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PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICALEVALUATION OF ANNONA SQUAMOSA L. STEM BARK EXTRACTS FOR MEMORY ENHANCING ACTIVITY

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ABSTRACT

The medicinal plant *Annona squamosa*L. is one of the traditionally important plant used for the treatment of various ailments. It belongs to the family Annonaceae. The synonyms of plant are Custard apple.The bark of the plant *Annona squamosa L.* is a powerful astringent & is stated to be given as a tonic. It is well distributed in tropical regions specially in India. This plant has been used as anti-epileptic, anti-helminthic, anti-bacterial, anti- spasmodic agent; it is also used in the treatment. The present study reports physicochemical characterization and memory enhancing activity ofextracts from *Annona squamosa* stem bark collected from local region of Nanded, Maharashtra, India. Different physical parameters like ash value, extractive value, loss on drying, solubility, etc. were evaluated for powdered drug.The extracts were obtained fromSoxhlet method byusing hot extraction method with the solvents pet-ether, methanol and ethyl acetate. Phytochemical standardization was undertaken todetect the presence of bioactive agents along with TLC and chemical tests.The *in –vivo*memory enhancing activity was evaluated by using radial maze arm and Morris water maze test in rats to measure the evaluation parameter of particular modal. From the acute toxicity studies as per reference standard and on the basis of the literature survey for ethyl acetate and methanolic extract of *Annona squamosa* extract of stem bark, maximum and minimum therapeutic experimental safe dose was found to be 200 mg/kg and 100 mg/kg respectively. The results suggest that ethyl acetate and methanolic extract of *Annona squamosa*L. stem bark extract possess memory enhancing activity, although themethanolic extract of *Annona squamosa* L. shows superior activity thanethyl acetate extract. This might probably due to presence of flavonoids and phenolic content and other phytoconstituents present in plant extract.

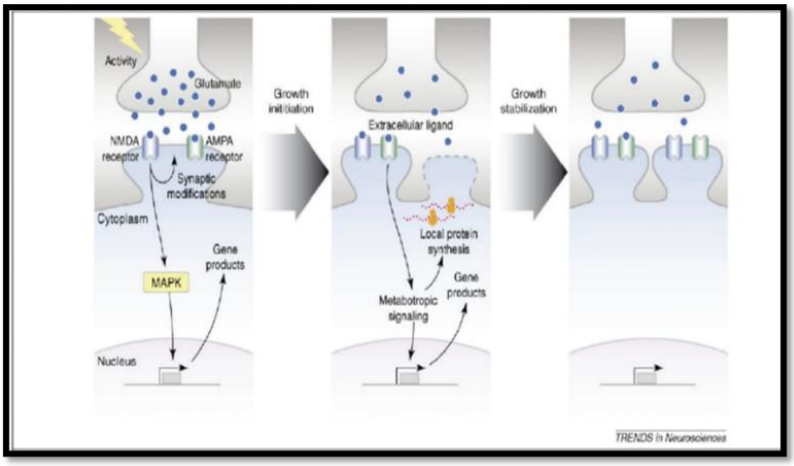
KEYWORDS: *Annona squamosa* L., Phytochemical Evaluation, Memory enhancing Activity.

INTRODUCTION

Learning is the process of an acquisition of information and skills, while subsequent retention of that information is called memory. Learning and memory together called as cognition. Memory is a fundamental mental process and without it we are capable of nothing. It is a faculty by which sensations, impressions and ideas are stored and recalled.

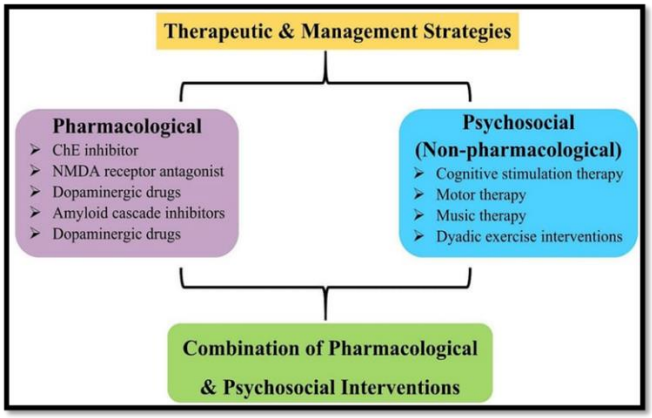
Learning and memory is one of the most intensively studied subjects in the field of neuroscience. Dementia (loss of memory) is a syndrome caused by disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, including memory, thinking, and orientation, and comprehension, calculation, learning capacity, language and judgment. Aging demographic transition is proceeding rapidly especially in India, china and Latin America, where dementia is rapidly becoming the major public health problem. Approximately 10% of the adults older than 65 years and 50% of the adults older than 90 years have dementia.

Mechanism of memory



Presynaptic activity that occurs with learning or stimulation leads to a release of glutamate onto NMDA and AMPA receptors, which depolarizes the membrane. This leads to a number of intracellular changes, including activation of transcription factors and translation of their downstream targets (left panel). These in turn lead to growth initiation, including protein synthesis, which then lead to the addition (middle panel) and stabilization (right panel) of new spines through insertion of new NMDAAMPA receptors. Targeting synaptic remodeling mechanisms to increase receptor insertion may be effective route for memory enhancement.

Treatment of memory



Processing of crude drug

The fresh stem barks of plant ***Annona Squamosa*** **L.** were subjected to shade drying and further crushed to coarse powder, and then the powder passed through mesh no. 14 and stored in airtight container for further use.

Crudedrug of Annona squamosa stem bark

Microscopic characteristics

Characters	Seeds	Leaves	Stem	Roots	Fruits
Colour	Black	Green	Green to Brown	Light brown - Dark brown	Greenish outside, whitish pulpy inside
Odour	Odourless	Characteristic	Characteristic	Odorless	Sweetish
Taste	Tasteless	Bitter	Slight Bitter	Bitter	Sweetish

Pharmacognostic evaluation of plant material

Extraction of plant material

Selection of solvent

On the basis extractive value and nature of phytochemical present in drug and literature review solvents were selected for the extraction of the Stem bark of *Annona Squamosa* L. like Petroleum ether, Ethyl acetate & Methanol.

Selection of extraction method

Study of literature survey revealed that most of the chemical constituents of the plant extract are heat stable and most of the researchers selected continuous hot extraction method for plant extraction, **Soxhlet extractor** is very essential with less time usage and with high efficiency, it issolvent penetrates faster to the plant and it is most convenient method. on that basis that **Soxhlet extraction method** was selected for extraction of stem bark powder of plant.

Extraction of *Annona squamosa* Linn of stem bark by using Soxhlet apparatus

Preparation of Ethyl acetate extract

Dried powdered plant was successfully extracted with Ethyl acetate by Soxhlet extractor apparatus according to the standard method till colorless solution was observed in siphon tube.

270 gm of the powdered plant and 1500 ml Ethyl acetate was used for extraction. After completion of extraction extract was cooled & evaporated by using Super fit Rotary evaporator. The extract was stored in air tight container. % Yield of extract was calculated.

powdered plant and 1500 ml petroleum ether was used for extraction. After completion of extraction extract was cooled & evaporated by using Super fit Rotary evaporator. The extract was stored in air tight container. % Yield of extract was calculated.

Preparation of Methanol extract

Dried powdered plant was successfully extracted with methanol by Soxhlet extractor apparatus according to the standard method till colorless solution was observed in siphon tube. 270 gm of the powdered plant and 1500 ml methanol was used for extraction. After completion of extraction extract was cooled & evaporated by using Super fit Rotary evaporator. The extract was stored in air tight container. % Yield of extract was calculated. The male and female wistar rats with 150-250 g body weight were selected for study by using memory enhancing models.

Animals used

Male and female wistar rats – 36

Route of administration:

Standard: Oral

Test: Oral

Grouping of animals

Animals Group Number	Drug & Dose
Group I	Animals were received CMC (0.5%)
Group II	Animals were received standard drug (Piracetam 200 mg/kg i.p.)
Group III	Animals were received 100 mg/kg dose of ethyl acetate extract of *Annona squamosa L.* stem bark (ASEAE 100 mg/kg oral)
Group IV	Animals were received 200 mg/kg dose of ethyl acetate extract of *Annona squamosa L.* stem bark (ASEAE 200 mg/kg oral)
Group V	Animals were received 100 mg/kg dose of methanolic extract of *Annona squamosa L.* stem bark (ASME 100 mg/kg oral)
Group VI	Animals were received 200 mg/kg dose of methanolic extract of *Annona squamosa L.* stem bark (ASME 200 mg/kg oral)

Grouping of animals

Memory Enhancing Activity:

1) MODEL-1 RADIAL ARM MAZE

Procedure

All animals were weighed up to 150-200g

The animals were train on daily basis in the maze to collect the food pellets

The animals were divided into 10 main groups, such as, control, standard and extract group with respective animals.

The control group received CMC the standard group receive the Piracetam (200mg/kg) and the extract group was receiving the extract of the plant with respective dose.

Food pellets (reward) place at the end of the arms. The animals were trained on daily basis in the maze to collect the food pellets the session was terminate after 8 choices. Rat was exposed to the maze daily with food pellets in a fixed arm following by respective drug treatment for the period of 7 days.

During the test, rats was feed once a day and their body weights maintained at 8 of their free- feeding weight to motivate the rat to run the maze.

The evaluation was carried out on 7th day 24 hr after the respective drug treatment where in food pellets was place in variable arm for evaluation of working memory. The rat has to obtain the maximum number of rewards with a minimum number of errors**. (Haans G vogel et.al 2007)**

Evaluation

The number of entries to baited arms was counted during the session.

No of entries in p zone.

MODEL -2 MORRIS WATER MAZE

Procedure

Wistar rat (150-200g) of approximately two months of age used as experimental animal.

The apparatus was a circular water tank filled to a depth of 20 cm with 18- 29°C water. Four points equally distributed along the perimeter of the tank serve as Starting locations.

The tank was divided in four equal quadrants and a small platform (19 cm height) was located in the center of one of the quadrants. The platform remains in the same position during the training days.

The rat was released into water and allowed 60-90 s to find platform. Animals usually receive 2-4 trials per day for 4-5 days until they escape onto the platform, well train rats escape in less than 10 sec.

Escape latency is recorded on the 6th to 9th day for each animal.

Afterwards on 10th day of drug administration the platform was removed and the rat was placed in any quadrant and allow to explore the target quadrant for 300 sec. The mean time spent in all the three quadrant was recorded. The mean time spent in target quadrant in search of missing platform was noted as an index of retrieval of memory. **(Haans G Vogel et.al 2007)**

Evaluation

The latency to reach the escape platform was measured during the training days.

The latency to reach the previous position of platform, the number of annulus crossing as well as the time the rat spent in the training quadrant was measured.

OBSERVATION & RESULTS

Observations for phytochemical qualitative analysis

Chemicaltests	Petether	Ethylacetate	Methanol
Test for Carbohydrate			
Molisch test	-	+	+
Fehling's test	-	+	+
Benedict'stest	-	-	+
Barford'stest	-	+	
Test for Proteins			
Biuret test	-	+	+
Millon's test	-	+	-
Test for proteinS.	-	-	+
Test for aminoacid			
Ninhydrin test (general test)	-	-	-
Test for Steroid			
Salkowski test	-	+	+
Liebermann Burchard Reaction	-	+	-
Test for Glycosides			
Legal's test	-	-	+
Keller-killiani test	-	+	+
Test for rsaponinGlycosides			
Foam test	-	-	+
Test for Flavonoids			
Shinoda test	-	+	+
Sulphuric acid test	-	+	+
Test for Tanninsandphenoliccompound			
Lead acetate test	-	+	+
5% Ferric chloride test	-	+	+
Dil. Iodine solution	-	-	-
Bromine water	-	-	+
Test for rAlkaloids			
Dragendroff's test	-	-	+
Wagner's test	-	+	+
Mayer's test	-	+	-
Hager's test	-	+	+

(+)Present, (-) Absent

Total phenolic content of AS stem bark extract

Sr. No.	Extract	Conc.µg/ml	Absorbance	TPC (mg/GAE/g)
1.	Petroleum ether	50	0.094±0.003	49.8
2.	Ethylacetate	50	0.111±0.002	58.8
3.	Methanol	50	0.139±0.004	73.7

Total Flavonoid Table content of AS stem bark extracts

Sr. No.	Extract	Conc.µg / ml	Absorbance	TFC(mg/QE/g)
1.	Petroleum ether	50	0.019±0.0017	42.6
2.	Ethylacetate	50	0.024±0.0029	53.9
3.	Methanol	50	0.034±0.0018	76.4

Pharmacological evaluation of *Annona squamosa* stem bark extracts

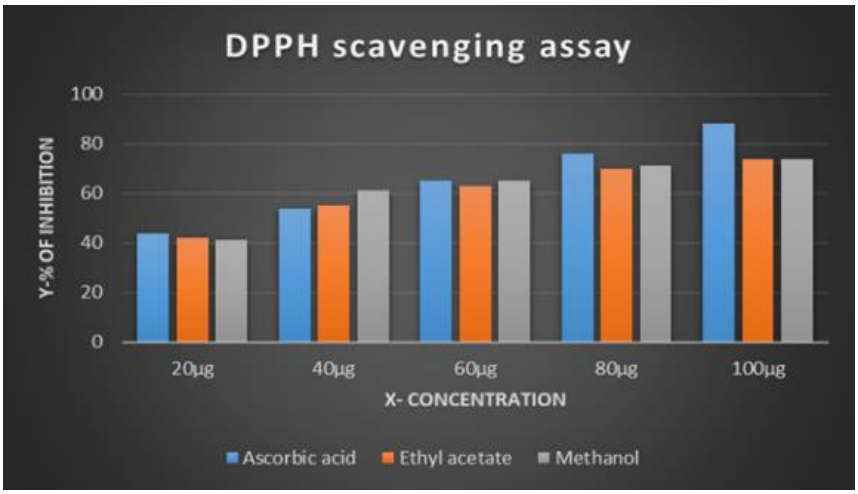
Safe dose calculation

The acute lethal study of *Annona squamosa* on rats shows that no animal died within 24 hours after treatment with the extract and the LDso was >5000mg/kg body weight. The biochemical analysis of AST, ALT, albumin and globulin shows no significant difference in any of the biochemical parameters examined in either the cor treated groups. damage to the tissue. Kidney congestion was seen in all rats treated with the extract. Extracts from *Annona squamosa* bark appear safer for usage in traditional medicine from the result of investigation. It is possible that variations observed were due to the quantitative variation of saponin in this plant parts reported that Annona squamosa is a common plant with many folklore claims, which has medicinal properties like antifertility, antitumor and antimicrobial activities in experimental mice and rats**. (Jamila salch*funsho et.al)**

Pharmacological screening of plant extracts for *In-vitro* anti oxidant activity

Table 16: (Absorbance of AS stem bark extracts at different concentrations for determination of Antioxidant activity)

Sr. No.	Conc. (µg/ml)	Absorbance		%Inhibition	
		Ethylacetate extract	Methanol extract	Ethyl acetate extract	Methanol extract
1.	20	0.474±0.0014	0.483±0.0017	42.12	41.02
2.	40	0.361±0.0008	0.315±0.0011	55.92	61.53
3.	60	0.296±0.0008	0.286±0.0011	63.85	65.07
4.	80	0.242±0.0017	0.234±0.0011	70.45	71.42
5.	100	0.209±0.0014	0.207±0.0005	74.48	74.72

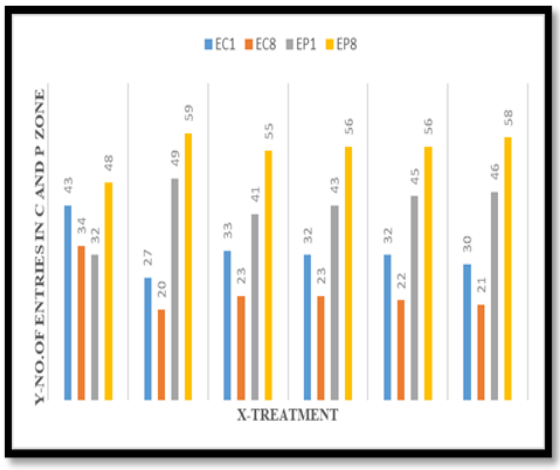


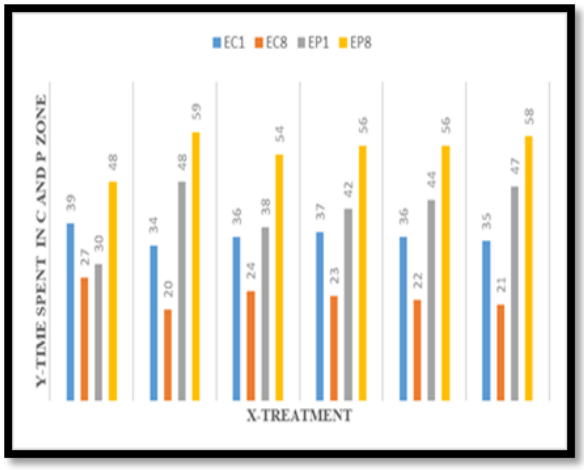
% of inhibition of AS stem bark extracts

***In-vivo* Memory enhancing activity**

❖ **Radial maze arm**

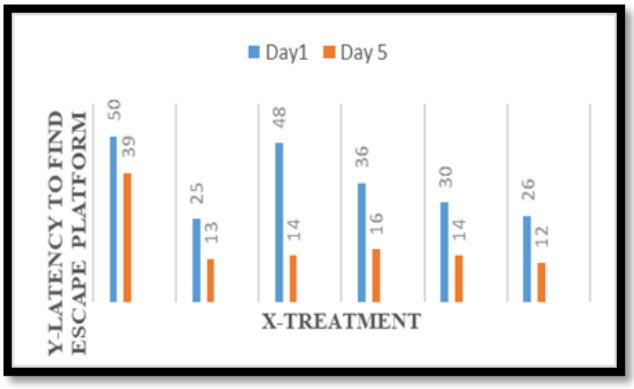
Group	Number of entries in				Time spent in			
	C Zone		P Zone		C Zone		P Zone	
	Day 1	Day 8	Day1	Day 8	Day 1	Day 8	Day 1	Day 8
Ctrl	43±0.408	34±0.516	32±0.557	48±0.494	39±0.516	27±0.477	30±0.557	48±0.494
Std	27±0.477**	20±0.365**	49±0.494**	59±0.428**	34±6.779**	20±0.365**	48±0.494**	59±0.428**
ASEAE-100	33±0.401**	23±0.210 **	41±0.365**	55±0.333**	36±0.333**	24±0.365**	38±0.428**	54±0.670**
ASEAE-200	32±0.223**	23±0.341**	43±0.333**	56±0.307**	37±0.333**	23±0.333**	42±0.428**	56±0.577**
ASME-100	32±0.333**	22±0.333**	45±0.333**	56±0.500**	36±0.428**	22±0.307**	44±0.666**	56±0.9545**
ASME-200	30±0.333**#	21±0.307**#	46±0.333**#	58±0.428**#	35±0.365**#	21±0.307**#	47±0.577**#	58±0.477**#

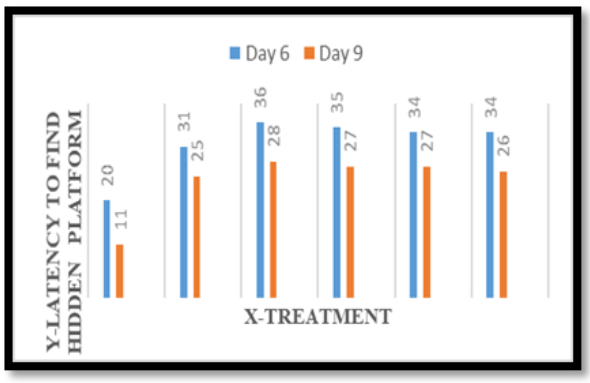


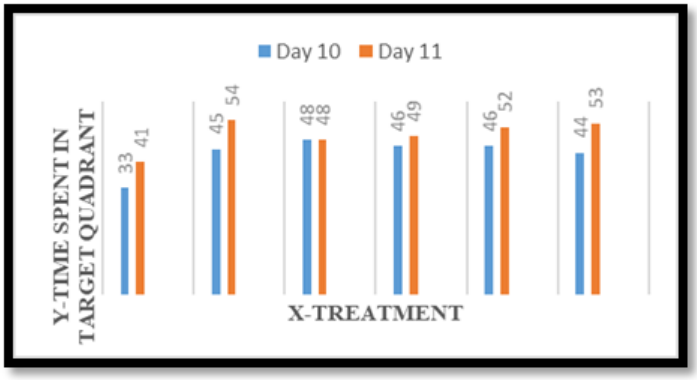


❖ **Water morris maze**

Group	Latency to find escape platfrom		Latency to find hidden platform		Time spent in target quadrant	
	Day 1	Day 5	Day 6	Day 9	Day 10	Day 11
Ctrl	50±0.600	39±0.477	20±0.477	11±0.557	33±0.365	41±0.494
Std	25±0.307**	13±1.335**	31±0.428**	25±0.365**	45±0.428**	54±0.333**
ASEAE-100	48±0.494**	14±0.477**	36±0.365**	28±0.307**	48±0.557**	48±0.421**
ASEAE-200	36±0.600**	16±0.619**	35±0.365**	27±0.428**	46±307**	49±0.333**
ASME-100	30±0.428**	14±0.210**	34±0.307**	27±0.365**	46±365**	52±0.365**
ASME-200	26±0.614**#	12±0.494**#	34±0.307**#	26±0.421**#	44±557**#	53±0.477**#







CONCLUSION

The method used to check the memory enhances potential of *AnnonasquamosaL*. stem bark extracts was Radial arm maze and Morris water maze as animal model. The study was done for 20 days and the parameter screened time in minute. The ASEAE and ASME at doses 100 mg/kg and 200 mg/kg and Piracetam 200 mg/kg all significantly improved memory compared with control 0.5% of CMC.

From the data obtained from the study, it was observed that group ASME-200 showed maximum entries in p zone i.e., 58±0.428**#. The same group showed maximum time spend in p zone 58±0.477**#. As well as time spend in p zone at dose ASME-100, ASEAE-200 And ASEAE-100 Represent 56±0.954**, 56± 0.577**, and 54± 0.670** respectively.

No. of entries in same group of animals in p zone at dose of ASME-100, ASEAE-200 and ASEAE-100 showed 58± 0.428**# ,56± 0.500** and 55 ±0.333**. All these values are compared with standard drug i.e piracetam 200 mg/kg.

Tha second modal is water maze the data obtained from the study revealed that the methanolic extract at dose 200 mg/kg showed decrease in latency to find escape platform**.** (12 ±0.494**#). The same group showed the time spend in target quadrant is (53±0.477**#). Where the other group that is ASME-100, ASEAE-200, and ASEA-100 exhibits 14±0.210**, 16±0.619** and 14±0.477** respectively. The time spend in target quadrant values are 52±0.365**, 49±0.333** and 48±0.421** respectively. All these values are compared with standard drug i,e piracetam 200mg/kg.

In-Vivo study has showed that ethyl acetate and methanolic extracts of *Annona squamosa* does possess significant memory enhancing activity with 100 mg/kg and 200 mg/kg. but high doses of the methanolic extract 200 mg/kg being more superior and showed significant to highly significant percentage inhibition (from $P < 0.05$ to $P < 0.001$) when compared with standard Piracetam. The finding of the present study revealed that *Annona squamosa* has potent memory enhancing activity.

So, it was concluded that the *Annona squamosa* L. stem bark extract can be one of the herbal remedies for the memory improvement.

REFERENCES

1. Abdalbasit, Adam Mariod et al., ("Antioxidant activity of different parts from Annona squamosa and Catunaregam nilotica methanolic extract", Acta Sci. Pol., Technol.Aliment, 2012; 11(3): 249-257.
2. Anuradha k et al, Assessment of memory deficits in psychiatric disorder: A systematic literature review .Journal of neurosciences in rural area practice, 2023; 15(2): 182-193.
3. Anshuman battachrya, Raja C., et al., The pharmacological properties of Annona squamosa linn, International journal of pharmacy & engineering, 2016; 4(2): 692-699.
4. Alok Nahata et al. Effect of *Convulvulus Pluricaulis* on Learning Behavior and Memory Enhancing Activity, Natural Product Research, 2008; 22: 1472-1482.
5. Arifia safira et al., A review of an important plant: Annona squamosa leaf, Pharmacogn J., 2022; 14(2): 456-463.
6. Arthur M. et al., what memory is for, Behavior and brain science, 1997; Vol.20: 1-55.
7. Carl F. Craver et al., Making of memory mechanism, Journal of the history of biology, 2003; Vol 36: 153-195.
8. Chengyao Ma et al., A review on Annona squamosa L. Phytochemical and biological activities, The American journal of Chinese Medicine, 2017; Vol. 45(5): 1-32.

9. C. K. Kokate, Pharmacognocy Vol- I & II, 47th edition, Nirali Prakashan, 2012; pp.7.9-7.11.
10. Debora O.D. Leite et al., A, Annona genus: traditional uses, phytochemistry and biological activities, Current Pharmaceutical Design, 2020; 26(33): 4056-4091.
11. Dinesh Dhingra et al., Memory enhancing activity of Glycyrrhiza glabra in mice, Journal of ETHNO-PHARMACOLOGY, 2004; 91(2): 361-365.
12. D.P.Singh et al., Anti-nociceptive and anti-inflammatory activity of Annona squamosa linn leaf extract in mice and rat, Research journal of pharmacognosy and phytochemistry, 2012; 4(3): 182-185.
13. EI Banna, H, et al, Some pharmacological and toxicological activities of Annona squamosa l. ethanolic extract, world journal of pharmacy and pharmaceutical sciences, 2016; 5(12): 188-198.
14. F. S. K. Barar, Essentials of Pharmacotheurapetics, S. Chand and Company Limited, New Delhi, 2000; pp. 83-90.
15. Gama mohan M.G. et al., Screening of antibacterial, antioxidant and phytochemical of leaf, stem and root extracts of Annona squamosa L. against pathogenic bacteria, Biological forum – An International Journal, 2023; Vol. 15(5): 960-969.
16. Hans Gerhard Vogel,"Drug discovery and evalution: Pharmacological assay (Drug effect on learning and memory) page no: 556-876.
17. Ivan Izquierdo et al., Separate mechanism for short and long term memory, Behavioral brain research, 1999; 103: pp 1-11.
18. Khandelwal K.R. Practical Pharmacognosy, Nirali Prakashan, 21st edition, 2010.
19. Kiranmai S. Rai, et al., Clitoria ternatea (linn) root extract treatment during growth spurt period enhances learning and memory in rats, Indian J Physiol Pharmacol, 2001; 45(3): 305-313.
20. Kokate C.K. et al., "A text book of pharmcognoy, 2004; 29: 317-318, 336-337, 542.
21. K D Tripathi et al., "Essentials of medical pharmacology" 6th edition, 2008.