Physicochemical, Phytochemical and Anti-anxiety Potential of Alcoholic of *Emblica officinalis* Gaertn Fruits

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Abstract

Emblica officinalis Gaertn (synonym *Phyllanthus emblica* Linn, Euphorbiaceae, *E. officinalis*) or Indian gooseberry, commonly known as, Amla, in Ayurveda, is unarguably the most important medicinal plant for prevention and treatment of various ailments. The objective of this study was to investigate pharmacognostical, phytochemical features and anxiolytic activity of alcoholic extracts of *E. officinalis* fruits. The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. The elevated plus maze test (EPMT), light and dark test (L and DT) and open field test (OFT) were used to assess the anxiolytic activity of the alcoholic extract of *E. officinalis* fruits in mice. The efficacy of extract (250, 500, 750mg/kg) was compared with standard anxiolytic drugs diazepam (1mg/kg). Phytochemical analysis of alcoholic extract revealed the presence of carbohydrates, tannins and phenolics, amino acids and proteins, flavonoids, glycosides, fixedoilsandfats. The alcoholic extract of *E. officinalis*(250, 500, 750mg/kg, p.o.) significantly increased the percentage of time spent and number of entries in open arm in EPMT. In L and DT, the extract produced significant increase in time spent, number of crossing and decrease in the duration of immobility in light box. In OFT, the extract showed significant increase in number of rearings, ambulation and decrease self grooming and fecal dropping, all of which are demonstrations of exploratory behavior. The results of the present study suggest that an alcoholic extract of *E. officinalis* may possess anxiolytic activity and provide a scientific evidence for its traditional claim.

Keywords: Anxiolytic, *Emblica officinalis* Gaertn, Elevated plus maze, Open field test, Light and dark test

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Introduction

Anxiety is considered a common emotional phenomenon in the human population, occurring in response to physiological and/or environmental factors. However, it can be a cause of disturbance in daily life and can express a pathological state [1]. Pharmacological treatment for anxiety disorder consists of the use of benzodiazepines and antidepressant drugs; however, although these treatments showing clinical efficacy, they have several problems. Benzodiazepines can lead to disturbing effects, such as amnesia, dependence liability, and sedation. The antidepressants can lead to sexual dysfunction, insomnia, and gastrointestinal disturbances [2, 3]. Thus, there is a need for the development of new anxiolytic drugs that lack these effects. In this context, medicinal plants continue to have anxiolytic potential [4]. Several traditionally used plants exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders, such as anxiety disorders [5]. In folk medicine, some species belonging to the family Fabaceae, such as *Erythrina falcata* and *Melilotus officinalis*, are known to possess anxiolytic action [6]. Moreover, Ribeiro et al. (2006) in recent studies revealed anxiolytic-like effects of another member of Fabaceae family, *Erythrina velutina*, in animal models of anxiety. This study showed that acute and chronic administration of the hydroalcoholic extract of stem bark from *E. velutina* impaired inhibitory avoidance in the elevated T-maze test, which suggests an anxiolyticlike effect [7].*E. officinalis* (*Euphorbiaceae*) commonly known as amla grow in the tropical areas of South-East Asia. The fruit of the plant is one of the most important medicinal ingredients used in Ayurveda, Siddha, Unani, Arabic, Tibetan, and various other folk systems for the management of myriad chronic ailments [8]. Experimental studies have shown potent antioxidant, analgesic, antipyretic, adaptogenic, immunomodulatory, and antiulcerogenic activities of the fruit of *E. officinalis* [8-10].The fruits are reported to contain thermostable vitamin C, minerals, amino acids, tannins, flavonoids, and other important phytochemicals which are believed to possess diverse pharmacological and biological effects [11]. Earlier studies have shown that the leaf extract possesses anti-inflammatory activities in the carrageenan and dextran-induced rat hind paw edema [12]. However, so far, its effect on CNS activity has not been studied. Therefore, we undertook the study to evaluate the anxiolytic potential of *E. officinalis*, by using different animal models.

Materials and methods

Plant materials

The fruits of *E. officinalis* were collected from Bhopal Region, Madhya Pradesh, India. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number **253/Saif./Sci./Clg/Bpl** was kept in Department of Botany, Saifia College of Science, Bhopal for future reference. Fresh fruits of plants were used for pharmacognostical studies. Fruits of *E. officinalis* were dried under shade and powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies

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Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this studywere of analytical grade.

Macroscopical characterization

The macroscopical description of *E. officinalis* fruits include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. were studied [13].

Physicochemical parameters

Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, loss of moisture content, swelling index were determined using standardprocedures [14,15].

Plant material fattening

Extraction

E. officinalis fruits powder was produced after shade drying at room temperature. The shade-dried plant material was coarsely ground up and put through a petroleum ether extraction process utilizing soxhlet equipment. The extraction process was continued until the material had been sufficiently defatted.

Extraction by soxhlation process

An *E. officinalis* fruits that has been defatted was exhaustively extracted with different solvent (chloroform, ethyl acetate and ethanol) by soxhletion method. The extract evaporated beyond their boiling points. The dried crude concentrated extract was weighed in order to calculate the extractive yield. When ready for analysis, it was then put into glass vials (6 x 2 cm) and stored in a refrigerator 4°C [16].

Phytochemical screening of the extract

Various phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were analysed qualitatively in the *E. officinalis* extract [17, 18].

Animals

Male Swiss albino mice (22-25 gm) were used in the study. This was done in order to avoid the influence of ovarian hormone fluctuations across the estrous cycle in female mice. The behavioral observations took place in sound proof rooms at the same period of the day to reduce the confounding influence of diurnal variation in spontaneous behavior. The registration number for the Institutional Animal Ethical Committee is (Reg. No. 1824/PO/RcBi/S/15/CPCSEA), and the animal experiment proposal number is IAEC-PBRI/IAEC/PN-19117. All procedures were performed in accordance with IAEC, constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, New Delhi. All the animals were obtained from the animal house of Pinnacle Biomedical Research Institute, Bhopal, where they were housed in groups of six mice per cages and maintained under standard environmental conditions: 25±2°C temperature, 12:12 hour light and dark cycle, and 45-55% relative humidity, with free access of food and water *ad libitum*. Food, not water, was withdrawn 6 hours before and during the experiment. All the experiments were carried out during the light period (0800:1600 hours).

Acute oral toxicity studies

Acute oral toxicity test was performed according to Organization for Economic Co-operation and Development (OECD) guideline test, ANNEX-423. Alcoholic extract of *E. officinalis* at different doses 5, 50, 300 and 2000 mg/kg was administered orally to female mice. The literature search of conventional LD_{50} tests shows that usually there is little difference in sensitivity between the sexes, but in those cases where differences are observed, females are generally slightly more sensitive than males. This was the reason behind choosing female mice for the toxicity studies [19]. The animals were observed for signs of toxicity such as hyperactivity, grooming, convulsions, sedation, and hypothermia continuously for 2 hours, and for mortality up to 24 hours, after administration of the doses.

Behavioral assessment of anxiolytic activity

Treatment schedule

The anxiolytic activity was examined by using the elevated plus maze (EPM) test, open field test (OFT), and light and dark test (L and DT). The animals were divided into five

groups, with each group consisting of six male mice. Group 1 received vehicle (normal saline); group 2 received diazepam (1 mg/kg); groups 3 to 5 received *E. officinalis* extract (250, 500 and 750 mg/kg).

Elevated plus maze test

The EPMT apparatus consisted of four arms elevated 30 cm above the floor, with each arm positioned at 90° relative to the adjacent arms. Two of the arms were enclosed with high walls (30 × 7 × 20 cm), and the other arms were connected via a central area (7 × 7 cm) to form a plus sign. The maze floor and the walls of enclosed arms were painted black. The room was illuminated with a 40-W lamp at the central platform. The animals were treated with vehicle, extract and diazepam, 60 min prior to the test. The experiment was performed between 0900 and 1400 hours, and the mice became accustomed to the dimly lit experimental laboratory for 30 min prior to behavioral testing. Each mouse was individually placed on the central platform facing toward an open arm. The frequency and duration of entries into the open and closed arms were observed for 5 min. An entry was counted when all four paws of the mouse entered an open or closed arm. Subsequently, the percentage of time spent (duration) in the open arms [100 × open/ (open + enclosed)] and percentage of the number of open arm entries (frequency, 100 × open/total entries) were calculated for each animal. The apparatus was thoroughly cleaned after each trial [20].

Open field test

The apparatus consisted of a wooden box (60 × 60 × 60 cm). The arena of the open field was divided into 16 squares (15 × 15 cm): the four inner squares in the center and 12 squares in the periphery along the walls. The experimental room was a sound attenuated, dark room. The open field arena was illuminated with a 40-W lamp, focusing on the field from a height of about 75-100 cm. After 60 min of treatment with vehicle, diazepam (1 mg/kg) and *E. officinalis* extract (250, 500 and 750 mg/kg), animals were placed individually in one of the corner squares and number of rearings, assisted rearings and number of squares crossed were observed for 5 min [21].

Light and dark test

The L and DT apparatus consisted of open top wooden box. Two distinct chambers, a black chamber (25 cm long × 35 cm wide × 35 cm deep), painted black and made dark by covering its top with black plywood, and a bright chamber (25 cm long × 35 cm wide × 35 cm deep), painted white and brightly illuminated with 40-W white light source, were placed 25 cm above the open box. The two chambers were connected through a small open doorway, (7.5 cm long × 5 cm wide) situated on the floor level at the center of the

partition. The mice were placed individually in center of the light box after 60 min of oral treatments and observed for 5 min [22].

Statistical analysis

All the results were expressed as mean ± SEM and the data were analyzed using one- way analysis of variance (ANOVA) followed by Dunnett's "*t*" test. A *P* value of <0.05 was considered as the level of significance.

Results

The crude extracts so obtained after the soxhletion extraction process was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. The yield of *E. officinalis* chloroform, ethyl acetate and ethanol extracts was 3.3, 6.7 and 7.9 % w/w respectively. Table 1 summarizes the morphological properties of *E. officinalis* fruits. The plants is a small to medium sized deciduous tree with an average height of 8-18 m, with thin light grey bark exfoliating in small thin irregular flakes, exposing the fresh surface of a different color underneath the older bark. Fruits are fleshy, almost depressed to globose shape, 5.3-5.7 g in weight, 4.5-5.0ml in volume. Their fruits are a light green to pale yellow colour, have characteristics odour, 2.1-

2.4 cm in diameter with astringent taste.*E. officinalis* fruits were shade dried and ground into powder to measure a number of physiochemical parameters, including, total ash value, water soluble ash, acid insoluble ash extractive soluble in alcohol, extractive soluble in water, loss on drying, foreign organic matter determination and foaming index (Table 2). Phytochemical investigation of various extract of *E. officinalis* revealed the nature of phytochemicals present in their fruits which are summarized in the table 3. It was found that they contain abundant amount carbohydrates; tannins and phenolics, amino acids and proteins, flavonoids, glycosides, fixedoilsandfats were found in alcoholic extracts. *E. officinalis* were found to contain fixed oils and fats and phytosterols in chloroform extract. Ethyl acetate extract was also containing flavonoids, phytosterols, fixed oils and fats steroids Table 3. The alcoholic extract of *E. officinalis* was orally administered to different groups of mice (three mice per group) at doses of 5, 50, 300 and 2000 mg/kg body weight, respectively. The animals were observed for 48 hours to study their general behavior and to detect signs of discomfort and nervous manifestations. As even the mice receiving the highest dose of *E. officinalis*(2000 mg/kg body weight, p.o.) did not show any mortality, dose levels at $1/8^{th}$ (250 mg/kg body weight, p.o.) and $1/2.6^{th}$ (750mg/kg body weight, p.o.) of this highest dose were selected for the anxiolytic activity. Diazepam treated mice showed significant increase ($P < 0.05$) in the number of open arm entries, time spent in open arms and the number of rears in

the open arm. They showed a reduction in the time spent in closed arm. *E. officinalis* extract treated mice exhibited significant increase ($P < 0.05$) in the number of open arm entries (250, 500 and 750 mg/kg), time spent in open arm, percentile ratio of open arm to total arm entries, the number of total arm entries, and the number of rears in the open arm entries, but decrease in time spent in closed arm Table 4. In the OFT, diazepam treated mice showed significant increase ($P < 0.05$) in the number of rearings, ambulation and decrease self grooming and fecal dropping during 5 min interval of test as compared to vehicle treated groups. *E. officinalis* extract treated mice (250, 500 and 750 mg/kg) also produced significant increase in the number of rearings ($P < 0.05$), ambulation and decrease self grooming and fecal dropping ($P < 0.01$) Table 5. Treatment with diazepam significantly increased the time spent ($P < 0.001$) in light box as well as the number of crossings ($P < 0.05$) between the light and dark boxes, whereas the time spent in dark box ($P < 0.001$) were significantly reduced. *E. officinalis* treated mice also showed significant increase ($P < 0.001$) in the time spent in light box and the number of crossings between light and dark boxes. However, the time spent in dark box ($P < 0.01$) were significantly reduced ($P < 0.05$) as compared to the vehicle treated group Table 6.

Discussions

Anxiety, like all emotions, has cognitive, neurobiological and behavioral components. It is a negative emotion that occurs in response to perceived threats that can come from internal or external sources and can be real or imagined [23]. The incidence of anxiety in the community is very high and associated with lot of morbidity [24]. Ethno medical and pharmacological knowledge about the plant under study would allow us to evaluate central nervous system activity, which could be used to treat anxiety type of disorders. The present work has shown that anxiolytic activity by the *E. officinalis*, as assessed by OFT, EPMT and light/dark box test. The EPMT is used to evaluate psychomotor performance and emotional aspects of rodents. Results obtained on the elevated plus maze after treatment with *E. officinalis* (250, 500 and 750 mg/kg) revealed anxiolytic activity, since increases in open arm entry parameters are the most representative indices of anxiolytic activity [25]. Time spent on the central platform appears to be related to decision making and/or risk assessment, and the total arm entries is a contaminated measure reflecting changes in anxiety or in general activity [26]. The anxiolytic-like activity was also observed in the light/dark box. L and DT is an ethological-based approach-avoidance conflict test and it is sensitive to drugs that

affect anxiety. In this test, the number of transitions between the light and dark compartments as well as the time spent in the light side is recognized as anxiety indices, despite the transition parameter being highly dependent on locomotor activity [27]. Mice treated with *E. officinalis* (250, 500 and 750 mg/kg) showed increase in the time spent in the light compartment and no changes in the numbers of shuttle crossings, confirming the activity upon the main anxiolytic parameter. The observed anxiolytic effect of *E. officinalis* may be due to the agonistic effect on GABA/benzodiazepine receptor complex, or antagonize the 5-HT1B receptor or agonize the 5-HT1A receptor [28, 29]. The OFT is used to evaluate the animal emotional state. The open field model examines anxiety related behavior characterized by the normal aversion of the animal to an open, brightly lit area. Thus, animals removed from their acclimatized cage and placed in environment express anxiety and fear, by showing alteration in all or some parameters. Anxiolytic treatments reduce such fearful behavior of animals in open field [30]. Statistical analysis of the data obtained from these experiments supported anxiolytic-like activity of *E. officinalis* extract at the doses (250, 500 and 750 mg/kg) as its effect shows significant increase in the number of rearings, ambulation and decrease self-grooming and fecal dropping as compared to the vehicle treated group, which indicates its anxiolytic- like effect.

Table 1: Morphological characteristic of *E. officinalis* fruits

S. No	Parameters	*E. officinalis* fruits
1	Shape	Depressed to globose
3	Size	2.1-2.4 cm in diameter
4	Odour	Characteristics
5	Taste	Astringent
6	Colour	Light Green to pale yellow
7	Foreign organic matter	No adulterants have been found

Table 2: Physiochemical analysis of powder of *E. officinalis* fruits

S. No.	Parameters	Observations
1	Total ash	5.9
2	Water soluble ash	2.64
3	Acid insoluble ash	0.85
4	Water-soluble extractive	16.6
5	Ethanol soluble extractive	12.8
6	Loss on drying (%)	22.1
7	Foreign organic matter determination	1.9
8	Foaming index	24 (ml)

Table 3: Phytochemical screening of *E. officinalis* fruits extracts

Phytoconstituents	Chloroform extract	Ethyl acetate extract	Alcoholic extract
Carbohydrates	-	-	+
Carbohydrates	-	-	+
Aminoacidsand			
Proteins	-	-	+
Flavonoids	-	+	+
Saponins	-	-	-
FixedoilsandFats	+	+	+
Alkaloids	-	-	-
Glycosides	-	-	+
Phytosterols	+	+	-
Carbohydrates	-	-	+

Table4: Effectof alcoholic extract of E. officinalis (AEEO)on EPM paradigm in mice

G. No.	Drug Treatment	Dose (mg/kg)	Number of entries (mean±SEM)		Time spent in sec (mean±SEM)	
			Open arm	Closed arm	Open arm	Closed arm
I	Control	0.05ml/10g	7.17 ±0.17	10.98±0.31	37.666±1.308	190.83 ±3.04
II	Diazepam	1	11.70±0.25***	6.56±0.34***	81.333±0.25***	129.66±2.210***
III	AEEO	250	7.02±0.20	9.9 ±0.15	45.00± 0.50**	159.5±2.31***
IV	AEEO	500	8.11±0.11**	8.1±0.09***	61.50± 0.21***	145.13±1.71***
V	AEEO	750	10.37± 0.21***	7.38 ±.0.21***	77.71±9***	136.6 ±1.96***

Values were mean ± S.E.M. for (n=6) expressed as time (in sec) of 6 animals in each group. * P<0.05, ** P<0.01 *** P<0.001 as compared to control

Table 5: Effectof*E. officinalis*(AEEO)on following parameters in open field test

G. No.	Drug Treatment	Dose (mg/kg)	Ambulation(N)	Rearing (N)	SelfGroomin g(N)	Activity inCentre (N)	Fecaldroppi ng(N)
I	Control	0.05ml/10g	32.16±1.352	6.67 ±0.34	5.67 ±0.42	2.17±0.33	2.17 ±0.31
II	Standard	1	41.45±1.30***	7.23±0.42	2.29±0.33***	3.18±0.30	1.19±0.32
III	AEEO	250	34.9±1.10	7.13±0.30	4.82±0.4014	2.50±0.34	1.30±0.30
IV	AEEO	500	50.3±1.21***	9.68±0.19**	3.75±0.187*	3.42±0.21	1.47±0.12
V	AEEO	750	69.96±1.18***	13.26±0.40***	2.71±0.29***	6.17±0.24**	1.19±0.21

Valuesweremean±S.E.M.for(n=6)expressedastime(insec)of6animalsineachgroup.*P<0.05,**P<0.01,***P <0.001vs.control

Table 6: Effect of E. officinalis (AEEO) on Light dark transition model

G. No.	Drug Treatment	Dose (mg/kg)	Time spent in min (Mean±SEM)		Number of Entries (Mean±SEM)	
			Dark	Light	Dark	Light
I	Control	0.05ml/10	7.21 ±0.19	0.5 ±0.31	4.51 ±0.24	1.34 ±0.22
II	Diazepam	1	4 .0 ± 0.26***	1.8 ±0.24*	13.0 ± 0.24	5. 49 ± 0.29***
III	AEEO	250	6.94 ±0.21	0.62 ±0.29	7.35 ±0.49**	1.6 ±0.40
IV	AEEO	500	5.32±0.19***	1.2 ±0.38	8.78±0.25***	2.8 ± 0.19**
V	AEEO	750	3.21±0.19***	1.81±0.23**	12.2±0.29***	4.3±0.19***

Valuesweremean±S.E.M.for(n=6)expressedastime(insec)of6animalsineachgroup.*P<0.05, **P<0.01,***P<0.001as compared to control

Conclusion

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, alkaloids, phenolic acids, essential oil, saponins and tannins possess activity against many CNS disorders [31]. A survey of the literature on *E. officinalis* revealed the presence of tannic acid, gallic acid, alkaloids, sterols, flavonoids, glycosides, hydrolyzable tannins and high–molecular-weight polyphenolic compounds. It is possible that the mechanism of anxiolytic action of *E. officinalis* could be mediated by synergistic action of these phytochemicals.The results obtained in this

study suggest that the alcoholic extract of *E. officinalis* possesses anxiolytic properties. Thus, *E. officinalis* has potential clinical applications in the management of anxiety disorders. Further investigations are warranted for elucidating the exact mechanism and bioactive compounds

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