Open access Journal International Journal of Emerging Trends in Science and Technology
DOI: https://dx.doi.org/10.18535/ijetst/v7i2.02

Pharmacogostical and Phytochemical Evaluation of *Grewia Asiatica* Linn. Leaves

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Abstract
Preliminary phytochemical study of G. asiatica Linn. leaf showed water soluble extractive (19.08 %) more than alcohol soluble extractive (12.40%). It also demonstrated pH, moisture content, total ash, water soluble ash, acid in soluble ash and sulphated ash 6.48, 28.18%, 2%, 1.75%, 0.6% and 5.65% respectively.Qualitative chemical examinations of ethanol and aqueous leaf extracts of G. asiatica Linn. revealed the presence of Carbohydrates, Flavonoids, Steroids, Glycosides, and Tannins & Phenolics. Successive petroleum ether and chloroform extract showed presence of steroids and glycosides. Aminoacids and Alkaloids were absent in all the extract.

Introduction:

Synonyms: *Grewia subinaequalis* DC.

Biological Source[1]: Drug consists of dried whole plant of *Grewia asiatica* Linn. belonging to family Tiliaceae.

Part used: bark, fruits, leaves[2]

Plant of *Grewia asiatica* Linn.

Vernacular names[3]

Sanskrit : Dharmana, Parusha

Bengali : Shakri, Phalsa

English : Phalsa

Gujrati : Phalsa

Hindi : Phalsa

Malayalam : Sataschi

Marathi : Daman, Damni, Karavarani

Tamil : Tadachit, Sadachi,Una, Tarra

Telugu : Phutiki, Charachi, Ettatada, Nulijana

Punjabi : Phalna, Pharua

Description[2,3]

A shrub or small tree, young parts stellately pubescent.

- **Bark:** Rough and gray.
- **Leaves:** Leaves are 7-17/6-12 cm, ovate or suborbicular, acute or subacuminate or cuspidate, sharply and often coarsely doubly serrate, subglabrous above, hairy-tomentose beneath, rounded or only slightly cordate at the base 5-6-7 nerved; petioles 6-12 mm long, thickened at the top; stipules nearly as long as the petioles, linear, lanceolate.
- **Flower buds:** Flower-buds broadly cylindric or clavate. Peduncles axillary, usually many, long, slender, far exceeding the petioles and often 3-4 times as long, sometimes 4 cm long.
- **Flowers:** Flowers large. Bracts beneath the pedicels lanceolate. Sepals about 10 cm. long, linear oblong, acute, stellately pubescent or tomentose. Petals yellow, oblong or ovate-oblong, jagged or entire, about 6 mm. long, not bifid, gland with a wide fleshy margin, pubescent towards the edges. Gonophore long. Stigma with 4 short, rounded lobes; style much thickened above.
- **Fruit:** Fruit red, globose, 6-8 mm. diameter; pyrenes 1-2, always 1- celled only.

Habitat: Drier woodlands and on most soils as well as drier vine thicketsand coastal regions".

Materials And Method:[4,5,6,7,8,9,10]

COLLECTION OF PLANT MATERIAL

Aerial parts of *Grewia asiatica* Linn. herbs growing in natural habitat in Rampura, Panchmahal, Gujarat, India, were collected in June, 2018.

Pharmacognostic Evaluation

Morphology:

The morphology or macroscopical description of a crude drug include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc.

Microscopic Evaluation

a) Transverse section of the leaf.

The Transverse sections were taken by placing the leaf portion cut along with the midrib in between the two flat surfaces of pith. Pith is usually piece of potato (about 3 X 1 X 1 cm) in which the longitudinal slit 2 cm deep was made, in to which the leaf was placed and section were taken as described below.

Using sharp razor held in the right hand, thin section was made the razor across the object in quick successions. Transferred the sections in to watch glass containing water, added chloral hydrate to these sections, boiled, filtered and the sections were stained with phloroglucinol & hydrochloric acid (1:1) and the same mounted in glycerin and observed under low power.

b) Powder microscopy

The coarse leaf and fruit powder was boiled with chloral hydrate for 5 minutes, and then stained with phloroglucinol and HCL (1:1) and observed for the microscopic features under high power (40 x).

Ash values

Total ash: Accurately weighed 2 g of the ground air dried material was placed in a previously ignited and tarred crucible (usually platinum or silica). Then the material was spread in an even layer and ignited it by gradually increasing the heat to 500- 600ºC (480ºC) until it was white, indicating the absence of carbon. It was cooled in desicator and weighed. If carbon free ash cannot be obtained in this manner, the crucible was cooled and residue was moisten with 2 ml of water or a saturated solution of ammonium nitrate. Then it was dried on a water bath or on a hot plate and ignited to constant weight. The residue was allowed to cool in desicator for 30 minutes, and it was weighed without delay. The percentage of total ashwas calculated with reference to air-dried plant material.

Acid insoluble ash: In the crucible containing the total ash, 25 ml of hydrochloric acid was added and covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and added this liquid to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible. It was dried on a hot plate and ignited to constant weight. The residue was allowed to cool in desicator for 30 minutes, and it was weighed without delay. The percentage of acid insoluble ash was calculated with reference to air-dried plant material.

Water soluble ash: In the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a

sintered-glass crucible or on an ash less filter paper and washed with hot water. The filter paper containing the insoluble matter was transferred to the original crucible and ignited in a crucible for 15 minutes at a temperature not exceeding 4500˚C. The residue was allowed to cool in desicator for 30 minutes, and it was weighed without delay. The weight of this residue was subtracted from the weight of total ash. The percentage of water soluble ash was calculated with reference to air-dried plant material.

Sulphated ash: Heated silica crucible to redness for 10 minutes; allowed to cool and weighed. Placed

one gram of air-dried powder in silica crucible, moistened with sulphuric acid, ignited gently again moistened with sulphuric acid and ignited at about 800^{0}C cooled and weighed, once again ignited for 15 minutes and weighed. The percentage of sulfated ash was calculated with reference to air-dried powder.

Extractive values

The determination of Extractive values helps to determine the amount of soluble constituents in a given amount of medicinal plant material, when extracted with solvents.

The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample.

Alcohol soluble extractive: Accurately weighed 4.0 g of coarsely powdered air dried material was placed in a glass-stoppered conical flask and macerated with 100 ml of the alcohol (ethanol or methanol) in closed flask for 24 hrs. It was shaken frequently for the first 6 hrs and allowed to stand for 18 hours. Then it was filtered rapidly taking care not to lose any solvent and then transferred 25 ml of the filtrate to tarred flat-bottomed dish and evaporated to dryness on water bath. It was dried at 105^{0}C for 6 hours, cooled in a desicator for 30 minutes and weighed without delay. The percentage of alcohol-soluble extractive was calculated with reference to air- dried drug.

Water soluble extractive: Accurately weighed 4.0 g of coarsely powdered air dried material was placed in a glass-stoppered conical flask and macerated with 100 ml of the water in closed flask for 24 hrs. It was shaken frequently for the first 6 hrs and allowed to stand for 18 hours. Then it was filtered rapidly taking care not to lose any solvent and then transferred 25 ml of the filtrate to tarred flat-bottomed dish and evaporated to dryness on water bath. It was dried at 105^{0}C for 6 hours, cooled in a desicator for 30 minutes and weighed without delay. The percentage of water-soluble extractive was calculated with reference to air-dried drug.

Total solid content

An accurately weighed quantity of the shade-dried of powder was taken in a tarred glass bottle and the initial weight was taken. The crude drug was heated at 105^{0}C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of the sample was calculated in percentage with reference to the shade-driedmaterial.

Determination of pH

The pH value of a solution was determined potentiometrically by means of a glass electrode, a reference electrode and a digital pH meter. The pH meter was operated according the manufacturer's instructions. First the apparatus was calibrated using buffer of 4, 9 and 7 pH. 1 g powdered extracts or 1 tablet or

capsule was taken and dissolved in 100 ml dimeneralized water. The electrodes were immersed in the solution and measured the pH.

Qualitative Phytochemical Evaluation

Extration of phytoconstituents

Leaves of *G. asiatica* Linn. were collected, shad dried and powdered first. Then 200g powder of leaf constituents of *G. asiatica* Linn. Were extracted by successive extraction using Petroleum ether (40-60), chloroform and ethanol successively by soxhlet apparatus.100g leaf powder are macerated with chloroform water for aqueous extraction for 7 days. The extracts were filtered, concentrated and air dried, weighed and percentage yield was determined.

Qualitative chemical identification

Qualitative chemical tests for identifying various phytoconstituents present were carried out on various extracts and powder *of G. asiatica* Linn. Leaf as follows.

1) **Tests for Alkaloids**

- **Mayer's Test(Potassium mercuric iodide solution):** To extract/sample solution, add few drops of Mayer's reagent, creamy white precipitate is produced.
- **Dragendroff's Test(Potassium bismuth iodide solution):** To extract/sample solution, add few drops of Dragendroff's reagent, reddish brown precipitate is produced.
- **Wagner's Test(Solution of Iodine in Potassium Iodide):** To extract/sample solution, add few drops of Wagner's reagent, reddish brown precipitate is produced.

2) **Tests for Glycosides**

- **General Test:** Extract 200 mg of the drug using 5 ml of dilute (10%) sulphuric acid and boil on water bath. After boiling add equal volume of water to the volume of NaOH used in the above test. Add 0.1 ml of Fehling's A and B until alkaline (red litmus changes to blue) and heat on water bath for two minutes. Note the quantity of the red precipitate formed represents the glycoside after acid hydrolysis.
- **Cardiac glycosides (Keller-kiliani Test):** The sample drug was treated with 1ml mixture of 1 volume of 5% $FeCl_3$ solution and 99 volume of glacial acetic acid. To this solution few drops of concentrated H_2SO_4 was added. Appearance of greenish blue color within few min. indicated the presence of cardiac glycosides.
- **Anthracene glycosides (Borntrager Test):** Boil 200 g of the test material with 2 ml of dilute H_2SO_4 in test tube for 5 min centrifuge or filter while hot, take supernatant/filtrate, cool and shake with an equal volume of dichloromethane. Separate the lower dichloromethane layer and shake with half its volume of dilute ammonia. A rose-pink to red colour indicate the presence of anthracene glycosides.

3) **Tests for Flavonoids**

❖ **Shinoda Test(Magnesium Hydrochloride reduction test):** To the extract solution add few fragments of magnesium ribbon and concentrated Hydrochloric acid drop wise, pink scarlet or crimson redcolor appears after few minutes.

4) **Test for Saponins**

❖ **Frothing Test:** The sample drug was vigorously shaken with distilled water and was allowed to stand for 10 minutes and classified for Saponin content as follows: Stable froth more than 1.5 cm indicated the presence of Saponin.

5) **Test for Tannins**

❖ **With $FeCl_3$:** The water extract of the sample drug was treated with alcoholic $FeCl_3$. Blue color indicated the presence of tannins.

❖ **With lead acetate:** With 5% lead acetate solution tannins give precipitate which turns red on addition of KOH solution on excess addition precipitate is dissolved.

6) **Tests for Sterols and Triterpenoids**

❖ **Libermann-Burchard test(10 ml acetic anhydride+10 ml conc. H_2SO_4+100 ml absolute alcohol):** Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from the side of the test tube, A brown ring at the junction of two layers and the upper layer turns green indicates the presence of sterols and formation of deep red color indicates the presence oftriterpenoids.

❖ **Salkowski's test:** Treat extract in chloroform with few drops of concentrated Sulfuric acid, shake well and allow to stand for some time, red color appears in the lower layer indicates the presence of sterols and formation of yellow colored lower layer indicating thepresence of triterpenoids.

7) **Test for Carbohydrates**

❖ **Molisch's test(α-nepthol in 95% alcohol):** Test solution with few drops of Molisch's reagent and 2ml of concentration sulphuric acid is added from the sides of the test tube, shows a purple ring at the junction of two liquids.

❖ **Barfoed's test:** Test solution treated with Barfoed's reagent on boiling on a water bath shows brick red precipitate.

❖ **Benedict's test:** Test solution treated with Benedict's reagent on boiling on a water bath shows reddish brown precipitate.

❖ **Fehling's test:** Equal volume of Fehling's A ($CuSO_4$ in distilled water) and Fehling's B (Potassium sodium tartarate + NaOH in distilled water) solutions was mixed and boiled for one minute. Add equal volume of test solution, heat on water bath for 5-10 minute. Yellow colour or

red precipitates indicates the presence of Sugar.

8) **Tests for Proteins**

- **Biuret Test:** Test solution treated with 40% sodium hydroxide and dilutes copper sulphate solution gives blue color.
- **Ninhydrin test(0.1% solution in n-butanol):** Test solution treated with ninhydrin reagent gives blue colour.

RESULT AND DISCUSSION:

Morphological evaluation

Table 1: Morphology of *G. asiatica* Linn. leaf

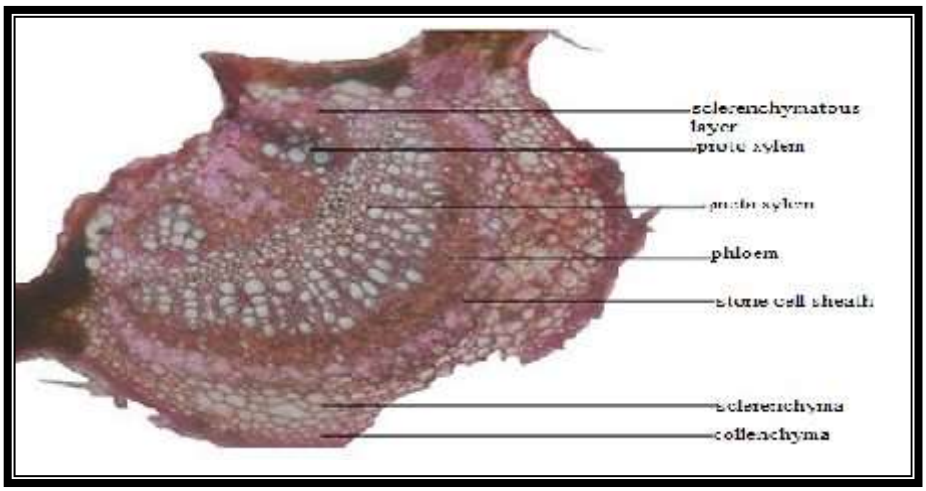
Sr. No	Features	Observation
1	Upper surface	Dark green
2	Lower surface	Light green
3	Odour	Aromatic when crushed
4	Shape	Variable in shapes like ovate or suborbicular, broadly cordate to ovate with oblique base
5	Size	7-17 cm long and 6-12 cm wide
6	Texture	Hairy
7	Colour of leaf	Green colour

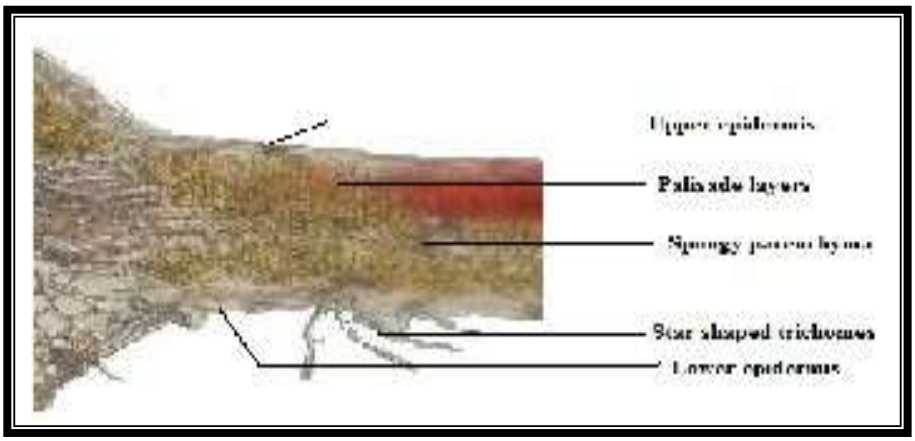
Table 2: Botanical evaluation of *G. asiatica* Linn. Leaf

Sr. No.	Leaf Portion	Observation
1	Apex	Acute
2	Margin	Serrate
3	Arrangement	Alternate
4	Leaf type	Simple
5	Venation	Reticulate
6	Midrib	Pale & many horizontal veins distinct
7	Surface	Hairy-tomentose
8	Petiole	Lanceolate
9	Lamina	Ovate
10	Base	Symmetric

Microscopic evaluation:

Transverse section of leaf





T.S of Midrib and Lamina region of *G. asiatica* Linn. Leaf

Transverse section of leaf through midrib shows single layered cuticularised papillose, upper and lower

epidermis with unicellular to multicellular stellate covering trichomes. Upper epidermis is followed by 3-4 layers of collenchyma having few pitted cells, 2 patches of chlorenchyma at the two ends of the collenchyma. Parenchymatous ground tissue contains few cell cavities. Two small vascular bundles are present at the two ends of the midrib towards the upper surface and a large central crescent shaped vascular bundle towards the lower surface having xylem surrounded by phloem. Bundles possess a sclerenchymatous bundle sheath. Mesophyll consists of 3-4 layers of palisade. Collenchyma and bundle sheath possess mucilage cells. Large subepidermal cells contain polygonal, solitary crystals at intervals. Cortex is parenchymatous having canals. Vascular cylinder consists of several vascular bundles enclosed by bundle sheath of phloem fibres. Medulla is composed of parenchymatous cells with few canals and pitted cells. Solitary or groups of starch grains are present in parenchyma cells.

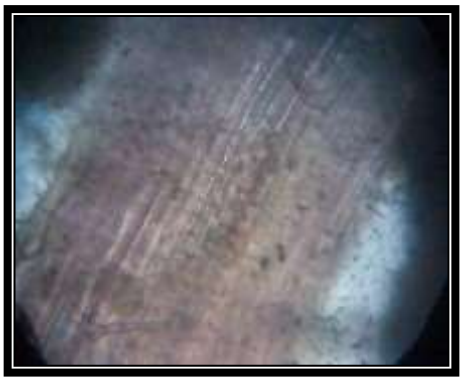
Powder study

Powder study of leaf of *G. asiatica* Linn. shows characteristic star shaped unicellular lignified trichomes, epidermal cells, anomocytic stomata, spiral shaped lignified xylem vessels, stone cells and square shaped calcium oxalate crystals.

Table 3 Powder study of *G. asiatica* Linn. leaf

Sr. No.	Features	Observation
1	Nature	Coarse powder
2	Colour	Greenish
3	Odour	Aromatic
4	Taste	Characteristic
Microscopic		
5	Stomata	Anomocytic
6	Trichomes	Star shaped unicellular covering trichomes
7	Xylem vessel	Lignified spiral shaped
8	Calcium oxalate crystal	Square shaped
9	Stone cell sheath	Irregular shaped compactly pecked

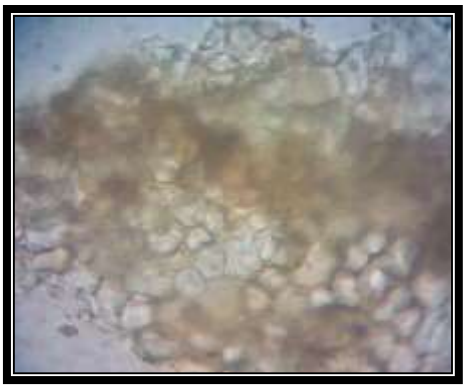
a) Star shaped trichome



b) Xylem vessels

c) Anomocytic stomata

d) Calcium oxalate crystal



e) Stone cells

f) Epidemal cells Powder

study of *G. asiatica* Linn. Leaf

Extractive Values and Ash Values

Table 4 : Physical Parameters of *G. asiatica* Linn. leaf

	leaf
Extractive value	
Water soluble (%w/w)	19.08 ± 0.26
Alcohol soluble(%w/w)	12.40 ± 0.14
pH	6.48
Moisture content(%w/w)	28.18 ± 0.45
Ash values (%w/w)	
Total ash	2.00 ± 0.34
Water soluble ash content	1.75 ± 0.18
Acid insoluble ash content	0.60 ± 0.19
Sulphated ash content	5.65 ± 0.22

Preliminary study of *G. asiatica* Linn. leaf showed water soluble extractive (19.08 %) more than alcohol soluble extractive (12.40 %). It also demonstrated pH, moisture content, total ash, water soluble ash, acid in soluble ash and sulphated ash 6.48, 28.18%, 2%, 1.75%, 0.6% and 5.65% respectively.

EXTRACTION AND PHYTOCHEMICAL INVESTIGATIONS

The powder of *G. asiatica* Linn. leaves were successively extracted with Petroleum Ether, Chloroform, Ethanol by soxhlet apparatus & Maceration with Chloroform water. The results are described in Table no 5

Table 5: Extractive values and physical characteristics of *G. asiatica* Linn. leaves extracts

Extract	**Extractive value (%)**	**Colour**	**Odour**	**Consistency**
Successive extraction of leaves				
Pet. Ether (40-60^{0}C)	06.75	Green	Characteristic	Semisolid
Chloroform	08.45	Dark green	Characteristic	Semisolid
Ethanol	12.60	Dark green	Characteristic	Semisolid
Aqueous	09.55	Brown	Characteristic	Semisolid

The petroleum ether *G. asiatica* leaf extract was semi-solid, green and the yield was 6.75%,

chloroform and ethanolic extracts were semi-solid, dark brown and the yield were 08.45% and 12.60% respectively and aqueous extract was semisolid, brown and the yield was 9.55%.

Qualitative chemical analysis of various extracts of *G. asiatica* Linn. Leaves.

Successive extract of *G. asiatica* Linn. Leaves were screened for various chemical investigation and results are illustrated in Table 6.

Table 6: Qualitative chemical analysis of various extracts of *G. asiatica* Linn.leaves

Nature	Pet. Ether	Chloroform	Ethanol	Aqueous
Alkaloids	-	-	-	-
Carbohydrates	-	-	+	+
Flavonoids	-	-	+	+
Amino acids	-	-	-	-
Steroids	+	+	+	+
Triterpenoids	-	-	-	-
Glycosides	+	+	+	+
Tannins & Phenolics	-	_	+	+

Qualitative chemical examinations of ethanol and aqueous leaf extracts revealed the presence of Carbohydrates, Flavonoids, Steroids, Glycosides, and Tannins & Phenolics. Successive petroleum ether and chloroform extract showed presence of steroids and glycosides. Amino acids and Alkaloids were absent in all the extracts.

REFERENCES:

1. Nadkarani KM, "Indian Materia Medica with ayurvedic, unani tibbi, siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & Indexes", Popular Prakashan Pvt. Ltd., Mumbai 1997; 1:593.
2. Prof. Mrs. Asima Chatterjee, Dr. Satyeshchandra Pakreshi, "The Treatise on Indian medicinal plants", Publications & information directorate, New Delhi 1992, 2:168-170.
3. Kirtikar KR and Basu BD, "Indian Medicinal Plants", International book distributors, Dehradun, India 1999; 13:388-389.
4. Evans WC and Trease, "Text Book of Pharmacognosy" 2002; 15th ed.; ELBS London.
5. Iyengar MA, "Pharmacognosy Lab Manual", Nirali Prakashan Pune 1998.
6. Khandelwal, "Practical Pharmacognosy" 1995, Nirali Publications Pune; 1st ed.
7. Ali R and Rab F, "Research needs and new product development from underutilized tropical fruits",

International society of Horticulture Science, XXV International Horticulture congress, ISN 518; Part 8.

8. "Quality controls methods for medicinal plant materials", World Health Organization, Geneva AITBS publisher and distributors, Delhi 2002; 28-30,38-40,64-73.
9. "Indian Pharmacopoeia", The Controller of Publication, Delhi 1996; Vol-2:A- 95, 736,A-81-83.
10. Khandelwal, "Practical Pharmacognosy" 1995; Nirali Publications Pune; 1st Edition: 146, 14