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"Phytochemical Investigation and Pharmacological Evaluation of *Moringa oleifera* Linn. Stem bark extracts for Memory enhancing Activity"

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1. Abstract :

The present study reports physicochemical characterization, antioxidant and Memory enhancing activity of extracts from *Moringa oleifera* L. stem bark collected from local region of Nanded, Maharashtra, India. The presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phytochemical analysis. The *In-Vivo* memory enhancing activity of *Moringa oleifera* L. stem bark was evaluated by radial arm maze model in rats using Piracetam as a standard. Both the extracts at 200mg/kg conc. showed significant to highly significant number of entries & time spent in P zone in radial arm maze. (from $P < 0.05$ to $P < 0.001$). The result suggests that *Moringa oleifera* L. stem bark extracts possess memory enhancing activity and this might be due to flavonids, phenolic compound, steroid and proteins present in extract.

Keywords : *Moringa oleifera* L. stem bark, Ethyl acetate and Ethanolic extract, Phytochemical screening, Antioxidant effect, *In-Vivo* Memory enhancing activity.

2. Introduction :

The analysis of the anatomical and physical basis of learning and memory is one of the great success of modern neuroscience. Learning is the process by which we acquire knowledge about the world. Learning refers to one or more less permanent change in behaviour which occurs as a result of practice, is a little better. Learning and memory are vital attributes of human intelligence. These processes underline the nature of our self awareness. The brain and the computer working similar fashion to store day to day happenings, incidents and visuals. Nootropic agents such as Piracitam, Pramiracitam, Aniracetam and Choline esterase inhibitors like Donepezil are being primarily used to improve memory, mood and behaviour.

Memory is understood as an informational processing system with explicit and implicit functioning that is made up of a sensory processor, short - term (or working) memory, and long-term memory.

3. Aim and objectives :

Aim : Phytochemical Investigation and Pharmacological Evaluation of *Moringa oleifera L.* Stem bark Extracts for Memory Enhancing Activity.

Objectives :

a. Phytochemical investigation and standardization of *Moringa oleifera* L. Stem bark extracts.
b. Acute oral Toxicity study of plant extracts.
c. *In-vitro* antioxidant activity of plant extracts.
d. Preclinical Pharmacological evaluation of plant extracts for Memory enhancing activity.

4. Materials and Methods :

4.1 Plant profile -

Moringa oleifera L. native to India, grows in the tropical and subtropical regions of the world. It is commonly known as "drumstick tree" or "horseradish tree". *Moringa* belongining to family Moringaceae is an effective remedy for malnutrition. *Moringa oleifera* can withstand both severe drought and mild frost conditions and hence widely cultivated across the world. *Moringa oleifera* L. is a valued medicinal plant in traditional folk medicine. Many pharmacological studies have shown the ability of this plant to exhibit analgesic, antiinflammatory, antipyretic, anticancer, hepatoprotective, cardiovascular, anti-obesity, diuretic, antiallergic, local anaesthetic properties.

Image 1: *Moringa oleifera* L. Plant

4.2 Physical Evaluation:

The dried stem bark of plant was used for determination of physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, moisture content (LOD).

4.3 Determination of Loss on Drying (LOD):

Procedure: Weigh about 2g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100◦c or 105◦c, until two consecutive weighing do not differ by more than 0.5 mg. Cool in a desiccator and weigh. The loss in weight is usually recorded as moisture.

4.4 Extraction of plant material:

Selection of Solvent - Study of literature survey revealed that leaves are aromatic and contain proteins, carbohydrates, fiber, flavonoids, quercetin, kaempferol on the basis of literature and solubility of chemical constituents, solvents selected for extraction were ,

a. Petroleum ether
b. Chloroform
c. Ethyl acetate
d. Acetone
e. Ethanol
f. Methanol

4.5 Preparation of Extracts:

Three extracts of powder of *Moringa oleifera* L. stem bark were prepared

- **Petroleum ether**
- **Ethyl acetate extract**
- **Ethanolic extract**

4.6 *In-Vivo* Memory enhancing activity :

Radial arm maze : *In-vivo* Memory enhancing activity of *Moringa oleifera* L. stem bark was carried out by using radial arm maze. In which Piracetam was used as standard drug. Test drug Ethanolic and Ethyl acetate extract was used, the rats were subjected to central zone of radial arm maze which is of 36 cm in diameter with eight radial arms, and each arm is 56 cm long, 5 cm wide with 2 cm height, along the length of the arm. Extracts were given to rats, once daily for period of 8 days. The evaluation was carried out from 1st to 8th day 30 min after the drug treatment wherein food pallets was placed in selected arm for evaluation of working of memory.

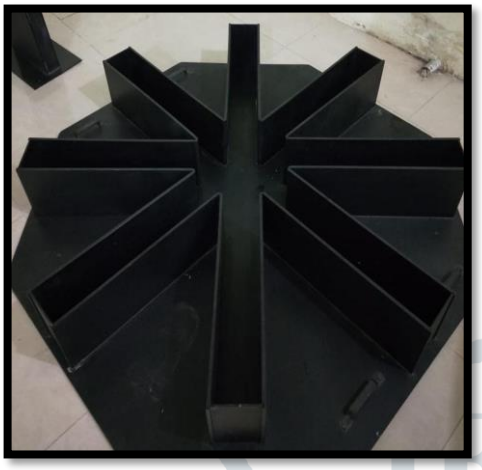


Image 2: Radial arm maze model

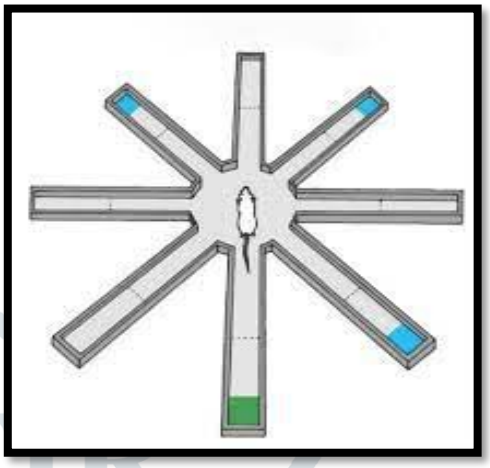


Image 3: Radial arm maze during experiment

5. Observation and results :

5.1 Determination of extractive values –

Table 1: Extractive values of different solvent

Sr.No	Solvent	Extractive value (%w/w)
1	Petroleum ether	**7.5%**
2	Chloroform	**8.5%**
3	Ethyl acetate	**14.5%**
4	Acetone	**3%**
5	Methanol	**14%**
6	Ethanol	**15.5%**

5.2 Phytochemical evaluation of *Moringa oleifera* L. stem bark extracts:

On the basis of literature survey most of the chemical constituents of the plant extract are **heat stable** and most of the researchers selected Soxhlet extraction. Therefore, same method i.e. **Soxhlet method** for extraction was selected for present research

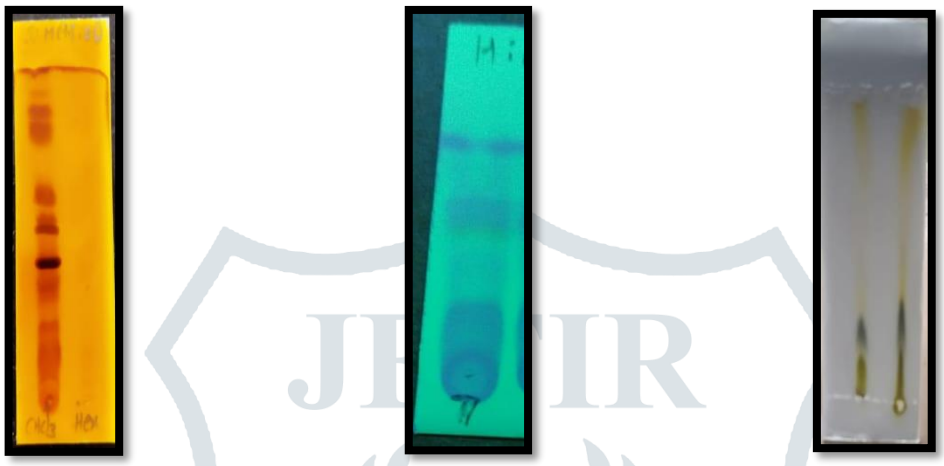
Table 2 : Physical parameters and yield of extracts

Sr. No.	Drug taken (gm)	Solvent used (ml)	Consistency	Colour of extract	Yield (gm)	% Yield (w/w)
1	260 gm	Petroleum ether	Sticky	Dark green	1.5	0.75%
2	260 gm	Ethyl acetate	sticky	Dark green	3.1	1.19%
3	260gm	Ethanol	sticky	reddish brown	4.7	1.8%

Above observation table reveals that Petroleum ether, Ethyl acetate and Ethanolic extracts have percentage yield **(0.75%), (1.19%) & (1.8%)** respectively. Highest yield was obtained for Ethanolic extract **(1.8%).**

5.3 TLC fingerprinting of extracts –

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. 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 120^{0}C for one hour.



5.4 Total Phenolic content of *Moringa oleifera* L.stem bark extracts

Table 3 : Results of Total Phenolic Content

Sr.No.	Extracts	Concentration (µg/ml)	Absorbance	% TPC
1	Ethyl acetate extract	100µg/ml	0.553 ± 0.0057	22.2
2	Ethanolic extract	100µg/ml	0.772 ± 0.0069	31.0

This observation table reveals that Ethyl acetate and Ethanol extract of MO have found phenolic content as 22.2 mg GAE/g DW, 31.0 mg GAE/g DW respectively. Ethanol extract shows more phenolic content than Ethyl acetate as per comparative evaluation of phenolic content of extracts.

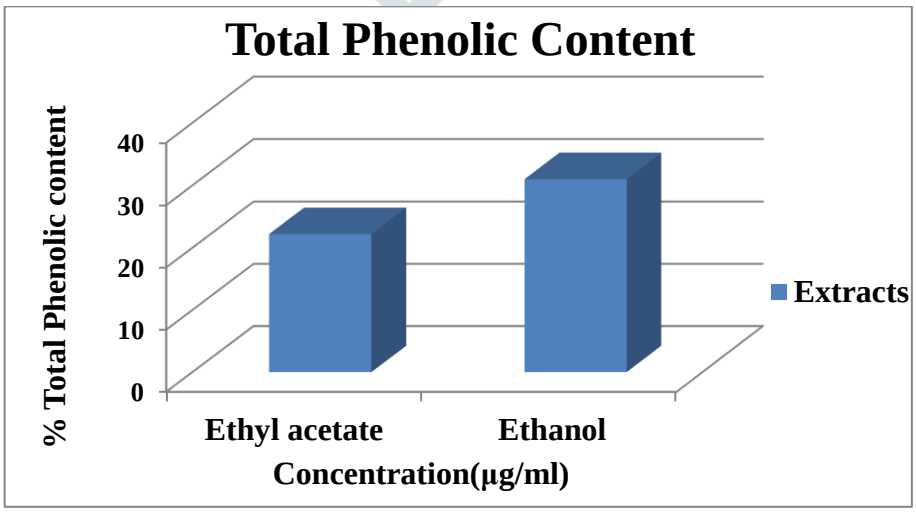


Chart 1: Total phenolic content of extracts

5.5 Total Flavonoid content of *Moringa oleifera* L. Stem bark extracts

Table 4: Total Flavonoid Content of MO stem bark extracts

Sr.No.	Extracts	Concentration (µg/ml)	Absorbance	TFC (mg Ru/g DW)
1	Ethyl acetate extract	100µg/ml	0.077 ± 0.0046	12.8
2	Ethanolic extract	100 µg/ml	0.093 ± 0.0017	15.5

Values represent mean ± SEM (n = 3)

The observation table reveals that Ethyl acetate and Ethanol extract of MO have flavonoid content as 12.8 mg Ru/g DW, 15.5 mg Ru/g DW respectively, Ethanol extract shows more flavonoid content than Ethyl acetate as per comparative evaluation of flavonoid content of extracts.

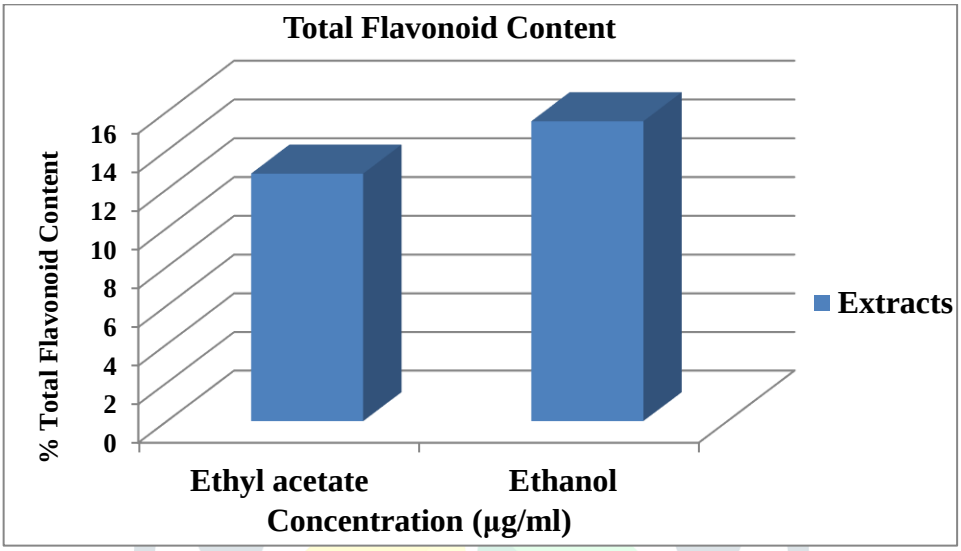


Chart 2 : Total Flavonoid Content of *Moringa oleifera* L. stem bark extract

5.6 Pharmacological evaluation of *Moringa oleifera* L. stem bark extracts

In-Vitro antioxidant activity

The antioxidant activity of *Moringa oleifera* L. was determined by *In -Vitro* methods such as DPPH Free radical scavenging assay method. The assay was carried out in triplicate and average value was considered. The results were compared with ascorbic acid as a reference standard.

DPPH(2,2- Diphenyl1'1 Picrylhydrazyl) radical scavenging activity:

Table 5: DPPH scavenging assay method of *Moringa oleifera* L. (Ethyl acetate and Ethanolic) stem bark extracts.

Sr.no.	Concentration (µg/ml)	% Inhibition		
		Ascorbic acid	Ethyl acetate	Ethanol
1	25	89.84%	54.1%	73.5%
2	50	92.76%	60.3%	75.8%
3	75	94.46%	66.4%	81.3%
4	100	96.30%	71.0%	85.2%
5	125	97.69%	75.6%	86.0%

In DPPH Scavening assay method, the % inhibition of ethyl acetate and Ethanolic stem bark extracts of *Moringa oleifera* L. at 517nm has been recorded at different concentration of 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml, 125µg/ml respectively. The results were compared with Ascorbic acid as a reference standard and both extracts showed very significant % inhibition close to reference standard. Among both extracts, Ethanol stem bark extract of *Moringa oleifera* shows highest % inhibition activity (86 % at 125µg/ml concentration).

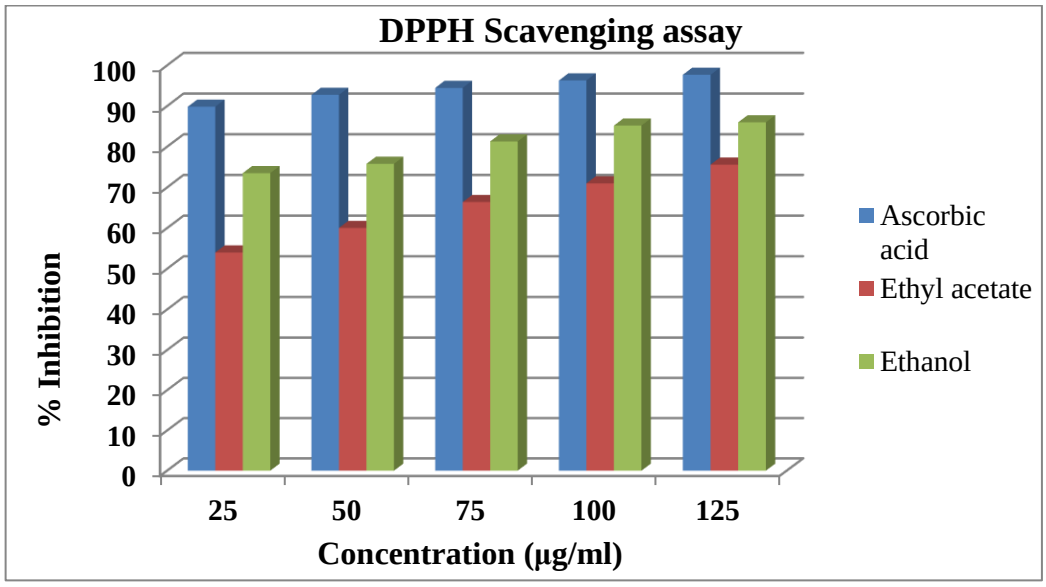


Chart 3 : DDPH scavenging assay

5.5 Safe dose calculation :

Acute toxicity studies conducted by many researchers for *Moringa oleifera* L. stem bark extracts as per standard references reveal that the administration of graded dose of extracts (up to a dose of 2000mg/kg) did nit produces any significant changes in behaviours such as alertness, motor activity, breathing, restlessness, diarrhoea, convulsions, coma and in appearance of the animals. No death recorded up to the concentration of 2000mg/kg body weight. The animals were physically active.

The result of such studies showed that in single dose; the plant extracts had no adverse effects, indicating that the medium lethal dose (LD50) could be greater than 2000mg/kg body weight in experimental small animals as in rats/mice. Accordingly, safe experimental dose considered for present investigation as ≤ 200mg/kg.

For acute oral toxicity studies literature survey has been carried out for referencing (LD50) range as per OECD (423) guidlines of selected plant extracts and accordingly $1/10^{th}$ of LD50 will be used as maximum experimental safe dose.

5.6 *In-Vivo* Memory enhancing activity

Table 6 : Number of entries in C and P zone for memory enhancing activity

Sr.No.	Group name with dose	Number of entries in C zone	
		Day 1	Day 8
1	Control	51 ± 1.958	32.75 ±1.25
2	Standard (Piracetam) 200mg	31 ± 0.91**	19.5 ± 0.64**
3	MOEA – 100mg	38 ± 1.58**	24.5 ± 0.64**
4	MOEA – 200mg	39.5 ±1.04**	25 ± 0.40**
5	MOE – 100mg	38.25 ±0.85**	23.2 ± 1.10**
6	MOE – 200mg	31.25 ± 1.60**#	21 ± 0.40**#

the values are represented as mean ± S.E.M. (n=6) for all groups and statistical singnificance between treated and control groups was analysed using One-way ANOVA, Followed by Tukey test * P<0.05-significant difference when compared to control , ** P <0.001-Highly Significant difference when compared to control, #-No Significant difference when compared to standard.

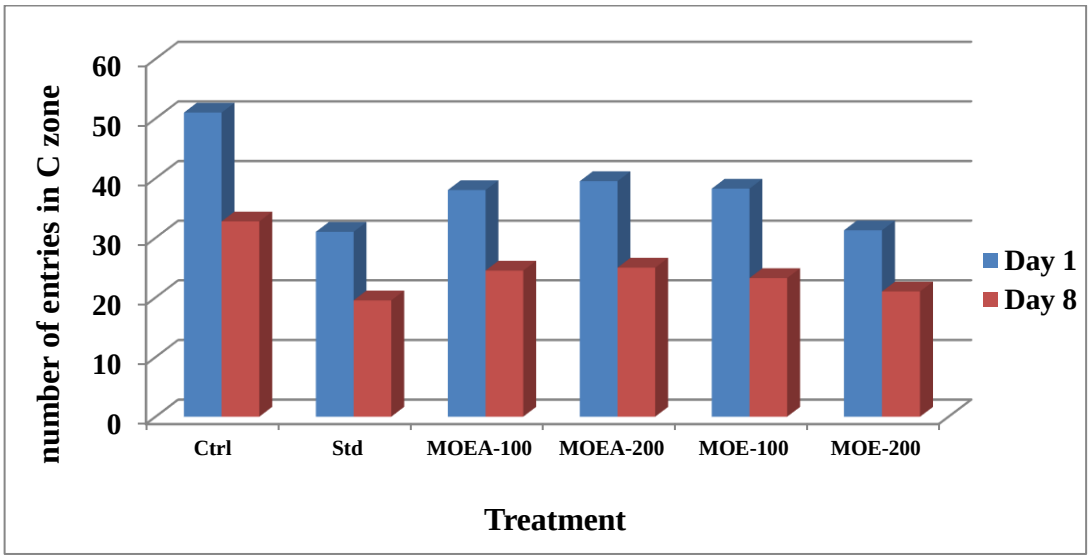


Chart 4 : Number of entries in C zone during experimental period.

The number of entries in C zone on day 8 when compared with day 1 it was found that MOEA-100, MOEA-200, &MOE-100, MOE-200 shows significant difference when compared with control .MOE-200 does show no significant difference when compared with standard.

Table 7 : Number of entries in P zone for Memory enhancing activity

Sr.No.	Group name with dose	Number of entries in P Zone	
		Day 1	Day 8
1	Control	11.25 ±0.75	27.75 ±0.62
2	Standard (Piracetam) 200mg	23.25 ±0.62**	39.75 ±0.47**
3	MOEA-100mg	18 ±0.40**	35.25 ±0.47**
4	MOEA-200mg	18.25 ± 1.03**	35.25 ± 1.03**
5	MOE-100mg	18.5 ± 0.64**	36.5 ±0.64**
6	MOE-200mg	23 ± 0.91**#	38.75 ±0.25**#

The values are represented as mean S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using one-way ANOVA, followed by turkey test. *P<0.05-significant difference when composed to control, **P<0.001 – significant difference when composed to control, #-No significant difference when compared to standard.

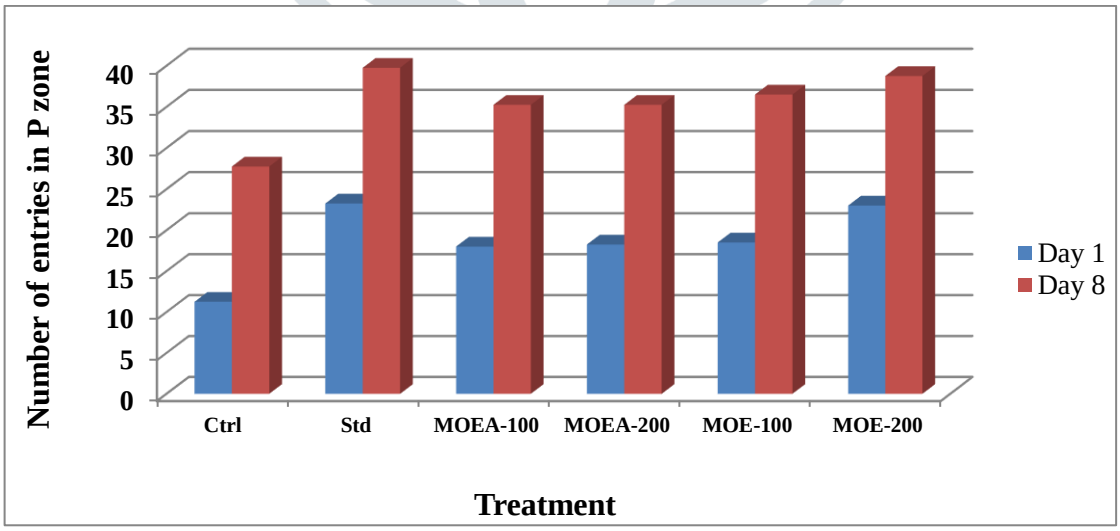


Chart 5 : Number of entries in P zone during experiment period

The number of entries in P zone on day 8 when compared with day 1 it was found that MOEA-100 &MOEA-200, MOE-100, MOE-200 shows highly significant difference as compared to control (P>0.001). All test does shows no significant difference when compared with standard.

Table 8: Time spent in C zone for memory enhancing activity

Sr.No.	Group name with dose	Time spent in C zone	
		Day 1	Day 8
1	Control	76.5 ± 1.44	59.25 ±1.652
2	Standard (Piracetam)200mg	60.5 ± 1.70**	24.5 ±1.55**
3	MOEA-100 mg	48 ± 1.82**	37.75 ±1.25**
4	MOEA-200 mg	48.5 ±2.95**	36.25 ±2.62**
5	MOE-100 mg	50.25± 0.75**	35.25 ±2.25**
6	MOE-200 mg	58.5 ± 1.84**#	25.25 ±1.43**#

The values are represented as mean ± S.E.M (n=6) for all group and significant between treated and control groups was analyzed using ANOVA, followed by turkey test. *P<0.05-significant difference when compared to control, ** P<0.001- Highly significant difference when compared to control, #-No significant difference when compared to standard.

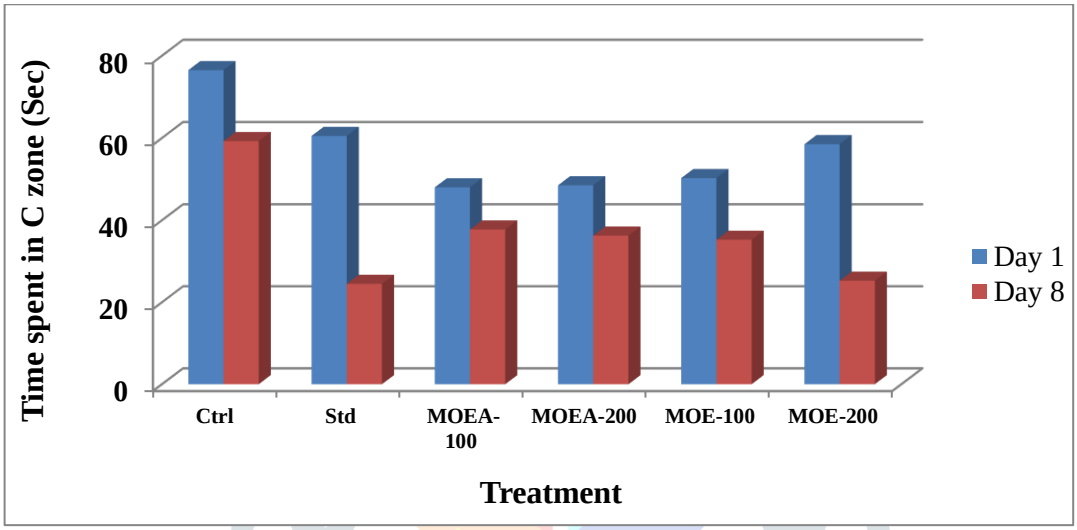


Chart 6 : Time spent in C zone during experimental period

The time spent in C zone on day 8 when compared with day 1 it was found that MOEA-100, MOEA-200 & MOE-100, MOE-200 shows highly significant difference as compared to control(P<0.001). The extract MOE-200 show no significant difference when compared with standard.

Table 9 : Time spent in P zone for Memory enhancing activity

Sr.No	Group name with dose	Time spent in P zone	
		Day 1	Day 8
1	Control	57.75 ± 0.85	63.75 ±. 1.49
2	Standard (Piracetam) 200mg	75 ± 0.81**	89 ± 1.47**
3	MOEA-100 mg	69.25 ± 0.85**	81.25 ± 1.88**
4	MOEA-200 mg	68.5 ± 0.64**	82.25 ± 1.25**
5	MOE-100 mg	70.5 ± 0.64**	82.5 ± 0.288**
6	MOE-200 mg	74 ± 1.58**#	88.25 ± 0.85**#

The values are represented as mean ± S.E.M (n=6) for all groups and statistical significance between treated and control groups was analyzed using One-way ANOVA, followed by turkey test. *P<0.05-significant difference when compared to control, **p<0.001 – highly significant difference when compared to control, #-No significant difference when compared to standard.

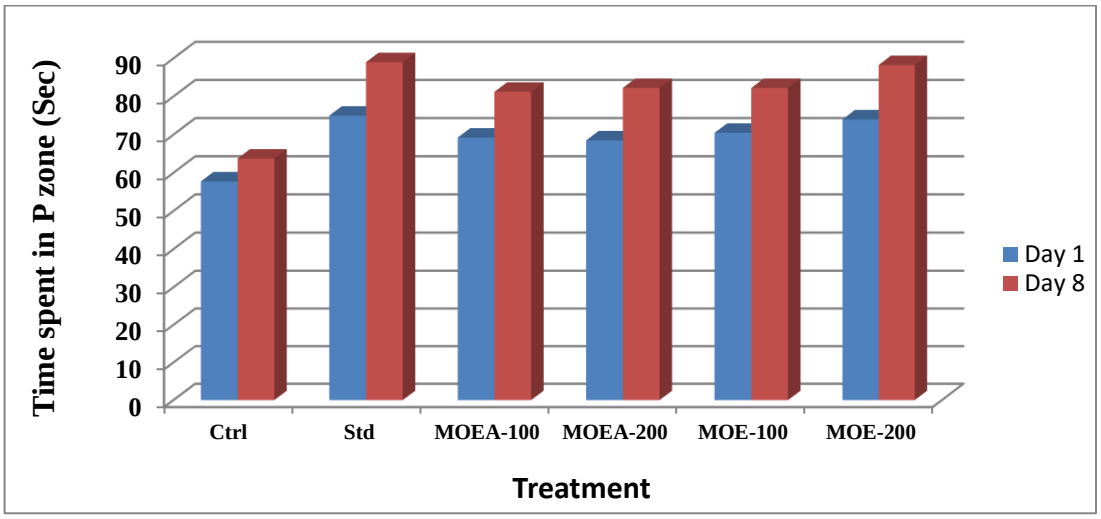


Chart 7 : Time spent in P zone during experiment period

The time spent in P zone on day 8 when compared with day 1 it was found that the MOEA-100, MOEA-200, MOE-100 and MOE-200 shows significant difference when compared to control ($P<0.05$) but, MOE-200 doses show significant difference when compared with standard.

6. Discussion –

Medicinal plants have been used for centuries as remedy for human diseases because they contain components of therapeutic values. In the last two decades of the century, the scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicine. The spots at Rf values of (Pet ether extract) **0.4, 0.9, 0.13, 0.15, 0.21, 0.23** and (ethyl acetate extract**) 0.2, 0.5, 0.12, 0.15**, **0.19** and (Ethanolic extract) **0.3, 0.5, 0.19** represents the presence of alkaloid, glycosides, tannins, flavanone, 6-hydroxy flavones in the extracts. Antioxidant property of *Moringa oleifera* L. stem bark extracts was carried out by using DPPH radical scavenging assay technique. By this method the percentage inhibition shown by Ethyl acetate and Ethanolic extracts were **75.6%** and **86%** respectively. Whereas standard Ascorbic acid showed **93.84%** percentage inhibition at 125 µg/ml. This provides evidence that ethanol extract of *Moringa oleifera* L. stem bark has potent antioxidant activity and it can be used as an antioxidant agent.

The memory enhancing activity of Ethyl acetate and Ethanolic extracts of *Moringa oleifera* L. stem bark was evaluated in rats by daily exposing them to the radial arm maze with the food pellet in a fix arm of maze. Food pellets were placed in a variable arm for evaluation of working memory. At the end of study, It was observed that group no. 6 i.e. ethanol extract treated group at dose of 200mg/kg showed maximum number of entries at P zone (38.75±0.25**#). The same group showed maximum time spent at P zone (88.25±0.85**) as well. All these values were compared with standard drug i.e. Piracetam at dose of 200mg/kg. From the results it was revealed that both extract i.e. Ethyl acetate and ethanolic showed effective memory enhancing activity. Although ethanol extract at 200mg/kg showed more superior and significant to highly significant (from $P < 0.05$ to $P< 0.001$) memory enhancing activity by using radial arm maze in rats.

7. Summary and conclusion :

Moringa oleifera L. stem bark contains several chemical constituents which are pharmacologically important as they have been proved to be beneficial in many specific diseases like, cancer, inflammation, infectious, diabetes, hepatotoxicity and many microbial attacks where its memory enhancing potential is claimed to be useful. The extracts of *Moringa oleifera* L. stem bark tested for memory enhancing activity by researchers. No methodical report on memory enhancing activity *of Moringa oleifera* L. stem bark was available.

In-Vivo study has showed that Ethyl acetate and Ethanolic extract *Moringa oleifera* L. does possess significant memory enhancing activity with 100mg/kg and 200mg/kg. High doses of the Ethanol extract 200mg/kg being more superior and showed significant to highly significant to highly significant percentage inhibition (from $P<0.05$ to $P<0.001$) when compared with standard Piracetam.

The finding of the present study reveals that *Moringa oleifera* L. stem bark has potent memory enhancing activity. Further study is required to evaluate the exact mechanism of memory enhancing effect of *Moringa oleifera* L. stem bark extracts.

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