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Research Article

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Phytochemical Studies of *Grewia umbellifera* in Different Solvent Fractions

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ABSTRACT

The present study attempt to evaluate the phytochemical studies of *Grewia umbellifera* stem crude drug. The phytochemical screening was carried out and the berberine alkaloid was quantified in different fractions of extract by HPLC. The result shows that the methanol extracts have higher concentration of berberine when compared to other solvent fractions. The present study reveals the standardization profile and characterization of berberine compound from *Grewia umbellifera*, which would be of immense value in botanical identification and authentication of plant drug and may help us in preventing its adulteration.

Keywords: *Grewia umbellifera*, berberine, phytochemical, standardization, characterization, HPLC.

ARTICLE INFO

CONTENTS

1. Introduction	134
2. Materials and Methods	135
3. Results and discussion	135
4. Acknowledgement.	137
5. Conclusion	137
6. References	137

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1. Introduction

The use of plant as medicine is an old as human civilization. Herbal medicines have being used by billions of people around the world for thousands of years. Herbal medicine is very popular in different system of medicines like Indian system of medicine, Chinese system of International Journal of Medicine and Pharmaceutical Research

medicine, the most known traditional system such as Ayurveda, Siddha, Unani and Homeopathy were being practiced all over the world and are going demand there years [1]. In recent years, interest on plant-based drugs has increased considerably. According to an estimate, around

80% of the world population still depends on herbal products from about 20,000 plants species, for their basic medicinal and health care requirement. This is emphasized with the toxicity and side effects of the prescribed allopathic medicines and lack of drugs for many chronic ailments [2]. It demands for an urgent need to investigate new herbals leads to overcome the needs. Annual growth rate between 5 – 15% for trade of plant based drugs and raw materials is indicative of growing demand for herbal drugs. But the quality control and quality assurance still remains a challenge because of the high variability of chemical compounds involved. The major problem of quality assurance of herbal medicine has been solved to a great extent with the help of chromatographic and spectral fingerprints analysis [3]. *Grewia umbelliferrea* is one such plant, which is widely used in indigenous system of medicine [4]. It is a large, glabrous, succulent, deciduous climbing shrub belonging to the family menispermaceae[5]. It is distributed throughout tropical India subcontinent, Sri Lanka and china, ascending to an altitude of 1200m. The stem of *Grewia umbelliferrea* is rather succulent with long filiform fleshy aerial roots from the branches. The bark is creamy white to grey, deeply left rosette like lenticels. The leaves are membranous and cordate. The flowers are small and yellow or greenish yellow [6].

Grewia umbelliferea (Tiliaceae) (GU) is herbaceous medicinal plant that has been distributed in Kanniya kumari district, Tamilnadu, India. The aerial plant part extract is much more useful in treatment like spleen damage, liver complications and cardio disorders. [7]. The large numbers of compounds have been isolated from the aerial parts and roots of *Grewia umbelliferrea*. GU is widely used in Ayurvedic system of medicine “Rasayanas” to the immune system and the body resistance against infections. In modern medicine *Grewia umbelliferrea* used for the treatment of general weakness, fever, dyspepsia, dysentery, gonorrhoea, urinary diseases, viral hepatitis and anaemia more recently, the immunomodulatory properties, antioxidant activity, anti-neoplastic, hypoglycemic activities have been reported [8,9]. The aim of the present research work was standardization of physical and chemical analysis and characterization of berberine from *Grewia umbelliferrea* stem extract in different solvent fractions.

2. Materials and Methods

Plant materials:

The stem part of *Grewia umbelliferrea* was collected fresh from Tirunelveli District area in Tamilnadu. The plant stem was authenticated by the Herbarium of Botany Directorate in National Institute of Herbal Science, Plant Anatomy Research Center, Chennai. A voucher specimen (No: TC08) was deposited in the center.

Preparation of plant extract:

The dried powdered stem of *Grewia umbelliferrea* was allowed to pass through SS sieve (20 mesh). It was defatted by treating with petroleum ether (60-80oC) and then extracted to exhaustion (soxhlet) with various solvents like methanol, water, chloroform and the excess solvent was removed under vacuum to get the solid mass.

Chemical materials: Acetonitrile, methanol, chloroform, petroleum ether, and other chemicals were used of AR grade (Merck, Darmstadt, Germany) and standard berberine from Sigma- Aldrich, Steinheim, Germany.

Standardization:

Physicochemical parameters were determined as per guidelines of WHO. Total ash, acid insoluble ash, water-soluble extractive value, alcohol soluble extractive value, heavy metals were determined by using standard procedures [10-12].

Microbial contamination screening:

For the safe use of the drug, microbial count, total yeast, and mould count are within the prescribed WHO limits by analysis through standard procedure [13].

Phytochemical studies: Phytochemical screening of *Grewia umbelliferrea* stem different solvent extract fractions was done for the presence of various phyto constituents by using standard procedure [14-17].

HPLC- conditions [18-20]: Injection volume- 20µl; Flow rate- 0.5ml/ min; Mobile phase- Acetonitril: water (60:40); Detection wave length- 265 nm; Mode- isocratic; Retention time (Rt)- berberine 5.15 min.

3. Results and Discussion

The parameters of powdered *Grewia umbelliferrea* stem like Total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value, heavy metals and microbial contamination was carried out and there result are shown in Table 1. The deterioration time of plant material depends upon the amount of water present in plant materials. If the water content is high, the plant can be easily deteriorated due to fungus. The loss on drying at 105°C in stem was found to be 3.31%. Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. Analytical results showed total ash value content was 17.6%. The negligible amount of acid insoluble siliceous matter present in the plant was 1.16%. The water soluble extractive value indicates the presence of sugar, acid and inorganic compounds. The alcohol soluble extractive value indicates the presence of polar constituents like phenol, alkaloids, steroids, glycosides, and flavonoids and other compounds the result given in Table 2. The various different extract fractions of *Grewia umbelliferrea* stem phytochemical testes result indicate the presence of wide range of phytochemical contents. Alkaloids are heterocyclic indole compounds, which have proved to be having pharmacological properties [21]. The presence of flavonoids and tannins in all the plants likely to be responsible for the free radical scavenging effects observed. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that are as primary antioxidant or free radical scavengers [22]. Flavonoids show anti allergic, anti-inflammatory, anti-cancer activity [23]. The present HPLC method was conducted to identifying and quantifying the berberine from *Grewia umbelliferrea* medicinal plants stem various different extract. Berberine peaks from solutions of various extract like pet- ether; methanol, aqueous and chloroform were identified by comparing their Rt values with these

obtained by chromatography of the standard under the same conditions.

The peaks of Rt 5.15min was observed in the chromatograms obtained from fractions like pet- ether, methanol, aqueous and chloroform, the chromatograms of standards and test samples are shown in Fig. 1 to 5

respectively. The berberine content of fractions of sample – I (pet- ether), sample –II (methanol), sample –III (aqueous), sample –IV (chlo roform) was 0.002% (W/W), 0.223% (W/W), 0.150% (W/W), 0.020% (W/W) respectively. The more amount of berberine was present in methanol extract when compared to other fractions.

Table 1: Physico-chemical parameters of *Grewia umbelliferrea* stem

Parameters	Results
Description	Light brown to dark brown powder
Loss on drying at 105°C (% W/W)	3.31
Total ash content (% W/W)	17.6
Acid insoluble ash (% W/W)	1.06
Water soluble extractive values (% W/W)	27.84
Alcohol soluble extractive values (% W/W)	35.76
Heavy metals	
Mercury	0.0153 ppm
Lead	0.3186 ppm
Cadmium	0.0347 ppm
Arsenic	0.0180 ppm
Microbiological analysis	
Test for <i>E.coli/g</i>	Absent
Test for <i>Salmonella/g</i>	Absent
Test for <i>Shigella.sp/g</i>	Absent
Test for <i>Entero bacterial sp.</i>	102 org g-1
Total <i>Heterotrophic</i> bacteria	112 x 104
Test for Yeast and Mould	19x101

Table 2: Phytochemical Screening for different solvent extract of *Grewia umbelliferrea* stem

Phytoconstituents	Pet ether	Methanol	Aqueous	Chloroform
Alkaloids	+	++	++	+
Carbohydrates	-	+	+	-
Glycosides	+	++	++	-
Protein & Amino acids	-	+	++	-
Fixed oils & Fats	-	+	-	+
Tannins	-	++	-	+
Saponins	-	+	+	-
Steroids (Phyto sterols)	+	++	-	-
Flavonoids	-	+	+	-
Phenols	+	++	+	+
Key: ++ means abundant;		+ Indicates presence; - indicates absence;		

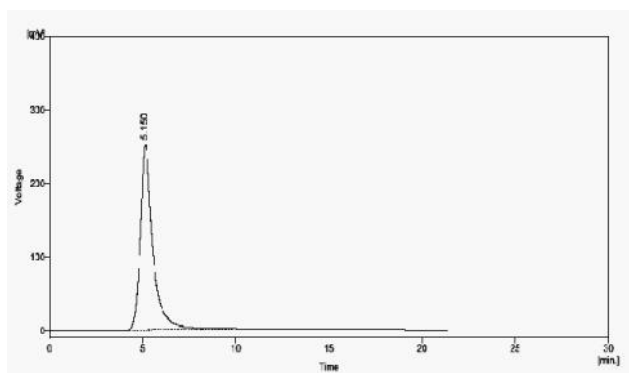


Figure 1: HPLC chromatogram of standard berberine. Berberine peak at the Rt 5.15 min. detected at a wavelength of 265 nm.

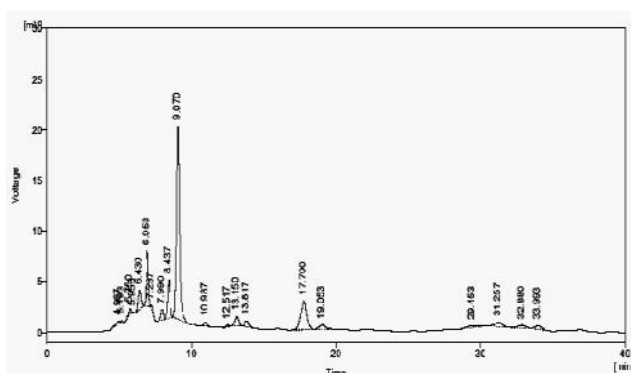


Figure 2: HPLC chromatogram of petroleum-ether extract of *Grewia umbelliferrea* peak at the Rt 5.15min. correspond to berberine detected at a wavelength of 265 nm.

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