International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Evaluation of Antidepressant Activity of *Apium Graveolens*

Veeresh Babu Pratap[1], Rahul Patil[2], Sowmya Amarthaluri[3], Gangaraju Mudunuri[4]

[1]Associate Professor, Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Hyderabad, Telangana, India
Corresponding Author Mail Id: *pratap.veeresh[at]gmail.com*
Phone No: 9493530528

[2, 3, 4]Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana – 500090, India

Abstract: ***Depression, a prevalent neuropsychiatric disorder, continues to pose significant challenges in both diagnosis and treatment. Natural compounds from plants have gained attention as potential sources for novel antidepressant agents. Apium graveolens, commonly known as celery, is rich in bioactive phytochemicals and has demonstrated various medicinal properties. This study aimed to investigate the antidepressant potential of Apium graveolens using widely employed preclinical models, the Tail Suspension Test (TST) and the Forced Swim Test (FST). Male albino mice were subjected to treatments with Apium graveolens extract at two different doses (200 and 400 mg/kg), and their behavior was assessed using TST and DST. The results demonstrated a significant reduction in immobility time in the treated groups, indicating a potential antidepressant effect. The findings suggest that Apium graveolens may possess anti - depressant activity, warranting further exploration of its mechanisms and potential use as a natural remedy for depressive disorders. This research contributes to the growing body of evidence supporting the use of plant - derived compounds in mental health therapeutics and underscores the importance of investigating the potential of traditional medicinal plants as sources of novel antidepressant agents.***

Keywords: Depression, *Apium graveolens*, Tail Suspension Test, Forced swim test, antidepressant

1. Introduction

Depression, a severe and incapacitating disease, can affect anyone, regardless of age or background. It has the power to strip individuals of their hope, ambition, and will to live, and its impact on society as a whole cannot be overstated [1, 2]. Studies have shown that between 1 - 4% of older adults experience major depression, while 4 - 13% experience minor depression [7]. Women, are at a higher risk of being affected than men, and the incidence of both major and minor depression doubles between the ages of 70 - 85 [8]. In recent years, there have been significant strides in the treatment of depression, with the development of drugs that can block the inactivation of two important neurotransmitters in the brain - serotonin and noradrenaline [2, 3]. Two classes of drugs that have been found to be effective in alleviating depression symptoms are monoamine oxidase inhibitors and monoamine reuptake blockers [5]. In cases where conventional drug therapy is not effective, electroconvulsive therapy is also an option [6].

It's important to consider pharmacologic interventions when treating depression in older adults, especially in severe cases where the patient is experiencing intense anxiety, anhedonia, insomnia, poor concentration, appetite disturbances, or suicidal ideation. The choice of antidepressant should be made based on the individual patient's symptoms, concurrent medical problems, medications currently taken, and potential side effects. While there is no perfect antidepressant that works for everyone without adverse effects, there is a need to develop safe and effective remedy which suits to majority of depressive patients.

Apium graveolens, commonly known as celery, is a versatile and nutritious vegetable that has been cultivated and enjoyed by humans for centuries. This biennial plant belongs to the Apiaceae family and is prized for its crisp stalks and distinctively fresh, earthy flavor. Celery is a rich source of essential vitamins and minerals, including vitamin K, vitamin C, potassium, and dietary fiber, making it a popular choice for those seeking a healthy and low - calorie addition to their diet. In addition to its culinary uses, celery has also found its place in traditional medicine due to its potential health benefits, such as its anti - inflammatory properties and potential to lower blood pressure. Whether used as a crunchy snack, an aromatic ingredient in soups and stews, or a garnish to enhance the presentation of dishes, *Apium graveolens*, or celery, remains a cherished and indispensable ingredient in the world of cuisine and nutrition.

Apigenin an important flavonoid found in Celery interacts with various neurotransmitter systems in the brain. It can modulate the activity of gamma - aminobutyric acid (GABA) receptors, which are essential for regulating depression. Hence it was intended to explore the antidepressant activity of *Apium graveolens* using *in vitro* and *in vivo* models.

2. Materials and Methods

Collection and air drying of plant material

Leaves of *Apium graveolens*were collected in the month of January from local market in KPHB colony, Hyderabad. It was authenticated by botanist from Department of Botany, Kukatpally Government College. The leaves were cleaned and dried under shade for about six days and powdered coarsely.

Preparation of extracts

Dried powdered leaves of *Apium graveolens* was kept for soxhlation for 8 hours by using Methanol as a solvent at a temperature of 50º C.

Preliminary phytochemical analysis
The phytochemical analysis of MEAG was done using standard procedures [26].

GC - MS analysis of methanolic extract of *Apium graveolens*
In the analysis, the initial column temperature was set at 35°C for 3 minutes. The temperature then increased by 8°C per minute until it reached a final temperature of 280°C. During this process, a 1µl sample was injected, vaporized, and carried through the column by helium gas at a rate of 1 ml/min. A Mass Spectrometry (MS) spectrum was captured at 70 eV. After column separation, components were further identified and analyzed using Flame Ionization Detection (FID). To identify the compounds, the spectrum of unknown substances was compared to the known compounds in the NIST MS 2.0 structural library, allowing determination of their names, molecular weights, and structures [27].

Acute toxicity studies
Acute toxicity studies were conducted in accordance with OECD guideline 425. Five female mice were subjected to oral administration of a 2000 mg/kg dose of a plant extract via gastric intubation. Continuous observation of the mice's behavioural, neurological, and autonomic profiles was carried out over the course of 24 hours. Lethality was monitored at 24 - hour mark.

Hydrogen Peroxide Scavenging Assay
A 40 mM H_2O_2 solution was mixed with phosphate buffer (pH 7.4). Various standard and test concentrations of H_2O_2 were added to the buffer, and absorbance at 230 nm was measured. Percentage of inhibition was calculated using the formula % scavenged (H_2O_2) = [(A_C - A_T) / A_C] x 100, where A_C is the control absorbance and A_T is the sample or standard absorbance [29].

Nitric Oxide Radical Scavenging Activity
A reaction mixture with sodium nitroprusside (5 mM) in phosphate - buffered saline was incubated with or without plant extract at different concentrations. Nitrite ion production was measured by adding Griess reagent, and absorption at 560 nm was recorded. The percentage of inhibition was determined as % scavenging activity = [(A_C - A_T or A_S) /A_C] ×100, with A_C as the control absorbance and A_T or $A_S$as the test or standard absorbance [30].

Animal Experimental design
A total of 24 male albino mice, weighing between 20 to 25 grams, were utilized and divided into 4 separate groups, each consisting of 6 mice. The study spanned 14 consecutive days, during which the mice had unrestricted access to food and water under standard living conditions. The housing and care of the mice strictly adhered to the guidelines of the CCSEA. All the experiments were Conducted in the Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad.

The study involved the administration of MEAG extract at doses of 200 and 400 mg/kg b. wt/day. Standard groups were treated with imipramine. A control group (Group 1) received a vehicle only, Data were expressed as mean ± SEM. When several treatments were compared, one - way ANOVA was used and post - hoc comparisons between vehicle and drug treated groups were made using Dunnett's multiple comparison test using Graph pad Prism Version 5. In all tests the criterion for significance was $P< 0.05$.

Estimation of Noradrenaline
In an in vitro study for anti - depressant activity and noradrenaline estimation, rats were sacrificed, and their brain tissue was homogenized with HCl - butanol. After centrifugation, the aqueous phase was used for analysis. To this, HCl, sodium acetate buffer, and iodine solution were added for oxidation. Na_2SO_3 was used to stop the reaction, followed by acetic acid. The sample was heated and then analyzed with a spectrofluorimeter to measure noradrenaline levels between 385–495 nm [31].

The tail suspension test
The tail suspension test Mice were hanged by their tails on the tail hanger, which was secured using sticky tape about 1 cm from the end of the tail. The hanger was secured in a black plastic box (20 x 20 x 45 cm) with a top front opening. The space between the hanger and the floor was around 40 cm. The mouse was held in the air by its tail for 6 minutes, and the immobility time was measured for last 4 minutes. The absence of all movement except that essential for respiration was characterized as the duration of immobility [32].

The forced swim test
In the forced swim test the mice were submerged in water up to a certain level and the temperature was maintained at around 25 - 28°C for an experiment. During a 6 - minute period, the mice were observed while floating in the water, and their time of immobility was recorded for last 4 minutes. Immobility was defined as complete stillness while passively floating in the water, with the head slightly above the water surface [32].

3. Results

Preparation of MEAG:
MEAG was obtained using soxhlation technique. The % yield of the extract was calculated using the below formula and the % yield was found to be 16.67 % w/w

$$\% \text{ yield of extract} = \frac{\text{Amount of extract obtaained (grams)}}{\text{Amount of powder used (grams)}} \times 100$$
$$= 100 \times \frac{25}{150}$$
$$= 16.67\% \text{ w/w}$$

Preliminary phytochemical analysis:
The phytochemical analysis of MEAG was done using standard procedures and the extract was found to contain alkaloids, flavonoids, glycosides, phenols, saponins, tannins, Carbohydrates, Proteins.

GC - MS studies of MEAG:
The identification of compounds was done on the basis of retention time, molecular formula, molecular weight and % of peak area. About 7 compounds were identified which includes alkaloids, flavonoids, phenols and coumarins. The

GC - MS spectrum was shown in figure1 and detailed analysis in table 1.

Acute toxicity studies:
After administration of a 2000 mg/kg dose of a plant extract via gastric intubation. Continuous observation of the mice's behavioural, neurological, and autonomic profiles was carried out over the course of 24 hours. Lethality was monitored at 24 hour mark. After 14 - day period, the animals were re - examined for any signs of toxicity.2000 mg/kg dosage did not result in any fatalities among the mice, the plant extract was deemed non - toxic. NO fatalities occurred following exposure to the plant extract in this study

***In Vitro* Antioxidant Assay:**

Hydrogen peroxide scavenging assay
The anti - oxidant activity of *Apium graveolens* was studied by using H_2O_2 scavenging assay. MEAG's IC_{50} value was found to be 26.5 µg/ml and standard ascorbic acid IC_{50} value was to be at 21.4 µg/ml.

The experiment was performed in triplicate, and the percentage of inhibition was represented as mean±SEM.

Nitric Oxide scavenging assay
The anti - oxidant activity of *Apium graveolens* was studied by using NOscavenging assay. MEAG's IC_{50} value was found to be 35.56µg/ml and standard ascorbic acid IC_{50} value was to be at 27.25µg/ml.
The experiment was performed in triplicate, and the percentage of inhibition was represented as mean ±SEM.

***In vitro* antidepressant activity**

Estimation of Noradrenaline
A low level of noradrenaline has been observed in non - treated depression - controlled rat when compared to normal ($p<0.001$). While in comparison with disease control, MEAG treated animals showed significant levels of noradrenaline level ($p<0.001$). The noradrenaline enhancing effect of 400 mg/kg of MEAG was found to be better than that of 200 mg/kg.

***In vivo* antidepressant activity**

Tail suspension test:
Tail Suspension method induced depression in the animals was indicated by high immobility time in control group (191 ± 2.08). Treatment with MEAG at 200 &400mg/kg doses significantly ($P < 0.0001$) reduced the immobility duration depicting its antidepressant potential. The effect of 400mg/kg (124 ± 3.02) was comparable to that of standard imipramine (121 ± 2.54).

Forced swim test:
Forced swim test induced depression in the animals was indicated by high immobility time in control group (130 ± 2.67). Treatment with MEAG at 400mg/kg, 200mg/kg doses significantly ($P < 0.001$, $P < 0.01$) reduced the immobility duration depicting its antidepressant potential. The effect of 400mg/kg (105 ± 3.17) was comparable to that of standard imipramine (94 ± 2.33).

4. Discussion

Depression remains a major global health concern, necessitating the continuous search for effective antidepressant agents. *Apium graveolens*, a well - known vegetable, has been traditionally used for various medicinal purposes. The present study aimed to explore its antidepressant potential using established behavioural models, the TST and FST.

By measuring immobility time, the Forced Swimming Test and tail suspension test are used to analyse depressive - like behaviour.

The key benefits of this approach are its ease of use and quick outcomes. It also makes for an effective screening method because it is responsive to a wide range of depression medications.

When animals get placed in situations like this, they initially try to escape but eventually give up and become immobile, which is seen as a symptom of "behavioural despair".

According to a report, apigenin has the potency to stop Monoamine Oxidase (MAO) from working. This process raises the brain's concentration of monoamines like norepinephrine, dopamine, and serotonin, which are linked to the disappearance of depressed symptoms.

MEAG's MAO - inhibiting potential, which is caused by the presence of apigenin in it, can be credited for its considerable antidepressant activity in the current study.

A common technique for evaluating prospective antidepressants is the tail suspension test. Being a quick, inexpensive, highly predictive, and high throughput screening test for antidepressant activity is one of its benefits. It's common to interpret an animal's motionless posture or cessation of struggle as an indication of depressive - like behaviour or "behavioural despair".

5. Conclusion

Apium graveolens demonstrates significant antidepressant activity as evidenced by its performance in the TST and FST. This research paves the way for further exploration of *Apium graveolens* as a potential natural remedy for depression, offering hope for individuals seeking alternative and complementary treatments for this widespread mental health condition.

Acknowledgement
Authors are grateful to the Principal and Management of Gokaraju Rangaraju College of Pharmacy for their encouragement and providing all the facilities required in conducting the work.

Conflict of Interest
All the authors have no conflicts of interest to declare

References

[1] Altar CA. Neurotrophins and depression. Trends in pharmacological sciences.1999 Feb 1; 20 (2): 59 - 62.
[2] Gillespie CF, Nemeroff CB. Hypercortisolemia and depression. Psychosomatic medicine.2005 May 1; 67: S26 - 8.
[3] Elhwuegi AS. Central monoamines and their role in major depression. Progress in Neuro - Psychopharmacology and Biological Psychiatry.2004 May 1; 28 (3): 435 - 51.
[4] Mongeau R, Blier P, De Montigny C. The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. Brain Research Reviews.1997 Apr 1; 23 (3): 145 - 95.
[5] Beck AT, Alford BA. Depression: Causes and treatment. University of Pennsylvania Press; 2009 Mar 25.
[6] Lisanby SH. Electroconvulsive therapy for depression. New England Journal of Medicine.2007 Nov 8; 357 (19): 1939 - 45.
[7] Obuobi - Donkor G, Nkire N, Agyapong VIO. Prevalence of Major Depressive Disorder and Correlates of Thoughts of Death, Suicidal Behaviour, and Death by Suicide in the Geriatric Population - A General Review of Literature. Behav Sci (Basel).2021; 11 (11): 142. Published 2021 Oct 21. doi: 10.3390/bs11110142
[8] Salk RH, Hyde JS, Abramson LY. Gender differences in depression in representative national samples: Meta - analyses of diagnoses and symptoms. Psychol Bull.2017; 143 (8): 783 - 822. doi: 10.1037/bul0000102
[9] Sansone RA, Sansone LA. Dysthymic disorder: forlorn and overlooked?. Psychiatry (Edgmont).2009; 6 (5): 46 - 51.
[10] Hall CA, Reynolds - Iii CF. Late - life depression in the primary care setting: challenges, collaborative care, and prevention. Maturitas.2014; 79 (2): 147 - 152. doi: 10.1016/j. maturitas.2014.05.026.
[11] Depression. U. S. Department Of Health And Human Services National Institutes of Health. NIH Publication No.21 - MH - 8079. Revised 2021
[12] Mulinari S. Monoamine theories of depression: historical impact on biomedical research. Journal of the History of the Neurosciences.2012 Oct 1; 21 (4): 366 - 92.
[13] Heninger GR, Delgado PL, Charney DS. The revised monoamine theory of depression: a modulatory role for monoamines, based on new findings from monoamine depletion experiments in humans. Pharmacopsychiatry.1996 Jan; 29 (01): 2 - 11.
[14] Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A, Young T, Praschak - Rieder N, Wilson AA, Houle S. Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. Archives of general psychiatry.2006 Nov 1; 63 (11): 1209 - 16.
[15] Goodwin GM. Depression and associated physical diseases and symptoms. Dialogues Clin Neurosci.2006; 8 (2): 259 - 265. doi: 10.31887/DCNS.2006.8.2/mgoodwin
[16] Smith KM, Renshaw PF, Bilello J. The diagnosis of depression: current and emerging methods. Comprehensive psychiatry.2013 Jan 1; 54 (1): 1 - 6.
[17] Kovich H, Kim W, Quaste AM. Pharmacologic Treatment of Depression. Am Fam Physician.2023 Feb; 107 (2): 173 - 181. PMID: 36791444.
[18] Potter WZ, Rudorfer MV, Manji H. The pharmacologic treatment of depression. New England Journal of Medicine.1991 Aug 29; 325 (9): 633 - 42.
[19] Gabriel FC, de Melo DO, Fráguas R, Leite - Santos NC, Mantovani da Silva RA, Ribeiro E. Pharmacological treatment of depression: A systematic review comparing clinical practice guideline recommendations. PLoS one.2020 Apr 21; 15 (4): e0231700.
[20] Kooti W, Ali - Akbari S, Asadi - Samani M, Ghadery H, Ashtary - Larky D. A review on medicinal plant of Apium graveolens. Advanced Herbal Medicine.2015 Jan 1; 1 (1): 48 - 59.
[21] Fazal SS, Singla RK. Review on the pharmacognostical& pharmacological characterization of Apium graveolens Linn. Indo Global Journal of Pharmaceutical Sciences.2012; 2 (1): 36 - 42.
[22] Kooti W, Daraei N. A review of the antioxidant activity of celery (Apium graveolens L). Journal of evidence - based complementary & alternative medicine.2017 Oct; 22 (4): 1029 - 34.
[23] Al - Asmari AK, Athar MT, Kadasah SG. An updated phytopharmacological review on medicinal plant of Arab region: Apium graveolens linn. Pharmacognosy reviews.2017 Jan; 11 (21): 13.
[24] Turner L, Lignou S, Gawthrop F, Wagstaff C. Investigating the factors that influence the aroma profile of Apium graveolens: A review. Food Chemistry.2021 May 30; 345: 128673.
[25] Salehi B, Venditti A, Frezza C, Yücetepe A, Altuntaş Ü, Uluata S, Butnariu M, Sarac I, Shaheen S, A. Petropoulos S, R. Matthews K. Apium plants: Beyond simple food and phytopharmacological applications. Applied Sciences.2019 Aug 29; 9 (17): 3547.
[26] Khandelwal K (2008) Practical Pharmacognosy. Nirali Prakashan, Pune.
[27] Mishra D, Patnaik S. GC - MS analysedphyto - chemicals and antibacterial activity of *Withaniasomnifera* (L.) Dunal extract in the context of treatment to liver cirrhosis. Biomedical and Pharmacology Journal.2020 Mar 28; 13 (1): 71 - 8.
[28] OECD guideline number – 425.
[29] Fernando CD, Soysa P. Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H2O2) scavenging activity of plant extracts. MethodsX.2015 Jan 1; 2: 283 - 91.
[30] Patel Rajesh M, Patel Natvar J. In vitro antioxidant activity of coumarin compounds by DPPH, Super oxide and nitric oxide free radical scavenging methods. Journal of advanced pharmacy education & research.2011; 1 (1): 52 - 68.
[31] V. T. Deshmukh, M. T. Deshmukh1, T. V. Deshmuk, P. K. Chede, M. M Abhyankar. Estimation of Monoamines concentration in rat brain after administration of Fluoxetine Hydrochloride Microsphere. Eur. Chem. Bull.2023, 12 (Special Issue 8), 1313 - 1319.

[32] Abdelhalim A, Karim N, Chebib M, Aburjai T, Khan I, Johnston GA, Hanrahan J. Antidepressant, anxiolytic and antinociceptive activities of constituents from Rosmarinus officinalis. Journal of Pharmacy & Pharmaceutical Sciences.2015 Sep 21; 18 (4): 448 - 59.

List of tables

Table 1: GC - MS analysis of *Apium graveolens*

S. No	Category	Mol. Wt. (g/mol)	Name of the compound	% of area	Retention time
1	Flavonoid	270.24	Apigenin	18.35	1.084
2	Phenols	464.4	Isoquercitrin	3.43	21.149
3		126.18	Celereoside	2.21	17.865
4	Furocoumarins	216.19	Bergapten	11.64	1.136
5		136.23	Camphene	2.04	16.492
6		136.23	Limonene	1.31	29.06
7	Terpenes	134.22	Cymene	1.51	29.634

Table 2: Effect of Methanolic extract of *Apium graveolens* on hydrogen peroxide scavenging assay

Compounds	Concentration	% Inhibition	IC_{50} value (µg/ml)
MEAG	10	22.6 ± 0.15	26.5
	20	43.4 ± 0.62	
	30	63.4 ± 0.39	
	40	70.4 ± 0.91	
	50	72.8 ± 0.49	
Ascorbic acid	10	26.9 ± 0.26	21.4
	20	48.9 ±0.75	
	30	62.8 ± 0.89	
	40	68.8 ± 0.32	
	50	74.9 ±0.61	

Table 3: Effect of Methanolic extract of *Apium graveolens* on Nitric Oxide scavenging assay

Compounds	Concentration	% Inhibition	IC_{50} value (µg/ml)
MEAG	10	24.51± 0.15	35.56
	20	31.03± 0.16	
	30	43.39± 0.48	
	40	54.54± 0.89	
	50	60.78± 0.78	
AscorbicAcid	10	36.84± 0.53	27.25
	20	48.71± 0.45	
	30	53.48± 0.67	
	40	58.62± 0.38	
	50	63.19± 0.69	

Table 4: Effect of methanolic extract of *Apium graveolens* on noradrenaline levels in rat brain

S. No	Groups	Mean ± SEM
1	Normal Control	146.68 ± 0.87
2	Disease Control	84.50 ± 1.06
3	MEAG (200 mg/kg)	98.14 ± 1.38
4	MEAG (400 mg/kg)	116.30 ± 1.02

Table 5: Effect of methanolic extract of *Apium graveolens* on mice in Tail suspension test

S. No	Groups	Mean ± SEM
1	Normal Control	191 ± 2.08
2	MEAG (200 mg/kg)	144 ± 3.75***
3	MEAG (400 mg/kg)	124 ± 3.02**
4	Imipramine (20 mg/kg)	121 ± 2.54*

Results were shown as Mean SEM, (n=6). For the statistical analysis, Dunnett's multiple comparison tests and one - way ANOVA were used. Comparisons were made between the values and the control group (***=p<0.001, **=p<0.01, and *=p<0.05).

Table 6: Effect of methanolic extract of *Apium graveolens* on mice in forced swim test

S. No	Groups	Mean ± SEM
1	Normal Control	130 ± 2.67
2	MEAG (200 mg/kg)	118.5 ± 1.68***
3	MEAG (400 mg/kg)	105 ± 3.17**
4	Imipramine (20 mg/kg)	94 ± 2.33*

Results were shown as Mean SEM, (n=6). For the statistical analysis, Dunnett's multiple comparison tests and one - way ANOVA were used. Comparisons were made between the values and the control group (***=p<0.001, **=p<0.01, and *=p<0.05).

List of figures

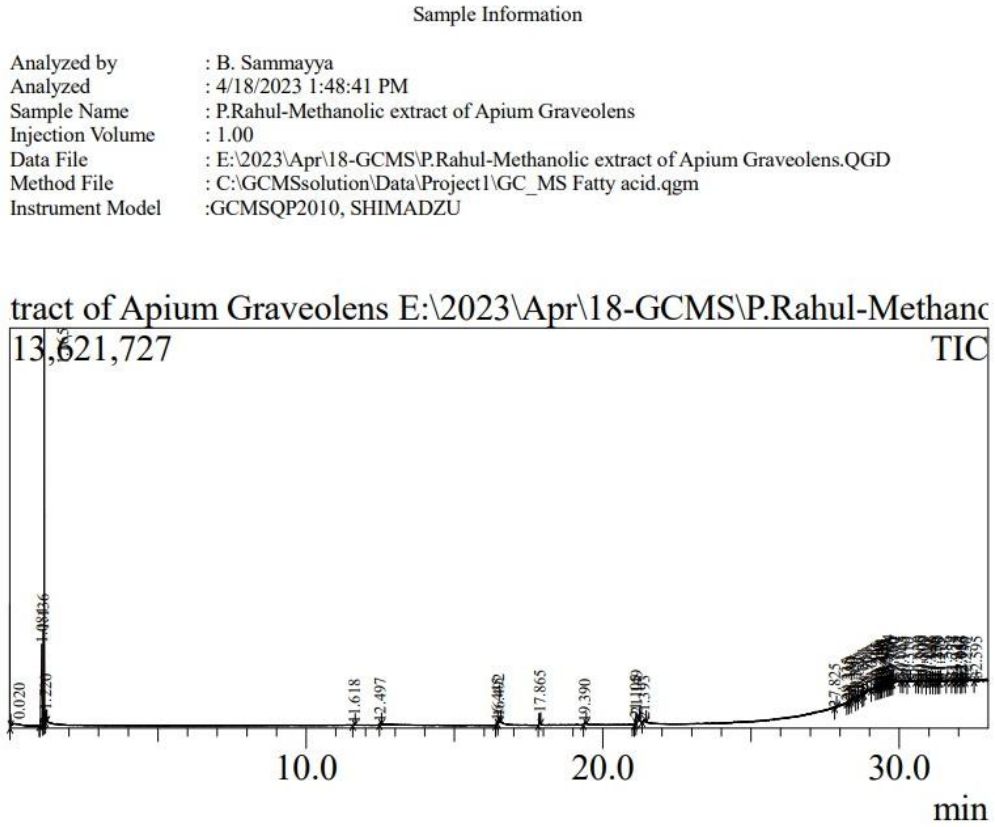

Figure 1: GCMS studies of Methanolic extract of *Apium graveolens (*MEAG)

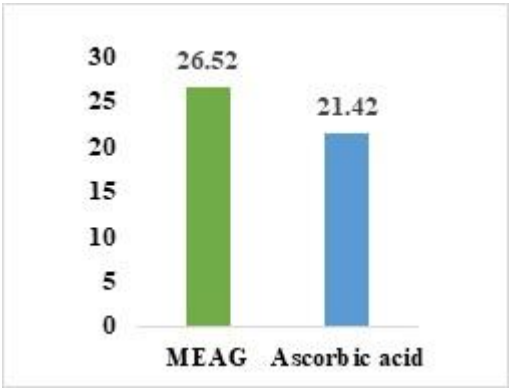

Figure 2: Graphical representation of IC_{50} value (µg/ml) of MEAG and ascorbic acid inhydrogen peroxide scavenging assay

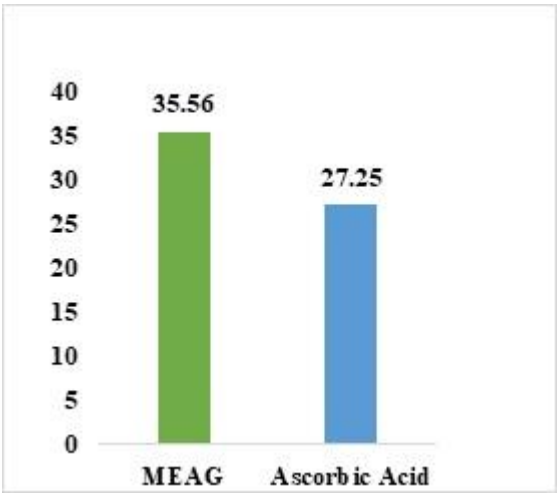

Figure 3: Graphical representation of IC_{50} value (µg/ml) of MEAG and ascorbic acid inNitric oxide scavenging assay

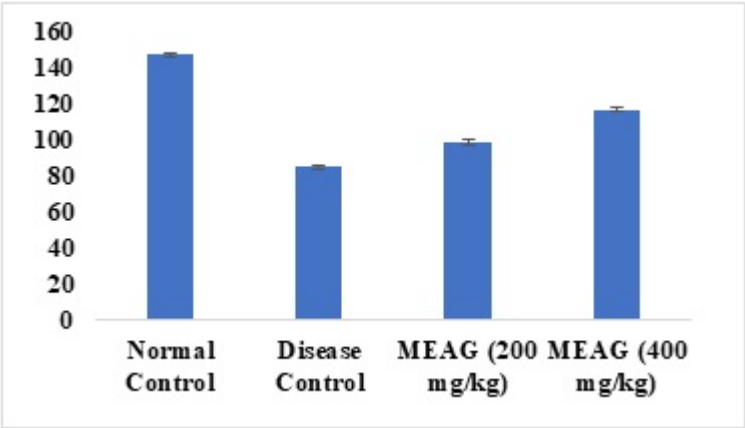

Figure 4: Graphical representation of effect of methanolic extract of *Apium graveolens* on noradrenaline levels in rat brain.

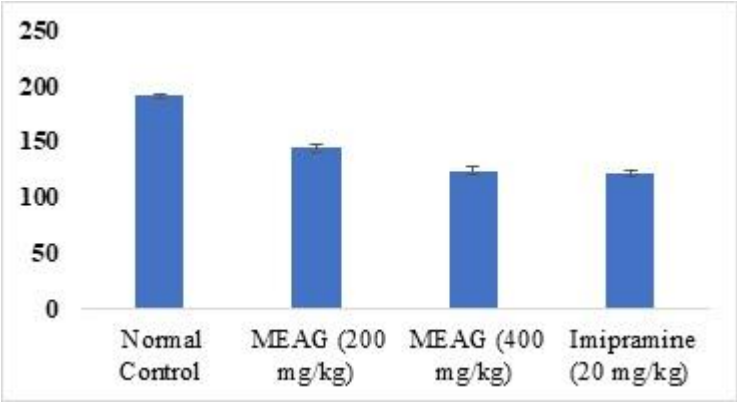

Figure 5: Graphical representation of effect of methanolic extract of *Apium graveolens* on mice in Tail Suspension Test

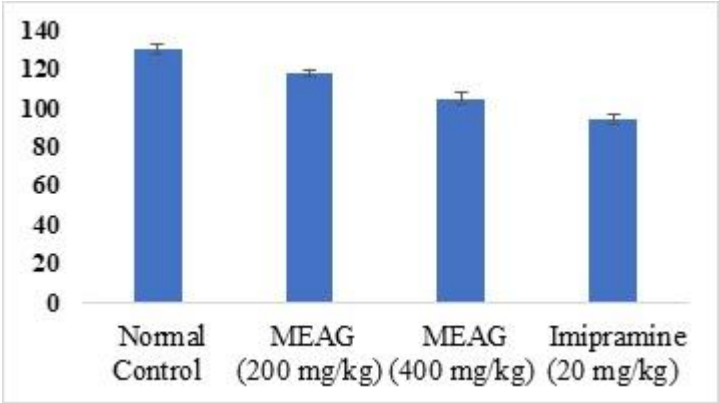

Figure 6: Graphical representation of effect of methanolic extract of *Apium graveolens* on mice in Forced Swimming Test