Research Article

Acetylcholinesterase Inhibitory and Cytotoxic Activity of Extracts and Isolated Compounds from *Strobilanthes ciliatus* Nees

Venkatachalapathi Senniyappan[1*], Ravi Subban[2], Sundaram Kaveri[2], Kumaresan Ramasamy[3]

[1*]Department of Chemistry, M.P. Nachimuthu M. Jaganathan Engineering College, Erode, Tamilnadu, India, 641202.

[2]Department of Chemistry, Karpagam Academy of Higher Education (Deemed to be University), Coimbatore, Tamilnadu, India, 641021.

[3]Department of Chemistry, Jai Shriram Engineering College, Tirupur, Tamilnadu, India, 641202.

***Corresponding author's E-mail:** venkiventure@gmail.com

Received: 18-08-2022; **Revised:** 23-10-2022; **Accepted:** 30-10-2022; **Published on:** 15-11-2022.

ABSTRACT

Strobilanthes ciliatus was used in the traditional medicine to treat central nervous system problems and cancer. This study aims to investigate the cytotoxic and inhibitory activity of acetylcholinesterase in extracts and isolated compounds of *S. ciliatus*. Chemical investigation of *S. ciliatus* led to isolation of two compounds. The isolated compounds were characterized by spectral studies like (IR, UV-Vis, MS and NMR) as lupeol and stigmasterol. The extracts and isolated compound have been evaluated for AChE inhibitory activity by Ellman's method and cytotoxicity on Hela and K562 cell lines by MTT method. The extracts and the isolated compounds showed moderate activities against acetylecholinesterase enzyme and HeLa and K562 cell lines.

Keywords: *Strobilanthes ciliatus*, AChE inhibition, Cytotoxicity, Lupeol, MTT assay.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2022.v77i01.025

DOI link: http://dx.doi.org/10.47583/ijpsrr.2022.v77i01.025

INTRODUCTION

The genus *Strobilanthes* (*Acanthaceae*) constitutes large number of species that are spread across the tropical region of the world, especially in tropical Asia. This genus is the second largest in the family with 300 species in tropical Asia. Out of which 39 species are endemic to the peninsular India[1,2]. *Strobilanthes ciliatus* is one of the endemic species with several beneficial therapeutic properties. This plant has been largely used in Ayurveda as a source of the drug "Sahacharya" which is used to treat anti-inflammatory, antimicrobial, antidiabetic, anticancer, analgesic, anti-inflammatory and anti-immunosuppressant and wound healing properties[3-6]. The aqueous extract of roots of *S. ciliatus* were screening for CNS and depressant activity[7]. The plant extract has shown that there is a good correlation between phenolic phyto constituents and antioxidant property by the DPPH° scavenging method[8].

Preliminary phytochemical analysis showed the presence of 20 phyto constituents in the petroleum ether extract of *S. ciliatus*. The phyto constituents present are phenolic compounds, steroids, flavonoids, tannins, triterpenoids and glycosides[9-12]. The plant has been reported to contain stigmasterol, lupeol, betulin, stigmasterol glucopyranoside and 4-Acetyl-2, 7- dihydroxy-1, 4, 8-diphenyl-octane 2, 5-dione[13]. Lupeol was quantified by HPTLC method and found to be a major compound in *S.ciliatus*.

The plant parts of the *S. ciliates* have been used traditionally used for the treatment of anticancer and CNS activities[3-7]. Hence the purpose of this study is therefore to detect the AChE inhibitory and cytotoxic activity of extracts and isolated compounds.

MATERIALS AND METHODS

General

The chemicals used for these experiments were of analytical grade acquired from m/s Sigma-Aldrich, Bangaluru, India. IR spectra were obtained using Perkin-Elmer instrument 1650 FT-IR spectrometer. ^{1}H and ^{13}C NMR were recorded on Bruker-400 spectrometer at 400MHz using tetramethylsilane as an internal standard and $CDCl_3$ as a solvent. The mass spectral data were obtained using Shimadzu LCMS instruments.

Preparation of plant extract

The aerial parts of the plant material (*S. ciliatus*) have been collected in Chittur, Palakad district, Kerala and the botanical identification conducted by botanical survey of India, Coimbatore. 2 kg of air dried aerial parts of the *S. ciliatus* was ground to coarse material and cold percolated repeatedly with petroleum ether for (72 x 3) hours followed by methanol. Then the extractions were filtered and concentrated by evaporation of solvent using rotary evaporator under reduced pressure at 40°C to yield residue A 3.6g (0.18%) and residue B 2.4g (0.12%) respectively.

The TLC has been carried out with the solvent system petroleum ether: ethyl acetate (8:2) for residue A. It showed the compound-1 at Rf value of 0.67.Similarly

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

residue B showed the compound-2 at Rf value of 0.54. The TLC analysis spots were developed in an iodine chamber.

The residue A was subjected to column chromatography over a silica gel as a stationary phase. Gradient elution was performed using mixture of petroleum ether and ethyl acetate. On further increase in the ratio of ethyl acetate, the compounds are isolated according towards their polarity. Elutes of 20ml were collected and homogenous nature of the segments were detected by TLC through silica gel-G plates. Fractions 9-18 yielded compound-1 (80mg). Similarly, residue B yields compound-2 (50 mg). The isolated compounds were characterized as lupeol (compound-1) and stigmasterol (compound-2) by comparing the values of the spectral data with the literature values[9,14].

Acetylcholinesterase inhibitory activity

Raw material

Acetylcholinesterase enzyme, 5,5'-thiobis-2-nitrobezoic acid(DTNB) and acetylthiocholine iodide (ATCI) were acquired from sigma aldrich.

Acetylcholinesterase inhibitory assay

Acetylcholinesterase inhibition was carried out for petroleum ether and methanolic extracts and isolated compounds from *S. ciliatus.* The AChE inhibitory effect of extracts and isolated compounds was screened by using Ellman's spectrophotometric method[15]. Acetylthiocholine iodide was cast-off as substrate. Each test tube was sampled with 2.81 ml of pH 8 phosphate buffer. Test solutions of various concertrations of 20µg, 40µg, 60µg, 80µg, 100µg were added and 30µl of enzyme was added. The mixture has been allowed to stand-up for 10min. DTNB reagent was added that produces the yellow colour anion of 5-thio-2-nitrobenzoic acid, followed by a 30 µl of substrate. This whole mixture was incubated for 20min and the absorbance measured at 412 nm.

The percentage of AChE inhibition can be determined as follows:

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{absorbance of substrate}}{\text{absorbance of control} \times 100}$$

In-vitro cytotoxic activity

In-vitro cytotoxic assay of the extracts and isolates was performed on K562 and HeLa cell lines by previously reported method[16,17].

MTT assay

The K562 and HeLa cells were sourced from the National Centre for Cell Sciences (NCCS), Pune, India. The K562 and HeLa cells were cultivated in an Eagle minimum essential medium with foetal bovine serum (FBS). For cytotoxic assay, the chambers were dispersed into sterile 96-well plates in a density of 10000 cells/well and nurtured at 370°C, 5.3% CO2 humidified air for 24h before adding extracts and isolated compounds. The extracts and isolated compounds were solubilized in dimethyl sulphoxide and diluted in a corresponding medium contains 1% FBS. After 24 h, this experiment was repeated under the same conditions with extracts and isolated compounds at various concentrations for 48 h. The commercial drug cisplatin assisted as a positive control and the triplicate was maintained for all concentrations. Phosphate buffer saline with 10ml of 3-[4,5-dimethylthiozol-2-yl]2,5-diphenyltetrazolium bromide (MTT) 5mg/ml has been added to each well and incubated at 37°C for 4h. Thereafter, the MTT-containing medium was removed and the formazan crystals formed were solubilized using 100ml of DMSO. The absorbance of test drug and control were recorded at 570nm.

$$\% \text{ cell inhibition} = \frac{100 - \text{absorbance of drug}}{\text{absorbance of control} \times 100}$$

RESULTS AND DISCUSSION

Acetylcholinesterase inhibitory activity

Acetylcholiesterase inhibitors are one among the class of drugs used for the treatment of Alzheimer's disease. These inhibitory tests intertwine with the function of cholinergic system to enhance remembrance and cognitive disorder in the patients by diminishing the deterioration of acetylcholine in the cerebral synapses. Nature is an inexhaustible reserve for delivering unique and complex components and biological compounds so far away, their chemical synthesis seem incredible and compounds from nature are inexpensive and harmless AChE inhibitors with greater efficiency[18,19].

Stigmasterol, lupeol and *S. ciliatus* extracts have been tested for acetylcholinesterase inhibition. The IC_{50} values were determined from the regression equation from the different concentrations of extracts and isolated compounds. Neostigmine is used as the standard substance. The IC_{50} values of 80, 90, 145 and 112mg/ml for lupeol, stigmasterol, petroleum ether extract and methanol extract of *S. ciliatus* respectively with standard neostigmine IC_{50} value 25mg/ml. (Figure. 1-4; Table. 1).

Table 1: Acetylcholine esterase inhibition of extracts and isolated compounds from *S. ciliatus*

Sample	IC_{50} value µg/ml
Petroleum ether extract	145
Methanol extract	112
Lupeol	80
Stigmasterol	90
Neostigmine	25

In-vitro cytotoxic activity

We have carried out MTT assay, a simple and most reliable approach, that evaluates the viability of the cells for screening antitumor activity and the results have been exhibited in the following Table 2.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

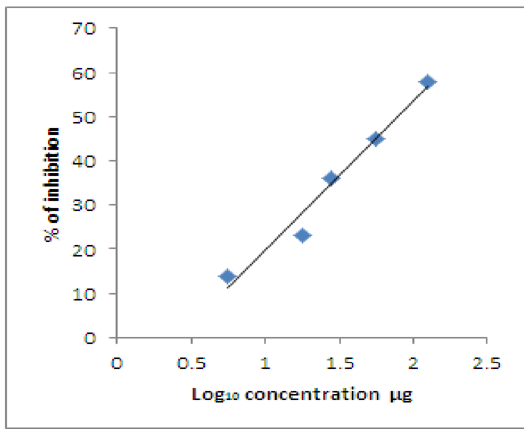

Figure 1: AChE inhibition of lupeol

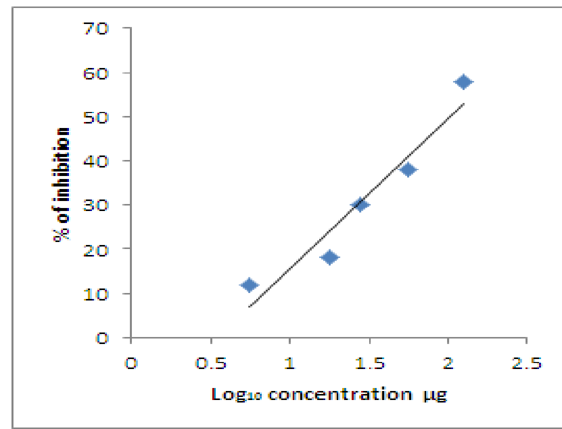

Figure 2: AChE inhibition of Stigmasterol

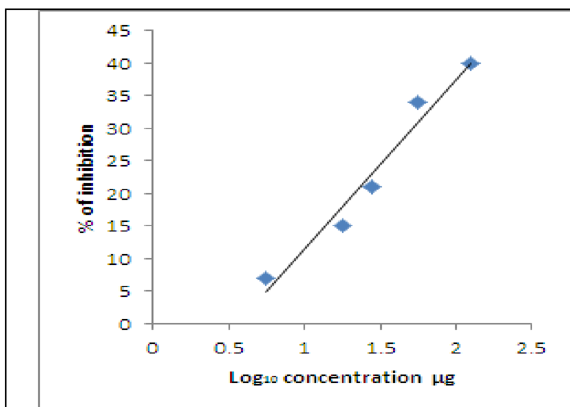

Figure 3: AChE inhibition of Petroleum ether extract of *S. ciliatus*

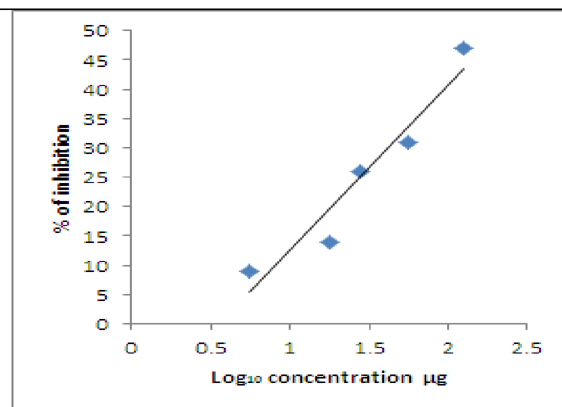

Figure 4: AChE inhibition of methanol extract of *S. ciliatus*

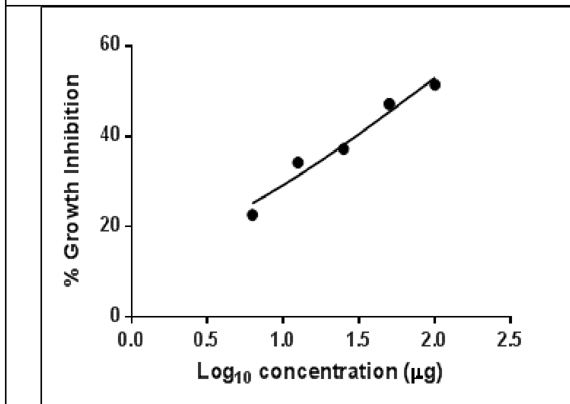

Figure 5: Cytotoxic activity of lupeol on K562 cell line

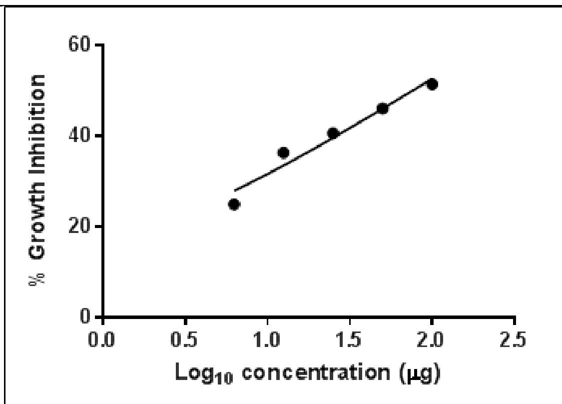

Figure 6: Cytotoxic activity of stigmasterol on K562 cell line

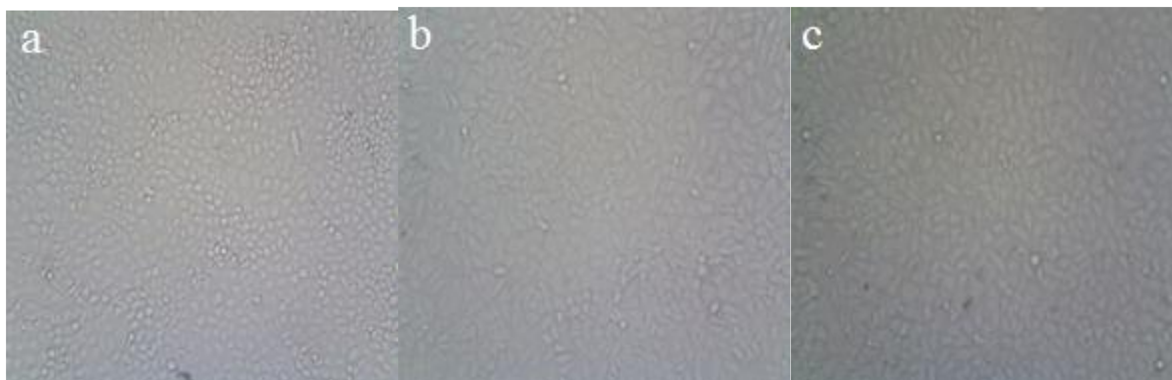

Figure 7: Morphological changes of K562 cell lines after the treatment of isolated compounds a) Untreated K562 cell lines b) Treatment with lupeol c) Treatment with stigmasterol

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

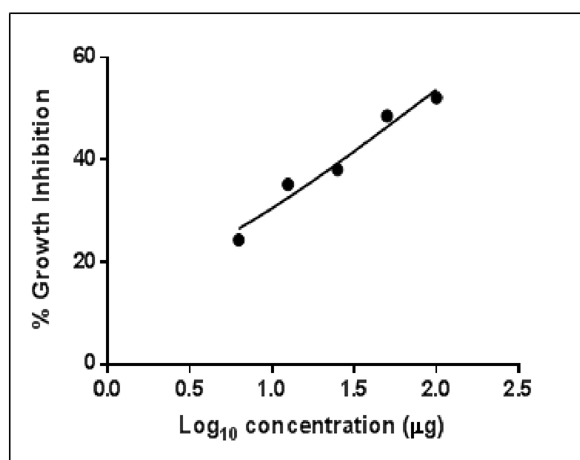

Figure 8: Cytotoxic activity of lupeol on HeLa cell line

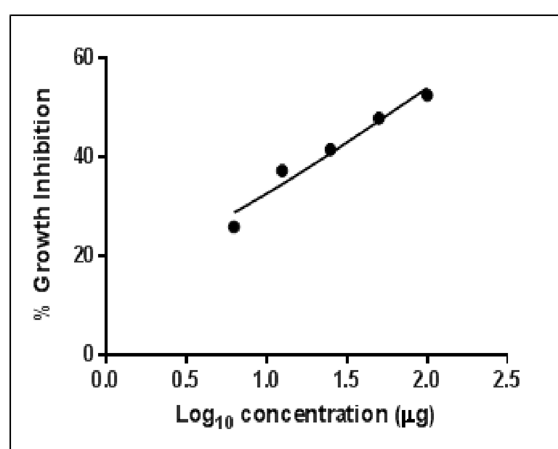

Figure 9: Cytotoxic activity of sigmasterol on HeLa cell line

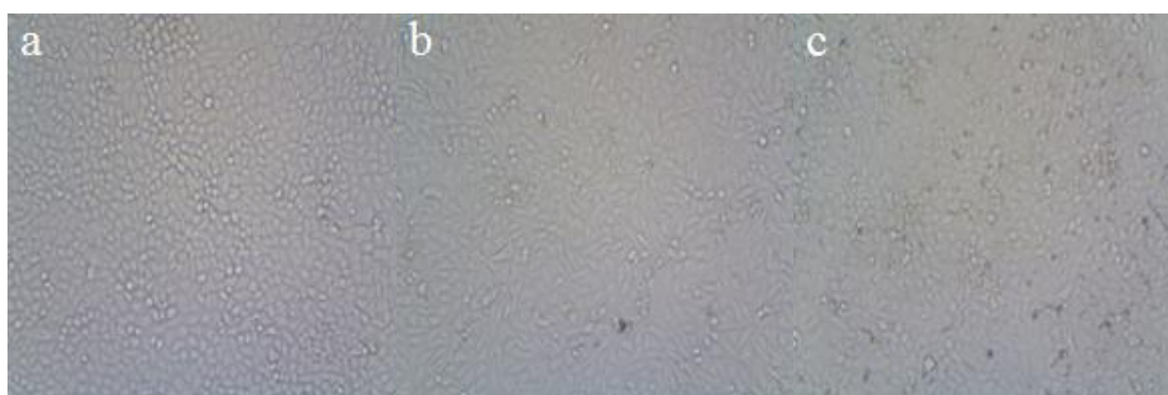

Figure 10: Morphological changes of HeLa cell lines after the treatment of isolated compounds a) Untreated HeLa cell lines b) Treatment with lupeol c) Treatment with stigmasterol

The extracts of aerial parts of *S.ciliatus* and isolated compounds were evaluated for their anti-cancer activity against HeLa and K562 cell lines. After incubation the cancer cells (HeLa and K562) viability with different concentrations of isolated compounds and extracts of *S. ciliatus* was measured. The hydroalcoholic extract of this plant reported to exhibit significant cytotoxic activity against MCF-7 cell line[20]. The extracts and the isolated compounds displayed dose-dependent cytotoxic effect on the K562 and HeLa cell lines and the IC_{50} values of petroleum ether and methanolic extract were determined as above 300 mg/rnl for both HeLa and K562 cancer cells. However, it is possible to obtain synergistic effect when we add some other cytotoxic active plant's extraction. This is the first report of this kind to test the *S. ciliatus* extracts for anticancer activity against K562 and HeLa cell lines. The isolated two compounds lupeol and stigmasterol showed comparable results with IC_{50} values of 76.54 and 74.98 g/ml for K562 (Figure. 5&6) and the IC_{50} value of 70.25 and 66.09 g/ml for HeLa cell lines (Figure. 8&9) respectively. The observation of the morphologic change of the HeLa and K562 cells shoewed changes in form and morphological characteristics (Figure 7&10).

Table 2: Cytotoxic activity of extracts and isolated compounds from *S.ciliatus* lupeol on HeLa and K562cell lines

Sample	IC_{50} µg/ml	
	HeLa cell line	**K562 cell line**
Petroleum ether extract	>300	>300
Methanol extract	>300	>300
Lupeol	70.25	76.54
Stigmasterol	66.09	74.98

CONCLUSION

The results provide comprehensive evaluation of cytotoxicity and AChE inhibitory activity of extracts from *Strobilanthes Ciliatus* using a variety of *in-vitro* assays. The extracts and isolated two compounds from *S. Ciliatus* showed moderate to comparable against Acetylcholinesterase enzyme and K562 and HeLa cell lines. Therefore, the results of this study provide an opportunity to obtain and identify the phytoconstituents of the extract of *S. Ciliatus* that can be assessed for the potential use in cancer therapy and AChE inhibition without any side effects.

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

REFERENCES

1. Warrier PK, Nambiar VPK, Raman Kutty C, Indian medicinal plants, Madras: Orient Longman ltd, 1994; 5: 142-145.
2. Thomas J, Joy PP, Mathew S, Skaria BP, Duethi PP, Joseph TS, Agronomic Practices for aromatic and medicinal plants, Calicut: Kerala Agricultural University, 2000; 124.
3. Nayar RB, Ravishankar B, Vijayan NP, Sasikala CK, Saraswathy VN, Anti-inflammatory effect of *Strobilanthes heyneanus* leaves a biochemical study, Journal of Research in Ayurveda & Siddha, 1988; 9: 49–58.
4. Ravishankar B, Nair RB, Sasikala CK, Sulochana S, Pharmacological screening of *Strobilanthes heyneanus* Nees. Root, Journal of Research in Ayurveda & Siddha, 1987; 8: 113–128.
5. Brahma SD, Sathishkumar MRN, In-vivo anti-inflammatory activity of *Strobilanthes Ciliatus* Nees, International Journal of Research in Pharmacy and Chemistry 2015; 5(4): 634-636.
6. Bharat S, Ram AS, Secondary Metabolites of Medicinal Plants: Ethnopharmacological Properties, Biological Activity and Production Strategies, 2020; 3.
7. Ravishankar B, Nair RR, Vijayan NP, Pankujavally PT, Sasikala CK, Sulochana S. Analgesic, anti- inflammatory and immunosuppressant effect of *Strobilanthes heyneanus* Nees. Stem, Journal of Research in Ayurveda & Siddha, 1987; 8: 53–61.
8. Christy KJ, Francis M, Aleykutty NA, Evaluation of In-Vitro Antioxidant Activity of Two Anti-Arthritic Plants by DPPH° Method, International Journal of Innovative Science and Research Technology, 2018; 3(9): 249-253.
9. Reneela P, Sripathi, Shubashini K, Triterpenoid and Sterol Constituents of *Strobilanthes ciliatus* Nees. Natural products, 2010; 6(1): 35-38.
10. Mathew G, Lincy J, Sony S, Evaluation of analgesic activity of ethanolic extract of *Strobilanthes ciliatus* Nees. The Pharma Innovation Journal, 2017; 6(7): 326-328.
11. Gomathi V, Rani S, Babitha MC, Phytochemical screening and GC-MS analysis of petroleum ether extract of *Strobilanthes Ciliatus* leaves, International Journal of Botany Studies, 2021; 6(5): 511-514.
12. Maria CF, Sellappan K, GC-MS Analysis of Metabolites in Leaf and Stem of Medicinally Important *Strobilanthes* Species from North Western Ghats of India, Phytomorphology, 2019; 69 (4): 67-79.
13. Anjana KN, Moola J, Nanjan C, Shijikumar PS, Phytochemical and Pharmacological Aspects of *Strobilanthes Ciliatus* Nees. (Bremek.): A Review, InternationalJournal of Research in Ayurveda and Phar macy, 2016; 7(4): 72-77.
14. Venkatachalapathi S, Subban R, Isolation and quantification of lupeol in *Strobilanthes ciliatus* Nees by HPTLC method, International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4(4): 405-408.
15. Ellman GL, Courtney KD, Andres V, Featherstone RM, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochemical Pharmacology, 1961; 7: 88-95.
16. Mosmann, T, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, The Journal of Immunological Methods, 1983; 65: 55-63.
17. Arung ET, Wicaksono BD, Handoko YA, Kusuma IW, Yulia D, Ferrry S, Anti- cancer properties of diethylether extract of wood from sukun (antocarpusaltilis) in human breast cancer (T47D) cells), The Journal of Immunological Methods, 2009; 8: 217-324.
18. Tayebeh N, Ahmad D, Antoni S, Eduardo SS, Samira S, Role of natural products for the treatment of Alzheimer's disease. European Journal of Pharmacology, 2021; 898: 173974.
19. Thaiane CDS, Thaís MG, Bruno ASP, Adriana LC, Antonio MAP, Naturally Occurring Acetylcholinesterase Inhibitors and Their Potential Use for Alzheimer's Disease Therapy, Frontiers in Pharmacology, 2018; 9: 1192.
20. Jayaraman S, Senthilkumar KL, Sivakumar R, Jagatheesh K, Arunachalam G, Evaluation of phytochemical and biological properties on *Strobilanthes ciliatus* Nees. Pharmanest, 2014; 5(1): 1821-1828.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

For any question relates to this article, please reach us at: globalresearchonline@rediffmail.com
New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.