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Preparation and Phytochemical Characterization of Grewia asiatica Fruit Extract by Successive Extraction Method

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KEYWORDS

Grewia asiatica, Successive extraction method, Phytochemical characterization, Alkaloids, Flavonoids, Antioxidant activity, DPPH radical scavenging.

ABSTRACT:

The present study aimed on the preparation and phytochemical characterization of Grewia asiatica fruit extract using the successive extraction method. Grewia asiatica, commonly known as Phalsa, is a tropical fruit rich in bioactive compounds with potential health benefits. The extraction process involved successive use of solvents with increasing polarity petroleum ether, benzene, chloroform, ethyl acetate and methanol. Each extract was subjected to qualitative phytochemical screening to identify and quantify various secondary metabolites, including alkaloids, flavonoids, tannins, phenols, saponins, and glycosides. The results indicated that the different extracts exhibited varying phytochemical profiles, with methanol extract showing the highest concentration of flavonoids, tannins, and phenols.

Introduction

Grewia asiatica Mast. (syn. G. subinaequalis DC), a member of the Tiliaceae family, produces edible fruits known for their astringent and stomachic properties. The unripe phalsa fruit is traditionally used to alleviate inflammation and is administered in cases of respiratory, cardiac, and blood disorders, as well as for reducing fever. Additionally, an infusion of the bark serves as a demulcent, febrifuge, and remedy for diarrhoea. The root bark finds application in treating rheumatism, while the leaves, known for their antibiotic action, are applied to the skin for managing eruptions [1-2].

Phalsa holds a significant position among India's indigenous fruits, attributed to its remarkable nutritional and medicinal attributes, early maturation, and resilience to diverse agro-climatic conditions. Consequently, it merits extensive cultivation and utilization in the future [3]. The ripe fruits, characterized by their highly delightful taste ranging from sour to sweet, possess a pleasing flavor and a refreshing effect. They contain approximately 50-60% juice, 10-11% sugar, and 2.0-2.5% acid, along with substantial quantities of vitamin A and C, and moderate amounts of phosphorus and iron. The appealing color of the fruit is likely attributed to the presence of delphinidin-3-glucoside and cyanidin-3-glucoside, as observed in analyses [4-5].

The plant is documented to possess a range of beneficial properties, including radioprotective, antibacterial, hepatoprotective, antipyretic, antiemetic, and antidiabetic activities. The fruit, known for its astringent and cooling nature, is utilized as a stomachic and is employed in the treatment of heart and blood disorders, as well as fevers [6-8]. Additionally, the fruits are recognized for their aphrodisiac qualities, ability to alleviate thirst and burning sensations, and efficacy in addressing biliousness and inflammation [9]. Fruit of G. asiatica is recognized for their diverse medicinal uses in treating conditions such as elephantiasis, inflammations, leprosy, leucoderma, diabetes fever, diarrhea, gout, rheumatoid arthritis, and bronchitis. In the realm of traditional medicine, particularly in Ayurveda, these leaves are endorsed as an antidiabetic remedy, presenting efficacy and cost-effectiveness compared to synthetic drugs [10-12].

Material and Methods

Grewia asiatica fruits were collected from the Gwalior district of Madhya Pradesh. The small fruits, resembling

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almost round drupes akin to blueberries, exhibit colors ranging from purple and crimson to cherry red when ripe. They are borne on 2- to 3-cm-long peduncles and cluster abundantly in open, branched arrangements. Individual fruits measure between 1.0 to 1.9 cm in diameter, 0.8 to 1.6 cm in vertical height, and weigh between 0.5 to 2.2 g. Ripening occurs gradually on bushes during the summer months. As the fruits mature, their skin transitions from light green to cherry red or purplish-red, ultimately adopting a dark purple or nearly black hue. The ripe fruit is adorned with a very thin, whitish blush and attains a soft texture. The identification and authentication of the plant were conducted from the Department of Botany, Institute of Basic Sciences, Bundelkhand University, Jhansi, Uttar Pradesh, India. All reagents used in the study were of analytical grade and procured from S.D Fine-Chem. Ltd., Mumbai, India.

Processing of Plant material

Following authentication, the fruits were air-dried at room temperature until completely devoid of moisture and then subjected to physical evaluation for various parameters [13-14].

Preparation of Grewia Asiatica Fruit extract

The fresh fruits were dried under shade, powered, pass through a 40 mesh sieve and stored in closed vessel for further use. The powder (180 g) was extracted successively with petroleum ether, followed by benzene, chloroform, ethyl acetate, and methanol, utilizing a Soxhlet extraction apparatus. After the solvent evaporation under reduced pressure with a vacuum rotary evaporator, the extractive values were determined [15-16]. The behavior of the powdered fruits was examined with various chemical reagents, and their fluorescence characteristics were observed under both ultraviolet and visible light. Preliminary phytochemical tests for different extracts were conducted employing specific reagents [17].

Ash and Extractive values

The determination of ash and extractive values involves a systematic procedure to assess the inorganic and soluble components of plant material. For ash content determination, a measured quantity (typically 2 to 5 grams) of the powdered plant material is accurately weighed and placed in a pre-weighed crucible. The sample is then gently heated over a Bunsen burner to eliminate volatile matter before being subjected to higher temperatures (usually 450-600°C) in a muffle furnace [18-19]. This process continues until a constant weight is achieved, ensuring the complete combustion of organic matter and resulting in a white or light-colored ash. After cooling in a desiccator to prevent moisture absorption, the crucible is reweighed to determine the ash content [20].

To determine extractive values, the plant material is successively extracted using different solvents such as petroleum ether, benzene, chloroform, ethyl acetate, and methanol in a Soxhlet extraction apparatus. After each extraction, the solvent is evaporated under reduced pressure using a vacuum rotary evaporator, and the extractive values are calculated. The behavior of the powdered plant material with various chemical reagents is then studied, and fluorescence characteristics are observed under ultraviolet and visible light, often compared with a reference color card for consistency. Preliminary phytochemical tests of different extracts are performed using specific reagents to identify and quantify various secondary metabolites present in the plant material [21-22].

Phytochemical Characterization

The successive extracts of petroleum ether, benzene, chloroform, ethyl acetate, and methanol extracts were subjected to various chemical tests for the identification of the phytoconstituents. The preliminary phytochemical screening of the plant material aimed to identify various plant constituents. Extracts obtained through successive solvent extraction underwent qualitative tests to detect alkaloids, carbohydrates, glycosides, proteins and amino acids, saponins, steroids, acidic compounds, mucilage, and fixed oils and fats [23-24]. This systematic screening process provided insights into the diverse chemical components present in the plant material, contributing to a comprehensive understanding of its phytochemical profile [25].

Results and Discussion

The investigation of the dried fruits of Grewia asiatica involved a meticulous and thorough analysis, adhering to standard procedures outlined by the World Health Organization (WHO) Guidelines. These guidelines provide a systematic framework for conducting analyses of various physicochemical parameters,

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ensuring a standardized and reliable approach to assessing the characteristics of dried fruits. The parameters selected for evaluation serve as crucial indicators that contribute to comprehending the overall quality, safety, and composition of Grewia asiatica fruits.

The physicochemical parameters subjected to scrutiny cover a broad spectrum, including but not limited to essential aspects such as ash content, extractive values, and phytochemical properties of the dried fruits. The determination of ash content is instrumental in understanding the inorganic residue left after the complete combustion of organic matter, providing insights into the purity and potential adulteration of the dried fruits. Extractive values, on the other hand, assess the quantity of active constituents extracted from the plant material, contributing to the understanding of its medicinal properties.

The exploration of phytochemical properties sheds light on the presence of specific chemical compounds in the dried fruits, such as alkaloids, flavonoids, tannins, and other bioactive components. This multifaceted analysis aligns with the broader goal of ascertaining the quality, safety, and therapeutic potential of Grewia asiatica fruits, making it a valuable resource in herbal medicine. The adherence to WHO Guidelines ensures the reliability and comparability of the obtained results, enhancing the scientific rigor of the study.

Ash Values

The determination of ash value holds significant importance as an analytical tool, playing a pivotal role in the identification of low-grade drugs, detection of exhausted drugs, and recognition of the presence of sandy or earthy matter in herbal or pharmaceutical samples. Ash value represents the residue remaining after the complete combustion of organic matter within a sample, offering valuable insights into the purity and quality of the substance under examination. In the specific context of the study, the results of ash values were meticulously documented for the tested samples, which may encompass dried fruits of Grewia asiatica among other materials. These recorded values, systematically presented in Table 1, serve as a comprehensive resource for understanding the mineral content and assessing the extent of inorganic impurities within the examined materials. Such insights contribute significantly to the overall evaluation of the quality, authenticity, and potential therapeutic efficacy of the substances under investigation.

Table 1. Physicochemical parameters of fruits of Grewia asiatica

Parameters	Values obtained (% w/w)
Total ash	5.25
Acid insoluble ash	1
Water soluble ash	204
Sulphated ash	0.65
Swelling index	0.1
Foaming index	Less than 100
Loss on drying	8.4

Extractive Values:

This method is designed to quantify the concentration of active constituents extracted from a specific quantity of medicinal plant material using various solvents. It proves particularly useful for materials where a suitable chemical or biological assay has not been established. The process involves treating the accurately weighed, air-dried medicinal plant material with a series of solvents, including petroleum ether, benzene, chloroform, ethyl acetate, and methanol. The resulting values, indicative of the extracted active constituents, have been systematically recorded and are available in Table 2. These recorded values offer valuable insights into the solubility and extractability of bioactive compounds from the medicinal plant material, contributing to the understanding of its pharmacological potential.

Table 3. Extractive values of fruits of Grewia asiatica

Solvents	Successive extractive value (% w/w)
Petroleum ether	1.65
Benzene	2.15
Chloroform	2.22
Ethyl acetate	3.68
Methanol	31.25

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The data in table represents the successive extractive values expressed as a percentage by weight (% w/w) for various solvents used in the extraction process. Each solvent, including petroleum ether, benzene, chloroform, ethyl acetate, and methanol, was employed sequentially to extract bioactive compounds from a given sample, possibly plant material. The extractive values indicate the proportion of the extracted material relative to the initial weight of the sample.

The extraction using petroleum ether resulted in a relatively lower percentage, suggesting a lower yield of extractable compounds with this solvent.The successive extraction with benzene yielded a slightly higher percentage compared to petroleum ether, indicating a comparatively higher extraction efficiency.The use of chloroform as a solvent resulted in a slightly higher extractive value, indicating its ability to extract a slightly greater quantity of bioactive compounds. Ethyl acetate extraction exhibited a higher percentage, suggesting a more efficient extraction and a higher yield of bioactive constituents compared to the previous solvents.Methanol extraction showed a significantly higher extractive value, signifying its efficacy in extracting a substantial amount of bioactive compounds. This high value may suggest the presence of a diverse range of polar compounds in the sample that are soluble in methanol.The higher extractive value in methanol indicates its suitability for extracting a broad spectrum of compounds from the sample.

Phytochemical screening

The preliminary phytochemical screening of Grewia asiatica fruits involved the evaluation of various phytoconstituents in different solvent extracts, namely petroleum ether, benzene, chloroform, ethyl acetate, and methanol. The results, presented in the table 3, provide a qualitative assessment of the presence or absence of specific phytochemicals in each extract. Notably, the extracts revealed the existence of alkaloids, proteins, amino acids, tannins, fats, fixed oils, acids, saponins, and gums/mucilages to varying degrees. The benzene and chloroform extracts exhibited the absence of glycosides and steroids, while the ethyl acetate and methanol extracts indicated their presence. The abundance of proteins and amino acids, as well as tannins, was notably high across all extracts, suggesting the potential medicinal and nutritional attributes of Grewia asiatica fruits. These findings contribute valuable insights into the phytochemical composition of the fruits, guiding further exploration of their therapeutic and functional properties.

Table 3. Preliminary Photochemical screening of fruits of Grewia asiatica

Phyto constituents	Petroleum ether extract	Benzene extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Alkaloids	-	-	+	+	+
Glycosides	-	-	-	-	+
Proteins and Amino acids	+	+	+	+	+
Carbohydrates	-	-	-	+	+
Tannins	+	+	+	+	+
Fats and Fixed oils	+	+	-	-	+
Saponins	+	+	+	-	-
Steroids	-	-	-	-	-

Conclusion

The generated data from this study holds significance in establishing the accurate identity and purity of plant parts, as well as in detecting potential adulteration. Through botanical authentication and examination of physicochemical parameters, a comprehensive understanding of the drug's quality can be obtained. These parameters serve as essential criteria for assessing the authenticity and purity of medicinal plant material. The reported data also contributes to identifying distinctive features of the drug, aiding in its characterization. The preliminary phytochemical study revealed that the presence of certain phytoconstituents is specific to selected species of Grewia asiatica Linn.

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As a result, further detailed screening becomes imperative to isolate these active constituents. This approach is crucial for scientifically proving the pharmacological responses of the plant, validating its traditional uses based on folklore. By isolating and characterizing these active compounds, a deeper understanding of the therapeutic potential of Grewia asiatica can be achieved, substantiating its traditional medicinal applications through rigorous scientific investigation.

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