Frontiers in Health InformaticsISSN-Online: 2676-7104 www.healthinformaticsjournal.com
2024; Vol 13: Issue 3 Open Access

PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITY OF *ADHATODA VASICA* (LEAVES) AND *VIGNA MUNGO* (SEEDS)

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Cite this paper as: Arabind Kumar, Dr. Jaya Sharma, Dr. Shamim Ahmad, Dr. Pankaj Sharma (2024) PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITY OF ADHATODA VASICA (LEAVES) AND VIGNA MUNGO (SEEDS). *Frontiers in Health Informatics, 13* (3),7204-7248

Abstract:
Biologically active compounds from natural sources are of interest as possible new drugs for different diseases. Over many centuries humans have been mining the bounties of nature for discovering natural products that have been used both for the treatment of all human diseases. *Adhatoda Vasica* (leaves) (Acanthaceae) and *Vigna mungo* (seeds) (*Leguminosae)* is widely used in the Indian system of medicine as an Asthma, bleeding, bronchitis, cough, diarrhea, dysentery, fever, flu, hysteria, neuralgia, rheumatic pain, swelling, TB, urinary disorders, vomiting etc. The plant was also reported for its diabetes, epilepsy, skin disorders and many more such activities. Here we characterized the molecules along with Phyto-pharmacological response were tested which is mentioned in the detailed.

Keywords: *Adhatoda Vasica, Vigna mungo* Clonidine, Antimicrobial activity, Qualitative analysis.

Introduction:

Memory is the human capacity to record, retain, and retrieve experiences over short or long periods. It is profoundly affected by factors such as chronic stress, negative emotions, aging, and conditions like schizophrenia and Alzheimer's disease (Kshirsagar, 2011). Its dementia and an excess of amyloid beta (Aβ) peptides, leading to the degeneration of brain cells and synapses in the cerebral cortex and certain sub-cortical regions (Youdim& Joseph, 2001).

Learning and memory are crucial cognitive functions that enable individuals to store and recall information from personal experiences. Learning involves acquiring new information or skills through experiences, while memory is the ability to retrieve this information when needed (Liu et al., 2009; Okano et al., 2000). Memory is integral to various neuronal pathways and neurotransmitter systems. Memory loss, often described as an organic brain disorder, is defined by a significant decline in intellectual ability that impairs work performance, social activities, or personal relationships, without severe function (Sharma et al., 2008).

Factors contributing to memory dysfunction include reduced cholinergic activity (Aisen, 2002). Current treatments for AD and memory disorders include nootropics like piracetam, pramiracetam, and aniracetam, as well as acetylcholinesterase inhibitors such as Donepezil, which aim to improve mood, behavior, and memory. However, these drugs often come with side effects that limit their use (Joshi & Parle, 2006).

In traditional Indian medicine, "Rasayana Chikitsa" therapy is believed to offer rejuvenation, delay aging, and enhance memory while boosting disease resistance. Rasayana herbs are thought to possess potent antioxidant properties that prevent free radical formation and lipid peroxidation in neuronal cell membranes. Notable herbs in this category include *Celastruspaniculatus, Clitoriaternatea, Centellaasiatica, Curcuma longa*, and *Ginkgo biloba*, which have been reported for their neuroprotective effects (Joshi & Parle, 2006). Additionally, *Withaniasomnifera* has been shown to influence the dopaminergic system, which can affect conditions like Parkinsonism (Dua et al., 2009).

Despite the isolate and characterize the active compounds responsible for their therapeutic effects. This will

help enhance their efficacy, reduce side effects, and integrate them into modern treatments. In light of this, *Adhatoda vasica* (leaves) and *Vigna mungo* (seeds) have been selected for evaluation of their memory-enhancing properties, or nootropic effects, to explore their potential benefits in cognitive health.

Medicinal Values of Black Gram:

According to Ayurveda, black gramme or Masha is a sweet-tasting yet hot-potent bean that helps calm and regulate Vata imbalances. Ayurvedic practitioners recommend that women of reproductive age incorporate urad dal into their daily diets to regulate hormones and improve reproductive systems. Our ancient medicine describes numerous ways to consume black gramme for total wellness. Try these simple home treatments with urad dal.

Material & Methods

1. Collection of Plant Material & Authentification

The plant parts were purchased from local market of Bundelkhand region. They were taxonomically identified by Head of the Department of Botany, Apex University, Jaipur and voucher specimens were deposited in laboratory.

2. Preparation of Extract by Successive Solvent Method

All the plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of *Adhatoda Vasica* (Leaves) and *Vigna Mungo (Seeds)*were extracted using petroleum ether, chloroform, methanol, and butanol and finally by water using Soxhlet's device. The whole extracts were completely dried by applying pressure, the respective extracts were subjected to weighing and % yield was determined (Mukherjee, 2002).

3. Estimation of different constituents

- **Determination of total Phenolic Content (TPC):**

The TPC of test extracts of plant parts measured using Folin-Ciocalteu reagent. The extracts of both plants were solubilized in distilled water. After that 100 µl of sample was mixed with Folin-Ciocalteu reagent (500 µl) with sodium carbonates (400 µl) and distilled water (5 ml). This solution was set aside at room temperature for 30 min, and the absorbance of solution was measured at 760 nm. Total phenolic content was completed using gallic acid as standard (Soniet al., 2018; Madaan*et al*., 2011).

- **Determination of total flavonoid content:**

The extracts of both plant parts in a quantity of 500 µl were mixed with ethanol (1.5 ml), aluminum nitrate (100 ml, 10 %), potassium acetate (100 ml, 1 M) and water (2.8 ml). The solution was reserved at ambient temperature for 40 min, and calculated the absorbance of solution using spectrophotometer. Total flavonoid content was recorded according to a standard established curve with Quercetin(Soniet al., 2018; Mohsen &Ammar 2008).

- **Determination of crude saponin content:**

In flask, about 20 gm of powder extract taken along with 10 ml of 20 % aqueous ethanol. This solution was frenzied for 4 hour with continuous stirring at 55^0 C. Then it was filtered and marc was extracted with 200ml of 20% ethanol. Afterwards, both extracts were mixed and let the solvent to evaporate till 40 ml remains. The remained concentrate was extracted with 20 ml of diethyl ether using separating funnel. Then aqueous layer was recovered while the ether layer was superfluous. Finally aqueous extract was purified by adding 60 ml n-butanol. Then it was washed twice with 10 ml of 5 % aq. NaCl. It was dried and percentage of saponin content was noted (Soniet al., 2018; Eleazu*et al*., 2012).

- **Determination of tannin content:**

Tannic acid stock solution (1mg/ml) was prepared by dissolving 100 mg of precisely weighed tannic acid in water. In this, 1-10ml of aliquots were prepared in which 0.5 ml of Folin-Denis reagent, 1 ml of Na2CO3 solution were added to each tube. All tubes adjusted to 10 ml with distilled water and mixed well then kept for 30 min. The absorbance was made at 760 nm against blank reagent (Soniet al., 2018; Polshettiwar*et al*., 2007).

- **Determination of alkaloid:**

Plant extract approx 1mg was liquefied in DMSO, in which 1 ml of 2N HCl was added & then filtered. Solution was taken to separating funnel and 5 ml of bromocresol green solution and 5 ml of phosphate buffer were mixed in it. This total content was shaken by adding 1, 2, 3 and 4 ml chloroform with continuous trembling with chloroform. Reference standard solution of atropine was prepared in same manner. The absorbance's for test & standard solutions were resolute beside the reagent blank at 470 nm with an UV- spectrophotometer Soniet al., 2018; Tambe&Bhambar 2014)

4. Assessment for antioxidant activity

- **Lipid Peroxidation Inhibitory Test**

➢ **β-carotene bleaching inhibition assay**

It is calculated as capability of β-carotene oxidative bleaching in carotene/linoleic acid combination with or without adjoining of a variety of extractives of both plants. In this, 6.0 mg ß-carotene was liquefied in 10 ml of $ChCl_3$ then 1ml was pipetted in glass filled of 20 mg of linoleic acid. From this, approx. 5 ml pipetted to reaction tube filled of extract in a diversity of concentration then blended homogenously. Sample absorptions were carried prior to and following incubation at 50ºC for 30, 60 & 120 minutes (Murdifin*et al*., 2017).

The β-carotene bleaching inhibition is estimated as-

$$\% \text{ Inhibition} = [1 - (AA(120) - AC(120)) / (AC(0) - AC(120))] \times 100$$

AA(120): sample absorbance at t = 30, 60 or 120 minute

AC(120): control absorbance at t = 30, 60 or 120 minute

AC(0): control absorbance at t = 0 min

➢ **Hydroxyl-radical ($OH^{\cdot}$) scavenging method**

The 1 ml of the reaction blend contained 100 µL of 2.8 mM 2-deoxyribose dissolved in phosphate buffer (10 mM) having pH 7.4, 500 µL solution of various concentrations of the extract (500n1000 µg/mL), 200 µL of 200 µM $FeCl_3$ and 1.04 µM EDTA (1:1 v/v), 100 µL of H_2O_2 (1.0 mM) and 100 µL of ascorbic acid (1.0 mM). After incubation time of one hour at $37^{O}C$, the amount of deoxyribose deprivation was measured by TBA reaction (Badmus*et al*., 2011; Halliwell*et al*., 1987).

The % inhibition was checked by using formula given below-

$$\text{Percentage Inhibition} = \frac{(100 - A_{Sample}) \times 100}{A_{Control}}$$

➢ **DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical scavenging activity**

In 2001, Mensor and collogues explained the free radical quenching potential of drugs by use of 1, 1-Diphenyl-2-picrylhydrazyl i.e. DPPH. Here, DPPH get adhered to antioxidant molecules and get reduced. The Color changes from deep violet to golden/light yellow which can be easily measured at 518 nm.

Here, 1 mL of 0.3 mM of DPPH solution was incorporated to 1mL of each and every test solutions and

then incubated for 30 min at room temperature and absorbance was taken at 518 nm. (Badmus*et al*., 2011). The % anti-oxidant potential was computed using formula-

$$\text{DPPH scavenging effect (\%)} = A_{Control} - A_{Sample} \times 100$$

$$A_{Control}$$

5. Preliminary *In-Vivo* Evaluation of different extracts

Animal Selection

The male/female wistar mice of amid 1 to 2 months of age weighing between 25-35 g were used procured from the approved animal house The rodents let liberated and fed with pallet diet (Lipton India Ltd, Mumbai, Ind.) with water *ad libitum.* All the laboratory conditions and animals were maintained as per CPCSEA guidelines throughout the experiments. The study design was as per CPCSEA guidelines.

6. Evaluation of Memory Enhancing Activity by Different Models Morris Water test

The various parameters and procedure for assessment of nootropic potential of mice were followed as reported by Domange*et al*., 2013; Morris, 1984; Parle & Singh, 2007.

The animals were divided in 10 groups with 6 in every cluster. The group 1 treated as control and group 2 as standard drug receiving physostigmine, 0.1 mg/kg *i.p.* Thenext groups 3 to 10 were treated with different extracts (chloroform, methanol, butanol and water) with a dose of 200 and 400 mg/kg, respectively for 15 successive days. On 11^{th} to 14^{th} days, EL was documented subsequent to 120 min of moieties/extracts administration. On day 15^{th}, after 120 min of drug administration,TSTQ was observed and calculated. Animals administered with physostigmine, EL and TSTQ were noted after 30 min of drug administration.

The treatment schedule was as follows-

Group-1 control vehicle

Group-2 Standard drug (physostigmine, 0.1 mg/kg *i.p.*)

Group-3 chloroform extract of *Vigna Mungo:*200 mg/kg

Group-4 chloroform extract of *Vigna Mungo*: 400 mg/kg

Group-5 methanolic extract of *Vigna Mungo*: 200 mg/kg

Group-6 methanolic extract of *Vigna Mungo*: 400 mg/kg

Group-7 butanolic extract of *Vigna Mungo* : 200 mg/kg

Group-8 butanolic extract of *Vigna Mungo*: 400 mg/kg

Group-9 water extract of *Vigna Mungo*: 200 mg/kg

Group-10 Mice were treated by water extract of *Vigna Mungo*: 400 mg/kg

In case of*Acorus calamus*, the procedure was same as followed above.

The various parameters and procedure for assessment of nootropic potential of mice were followed as reported previously (Domange*et al*., 2013; Morris, 1984; Parle & Singh, 2007).

The treatment schedule was as follows-

Group-1 control vehicle

Group-2 Standard drug (physostigmine, 0.1 mg/kg *i.p.*)

Group-3 Chloroform extract of *Adhatoda vasica*: 200 mg/kg

Group-4 Chloroform extract of *Adhatoda vasica*: 400 mg/kg

Group-5 Methanolic extract of *Adhatoda vasica*: 200 mg/kg

Group-6 Methanolic extract of *Adhatoda vasica*: 400 mg/kg

Group-7 Butanolic extract of *Adhatoda vasica*: 200 mg/kg

Group-8 Butanolic extract of *Adhatoda vasica*: 400 mg/kg

Group-9 Water extract of *Adhatoda vasica*: 200 mg/kg

Group-10 Water extract of *Adhatoda vasica*: 400 mg/kg

Elevated-Plus-Maze (EPM)

In this model, the equipment have 2 open and 2 close arm of equal dimensions (20cm X 7cm) with close arm have 30 cm elevations. The all arms are joined centrally in a square. All the walls and base are colored with black paint and placed in sound proof room. During assessment, rodents were placed with their head facing open arm. During whole experimentation, trouble from external stimuli are avoided which may cause anxiety.

The calculations were made for percentage of time given in the open arms and number of open arm entries using formula-[100 × open/ (open + enclosed)] and (100 × open / total entries), respectively (Nishino *et al*., .2008).

One hour after administration of vehicle, diazepam & different extracts of *Adhatoda vasica (Leaves) and Vigna Mungo (Seeds)*(chloroform, methanol, butanol and water) were assessed for memory enhances or behavior studies using elevated plus-maze test. Test extracts of both plants were used in a dose of 200 & 400 mg/kg.

In this study, vehicle and diazepam treated mice groups were same in case of *Adhatoda vasica* (Leaves) and *Vigna Mungo (Seeds)*. No separate mice or groups were used in case of both plants.

Biochemical Estimation

Collection of Brain Sample

The animals were sacrificed after 15th day of Morris water maze by cervical dislocation method. The brains were carefully removed and were weighed immediately. The brain was homogenized at 3000 rpm for about 10 min using temperature 4^0 C by addition of 10 vol. of 0.1M phosphate buffer (pH-8) using refrigerated centrifuge (Remi, Mumbai). The obtained unclear liquid was used in the estimation of brain acetyl cholinesterase activity (Ellman *et al*., 1961.).

Assessment of Acetyl cholinesterase effect

In glass test tube, having 2.6ml of phosphate-buffer and 5,5-dithiobis-2-nitrobenzoic acid reagent (0.1 ml), 0.4 ml of brain homogenate was added and the absorbance was noted at 412 nm. To this, 0.02 ml of Ach-iodide solution added and one more absorbance was taken 15 min afterwards. The alterations in absorbance/ minute were computed. (Ellman*et al*., 1961).

7. Bioactivity directed separation of Phytoconstituents by Column Chromatography

The column is simply a glass tube with a stopcock at the bottom. The column is packed with the stationary phase and sample or impure product or plant extract is placed over the top of the stationary phase.

The eluting solvent (mobile phase) is run through the column; the compounds from the plant extract slowly pass down through the stationary phase. The time required to travel each compound depends upon number of factors such as particle size of the stationary phase material, the polarity of mobile phase and elution flow rate. Each compound will partition between the mobile and stationary phases differently depending upon the affinity towards the two phases. As eluting solvent drips out from the bottom of the column, it is collected in flask, called as fraction and subjected to further analysis and if required, further purification (Still and Kahn 1978).

8. Isolation of Phytoconstituents from Solvent plant extracts

The solvent extract of were subjected to column chromatography on silica gel with the gradient elution using varying proportions of solvents. Chromatographic separation was performed under following conditions.

Column	:	Glass
Dimensions	:	3.5 cm diameter, 90 cm length
Stationary phase	:	silica gel 60-120#
Mobile phase	:	n-hexane and Chloroform *Adhatoda Vasica* (Leaves)&
		Petroleum ether: dichloromethane (DCM)
		Vigna Mungo (Seeds)
Flow rate	:	5-7 ml per minute
Fraction size	:	50 ml
Elution mode	:	gradient
No. of fraction collected:		250

The chloroform extract of *Adhatoda Vasica* (Leaves) was loaded on to the glass column packed with silica gel (60-120 mesh) dissolved in n-hexane. Stepwise gradient elution was carried out by using n-hexane & chloroform. Total of 250 fractions were collected of 50 ml each, and pooled similar fractions as per their thin layer chromatographic pattern. The compounds being isolated were purified by re-crystallization from methanol. The isolated compound was characterized using different spectral techniques (^{1}H-NMR, ^{13}C-NMR, IR and MS analyses) to reveal their identity. The same procedure was followed for *Vigna Mungo (*Seeds).

9. Characterization of isolated compounds

Solubility was determined according to polarity of solvent and Thiele tube apparatus was used for melting point apparatus (Furniss*et al*., 2007).

The structural identification was carried out by using various spectroscopic methods i.e. IR, NMR, Mass and compared with previously reported structures.

11.Acute toxicity studies for dose determination

Graphical method (Miller &Tainter) was employed to compute LD_{50} of isolated compound. Overnight fasted mice were alienated into 5 groups; 10 mice (5 male & 5 female) in every group. The 5 dose levels were selected such that one dose with no mortality and a higher dose with 100% mortality; other 3 doses were in between. Animals were observed for first 2 hrs and then subsequently for 6th hrs for any toxic symptoms. After 24 hrs, digit of deceased mice was calculated and % mortality was noted. The probit values were plotted contiguous to log dose and then dose equivalent to probit 5 was calculated. The activity was assessed using the 1/10th of LD_{50}. (Randhawa, 2009).

8. Nootropic activity of compound -1 (*Adhatoda Vasica*- Leaves)

Morris –Water-Maze (MWM) (Domange *et al*., 2013; Morris, 1984; Parle & Singh, 2007).

Mice of either sex divided into 8 groups having 6 in each. Groups are divided as given below with total schedule of 15 days. The EL was recorded after 120 min of drug administration from 11th day to 14th day.

On 15thday, TSTQ was calculated after 120 min of drug administration. In case of animals treated with physostigmine, EL and TSTQ was noted after 30 min after commencement of experiment. Here animals got drug treatment as follows-

Group-1: control Group

Group-2 Standard drug (physostigmine, 0.1 mg/kg *i.p.*)

Group-3: Compound -1: 1.5 mg/kg

Group-4: Compound -1: 03 mg/kg

Group-5: Scopolamine: 0.4 mg/kg

Group-6: Scopolamine: 01 mg/kg

Group-7: Combination of Scopolamine + Compound -1 (03+0.4 mg/kg)

Group-8: Combination of Diazepam + Compound -1 (03+01 mg/kg)

9. Nootropic activity of moiety (*Vigna Mungo - Seeds)*

Morris-Water-Maze (MWM) (Domange *et al*., 2013; Morris, 1984; Parle & Singh, 2007).

Mice of either sex divided into 8 groups having 6 in each. Groups are divided as given below with total schedule of 15 days. The EL was recorded after 120 min of drug administration from 11th day to 14th day.

On 15thday, TSTQ was calculated after 120 min of drug administration. In case of animals treated with physostigmine, EL and TSTQ was noted after 30 min after commencement of experiment. Here animals got drug treatment as follows-

Group-1 Control group

Group-2 Standard drug (physostigmine, 0.1 mg/kg i.p.)

Group-3 Compound-2: 10 mg/kg

Group-4 Compound-2: 20 mg/kg

Group-5 Scopolamine: 0.4 mg/kg

Group-6 Scopolamine: 01 mg/kg

Group-7 Combination of Scopolamine + Compound-2 (20+0.4 mg/kg)

Group-8 Diazepam + Compound-2 (20+01 mg/kg)

Measurement of Locomotor activity

On 15th day, all treated eight groups were tasked in performing locomotor activity using Actophotometer (INCO, Ambala) (Domange*et al*., 2013; Morris, 1984; Parle & Singh, 2007).

Biochemical Estimation

Collection of Brain Sample

The rodents were killed after 15th day of MWM by cervical dislodgment technique. The brains carefully removed, weighed then homogenized in 10 volumes of 0.1 M phosphate buffer (pH 8) using a glass homogenizer at 3000 rpm for 10 min at 4^{0} C using refrigerated centrifuge (Remi, Mumbai). The obtained cloudy liquid was utilized to evaluate brain AChE- activity (Ellmann*et-al*., 1961.).

Evaluation of Acetyl cholinesterase potentials-

Here, in 2.6 ml of phosphate buffer & 5, 5-dithiobis-2-nitrobenzoic acid reagent (0.1 ml), 0.4 ml of brain homogenate was mixed & absorbance noted at 412 nm. To this, 0.02 ml of acetylcholine-iodide was added & once more absorbance was noted 15 min afterwards. Changes in absorbance for each minute were calculated (Ellman*et al*., 1961).

10. Statistical Analysis

The estimation of consequences was articulated in mean ± SEM. The outcomes were explored using ANOVA followed by Dunnet's "t" test to conclude the statistical worth. $p< 0.05$ was selected as the pinnacle of implication.

Results and discussion

Phytoconstituents Estimation

UV spectroscopic analysis was conducted to determine tannin, and alkaloid contents in extracts from *Adhatoda Vasica* and *Vigna mungo*. The flavonoid content was measured at approximately 88% in *Adhatoda Vasica* and 50% in *Vigna mungo*, with values corresponding to 88 mg and 50 mg of Quercetin equivalent per gram of dry sample, respectively, based on a Quercetin standard curve ($y = 0.005x - 0.05$, $R^2 = 0.997$). Phenolic content, which influences enzyme activity through interactions with various biomolecules, was estimated using a gallic acid standard curve ($y = 0.004x - 0.11$, $R^2 = 0.986$), revealing 82% and 75% phenolic content in *Adhatoda Vasica* and *Vigna mungo*, respectively. The total saponin content was determined to be 2.48% in *Adhatoda vasica* and 2.32% in *Vigna mungo*. Additionally, the tannin content in *Adhatoda vasica* was found to be higher at 75.74% w/w, compared to that in *Vigna mungo*. Alkaloid content, expressed as atropine equivalents in mg of AE/g of extract ($y = 0.011x + 0.013$, $R^2 = 0.990$), was 93% in *Vigna mungo* and 65.82% in *Adhatoda Vasica*.

Table No.01: Standard curve of Gallic acid

S. No	Conc. (µg/ml.)	Abs.
1.	100	0.4
2.	200	0.7
3.	300	1.4
4.	400	1.8
5.	500	2.2

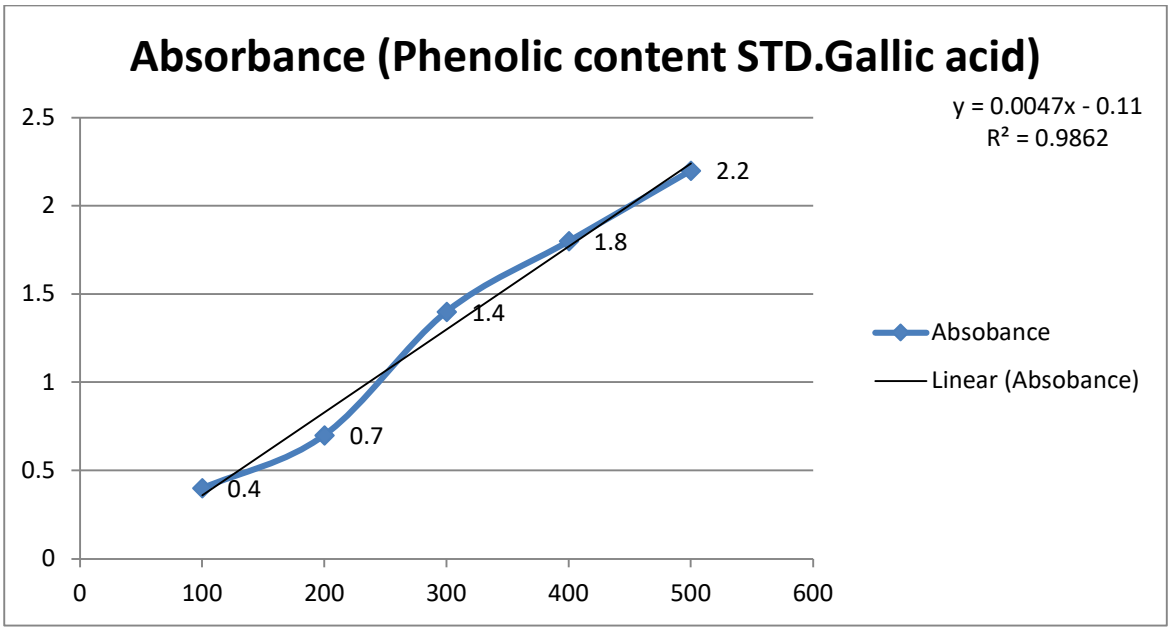

Figure No. 1: Standard curve of Gallic acid

Table No.02: Standard curve of quercetin

S. No	Conc (µg/ml.)	Abs.
1	100	0.5
2	200	1.12
3	300	1.59
4	400	2.22

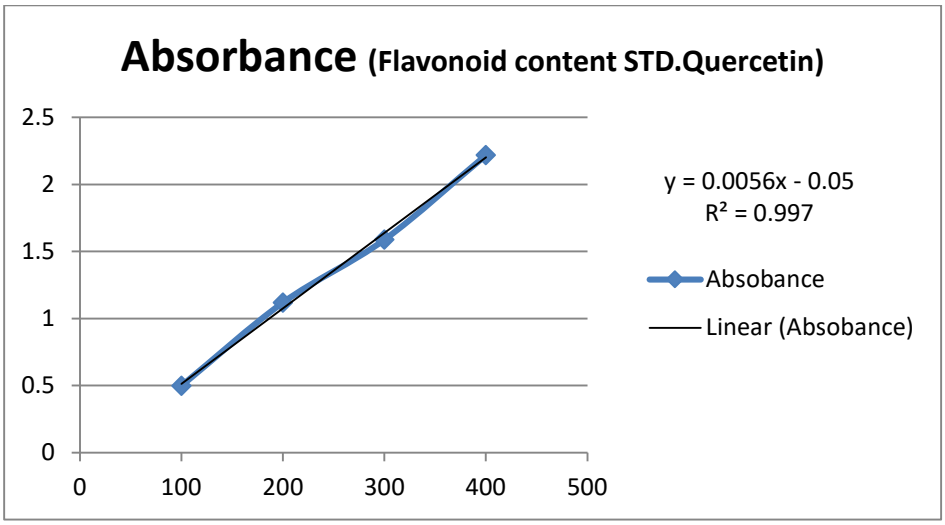

Figure No.02: Standard curve of Quercetin

Table No. 03: Standard curve of Atropine

Conc. (µg/ml.)	Abs.
0	0
20	0.24
40	0.5
60	0.65
80	0.8
100	1.11
120	1.42
140	1.65

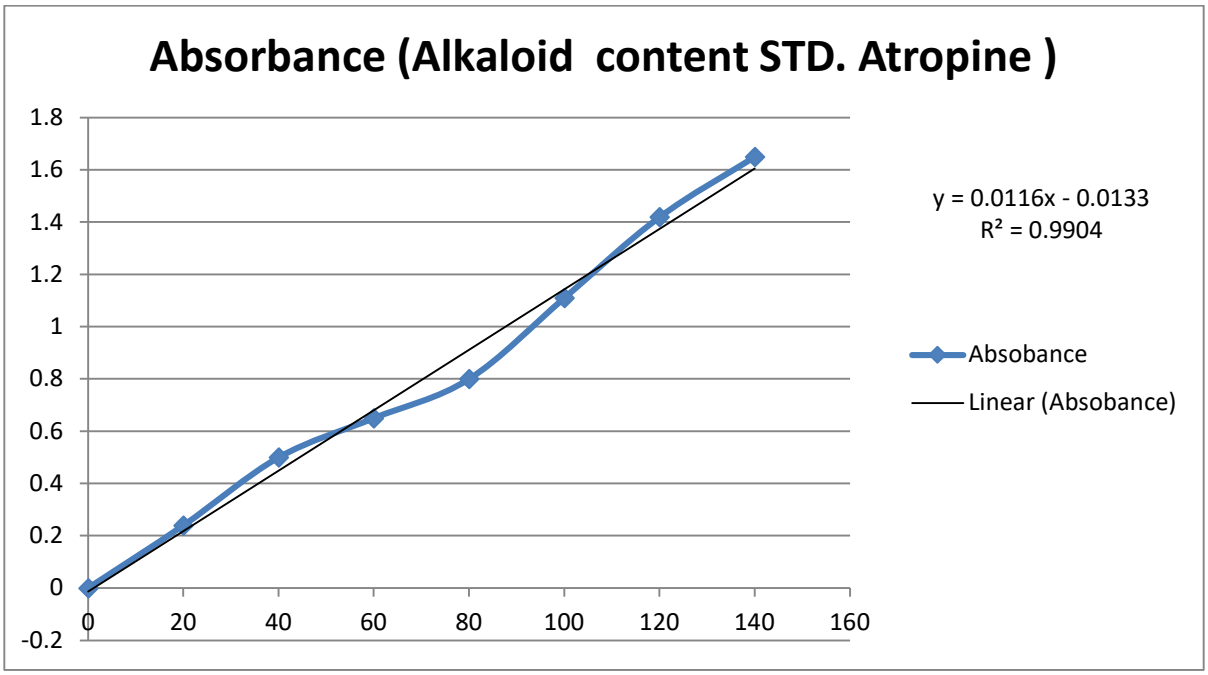

Figure No.3 : Standard curve of Atropine

Table No.04: Standard curve of Tannic acid

Conc.(µg/ml.)	Abs.
10	0.21
20	0.42
30	0.65
40	0.79
50	1.1
60	1.3
70	1.58
80	1.9

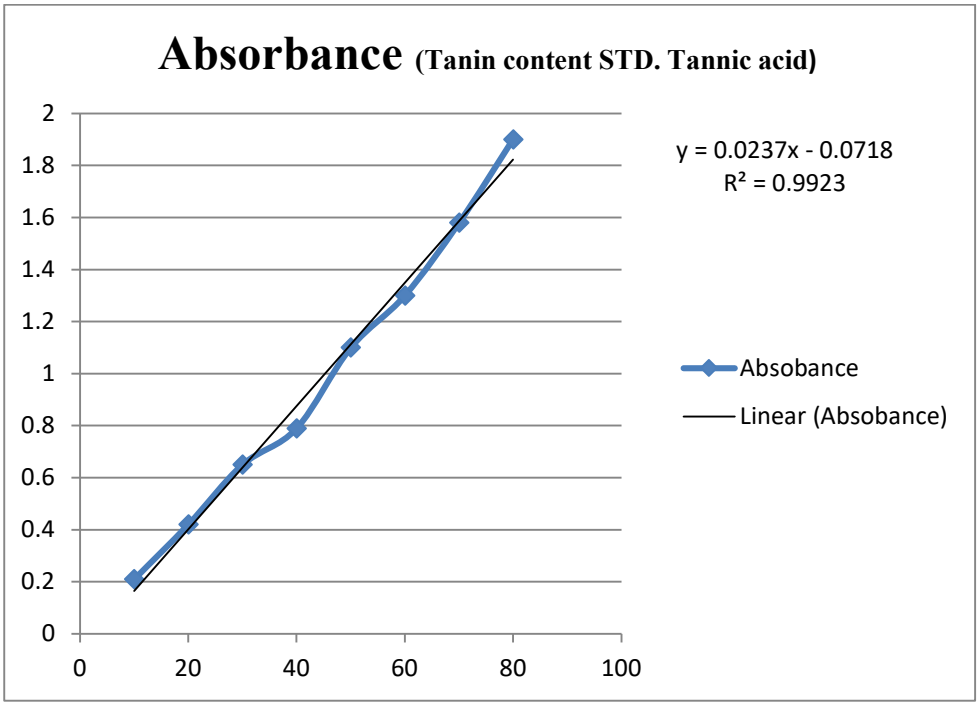

Figure No.4: Standrad curve of Tannic acid.

Table No. 05: Estimation of phytoconstituents in leaves of *Adhatoda vasica*

S. No.	Chemical constituents	Conc. of test solution (µg/ml.)	ABS.	Conc. Found (µg/ml.)	% Conc. Present in test solution
1	Alkaloid cont.	40	0.4	37.55	93
2	Flavonoids cont.	100	0.2	50	50
3	Phenolic cont.	100	0.19	75	75
4	Tannin cont.	50	0.8	37.87	75.74
5	Saponin cont.	------	------	------	2.48

Table No. 06: Estimation of phytoconstituents in *Vigna Mungo* (Seeds)

S. No.	Chemical constituents	Conc. of test solution (µg/ml.)	ABS.	Conc. Found (µg/ml.)	% Conc. Present in test solution
1	Alkaloid cont.	40	0.28	26.62	65.82
2	Flavonoids cont.	100	0.39	88	88
3	Phenolic cont.	100	0.22	82	82
4	Tannin cont.	50	0.66	31.78	63.56
5	Saponin cont.	------	------	------	2.32

Antioxidant potential estimation of various extracts of *Adhatoda Vasica*

- **β-carotene bleaching inhibition technique**

The findings indicate that *Adhatoda Vasica* extracts inhibited the oxidation of β-carotene/linoleic acid, with the inhibition effectiveness increasing alongside extract concentration. This effect can be attributed to the polarity difference between the extract and β-carotene. Previous research has shown that the lower polarity of vitamin E enhances its solubility in the lipid phase, making it more effective in protecting linoleic acid.

Table No. 07: Effect of different extracts on β-carotene bleaching inhibition method

S. No.	Extracts	Concentration	Time		
			30 Min	**60 Min**	**120 Min**
1	Chloroform Extracts	20 µg/ml	52.33±4.49	38.94 ± 3.33	29.33 ± 3.33
		40 µg/ml	60.14±3.55	49.33 ± 3.55	30.91 ± 3.55
		60 µg/ml	65.22±3.49	50.39 ± 2.56	42.33 ± 3.11
		80 µg/ml	70.10±1.22	59.31 ± 1.44	45.21 ± 3.33
2	Methanolic	20 µg/ml	40.11±2.33	18.22± 3.13	16.22 ± 3.71

	Extracts	40 µg/ml	42.33±3.23	20.54 ± 3.71	18.33 ± 3.31
		60 µg/ml	45.77±3.33	22.35 ± 2.44	21.33 ± 3.48
		80 µg/ml	49.30±1.13	25.31 ± 3.36	22.25 ± 3.68
3	Butanolic Extracts	20 µg/ml	33.13±2.44	15.22± 3.12	12.23 ± 3.44
		40 µg/ml	35.22±3.35	18.33 ± 3.33	14.11 ± 3.55
		60 µg/ml	38.71±3.45	20.25 ± 2.66	18.32 ± 2.48
		80 µg/ml	40.41±2.13	22.44 ± 3.66	21.27 ± 2.77
4	Water Extracts	20 µg/ml	30.22±2.33	15.33± 3.33	13.43 ± 3.55
		40 µg/ml	32.44±3.44	16.77 ± 3.88	15.66 ± 2.98
		60 µg/ml	35.44±3.55	19.23 ± 3.77	17.32 ± 3.55
		80 µg/ml	38.43±2.44	21.58 ± 3.44	20.64 ± 3.68
5	Vitamin E	4 µg/ml	30.34±2.10	33.45 ± 2.22	15.45 ± 2.21
		6 µg/ml	37.88±2.56	32.86 ± 3.95	20.45 ± 2.33
		8 µg/ml	69.113±2.35	58.21 ± 2.22	40.26 ± 3.63
		10 µg/ml	84.21±3.11	73.21 ± 3.55	60.22 ± 3.44

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capability calculated as IC_{50} (Table No. 11) exposed that, chloroform, methanol, butanol and water extracts have IC_{50} of 47, 100, 200 and 150µg/mL correspondingly. This consequence implied that chloroform and methanol extracts have highest OH ˙radical scavenging capacity as compared to butanolic and water extracts. Since chloroform extract had very lowest IC_{50} as compared to other extracts so it was considered as best extract for the further evaluation.

Table No. 08: Effect of different extracts on Hydroxyl radical scavenging activity

S. No.	Concentration (µg/mL)	Chloroform (%)	Methanol (%)	Butanol (%)	Water (%)
1.	50	53.78±2.33	48.22±2.44	40.22±2.44	48.38±2.34
2.	100	57.21±3.21	50.24±3.34	42.21±3.56	49.11±3.11
3.	150	65.78±3.45	55.44±3.78	46.28±3.31	50.78±3.51
4.	200	74.34±3.66	58.34±3.99	50.56±3.55	53.21±3.87
5.	250	82.76±3.55	62.31±3.51	53.76±3.48	60.99±3.53

Values are uttered as the mean of triplicate

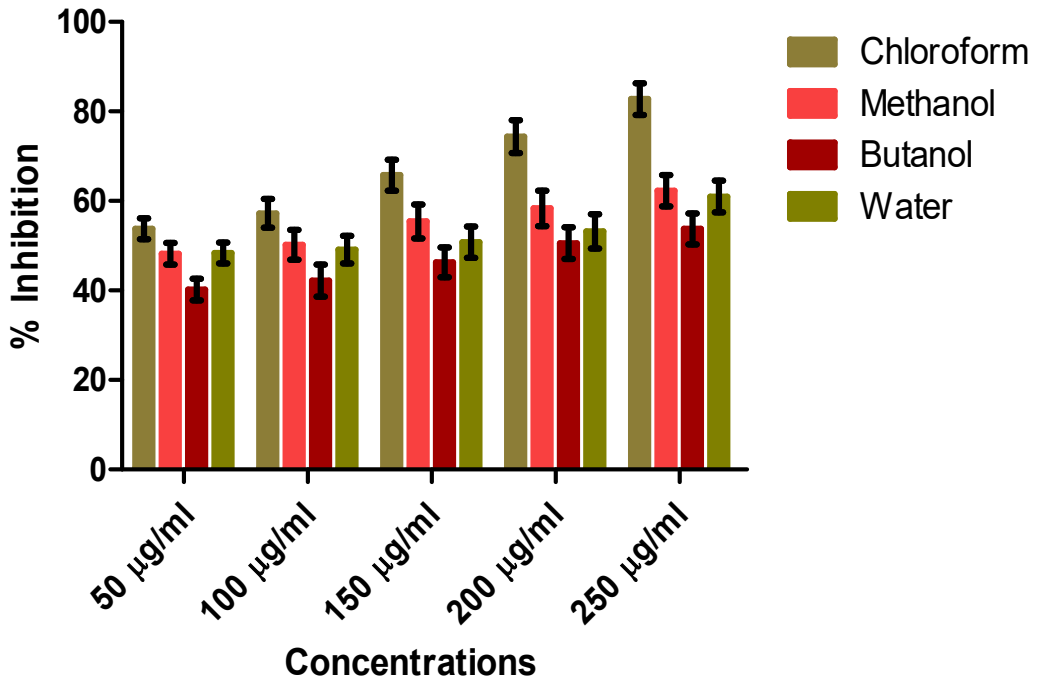

Figure No.5: Effect of different extracts on Hydroxyl radical scavenging activity

DPPH scavenging aptitude analyzed as IC_{50} from Table No 12 and 13 illustrate that chloroform extract have IC_{50} of 36 µg/mL and ascorbic acid have (3.2 µg/mL), gallic acid (3.5µg/mL), methanol (40µg/mL), butanolic (47µg/mL) and water (45µg/mL) extracts. The result revealed that chloroform extract had the highest DPPH scavenging ability.

Table No. 09 : Effect of different extracts on Percentage DPPH scavenging activities

S. No.	Concentration (µg/mL)	Chloroform (%)	Methanol (%)	Butanol (%)	Water (%)
1.	10	28.23±2.11	22.55±2.12	25.01±2.42	26.33±2.31
2.	20	33.11±3.32	26.26±3.15	28.11±3.22	29.11±2.14
3.	30	41.78±3.45	36.14±3.78	32.28±3.11	33.12±2.51
4.	40	55.37±3.55	50.44±3.33	42.56±3.24	45.31±3.44
5.	50	62.76±3.12	52.31±2.11	53.11±3.22	54.17±2.66

Values are expressed as the mean of triplicate

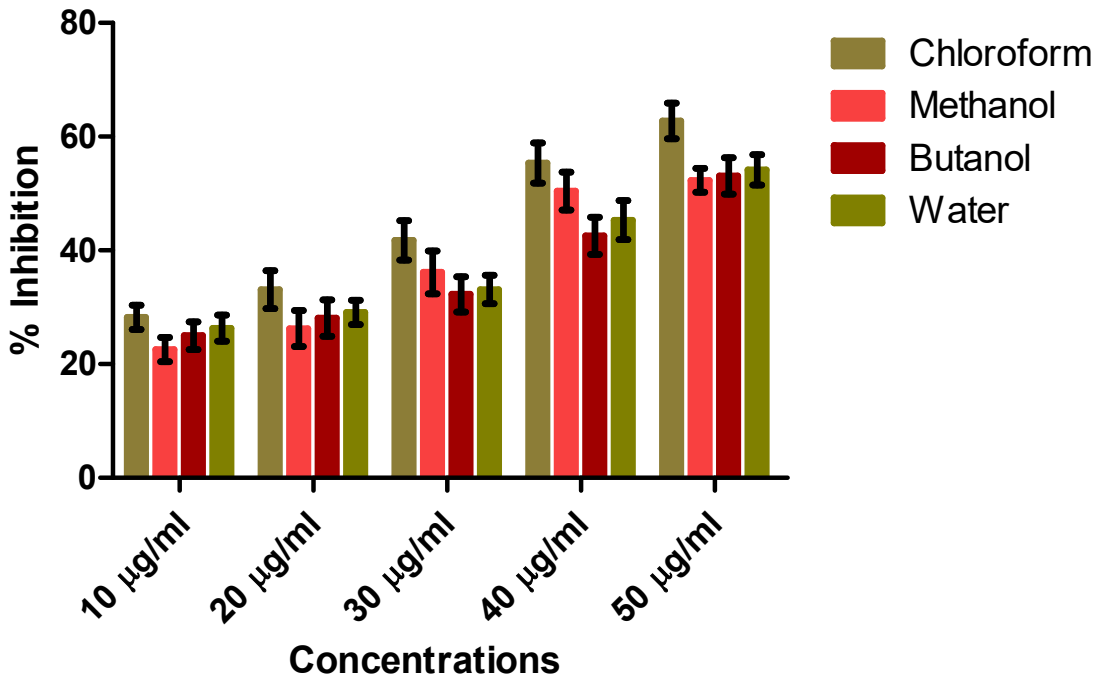

Figure No. 6: Effect of different extracts on Percentage DPPH scavenging activities

Table No. 10: DPPH radical scavenging effect in percentage (standard)

S. No.	Concentration (µg/mL)	Vitamin C (%)	Gallic Acid (%)
1.	2	47.98±2.33	43.66±3.56
2.	3	49.22±3.67	48.77±3.77
3.	4	62.33±3.28	55.22±3.11
4.	5	70.38±3.78	60.78±3.59

Antioxidant potentials of various extracts of seeds of *Vigna Mungo*

Table No. 11: Effect of different extracts on β-carotene bleaching inhibition method.

SN	Extracts	Conc.	Time		
			30 Min	60 Min	120 Min
1	Chloroform Extracts	20 µg/ml	51.32±4.9	37.14 ± 2.43	28.35 ± 3.22
		40 µg/ml	61.12±2.6	48.13 ± 3.78	31.91 ± 2.33
		60 µg/ml	65.22±3.9	51.22 ± 2.78	43.13 ± 2.33
		80 µg/ml	71.10±1.4	60.31 ± 1.24	46.31 ± 3.68
2	Methanolic	20 µg/ml	42.13±2.4	19.24± 4.22	18.22 ± 3.44

	Extracts	40 µg/ml	43.33±3.6	22.22 ± 3.71	19.32 ± 3.55
		60 µg/ml	46.71±3.4	23.35 ± 2.55	22.31 ± 3.46
		80 µg/ml	50.31±1.2	24.32 ± 3.46	24.25 ± 3.77
3	Butanolic Extracts	20 µg/ml	32.13±2.4	16.22± 3.14	13.21 ± 3.67
		40 µg/ml	36.33±3.2	19.33 ± 3.22	15.11 ± 4.51
		60 µg/ml	39.71±3.1	22.22 ± 2.64	19.32 ± 3.42
		80 µg/ml	41.52±2.4	23.44 ± 4.66	21.27 ± 2.77
4	Water Extracts	20 µg/ml	32.21±2.4	16.32± 3.46	14.47± 3.88
		40 µg/ml	32.44±3.4	16.77 ± 3.88	16.68 ± 3.56
		60 µg/ml	36.42±3.8	20.23 ± 3.68	19.32 ± 3.89
		80 µg/ml	39.43±2.5	22.58 ± 3.43	22.64 ± 3.70
5	Vitamin E	4 µg/ml	30.34±2.10	33.45 ± 2.22	15.45 ± 2.21
		6 µg/ml	37.88±2.5	32.86 ± 3.95	20.45 ± 2.33
		8 µg/ml	69.113±2.3	58.21 ± 2.22	40.26 ± 3.63
		10 µg/ml	84.21±3.1	73.21 ± 3.55	60.22 ± 3.44

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capability computed as IC_{50} from Table No. 15 divulges that, chloroform, methanol, butanol and water extracts have IC_{50} values of 50 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, correspondingly. These results oblique that chloroform along with methanol extracts have the highest OH‘radical scavenging capability when compared to butanolic and water extracts. Since chloroform extract had very lowest IC_{50} as compared to other extracts so it was considered as best extract for the further evaluation.

Table No. 12: Effect of different extracts on Hydroxyl radical scavenging activity

S. No.	Concentration (µg/mL)	Chloroform (%)	Methanol (%)	Butanol (%)	Water (%)
1.	50	50.72±2.32	49.21±2.43	42.22±2.14	49.38±2.34
2.	100	58.21±3.21	50.24±3.38	44.26±3.26	49.11±1.11
3.	150	66.28±3.78	58.41±3.55	48.28±3.78	50.78±4.51
4.	200	76.32±3.89	60.34±3.88	50.46±3.45	55.21±3.57
5.	250	82.76±3.55	64.32±3.51	56.76±3.18	62.99±3.13

Values are expressed as the mean of triplicate

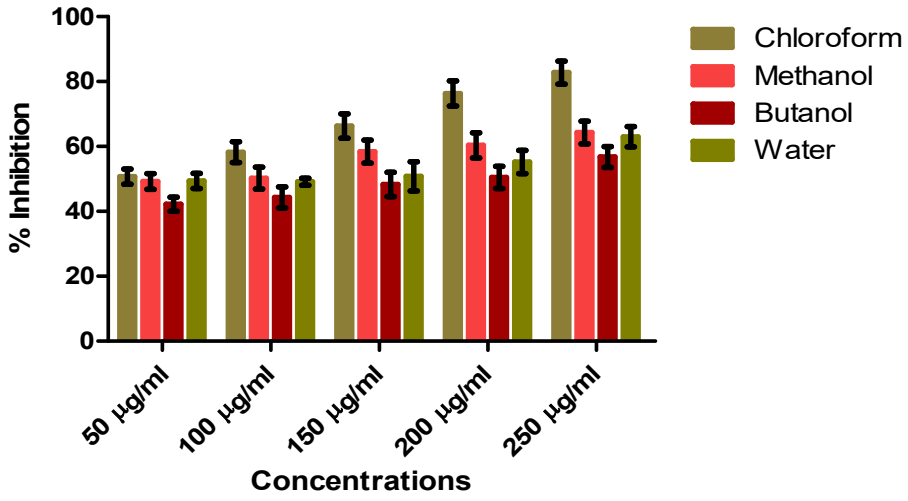

Figure No. 07: Effect of different extracts on Hydroxyl radical scavenging activity

DPPH scavenging activity

DPPH rummaging capacity determined as IC50 from Tables No 16 shows that chloroform remove has IC50 of 36 µg/mL followed by ascorbic corrosive (3.2µg/mL), gallic corrosive (32µg/mL), methanol (40µg/mL), butanolic (42µg/mL) and water (44µg/mL) extricates. The outcome uncovered that chloroform extricate has the most noteworthy rummaging capacity.

Table No. 13: Different extract express By DPPH Action

S. No.	Concentration (µg/mL)	Chloroform (%)	Methanol (%)	Butanol (%)	Water (%)
1.	10	27.23±2.44	21.55±3.14	26.01±2.42	28.31±2.44
2.	20	34.11±3.44	26.26±3.17	29.18±2.22	28.11±2.22
3.	30	48.48±2.45	38.14±2.17	33.21±4.11	38.11±2.55
4.	40	56.37±4.66	50.22±1.22	48.58±4.24	47.61±3.11
5.	50	64.76±3.55	53.31±2.55	55.12±1.22	57.77±2.78

Values are expressed as the mean of triplicate

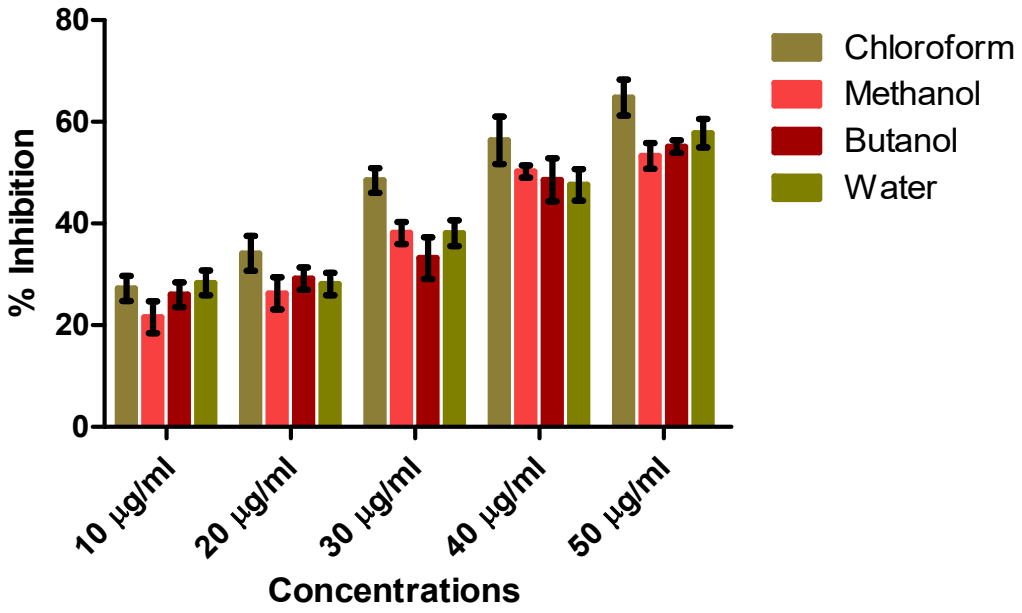

Figure No. 08: Effect of different extracts on Percentage DPPH scavenging activities

Evaluation of Memory elevating action of several actions

Morris Water Test for Various Concentrates of Leaves of *Adhatoda Vasica*

The information introduced in Tables 19 and 20 propose areas of strength for a between get away from dormancy (EL) and time spent in the objective quadrant (TSTQ) A decrease in EL joined with an expansion in TSTQ in the Morris Water Labyrinth test shows an improvement in mental capabilities. Mice were managed different plant concentrates and Physostigmine (0.1 mg/kg, i.p.) for 15 successive days. This treatment fundamentally decreased EL from the eleventh to the fourteenth day and expanded TSTQ on the fifteenth day, contrasted benchmark group, demonstrating a noticeable upgrade in learning and memory. Among the tried concentrates, the chloroform separate delivered the main outcomes, prompting a significant decrease in EL ($P<0.001$) and a huge expansion in TSTQ, outflanking the vehicle.

Table 14. Effect of different extract on EL of mice using MWM

Treatment Schedule	EL (Sec) Day 11	EL (Sec) Day 12	EL (Sec) Day 13	EL (Sec) Day 14
Normal Control	93.22 ± 1.11	94.21 ± 1.13	94.48 ± 1.66	93.12 ± 2.28
Physostigmine, 0.1 mg	92.32 ± 1.21	89.11 ± 1.24*	85.18 ± 1.41**	78.32 ± 2.19 ***
Chloroform Extract, 200 mg/kg	92.41 ± 1.09	88.15 ± 1.21 *	83.51 ± 1.16 **	78.31 ± 1.58 ***
Chloroform Extract, 400 mg/kg	92.11 ± 1.29	85.25 ± 1.31 *	81.30 ± 1.41 **	80.52 ± 1.32 ***
Methanol Extract, 200	92.42 ± 1.08	93.41 ± 1.22	94.18 ± 1.47	93.11 ± 1.78

mg/kg				
Methanol Extract, 400 mg/kg	92.31 ± 1.42	92.51 ± 1.78	94.58 ± 1.90	93.47 ± 1.87
Butanolic Extract, 200 mg/kg	93.67 ± 1.57	90.31 ± 1.22	88.28 ± 1.77 *	85.32 ± 1.70 **
Butanolic Extract, 400 mg/kg	92.56 ± 1.35	92.11 ± 1.27	86.32 ± 1.88 *	84.18 ± 1.76 **
Water Extract, 200 mg/kg	94.12 ± 1.34	93.41 ± 1.21	93.28 ± 1.88	93.10 ± 1.28
Water Extract, 400 mg/kg	93.46 ± 1.76	93.20 ± 1.83	94.18 ± 1.72	94.13 ± 1.66
Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control				

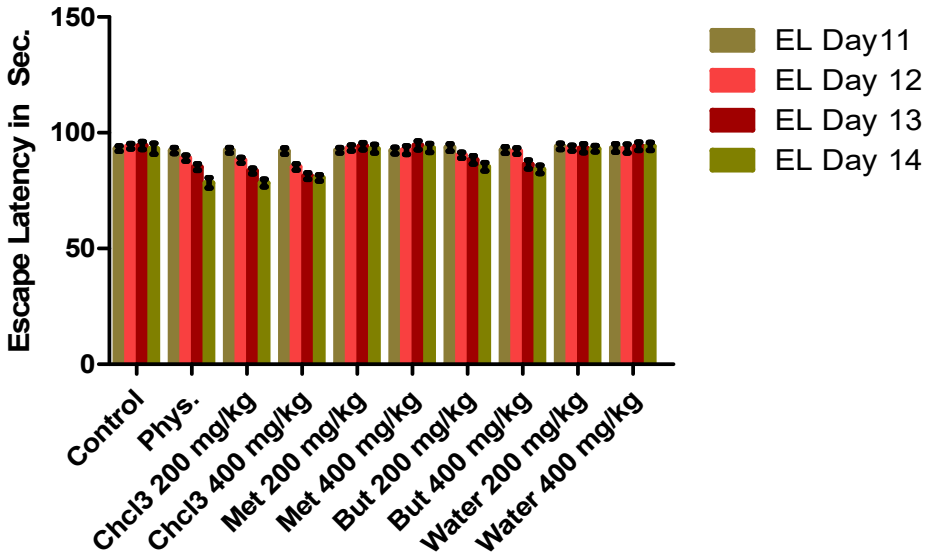

Figure No. 09: Effect of different extract on EL of mice using MWM

Table 15. Effect of different extract on TSTQ of morris water maze

Treatment Schedule	Time spent (sec) in target quadrant (15thday)
Normal Control	45.42 ± 2.23
Physostigmine, 0.1 mg	98.21±2.15***
Chloroform Extract, 200 mg/kg	99.31±2.45***
Chloroform Extract, 400 mg/kg	101.45±2.98***
Methanol Extract, 200 mg/kg	58.35±1.26*
Methanol Extract, 400 mg/kg	60.21±1.78*

Butanolic Extract, 200 mg/kg	62.42±2.87**
Butanolic Extract, 400 mg/kg	65.36±2.64**
Water Extract, 200 mg/kg	63.89±1.74**
Water Extract, 400 mg/kg	65.68±1.64**

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

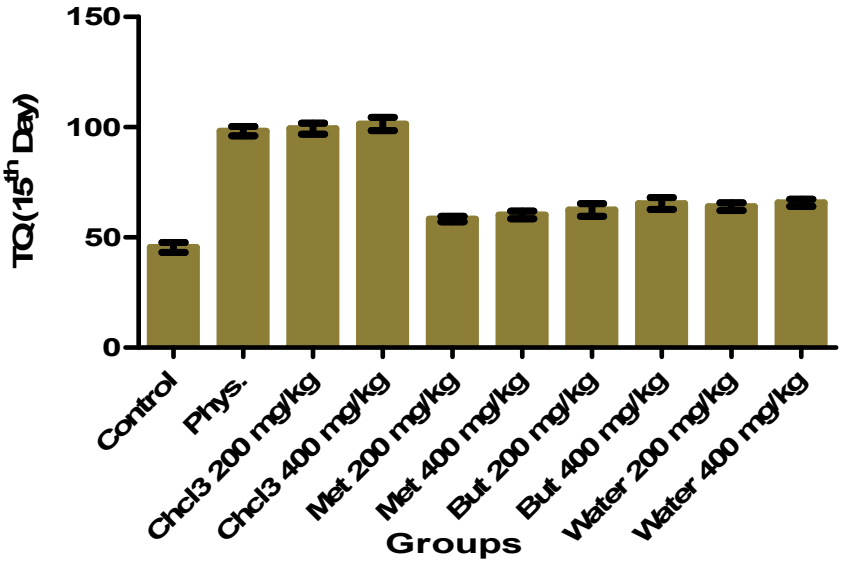

Figure No. 10: Action define extract in TSTQ of Morris Water Maze

Table No. 16: Effect of various extracts on Brain Acetyl cholinesterase activity of mice

Treatment Schedule	Acetyl cholinesterase activity (mol/l per min × 10^{-6}/g of tissue)
Normal Control	0.061 ± 0.010
Physostigmine, 0.1 mg	0.019±0.002***
Chloroform Extract, 200 mg/kg	0.021±0.005***
Chloroform Extract, 400 mg/kg	0.020±0.008***
Methanol Extract, 200 mg/kg	0.050±0.006*
Methanol Extract, 400 mg/kg	0.046±0.006*
Butanolic Extract, 200 mg/kg	0.045±0.007**
Butanolic Extract, 400 mg/kg	0.042±0.005**
Water Extract, 200 mg/kg	0.041±0.004**
Water Extract, 400 mg/kg	0.038±0.002**

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

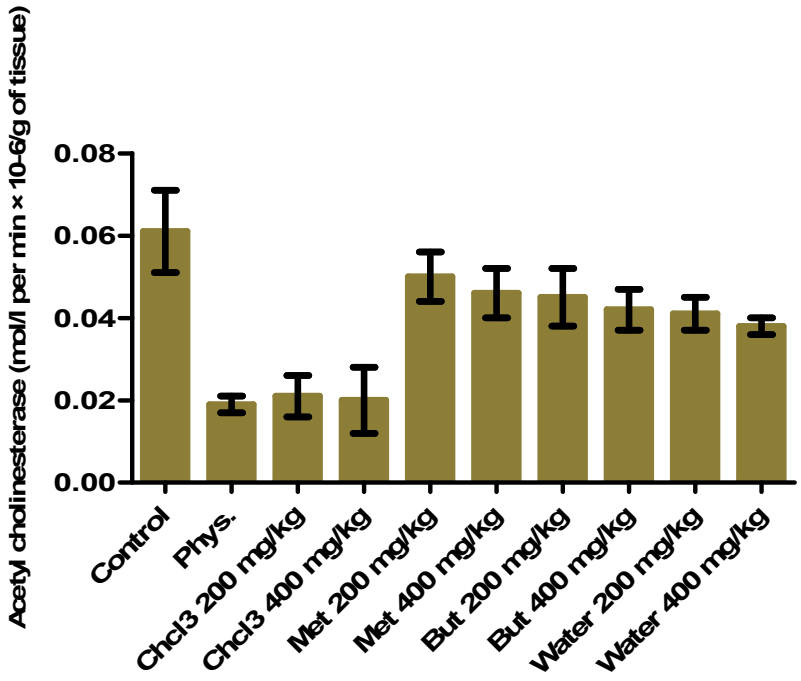

Figure No. 11: Effect of different extracts on brain Acetyl cholinesterase activity of mice

Morris Water Test for Different Extracts of *Vigna Mungo seeds*

According to results got which were referenced in Table No. 22 and 23, Learning and memory are related with EL and TSTQ. Decline of EL with expansion in TSTQ by mice in MWM demonstrates upgrade of learning and memory.

Table No. 17: Effect of different extract on EL of mice using MWM

Treatment Schedule	EL (Sec) Day 11	EL (Sec) Day 12	EL (Sec) Day 13	EL (Sec) Day 14
Control	93.22 ± 1.11	94.21 ± 1.13	94.48 ± 1.66	93.12 ± 2.28
Physostigmine, 0.1 mg	92.32 ± 1.21	89.11 ± 1.24*	85.18 ± 1.41**	78.32 ± 2.19 ***
Chloroform Extract, 200 mg/kg	91.31 ± 1.09	87.15 ± 2.21 *	82.41 ± 1.33 **	77.21 ± 1.48 ***
Chloroform Extract, 400 mg/kg	91.11 ± 1.26	84.15 ± 1.31 *	80.10 ± 1.32 **	81.32 ± 1.22 ***
Methanol Extract, 200 mg/kg	91.32 ± 1.38	92.31 ± 1.33	93.13 ± 1.33	93.22 ± 1.44
Methanol Extract, 400 mg/kg	92.31 ± 1.22	91.33 ± 1.54	92.33 ± 1.32	92.42 ± 1.55
Butanolic Extract,	92.47 ± 1.44	91.34 ± 1.11	87.18 ± 1.55 *	84.32 ± 1.67 **

200 mg/kg				
Butanolic Extract, 400 mg/kg	92.46 ± 1.32	92.22 ± 1.23	85.11 ± 1.83 *	83.18 ± 1.66 **
Water Extract, 200 mg/kg	93.11 ± 1.22	93.58 ± 1.26	93.66 ± 1.55	93.15 ± 1.21
Water Extract, 400 mg/kg	93.33 ± 1.68	93.21 ± 1.43	93.18 ± 1.77	93.18 ± 1.88

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

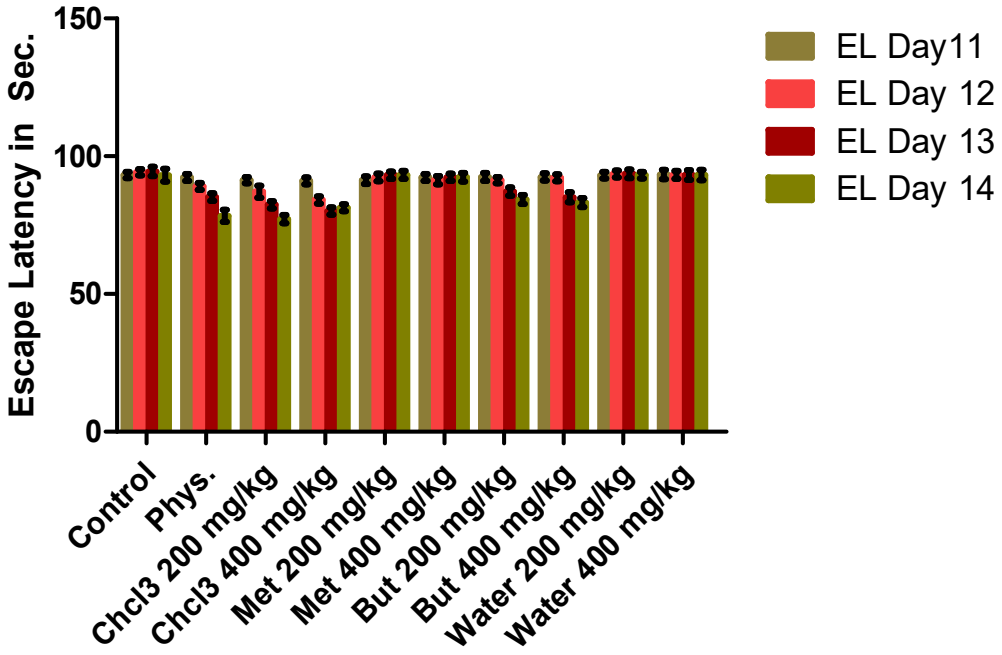

Figure No. 12: Effect of different extract on Escape Latency (EL) of mice using Morris Water Maze

Table No. 18: Effect of different extract on TSTQ of MWM

Treatment Schedule	TSTQ (Sec) (15thday)
Normal Control	45.42 ± 2.23
Physostigmine, 0.1 mg	98.21±2.15***
Chloroform Extract, 200 mg/kg	98.13±2.56***
Chloroform Extract, 400 mg/kg	103.31±2.77***
Methanol Extract, 200 mg/kg	57.38±1.77*
Methanol Extract, 400 mg/kg	61.26±1.66*
Butanolic Extract, 200 mg/kg	63.12±2.97**
Butanolic Extract, 400 mg/kg	66.16±2.33**

Water Extract, 200 mg/kg	64.89±1.84**
Water Extract, 400 mg/kg	65.55±1.24**

Values are expressed as mean±SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

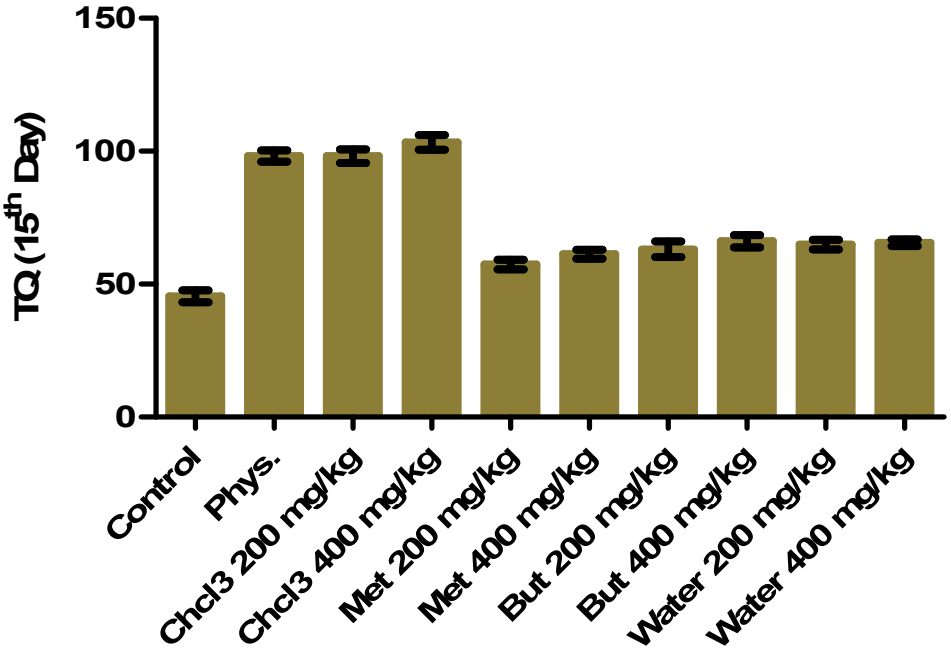

Figure No. 13: Effect of various extract on time spent in target quadrant (TSTQ) of Morris Water Maze

Isolation and Characterization of compound- I from chloroform extract of *Adhatoda Vasica*

The chloroform extract underwent column chromatography utilizing silica gel (60-120 mesh size) as the stationary phase. Gradient elution was carried out by starting with n-Hexane and gradually increasing the concentration of chloroform. Approximately 10 ml of each fraction was collected, and similar fractions were combined based on their TLC patterns. Fractions 76-98, eluted with a mixture of n-Hexane and chloroform in a 9:1 ratio, displayed a single spot on TLC. Consequently, separed moiety pooled to obtained compound-I. Compound-I was then recrystallized using cold methanol. Finally, compound-I was subjected to spectral analysis, including IR, NMR, and mass spectroscopy, to determine its physicochemical properties.

Table No: 19: Physicochemical Properties of Compound-I

S.N.	Parameter	Physicochemical Properties
1	Source	Isolated from Fraction 76-98 of n-hexane and Chloroform (9:1)
2	TLC solvent System	*n*-hexane: ethyl acetate, 8:2
3	R_f Value	0.86
4	Detecting reagent	UV Chamber, Vaniline-Sulphuric acid spray and anisaldehyde spray
5	Color	Yellowish
6	Nature	Crystalline needle shape

7	Melting Point	78-80°C

Here, isolated compound gave positive Dragendorff's test for alkaloid. The compound obtained is yellowish needle shaped crystal & melting point was found to be at 78-80°C. Mass spectrum revealed the parent molecular ion $[M+H]^+$ peak at m/z – 187.9 (M+1), 186.5, 178.1 which corresponds to the molecular formula $C_{11}H_8O_3$. The IR, and ^{1}H-NMR data of Compound are as follow:

IR spectral data of compound-I

IR spectrum of showed frequencies at 3456 cm^{-1} indicating the presence of O-H hydroxyl group while 2921 and 2866 cm^{-1} indicated the presence of C-H aromatic and methyl group. The peak at 1508 cm^{-1} indicated the presence of C=C in conjugation and the absorption peaks at 1045 and 1263 cm^{-1} indicated the presence of C-OH. The peak at 1643 cm^{-1} corresponds to the presence of aromatic ketone group. The absorption signal at1458 cm^{-1} indicated the presence of C-CO-C i.e ketone skeleton. The peak at 684 cm^{-1} indicated O-H bending. The comparison of IR spectrum of compound I with the reported spectrum of plumbagin is given in following table.

Table No.20: Comparison of observed & reference values of IR

S. No	Bond	Reported (cm⁻¹)	Observed (cm⁻¹)
1.	C–H (aromatic)	2990 - 3100	2930
2.	C=C (aromatic)	1460, 1610	1512
3.	O–H (hydroxyl)	3510 - 3620	3460
4.	C–O (carbonyl)	1060, 1270 - 1360	1050, 1275
5.	C=O (ketone/dioneAr–C=O)	1690 - 1710	1650
6.	C–CO–C (ketone skeleton)	1225 - 1335	1462
7.	O–H bending	660 - 760	690
8.	C–H (Ar–CH_3)	2910 - 3010	2870

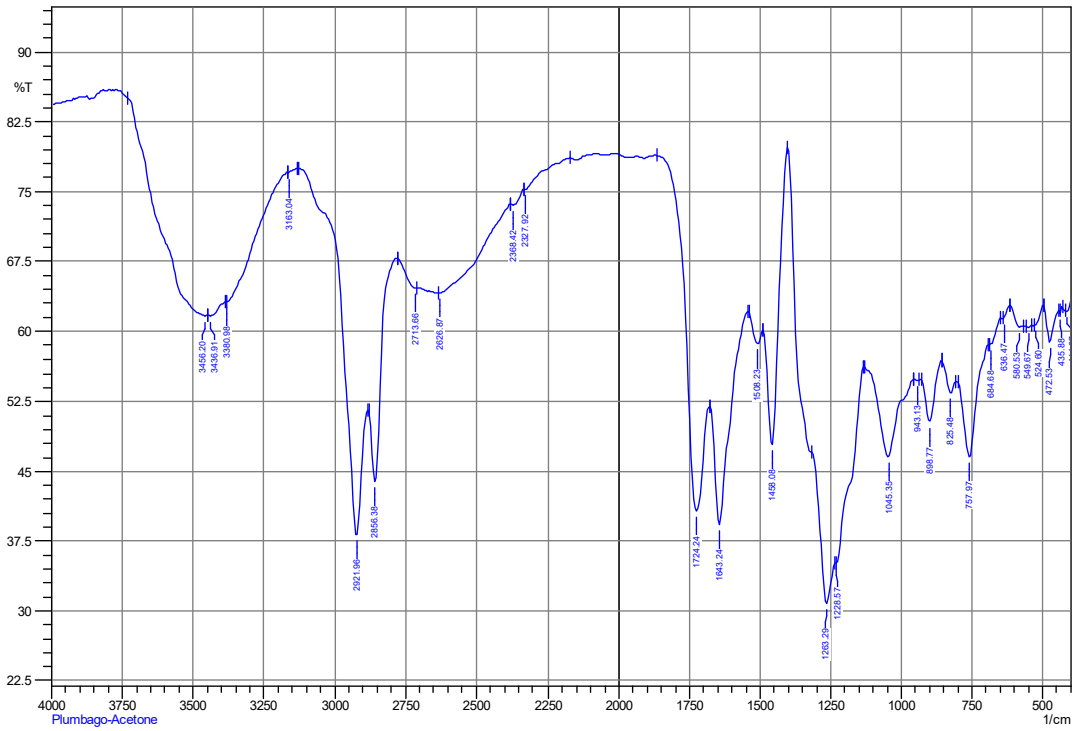

Figure No. 14: IR Spectra of isolated compound I

NMR of compound-I

^{1}H NMR spectrum, analysis of the compound I showed peaks in between δ 0 to 2.5 and δ 6.5 to 7.5. Both groups of peaks were identified as acyclic aliphatic compounds. An α-mono substituted aliphatic group of possible chemical constituent in $CDCL_3$ showed a single peak at 2.59 δ, two doublet strong bands at (5.64 and 7.74) δ, multiplate peaks at (7.58) δ. The ^{1}H NMR signal of compound-I is as follows. ^{1}H NMR ($CDCl_3$) for Compound I δ: 2.59 (3H, s, Me-2), 5.64 (1H, s, H-3), 7.74 (1H, m, H-6), 7.58 (2H, m, H-7, 8).

Table No. 21*:* Comparison of observed & reference values of NMR

Values(ppm) (observed)	**Values(ppm) (Reported)**
δ = 2.59, 3H, s, CH_3	δ = δ 2.19 (3H, s, Me-2)
δ = 5.64, s, 1H, Aromatic -C-OH	δ = 6.81 (1H, s, H-3)
δ = 7.74, d, *J* = 8.2 Hz, 2H, CH	δ = 7.25 (1H, m, H-6)
7.58, s, 1H, CH)	7.62 (2H, m, H-7, 8)
7.19 (s, 1H, CH)	7.47 (s, 1H, CH)

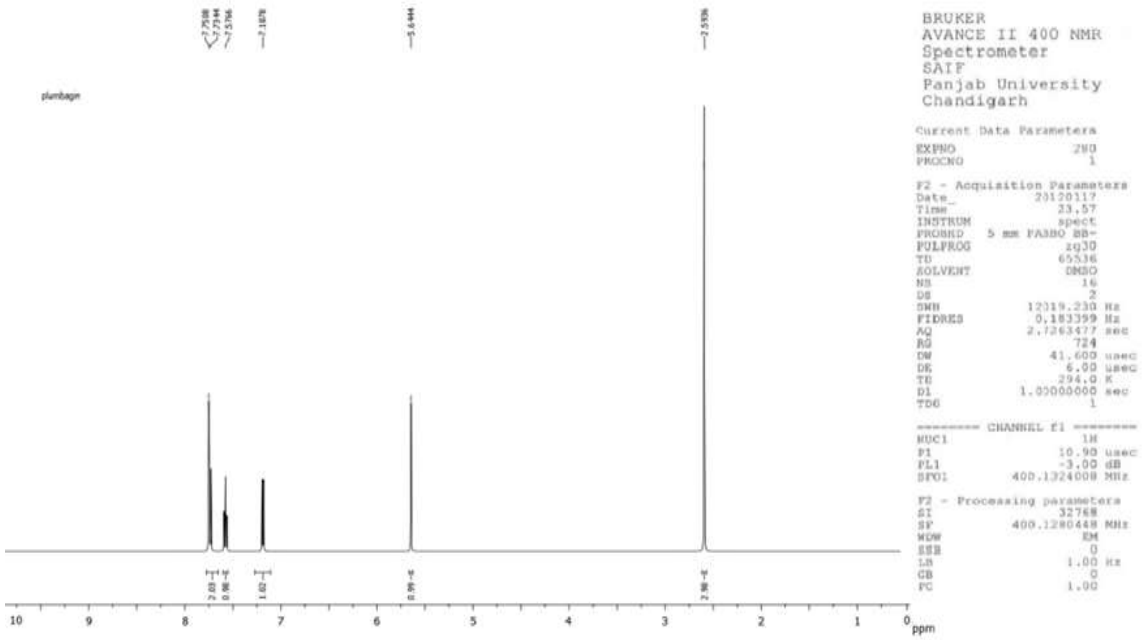

Figure No. 15: ^{1}H-NMR Spectra of Compound-I

Mass Interpretation

Isolated compound showed M/z value and molecular weight as– 187.9 (M+1), 186.5, 178.1. The standard peak reported is maximum at m/z 188.1794 which confirms the compound as plumbagin

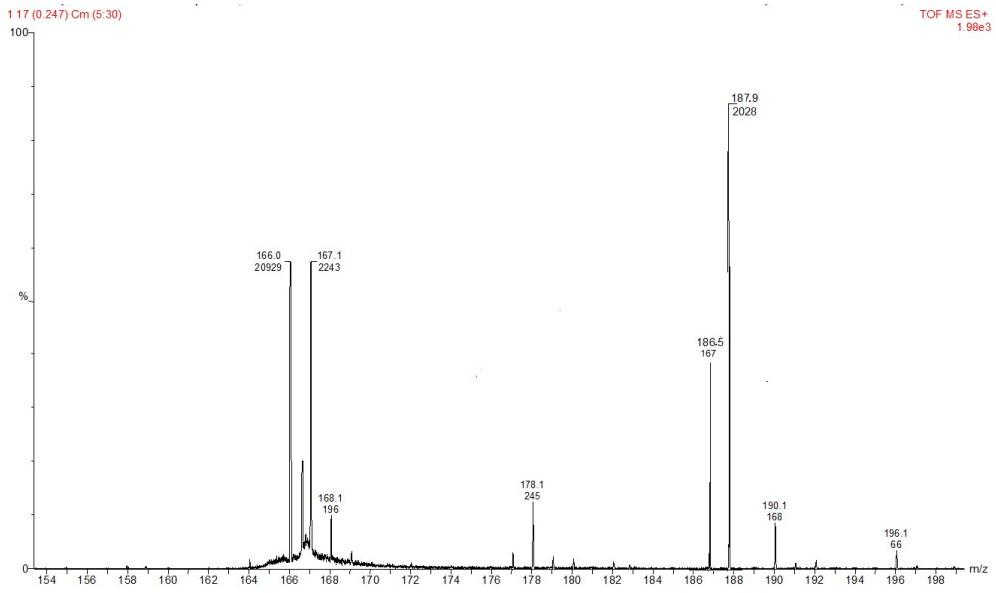

Figure No. 16: Mass Spectra of Compound-I

On the basis of foregoing account of the IR, ^{1}H-NMR and mass spectral data of isolated compound and also supported by the strong Dragendorff'stest for alkaloid the isolated compound seems to be plumbagin.

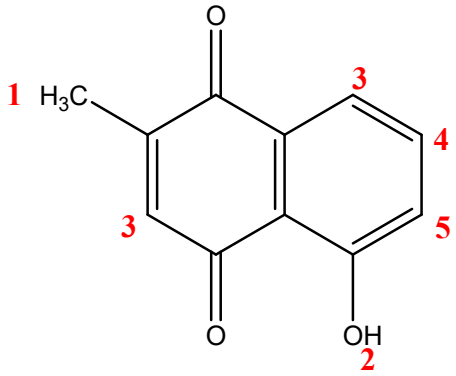

Figure No. 17: Structure of Plumbagin

Isolation and Characterization of compound-II from chloroform extract of *Seeds of Vigna Mungo*

The chloroform extract was analysed using column chromatography with silica gel (60-120 mesh size), and elution was performed using a gradient elution approach with petroleum ether: dichloromethane (DCM) (10% increments of DCM), and finally with DCM. Fractions 104-123 were eluted with DCM and ethanol (9:1) and combined together to form fraction C, which was crystallised by adding a few drops of cold methanol. Further fractionation with DCM and ethanol produces fractions without spot-on TLC. The physicochemical properties of the produced compound are as follows.

Table No: 22: Physicochemical Properties of Compound-II

S.N.	Parameter	Physicochemical Properties
1	Source	Isolated from Fraction 76-98 of n-hexane and Chloroform (9:1)
2	TLC solvent System	*n*-hexane: ethyl acetate, 9:1
3	Rf Value	0.88
4	Detecting reagent	UV Chamber, Vaniline-Sulphuric acid spray and anisaldehyde spray
5	Color	Yellowish
6	Nature	Crystalline needle shape
7	Melting Point	78-80°C

Here, isolated compound-II gave positive Libermann-Burchard test for steroids. The compound obtained is off-white crystalline & melting point was found to be at 158 to 162°C. Mass spectrum revealed the parent molecular ion $[M+H]^+$ peak at m/z 426 which corresponds to the molecular formula $C_{30}H_{50}O$.

IR spectral data of Compound-II

The IR spectrum showed frequencies 3442 cm^{-1} which indicated the presence of hydroxyl group. Peak at 3056 cm^{-1} indicated the presence of C–H in conjugation and absorption peaks at 1643, 1483 and 1359 cm^{-1}shown unsymmetrical ethylene double bond, aromatic rings and aromatic $–CH_3$ group, respectively.

Table No 23: Comparison of observed & reference value of Compound II

S. No	Bond	Reported (cm^{-1})	Observed (cm^{-1})
1.	C –H (aromatic)	3035	3056
2.	C = C (aromatic)	1665	1643
3.	O – H hydroxyl	3446	3442
4.	C – H (aromatic methyl Ar – CH_3)	1369	1359
5.	Aromatic Ring	1452	1483

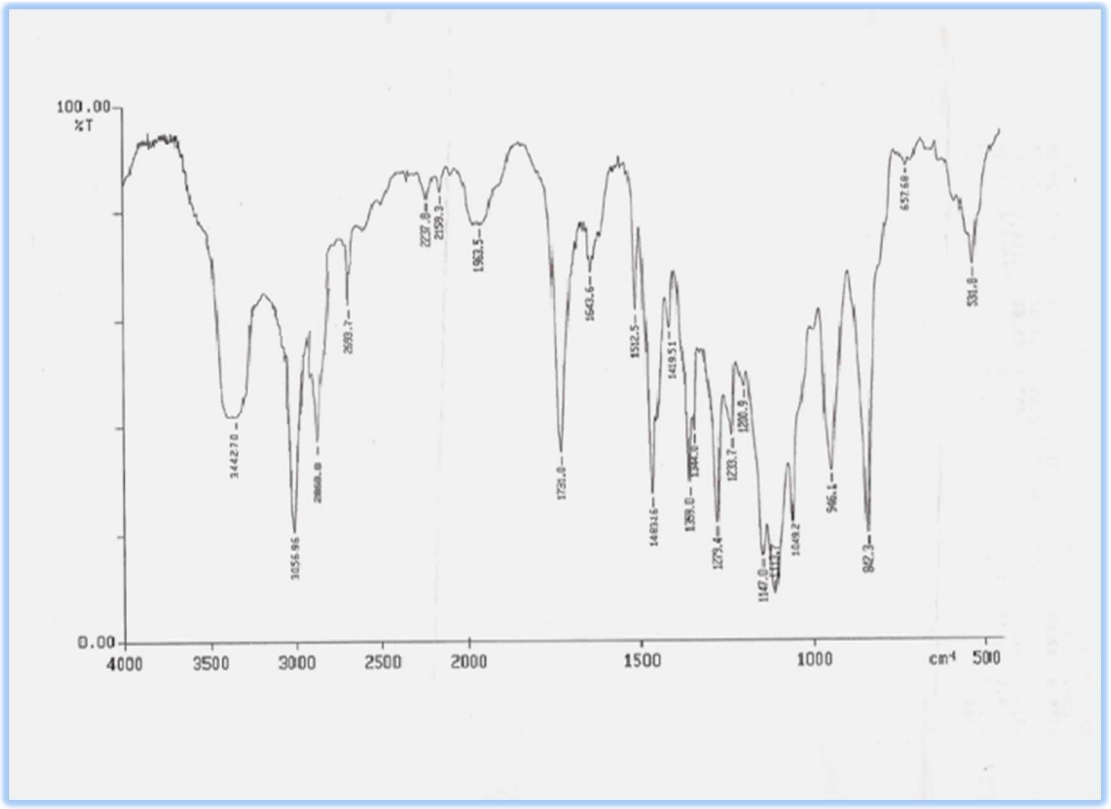

Figure No. 18: IR Spectra of isolated Compound-II

^{1}H-NMR

The ^{1}H-NMR spectrum shows a typical signal for the hydroxyl proton H-3 of a terpenoids nucleus that appears in the area around δ 3.47 ppm and the signal at 12 5.40 ppm is the proton signal bound to the substituted C-olefin (H-5). Proton signals at 36 1.32 ppm (1H, H- 19) and δ 0.88 ppm (1H, H-20) indicate the position of the methyl group (C-29) bound to the C-19 atom (which distinguishes it from the β-isomer of amyrin). The signal at δ 1.32 ppm indicates the position of a methyl group at C-19

Table No 24: Comparison of ^{1}H-NMR data of isolated compound-II

Position	α-Amyrin (Standard) (ppm)	Observed Values (ppm)
1.	1.70 (2H, m)	1.64
2.	2.05 (2H, m)	2.20
3.	3.25 (1H, dd, J=4.8, 11.2)	3.50
4.	0.76 (1H, d, J=11.8)	0.82
5.	1.60 (2H, m)	1.52
6.	1.38 (1H, m)	1.34
7.	1.56 (1H, m)	1.41
8	1.93 (2H, m)	1.82
9.	5.14 (1H, t, J=3.8)	5.42
10.	1.63 (2H, t, J=4.2)	1.60
11.	1.85 (2H, t, J=4.9)	1.87
12.	1.33 (1H, s)	1.32
13.	1.38 (1H, m)	1.34
14.	0.89 (1H, m)	0.90
15.	1.41 (2H, m)	1.40
16.	1.43 (2H, t, J=10.1)	1.42
17.	1.01 (3H, s)	1.15
18.	0.97 (3H, s)	1.10
19.	0.99 (3H, s)	1.05
20.	1.03 (3H, s)	1.02
21.	1.09 (3H, s)	1.12
22.	0.82 (3H, s)	0.82
23.	0.81 (3H, d, J=3.45)	0.82

24.	0.93 (3H, d, J=5.8)	0.95

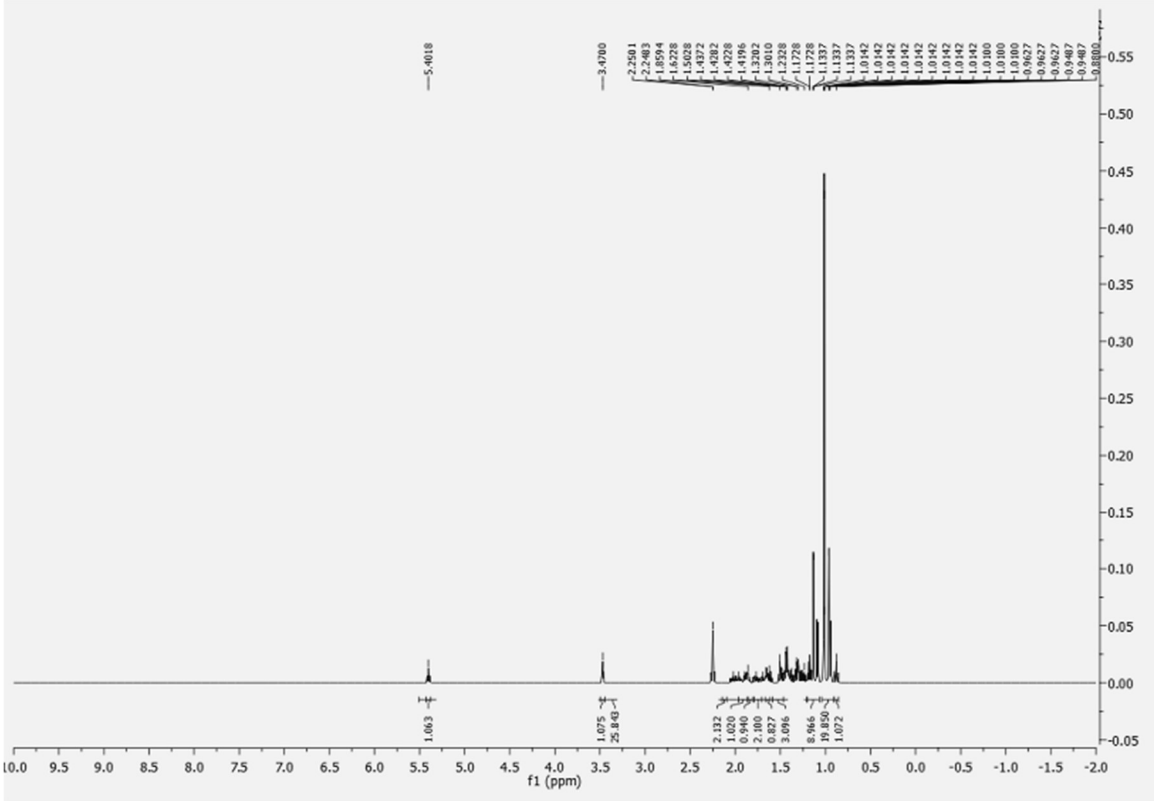

Figure No. 19: ^{1}H-NMR Spectra of isolated Compound-II

^{13}C-NMR of isolated Compound-II

The 13^{13}13C-NMR spectrum reveals 30 distinct carbon signals. Among these, the signals at δ 143.02 and δ 125.42 ppm correspond to the C-12 and C-13 atoms involved in double bonds, while the signal at δ 78.56 ppm arises from carbon atoms bonded to hydroxyl groups adjacent quaternary carbons. The methyl carbon signals are observed at δ 27.72, δ 29.16, δ 23.35, δ 19.07, δ 17.93, δ 17.04, δ 15.16, and δ 17.04 ppm. The methylene carbon signals appear at δ 39.67, δ 38.71, δ 32.86, δ 29.16, δ 27.72, δ 27.63, δ 25.87, δ 23.35, and δ 18.79 ppm. Additionally, the methine carbon signals are found at δ 78.56, δ 57.72, δ 54.68, δ 46.51, δ 38.71, and δ 39.67 ppm. Finally, the signals at δ 40.20, δ 38.39, δ 32.86, and δ 37.47 ppm correspond to quaternary carbon atoms.

Table No 25: Comparison of ^{13}C NMR data of isolated compound-II

Position	α-Amyrin (standard) (ppm)	Compound-II(ppm)
1.	38.93	38.71
2.	28.25	27.72
3.	79.19	78.56
4.	38.73	38.39
5.	55.32	54.68
6.	18.53	18.79

7.	33.08	32.86
8.	40.15	40.20
9.	47.85	46.51
10.	37.04	37.47
11.	23.52	23.35
12.	124.55	125.42
13.	139.72	143.02
14.	42.22	40.20
15.	27.42	27.63
16.	26.76	25.87
17.	33.9	32.86
18.	59.19	57.72
19.	39.81	38.71
20.	39.76	39.67
21.	31.41	29.16
22.	41.68	39.67
23.	28.28	29.16
24.	15.79	16.05
25.	15.84	16.01
26.	17.08	17.04
27.	23.42	23.35
28.	28.91	27.72
29.	17.63	17.93
30.	21.57	19.60

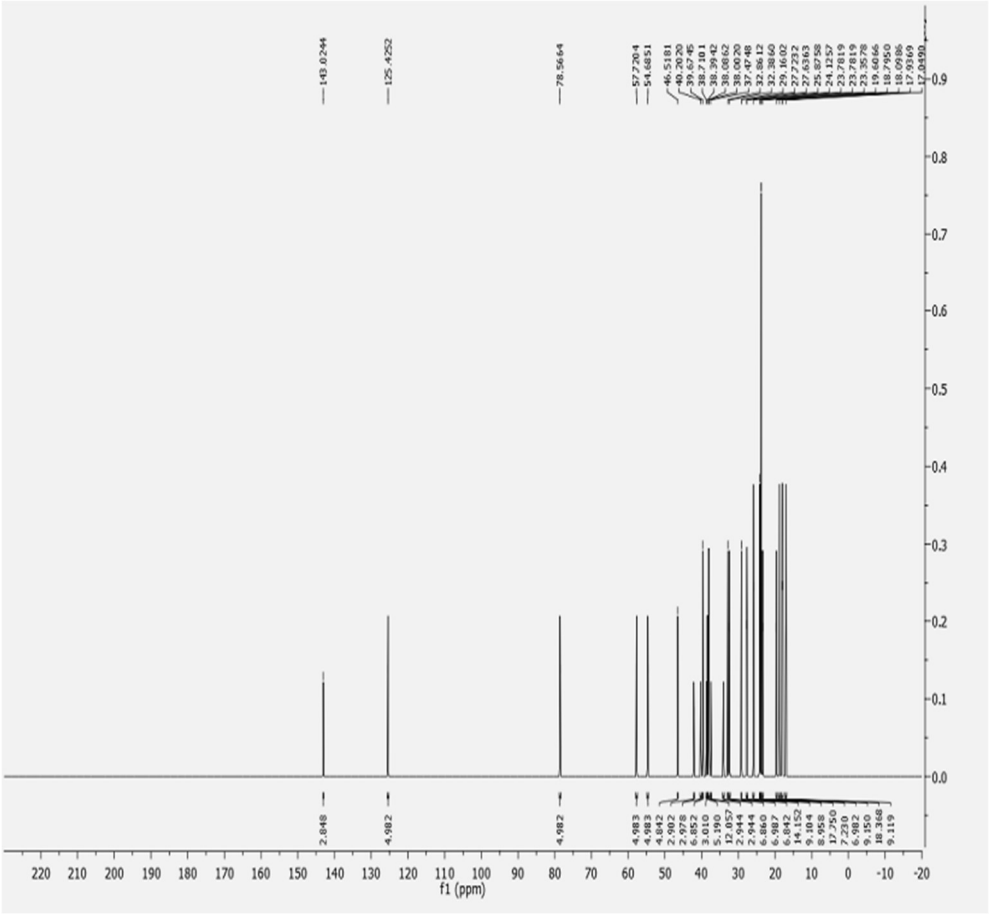

Figure No. 20: ^{13}C-NMR Spectra of Compound II

Mass Spectroscopy

Mass spectrum showed a parent molecular ion $[M+H]^+$ peak at m/z 426 respectively which corresponds to the molecular formula $C_{30}H_{50}O$. The standard peak reported is maximum at m/z 426.729 which confirms the compound as ά-amyrin.

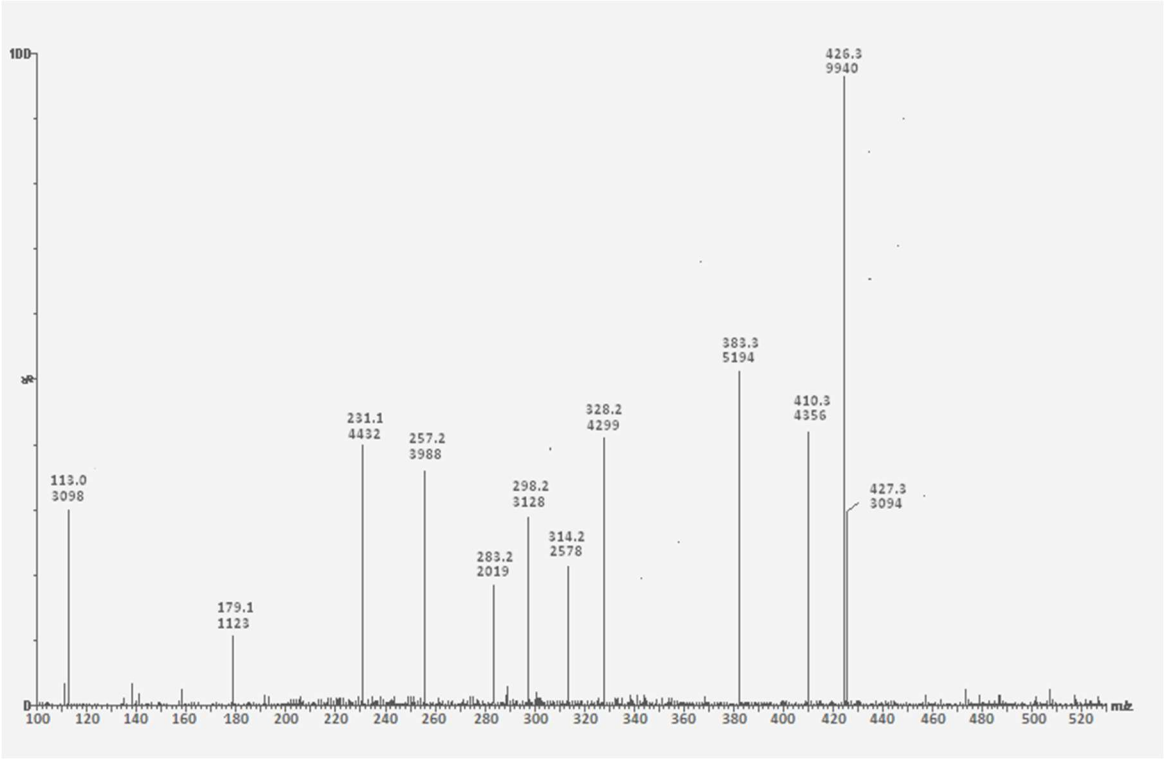

Figure No. 21: Mass Spectra of isolated Compound-II

On the basis of foregoing account of the IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data of Compound II and also supported by the strong positive Liebermann-Burchard test for triterpenoids. The isolated compound seems to be α- amyrin.

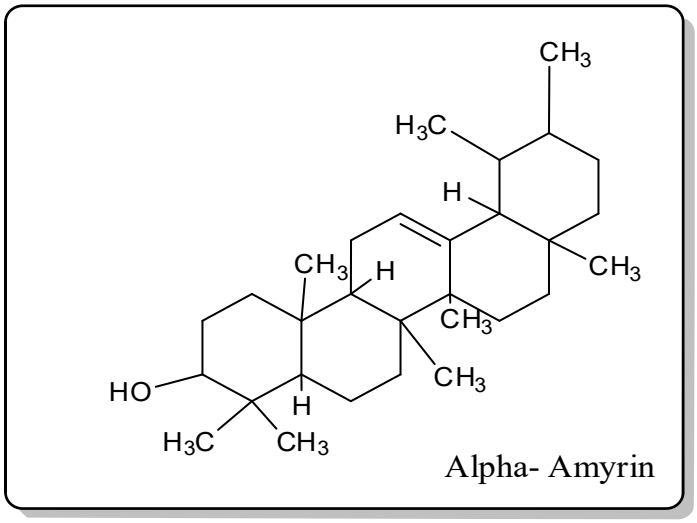

Fig. 22: Structure of α-amyrin

Pharmacological Evaluation of isolated Plumbagin

Effect of Plumbagin on EL & TSTQ

Escape latency and TSTQ is associated with learning and memory and decrease of EL and increase of TSTQ

by mice in MWM indicates improvement of learning and memory. The Plumbagin (1.5 & 3 mg/kg) and Physostigmine (0.1mg/ kg, *i.p.*) fed for 15 consecutive days. After administration, they significantly decreased EL of mice from 11thto 14thday and augmented TSTQ by mice on 15thday as compared to the control, thus illustrated learning and memory enhancement effect. Mice treated by Plumbagin in a dose of 3 mg/kg appreciably upturned scopolamine-and diazepam-induced amnesia as compared to scopolamine and diazepam treated animals.

Table No. 26 : Effect of Plumbagin on EL of mice using Morris Water Maze

Treatment Schedule	EL (Sec) Day 11	EL (Sec) Day 12	EL (Sec) Day 13	EL (Sec) Day 14
Normal Control	92.24 ± 1.29	94.44 ± 1.23	94.31 ± 1.36	93.34 ± 2.19
Physostigmine, 0.1 mg	93.22 ± 1.31	88.14 ± 1.26*	84.33 ± 1.53**	77.42 ± 2.29 ***
Plumbagin (1.5 mg/kg)	92.31 ± 1.59	87.15 ± 1.45 *	82.41 ± 1.36 **	79.23 ± 1.24 ***
Plumbagin (3 mg/kg)	93.42 ± 1.32	84.51 ± 1.62 *	80.30 ± 1.38 **	77.52 ± 1.86 ***

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

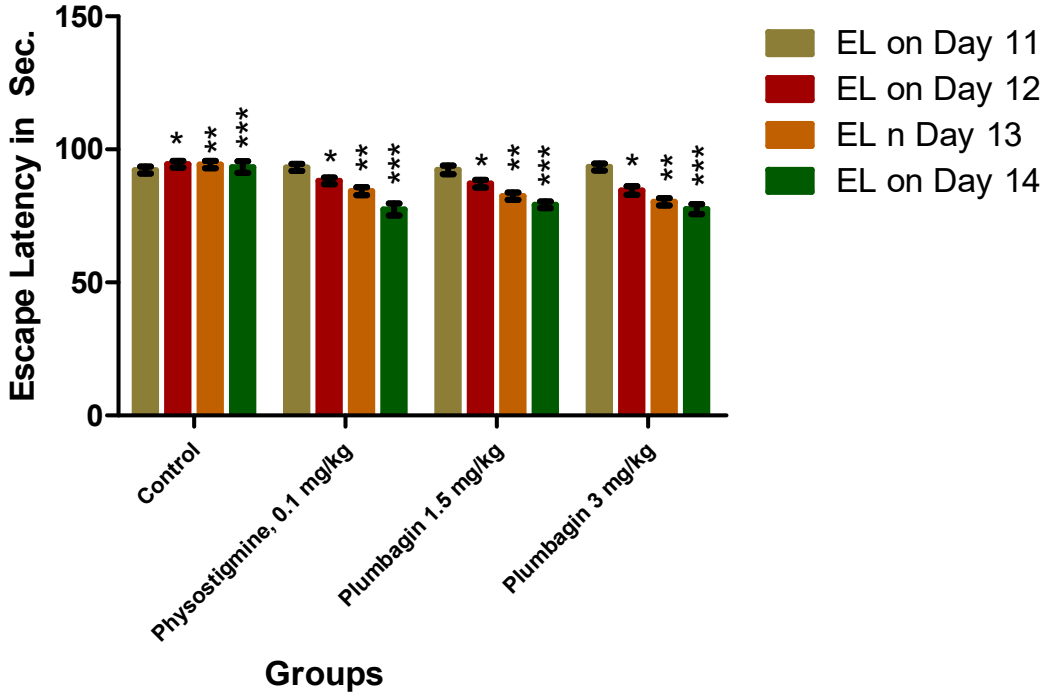

Figure No. 23: Effect of Plumbagin on Escape Latency (sec) on different days

Table No. 27: Effect of Plumbagin on TSTQ of Morris Water Maze

Treatment Schedule	Time spent (sec) in target quadrant (15th day)
Normal Control	46.72 ± 2.51
Physostigmine, 0.1 mg	102.21±2.55***
Plumbagin (1.5 mg/kg)	88.56±2.59***
Plumbagin (3 mg/kg)	105.32±2.58***
Scopolamine (0.4 mg/kg)	55.21±1.56*
Diazepam (1 mg/kg)	58.21±1.37*
Plumbagin + scopolamine (3+0.4 mg/kg)	77.32±2.67**
Plumbagin+ diazepam (3+1 mg/kg)	85.56±2.34**

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

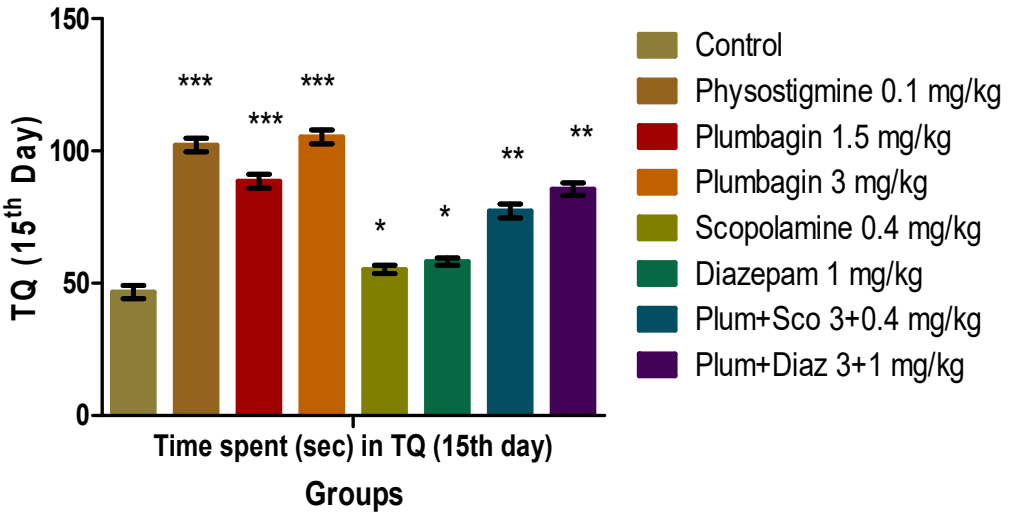

Figure No.24: Effect of Plumbagin on TQ on 15th Day

Effect of Plumbagin on Locomotor activity of mice

There was a noteworthy modification in Locomotor activity in mice treated by Plumbagin (1.5 & 3 mg/kg) and physostigmine as measure up to vehicle treated control

Table No. 28: Effect of Plumbagin on Locomotor activity of mice

Treatment Schedule	Locomotor activity counts / 5 min
Normal Control	210.40 ± 2.14
Physostigmine, 0.1 mg	410.15±2.31***
Plumbagin, 1.5 mg/kg	280.44±1.72**

Plumbagin, 3 mg/kg	326.79±2.47***

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

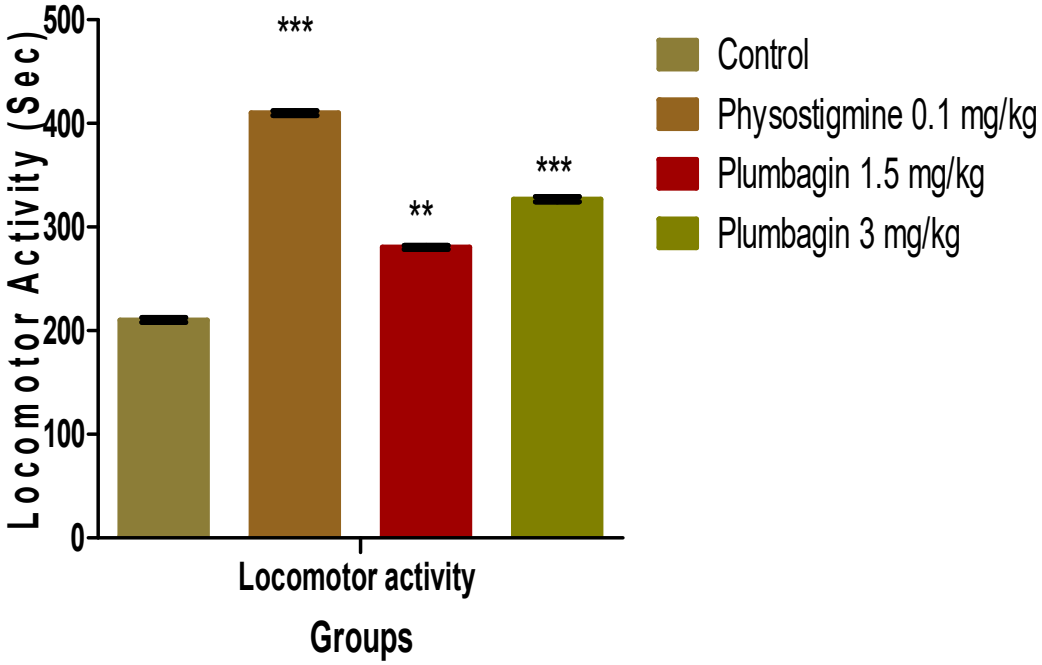

Figure No. 25: Effect of Plumbagin on Locomotor activity in mice

Impact of Plumbagin on mind Acetylcholine esterase movement of mice

Creatures treated by Plumbagin and physostigmine for 15 progressive days delivered a huge diminish in mind Acetyl cholinesterase movement when contrasted with control bunch. Mice treated by Plumbagin in a portion of 3 mg/kg showed a profoundly critical diminishing impact on mind Acetyl cholinesterase movement contrasted with rodents of control bunch. Results were communicated in Table No. 33.

Table No. 29: Effect of Plumbagin on brain Acetyl cholinesterase activity of mice

Treatment Schedule	Acetyl cholinesterase activity (mol/l per min × 10^{-6}/g of tissue)
Normal Control	0.068 ± 0.010
Physostigmine, 0.1 mg	0.021±0.007***
Plumbagin, 1.5 mg/kg	0.036±0.002***
Plumbagin, 3 mg/kg	0.026±0.008*

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

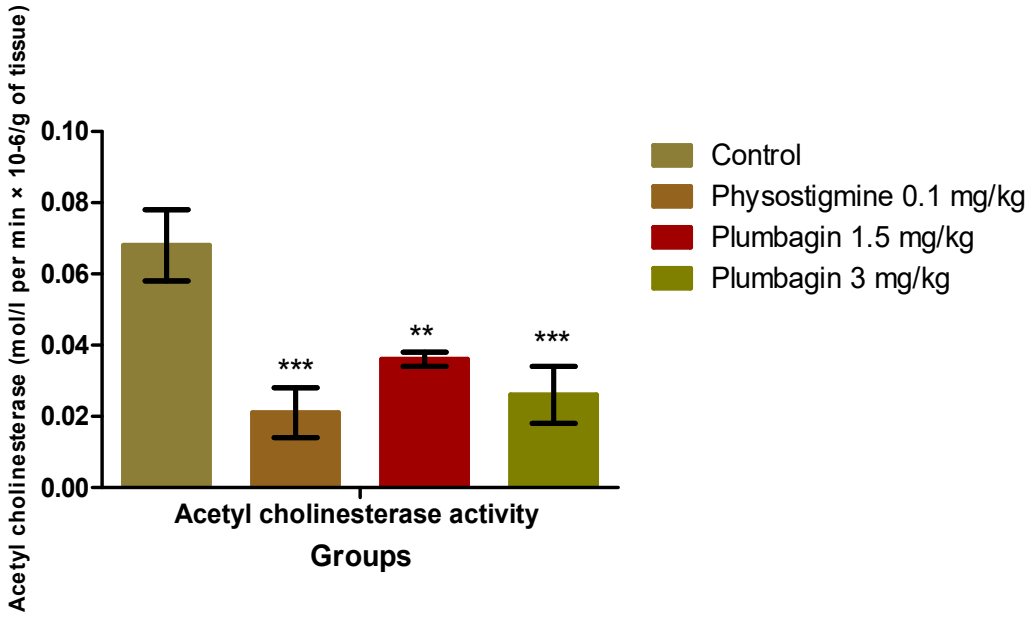

Figure No.26: Effect of Plumbagin on Acetyl cholinesterase level in brain

Pharmacological Evaluation of isolated α-Amyrin

Effect of α-Amyrin on EL & TSTQ

Get away from idleness (EL) and time spent in the objective quadrant (TSTQ) are key marks of learning and memory, with a diminishing in EL and an expansion in TSTQ in the Morris Water Labyrinth (MWM) reflecting mental improvement. In our review, α-Amyrin (directed at portions of 10 and 20 mg/kg) and Physostigmine (0.1 mg/kg, i.p.) were given to mice for 15 sequential days. Following treatment, there was a huge decrease in EL from days 11 to 14 and an expansion in TSTQ on the fifteenth day contrasted with the benchmark group, exhibiting improved learning and memory. Furthermore, rodents treated with 20 mg/kg of α-Amyrin showed a noticeable inversion of scopolamine-and diazepam-initiated amnesia when contrasted with the gatherings treated with scopolamine and diazepam alone.

Table No. 30: Effect of α-Amyrin on EL of mice using Morris water Maze

Treatment Schedule	EL (Sec) Day 11	EL (Sec) Day 12	EL (Sec) Day 13	EL (Sec) Day 14
Normal Control	92.24 ± 1.29	94.44 ± 1.23	94.31 ± 1.36	93.34 ± 2.19
Physostigmine, 0.1 mg	93.22 ± 1.31	88.14 ± 1.26*	84.33 ± 1.53**	77.42 ± 2.29 ***
α-Amyrin (10 mg/kg)	91.21± 1.33	86.31 ± 1.25 *	81.53± 1.44 **	76.31 ± 1.64 ***
α-Amyrin (20 mg/kg)	92.33 ± 1.64	83.41 ± 1.34*	79.36 ± 1.45**	73.52 ± 1.66 ***

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

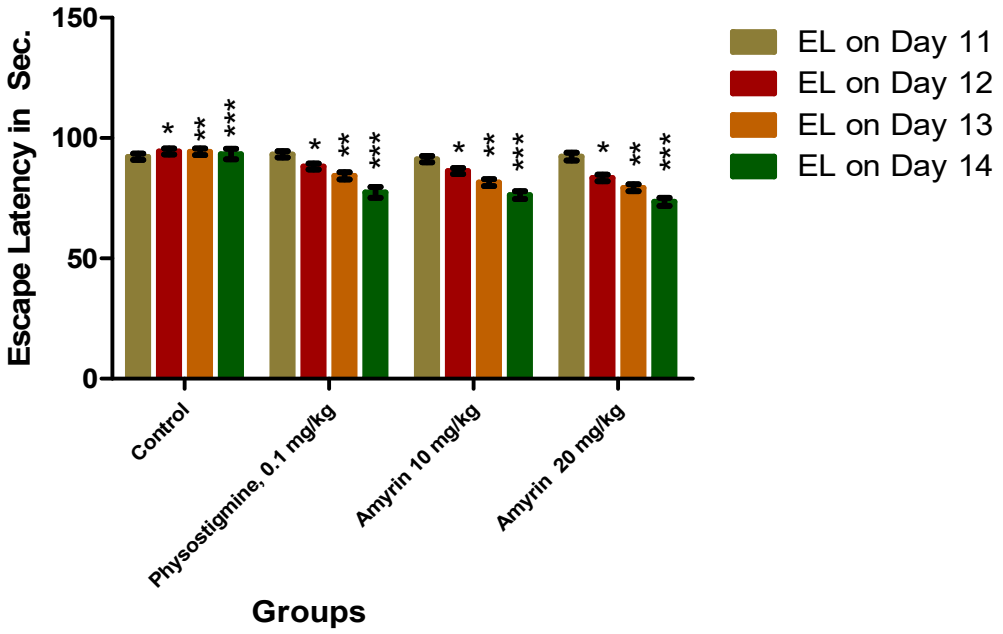

Figure No.27: Effect of α-Amyrin on Escape Latency (sec) on different days

Table No. 31: Effect of α-Amyrin on TSTQ of Morris Water Maze

Treatment Schedule	TSTQ (Sec) (15thday)
Normal Control	46.72 ± 2.51
Physostigmine, 0.1 mg	102.21±2.55***
α-Amyrin (10 mg/kg)	89.12±3.41***
α-Amyrin (20 mg/kg)	107.43±3.66***
Scopolamine (0.4 mg/kg)	55.21±1.56*
Diazepam (1 mg/kg)	58.21±1.37*
α-Amyrin + scopolamine (20+0.4 mg/kg)	80.87±3.88**
α-Amyrin + diazepam (20+1 mg/kg)	86.76±3.91**

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

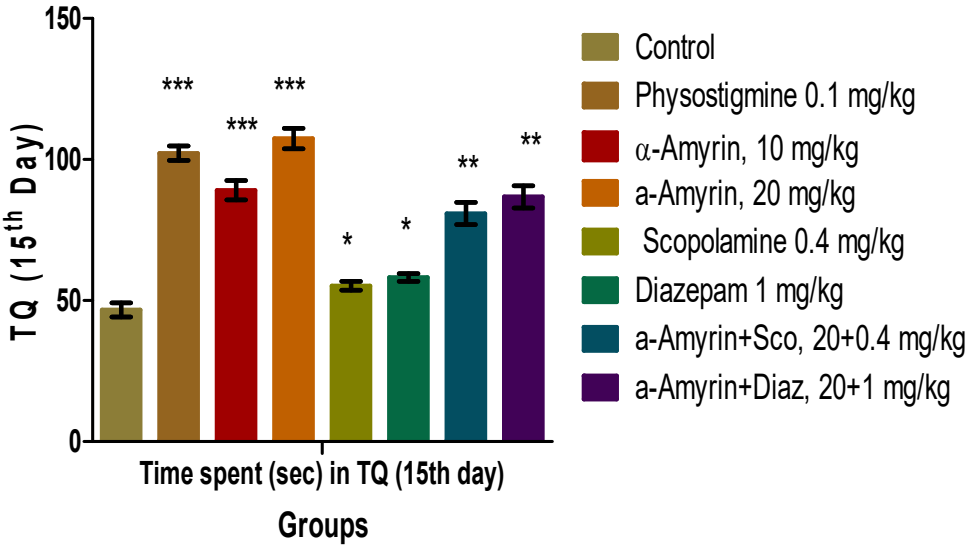

Figure No. 28: Effect of α-Amyrin on TQ on 15th Day

Effect of α-Amyrin on Locomotor activity of mice

There was a noteworthy transformation in Locomotor activity in mice treated by α-Amyrin (10 & 20 mg/kg) and physostigmine as compared to vehicle treated control,

Table No. 32: Effect of α-Amyrin on Locomotor activity of mice

Treatment Schedule	Locomotor activity counts / 5 min
Normal Control	210.40 ± 2.14
Physostigmine, 0.1 mg	410.15±2.31***
α-Amyrin, 10 mg/kg	288.36±3.63**
α-Amyrin, 20 mg/kg	332.64±3.66***

Values are expressed as mean± SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

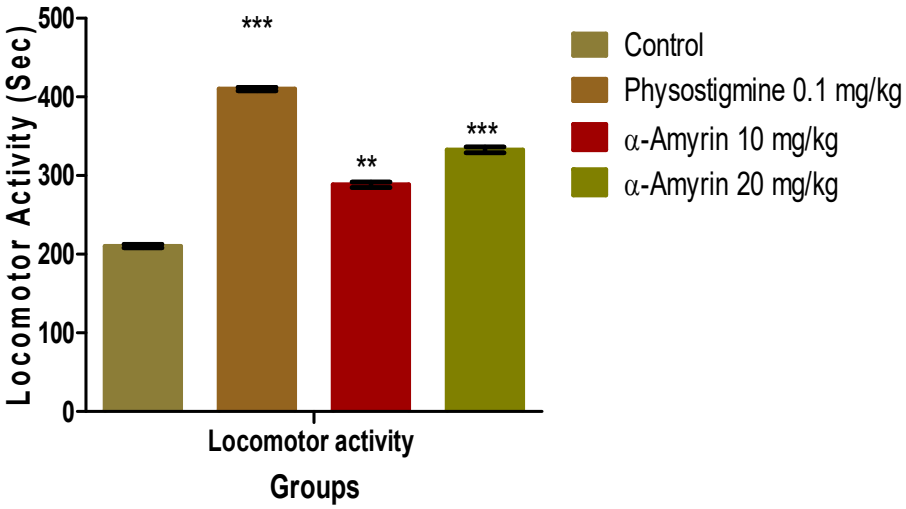

Figure No. 29: Effect of α-Amyrin on Locomotor activity in mice

Effect of α-Amyrin on brain Acetylcholine esterase activity of mice

Creatures treated by α-Amyrin and physostigmine for 15 progressive days made an imperative decline in mind Acetyl cholinesterase movement when contrasted with control bunch. Mice took care of with α-Amyrin in a portion of 20 mg/kg showed a really important diminishing impact on mind Acetyl cholinesterase action contrasted with rodents of control bunch.

Table No.33: Effect of α-Amyrin on brain Acetyl cholinesterase activity of mice

Treatment Schedule	Acetyl cholinesterase activity (mol/l per min × 10^{-6}/g of tissue)
Normal Control	0.068 ± 0.010
Physostigmine, 0.1 mg	0.021±0.007***
α-Amyrin, 10 mg/kg	0.032±0.014***
α-Amyrin, 20 mg/kg	0.025±0.012***

Values are expressed as mean±SEM, n=6 in each group; * p<0.05, compared to control ** p<0.01, compared to control. *** p<0.001, compared to control

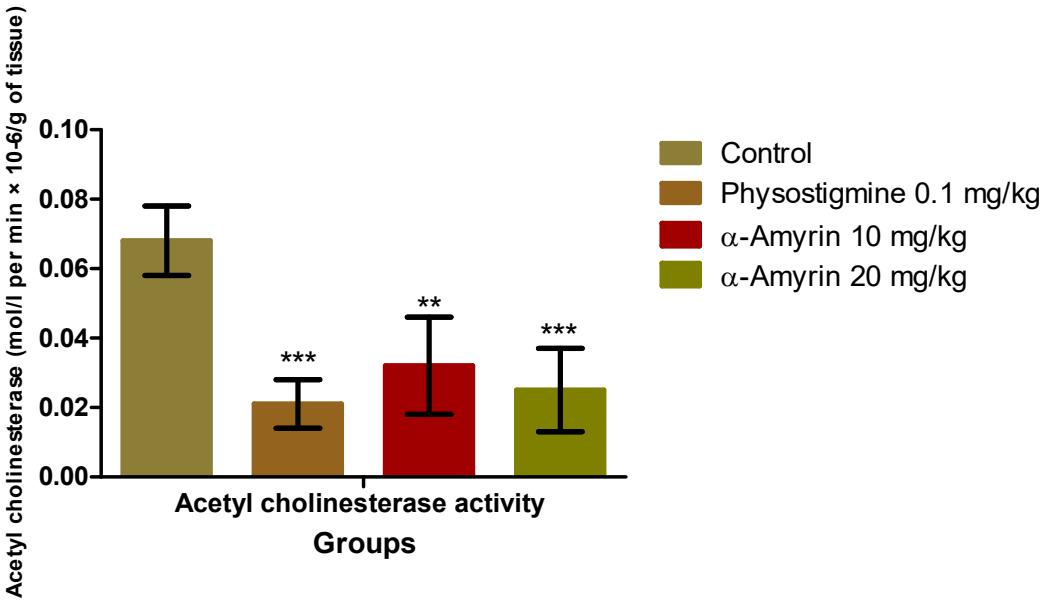

Figure No.30: Effect of α-Amyrin on Acetyl cholinesterase level in brain

Conclusion and Future Investigation:

The demand for herbal medication isolated from plant parts are being increasing to treat ample diversity of diseases, although relatively modest associate about their mode of action is existing. The Ayurveda had come in light and developed interest to researchers for phytopharmacological evaluation.
Hence, by keeping in view, the two plants i.e. *Adhatoda vasica* (Leaves) and *Vigna Mungo* (Seeds) were selected for evaluation of memory enhancing activity (Nootropics).
In our beginning study, the shed dried powder of both plants were extracted sequentially using petroleum ether, chloroform, methanol, butanol and water. After drying, it was evaluated for presence of various constituents. From study, we found the presence of various essential phytoconstituents which may responsible for memory enhancing such as terpenoids, alkaloids glycosides, phenolic compounds, flavonoids, tannins fatty acids and steroids. After that, antioxidant assays were carried out for different extracts by hydroxyl radical scavenging activity, lipid peroxidation and by DPPH methods

β-carotene bleaching inhibition method

In case of *Vigna Mungo*, we found that, among all extracts, chloroform extract showed best inhibitory activity. Then methanolic extract showed higher activity as compared to butanolic and water extracts. Vitamin E as standard was used in this assay and 84% inhibition was found to be at 30 minutes. Chloroform extract also showed 70% inhibition at 30 minutes which was reduced to 45% at the time of 120 minutes.

In case of *Adhatoda vasica*, the same i.e. chloroform extract showed best inhibitory activity. Then methanolic extract showed higher activity as compared to butanolic and water extracts. Vitamin E as standard was used in this assay and 84% inhibition was found to be at 30 minutes. Chloroform extract also showed 71% inhibition at 30 minutes which was reduced to 46% at the time of 120 minutes.

Hydroxyl Scavenging Activity

In case of *Vigna Mungo*, Hydroxyl radical scavenging capability calculated as that chloroform, methanol, butanol and water extracts having IC_{50} values of 47 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, respectively. From the observations, it was concluded that chloroform and methanol extracts possesses highest $OH^{\cdot}$radical scavenging activity when compared with water and butanolic extracts.

In case of *Adhatoda vasica*, IC_{50} reveals that chloroform; methanol, butanol and water extracts have IC_{50} values of 50 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, respectively. These observations implied that chloroform and methanol extracts have highest OH° radical scavenging capability as compared to butanolic and water extracts.

DPPH Free Radical Activity

In case of *Vigna Mungo*, DPPH scavenging ability calculated as IC_{50} shows that chloroform extract has IC_{50} of 36 µg/mL trail by ascorbic acid (3.2 µg/mL), gallic acid (3.5µg/mL), methanol (40µg/mL), butanolic (47µg/mL) and water (45µg/mL) extracts. The result revealed that chloroform extract had the highest DPPH scavenging ability.

In case of *Adhatoda vasica*, DPPH scavenging ability calculated as $IC_{50,}$ shows that chloroform extract has IC_{50} of 36 µg/mL followed by ascorbic acid (3.2 µg/mL), gallic acid (32µg/mL), methanol (40µg/mL), butanolic (42µg/mL) and water (44µg/mL) extracts. The result revealed that chloroform extract had the highest DPPH scavenging ability among all extracts.

Learning and memory are associated with escape latency (EL) and time spent in target quadrant (TSTQ). The *Vigna Mungo* produced decline of EL and augment of TSTQ by rodents in MWM indicated upgrading of learning and memory and vice-versa. Various extracts and Physostigmine (0.1mg/ kg, *i.p.*) given for 15 consecutive days appreciably decreased EL of mice from 11^{th} to 14^{th} day and augment TSTQ by mice on 15^{th} day as compared to the control, thus showed significant enhancement of learning and memory. Among all the extracts, chloroform extract showed an exceedingly noteworthy outcome on EL and TSTQ. Chloroform extract significantly declined ($P<0.001$) EL and significantly improved TSTQ as match up to vehicle treated control.

In our study, all the extracts in both doses were administered for 15 consecutive days significantly pick up learning and memory of rodents. Memory improving effects of extracts were comparable to physostigmine. MWM was used as a behavioral model for assessment of learning and memory. Chloroform extract of both plants significantly decreased EL during training and it significantly increased TSTQ during recovery, indicating enhancement of learning and memory.

The introductory phytochemical study showed the burly proof of alkaloids and triterpenes in chloroform extract. So, the presence of Plumbagin and α-amyrin were employed to detect the probable mechanism of memory enhancing.

Acetylcholine is measured as the very imperative neurotransmitter engaged in the functioning of cognition. In previous discussion regarding plumbagin, which is the main active constituent of chloroform extract may be responsible for enhancement of memory. This is due to its inhibition of acetyl cholinesterase which is accountable for augment in echelon of brain acetylcholine.

In case of *Adhatoda vasica*, chloroform extract also significantly reduced the cholinesterase enzymes in brain. From the phytochemical screening, it was cleared that chloroform extract shows the presence of Triterpenoids, and the α-amyrin is the main triterpenoids present in chloroform extract. So from study, it may be concluded that memory enhancing effect of chloroform extract may be due to availability of these triterpenoids.

In our study of *Vigna Mungo*, the vehicle-treated mice (10 ml/kg, p. o. normal saline) gave more time in closed arm and illustrated fewer entries in open arm as compared to closed arm of the maze in period of 5 min. Rodents treated with diazepam (1 mg/kg, p. o.) showed noteworthy ($P < 0.001$) augment in the proportion of open arms entries as well as time spent in open arm whereas, in closed arm number of entries and time spent were significantly ($P < 0.001$) decreased. The chloroform extract of *Vigna Mungo* in 200 and 400 mg/kg, p.o. respectively exhibited noteworthy ($P < 0.01$) boost in the percentage of number of open arm entries and time spent in open arm while, in the closed arm number of entries and time spent was significantly ($P < 0.01$) reduced as compared to vehicle-treated group.

In case of *Adhatoda vasica*, chloroform extract of *Adhatoda vasica* in 200 and 400 mg/kg, p.o. respectively

exhibited significant ($P < 0.01$) augment in the proportion of number of open arm entries and time spent in open arm whereas, in the closed arm number of entries and time spent was significantly ($P < 0.01$) reduced as match up to vehicle-treated cluster.

Bioactivity guided isolation was performed to split phytoconstituents from bioactive chloroform extract of *Vigna Mungo* by pertaining diversity of mobile phases.

Fractions (F2) eluted showing just one spot on TLC (*n*-hexane: chloroform, 9:1, R_f-0.88) were pooled and evaporated to aridness, yielding plumbagin. Subsequent to isolating active compound, melting point of plumbagin was traced by capillary tube technique. The spectral fortitude of compound was carried out by various spectral methods i.e. IR, NMR, Mass spectra were followed.

Bioactivity guided isolation was also performed for *Adhatoda Vasica* and fraction 104-123, eluted with Petroleum ether: Dichloromethane (9:1) were mixed together to give an active compound, which was crystallized out as compound on addition of a few drop of cold methanol.

In our investigations, mice treated by Plumbagin (1.5 & 3 mg/kg, *p.o.*) administered for 15 consecutive days appreciably upturned amnesia induced by scopolamine and diazepam in mice. The plumbagin (1.5 & 3 mg/kg) drastically reduced acetyl cholinesterase level in brain of mice as compared to the control.

In whole investigation, the behavioral model employed was Morris water maze for assessing memory and learning. The α-amyrin considerably picks up learning and memory of experimental animals in pertinent test along with decreased the altitude of acetyl cholinesterase enzyme.

The results and observations from this study have exposed that extracts and phytoconstituents exhibited significant memory enhancing activity.

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