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PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF *AEGLE MARMELOS* LEAVES FOR SELECTIVE PSYCHOTROPIC ACTIVITY

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Keywords:

Aegle marmelos, Phytochemical, Hydroalcoholic, Anti-anxiety, Antidepressant

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ABSTRACT: *Aegle marmelos*, commonly known as Bael or Bilva, belonging to family Rutaceae, has been a tree of humongous significance since time immemorial. It has been used tremendously in ethnomedicine and ethnobotany owing to its spiritual connection. There are testimonials of its prolific use in Ayurveda, Unani and Siddha system of medicine. People also have found this tree of utility owing to its ecological and economic perspective. Almost all the parts of this tree are beneficial and have been used for various reasons. This research was undertaken to ascertain anti-anxiety and antidepressant responses in the hydroalcoholic leaf extract of *Aegle marmelos* employing the Elevated plus Maze for anxiety and Tail Suspension Test model for depression. Phytochemical screening of the prepared extract was also performed to recognize the phytochemicals possibly accountable for the selective psychotropic activities in the plant. A hydroalcoholic leaf extract using 50% ethanol was prepared and screened for presence of different phytochemicals and was found to comprise alkaloids, carbohydrates, flavonoids, tannins, saponins, terpenoids, coumarins, phenols, and reducing sugars. The findings revealed that AME 200 mg/kg and 400 mg/kg ameliorated the anxiolytic response by increasing the total time spent and number of entries made in the open arms in the elevated plus maze compared to the control. Also, the AME at 400 mg/kg dose, showed a dose-dependent, comparable response to the standard drug diazepam 2 mg/kg. Also, when AME was administered at 200 mg/kg and 400 mg/kg orally in the mice in the TST, it caused marked reduction in the total time of immobility in mice compared to the control. Also, the AME 400 mg/kg dose showed a dose-dependent and comparable response to the standard drug Fluoxetine 20 mg/kg. Thus, *Aegle marmelos* hydroalcoholic leaf extract is inferred to possess anxiolytic and antidepressant therapeutic responses and can serve as a potential agent against the available synthetic marketed preparations.

INTRODUCTION: With the advancement of technology and hastily changing lifestyle, there is an unceasing entail to corroborate own abilities in work and society and people are inadvertently making them fall in the grip of psychotropic diseases.

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The prominent diseases are anxiety and depression. Anxiety is an innate emotional state of mind which enables a person to perform many of his routine tasks. A little anxious state helps stimulate the mind and muster courage to face daily life challenges [1].

However, anxiety beyond the desirable state can lead to a continuous state of unrest and affect a person's psychological and physiological state to the extent of hindering his routine life. The anxiety disorder can be exhibited by continuous disturbances in a person's thoughts, behavior, mood and physiological activities.

It comprises panic disorders, social phobia, post-traumatic stress disorder, specific phobia, agrophobia, generalized anxiety disorder, disorders generated by generalized medical conditions, obsessive-compulsive disorder, substance-induced anxiety disorder, and acute stress disorder [2].

Depression is a severe mental sickness that affects people of all ages globally and can be counted as a major contributor to the load of ailments worldwide. A survey conducted by the World Mental Health in seventeen countries stated that one out of every twenty people was affected by depression. It adversely affects the way a person thinks, feels, behaves, and acts. It is normal and natural human behavior to become tense in some situations and start behaving negatively. When this situation prolongs and starts hampering a person's routine to the extent that they cannot come out of it and become intensely pessimistic and hopeless about life, it becomes a case of depression. It comprises major depression, bipolar disorder, persistent depressive disorder, perinatal depression, seasonal affective disorder, and depression with psychosis symptoms [3, 4].

There are numerous synthetic preparations available that can provide substantial relief, but kindle various side effects; hence there is a consistent necessitate for herbal remedies that have minimal side effects. *Aegle marmelos*, commonly known as Bael, belongs to the family Rutaceae. *Aegle marmelos* have been used traditionally for a long time by the indigenous people to heal and treat several diseases and disorders. It has been used extensively in Ayurveda [5, 6, 7, 8, 9], Unani [10, 11] and Siddha [10] system of medicine owing to its immense medicinal properties. It has enormous economic and ecological value. It was found that various pharmacological activities were exhibited by different parts of this tree, like, anti-cancer [12], analgesic [13], antipyretic [14], anti-inflammatory [15], anti-malarial [16], antioxidative [17], hepatoprotective [18], antidiabetic [19], antimicrobial [12], antiulcer [20], gastroprotective [21], antidiarrheal [22], cardio-protective [23], radioprotective [24], anti-spermatogenic [25], antiproliferative [26], anti-hyperlipidemic [27], immunomodulatory [28], anti-thyroid [29] *etc*. Several phytochemicals were segregated from different parts of *Aegle marmelos*, like glycosides, terpenoids, alkaloids, coumarins, carbohydrates, saponins, phenols, sterols, gums and mucilage, tannins *etc*., which were thought to have attributed to their pharmacological responses.

All parts of the plant are found to contain many phytoconstituents of therapeutic value. Alkaloids isolated from the leaves are O-(3,3-dimethylallyl) halfordinol, Aegelin, marmeline, N-2-ethoxy-2-(4-methoxyphenyl) ethylcinnamamide, aegelinosides A and B, N-2-methoxy-2-[4-(3',3'-dimethyl-allyloxy) phenyl], anhydomarmeline, N-2-methoxy-2-(4-methoxyphenyl) ethylcinnemamide. Phenylpropanoids that are found are hydro-coumarins, lignans, phenylpropenes, Aegelinine, and Marmesin. Marmelosin, marmesinine, rutin, and beta-sitosterol-beta-D-glucoside are also found in the leaves.

Among the terpenoids are alpha-phellendrene, p-cymene, p-menth-1-en-3, 5-diol, gamma-sitosterol and limonene. Other constituents found present in the leaves are rutaretin, montanine, N-p-cis-and trans-coumaroyltyramine, betulinic acid, valencic acid, 4-methoxybenzoic acid and trans-cinnamic acid [30, 31, 32]. The Bael fruit has been used in ethnomedicine for ages as a tonic for the brain, but very little research has been done in investigating the psychotropic responses in other parts of this tree. Hence, this research was undertaken to ascertain anti-anxiety and antidepressant responses in its leaves.

FIG. 1: TRIFOLIATE BAEL LEAVES

FIG. 2: *AEGLE MARMELOS* IN FRUITING PERIOD

MATERIAL AND METHODS:

Collection of Drugs: The mature green leaves of *Aegle marmelos* were collected in February from the B-Block, Shastri Nagar locality of Ghaziabad. The sample of plant leaves was identified and authenticated by Dr. Vijay Malik, Head of the Department of Botany at Chaudhary Charan Singh University, Meerut, and the voucher specimen was preserved at the herbarium of the department, Ref: Bot/654/ Dt. 25.02.2022. The leaves were thoroughly washed with water to remove any dust and debris and then dried completely in the shade for two-three days. The leaves were crushed coarsely, then powdered in a grinder, and kept in an airtight container until used.

Experimental Animals: The experimental work involving pharmacological activity was performed on the mice after procuring the authentication by the Committee for the Purpose of Control and Supervision of Experiments on Animals CPCSEA/Institutional Animal Ethics Committee IAEC having CPCSEA registration no. 1207/c/08/CPCSEA and IAEC Approval no. TAEC/PH-21/TIPER/153. Swiss Male Albino mice were furnished from the Central Animal Facility at All India Institute of Medical Science, New Delhi, and kept at the animal house facility at Translam Institute of Pharmaceutical Education and Research, Meerut, Uttar Pradesh. The mice were provided with a standard pellet diet and water *ad libitum* and housed in cages in the animal facility of Translam Institute at normal room temperature and provided with a 12-hour dark and 12-hour light cycle.

Standard Drug used: For the Anti-Anxiety activity using Elevated plus Maze, the Standard drug Diazepam was taken from Indian Pharmacopoeia Commission, Ghaziabad and for the Anti-Depression activity using Tail Suspension Model, the Standard drug Fluoxetine was procured from the market.

Extraction: Shade-dried leaves of *Aegle marmelos* (AM) were taken and coarsely powdered. 500g of powdered leaves were taken and extracted with 50 percent ethanol by cold maceration method, keeping the drug in borosilicate beakers, covered by aluminum foil. It was kept from sunlight for a week in normal room temperature conditions to avoid evaporation of the solvent [33]. After one week, it was filtered by Whatman filter paper 1, and the solvent was evaporated using the rotary evaporator. 139.05g of extract was obtained. The yield percentage of the extract was calculated as (Actual / Theoretical) * 100, where *Actual* is the quantity of extract obtained, and *Theoretical* is the quantity of powder taken initially. The extract was stored under refrigeration at four degrees centigrade until further use [34].

Macroscopic Characters of *Aegle marmelos* Leaf: The fresh leaf of AM was examined for various macroscopic or organoleptic characteristics based on shape, size, color, odor, taste, and texture, and the results were noted.

Phytochemical Analysis: Detection of Glycosides: To 1 ml of filtrate, 1.5 ml glacial acetic, then a few drops of 5% ferric chloride were added. To this, concentrated sulphuric acid was added along the sides of the test tube. Reddish brown color appearing at the interface of the two layers and bluish green color appearing in the upper layer will indicate the presence of cardiac glycosides.

Detection of Alkaloids: 1ml of test solution was treated with a few drops of acetic acid and1-2 ml of Dragendroff's Reagent (Potassium Bismuth Iodide solution). An orangish-red colored precipitate will indicate the presence of Alkaloids in the extract. 1-2 ml of test solution was treated with a few drops of Mayer's Reagent (Potassium mercuric iodide solution). A creamy white or yellowish precipitate will indicate the presence of alkaloids in the test sample.

Detection of Carbohydrates: To 2ml of test solution, 2 drops of Molisch's Reagent (Solution of Alpha naphthol in ethanol) were added. 2ml of Concentrated Sulphuric acid was added to this along the sides of the test tube. The purple to violet colored ring appearing at the junction of the two layers will indicate the presence of carbohydrates in the test solution.

Detection of Proteins:

Biuret test: 2ml of test solution was taken in a test tube. To this, 2 ml of 3% Sodium Hydroxide and a few drops of 1% Copper Sulfate solution were added. Solution turning bluish violet color will indicate the presence of protein in the test sample.

Detection of Starch: To 2ml of the test solution, a few drops of Dilute Iodine solution were added. The blue color will appear, which disappears on heating the solution but re-appears on cooling will indicate the presence of starch in the test sample.

Detection of Flavonoids: To 2ml of the test solution, a few drops of 1% Ammonia Solution were added. The formation of yellow color will indicate the presence of Flavonoids in the test solution.

Detection of Tannins: To 0.5ml of the test sample, 1ml of water and 1-2 drops of Ferric Chloride were added. The appearance of a blue-black color will indicate the presence of tannins in the sample.

Detection of Steroids:
Liebermann-Burchard test: To 2ml of the test sample, a few drops of acetic anhydride were added, and the solution was boiled and cooled. To this, a few drops of concentrated Sulfuric Acid were added along the sides of the test tube. The presence of greenish yellow fluorescence in the upper layer and the formation of a brown ring at the interface of two layers will indicate the presence of steroids in the sample.

Detection of Saponins: To 1ml of the test sample, 1ml of water was added, and the solution was shaken. Persistent foaming will indicate the presence of Saponins in the sample.

Detection of Terpenoids:
Salkowski Test: To 2ml of the test sample, 2ml of chloroform was added. To this, a few drops of Concentrated Sulfuric Acid were added along the sides of the test tube to create a layer. A reddish-brown color that appears at the junction of the two layers will confirm the presence of terpenoids in the sample.

Detection of Gums: To 1ml of the test sample, 3ml of Dilute Hydrochloric acid were added dropwise. The red color will indicate the presence of gums in the sample.

Detection of Phenols: To 1ml of the test sample, a few drops of 1% Ferric Chloride solution were added. The formation of blue, violet, green or red colors will indicate the presence of Phenols ions in the test sample.

To 2ml of the aqueous test sample, 3ml of 10% Sodium Hydroxide solution was added. The appearance of yellow color will indicate the presence of Coumarins in the test sample.

Detection of Reducing Sugars: To 3ml of the aqueous test sample, a few drops of both Fehling Solution A and B were added. The appearance of an orange-red precipitate will indicate the presence of reducing sugars in the test sample.

Experimental Design: A total of four groups of Swiss male albino mice, each weighing between 25-35 gm, were made. Each group contained six mice. Group I was given distilled water and served as a control group. Group II and III were given *Aegle marmelos* leaf extract (AME) at a dose of 200 mg/kg and 400mg/kg by the oral route of administration, respectively. Group IV was given the standard drug Diazepam at a dose of 2 mg/kg by the oral route of administration for the anti-anxiety activity (30) and Fluoxetine 20 mg/kg for the antidepressant activity. The Effective Dose of AME was calculated based on a literature survey of acute toxicity studies done on AM which suggests that AM has no toxicity reported till 1750mg/kg and has LD_{50} at 2250mg/kg [35, 36].

Anti-anxiety Activity: Elevated Plus Maze (EPM) was first delineated by Handley and Mithani in 1984 and was validated by Pellow et al. in 1985 [37]. It has been used for over ten years as a behavioral model for determining. The anti-anxiety activity of AM. The EPM apparatus is a plus-shaped maze comprising two wooden arms crossing each other. The EPM used in this study was a model made with slight modifications to the original. Two open arms (28.5×8 cm) lay perpendicular to two close arms (28.5×8×18 cm) and merge at the center (8×8 cm). The closed arms are elevated to prevent the rodent from falling.

The EPM was elevated to a height of 29cm above the ground. This maze is built on the concept of the natural disinclination of rodents for heights and opened spaces, which induces anxiety in them. Hence, they tend to enter more in the closed arms and spend more time there than in the open arms, despite their innate provocation for exploration of a novel area [38]. Each mouse was placed for five minutes in the plus maze, facing towards the open

arm, one hour after administration of the extract to examine them for the anti-anxiety effect. The observation was made at a distance of at least two meters. Care was taken to avoid any distractions or sounds while observing the mice avoid interference from external stimuli from creating more anxiety in them. Each mouse was observed for the total time spent in each arm and number of open and closed arms entries, respectively. Entry in an arm was denoted by all four feet of the mouse on that particular arm [39]. After each observation period of five minutes, the mouse was placed back in the cage, and the surface of the EPM was cleaned thoroughly with 70% ethanol to remove any odor, which may interfere with the next observation [40]. The mice were not given prior exposure to the EPM other than the experimental duration, so there are no biased results obtained [41].

Antidepressant Activity: The Tail Suspension Test (TST) is a behavioral model used to determine the Antidepressant activity of *Aegle marmelos*. This model was designed by Steru *et al.* in the year 1985. The model is based on the concept, "hanging the body upside down will instill depression after a period of agitation to rescue from the situation and finally, the body will become tired of struggling and ends up in immobility". Based on the original method by Steru *et al.*, the TST was carried out to determine the antidepressant activity, which involved suspending the mice upside down from the edge of a table by putting tape on their tails, 2cm away from the tip, restricting their chances of getting upright [42]. The distance between the edge of a table and the floor was maintained at 50 cm. All the mice were acoustically and visibly secluded from each other to prevent any biased results [43]. After a period of agitation, after two minutes, the total time of immobility was noted down. Mice were immobile when their bodies were completely motionless and hanged passively. The total time given to each mouse was six minutes. After this, the mice were carefully removed and kept back in their cage.

Statistical Analysis: All the results of the Anti-anxiety activity using the EPM model and Antidepressant activity using the TST were expressed as Mean ± SEM and statistically analyzed on the Graph Pad Prism 9.4.0 (673) using One Way ANOVA followed by Tukey's Multiple comparisons, keeping sample size equal to six. P values <0.0001 were considered highly significant.

RESULTS AND DISCUSSION:

Extraction: The yield percentage of the hydroalcoholic extract of *Aegle marmelos* leaves (AME) was calculated to be 27.81%.

Macroscopic characters of *Aegle marmelos* (am) leaf:

Shape	Trifoliate leaves, each leaflet lanceolate to ovate
Size	2-5 cm wide, 4-10 cm long leaflets
Color	Mature leaves are light green to dark green; new foliage is pinkish to maroon
Odor	Aromatic when chopped
Taste	Bitter
Texture	Mature leaves are smooth; young foliage is smooth and glossy

Phytochemical Analysis:

Glycosides	Absent
Alkaloids	Present
Carbohydrates	Present
Proteins	Absent
Flavonoids	Present
Tannins	Present
Steroids	Absent
Saponins	Present
Terpenoids	Present
Gums	Absent
Phenols	Present
Reducing Sugars	Present

Data of Anti-anxiety Activity using EPM Model:

TABLE 1: DATA OF TOTAL TIME (300 SECONDS) SPENT IN OPEN AND CLOSED ARMS BY THE FOUR TREATMENT GROUPS EXPRESSED AS MEAN ± SEM, SAMPLE SIZE N=6.

Treatment groups	Total time spent in open arms (sec) (Mean ± SEM)	Total time spent in closed arms (sec) (Mean ± SEM)
Control (Distilled water 10ml/kg)	27.16 ± 1.42	251.5 ± 3.31
AME 200 mg/kg	208.3 ± 3.69****	70 ± 0.577****
AME 400 mg/kg	234.5 ± 1.17****	51.33 ± 0.66****
Standard (Diazepam 2mg/kg)	252.16 ±1.137****	32.5 ± 0.76****

The p value expressed as **** are significant values where p<0.0001 as compared to control.

TABLE 2: DATA OF NUMBER OF OPEN AND CLOSED ARM ENTRIES OF THE FOUR TREATMENT GROUPS, EXPRESSED AS MEAN ± SEM, TAKING SAMPLE SIZE N=6.

Treatment groups	No. of entries in open arm (Mean ± SEM)	No. of entries in closed arm (Mean ± SEM)
Control (Distilled water 10 mg/kg)	3.166 ± 0.307	8.16 ± 0.307
AME 200 mg/kg	6.166 ± 0.307****	5.0 ± 0.258****
AME 400 mg/kg	8.66 ± 0.21****	3.5 ± 0.341****
Standard Diazepam 2 mg/kg	10.33 ± 0.42****	1.83 ± 0.166****

The p value expressed as **** is significant values as compared to control, where p<0.0001.

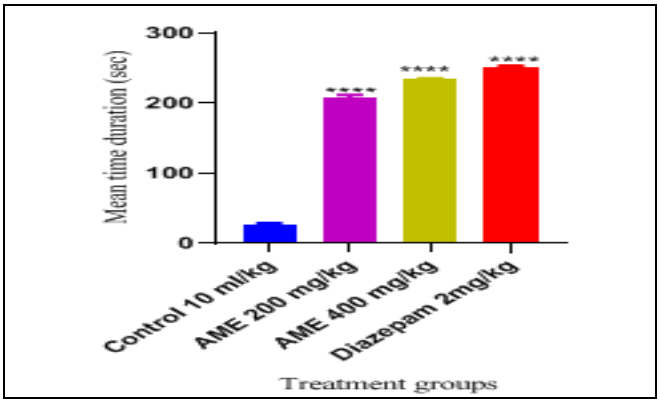

FIG. 3: GRAPH OF TREATMENT GROUPS AGAINST TOTAL TIME SPENT IN SECONDS IN OPEN ARMS. Results are expressed as Mean±Sem, n=6, statistically analyzed by one-way ANOVA followed by tukey's multiple comparison test. The p-value denoted as **** is significant (p<0.0001) as compared to the control.

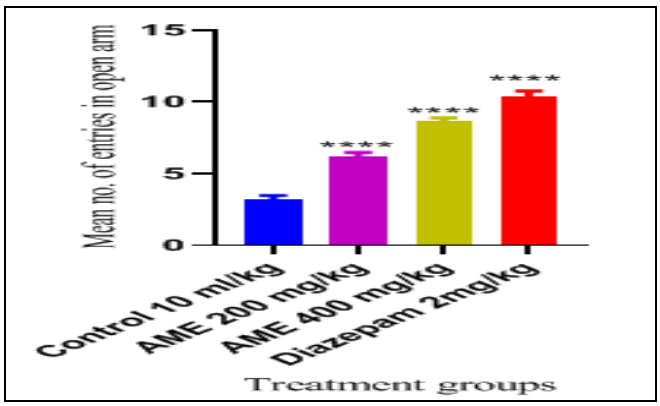

FIG. 4: GRAPH OF TREATMENT GROUPS AGAINST NUMBER OF ENTRIES IN OPEN ARMS. Results are expressed as Mean±Sem, n=6, statistically analyzed by one-way ANOVA followed by tukey's multiple comparison test. The p-value expressed as **** is significant values where p<0.0001 as compared to control.

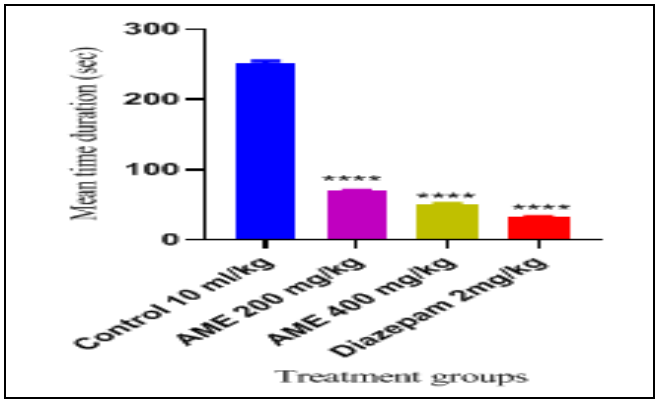

FIG. 5: GRAPH OF TREATMENT GROUPS AGAINST TOTAL TIME SPENT IN SECONDS IN CLOSED ARMS. Results are expressed as Mean±SEM, n=6, statistically analyzed by One Way ANOVA followed By Tukey's Multiple Comparison Test. The p-value expressed as **** is significant values where p<0.0001 as compared to control.

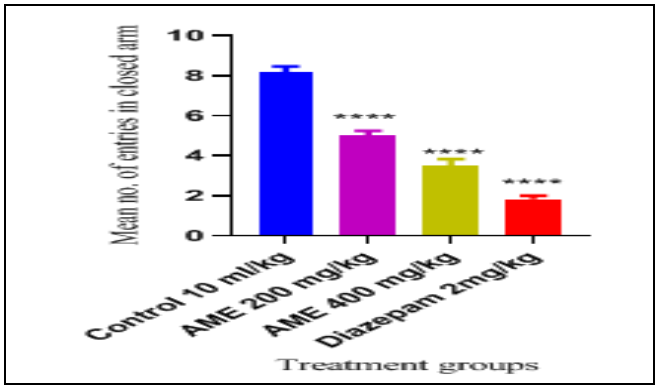

FIG. 6: GRAPH OF TREATMENT GROUPS AGAINST NUMBER OF ENTRIES MADE IN CLOSED ARMS. Results are expressed as Mean±SEM, n=6, statistically analyzed by One Way ANOVA followed By Tukey's Multiple Comparison Test. The p-value expressed as **** is significant value where p<0.0001 as compared to control.

Outcome of the Anti-anxiety Activity in EPM Model: Elevated plus maze model was employed to assess the anti-anxiety activity of AME at doses 200 and 400 mg/kg in mice. Significant anxiety induction was observed in the control group, as shown in **Tables 1** and **2**, respectively. Treatment of mice with AME 200 and 400 mg/kg orally showed marked escalation in total time spent (208.3 sec, 234.5 sec) and number of entries (6.166, 8.66) in the open arms and depletion in the total time spent (70 sec, 51.33 sec) and number of entries (5.0, 3.5) in the closed arm respectively. In addition, treatment of mice with the standard drug diazepam 2 mg/kg showed a significant increase in the total time spent (252.16 sec) and a number of entries (10.33) in the open arm and a decrease in total time spent (32.5 sec) and number of entries (1.83) in closed arm respectively when compared with the control group. This indicates a prominent anxiolytic response of AME when administered in doses 200 and 400 mg/kg in the mice. Significant p values p<0.0001 were obtained when AME 200mg/kg, 400mg/kg, and Standard Diazepam 2mg/kg groups were compared with the Control group for total time spent and the number of entries made in both the arms.

Data of Antidepressant Activity using TST:

TABLE 3: RESULTS OF TIME OF IMMOBILITY IN THE FOUR TREATMENT GROUPS, EXPRESSED AS MEAN ± SEM, TAKING SAMPLE SIZE

Treatment groups	Immobility time in seconds
	Mean ± SEM
Control (Distilled water 10 ml/kg)	233.5 ± 2.09
AME 200mg/kg	217.3 ± 3.56**
AME 400/kg	186.5 ± 1.17****
Standard Fluoxetine 20mg/kg	113.5 ± 3.61****

N=6. The p-value **** denotes p< 0.0001 was considered highly significant and ** denotes p=0.0031 was considered less significant compared to the Control group.

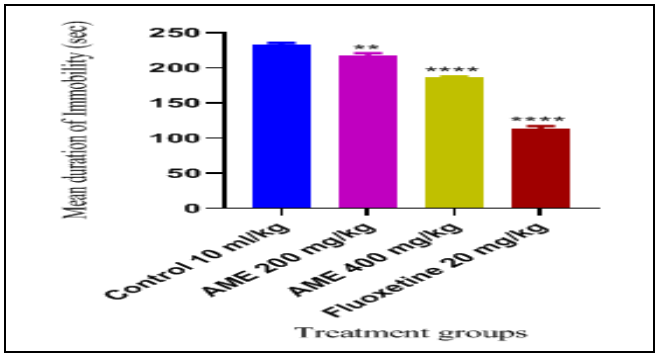

FIG. 7: GRAPH OF TREATMENT GROUPS AGAINST MEAN IMMOBILITY TIME IN SECONDS. Results of total time of immobility in the four treatment groups, expressed as Mean ± SEM, statistically analyzed using One Way ANOVA followed by Tukey's multiple comparison, taking sample size n=6. The p-value **** p< 0.0001 was considered highly significant and ** denotes p=0.0031 was considered less significant as compared to the Control group.

Outcome of the Antidepressant Activity in the TST Model: Treatment of mice with AME 200 and 400 mg/kg orally significantly decreased the mean duration of immobility (217.3 sec, 186.5 sec), respectively as compared to the control group given distilled water 10 ml/kg (233.5 sec) in the tail suspension method conducted to assess the antidepressant activity. Also, the standard drug fluoxetine administered at 20 mg/kg remarkably reduced the mean immobility time (113.5 sec) as compared to the control group. The results show dose-dependent marked antidepressant activity in AME 400 mg/kg comparable to the standard fluoxetine 20 mg/kg. Significant p values $p<0.0001$ were obtained when AME 400mg/kg and Standard Fluoxetine 20mg/kg groups were compared with that of the Control group treated with distilled water. A less significant p-value of $p=0.0031$ was obtained when AME 200mg/kg group was compared with that of the Control group treated with distilled water. Significant p values $p<0.0001$ were obtained when results of AME 200mg/kg and AME 400mg/kg groups were compared with that of the Standard Fluoxetine 20mg/kg group.

DISCUSSION: *Aegle marmelos,* commonly known as Bael or Bilva, has been known for ages for its tremendous medicinal benefits, spiritual significance, ecological relevance, and economic perspective. Almost all parts of this tree have been studied by researchers over many years for the massive therapeutic value they carry, like anti-cancer, analgesic, antipyretic, anti-inflammatory, anti-malarial, antioxidant, hepatoprotective, antidiabetic, antimicrobial, antiulcer, gastro-protective, antidiarrheal, cardioprotective, radio-protective, anti-spermatogenic, *etc*. There has been extensive research going on this tree to prove more of its medicinal properties, isolate the phytoconstituents present in it to prove and establish their association with various pharmacological activities displayed by the plant, elucidate the structure of various phytoconstituents, and validation of methods used in the research work. This will pave the way for clinical trials of the drug; after successful completion, the industry can formulate a medicine for treating those ailments too. In the book 'Food and Brain Health', it is stated that AM had been proved to be a powerful drug for treating various disorders of the brain due to the existence of various phytoconstituents in it and could be used as a potent psychotropic drug in the future without side effects unlike the synthetic standard medicines [44]. In recent research on AM, it was found that the essential oil procured from its leaves, when administered at dose 25-100mg/kg, i.p. displayed notable anti-anxiety and antidepressant activity at all the doses and observable anti-convulsant and sedative effects at its higher doses. These activities were a result of the serotonergic and GABAergic pathways [45]. There has been very little research on AM to prove its use in treating anxiety and depression, despite its ethnomedical use as a brain tonic. Hence, the present study was conducted to ascertain its hydroalcoholic leaf extract's anxiolytic and antidepressant activities.

Elevated Plus Maze was first delineated by Handley and Mithani in 1984 and was validated by Pellow *et al.* in 1985 [37]. It has been used for over ten years as a behavioral model for the determination of Anti- Anxiety activity of AM; hence, it was used as a preferred method for ascertaining the anxiolytic effect in the hydroalcoholic leaf extract of AM. The tail suspension method, devised by Steru et al. in 1985, is one of the safest and most popular behavioral models for assessing antidepressants. Hence, it was employed to discern the antidepressant effects of the hydroalcoholic leaf extract of AM.

In the present study, when an Anti-anxiety test was conducted on mice on the EPM using the hydroalcoholic extract of the leaves of AM, it was found that the oral administration of AME 200mg/kg and 400mg/kg in the mice remarkably augmented the total time spent and several entries made in the open arms, and attenuated the total time spent and a number of entries made in the closed arms, when compared with the control group treated with distilled water 10ml/kg. Interestingly, AME 400mg/kg group showed a dose-dependent, closely comparable result with the Standard Diazepam 2mg/kg group. When the mice were treated with AME 200 and 400 mg/kg, the mean duration of immobility decreased significantly compared to the control group treated with distilled water 10 ml/kg. However, AME 400mg/kg group showed dose-dependent, closely comparable results with the standard fluoxetine 20mg/kg group.

The obtained results indicated that when administered in mice at a dose of 400mg/kg, hydroalcoholic extract of AM leaves showed notable anxiolytic response in the elevated plus maze model and antidepressant effect in the tail suspension test, respectively, as compared to a dose 200mg/kg. The presence of phytochemicals like alkaloids, coumarins, phenols, terpenoids, tannins, and flavonoids may have been attributed to the anxiolytic and antidepressant activities exhibited by the hydroalcoholic extract of AM leaves. Scopoletin, a phenolic coumarin isolated from various plants, including AM, exhibited the therapeutic potential to treat anxiety, epilepsy, depression, and Alzheimer's disease, owing to its neuroprotective, antiadrenergic, anticholinesterase, antioxidant, anti-dopaminergic and other properties [46], thus presence of phytoconstituent like coumarin can also be responsible for the anxiolytic and antidepressant activity of AM leaves. Anxiolytic activity of AM leaves was exhibited possibly owing to GABA-dependent facilitation of various phytoconstituents present in the drug *viz*. flavonoids, saponin, marmesinin, tannic acid, phenols, *etc* [47, 48]. It can also be assumed that the leaf extract of AM showed remarkable anxiolytic and antidepressant activity, probably due to a rising level of monoamines at the postsynaptic sites [39]. The psychoneuropharmacological activities found in the leaves of AM can also be attributed to the serotonergic and GABAergic pathways [45].

CONCLUSION: In the present research, it can be concluded that when the hydroalcoholic leaf extract of Aegle marmelos was administered to the mice at doses 200 and 400 mg/kg orally to discern its anti-anxiety effect by employing an elevated plus maze, it exhibited marked response in its higher dose 400 mg/kg than its lower dose, which was comparable with the standard drug diazepam 2 mg/kg. Also, AME showed a prominent response at its higher dose 400 mg/kg than its lower dose, comparable to standard drug fluoxetine 20mg/kg, using a tail suspension test to determine the antidepressant activity. Thus, it can be deduced that hydroalcoholic AME 400 mg/kg has marked dose-dependent anxiolytic and antidepressant responses. The hydroalcoholic extract was a rich amalgamation of phytoconstituents like alkaloids, carbohydrates, saponins, reducing sugars, coumarins, phenols, terpenoids, tannins, and flavonoids, which may have attributed to the anxiolytic and antidepressant activities found in the plant leaves. This project has been done with acute studies employing fewer animals, and there might have been chances of biased readings due to behavioral aspect of animals; thus there are ample opportunities for further research on this plant employing more models and a large sample size for a detailed outcome. Since very few studies have been conducted to demonstrate these psychotropic activities in the leaves of *Aegle marmelos*, it can open grounds for further research to investigate and isolate the phytoconstituents which would be accountable for such responses. This can facilitate the production of herbal anxiolytic and antidepressant formulations, which would be equally efficacious as the synthetic medicines available on the market, but with minimal side effects.

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