INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHARMACOGNOSTIC INVESTIGATIONS ON LEAVES OF *GREWIA ASIATICA* LINN.

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Article Received on: 13/04/18 Approved for publication: 29/05/18

DOI: 10.7897/2230-8407.09578

ABSTRACT

Grewia asiatica, locally known as phalsa, is well known for its nutritional and therapeutic attributes. The plant is believed to possess antipyretic, antidiabetic, analgesic, antibiotic and antimicrobial properties. Any plant which possesses medicinal properties should be subjected to detailed pharmacognostic study so that its proper identification can be done and its adulteration with other drugs can be prevented. The main objective of present study was to carry out various pharmacognostic parameters on the leaves of *Grewia asiatica* Linn. Various pharmacognostic parameters such as macroscopic, microscopic, physiochemical and phytochemical were studied on the leaves of *Grewia asiatica* Linn. Microscopic evaluation showed the presence of rectangular shaped epidermal cells with thin, cuticle wall and starch grains, unicellular to multicellular stellate covering trichomes and calcium oxalate crystals. The physicochemical analysis comprises of ash value, moisture content and fluorescence analysis. Phytochemical screening of different solvent extracts of the leaves revealed the presence of alkaloids, flavonoids, glycosides, carbohydrates, saponins and tannins. TLC fingerprinting of petroleum ether, chloroform and methanol extract was carried out to determine the presence of various phytoconstituents. This investigation will be useful in determination of identity, purity and quality of *G. asiatica* plant which will be useful in carrying out research on this plant in future.

Keywords: *Grewia asiatica,* Macro-microscopic, Physicochemical, Phytochemical investigation

INTRODUCTION

In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent[1]. The use of herbal medicine has increased remarkably in line with the global trend of people returning to natural therapies[2]. The plant raw material is susceptible to variation due to several factors, i.e., the identity of the plant, seasonal variation, drying and storage conditions, the genotypic and chemotypic variations[3]. WHO acknowledges that pharmacognostic standards should be proposed as a protocol for the identification of herbal drugs, and authenticated raw material should be the basic starting point for developing a botanical product. The safety and efficacy of herbal products are dependent upon their standardization [4]. Based on the above facts, the present study entailed pharmacognostic characteristics of leaves of *Grewia asiatica.*

Grewia asiatica, locally known as phalsa is well known for its nutritional and therapeutic attributes. Various parts of the plant has been reported for possessing several therapeutic utilities i.e., , hepatoprotective, appetizer, aphrodisiac and in curing inflammation, heart and blood disorder, fever, rheumatism, pustular eruptions, and diarrhoea[5]. *Grewia asiatica* has a high content of antioxidants like vitamin C, total phenolics, flavonoids, tannins and anthocyanins[6]. The leaves of *Grewia asiatica* have been reported to possess various pharmacological activities such as anti-malarial, anti-emetic, anti-platelet, anti-microbial, anti-diabetic and anti-cancer activities[7-10].

MATERIAL AND METHODS

Plant Material

Grewia asiatica leaves were procured from the local area of Amritsar located in Punjab through supplier. The botanical identity of the plant was verified by Department of Botanical and Environmental Sciences (voucher specimen No. 1041), Guru Nanak Dev University, Amritsar (Punjab).

Organoleptic and Microscopic evaluation

Organoleptic and microscopic analysis of leaves was done for estimation of color, odor, taste and internal structure of drug. Microscopic evaluations of tissues were supplemented with micrographs. Photographs of different magnifications were taken with Pentax MV1 microscopic Unit. For normal observations bright field was used.

Physiochemical analysis

The dried powdered leaves were subjected to physiochemical analysis including ash value, loss on drying and fluorescence analysis [11].

Phytochemical Screening

Petroleum ether, chloroform and methanol extracts were prepared by subjecting powdered leaves to Soxhlet apparatus. All the extracts were subjected to preliminary phytochemical screening to detect the presence of various phytochemicals viz. alkaloids, flavonoids, tannins, glycosides, carbohydrates and saponins[12].

TLC fingerprinting

Precoated TLC plates were used for development of chromatogram. Chromatograms were observed in visible light, then under short wave and long wave ultraviolet light.

RESULTS

Organoleptic evaluation

The organoleptic evaluation of leaf of *Grewia asiatica* showed that the leaf has dark green colour having characteristic odour and acrid taste. The size of leaf is 18.5cm in length and 15.2 cm in width and it has cordate to ovate shape. The results of organoleptic evaluation are shown in Table 1.

Powder microscopy

Powder of *G. asiatica* leaves when observed under 10x magnification revealed the presence of rectangular shaped epidermal cells with thin, cuticle wall and starch grains (Figure 1), elongated unicellular to multicellular stellate covering trichome (Figure 2), polygonal and cuboidal calcium oxalate crystals (Figure 3), unicellular covering trichome, multicellular head and unicellular stalk of a glandular trichome (Figure 4).

T.S. microscopy

The thin transverse section of the leaf through midrib revealed the presence of parenchymatous ground tissue containing few cell cavities, upper epidermis was followed by 3-4 layers of collenchyma cells, lignified tissues and vascular bundles. (Figure 5)

Physicochemical evaluation

The air dried powdered leaves of *G. asiatica* were subjected to physical parameters and the results obtained are briefed in the Table 2.

Preparation of Extracts

The percentage yield of each of the four extracts i.e. Petroleum ether, chloroform, methanol and aqueous are calculated and the results obtained are reported in Table 3.

Phytochemical Screening

The phytochemical screening of successive extracts was carried out by using various chemicals and reagents and its results were reported in Table 4. In the present work, preliminary phytochemical screening of the various extracts of leaves revealed the presence of alkaloids, flavonoids, glycosides, tannins carbohydrates and saponins.

Fluorescence analysis

Fluorescence analysis of the air dried powder of *G. asiatica* was carried out by observing it under the day light, short UV (254 nm) and long UV (365 nm) in an ultraviolet chamber. The colors observed in different solvents under different ultraviolet radiations are summarized in Table 5.

TLC fingerprinting

TLC fingerprinting analysis was done for petroleum ether; chloroform and methanol extract (Fig. 6). Results of TLC fingerprinting profiles are shown in Table 6. Thin layer chromatography fingerprinting was done in order to identify the various chemical constituents present in each of them.

Table 1: Organoleptic features of leaves of *G. asiatica*

Condition	Leaf
Color	Dark green
Odour	Characteristic
Taste	Acrid
Size	18.5 cm in length and 15.2 cm in width
Shape	Ovate with coarsely toothed margin, pointed at apex and oblique base

Table 2: Physical parameters of leaves of *G. asiatica*

S.NO	Physical parameter	% (with reference to air dried drug)
1.	Total ash	2.43
2.	Acid insoluble ash	0.19
3.	Water soluble ash	2.24
4.	Loss on drying	12

Table 3: Physical appearance and percentage yield of extracts

Extract	%yield (w/w)
Petroleum ether	9.47
Chloroform	8.21
Methanol	17.68
Aqueous	13.47

Table 4: Results of preliminary phytochemical screening of extracts

Test reagent used	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
1. Alkaloids				
Mayer's reagent	-	+	-	-
Hager's reagent	-	+	-	-
Wagner's reagent	-	+	-	-
2. Carbohydrates				
Molish reagent	-	-	+	+
Fehling solution A	-	-	+	+
Fehling solution B	-	-	+	+
Benedict solution	-	-	+	+
3. Proteins				
Biuret test	-	-	-	-
Millon's test	-	-	-	-
4. Phytosterols				
Libermann– Burchard s test	+	+	-	-
Salkowski test	+	+	-	-
5. Saponins				
Foam test	-	-	+	+
6. Flavonoids				
Lead acetate test	-	-	+	+
Shinoda test	-	-	+	+
NaOH test	-	-	+	+
7. Glycosides				
Borntrager test	-	-	+	+
Keller – Killani test	-	-	+	+
Legal' s test	-	-	+	+
8.Tannins				
Ferric chloride test	-	-	+	+

Table 5: Results of fluorescence analysis of leaves of *G. asiatica*

S.NO	Chemical treatment	Short UV (254nm) Fluorescence	Long UV (365nm) Fluorescence	Visible /day light (color
1.	Powder + petroleum ether	Nil	Nil	green
2.	Powder +conc. Nitric acid	Nil	Nil	brown
3.	Powder +conc. Ammonia	Nil	Nil	green
4.	Powder + conc. HCL	Nil	Nil	green
5.	Powder +conc. Sulphuric acid	Nil	Nil	Yellowish green
6.	Powder + methanol	Nil	Nil	green
7.	Powder + chloroform	Nil	Nil	green
8.	Powder + acetic acid	Nil	Nil	Yellowish brown

Table 6: Results of TLC fingerprinting of extracts

Extract	Mobile phase	No. of spots
Petroleum ether extract	Hexane: Ethyl acetate (8:2)	5
Chloroform extract	Hexane: Ethyl acetate (9:1)	2
Methanol extract	Ethyl acetate: hexane (9:1)	5

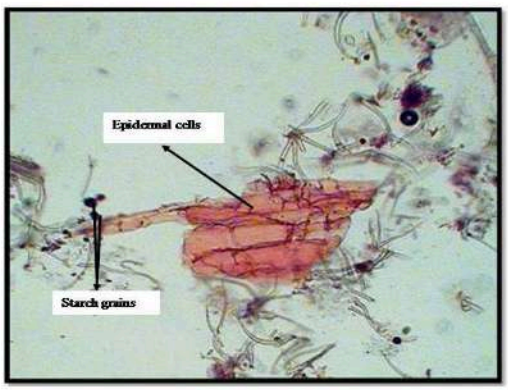

Figure 1: Powder microscopy showing epidermal cells and starch grains

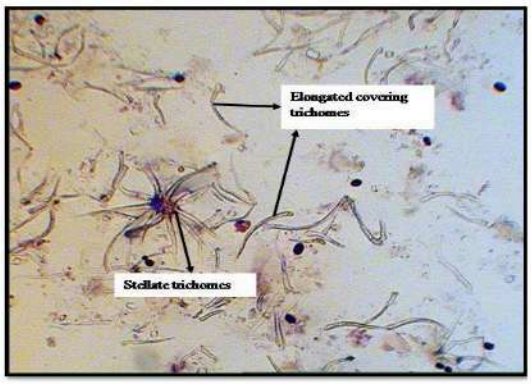

Figure 2: Leaf powder microscopy depicting stellate covering trichomes and elongated covering trichomes

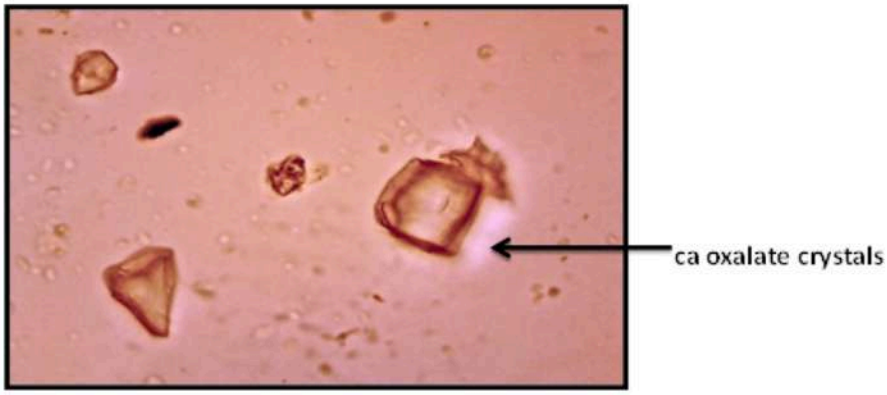

Figure 3: Leaf powder microscopy showing cuboidal calcium oxalate crystals

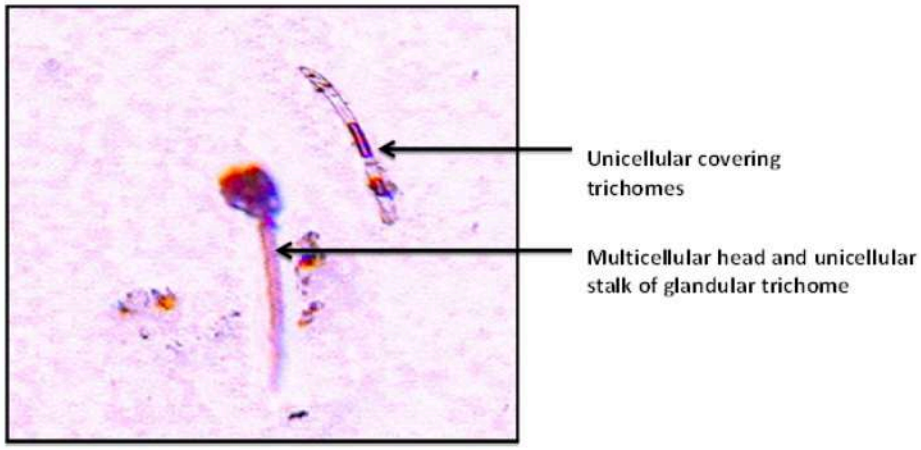

Figure 4: Leaf powder microscopy showing unicellular covering trichome and multicellular head and unicellular stalk of glandular trichomes

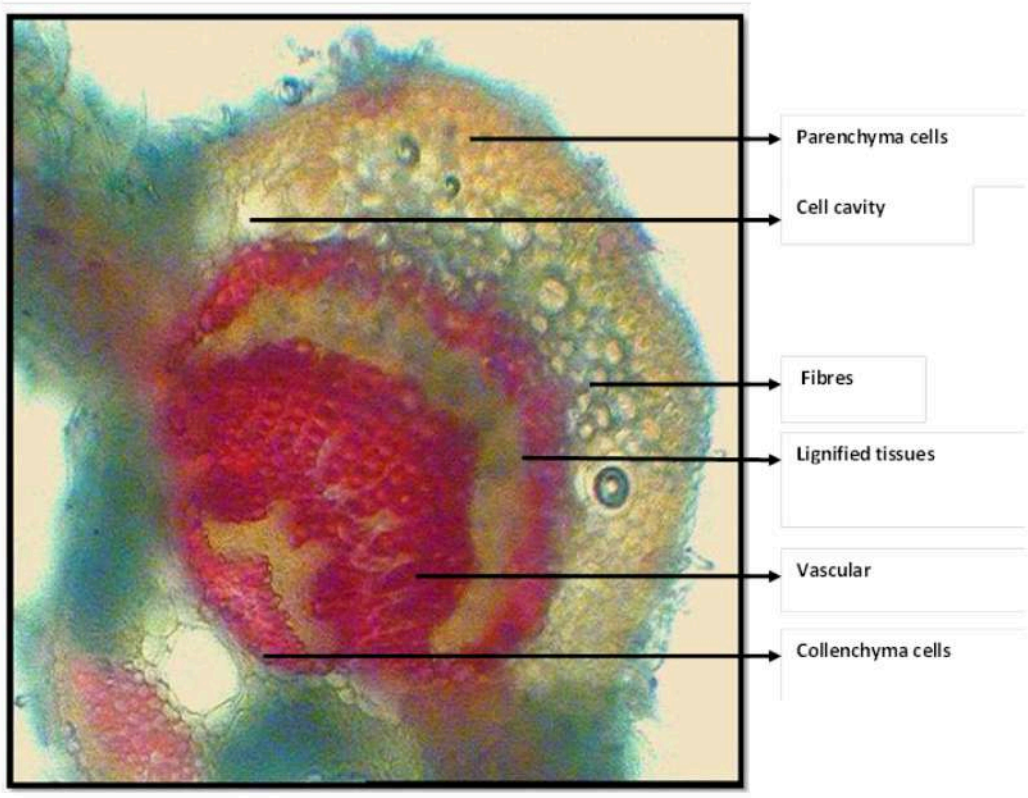

Figure 5: T.S of the leaflet through midrib along with parenchyma cells, collenchyma cells, vascular bundles, lignified tissue

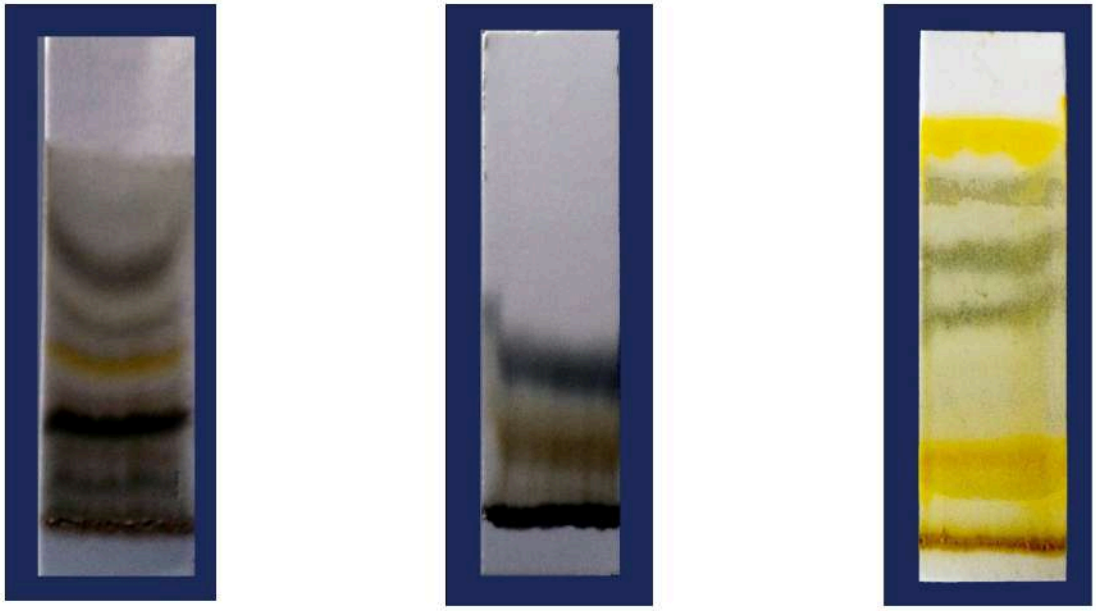

Figure 6: TLC fingerprint profile of A- petroleum ether, B- chloroform and C- methanol extract

DISCUSSION

Standardization of crude drug is an integral part of its correct identity. The results of these investigations could therefore serve as an important tool towards proper identification, collection and investigation of *G. asiatica*. The micromorphological features of leaves, which are the inherent diagnostic features of a plant, create a confirmed discrepancy from other members of genera. Physical evaluation is also required in standardization and is important towards the judgement of identity, purity and quality of plant. Different chemical compounds such as tannins, flavonoids and glycosides were detected in this plant implying towards its broader use for health care. The study of various pharmacognostic parameters, i.e., ash value, moisture content, fluorescence analysis, phytochemical screening and TLC fingerprinting will be helpful in authentication of the plant *G. asiatica*. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is cost effective and convenient approach. Hence, the present investigation will help in ensuring reproducible quality of the plant.

CONCLUSION

The present study will be helpful in determination of quality, purity and efficacy of the plant G. asiatica. These pharmacognostic parameters will ensure plant identity; lay down standardization parameters which will be helpful in preventing adulteration of genuine species. This investigation will also be useful in utilization of this ethno medicinal plant for development of new herbal products and drug discovery. Further studies on pharmacological activities and isolation of phytoconstituents from this promising plant will help in discovery of new chemical entities.

ACKNOWLEDGMENT

The authors are grateful to Department of Pharmaceutical sciences, Guru Nanak Dev University, Amritsar for providing the financial assistance.

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Cite this article as:

Shabnampreet Kaur *et al*. Pharmacognostic investigations on leaves of *Grewia asiatica* Linn. Int. Res. J. Pharm. 2018;9(5):85-90 http://dx.doi.org/10.7897/2230-8407.09578

Source of support: Guru Nanak Dev University, Amritsar, India, Conflict of interest: None Declared