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Pharmacopoeial Standardization of *Hibiscus rosa sinensis* Linn.

Gupta V[1*], Bansal P[1], Garg A[2], Meena AK[1]

[1]*National Institute of Ayurvedic Pharmaceutical Research, Patiala (Punjab), India*
[2]*Department of Pharmacognosy, S. B. S. College of Pharmacy, Patti, Punjab, India*

ABSTRACT
The present communication attempts to investigate pharmacognostical and phytochemical details of *Hibiscus rosa-sinensis,* Linn. (*Malvaceae*). Results of microscopic studies of leaf show chained midrib of leaf, small and numerous epidermal cells, calcium oxalate crystals and absence of trichomes on both upper and lower surface. The Preliminary phytochemical analysis revealed presence of carbohydrates, alkaloids, flavonoids, saponins, proteins and amino acids in chloroform and alcoholic extract out of six extraction solvents used for these studies. HPTLC studies reveal that alcoholic extract gives 8 spots and chloroform extract depicts 5 spots on the TLC plate. Powdered drug analysis after treatment with 17 different reagents emitted various colour radiations under UV and visible light which may provide a lead in identification of the drug in powder form. The study revealed specific identities for *Hibiscus rosa- sinensis,* Linn which may play a key role in identification of plant and can be useful in standardization of the herbal drugs.

Keywords: *Hibiscus rosa-sinensis,* pharmacognosy, phytochemical, HPTLC fingerprint, *Malvaceae*.

INTRODUCTION

Medicinal plants have been used in virtually all cultures as a source of medicine, since times immemorial. Herbal Medicine is still the mainstay of health care in several developing countries. The widely used herbal remedies and health care preparations as described in ancient texts such as the Vedas and the Bible are obtained from commonly used traditional herbs and medicinal plants. The medicinal properties of these botanicals are being better understood and are attributable to the phytochemicals that specific plants contain. The efficacy and safety of herbal products therefore rely on the quality and proper identification of the raw material or the original plant source. One major obstacle that might impair the potential use of traditional medicine as medicine of choice is the lack of standardization. Adulterations and substitutions are common in raw material trade of medicinal plants. Unintentional adulterations also exist in herbal raw material trade due to various reasons such as confusion in vernacular names between indigenous systems of medicine and local dialects, lack of knowledge about the authentic plant, non-availability of the authentic plant, similarity in morphology and /or aroma or careless collection. [1] To avoid this accurate authentication is very important to prevent the adulteration of target plant with other plant species. The techniques used in past for the standardization of botanical preparations like HPLC, TLC, HPTLC, UV spectroscopy, mass spectroscopy, gas chromatography, infrared and NMR spectroscopy have limitations because the compositions and relative amount of chemicals in a species varies with growing conditions, harvesting periods, post-harvest processes and storage conditions. This can be misleading if the samples are deliberately adulterated with a marker compound. Also, it is difficult to distinguish closely related species due to similar chemical compounds. [2] Identification of plants with botanical verifications is essential as contamination due to misidentification of plant species or parts is common. Therefore, it becomes necessary to develop more effective, accurate, reliable and sensitive methods for the authentication of herbs. In the present study an effort has been made to establish physicochemical, pharmacognostic, and phytochemical parameters which could be helpful in identification of the authentic plant samples and differentiating it from adulterants.

Hibiscus rosa-sinensis, Linn. (*Malvaceae*) known as China rose is an important medicinal plant. [3] It is an evergreen woody, glabrous, showy shrub 5-8 feet in height. Leaves are bright green, short petiolated, ovate or lanceolate, more or less acuminate; irregularly and coarsely serrated towards the top, entire near base, glabrous on both sides, a few minute stellate hairs on the nerves beneath stipules, lanceolate-subulate and glabrous. Pedicels are axillary, solitary, and longer than the leaves and joined above the middle. Flowers are solitary, axillary, bell shaped, large, 4-6 inches in diameter with pistil and stamens projecting from centre. [4] Leaves are used as emollient, anodyne, and laxative in Ayurveda. [5-6] In South Asian traditional medicine, various

***Corresponding author: Mr. V. Gupta,**
National Institute of Ayurvedic Pharmaceutical Research, Patiala (Punjab), India
E-mail: vikas_4308@rediffmail.com

parts of the plant is used in the preparation of a variety of foods. [7] The flowers have been reported in the ancient Indian medicinal literature to have beneficial effects in heart diseases, mainly in ischemic disease [8] and used in folklore medicine as demulscent, emollient, refrigerant, aphrodisiac, brain tonic and cardio tonic. A decoction of flowers is also useful in bronchial catarrh, menorrhagia, and fertility control. [9-10] The extracts showed hair growth potential [11], anticonvulsive activity [12] and hypoglycemic activity. [13]

MATERIAL AND METHOD

Fresh leaves collected from Botanical garden of S.B.S. college of Pharmacy, Patti, Punjab were preserved in 70 % ethyl alcohol for histological studies. Voucher herbarium sample along with the voucher crude drug sample (voucher number- 0383/S.R.) is preserved at the herbarium of Department of Botanical sciences, GNDU, Amritsar. Botanical identification was carried out using local floras. [14-15] Free hand sections were used for histological studies. Quantitative microscopy was done as per the standard methods described by TE Wallis. [16]

Physicochemical studies like total ash, acid insoluble ash, water soluble ash, pH of 1 % w/v solution of aqueous extract, swelling index and successive extractive values were carried out by soxhlet extraction method as per the WHO guidelines. [17] The fluorescence behavior of the powder drug in the visible light and ultraviolet light were carried out by soaking the powder in different reagent solutions and viewing under the light of required wavelength in a UV chamber. [18-19] Preliminary Phytochemical analysis [18, 20] and high performance thin layer chromatography [21-22] were carried out from shade dried powder as per standard methods.

RESULT AND DISCUSSION

Microscopic Characters

Transverse section of midrib of leaf showed chained, small and numerous epidermal cells. The mesophyll layer is irregular and comprised of 6-7 layers. Cells of parenchyma varied greatly in shape and size and were sometimes, elongated or lobed. The xylem vessels were numerous, very big in size and circular in shape. Phloem vessels were small in size, numerous and circular in shape. Calcium oxalate crystals were dark stained and numerous in mesophyll parenchyma. Trichomes were absent on both upper and lower surface. Transverse section of lamina showed cuticle and thick walled cells in upper and lower epidermis. Epidermal cells were large in size, elongated and compact. Palisade parenchyma showed 3 or 4 layers of large, compact and dark cells. Dark stained crystals were present in mesophyll layer. The spongy mesophyll was wider comprising of 6-8 layers of lobed tightly interconnected cells. Trichomes were absent on both upper and lower surfaces. Vascular bundles had compact parallel rows of xylem vessels and fibres. (Fig. 1, 2)

Physicochemical parameters

The percent of loss on drying, total ash, acid insoluble ash, water soluble ash, pH of 1 % w/v solution of aqueous extract and swelling index has been shown in Table 1. A known quantity of dried leaf powder was extracted in a soxhlet apparatus with petroleum ether (60-80ºC), benzene, chloroform, ethyl acetate and methanol (95 %) and finally macerated with distilled water for 24 hours successively and the % of respective extractive values have been shown in Table 1.

Preliminary Phytochemical Screening

The successive extracts were tested for different constituents. The alcoholic and chloroform extracts revealed presence of alkaloids, glycosides, flavonoids, proteins and amino acids (Table 2).

Table 1: Physicochemical parameters of leaves of *H. rosa-sinensis*

S. No.	Parameters	Average Values
1	Total ash (%)	7.75
2	Acid-insoluble ash (%)	0.75
3	Water soluble ash (%)	6.32
4	pH (1% w/v aqueous extract)	7.92
5	Swelling index	6.33
6	Loss on drying (%)	4.93
Extractive value (%)		
1	Petroleum ether	1.45
2	Benzene	2.60
3	Chloroform	2.80
4	Ethyl acetate	3.20
5	Methanol	15.60
6	Distilled water	5.30

Table 2: Preliminary phytochemical Screening of leaf powder of *H. rosa-sinensis*

S. No	Test	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Methanol	Water
1.	**Alkaloids**	+ ve	+ ve	+ ve	+ ve	+ ve	- ve
2.	**Quinone**	- ve	- ve	- ve	- ve	- ve	- ve
3.	**Coumarin**	- ve	- ve	- ve	- ve	- ve	- ve
4.	**Flavonoids**	- ve	- ve	+ ve	+ ve	+ ve	+ ve
5.	**Steroid**	+ ve	- ve	- ve	- ve	- ve	- ve
6.	**Phenol**	- ve	- ve	- ve	- ve	- ve	+ ve
7.	**Tannins**	- ve	- ve	- ve	- ve	- ve	- ve
8.	**Glycosides**	+ ve	+ ve	+ ve	+ ve	+ ve	- ve
9.	**Terpenoids**	- ve	- ve	- ve	- ve	- ve	+ ve
10.	**Proteins**	- ve	+ ve	+ ve	+ ve	+ ve	+ ve
11.	**Amino acids**	- ve	+ ve	+ ve	+ ve	+ ve	+ ve

Table 3: Ultra-violet analysis of leaf powder of *H. rosa-sinensis*

Treatment	Visible Light	UV(254 nm)	UV (366 nm)
Drug Powder	Light green	Dark green	Blackish Brown
Sulfuric acid (conc.)	Dark green	Black	Black
Sulfuric acid (dilute)	Light brown	Greenish black	Black
Hydrochloric acid (conc.)	Green	Black	Black
Hydrochloric acid (dilute)	Brown	Greenish black	Black
Acetic acid	Brown	Greenish black	Black
Nitric acid (dilute)	Brown	Greenish black	Black
Methanol	Green	Dark green	Black
Ethanol	Brown	Brownish Black	Black
Chloroform	Brown	Dark green	Black
Petroleum ether	Green	Greenish black	Black
Distilled. Water	Brown	Dark green	Black
10% NaOH	Brown	Green	Black
5% Iodine	Greenish brown	Dark green	Black
Picric Acid	Green	Greenish red	Black
$FeCl_3$ solution	Brown	Dark green	Black
Ammonia solution	Brown	Green	Greenish black

HPTLC study

The alcohol and chloroform extracts were used to carry out HPTLC. For alcohol extract solvent system with toluene**:** ethyl acetate**:** acetic acid (9.5**:** 8**:** 5.2) and for chloroform extract toluene: ethyl acetate: acetone (8: 1: 3) was used. Alcoholic extract demonstrated 8 spots of R_f value of 0.03,

0.05, 0.12, 0.23, 0.48, 0.59, 0.64, 0.73 and 0.85 a.(Fig. 3) and chloroform extract showed 5 spots of R_f value of 0.06, 0.20, 0.46, 0.80 and 0.93 (Fig. 4).

Fluorescence Analysis

Powdered drug under UV and visible light when treated with different reagents emitted various colour radiations which help in identifying the drug in powder form. (Table 3)

CONCLUSION

This study presents a set of diagnostic characters of *Hibiscus rosa-sinensis*, Linn. that will help to identify the drug in fragmentary condition as well as in whole form. The results of parameters for preliminary phytochemical screening, UV analysis and HPTLC studies can act as biomarkers for identification and authentification of raw drug samples and play an important role in quality control and prevention of adulteration.

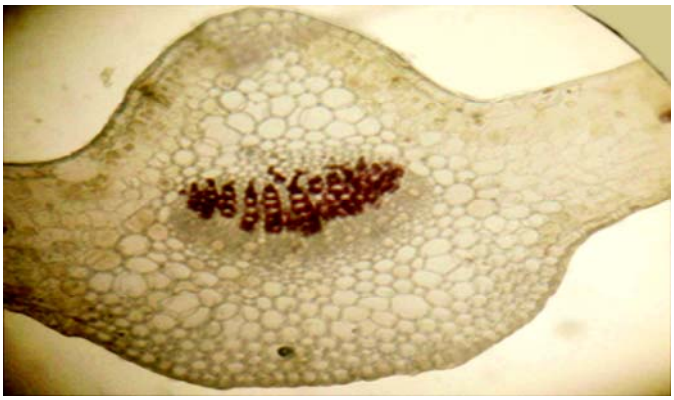

Fig. 1: T. S. of midrib of *H. rosa-sinensis* Leaf

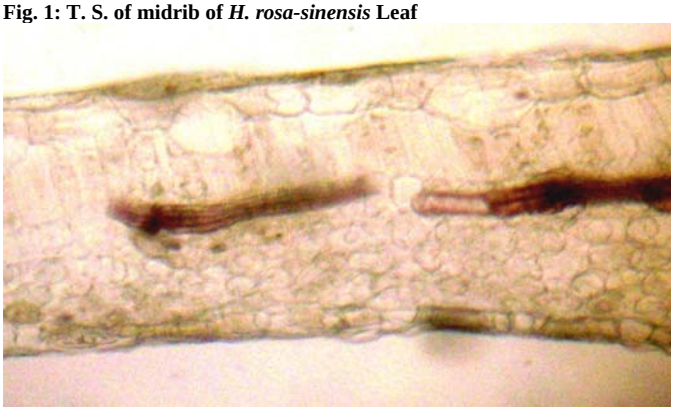

Fig. 2:T. S. of lamina of *H. rosa-sinensis* Leaf

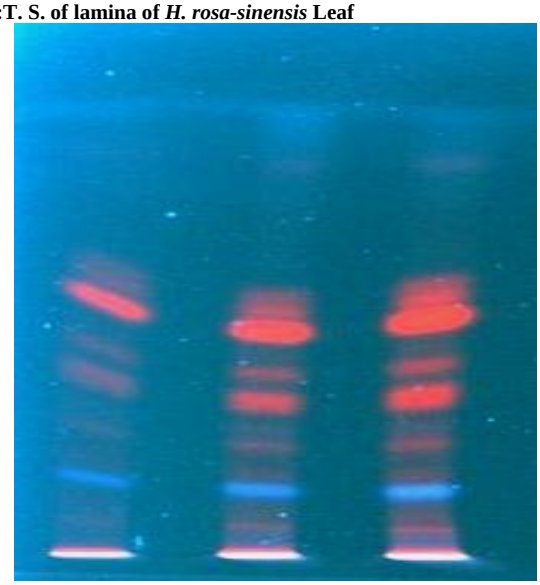

Fig. 3: Alcoholic extract chromatography

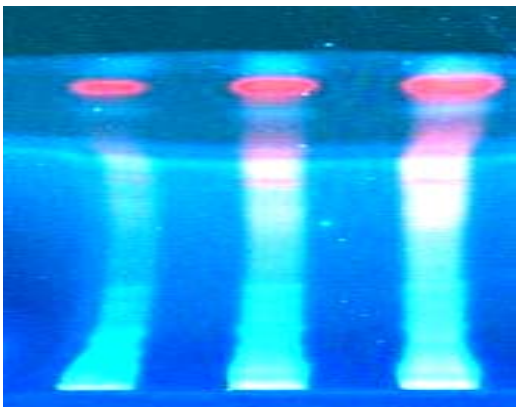

Fig. 4:Chloroform extract chromatograph

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