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Exploring the Antioxidant and Anxiolytic Biological Activities of *Spinacea Oleracea* Seed Extract

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ABSTRACT

Spinacia oleracea is a green leafy vegetable widely consumed for its nutritional value. The present research work is focused on *spinacia oleracea* seed extract for evaluation of in vitro antioxidant and in vivo anxiolytic activities. Spinach leaves antioxidant properties are well documented but the bioactive potential of seeds remains unexplored. Hence this research work aims to explore the antioxidant and anxiolytic effects of *Spinacia oleracea* seed extract using standard in vitro and in vivo methods. The in vitro antioxidant potential of the *spinacia oleracea* seed extract at dose of 50,100,300 and 500µg/ml were assayed using hydroxyl radical scavenging, nitric oxide, and DPPH radical scavenging assay and results are compared with standard reference drug Gallic acid at concentration 2.5µg/ml. In behavioral studies, *spinacia oleracea* seed extract at dose of 75mg/kg and 150mg/kg was tested on albino mice subjected to standard behavioral paradigms such as elevated plus maze, open field test, hole board test, and y-maze. The results of these effects were compared to standard anxiolytic drug diazepam. The results obtained were encouraging and indicated significant antioxidant and anxiolytic effect. The FTIR studies revealed that the seed extract was rich in phenolic compounds known for its antioxidant property.

Keywords: *Spinacia oleracea*; antioxidant; DPPH; anxiolytic; elevated plus maze; FTIR

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INTRODUCTION

Anxiety is a complex emotional and physiological response characterized by feelings of tension, worry, and physical changes such as increased heart rate and restlessness[29,32]. While mild anxiety can serve as a normal and adaptive response to everyday challenges, excessive or persistent anxiety may interfere with an individual's daily functioning and quality of life[30,31]. Anxiety disorders represent one of the most prevalent mental health conditions globally, affecting over 264 million people across all the age groups, according to the world health organization. *Spinacia oleracea*[39,41,47] is a green leafy vegetable known for its nutritional benefits belongs to the family amaranthacea commonly known as spinach and Hindi palak[6,7,35]. This is abundantly grown in all parts of Andhra pradesh[8,34,35]. The natives of surampalem and naykampalli are using the seeds of *Spinacea oleracea* plant for nervousness related problems, based on the folkloric usage of this plant, a study was taken up to evaluate the possible biological activities like in vitro antioxidant and anxiolytic activity.

MATERIALS AND METHOD

Collection of plant material:

The seeds of *Spinacia oleracea*[42,47] were collected from different parts of East Godavari district, Andhra Pradesh.

Extraction

The obtained seeds were dried under shade and powdered. The coarsely powdered seeds of *Spinacia oleracea*[43,44,45] were subjected to successive extraction using ethanol in a Soxhlet apparatus. The resulting extract was concentrated by removing the solvent through distillation, followed by evaporation on a water bath to obtain a dried extract and stored in a dessicator for further analysis.

Experimental animals

Albino mice of either sex, weighing 20-25g were used. Three mice in each group were housed in a cage under standard laboratory conditions and acclimatized for 7 days before starting the experiment. The animal protocol was approved by the institutional animal ethics committee (IAEC) and the animals were taken care as per CPCSEA guidelines.

FTIR studies

FTIR spectroscopy was performed to characterize the functional groups present in the *Spinacia oleracea* seeds extract. The spectra were recorded in the range of 4000-400 cm^{-1}at a resolution of 4 cm^{-1} using an FTIR spectrometer. The resulting spectra were analyzed for the identification of

characteristic peaks corresponding to specific functional groups, provided insights into chemical constituents of the extract.

Determination of in vitro antioxidant activity using DPPH scavenging method

DPPH (1,1 diphenyl, 2-picryl hydrazyl) was used to study the free radical scavenging effect of *Spinacia oleracea* seed extract[1,2,3]. Different concentrations of extract (50,100,300,500 µg/ml) were prepared in methanol. 1 ml of extract at various concentrations (50,100,300,500 µg/ml) is added to 0.1 ml of prepared DPPH solution and kept in dark for 30 minutes. Incubated samples were checked for absorbance at 517nm using the following equation and expressed as percent inhibition and the obtained results were compared with that of the standard Gallic acid of concentration (2.5µg/ml)[1,4,5].

$$\% \text{ RSA} = [(A_0\text{-}A_1/A_0)] *100$$

Where

A_0 indicates control absorbance

A_1 indicates sample absorbance

Hydroxyl radical scavenging assay method:

For each individual concentration of extract, 1ml of the reagent solution was added[11], followed by the sequential addition of 1 ml of 1.5Mm $Feso_4$, 0.7 ml of 6mM H_2O_2, and 0.3ml of 20mM sodium salicylate[12]. The resulting mixture was incubated for approximately 1 hour[13], after which the absorbance was measured at 562nm and % inhibition is calculated by using the formula and compared with that of standard Gallic acid(2.5µg/ml)[14].

$$\% \text{ RSA} = [\ (A_0\text{-}A_1/A_0)] *100$$

Where,

A_0 indicates control absorbance

A_1 indicates sample absorbance

Nitric oxide scavenging assay

For each individual concentration of the extract, 0.5ml of phosphate-buffered saline and dissolved sodium nitroprusside were added and thoroughly mixed[17,18]. The mixture was then incubated at 25^0C for 2 hours. Following incubation, 0.5ml of the reaction mixture was withdrawn from each sample and mixed with 0.5ml of Griess reagent. The resulting solution was measured at 546nm[19]. The percentage of nitric oxide scavenged by the test extract was then calculated using the formula and compared with standard Gallic acid(2.5µg/ml)[20,21].

$$\% \text{ RSA} = [\ (A_0\text{-}A_1/A_0)] *10$$

Where,

A_0 indicates control absorbance

A_1 indicates sample absorbance

Determination of in vivo anxiolytic activity by behavioral tests

Experimental design:

The animals were divided into four groups, each group containing 3 mice.

Group 1: control

Group 2: animals treated with low dose of extract (75mg/kg)

Group 3: animals treated with high dose extract (150mg/kg)

Group 4: animals treated with standard drug diazepam (1 mg/kg)

Elevated plus maze test

The elevated plus maze (EPM) is a widely utilized behavioral model for assessing anxiety-like behavior in rodents. In this study group 2 and group 3 were administered a low and high dose of the test extract, respectively for 7 consecutive days. Group 4 received diazepam (1mg/kg) one hour prior to the experiment as a standard anxiolytic reference. On 7th day, the behavioral activity of each group was assessed and compared based on parameters such as the number of entries and time spent in open arms, as well as the number of entries and time spent in the closed arms of the maze.

Open field test

The apparatus consists of a contained area with surrounding walls to prevent the animal from escaping[58], typically marked with a grid to distinguish center and peripheral squares. Animals were divided into four groups. Group 2 and Group 3 received low and high dose of test extract, respectively for 7 consecutive days. On the 7th day, one hour after extract administration, the animals were placed in a corner of the open field apparatus, and their behavior was recorded for 5 minutes. Parameters observed included the number of center square crossings, peripheral square crossings, and readings. As a standard, diazepam was administered to group 4 and the same behavioral parameters were recorded.

Hole board apparatus

The apparatus consists of an open field arena equipped with 16 evenly spaced holes each measuring 3 cm in diameter. Animals were divided into four groups and treated with their respective test and standard substances. One hour following the administration of the extract, each animal was individually placed on a corner of the apparatus. The number of head dipping behaviors exhibited by each animal was recorded for 5 minutes.

Y-maze test

The apparatus consists of three identical arms arranged at 120-degree angles, forming a y-shaped structure. Animals were divided into four groups and treated with their respective substances. Group 2 and group 3 received low and high dose of the test extract for 7 consecutive days, while group 4 received diazepam as the standard drug. On the 7th day, one hour after extract administration for group 2 and group 3 and 30 minutes post diazepam administration for group 4, each animal was placed at the center of the maze and allowed to explore freely for 5 minutes and recorded the number of visits into three arms.

RESULTS AND DISCUSSION

FTIR findings

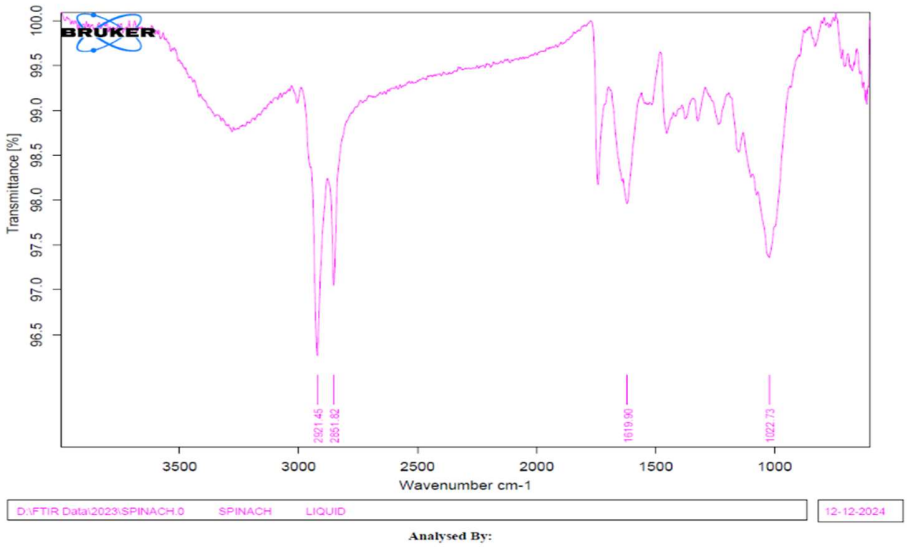

Figure: 1 FTIR spectral peaks of *Spinacia oleracea*

FTIR analysis was carried out to identify the functional groups present in the *Spinacia oleracea* seed extract and to confirm its bioactive compound The FTIR spectrum of the ethanolic seed extract of *Spinacia oleracea* reveals a complex mixture of bioactive constituents: a broad absorption around 3300 cm^{-1} indicates extensive hydrogen bonded O–H and N–H stretching, characteristic of phenolic compounds and proteins; prominent bands at 2920 and 2850 cm^{-1} arise from asymmetric and symmetric C–H stretching of –CH_2– groups in long chain fatty acids, while a sharp ester carbonyl stretch at 1740 cm^{-1} further confirms lipid content. In the mid-region, the Amide I (≈1650 cm^{-1}) and Amide II (≈1540 cm^{-1}) bands attest to peptide backbones, and bending vibrations near 1450 cm^{-1} reinforce the presence of aliphatic chains. Below 1300 cm^{-1}, strong C–O–C and C–O stretching vibrations (≈1240 and 1075 cm^{-1}) point to polysaccharides or glycosides linkages, while the fingerprint region (900–600 cm^{-1}) displays aromatic C–H bends and C–C skeletal modes associated with phenolic rings as shown in the Figure 1. Collectively,

these features demonstrate that the seed extracts rich in phenolics, proteins, lipids, and carbohydrates, consistent with its known antioxidant, nutritional, and structural properties.

Antioxidant activity

The antioxidant effect of ethanolic seed extract of *Spinacia oleracea* was evaluated using standard protocols hydroxyl radical scavenging, nitric oxide scavenging, and DPPH method[1,3]. The IC_{50} values so obtained were compared with standard reference Gallic acid (2.5µg/ml)[2,4].

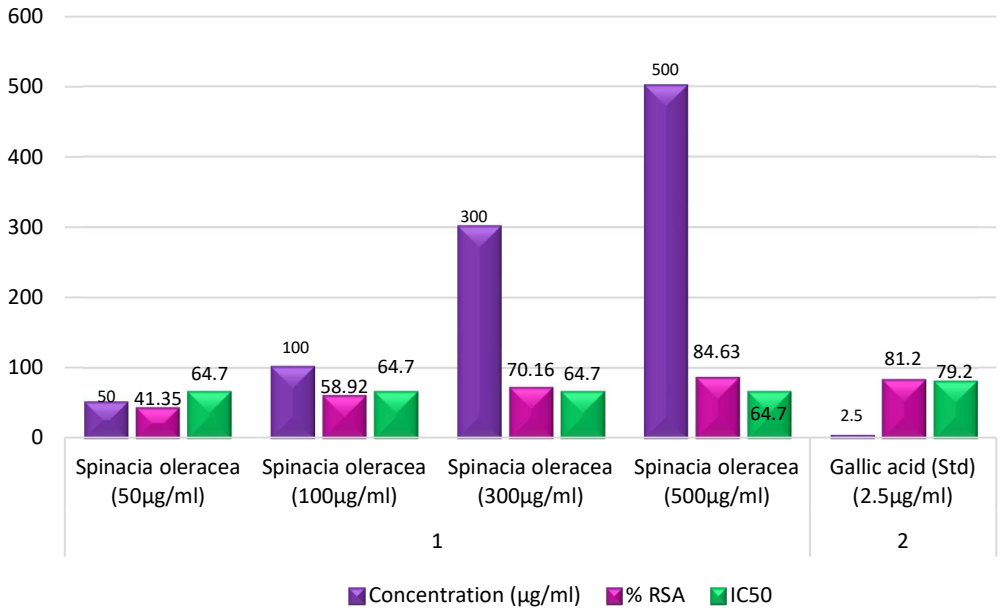

Figure: 2 Hydroxyl radical scavenging activity

The above graph indicates the hydroxyl radical scavenging activity of *Spinacia oleracea* seed extract at different concentrations (50,100,300,500µg/ml) compared to a standard antioxidant, Gallic acid (2.5µg/ml). The results are represented in terms of % Radical scavenging activity (%RSA) and IC_{50} values. The data indicate a concentration -dependent increase in %RSA for *Spinacea oleracea.* It is shown in Figure 2.

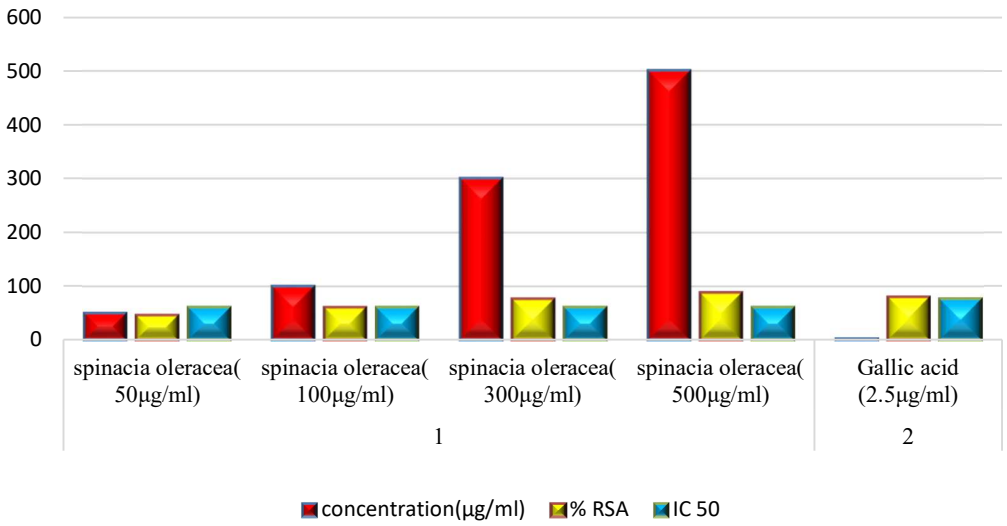

Figure: 3 DPPH radical scavenging activity

The above graph presents the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *Spinacia oleracea* seed extract at different concentrations (50,100,300,500 µg/ml), compared to the standard antioxidant, Gallic acid(2.5µg/ml). The results displayed in terms of concentration, % radical scavenging activity (%RSA), IC_{50} values. A concentration dependent increase in %RSA was observed with increasing concentrations of *spinacia oleracea* extract. At the highest concentration (500µg/ml), the extract showed the greatest %RSA, indicating enhanced antioxidant activity. In comparison, Gallic acid, used as a reference standard at 2.5µg/ml, exhibited superior %RSA and a significantly lower IC_{50} value. It is shown in Figure 3.

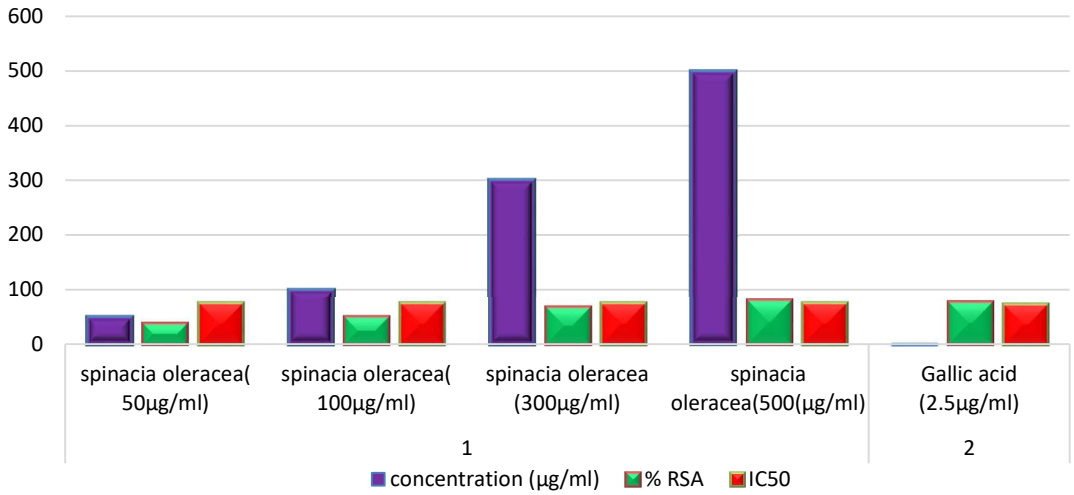

Figure: 4 Nitric oxide scavenging assay

The above graph presents the Nitric oxide scavenging activity of *Spinacia oleracea* seed extract at different concentrations (50,100,300,500 µg/ml), compared to the standard antioxidant, Gallic acid(2.5µg/ml). The results displayed in terms of concentration, % radical scavenging activity (%RSA), IC_{50} values. A concentration dependent increase in %RSA was observed with increasing concentrations of *Spinacia oleracea* extract. The highest activity noted at 500µg/ml, indicating that the extract becomes more effective at higher doses. It is shown in Figure 4.

Anxiolytic activity

The anxiolytic activity was assessed using elevated plus maze, open field apparatus, hole board test, y-maze. All these methods are widely acceptable for the screening of behavioral studies. Hence we have taken up the experiment using these standard protocols.

Elevated plus maze

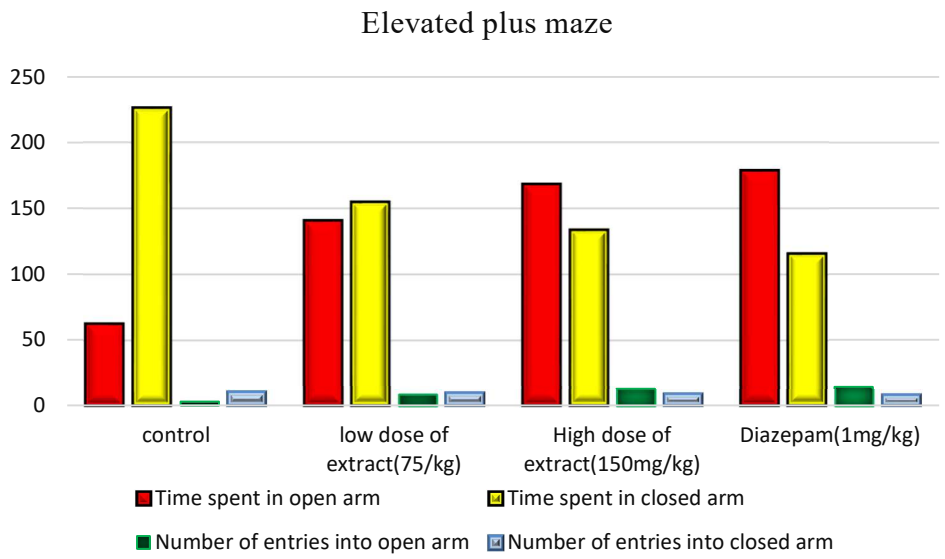

Figure: 5 Effect of ethanolic seed extract of *Spinacia oleracea* on mice using elevated Plus maze.

The above graph demonstrates that *Spinacia oleracea* seed extract exhibits dose-dependent anxiolytic activity in mice. The higher dose (150 mg/kg) closely mimics the anxiolytic profile diazepam. These findings support the efficacy of *Spinacia oleracea* in modulating anxiety-related behavior through elevated plus maze performance has shown in Figure 5.

Open field test

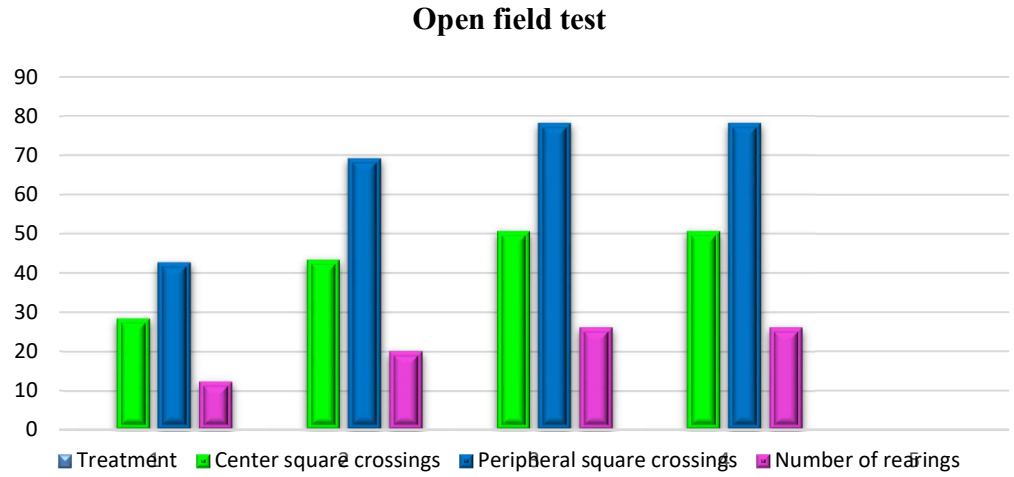

Figure: 6 Ethanolic seed extract of *Spinacia oleracea* on albino mice in open field test.

The above graph indicates that the ethanolic seed extract of *Spinacia oleracea* enhances exploratory behavior and reduces in a dose-dependent manner in albino mice. The high dose (150 mg/kg) produced effects similar to the standard anxiolytic, Diazepam, as evidenced by increased center crossings and rearing activity which is shown in Figure 6.

Hole board test

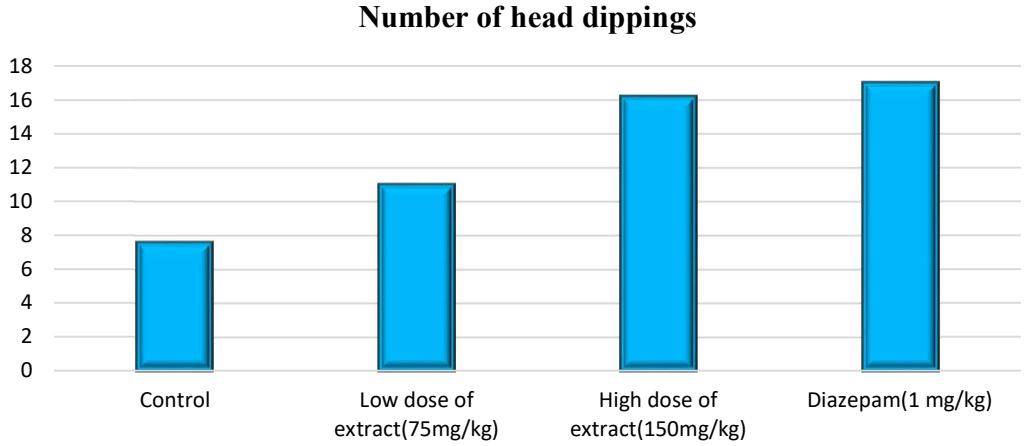

Figure: 7 Effect of ethanolic seed extract of *Spinacia oleracea* on albino mice in hole board apparatus.

The results from the hole board test indicates that *Spinacia oleracea* seed extract promotes anxiolytic activity in a dose dependent manner. The high dose (150 mg/kg) substantially increased head-dipping behavior and it is indicated in Figure 7.

Y-maze

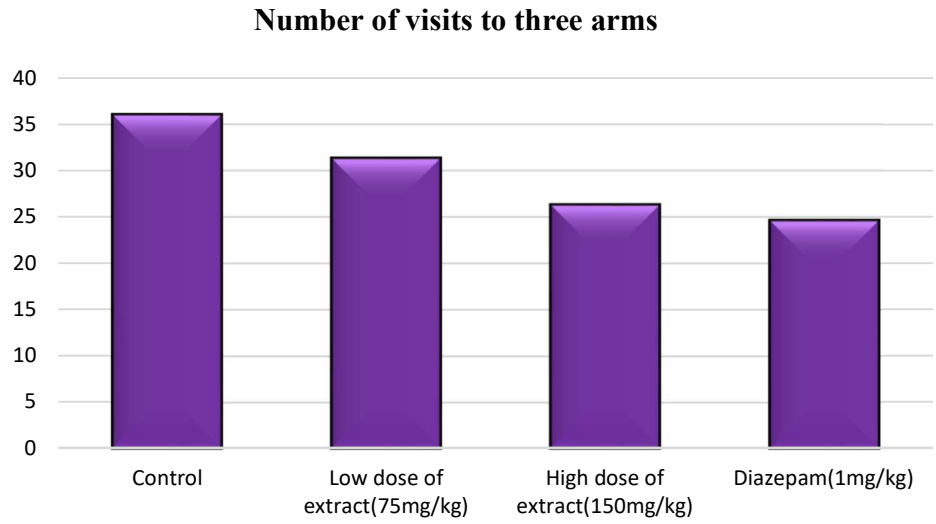

Figure: 8 Effect of ethanolic seed extract of *Spinacia oleracea* on albino mice on Y-maze.

The above graph represents that *Spinacia oleracea* seed extract reduces the number of arm entries in a dose-dependent manner, similar to the standard anxiolytic diazepam which is shown in Figure 8.

Hydroxyl radical scavenging assay

Table: 1: Effect of ethanolic seed extract of *Spinacia oleracea* seed extract on hydroxyl scavenging assay.

Sample	Concentration (µg/ml)	Hydroxyl Radical Scavenging Assay % RSA	IC 50 (µg/l)
Ethanolic seed extract of *Spinacia oleracea*	50	41.35 ± 0.33	38.41
	100	58.92 ± 0.47	
	300	70.16 ± 0.52	
	500	84.63 ± 0.41	
Gallic acid	2.5	81.20±0.44	79.20

Values are expressed as mean ± SEM, n = 3

The ethanolic seed extract of *Spinacia oleracea* demonstrated significant hydroxyl radical scavenging activity in a concentration-dependent manner, with %RSA increasing from 41.35 ± 0.33% at 50 µg/mL to 84.65 ± 0.41% at 500 µg/mL. The IC_{50} value of the extract was calculated to be 38.41 µg/mL, indicating potent antioxidant capacity. When compared to the standard Gallic acid, which exhibited 81.20 ± 0.44% RSA at a much lower concentration (2.5 µg/mL) with an IC_{50} of 79.20 µg/mL, the extract showed comparable efficacy at higher concentrations as listed in Table 1. These findings suggest that *Spinacia oleracea* seeds possess considerable hydroxyl radical scavenging potential and could serve as a natural antioxidant source.

DPPH scavenging assay

Table: 2 Effect of ethanolic seed extract of *Spinacia oleracea* on DPPH scavenging assay

Sample	Concentration (µg/ml)	DPPH Scavenging Assay	
		% RSA	IC 50 (µg/l)
Ethanolic seed extract of Spinacia oleracea	50	45.80 ± 0.40	
	100	60.75 ± 0.55	62.40
	300	76.30 ± 0.45	
	500	87.90 ± 0.60	
Gallic acid	2.5	79.50±0.57	76.50

Values are expressed as mean, ±SEM, n = 3

The antioxidant potential of the ethanolic seed extract of *Spinacia oleracea* was evaluated using the DPPH radical scavenging assay at varying concentrations (50–500 µg/mL). The results revealed a concentration-dependent increase in radical scavenging activity. At 50 µg/mL, the extract exhibited 45.80 ± 0.40% radical scavenging activity, which increased to 60.75 ± 0.55%, 76.30 ± 0.45%, and 81.90 ± 0.60% at concentrations of 100, 300, and 500 µg/mL, respectively. The IC_{50} value of the extract was found to be 62.40 µg/mL, indicating significant antioxidant potential. For comparison, Gallic acid, used as the standard, showed a scavenging activity of 79.50 ± 0.57% at 500 µg/mL, with an IC_{50} value of 76.50 µg/mL. as listed in Table 2. Notably, the extract exhibited a slightly lower IC_{50} value than the standard, suggesting that *Spinacia oleracea* seed extract possesses potent free radical scavenging activity, potentially attributable to its rich phytochemical content.

Nitric oxide scavenging assay

Table: 3 Effect of ethanolic seed extract of *Spinacia oleracea* on nitric oxide scavenging assay

Sample	Concentration (µg/ml)	Nitric Oxide Scavenging Assay	
		% RSA	IC 50 (µg/l)
Ethanolic seed extract of *Spinacia oleracea*	50	38.50 ± 0.60	
	100	50.20 ± 0.55	74.85
	300	67.30 ± 0.45	
	500	80.10 ± 0.50	
Gallic acid	2.5	76.50±0.59	72.80

Values are expressed as mean, ±SEM, n = 3

The nitric oxide scavenging activity of the ethanolic seed extract of *Spinacia oleracea* was assessed at various concentrations (50–500 µg/mL). The extract demonstrated a dose-dependent increase in scavenging potential. At 50 µg/mL, the extract exhibited 38.50 ± 0.60% scavenging activity, which progressively increased to 50.20 ± 0.55%, 67.30 ± 0.45%, and 80.10 ± 0.50% at concentrations of 100, 300, and 500 µg/mL, respectively. The IC_{50} value was determined to be 74.85 µg/mL, indicating notable nitric oxide inhibitory activity. In comparison, Gallic acid used as the standard exhibited 76.50 ± 0.59% scavenging activity at 500 µg/mL with an IC_{50} of 72.80

µg/mL as listed in table 3. The results suggest that the ethanolic extract of *Spinacia oleracea* possesses strong nitric oxide radical scavenging capacity, likely due to its bioactive phytoconstituents.

Elevated plus maze

Table: 4: Effect of ethanolic seed extract of *Spinacia oleracea* seed extract on albino mice in elevated plus maze.

Treatment	Control	Lower dose of extract (75mg/kg)	Higher dose of extract (150mg/kg)	Diazepam (1mg/kg)
Time spent in open arm	62.3 ± 0.88	140.4 ± 1.06	168 ± 0.47	178.5±0.5
Time spent in closed arm	225.9± 1.08	154.4 ± 0.66	133.3 ± 0.72	115.3±0.48
No. of entries in open arm	3 ± 0.47	8.3 ± 0.98	12.6 ± 0.56	14±0.47
No. of entries in closed arm	10 ± 1.24	9.3 ± 0.72	8.6 ± 0.73	8±0.27

All values are expressed as Mean ± SEM, n=3, P<0.001 when compared with standard values.

The anxiolytic activity of the ethanolic seed extract of *Spinacia oleracea* was evaluated using the elevated plus maze (EPM) model in albino mice. The control group showed minimal exploration of the open arm, spending 62.3 ± 0.88 seconds in the open arm and 225.9 ± 1.08 seconds in the closed arm, with 3 ± 0.47 entries into the open arm and 10 ± 1.24 entries into the closed arm. Treatment with the lower dose (75 mg/kg) of the extract significantly increased open arm activity, with mice spending 140.4 ± 1.06 seconds in the open arm and 154.4 ± 0.66 seconds in the closed arm. The number of entries was 8.3 ± 0.98 in the open arm and 9.3 ± 0.72 in the closed arm. At the higher dose (150 mg/kg), mice exhibited enhanced anxiolytic behavior, spending 168 ± 0.47 seconds in the open arm and 133.3 ± 0.72 seconds in the closed arm. The number of entries increased to 12.6 ± 0.56 for the open arm and 8.6 ± 0.73 for the closed arm. The standard drug diazepam (1 mg/kg) showed the most significant effect, with 178.5 ± 0.5 seconds spent in the open arm, 115.4 ± 0.49 seconds in the closed arm, 14 ± 0.47 entries in the open arm, and 8 ± 0.27 entries in the closed arm which was listed in above Table

Open field test

Table 5: Effect of ethanolic seed extract of *Spinacia oleracea* on mice in open field test.

Treatment	Centre square crossings	Peripheral square crossings	Number of Rearings
Control	28.3 ± 0.98	42.6 ± 0.98	12.3 ± 0.47
Lower dose of extract (75mg/kg)	39.3 ± 0.75	59.3 ± 1.65	18.3 ± 1.96
Higher dose of extract (150mg/kg)	43.3 ± 0.74	69 ± 1.69	20 ± 0.96
Diazepam (1mg/Kg)	50.6 ± 0.72	78 ± 1.24	26 ± 0.71

All values are expressed as Mean ± SEM, n=3, P<0.001 when compared with standard values

The anxiolytic-like behavior of the ethanolic seed extract of *Spinacia oleracea* was further evaluated using the open field test in mice. Parameters assessed included the number of center square crossings, peripheral square crossings, and rearings. In the control group, mice exhibited 28.3 ± 0.98 center square crossings, 42.6 ± .98 peripheral square crossings, and 12.3 ± 0.47 rearings, indicating typical exploratory activity with limited anxiety reduction. Mice treated with the lower dose (75 mg/kg) of *S. oleracea* extract showed moderate improvements in exploratory behavior, recording 39.3 ± 0.75 center crossings, 59.3 ± 1.65 peripheral crossings, and 18.3 ± 1.96 rearings. The higher dose (150 mg/kg) significantly enhanced activity levels, with 43.3 ± 0.74 center crossings, 69 ± 1.69 peripheral crossings, and 20 ± 0.96 rearings, suggesting a dose-dependent anxiolytic effect. Treatment with the standard drug diazepam (1 mg/kg) resulted in the highest exploratory behavior, with 50.6 ± 0.72 center crossings, 78 ± 1.24 peripheral crossings, and 26 ± 0.71 rearings, confirming its potent anxiolytic effect as shown in Table 5.

Hole board test

Table 6: Effect of ethanolic seed extract of *Spinacia oleracea* seed extract on albino mice in hole board apparatus.

Treatment	No. of Heads Dipping
Control	7.6 ± 1.18
Lower dose of extract (75mg/kg)	11 ± 1.12
Higher dose of extract (150mg/kg)	16.2 ± 0.95
Diazepam (1mg/Kg)	17 ± 0.47

All the values are expressed as Mean ± SEM, n=3, P<0.001 when compared with the standard values.

The anxiolytic activity of the ethanolic seed extract of *Spinacia oleracea* was further assessed using the head dipping test in mice. The number of head dippings was recorded as an indicator of exploratory behavior and anxiety. In the control group, mice exhibited 7.6 ± 1.18 head dippings, reflecting lower exploratory activity and higher anxiety levels. Administration of the lower dose (75 mg/kg) of *S. oleracea* extract resulted in a moderate increase in head dippings (11 ± 1.12), indicating reduced anxiety and increased exploratory behavior. A more significant anxiolytic effect was observed with the higher dose (150 mg/kg) of the extract, which produced 16.2 ± 0.95 head dippings substantially higher than both the control and lower dose groups. The standard anxiolytic drug diazepam (1 mg/kg) exhibited the greatest increase in exploratory behavior with 17 ± 0.47 head dippings, serving as a benchmark for comparison as shown in Table6. These results indicate that *Spinacia oleracea* seed extract enhances exploratory activity in a dose-dependent manner, demonstrating significant anxiolytic potential similar to that of diazepam.

Y-maze test

Table: 7 Effect of ethanolic seed extract of *Spinacia oleracea* on albino mice in y-maze

In y-maze, after 7 days treatment of animals with test extract, there is a decrease in number of visits to three arms is observed with the high dose of extract.

Treatment	Number of visits to three Arms
Control	36 ± 0.47
Lower dose of extract (75mg/kg)	31.3 ± 1.18
Higher dose of extract (150mg/kg)	26.3 ± 0.72
Diazepam (1mg/Kg)	24.6 ± 0.54

All the Values are express as mean, ±SEM, n = 3, P>0.001, when compared with the standard values.

The Y-maze test was conducted to evaluate the effect of *Spinacia oleracea* ethanolic seed extract on spatial working memory and exploratory behavior in albino mice. The number of visits to the three arms of the maze was recorded as an index of spontaneous alternation behavior. In the control group, mice made an average of 36 ± 0.47 visits to the arms, indicating normal exploratory behavior and baseline cognitive function. Following treatment with the lower dose (75 mg/kg) of the extract, the number of arm visits decreased to 31.3 ± 1.18, and suggesting mild reduction in spontaneous alternation. A more pronounced reduction was observed with the higher dose (150 mg/kg), which resulted in 26.3 ± 0.72 visits, indicating reduced locomotor activity and potentially enhanced anxiolytic or sedative-like effects. Mice treated with diazepam (1 mg/kg) showed the lowest number of arm visits (24.6 ± 0.54), consistent with its known CNS depressant and anxiolytic properties. These findings suggest that *Spinacia oleracea* extract, particularly at higher doses, may exert anxiolytic or sedative effects, reducing excessive locomotor activity in mice. It is shown in Table 7.

DISCUSSION

The results obtained from the studies indicate that the ethanolic seed extract of *Spinacia oleracea* possesses significant antioxidant and anti-anxiety activity.

FTIR analysis was carried out to identify the functional groups present in *Spinacia oleracea* seed extract and to confirm its bioactive compounds. The spectrum showed broad absorption band at around 3300 cm^{-1}, which is typically associated with indication of flavanoids and tannins. Overall, the FTIR analysis confirmed the presence of hydroxyl, carbonyl, and aromatic functional groups, which are characteristic of polyphenols and flavanoids as shown in the figure 1. The constituents may contribute to the observed antioxidant and anxiolytic properties of the extract.

The finding from the antioxidant activity clearly demonstrates the results clearly demonstrate a concentration dependent increase in radical scavenging activity by all the standard methods. The possible free radical scavenging activity observed in this research work can be implicated due to the presence of important phytoconstituents, phenolic compounds and flavanoids in the *Spinacia oleracea* seed extract. This can be aligned from the findings of the experiment that flavanoids may play a key role with reference to antioxidant activity.

The current study evaluated the anxiolytic potential of the ethanolic extract of *Spinacia oleracea* seeds using validated behavioral models, including the elevated plus maze (EPM), open field test (OPT), hole board test (HBT), and Y-Maze. The higher dose of extract exhibited significant anxiolytic activity, as indicated by increased time spent in open arms (EPM), enhanced exploratory behavior (HBT). These behavioral activities reflect reduced anxiety-like behavior, comparable to the effects observed with standard anxiolytic agents. The anxiolytic effects observed may be attributed to the phytoconstituents present in the *Spinacia oleracea* extract, particularly flavanoids, alkaloids and phenolic compounds. The extracts effects were dose-dependent, with higher dose producing more pronounced anxiolytic behavior.

CONCLUSION

The present study demonstrates that the ethanolic extract of *Spinacia oleracea* seeds exhibits significant antioxidant and anxiolytic activities, suggesting its potentials a natural therapeutic agent. The extract showed strong free radical scavenging capacity in in vitro assays, indicating the presence of potent antioxidant photochemical such as flavanoids and phenolic compounds. Furthermore, behavioral models like the elevated plus maze, open field, and hole board test revealed notable anxiolytic effects, as evidenced by increased exploratory behavior and reduced anxiety-like responses. These findings support the traditional use of *Spinacia oleracea* in managing oxidative stress and anxiety related disorders and warrant further studies to isolate and characterize the specific bioactive compounds responsible for these effects.

ANIMAL ETHICS APPROVAL

All animal experiments were carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. The animals were maintained, controlled and studied according to approved guidelines and regulations. During this period of study, Mice did not exhibit any adverse changes in their behavior and no signs of morbidity or mortality were observed. The experiments were carried out as per the guidelines laid down by IAEC as per the provisions made by CPCSEA. (REG.No. 1269/a/10/CPCSEA).

CONFLICT OF INTEREST:

The authors declare that they have no competing interests.

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