

***In Vitro* Acetylcholinesterase Inhibition of *Acorus calamus* (Lubigan) Rhizome Fractions for Alzheimer's Disease**

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder which neurons degenerate or lose their structure and function leading to memory impairment and cognitive deficiency due to decreased levels of acetylcholine via acetylcholinesterase (AChE). Substances that inhibit and reduce the activity of this enzyme were found to ameliorate the symptoms of AD. *Acorus calamus* (Lubigan), is an herb that has been used for memory loss and reported to have neuroprotective activity. This study focuses on the potential *in vitro* acetylcholinesterase inhibitory activity of the plant's rhizomes. Maceration of the powdered rhizomes of *Acorus calamus* was done with ethanol. The extract was then subjected to solvent-solvent extraction using hexane, carbon tetrachloride, chloroform, and water. Phytochemical screening revealed phenols, tannins, flavonoids, and alkaloids in the chloroform fraction (LRChF) while the other fractions and the ethanolic extract had the same constituents with additional higher alcohols, sterols, triterpenes, essential oils, and steroids. The samples were assayed using Ellman's method on microwell-plates to determine the percent inhibition and inhibitory concentration (IC₅₀) with Donepezil as the positive control. All samples inhibited AChE to some degree. LREE at 1000 µg/mL had the highest significant percent inhibition across all test samples while LRChF and LRWF at 1000 µg/mL were found to be comparable to donepezil at 500 µg/mL. Among the fractions, the LRWF had the most potent inhibitory activity at 129.1 µg/ml [95% CI: 121.9 to 136.3]. In view of the results obtained, *Acorus calamus* may be further developed as an alternative acetylcholinesterase inhibitor for the management of AD.

Keywords: *Acetylcholinesterase inhibitor; Alzheimer's disease; Ellman's Method; Solvent-solvent partitioning*

1. INTRODUCTION

1.1 Rationale

Memory is the faculty of the mind for the retention of information and learning that is used to adapt environmental responses (Aakondi, Alikatte, Palle, Veerareddy, & Yerragunta, 2012). Dementia is a serious mental illness that can cause a lot of progressive diseases which worsens as time passes and affect one's memory, thinking, and behavior. One of the most common causes of dementia is Alzheimer's disease (AD). It is a neurodegenerative disease that mainly affects the elderly population over 65 years old (Duncan, Elgorashi, Elisha, Eloff, & Hussein, 2012). Progressive loss of memory, deterioration of virtually all intellectual functions, decreased speech function, and disorientation characteristic of this condition (Alzheimer's Association, 2015).

AD is a major public health concern. The World Health Organization (WHO) is the top seven ailments disabling the general population, and it is estimated to cost the world some \$ 1 trillion in 2018. There was a total of 35.6 million people worldwide who have dementia in 2010, and it is expected to double every twenty years (WHO, 2013). By 2030, it is estimated to increase to 65.7 million and 115.4 million by 2050. There are nearly 7.7 million new cases of dementia worldwide each year, implying that there is one new case every four seconds. AD and other dementias in the Philippines have increased by 43.3% since 1990 represents an average of 1.9% a year, and an annual mortality rate per 100,000 people with dementia (Abaekwuwe et al. 2018).

The first area in the brain affected by Alzheimer's disease is the hippocampus which helps develop new memories. It is the part of the brain where memories are processed for storage into long-term memory. In the presence of AD, there is atrophy or shrinkage of the hippocampal areas which explains why impairment of memory is one of the early symptoms of AD (Anand & Dhikav, 2012). AD is caused by progressive damage to brain cells and subsequent loss of chemicals known as neurotransmitters. AD is thought to be caused by the abnormal build-up of proteins such as amyloid and tau in and around brain cells. Amyloid deposits form plaques around brain cells while tau deposits form tangles within brain cells (Hyman & Spire-Jones, 2014). As brain cells become affected, there is also a decrease in neurotransmitters involved in sending messages or signals between brain cells. One of the neurotransmitters being affected is acetylcholine. Levels of acetylcholine are usually low in the brains of people with AD (Adewusi, & Steenkamp, 2011). Acetylcholine enhance encoding by increasing the strength of afferent input relative to feedback, by contributing to theta rhythm oscillations, by activating intrinsic mechanisms for persistent spiking, and by increasing the modification of synapses (Hasselmo, 2009).

Acetylcholinesterase (AChE) is a key enzyme in the regulation of cholinergic activity in the nervous system. AChE inhibitors enhance

cholinergic transmission with modest and transient therapeutic effects. One of the primary uses of acetylcholinesterase inhibitors is to decrease the breakdown of acetylcholine. It helps to slow down the progression of AD and is useful in preventing the formation of β -amyloid plaques and modulation of the APP (Amyloid Precursor Protein) processing (Bolognesi, Matera, Minarini, Rosini, & Melchiorre, 2009). A primary therapeutic target for the AD treatment is the inhibition of acetylcholinesterase (AChE). There is still no cure for AD since its discovery. AD destroys the brain cells that produce acetylcholine thereby reducing the number of available cells that deliver messages to other cells. By inhibiting acetylcholinesterase, it will boost cholinergic neurotransmission in forebrain regions and compensate for the loss of functioning brain cells (Kandel, Dudai & Mayford, 2014).

Medications currently approved by the Food and Drug Administration (FDA) to treat the cognitive manifestations of AD and improve the life quality of the patients are Donepezil, Rivastigmine, and Galantamine. Donepezil is approved to treat all stages of Alzheimer's disease and it is a specific and reversible inhibitor of AChE. Donepezil is approved in more than ninety countries around the world for the treatment of mild to moderate Alzheimer's disease, and in the United States, Japan, Canada, and several other countries, it is approved for the treatment of severe AD (Seltzer, 2007). Galantamine is approved for mild-to-moderate stages, and Rivastigmine is approved for mild-to-moderate Alzheimer's. Side effects of these drugs are commonly nausea, vomiting, loss of appetite and increased frequency of bowel movements (Alzheimer's Association, 2015).

Acorus calamus, which is commonly known as Lubigan is a perennial herb that belongs to the Acoraceae family. In the Indian and Chinese health system, it has been used to treat diseases especially the Central Nervous System (CNS) abnormalities. The rhizome of Lubigan is widely used in the treatment of several ailments like epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh; intermittent fevers glandular, abdominal tumors, kidney and liver troubles, rheumatism, sinusitis and eczema (Devaki, Nirupama, Nirupama, & Yajurveda, 2015). In the Philippines, Lubigan grows mainly in swamps, marshes and riverbanks. It is used in the treatment of rheumatic arthritis, and leg pains. It is also used for fever, tonic dyspepsia, indigestion and gastritis and masticatory for toothache also as a stimulant and tonic (Dastmalchi, Dorman, & Vuorela, Hiltunen, 2007).

Recently, a study conducted by Abaekwuwe et al. (2018) reported that the ethanolic rhizome extract of Lubigan has a neuroprotective activity in terms of cognitive function as to retention and familiarity using Elevated plus Maze and Novel Object Recognition Test According to Abaekwuwe, et al. (2018), there is no significant difference between ethanolic rhizome extract of Lubigan and Donepezil hydrochloride in controlling the

parameters indicative of neuroprotective property. However, there were no studies focusing on its possible mechanism of action. It is in this light that the researchers have chosen to continue this study. The current study proposes to explore the potential of Lubigan rhizome extract as an acetylcholinesterase inhibitory drug.

2. METHODOLOGY

2.1 Sample Collection, Preparation, and Extraction

The rhizomes of Lubigan were harvested at a farm in the community of Catiningan, Socorro, Oriental Mindoro province, from September to October 2018. The plant material was identified and authenticated by the Bureau of Plant Industry (BPI) at San Andres Street Malate, Manila. The freshly collected rhizomes were garbled, cut into small pieces, and dried in an oven at 55°C to constant weight. After drying, the sample was pulverized and milled. The sample was macerated in 70% ethanol for 48 hours before it was filtered. The filtrate was then concentrated using a rotary evaporator (Eyela SB-1100) and was subjected to the water bath until it reached a semi-solid consistency. The extract was kept in a tight, light-resistant container and stored in a cool dry place.

2.2 Solvent-Solvent Partitioning

The solvent-solvent partitioning was performed to further separate the different components of the Lubigan rhizome ethanolic extract. As shown in Figure 1, five milligrams of the ethanolic extract was triturated with ethanol. The prepared solution was fractionated successfully using solvents of increasing polarity: hexane, carbon tetrachloride, and chloroform. The residue was kept as the water-soluble fraction. All fractions were evaporated to dryness by using rotary evaporator and kept in airtight containers for further analysis.

2.3. Thin Layer Chromatography and Phytochemical Screening using TLC Spray Reagents

Phytochemical analysis of the Lubigan rhizome was conducted using Thin Layer Chromatography (TLC) to further elute the components of the extract and its fraction. These were later exposed to different spray reagents to identify the constituents present.

The procedures on the Thin Layer Chromatography were adapted from Aguinaldo et al. (2005). Silica Gel G was used as stationary phase while varying solvent combinations of increasing polarity was used as the mobile phase (Ajayi, Asuzu, & Ode, 2011). Several solvent systems were used to better elute the constituents present.

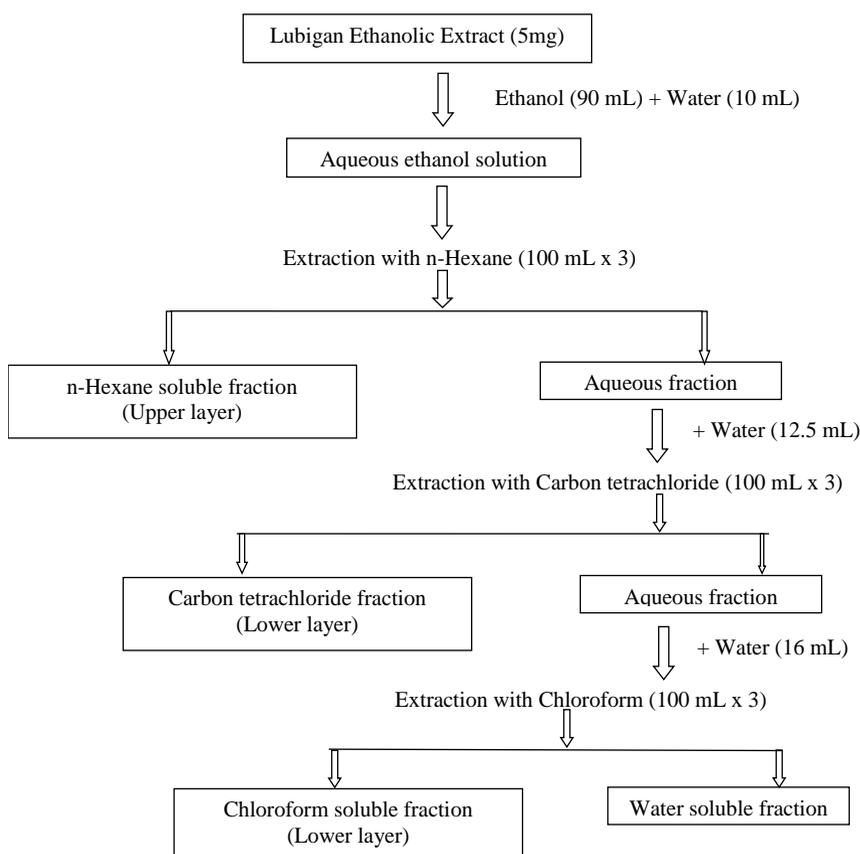


Figure 1. Schematic Diagram on Solvent-solvent Partitioning (Alejandro, 2018; Muhit et al., 2015; Kupchan, Tsou, & Sigel, 1973)

2.3. Microwell-plate Assay using Ellman's Method

Acetylcholinesterase inhibitory activity was measured according to a slightly modified spectrophotometric method (Ellman, Courtney, Andres, & Featherstone, 1961). Acetylthiocholine iodide (EC 217-474-1) was used as a substrate to assay acetylthiocholine, and 5,5'-Dithiobis[2-nitrobenzoic-acid] (DTNB) was used for the measurement of cholinesterase activity. Twenty (20) μL of the test sample, 10 μL of 3 mM DTNB, and 20 μL of acetylcholinesterase (EC 3.1.1.7) were mixed in 140 μL of 100 mM sodium phosphate buffer (pH 8.0) and was incubated for 15 minutes at 25° C. The reaction was initiated with the addition of 10 μL 15mM acetylthiocholine. The hydrolysis of acetylthiocholine was monitored by the formation of yellow 5- thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine at a wavelength of 405 nm. The rate of hydrolysis of AChE was measured over 15 min using a 96-well microplate

reader. Donepezil was used as a positive control. The concentrations of test compounds that inhibit the hydrolysis of a substrate (acetylthiocholine) by 50% (IC_{50}) are computed by monitoring the effect of increasing concentrations of these compounds in the assays on the inhibition values (Farag, Ezzat, Salama, Tadros, & Senya, 2014). Figure 2 shows a flowchart depicting the rationale behind the Ellman's method. Tests were performed using four different concentrations of each test sample in triplicate across three trials.

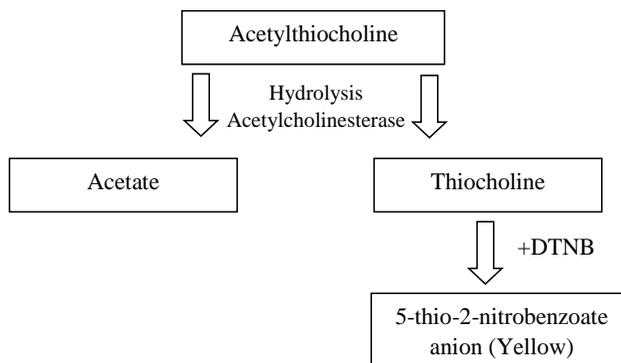


Figure 2. Ellman's Method Flowchart

2.4 Data Analysis

The values obtained for the percent inhibition are presented as mean \pm SEM and evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests. Data for the Ellman's Method (3 independent trials in triplicates) were computed with a level of significance of $p < 0.05$.

The IC_{50} values were identified and estimated using the software program GraphPad Prism version 7.0. The concentration of samples that causes 50% inhibition of the AChE activity (IC_{50}) was calculated via four parameter equation (4PL).

Computation for the determination of IC_{50} :

$$\text{Acetylcholinesterase inhibition} = \left[1 - \frac{\text{Absorbance sample}}{\text{Absorbance Control}} \right] \times 100$$

Four parameter equation

$$y = d + \frac{a-d}{1+(x/c)^b}$$

Where:

x = the independent variable (IC_{50})

y = the dependent variable

c = the point of inflection

a = the minimum value that can be obtained

d = the maximum value that can be obtained

b = Hill's slope of the curve

3. RESULTS AND DISCUSSION

3.1. *Phytochemical Screening*

Using normal phase thin layer chromatographic plates, the ethanolic extract was eluted using chloroform, ethyl acetate and hexane (7:7:2) as a solvent system, while for the hexane fraction, hexane and ethyl acetate (3:5) was used. For the carbon tetrachloride fraction, toluene and chloroform (9:11) had the best separation of spots. Toluene and chloroform (9:15) was used for the chloroform fraction and lastly, the water fraction was separated using methanol and ethyl acetate (7:4). Table 1 contains the summary of the results for the phytochemical screening using Thin Layer Chromatographic Spray Reagents.

Phenols, tannins, and flavonoids were present in all the samples after exposure to the potassium ferricyanide-ferric chloride spray reagent. Furthermore, in the vanillin-sulfuric acid spray reagent, all samples except for the chloroform fraction showed the presence of higher alcohols, phenols, steroids, essential oils. All samples had positive results for the Antimony (III) chloride and Dragendorff's reagent which detected the presence of flavonoids, phenols, and alkaloids respectively.

According to the study of Konrath, Passos, Klein- Júnior, and Henriques (2013), the anti-acetylcholinesterase activity of alkaloids, together with their structural diversity and physicochemical properties, makes them a good candidate agent for the treatment of AD. It is due to their complex nitrogen-containing structures. Nitrogen-containing compounds, especially alkaloids from herbal sources have been so far proven to exert anticholinesterase activity. Plants containing alkaloids have been traditionally used as a tea for elderly people suffering from dementia.

According to Stefani and Rigacci (2013), natural phenolic substances are present in many plants and many epidemiological studies show that there is significantly reduced the incidence of age-related diseases including amyloid diseases particularly those implying neurodegenerative conditions suggesting the use of such substances as preventative agents.

According to the study of Abaekwuwe et al. (2018), evidence from epidemiological and human intervention studies and animal studies have also suggested that tannin-rich plant is effective at reversing neurodegenerative pathology and age-related declines in neurocognitive performance. Tannins also exert several biological effects such as antioxidant, anti-microbial, anti-cancer, and cardioprotective properties.

Flavonoids may represent important precursor molecules in the quest to develop a new generation of brain enhancing drugs due to the intense interest in the development of drugs capable of enhancing brain. The neuroprotective actions of flavonoids involve several effects in the brain, including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation and the potential to

promote memory, learning and cognitive function (Abaekwuwe et al., 2018).

The constituents and various extracts of the LREE are significant sources for treating various diseases. Including neuroprotective, anticonvulsant, antioxidant, and hypolipidemic properties (Abaekwuwe et al., 2018).

Table 1
Summary of Phytochemical Screening by TLC

<i>Constituents tested</i>	<i>Spray Reagent</i>	<i>Positive Result</i>	<i>EtOH Extract</i>	<i>Hex Fraction</i>	<i>CCl4 Fraction</i>	<i>CHCl3 Fraction</i>	<i>Water Fraction</i>
Phenols, Tannins, Flavonoids	$K_3Fe(CN)_6$ $FeCl_3$	Blue spots	+++	+++	+++	+++	+++
Higher Alcohols, Phenols, Sterols, Triterpenes Essential oils	Vanillin-Sulfuric acid	Blue-violet wide range of colors	+++	++	+++	(-)	+++
Sugars	α -naphthol-sulfuric acid	Blue spots	(-)	(-)	(-)	(-)	(-)
Indole	Van-urk Slakowski Test	Blue-violet spots	(-)	(-)	(-)	(-)	(-)
Anthraquinones	Mg (Ac) ₂ in methanol	Orange violet	(-)	(-)	(-)	(-)	(-)
Cardenolides	Kedde's reagent	Blue to red-violet	(-)	(-)	(-)	(-)	(-)
Flavonoids, steroids	Antimony (III) chloride	Intense yellow	+	+	+	(-)	+
Alkaloids	Dragendorff's reagent	Brown orange	+++	+++	+++	+++	+++
Anthraquinon, Coumarines, Anthrones, and Phenols	Borntranger's Reagent	Anthraquinones (orange) Antrones (Yellow) and Coumarins (Blue)	(-)	(-)	(-)	(-)	(-)

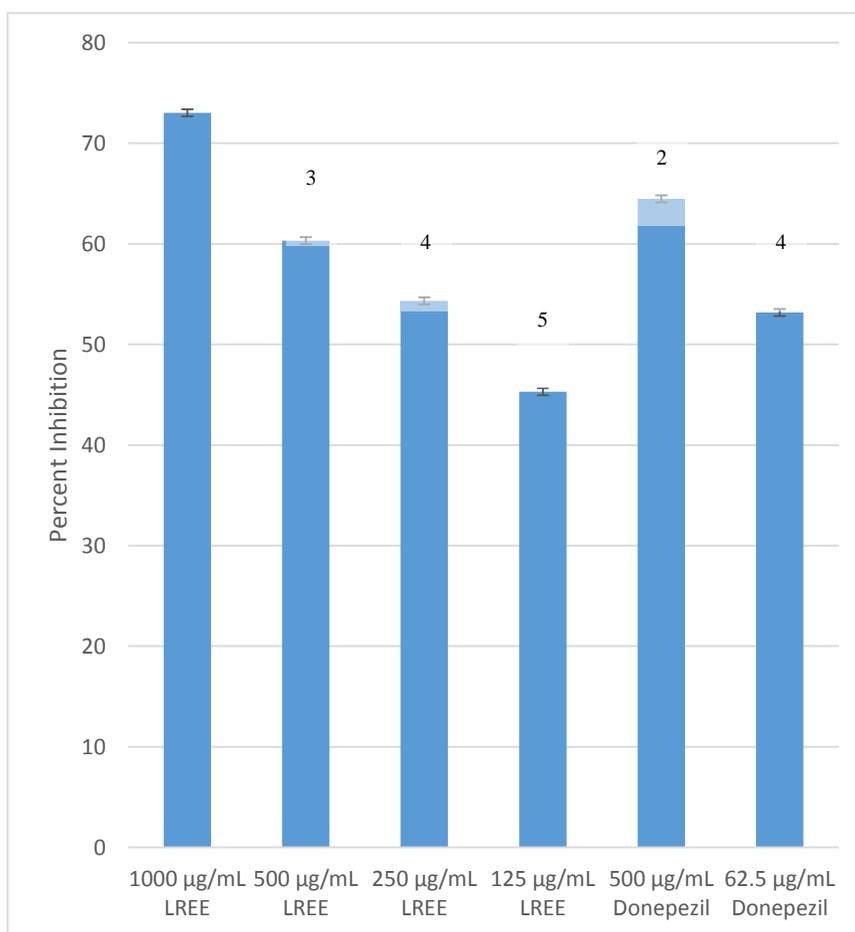
Legend: +++ very strongly positive, ++ strongly positive, + positive, (-) negative

3.2. *In vitro* Acetylcholinesterase Inhibitory Assay

The inhibitory effect of the Lubigan Rhizome's ethanolic extract and its different fractions against AChE was determined using Acetylthiocholine iodide as substrate and compared with a commercial AChE inhibitor, donepezil.

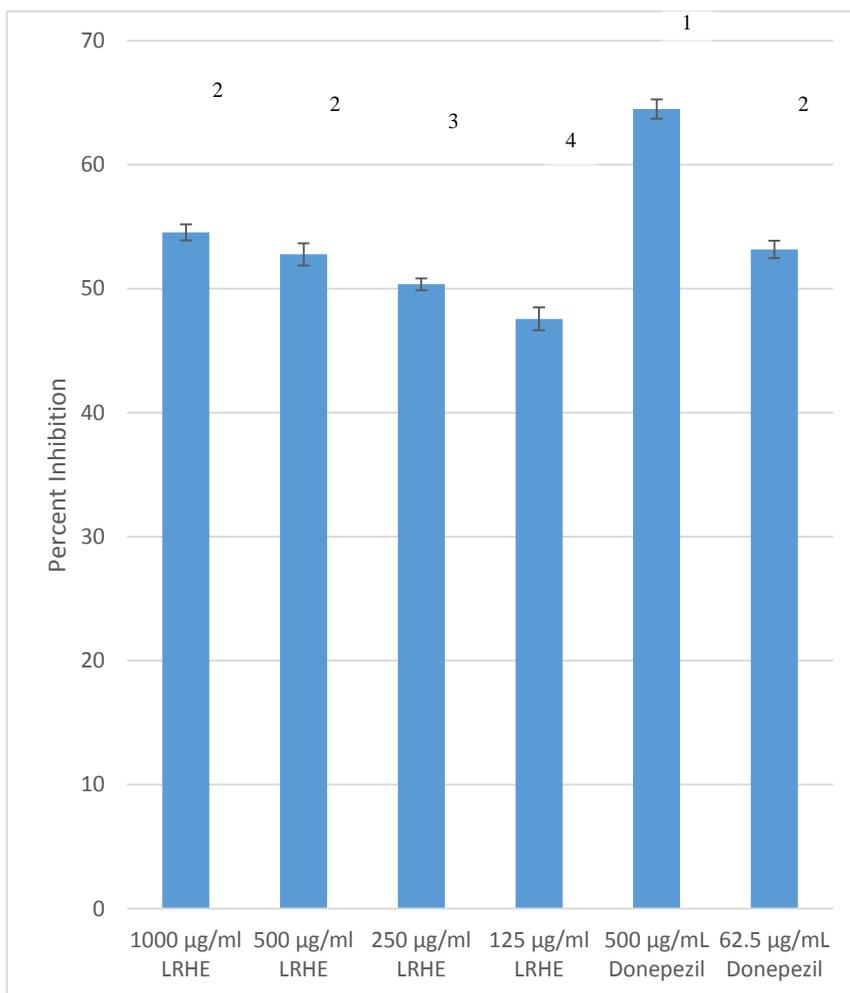
3.2.1 Percent inhibitions against AChE for the Lubigan Rhizome ethanolic extract and its fractions against donepezil

The ethanolic extract of the Lubigan Rhizome (LREE) was able to inhibit AChE activity by $71.72 \pm 0.35\%$, $57.02 \pm 0.92\%$, $54.40 \pm 1.12\%$, and $45.09 \pm 0.16\%$ at concentrations 1000, 500, 250, and 125 $\mu\text{g/mL}$ respectively. The LREE at a concentration of 1000 $\mu\text{g/mL}$ had a higher percent inhibition compared to the highest concentration of donepezil ($60.19 \pm 0.82\%$ at 500 $\mu\text{g/mL}$) while its lowest concentration ($51.72 \pm 0.74\%$ at 62.5 $\mu\text{g/mL}$) was comparable to 250 $\mu\text{g/mL}$ of LREE. Comparison is shown in Figure 3.



Each value is expressed as mean \pm SEM. Values with different symbols (1, 2, 3, 4, and 5) are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

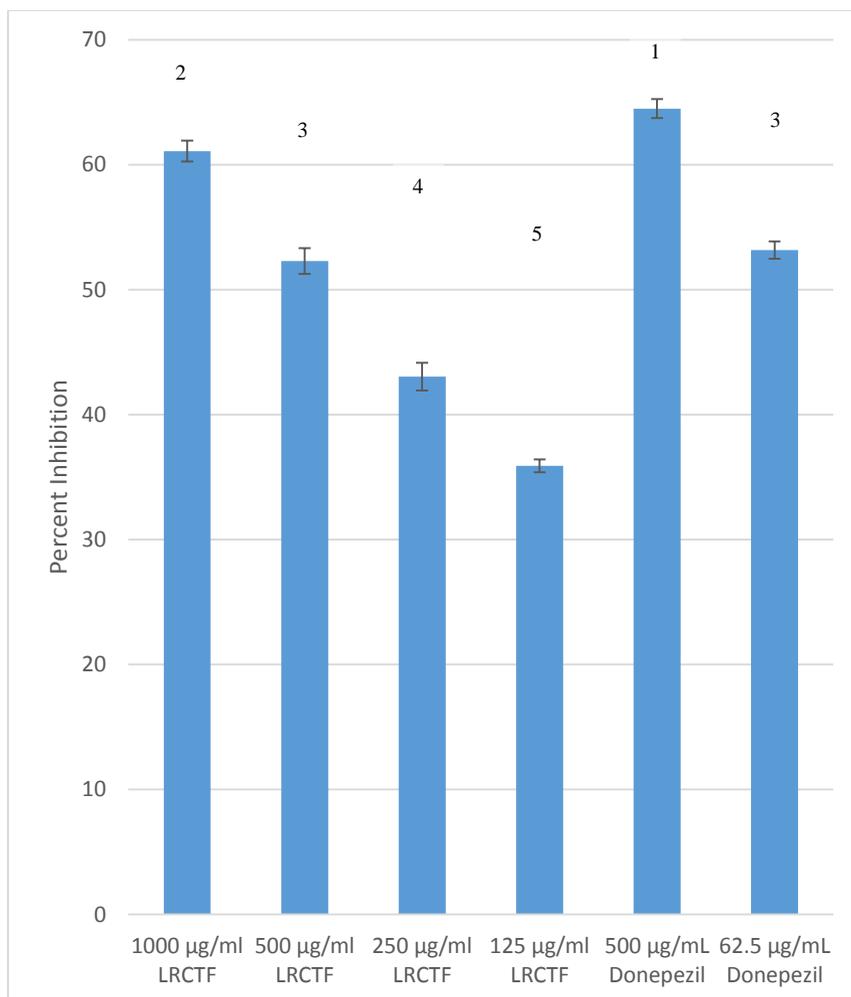
Figure 3. Percent Inhibition of the Lubigan Rhizome Ethanolic Extract (LREE) vs Donepezil



Each value is expressed as mean \pm SEM. Values with different symbols (1, 2, 3, and 4) are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

Figure 4. Percent Inhibition of the Lubigan Rhizome Hexane Fraction (LRHF) vs Donepezil

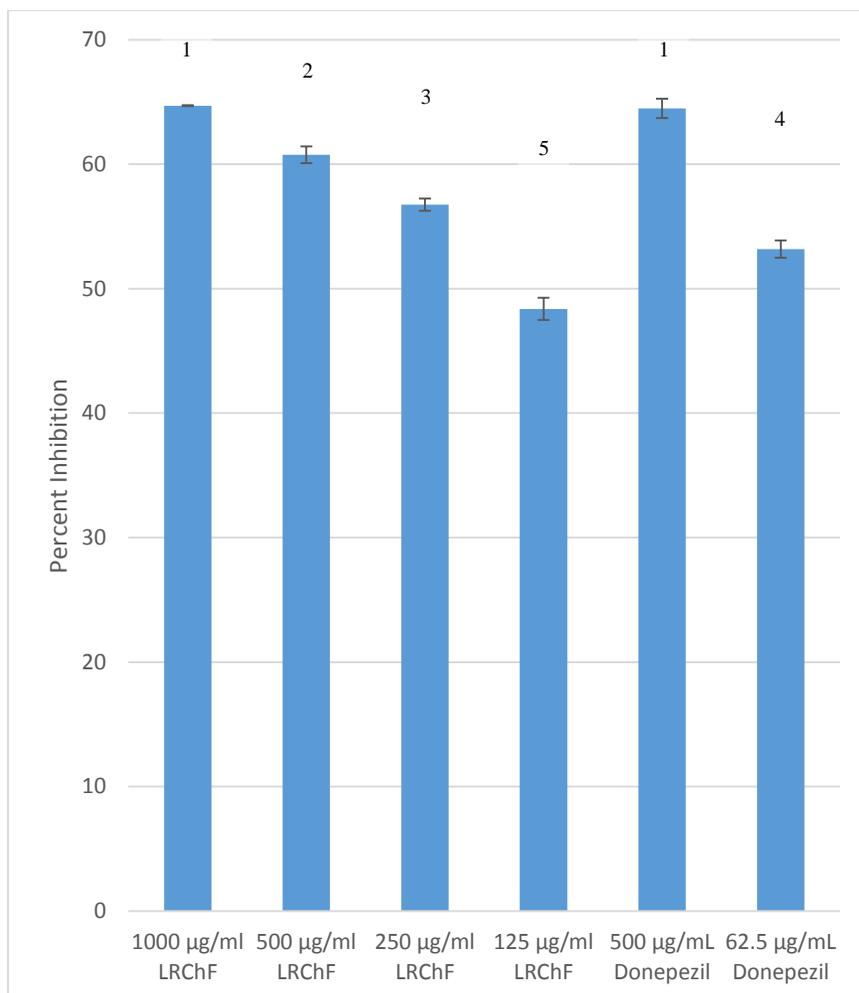
For the Lubigan Rhizome Hexane Fraction (LRHF) the percent inhibitions are shown in Figure 4. AChE was inhibited by $54.53 \pm 0.65\%$, $52.76 \pm 0.89\%$, $50.35 \pm 0.48\%$, and $47.56 \pm 0.92\%$ at concentrations 1000, 500, 250, and 125 $\mu\text{g/mL}$ respectively. Donepezil at a concentration of 500 $\mu\text{g/mL}$ had the highest percent inhibition ($60.19 \pm 0.82\%$) while the LRHF was comparable to 62.5 $\mu\text{g/mL}$ of donepezil ($51.72 \pm 0.74\%$) at both 1000 and 500 $\mu\text{g/mL}$.



Each value is expressed as mean \pm SEM. Values with different symbols (1, 2, 3, 4, and 5) are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

Figure 5. Percent Inhibition of the Lubigan Rhizome Carbon Tetrachloride Fractions (LRCTF) vs Donepezil

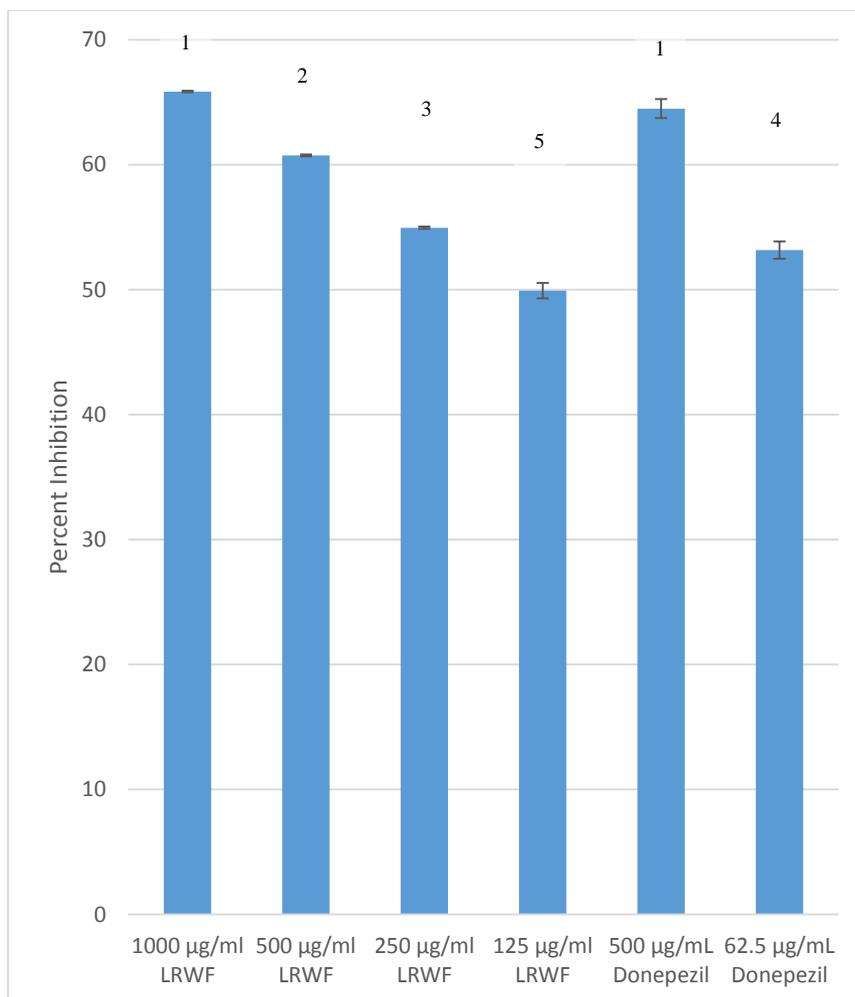
The highest concentration of donepezil also had the highest percent inhibition of AChE as compared to all of the Lubigan Rhizome Carbon Tetrachloride Fractions (LRCTF) as shown in Figure 5. However, the 1000 µg/mL LRCTF had a higher percent inhibition ($61.08 \pm 0.84\%$) compared to that of donepezil at its lowest concentration ($51.72 \pm 0.74\%$ at 62.5 µg/mL). Furthermore, donepezil at 62.5 µg/mL was found to be comparable to LRCTF at a concentration of 500 µg/mL ($52.30 \pm 1.03\%$). The percent inhibitions of the LRCTF at 250 and 125 µg/mL were found to be $43.03 \pm 1.11\%$ and $35.90 \pm 0.52\%$ respectively.



Each value is expressed as mean \pm SEM. Values with different symbols (1, 2, 3, 4, and 5) are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

Figure 6. Percent Inhibition of the Lubigan Rhizome Chloroform Fraction (LRChF) vs Donepezil

Figure 6 shows the percentage inhibition of AChE for the Lubigan Rhizome Chloroform Fraction (LRChF) at a concentration of 1000 µg/mL was found to be $64.69 \pm 0.04\%$ and had no significant difference to that of Donepezil at 500 µg/mL ($60.19 \pm 0.82\%$). Both the 500 and the 250 µg/mL of the LRChF showed significantly higher percent inhibitions for AChE ($60.76 \pm 0.68\%$ and $56.76 \pm 0.49\%$, respectively) than that of the lowest concentration of donepezil ($51.72 \pm 0.74\%$ at 62.5 µg/mL). The 125 µg/mL concentration of the LRChF had the significantly lowest percent inhibition ($48.38 \pm 0.89\%$) compared to all the test samples.



Each value is expressed as mean \pm SEM. Values with different symbols (1, 2, 3, 4, and 5) are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

Figure 7. Percent Inhibition of the Lubigan Rhizome Water Fraction (LRWF) vs Donepezil

Following the trend of the LRChF, the Lubigan Rhizome Water Fraction (LRWF) as depicted in Figure 7. The percent inhibition against AChE by LRWF at a concentration of 1000 $\mu\text{g/mL}$ was $65.85 \pm 0.06\%$ and had no significant difference to that of Donepezil at 500 $\mu\text{g/mL}$ ($60.19 \pm 0.82\%$). Both the 500 and the 250 $\mu\text{g/mL}$ of the LRWF also showed significantly higher percent inhibitions for AChE ($60.74 \pm 0.69\%$ and $54.95 \pm 0.09\%$, respectively) than that of the lowest concentration of donepezil ($51.72 \pm 0.74\%$ at 62.5 $\mu\text{g/mL}$). The 125 $\mu\text{g/mL}$ concentration

of the LRChF had the significantly lowest percent inhibition ($49.92 \pm 0.62\%$) compared to all the test samples.

Compared to all the test samples, the LREE at 1000 $\mu\text{g/mL}$ had the highest significant percent inhibition. While the LRChF and LRWF at 1000 $\mu\text{g/mL}$ were found to be comparable to donepezil at 500 $\mu\text{g/mL}$.

3.2.1 IC_{50} estimates for the Lubigan Rhizome Ethanolic extract and its fractions against donepezil

After obtaining the different percent inhibitions of the different test samples. The IC_{50} values across the 3 trials (each performed in triplicate) was computed using the four-parameter equation. The results are reflected in Table 2. IC_{50} values are used to measure the potency of a substance against AChE. The lower the IC_{50} value, the higher the substance potency.

Table 2

The IC_{50} estimate of the Lubigan Rhizome Ethanolic Extract, its Fractions, and Donepezil

Sample	IC_{50} ($\mu\text{g/ml}$)	Range (CI: 95%)	R^2
Ethanolic Extract	185.7	171.7 to 199.5	0.9946
Hexane Fraction	246.1	201.1 to 295.4	0.9958
Carbon tetrachloride Fraction	415	389.4 to 443.1	0.9944
Chloroform Fraction	131.5	114.9 to 147.6	0.9964
Water Fraction	129.1	121.9 to 136.3	0.9993
Donepezil	8.726	6.24 to 11.14	0.9932

Donepezil has the highest potency with the lowest IC_{50} estimate at 8.726 $\mu\text{g/ml}$ [95% CI: 6.24 to 11.14] followed by the water (LRWF) and chloroform (LRChF) fractions at 129.1 $\mu\text{g/ml}$ [95% CI: 121.9 to 136.3] and 131.5 $\mu\text{g/ml}$ [95% CI: 114.9 to 147.6] respectively. The ethanolic extract of the Lubigan Rhizome had an estimated IC_{50} of 185.75 $\mu\text{g/ml}$ [95% CI: 171.7 to 199.5] while the hexane fraction (LRHF) had an estimated IC_{50} of 246.1 $\mu\text{g/ml}$ [95% CI: 201.1 to 295.4]. The carbon tetrachloride fraction (LRCTF) had the highest estimated IC_{50} at 415 $\mu\text{g/ml}$ [95% CI: 389.4 to 443.1] and thus the least potent of the test samples.

4. CONCLUSION

The LRChF was found to contain phenols, tannins, flavonoids, and alkaloids. LREE and LRWE had the same constituents as LRChF but with additional higher alcohols, sterols, triterpenes, and essential oils, steroids. LREE at 1000 µg/mL had the highest significant percent inhibition for AChE across all test samples while LRChF and LRWF at 1000 µg/mL were found to be comparable to donepezil at 500 µg/mL. Among the fractions of the Lubigan rhizome, the LRWF had the most potent inhibitory activity at 129.1 µg/ml [95% CI: 121.9 to 136.3] but was not as potent as donepezil (8.726 µg/ml [95% CI: 6.24 to 11.14]). From the data gathered, the LREE and its fractions are potential sources of active constituents which may be used to inhibit AChE for AD.

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