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PRELIMINARY STUDY ON THE ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *IPOMOEA MURICATA* (LINNAEUS) JACQUIN

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Ipomoea muricata Linn. Jacquin; acetylcholinesterase; cholinergic tone; Alzheimer's disease.

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ABSTRACT: Acetylcholinesterase (AChE), a member of α/ß hydrolase protein super family, is the key enzyme in the breakdown of acetylcholine (ACh) - an important neurotransmitter. Inhibition of this enzyme has been considered an auspicious strategy for the treatment of various neurological disorders such as Alzheimer's disease (AD). In this study, crude methanolic Ipomoea muricata seed extract was subjected for analysis of its potential AChE inhibitory activity using a microplate assay based on Ellman's method. I. muricata's inhibitory activity was compared using Donepezil, a prescription drug for AD treatment. Two time intervals were used: 40 minutes and 24 hours. Based on the findings, Donepezil exhibited greater potency (IC50 > 0.005 µg/mL) at both time intervals in contrast to I. muricata (IC50 = 39.67 µg/mL) at 40 minutes and (IC50 > 57.44 µg/mL) at 24 hours. It is important to note that the crude methanolic seed extract of I. muricata has been shown to cause moderate but persistent inhibition of AChE in comparison to Donepezil which has shown a strong but short-lived action. With this concludes that in the near future followed by further studies, I. muricata can be a potential herbal alternative in the symptomatic treatment of AD.

INTRODUCTION: Alzheimer's disease (AD) is the major form of senile dementia, characterized by neuronal loss, extracellular deposits, and neurofibrillary tangles. It is the most common form of dementia which contributes to 60-70% of the cases with an estimated number of 35.6 million affected individuals worldwide according to the WHO 2012 report "Dementia: a public health priority". These numbers are expected to double by 2030 and triple by 2050 with the greatest surges attributable to low and middle income countries [1, 2].

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The disease is accompanied by a profound loss of cholinergic tone, and acetylcholine (ACh) levels in the brain, which were hypothesized to be responsible for the cognitive decline observed in AD [1]. Also, acetylcholinesterase (AChE) activity is found to be enhanced around amyloid deposits (which are toxic to the brain) in the very early stages of amyloid deposition.

These events are then followed by the hydrolysis of the remaining ACh molecules in the synapse by AChE thereby impairing cholinergic neurotransmission. Since cholinergic function is required for short term memory, any cholinergic deficit in AD patients is believed to be responsible for much loss of intellectual abilities. Hence, blocking ACh degradation through the use of AChE may potentially reduce the severity of

cognitive loss and slow down the progression of the disease [3, 4, 5, 6, 7].

Cholinesterase inhibitors are one of the classes of compounds to date that have consistently proven to be efficacious in managing the cognitive and functional symptoms of AD. Their administration often leads to the increased availability of ACh in the synaptic cleft leading to improved communication between nerve cells that utilize them as chemical messengers. This in turn improves cognitive performance and temporarily eases cognitive symptoms commonly associated with AD. Currently, three of these medications have been approved for the symptomatic treatment of AD namely Donepezil, Rivastigmine, and Galantamine [8, 9].

Donepezil (Aricept) is an oral, highly selective, reversible acetylcholinesterase inhibitor (AChEI) that inaugurates a new class of AChEI's with longer and more selective action. Administration results to increased ACh levels in the synapse leading to an improved cholinergic tone which in turn controls the symptoms associated with AD. However, as with any other AChEI that increase the levels of ACh in the brain, they can also increase ACh in the periphery causing potential side effects. Adverse events reported include nausea, vomiting, and diarrhea [10].

Taking into account these side effects, many research groups have averted their gaze into developing new, more effective, targeted therapeutics for the treatment of AD that have fewer adverse reactions. For this reason, the gain in popularity of harnessing naturally-occurring compounds from plants became undeniable in the recent years and not without a reason that there is a possibility to slow down the brain's degeneration caused by AD [5]. Several reviews regarding newly discovered AChEI's found in plants, fungus, and marine organisms have been published throughout the years. Majority of these AChEIs belong to the alkaloidal group, including indole, isoquinoline, quinolizidine, piperidine, physostigmine and its analogs, xanthostigmine and its analogs, lycorine, carbazole, and steroidal alkaloids. On the other hand, potent non-alkaloidal AChEI's have also been found from natural sources such as terpenoids, flavonoids, and other phenolic compounds [11, 12, 13, 14, 15].

Ipomoea muricata (Linn.) Jacquin of the family Convolvulaceae, locally known as "tonkin/tunkin" [16], has various therapeutic applications such as being used as a purgative, febrifuge, spasmolytic, hypotensive, antibacterial and antifungal agent [17, 18]. In addition, the Dominican parochial community has been known to exploit its seeds, stems, and leaves for the treatment of skin ailments for instance chronic and gangrenous wounds, cuts, and blisters due to burns [19]. It is found effective in the treatment of pain, insect and snake bites, ulcer, tumor, and cancer [20].

I. muricata contains an array of biologically active compounds in which alkaloids comprise of (0.49%). For example, Lysergol, which constitutes ~53% of these alkaloids, has been demonstrated to have hypotensive, psychotropic, and uterus and intestine-stimulating properties.

Also, Chanoclavine, which comprises ~37% of these alkaloids, may have AChE inhibitory capability according to a literature but its mechanism still remains unclear [18]. The seeds were reported to contain ethyl caffeate and muricatin that have antibacterial activity [20]. The presence of ipomine, ipalbidine, ipalbine, and ipalbinium [21] was also confirmed in which ipalbidine displayed analgesic activity [22, 23]. Lastly, caffeic acid which can be found in the seeds of *I. muricata* has been known to be hepatoprotective [24] and is effective in scavenging 2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ABTS•+, 1,1-diphenyl-2-picryl hydrazyl radical (DPPH•) , and superoxide anion radical. Alongside, *I. muricata* has high total reducing power and metal chelating activity on ferrous ions [25]. Following this, a recent study showed that crude *I. muricata* extract bears strong antioxidant activity against DPPH•, nitric oxide, and lipid peroxides [26]. The study aimed to investigate the probable AChE inhibition of the crude methanolic seed extract of *I. muricata* Linn. Jacquin.

MATERIALS AND METHODS:

Plant Material: Seeds were given by the director of the Research Center for the Natural and Applied

Sciences (RCNAS), Prof. Mafel C. Ysrael Ph.D. for study. Seeds were collected from Bocaue, Bulacan, Philippines and were identified by botanist Ophelia S. Laurente M.Sc. of University of Santo Tomas, Research Center for the Natural and Applied Sciences, Manila, Philippines. A voucher specimen (USTH-012720) was deposited at the University of Santo Tomas Herbarium.

Chemicals and Reagents:
Analytical grade solvents were used in all of the experiments and were purchased from RCl Labscan Limited, Bangkok, Thailand. All standards and reagents used in the study were purchased from Sigma-Aldrich (Singapore) and Merck (Germany).

Extraction:
Two hundred grams of *I.* muricata seeds were made into a coarse powder and were later subjected to solvent extraction by adding 500mL methanol. It was then placed on an orbital shaker (J.P. Selecta Rotabit) at 135 opm for approximately 6-8 hours and was left standing at room temperature for a day. The extract was filtered using Whatman Filter Paper Grade no. 1 and concentrated by using a rotary evaporator (EYELA). The extraction was repeated thrice. The accumulated extract was further concentrated by placing it under a fume hood for a week. Extraction yield was 17.15%. The concentrate was then transferred in an amber bottle and stored at -20°C until use.

Standard Curve Preparation:
Analytical grade L-cysteine hydrochloride monohydrate was used as a standard. The reaction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) with the liberated thiocholine from the hydrolysis of acetylthiocholine may be estimated in a sample by comparison to a standard curve comprising known concentrations of sulfhydryl-containing compounds such as cysteine. Stock standard solution were initially made by mixing 250µL of a standard concentration, 50µL of 3.96mg/mL DTNB, and 2.5mL of 0.1M sodium phosphate buffer pH=8.0 with 0.1mM ethylenediaminetetraacetic acid (EDTA) then was incubated at room temperature for 15 minutes. 200µL for each concentration mixture was transferred in a 96-well microplate and was read at 412nm using Corona Electric SH1000 microplate reader [27].

Enzyme Inhibition Assay:
AChE activity was determined by adapting and modifying the colorimetric method described by Ellman *et al* .[28], specifically for microplate analysis. The enzyme hydrolyzes the substrate acetylthiocholine resulting to the formation of thiocholine which reacts to Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate (TNB) which can be detected at 412nm. For the tests, 150µL of 0.1M sodium phosphate buffer pH=8.0 with 0.1mM EDTA, 10µL (3.96mg/mL) DTNB, 10µL of the test compound/control, and 20µL of (0.1U/mL) AChE was placed in a 96-well microplate and was incubated at room temperature for 15 minutes. The reaction was initiated by the addition of 10µL (1.5mg/mL) acetylthiocholine iodide. The hydrolysis process was confirmed by the formation of TNB from the interaction of DTNB with thiocholine, producing a yellow coloration. The rate of hydrolysis was monitored by Corona Electric SH1000 microplate reader at 412nm for 40 minutes with 2 minute intervals and at the 24th hour of the assay.

The experiment was done in triplicates to ensure the repeatability of the results. The median inhibitory concentration (IC_{50}) was determined by linear regression analysis.

Statistical Analysis:
One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used to determine if there are significant differences between the concentrations of the crude methanolic seed extract of *I. muricata* in inhibiting AChE with $p<0.05$ being considered as significant. Graph Pad Prism software was used.

RESULTS AND DISCUSSION: AD is a neurological disorder characterized by the death of brain cells that causes memory loss and cognitive decline in affected individuals. Although the exact mechanism remains unknown, a complex interplay of genetics, environment and aging are likely the culprits in the course of disease progression. Given the enormity of this field, this research paper

mainly focuses on AChE and its inhibition and a slight deviation to oxidative stress, both shown involved in areas of study.

The crude methanolic extract from the seeds of *I. muricata* were analyzed together with 0.005µg/mL Donepezil, a potent reversible AChE inhibitor, as positive control in order to assess its stance in contrast to AD prescription drugs. The results were expressed in rate of TNB formation (mmol/min), percent inhibition (%) and as median inhibitory concentration (IC_{50}) values.

Fig.1 shows the buildup in the production of TNB over time. A slower buildup of TNB signifies a slower hydrolysis of the substrate acetylthiocholine. The hydrolysis process generates thiocholine which reacts with DTNB to produce TNB, a colored product which can be detected at 412nm [28]. The negative control which contains methanol as sample has shown the greatest surge in the rate of formation of TNB (0.0508mmol/min) representing uninhibited AChE activity. On the other hand, 0.005 µg/mL of Donepezil showed the least increase in TNB formation (0.0049mmol/min) demonstrating the greatest inhibition of AChE which was followed by 100µg/mL of the crude methanolic seed extract of *I. muricata* (0.0174mmol/min) with a concentration dependent inhibition.

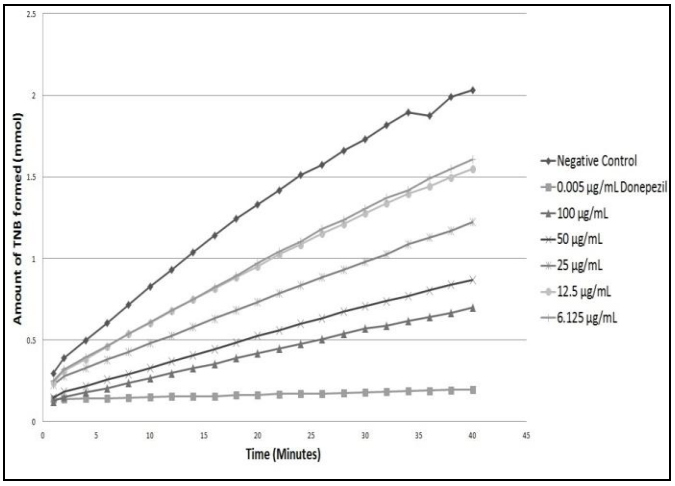

FIG.1: KINETIC ANALYSIS OF THE ACETYLCHOLINESTERASE INHIBITION OF *I. MURICATA* IN COMPARISON TO DONEPEZIL.

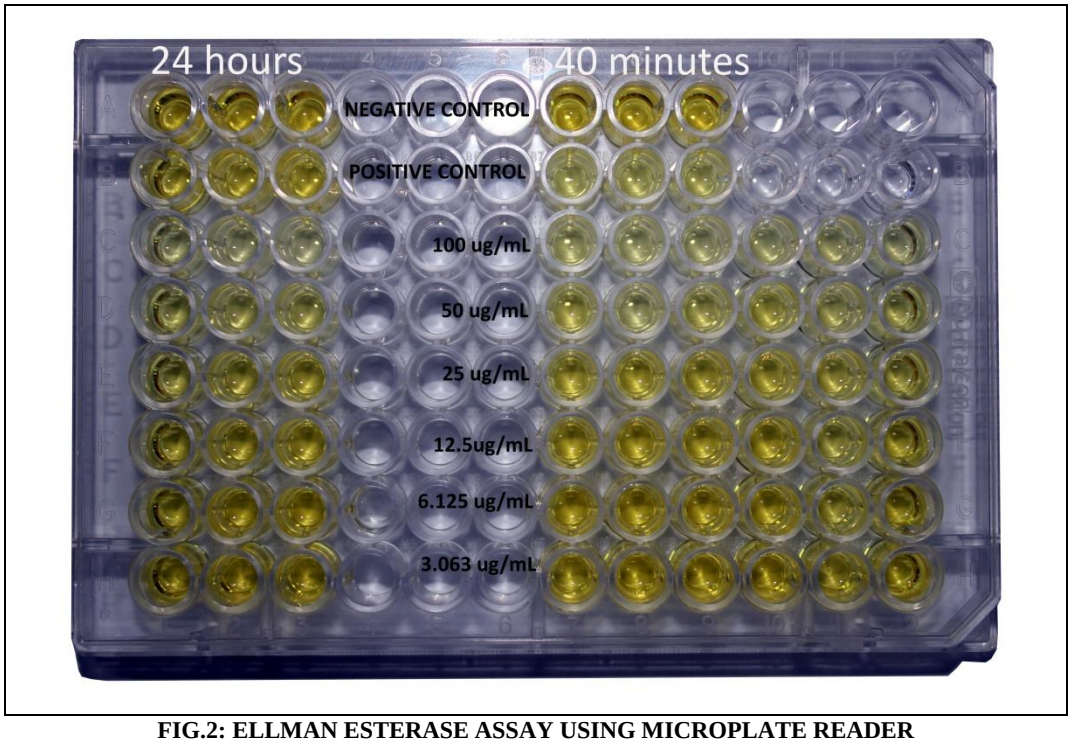

FIG.2: ELLMAN ESTERASE ASSAY USING MICROPLATE READER

In relation to the development of yellow color as a result of TNB creation as shown in Figure 1, a lighter colored well implies less TNB formed as a result of the slower hydrolysis of acetylthiocholine indicating AChE inhibition. As shown in **Fig.2**, Donepezil achieved close to colorless wells followed by the light tinges of yellow of the various concentrations of crude *I. muricata* extract.

This has shown that Donepezil prevented the formation of thiocholine (a product in the hydrolysis of acetylthiocholine) that reacts with DTNB to form TNB that produces the yellow coloration to a greater extent in contrast to the crude extract. On the other hand, the color of Donepezil wells became more intense in comparison to 100µg/mL of the extract that maintained its color at the 24th hour mark indicating the extract's stability and AChE inhibitory persistence.

Fig.3 shows the highest inhibitory activity recorded was that of Donepezil (positive control) having 90.36% inhibition at 40 minutes. It was subsequently followed by *I. muricata* at 100µg/mL having 65.62% inhibition with decreasing inhibitory activity as the concentration decreases having a concentration dependent behavior. The extract's level of inhibition is considered mild in comparison to pure isolated AChE inhibitors. However, it is important to note that 100µg/mL of the extract maintained its inhibitory activity (64.94%) in a span of 24 hours compared to Donepezil which declined (58.17%) over the course of the assay. At the same time, concentrations $\leq$ 100µg/mL had decreased inhibitory activity presenting the same behavior as the positive control.

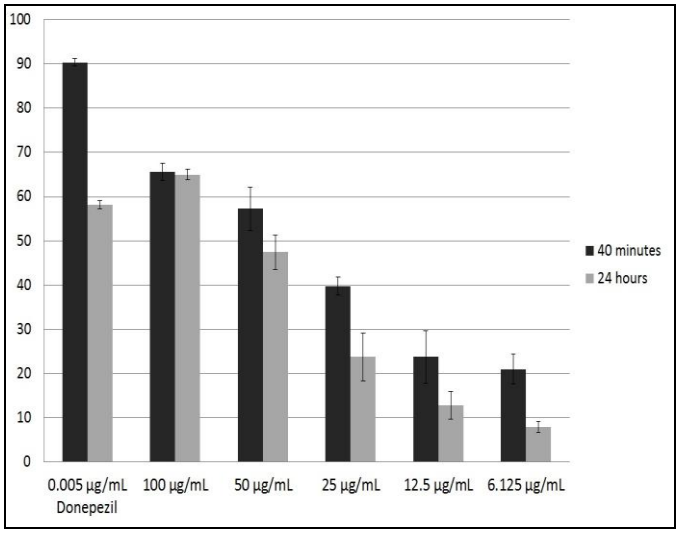

FIG.3: ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF DIFFERENT CONCENTRATIONS OF *I. MURICATA* AT DIFFERENT ASSAY TIMES.

A lower IC_{50} value means greater potency and better inhibition of the enzyme. Based on interpolated values: IC_{50}=39.67µg/mL (40 minutes) and IC_{50} = 57.44µg/mL (24th hour) in comparison to Donepezil IC_{50} < 0.005µg/mL at both time intervals, it has been shown unequivocally that Donepezil has higher potency and better AChEI activity. After all, the methanolic sample used is of crude nature and purification may most likely increase its effectiveness and strength.

One of the characteristic neuropathological alterations that occur in AD is the decrease of AChE activity in both cholinergic and noncholinergic neurons in the brain[4] Despite this loss of AChE activity in the brain, AChE activity is found to be enhanced around amyloid plaques, promoting the assembly of amyloid beta-peptides into fibrils and intensifying the cytotoxicity of these peptides [9]. Accompanied by the decrease of ACh synthesis, cognitive impairment is further exacerbated by the hydrolysis of the remaining ACh in the synapse by the enzyme AChE. Hence, restoration of cholinergic function by inhibiting AChE to increase ACh levels in the synapse may potentially reduce the severity of cognitive loss and slow down the progression of the disease [7].

Current approved AChEIs for AD treatment increase ACh levels in the synapse which results to the temporary improvement of cognitive function while at the same time increase ACh levels in the periphery causing unwanted side effects that may develop to clinically significant problems in the long run [11]. Consequences of AChEI administration has resulted to the need for the development of targeted effective therapeutics with fewer adverse reactions. Herbal medication has gained the attention of the scientific community as medicinal herbs contain various phytochemicals which may hold the answer for this overwhelming disease. In addition, these herbs are inexpensive and can easily be obtained which gives us the possibility to reduce the costs attributed to medications used for AD treatment [5].

Based on the findings, the crude methanolic seed extract of *I. muricata* has been shown to cause moderate but persistent inhibition of AChE in comparison to Donepezil which has shown strong but short-lived action. Chanoclavine which has been reported to be present in the seeds of *I. muricata* is suspected to be responsible for

inhibiting AChE [18]. Several species in the *Ipomoea* family have been discovered to exhibit potent anticholinesterase activity *in vitro* such as the leaves of *Ipomoea asarifolia* [30] and *Ipomoea aquatica* Forsk [30]. In addition, isquinoline alkaloids isolated from *Hippeastrum* species demonstrated significant AChE inhibitory activity which also opens the possibility of the active component in *I. muricata* being an alkaloid [31].

Admonishing the fact that the medium used in this study are seeds rather than using leaves makes *I. muricata* unique among its family. This may also explain why *I. muricata* presents this kind of inhibitory activity and may contain several similar to identical biochemicals responsible for these effects as the other species of *Ipomoea*. Despite all of this, the underlying mechanism on how the plant executes its AChE inhibitory activity *in vivo* as well as the responsible biological components remains unclear and need further studies. Overall, the crude methanolic seed extract of *I. muricata* should be considered for its application in the prevention and management of neurodegenerative disorders such as AD.

CONCLUSION: *I. muricata's* moderate but persistent inhibitory activity implies that it can be a possible natural alternative source for an AChE inhibitor even though it is less potent compared to Donepezil that has intermittent action. Then again, it is imperative to consider that the methanolic extract used is crude which when purified, is expected to display higher inhibitory activity and better potency. Further studies regarding the responsible active component that inhibits AChE, the type of interaction that exists between the inhibitor and the enzyme, the use of brain tissue homogenates and whether it can pass through the blood brain barrier are recommended. With this concludes that in the near future *I. muricata* can be used as a potential herbal alternative in the symptomatic treatment of AD.

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REFERENCES:

1. Lombardo S and Maskos U: Role of the nicotinic receptor in Alzheimer's disease pathology and treatment. Neuropharmacology 2015; 96B:255-256.
2. Duthey B: Update on 2004 Background Paper Written by Saloni Tanna, Background Paper 6.11 Alzheimer's Disease and other Dementias. Priority Medicines for Europe and the World "A Public Health Approach to Innovation". 2013.
3. Sberna G, Sáez-Valero J, Beyreuther K, Masters CL and Small DH: The Amyloid , 8-Protein of Alzheimer's Disease Increases Acetylcholinesterase Expression by Increasing Intracellular Calcium in Embryonal Carcinoma P19 Cells. Journal of Neurochemistry 1997; 69(3):1177-1184.
4. Dumas JA and Newhouse PA: The cholinergic hypothesis of cognitive aging revisited again: Cholinergic functional compensation, Pharmacology. Biochemistry and Behavior 2011; 99(2):254-261.
5. Singh N, Pandey BR and Verma P: An overview of Phytotherapeutic Approach in Prevention and Treatment of Alzheimer's Syndrome & Dementia. International Journal of Pharmaceutical Sciences and Drug Research 2011; 3(3):162-172.
6. Singh M, Kaur M, Kukreja H, Chugh R, Silakari O and Singh D: Acetylcholinesterase inhibitors as Alzheimer therapy: From nerve toxins to neuroprotection. European Journal of Medicinal Chemistry 2013; 70:165-188.
7. Karimi G, Iranshahi M, Hosseinalizadeh F, Riahi B and Sahebkar A: Screening of Acetylcholinesterase Inhibitory Activity of Terpenoid and Coumarin Derivatives from the Genus *Ferula*. Pharmacologyonline 2010; 1:566-574.
8. Dall'Acqua S: Plant-derived acetylcholinesterase inhibitory alkaloids for the treatment of Alzheimer's disease. Botanics: Targets and Therapy 2013; 3:19-28.
9. Čolović MB, Krstić DZ, Vasić V, *et al.*: Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Current Neuropharmacology 2013; 11:315-335.
10. Camryn B and Sabbagh M: Broader Considerations of Higher Doses of Donepezil in the Treatment of Mild, Moderate, and Severe Alzheimer's Disease. International Journal of Alzheimer's Disease 2012; 1-4.
11. Houghton PJ, Ren Y and Howes MJ. Acetylcholinesterase inhibitors from plants and fungi. Natural Product Reports 2006; 23:181-199.
12. Williams P, Sorribas A and Howes MJ: Natural Products as a source of Alzheimer's drugs leads. Natural Product Reports 2011; 28:48-77.
13. Mukherjee PK, Kumar V, Mal M and Houghton PJ: Acetylcholinesterase inhibitors from plants. Phytomedicine 2007; 14:289-300.
14. Orhan G, Orhan I, Subutay-Oztekin N, Ak F and Sener B: Contemporary anticholinesterase pharmaceuticals of natural origin and their synthetic analogues for the treatment of Alzheimer's disease. Recent Patents on CNS Drug Discovery 2009; 4:43-51.
15. Murray AP, Faraoni MB, Castro MJ, Alza NP and Cavallaro V: Natural AChE Inhibitors from Plants and their Contribution to Alzheimer's Disease Therapy. Current Neuropharmacology 2013; 11:388-413.

16. Ysrael M: Tunkin herbal drug: a multidisciplinary approach to development. Clinical Hermorheology and Microcirculation 2003; 29:247-251.
17. Maurya A and Srivastava SK (2009). Large-scale separation of clavinet alkaloids from *Ipomoea muricata* by pH-zone-refining centrifugal partition chromatography. Journal of Chromatography B 2009; 877(18-19):1732-1736.
18. Ramesh S, Rajan R and Santhanam R: Freshwater Phytopharmaceutical Compounds. CRC Press Taylor & Francis Group 2013.
19. Peter KV: Underutilized & Underexploited Horticultural Crops (Vol. 4). New India Publishing Agency 2008.
20. Nonato MG, Guevarra BQ and Toia RF: "Two antibacterial compounds from *Ipomoea muricata* (L) Jacq (Convolvulaceae)". Acta Manilana 1986; 35:7-14.
21. Guevarra BQ: The alkaloids of *Ipomoea muricata* (L.) Convolvulaceae. Acta Manilana 1988; 3:51.
22. Khana SN and Gupta PC: The structure of a galactomannan from the seeds of *Ipomoea muricata.* Phytochem 1966; 6:605-609.
23. Ysrael MC, Waterman PG and Nonato MG: The Complete NMR Assignment of the Alkaloids of *Ipomoea muricata* (L.) Jacq. Convolvulaceae. Journal Kapisanang Kimika ng Pilipinas 2000; 16(1):25-30.
24. Jayanthi R and Subash P: Antioxidant Effect of Caffeic Acid on Oxytetracycline Induced Lipid Peroxidation in Albino Rats. Indian Journal of Clinical Biochemistry 2010; 371-375.
25. Gulcin I: Antioxidant activity of caffeic acid (3,4-dihydrocinnamic acid). Toxicology 2006; 217:213-220.
26. Tan Casis ML, Gerona MLG and Santiago LA: *Ipomoea muricata* (Linn.) Jacq. Methanolic Extract Displays Prooxidant and Antioxidant Activities *in vitro.* Undergraduate Thesis Biochemistry, University of Santo Tomas 2015.
27. Wu C and Allis DC: Chromatin and Chromatin Remodeling Enzymes. Elsevier Academic Press 2004.
28. Ellman GL, Courtney KD, Andre Jr. V and Featherstone RM: A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 1961; 7:27-30.
29. Feitosa CM, Freitas RM, Luz NNN, Bezerra MZB and Trevisan MTS: Acetylcholinesterase inhibition by somes promising Brazilian medicinal plants. Brazilian Journal of Biology 2011; 71(3):783-789.
30. Dhanasekaran S, Perumal P and Palayan M: *In-vitro* Screening for acetylcholinesterase enzyme inhibition potential and antioxidant activity of extracts of *Ipomoea aquatic* Forsk: therapeutic lead for Alzheimer's disease. Journal of Applied Pharmaceutical Science 2015; 5(2):12-16.
31. Pagliosa LB, Monteiro SC, Silva KB, Andrade JP, Dutilh J, Bastida J, Cammarota and M, Zuanazzi JAS: Effect of isoquinoline alkaloids from two *Hippaestrum* species on *in vitro* acetylcholinesterase activity. Phytomedicine 2010; 17:8-9.

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