EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 12
Issue: 11
Year: 2025

EVALUATION OF NOOTROPIC ACTION OF METHANOLIC FRACTION FROM ETHYLACETATE EXTRACT OF *GREWIA HIRSUTA* BARK

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DOI: https://doi.org/10.5281/zenodo.17539282

How to cite this Article: Swadha Kumari Sah*1, Ashish Chaurasia1. (2025). EVALUATION OF NOOTROPIC ACTION OF METHANOLIC FRACTION FROM ETHYLACETATE EXTRACT OF GREWIA HIRSUTA BARK. European Journal of Biomedical and Pharmaceutical Sciences, 12(11), 163–167.

Article Received on 10/10/2025 **Article Revised on 30/10/2025** **Article Published on 01/11/2025**

ABSTRACT

The objective of the present work was to prepare the ethylacetate extract of bark of *Grewia hirsuta* and obtain the methanolic fraction of the extract and evaluate its nootropic potential in rodent. The extraction yield of the bark of *Grewia hirsuta* in methanol was found to be 17.47% w/w. The preliminary phytochemical analysis suggest the presence of phenolics, tannins, flavonoids, protein, sterol and terpenes in the ethylacetate extract. The ethylacetate extract of *Grewia hirsuta* was quantified for the total phenolic content. The total phenolic content of ethylacetate fraction was found to be 154.87 ± 1.416 GAE mg/g. The extract was fractionated with methanol using a separating funnel and the methanolic fraction was test found to test positive for flavonoids and phenolics. The methanolic fraction was evaporated to obtain dark yellow powder in 21.6 % w/w yield. The spectral analysis of the methanolic fraction revealed the presence of three components in UV spectra (λmax 305.0, 261.0 and 224.0 nm). The FTIR spectra suggested hydroxyl, amine, carbonyl and aromatic functionalities in the fraction. The methanolic fraction of the ethylacetate extract of *Grewia hirsuta* bark was subjected to evaluation of nootropic potential using morris water maze test at dose levels of 200 and 400 mg/kg/p.o against scopolamine induced amnesia. The mice treated with 400 mg/kg MFGH required only 15.50 ± 1.378 seconds on the 4th day to find the hidden platform as compared to 50.83 ± 2.228 for the scopolamine treated mice.

KEYWORDS: *Grewia hirsuta*, nootropic, scopolamine, flavonoids, fractionation.

INTRODUCTION

Dementia has been a key factor in several syndromes like Alzheimer's disease and Parkinson's disease.[1] Aging, stressful conditions, reduced brain metabolism, high oxidative stress levels, inflammation or reduced plasticity has been hypothesized to be involved in cognitive dysfunction associated with neurodegenerative disorders such as Alzheimer's (AD) or Parkinson's disease (PD).[2,3] Plant-derived natural products are significant as sources of medicinal agents and models for the design of new remedies. Lately, research has been directed to traditional folk medicines as they are generally characterized by high acceptability and good toleration. Several reports mention the role of oxidative stress in dementia.[4]

Grewia hirsuta belongs to Malvaceae family and is rich in constituents like α-curcumene, sesquiterpenes, sesquiterpene alcohol, undecanoic acid, tetradecanoic acid, myristic acid, palmitic acid, oleic acid, linoleic acid, gingerol, and ephedrine.[5,6] *Grewia* has been reported to have strong antioxidant action along with several central nervous system activities.[7-11] In light of the above facts, it was envisioned to explore and scientifically validate the nootropic properties of *Grewia* bark extracts. The objective of the present investigation is to evaluate the nootropic capability of the methanolic fraction of ethylacetate extract of *Grewia hirsuta* bark.

MATERIAL AND METHODS

Collection and preparation of the plant material

The bark of *Grewia hirsuta* were purchased from Indian jadibooti ecommerce site, and authenticated by botanist at RB Science, Bhopal. The authenticated bark was powdered using a blender at low speed, passed through

sieve number 80 and stored in air tight container until taken for further processing.

Extraction of phytoconstituents[12]

The powdered bark were used for the extraction process by soxhlet method. 96 g of powdered bark was evenly placed in the extractor to soxhlet apparatus and defatted with petroleum ether. The defatted marc (84.7g) was dried and extracted with ethylacetate. Ethylacetate (300 mL) was flown down the powder and extraction was carried out by hot continuous extraction method for 9 h. The extract was filtered through Whatman filter and concentrated using rotary vacuum evaporator. The resinous extract was collected and stored in desiccator to remove the excessive moisture**.** The dried extracts were stored in desiccators for further processing.

Preliminary phytochemical screening[13]

The extract was evaluated by qualitative phytochemical screening in order to identify the type of plant secondary metabolites present in it. The screening was performed for triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids. The color intensity or the precipitate formation was used as analytical responses to these tests.

TLC of the ethylacetate extract

The ethylacetate extract was dissolved in small volume of ethyl acetate and a spot was placed on the precoated TLC plate (silica gel F_{254}). The plate was placed in developing chamber saturated with mobile phase (toluene:ethyacetate:formic acid, 4.5:4.5:1 for phenolics and ethylacetate: formic acid: acetic acid: water, 100:11:11:27 for flavonoids). The solvent was allowed to run upward through the plate until 1 cm below the top of the plate. The plate was removed, air dried and visualized in UV cabinet.

Total Phenolic Content

In order to determine the total phenolic content, 10 mg of the ethylacetate fraction (extract) was mixed with 10 mL methanol. 100 µL of sample was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After 3 min, 300 µL of 20% aqueous Na_2CO_3 solution was added to it and the mixture was allowed to settle for 2 h [14]. The absorbance was measured at 760 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (20-100 ppm) were treated similarly to obtain the calibration curve. The control solution contained 200 µL of water and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Fractionation of the extract with methanol

The extract was suspended in 25 mL of distilled water with sonication. This solution was placed in a separating funnel and 25 mL of methanol was added to it. The mixture was shaken vigorously for 30 min and allowed to stand for 2 h. The methanol layer was separated and studied further. The phytochemical screening of the methanolic fraction was done as per previously reported methods.

UV Spectral Analysis of the fraction

In a clean and dry test tube, 0.1 mL of the methanolic fraction was diluted with methanol to a volume of 5 mL and the solution was scanned using UV visible spectrophotometer from 200-800 nm. The absorption spectrum was obtained.

FT-IR Spectral Analysis of the fraction

The dried methanolic fraction was scanned in the range of 400 to 4000 cm-1 using a FT-IR spectrophotometer and the stretching and bending vibrations were observed.

Pharmacological Evaluation of the methanolic fraction

Animal

The male/female albino mice of amid 1 to 2 months of age weighing between 25-35 g were used procured from approved suppliers from Bhopal. The rodents were allowed free access pallet diet (Lipton India Ltd, Mumbai, Ind.) and water *ad libitum*. All the laboratory conditions and animals were maintained as per CPCSEA guidelines throughout the experiments.

Acute Toxicity study

The short- and long-term toxic effects of both drugs and their extracts were performed within prescribed guideline set by OECD guideline no. 423.[15]

Grouping of animal for treatment

The animals (albino mice, 25-35 g, 4 to 8 weeks) were divided in 4 groups with 6 animals in each group. **Group 1** control vehicle (0.9% NaCl); **Group 2** was injected with scopolamine (SCOP) 2mg/kg intraperitoneally for 21 days; **Group 3** and **4** were administered with methanolic fraction of *Grewia hirsuta* (MFGH) at dose of 200 and 400 mg/kg/p.o respectively and injected with SCOP (2 mg/kg) for 21 days.

Morris water maze test[16,17]

Spatial learning and memory were assessed using the Morris water maze previously described. Briefly, the testing system was composed of a black circular pool (150 cm in diameter and 30 cm deep) filled with water (temperature 20±2 °C) and surrounded by extra maze distal visual cues of different shape, size and color. The pool was divided in four quadrants. A black circular hidden platform was placed in the northwest (NW) quadrant 2 cm under the water surface so that mice could escape from swimming. Experimental mice were screened for their swimming ability by recording the latency to reach the visible platform. Mice were trained to exit the water tank onto the platform by using the visual cues. Each mice was placed inside the water tank facing the tank wall, at one of the four randomly selected entry points, once in every block of four trials. The starting position was changed randomly for each trial and

the animal was allowed to search for 60 s to find the hidden platform. Mice were guided to the platform, if failed to find the platform within 60 s. At the end of the trials, the mice were allowed to remain on the platform for 30 s.

RESULTS AND DISCUSSION

Extraction Yields and phytochemical screening

On defatting with petroleum ether 7.91% of fatty material was separated from the bark. The extraction yield of the bark of *Grewia hirsuta* in ethylacetate was found to be 17.47% w/w. The extract was resinous and dark brown in color. The findings of the phytochemical analysis suggest the presence of sterols, phenolics and tannins, proteins, flavonoids and terpenes in the ethylacetate extract of the bark.

TLC of the extract

The extract was subjected to TLC analysis for detecting flavonoids and phenolics in the same. The TLC of the extract in solvent used for detection of phenolics resulted in the separation of 4 distinct components whereas in solvent used for detection of phenolics resulted in the separation of 2 components in the ethylacetate extract (Figure 1). On the other hand the petroleum ether extract revealed 1 phenolic and no flavonoids.

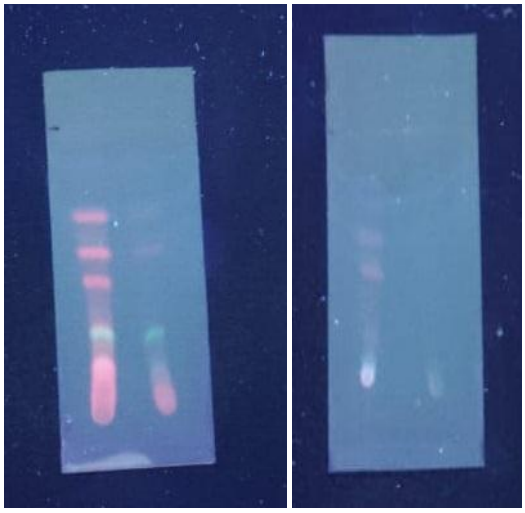

Figure 1(A): TLC for phenolics (B) TLC for flavonoids.

Total Phenolic content

The ethylacetate extract of *Grewia hirsuta* was quantified for the total phenolic content. Standard curve of gallic acid was plotted in distilled water. The result of the total phenolic content of the extract examined using Folin-Ciocalteu method. The total phenolic content of ethylacetate extract was found to be 154.87 ± 1.416 GAE mg/g.

Fractionation with methanol and phytochemical screening

The methanolic fraction was evaporated to obtain dark yellow powder in 21.6 % w/w yield. The fraction tested positive for flavonoids and phenolic components.

UV analysis of the methanolic fraction

The UV spectrum of the methanolic fraction revelaed three thrifts in absorbances with absorption maxima at 305.0 nm, 261.0 nm and 224.0 nm respectively, suggesting separation of maximum phenolics in methanol (Figure 2).

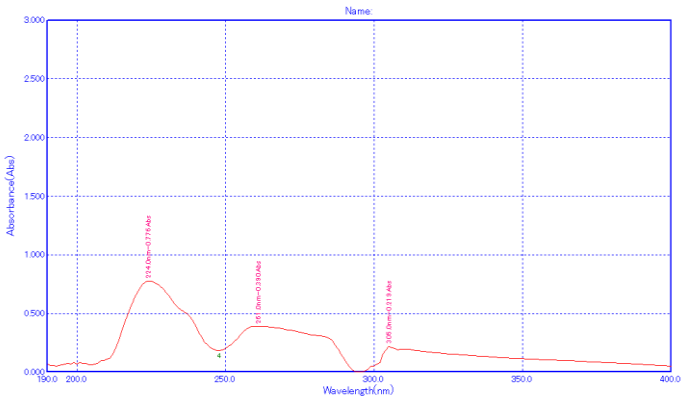

Figure 2: UV Spectrum of methanolic fraction.

FTIR analysis of the methanolic fraction

The FTIR spectrum suggested the presence of hydroxyl, amine, carbonyl functional groups along with aromatic rings in the compound (Figure 3), suggesting phenolics in the fraction.

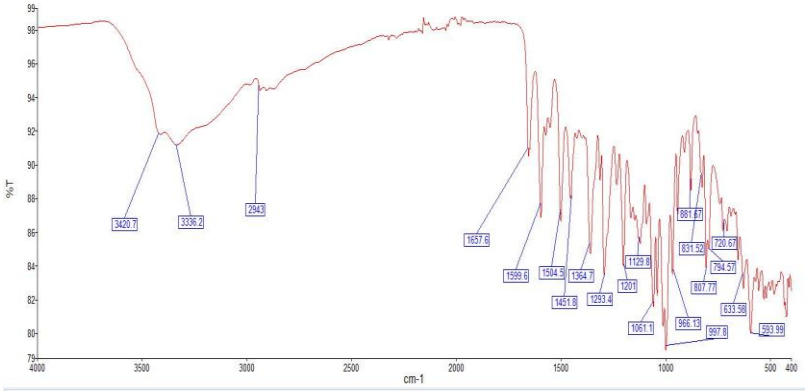

Figure 3: FTIR Spectrum of methanolic fraction.

Acute toxicity Study

There was no sign and symptoms or any toxic effects in rodents for both plants even at higher dose of 2000 mg/kg body weight. Thus, 1/10th of maximum dose was selected as effectual dose. The cut off value of 200 & 1/5th dose i.e 400 mg/kg were chosen for evaluation of memory enhancing activity.

Nootropic assessment in Morris water maze method

MWM (Morris water maze) tests show that average latency to find the hidden platform by experimental mice. Our observations indicate that all experimental groups learned to find hidden platform in four experimental days. This implies that all experimental mice learn to escape swimming by searching hidden platform using visual cues. Results of MWM test in experimental animals confirmed a significant effect of Scopolamine-toxicity, MFGH (200 mg/kg) + SCOP, MFGH (400 mg/kg) + SCOP compared to control on latency to acquire hidden platform. In Two-way ANOVA confirmed a significant interaction between treatment (control vs. SCOP treated) × trial days ($p<0.0001$) and SCOP + MFGH treated × trial days ($p<0.0001$). (Table 1, Figure 4).

Water maze tasks were performed to evaluate effect of methanolic fraction of the ethylacetate extract of *Grewia hirsuta* bark treatment on the spatial memory abilities. Each data point represents the mean (±SD) latency of the trials for a minimum of six mice performed each day. The mice treated with 400 mg/kg MFGH required only 15.50 ± 1.378 seconds on the 4th day to find the hidden platform as compared to 50.83 ± 2.228 for the scopolamine treated mice.

Table 1: Time to reach hidden platform in morris water maze test.

Treatment	Dose (mg/kg)	Time to reach platform (sec)			
		Day 1	Day 2	Day 3	Day 4
Vehicle	0.5 ml/kg, i.p.	39.66 ± 2.338	32.50 ± 3.449	30.50 ± 1.048	27.16 ± 2.228
SCOP	2 mg/kg, i.p.	65.33 ± 3.444	61.83 ± 2.714	57.50 ± 3.146	50.83 ± 2.228
MFGH	200 mg/kg, p.o.	36.16 ± 2.483	30.83 ± 1.940	28.33 ± 1.366	23.66 ± 1.211
	400 mg/kg, p.o.	23.83 ± 1.940	21.33 ± 1.211	19.00 ± 1.264	15.50 ± 1.378

Values represent means±SD (n = 6), ***($p<0.001$)

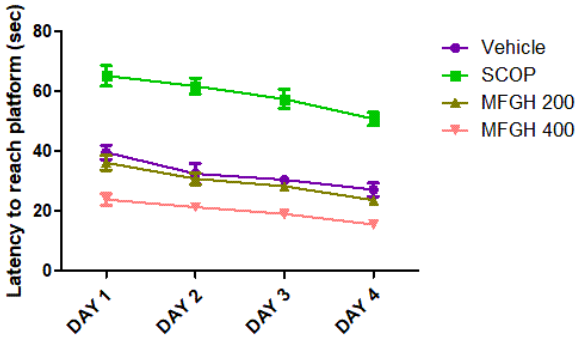

Figure 4: Latency to reach platform.

CONCLUSION

The objective of the present study was to assess the nootropic potential of methanolic fraction of ethylacetate extract of bark of *Grewia hirsuta* using the animal models. The results obtained led to the conclusion that *Grewia hirsuta* bark are a good source of potential flavonoids and phenolic. The ability to reverse the scopolamine induced amnesia by the fraction makes it a subject for further investigation to deduce the mechanism involved and optimize the nootropic potential of the plant.

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