RESEARCH ARTICLE

Evaluation of Anti-oxidant Activity of Different Extracts of *Plumbago zeylanica* Linn. & Development of Chromatographic Fingerprint of Chloroform Extracts

Kunal Gupta*, Naveen K Choudhary

Faculty of Pharmacy, Mandsaur University, Mandsaur, Madhya Pradesh, India

Received: 05th March, 2024; Revised: 26th May, 2024; Accepted: 03rd July, 2024; Available Online: 31st August, 2024

ABSTRACT

The aim of the present investigation is to Evaluation of Anti-oxidant Activity of Different Extracts of *Plumbago zeylanica* Linn. & Development of Chromatographic Fingerprint of Chloroform Extracts. The roots of *Plumbago zeylanica* Linn. were purchased from the local market. The roots were taxonomically identified by Senior Scientist at KNK college of Horticulture. Accurately weighed quantity of roots of *Plumbago zeylanica* Linn. were extracted using petroleum ether (only for removal of fats and lipids), chloroform, methanol, butanol and finally water by soxhlet apparatus for 72 h. Qualitative chemical tests of chloroform, methanol, butanol and water extracts were subjected to various chemical tests to detect various phytoconstituents. β-carotene oxidative bleaching in β-caroten/linoleic acid mixture with and without the addition of different extract of plant. DPPH free radical scavenging activity of the test solutions was determined using DPPH photometric method. Anisaldehyde sulphuric acid and vanillin sulphuric acid and heated at 115^0C for 5 minutes. Solvent systems; *n*-hexane: ethyl acetate, 7:3 were found to be most satisfactory solvent system for chloroform extracts for root respectively. Vitamin E as standard was utilized in this measure and 84% hindrance was viewed as at 30 minutes. Chloroform separate likewise showed 70% restraint at 30 minutes which was decreased to 45% at the hour of 120 minutes. Hydroxyl revolutionary ability to rummage determined as IC_{50} from the trial information and it uncovers that chloroform, methanol, butanol and water removes have IC_{50} upsides of 46 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, individually. DPPH scavenging capacity determined as IC_{50} and it shows that chloroform separate has IC50 of 36 µg/mL followed by ascorbic corrosive (3.1µg/mL), gallic corrosive (3.4µg/mL), methanol (41µg/mL), butanolic (46µg/mL) and water (44µg/mL) extricates. The present findings are momentous for the development of alternative, inexpensive and perhaps safer strategies for the handling of diseases.

Keywords: Anti-oxidant Activity, *Plumbago zeylanica* Linn., Chromatographic Fingerprint, Chloroform Extracts, *Bacopa monniera.*

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.25

How to cite this article: Gupta K, Choudhary NK. Evaluation of Anti-oxidant Activity of Different Extracts of *Plumbago zeylanica* Linn. & Development of Chromatographic Fingerprint of Chloroform Extracts. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1256-1261.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The Indian arrangement of medication is loaded down with restorative plant professed to advance learning memory and insight. The plants like *Bacopa monniera*, *Withania somnifera*, *Tinospora cordifolia* and *Acorus calamus* affect mental significance of the cerebrum. These plants have been assembled under the general class of Medhaya Rasayana that is substance/specialists that go against the degenerative changes related with maturing and are beneficial in advancing mind [1]. By and large, Physostigmine, the first of its sort Hurt inhibitor has been utilized to further develop memory and mental reason for patients with Promotion. In both present moment, long haul fake treatment prohibited examinations, Physostigmine extensively upgraded proportion of memory and cognizance. Be that as it may, the clinical worth of this specialist is diminished because of its short half life ($t_{1/2}$) of 30 min. It is guessed that almost three fourths of the home grown drugs utilized wide-coming to were found following leads from neighborhood medication. As indicated by WHO around 25% of current meds are plummeted from plants previously utilized customarily. Numerous others are manufactured analogs based on model mixtures confined from plants. Nearly, 70% present day meds in India are resulting from normal items[2]. The fundamental purposes of plants in medication will draw out from now on, as a wellspring of gainful specialists, and as unrefined substance base for the hauling out of semi-

*Author for Correspondence: lakhawatsudarshan@gmail.com

manufactured synthetic mixtures like beauty care products, scents and food ventures. Positive gathering of medical services plant-determined items has been followed to their expanding getting and use in the restorative business as well as to steadily expanding public costs in the everyday duration of individual wellbeing and prosperity. However the worth of natural requires extension of value wisdom in regard of the assessment related confirmations, providing the interest for botanicals and herbals is a functioning business. Vegetable oils are fundamental in social gathering worldwide dietary requests and are used for some food sources and other modern purposes [3]. The *Plumbago zeylanica* L. roots (Plumbaginaceae) is included in "Rasayana"- an Ayurvedic ancient system of medicine for rejuvenation. It also contains Plumbagin as active constituent which is alkaloid which may act as nervine tonic. The aim of the present investigation is to evaluate the antioxidant of different extracts and to develop the fingerprint chromatographic study of active extracts.

MATERIAL AND METHODS

Procurement of Plant Materials & Authentification

The roots of *Plumbago zeylanica* Linn. were purchased from the local market. The roots were taxonomically identified by Senior Scientist at KNK college of Horticulture. The herbarium sheets were submitted in Department of Pharmacognosy for the future as references.

Preparation of Extract by Hot Method

The roots of *Plumbago zeylanica* Linn. were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of roots of *Plumbago zeylanica* Linn. were extracted using petroleum ether (only for removal of fats and lipids), chloroform, methanol, butanol and finally water by soxhlet apparatus for 72 h. The extracts were dried completely under reduced pressure. After drying, the respective extracts were weighed and percentage yield was determined [4].

Preliminary Phytochemical Tests

Qualitative chemical tests of chloroform, methanol, butanol and water extracts were subjected to various chemical tests to detect various phytoconstituents [5, 6].

Evaluation of Antioxidant Acivity of Different Extracts

Lipid peroxidation inhibitory test

- *β-carotene inhibition method*

This assay is based on the capacity of β-carotene oxidative bleaching in β-caroten/linoleic acid mixture with and without the addition of different extract of plant, the method described by Kikuchi and Kitamura [7] with a slight modification.13 Briefly, 6.0 mg ß-carotene was dissolved in 10 ml of chloroform, then 1 ml of solution pipette to glass filled of 20 mg linoleic acid. 5 ml of mixture then pipette to reaction tube filled of extract in a range of concentration, mixed homogenously. Sample absorptions were conducted before and after incubation at 50^{O}C for 30, 60, and 120 minutes [8].

β-carotene bleaching inhibition percentage was calculated by the following formula:

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

Hydroxyl radical (OH) scavenging activities

One mL of the reaction mixture contained 100 µL of 2.8 mM 2-deoxyribose (dissolved in phosphate buffer (10 mM), pH 7.4), 500 µL solution of various concentrations of the extract (500n1000 µg/mL), 200 µL of 200 µM FeCl3 and 1.04 µM EDTA (1:1 v/v), 100 µL of H2O2 (1.0 mM) and 100 µL of ascorbic acid (1.0 mM). After incubation time of 1 hour at 37^{O}C, the amount of deoxyribose degradation was measured by TBA reaction [9]. The % inhibition of hydroxyl radical was calculated by using following formula.

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

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

DPPH free radical scavenging activity of the test solutions was determined using DPPH photometric method. When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced. The modification in color from deep violet to golden/light yellow can be measured at 518 nm. Briefly, 1 mL of 0.3 mM of DPPH solution was added to 1 mL each of the test solutions, and was incubated in the dark at room temperature for 30 min [9]. The absorbance values were read at 518 nm, and converted into percentage antioxidant activity, using the below mentioned formula:

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

Chromatographic Study

Chromatographic separation techniques are separation methods in which the components of an extracts are scattered between two phases, i.e. stationary and mobile. The separation process is based on adsorption or may be based on differences in the physico-chemical properties of the molecules such as size, mass, volume etc [4].

Thin Layer Chromatography (TLC)

Chromatographic separation of chloroform extracts of P. zeylanica Linn.

TLC for the separation of a range of bioactive compounds from chloroform extracts of root parts were developed to find out the possible number of compounds present in them.

A number of developing solvent systems were tried during the study, but solvent system showed best separations were used for the separation study. Each time plate was sprayed with Anisaldehyde sulphuric acid and vanillin sulphuric acid and heated at 115^{0}C for 5 minutes. Solvent systems; *n*-hexane: ethyl acetate, 7:3 were found to be most satisfactory solvent system

for chloroform extracts for root respectively. After development of plates, they were air-dried and number of spots, color and R_f values were recorded [4].

HPTLC of chloroform extract of P. zeylanica

The sample was dissolved in 10 ml of chloroform, sonicated for 10 minutes, filtered and applied on TLC plates (5×10 cm) in 2 tracks (2 µl and 4µl for chloroform extract) in the form of band. One side of twin trough chamber was charged with the solvent system *n*-hexane: ethyl acetate, 7:3 for chloroform extracts and allowed to equilibrate for 10 minutes. Plate was then scanned at 254 and 366 nm for chloroform extract. Photos of the plate were taken at 254 nm and 366nm. Number of spots, color, R_f values and % relative areas were recorded [10].

Statistical Analysis

The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test.

RESULTS

Extractive Value

Dried roots of *Plumbago zeylanica* Linn. were extracted using petroleum ether, chloroform, methanol, butanol and water (Table 1).

Preliminary Phytochemical Screening

The results are presented in the table 2.

Antioxidant Activity Evaluation

β-carotene inhibition method

The outcome shows that *Plumbago zeylanica* separate hindered β-carotene/linoleic corrosive oxidation and moderate movement occurring next to with extract fixation. In this movement, we found that chloroform extricate among all concentrates showed best inhibitory action. Then, at that point, methanolic remove showed higher action when contrasted with butanolic and water separates. Vitamin E as standard was utilized in this measure and 84% hindrance was viewed as at 30 minutes. Chloroform separate likewise showed 70% restraint at 30 minutes which was decreased to 45% at the hour of 120 minutes.

Table 1: Various concentrates with their % yield (in gm)

S. No.	*Extracts*	*Color of dried extracts*	*Consistency of dried extracts*	*% Yield (W/W)*
1	Chloroform extracts	Dark Green	Sticky	11 %
2	Methanolic extracts	Dark Green	Dried	12 %
3	Butanolic extracts	Dark Orange	Dried powdered	7 %
4	Water extracts	Dark Brown	Sticky	10 %

Table 2: Qualitative chemical investigation

S. No	*Phytoconstituents*	*Chemical Tests*	*Plumbago zeylanica Linn.*
1	Alkaloids	Dragendorff's test	+
		Mayer's test	+
2	Amino Acid	Millon's test	+
		Ninhydrine test	-
3	Flavonoids	Shinoda test	+
		Zinc hydrochloride test	_
4	Phenolics (Tannins)	Gelatin test	+
		Ferric chloride test	+
5	Protein	Biuret test	+
6	Triterpenoids & Steroids	Libermann-Burchard test	+
7	Anthraquinone glycosides	Borntrager's test	+
		Modified Borntrager's test	+
9	Coumarin glycosides	-------	-
10	Saponin glycosides	-------_____	+

Hydroxyl radical scavenging activity

Hydroxyl revolutionary ability to rummage determined as IC_{50} from the trial information and it uncovers that chloroform, methanol, butanol and water removes have IC_{50} upsides of 46 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, individually **(Figure 1)**. These outcomes suggested that chloroform and methanol separates have the most noteworthy OH^{o} extremist rummaging skills when contrasted with butanolic and water extricates. Since chloroform separate had extremely least IC_{50} when contrasted with different concentrates so it was considered as best concentrate for the further assessment.

DPPH scavenging activity

DPPH scavenging capacity determined as IC_{50} and it shows that chloroform separate has IC50 of 36 µg/mL followed by ascorbic corrosive (3.1µg/mL), gallic corrosive (3.4µg/

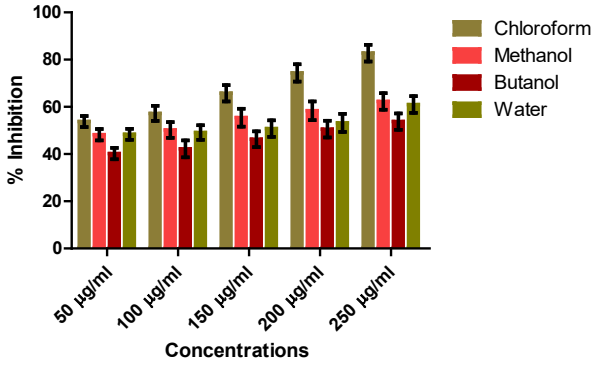

Figure 1: Effect of different extracts on Hydroxyl radical scavenging activity

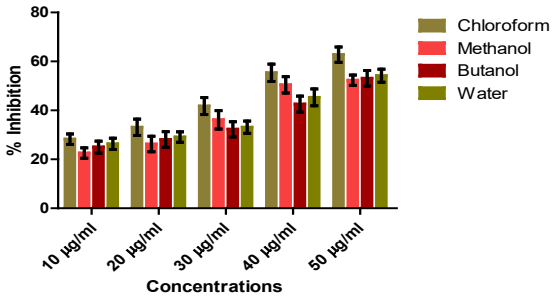

Figure 2: Effect of different extracts on Percentage DPPH scavenging activities

Table 3: Percentage (%) DPPH radical scavenging effect (standard)

S. No.	*Concentration (µg/mL)*	*Vitamin C (%)*	*Gallic Acid (%)*
1	2	47.44±2.24	43.76±3.51
2	3	49.67±3.11	48.98±3.71
3	4	62.89±3.29	55.25±3.45
4	5	70.11±3.72	60.76±3.22

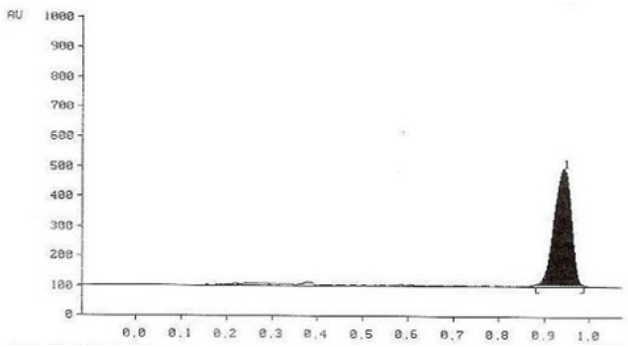

Figure 3: HPTLC of standard Plumbagin

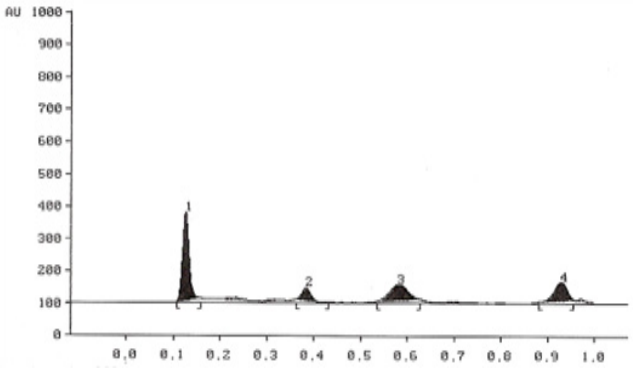

Figure 4: HPTLC of chloroform extract of Plumbago zeylanica Linn

mL), methanol (41µg/mL), butanolic (46µg/mL) and water (44µg/mL) extricates **(Figure 2 and Table 3)**. The outcome uncovered that chloroform extricate had the most elevated DPPH searching capacity.

HPTLC investigation of chloroform concentrate of Plumbago zeylanica Linn.

HPTLC finger impression of dynamic chloroform portion uncovered a few pinnacles. HPTLC profile of chloroform extricate under UV at 254 and 366 nm was recorded. The dynamic chloroform separate uncovered 4 significant spots, individually with Rf values in scope of 0.11 to 0.88 for 4 µL application volume separately **(Figure 3, 4)**. The virtue of test was affirmed by looking at the retention spectra toward the beginning, center and end position of the groups displayed in Table 4. The relating HPTLC chromatograms are addressed in figure and table.

DISCUSSION

Natural medications coming about due to establish eliminates are generally logically used to treat a wide assortment of disorders, though fairly honest accomplice about their technique for action is existing. There is a making interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine. In the preliminary survey, dried powders of picked plants were removed by using oil ether, chloroform, methanol, butanol in conclusion water. The concentrates were dried and assessed for the presence of various powerful constituents. The concentrates showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds, tannins, steroids and unsaturated fats.

Lipid peroxidation is an accumulated effect of responsive oxygen species (ROS), which prompts disintegrating of normal systems. It may be begun by brief free radicals, which eliminate an allylic hydrogen particle from a methylene social event of polyunsaturated unsaturated fat side chains. This is joined by bond defer that results in change by diene structure course of action. The lipid fanatic then, takes up oxygen to shape peroxy species[11]. Oxygen progressives and other responsive kind are made in regular structures either as symptoms of oxygen decline or by xenobiotic catabolism [12]. These ROS like superoxide anion (O2-), hydroxyl progressives (Charitable.), nitric oxide (NO) and peroxy progressive (ROO.) are inconsistent and can pursue key biomolecules like lipids, proteins and nucleic acids [13].

β-carotene whitening obstacle strategy was deliberate considering the limit of a malignant growth counteraction specialist to tone down orange assortment diminishing of

Table 4: Rf values and relative level of mixtures from chloroform extracts

S. No.	*Volume*	*Peak*	*Start R_f values*	*Start Height*	*Max %*	*End R_f*	*% Area*	*Assigned substance*
1	4 µL	1	0.11	273.4	65.82	0.16	48.08	Sub 1
2	4 µL	2	0.36	39.3	9.46	0.43	9.04	Sub 2
3	4 µL	3	0.54	47.5	11.42	0.63	23.32	Sub 3
4	4 µL	4	0.88	55.3	13.30	0.96	19.56	Sub 4

β-carotene as a result of the oxidation occurred in linoleic destructive/β-carotene mix [14]. β-carotene is especially open to free progressive outlined by linoleic destructive oxida¬tion. Linoleic destructive free radical shaped when gurgled will attract hydrogen particle of methylene diallylic, then, twisted peroxide cost progressive organizations con¬jugated twofold commitment of β-carotene which is answerable for its carotenoid orange assortment which range at 400-500 nm. Late assessments definite that lower limit of vitamin E achieve prevalent breaking down in lipid stage and more capable in shielding linoleic destructive [15].

Assuming that there ought to emerge an event of *P. zeylanica*, we found that chloroform separate among all concentrates showed best inhibitory activity. Then methanolic remove showed higher development when stood out from butanolic and water isolates. Vitamin E as standard was used in this action and 84% deterrent was seen as at 30 minutes. Chloroform eliminate moreover showed 70% deterrent at 30 minutes which was reduced to 45% at the hour of 120 minutes. Hydroxyl progressives are completely analyzed to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atoms from polyunsaturated unsaturated fat, which accomplishes peroxidic reactions of film lipids [16] and moreover, from all of the carbon molecule of the sugar moiety of DNA making oxidative pound up DNA. In case of *P. zeylanica*, Hydroxyl progressive capacity still up in the air as that chloroform, methanol, butanol and water eliminates have IC50 potential gains of 46 µg/mL, 100µg/mL, 200 µg/mL and 150µg/mL, independently. DPPH is a free limit predictable at room temperature, and produces a purple assortment game plan in methanol. It is united inside seeing cell support molecule, prompting a yellowish methanol plan. One of the parts stressed in cell support development measure is the wellness of a molecule to give a hydrogen bit to a progressive, and the propensity of the hydrogen gift is the essential variable drew in with free fanatic scavenging [17-19]. In case of *P. zeylanica*, DPPH not entirely set in stone as IC50 shows that chloroform remove has IC50 of 36 µg/mL followed by ascorbic destructive (3.1 µg/mL), gallic destructive (3.6µg/mL), methanol (40µg/mL), butanolic (4.6µg/mL) and water (4.4µg/mL) eliminates. The result uncovered that chloroform remove had the most raised DPPH scrounging limit. Bioactive chloroform concentrate of the two plants were then attempted artificially to know the presence of different compound constituents. Careful attention and HPTLC studies were in like manner performed to realize the amount of constituents present in both the chloroform eliminates and to spread out finger impression profile. In the ongoing assessment, phytochemical screening showed the presence of alkaloids and terpenoids in chloroform remove. Consideration revelations were in simultaneousness with the data of abstract engineered tests and the spots brand name for alkaloids and terpenoids were taken note.

CONCLUSION

The present findings are momentous for the development of alternative, inexpensive and perhaps safer strategies for the handling of diseases. Herbal medicines resulting from plant extracts are being increasingly utilized to treat a wide diversity of diseases, although relatively modest associate about their mode of action is existing. There is a creating interest in the pharmacological assessment of different plants utilized in Indian conventional frameworks of medication.

REFERENCES

1. Sharma M, Gupta YK. Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life Sciences. 2001;68(9): 1021-1029. https://doi.org/10.1016/S0024-3205(00)01005-5
2. Deb D, Nayak V, Bairy KL, Rao M, Shetty J, Hedge MV, Koshy SS. Antiamnesic and Neuroprotective Effects of Low Dose of Ramipril and Losartan in Scopolamine Induced Amnesia model of Dementia. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2013;4(1):1174-1182.
3. Idouraine A, Kohlhepp EA, Weber CW. Nutrient Constituents from Eight Lines of Naked Seed Squash (Cucurbita pepo L.). Journal of Agricultural & Food Chemistry. 1996;4:721–724.
4. Madaan R, Bansal G, Kumar S, Sharma A. Estimation of total phenols and flavonoids in extracts of Actaea spicata roots and antioxidant activity studies. Indian Journal of Pharmaceutical Science. 2011;73:666-669. doi: 10.4103/0250-474X.100242
5. Kokate, C.K., 1996, *Practical Pharmacognosy*. Delhi, Vallabh Prakashan.
6. Khandelwal, K.R., 2006. *Practical Pharmacognosy.* Pune, Nirali Prakashan.
7. Kikuchi A, Kitamura K. Simple and Rapid Carotene Bleaching Tests for the Detection of Lipoxygenase Isozymes in Soybean Seeds. Japanese Journal of Breeding. 1987;37(1):10-16.
8. Murdifin M, Pakki E, Alam G, Manggau AM, Muslimin L, Rusdi M, Wahyudin E. Lipid Peroxidation Inhibitory Activity *In vitro* of Mezzetia parviflora Becc. Wood Bark Polar extract. Pharmacognosy Journal. 2017; 9(2): 171-175. DOI:10.5530/pj.2017.2.28
9. Gupta S, Ahirwar D, Sharma NK, Jhade D, Ahirwar B. Effect of plumbagin free alcohol extract of Plumbago zeylanica Linn root on reproductive system of female wistar rats. Asian Pacific Journal of Tropical Medicine. 2011:978-984. https://doi.org/10.1016/S1995-7645(11)60230-7
10. Tambe VD, Bhambar RS. Estimation of total Phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* Linn. Wood Extracts. Journal of Pharmacognosy & Phytochemistry 2014;2:41-44.
11. Achliya SG, Barabde U, Wadodkar S, Dorle A. Effect of Bramhi Ghrita, an polyherbal formulation on learning and memory paradigms in experimental animals, Indian Journal of Pharmacology. 2004;36(3):159-162.
12. Adriana L, Da S, Angelo LP, Juliana GF, Barbara SM, Domingos S, Nunes and Elaine Elisabetsky. Promnesic effects of Ptychopetalum olacoides in aversive and non-aversive learning paradigms, Journal of Ethnopharmacology. 2007;109(3):449-457.
13. Ashwalayan VD, Singh R. Reversed Effect of Asparagus racemosus wild Root Extract Memory Deficits of Mice. International Journal of Drug Development and Research. 2011;3(2):314-323.
14. Kshirsagar SN. Nootropic Activity of Dried Seeds kernels of Caesalpinia crista Linn against Scopolamine Induced Amnesia in Mice. International Journal of Pharma Tech Research. 2011;3(1):104-109.

15. Kulkarni KS, Kasture SB, Mengi S. Efficacy study of *Prunus amygdalus* (almond) nuts in scopolamine-induced amnesia in rats. Indian Journal of Pharmacology. 2010;42(3):168-173. doi: 10.4103/0253-7613.66841
16. Lu SH, Wu JW, Liu HL. The Discovery of potential acetylcholinesterase inhibitors: A Combination of pharmacophore modeling, virtual screening, and molecular docking studies. Journal of Biomedical Science. 2011;18(8):1-13. https://doi.org/10.1186/1423-0127-18-8
17. Luiz FL, Claudia MS, Cassia VR, Larissa DB, Villas-Boas, Cid A. Moraes Santos and Rubia M.W. de Oliveira, Possible involvement of GABAA-benzodiazepine receptor in the anxiolytic-like effect induced by Passiflora actinia extracts in mice. Journal of Ethnopharmacology. 2007;111 (2):308-314. doi: 10.1016/j.jep.2006.11.021
18. Jawarkar SG, Game MD. Synthesis, Characterization and Antimicrobial Evaluation of New Substituted Thiazolidinones Bearing Triazole Moiety. International Journal of Pharmaceutical Quality Assurance. 2024;15(2):796-802. DOI: 10.25258/ijpqa.15.2.39
19. Bhadauria P, Rathore KS. Isolation and Identification of Phenolic Profiles in Selected Himalayan Wild Berries and Determination of their Antimicrobial Activity. International Journal of Pharmaceutical Quality Assurance. 2023;14(2):310-315. DOI: 10.25258/ijpqa.14.2.12