

Pharmacological Evaluation of *Antidesma ghaesembilla* Gaertn Fruits for Central Nervous System Depressant Activity

[Evaluación farmacológica de frutos de *Antidesma ghaesembilla* Gaertn como depresores del sistema nervioso central]

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Abstract

The objective of this study is to investigate the anxiolytic and sedative activities of the methanol and chloroform extracts of *Antidesma ghaesembilla* fruits at the dose of 400 mg/kg bw using rodent behavioral models such as thiopental sodium-induced sleeping time, hole cross method and open field process for sedative and its anxiolytic activity was evaluated using the elevated plus maze (EPM) methods. In case of thiopental sodium-induced sleeping time, both extracts exhibited dose dependent suppression of motor activity, exploratory behavior (in open field and hole cross method) and prolongation of thiopental sodium-induced sleeping time in mice, where maximum effect was shown by the methanol extract. In EPM test, the methanolic extract significantly increased exploration to and time spent by the treated mice in EPM open arms in a way similar to that of diazepam, but the chloroform extract was found to produce moderate activity. These significant results may justify the scientific basis for use of this plant in traditional medicine as a modality for anxiety and related disorders.

Keywords: Medicinal plant, *Antidesma ghaesembilla*, sedative, anxiolytic, elevated plus maze

Resumen

El objetivo de este estudio fue la investigación de las actividades ansiolíticas y sedantes de los extractos clorofórmicos y metabólicos de los frutos de *Antidesma ghaesembilla* a las dosis de 400 mg/kg pp utilizando modelos de comportamiento de roedores, tales como el tiempo de sueño inducido por tiopental sódico, el método de "hole cross" (cruce de un agujero) y el campo abierto para evaluar sedación, y la actividad ansiolítica fue evaluada utilizando el método del laberinto elevado (elevated plus maze, EPM). En el caso del sueño inducido por tiopental sódico, ambos extractos exhibieron una supresión dosis dependiente de la actividad motora, de la actividad exploratoria (en el método de campo abierto y "hole cross") y prolongación del tiempo de inducción de sueño inducido por tiopental en ratones, con efectos máximos mostrados para el extracto metabólico. En el ensayo de EPM, el extracto metabólico aumentó significativamente el tiempo de exploración y el tiempo consumido en el laberinto de una manera similar al diazepam, pero el extracto clorofórmico se encontró que produjo solo una moderada actividad. Estos resultados significativos pueden justificar una base científica para el uso de plantas en medicina tradicional para tratar la ansiedad y desórdenes relacionados.

Palabras Clave: Planta medicinal, *Antidesma ghaesembilla*, sedante, ansiolítico, laberinto elevado.

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INTRODUCTION

Anxiety and depression are the most common and complex problems worldwide. It is reported that more than 20% of the adult population suffer from these conditions at some stage during their life (Abid *et al.*, 2006; Wattanathorn *et al.*, 2007). Since long the benzodiazepines remain to be the most frequently prescribed synthetic drugs of choice for acute anxiety and other related disorders including depression, epilepsy and insomnia, but chronic use of these drugs have serious side effects ranging from respiratory, digestive and immune system dysfunctions to deterioration of cognitive function, physical dependence and tolerance (Dhawan *et al.*, 2003). In respect to anxiety and sedation, effective plant remedies are likely to have advantages over benzodiazepines by not having serious side effects mentioned above. In this context, there has been a revival of interest in medicine from natural sources (mainly from plant kingdom) with the hope that drugs of plant origin will have significantly lesser side effects than that observed with synthetic drugs while exhibiting comparable efficacy. The effectiveness of traditional medicines in treating such disorders is well documented (Fennell *et al.*, 2004; Gelfand *et al.*, 1985). *Antidesma ghaesembilla* (Family: Phyllanthaceae) is a large black tree, indigenous from Australia and Southeast Asia (Gumede, 1990) and grows mostly in India, Bangladesh, China and Myanmar. The fruits of this plant are one seeded true fruits generally cluster in habit and occur mostly in summer season. The ripe fruits of *A. ghaesembilla* are used as a seasoning agent in fish and meat preparations (Hedrick, 1919; Nazaruddin, 2010). Traditionally, this plant is used as a folk medicine for sedation among the tribes although it is not yet included in any literature. Hence, we decided to investigate the sedative and hypnotic effects of the methanol and chloroform extracts of dried fruits of *A. ghaesembilla*.

MATERIALS AND METHODS

Drugs and chemicals

The following drugs and chemicals were used in this study: Diazepam (Square Pharmaceutical Ltd., Bangladesh), thiopental sodium (Gonosashto Pharmaceuticals Ltd., Bangladesh), methanol and chloroform (Sigma Chemicals Co., USA).

Plant material

The plant samples were collected from the forests of Chittagong and Chittagong Hill Tracts in May 2010 when fruits were in their maximum productivity. The plant was identified by Dr. Shaikh Bokhtear Uddin, Associate Professor, University of Chittagong and a voucher specimen (SUB316ctgUH), has been deposited in the Department of Botany, University of Chittagong, Chittagong, Bangladesh. The fruits were thoroughly washed with water and dried in a shade at room temperature for 7 days; after that they were dried in an oven at 40° C for the next 2 days to facilitate grinding.

Preparation of plant extracts

The dried fruits were powdered coarsely and about 500 g of powdered material was separately macerated in chloroform (2 L) and methanol (2 L) at room temperature for seven days accompanying occasional shaking and stirring. The whole mixture was then filtered and the filtrate thus obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterlin Ltd, UK) to get a viscous mass. The viscous mass was then kept at room temperature under a ceiling fan to get the dried extracts.

Animals

White albino mice (Swiss-webstar strain, 20-35 g body weight [bw]) bred in the animal house of Jahangirnagar University, Bangladesh was used for the experiments. The procedures in this study for animal handling were performed in accordance with the Animal Resources Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B) and approved by the Institutional Ethical Review Committee. All efforts were made to minimize animals' suffering and to reduce the number of animals used in the experiments. The animals were provided with standard laboratory food and tap water *ad libitum* and maintained at natural day-night cycle. All the experiments were conducted in an isolated and noiseless condition. The test animals were divided into two groups for two different extracts at the dose of 400 mg/kg bw. The animals were acclimatized to laboratory condition for one week prior to experimentation.

Preliminary phytochemical screenings

The extracts were screened for the presence of various chemical classes of constituents (alkaloids, tannins, glycosides, steroids, terpenoids, flavonoids, carbohy-

drates, and saponins) using standard procedures (Ghani, 2003).

Acute toxicity test

LD₅₀ value was estimated by "Acute Toxicity Test". Swiss Albino mice of 4-5 weeks old, weighing 20-25 g were used for this study. The test extract (methanol) was dissolved in distilled water with the help of Tween 80 and administered orally to six groups of mice (n = 5) at different doses (200, 400, 1000, 2000, 4000, and 6000 mg/kg bw). LD₅₀ was evaluated by recording mortality after 24 hours (Litchfield and Wilcoxon, 1949).

Thiopental sodium-induced sleeping time test

The animals were randomly divided into four groups consisting of five mice in each group. The test groups received two different extracts from fruits of *A. ghaesembilla* at 400 mg/kg b.w. orally, while the mice of the positive control group were treated with diazepam (1 mg/kg) and the negative control mice with vehicle (1% Tween 80 in water). Thirty minutes later, thiopental sodium (40 mg/kg) was administered to each mouse to induce sleep. The animals were observed for the latent period (time between thiopental sodium administrations to loss of righting reflex) and duration of sleep, time between the loss and recovery of reflex (Ferrini *et al.*, 1974).

Hole cross test

The experiment was carried out as described by Takagi *et al.*, (1971). A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm in diameter was made at a height of 7.5 cm at the center of the cage. The animals were divided into negative control, positive control, and test groups containing five mice in each group. The test groups received the extracts of *A. ghaesembilla* at a dose of 400 mg/kg b.w. orally whereas the negative control group received vehicle (1% Tween 80 in water) and positive control group was given diazepam (1 mg/kg b.w.). The number of passage of a mouse through the hole from one chamber to another was counted for a period of 3 min at 0, 30, 60, 90, and 120 min after oral administration of the test drugs.

Open field test

In open field test, the animals were divided into negative control, positive control, and test groups containing five mice in each group. The test groups received both extract at 400 mg/kg bw orally, the negative control group received vehicle (1% Tween 80 in water) whereas positive control group received Diazepam (1 mg/kg bw). The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 min after oral administration of the test drugs (Gupta *et al.*, 1971).

Elevated plus maze test

The apparatus consisted of two open arms (5 × 10 cm) and two closed arms (5 × 10 × 15 cm) radiating from a platform (5 × 5 cm) to form a plus-sign figure. The apparatus was situated 40 cm above the floor. The open arms edges were 0.5 cm in height to keep the mice from falling and the closed-arms edges were 15 cm in height. Sixty minutes after administration of the test drugs, each animal was individually placed in the center of the elevated plus maze and were allowed 5 min for free movement. Next, the number of open and enclosed arm entries, and time spent on open arms were manually registered (Pellow and File, 1986). Entry into an arm was defined as the point when the animals placed all four paws into the arm. The procedure was conducted in a sound attenuated room and observations made from an adjacent corner (Hogg, 1996).

Statistical analysis

The experimental results have been expressed as the mean ± SEM (Standard Error of Mean). Data have been calculated by one way ANOVA followed by Dunnett 't' test using SPSS software (version 10). P values < 0.05 were considered significant.

RESULTS

Preliminary phytochemical screenings

Results of the phytochemical analysis revealed the presence of glycosides, steroids, flavonoids and terpenoids in both methanol and chloroform extracts of *A. ghaesembilla* as depicted in Table 1.

Table 1
Result of phytochemical screening of *A. ghaesembilla* extracts

Extracts	Carbohydrates	Glycosides	Saponins	Steroids	Alkaloids	Tannins	Flavonoids	Terpenoids
Methanol	-	+	-	+	-	+	+	+
Chloroform	-	+	-	+	+	-	+	+

(+) denotes for presence and (-) denotes absence.

Acute toxicity test

Acute toxicity studies revealed the LD₅₀ as 4.0 g/kg for oral route. No visible signs of delayed toxicity and mortality were observed when the animals were monitored for 7 days.

Thiopental sodium-induced sleeping time test

In thiopental sodium-induced hypnosis test, methanol

extract of the dried fruits of *A. ghaesembilla*, at the dose of 400 mg/kg, induced the sleep at an earlier stage as compared to standard diazepam but the chloroform soluble extract exhibited moderate hypnotic activity. However, both extract at 400 mg/kg b.w. prolonged the duration of sleeping time in test animals when compared to control (Figure 1).

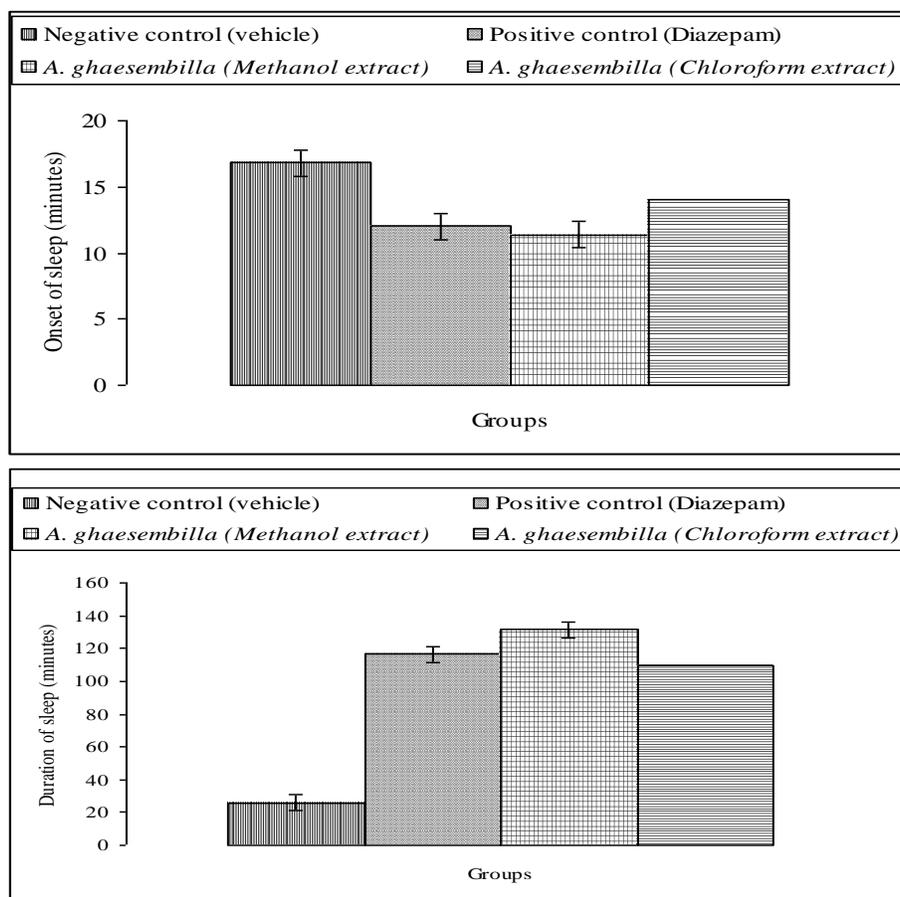


Figure 1

Effect of methanol and chloroform extracts of dried fruits of *A. ghaesembilla* on thiopental sodium-induced sleeping time in mice. Values are mean \pm SEM ($n = 5$); $p < 0.05$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse].

Hole cross test

The chloroform and methanol extracts at 400 mg/kg (b.w), produced significant ($p < 0.05$) decrease of locomotion from its initial value during the period of

experiment (Figure 2). Maximum suppression of locomotor activity was displayed by methanol extract, which was comparable to the reference drug diazepam.

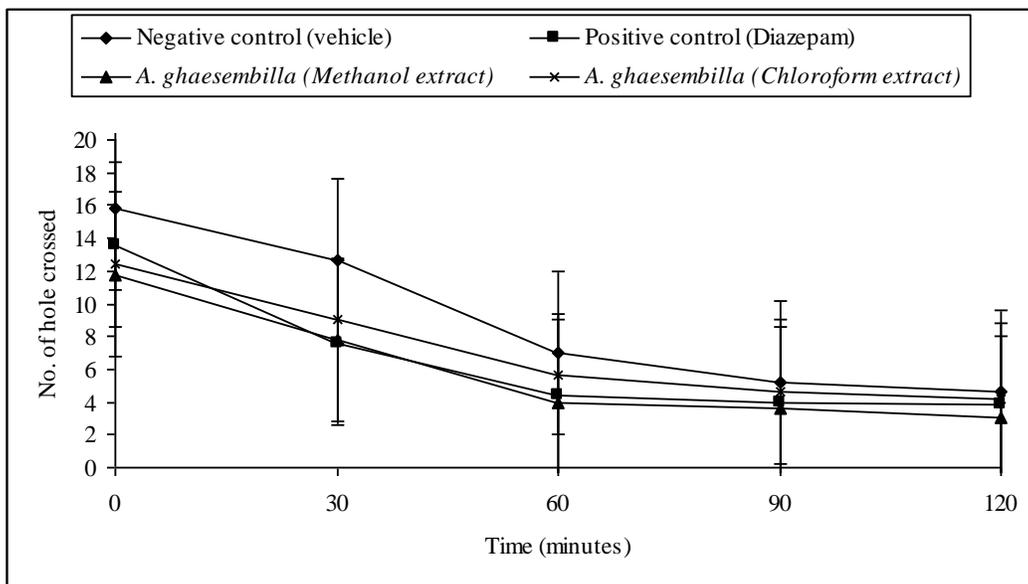


Figure 2

Effect of methanol and chloroform extracts of the dried fruits of *A. ghaesembilla* on Hole cross test in mice. Values are mean \pm SEM ($n = 5$); $p < 0.05$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse].

Open field test

The number of squares traveled by the mice was suppressed significantly from the second observation period at the dose level (400 mg/kg b.w.) for both

extracts. The results were statistically significant (Figure 3). The locomotor activity decreased in the following order: methanol>chloroform.

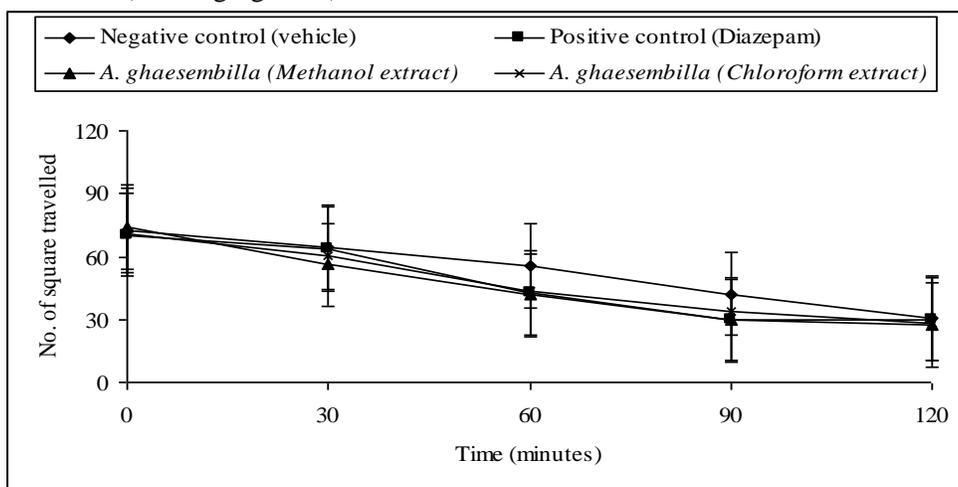


Figure 3

Effect of methanol and chloroform extracts of the dried fruits of *A. ghaesembilla* on Open field test in mice. Values are mean \pm SEM ($n = 5$); $p < 0.05$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse].

Elevated plus-maze test

The methanol extract at the dose of 400 mg/kg b.w. significantly increased the percentage of entries

of mice into the open arms, and the percentage of time spent (Table 2) in the open arms of the elevated plus-maze.

Table 2
Effect of methanol and chloroform extracts of *A. ghaesembilla* on the percentage of entries and the time spent in open arms of the elevated plus-maze during the 5-min test session.

Animal group	% No. of entry into open arm	% Time spent in open arm
I (Negative Control)	55.88 ± 2.133	51.93 ± 8.243
II (Positive control: Diazepam)	76.28 ± 1.847	79.39 ± 5.749
III <i>A. ghaesembilla</i> (methanol extract)	74.40 ± 1.324	81.62 ± 6.522
IV <i>A. ghaesembilla</i> (chloroform extract)	61.04 ± 3.43	68.33 ± 3.757

Values are mean ± SEM ($n = 5$); $p < 0.01$, Dunnet test as compared to control [Vehicle = 0.4 mL/mouse].

DISCUSSION

The studies have examined some neuropharmacological activities of methanolic and chloroform extracts of *A. ghaesembilla*. The plant extracts possessed central nervous system depressant activity as indicated by the decrease in exploratory behaviour in mice. It also showed a marked sedative effect as indicated by the reduction in gross behaviour and potentiation of thiopental induced sleeping time. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both (Nyeem *et al.*, 2006). Moreover, the study on locomotor activity, as measured by hole cross and open field tests, showed that both extracts of the dried fruits of *A. ghaesembilla* (400 mg/kg) decreased the frequency and the amplitude of movements. Since, locomotor activity is a measure of the level of excitability of the CNS (Mansur *et al.*, 1980), this decrease in spontaneous motor activity could be attributed to the sedative effect of the plant extracts (Rakotonirina *et al.*, 2001; Ozturk *et al.*, 1996). Both the extracts significantly decreased the locomotion in mice. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 5th observation period (120 min). Maximum depression of locomotor activity was observed from the 3rd (60 min) to 5th (120 min) observation period.

However, the anxiolytic effect was evidenced by elevated plus maze test which has been recognized as a valuable model to predict anxiolytic effects of drugs in rodents (Lister, 1987). An anxiolytic effect is suggested when the test drug increases open arms entries without altering the total number of arm entries. Although the chloroform extract at 400 mg/kg bw, in mice did not display significant increase in the percentage of entries into open arms, it is of interest to note that the methanol extract at the same dose showed a significant increase in the percentage of time spent in the open arms of the maze, slightly larger to the effects observed following treatment with the reference anxiolytic drug diazepam. Anxiety may be represented by an avoidance of the open arm and immobility of an animal placed in the EPM that exhibited by the methanol extract. The beneficial effects of medicinal plants result from the combination of secondary metabolites present in the plant, through additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process (Briskin, 2000). According to Kaufman *et al.*, (1999), preliminary phytochemical analysis in this study revealed the presence of alkaloids, tannins, glycosides, steroids, flavonoids and tannins. These secondary metabolites especially flavonoids individually or in combination, might

account for the observed pharmacological effects of this plant. However, many flavonoids were found to be ligands for the gamma aminobutyric acid type A (GABA_A) receptors in the central nervous system (CNS), which led to the hypothesis that they act as benzodiazepine like molecules. Thus, the sedative and anxiolytic effects exhibited by the *A. ghaesembilla* extracts might be due to the interaction of flavonoids with the GABA/benzodiazepine receptor complex in brain (Trofimiuk *et al.*, 2005). This is supported by their behavioral effects in animal models of anxiety, sedation and convulsion (Marder and Paladini, 2002; Johnston, 2005). Electrophysiological experiments with flavone and flavanone derivatives have shown that some of them can modulate GABA-generated chloride currents, either positively or negatively. Due to the increased knowledge of the diversity of GABA_A receptor sub-types, the number of studies with cloned receptors of defined subunit composition has recently risen, and experiments with some natural and synthetic flavones and flavonones have shown that they can modulate gamma aminobutyric acid (GABA)-generated chloride currents, either positively or negatively (Goutman *et al.*, 2003; Campbell *et al.*, 2003; Kavvadias *et al.*, 2004). Thus the decreased spontaneous motor activity could be attributed to the CNS depressant activity of the fruits of *A. ghaesembilla*. Lethal dose was found to be about 4.0 g/kg (per oral) in Swiss Albino mice because the experimental animal showed high toxicity on body weight or general appearance, at this dose level.

The results from the experiments confirmed that the methanol extract of *A. ghaesembilla* fruits possesses strong sedative and anxiolytic potential where as chloroform has moderate effect. Therefore this justifies its use in traditional medicine in the management of anxiety and related neuropsychiatric disorders.

CONCLUSION

Based on the results of the present studies, it can be concluded that the extracts of *A. ghaesembilla* possess significant neuropharmacological activity. Using behavioral pharmacology models, we have confirmed that *A. ghaesembilla*, in particular the methanol extract of the fruits possesses strong sedative and anxiolytic potential. Therefore, this plant extract could have significant therapeutic utility for the treatment of anxiety and related neuropsychiatric disorders. Furthermore, evidence obtained from the present study may justify the use of this plant in traditional medicine

for the treatment of excited mental disorders such as psychosis, insanity, epilepsy. However, the study conducted here are preliminary in nature. So further studies to be conducted with this plant extracts for better understanding of the pharmacological activities, mechanism of action as well as to isolate the active compound(s) responsible for these properties.

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