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**Viani Anggi**  
College of Pharmaceutical  
Sciences Pelita Mas Palu,  
Central Sulawesi 94111,  
Indonesia

**Muthmainah Tuldjanah**  
College of Pharmaceutical  
Sciences Pelita Mas Palu,  
Central Sulawesi 94111,  
Indonesia

**Dina Novita**  
College of Pharmaceutical  
Sciences Pelita Mas Palu,  
Central Sulawesi 94111,  
Indonesia

**Erick Budiawan**  
College of Pharmaceutical  
Sciences Pelita Mas Palu,  
Central Sulawesi 94111,  
Indonesia

**Niluh Puspita Dewi**  
College of Pharmaceutical  
Sciences Pelita Mas Palu,  
Central Sulawesi 94111,  
Indonesia

**Correspondence Author;**  
**Priyanka Chouhan**  
College of Pharmaceutical  
Sciences Pelita Mas Palu,  
Central Sulawesi 94111,  
Indonesia

## The aphrodisiac activity of water fractions of clove plant leaves (*Syzygium aromaticum* L.) On wistar rats (*Rattus norvegicus*)

**Viani Anggi, Muthmainah Tuldjanah, Dina Novita, Erick Budiawan and Niluh Puspita Dewi**

### Abstract

**Introduction:** The clove plant belongs to the Myrtaceae family which has many pharmacological activities and has been used traditionally for various therapies, one of which is as an aphrodisiac.

**Purpose:** This study aims to determine the aphrodisiac activity of the aqueous fraction of clove leaves to prove its traditional use.

**Method:** The active compound of clove from the Myrtaceae family was carried out using liquid liquid fractionation to extract water-soluble compounds of flavonoids, alkaloi, tannins, saponins and steroids in these plants. Aphrodisiac activity was determined by calculating the parameters Mount Latency (ML), Intromission Latency (IL), Ejaculation Latency (EL), Mount Frequency (MF), Intromission Frequency (IF), Ejaculatin Frequency (EF) of the rats.

**Results:** Based on the tests conducted, the water fraction of clove leaves in male white rats at a dose of 250 mg/kgBB has aphrodisiac activity in parameters with a mean value of Mount Latency (ML) (25.60 Seconds), Intromission Latency (IL) (30.80 seconds), Ejaculation Latency (EL) 5.32 Minutes, Mount Frequency (MF) (13.40 Seconds) Intromission Frequency (IF) (11.80), Ejaculation Frequency (EF) (1.00) when compared to control normal but not more than positive control. Conclusion: the leaves of the clove plant not have aphrodisiac activity.

**Keywords:** Aphrodisiac, clove leaves, ejaculation

### Introduction

Sexual disorder is a condition where there are difficulties when having sex, sexual disorder is a life-threatening emergency (Indrisari, 2018) [2]. This disorder affects 10% to 52% of men and 25% to 63% of women, male sexual dysfunction such as impotence and erectile dysfunction (ED) has become a very important health problem because it affects quality of life. This sexual dysfunction affects about 20 - 30 million men or occurs in about 1 in 10 men, which increases with age and in people with diseases such as diabetes, heart disease, stress and insomnia (Kusumawati *et al.* 2021) [1].

Sexual dysfunction occurs due to a decrease in total Leydig interstitial cells, around 40%, decreased pulsatile lutenizing hormone secretion, free testosterone levels will decrease by around 1.2% every year (Andini). Sexual dysfunction can be influenced by two aspects, namely aspects of the soul or mental and body. Mental or mental factors such as worrying thoughts, mental pressure, excessive thinking, fear of sexual failure, while external factors from the body are lack of exercise, smoking habits, hereditary and disease consequences. This problem tends to be serious and will have an impact on the lives of sufferers and their partners.

One way to treat sexual disorders is by using plants that are efficacious as aphrodisiacs, which function as stimulants to increase sexual arousal. Aphrodisiac functions as a sexual stimulant and can increase low libido. Decreased libido shows a disinterest in sexual activity caused by erectile dysfunction and impotence. decreased libido can be overcome with drugs that can increase sexual desire (Numlil Khaira Rusdi *et al.*, 2018) [4].

One of the plants that people can use to treat aphrodisiacs is the clove plant (*Syzygium aromaticum* L.). Literature on clove gardens is still lacking because research on cloves is still lacking in interest. Aphrodisiacs are substances that can increase sexual desire, clove plants (*Syzygium aromaticum* L.) contain alkaloids, tannins, saponins, flavonoids and steroids. Flavonoids and alkaloids are active compounds with aphrodisiacs. (Rizka, 2022) [10].

Previous research conducted by Arifah Sri Wahyu on testing the aphrodisiac activity of clove

bud essential oil can increase sexual desire in male white rats (Sriwahyuni, 2010) [11]. The results of research conducted by Widayarni 2012 stated that clove flower bud extract can increase libido in sexual activity. Based on the literature regarding the leaves of the clove plant (*Syzygium aromaticum* L) there is still very little. Therefore, researchers are interested in further researching the secondary metabolite compounds contained in the leaves of the clove plant (*Syzygium aromaticum* L.) at a dose of 250 mg/kg BW to determine the aphrodisiac effect of the extract by looking at several parameters, namely the mount latency (ML), intromission latency (IL), ejaculation latency (EL), mount frequency (MF), intromission frequency (IF), and ejaculation frequency (EF).

## Research Methods

### Tools and materials

The equipment and materials used in the experiment included a rotary vacuum evaporator (Rotary Evaporator REV-3000A, Infitek Co. Ltd., Shandong, China), refrigerated microcentrifuge (CFGR-B16.5B/CFGR-17B, Infitek Co. Ltd., Shandong, China), a 3 ml oral sonde, a 3 ml injection syringe, spot plates, test tubes, gram scales, analytical scales (KERN ADB 200-4, Lab Friend Pty. Ltd., Australia), distilled water, anhydrous acetic acid (Sigma Aldrich, St. Louis, MO, USA), aluminum foil, Dragendrof R (SinarLab, Jakarta, Indonesia), ethyl acetate (Sigma Aldrich, St. Louis, MO, USA), 96% ethanol (Sigma Aldrich, St. Louis, MO, USA), FeCl<sub>3</sub> (Sigma Aldrich, St. Louis, MO, USA), gelatin (SinarLab, Jakarta, Indonesia), HCL (PT Lamurindo, Jakarta, Indonesia), gloves, mask, 10% NaOH (Merck, Kenilworth, NJ, USA), 10% NaCl (Sigma Aldrich, St. Louis, MO, USA), 0.5% Na-CMC (Sigma Aldrich, St. Louis, MO, USA), plastic wrap, *Syzygium aromaticum* L powder, styrofoam, copper acetate, and X-Gra.

### Plant Identification

Identification of *Syzygium aromaticum* L was carried out at Laboratorium Herbarium Tadulako University (078/UN.28.UPT-SDHS/LK/2022). The results of plant identification showed that the test material used was *Syzygium aromaticum* L

### Ethics Assessment

Ethical studies are given before testing animals are given treatment which aims to ensure that research procedures and methods are in accordance with applicable animal ethics. The ethical study of test animals was carried out by the ethical review commission of the Faculty of Medicine, Tadulako University. The results show that the research methods and procedures have passed the Ethics License No. 1692/UN 28.1.30/KL/2023.

### Research Test Animals

The test animals used were male white rats (*Rattus norvegicus*) of the Wistar strain with inclusion criteria being approximately 3-4 months old, body weight 180 - 250 grams, male and female, white fur color, healthy body condition (active and not disabled). The research population included male white rats (*Rattus norvegicus*) obtained from the animal testing provider of the Faculty of Mathematics and Natural Sciences, Tadulako University.

**Treatment of test animals:** 15 male white mice and 30 female white mice were adapted separately for 14 days in the laboratory by being caged at normal environmental temperature and given standard food and drink and weighing the mice. Male White Rats were divided into 3 groups, group A (normal control), B (Positive control), and group C (water fraction).

### Preparation Simplicia powder manufacture

The simplicia powder was extracted by maceration method using 96% ethanol as a dispersion agent (3x24 hours). Simplicia powder was weighed as much as 400 grams. The extract obtained was then thickened using a rotary evaporator. The viscous extract obtained was then tested qualitatively (phytochemical screening) to see which secondary metabolites were found in plants.

### Preparation of water fraction

Fractionation of clove leaf extract was made using the liquid-liquid partition technique. As much as 10 grams of clove leaf ethanol extract was dissolved using 100 ml of distilled water. After that it was placed in a separating flask then added 100 ml of N-Hexane, shaken slowly for about 15 minutes, then allowed to stand until a separation was formed between the N-Hexane extract and the residue. The N-Hexane extract was separated by a layer of residue after which it was partitioned again until the solution produced a clear color, after which 100 ml of ethyl acetate was added to the residue, then the same treatment was carried out as the N-Hexane fraction. Then the residue of ethyl acetate is evaporated to obtain a thick extract for the water fraction (R. M. Kusumawati, 2022) [5].

### Phytochemical Separation Test

Preliminary phytochemical testing is used to detect the presence of secondary metabolites according to group and as initial data it is necessary to know the useful activity of a plant in simplicia or extracts. Tests were carried out on alkaloids, saponins, flavonoids and steroids and were carried out quantitatively with differences in color or precipitate. Clove leaf thick extract (*Syzygium aromaticum* L.) obtained was then identified qualitatively in the form of a phytochemical screening test. Examination was carried out to determine the presence or absence of secondary metabolite compounds contained in the water fraction of clove leaves (*Syzygium aromaticum* L.) (Wael *et al.*, 2023) [6].

### Manufacture of Sodium Carboxymethyl Cellulose suspension

0.5 gram of sodium carboxymethyl cellulose was weighed and then placed in a mortar then added 10 ml of heated distilled water, then stirred until a transparent and completely mixed result was obtained. After that the Na CMC solution was put in a 100 ml volumetric flask. The volume was made up using distilled water up to 100 ml (Jumain *et al.*, 2019) [7].

### Preparation Suspension

The water fraction of clove leaves (*Syzygium aromaticum* L.) for a dose of 250 mg/kg BW was weighed 0.5 gram, then dissolved using 0.5% Na CMC solution and added to 25 ml.

**Aphrodisiac Test Parameters:** Some of the parameters used in aphrodisiacs is Mount Latency (ML) is the time from introduction when the female rat goes to the first mount, Intromission Latency (IL) is the time from the introduction of female rats until the first intromission (vaginal penetration) occurs. Ejaculation latency (EL) is the time it takes from the initial intromission to the release of sperm. Mount Frequency (MF) is the total mount before ejaculation occurs. Intromission Frequency (IF) is the total intromission that occurs before ejaculation. Ejaculation Frequency (EF) Total ejaculation during (30 minutes).

The SPSS statistical data examination application involving homogeneity, normality, (One-Way ANOVA) and non-parametric (Kruskal Wallis) tests. When the data is normally distributed and homogeneous, one way ANOVA statistical analysis is used, then it is continued with the Duncan or Tukey test to find out the differences between treatments. When the data obtained was abnormal or non-homogeneous, it was then analyzed using the non-parametric statistical Kruskal Wallis test and continued using the Mann Whitney test. Parametric differences were carried out to see differences in the treatment groups.

## Data Analysis

## Results and Discussion

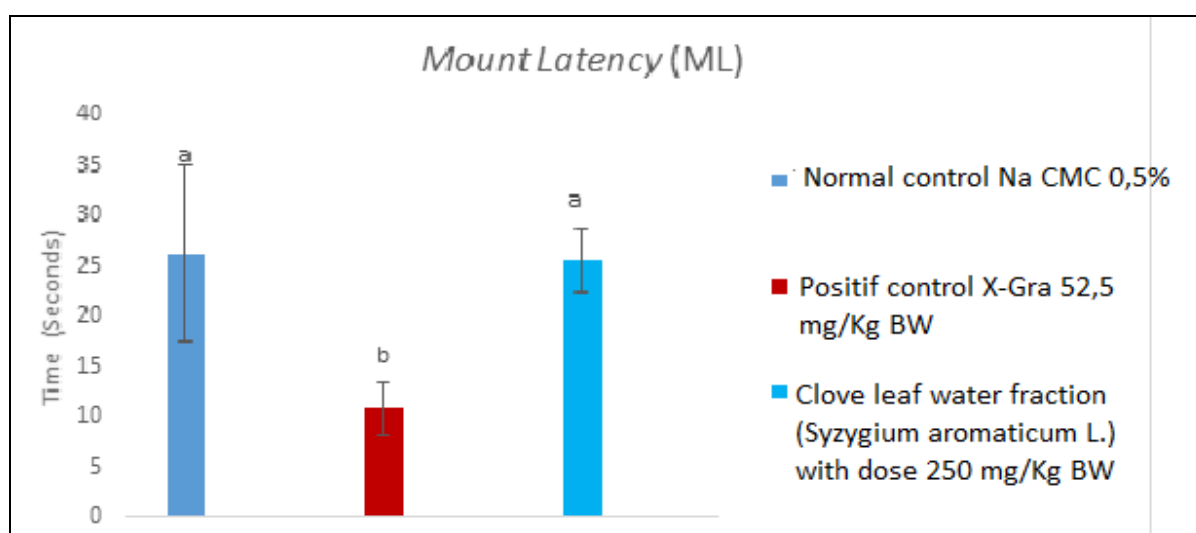
**Table 1:** Phytochemical Test Results of Clove Plant Leaves

Testing	Reactor	Observation	Results
Alkaloid test	5ml Hydrochloric acid 2n + 3 drops dragendr of (brown-black precipitate)	A brick red orange yellow precipitate was formed	-
Flavonoid test	10ml aquadest+1ml ethanol+ Magnesium +10 ml hydrochloric acid P (yellow or orange color forms)	The formation of orange yellow color	+
Saponin test	10 ml hot water+ shake+ hydrochloric acid 2N (forms 1-10cm foam)	Formed foam	+
Tanin test	20ml hot water + 3 drops of 10% NaCl + FeCl <sub>3</sub> (Formed black blue or greenish black)	Formed a dark blue color	+

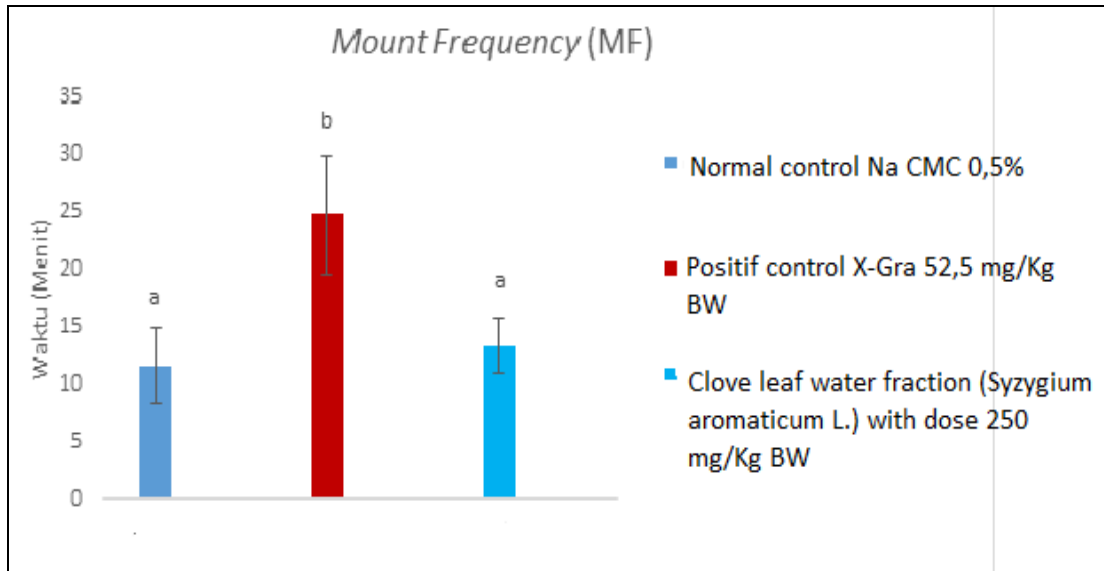
**Table 2:** Results of aphrodisiac effect testing on male white rats.

Parameter	Control Group		Treated group	p-value
	Healthy	X-gra		
<sup>(y)</sup> ML	35.00 ± 1.304 <sup>b</sup>	10.80 ± 2.683 <sup>a</sup>	25.60 ± 3.209 <sup>b</sup>	0.000
<sup>(z)</sup> IL	46.60 ± 2.881 <sup>a</sup>	14.80 ± 2.683 <sup>b</sup>	30.80 ± 3.114 <sup>c</sup>	0.000
<sup>(y)</sup> EL	4.55 ± 0.561 <sup>b</sup>	5.924 ± 0.653 <sup>a</sup>	5.326 ± 0.233 <sup>a</sup>	0.010
<sup>(z)</sup> MF	11.60 ± 3.362 <sup>a</sup>	24.80 ± 5.167 <sup>b</sup>	13.40 ± 2.408 <sup>a</sup>	0.000
<sup>(z)</sup> IF	9.20 ± 2.280 <sup>a</sup>	22.20 ± 5.020 <sup>b</sup>	11.80 ± 1.924 <sup>a</sup>	0.000
<sup>(y)</sup> EF	1.00 ± 0.00 <sup>a</sup>	2.00 ± 0.00 <sup>b</sup>	1.00 ± 0.00 <sup>a</sup>	0.003

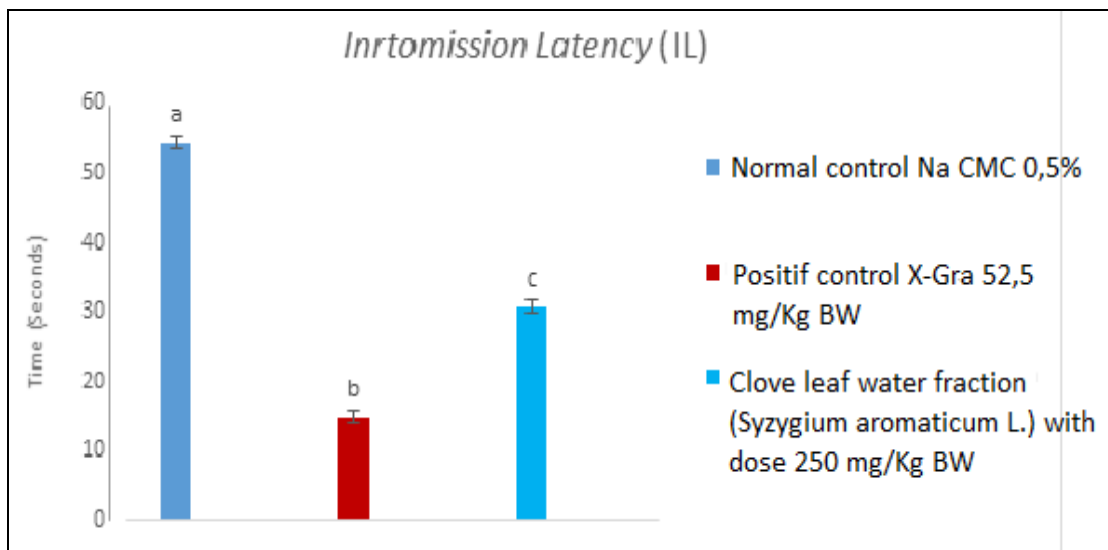
Note: (y) = Kruskal-Wallis test. (z) = One-way ANOVA test. ± = Standard deviation. P < 0.05 = Different. P > 0.05 = Not different. Different subscript letters in one column indicate significant differences between groups (p < 0.05). ML= Mount latency. IL= Intromission latency. EL= Ejaculation latency. MF= Mount frequency. IF= Intromission frequency. EF= Ejaculation frequency.



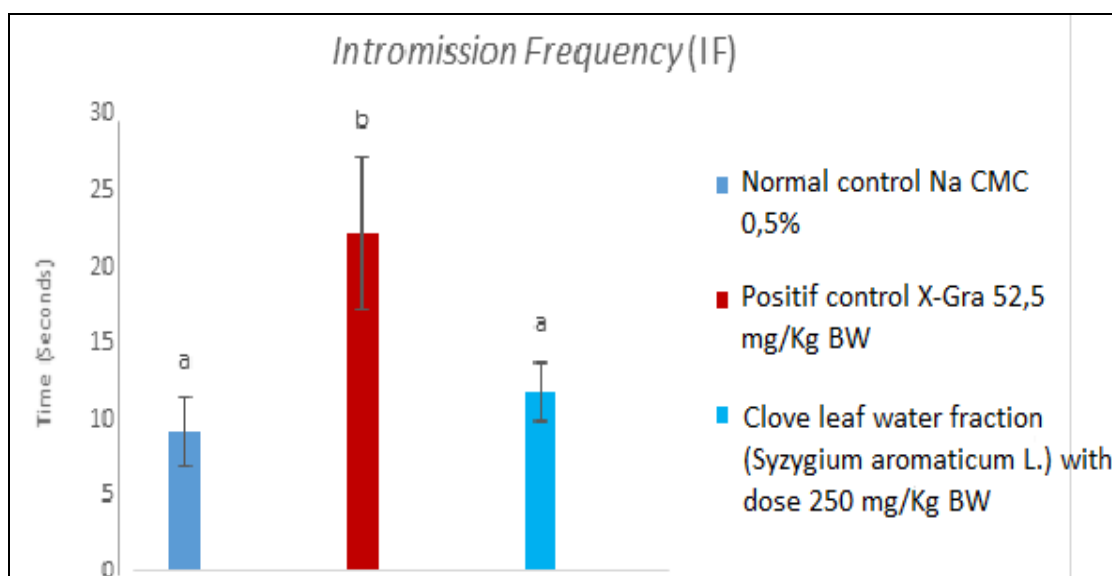
**Graph 1:** Parameters Mount Latency (ML) of water fraction of clove leaf



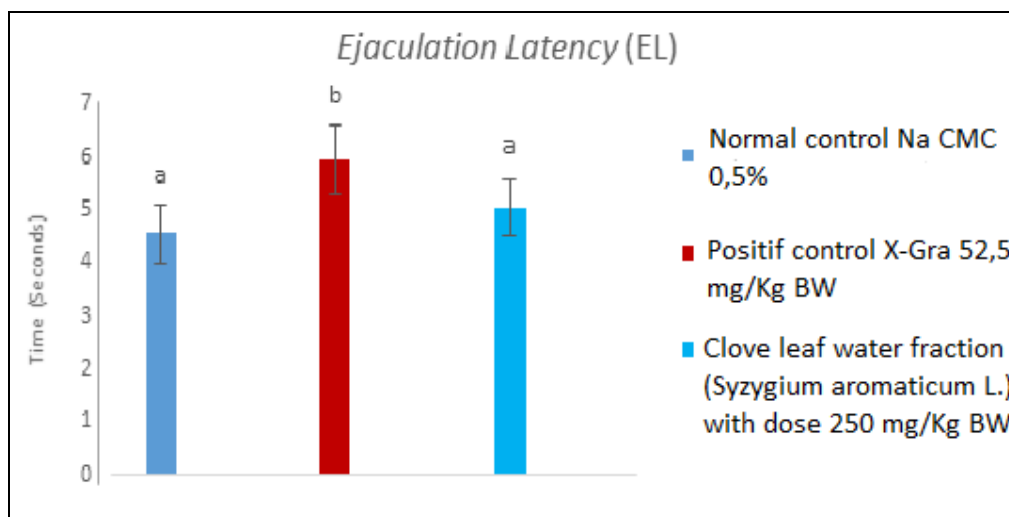
**Graph 2:** Parameters Mount frequency (MF) of water fraction of clove leave



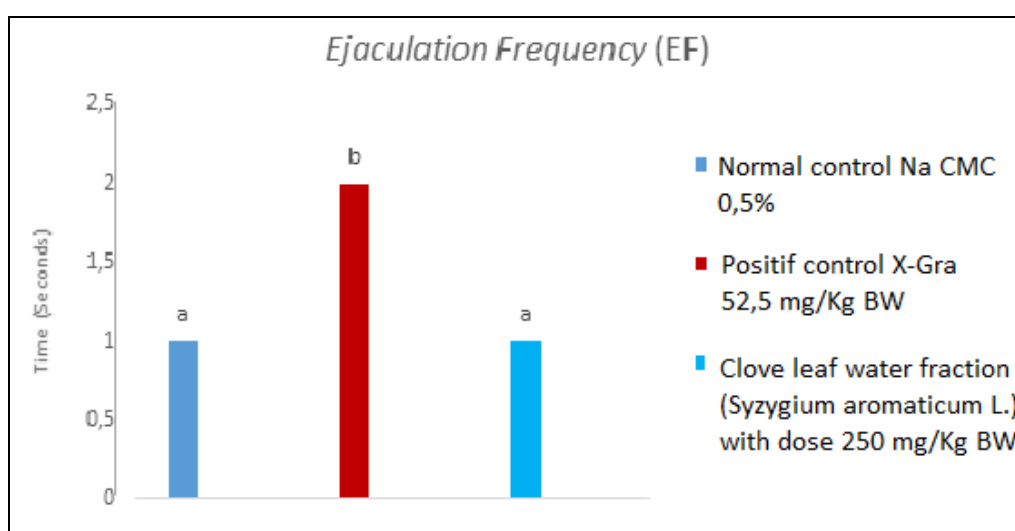
**Graph 3:** Parameters Intromission Latency (IL) of water fraction of clove leave



**Graph 4:** Parameters Intromission frequency (IF) of water fraction of clove leave



Graph 5: Parameters Ejaculation latency (EL) of water fraction of clove leaf



Graph 6: Parameters Ejaculation Frequency (EF) of water fraction of clove leaf

## Discussion

This study aims to evaluate aphrodisiac activity in giving the water fraction of clove plant leaves at a dose of 250 milligrams / kg body weight. The plants used are detimitated to determine the plants used are indeed clove leaves (*Syzygium aromaticum* L.). Clove leaves (*Syzygium aromaticum* L.) are extracted by maceration using a 96% ethanol filter liquid. The concentrated extract produced using a vacuum rotary evaporator was obtained 90.2% with a 24.5% soak and fractionation was obtained fraction 9.23 with a result of 2.30% immersion. The maceration procedure was selected because it avoided the formation of destruction of compounds contained in the water fraction of clove leaves. After that, phytochemical screening tests were tried to recognize the content of compounds in the water fraction of clove leaves. The results of phytochemical screening show that the water fraction of clove leaves contains alkaloids, tannins, flavonoids, saponins and does not contain steroids. The results of phytochemical tests can be seen in table 1. This is in line with research (Nurhadianty *et al*, 2017) [8].

This research uses male white rats (*Rattus norvegicus*) as test animals which is a category of mice that are universally used for research. Rats are used as test animals because they

have genetic similarities, biological criteria that are similar to humans. The mice purchased are male white rats, this is because male white rats can share the results of more constant studies because they are not affected by the presence of menstrual cycles and pregnancy such as in females. This research tried to observe the intimate activities of male white rats. The parameters observed in this research are Mount Frequency (MF), Mount Latency (ML), Intromission frequency (IF), Intromission Latency (IL), Ejaculation Frequency (EF), Ejaculation Latency (EL), Mount Frequency (MF) is the real number of mountings of male rats against benthine rats within 30 minutes after being placed in the test chamber (Adelati *et al.*, 2016) [9]. The normality and homogeneity test results showed that the Mount Latency (MF), Intromission Frequency (IF), Mount Frequency (ML), Interomimission Latency (IL) and Ejaculation Latency (EL) data were normally distributed and homogeneous ( $p > 0.05$ ). Therefore, testing was carried out using the One Way Anova test. Meanwhile, the Ejaculation Frequency (EF) parameter is not normally distributed and homogeneous ( $p < 0.05$ ). Therefore, testing was carried out using the Kruskal wallis test.

In the ML (Mount Latency) parameter, which is the time from when male rats are paired with female rats until male



rats first mount female for 30 minutes. In this parameter can be seen in table 4.2 the lowest value was found in the positive group with an average value of 10.80, while in the treatment group got an average value of 25.60, and in the normal group got an average value of 26.2. The above results mean that the positive group has more effect than the water fraction and the normal group. Then the results obtained in the mount latency (ML) parameter comparison between all control groups gave significantly different results between the water fraction and the positive group, the normal group and the positive group, while the normal group and the water fraction did not differ significantly. This means that the application of the water fraction of clove leaves (*Syzygium aromaticum* L.) has no effect on the time of mounting in male rats.

The MF (Mount Frequency) parameter is the time it takes for male rats from being put into a chamber containing male rats from being put into a chamber containing female rats until the first ride for 30 minutes. In this parameter, it can be seen in table 4.2 the highest value in the positive group with an average value of 24.80, while in the water fraction get an average value of 13.40, and the normal group 11.60. The above results mean that the positive group has more effect than the water fraction and the normal group. Then the results obtained in the Mount Frequency (MF) parameter comparison between all control groups gave significantly different results between the water fraction and the positive group, the normal group and the positive group, while the normal group and the water fraction did not differ significantly. This means that the administration of the water fraction of clove leaves (*Syzygium aromaticum* L.) at a dose of 250 mg / kg BB has no effect on the time of mounting in male rats.

Mounting is a parameter that can describe an increase in MF value, and a decrease in ML value. This showed that the administration of the water fraction of clove leaves (*Syzygium aromaticum* L.) at a dose of 250 mg / kg BB did not have a significant effect in increasing sexual motivation of male white rats Wistar strains. The intromission frequency (IF) parameter is the number of intromissions performed by male rats before ejaculation for 30 minutes. In this parameter, the highest average frequency of intromission was the positive group with an average value of 22.20, while in the water fraction it got an average value of 11.80, and normal controls got a value of 9.20. The above results mean that giving the clove leaf water fraction at a dose of 250 mg / kg body weight has no effect on the intromission of male white rats. Then the results obtained in the Intromission Frequency (IF) parameter comparison between all control groups gave significantly different results between the water fraction and the positive group, the normal group and the positive group, while the normal group and the water fraction did not differ significantly. This means that the administration of the water fraction of clove leaves (*Syzygium aromaticum* L.) at a dose of 250 mg / kg BB has no effect on the time of mounting in male rats.

Intromission Latency (IL) is the time from when a male rat is paired with a female mouse until the male rat first intromissions for 30 minutes. In this parameter, the lowest value was found in the positive group with an average value of 14.80, while in the water fraction it got an average value of 30.80, and in the normal group it got a value of 54.4. The above results mean that giving the clove leaf water fraction at a dose of 250 mg / kg body weight has no effect on the

intromission of white antan rats. Then the results obtained in the Intromission Latency (IL) parameter comparison between all control groups gave significantly different results in each control group. This means that the administration of the water fraction of clove leaves (*Syzygium aromaticum* L.) at a dose of 250 mg / kg BB has no effect on the time of mounting in male rats. Intromission is a parameter that can describe an increase in sexual performance with a decrease in IL value, and an increase in IF value. In this study, the results showed no decrease in IL value and increase in IF value in the clove leaf water fraction group with a dose of 250 mg / kg BB. This showed that the water fraction group had no significant effect on the sexual performance of male white rats.

The Ejaculation Latency (EL) parameter is the time of first intromission from the number of intromissions to ejaculation. In this parameter, the highest value was found in the positive group with an average value of 4.55, while in the water fraction it had an average value of 5.925, and in the group it had an average value of 5.326. The above results mean that clove leaf water fraction at a dose of 250 mg / kg BB has no effect on Ejaculation of male white rats. Then the results obtained in the Ejaculation Latency (EL) parameter comparison between all control groups gave significantly different results in the normal group and positive group, normal group and water fraction, while in the normal group and water fraction did not differ significantly. This means that the administration of the water fraction of clove leaves (*Syzygium aromaticum* L.) at a dose of 250 mg / kg BB has little effect on the time of Ejaculation in male rats.

The Ejaculation Frequency (EF) parameter is the last parameter seen in this study by calculating the number of ejaculations that occur over a 30-minute period. In this parameter, the highest value was in the positive group with an average value of 2.00, while in the water fraction it got an average value of 1.00, and in the normal group it got an average value of 1.00. The above results mean that giving the water fraction of clove leaves at a dose of 250 mg / kg body weight has no effect on the Ejaculation of male white rats. Then the results obtained on the Ejaculation Frequency (EF) parameter comparison between all treatment groups gave significantly different results in the normal group and positive group, normal group and water fraction, while in the normal group and water fraction did not differ significantly. This means that the administration of the water fraction of clove leaves (*Syzygium aromaticum* L.) at a dose of 250 mg / kg BB has no effect on the time of ejaculation in male rats. In this study, what was considered was the hormone testosterone. This hormone serves to stimulate the development of reproductive organ activity. Testeron hormone together with FSH (Folicle Stimulating Hormone) and LH (Luteinizing Hormone) is responsible for spermatogenesis, sperm maturase and increasing fructose excretion by seminal vesicles as the main nutrients of spermatozoa. It is possible that the content of saponins, flavonoids, and steroids or all three can stimulate the excretion of gonadotropins FSH (Folicle Stimulating Hormone) and LH (Luteinizing Hormone) and testosterone. These three hormones increase the excretion of fructose by the seminal vesica as the main nutrient of spermatozoa.

Based on the results of research that has been found that aphrodisiac activity has no effect on the water fraction at a dose of 250 mg / kg BB. This study did not have an effect

because it was not detected that the presence of steroid compounds functions to affect sexual activity through the mechanism of action to replace cholesterol in synthesizing testosterone. Testosterone is synthesized from a cholesterol precursor known by the name of pregnolone. Pregnenolone will then be converted into progesterone which will act as a precursor in inducing the formation of androgens such as testosterone (Hafez, 2000). Natural products that are reported to act as PDE-5 inhibitors include terpenoids (Forskolin from *Coleus forskohlii*).

### Conclusion

1. The water fraction of clove leaves (*Syzygium aromaticum* L.) contains secondary metabolite compounds namely alkaloids, flavonoids, saponins, tannins and does not have steroid compounds.
2. The administration of clove leaf water fraction (*Syzygium aromaticum* L.) at a dose of 250 mg/kgBB did not have aphrodisiac activity with average indigo on the parameters of Mount Latency (ML) (25.60), Intromission Latency (IL) (30.80 Seconds), Ejaculation Latency (EL) (5.326 Minutes), Mount Frequency (MF) (13.40 Seconds) Intromission Frequency (IF) (11.80), Ejaculation Frequency (EF) (1.00).

### Suggestion

Further research is needed on the impact of giving the water fraction of clove leaves (*Syzygium aromaticum* L.) at varying doses and further testing on the toxicity of the water fraction of clove leaves (*Syzygium aromaticum* L.) In order to be developed into herbal supplements so that clinical trials can be carried out in patients with sexual disorders (Rizka 2022) <sup>[10]</sup>.

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