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# THE STETH

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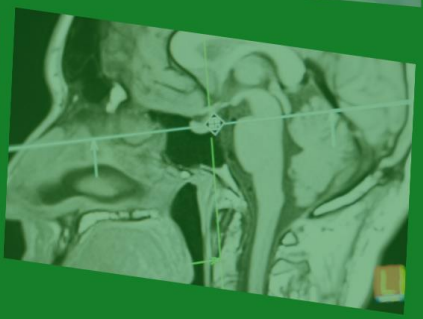

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# In vitro anti-acetylcholinesterase and antioxidant properties of *Cordia dichotoma* (Boraginaceae) polyphenolic leaves extract

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## Abstract

Alzheimer's disease (AD) is a progressive brain disorder which slowly destroys memory, thinking skills and behavior. AD presents with a sequence of pathophysiological events (such as amyloid beta overproduction, aggregation of tau protein inside the cell, acetylcholine deficiency and production of free radicals), that involves common cellular signaling pathways and leads to neural network dysfunction which gives rise to failure of neurotransmission, cell death and decrease in cognitive function. This study was done to determine possible anti-acetylcholinesterase and antioxidant activity of *Cordia dichotoma* extract. The total phenolic content of crude, DCM, ethyl acetate, methanol and aqueous extract were calculated spectrophotometrically using Folin- Ciocalteau's method and gallic acid was used as the standard. Result indicated that ethyl acetate extract had the highest gallic acid equivalent with 307.67$\mu$g. The antioxidant activity of the extracts were determined using DPPH radical scavenging capacity. Ethyl acetate, methanol, and aqueous extracts obtained the highest percent inhibition, compared to the ascorbic acid. Ethyl acetate, methanol, and aqueous extracts were evaluated its inhibitory effects against acetylcholinesterase and all extract displayed inhibitory activity with IC50 of 549.10 mg/mL, 453.80 mg/mL, and 664.50 mg/mL, respectively, compared to galantamine. Antioxidant property of the extracts that elicit anti-acetylcholinesterase were further characterize by FRAP assay and compared to ascorbic acid. Result indicated that ethyl acetate extract has the highest ferric reducing power with 464.77$\mu$g.

***Keywords:*** anti-acetylcholinesterase, antioxidant, Cordia dichotoma, Alzheimer's disease

## INTRODUCTION

In the projected future, neurodegenerative diseases are to be considered as the second leading cause of death following cardiovascular disease worldwide (Giaume, Saez, Song, Leybaert, & Naus, 2017). Alzheimer's disease is a degenerative disease that impairs the brain by deterioration over time (Association, 2019). This is the most common type of depleted cholinergic transmission and dementia seen in elderly population aging 65 years old and above (Tellechea, Pujol, Echeveste, Arbizu, & Riverol, 2016). However, teenagers and younger groups in their 20's can also be affected, where most of these are due to genetic abnormalities. A study showed that deaths from AD was counted to 121,404, making it the sixth leading cause of mortality in the United States in 2017 (Association, 2019; Isaacson et al., 2018; Ulep, Saraon, & Mclea, 2019).

Of all the organs inside our body, it is our brain that greatly experiences the effect of free radical damage due to its high oxygen consumption and its low availability of antioxidants (Pereiraa et al., 2014). With Alzheimer's disease, free radicals are processed in a great degree and play a role in its pathogenesis, which is due to oxidative stress (Gali & Bedjou, 2018). Several ideas were hypothesized to determine the clinical origin of AD. Examples of which are: the amyloid beta overproduction hypothesis, acetylcholine deficiency hypothesis and the tau hypothesis (Chen, 2018; Zvěřová, 2019). AD's neuropathology has a distinctive nature of the build-up of amyloid-beta peptides outside the cell and the aggregation of tau proteins inside the cell (Calderon- Garciduenas & Duyckaerts, 2018; Crews & Masliah, 2010) that produces effects caused by the depletion of cholinergic transmission resulting to decreased cognitive function (Emir, Emir, Bozkurt, & Somer, 2019).

Alzheimer's disease can also be precipitated by external health factors like oxidative damage (Orhan, Akkol, Suntar, & Yesilada, 2019). Oxidative stress is a condition induced by metabolic processes of molecules such as reactive oxygen (ROS) and nitrogen species (RNS), which are known hallmarks of oxidative stress. Imbalance and immoderate production of these two can harm DNA, proteins and lipid oxidation processes. This may result to cellular failure and may lead to neuronal death (Pereiraa et al., 2014). Antioxidants have an important role in the prevention of harmful effects of free radicals by neutralizing them. They also prevent formation of free radicals by separating transition metals like copper and iron (Gali & Bedjou, 2018). The use of antioxidants can potentially slow down AD progression and neurodegeneration (Eruygur et al., 2018).

Present medications for AD only alleviate symptoms associated with it (Cummings, Lee, Ritter, & Zhong, 2018). Some of these medications are acetyl cholinesterase inhibitors, which includes galantamine, rivastigmine and donepezil. These drugs help central cholinergic activity by reducing the physiologic breakdown of acetylcholine (ACh) by the enzyme acetylcholinesterase (AChE) in the synaptic cleft. Non-pharmacologic techniques and behavioral strategies are the prioritized options to treat neuropsychiatric symptoms in AD dementia (Atri, 2019; Steffany, Fernández, Maria, & Ribeiro, 2018). Significant side effects include lack of appetite, loose stool, nausea and vomiting, peptic ulcer disease, vivid dreams at night, dehydration, bradycardia, rash and seizures (Mehta, Adem, & Sabbagh, 2012).

The *genus Cordia* is classified under the family Boraginaceae with about 300 species identified worldwide, mostly in tropical and subtropical regions (Rahman & Akhtar, 2016; Thirupathi et al., 2008). *Cordia dichotoma* (Figure 1) is a 3-4 m medium sized deciduous tree that can be found in the Philippines, where it is locally known as 'Anonang' (Ragasa et al., 2015). In the past years, aerial parts of *C. dichotoma* are often used for medicinal purposes and, until today,are considered useful for the treatment of several ailments (Rahman & Akhtar, 2016). Previous studies have shown that the aerial parts of *C. dichotoma* exhibit pharmacologic activities such as antidiabetic, anthelmintic, antidysentery, antilarvicidal, immuno- modulator, hepatoprotective, antidyspepsia, anti-inflammatory, anti-ulcer, diuretic and laxative (Ragasa et al., 2015). A phytochemical review of written works revealed the presence of flavonoid, steroid, phenolic compounds, alkaloid, cardiac glycosides, carbohydrate and saponin (Rahman & Akhtar, 2016).

However, none of the studies conducted on C. dichotoma include the anti- acetylcholinesterase property of its leaf extract, which is the main goal of this study. This study also aims to quantify the total phenolic content of each extract using the Folin-Ciocalteau method. In relation to oxidative stress in Alzheimer's disease, 1- diphenyl-2-picryl-hydrazyl (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) assays were used as parameters in detecting antioxidant activities.

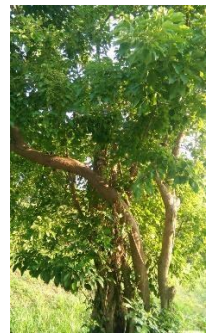

**Figure 1. Anonang, *Cordia dichotoma* tree.**

## MATERIALS AND METHOD

### Collection and Authentication of Plant Material

The leaves of *C. dichotoma* were collected at Barangay Ticub, Laurel, Batangas. The plant sample was validat ed and authenticated at University of Santo Tomas Research Center for the Natural & Applied Sciences, UST Herbarium.

### Solvents and Reagents

Methanol (TG), Dichloromethane (AR), Ethyl acetate (AR), Methanol (AR), and Hexane (AR) were purchased from RTC Laboratories, Services and Supplies House. Folin-Ciocalteau's reagent was obtain from Loba Chemie. Acetylcholinesterase enzyme, Galantamine, DTNB and Acetylthiocholine iodide were purchased from Sigma-Aldrich.

### Extraction and Partition

The collected leaves of *C. dichotoma* were air-dried (605g) at room temperature for 3 weeks. The fine dried leaves were percolated with hexane for 3 days and 80% methanol for 6 days (Imam, Alhaji, Adeniyi, Fulatan, & Peret, 2016; Singh, Lawania, Mishra, & Gupta, 2010). The 20.49 L collected crude methanolic extract was concentrated (68.68g with 9.94% yield) using a water bath. The concentrated crude methanolic extract was successively partitioned with dichloromethane (DCM), ethyl acetate, methanol and distilled water through a separatory funnel (Sócrates & Silva-júnior, 2019). Partition yield to DCM extract, ethyl acetate extract, methanol extract and aqueous extract, that was then further concentrated until solid.

**Total Phenol Quantification (Orhan et al., 2019)**

Determination of the phenol content of the different fractions were established by applying the Folin-Ciocalteau method described in Orhan et al. with slight modifications. Different concentrations of gallic acid (500, 250, 125, 62.5 and 31.25 µg/ml) were obtained to prepare the standard curve. All six different extracts were prepared to 1000, 500, 250, 125, 62.5 and 31.25 µg/ml concentrations. The gallic and extract dilutions were prepared with 1 ml of distilled water and 1 ml DMSO, respectively. In a 96- microwell plate, 200 µl of standard and extract were mixed with 800 µl of Folin- Ciocalteau's reagent and incubated for 5 mins. Afterwards, it was mixed with 600 µl sodium carbonate and left to stand at room temperature for 2 hours. Absorbance was measured through a 96-well plate reader at 760 nm and expressed as means of triplicates.

**1,-Diphenyl-2-picryl-hydrazyl Scavenging Activity (Sailaja, Bharathi, & Kvsrg, 2010)**

Antioxidant activity of the different extracts were examined using DPPH stable free radical. Ascorbic acid (125, 62.5, 31.25,15.63, and 7.81 µg/ml) was used as the standard. Each solution of the extract with different concentrations 1000, 500, 250, 125, and 62.5 µg/ml were added to 100µM of DPPH in methanol and the tubes were kept at ambient temperature for 20 min and the absorbance was measured at 516 nm. The sample's ability to scavenge DPPH radical was determined using the following formula:

$$\text{DPPH Scavenging effect} = \frac{[(A0 - A1)]}{A0} \times 100$$

A0 : absorbance of the control

A1 : absorbance of test samples

The IC50 of the standard and the different extracts were computed using GraphPad Prism V.7.0 Software.

**Anti-Acetylcholinesterase Assay (Dash & Sahoo, 2017; Hacıbekiroglu & Kolak, 2015)**

This assay was performed by the modified method of Dash & Sahoo (2017). In a 96-microwell plate, 20 µl of different concentrations of extract and standard galantamine (1000, 500, 250, 125, and 62.5 µg/ml) was added to 40 µl of 50 mM Tris-HCl buffer (pH 8.0) and 20 µl of acetylcholinesterase (AChE) was added, followed by 100 µl of 10 mM 5,5′-dithiobis- 2- nitrobenzoic acid (DTNB) and was incubated for 15 minutes at 25°C. Absorbance was taken at time zero without

colorimetric solution. Then, 20 µl of acetylthiocholine iodide was added. Absorbance was taken at time zero with colorimetric solution and every 5 minutes until 20 minutes at 412 nm in triplicate samples. A 50 mM Tris-HCl buffer served as the negative control, with and without enzyme. The IC50 of the standard and different extract were computed using GraphPad Prism V.7.0 Software.

**Ferric Reducing Power Determination (Jemli et al., 2016)**

The ferric reducing capacity of extracts was investigated by using the potassium ferricyanide-ferric chloride method. Briefly, 250 µl of the extracts (1000 µg/ml) were mixed with 312.5 µl of 1 M phosphate buffer saline (pH 6.6) and 312.5 µl of 1% potassium ferricyanide was vortex ed and then incubated for 20 minutes at 50 °C. Then, 312.5 µl of 10% trichloroacetic acid was added. The tubes were centrifuged for 10 minutes at 3,000 rpm. In a 96-microwell plate, 100 µl of supernatant layer were mixed with 50 µl distilled water and 20 µl of 0.1% of ferric chloride was added and left to stand for 10 minutes at room temperature. The test was read in triplicates and the absorbance of the reaction mixtures was measured at 610 nm. Ascorbic acid (125, 62.5, 31.25, 15.63, and 7.81 µg/ml) was used as the standard

## RESULTS

**Plant Extraction**

The *C. dichotoma* concentrated extracts were weighed and percentage yield was computed using the formula:

$$\% \text{ yield} = \frac{\text{wt.of concentrated extract}}{wt.of\ dried\ raw\ material} \times 100$$

**Table 1**
**Different *C. dichotoma* leaves extract and itscalculated percentage yield**

| Extracts of *C. dichotoma* | % yield |
|---|---|
| DichloromethaneEthyl acetate | 0.17% |
| Methanol | 0.20% |
| Aqueous | 0.34% |
| | 2.01% |

**Total Phenolic Content**

The results are summarized in Table 2. Ethyl acetate extract contains the highest phenolic compounds, followed by DCM, Methanol and aqueous extract. The gallic acid served as the phenol compound and compared with the extracts in order to compute the gallic acid equivalent (GAE)using the standard curve in Figure 2.

**Table 2**
**GAE of *C. dichotoma* leaves extracts.**

| Extracts of *C.dichotoma* | Absorbance | GAE y=0.0066x+0.2487 |
|---|---|---|
| Crude | 0.14 | -15.81 $\mu$g |
| DCM | 0.48 | 35.25 $\mu$g |
| Ethyl acetate | 2.28 | 307.67 $\mu$g |
| Methano | 0.42 | 25.65 $\mu$g |
| lAqueous | 0.52 | 41.11 $\mu$g |

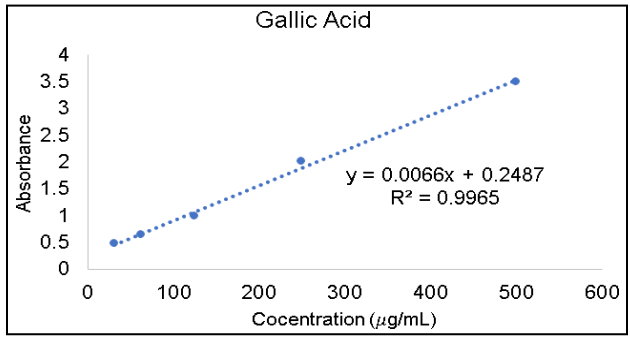


**Figure 2. Standard calibration curve of Gallic acid**

**DPPH Scavenging Activity**

DPPH scavenging activity of standard is summarized at Figure 3 with an IC50 of 15.53 mg/mL. Different plant extracts are summarized at Figure 4. Aqueous, methanol and ethyl acetate extract obtained the highest percent inhibition and had an IC50 of 103.30mg/mL, 159.80 mg/mL and 223.60 mg/mL, respectively.

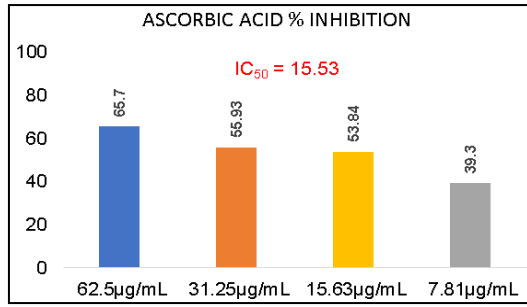


**Figure 3. Graphical representation of percentinhibition of Ascorbic acid.**

**Anti-Acetylcholinesterase Activity**

Ethyl acetate, methanol and aqueous extract of *C. dichotoma* obtained an IC50 of 549.10 mg/mL, 453.80 mg/mL and 664.50 mg/mL, respectively, compared to the standard drug, Galantamine that has 441.00 mg/mL IC50 (Figure 5). Among the 3 extracts, methanol has shown the highest percent inhibition and lowest IC50. Sample with the lowest IC50 indicates that it is more potent than the other samples. IC50 of the extracts had the difference of 102.80 mg/mL – 223.50 mg/mL from Galantamine.

**Ferric Reducing Antioxidant Power**

The ethyl acetate extract gave the highest ferric reducing power with 464.77$\mu$g, while methanol and aqueous extract has 220.05$\mu$g and 222.44$\mu$g, respectively (Table 3). The FRAP of the extracts were compared to the standard curve of ascorbic acid in Figure 6. Extracts were subjected to this assay to further characterized the 3 extracts that have shown anti-acetylcholinesterase inhibitory effects.

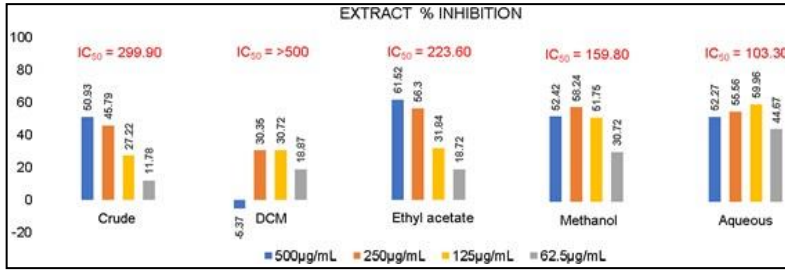

**Figure 4. Graphical representation of percent inhibition of *C. dichotoma* extracts.**

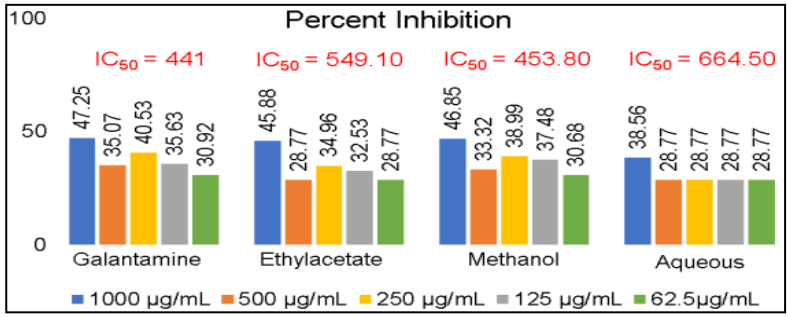


**Figure 5. Graphical representation of Anti- acetylcholinesterase activity of *C. dichotoma.***

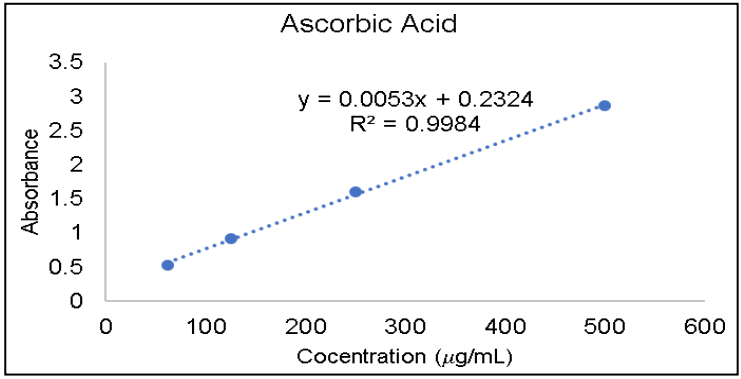


**Figure 6. Standard calibration curveof Ascorbic acid.**

**Table 3**
**Ferric Reducing Power of different extracts of *C. dichotoma.***

| Extracts of *C. dichotoma* | Ferric Reducing Power |
|---|---|
| Ethyl acetate | 464.77 µg |
| Methanol | 220.05 µg |
| Aqueous | 222.44 µg |

## DISCUSSION

Each extract of *C. dichotoma* possess a high amount of phenolics compounds. DPPH scavenging activity have shown that ethyl acetate, methanol and aqueous extracts of *C. dichotoma* displayed an antioxidant effect. It is proven in different studies that phenolics displayed a potent antioxidant property (Ahangarpour, Sayahi, & Sayahi, 2018; Cesari, Mutelet, & Canabady - rochelle, 2019; Irondi et al., 2019).

The *in vitro* anti-cholinesterase assay suggests that the same extract mentioned above can induce anti-acetyl cholinesterase effect.

To further differentiate and characterize the three extracts, the said extracts were subjected to another antioxidant assay. FRAP assay revealed that ethyl acetate extract possessed the highest ferric reducing power.

According to studies, acetylcholinesterase deficiency was one of many hypotheses on pathophysiological events of AD. It is also strongly associated with oxidative stress, which cause cellular failure and neural damage (Orhan et al., 2019; Pereiraa et al., 2014).

The currently available treatment of AD includes acetylcholinesterase inhibitors. Inhibiting acetylcholinesterase increases the overall levels of acetylcholine, which help alleviate the characteristic symptoms of AD.

Previous studies revealed that oxidative stress enhances the buildup of peptide plaques, which causes AD. Therefore, antioxidants can also be helpful in ameliorating the symptoms of the disease (Kumar & Singh, 2015).

Counter-intuitively, this data does not seem to directly correlate with the levels of phenolic compounds with antioxidant activity . Extraction of phenols used by the researchers was non-specific and based on increasing polarity of the solvent. However, phenols belong to highly diversified group of phytochemicals. In plants, it includes phenolic acids, tannins, coumarins, flavonoids, stilbenes, lignans and lignins. Extraction of phytochemicals depends on the nature of it (Chandrasekara, 2018). It is possible that the phenols that was read in quantification of phenolic content are different from phenols that exerted antioxidant activity in DPPH assay

## CONCLUSION

Result have shown that each extract of *C. dichotoma* leaves displayed relatively high number of phenolic compounds. Ethyl acetate, methanol and aqueous extract also showed inhibitory effect against oxidation reaction and acetylcholinesterase that are compared to their respective standard drug. Ethyl acetate displayed the highest ferric reducing power compared to ascorbic acid.

## RECOMMENDATION

The researchers would like to recommend more studies in *C. dichotoma* leaves extract and further isolation of compound present in the leaves extract to ascertain the mechanism behind its anti-cholinesterase and antioxidant property.

## REFERENCES

Ahangarpour, A., Sayahi, M., & Sayahi, M. (2018). The antidiabetic and antioxidant properties of some phenolic phytochemicals:A review study. Diabetes & Metabolic Syndrome: Clinical Research & Reviews https://doi.org/10.1016/j.dsx.2018.11.051

Association, A. (2019). 2019 Alzheimer ' s disease facts and figures. Alzheimer's & Dementia, 15(3), 321–387. https://doi.org/10.1016/j.jalz.2019.01.010

Atri, A. (2019). The Alzheimer's Disease Clinical Spectrum. Medical Clinics of NA, 103(2), 263–293.https://doi.org/10.1016/ j.mcna.2018.10.009

Calderon-Garciduenas, A. L., & Duyckaerts, C. (2018).Handbook of Clinical Neurology. Alzheimer disease, 145. https://doi.org/10.1016/B978-0-12-802395-2.00023-7

Cesari, L., Mutelet, F., & Canabady-rochelle,L. (2019). Antioxidant properties of phenolic surrogates of lignin depolymerisation. Industrial Crops & Products, 129(December 2018), 480– 487. https://doi.org/10.1016/j.indcrop.2018.12.010

Chandrasekara, A. (2018). Phenolic Acids. Encyclopedia of Food Chemistry. Elsevier. https://doi.org/10.1016/B978 - 0-12-814026-0.22395-0

Chen, Y. G. (2018). Research Progress in the Pathogenesis of Alzheimer ' s Disease. Chinese Medical Journal,131(13). https://doi.org/10.4103/0366-6999.235112

Crews, L., & Masliah, E. (2010). Molecular Mechanisms of Neurodegeneration in Alzheimer ' s Disease. Oxford Journals, (858), 1–41.

Cummings, J., Lee, G., Ritter, A., & Zhong, K. (2018). Alzheimer's disease drug development pipeline : 2018. Alzheimer's & Dementia: Translational Research & Clinical Interventions, (May), 1–16. https://doi.org/10.1016/j.trci.2018.03.009

Dash, U. C., & Sahoo, A. K. (2017). In vitro antioxidant assessment and a rapid HPTLC bioautographic method for the detection of anticholinesterase inhibitory activity of Geophila repens. Journal of Integrative Medicine, 15(3), 231–241. https://doi.org/10.1016/S2095- 4964(17)60326-1

Emir, C., Emir, A., Bozkurt, B., & Somer, N.(2019). Phytochemical constituents from Galanthus alpinus Sosn. var. alpinus and their anticholinesterase activities. South African Journal of Botany, 121, 63-67. https://doi.org/10.1016/j.sajb.2018.10.0 21

Eruygur, N., Koçyiğit, U. M., Taslimi, P., Ataş,M., Tekin, M., & Gülçin, İ. (2018). Screening the in-vitro antioxidant, antimicrobial, anticholinesterase, antidiabetic activities of endemic Achillea cucullata (Asteraceae) ethanol extract. South African Journal of Botany.https://doi.org/10.1016/j. sajb.2018.04.001

Gali, L., & Bedjou, F. (2018). Antioxidant and anticholinesterase effects ofthe ethanol extract, ethanol extract fractions and total alkaloids from the cultivated Ruta chalepensis. South African Journal of Botany https://doi.org /10.1016 /j.sajb.2018.04.011

Giaume, C., Saez, J. C., Song, W., Leybaert, L., & Naus, C. C. (2017). Connexins and pannexins in Alzheimer's Disease. Neuroscience Letters. https://doi.org/10.1016/j.neulet. 2017.09.006

Hacıbekiroglu, I., & Kolak, U. (2015). Screening antioxidant and anticholinesterase potential of Iris albicans extracts. Arabian Journal of Chemistry, (2015) 8, 264–268.

Imam, A., Alhaji, M. I., Adeniyi, S., Fulatan, S. U., & Peret, D. (2016). Evaluation of anti-inflammatory , antibacterial and cytotoxic activitie of Cordia africana leaf and stem bark extracts. Bayero Journal of Pure and Applied Sciences, 9(1), 228–235.

Irondi, E. A., Adegoke, B. M., Effion, E. S., Oyewo, S. O., Alamu, E. O., & Boligon, A.A.(2019). Enzymes inhibitory property,

antioxidant activity and phenolics profile of raw and roasted red sorghum grains in vitro. Food Science and Human Wellness.https://doi.org/10.1016/j.fshw.2019.03.012

Isaacson, R. S., Ganzer, C. A., Hristov, H., Hackett, K., Caesar, E., Cohen, R., … Krikorian, R. (2018). The clinical practice of risk reduction for Alzheimer ' s disease : A precision medicine approach. Alzheimer's & Dementia, 1– 11. https://doi.org/10.1016/j.jalz.2018.08.004

Jemli, M. El, Kamal, R., Marmouzi, I., Zerrouki, A., Cherrah, Y., & Alaoui, K. (2016). Radical-scavenging activity and ferric reducing ability of Juniperus thurifera ( L .), J . oxycedrus ( L .), J . phoenicea ( L .) and Tetraclinis articulata ( L .). Advances in Pharmacological Sciences, 2016.

Kumar, A., & Singh, A. (2015). Review article a review on Alzheimer ' s disease pathophysiology and its management : an update. Pharmacological Reports, 67(2),195–203. https://doi.org/10.1016/j.pharep.2014.09.004

Mehta, M., Adem, A., & Sabbagh, M. (2012). New Acetylcholinesterase inhibitors for Alzheimer ' s Disease. International Journal Of Alzheimer's Disease, 2012. https://doi.org/10.1155/2012/728983

Orhan, I. E., Akkol, E. K., Suntar, I., & Yesilada, E. (2019). Assessment of anticholinesterase and antioxidant properties of the extracts and (+)- catechin obtained from Arceuthobium oxycedri (D.C.) M. Bieb (dwarf mistletoe). South African Journal of Botany, 120, (2019), 309–312.

Pereiraa, R. P., Boligon, A. A., Appel, A. S., Fachinetto, R., Ceron, C. S., Tanus - Santos, J. E., … Rocha, J. B. T. (2014). Chemical composition, antioxidant and anticholinesterase activity of Melissa officinali. Industrial Crops and Products, 53, (2014), 34– 45.

Ragasa, C. Y., Jr, V. E., Reyes, M. M. D. L.,Mandia, E. H., S, M. C., Brkljača, R., & Urban, S. (2015). Chemical Constituents of Cordia dichotoma G . Forst . Journal of Applied Pharmaceutical Science, 5(Suppl 2), 16–21. https://doi.org/10.7324/JAPS.2015.58.S3

Rahman, A., & Akhtar, J. (2016). Phytochemistry and pharmacology of traditionally used medicinal plant Cordia dichotoma Linn ( Boraginaceae ). Biotechnology and Pharmacy, 10(April), 186–193.

Sailaja, B., Bharathi, K., & Kvsrg, P. (2010). Evaluation of Ruellia

tuberosa L . for antiurolithiatic and antioxidant activities in rats. Journal of Pharmaceutical Research, 9(2), 70–75.

Singh, R., Lawania, R. D., Mishra, A., & Gupta, R. (2010). Role of Cordia dichotoma seeds and leaves extract in degenerative disorders. International Journal of Pharmaceutical Sciences Review and Research, 2(1), 21–24.

Sócrates, E., & Silva-júnior, A. A. (2019). Protective effect of aqueous extract, fractions and phenolic compounds of Hancornia speciosa fruits onthe inflammatory damage in the lungs of mice induced by Tityus serrulatus envenomation. Toxicon. https://doi.org/10.1016/j.toxicon.2019.03.018

Steffany, S., Fernández, M., Maria, S., & Ribeiro, L. (2018). Nutrition and Alzheimer Disease Nutrients Food Dietary pattern Alzheimer disease Prevention Management. Clinics in Geriatric Medicine. https://doi.org/10.1016/j.cger.2018.06 012

Tellechea, P., Pujol, N., Echeveste, B., Arbizu, J., & Riverol, M. (2016). Early- and late-onset Alzheimer disease: Are they the same entity? Neurología (English Edition). https://doi.org/10.1016/j.nrleng.2015.08.009

Thirupathi, K., Kumar, S. S., Raju, V. S., Ravikumar, B., Krishna, D. R., & Mohan, G. K. (2008). A review of medicinal plants of the genus Cordia : Their chemistry and pharmacological uses. Journal of Natural Remedies, 8, 1–10.

Ulep, M. G., Saraon, S. K., & Mclea, S. (2019). Alzheimer Disease. TJNP: The Journal for Nurse Practitioners, 14(3), 129–135. https://doi.org/10.1016/j.nurpra.2017.10.014

Zvěřová, M. (2019). Clinical aspects of Alzheimer's disease Martina. Clinical Biochemistry. https://doi.org/10.1016/j.clinbiochem.20 19.04.015