

CNS-Antidepressant Activity of Crude Extracts and Different Fractions of Stem Bark of *Acacia Nilotica*

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Abstract: According to the World Health Report, approximately 450 million people suffer from a mental or behavioral disorder. This amounts of 12.3% of the global burden of disease, and will rise to 15% by 2020. Depression is most prevalent mental disorder and it is recognized to be symptomatically, psychologically and biologically heterogeneous. The crude methanolic extract of *Acacia nilotica* bark with different soluble partitionates were subjected to investigate for the evaluation of analgesic, hypoglycemic, CNS depressant and antidiarrheal activity on mice and thrombolytic, antihelmentic, antimicrobial, antioxidant along with cytotoxicity different in vivo experiment. The crude methanolic extract of bark of *Acacia nilotica* were evaluated for antidepressant activity showed insignificant value.

Keywords: *Acacia Nilotica*, CNS-Antidepressant Activity, Stem Bark and Methanolic Extract

1. Introduction

According to the World Health Report, approximately 450 million people suffer from a mental or behavioral disorder. This amounts of 12.3% of the global burden of disease, and will rise to 15% by 2020 [1]. Depression is most prevalent mental disorder and it is recognized to be symptomatically, psychologically and biologically heterogeneous. In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamino oxidase [2], selective serotonin reuptake inhibitors (SSRIs) [2] and selective noradrenalin reuptake inhibitors [2]. Basic neuroscience offers the promise identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drug. This consideration leads to search for new antidepressant agents that have a fast onset of action with less side effect [3].

Therefore the present study has undertaken to investigate the effect of methanolic extract of bark of *Acacia nilotica*.

2. The Plant Family: Fabaceae [4] (a, b)

The plant under investigation is *Acacia nilotica* belongs to the family Fabaceae. The Fabaceae, also called Leguminosae or bean and pea family, is the third largest family in terms of agricultural and economic importance [5].

3. Growth Pattern

Legumes vary in habit from annual and perennial herbs to shrubs, trees, vines/lianas, and even a few aquatics. Ranging in size from some of the smallest plants of deserts and arctic/alpine regions to the tallest of rain forest trees, legumes are a conspicuous, and often dominant, component of most of the vegetation types distributed throughout temperate and tropical regions of the world [6, 7]. Over the past 30 years, the study of legume classification and biology has benefitted from major advances in understanding of the morphology, evolution and systematics, and ecology of the family [8].

4. Taxonomy [9]

Taxonomically, Fabaceae has been traditionally divided into three subfamilies

- (1) Caesalpinioideae
- (2) Mimosoideae
- (3) Papilionoideae

4.1. Agricultural & Economic Importance of Legumes

Legumes have demonstrated agricultural importance for thousands of years, beginning with the domestication of lentils (*Lens esculenta*) in Iran dating to 9,500 to 8,000 years ago, their use as a food source during the prehistory of North and South America (beans, more than 3,000 years ago), and their use by the Roman Empire as a food source and for soil improvement [10, 11].

4.2. The Plant: *Acacia Nilotica* [12-15]

Acacia nilotica is also known as Gum Arabic tree, Babul, Egyptian thorn, or Prickly Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 2000 m and withstand at extreme temperature (>50°C) and air dryness. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India.

4.3. Synonyms

ACAR11: *Acaciaarabica* (Lam.) Willd.
MINI2: *Mimosanilotica* L.

4.4. Taxonomical Classification

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta– Vascular plants
Superdivision	Spermatophyta– Seed plants
Division	Magnoliophyta– Flowering plants
Class	Magnoliopsida– Dicotyledons
Subclass	Rosidae
Order	Fabales

Family	Fabaceae– Pea family
Genus	Acacia Mill. – acacia
Species	Acacianilotica (L.) Willd. ex Delile – gum arabic tree

4.5. Plant Description

Acacia nilotica is a single stemmed plant with a well-developed deep root system. The average height of the plant has been 15-18 m in height and 2-3 m in diameter. Pods are 7-15 cm long, green and tomentose (when immature) or greenish black (when mature), indehiscent, deeply constricted between the seed giving a necklace appearance. Seeds are 8-12 per pod, compressed, ovoid, dark brown shining with hard testa [16]. The leaves are bipinnate, pinnate 3-10 pairs, 1.3- 3.8 cm long, leaflets 10-20 pairs, and 2-5mm long [17]. Flowers are globular heads, 1.2-1.5 cm in diameter of a bright golden yellow color, develop either in axillary or whorl pattern on peduncles 2-3 cm long located at the end of branches [18]. Stems are usually dark to black colored, deep longitudinal fissured, grey-pinkish slash, exuding a reddish low quality gum [18]. The bark a tinge of orange and/or green (young tree), but older trees have dark, rough bark and tend to lose their thorns [19]. Thorns are thin, straight, light grey exist in axillary pairs (usually 3-12), 5-7.5 cm long in young trees. Root is generally of brown color in older and whitish in younger regions. The gum has a moisture content of about 13% and is slightly dextrorotary [20].

4.6. Growth Pattern and Germination

Acacia nilotica is a tropical species found throughout India and occurs from sea-level to over 2000 m altitude. Prickly Acacia germinates in rainfall in the wet season. But some seeds may still germinate up to 15 years after seed drop. Seedlings grow rapidly near water but more slowly in open grasslands. It grows in average annual temperatures range from 15–28°C, being frost sensitive when young and withstanding daily maximum temperatures of 50°C [21-27].

5. Some Common Medicinal Uses of Different Parts of *Acacia Nilotica*

Table 1. Some common medicinal uses of different parts of *Acacianilotica*.

Part used	Uses
Root	The roots are used against cancers and/or tumors (of ear, eye, or testicles), tuberculosis and indurations of liver and spleen [16].
Leaf	Chemopreventive, antimutagenic, anti bacterial, anticancer, astringent, anti microbial activity Tender leaves are used to treat diarrhea, Aphrodisiac, dressing of ulcers, anti-inflammatory and Alzheimer's diseases [17].
Gum	Astringent, emollient, liver tonic, antipyretic and antiasthmatic [18].
Stem bark	Anti bacterial, antioxidant, anti-mutagenic, cytotoxic bark is used as astringent, acrid cooling, styptic, emollient, anthelmintic, aphrodisiac, diuretic, expectorant, emetic, nutritive, in hemorrhage, wound ulcers, leprosy, leucoderma, small pox, skin diseases, biliousness, burning sensation, toothache, leucoderma, dysentery and seminal weakness. The trunk bark is used for cold, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leukoderma [19].
Seeds	Spasmogenic activity and antiplasmodial activity [20].
Pods	Anti hypertensive and antispasmodic, anti-diarrhoeal, astringent, anti-fertility and against HIV-1 PR, Inhibited HIV-1 induced cytopathogenicity, antiplatelet aggregatory activity and anti oxidant [21].

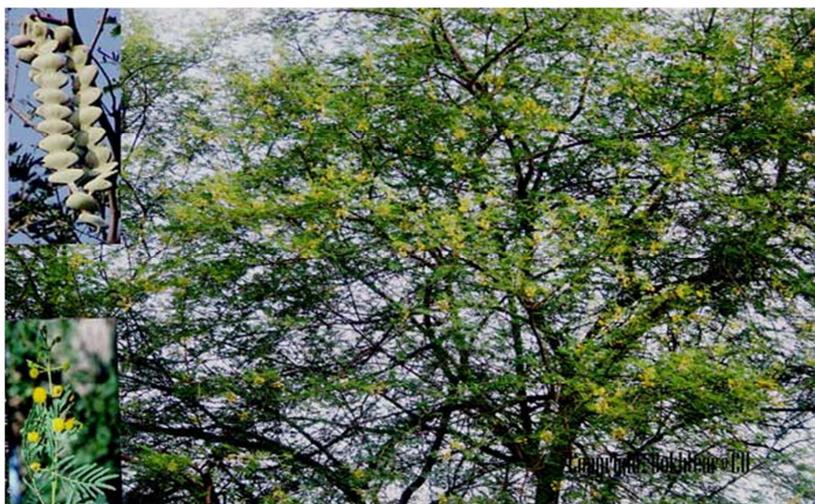


Figure 1. Whole tree of *Acacia nilotica*.

6. Materials for Partitioning and Extract Preparation

Glass wares: Distilled machine, Conical flasks (250 ml, Beakers (100 ml, 500 ml), Test tubes, Funnels, Measuring cylinders, Pipettes, Automatic pipette puller.

Solvents: n-Hexane, Carbon tetrachloride (CCl_4), Dichloromethane (CH_2Cl_2), Ethyl acetate ($\text{CH}_2\text{CH}_3\text{OOCCH}_3$), Methanol, Acetic acid, Ethanol and Distilled Water.

Filter aid: Filter Paper (Whatman no. 1) and Normal Cotton.

Equipments: Rotary vacuum evaporator, Electronic balance, Table-top UV detector (252 & 366 nm), Grinding machine, Oven, Solvent distillation plant and Distilled water plant.

6.1. Collection and Preparation of Plant Material

Plant sample (bark) of *Acacia nilotica* was collected from Pabna, Bangladesh in April 2012. Then proper identification of plant sample was done by an expert taxonomist. The bark was sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried bark was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Faculty of Pharmacy; University of Dhaka.

6.2. Extraction of the Plant Material

About 950 gm of the powdered material was taken in separate clean, round bottomed flask (4.5 liters) and soaked in 5 liter of methanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 21 days accompanying occasional shaking and stirring.

The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 39°C with a Heidolph rotary evaporation. The concentrated extract was then air dried to solid residue. The weight of the crude methanol extract obtained from the powdered whole plant was 22 gm.

6.3. Solvent-Solvent Partition of Crude Extract

Solvent-solvent partitioning of crude methanolic extract was done following Modified Kupchan Partition [35].

6.4. Preparation of Mother Solution

5 gm of methanol extract was triturated with 90 ml of methanol containing 10 ml of distilled water. The crude extract was dissolved completely. This was the mother solution which was partitioned off successively by four solvents of different polarity. In subsequent stages each of the fractions was analyzed separately for the detection and identification of compounds having antibacterial, cytotoxic, antioxidant and other pharmacological properties.

6.5. Partition with N-hexane

The mother solution was taken in a separating funnel. 100 ml of the n-hexane was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice ($100\text{ ml} \times 3$). The n-hexane fraction was then air dried for solid residue.

6.6. Partition with Carbon Tetrachloride

To the mother solution left after partitioning with n-hexane; 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with carbon tetrachloride (CCl_4). The process was repeated thrice ($100\text{ ml} \times 3$). The carbon tetrachloride fraction was then air dried for solid residue. The aqueous fraction was preserved for the next step.

6.7. Partition with Dichloromethane

To the mother solution that was left after partitioning with petroleum ether and carbon tetrachloride; 16 ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with dichloromethane (CH_2Cl_2) ($100\text{ ml} \times 3$). The dichloromethane soluble fractions were collected together

and air dried. The aqueous fraction was preserved for the next step.

6.8. Partition with Ethyl Acetate

To the mother solution that was left after washing with

petroleum ether, carbon tetrachloride and dichloromethane; was then taken in a separating funnel and extracted with ethyl acetate (100 ml × 3). The ethyl acetate soluble fractions were collected together and air dried.

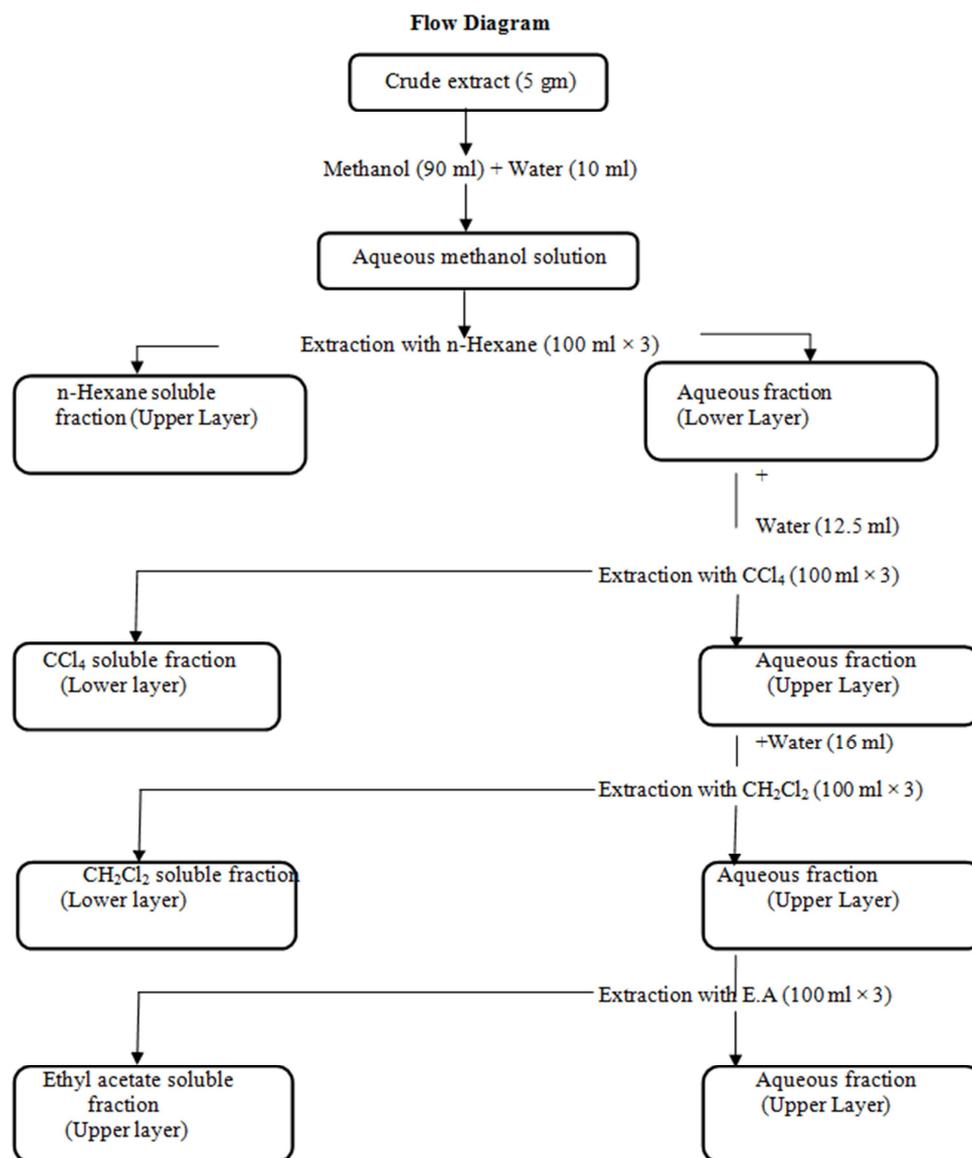


Figure 2. Schematic representation of the modified Kupchan Partitioning of methanolic crude extract of *Acacia nilotica*.

After evaporation the weight of the different fractions obtained are as follows:

Table 2. Amount of fractions after fractionation of crude methanolic extract.

Fraction	Weight
n-Hexane soluble fraction	0.50 g
Carbon tetrachloride soluble fraction	0.90 g
Dichloromethane soluble fraction	1.25 g
Ethyl acetate soluble fraction	1.80 g

7. Principle

In this method test group received the methanolic crude

extract, ethyl acetate fraction and control group received vehicle (1% Tween 80 normal saline). Thirty minutes later phenobarbitone was administered to each mouse to induce sleep. The animals were observed for the latent period and duration of sleep [22].

8. Experimental Animal

Swiss albino mice of either sex weighing 25-30 g were obtained from the Animal house of Jahangirnagar University. The animals were housed under standard laboratory conditions (relative humidity 55-65%, r.t. $23.0 \pm 2.0^\circ\text{C}$ and 12 h light: dark cycle). The animals were fed with standard

diet and water.

As it was difficult to observe the biologic response of five mice at a time receiving same treatment, it was quite necessary to identify individual animal of a group during the treatment. The animals were individualized in the following way (Figure 3) and marked as M-1=Mice 1, M-2=Mice 2, M-3=Mice 3, M-4=Mice 4 and M-5=Mice.

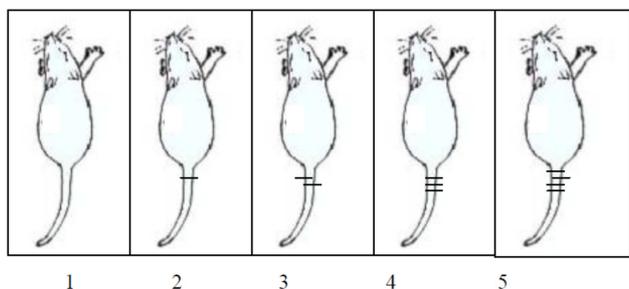


Figure 3. Identification of each test animals per group.

9. Experimental Design

Fifteen experimental animals were randomly selected and divided into three groups denoted as group-I, group-II(A), group-II(B) consisting of 5 mice in each group. Each group received a particular treatment. Prior to any treatment, each mouse was weighed properly and the doses of the test samples and control material were adjusted accordingly. As it was difficult to observe the biologic response of five mice at a time receiving same treatment, it was necessary to identify individual animal of a group during the treatment. The animals were individualized by keeping the separate cages.

10. Drugs

Pure phenobarbitone sodium was obtained from Incepta Pharmaceuticals Ltd. Bangladesh. Normal saline water was obtained from Beximco pharmaceuticals Ltd, Bangladesh.

11. Preparation of Test Materials

In order to administer the methanol (crude) extract at doses of 400 mg/kg body wt and 200 mg/kg body wt of mice, 120 and 60 mg of the extract were measured respectively and triturated in unidirectional way by adding of 2, 3 drops of Tween-80 (A suspending agent) and 50-100 micro litre of DMSO. After proper mixing of extract, suspending agent and DMSO, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture.

Table 4. Screening of hypnotic activity by measuring the onset of sleep and total sleeping time after intraperitoneal administration of phenobarbitone sodium.

Animal group	Time of onset of sleep (Min)					Total sleeping time (Min)				
	M 1	M 2	M 3	M 4	M 5	M 1	M 2	M 3	M 4	M 5
Control	25	13	17	15	20	60	75	58	62	45
Crude extract	18	21	20	19	24	52	65	45	61	57
Ethyl acetate fraction	22	15	18	25	20	62	55	49	71	60

M 1, M 2, M 3, M 5 denotes first, second, third, fourth and fifth mice respectively.

Table 3. Particulars of test materials used in the Phenobarbitone induced sleeping time test.

Test Samples	Group	Identification	Dose (mg/kg)	Route of administration
1% Tween-80 in normal saline	I	Control group	5 ml/kg	Oral
Control	II	Positive control	1.0	i.p.
MEAN	III	Test group	400	Oral
EAAN	IV	Test group	400	Oral

MEAN=Methanolic extract of *Acacia nilotica*, EAAN= Ethylacetate fraction of *Acacia nilotica*

12. Procedure

Phenobarbitone induced Sleeping test was carried out according to the method of Williamson et al. (1996). The test animals were divided in three groups consisting of five mice per group. Group I was the control group where as group III and IV were experimental groups. The experimental groups were administered with test samples prepared with normal saline water and tween-80 at doses of 400 mg/kg body weight, while the control group was administered normal saline water containing 1% Tween 80 solution. Thirty minutes later Phenobarbitone sodium (25mg/kg body weight) was administered intraperitoneally to all the groups to induce sleep. The onset of sleep and total sleeping time were recorded for both control and treated groups.

13. Statistical Analysis

All values were expressed as the mean \pm standard error of the mean (SEM) and the results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's test by using SPSS ver.16.

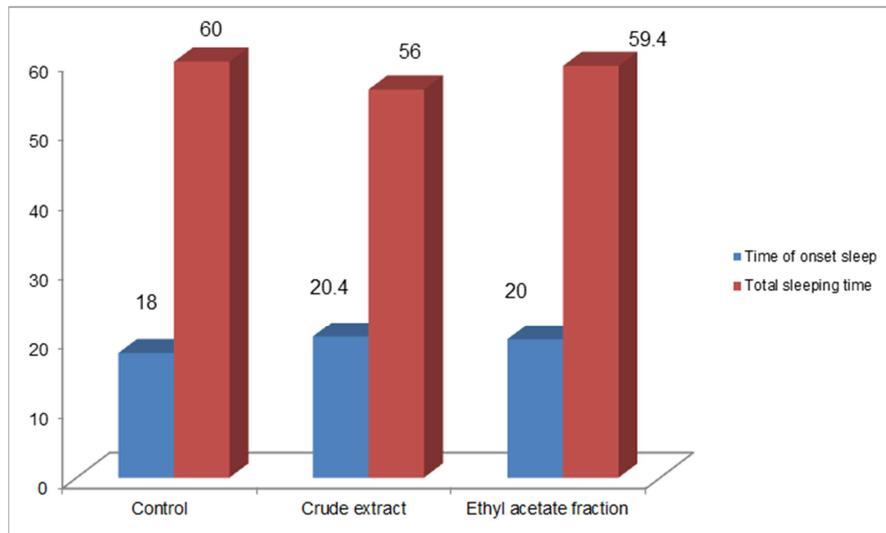
14. Result

The methanolic extract of *Acacia nilotica* bark has no effect on phenobarbitone sodium-induced sleeping time. The time of onset of sleep was 18 min in control group whereas in experimental group it was 20.4 min for crude extract and 20 min for ethyl acetate fraction group. The total sleeping time was 56 min and 59.4 min. for crude extract and ethyl acetate fraction respectively, while it was 60 min in control group.

From this study it has been showed that methanol extracts and ethyl acetate fraction of *Acacianilotica* has no antidepressant activity on mice.

Table 5. Effect of methanol extract and ethyl acetate fraction of *Acacia nilotica* on phenobarbitone sodium-induced sleep.

Group	Time of onset sleep	Total sleeping time
Control	18 ± 2.10	60 ± 4.79
Crude extract	20.4 ± 1.03	56 ± 3.492
Ethyl acetate fraction	20 ± 1.703	59.4 ± 3.668

**Figure 4.** Phenobarbitone induced sleeping time of *Acacia nilotica* bark extract.

15. Discussion

Depression is an important psychiatric disorder that affects individuals' quality of life and social relations directly. Depression is characterized by emotional symptoms such as hopelessness, apathy, loss of self-confidence, sense of guilt, indecisiveness, and amotivation, as well as biological symptoms like psychomotor retardation, loss of libido, sleep disturbances, and loss of appetite. When the symptoms are very severe, major depression is considered.

The methanolic extract of *Acacia nilotica* bark has no effect on phenobarbitone sodium-induced sleeping time. As it can not prolong the sleeping time so the finding from the experiment suggests that the methanolic extract of *Acacia nilotica* has no significant sedative effects.

16. Conclusion

The present study provides the first evidence indicating that methanolic extract of *Acacia nilotica* didn't show significant antidepressant activity. Further research is required to know more about antidepressant activity using Forced Swim Test and Tail Suspension Test.

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