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Evaluation of nootropic action of ethylacetate fraction from methanolic extract of Grewia Hirsuta bark.

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ABSTRACT

The objective of the present work was to prepare the methanolic extract of bark of Grewia hirsuta and obtain the ethyacetate fraction of the extract and evaluate its nootropic potential in rodent. The extraction was done by soxhlet apparatus and qualitative analysis of phytochemicals was done. The extraction yield of the bark of Grewia hirsuta in methanol was found to be 11.8% w/w. The fractionation of extract was achieved with ethylacetate using separating funnel. The ethyl acetate fraction was evaporated to obtain dark yellow powder in 18.2% w/w yield. The fraction tested positive for flavonoids and phenolic components. The ethylacetate fraction of methanolic extract of Grewia hirsuta was quantified for the total phenolic content. The total phenolic content of ethylacetate fraction was found to be 138.52 ± 3.511 GAE mg/g. The ethylacetate fraction of the methanolic extract of Grewia hirsuta bark was subjected to evaluation of nootropic potential using elevated plus maze test and morris water maze test at dose levels of 200 and 400 mg/kg/p.o against scopolamine induced amnesia. EAGH (200 and 400 mg/kg, p.o.) were found to be significantly improving the open arms activity (both the parameters) compared to Scopolamine treated group in the elevated plus maze test. The mice treated with 400 mg/kg EAGH required only 15.66 ± 1.211 seconds on the 4th day to find the hidden platform as compared to 51.16 ± 2.714 for the scopolamine treated mice.

***Keywords**: Grewia hirsuta, morris water maze, elevated plus maze, extraction, fractionation*

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1. INTRODUCTION

Grewia hirsuta Vahl, commonly called as nagabala is a shrub of the family Malavaceae, is reported to possess several secondary metabolites like α-curcumene, sesquiterpenes, sesquiterpene alcohol, undecanoic acid, tetradecanoic acid, myristic acid, palmitic acid, oleic acid, linoleic acid, gingerol, ephedrine 4-methoxy kaempferol, vanillic acid, syringic acid, ferulic acid, coumaric and gentisic acid.1 In several culture across the world, the plant is used for its medicinal purposes in treated fever, digestive disorders and inflammation. It has also been used as skin and nervine tonic by tribal people and folkoric practitioners.2 It has been scientifically explored for its anti-oxidant3, anti-microbial4, anti-diabetic5, and immunomodulatory6 activities.

Memory is the ability to register and retain events for longer period of times. Conditions like stress, emotion, amnesia, anxiety, dementia, schizophrenia and Alzheimer's disease (AD) may trigger a loss of memory.7,8 Dementia has been a key factor in several syndromes like Alzheimer's disease and Parkinson's disease. 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 AD or Parkinson's disease (PD).9,10

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. Grewia has been reported to have antioxidant action. 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 ethylacetate fraction of methanolic extract of Grewia hirsuta bark.

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2. MATERIAL AND METHODS

Collection, identification 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

The powdered bark were used for the extraction process by maceration method. 108 g of powdered bark was evenly placed in the extractor to soxhlet apparatus. Methanol (350 mL) was flown down the powder and extraction was carried out by hot continuous extraction method for 14 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.[11]

Preliminary phytochemical screening

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.[12]

Fractionation of the extract with ethylacetate

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

Total phenolic content (TPC)

In order to determine the TPC, 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. The absorbance was measured at 760 nm with a UV-Vis spectrophotometer.[13] Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Pharmacological Evaluation of the extract

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 (TIT-PY/2024/30).

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.[14]

Grouping of animal for treatment

The animals (albino mice, 25-35 g, 4 to 8 weeks) were divided in 6 groups with 6 animals in each group. The grouping and treatment per group is presented below.

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 ethylacetate fraction of *Grewia hirsuta* (EAGH) at dose of 200 and 400 mg/kg/p.o respectively and injected with SCOP (2 mg/kg) for 21 days. (For morris water maze study); Group 5 and 6 were administered with ethylacetate fraction of *Grewia hirsuta* (EAGH) at dose of 200 and 400 mg/kg/p.o respectively and injected with SCOP (2 mg/kg) for 21 days. (For elevated plus maze study)

Morris water maze test

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 test was performed on four consecutive days (8 trials per day). 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 was allowed to remain on the platform for 30 s. Morris water maze training was recorded using a web camera mounted to the ceiling. Recording was performed from 11:00AM to 2:00PM to exclude variations in performance resulting from circadian rhythmicity.[15,16]

Elevated plus maze test

The Elevated plus-maze comprised of two open (50cm × 10 cm) and two enclosed (50cm × 10 cm×40 cm) arms that radiated from the central platform (10cm × 10 cm) to form a plus sign. The maze was constructed of black acrylic sheet. The plus maze was elevated to a height of 50 cm above from the floor level by a single central support. All the four arms consist of infra-red beams fitted at regular distance. The experiment was conducted during the dark phase of the light cycle (9:00 – 14:00 h).The trial was started by placing an animal on the central platform of the maze facing an open arm. During the 5 min experiment, behavior of mice was recorded as (i) preference of the mice for its first entry into the open and closed arms, (ii) the numbers of entries into the open or closed arms, and (iii) time spent by the mice in each of the arms. The mice were considered to have entered an arm when and four paws were on the arm. The apparatus was cleaned thoroughly between trials with damp and dry towels. All behavioral recording were carried out with the observer unaware of the treatment of the mice had received.[17,18]

3. RESULTS AND DISCUSSION

Extraction Yields

The extraction yield of the bark of *Grewia hirsuta* in methanol was found to be 11.8% w/w. The extract was resinous and dark brown in color.

Phytochemical Screening

For detecting the occurrence of alkaloids, glycosides, tannins, saponins, flavonoids and terpenoids in the extracts, a small fraction of the dried extract was subjected to the phytochemical testing procedures by resuspending a small amount of each extract suitably into the methanol. The findings of the phytochemical analysis suggest the presence of phenolics and tannins, proteins and flavonoids in the methanolic extract of the bark.

Fractionation with ethylacetate and phytochemical screening

The ethyl acetate fraction was evaporated to obtain dark yellow powder in 18.2% w/w yield. The fraction tested positive for flavonoids and phenolic components.

Total Phenolic content

The ethylacetate fraction of methanolic 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 fraction was found to be 138.52 ± 3.511 GAE mg/g.

Pharmacological evaluation

A 2000 mg/Kg dose was found to be safe in test animals and the dose for study was selected as per guidelines.

Nootropic assessment in elevated plus maze paradigm

Scopolamine treated group significantly increased close arm entries and time spent. EAGH (200 and 400 mg/kg, p.o.) were found to be significantly improving the open arms activity (both the parameters) compared to SCOP treated group (Table 1). The time spent in open arm by the mice treated with vehicle was not significant while EAGH exhibited a significant result compared to SCOP ($p<0.0001$) in Two way ANOVA. The number of movements in open arm on administration of EAGH (400 mg/kg) was found to be 6.83 ± 0.752 as compared to a meager movement of 1.33 ± 0.516 on Scopolamine treated mice.

Table 1 Memory assessment in elevated plus maze paradigm

Treatment	Dose (mg/kg)	Number of open arm entries	Number of close arm entries
Vehicle	0.5 ml/kg, i.p.	1.83 ± 0.752	4.80 ± 0.408

SCOP	2 mg/kg, i.p.	1.33 ± 0.516^{ns}	6.50 ± 1.048$^{###}$
EAGH	200 mg/kg, p.o.	5.33 ± 0.816***	2.33 ± 0.516***
	400 mg/kg, p.o.	6.83 ± 0.752***	1.33 ± 0.516***

Values represent means±SD (n = 6). ^{ns}- not significant; ***(p<0.001 vs SCOP), $^{###}$(p<0.001 vs vehicle)

The elevated plus-maze exploration was originally validated as a predictive test of rodent, anxiety-like behavior wherein rodent prefers to remain in the closed arms than open arm. Effect of the ethylacetate fraction of the methanolic extract of *Grewia* hirsuta bark on scopolamine- induced learning and memory impairment in rodents showed positive response in elevated plus-maze.

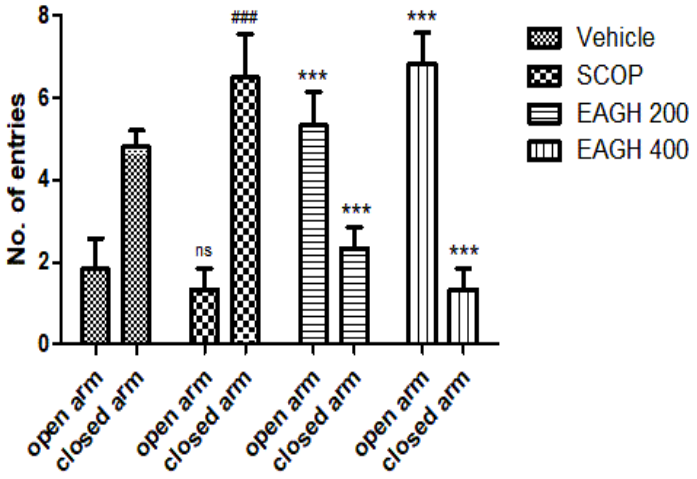

Figure 1 Effect of EAGH on mice in EPM test

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, EAGH (200 mg/kg) + SCOP, EAGH (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 + EAGH treated × trial days (p<0.0001) (Table 2, Figure 2).

Water maze tasks were performed to evaluate effect of ethylacetate fraction of the methanolic 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 EAGH required only 15.66 ± 1.211 seconds on the 4th day to find the hidden platform as compared to 51.16 ± 2.714 for the scopolamine treated mice.

Table 2 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.	39.16 ± 2.041	32.16 ± 3.544	30.83 ± 1.329	27.33 ± 2.338

SCOP	2 mg/kg, i.p.	64.83 ± 3.544	61.5 ± 2.664	57.83 ± 2.786	51.16 ± 2.714
EAGH	200 mg/kg, p.o.	35.83 ± 2.316	30.33 ± 1.966	28.5 ± 1.643	24.00 ± 1.264
	400 mg/kg, p.o.	24.16 ± 1.834	20.83 ± 1.940	19.16 ± 1.169	15.66 ± 1.211

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

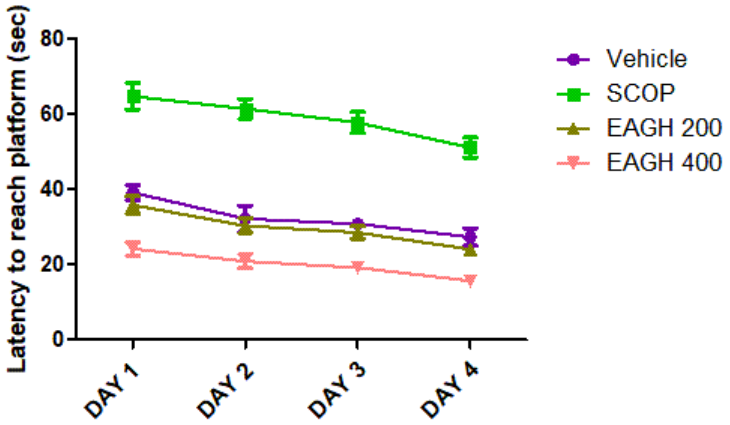

Figure 2 Latency to reach platform

4. CONCLUSION

The objective of the present study was to assess the nootropic potential of ethylacetate fraction of methanolic 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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