COGNITIVE AND NEUROCHEMICAL EFFECTS OF *AEGLE MARMELOS L.* STEM BARK EXTRACTS IN WISTAR RATS: IMPLICATIONS FOR ANTIDEPRESSANT POTENTIAL

Shriniwas K. Sarje[1]*, Vijay N. Gunjkar[2], Mangal L. Pawde[3], Anup T. Sharma[4], Sagar. N. Firke[5].

ABSTRACT:
The contemporary era is characterized by its swift pace, and while allopathic medicines undoubtedly offer rapid outcomes, their efficacy is accompanied by a range of adverse effects and contraindications. Conversely, botanical resources present a promising alternative, marked by minimal to negligible side effects and a propensity to address underlying issues. Among these botanical resources, *Aegle marmelos*, a member of the *Rutaceae* family, holds particular prominence within the realm of medicinal applications.
This study centers on the meticulous exploration of phytochemical attributes and the rigorous evaluation of pharmacological dynamics associated with *Aegle marmelos*, colloquially recognized as "bael" in India. The stem bark of *Aegle marmelos* was meticulously sourced from the local environs of Nanded, Maharashtra. Methodologically, phytochemical constituents were extracted via a Soxhlet extraction approach, utilizing ethyl acetate and methanol solvents. Focusing on the antidepressant potential, the efficacy of *Aegle marmelos L.* stem bark was gauged through the application of the despair swim test and tail suspension test. The standard comparator in this context was imipramine (10mg/kg). Notably, both extracts at a concentration of 200mg/kg exhibited marked to highly significant outcomes, signifying a noteworthy reduction in immobility time (ranging from $P<0.005$ to $P<0.001$).
In essence, this investigation underscores the multifaceted attributes of *Aegle marmelos*, unraveling its potential as a therapeutic entity. The pursuit of this botanical resource extends to the systematic delineation of ethyl acetate and methanolic extracts, complemented by meticulous phytochemical screening and a nuanced evaluation of antioxidant and antidepressant potential. The underpinning methodologies, namely the despair swim test and tail suspension test, collectively contribute to a comprehensive understanding of Aegle marmelos' therapeutic capacities.

Key words: *Aegle marmelos*, ethyl acetate and methanolic extracts, phytochemical screening, antioxidant activity, antidepressant activity, despair swim test, tail suspension test.

[1, 3]Dept. Of Pharmacology, Nanded Pharmacy College, Nanded.
[2, 4, 5]Dept. Of Pharmaceutics, Nanded Pharmacy College, Nanded.

***Corresponding Author: -** sksarje2020@gmail.com , shriniwas.sarje@gmail.com

INTRODUCTION

Depression is a chronic debilitating disease. It's a major psychotic illness characterized by intense despair, sadness, loss of concentration, mental slowing, pessimistic worry and lack of pleasure. It is associated with physical symptoms like insomnia or hypersomnia, altered eating pattern with anorexia, weight loss and over eating. According to World Health Organization (WHO) over 4.4 % of global population suffer from depression.
Mental depressions are mainly two types, specifically unipolar depression and bipolar depression. In unipolar depression, mood swings are constantly in the identical track and is conjoint (about 75% of cases) non familial, evidently accompanying with traumatic life events and complemented by indicators of anxiety and agitation. The following type is bipolar depression (about 25% of cases) occasionally also called as endogenous depression, displays a familiarized pattern, dissimilar to exterior stresses and frequently seems in premature grown-up life, results in vacillating depression and mania over a period of a few weeks. Although a number of synthetic drugs are being used as the standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment, these common adverse effect include dry mouth, fatigue, gastrointestinal or respiratory problems, anxiety, agitation, drowsiness, and cardiac arrhythmias. Several drug-drug interactions can also occur. These conditions create an opportunity of alternative

treatment for depression by the use of medicinal plants.

MATERIALS AND METHOD

Collection of Plant Material:

The fresh stem barks of *Aegle marmelos L.* were collected from local region of Nanded city and plant was authenticated at department of Botany and horticulture, Yeshwant Mahavidyalaya, Nanded. Collection, authentication, identification, processing and storage have been done according to standard procedure for the plant material. (The Indian Pharmacopoeia, 2007).

Preparation of phytochemical Extract:

The collected fresh stem barks of plant *Aegle marmelos* were subjected to shade drying and further crushed to powder, and then the powder is passed through the mesh No. 14 sieve, stored in air tight container. Powered 300g stem bark was extracted with pet. Ether, ethyl acetate and methanol solvents using Soxhlet extraction method.

Preliminary Phytochemical analysis:

The extracts obtained by successive extraction method were subjected to quantitative tests for the identification of various secondary metabolites such as carbohydrates, proteins, tannins, Saponins, steroids, flavonoids and glycosides. Phytochemical examinations were carried out for all the extracts as per standard methods. (K. R. Khandelwal, 2010).

Test for carbohydrates:

a) Molisch's test: To the extract, add few drops of alcoholic α-napthol. Then add few drops of concentrated sulphuric acid through sides of test tube; purple to violet color ring appears at the junction.
b) Barfoed's test: 1ml of extract is heated with 1ml of Barfoed's reagent, if red cupric oxide is formed monosaccharide is present. Disaccharides on prolong heating (about 10 min.) may also cause reduction, owing to partial hydrolysis to monosaccharide.
c) Fehling's test: 1ml of Fehling's A & Fehling's B solution were taken in test tube, boiled for one minute equal volume of aqueous extract solution was added and heated in boiling water bath for 5-10 min formation of brick red ppt indicated the presence of carbohydrate.
d) Benedict's test: Equal volume of benedicts reagent and test solution was taken in test tube and heated in boiling water bath for 5 min formation of red colored solution indicates the presence of carbohydrates.

Test for proteins:

a) Biuret test (general test): To 3 ml of test solution, add 4 % sodium hydroxide add few drops of 1 % copper sulphate solution, violet or colour appears.
b) Million's test (for proteins): Mix 3 ml of test solution with 5 ml Millon's reagent. White precipitate warm precipitate turns brick red or the precipitate dissolves giving red coloured solution.

Test for alkaloids:

a) Dragendoff's test: To the extract, add Dragendoff's reagent, (Potassium Bismuth Iodide) reddish brown precipitate indicates presence of alkaloids.
b) Mayer's test: The extract on treatment with Mayer's reagent give cream coloured precipitate indicates presence of alkaloids.
c) Wagner's test: Filtrates on treatment with Wagner's reagent give reddish brown precipitate indicates presence of alkaloids.
d) Hager's test: Filtrates were treated with Hager's reagent to produce yellow precipitate indicates presence of alkaloids.

Test for glycosides:

a) Keller killani test: To 2 ml extract , add glacial acetic acid, one drop 5%FeCl3 and conc. H2SO4. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green.
b) Borntrager's test: To the extract, add dil. H2SO4. Boil and filter. To cold filtrate, add equal volume benzene. Shake well. Separate the organic solvent. Add ammonia. Ammoniacal layer turns pink or red.
c) Modified borntrager's test: To the extract, add 5 ml 5 % FeCl3 and 5 ml dil. HCl. Heat for 5 min in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate organic layer, add equal volume dilute ammonia. Ammoniacal layer shows pinkish red colour.

Test for flavonoids:

a) Shinoda test: Extract treated with few magnesium turning and few drops of concentrated hydrochloric acid gives pink scarlet, crimson red or occasionally green to blue colour after few minutes.
b) Sulphuric acid test: On addition of sulphuric acid (66% or 80%) flavones and flavono

dissolve into it and gives a deep yellow solution. Chalcones and aurones give red or red bluish solutions. Flavanes give orange to red colours.

Test for Phenolic compounds (Tannins):

a) Ferric chloride test: Extract treated with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.
b) Lead acetate test: Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

Test for Steroids and Triterpenoids:

a) Salkowski test: Treat the extract with few drops of concentrated sulphuric acid red color at lower layer indicates presence of steroids and formation of yellow colored lower layer indicates presence of triterpenoids.
b) Libermann-Burchard test: Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction two layer turns green which shows presence of steroids and formation of deep red color indicates presence of triterpenoids.

Test for Amino acids:

a) Ninhydrin test: To the extract, add Ninhydrin solution, boil, violet colour indicates presence of amino acid.

Thin layer chromatography (TLC):

Thin layer chromatography is a method of analysis in which the stationary phase, a finely divided solid, is spread as a thin layer on a rigid supporting plate and the mobile phase, a liquid, is allowed to migrate across the surface of the plate by capillary action by gravity or pressure. TLC separation takes place in the open layer with each component having the same total migration time but different migration distance. Numerous fixed sorbents have been used, including Silica gel, Cellulose, Polyamide, Alumina, Ion exchange and chemically bonded silica gel. Mobile phase consists of a single solvent or a mixture of solvents. The stationary phase of the TLC is prepared using various techniques such as pouring, dipping and spraying. The prepared plates are allowed for setting (air drying). This is done to avoid cracks on the surface of adsorbent. After setting the plates are activated by keeping in an oven at 100 to 1200C for one hour. Activation of TLC plates is nothing but removing water/moisture and other substances from the surface of any absorbent, by heating at temperature around 1100C so that adsorbent activity is retained. TLC studies were carried out using various extracts to confirm the presence of different Phytoconstituents in the extract. (Harborne J., 1998).

Determination of Total Phenolic Content:

The total phenols of all extracts were measured at 765 nm by Folin Ciocalteu reagent (McDonald et al., 2001). The dilute methanolic extract (0.5 ml of 1:10 g ml -1) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4 ml, 1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg l -1 solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed In terms of gallic acid equivalent (mg g –1 of dry mass), which is a common reference compound.

Determination of Total Flavonoid Content:

Aluminium chloride colorimetric technique was used for flavonoids estimation (Chang et al., 2002). Each extract (0.5 ml of 1:10 g ml -1) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with a double beam UV/Visible spectrophotometer, SHEMADZU (Japan). The calibration curve was plotted by preparing the quercetin solutions at concentrations 12.5 to 100 g ml -1 in methanol.

SAFE DOSE CALCULATION:

Acute toxicity is involved in estimation of LD50. As per reported reference, the acute toxicity studies were carried out in accordance with OECD test guideline 423: acute oral toxicity- acute toxic class method. The stem bark extracts of *A. marmelos* was found to be safe up to 2000 mg/kg body weight after oral administration of the test compound. Accordingly, maximum therapeutic experimental dose was calculated as 100 mg/kg and 200 mg/kg. ***(Birudu R.B. et.al 2020 and mule V. S. et.al. 2011).***

IN – VITRO ANTI-OXIDANT STUDY:

DPPH (2, 2-dipheny 1, picryl- hydrazyl) free radical scavenging activity

Principle: DPPH assay method is based on the reduction of Methanolic solution of colored free radical DPPH by free radical scavenger. DPPH composed of stable free radical molecules. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517nm and also for a visible deep purple colour. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is expressed as effective concentration EC50.

Procedure: The ability of Aegle marmelos stem bark to scavenge DPPH radicals was measured according to the method as previously described. Initially, absorbance of DPPH solution (0.1 Mm in methanol) were read at wavelength 517nm.various concentrations of extracts (25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml, 125µg/ml) were mixed with 1 ml of 0.1 Mm DPPH radical solution in methanol and made up final volume of 10ml with methanol. A similar solution without samples was applied as a control. The mixture was shaken vigorously and incubated in the dark for 30min. at room temperature. For each concentrations, the assay was run in triplicate. Gallic acid and ascorbic acid was used as a standard. The absorbance was measured at wavelength 517 nm by using uv-visible spectrophotometer.

IN-VIVO ANTIDEPRESSANT ACTIVITY:

In the present study, the antidepressant activity of *Aegle marmelos* was evaluated using despair swim test method and tail suspension test.

Drug used: Imipramine (10mg/kg) was used as a standard drug in the dose of 10 mg/kg and administered by oral route.

Selection of animals: For the study Wistar rats of either sex, of weight 150–200 gm and mice of weight 20-25gm were used.

Despair Swim Test Model:

Procedure:

Wistar rats of either sex weighing 150–200 g was used. They was brought to the laboratory at least one day before the experiment and was housed separately in Makrolon cages with free access to food and water. Naive rats was individually forced to swim inside a vertical cylinder (height: 40 cm; diameter: 18 cm, containing fresh water to a height of 15 cm and maintained at 25 °C). Rats placed in the cylinders for the first time was initially highly active, vigorously swimming in circles, trying to climb the wall or diving to the bottom. After 2–3 min activity begins to subside and to be interspersed with phases of immobility or floating of increasing length. The total duration of immobility was recorded in next 4 minutes of total 6 min test. After 6 min in the water the rats was removed and dried with cloth before being returned to their home cages. The water is changed after each test because urine and the other chemicals released by the first rat will affect the swimming pattern of the next rat. An animal is judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. Plant extracts or standard drug Imipramine (10 mg/kg) is administered one hour prior to testing. The similar procedure was conducted on 1st and 8th days of experiment.

Animal Grouping and drug administration:

Group I: Control 0.5% Dimethyl sulfoxide orally.
Group II: Standard (Imipramine 10 mg/kg) orally.
Group III: *Aegle marmelos L.*stems bark ethyl acetate extract (AMEA) 100 mg/kg orally.
Group IV: *Aegle marmelos L.*stems bark ethyl acetate extract (AMEA) 200 mg/kg orally.
Group V: *Aegle marmelos L.*stems bark methanol extract (AMME) 100 mg/kg orally.
Group VI: *Aegle marmelos L.*stems bark methanol extract (AMME) 200 mg/kg orally.

Evaluation:

Evaluation was done on the basis of duration of immobility time in sec in total 6 min test on respective days of experiment i.e. on day 1st day and 8th day.

Tail Suspension Test:

Procedure: Male albino mice weighing 20–25 g was used preferentially. They were housed in plastic cages for at least 10 days prior to testing in a 12 h light cycle with food and water freely available. Animals was transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. Groups of 06 animals are treated with the test compounds or the vehicle by oral injection 30 min prior to testing. For the test the mice are suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility is recorded for a

period of 5 min. Mice are considered immobile when they hang passively and completely motionless for at least 1 min.

Animal Grouping and drug administration:
Group I: Control 0.5% Dimethyl sulfoxide orally.
Group II: Standard (Imipramine 10 mg/kg) orally.
Group III: *Aegle marmelos L*. stems bark ethyl acetate extract (AMEA) 100 mg/kg orally.
Group IV: *Aegle marmelos L*. stems bark ethyl acetate extract (AMEA) 200 mg/kg orally.
Group V: *Aegle marmelos L.* stems bark methanol extract (AMME) 100 mg/kg orally.
Group VI: *Aegle marmelos L*. stems bark methanol extract (AMME) 200 mg/kg orally.

Evaluation:
The percentage of animals showing the passive behavior is counted and compared with vehicle treated controls.

OBSERVATIONS AND RESULTS:

Table 1: Preliminary Phytochemical analysis

Test for	Petroleum Ether	Ethyl acetate	Methanol
Carbohydrate			
a. Molisch's test:	+	+	+
b. Fehling's test:	+	+	+
c. Benedict's test:	+	+	+
d. Barfoed's test:	-	-	+
Proteins			
a. Biuret test:	-	+	+
b. Millons test:	-	+	+
Amino acids:			
a. Ninhydrin test	_	+	+
Steroid			
a. Salkowaski test	_	+	+
b. Liebermann-burchard test	_	+	+
Glycosides			
a. Keller killani test	_	+	+
b. Borntrager's test	_	+	+
c. Modified borntrager's test	_	+	+
Flavonoids			
a. Shinoda test	_	+	+
b. Sulphuric acid test	+	+	+
Tannins and phenolic compound			
a. Lead Acetate Test	_	+	+
b. Fecl3 test	+	+	+
Alkaloids			
a. Hager's test	+	+	+
b. Wagner's test:	+	_	+
c. Dragendroff's test:	+	+	+
d. Mayer's test:	-_	+	+

(+); Present, (-); Absent

Above observation table shows the presence of various phytoconstituents in the all three extracts. (i.e. pet. Ether , ethyl acetate and Methanol).

Confirmation of phytoconstituents with Thin Layer Chromatography:

Table 2: Results of TLC. Profile of extracts.

Sr. No	Extracts	Solvent System	Spraying reagent	Rf values	Colour
1	Petroleum Ether	Benzene: ethyl acetate: methanol 7:2:1	10% H2SO4	0.88	Green
2	Ethyl acetate	Toluene: ethyl acetate: acetone:	10% H2SO4	0.19 0.56	Yellow Grey Faint blue

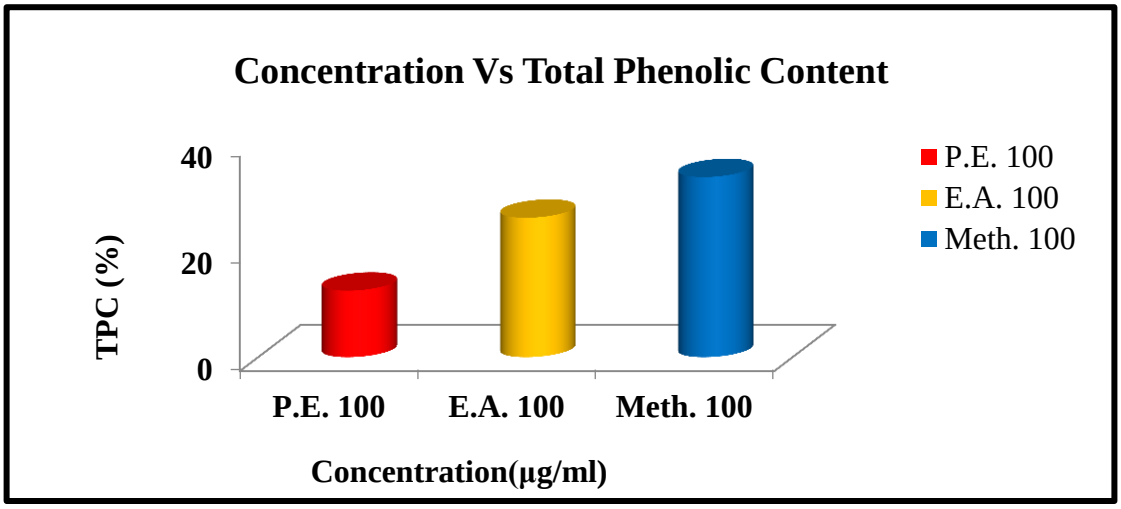
		methanol 3:4:2:1		0.71 0.87	yellow
3	Methanol	benzene: ethyl acetate: chloroform 5:1:4	10% H2SO4	0.24 0.66	Dark green yellow

Table 3: Total phenolic content of *Aegle marmelos* stem bark extracts

Sr. No.	Concentration (µg/ml)	Extracts	Absorbance at 765nm	TPC (mg/GAE/g DW)
1	100	Pet. ether	0.149	12.41±0.31
2	100	Ethyl acetate	0.312	26± 0.40
3	100	Methanol	0.403	33.58± 2.58

(N=3) Note: GAE/g DW denotes Gallic Acid Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, ethyl acetate and Methanol have Phenolic content as 12.41 (mg/GAE/g DW), 26 (mg GAE/g DW), 33.58 (mg GAE/g DW) respectively. Methanol extract shows more phenolic content than Petroleum ether and ethyl acetate as per comparative evaluation of phenolic content of extracts.



Graph 1: Total Phenolic Content of Extracts.

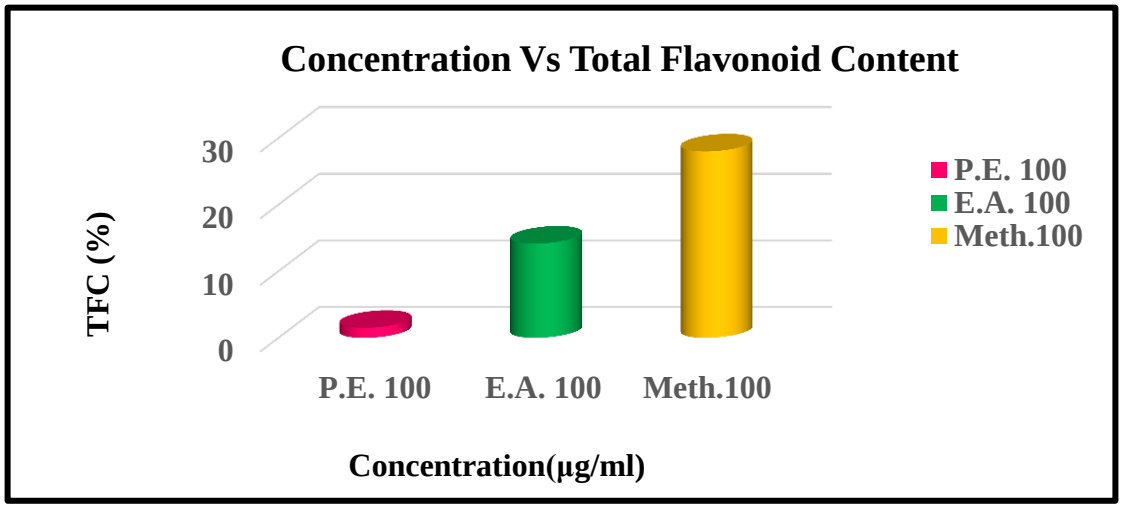
The concentration absorbance calibration curve for sequentially and separately prepared stock solution of standards of Gallic acid solution was taken. The absorbance measured at 765 nm for 20, 40, 60, 80, 100 µg/ml concentration Gallic acid solution are in a range of 0.271 to 1.232 within the range of concentrations, the calibration curve of Gallic acid has clearly exhibited linearity. Above table indicate that the Methanolic extract contain more phenolic content (33.58 mg GAE/g DW) than ethyl acetate and Petroleum extract (26 mg GAE/g DW, 12.41 mg GAE/g DW) respectively equivalent to Gallic acid.

Table 4: Total Flavonoid Content of *Aegle Marmelos* Stem Bark Extracts

Sr. No.	Concentration (µg/ml)	Extracts	Absorbance at 510nm	TFC (mg/RUE/g DW)
1	100	Pet.ether	0.009	1.5± 0.05
2	100	Ethyl acetate	0.085	14.16 ± 0.27
3	100	Methanol	0.168	28± 0.57

(N=3) Note: Ru/g DW denotes Rutin Equivalent per gram dry weight.

Above observation table reveals that Petroleum ether, ethyl acetate and Methanol have Flavonoid content as 1.5 (mg Ru/g DW), 14.16 (mg Ru/g DW), 28 (mg Ru/g DW) respectively. Methanol extract shows more Flavonoid content than Petroleum ether and ethyl acetate as per comparative evaluation of Flavonoid content of extracts.



Graph 2: Total Flavonoid Content of Extracts.

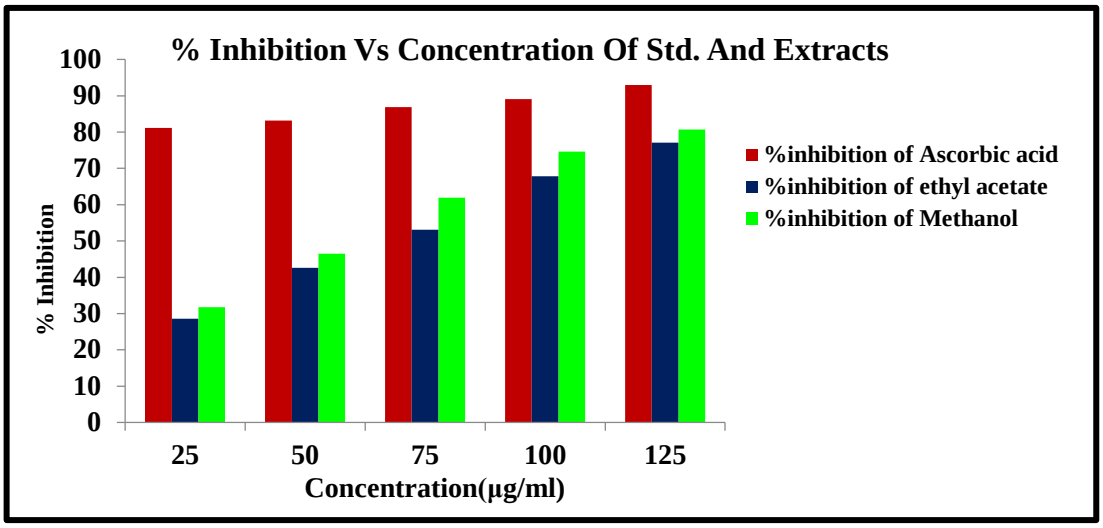
The calibration curve for sequentially and independently prepared stock solution of rutin that depicts the concentration of rutin against the absorbance at 510 nm. The absorbance values increased proportionally upon increasing the concentration of rutin from 10 µg/ml to 50 µg/ml.

Above table indicates that the Methanol extract contain more flavonoids (28 mg Ru/g DW) of extract than ethyl acetate and Petroleum ether extract (14.16 mg Ru/g DW and 1.5 mg Ru/g DW) respectively equivalent to rutin.

Table 5: % Inhibition of Aegle marmelos stem bark extracts

Conc. (µg/m l)	Absorbance Of ethyl acetate extract	% inhibition of ethyl acetate extract	Absorbance of methanol extract	% inhibition of methanol extract	Absorbance of Ascorbic acid	% inhibition of Ascorbic acid
25	0.315±0.004	28.57	0.301±0.001	31.74	0.083±0.006	81.17±0.23
50	0.253±0.001	42.63	0.236±0.008	46.48	0.074±0.008	83.21±0.34
75	0.207±0.002	53.06	0.168±0.001	61.90	0.058±0.0011	86.84±0.55
100	0.142± 0.0005	67.80	0.112±0.001	74.60	0.048±0.013	89.11±0.19
125	0.104±0.002	77.09	0.085±0.002	80.72	0.031±0.012	92.97±0.18

Above table indicates that the Methanol extract contain more antioxidant content (31.74µg/ml, 46.48 µg/ml, 61.90 µg/ml, 74.60 µg/ml, 80.72µg/ml) of extract than ethyl acetate extract (28.57 µg/ml, 42.63 µg/ml, 53.06 µg/ml, 67.80 µg/ml, 77.09 µg/ml) respectively at concentration 25, 50, 75, 100, 125 µg/ml.



Graph 3: Effect of % Inhibition of Extracts with Standard

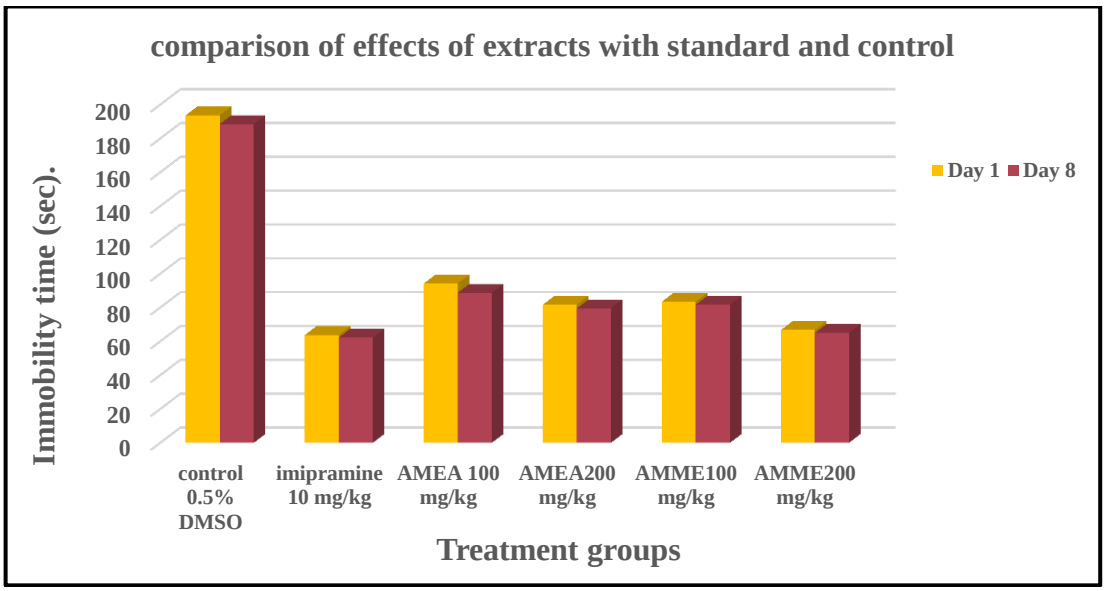
Pharmacological Screening of Aegle marmelos Linn. Stem bark Extracts:

DESPAIR SWIM TEST MODEL:

Table 6: Effect of Day 1 and Day 8 AMEA and AMME (100mg/kg and 200mg/kg) on Immobility time in Despair Swim test model on wistar rats

Treatment Group	Despair swim test (Immobility time in sec)	
	Day 1	Day 8
Control	138±2.8	135.25± 3.44
Imipramine 10 mg/kg	56.75±0.6**	55.25±0.629**
AMEA 100 mg/kg	77.25±1.1**	75.5±1.041**
AMEA 200 mg/kg	70.75±0.7**	68±0.7**
AMME100 mg/kg	74.25±0.8**	71±0.91**
AMME200 mg/kg	62.25±0.8**#	62.25±0.47**#

Values are expressed as mean±SEM (n=6)**p<0.001,*<0.05v/s vehicle (one-way ANOVA followed by tukey's test),#p>0.05 non-significant difference when compared with standard .



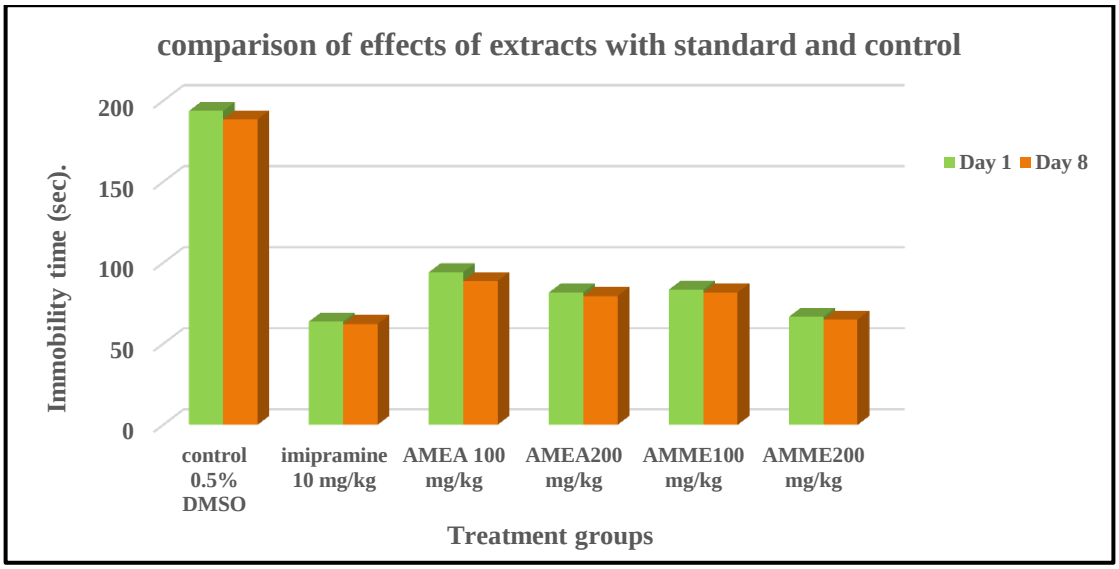
Graph 4: Comparative Effect of Extracts with Std. And Control On Immobility Time in Despair Swim Test Model on Wistar Rats

TAIL SUSPENSION TEST:

Table 7: Effect of Day 1 and Day 8 AMEA and AMME (100mg/kg and 200mg/kg) on Immobility time in tail suspension test on mice.

Treatment Group	Tail suspension test (Immobility time in sec)	
	Day 1	Day 8
Control	193.75±0.75	188.5± 0.64
Imipramine 10 mg/kg	63.75±1.03**	62.25±0.85**
AMEA 100 mg/kg	94.25±1.2**	88.75±0.6**
AMEA 200 mg/kg	81.75±0.6**	79.5±0.64**
AMME100 mg/kg	83.5±0.8**	81.75±0.8**
AMME200 mg/kg	66.75±0.4**#	65±0.4**#

Values are expressed as mean±SEM (n=6)**p<0.001,*<0.05v/s vehicle (one-way ANOVA followed by tukey's test),#p>0.05 non-significant difference when compared with standard.



Graph 5: Comparative Effect of Extracts with Std. And Control On Immobility Time in Tail Suspension Test on Wistar Rats.

DISCUSSION

In the present study we have selected a plant namely *Aegle marmelos (Linn.)* which is commonly known as Bael. And Belongs to the *Rutaceae* family has been widely employed in indigenous systems of Indian medicine due to its many therapeutic characteristics. the pharmacological studies revealed that the plant shows many pharmacological activities including CNS effects (muscle relaxant, anxiolytic, in treatment of Alzheimer's disease), anti-oxidant, anti-inflammatory, analgesic, antiasthmatic, antimicrobial effect. To the best of our knowledge, no scientific data regarding the antidepressant effect of *Aegle marmelos L.* stem bark. Thus the present study was undertaken for comparative evaluation of *Aegle marmelos L.* stem bark ethyl acetate & methanolic extracts for antidepressant activity on wistar rats. In the present study we investigated the pharmacognostic characteristics of *Aegle marmelos L.* stem bark powder were determined . Preliminary phytochemical evaluation of all three extracts of (i.e. Petroleum ether, ethyl acetate and Methanol) were carried out for the determination of presence of phytoconstituents. Extracts shows presence of alkaloid, flavonoids, steroids, protein, carbohydrates, tannins and phenolic compounds. Acute toxicity study were carried out as per literature survey by referencing LD50 range upto the doses of 2000 mg/kg of selected plant extracts at doses 100 mg/kg and 200 mg/kg as per OECD guidelines No. 423. The result of the present study revealed that the stem bark extracts of A. marmelos, shows presence of flavonoid and phenolic compounds, exhibited the best antioxidant activity. Despite widespread use of *A. marmelos* as folklore medicines in India, the literature contains few reports on its antioxidant activity. In present experiment, we conceded out a systematic record on the total phenolic content, total flavonoid content anti-oxidant content in pet. Ether, ethyl acetate and methanolic extract of selected parts of *A. marmelos.*

Experimental Despair Swim Test model and tail suspension test was used to test antidepressant

activity of ethyl acetate and methanolic extract of plant material. The AMEA and AMME at doses (100 mg/kg and 200 mg/kg) and Imipramine (10 mg/kg) all significantly reduced Immobility time compared with Control (0.5% Dimethyl sulfoxide orally) wistar rats $p< 0.05$ to P0.05 was considered non-significant (ns). Post hoc analysis Turkey's multiple comparisons test found that AMME 200 mg/kg has significant difference when compared to control and activity is same like standard (Imipramine 10 mg/kg) in DST and TST. Aegle marmelos L. stem bark exhibited a slight but nonsignificant dose-dependent decrease in immobility. The results indicated that AM showed significant antidepressant-like effects in the DST and TST. On Day 1 the oral administration of AMEA and AMME (100 mg/kg, 200 mg/kg) and Imipramine (10 mg/kg) shows significant decrease in duration of immobility compared to day 8. On day 8 AMME (200mg/kg) shows less significant difference when compared to control and shows non-significant difference when compared to standard (imipramine 10mg/Kg).

CONCLUSION

In pursuit of elucidating the antidepressant potential of *Aegle marmelos*, the stem bark was judiciously chosen as the focal point. Consequent to this selection, three distinct extracts were meticulously prepared through a sequential process that involved initial defatting using petroleum ether followed by subsequent extraction employing ethyl acetate and methanol. These derived extracts were then subjected to a comprehensive battery of assessments encompassing phytochemical estimation, antioxidant activity evaluation, and antidepressant profiling.

The preliminary phytochemical analysis undertaken on the three extracts aimed to ascertain the presence of diverse phytoconstituents. The outcomes of this analysis unveiled the prevalence of flavonoid and phenolic compounds within the stem bark extract of *A. marmelos*. This particular extract exhibited the most robust antioxidant activity among the evaluated extracts. Notably, while *A. marmelos* has a well-established history in traditional Indian medicine, only limited literature addresses its antioxidant attributes. In this study, we embarked on a systematic exploration, meticulously documenting the relative efficacy of free radical scavenging within the pet ether, ethyl acetate, and methanolic extracts of the designated segment of *A. marmelos.*

The experimental assessment of the plant extracts was conducted using the despair swim test model and the tail suspension test, both serving as valid indicators of in vivo antidepressant potential. Both extracts underwent rigorous testing for their capacity to induce an antidepressant response in vivo. The findings gleaned from this experimentation revealed the prominent antidepressant effects of all extracts at doses of 100 mg/kg and 200 mg/kg relative to the subjects' body weight. This effect was duly contrasted against the established benchmark, the antidepressant standard drug Imipramine (10 mg/kg). Among the extracts, the methanolic derivative at the 200 mg/kg dose exhibited the most pronounced outcome, outperforming the other ethyl acetate extracts.

While constituting an essential foundational endeavour, it's imperative to acknowledge that this study serves as a preliminary stride. Subsequent investigations operating at a molecular stratum are imperative to unravel the precise active constituents responsible for the observed antidepressant activity. This progressive endeavour will shed light on the intricate mechanisms underlying *A. marmelos'* antidepressant potential, offering a profound understanding of its therapeutic utility.

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