INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHYTOCONSTITUENTS PROFILE OF *CLITORIA TERNATEA* BY GC-MS AND ITS AGE-RELATED ANTICHOLINERGIC ACTIVITY AGAINST ALUMINUM AND RESTRAINT STRESS

Chandana Naga Durga Maha Lakshmi [1], Jaya Prasanthi Rantham Prabhakara [2], Kondammagari Saritha [1], Borelli Deva Prasad Raju [3], Nannepaga John Sushma [1]*
[1]Department of Biotechnology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, India
[2]Department of Zoology, Sri Venkateswara University, Tirupati, India
[3]Department of Physics, Sri Venkateswara University, Tirupati, India
*Corresponding Author Email: johnsushma@gmail.com

Article Received on: 03/01/18 Approved for publication: 02/02/18

DOI: 10.7897/2230-8407.09224

ABSTRACT

Neurodegenerative diseases are associated with brain aging which leads to impaired cholinergic dysfunction. In the current study, we aimed to identify the neuroprotective effect of *Clitoria ternatea* (CT) methanolic leaf extract against restraint stress (RS) and aluminum (AlM) induced age-related toxicity in cerebellum of young and adult rats. In CT methanolic leaf extract 9- phytoconstituents such as 4H-1-Benzopyran-4-one, 7-hydroxy-2-(4-hydroxyphenyl); Cyclopentaneundecanoic acid, methyl ester; Phytol; Methyl Isostearate; 12-Methyl-E, E-2,13-Octadecadien-1-ol; Cyclopropaneoctanoic acid, 2-{[2 pentylcyclopropyllmethyl}, methyl ester; Ethanol,2-[9-octadecenyloxy], [Z]; Octadecanoic acid, 5,9,13,17-tetramethyl, methyl ester; Hyocholic acid were identified using Gas chromatography-mass spectrometry (GC-MS), their structures identified based on NIST data, and biological activities were specified. Further, the young (3 months age) and adult rats (12 months age) were randomized into eight groups, control, AlM, RS, CT, RS+AlM, AlM+CT, RS+CT and RS+AlM+CT administered groups. Both the age group rats were restrained in a restraint chamber for 60 min/day/30 days, AlM (100 mg/kg/30 days) and CT leaf extract (50 mg/kg/30 days) were administered orally according to their respective groups. RS and AlM administration showed significant reduced ACh and AChE Cholinergic markers levels in cerebellum compared to control group. Whereas co-administration of CT methanolic leaf extract with RS and AlM showed enhanced levels of ACh and AChE compared to RS and AlM alone treated groups. These data suggest that the rich source of phytoconstituents of CT methanolic leaf extract are responsible for anti-cholinergic and neuroprotective effects against RS and AlM age-related toxicity.

Keywords: Aluminum, Restraint stress, *Clitoria ternatea,* GC-MS, Cerebellum, Cholinergic markers.

INTRODUCTION

Modern human life style gives the circumstantial evidences on psychological stress conditions and also exposure to environmental toxicants have been strongly associated with brain age-related neurodisorders such as Alzheimer's disease. Human brain which performs multiple mechanistic function may facilitate the age-related impairment in cognitive function[1], impairment of short-term memory, formation of amyloid rich senile plaques and neurofibrillary tangles are major symptoms associated with AD.

Stress and aluminum exposure has become increasing in our day-to-day life, and strongly associated with aging related neurodisorders. Aluminum (Al) a well-known environmentally available neurotoxicant gains an easy access into the central nervous system (CNS) and has been reported to alter the blood–brain barrier (BBB) under normal physiological conditions and accumulates in the different brain regions[2]. It has been reported to be involved in the etiology of several neurodegenerative diseases such as Alzheimer's disease[3]. Long-term Al-administration inflicts in oxidative stress resulting in biochemical changes proposed to accelerate the aging damage in brain regions[4]. Stress is a homeostatic challenge with physical and psychological ramifications and particularly negative effect on the learning and memory proces[5,6]. Stress promotes to attributable stimuli in oxidation process and free radical generation that causes neuromolecular damage to aging[7] and neuropathogenic death in AD.

It is very necessary to identify and control the stress and Aluminum induced neurotoxicity before it worsens into strong age-related neurodisorders such as Alzheimer's disease. The use of plants with rich phytoconstituents as sources of pharmacological products to enhance health defense is currently of great interest[8]. There has been an exponential growth in study of pharmacological properties of *Clitoria ternatea* (CT) for its promising future development. Traditionally CT was used as reputed drug of ayurveda as nervine tonic for memory enhancing, gaining more attention in neuroscience[9]. It consists of tannins, resins, taraxerol, alkaloids, flavonoids, saponins, proteins and anthocyanin phytoconstituents[10]. A range of CT extracts exhibited extensive biological and pharmacological activities specifically nootropic, anxiolytic, anticonvulsant, sedative, anti-pyretic, anti-inflammatory, anti-diabetic, anti-oxidative, anti-stress[11], immunomodulatory, larvicidal, proteolytic, antihelmintic, diuretic, anti-microbial and memory enhancing[12]. So therefore, the main aim of this study was to investigate natural supplementation of CT as herbal medicine, forms major therapeutic strategies to prevent aging related neurodegeneration against AlM and RS. No study is available regarding the possible mechanism of action on CT methanolic leaf extract in counteracting aluminum and stress induced aging related anticholinergic activity in brain cerebellum.

MATERIALS AND METHODS

The research study has been carried out during the period of January 2014 to August 2016. During this period the collection of plant, procurement of chemicals and experimental animals was done to carry out the research work with ethical committee approval, etc.

Preparation of *Clitoria ternatea* leaf extract

The CT plants were obtained from surrounding areas of Tirupati, Andhra Pradesh, India and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati. Voucher specimens (no. 1160) were confirmed and deposited at the herbarium of Sri Venkateswara University, Tirupati. The plants were thoroughly washed with double distilled water, leaves were separated and dried under shade dust-free condition for one week at room temperature. Then plant material was ground into fine powder. Finally, powdered plant material was extracted with 60% methanol. The mixed solution was left on constant magnetic stirring at room temperature for 72 hr. The extract was filtered, and dried using vaccum desiccator, and the powder yield was stored at 4^0C for further experiments.

GC-MS analysis of CT methanolic leaf extract

GC-MS analysis of CT methanolic leaf extract was performed using JEOL GCMATE II GC-MS Spectrometer (with system is a high resolution, double focusing instrument. Maximum resolution: 6000, Maximum calibrated mass: 1500 Daltons). Helium was used as the carrier gas and the temperature programming was set. 2 µL sample was injected with splitless mode. Mass spectra were recorded over 200-1200 TIC range with electron impact ionization energy 70 eV. The total running time for a sample is 30 min. The relative peak percent (%) amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbo mass. National Institute Standard and Technology (NIST) database has been used for the interpretation of mass spectrum GC-MS, NIST was having more than 62,000 patterns of compound data library. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the materials were ascertained.

Animals

Male wistar strain rats of young (3-months) and adult (12-months) age groups, weighing 150 ± 200g and 300 ± 350g procured from an authorized vendor (Sri Venkateswara Enterprises, Bangalore, India), were used in the study. Rats were acclimatized in the lab for one week in order to adopt the laboratory conditions, the animals were randomized into eight groups, each group containing six animals housed in a polypropylene cages (47x34x20cm) with sterile paddy husk as bedding and maintained at 22-25^{o}C regulated temperature, with a light/dark cycle (12h/12h). The rats were fed with standard rat chow (Sri Venkateswara Enterprises, India) and water *ad libitum*.

Experimental design

Experimental protocols were approved by the institutional ethical committee (CPCSEA Registration No. **1677/PO/a/12/IAEC-Feb-14/03**). Young and adult albino rats were equally randomized into eight groups as follows.

Group I: Control administered group: with (0.9%) saline solution

Group II: Aluminium -maltolate (AlM) administered group: AlM was dissolved in (0.9%) saline solution and administered orally at a dose of 100 mg/kg/b.wt./30 days.

Group-III: Restraint stress (RS) treated group: immobilization stress was given for 1hr inside the cylindrical steel tube (7 cm diameter, 17.5 cm along with holes for ventilation) at room temperature during the early phase of the light cycle for 30 days.

Group-IV: *Clitoria ternatea* (CT) treated group: CT methanolic leaf extract was Administered orally at a dose of 50 mg/kg/b.wt./30 days.

Group-V: RS+AlM treated group: immobilization stress period was given for 1hr and After that, AlM was administered orally at a dose of 100mg/kg/b.wt. for 30days.

Group-VI: AlM+CT administered group: AlM (100mg/kg/b.wt.) and CT methanolic leaf extract (50mg/kg/b.wt.) were administered orally with 1hr time interval for 30 days.

Group-VII: RS+CT treated group: immobilization stress induced for 1hr, and after that CT methanolic leaf extract at (50 mg/kg/b.wt.) was administered orally for 30 days.

Group-VIII: RS+ AlM+ CT administered group: immobilization stress was given for 1hr inside the cylindrical steel tube at room temperature and after that the animals were administered with AlM(100mg/kg/b.wt.) and CT methanolic leaf extract at (50 mg/kg/b.wt.) orally for 30 days.

Tissue collection and preparation of tissue homogenates

Rats of each group were sacrificed by cervical dislocation and dissected after the treatment period. The brain tissues were immediately removed, the cerebellum was dissected on ice cold glass plate and homogenate was prepared. Tissue homogenate was made in 50 mM phosphate buffer containing 0.1 mM EDTA using homogenizer and centrifuged at 10,000 rpm for 15 min at 4^0C, the supernatants thus obtained were used for the estimation of various biochemical analysis.

Acetylcholine (ACh) content and Acetylcholinesterase (AChE) activity

Cholinergic dysfunction was assessed in terms of acetylcholine (ACh) content and acetylcholinesterase (AChE) activity. The measurement of ACh levels in cortex was performed according to the method as given by[13] Augustinson, 1957 and AChE by [14] Ellmann et al. 1961. The colour change was read at an absorbance of 540 nm for ACh and 412 nm for AChE. The ACh content was expressed as µ moles of acetylcholine/gm wet weight of tissue. The AChE enzyme activity was expressed as µ moles of acetylthiocholine hydrolyzed /mg protein/hr.

Statistical Analysis

The results were expressed as means ± standard deviation (SD) of n=6. The statistical significances of data were determined using one-way analysis of variance (ANOVA) followed by Dunnett test, $p< 0.0001$ was regarded as significant.

RESULTS AND DISCUSSION

GC-MS spectrometer is a well-organized and widely used technique in the fields of environment and life sciences, for the effective separation of compounds and to analyze mixtures with high sensitivity. It is applied for the identification of volatile profile of bioactive compounds[15] in various fields, such as measurement of dioxin, bioactive compounds from

phytosamples, organic compounds in drinking water, and concentration of drug in blood, as well as a tool for developing new pharmaceutical products. The MS instrument provides specific results, but produces uncertain qualitative results, Gas chromatograph (GC) coupled to a mass spectrometer (MS) system was used to determine the composition of samples eluted at different times to identify the nature and structure of the compounds[16,17]. Previously GC-MS studies shown the presence of 7compounds and their biological activities were identified in both CT ethanolic and methanolic extracts[18,19]. In the present study, 9- phytoconstituents like 4H-1-Benzopyran-4-one, 7-hydroxy-2-(4-hydroxyphenyl); Cyclopentaneundecanoic acid,-methyl ester; Phytol ; Methyl Isostearate; 12-Methyl-E, E-2,13-Octadecadien-1-ol; Cyclopropaneoctanoic acid, 2-{[2 pentylcyclopropyllmethyl}, methyl ester; Ethanol,2-[9-octadecenyloxy], [Z]; Octadecanoic acid, 5,9,13,17-tetramethyl, methyl ester; Hyocholic acid were identified from CT methanolic leaf extract by using a gas chromatograph-mass spectrograph (GC-MS) (Figure. 1) and their structures were given in Table-1.

This identification was done by comparing mass spectra on both columns with phytochemicals. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table-2). This investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents from CT leaf extract and they are responsible for many biological activities were mentioned in Table-3, such as anti-inflammatory, antibiotic, antiseptic chemotherapeutics, used as drugs for dermatological and skeletal disorders, antimicrobial and also with anti-tumor activities[20,21], Most of the compounds belong to the group of antioxidant agents[22] . Hence the present identification of bioactive profile of CT methanolic leaf extract could be effectively utilized for the development of CT based traditional medicines. Further investigation needs to elute novel active compounds from the CT which may create a new way to treat many incurable diseases.

Psychological stress conditions and exposure to various environmental toxicants accompanies to brain aging which is characterized by deterioration of cognitive function, including learning and memory[23]. The natural supplementation of herbal medicine offers several options to modify the progress and symptoms of neurological disorders and play new trends in their scientific and commercial significance towards health-relevant areas[24]. In the present investigation, in order to determine the age-related neurodegeneration induced by both RS exposure and environmental neurotoxicant such as AlM were administered to the experimental animals of young and adult male wistar rats. Al has been reported to be involved in neurodisorders such as Alzheimer's disease[25,26,27]. Young and adult rats that were exposed to RS and AlM resulted in significant reduction of ACh and AChE activities in cerebellum. It was also observed that the decreased cholinergic marker levels were more pronounced in adult rats compared to young rats (Figure 2 & 3). This is due to the AlM and RS showing adverse effect on the cholinergic pathway of neurotransmitters by decreasing ACh and AChE activities.

In cholinergic neuronal system ACh neurotransmitter plays a prominent role in memory regulation and cognitive function in brain, and it will be degraded by AChE enzyme. The cholinotoxic effects of Al are exerted by blocking Acetyl CoA-which is responsible for ACh synthesis[28]. In the present study ACh levels were decreased in AlM and RS of both the age groups compared to control. Previous reports also demonstrated that substantial neocortical deficits in the enzyme responsible for the synthesis of ACh and ChAT (choline acetyltransferase) would lead to neurochemical abnormality[29,30]. Subsequent discoveries in reduced choline uptake, ACh release and loss of cholinergic perikarya from the nucleus basalis of meynert confirmed a substantial presynaptic cholinergic deficit. Alterations in these cholinergic markers in brain regions have been implicated in cognitive deficit associated with aging and neurodegenerative disease like Alzheimer's disease.

CT administration has revealed its tremendous neuroprotective benefits and provided evidence against aging induced decline in cholinergic levels and its relation to neurodisorders induced by AlM and RS (Figure 2 & 3). Both are able to influence the cellular metabolism and stress related pathway in neuro-cell and ultimately brain. Therefore, CT leaf extract with rich phytoconstituents can easily incorporated into clinical trials in aging neurodegenerative studies. In conclusion, RS and AlM caused significant cholinergic damage in the present study in both young and adult cerebellum of albino rats compared to control, these results are in conformity with our previous research annotations about Al induced neurotoxicity by[31] Sushma et al. 2014. However, cholinergic change was more pronounced in adult rats compared to control. In CT administered group the alterations were not significant and the results are almost similar to control is observed. Hence from the study it is declared that RS and AlM pro-oxidant properties have potentiated to enhance the cholinergic levels. The results suggested that CT has pivotal role on aging related neuroprotection of cholinergic activity against RS and AlM intoxicated of rat cerebellum. Further isolation of specific bioactive compounds and its activity on target gene molecular studies is needed to determine possible role of CT in cell survival.

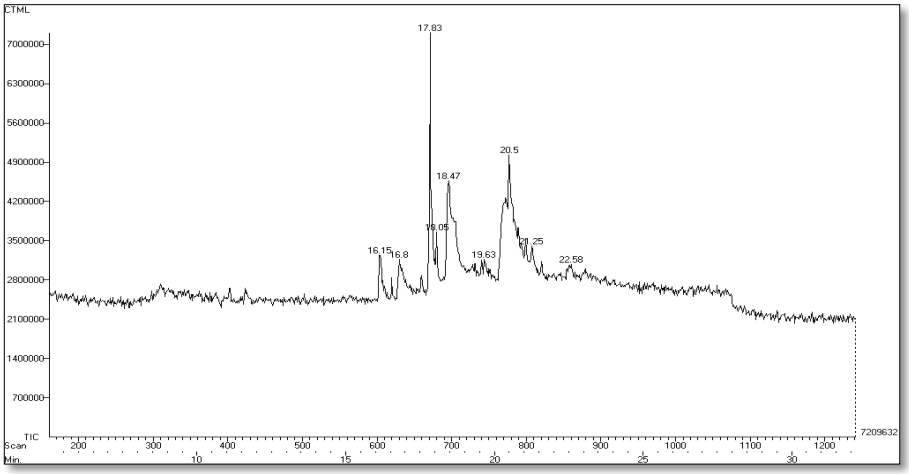


Figure 1: Bioactive compounds identified in the CT methanolic leaf extract by GC-MS spectroscopy.

Table 1. NIST data of Bioactive compounds structure and name identified in the CT methanolic leaf extract by GC-MS spectroscopy

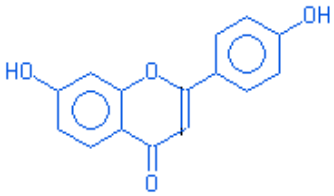
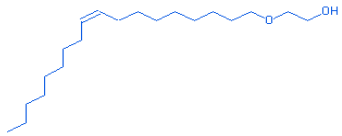
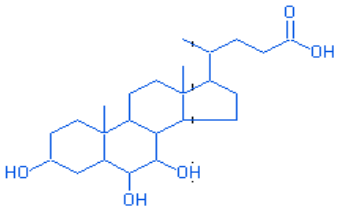
Compound Structure and Name		
1  4H-1-Benzopyran-4-one, 7-hydroxy-2-(4-hydroxyphenyl)	2  Cyclopentaneundecanoic acid, methyl ester	3  Phytol
4  Methyl Isostearate	5  12-Methyl-E, E-2,13-Octadecadien-1-ol	6  Cyclopropaneoctanoic acid, 2-{[2-pentylcyclopropyllmethyl}, methyl ester
7  Ethanol,2-[9-octadecenyloxy], [Z]	8  Octadecanoic acid, 5,9,13,17-tetramethyl, methyl ester	9  Hyocholic acid

Table 2. NIST data on Bioactive compounds identified in the CT methanolic leaf extract by GC-MS spectroscopy

Peak no.	**RT**	**Name of the Compound**	**Molecular formula**	**MW**	**Peak Area %**
1	16.8	4H-1-Benzopyran-4-one, 7-hydroxy-2-(4-hydroxyphenyl)	$C_{15}H_{10}O_4$	--	7%
2	16.15	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268.434	20.6%
3	17.83	Phytol	$C_{20}H_{40}O$	296.539	44.5%
4	18.05	Methyl Isostearate	$C_{19}H_{38}O_2$	298.511	19.6%
5	18.47	12-Methyl-E, E-2,13-Octadecadien-1-ol	$C_{19}H_{36}O$	280.496	22.4%
6	19.63	Cyclopropaneoctanoic acid, 2-{[2-pentylcyclopropyllmethyl}, methyl ester	$C_{26}H_{50}O_2$	394.674	7.8%
7	20.5	Ethanol,2-[9-octadecenyloxy], [Z]	$C_{20}H_{40}O_2$	312.538	20%
8	21.25	Octadecanoic acid, 5,9,13,17-tetramethyl, methyl ester	$C_{23}H_{46}O_2$	354.610	5.8%
9	22.58	Hyocholic acid	$C_{24}H_{40}O_5$	408.579	4.8%

Table 3. Pharmacological activity of bioactive compounds identified in the methanolic leaf extract of *Clitoria ternatea* by GC-MS.

Peak No.	COMPOUND NAME	COMPOUND NATURE	ACTIVITY
1	4H-1-Benzopyran-4-one, 7-hydroxy-2-(4-hydroxyphenyl)	Flavone (Di-substituted flavones) Aromatic compound	Anti-inflammatory Antibiotic, antiseptic chemotherapeutics Drug for dermatological and skeletal disorders Antimicrobial
2	Cyclopentaneundecanoic acid, methyl ester	Fatty acid	Antimicrobial
3	Phytol	Diterpene alcohol	Antimicrobial Anti-inflammatory Anticancer Diuretic Antiasthmatics
4	Methyl Isostearate	-	Drug used for dermatological disorders, treating wounds, ulcers, burns, scars, keloids. Antipruritics, non-central Analgesic, antipyretic or Anti-inflammatory agents.
5	12-Methyl-E, E-2,13-Octadecadien-1-ol	Œ-Lenoic acid	Antihistaminic, antioxidant, Anagelsic, Anesthetic, Allergenic, Antibacterial, Anticonvulsant, Anti-salmonella, Antiseptic.
6	Cyclopropaneoctanoic acid, 2-{[2-pentylcyclopropyllmethyl}, methyl ester	Ether compounds Sesquiteroene	Antimicrobial Anti-tumor, analgesic
7	Ethanol,2-[9-octadecenyloxy], [Z]	-	Drug for genital, sexual disorders, antiabortive agent, Antiacne agents Antiinfective, antibiotics, Astiseptics, chemotherapeutic, Antiparasitic, antineoplastic agent
8	Octadecanoic acid, 5,9,13,17-tetramethyl, methyl ester	Saturated fatty acid	Cosmetic, flavor, Hypocholesterolemic, Lubricant, perfumery, Non-Steroidal liquid crystal Compound
9	Hyocholic acid	Bile acid Steroid derivatives	Essential for the absorption of Hydrophobic nutrients, dietary fats and vitamins, Modulate the bile flow and Lipid secretion

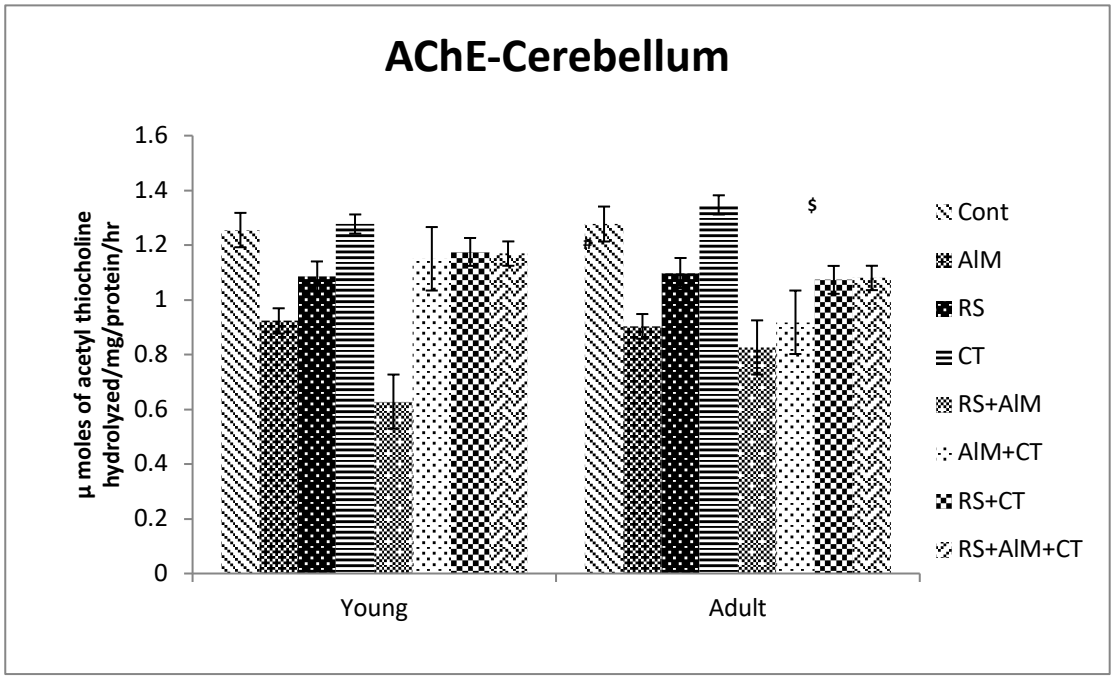


Figure 2: Effect of CT on activities of Acetylcholinesterase activity (AChE) in brain cerebellum of 3&12 months old rats exposed to AlM & RS. Each column represents the mean ± SD (standard deviation of the mean, n=6) in micro moles of acetylcholine /gm wet weight of tissue. *p<0.0001, #p<0.001, significant change with respect to control, $p<0.001 $$p<0.005, **p<0.0001, @p<0.0002 significant change with respect to AlM and RS (student t-test).

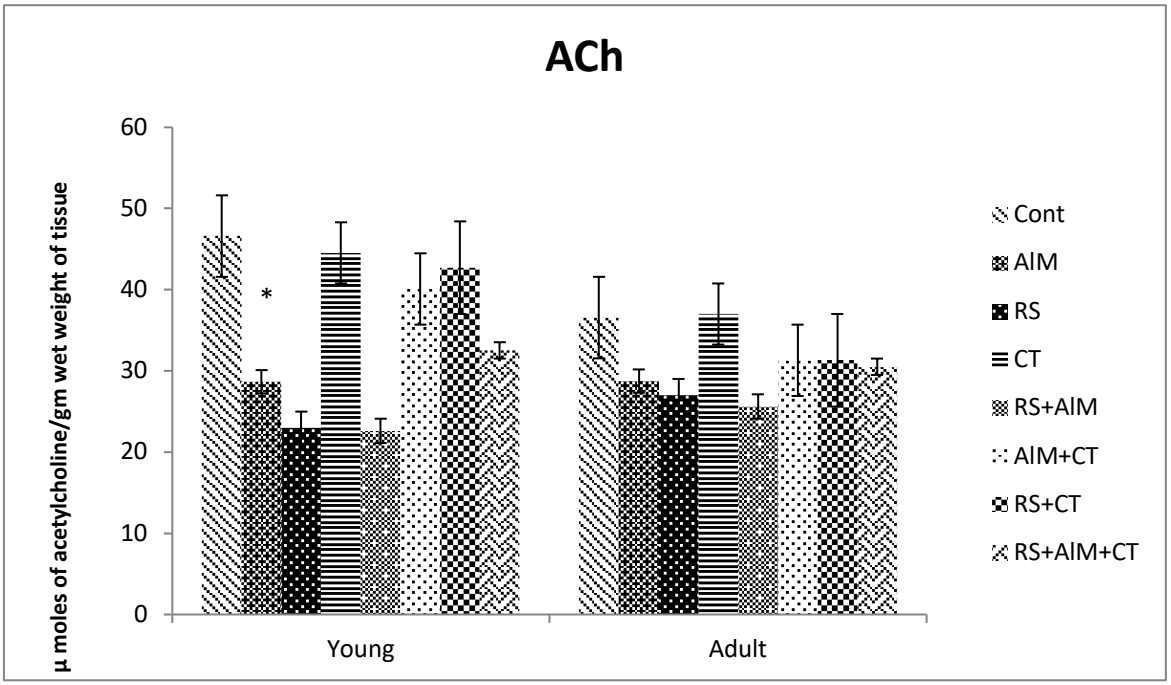


Figure 3: Effect of CT on activities of Acetylcholine content (ACh) in brain cerebellum of 3 & 12-months old rats exposed to AlM & RS. Each column represents the mean ± SD (standard deviation of the mean, n=6) in micro moles of acetylcholine /gm wet weight of tissue. *p<0.0001 significant change with respect to control, $^{\#}$p<0.001, **p<0.0001, $^{@}$p<0.0005 significant change with respect to AlM and RS (student t-test).

CONCLUSION

It is concluded that the *Clitorea ternatea* leaf extract possesses potential ameliorating age-related neuroprotective activity by enhancing the activity of cholinergic neurotransmitter levels beneficial for the improvement of brain cerebellum against stress and aluminum induced neuro-cholinergic damage. Further study should focus on molecular studies to elucidate the mechanisms underlying the protective effects of *Clitorea ternatea*.

ACKNOWLEDGEMENTS

The corresponding author, Dr. N. John Sushma is highly grateful to DST-SERB for providing financial assistance in the form of major research project with a vide reference number (SB/EMEQ-341/2013 dated: 17/10/2013) to carry out this research work.

REFERENCES

1. Siriporn C, Sukumal C. Inhibition of Hippocampal Estrogen Synthesis by Reactive Microglia Leads to Down-Regulation of Synaptic Protein Expression. Neuro Toxicology. 2015; 46:25-34.
2. Zatta P, Ibn-Lkhayat-Idrissi M, Zambenedetti P, Kilyen M, Kiss T. Brain Research Bulletin. 2002; 59:41-45.
3. Kawahara M, Muramoto K, Kabayashi K, Mori H, Kuroda Y. Aluminum Promotes the Aggregation of Alzheimer's Amyloid β-protein In Vitro. Biochemical and Biophysical Research Communications. 1994; 198:531-535.
4. Bharati P, Vasudevaraju M, Govindaraju A, Palanisamy P, Sambamurti K, Rao KSJ. Molecular toxicity of Aluminium in Relation to Neurodegenation. Indian Journal of Medicinal Research. 2008; 128:545-556.
5. Aleisa AM, Alzoubi KH, Gerges NZ, Alkadhi KA. Chronic Psychosocial Stress-Induced Impairment of Hippocampal ltp: Possible Role of BDNF. Neurobiology of Disease. 2006; 22:453-462.
6. McEwen BS, Sapolsky RM. Stress and Cognitive Function. Current Opinion of Neurobiology. 2005; 5(2):205-216.
7. Sohel RS, Forster MJ. Coenzyme Q Oxidative stress and aging. Mitochondria. 2007; 7:103-111.
8. Marziyeh Valifard, Sasan Mohsenzadeh, Bahman Kholdebarin. Salinity Effect on Phenolic Content and Antioxidant Activity of Salvia Macrosiphon. Iranian Journal of Science and Technology, Transaction A: Science. 2017; 41(2):295-300.
9. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian Medicinal Plants for Acetyl Cholinesterase Inhibitory Activity. Phytotherapy Research. 2007; 21:1142-1145.
10. Mukherjee PK, Kumar V, Kumar NS, Heinrich M. The Ayurvedic Medicine *Clitoria ternatea*-from traditional use to Scientific Assessment. Journal of Ethnopharmacology. 2008; 120:291-301.
11. Jain NN, Ohal CC, Shroff SK, Bhutada RH, Somani RS, Kasture VS, Kasture SB. *Clitoria ternatea* and the CNS. Pharmacology Biochemistry and Behavior. 2003; 75:529-536.
12. Giurgea C. The Nootropic Approach to the Pharmacology of the Integrative Action of the Brain. Conditional Reflex. 1973; 8:108-115.
13. Augustinson KB. Methods of Biochemical Analysis. 1957; 5:1.
14. Ellman GL, Courtney KD. A New and Rapid Colorimetric Determinations of Acetylcholinesterase Activity. Biochemical Pharmacology. 1961; 7:88-90.
15. Amarvani P, Kanjikar, Aruna LH, Ramesh L Londonkar. Novel Investigation of In-Vitro Anti-Diabetic and Volatile Profile of Bioactive Compounds Present in Methanol Extract of *Ficus krishnae*. International Journal of Chemtech Research. 2017; 10(9):220-228.
16. Amarvani P Kanjikar, Ramesh L Londonkar. GC-MS Analysis Of Bioactive Compounds In Petroleum Ether Extract of *Ficus krishnae* . International Research Journal of Pharmacy. 2017; 8 (10):178 -181.
17. Soheil Mohseni, Ali Shokuhi Rad. Determination of Compositions of Thymus Pubescens; The Comparison of Different Solvents Towards Extraction. Iranian Journal of

Science and Technology, Transaction A: Sciences. 2017; pp:1-6.
18. Sarumathy K, Dhana Rajan MS, Vijay T, Jayakanthi. Evaluation of Phytoconstiuents, Nephro-Protective and Antioxidant Activities of *Clitoira ternatea*. Journal of Applied Pharmaceutical Sciences. 2011; 1(05):164-172.
19. Swathi Deshmukh, Varsha Jadhav. GC-MS studies of *Clitoira ternatea* L. –A Medicinally Important Plant. Indo American Journal of Pharmaceutical Research. 2013; 3(7):5394-5399.
20. Gomathi Rajashyamala L, Elango V. Identification of Bioactive Components and its Biological Activities of Evolvulus Alsinoides linn. –A GC-MS study. International Journal of Chemical Studies. 2015; 3(1):41-44.
21. Leena P, Zeinul Hukuman NH, Jisha M. In vitro Antimicrobial Efficacy and GC-MS Analysis of Bioactive Components from Lepidagathis keralensis (Acanthaceae). World Journal of Pharmaceutical Research. 2016; 5(12):937-948.
22. Yoshida Y, Nihi E. Antioxidant Effect of Phytosterol and its Compounds. Journal of Nutritional Science and Vitaminology (Tokyo). 2003; 43:277-280.
23. Papandeeou MA, Maria Tsachaki. Spiros Efthimiopoulos, Cordopatis P. Memory Enhancing Effects of Saffron in Aged Mice are Correlated with Antioxidant Protection. Behavioral Brain Research. 2011; 219:197-204.
24. Abascal K, Yarnell E. Alzheimer's Disease: Part 2-A Botanical Treatment Plan. Alternative and Complementary Therapies. 2004; 10:67-72.
25. Esparza JL, Garcia T, Go´mez M. Role of Deferoxamine on Enzymatic Stress Markers in an animal Model of Alzheimer's Disease After Chronic Aluminum Exposure. Biological Trace Element Research. 2011; 141:232-245.
26. Dai Cheng, Yu Xi, Jiankang Cao, Dongdong Cao, Yuxia M, Weibo Jiang. Protective Effect of Apple (Ralls) Polyphenol Extract Against Aluminum-Induced Cognitive Impairment and Oxidative Damage in Rat. Neuro Toxicology. 2014; 45:111-120.
27. Karuna A, Talpate Uma A, Bhosale Mandar R, Zambare Rahul S Somani Neuroprotective and Nootropic Activity of *Clitorea Ternatea* Linn. (Fabaceae) Leaves on Diabetes Induced Cognitive Decline in Experimental Animals. Journal of Pharmacy and Bioallied Science. 2014; 6(1):48-55.
28. Bielarczyk H, Tomaszewicz M, Szutowicz A. Effect of Aluminium on Acetyl- Coa and Acetylcholine Metabolism in Nerve Terminals. Journal of Neurochemistry. 1998; 70: 1175-1181.
29. Bowen DM, Smith CB, White P. Neurotransmitter-related Enzymes and Indicates of Hypoxia in Senile Dementia and Other Abiotrophies. Brain. 1976; 99:459-96.
30. Davies P, Maloney AJF. Selective lossal of central cholinergic neurons in Alzheimer's disease. Lancet. 1976; ii: 1403.
31. John Sushma Nannepaga, Mallikarjuna Korivi, Madhavi Tirumanyam, Mahitha Bommavaram Chia-Hua Kuo. Neuroptotective Effects of *Bacopa monniera* Whole-Plant Extract Against Aluminum-Induced Hippocampus Damage in Rats: Evidence from Electron Microscopic Images. Chinese Journal of Physiology. 2014; 57(5):279-285.

Cite this article as:

Chandana Naga Durga Maha Lakshmi *et al.* Phytoconstituents profile of *Clitoria ternatea* by GC-MS and its age-related anticholinergic activity against aluminum and restraint stress. Int. Res. J. Pharm. 2018;9(2):38-44 http://dx.doi.org/10.7897/2230-8407.09224

Source of support: DST-SERB, Conflict of interest: None Declared