Original Article

An Evidence Based Phytochemical Analysis And In-Vivo Pharmacological Evaluation Of Amaranthus Spinosus Whole Plant Extract For The Enhancement Of Memory And Cognitive Behaviour

Arti Gautam[1], Annie Gupta[2]*, Lal Chand Pal[3], Lubna Azmi[4], ChV Rao[3]

[1]Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences (SHUATS), Naini, Prayagraj, Uttar Pradesh, India, (211007)
[2]Lloyd Institute of Management and Technology, Plot No.-11, Knowledge Park II, Greater Noida, Uttar Pradesh, India-201306
[3]Pharmacognosy and Ethnopharmacology Division, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India, 226001
[4]Institute of Pharmaceutical Science, University of Lucknow, Lucknow, Uttar Pradesh India, 226031

***Corresponding Author.**
1. Annie Gupta
Associate Professor Lloyd Institute of Management and Technology,
Plot No.-11, Knowledge Park II, Greater Noida, Uttar Pradesh, India-201306 Email:anniegupta0601@gmail.com
DOI: 10.47750/pnr.2022.13.S07.624

Abstract

Background: An increasing prevalence of disorders such as Alzheimer's is still to be addressed through effective synthetic-based therapeutics which are not hazardous.

Objective: The aim of this research was to study the phytochemicals, physiochemical and memory enhancing activity Amaranthus spinosus.

Material and Methods: At different doses of 250-2000 mg/kg body weight, an ethanolic extract of the whole plant was extracted and used for physiochemical, phytochemical, and acute oral toxicity studies. For memory enhancement evaluation, two doses (250 and 500 mg/kg body weight p.o) were given to each group of animals for seven days. Histopathology was also performed. Shankpushpi syrup 200 mg/kg body weight was taken as standard.

Results: A phytochemical investigation of the entire plant extract reveals tannins account for 6.07 percent, saponins for 53 percent, alkaloids for 13.14 percent, proteins for 16.76 percent, and glycosides for 63.2 percent. Total phenolic content (TPC) was 2.81 in GAE, g/100 g, and total flavonoid content (TFC) was found 18.4 QE, g/100 g extracts. The lethal dose (LD50) of A. spinosus ethanolic extract was determined to be >2000 mg/kg, showing that it would be very safe when given acutely. When compared to the control group (37.80±0.51, 705.75±12.56), the dose of ethanolic extract of A. spinosus 500 mg/kg p.o reduced the number of errors and latency (32.79±0.54, 469.91±10.29). In a histopathological investigation, the control group's brain section was compared to the treatment group's brain section, and there were apparent alterations in the number of pyramidal cells.

Conclusion: This study demonstrates memory enhancer potential of Amaranthus Spinosus extract.

Keywords: Amaranthus spinosus; amaranthaceae; alkaloids; glycosides; flavonoids; memory; shankhpushpi

INTRODUCTION

A. spinosus Linn. (Family: Amaranthaceae) is commonly referred to as "Kate Wali Chaulai (Kanatabhajii)" in tr aditional medicine. Amaranth is a worldwide genus of herbs that belong to the Amaranthaceae family1. Amaran thus extracts have been used to treat a variety of ailments in traditional Indian, Nepalese, Chinese, and Thai med icine, including urinary infections, gynaecological diseases, diarrhoea, pain, respiratory disorders, diabetes, and as a diuretic2. Amaranthus spp. is one of the plants whose natural crude extracts have been employed in traditio nal medicine to treat a variety of diseases, but its full restorative potential has yet to be discovered. With numerous evaluations revealing the nutraceutical qualities of Amaranth[3].In recent years, the focus of research on amaranthus as well as its health promoting properties has surged. Thousands of active bioactive molecules have been identified and evaluated for pharmacological activity in vivo and in vitro, such as hepatoprotective[4-5], antibacteria[l7], analgesic[8], anthelminitic[9], antimalarial[10], antideppresent[11], immunomodulatory[12], anti-peptic ulcer[13], antioxidant and chemoprotective activity[14] in the last decade. Alkaloids, terpenes, sugars andglycosides, were reported as the principal phytochemical constituents in the roots of A. Spinosus[15]. rutin[16-17], and, quericitin[18] is a lignan glycoside called amaranthoside. The amaricin-a coumaroyl adenosine stigmasterol glycoside[19] is a coumaroyl adenosine stigmasterol glycoside.

Around 24 million people worldwide suffer from dementia, with AD20 constituting the bulk of cases. Oxidative stress aggregation causes nucleic acid and protein destruction. Mitochondrial dysfunction in the brain leads to cognitive and neurological problems.In Spain, a study of 1,637 persons over the age of 64 was done to look into subjective memory issues (SMC). SMC was found in 524 people (32.4 percent). The prevalence of SMC is linked to cognitive performance, mood, sex, education, and age. SMC has been reported in 24% of adults between the ages of 65 and 69. People aged 90 and up have a 57 percent rise in SMC. In persons with anxiety and depression, SMC is 52.8 percent[21].Various plant extracts and their bioactivities with antiamnesic effects on diverse neurotransmitter systems have also been reported[22], mostly from in vitro or in vivo models. The purpose of this study was to see how an ethanolic extract of the whole plant of A. spinosus improved learning and memory in mice.

MATERIALS AND METHODS

Preparation of A. spinosus ethanolic extract from plant material: The plant of A. spinosus was collected from Andhra Pradesh in month of November 2016and identified by K. Madhava Chetty of Venkateswara University in Tirupati, India. The voucher specimen number is 1098. Plant materials were collected, separated into roots and stems, cleaned thoroughly in tap water until the soil was clear, and then dried in the open air. After three days, mechanical grinding was used to turn the dried materials into a powder. About 10 g of sample was mixed with 100 ml of ethanol analytical grade and distilled water and stirred continuously for 72 hours at room temperature using a rotator magnetic stirrer.The plant extracts were filtered twice or three times using no. 42 what man filter paper after three days. T he filtrate was collected into airtight, sterilised, and labelled bottles then stored at 4°C for future use.

Phytochemical analysis: Standard phytochemical screening protocols were used to examine tannins, alkaloids, glycosides, flavonoids, and phenolics. [23].

Qualitative analysis of phytochemicals

Alkaloids: The extract sample were warmed in a boiling water bath with 2 percent HCL, cooled, filter, and reacted with Mayer's reagent after the extract was evaporated by dehydration. Following that, the sample was inspected for any yellow precipitation or turbidity[23].

Flavonoids: To a total of 4 mL of extracts, 1.5 mL of 50% methanol was added. After heating, add magnesium powder and a few drops of strong HCL. The presence of flavonoids is indicated by a pink or red colour[23].

Tannins: A quantity of the extracted amount was diluted 1:4 with double distilled water, and a few drops of 10% ferric chloride solution were added. Tannins are indicated by a blue or green color[24].

Saponins: The heat was applied to a little amount of ethanolic extract. The mixture was filtered and 2.5 mL of the filtrate was mixed with 10 mL distilled water in a test tube, shaken vigorously for 30 seconds, and foaming was observed[23].

Glycosides:
Fehling's reagent was added to the ethanolic extract and heated for 2 minutes. The presence of glycosides is indi cated by a brick red hue.

Quantitative analysis, estimation of alkaloids contents: The extract was evaluated for alkaloid content[25].

Estimation of tannin content: Placing a 500 mg extract in a 50 ml flask and weighing it. After that, 50 mL of distilled water was mixed, and the mixture was stirred for an hour. The material was filtered into a 50 ml volumetric flask with the volume regulated to the desired amount. 5 ml of the clear supernatant and 2 ml of 0.1 M ferric chloride were aliquoted into a test tube. The absorbance was taken with a spectrophotometer at 395 nm after 10 minutes[26].

Evaluation of saponin content: Saponins content was evaluated by the reported method[27].

Estimation of glycosides: The glycosides content of the extract was determined by dissolving 5.0 g of the extract in 50 ml of 50% H_2SO_4 in glass vials. After 15 minutes in boiling water, 5 ml of Fehling solution was added and the mixture bubbled. The presence of glycosides was revealed by the appearance of a crimson precipitate in the extract examined. The glycoside percentage was determined[24].

Total phenolic contents and total flavonoid contents: Total phenolic content (TPC) was evaluated as Gallic acid equivalent (GAE/gram extract) using the folin-ciocalteu reagent. Using a modified approach, the total flavonoid contents (TFC) in whole plant extracts were calculated, with quercetin serving as a standard and measured as quercetin equivalent (QE/gram extract).

Animals: In the current investigation, Swiss albino mice (30–35 g) of both sex were used. The space was well-ventilated (> 10 air changes per hour) and completely fresh. A 12-hour light/dark photoperiod was kept. Room temperature and relative humidity were adjusted according to CPCSEA recommendations to maintain a range of 22 to 20 degrees Celsius and 40 to 80 percent, respectively, and the animals were given a routine pellet diet and water ad libitum.All tests were carried out in the morning, in accordance with current research laboratory animal care guidelines and ethical guidelines for experimental pain in conscious animals[28].The research was carried out after the Institutional Animal Ethics Committee approved the protocol an d in accordance with the rules of the Committee for the Reason of Guidance and Regulation of Animal Experim ents (CPCSEA), New Delhi (Reg. No. 1732/GO/Re/13/CPCSE).

Acute toxicity studies: The mice were placed into five groups of five animals each. The animals were given p.o. dosages of the ethanolic extract in increments of 250, 500, 1000, and 2000 mg/kg. Orally, 0.1 mL of distilled water was given to the control group. The animal were monitored for 24 hr after treatment for death, behavioural abnormalities (restlessness, dullness, and agitation), and toxicity.

Dosing: A. spinosus at 250 and 500 mg/kg p.o., as well as renowned memory enhancers such as shankhpushpi syrup 200 mg/kg (Dabur pharma. India) and Tween 80 (Ranchem, India), were given orally twice daily for seven days for memory and learning experiments in two models. Animals in the control group were given a suspension of 1 percent carboxymethyl cellulose in distilled water (1 ml of 1%, w/v, p.o. body weight).

Group I Control group served as healthy mice

GroupII Positive control group (Std group) was given liquid formulation (ShankpushpiSyrup) 200 mg/kg,b.w
GroupIII Test group given as ethanolic extract of A. spinosus 250mg/kg,b.w
GroupIV Test group given as ethanolic extract of A. spinosus 500mg/kg,b.w

Elevated plus-maze test: The plus-maze is a simple, quick, and time-saving method. There was no requirement for prior training or unpleasant stimuli (sound or light). It is a predictable and dependable method for testing cognition in Alzheimer's disease and the effect of treatment response to senile dementia. When animals are exposed to a novel maze, they involvement an approach-avoidance conflict that is stronger in the open arm than in the closed arm. Mice have an aversion to high and wide gaps and prefer enclosed arms, therefore they spend more time in enclosed arms. The plus maze is made up of two opposed open arms (50times10 cm) that are crossed by two locked arms of the same proportions with 40 cm high walls. A central square connects the arms (10. times.10 cm). Individual animals were put at one end of an extended arm, facing away from the central square. The time it took the animal to travel from an open arm to one of the closed arms was measured as the "initial transfer delay' (ITL). After allowing the animal to explore the maze for 30 seconds after recording the ITL, the mice were placed similarly on the open arm and the retention latency was noted again, which was labelled as 'first retention transfer latency" (1.sup.st RTL) and'second retention transfer latency" (2.sup.st RTL) (2.sup.nd RTL)[29, 30].

Radial arm-maze test: The animals were fed at a rate of around 80% of their adlibitum during radial arm–maze training and trained for five days to navigate a radial arm maze. Extending from an octagonal centre platform (brown, wood, 60x10 cm). The maze was placed in the middle of a darkly lit (15 x 10 feet) room with a plethora of posters and antiques adorning the walls. The animals were baited with candy pieces and placed in the middle of the maze, with all eight legs accessible. The animals were detached from the maze after examining all of the arms. Arms were only rebaited after the animals had entered the arm, and the maze was cleaned with a 50 percent alcohol solution between animals[31, 32].

Histopathological examination: After a seven-day administration of the entire subject group, the brain was dissected out and stored in a 10% formalin solution for three days. A portion of the brain was removed for histological examination after 3, 5, and 15 minutes. The slides for the histology studies are ready.

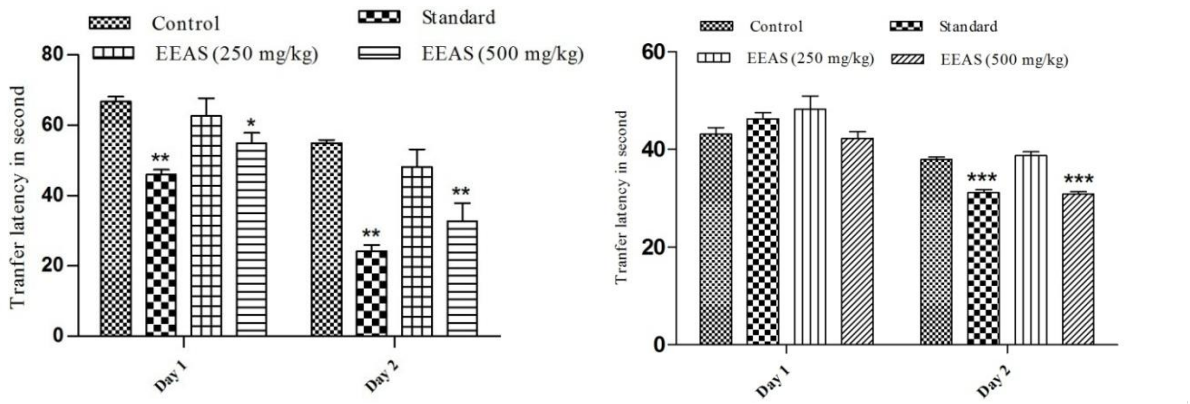
Statistical analysis: The turkey-Kramer test was used to statistically analyse all of the results, which were expressed as mean standard error of mean (S.E.M.). The graph pad instat 3.06 tool, which was installed on a Windows 10 PC, was used to generate and analyse the data (Microsoft Corp.).

RESULTS AND DISCUSSION

The investigation of plant extract confirmed the existence of medicinally active phytocompounds. The phytochemical properties of A. spinosus revealed the presence of Alkaloids, Tannins, Saponins, and Glycosides. The quantifiable calculation of crude chemical contents in A. spinosus revealed glycosides 53.2 ± 0.80 saponins 63.0 ±0.50, tannins 6.07 ±0.93, and alkaloids 13.14 ± 0.86, with saponins being the most abundant physiochemical parameters of whole plant extracts of A. spinosus. The total ash content was 06.8 percent, the acid insoluble ash content was 01.20 percent, the water-soluble ash content was 01.60 percent, the alcohol-soluble extractive value was 06.65 percent, the water-soluble extractive value was 12.25 percent, and the moisture content was 08.90 percent. The percentage yield of A. spinosus ethanolic extract of the whole plant revealed the existence of total phenolic content (TPC 2.81 ± 0.2 g/100 g) and total flavonoid content (TFC 18.4 ± 0. 30 g/100 g of DM) at the 80 percent ethanolic extract and 1.4±0.5 TPC, 2.78±0.20 at the 100 percent ethanolic extract. TFC values are the mean standard deviation of samples evaluated independently in triplicate. *Total phenolic content is expressed in gallic acid equivalents; total flavonoid content is expressed in quercetin equivalents.

The acute toxic effect of A. spinosus ethanolic extract on rats resulted in no death within 24 hours of treatment v ia either oral or intravenous routes. The oral minimum lethal dose (LD50) of A. spinosus ethanolic extract was d etermined to be >2000 mg/kg, demonstrating the plant extract's relative safety when taken immediately. The mai

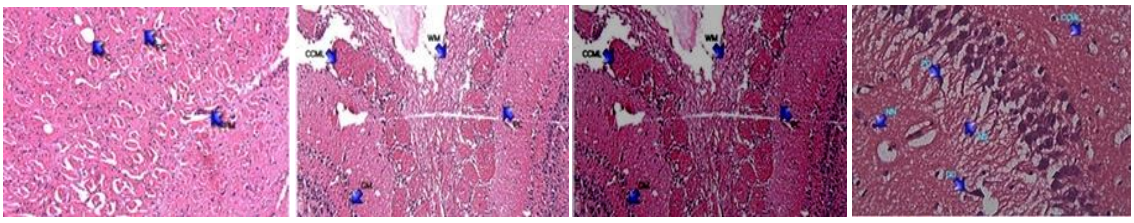
n symptoms of toxicity observed within the first 2 hours included moderate lethargy in some of the animals at th e high dose provided orally at 2000 mg/kg.



(a) (b)

The impact on transfer latency (using elevated plus-maze) The first day's TL showed animal learning behaviour, but the second day's TL reflected information retention or memory. Oral administration of A. spinosus extract (250 mg/kg) for seven days had no significant effect on TL. In both tests, the higher dose (500 mg/kg) P< 0.05* and P< 0.01**, P< 0.001***, ns = P> 0.05 were regarded as significant when compared to the group I control (Fig 1 a, b).

The transverse section of the brain section revealed a better description of pyramidal cells in grey matter and white matter than the average populated pyramidal cells in mice.



(a) (b) (c) (d)

500mg/kg group. Pyramidal cells(PC),cerebreller cortex of molecular layer(CCML), white matter(WM), Gray matter(GM), neuron synapses(NS),ethanolic extract of A. spinosus(EEAS).

The T.S. of a brain segment revealed a large number of pyramidal cells in Fig. 2a, although the homogeneity was disturbed. The T.S. of the brain revealed distant features of white matter and grey matter under the white matter. This molecular layer consists of the pyramidal Fig. 2c junction of the cerebrellar cortex. In a histological investigation, the control group's brain section was compared to the treatment group's brain section, and there were noticeable alterations in the number of pyramidal cells. The population of pyramidal cells, grey matter, and white matter in the control group animals was average. The number of pyramidal cells increased in the extract of A. spinosus (250, 500 mg/kg) treated groups, and the interface of the cerebrellar cortex molecular layer revealed neuron connections.The whole plant of A. spinosus contains phytoconstituents such as fixed oils and lipids, mucilage, phenolic compounds, protein amino acids, carbohydrates, glycosides, gum, tannins, and saponins. The potential of flavonoid contents to regulate intracellular signals, encouraging cellular survival[33], can protect the brain. Phytochemical examination is a crucial step in determining the drug's identity as well as its quality and purity. Impurities such as inorganic salts, carbonates, phosphates, silicates, and silica are detected using ash values.The fact that preadministration with A. spinosus for 8 days enhanced IR and lowered TL suggested that

healthy mice had improved learning and memory. A. Spinosus also boosted IR while lowering TL. Scopolamine treated mice showed preservation of the learning and memory processes[34]. As behavioural models for evaluating learning and memory, the elevated plus maze and the radial arm-maze test were used. These models are commonly used to assess medication effects on learning and memory[35, 36]. Reduced transfer latency on the second day (i.e., 24 hours after the first trial) suggested improved memory in the elevated plus maze, and vice versa. Out of the two therapeutic concentrations of ethanolic extract of A. spinosus (250 and 500 mg/kg, b.w), the higher dose (500 mg/kg) was found to be significantly different from the control P*< 0.05 and P**< 0.01, P***< 0.001, ns = P> 0.05, so the higher dose (500 mg/kg) was used to elucidate the potential mechanisms of memory enhancing activity.

Shankhpushpi is an Ayurvedic medicine that works on the central nervous system, increasing memory and restoring intelligence. Because of its widespread use as a brain stimulation and memory enhancer, it is referred to as a rasayana in Ayurveda. It's a wonderful brain tonic that helps you improve your ability and capacity while also revitalising your neurological system. It improves learning, memory, intelligence, focus, and recall abilities[37]. Both shankhpushpi and A. spinosus extract meet one of the most important requirements for pharmacological activity: memory improvement in the absence of cognitive impairment.However, more research employing other experimental paradigms is needed to demonstrate A. spin osus's nootroic capability in the therapy of diverse cognitive diseases. By analysing the protective effects in seve n days, we were able to show that 500 mg/kg ethanolic extract of A. spinosus improved learning and memory in mice significantly in both the proprioceptive behaviour tests used. Proprioceptive behaviour tests have the stimu lus outside the body, and interoceptive behaviour tests have the stimulus inside the body.

CONCLUSION

It was discovered that the edible plant species A. spinosus, which belongs to the underused plant family, contains a wealth of useful ingredients that are beneficial to one's health. The beneficial effects of this plant will aid researchers in identifying the important area of neurodegenerative disease, which will aid in identifying the medicine and determining its quality and purity. It's likely that A. spinosus ethanolic extract has memory-enhancing properties. The current study adds to the scientific evidence supporting their usage in traditional medicine.

SIGNIFICANT STATEMENT

- Several secondary metabolites and vitamins have been shown to be helpful to health and have increased the production of A. spinosus as a healthy food.
- The acute oral toxicity investigation revealed that A. Spinosus has a safe lethal dose (LD50) for further suba cute and chronic toxicity studies.
- • This research identifies herbal formulations that may be useful in overcoming problems associated with neurodegenerative illness.

ACKNOWLEDGMENTS

The Authors are thankful to Pharmacognosy and Ethnopharmacology NBRI Lucknow for providing the necessary facilities and encouraging throughout the study.

CONFLICT OF INTEREST

Authors state that there are no conflicts of interest.

REFERENCES

1. Erum, S, M.,Naeemullah, S., Masood, A., Qayyum., and M, A, Rabbani.,2012. "Genetic Divergence in Amaranthus collected from Pakistan." The Journal of Animal & Plant Sciences., 22(3): 653-658.

2. Baral, M., Datta, A., Chakraborty, S., Chakraborty, P., 2011. Pharmacognostic studies on stem and leaves of Amaranthus spinosuslinn. Int J Appl Biol Pharm., 2:41-7.
3. Jose, AHOJ., Ana, PBR.,2011. Amaranth: a pseudo-cereal with nutraceutical properties. CurrNutr Food Sci., 7:1-9.

4. Venskutonis, PR., Kraujalis, P., 2013. Nutritional components of Amaranth seeds and vegetables: a review on composition, properties, and uses. Comp Rev Food Sci Saf., 12:381-412.
5. Zeashan, H., Amresh, G., Singh, S., Rao, CV., 2010. Protective effect of Amaranthus spinosus against D-galactosamine/lipopolysaccharide-induced hepatic failure. Pharm Biol 2010;48:1157-63
6. Mishra, SB., Verma, A., Mukerjee, A., Vijayakumar, M., 2012. Amaranthus Spinosus L. (Amaranthaceae) leaf extract attenuates streptozotocin-nicotinamide induced diabetes and oxidative stress in albino rats: a histopathological analysis. Asi Pac J Trop Biomed., 16:47-52.
7. Bulbul, IJ., Nahar, L., Ripa, FA., Haque, O., 2011. Antibacterial, cytotoxic and antioxidant activity of chloroform, n-hexane and ethyl acetate extract of plant Amaranthus spinosus. Int J Pharm Tech Res., 3:1675-80
8. Taiab, MJA., Nazmul, Q., Asif, AM., Amran, HM., Shams-Ud-Doha KM., Apurba, SA., 2011. Analgesic activity of extracts of the whole plant of Amaranthus spinosuslinn. Int J Drug Dev Res., 3:189-93.
9. Baral, M., Chakraborty, S., Chakraborty, P., 2010. Evaluation of anthelmintic and anti inflammatory activity of Amaranthus spinosus Linn. Int J Current Pharm Res., 2: 44-47.
10. Hilou, A., Nacoulmaa, O.G., Guiguemdeb, T.R., 2006. In vivo antimalarial activities of extracts from Amaranthusspinosus L. and BoerhaaviaerectaL. in mice. JEthnopharmacol., 103: 236-240.
11. Kumar, BSA., Lakshman, K., Velmurugan, C., Sridhar, S., Gopisetty, S.,2014. Antidepressant activity of methanolic extract of Amaranthus spinosus. Basic Clin Neurosci., 5(1):11-7.
12. Tatiya, A.U., Surana, S.J., Khope, SD., Gokhale, SB., Sutar, M.P., 2007. Phytochemical investigation and Immunomodulator activity of Amaranthus spinosus Linn. Indian J Pharm Ed Res., 41: 337-341
13. Ghosh, D., Mitra, P., Ghosh, T., Mitra. P., 2013. Anti-peptic ulcer activity of the leaves of Amaranthus spinosus in rats. Mintage J Pharma Med Sci., 2:152-3.
14. Kumar, BSA., Lakshman,Kuruba., Jayaveera,Koralkonta, Narasimha., Shekar,Devangam,Sheshadri., Nandeesh,Rudrappa., Velmurugan,Chinnasamy.,2010. Chemoprotective and antioxidant activities of methanolic extract of Amaranthus spinosus leaves on paracetamol induced liver damage in rats. AMS., 39(2):68-74.
15. Jhade, D., Ahirwar, D., Jain, R., Sharma, NK., Gupta, S., 2011.Pharmacognosticstandardization, physico- and phytochemical evaluation ofAmaranthusSpinosus Linn. root. J Young Pharm., 3:221-5.
16. Vijay L.S, Sathe P.A., Baing M.M., Singh G.R, Lakshmi, S.N., 2007. Determination of Rutin in Amaranthus spinosusLinn. whole plant powder by HPTLC. Chromatographia., 65: 767-769.
17. Vijay, L.S., Sathe, P.A., Vaidya, V.V., Manisha M. Baing, Ganesh, R., Singh, RP-LC.,2008. Determination of Rutin in Amaranthus spinosus Linn. Whole Plant Powder. Chromatographia., 67: 189-191.
18. Gul, T., Singh, K., Srivastava, A., Ahirwar, V., 2011. Biochemical alteration of methanolic extract of Amaranthus spinosus on liver of Sprague Dawley rats. Int J Biol Med Res., 3:365-9.
19. Haq, A., Malik, A., Haq, A., Khan, S.B., Shah, M.R., Muhammad, P., 2004. Spinoside, New Coumaroyl Flavone Glycoside from Amaranthus spinosus. ArchPharmacal Res., 27: 1216-1219.
20. Ballard, C., Gauthier, S., Corbett, A., Brayne, C., Aarsland, D., Jones, E., 2011. Alzheimer's disease. Lancet., 377:1019-1031.
21. Montejo, P., Montenegro, M., Fernandez, M., Maestu, F., 2011. Subjective memory complaints in the elderly: Prevalence and influence of temporal orientation, depression and quality of life in a population-based study in the city of Madrid.Aging Ment Health., 15:85-96.
22. Shafer, T.J., Meyer, D.A., 2005. Crofton KM. Developmental neurotoxicity of pyrethroid insecticides: Critical review and future research needs. Environ Health Perspect., 113:123–136.
23. Das, S., 2012. Systematics and taxonomic delimitation of vegetable grain and weed amaranths: a morphological and bio-chemical approach. Genet Resour Crop Evol., 59: 289-303.
24. Fransen, M., Crosbie, J., Edmonds, J., 2001. Physical therapy is effective for patients with osteoarthritis of the knee: a randomized controlled clinical trial. J Rheumatol., 28: 156-164.
25. Drzewiecki, J., Delgado-Licon E., Haruenkit., 2003. Identification and differences of totalproteins and their soluble fractions in some pseudocereals based on electrophoretic pattern. Journal of Agriculture and Food Chemistry, 51: 7798-7804.
26. Fasuyi, A.O., 2007.Amaranthus cruentusleaf meal as a protein supplement in broiler finisher diets part 1. Performance and nitrogen utilization. Afr J Food Agric Nutr Dev 7: 1-15.
27. Gotor, E., Irungu, C., 2010. The Impact of Biodiversity International's African Leafy vegetables Programme in Kenya. Impact Assessment and Project Appraisal., 28: 41-55.
28. Zimmerman, M., 1983. Ethical guidelines for investigation of experimental pain in conscious animals Pain., 16:109-10.
29. Dhingra, D., Parle, M., Kulkarni, SK., 2004. Neurochemical Basis of Learning and Memory. Indian J. Pharm. Sci., 66:371-6.
30. Milind,Parle., Mani,Vasudevan., 2007. Memory enhancing activity of Abana: An Indian Ayurvedic poly herbal formulation. Journal of health Science., 53:43-52.
31. Vogel, H.G.,2002. Drug Discovery and Evaluation; Pharmacological Assays. Springer - Verleg. Germany., 619-43.
32. Ganguly, R., 2008. Guha, D., Alteration of brain monoamines and EEG wave patterns in rat model of Alzheimer's disease and protection by Moringa oleifera. Indian J Med Res., 744-51.
33. F,Dajas., F, Rivera-Megret., F, Blasina et al., 2003. "Neuroprotection by flavonoids," Brazilian Journal of Medical and Biological Research., 36 (12): 1613–1620.
34. K, S, Kulkarni., S, B,Kasture., and S, A,Mengi., 2010. "Efficacy study of Prunusamygdalus (almond) nuts in scopolamine-induced amnesia in rats, Indian Journal ofPharmacology., 42(3): 168–173.

35. Itoh, J., Nabeshima, T. and Kameyama, T., 1990. “Utility of an elevated plus-maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock,” Psychopharmacology., 101:27–33.
36. Morris, R., 1984. “Developments of a water-maze procedure for studying spatial learning in the mice,” Journal of Neuroscience Methods., 11:47–60.
37. Manish,Bhatiya.,2012.Shankpushpi: A cognition-boosting Ayurvedic medicine, International Journal of Natural Product Science., 1:163