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The Effect of Ethanolic Extract of *Pandanus tectorius* Leaves on Spatial Memory Ability and Gcms Analysis of Potentially Therapeutic Compounds

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ABSTRACT

Free radicals are known to damage the hippocampus and will decrease spatial memory. This condition will decrease cognitive function. This study focused on discovering the ability of the ethanolic extract of *Pandanus tectorius* leaves (EEPTL) to increase spatial memory and its active compounds using GC-MS. This study used twenty-five male mice (*Mus musculus*) divided into five groups. Three groups were given EEPTL orally using 100, 200, and 300mg/kg BW doses; one negative control (ethanol 10%) and one positive control (donepezil 0.65mg/kg BW). Each group was induced using ethanol 10% for three weeks (0.5mL/day) and tested for spatial memory using the Y-maze method. The result showed that EEPTL significantly increases mice's spatial memory ability ($P<0.005$). The most effective EEPTL dose is 300 mg/kg BW. GC-MS results showed that some active compounds in EEPTL are palmitic acid, stearic acid, coumarin, and 3-benzoyl-4-phenyl-ethyl linoleic. These results indicate that EEPTL increases spatial memory and contain several fatty acids, volatile oil, and phenols.

Keywords: *Pandanus tectorius*; spatial memory; cognitive learning; Y-maze method

INTRODUCTION

Memory and cognitive function are essential for human life. It is because humans need to learn, organize and use knowledge. Memory makes humans able to design thought into action in daily life.

There are 46.8 million people worldwide who have cognitive impairments like dementia, according to the 2015 World Alzheimer Report. Every 20 years, this number doubles and will eventually reach 131.5 million in 2050. Heretofore, Indonesia does not possess national prevalence data for cognitive disorders. However, one study in Yogyakarta and Bali showed that the prevalence of neurocognitive disorders in that area reached 20.1% and 32.6%, respectively (Suswanti et al., 2020).

Medicinal plant utilization was based on empirical data and awareness that plants can synthesize various chemical compounds with different therapeutical functions. One Indonesian plant that has the potential as a medicine is *Pandanus tectorius,* locally known as "Pandan Tikar." This plant has a high antioxidant content (Omodamiro & Ikekamma, 2016). The phytochemical screening of its leaf extract showed tannins, glycosides, saponins, sterols, triterpenoids, and flavonoids (Bhatt & Bhatt, 2016). Flavonoids are potent antioxidants. Other studies have shown that flavonoid compounds from the ethyl acetate fraction of *Pandanus tectorius* leaves have neuroprotective properties (Bhatt & Bhatt, 2016).

Based on the explanation above, the content of *Pandanus tectorius* as an antioxidant is frequently studied. But an inadequate number of studies about *Pandanus tectorius* phytochemical contents as neuroprotectants.

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METHODOLOGY

Materials and Methods

This study used several instruments: a maceration apparatus, rotary evaporator, analytical balance (Fujitsu FS-AR series), separatory funnel, stirrer, and GCMS (GCMS QP2010 SE Shimadzu).

The main ingredient used is *Pandanus tectorius* leaves obtained from Camplong, Fatuleu Sub-District, Kupang Regency, East Nusa Tenggara Province. The chemicals used were: Donepezil (Aricept® 10 mg), ethanol 96%, chloroform, sodium hydroxide, ethyl acetate, n-Hexane (Emsure® Merck), sulfuric acid, hydrochloric acid (PT Smart Lab Indonesia), ferric chloride and Na-CMC (PT. Kimia ARD Yogyakarta), and distilled water (One Lab Waterone™).

Study Design

This study is an experimental study using a pretest-posttest control group approach and BALB/c male mice as test animals (average weight 25 grams) aged 2-3 months. The ethical commission of Universitas Citra Bangsa has approved this research with ethical clearance number: 017/EC/KEPK/FK/2022.

Subject and Sample Preparation

Male mice were placed into five groups, with five mice in each group. Each mouse was induced using 0.5 mL ethanol 10% orally daily for one week. In the next two weeks, group 1 (negative control) was given only 0.5 mL ethanol 10% orally, and group 2 (positive control) was given ethanol 10% and 0.65 mg/kg BW donepezil orally. Group 3 received 0.5mL ethanol 10% and 100 mg/kg BW ethanolic extract of Pandanus tectorius leaves (EEPTL), while group 4 was given 0.5mL ethanol 10% and 200 mg/kg BW EEPTL. Group 5 received 0.5 mL of 10% ethanol and 300 mg/kg BW EEPTL. Donepezil and EEPTL were prepared using 0.5% CMC as a suspending agent and were given orally.

Male mice were obtained from Jean Pet Store, Surabaya. The mice were acclimatized for seven days while still being given standard feed and in a light room (12 hours of light). All tests on animals were performed according to the UCB's Committee of Ethics Guideline.

Extraction

Seven hundred grams of *Pandanus tectorius* leaves were extracted using 7 L of ethanol 96% at ambient temperature in the following five days. Hereafter, the extract was filtered to separate from the simplisia and evaporated using a rotary evaporator at 40^{o}C.

Chemical Compounds Identification

Chemical compounds in *Pandanus tectorius* leaves were identified using GCMS (GC-MSQP2010 SE Shimadzu) by injecting 1 mL of each extract into the instrument. This procedure was performed in the Laboratory of Integrated Research, Ahmad Dahlan University, Yogyakarta.

Y Maze Cognitive Test

Y Maze consisted of three arms (like the letter Y) and different lengths for each arm (40x 9x 16 cm) (Ahmed, 2021). The angle between each arm is 120^{o}, and each mouse was given 5 minutes to explore the maze. Mice were put into the middle of the maze and moved spontaneously to the three arms of the maze. The result will be analyzed using the formula: the number of alternations divided by the number of possible triads x 100%.

Statistical Analysis

Statistical analysis used SPSS ver 22. Data processing was performed to get the average and standard deviation (SD). Processed data were then compared using one-way ANOVA and post hoc Tukey analysis.

RESULT AND DISCUSSION

The result of *Pandanus tectorius* identification

The plant used in this study underwent an identification process in the Biology Laboratorium, Faculty of Agriculture, Department of Agrotechnology, Nusa Cendana University. The identification paper with number No: 136/UNI5.15.20/TU2022 established that the plant used in this study is *Pandanus tectorius.*

EEPTL phytochemical screening test results.

The phytochemical screening *Pandanus tectorius* showed that this plant contains saponin, tannin, terpenoid, alkaloid, and flavonoid compounds. These are congruous with Kumar *et al*. (2011), that showed *Pandanus tectorius* leaves are rich in phenolic compounds such as alkaloids, saponins, and flavonoids. Flavonoids are well-known as a neuroprotector, regenerate nerves and improve cognitive function. They inhibit butyrylcholinesterase (BChE), β secretase, acetylcholinesterase (AChE), and free radicals (Ayaz et al., 2019). In addition, flavonoids activity as a neuroprotective against neurotoxins (Vauzour et al., 2008). Saponins protect neurons by reactivating the cell's natural antioxidant mechanism (Xinxin et al., 2014). The alkaloids in EEPTL inhibit ROS and increase the potential of the mitochondrial membrane, preventing degradation and oligomerization of β-amyloid plaques (Tan, 2022).

Gas Chromatography-Mass Spectrometry (GCMS) Active Compounds Identification Result

Qualitative and quantitative analyses of EEPTL were done in the Laboratory of Integrated Research, Ahmad Dahlan University. The resulting paper with number No:09/LHU-LAMDA/X/2022 showed that there are 36 active chemical compounds in EEPTL. These active compounds showed in Table I.

There are ten compounds with large area percentages (bold letters in Table I): 13-Octadecenal, (Z)- (32.28%); palmitic acid (16.13%); methyl stearate (8.93%); 9-octadecenoic acid, 12-(acetyloxy)-, methyl ester, [R-(Z)]- (6.63%); Hexadecanoic acid (CAS), Octadecenoic acid (6.34%); 2-Hexadecene,

Table Ia. GCMS Active Compounds Identification Result

No	RT (min)	Mol. Mass	Tentative identification	Mol. Formula	Compound class	% Area
1	8.325	192	Vitispirane	C13H20O	Volatile oil	0.53
2	13.832	192	Bicyclo[4.4.0]dec-5-en-4-one-1-carboxylic acid	C11H14O3	carboxylic acid	0.29
3	14.443	220	BUTYL HYDROXY TOLUENE	C15H24O	Phenol	1.14
4	19.868	210	3-oxo-7,8-dihydro-.alpha.-ionol	C13H22O2	Ketone Alcohol	0.31
5	19.960	264	3,5-di(t-butyl)-4-hydroxybenzyl alcohol ethyl ether	C15H23NO3	Hydrocarbon	0.37
6	20.685	282	9-Octadecenoic acid (Z)- (CAS)	C18H34O2	Fatty acid	0.35
7	**21.051**	**278**	**Neophytadiene**	**C20H38**	**Terpenoid**	**2.49**
8	21.104	280	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	C20H40O	Volatile oil	0.26
9	21.214	268	2-Pentadecanone, 6,10,14-trimethyl- (CAS)	C18H36O	Volatile oil	0.56
10	21.352	250	1-Octadecyne	C18H34	Fatty acid	0.55
11	21.414	208	Pluchidiol	C13H20O2	Alcohol	0.73
12	21.565	296	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O		1.09
13	21.854	242	PENTADECANOIC ACID	C15H30O2	Fatty acid	0.23
14	**22.136**	354	**9-Octadecenoic acid, 12-(acetyloxy)-, methyl ester, [R-(Z)]- (CAS)**	**C21H38O4**	**Fatty acid methyl ester**	**6.63**
15	22.814	284	Hexadecanoic acid, ethyl ester (CAS)	C17H34O2	Fatty acid ester	1.26
16	**22.971**	**256**	**Hexadecanoic acid (CAS)**	**C17H34O2**	**Fatty acid**	**16.13**
17	23.130	284	Heptadecanoic acid, methyl ester (CAS	C17H34O2	Fatty acid methyl ester	1.31
18	**23.860**	296	10-Octadecenoic acid, methyl ester (CAS)	C19H26O2	Fatty acid methyl ester	8.93
19	**23.901**	348	8,11,14-Docosatrienoic acid, methyl ester (CAS)	C23H40O2	Fatty acid methyl ester	2.58
20	**24.020**	**296**	**2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)**	**C20H40O**	**Diterpen**	**5.07**
21	24.267	170	p-Menth-8(10)-ene-2,9-diol (CAS)	C10H18O2	Alcohol	0.32
22	24.390	308	Ethyl linoleate	C20H36O2	Fatty acid	1.54
23	**24.579**	**266**	**13-Octadecenal, (Z)-**	**C18H34O**	**Fatty acid**	**32.28**
24	**24.713**	**284**	**Octadecanoic acid (CAS)**	**C18H36O2**	**Fatty acid**	**6.34**
25	**24.955**	**294**	**9,12-Octadecadienoic acid, methyl ester, (E, E)- (CAS)**	**C19H34O2**	**Fatty acid**	**2.16**
26	25.123	243	8-Nitro-11-dodecanolide	C12H21NO4	Ester	0.65
27	25.212	330	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)	C19H38O4	Fatty acid	0.59
28	25.256	248	1,E-11,Z-13-Octadecatriene	C18H32	Amina	1.11
29	25.445	114	2-Heptanone (CAS)	C17H14O	Ketone	0.53
30	25.583	568	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)	C35H68O5	Fatty acid	0.52
31	25.765	326	EICOSANOIC ACID, METHYL ESTER	C21H42O2	Fatty acid	0.30
32	26.398	312	Eicosanoic acid (CAS)	C20H40O2	Fatty acid	0.43
33	26.910	266	9-Octadecenal, (Z)- (CAS)	C18H34O	Fatty acid	0.33
34	31.675	326	Benzene, 1,4-bis(3-formylcyclohexan-2-one-1-yl)-	C20H22O4	Benzene	0.31

Table Ib. GCMS Active Compounds Identification Result

No	RT (min)	Mol. Mass	Tentative identification	Mol. Formula	Compound class	% Area
35	**33.462**	**410**	**2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (CAS)**	**C30H50**	**Ether**	**1.83**
36			Coumarin, 3-benzoyl-4-phenyl- (CAS)	C22H14O3		

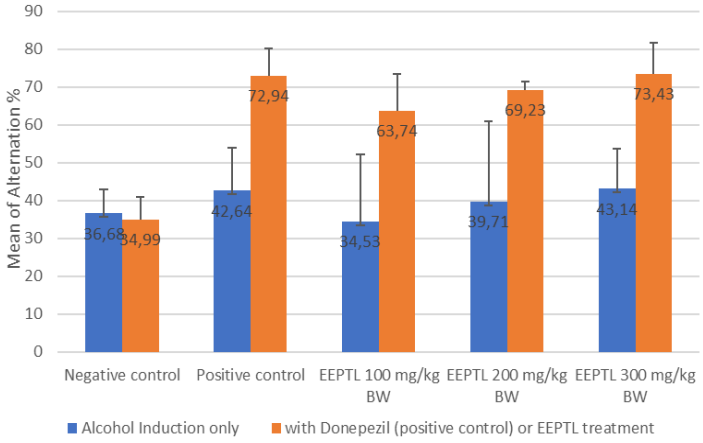

Figure 1. Y maze test result

3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]- (5,07 %); 8,11,14-Docosatrienoic acid, methyl ester (2,58%); Neophytadiene (2.49%); 9,12-Octadecadienoic acid, methyl ester, (E, E)- (2,16%); and Ethyl linoleate (1.54%).

EEPTL effect on a Y maze test

The outcome of EEPTL treatments, donepezil (positive control), and ethanol 10% (negative control) on the spatial memory ability of mice are presented in Figure 1.

The histogram in Figure 1 shows that after ethanol induction for 14 days, there was an exploration decrease of mice in all three arms. The average decrease in mice exploration on three arms of Y-maze, in sequence: test group 1 (34.53), negative control (38.68) < test group 2 (39.71) < donepezil group (42.64) < test group 3 (43.14). The deflation of the average value of mice exploration in the Y-maze arm indicates a decrease in the ability of cognitive function in mice. The administration of alcohol reduces the generation of hippocampal neurons and results in reduced spatial memory function (Yunus & Sari, 2012). Consistent alcohol consumption in rats for around 20 days resulted in impaired spatial learning (Du et al., 2019). Prolonged alcohol consumption will cause the formation of free radicals, which will react with fats, proteins, and cellular nucleic acids resulting in local damage and organ dysfunction (Toyokuni, 1999). The frontal lobe is a part of the brain that is highly susceptible to damage if exposed to alcohol. The damage to the frontal lobe will cause disturbances such as decreased cognitive function (Crews et al., 2009).

Administration of EEPTL for 14 days significantly increased spatial memory abilities. This effect is indicated by the average increase in the exploration of mice in 3 arms. The positive control was the highest (30.30), followed by the 300 mg/kg BW dose group (30.29), 200 mg/kg BW dose group (29.52), and 100 mg/kg BW dose group (29.21%). Ethanol 10% and 5% CMC were given to the negative control group, and there was a decrease in mice exploring the arms with an average of - 3.70. The standard deviation (SD) shows the uniformity of data distribution from the results of each mouse in one group. A small SD value indicates that the performance of each mouse in one group is not significantly different and that the average results for each group are accurate. The statistical results revealed a significant difference between the EEPTL groups and the negative control ($p<0.05$) but no difference between the positive control and the EEPTL groups.

The spatial memory restoration after donepezil administration could be due to its ability to inhibit AChE. This enzyme rapidly breaks acetylcholine, increasing acetylcholine concentration in the central nervous system. Coumarin, 3-benzoyl-4-phenyl-, a compound found in the EEPTL, has activity in improving cognitive function. The benzopyrene group in the coumarin structure allows this compound to inhibit the accumulation of AChE-induced β-amyloid and AChE (Yusufzai et al., 2018). Furthermore, EEPTL tested positive for flavonoid, famous for its antioxidant characteristic. This compound can protect neurons from the neurotoxic effect of ROS that can damage neurons. Additionally, flavonoid protects blood vessels in the brain and inhibits cholinesterases (Ayaz et al., 2019; Vauzour et al., 2008)

In addition, EEPTL contained several other active compounds, such as palmitic acid, stearic acid, 3-benzoyl-4-phenyl- (CAS), and ethyl linoleic. Palmitic acid is naturally found in fatty acids in the brain. Hosseinzadeh et al. (2007) proved that giving palmitic acid in rat feed can improve cognitive function. This effect is due to the role of palmitic acid in the process of protein palmitoylation of nerve proteins such as GAP-43, NMDA receptors, and AMPA receptors. Ethyl linoleate potentially inhibits the AChE (Kissling et al., 2005). Stearic acid shields cortical neurons from oxidative stress. This ability is manifested by increasing internal antioxidant enzymes. Stearic acid's neuroprotective effects seem to be mediated by peroxisome proliferator-activated receptor-γ (PPARγ) and the synthesis of new proteins in cortical neurons (Wang et al., 2007). These compounds' combination in EEPTL seems to produce its neuroprotective and neurogenerative activities.

CONCLUSION

EEPTL can improve the spatial memory ability of mice. The most effective EEPTL dose is 300 mg/kg BW. Compounds in EEPTL that seem to exhibit neuroprotective and neurorestorative effects are 13-Octadecenal, (Z)- (32.28%); palmitic acid (16.13%); methyl stearate (8.93%); 9-Octadecenoic acid; 12-(acetyloxy)-, methyl ester, [R-(Z)]- (6.63%); hexadecanoic acid (CAS), Octadecenoic acid (6.34%); 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*, R*-(E)]]- (5,07 %); 8,11,14-Docosatrienoic acid, methyl ester (2,58%); Neophytadiene (2.49%); 9,12-Octadecadienoic acid, methyl ester, (E, E)- (2,16%); and ethyl linoleate (1.54%).

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