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In Vitro Antioxidant Activity and Total Phenolic Analysis of Grewia Multiflora Juss

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ABSTRACT: This study was conducted to evaluate the antioxidant activity and total phenolic content of Grewia multiflora Juss. extracts obtained using solvents of different polarities, namely chloroform, acetone, methanol, water and also methanol (70%). Fresh leaves of Grewia multiflora Juss. (5 kg) were collected from the Meru Matha area near the Subarnarekha River in the biodiversity region of the Similipal forest, Mayurbhanj district.The extracts were prepared and their percentage yields were determined. Antioxidant activity was assessed using the DPPH free radical scavenging assay, while total phenolic content was estimated by the Folin–Ciocalteu method. Among the tested extracts, the methanol (70%) extract exhibited the strongest antioxidant activity, with the lowest IC50 value of 95.08. This activity correlated with its highest total phenolic content (115.48±3.72mg GAE/g) and greatest extraction yield (17.35%). In comparison, the methanol extract showed moderate antioxidant activity with an IC50 value of 205.96, a total phenolic content of 108.47±3.12 mg GAE/g, and a lower extraction yield of 9.18%. The chloroform and acetone extracts demonstrated the weakest antioxidant activity, which corresponded with their low phenolic content and minimal extraction yield. These findings suggest that solvent polarity significantly influences the extraction efficiency, phenolic composition, and antioxidant potential of Grewia multiflora Juss., indicating that the plant may serve as a promising natural source of antioxidant compounds.

INTRODUCTION

Free radicals are major contributors to oxidative stress, which can cause cellular damage, accelerate aging, and promote the development of various degenerative diseases [1]. Antioxidants play an important role in scavenging free radicals and reducing their harmful effects on the body [2]. Traditional and folk medicines have been used for centuries as home remedies and remain an important part of healthcare practices in many communities. The evolution of complementary and alternative medicine, as well as the discovery of several modern therapeutic agents, has its roots in traditional medicinal systems. Medicinal plants serve as valuable sources for pharmacological research and drug development. It is estimated that nearly 70% of pharmaceutical compounds used in India are derived directly or indirectly from natural sources. Plant-derived phytochemicals have contributed significantly to the treatment of pain and various diseases, and many bioactive organic compounds originate from higher plants. Although synthetic medicines are widely available, the use of herbal medicines continues to increase because of their therapeutic potential and natural origin. Between 1983 and 1994, approximately 41% of newly approved drugs were derived from natural resources such as plants, microorganisms, and marine organisms. Despite advances in synthetic drug discovery, natural products continue to provide numerous clinical candidates and novel

therapeutic agents. Marine algae, for example, contain phenolics, flavonoids, and carotenoids that possess strong antioxidant properties and have potential applications in pharmaceutical and cosmetic industries. Among medicinal plants, *Grewia multiflora* Juss. has attracted attention due to its traditional therapeutic uses. Tribal communities, local healers, and vaidyas traditionally use the leaf juice of *Grewia multiflora* for the management of Type-2 diabetes mellitus and jaundice. The plant is commonly known as "Bhansuli" in Hindi and "Hadjodi" in Odiya. *Grewia multiflora* grows abundantly in the Similipal forest biosphere and other regions of India as well as different parts of the world. Traditionally, its leaves are used for liver protection, appetite stimulation, treatment of heart ailments, reduction of fever, management of rheumatic conditions, diarrhea, and diabetes. Phytochemical investigations of the leaves have revealed the presence of flavonoids, saponins, glycosides, terpenoids, steroids, and phenolic compounds. Due to these bioactive constituents, leaf extracts of *Grewia multiflora* Juss. have been extensively studied for their pharmacological activities [3].

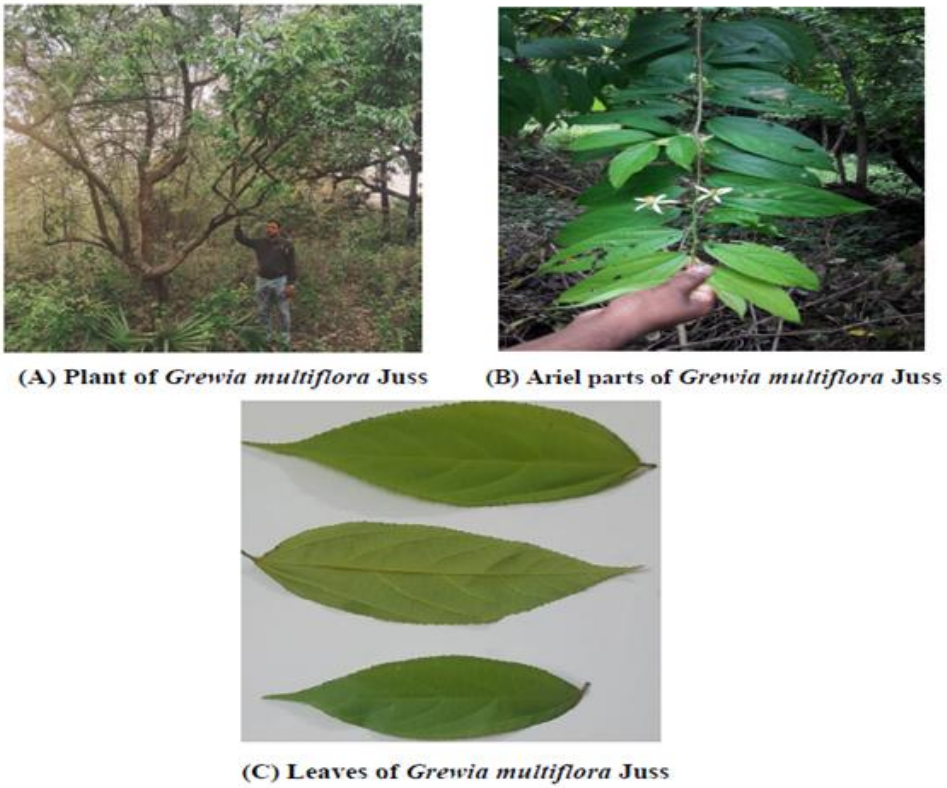

Fig. 1: Photograph of (A) Whole plant, (B) Ariel parts, (C) Leaves of Grewia Multiflora Jus

MATERIALSAND METHODS

Leaves of *Grewia multiflora* Juss were plucked freshly(5kg) from the Meru Matha area near Subarnarekha River in the biodiversity territory of Similipal forest, Dist- Mayurbhanj, Odisha in August 2018 (Figure1). We took the help of Botanical survey of India, Howrah, WB for authentication. A sample specimen was deposited in that herbarium. The chemicals used include 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder (Sigma Aldrich), FollinCiocalteu (Merck), Na_2CO_3(Merck), methanol pa (Merck), n-hexane pa (Merck), ethyl acetate (Merck), ascorbic acid (Merck), and distilled water.The primary instruments employed were a UV-Vis spectrophotometer (Shimadzu), shaker, analytical balance (CHQ), rotary evaporator (B-One), oven (Memmert), and micropipette (Socorex). Solvents of varying polarities, including chloroform, acetone, methanol and water were used for extraction. A part of the residue remained after chloroform extraction was separately extracted with methanol (70%) [methanol:water=70:30]. Prepared extracts were used to evaluate the antioxidant activity potential of *Grewia multiflora*. The selection of these solvents was based on their ability to dissolve different classes of phytochemical constituents present in the plant. The antioxidant activity of the extract was assessed in vitro using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

method. This method is widely employed for evaluating the free radical scavenging ability of pure antioxidant compounds as well as herbal extracts and natural products rich in phenolic constituents [4].

Sample Preparation

Preparation of *Grewia multiflora* samples was performed according to the method described by Sofiana *et al.*,2021. Fresh samples of *Grewia multiflora* were thoroughly washed with clean water to remove impurities and reduce surface moisture, followed by air-drying at room temperature for 2–5 days. The dried samples were then cut into small pieces of approximately 0.2–0.5 cm in size, and 500 g of the material was weighed separately for each solvent extraction. Extraction of bioactive compounds from the dried samples was carried out using the soxhlation technique with solvents of different polarities, namely chloroform, acetone, methanol and water. A portion of the residue remained after chloroform extraction was separately extracted with methanol (70%). Each extraction process was conducted for 3 × 24 hours. After filtration, the remaining plant residue was re-macerated for an additional 2 times. The collected filtrates from each solvent were concentrated using a rotary evaporator. The resulting concentrated extracts were weighed and subsequently subjected to evaluation for total phenolic content and antioxidant activity [5].

Determination of Total Phenolic Content:

Total phenolic content (TPC) of each *Grewia multiflora* extract was determined according to the method of Qurniasih *et al.,* 2022. Briefly, 5 mg of the extract was dissolved in 2 mL methanol, followed by addition of 5 mL distilled water and 0.5 mL Folin–Ciocalteu reagent. After 5 min, 1 mL of 5% Na_2CO_3 was added, and the mixture was homogenized and incubated for 1 h in the dark. Absorbance was measured at 510nm using a UV–Vis spectrophotometer. Total phenolic content was calculated from the gallic acid calibration curve and expressed as mg gallic acid equivalent per gram of extract (mg GAE/g extract) [6].

$$C = \frac{a\,xV/1000}{G} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, C is total phenolics, a is the gallic acid concentration (mg/L), V is the volume of test solution (ml), G is the mass of extract (grams), and 1000 is a correction factor.

Antioxidant Activity Test:

Considering the total phenolic content of the extracts, methanol (70%) and methanol extracts were selected and subjected to antioxidant activity test. Antioxidant activity was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, with ascorbic acid used as the positive control. Standard solutions of ascorbic acid were prepared at concentrations of 25, 50, 100 µg/mL. Similarly, each plant extract was prepared at concentrations of 25, 50, 100 µg/m [7].

The DPPH solution (0.1 mM) was prepared by dissolving 0.02 g of DPPH in 50 mL of the respective solvent used for extraction. For the assay, 2 mL of each sample solution was mixed with 1 mL of DPPH solution. The positive control was prepared by mixing 2 mL of ascorbic acid solution with 1 mL of DPPH solution in ethanol. All mixtures were homogenized using a vortex shaker and incubated in the dark at room temperature for 30 minutes [8]. The absorbance of the reaction mixtures was measured at 517 nm using a U-1240 Shimadzu UV–Visible spectrophotometer, with methanol serving as the blank solution. The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{absorbance\ blank - absorbance\ sample}{absorbance\ sample}\ x\ 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

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RESULTS

Yield of *Grewia multiflora* Extract

The results of the extract yield using the solvents can be seen in Table (1).

Table 1: Results of the extract yield of *Grewia multiflora*.

Extract samples	Yield (%)	Color
Chloroform	1.18	Greenish black
Acetone	2.03	Straw green
Methanol	9.18	Brownish green
Water	7.62	Greenish black
Methanol (70%)	17.35	Brownish green

Total Phenolic Content:

Analysis of total phenol content is shown in Table (2).

Table 2: Total phenolics of *Grewia multiflora* crude extract.

Extractsamples	Total Phenolic Content (mg GAE/g)
Chloroform	0.61±0.03
Acetone	28.13±1.48
Methanol	108.47±3.12
Water	1.14±0.42
Methanol (70%)	115.48±3.72

Antioxidant activity testing:

The results of the antioxidant test of *Grewia multiflora* using the DPPH method are depicted in Table (3).

Table 3: Antioxidant activity of *Grewia multiflora* extract using the DPPH method**:**

Extract	**Amount [µg/mL]**	**% inhibition ±SD**	**IC_{50}**
Methanol	25	16.8 ± 0.36	205.96
	50	34.68 ± 0.13	
	100	58.47 ± 0.58	
Methanol (70%)	25	21.24 ± 0.67	95.8
	50	45.63 ± 1.09	
	100	77.53 ± 1.1	
Ascorbic acid	25	71.6 ± 1.17	11.2
	50	77.53 ± 1.1	
	100	90.38 ± 0.87	

*Antioxidant Category (**Molyneux, 2004**): Very strong(<50), Strong(50-100), Week(100-200), Very weak (>200)+

DISCUSSION

Grewia multiflora contains bioactive compounds with different polarities, including polysaccharides, phenolics, flavonoids, terpenoids, sterols, and carotenoids [9]. Therefore, solvents with varying polarities such as chloroform, acetone, methanol, water and also methanol (70%) were used to obtain a broader range of phytochemicals. Methanol, being a polar solvent, effectively extracts hydrophilic compounds such as phenolics, flavonoids, and polysaccharides, while chloroform mainly extracts lipophilic constituents such as sterols and carotenoids [10-11].

% Yield:

Among the extracts, methanol (70%) followed by methanol produced the highest yield due to its ability to dissolve both polar and some semi-polar compounds Water and Acetone showed moderate extraction efficiency, whereas chloroform gave the lowest yield because lipophilic compounds occur in smaller quantities compared to phenolics and polysaccharides.

Total Phenolic Content (TPC)

The methanol extract showed the highest total phenolic content (115.48±3.72 mg GAE/g), followed by the methanol extract (108.47±3.12 mg GAE/g), acetone extract(28.13±1.48 mg GAE/g), water extract (1.14±0.42 mg GAE/g) and chloroform extract (0.61±0.03 mg GAE/g). These findings indicate that methanol (70%) is the most efficient solvent for extracting phenolic compounds from *Grewia multiflora*. Total phenolic content was determined using the Folin–Ciocalteu method, where phenolic compounds reduce the Folin reagent to form a blue-colored complex measured using UV–Vis spectrophotometry [12]. Phenolic compounds are strongly associated with antioxidant and photoprotective activities, explaining the superior antioxidant performance of the methanol (70%) extract [13]. Previous studies have also demonstrated a positive correlation between total phenolic content and antioxidant activity [14]. Thus, the polarity of the extraction solvent significantly influences the phytochemical composition and biological activity of *Grewia multiflora* extracts [15].

Antioxidant activity

The antioxidant activity evaluated by the DPPH assay revealed that the methanol (70%) extract exhibited the strongest activity with the lowest IC_{50} value (95.8), followed by the methanol extract (205.96). A lower IC_{50} value indicates stronger free radical scavenging activity [16]. The high antioxidant potential of the methanol (70%) extract may be attributed to the presence of phenolic compounds which are known for their ability to donate hydrogen or electrons to neutralize free radicals[17].Phenolic compounds possess hydroxyl (–OH) groups that stabilize free radicals by hydrogen donation, there by interrupting oxidative chain reactions and enhance free radical scavenging capacity [18]. In contrast, nonpolar extracts mainly contain lipophilic compounds, which generally exhibit weaker antioxidant activity in DPPH-based assays [19].

CONCLUSION

Extraction of *Grewia multiflora* using different solvents demonstrated significant variations in antioxidant activity and phytochemical content. Among the solvents tested, methanol (70%), a polar solvent, exhibited the highest antioxidant activity with an $IC_{50\ value}$ of 95.08, indicating moderate antioxidant potential. The methanol (70%) extract also showed a higher extraction yield and greater total phenolic content, which may have contributed to its enhanced antioxidant activity. In comparison, the methanol extract produced a lower yield than the methanol (70%) extract but was likely able to dissolve semi-polar compounds that were more efficient in absorbing ultraviolet (UV) light. On the other hand, the acetone, water and chloroform extracts have less phenolic content. As a non-polar solvent, chloroform is generally more effective in extracting lipophilic compounds that are associated with biological mechanisms in living systems. Overall, the findings indicate that the choice of solvent plays a crucial role in the extraction efficiency, bioactive compound profile, and antioxidant potential of *Grewia multiflora* extracts.

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