



Research Article

Measurement Of Dementia Using Medicinal Plants For Cognitive Impairment

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Abstract

Dementia is a condition that is more widespread in aged. It involves a decline in their ability to think, or cognition, and memory. The present study was conducted to evaluate the presence of bioanalytes using different methods in the edible part extracts of *Tinospora cordifolia* by ELISA method. Enzymatic assay of esterase, choline esterase and acetylcholine esterase were detected as 36.67, 17.43 and 65.45 % in triplicates assay. Analysis of the edible parts leaves *Tinospora cordifolia* confirmed the presence of total phenolics content were found to be greater in support of drug development against deficits of learning and memory. Our finding suggests that leaves parts use in *Tinospora cordifolia* are proficient with redox potentials, immunostimulation and for cognitive impairment.

Keywords: Dementia, total phenolics, leaves, *Tinospora cordifolia*

Introduction

Dementia is a neurodegenerative disease dis-

ressing older adults. Neurodegenerative diseases are conventional to be second most all-inclusive cause of death among aged by the 2040s (Ansari et al, 2010). Cognitive and neural dysfunctions ensue due to accumulation of oxidative damage in the way of biochemical anomalies as nucleic acid, protein and mitochondria in the brain. Amongst the strategies for neuroplasty, plant based chemicals or phytotherapy may possibly characterize a helpful therapy in chemoprevention of neurological diseases. As a complementary and alternative therapy, herbal medicine refers to the medicinal use of plant components such as roots, stems, flowers, leaves, fruits and seeds for their curative properties. Acetylcholinesterase (AChE) is a hydrolyse that plays a vital role in cholinergic transmission by catalyzing the rapid hydrolysis of the neurotransmitter acetylcholine (ACh) (Gabrovska et al, 2008). Natural products might slow the progression of Alzheimer's disease (AD) by simultaneously protecting neurons from oxidative stress and acting as cholinesterase inhibitors (Costa et al, 2013). Herbal products contain a variety of bioactive substances include alkaloids, terpenoids, phenolics, saponins and flavanoids. Among these phenolics substance possesses biologically active for unrivaled discourse (Hossain et al, 2009). The potential effects on various extracts of leaf, stem and roots of *T. cordifolia*, viz., hepatoprotective effect, antioxidant effect, effect against infection, immunomodulatory activity, antistress activity, gastrointestinal protective activity and other properties of therapeutic importance, like learning memory, antiinflammatory and antiallergic properties effects (Panchabhai et al, 2008). The present study reveals the biomolecules and total phenolic content showed their bioactive potentials and neuroprotective effects and other related diseases like cognitive impairment. The medicinal plants are confirmed for their usefulness in Alzheimer's disease and memory deficits.

Materials and Methods

Plant Materials

T. cordifolia is a large, glabrous, deciduous climbing shrub, belongs to the family Menispermaceae, native to India. *Tinospora cordifolia* leaves were collected from Herbal Garden of St. Josephs

College (Autonomous), Trichy. It is brought to the Crop Improvement division, Dhanalakshmi Srinivasan Agriculture College, Perambalur in cool and dry containers. Leaves were washed with water to eliminate sediments, dried in the shade so as to prevent the composition of enzymatic assay. It was then powdered using blender and stored in dry container for further use.

The *Tinospora cordifolia* leaves powdered was cold macerated with 50% Methanol and acetone precipitation (1:1 ratio) and centrifuge 15000 rpm for 10 mins. The supernatant is used for analysis of enzymatic assay of esterase, choline esterase and acetylcholine esterase through ELISA method.

MMSE (Mini-Mental State Examination):

According to Preston, 1986, Psychosomatic index of Mini-Mental State Examination is used to screen patients for cognitive impairment, track changes in cognitive functioning over time, and oftentimes to assess the effects of therapeutic agents on cognitive function. Diagnostic accuracy estimates (sensitivity, specificity, positive and negative predictive power) of MMSE cut-scores in detecting cognitive dysfunction.

Enzymatic Assay

The AChE inhibition assay was carried out in a multi-well plate using a modified method, as described by Ellman et al. (1961). Electric eel acetylcholinesterase was used, while acetyl thiocholine iodide (ATCI) was used as the substrate of the reaction. 5,5-dithiobis(2-nitrobenzoyl) acid (DTNB) was used for measurement of AChE activity. Briefly, 150 μ L of 0.1 M sodium phosphate buffer (pH 8.0), 10 μ L test compound solution, and 20 μ L of enzyme solution (0.09 units/mL) were mixed and incubated for 15 min at 25 °C. 10 μ L of DTNB (10 mM) was then added and reaction was initiated by the addition of substrate (10 μ L of ATCI, 14 mM solution). The hydrolysis of the ATCI can be measured by the formation of the product, 5-thio-2-nitrobenzoate, a colored anion formed by the reaction of DTNB and thiocholine, which is released by enzyme hydrolysis. Absorbance was measured at 412 nm (Shimadzu, 1200, Japan) after 10 min. Tacrine, a standard AChE inhibitor, was used as positive control.

The percent of acetylcholinesterase inhibition was calculated as following: % Inhibition = $100 - [\text{Absorbance of the test compound} / \text{Absorbance of the control}] \times 100$

Esterase Activity was extracted from young leaves were collected individually homogenized with a glass rod in an Eppendorf microcentrifuge tube using 40 μ L 0.1 M Tris-HCl buffer at pH 8.5, containing 6% PVP-40, 0.1% ascorbic acid, 0.2% EDTA and 0.5% β -mercaptoethanol. After homogenization, the samples were centrifuged at 25,000 rpm for 30 min at 4°C in a microcentrifuge; the supernatant (20 μ L) was used for analyses. It was measured at its maximum absorption of 600 nm by using UV-260 Shimadzu spectrophotometer. For the calibration of the 1-naphthol product, the procedure of van Asperen (1962) was followed.

Total Phenolics Assay:

Dry plant material (500 mg) was weighed in to a test tube. Add 10ml of 80% aqueous methanol and suspension was stirred slightly. The tubes were sonicated for 5 mins and centrifuged for 10 mins (2000g) for 2 mins, and finally the supernatants were collected and assayed using Folin ciocalteu's reagent in addition of 2 ml of sodium carbonate mixed and allowed to stand for 30 mins. The concentration of total phenolics in leaves extract of *Tinospora cordifolia* was detected at 765 nm. It is expressed as tannins equivalents in milligrams per gram (Ainsworth et al, 2007).

Statistical Analysis

Data are expressed as mean \pm standard deviation (SD). All analysis was carried out in at least three replicates for each sample. Results were analyzed statistically using SPSS 15.0 (San Diego, CA, USA). A value of $p < 0.05$ was considered statistically significant.

Results

However, sensitivity and specificity measures are vital to ascertain the diagnostic validity of test measures such as the MMSE (Mini mental state examination), the diagnostic utility of a particular score earned by a particular patient is represented by the test's predictive values. An optimal balance between sensitivity (.89) and specificity (.91) was obtained with a cut-score of 27 in elderly cases. This yielded only slight improvement in the overall correct classification rate (91%) but identified 35 of the 50 dementia patients who were missed using the traditional cutoff. The cut score of 27 yields a likelihood ratio of 9.6, indicating that elder cases with an MMSE score of 25 and with complaints of cognitive decline (self- or other-report) are nearly 10

times more likely to have dementia than those who obtain a score of 27 or improved. MMSE is the DSM-IV psychoanalysis used to assess the cut score for sensitivity and specificity of deficits in the mental status sheet is early detection of possible dementia.

Table.1 Psychosomatic index for Sensitivity and Specificity Estimates for Detecting Dementia

Demographic chart	40-50 years	60-70 years	70-80 years
MMSE Cut Score < 27			
Sensitivity	0.80 (0.75-0.84)	0.89 (0.85-0.90)	0.66 (0.61-0.71)
Specificity	0.89 (0.85-0.92)	0.91 (0.88-0.95)	0.79 (0.82-0.90)

Data are expressed as mean \pm standard deviation (SD). All analysis was carried out in at least three replicates for each sample.

Table.2 Bioenzymatic analytes in leaves extract of *Tinospora cordifolia* using ELISA method

Enzymes (μ g/ml)	40-50 years	60-70 years	70-80 years
esterase	12.36 \pm 0.56	9.58 \pm 0.24	6.45 \pm 0.12
Choline esterase	15.66 \pm 1.02	18.25 \pm 1.85	25.32 \pm 2.87
Acetyl choline esterase	36.67 \pm 1.32 ^a	65.45 \pm 1.68 ^a	17.43 \pm 1.26
Total phenolics assay (mg/g gallic acid equivalent)	9.58 \pm 0.24	12.36 \pm 0.56 ^a	6.45 \pm 0.12

Data are expressed as mean \pm standard deviation (SD). All analysis was carried out in at least three

replicates for each sample.

*value of $p < 0.05$ was considered statistically significant.

The findings of this study confirmed the effectiveness of certain Indian traditional medicine plants on enhancing memory and learning or in the treatment/prevention of AD. Some Indian traditional medicine plants like *Tinospora cordifolia* showed improving effects on memory and the treatment of AD in clinical trials shows that total phenolic content 12.36 μ g/ml in upper age groups. Enzymatic analytes findings suggest that acetyl cholinesterase activity possesses more significant as 65.45 μ g/ml in the age of 60-70 years which elicits the favorable pharmacological profile in the treatment of Alzheimer's disease type of dementia. A few cases, active principles conscientious for the efficacy of these plants on memory were also firmed.

Discussion

The present study was considered in elderly cases that are more prevalence towards dementia followed by DSM-IV and a few herbs were evaluated in clinical trials on AD. Furthermore, there are insufficient or no investigations on certain herbal medicines used in Indian traditional medicine to confirm their effectiveness on memory and learning (Shekhar et al, 2014). Therefore, further experimental and clinical studies are necessary to evaluate the effectiveness of these plants on memory and AD as well as determining their active components (Gupta and Gupta, 1997). In conclusion, these findings suggest that these plants which were reported to have acetyl cholinesterase activity may have favorable pharmacological profile in the treatment of Alzheimer's disease (Singh et al, 2003). Ever since, Acetylcholine plays a crucial role in cognitive function plus learning and memory and is obvious that the acetyl cholinesterase activity has memory enhancing properties.

Reference

Panchabhai TS UPK, Rege N N. Validation of Therapeutic Claims of *Tinospora cordifolia*: A Review. *Phytother Res.* 2008; 22:425–41.

Hossain Md. Mokarram SMRH, Akter Raushanara Md., Islam Nurul MJR, Saha Moni Rani, Hoque Md. Ehsanul, Mazumder SR. Evaluation of analgesic and neuropharmacological properties of the aerial part of *Tinospora cordifolia* Miers. in mice. *Stanford Journal of Pharmaceutical*

Sciences. 2009;2(2):31–37.

Gabrovska, K.; Marinov, I.; Godjevargova, T.; Portaccio, M.; Lepore, M.; Grano, V.; Dianoc N; Mita, G.M. The influence of the support nature on the kinetics parameters, inhibition constants and reactivation of immobilized acetylcholinesterase. *Int. J. Biol. Macromol.* 2008, 43, 339–345. 6.

Costa, P.; Gonçalves, S.; Valentao, P.; Andrade, P.B.; Romano, A. Accumulation of phenolic compounds in in vitro cultures and wild plants of *Lavandula viridis* L'Hér and their antioxidant and anti-cholinesterase potential. *Food Chem. Toxicol.* 2013, 57, 69–74.

Ellman, G.L., Courteney, K.D., Valentino, A.J., Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 1961, 7, 88–95

Gupta, A.; Gupta, R. A survey of plants for presence of cholinesterase activity. *Phytochemistry* 1997, 46, 827–831.

Singh SS, Pandey SC, Srivastava S, Gupta VS,

Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora Cordifolia* (Guduchi) *Indian J Pharmacol.* 2003;35:83–91.

Shekhar C, Kumar S. (Kinetics of Acetylcholinesterase Inhibition by an Aqueous Extract of *Mentha longifolia* Leaves). *Int. J. Pharm. Sci. Drug Res*, 2014; 6(4): 348-50.

van Asperen, K. 1962. A study of housefly esterases by means of a sensitive colorimetric method. *J. Insect. Physiol.* 8:401-416.

American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders* (4th edition). Arlington, US: American Psychiatric Publishing Inc., 2000

Preston G . Dementia in elderly adults. Prevalence and institutionalization. *J Gerontol* 1986;41:261–7.

Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin- Ciocalteu reagent. *Nat Protoc.* 2007; 2(4):875–877.