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Original Article

ANTIDEPRESSANT EFFECTS OF FRESH JUICE, ETHYL ACETATE, AND N-HEXENE FRACTION OF UNRIPE AMBON BANANA PEELS ON MICE (*MUS MUSCULUS*) INDUCED BY UNPREDICTABLE CHRONIC MILD STRESS

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ABSTRACT

Objective: This study aims to determine the effects of fresh juice, ethyl acetate fraction, and n-hexene fraction of unripe Ambon banana peel as an antidepressant on mice (*Mus musculus*) and to determination of Brain-derived neurotrophic factor (BDNF) levels in the hippocampus.

Methods: The animals weighing 20-30 g were induced stress by the UCMS (Unpredictable Chronic Mild Stress) method for five weeks with circadian disturbances, wet cages, wet bedding, empty cages, tilted cages, predator sounds, iced water, and restraints. Twenty-five mice were randomly divided into five groups, namely the negative control group (distilled water) for 15 days, and positive control group (Sertraline 9.1 mg/kg BW) for 15 days, fresh juice, ethyl acetate fraction, and n-hexene fraction of unripe Ambon banana peel were given 10 mg/kg BW for 15 days. Duration of immobility time with the FST and TST methods were measured. The brain organs histopathology was evaluated and BDNF protein expression was measured by Enzyme-Linked Immunosorbent Assay (ELISA).

Results: Results showed that Fresh juice, ethyl acetate, and n-hexene fraction of unripe Ambon banana peel significantly reduced the immobility time ($p<0.01$) in the FST test by 56.63, 85.48, and 94.30 % and TST test, respectively, 57.16, 84.92, and 94.56%. Cell damage in the positive control group showed no significant difference with n-hexene fraction group ($p = 0.098$). BDNF expressed on groups positive, ethyl acetate, and n-hexene fractions. Meanwhile, negative control and fresh juice, BDNF proteins were unexpressed.

Conclusion: n-hexane fraction has significant results with the positive control group on mice induced by mild chronic stress.

Keywords: Banana peel, Antidepressant, TST, FST, BDNF

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INTRODUCTION

Changes in mood, physical health, and behaviour are the symptoms of mental disorders, also known as depression. It can lead to a decrease in work productivity and disruption in social relationships [1-4]. Neurotransmitters that can cause depressive disorders include norepinephrine (NE), serotonin (5-HT) and dopamine [5, 6]. Meanwhile, several factors that can trigger depression include genetics, environment, biochemistry and psychology, but sometimes it can emerge without any clear reason or trigger.

The patient's symptoms can be minimized using antidepressant medication. Selective Serotonin Reuptake Inhibitors (SSRI) [7], Serotonin Norepinephrine Reuptake Inhibitors (SNRI), Tricyclic Antidepressants (TCA), and Monoamine Oxidase Inhibitors (MAOI) are the main types of frequently used antidepressant drugs [8]. This drug can cause nausea, vomiting, dry mouth, urinary retention, visual disturbances, constipation, tachycardia, orthostatic hypotension, and nausea [9]. The side effects of antidepressant drugs can affect the life quality of the patient. This can lead to the non-compliance of the patient with treatment. An alternative therapy that can be used to avoid these side effects is herbal medicine from several types of plants, one of which is banana peel (*Musa paradisiaca* L.) [10–13].

Previous research revealed that acetone extract from young Ambon banana peel at a dose of 200 mg/Kg BW could provide an antidepressant effect by increasing tryptophan levels in the brain of mice by 30.59 µg/g. This can significantly increase the maintenance duration in Open Field Testing (OFT) by 36.79% and reduce immobility time in FST by 17.36% and TST by 24.11% against the mice with chronic stress [12].

The compounds in banana peel are minerals, polyphenols, tannins, vitamin C, phytic acid, leucine, valine, phenylalanine, and threonine [14–16]. Ethanolic extract of Cavendish banana peels showed (*Musa acuminata* Cavendish) potency as a complementary therapy for anxiety disorder [17]. In the present study, banana peels increased the time spent in a lightbox and open arm, suggesting anxiolytic effects. A significant decrease in immobility time was observed in FST in banana peel-treated animals, suggesting antidepressant-like effects. Banana peels showed that it can decrease time to reach the platform in both short-term and long-term memory tests suggesting increased memory function in treated animals as compared to control animals. The activities of all antioxidant enzymes were significantly different ($p<0.05$) in banana peel-treated animals than control. It is concluded that banana peel has anti-anxiety and antidepressant effect as well as strengthens memory possibly via its antioxidant mechanism. Therefore, it is recommended that supplementation of bananas could take a vital role in stress (anxiety and depression) relief and increase memory function possibly by phytoantioxidants [18].

The tryptophan level of bananas is higher than that of other fruits (26.15±0.37 µg/g) [19]. Banana peel powder has a higher tryptophan concentration (1000 µg/g) than other plant parts, according to a prior study [20]. Based on the tryptophan concentration, banana peels were chosen. Unripe banana peels have more tryptophan than ripe. Free tryptophan can cross the blood-brain barrier (BBB) when it gets close to it [21]. Histidine, isoleucine, methionine, phenylalanine, threonine, tyrosine, and valine are among the major neutral amino acids that tryptophan competes with for the cerebrovascular transport system. The large hydrophobic side chain of tryptophan, however, gives it a greater affinity for that transport route. Thus, the ratio of tryptophan to other amino acids that compete for the same transporter is used to express the bioavailability of tryptophan. It is possible to increase the amount of tryptophan or decrease the amount of other competing amino acids to increase the concentration of tryptophan accessible for serotonin production [22].

The banana peel (*Musa balbisiana Colla*) showed that the immobility time in TST was significantly reduced in the groups supplemented

with 15% and 30% banana peel floss, respectively. We observed a significant association between serotonin and cortisol levels and also between the duration of immobility time in TST and serotonin levels. The administration of banana peel floss caused significant changes in plasma serotonin concentrations, implying that dietary fiber, tryptophan, and bioactive components in banana peel floss can reduce stress-induced depression by regulating cortisol levels and increasing serotonin levels [23].

Tryptophan and combination monoamine oxidase inhibitors are clinically useful [24]. RCTs study showed that taking 0.14-3 g of tryptophan per day could improve the mood of healthy individuals [25]. Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays a key role in neuronal survival and development and is involved in several diseases, including Alzheimer's disease and major depression. Many studies suggest that reduced BDNF levels are associated with an increased incidence of depressive symptoms, neuronal loss, and cortical atrophy [26].

Based on previous research, this research aimed comparison of antidepressant effects of fresh juice, ethyl acetate fraction, and n-hexene fraction Ambon banana peels (*Musa paradisiaca* L.). The antidepressant effect used by mice animal models that induced by UCMS. Duration of immobility time with the FST (forced swimming test) and TST and BDNF levels were measured.

MATERIALS AND METHODS

Material

Materials used during the study were unripe Ambon banana peels aged±3 mo obtained from Tuntang Area, Semarang Regency, male mice aged 3-4 mo and weighed 25-30 g (Pharmacology Laboratory, Faculty of Pharmacy, UMS), distilled water, methanol (analytical grade, Merck), ethanol 96% (analytical grade, Merck), CMC Na (Merck), Sertraline obtained from Sarika Pharmacy Semarang, magnesium powder (analytical grade, Merck), Dragendorff reagents, Wagner reagents and Hager reagents, and BDNF ELISA Kit (ABclona). The tools used analytical scales (Ohaus), glassware (Pyrex), a water bath, a stopwatch, a vacuum rotary evaporator (Heidolph), a mice cage, one set FST test (44 x 20.5 x 23 cm aquarium with climbing net), one set of TST test (clamp pole for the tail of tested animals), UCMS induction tool, a set of surgical tools, microtube, and ELISA reader (Biotek).

Methods

The protocol study was evaluated and approved by the Ethics Committee of Health Research of the Faculty of Medicine Universitas Muhammadiyah Surakarta (No.4824/A.1/KEPK-FKUMS/III/2023). Test animals were given treatment in a room with a temperature of 20 °C and humidity of 75%.

Extraction and fractionation of ambon banana peels

Fresh juice was obtained by placing 100 g of banana peel into a juicer. Fresh juice is then filtered through filter paper. Meanwhile, extraction was carried out by maceration with a ratio of (1:5) Ambon banana fruit peels with 96% methanol as a solvent for 3 days. The liquid extract is then evaporated by a rotary evaporator. Fractionation was performed by liquid-liquid extraction (LLE) method, using ethyl acetate and n-hexene as solvents with a ratio 1:1. The fractions obtained were ethyl acetate fraction and n-hexene fraction. The yield of extract is calculated using the formula:

$$\%\text{yield of extract} = \frac{\text{Extract weight}}{\text{Powder weight}} \text{ x } 100\%$$

The yield of the fraction is calculated using the formula:

$$\%\text{ yield of fraction} = \frac{\text{Fraction weight}}{\text{Extract weight}} \text{ x } 100\%$$

Phytochemical screening of Ambon banana peels

- **Alkaloid test**

A 2 ml sample was put into a test tube and mixed with 5 ml of chloroform and 5 ml of ammonia prior to be heated, shaken and filtered. 5 drops of H_2SO_4 were added to each filtrate and then shaken and left. The top of each filtrate was taken and tested with Mayer, Wagner and Dragendorff reagents. The formation of a white precipitate in Mayer's reagent, a brown precipitate in Wagner's reagent, and orange precipitate in Dragendorff reagent indicated the presence of alkaloids [12, 14].

- **Flavonoid test**

The sample was weighed at 0.5 g, and added with 10 ml of distilled water. It was then heated over a water bath, filtered and dissolved into 1 ml of 96% ethanol. The sample was added with magnesium powder and dissolved into 10 ml of concentrated HCl solution. The color change from yellow, orange to purple-red indicated the positive flavonoids [15].

Induction of UCMS

Twenty-five mice divided into 5 groups:

- Negative control: administration of CMC Na 0.5% orally for 15 d
- Positive control: administration of Sertraline at the dose of 9.1 mg/kg BW orally for 15 d.
- Fresh juice of banana peel: administration of fresh juice of banana peel with the dose of 10 mg/kg BW orally for 15 d.
- Ethyl acetate fraction of banana peel: administration of ethyl acetate fraction of banana peel at a dose of 10 mg/kg BW orally for 15 d.
- n-hexene fraction of banana peel: administration of n-hexene fraction of banana peel with the dose of 10 mg/kg BW orally for 15 d.

The time used to conduct stressor exposure was 36 d. Each stressor was carried out 4 times in 36 d. There were nine different kinds of stressors used. Based on table 1, the stressors were randomly assigned, and one type of stressor was used for technical implementation throughout the course of one day.

Table 1: UCMS induction procedure [27]

Stressor	Time	Remarks	Day of induction
Circadian disorder	2 x 12-hour cycle	Constantly dark or light	1,10,19,28
Circadian disorder	(Cycle per 30 min within 8 h)	Alternately dark or light	2,11,20,29
Wet Bedding	1-6 h	125 ml water was added into bedding	3,12,21,30
Wet Cage	15-30 min	Placement into the cage without bedding filled with water 1 cm high (water temperature should be at approximately 30 °C)	4,13,22,31
Empty Cage	1-6 h	Mice were placed into the cage without bedding	5,14,23,32
Sloping Cage	1-4 h	Placement into the cage with the tilt position	6,15,24,33
Predator Voice	30 min	Broadcasting the voice of dog and cat	7,16,25,34
Iced Water	5 min	Immersion in cold water	8,17,26,35
Restraint	4 h	In a tube 4 cm in diameter and 10 cm in length	9,18,27,36

- **FST method**

A transparent cylindrical tube with a diameter of 20 cm and a height of 50 cm filled with water to a height of 35 cm at a controlled temperature (25 °C) was used to make the house mice swim. The time used to swim for the mice was 5 min and the duration of immobility was observed in the last 3 min. The mice were immobilized if they did not move and only made some movements

to keep their heads above water, while the mobility of house mice could be seen when swimming and climbing. The pretest and post-test results are shown on the immobility time parameter [28, 29].

- **TST method**

The table was horizontally positioned with a 50-cm long rod used to hang the house mice's tail from the base to the tip of the wood (1 cm). The parameters for the duration of immobility time for house mice in the TST test were measured for 5 min before and after the test. A stopwatch was used to measure the time when mice stopped moving (immobility time) and the duration of immobility (mobility time) [11].

- **Hippocampus histopathology**

Surgery was carried out through the dislocation of the tested animal, and the hippocampus part of the brain was then taken. The organs subsequently were preserved in 4% Normal Buffer Formalin (NBF) solution. Furthermore, the histopathology of hippocampal cell damage was observed and calculated.

- **Determination of brain-derived neurotrophic factor (BDNF)**

The blood of the tested animals was taken from the eye vein. The blood of the tested animals was centrifuged at a speed of 10,000 rpm within 20 min, and then the serum was taken. BDNF protein level in serum was analyzed by means of the Sandwich ELISA KIT BDNF ABclonal and read using an ELISA reader [26].

- **ELISA measurement**

Lyophilized standard solutions with concentrations of 1500, 750, 375, 187.5, 93.7, 46.8, 23.4, and 0 pg/ml were employed as the standard standards. Wells were prepared with 96 wells that had been cleaned using 350 µl** of buffer per well. To the blank well, add 100 µl** of Standard/sample Diluent (R1). Apply the included sticky strip on top. Incubate at 37 °C for 2 h. 15 min prior to usage, prepare the Concentrated Biotin Conjugate Antibody (100x) Working Solution. In each well, add 100 µl** of Working Biotin Conjugate Antibody, then cover with a sticky seater. Incubate at 37 °C for 1 hour. Before using, prepare the Streptavidin-HRP concentrate (100x) 15 min in advance. Put 100 µl** of Working Streptavidin-HRP into each well, then cover with the fresh adhesive that was supplied. Incubate at 37 °C for half an hour. Put one hundred microliters of TMB (3',5,5'-Tetramethylbenzidine) substrate into every well. Incubate at 37 °C for 15 to 20 min. Defend against light. Use a microplate reader set to 450 nm to measure the optical density of each well after adding 50 µl** of Stop Solution in 5 min.

Data analysis

The analysis of two paired samples was determined by the paired T-test. The results analyzed using T-paired were the 5 w UCMS stressor in pretest-post test data (basalt before UCMS induction and after UCMS induction) as well as the duration of immobility time (IT) in the TST test and FST test. Each sample on day 0 (pretest) was compared with day 15. The results of the post-test data are the observational data on antidepressant therapy. Differences in results between test groups for each antidepressant measurement can be concluded through data analysis with SPSS software version 25 using MANOVA-LSD with a confidence level of 95%.

RESULTS AND DISCUSSION

Extraction and fractionation yield

Extraction using the maceration method produced a thick methanol extract of unripe Ambon banana peel (69.85 g with a yield of 17.46%). The yield of the ethyl acetate and n-hexene fraction were 3.99 and 7.40%.

Phytochemical screening

Identification of secondary metabolites of unripe Ambon banana performed on tube reaction using several reagents. The results of the phytochemical screening (table 2), showed that the compounds contained in unripe Ambon banana peels were alkaloids and flavonoids. These results are in accordance with research conducted by Alamsyah [13, 30, 31] stating that Ambon banana peel contained alkaloid and flavonoid compounds. It should reveal the findings of the works.

Table 1*:* Phytochemical content of unripe Ambon banana peel based on test tube

Chemical test	Reference	Fresh juice	Ethyl acetate fraction	n-Hexene fraction	Remark
Alkaloid	Mayer: White precipitate	White precipitate	White precipitate	White precipitate	+
	Wagner: Brown precipitate	Brown precipitate	Brown precipitate	Brown precipitate	+
	Dragendorff: Orange	Orange precipitate	Orange	orange precipitate	+
Flavonoid	Yellow, orange to dark red to purple	Light yellow	Dark yellow	Light yellow	+

Remark: (+): containing compound

Antidepressant UCMS induction

The UCMS method is a method for animal depression by providing chronic environmental and psychological stress stimuli by imitating the stressors of everyday human life (table 1). This model was selected considering that it can provide a model of depression that can mimic everyday stress in human life and induce anhedonia, which is a core symptom of depressive disorders as mentioned in the Diagnostic Manual and Statistics of Mental Disorders IV (DSM-IV) [27]. Table 3 and 4 present the antidepressant test results.

Table 2: Antidepressant test based on FST method (n=5)

Treatment group	Baseline before UCMS induction	after UCMS induction	Treatment on 16 d
	Immobility time (IT) x̄±SD (min)		
Negative Control	1.1±0.2*	3.3±0.2*	3.0±0.3*
Positive Control	1.4±0.4	3.2±0.1	0.2±0.1
Fresh Juice	1.1±0.4*	3.3±0.1*	1.4±0.3*
Ethyl Acetate Fraction	1.4±0.3*	3.6±0.2*	0.5±0.1*
N-Hexene Fraction	1.6±0.5	3.6±0.3	0.2±0.1

FST=forced swimming test; UCMS =unpredictable chronic mild stress, remark: (* $p<0.05$)

Based on the graph above, a significance value of p=0.000 was obtained between the baseline group before UCMS induction and the baseline after UCMS induction. The success of the FST method of antidepressant therapy was determined by comparing IT after induction with IT on the 16th day of treatment. The results of immobility time in the baseline group after therapy on the negative

control had a significance of p>0.05. The 16th day treatment group showed that the negative control group had a significance of p=0.000 with the positive control group, fresh juice, ethyl acetate fraction, and n-hexane fraction. Negative control serves to prove that all treatments given to test animals do not affect the antidepressant test. The results of antidepressant therapy in the ethyl acetate fraction group had a significance value of p=0.013 with the n-hexane fraction group. In contrast, the significant results in the fresh fruit juice and ethyl acetate fraction groups had a value of p=0.000 compared to the positive control. It can be concluded that the possibility of fresh fruit juice and ethyl acetate fraction having antidepressant activity is very low, while the n-hexene fraction group has a significance value of p=0.750 compared to the positive control. The fresh fruit juice group, ethyl acetate fraction, and n-hexene fraction have a significance value of p<0.05; meaning that the three samples are significantly different.

Table 3: Antidepressant test based on TST method (n=5)

Treatment group	Baseline before UCMS induction	After UCMS induction	Treatment on 16 d
	Immobility time (IT) x̄±SD (min)		
Negative Control	1.1±0.3	3.2±0.1	3.0±0.3
Positive Control	0.8±0.6*	3.5±0.2*	0.3±0.1*
Fresh Juice	1.1±0.4	3.2±0.1	1.4±0.2
Ethyl Acetate Fraction	1.3±0.2	3.0±0.3	0.6±0.3
N-Hexene Fraction	1.6±0.4*	3.3±0.3*	0.2±0.0*

UCMS =unpredictable chronic mild stress TST: tail suspension test; (*p<0.05)

Based on the t-Test, the results showed that p-value was 0.000, indicating a difference in treatment between before induction and after induction and a difference between the IT after basalt induction and the IT on day 16 of the TST test fig. 2B). Serotonin levels could be increased by administering positive control drugs such as sertraline as indicated by a significant decrease (p = 0.000) in the duration of the immobility time of TST method for 15 d. The administration of fresh juice, ethyl acetate fraction, and n-hexene fraction showed significant differences with the negative control (p=0.005). In the TST test (fig. 2A), the fresh juice (p=0.000) and ethyl acetate fraction (p=0.024) groups showed a significance of<0.05 when compared with sertraline, while n-hexene fraction group showed p=0.298 compared with sertraline. This showed the very little possibility of fresh juice and ethyl acetate fraction to have an antidepressant therapeutic effect. The n-hexene fraction was thought to be capable of increasing brain neurotransmitter levels by inhibiting MAO and increasing serotonin from tryptophan supply, thereby reducing depression as seen in the long decrease in IT using the TST method during the 15-day administration.

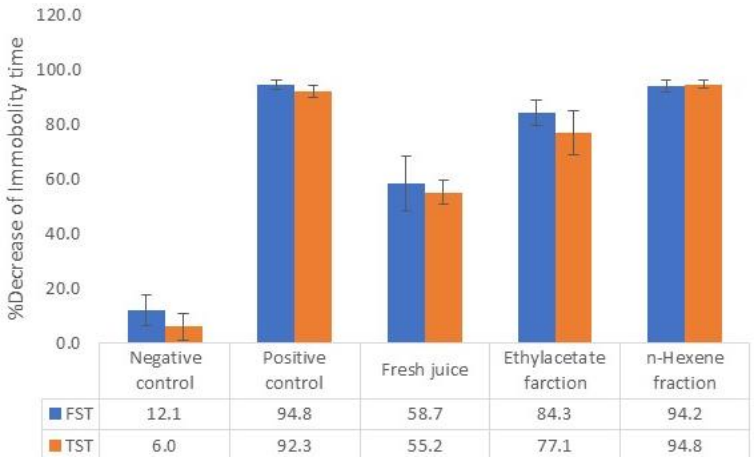

Fig. 1: Bar chart of percentage in the decrease of immobility time (%) of groups based on FST and TST

As shown from the graph (fig. 1), the results obtained showed that all samples were assumed to have antidepressant activity as seen in the % decrease in IT between the negative control group and each sample. The percentage of the negative control group was significantly different (p<0.05) from the sertraline, fresh juice, ethyl acetate fraction and n-hexene fraction groups. The sertraline group showed a % reduction of 94.74% (FST) and 91.29% (TST) in which, when compared with each sample, it showed a significance of>0.05, meaning that it was not significantly different from the sertraline group. The greatest reduction in IT was shown in n-hexene fraction, i. e. 94.3% (FST) and 94.56% (TST), while fresh juice had an IT reduction of 56.63% (FST) and 57.16% (TST) and ethyl acetate fraction of 85.48% (FST) and 84.92% (TST).

Histopathology of hippocampus

The hippocampal histopathology was used to assess the damage of the hippocampal brain organ. In addition, the hippocampus was also used for determining the expression of the BDNF protein [32].

Studies *in vivo* showed that BDNF is involved in the regulation of hippocampal neurogenesis. Binding of BDNF to the Tropomyosin receptor kinase B (TrkB) receptor can stimulate neuronal differentiation and dendritic morphogenesis in the sub-granular region of hippocampus, while decreasing BDNF does not cause a significant reduction in the number of neurons. This then can cause the inhibition of dendritic arborization and worsen the synaptic plasticity. The reduction in BDNF concentration induced in hippocampal neurons is associated with decreased gene expression [33].

The position at which BDNF binds to TrkB receptors determines the exact regulation of its release and function. Following its mature cleavage, BDNF can be transported to postsynaptic dendritic locations and presynaptic boutons, where it is stored in dense core vesicles. SNARE proteins including SNAP25, SNAP47, and synaptobrevin-2 (Syb2) aid in the release of BDNF upon arrival at the synapse. After being released into the extracellular space, BDNF

interacts with transsynaptic and cis-TrkB receptors locally. Different receptor locations have distinct effects on processes like synaptogenesis and synaptic plasticity. BDNF can trigger downstream TrkB signaling pathways and be endocytosed into an endosome containing BDNF-TrkB to be trafficked for additional intracellular signaling or receptor recycling once it connects to its high-affinity TrkB receptor [34]. Table 5 shows the hippocampal histopathology results. The results showed that the negative control group had the highest cell damage, which was 60±3, while the group with the least cell damage was the positive control group. Cell damage in the negative control group had a significance of $p<0.01$ with the positive control group and the n-hexane fraction group, while the significance results in the fresh juice group compared to the ethyl acetate fraction were $p<0.05$. The number of normal cells in the negative control group had a significance of $p<0.01$ with the positive control group and the n-hexane fraction group, while the significance results in the fresh juice group compared to the ethyl acetate fraction were $p<0.05$.

The number of cell damages (cell necrosis) given the positive control was found fewer than the negative control. It occurred as sertraline can increase the synthesis of suppressive serotonin in serotonergic neurons in the hippocampus, thereby repairing cell damage [30]. Cell damage is characterized by changes in the cell nucleus in the form of pyknosis (the cell nucleus shrinks), karyorrhexis (the cell nucleus that is destroyed into segments), and karyolysis (the lysed cell nucleus). Cell damage can cause denaturation of cytoplasmic proteins giving necrotic cells a blurry and intense cytoplasmic eosinophilia. Necrosis is a typical initial response to hypoxia, ischemia, or toxic injury. The mechanism of cell damage is when the cell experiences an initial injury or cellular acidosis and then experiences the denaturation of structural proteins and lysosomal enzymes in the cell, which will cause proteolytic disintegration of the entire cell, but protein denaturation causes the proteolytic disintegration of the cell delayed so that nucleic acid degradation is not hindered [31].

Table 5: Cell damage histopathology of hippocampus (n=3)

Groups	Number of cell damages	Number of normal cells
	x̄±SD	
Negative Control	60±3**	40±3**
Positive Control	27±4**	73±4**
Fresh Juice	50±3*	50±3*
Ethyl Acetate Fraction	45±4*	55±4*
N-Hexene Fraction	34±7**	66±7**

(* $p<0.05$, **$p<0,01$)

Cell damage can be quantified by assessing nuclear changes such as pyknosis (nuclear shrinkage) and karyorrhexis (nuclear fragmentation). Severity of damage divided into 3 categories, namely, Mild Damage:<10% of cells show pyknosis/karyorrhexis. Moderate Damage: 10–30% of cells exhibit these features; Severe Damage:>30% of cells are affected [35]. The number of cell damages, when given the negative control, showed that the results were significantly different with positive control and n-hexene fraction ($p<0.05$), while the number of cell damages when given the positive control showed a significance value of $p>0.05$ compared to n-hexene fraction group ($p= 0.098$) (table 5). This showed that the n-hexene fraction had an equal effect as the positive control. The fresh juice and ethyl acetate fraction groups had a significance of $p<0.05$; it indicated the possibility of fresh juice and the ethyl acetate fraction having a low antidepressant effect.

Correlation of brain cell damage and brain weight

The data on average brain weight after treatment was statistically analyzed. The normality results showed that the data were normally distributed $p>0.05$. The result was $p = 0.000$. The results of the significance of brain weight in the fresh juice group had a p-value of>0.05 compared to the negative control, while the brain weight of the n-hexene fraction group had a significance value of>0.05 compared to the positive control (table 6). Fresh juice, ethyl acetate fraction and n-hexene fraction had a significance value of<0.05, which indicated that the three samples were significantly different.

Table 6: Brain weight after treatment (n=3)

Groups	Brain weight x̄±SD (g)
Negative control	0.39±0.06
Positive control	0.65±0.05*
Fresh juice	0.39±0.03
Ethyl acetate fraction	0.49±0.09
n-Hexene fraction	0.63±0.04*

Remark: (*): No significant difference in the Post Hoc test

In this study, a Chi-Square test was carried out purposely to determine the correlation between cell damage and brain weight. Based on statistical tests, the results showed that the p-value was<0.05, which showed that damage to brain cells can affect brain weight. The higher the number of cell damages, the lower the brain weight. This was shown in the risk estimate value. These results showed that damage to brain cells had 20 times more tendency to reduce brain weight. This is also confirmed by research of Marhounova [36] stating that brain damage can affect brain weight for a change in the number of neurons.

Fig. 4A shows the normal cells and damaged cells in the normal control group, when compared with fig. 4B, fig. 4C, fig. 4D, and fig. 4E, a significant difference can be seen in which in the group given distilled water, there were more cell damages than in the group of sertraline, fresh juice, ethyl acetate fraction and n-hexene fraction. Based on the histopathological description of the hippocampus of house mice, it showed differences in cell damage between house mice given negative control and sertraline mice and each sample.

Concentration of brain-derived neurotrophic factor (BDNF)

Neurotrophin-3, neurotrophin-4/5, and nerve growth factor are also members of the same family of growth factors as BDNF. A precursor molecule known as pro-BDNF is created in the cell, transformed into mature BDNF form by post-translational cleavage, and then released into the extracellular environment. Both mature and pro-BDNF are physiologically active molecules that function by binding to particular receptors found in the membrane and nucleus of cells [46]. BDNF attaches itself to the receptors for p75 neurotrophins (p75NTR) and tyrosine kinase B (TrkB). Compared to pro-BDNF, which has a higher affinity for p75NTR, BDNF has a significantly higher affinity for the TrkB receptor. The activation of p75NTR results in apoptotic and neurodegenerative processes, whereas the activation of the TrkB receptor has a protective and anti-apoptotic impact. The majority of the neurons in the hippocampus express the TrkB receptor [37].

BDNF expression could be identified in the samples of positive control, ethyl acetate fraction, and n-hexene fraction. Based on statistical results, both ethyl acetate fraction and n-hexene fraction had a significance of $p>0.05$ compared to positive control (table 7). As shown in fig. 4, the n-hexene fraction became the sample that had the highest BDNF expression, i. e. 505.40 pg/ml compared to other groups. The n-hexene fraction is a non-polar compound which will be folded by proteins containing most of the non-polar residues (~80%) in the interior of the protein surface.

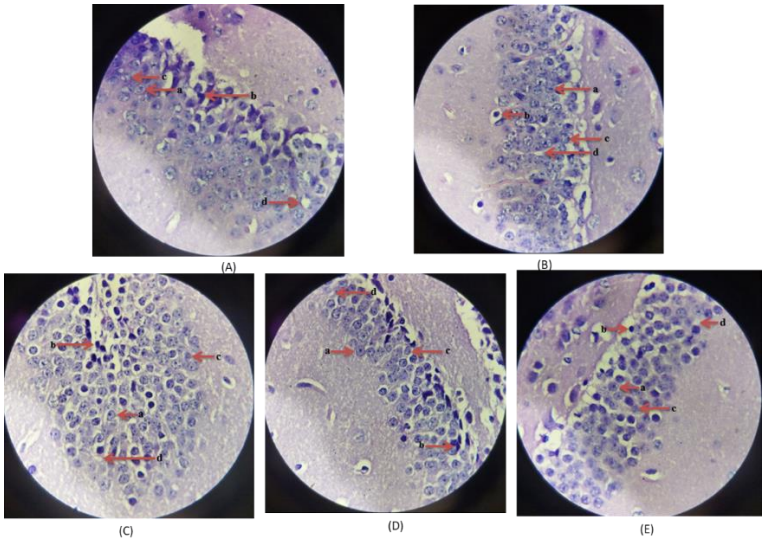

Fig. 4: Hippocampus histopathology of normal cells, pyknosis cells, karyorrhexis cells, and karyolysis cells. (Magnification 1000x); (A) Negative control, (B) Positive control, (C) Fresh juice, (D) Ethyl acetate fraction, (E) n-Hexene fraction; a: Normal Cells; b: Pyknosis; c: Karyorrhexis; d: Karyolitic

Table 7: Concentration and expression of brain-derived neurotrophic factor (BDNF) of groups

Group	x̄±SD level (pg/ml)	Remark
Negative control	0	Unexpressed
Positive control	196.75±304.84	Expressed
Fresh Juice	0	Unexpressed
Ethyl acetate fraction	46.51±21.26	Expressed
n-Hexene fraction	505.40±306.74	Expressed

Note: n= 5

This causes a protein to maintain its integrity [38]. Hydrophobic interactions of protein structures play a role in regulating protein folding and stability. The mechanism for increasing BDNF expression can occur through the TrkB receptor. The n-hexene fraction stimulates 5-HT1A and 5-HT2A, which can increase 5-HT levels in several areas of the hippocampus. The high levels of 5-HT promote the expression of CREB (cAMP-response element binding), thereby stimulating an increase in the mRNA and protein of the TrkB receptor. This causes BDNF mRNA and protein expression levels to increase [39].

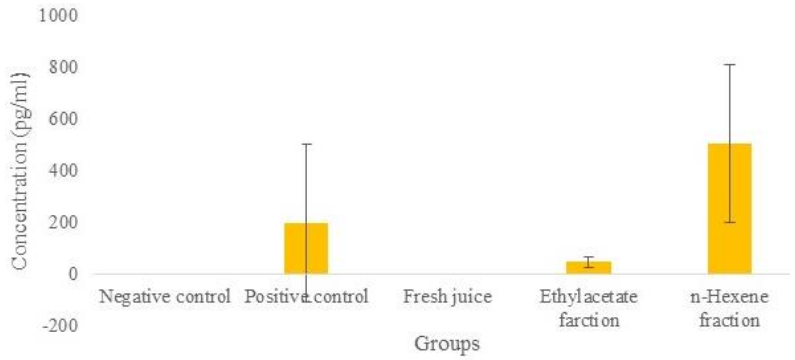

Fig. 5: Bar chart BDNF (brain-derived neurotrophic factor) concentration of groups, N=5

The n-hexene fraction is a class of non-polar compounds that have high levels of BDNF protein expression since these compounds are able to break the blood-brain barrier (BBB). This can be known by the mechanism of substrates transfer from the systemic circulation across the endothelial cells and into the brain parenchyma. Carrier substances work centrally on their substrates [33].

DISCUSSION

In humans, depression can occur due to a decrease in the production of neurotransmitters, i. e. 5-HT and NE in the brain. Serotonin plays an important role in eliminating fear when the house mice swim and regulates locomotor movements to move from one place to another. NE, meanwhile, is a molecule that plays a role in the stress response when the house mice swim to stay focused and control emotions [40].

The antidepressant effect of various plant alkaloids has been reported in the literature [41]. Berberine administration significantly decreased immobility and increased climbing behavior in the FST [42]. Nonaine, liriodenine, and nornuciferine showed that repeated treatment with this plant produced an antidepressant-like

action in mice [43]. Protpine reduced the immobility time in the TST and thus could be effective for the moderate state of depression [44]. In addition to all those, pramipexole is a non-ergoline alkaloid showed significant clinical efficacy in a double-blind, placebo-controlled study in bipolar and unipolar depressive patients [45].

The work mechanism of antidepressants in unripe Ambon banana peels comes from alkaloid compounds that can inhibit MAO (monoamine oxidase) and supply tryptophan so that serotonin levels in the synaptic cleft are high and make postsynaptic receptors saturated. Meanwhile, flavonoid compounds with the work mechanism of flavonoid compounds can increase 5-HT, NE and BDNF [1]. BDNF was detected in serum instead of hippocampus tissue in this investigation. A number of factors that interact with the central nervous system may have an impact on the amount of circulating BDNF in serum, which may represent the amounts of BDNF released by different body cells, including the brain.

Research by Omar [46] revealed that the alkaloid compound in the form of 1,3,7,9-tetramethyluric acid showed antidepressant activity by increasing BDNF regulation in the hippocampus and modulating the function of the HPA axis and could improve disorders and disorders of monoamine neurotransmitter metabolism in the brain [46].

Various natural chemical agents have been shown to alleviate depression, one of which is flavonoids. These compounds can be used as alternative and complementary therapies for depression. A variety of mechanisms are proposed to explain the anti-depressant potential of flavonoids, including modulation of the neurotransmitter system and regulation of dopaminergic, noradrenergic, and serotonergic pathways in the CNS or neurotrophic factors [43].

Research by Ali [47] explained that flavonoid compounds in the type of flavonols such as flavan-3-ols, flavonols, flavones, anthocyanins, and proanthocyanidins can function as antidepressants with a working mechanism of increasing BDNF levels in the brain's hippocampus and increasing the levels of monoaminergic neurotransmitters such as serotonin, noradrenaline (NA), and dopamine (DA). Neurotransmitters are synthesized in presynaptic nerve endings, stored in vesicles and released in the synaptic wall (the space between presynaptic and postsynaptic nerve endings) in response to certain stimuli. This causes the transmission of nerve impulses from one neuron to another [48].

The parameter in this study was immobility time (IT) as the time calculated when the house mice were not moving. Changes in IT can indicate depressive behavior in house mice 28. The greater the IT, the higher the level of depression in house mice [49]. IT changes in the antidepressant activity test can be seen using the FST and TST methods. Research by Varghese [50] showed that IT success is marked by a reduction in immobility of approximately 80%.

FST is a method of measuring the effects of antidepressant drugs in experimental animals [51]. The efficacy of antidepressant drugs is measured through shorter immobility times compared to groups of animals that are not given antidepressant drugs or extracts that have antidepressant properties [28]. This parameter was selected as it was closely related to the behavior of tested animals that led to depression. FST could affect serotonin in the brain because the house mice were required to do the forced swimming. This behavior resulted in dysregulation of physiological arousal and emotional feelings [52–54].

TST is a test used to evaluate antidepressant activity with an aim to obtain optimal conditions for tested animals in a state of depression. This method is more sensitive for detecting Selective Serotonin Reuptake Inhibitor (SSRI) antidepressant activity and it has high validity for screening and analyzing antidepressant activity [55–58]. This test is based on the fact that animals experience an imbalance in the neurotransmitters 5-HT and NE due to tail hanging.

The antidepressant effect via its antioxidant mechanism is the basis for the dose of 10 mg/Kg BW. Through antioxidant activity, banana peel with an IC_{50} value of 251.47 µg/ml has been shown in previous studies to have antidepressant potential [23]. A dose of 10 mg/Kg BW was derived by converting the IC_{50} value to a dose for mice. The production of oxidative stress is linked to major depression. Increased exposure to or generation of Reactive Oxygen Species (ROS) combined with weakened antioxidant defenses can result in oxidative stress, which can harm proteins, DNA, and lipids [59].

This imbalance then causes the house mice to lose interest in activities 36 and it shows that the house mice are depressed. Phosphorylated CREB levels were shown to be low in the hippocampus and prefrontal cortex of people with significant depression, according to postmortem tests; however, phosphorylated CREB levels were restored by long-term antidepressant treatment [60]. Increased CREB expression in the hippocampal dentate gyrus produced antidepressant-like effects in the FST and TST methods in an animal model of acquired helplessness [61]. BDNF, a potent trophic factor that controls synaptic plasticity and maintains the structure of neurons, is produced in greater amounts when CREB is activated [62]. Patients with major depressive illness may see an increase in brain BDNF levels or a return to normal after using antidepressants [63]. Neuroplasticity and antidepressant-like effects are mediated by cAMP/CREB/BDNF signaling in neonatal mice neurons [64]. The limitation of this study is the assessment of the stress induction behavior of UCMS. This induction is able to produce preference disorders because it is qualitative, namely by providing stress with various environmental conditions. Another limitation is the size of the serum which is too small, making it difficult to obtain results in the analysis.

CONCLUSION

The administration of antidepressant fresh juice, ethyl acetate fraction, and n-hexene fraction have antidepressant activity in mice induced by mild chronic stress, based on FST and TST methods. In addition, BDNF protein expression in the hippocampus of white male house mice was identified in the groups that received the ethyl acetate fraction and n-hexene fraction of unripe Ambon banana peel (*Musa paradisiaca* L.). The study presents promising findings on banana peel fractions as antidepressants, particularly the n-hexene fraction. Preclinical testing will be required in the future to identify the mechanism of action of boosting TrKB receptors in the hippocampus by isolating alkaloid and flavonoid components from banana peels.

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AUTHORS CONTRIBUTIONS

AS: responsible for conceptualization, methodology, formal analysis, and writing the original draft. ASW: conceptualization, supervision, and review and editing. RP: played a role in collecting, writing review and editing.

CONFLICT OF INTERESTS

Declared none

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