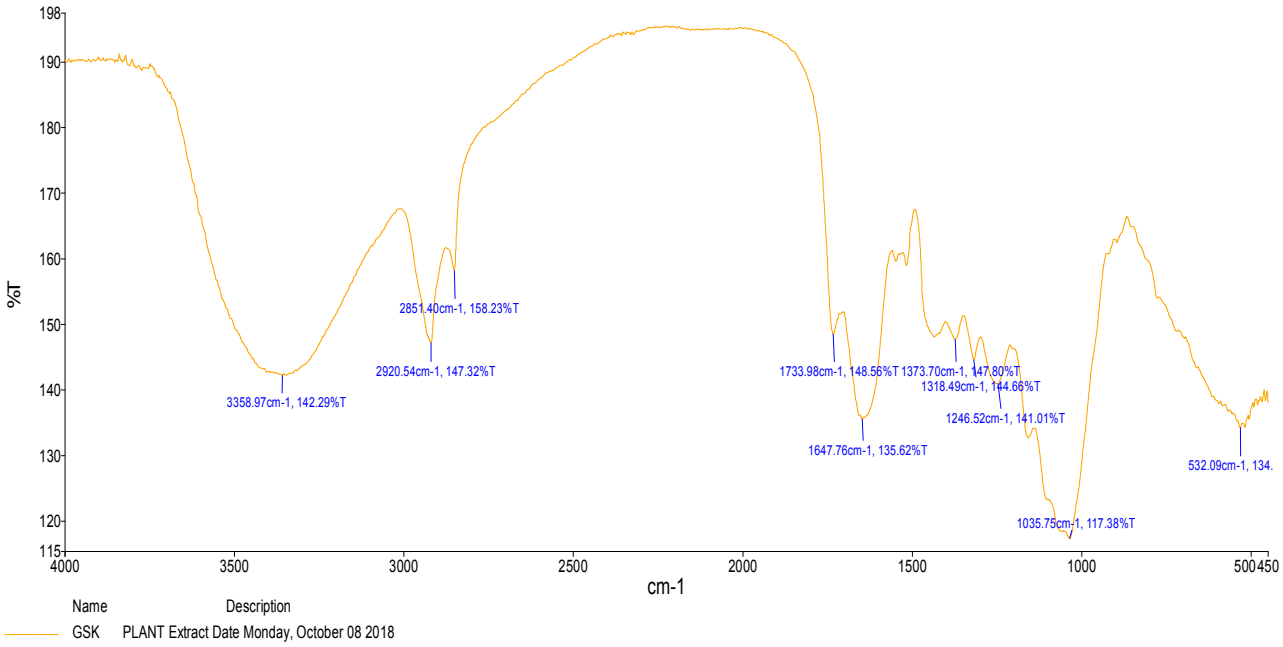

Fig. 5. FTIR spectrum of leaves of *G. sylvestre*.

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 FITR 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.

ii. Powder + Nitric acid + Ammonia → Green fluorescence under 254 nm.

iii. Powder + Ferric chloride → Dark Black colour under 365 nm.

iv. Powder + NaOH (aq.) → Black colour under 365 nm.

FTIR markers

I. Unique peak for alkyl halides (C-Br stretching).

II. Peak for N-O absent.

III. Presence of C=C stretching for alkene.

IV. Unique peak for 1,2,3-tridistributed C-H bending present.

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.

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.

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.

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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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