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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *VIGNA RADIATA* STEM BARK EXTRACTS FOR ANTIAMNETIC ACTIVITY

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ABSTRACT

The present study reports physicochemical characterization, antioxidant and Antiamnetic activity of extracts from *vigna radiata* stem bark collected from local region of Nanded, Maharashtra, India. Different physical parameters like ash values, extractive value, Loss on drying, solubility etc were evaluated for powdered drug. The extracts were obtained from Soxhlet method by using ethyl acetate and methanol as solvents for extraction and subjected for preliminary physicochemical evaluation and antioxidant studies. Total phenolic and flavonoids content were also analyzed. The presence of primary and secondary metabolites such as carbohydrate, proteins, alkaloids, phenolic compounds, saponins was confirmed through preliminary phyto-chemical analysis. DPPH free radical scavenging assays showed strong antioxidant activities with increase in concentration of ethyl acetate and methanol stem bark extracts. Maximum percentage inhibition i.e. 80.97% was shown by ethyl acetate extract at concentration of 150 µg/ml and was compared with Ascorbic acid as reference standard. The *In-Vivo* Antiamnetic activity of *vigna radiata stem* bark was evaluated by radial arm maze model in rats using Piracetam as a standard. Scopalamine (1mg/kg) used as inducing agent. Both the extracts at 200mg/kg concentrations showed significant to highly significant number of entries & time spent in P zone (from $P < 0.05$ to $P < 0.001$). The result suggests that *vigna ridiata* stem bark extracts possess Antiamnetic avtivity and this might be due to flavonids. Phenolic compound, steroid and proteins present in extract.

Keywords: *Vigna radiata,* ethyl acetate and Methanolic extract, Phytochemical screening, Antioxidant effect, Antiamnetic activity.

I. INTRODUCTION:

Amnesia is when a person can no longer memorize or recall information that is stored in memory. It is very rare, despite being a popular theme for movies and books. Being a little forgetful is completely different to having amnesia. Amnesia refers to a large-scale loss of memories that should not have been forgotten.These may include important milestones in life, memorable events, key people in our lives and vital facts we have been told or taught.
A French psychologist Theodule-Armand Ribot was among the first scientists to study amnesia. Because of this, medical experts started to call the gradients of memory loss as Ribot gradients.

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Areas include the medial temporal lobe structures comprising the hippocampus, the amygdala, the entorhinal cortex and the perirhinal cortex. A second brain region associated with amnesia is the midline diencephalic region, which includes the mamillary bodies of the hypothalamus and the dorsomedial nucleus of the thalamus. Causes of damage to these diencephalic structures include stroke, brain tumours in this region [1].

In recent years, the physiological functionality of mung bean has received attention, particularly with respect to the content of anti-angiotensin I-converting enzyme and to antitumor, antioxidant, anti-diabetic, and anti-melanocyte effects. Mung bean starch is also considered to be the most suitable raw material for starch noodle-making. The Stem bark of *Vigna radiata* contain kaempferol, Rhamnetin, Kaempferitrin, Gallic acid, Vanillic acid, Scopolin, Rhododendrin, Ellagic acid, Quercetin, Quercetin 3-O-glucoside 4-Hydroxybenzoic acid, Syringic acid, p-Coumaric acid, Caffeic acid,Feruloyl glucose, Ferulicacid, Chlorogenic acid, Sitosterol ferulate [2].

The aim of the article is Phytochemical and Pharmacological evaluation of *Vigna Radiata* Stem bark extracts for Antiamnetic activity

II. MATERIAL AND METHODS:

1. Collection, identification and authentication of plant material

Fresh leaves were collected in the month of October from local region of Nanded district and the plant was authenticated by Dr. S. S. Bodke, Associate Professer & Head of Department of Botany & Horticulture, Yeshwant Mahavidyalaya, Nanded. A voucher specimen of plant was preserved in the herbarium (NPC/M. Pharm/herbarium/2019-20/H-4) for further reference. Collection, authentication, identification, processing and storage have been done according to standard procedure for the plant material.

Processing of crude drug:

The collected leaves were dried under shade, segregated and further crushed to coarse powder by mechanical grinder and the powder was passed through No. 14 sieve.

2. Preparation of Extracts:

Three extracts of *Vigna radiate bark* powder were prepared

1. Pet ether
2. Ethyl acetate extract
3. Methanol extract

The extract obtained and the dried mass was weighed and recorded. The percentage of yield was calculated.

$$\%\ \text{yield} = \frac{\text{Wt. of extract}}{\text{Wt. of powdered drug}} \times 100$$

A. Preparation of Ethyl acetate extract

Ethyl acetate extract of powdered leaves was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube.

300 gm of the powdered and 1000 ml Ethyl acetate was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

B. Preparation of Methanol extract

Methanolic extract of powdered leaf was prepared in Soxhlet extractor according to the standard method till colorless solution was observed in siphon tube. 150gm. of the powdered and1000 ml Methanol was used for extraction. After completion of extraction extract was cooled and dried. The extract was stored in air tight container till use. Percentage yield of extract was calculated.

III. PHYTOCHEMICAL EVALUATION:

A. CHEMICAL TEST [3]:

1. Detection of alkaloids:

Extracts were dissolved individually in dilute HCl and filtered.

Dragendorff's test:

To 2-3 ml Filtrate, add few drops of Dragendorff's reagent. Orange brown Ppt. formed indicates the presence of alkaloids.

Hager's test:

To 2-3 ml Filtrate Hager's reagent Formation of yellow precipitate indicates the presence of alkaloids

Tannic acid test:

Test solution treated with tannic acid solution gives buff colored precipitate the presence of alkaloids.

2. Detection of proteins & amino acid:

Million's test:

Mix 3 ml test solution with 5 ml Million's reagent. White precipitate warm precipitate turns brick red precipitate dissolves giving red colored solution indicates the presence of protein.

Ninhydrin test:

To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

Biuret test:

To 3 ml test solution adds 4% NaOH and few drops of 1% Copper sulphate solution. Violet color appears.

3. Detection of carbohydrates:

Extracts were dissolved individually in 5 ml of distilled water and filtered. The filtrates were used to test for the presence of carbohydrates

Molish's test:

Filtrates were treated with 2 drops of alcoholic α - naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Doi: 10.2016-37245516/; DOI Link:: http://doi-ds.org/doilink/08.2020-49964865/

Barfoed's test:
Mix equal volume of Barfoed's reagents and test solution. Heat for 1-2 min in boiling water bath and cool Red precipitate is observed

Benedict's test:
Filtrates treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's test:
Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A &B solutions. Formation of red precipitate indicates the presence of reducing sugars.

4. **Detections of glycosides:**
Extracts were hydrolysed with diluted HCl, and then subjected to test for glycosides.

Modified Borntrager's Test:
Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink color in the ammonical layer indicates the presence of anthranol glycosides.

5. **Detection of tannins:**
To 2-3 ml of aqueous or alcoholic extract, add few drops.

5% Ferric chloride test: deep blue – black color
Lade acetate sol. Test: White precipitate

6. **Detection of Flavonoids:**

Lead acetate test:
Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Shinoda test:
To dry powder or extract add 5 ml 95% ethanol few drops conc. HCL and .0.5 gm. magnesium turnings. Orange, pink, red to purple color appears. Add t-butyl alcohol before adding the acid to avoid accidents from a Violent reaction and magnesium, only flavones give a deep red to magenta color while flavones and flavones give weak pink to magnetic color is observed.

7. **Detection of phytosterols:**

Salkowski's test:
Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

8. **Detection of Saponin:**

Foam test:
Shake the drug extract or dry powder vigorously with water. Persistent foam observed indicates the presence of saponin.

IV. Development of TLC fingerprints [3]:

1. **Introduction:**
Thin layer chromatography is a method of analysis in which the stationary phase, a finely divided solid, is spread as a thin layer on a rigid supporting plate and the mobile phase, a liquid, is allowed to migrate across the surface of the plate by capillary action by gravity or pressure. TLC separation takes place in the open layer with each component having the same total migration time but different migration distance. Numerous fixed adsorbents have been used, including Silica gel, Cellulose, Polyamide, Alumina, Ion exchange and chemically bonded silica gel. Mobile phase consists of a single solvent or a mixture of solvents.

The stationary phase of the TLC is prepared using various techniques such as pouring, dipping and spraying. The prepared plates are allowed for setting (air-drying). This is done to avoid cracks on the surface of adsorbent. After setting the plates are activated by keeping in an oven at 100 to 120^{0}C for one hour. Activation of TLC plates is nothing but removing water/moisture and other substances from the surface of any absorbent, by heating at temperature around 110^{0}C so that adsorbent activity is retained. TLC studies were carried out using various extracts to confirm the presence of different phytoconstituents in the extract.

Analysis
In TLC qualitative analysis of the unknown compound is done by comparing the R_f values. As solutes never travel the full length of the stationary phase in TLC all the R_f value depends on the amount of the stationary phase, the humidity, layer thickness, solvent quality, saturation of chamber, development distance, temperature, amount of substance added, and the presence of impurities.

$$R_f = \frac{\text{Distance from origin to the point of maximum intensity}}{\text{Distance from origin to the solvent front}}$$

R_f = Retention factor

2. **Total Phenolic Content [4, 5]:**
Total Phenolic Content was determined by using the **Folin-Ciocalteu assay**. An aliquot (1m) of extract or standard solution of Gallic acid [2, 4, 6, 8, 10µg/ml] was added to 10 ml of volumetric flask, containing 9ml of distilled water. A blank reagent using distilled water was prepared. 0.5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 2 ml of 2% $NaHCo_3$ solution was added to the mixture. The volume was then made up to the mark. After incubation for 120 minutes at room temperature, the absorbance against the reagent blank was determined at 746 nm with an UV-Visible spectrophotometer.

3. **Total Flavonoids Content [5, 6]:**
Total Flavonoid Content was measured by the alluminium trichloride colorimetric assay. An aliquot (1ml) of extracts or standard solutions of Rutin (50, 100, 150, 200 and 250µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$,

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after five minutes 0.3 ml 10 % $AlCl_3$ was added. After five minutes, 2 ml 1M NaOH was added and the volume was made up to 10 ml with distilled water. The solution was mixed and absorbance was measured against the blank at 258 nm.

4. *IN VITRO* ANTI-OXIDANT ACTIVITY [6, 7]:

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.

2,2 Diphenyl- 1 picryl-hydrazylradical scavenging (DPPH) Activity:

Principle:

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a color change from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 1, 1- diphenyl-2-picryl-hydrazyl.

Reagents Required:

1) DPPH
2) Pure Methanol

Preparation of samples and standard solutions:

Accurately weighed 10 mg of Acetone and Methanolic extracts and the standard ascorbic acid and dissolved separately in 10 ml of phosphate buffered saline. These solutions were serially diluted with methanol to obtain the lower dilutions.

Procedure:

The reaction mixture (3.0 ml) consists of 1 ml of 0.1mM DPPH solution in methanol was mixed with 1 ml of drug solution and 1.0 ml of methanol. The reaction mixture was vortexes and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample was served as control.

The percentage of inhibition can be calculated using the formula:

$$\%\ \text{inhibition} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where,

$A_{control}$: Absorbance of control.

A_{test}: Absorbance of test.

V. Animal used:

Wistar rats of either sex, of weight 150-200gm were selected to carry out this study and it was carried after taking the consent and approval from the the institutional ethical committee [**1613/PO/Re /S/12/CPCSEA Dt. (9/2/2020)].**

Test group:

For the study seven groups of animals were made. Each group consisted with six rats.

Route of administration: Oral route and ip. Route of administration.

Housing Condition:

Animals were housed seven groups in separate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly. Animals were divided randomly into six treatment groups; each group consisting of six rats

Methodology:

The rats were fasted overnight and maintained 85% of its total diet weight. Animals were divided randomly into six treatment groups; each group consisting of six rats, each treatment group received orally the extracts of *Vigna radiata* stem bark in a dose of 100 mg/kg and 200mg/kg of both the extract as per the group. Extracts was given to rats, once daily for period of 8 days and daily evaluation was done. The end point will be taken as per the number of entries in P zone and time spent in P zone. The mean of number of entries and time spent in P zone for each group is calculated.

Evaluation

Evaluation was done on the basis of time spend in C zone and selected arms P zone and number of entries in C zone and selected arms P zone.

VI. Antiamnetic Activity [7].

In-vivo Antiamnetic activity of *Vigna radiata* steam bark was carried out by using radial arm maze. In which Piracetam was used as standard drug and the scopolamine was used for creating amnesia in wistar rat by imparing its memory. Test drug methanolic 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 43 cm long, 15 cm wide with 12 cm height and has small black plastic cups mount at 30cm from the central hub. Each rat maintain at 85% of its total diet weight was exposed to the maze with the food pellet in a fix arm followed by respective drug treatment for the period of 7 days. And on each day the evaluation was done.

The scopolamine test [8].

Experiments were carried out on adult albino rats. Albino rats (150-200gm) were use in these experiments.

Doi: 10.2016-37245516/; DOI Link:: http://doi-ds.org/doilink/08.2020-49964865/

Amnesia was induced by scopolamine injection (1mg/kg, i.p.) 30 min before the behavioral experiments. Rats were divided into seven groups.
Administration of Scopolamine and extract in experimental groups was done before the start of behavioral testing and during behavioral testing which provoke the cognitive impairment in rats.

VII. ANTIAMNETIC MODEL:

1. RADIAL ARM MAZE [9].

Rational and Purpose:

The radial arm-maze allows the study of spatial reference and working memory processes in the rat.
In reference memory procedures, information was useful for many sessions/days and may usually be needed during the entire experiment.
On the contrary, working memory procedures have a major temporal component as the information presented in the maze (arms baited) was useful for one session but not for subsequent ones; the rat has to remember the information during a delay interval (min. to hours).
Correct choices in the radial arm-maze were rewarded by food.

In vivo Antiamnetic activity:

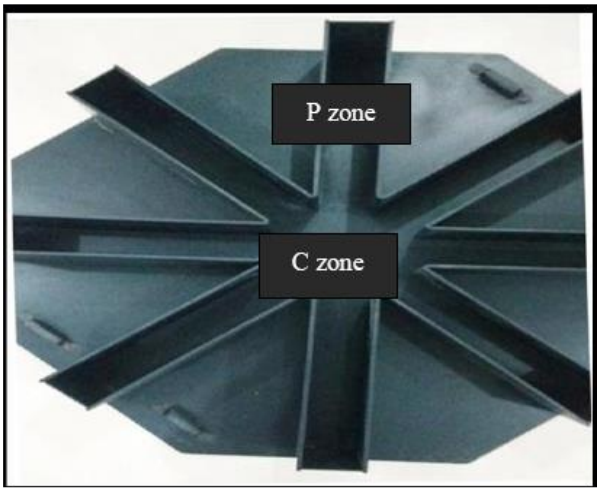

Fig. 1: Radial arm maze

Procedure:

The apparatus is a wooden elevated eight-arm radial maze with the arms extending from a central platform 26 cm in diameter. Each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm. Rats of either sex (150-200) gm were used.
The maze was well illuminated and numerous cues were present food pellets (reward) were placed at the end of the arms.
During the test, rats will be placed at the end of their bodyweights maintained at 85% of their free feeding weight to motivate the rat to run the maze. The animals were trained on daily basis in the maze to collect the food pellets.
The session was terminated after 8 choices and the rat has to obtain the maximum number of rewards with a minimum number of errors.
Following evaluation parameter consider for the evaluation of memory i.e Time spent in C zone and selected arms P zone and number of entries in C zone and selected arms P zone.

Evaluation:

The evaluation was carried out on 8th day 24 hr after the respective drug treatment where food pellets were place in variable arm for evaluation of working memory.
The Number of errors (Entries to non-baited arms) was counted during the session.

Animal Grouping and drug administration:

Wistar rats of either sex weighing 150-200gm., obtained from animal house of college.

The Animals were randomly divided into seven groups of six animals in each as follows-

Table 1: Grouping of animals

Sr no.	Groups	Animals (*Wistar rats*)
1	Control group (Vehicle-treated)	Six rats
2	Standard (Piracetam 200 mg/kg ip)	Six rats
3	Scopolamine (1mg/kg ip)	Six rats
4	*Vigna radiata* Ehtyl acetate extract + Scopolamine (100mg/kg orally)	Six rats
5	*Vigna Radiata* Ethyl acetate extract + Scopolamine (200mg/kg orally)	Six rats
6	*Vigna Radiata* Methanolic extract +Scopolamine (100mg/kg orally)	Six rats
7	*Vigna Radiata* Methanolic extract+Scopolamine. (100mg/kg orally)	Six rats

VIII. STATISTICAL ANALYSIS

The data were expressed as mean + standard of mean (SEM). Statistical analysis was performed by one way analysis of variance (ANOVA).

Doi: 10.2016-37245516/; DOI Link:: http://doi-ds.org/doilink/08.2020-49964865/

IX. RESULTS:

1. Phytochemical tests of *Vigna radiata* stem bark extract:

Table 2: Observations for Phytochemical qualitative analysis

Sr. no	Test for	Pet ether	Ethyl acetate	Methanol
1	Alkaloids			
	Dragendoff's	-	**+**	**+**
	Hager's test	-	**-**	**-**
2	Proteins			
	Millon's test	+	+	+
	Biuret test	-	+	+
3	Carbohydrate			
	Molish's test	+	+	+
	Barfoed's test	+	+	+
	Benedicts test	+	+	+
	Fehling's test	+	+	+
4	Glycosides			
	Borntrager's test	-	-	+
	Keller killani test	+	+	+
5	Tannins and Phenolic comp.			
	Ferric chloride test	-	-	+
	Lead acetate sol^{n}test:	-	-	-
	Dil.Nitric acid test	+	-	+
6	Flavonoids			
	Alkaline test	-	+	+
	Shinoda test	-	+	+
8	Steroids			
	Salwoski test	-	+	+
	Libberman test	-	-	+
9	Amino acid			
	Ninhydrin test	+	+	+
	Tyrosin test	+	-	-
	Million's test	-	+	+

A-Acetone Extract

B-Methanolic Extract

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Fig. 2: TLC plates of A -Ethyl acetate and B-Methanolic extracts

Table 3: Results of TLC profile of extracts:

Sr. No.	Extracts	Solvent systems	Proportions	Spraying Reagent	R_f Values
1.	Ethyl acetate extract	Ethyl acetate:Methanol	(1:1)	Sulphuric acid	0.43 0.30 0.18
2.	Methanol extract	Ethyl acetate:Methanol	(1:1)	Sulphuric acid	0.71 0.60 0.34

Table 4: Total phenolic content and flavonoid content of *Vigna radiata* stem bark extracts

Sr. No.	Conc. µg/ml	Extracts	Phenolic content (mg GAE/g DW)	Flavonoid content (mg RE/g DW)
1	100	Petroleum ether	26.77 ± 0.18	39.00 ± 0.19
2	100	Ethyl acetate	33.42 ± 0.37	51.44 ± 0.16
3	100	Methanol	58.39 ± 0.21	61.83 ± 0.60

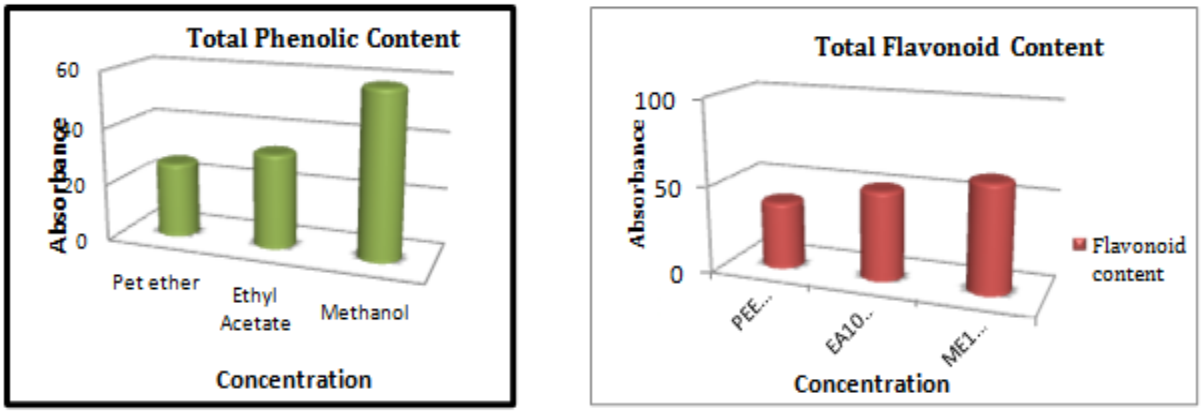

Chart 1: Total Phenolic and Flavonoid Content of *Vigna radiata* stem bark extracts

Doi: 10.2016-37245516/; DOI Link:: http://doi-ds.org/doilink/08.2020-49964865/

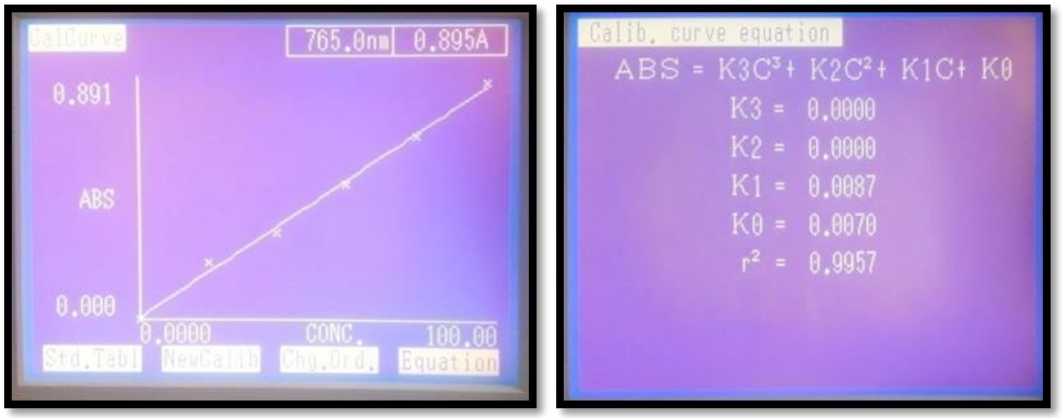

Fig. 3 Calibration Curve of Gallic acid

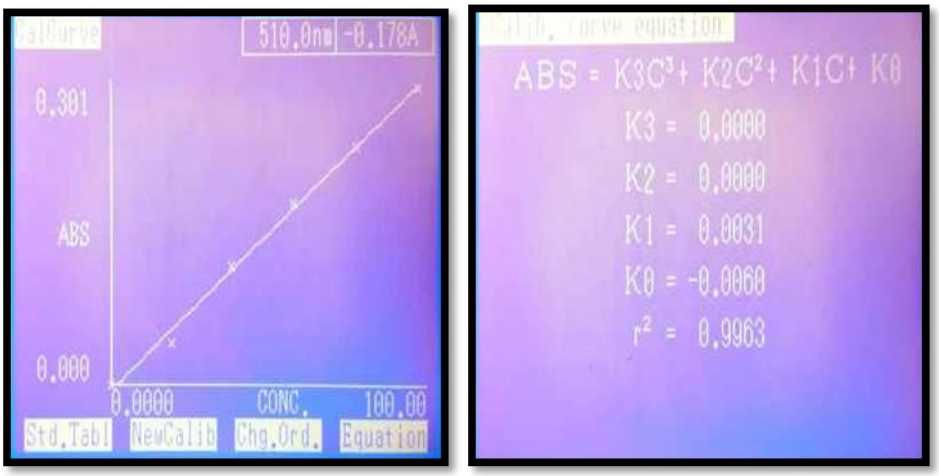

Fig. 4 Calibration Curve of Rutin

***In-vitro* Anti-Oxidant Activity**

The antioxidant activity of *Vigna radiata* was determined by *in-vitro* methods such as, DPPH free radical scavenging assay method. The assays were carried out in triplicate and average value was considered. The results were compared with Ascorbic acid as a reference standard.

Table 5: DPPH (2, 2-Dipheny1, 1-Picrylhydrazyl) radical scavenging activity

Sr. No.	Concentration in µg/ml	Ascorbic acid % inhibition	Gallic acid % inhibition	Rutin % inhibition
1	25	62.62 ± 0.23	41.06 ± 0.33	41.47 ± 0.19
2	50	73.51 ± 0.22	54.00 ± 0.26	51.54 ± 0.17
3	75	81.93 ± 0.21	76.18 ± 0.27	74.53 ± 0.25
4	100	87.88 ± 0.04	80.90± 0.27	79.05 ± 0.31
5	125	95.07 ± 0.25	91.17 ± 0.28	93.42 ± 0.31

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Table 6: Comparative DPPH Scavenging assay method of *Vigna radiate*

Sr. No.	Conc. µg/ml	Petroleum ether % inhibition	Ethyl acetate % inhibition	Methanol % inhibition	Ascorbic acid % inhibition
1	25	53.62 ± 0.19	56.11 ± 0.35	58.34 ± 0.35	62.62 ± 0.23
2	50	60.93 ± 0.36	59.89 ± 0.32	69.84 ± 0.06	73.51 ± 0.22
3	75	68.84 ± 0.27	76.31 ± 0.36	79.08 ± 0.25	81.93 ± 0.21
4	100	74.00 ± 0.35	82.88 ± 0.34	86.26 ± 0.31	87.88 ± 0.04
5	125	78.01 ± 0.28	88.90 ± 0.33	90.89 ± 0.31	95.07 ± 0.25

DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity Concentration Vs % inhibition

The grapgh reveals that all among all 3 extracts of *Vigna radiata* Stem bark, Methanol extract have comparable percent DPPH scavenging activity (90.89%) in comparison to standard ascorbic acid (95.07%). Methanolic extract shows better activity than the petroleum ether & Ethyl acetate extract.

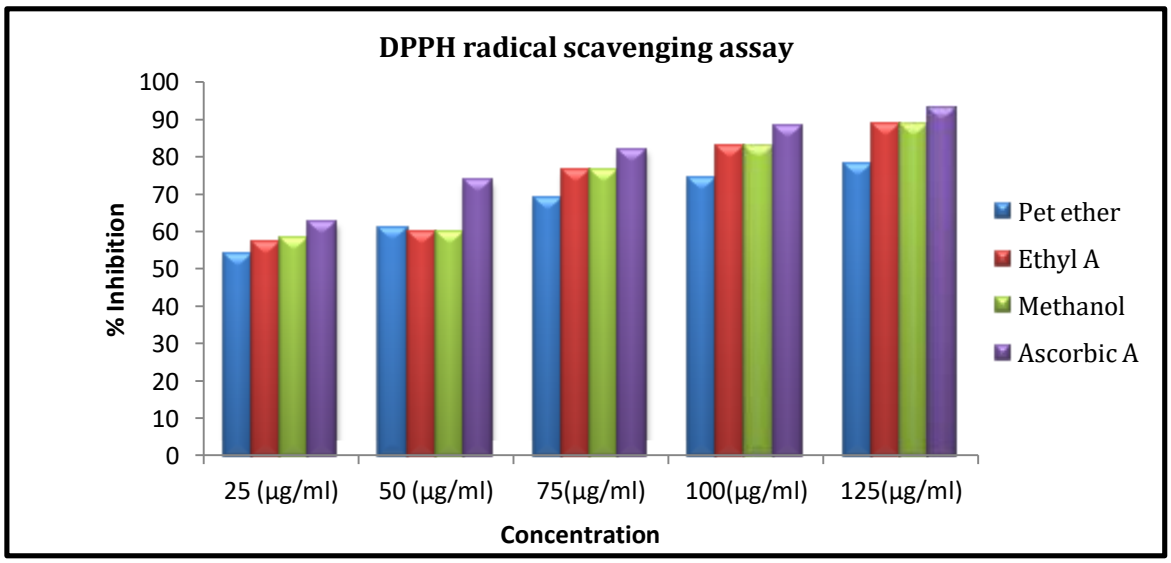

Chart 2: DPPH scavenging activity of *Vigna radiata* stem bark extracts

In DPPH scavenging activity, all the three extracts showed decrease in absorbance and increase in percentage inhibition as the concentration of extract was increased.

All three extracts showed better activity at 125mg/ml & Methanolic extract *of Vigna radiata* stem bark has the maximum (90.89% inhibition) anti-oxidant activity as compared to pet ether and ethyl acetate extract.

***In- Vivo* Antiamnetic activity:**

Number of entries in C and P zone of rats for Antiamnetic activity of *Vigna radiata* Ethyl acetate (VREA) and methanolic (VRME) stem bark extracts.

Table 7: Number of Entries in C zone

Sr. no.	Group name	Number of Entries in C zone	
		Day 1	Day 8
1	Std	12.16±0.47	10.5±0.42**
2	Ctrl	21.5±0.92	18.55 ±0.42
3	Scopolamine	11.3±1.4	12.66 ±1.211
4	VREA-100+Sco.	19.16±0.47	16.5±0.42*
5	VREA-200+Sco.	18.16±0.30	15.83±0.47*
6	VRME-100+Sco.	16.16±0.47	14.83±0.30**
7	VRME-200+Sco.	14.16±0.47	12.16±0.60**#

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 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, Δ-Significant difference when compared to Standard but more activity.

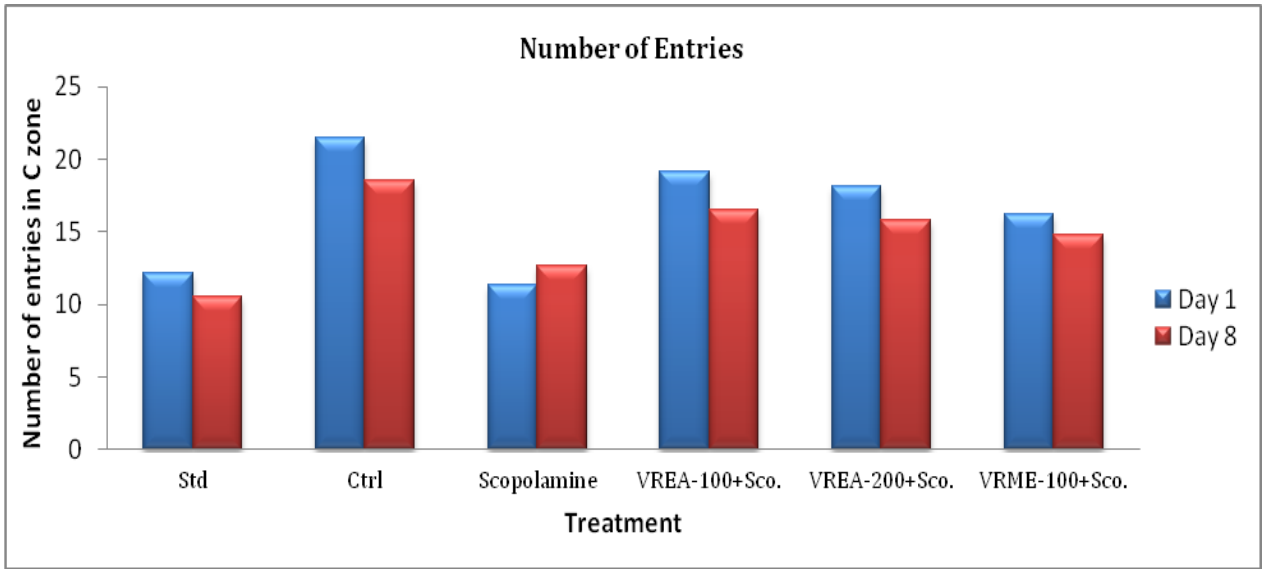

Chart 3: Number of entries in C zone of rats during experimental period

The number of entries in C zone on day 8 when compared with day 1 it was found that VREA-100, VREA-200 & VRME-100, VRME-200 shows no significant difference as compared to control but standard shows highly significant difference on day 8. All test doses shows no significant difference when compared with Standard.

Table 8: Number of Entries in P zone

Sr.no.	Group name	Number of Entries in P zone	
		Day 1	Day 8
1	Std	8.66±0.21	10.5±0.42**
2	Ctrl	3.5±0.22	3.83±0.16
3	Scopolamine	4.6 ±0.16	8.6±0.23
4	VREA-100+Sco.	4.66±0.21	5.5±0.22*
5	VREA-200+Sco.	5.16±0.30	6.33±0.22*
6	VRME-100+Sco.	7.16±0.30	7.33±0.42**
7	VRME-200+Sco.	8.16±0.30	9.16±0.47**#

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 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, Δ-Significant difference when compared to Standard but more activity.

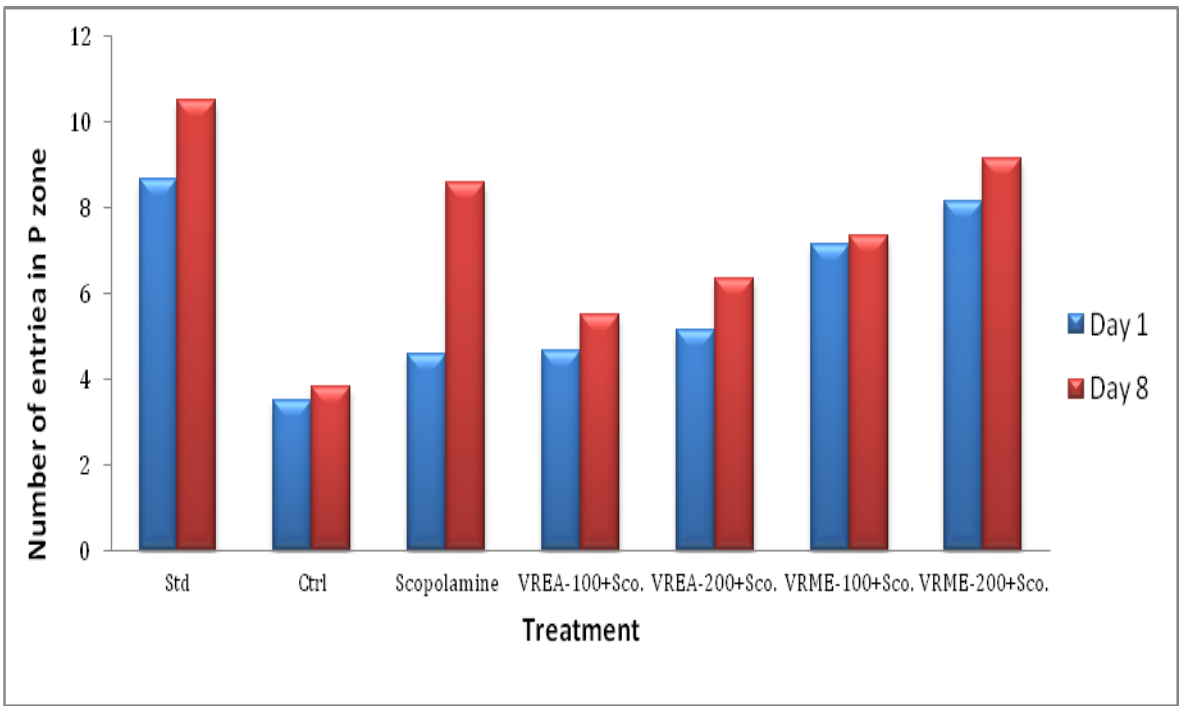

Chart 4: Number of entries in P zone of rats during experimental period

The number of entries in P zone on day 8 when compared with day 1 it was found VREA-100, VREA-200 & VRME-100, VRME-200 shows highly significant difference as compared to control (P<0.001). All test doses shows no significant difference when compared with Standard.

Table 9: Time spent in C and P zone of rats for Antiamnetic activity of *Vigna radiata* (Ethyl acetate and Methanolic) stem bark extracts.

Sr. No	Group name	Time spent in C zone	
		Day 1	Day 8
1	Ctrl	32.33±0.55	30.33±0.42**
2	Std	65.33±1.25	66.16±1.51
3	Scopolamine	26.5±1.61	32.66±1.3
3	VREA-100+ Sco.	46.16±0.47	53.16±0.54**
4	VREA-200+ Sco.	42.16±1.16	43.16±0.47**
5	VRME-100+ Sco.	40.5±0.76	35.16±0.47**
6	VRME-200+ Sco.	36±0.57	33.16±0.47**#

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 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, Δ-Significant difference when compared to Standardbut more activity.

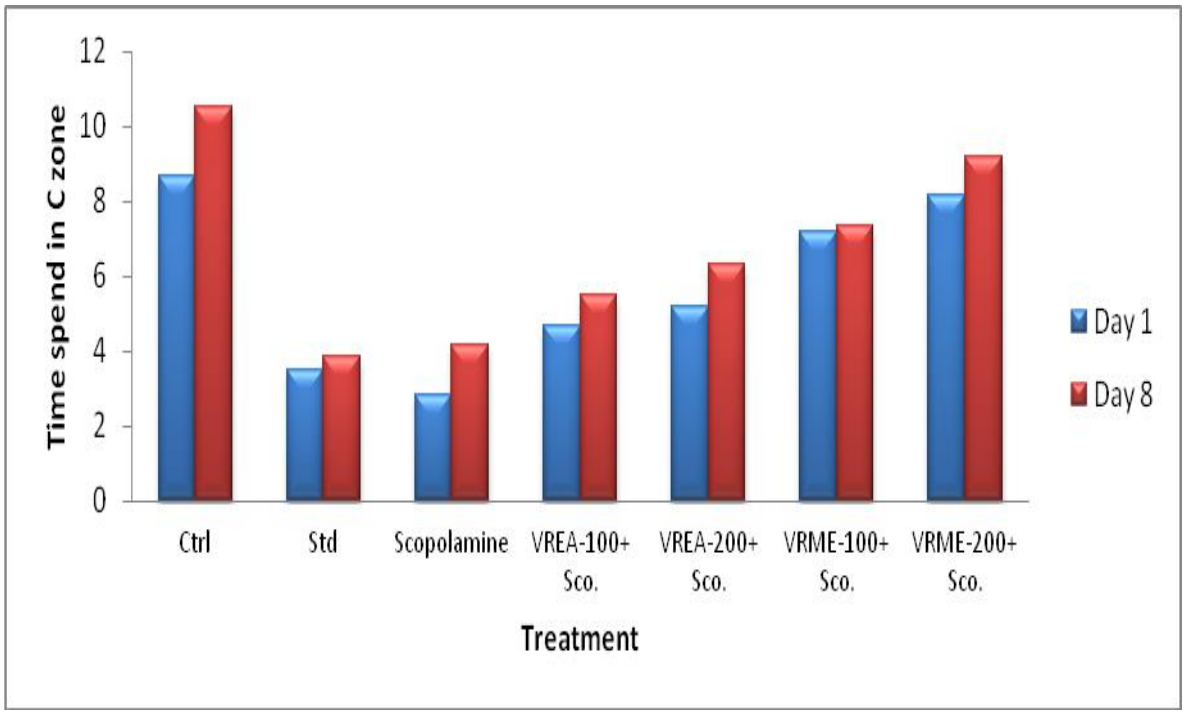

Chart 5: Time spent in C zone of rats during experimental period

The time spent in C zone on day 8 when compared with day 1 it was found that the VREA-100 and VRME-100 shows significant difference when compared to control(P<0.05) but VREA-200 & VRME-200 shows highly significant difference (P<0.001). All test doses shows significant difference when compared with Standard.

Table 10: Time spent in P zone

Sr. No	Group name	Time spent in P zone	
		Day 1	Day 8
1	Ctrl	145.83±0.87	170.33±1.72**
2	Std	112.33±1.58	137.33±0.66
3	Scopolamine	109.34±1.60	117.34±0.58
3	VREA-100+ Sco.	134.16±0.65	160.16±0.65**
4	VREA-200+ Sco.	138.16±0.47	163.16±0.65**
5	VRME-100+ Sco.	139.16±0.60	165.5±0.99**
6	VRME-200+ Sco.	143.66±0.71	167±0.57**#

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 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, Δ-Significant difference when compared to Standard but more activity.

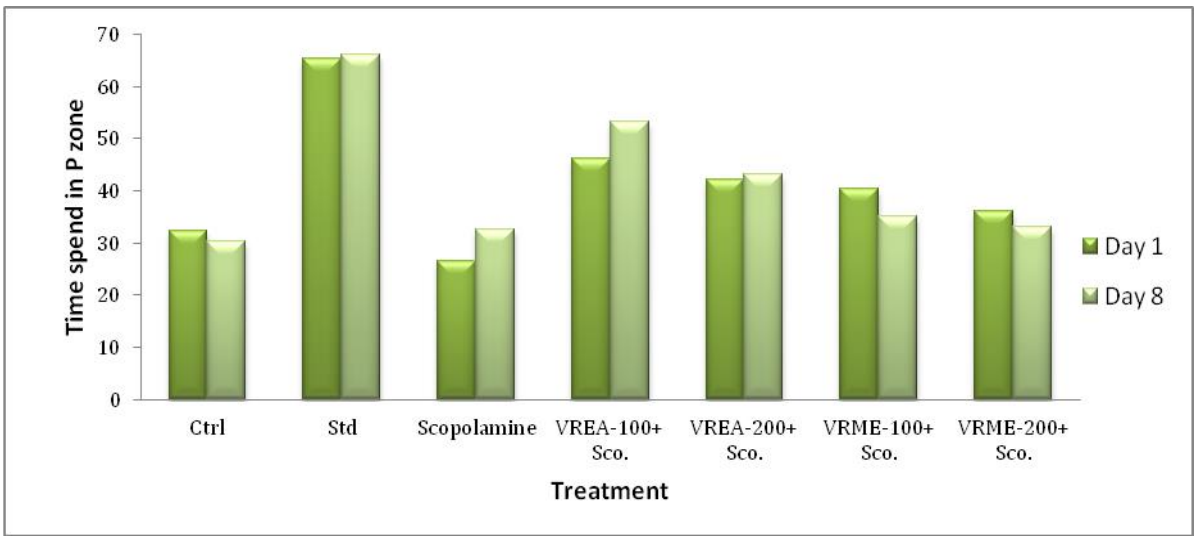

Chart 7: Time spent in P zone of rats during experimental period

The time spent in P zone on day 8 when compared with day 1 it was found that VREA-100, VREA-200 & VRME-100, VRME-200 shows highly significant difference as compared to control (P<0.001). All test doses shows no significant difference when compared with Standard.

X. DISCUSSION:

In the last two decades of the century, the scientists are sincerely trying to evaluate many plant drugs used in traditional system of medicine. Different parts of this plant have been reported to possessanti-inflammator, anti-oxidant hypolipidaemic, antiobesity and antimicrobial activity [10].The stem bark are cooling, emollient, anti-pyretic, hypoglycemic, diuretic, Laxative, digestible, anthelminthic, urinary concretions, sore throats, pain in the joints, flatulence throat. Traditionally it has been reported that, *Vigna radiata* stem bark may exhibit anti-inflammatory potential so it was selected for evaluation of anti-inflammatory studies [11].

Preliminary phytochemical evaluation of ethyl acetate & methanoli extracts was carried out for the determination of presence of phytoconstituents along with TLC fingerprinting. Both extracts showed presence of alkaloid, glycosides, tannins, carbohydrates, flavonoids, and saponins. The spots at R_f values (pet ether) 0.07, 0.25, 0.12 and (Ethyl acetate extract) 0.5, 0.37, 0.3, 1.1(Methanolic extract) 0.57, 0.61, 0.5represents the presence of kaempferol, dihydroflavonols, Vanillic acid, quercetin, Formononetin, in the extracts.

Antioxidant property of *Vigna radiata* stem bark extracts was carried out by using DPPH radical scavenging assay technique. In this method percentage inhibition of test sample was calculated and compared with percentage inhibition of standard (ascorbic acid). All among all 3 extracts of *Vigna radiata* stem bark, Methanol extract have comparable percent DPPH scavenging activity (90.89%) in comparison to standard ascorbic acid (95.07%). This provides evidence that Ethyl acetate and Methanol extract of *Vigna radiata* stem bark has potent antioxidant activity and it can be used as an antioxidant agent.

In-vivo Antiamnetic activity of *Vigna radiata* stem bark extracts was evaluated by using the Radial arm maze and by using Wistar rats as an animal model. The Antiamnetic activity of ethyl acetate and methanolic extracts of *Vigna radiata* 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. It is characterized by increase in latency to find the food and time spent in selected arm. The results were drawn by evaluating time spent & number of entries in P zone. The results showed that the highest dose (200mg/kg) of both the extracts, showed highly significant memory enhancing activity when given orally in daily single dose. The findings suggest effect of two different doses of both the extracts (100mg/kg and 200mg/kg) were probably mediated through ability of the animals to cause a significant decrease in number of errors and increase in latency to find the food and time spent in selected zone as well. At the end of the study it was observed that group no. 7 i.e. Methanol extract treated group at dose of 200mg/kg showed maximum number of entries at P zone **(9.16±0.47**#),** Group no.7 (Methanol at dose of 200mg/kg) and group no. 6 (Methenol at dose of 100mg/kg) represent **9.16±0.47**#, 7.33±0.42****for number of entries in P zone. Time spend of these groups at P zone were found to be **117.34±0.58, 160.16±0.65**, 163.16±0.65**, 165.5±0.99**, 167±0.57**#** respectively. 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 methanolic showed effective Antiamnetic activity. Although Methanolic extract at 200 mg/kg showed more superior and significant to highly significant (from $P < 0.05$ to $P < 0.001$) Antiamnetic activity by using radial arm maze in rats.

XI. SUMMARY AND CONCLUSION

Phytochemical study revealed that presence of Carbohydrates, Alkaloids, Glycosides, protein, phenol and flavonoid and was further confirmed by studying the TLC fingerprinting and Total phenol and flavonoid content. The results obtained in present study indicate that V.R. Eth. A. and V. R. Methanol. Extracts have significant free radical scavenging activity. The overall antioxidant activity of these extracts might be attributed to its flavonoids, phenolic and other phytochemical constituents. These could be source of natural antioxidant and have greater importance or showing oxidative stress related degenerative diseases.

Vigna radiata stem bark contain several chemical constituents which are pharmacologically important as they have been proved to be beneficial in many specific diseases like Depression, Anti-inflammatory, Anti-bacterial, Anti-oxidant, Anti-tumor, Anti-fungal, Antiviral and many microbial attacks where its Antiamnetic potential is claimed to be useful. The extracts of *Vigna radiate* stem bark tested for Antiamnetic activity by researchers. The present study aimed at evaluating the *In-vivo* Antiamnetic activity of *Vigna radiata* stem bark extract in rats. Ethyl acetate and methanolic extracts were prepared by the hot extraction process, i.e. by using Soxhlet apparatus.

The finding of the present study reveals that *Vigna radiata* stem bark has potent Antiamnetic activity. Further study is requiring evaluating the mode of action of Antiamnetic effect of *Vigna radiata* stem bark extracts.

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XIII. REFERENCES:

[1]. Abhinav Kanwal Jogender Mehla, Madhusudana Kunchal, Vegi Ganga Modi Naidul, Yogendra Kumar Gupta, Ramakrishna Sistla, Anti-Amnesic Activity of Vitex Negundo in Scopolamine Induced Amnesia in Rats Pharmacology & Pharmacy, 2010, 1: 1-8.

[2]. Kumar Ganesan; A critical review on phytochemical profile and health promotingeffects of *mung bean (Vigna radiata)*, Food Science and Human Wellness 2018, 7: 11–33.

[3]. Khandelwal K. R. Practical Pharmacognosy NiraliPrakashan, 21th edition, 2010, 25:1-25.9.

[4]. Biju J, Sulajman CT, Satheesh G, Reddy VR. Total Phenolics and Flavonoids in Selected Medicinal Plants from Kerala. Int J of Phar and Pharm. Sci., 2014, 6(1): 406-408.

[5]. US, Muhammad MA, Azwani MS, Nasyriq A. Determination of Total Phenolics, Flavonoids Content and Free Radical Scavenging Activities of Common Herbs and Spices. J of Pharmacognosy and Phytochemistry, 2014, 3(3): 104-108.

[6]. Dildar A, Muhammad MK, Ramsha S. Comparative Analysis of Phenolics, Flavonoids, Antioxidant and Antibacterial Potential of Methanolic, Hexanic and Aqueous Extracts from Adiantum Caudatum Leaves. Antioxidants, 2015, 4: 394-409.

[7]. Reena Randhir, Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated *mung bean* sprouts in response to peptide and phytochemical elicitors process biochemistry, 2004, 39: 637-646.

[8]. H. Gerhard Vogel, Drug discovery & evaluation, Pharmacological assays, II edition, 2002, 936-944.

[9]. Samira Rohanama Zhara Robiei, Zahra Alibabbaei et al. antiamnetic activity on C. aurantium flowers extract against Scopolamine-induced memory impairments in rats, Neural Sci., 2015, 6: 553-560.

[10]. Lee SJ, Lee JH, Lee HH, Lee S, Kim SH, Chun T, Imm JY. Effect of mung bean ethanol extract on pro-inflammtory cytokines in LPS stimulated macrophages. Food Sci Biotechnol., 2011, 20(2): 519–524.

[11]. Dongyan Tang, Yinmao Dong, Hankun Ren , Li Li and Congfen He. A review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts *Vigna radiate*, Tang *et al.* Chemistry Central Journal 2014, 3: 8-4.