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# EVALUATION OF APHRODISIAC ACTIVITY OF ETHYL ACETATE FRACTION OF CLOVE LEAVES (*Syzygium aromaticum L.*) IN MALE WHITE RATS (*Rattus norvegicus*)

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## ABSTRACT

Clove leaves contain antioxidant compounds such as saponins, flavonoids, tannins, sterols, and essential oils. Among those chemical constituents, eugenol, eugenol acetate, caryophyllene, and sesquiterpenes were believed to have aphrodisiac activity. This study aims to investigate the potential aphrodisiac effects of the ethyl acetate fraction of clove leaves (*Syzygium aromaticum L*) administered at a dose of 250 mg/kg BW. The research employed an experimental method with a modified post-test randomized controlled group design and utilized a total of 45 white rats, consisting of 15 males and 30 females, divided into three treatment groups in a 1:2 ratio. Each group consisted of 5 male rats and 10 female rats. The three groups included the healthy control group (Na-CMC 0.5%), the positive control group (treated with X-Gra 52.5 mg/kg body weight) and the treatment group receiving clove leaf extract samples at a dosage of 250 mg/kg BW. The results indicate that the ethyl acetate fraction of clove leaves contains steroids and alkaloids with mount latency (ML), intromission latency (IL) ejaculation latency (EL), mount frequency (MF), intromission frequency (IF) and ejaculation frequency (EF) of 11.40 s, 15.80 s, 6.228 s, 23.60, 14.60, and 1.60 s, respectively. Based on these findings, it can be concluded that it has the potential to be used as an aphrodisiac agent.

**Keywords:** Aphrodisiac, *Syzygium Aromaticum* L., ethyl acetate fraction, clove leaves, ejaculation frequency

## INTRODUCTION

The natural physiological urge for sexual activity is a common human drive. It is worth noting that sexual disorders tend to have a greater impact on men compared to women, affecting approximately 10% of people across all age groups. However, the majority of cases, more than 50%, occur in men aged between 50 and 70. Among this group, 40% exhibit reduced levels of Leydig cells and Luteinizing Hormone (LH). These conditions, such as erectile dysfunction, impotence, and infertility, can collectively result in a decreased libido, characterized by a diminished interest in sexual activity. To address low libido, aphrodisiacs, which are substances or medications that boost sexual desire, can be employed (Rusdi et al., 2018).

Among the traditional medicinal plants known for their aphrodisiac properties are clove leaves. Clove leaves are rich in saponins, flavonoids, tannins, sterols, and essential oils, which act as natural antioxidants (Budiman et al., 2022). In addition, clove leaf oil contains phenolic compounds like eugenol and eugenol acetate, along with caryophyllene and sesquiterpenes, which are believed to have aphrodisiac properties that enhance arousal (Thakur et al., 2009).

The mechanism of aphrodisiacs involves a multifaceted interplay of physiological, psychological, and biochemical factors. Aphrodisiacs function by targeting various pathways that influence sexual desire and arousal (Ali et al., 2013). These substances may impact neurotransmitters, such as dopamine and serotonin, which are associated with mood and

pleasure, thereby enhancing the overall mood and receptivity to sexual stimuli. Additionally, aphrodisiacs can stimulate blood flow, leading to increased genital sensitivity and improved sexual performance (Calabrò et al., 2019). Some aphrodisiacs act as vasodilators, promoting the relaxation of blood vessels and increasing circulation to the genital area. Moreover, certain compounds in aphrodisiacs may mimic sex hormones or enhance their release, contributing to heightened libido and sexual response (Ali et al., 2013).

Aphrodisiac activity is intricately linked to various parameters that gauge sexual behavior and libido. These parameters, including mount latency, intromission latency, ejaculation latency, mount frequency, intromission frequency, and ejaculation frequency, provide crucial insights into the effects of aphrodisiacs on sexual behavior (Ramachandran et al., 2004; Singh et al., 2012). Mount latency and intromission latency measure the time it takes for an animal to initiate sexual activity, while ejaculation latency records the time until ejaculation. Aphrodisiacs can significantly impact these parameters by reducing the time it takes to engage in sexual acts and reach ejaculation, indicating increased sexual desire and responsiveness. Furthermore, mount frequency and intromission frequency reflect the frequency of sexual attempts and successful sexual interactions, respectively, while ejaculation frequency counts the number of ejaculatory events during a sexual encounter. Aphrodisiacs can lead to an increase in these parameters, suggesting a higher level of sexual motivation, more prolonged sexual activity, and enhanced sexual performance (Singh et al., 2012).

Previous studies conducted using a 50% ethanolic extract of clove flower buds,ingested at doses of 100, 250, and 500 mg/kg, exhibited an aphrodisiac effect on male rats (Tajuddin et al., 2004). In contrast, an evaluation of the aphrodisiac effect of young *Areca catecu* nut seed extract on male rats at a dose of 200 mg/kg body weight revealed that the extract contained 39.8% polyphenols and did not exhibit any aphrodisiac effects (Rahman et al., 2020). In this study, 96% ethanol extract was employed, which has the potential to extract wide range of compounds (Tassa et al., 2023). The fractionation method used in this research utilizes ethyl acetate, with a specific focus on attracting sterols and phenols that are suspected to have aphrodisiac properties. Furthermore, the use of the ethyl acetate fraction aims to explore its potential as an aphrodisiac.

Based on this background, we aimed to determine the afrodicsiac activity of the ethyl acetate fraction of clove leaves (EAFCL) against the libido of male white rats (*Rattus norvegicus*) at a dose of 250 mg/kg body weight by measuring several parameters, namely ejaculation frequency (EF), intromission latency (IL), mount frequency (MF), ejaculation latency (EL), intromission frequency (IF), and mount latency (ML).

## RESEARCH METHODS

### Equipment and Materials

The equipment and materials used in the experiment included a rotary vacuum 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, LabFriend 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), $FeCl_3$ (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.

**Research Procedure**

1. **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*.

2. **Ethyl Acetate Fractionation**

The fractionation of ethanolic extract from clove leaves was performed using the liquid-liquid partition method. Initially, 10 grams of the clove leaf ethanol extract were dissolved in 100 ml of water. This mixture was then transferred to a separating funnel, and 100 ml of ethyl acetate was added. It was gently shaken for 15 minutes and allowed to settle until a clear separation occurred between the n-hexane fraction and the water phase. Subsequently, the n-hexane fraction was carefully separated from the aqueous layer, and this partitioning step was repeated up to three times until the solution became clear.

Accordingly, the aqueous fraction from the previous n-hexane partitioning was transferred to a separate separating funnel, and 100 ml of ethyl acetate was added. The mixture was agitated for 15 minutes and allowed to settle, resulting in a separation between the ethyl acetate fraction and the aqueous phase. The ethyl acetate fraction was separated from the aqueous layer and, again, partitioned up to three times until the solution became clear. Following these steps, the liquid ethyl acetate fraction was subjected to evaporation, yielding a viscous extract (Nuari et al., 2017).

3. **Preparation of 0.5% Na-CMC Colloidal Solution**

A total of 0.5 grams of sodium carboxymethyl cellulose (Na-CMC) was carefully weighed and then sprinkled into a mortar containing 10 ml of heated distilled water. The mixture was stirred diligently until a transparent and homogeneous mass was achieved. Subsequently, the Na-CMC solution was transferred into a 100 ml measuring flask, and additional distilled water (aquades) was added to bring the solution volume up to the 100 ml mark, ensuring it reached the desired volume.

4. **Extract Preparation**

To prepare the suspended extract, a 100 ml dispersion of 0.5% Na-CMC was prepared. For the X-Gra preparation, 0.5 grams of X-Gra was blended with 0.5% Na CMC until a homogeneous mixture was obtained. The resulting mixture were then transferred into a measuring flask, and an additional 0.5% Na CMC solution was added to reach a total volume of 100 ml. The entire solution was homogenized thoroughly (Rusdi et al., 2018; Sutoro et al., 2022).

5. **Aphrodisiac effects testing**

All experiments were rigorously conducted in full compliance with the animal welfare guidelines established by the CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) (Sahu et al., 2023). Additionally, they received prior approval from the Research Ethics Committee at the Faculty of Medicine, Tadulako University, with the official approval number 2576/UN.28.1.30./K/2019. The test procedure involved the administration of the suspended extract and Na CMC dispersion for a duration of 30 days. Two days prior to testing, female rats were administered estradiol valerate as adopted from (Brawer et al., 1978). The male rat was placed in a cage situated in a quiet room illuminated with red light (75 W) for a 5-minute adaptation period. Subsequently, female rats were introduced into the cage, maintaining a ratio of one male to two females (1:2). The interactions were observed and recorded for a duration of 30 minutes using a recording device.

The observations were conducted and calculations were made following the standard method (Singh et al., 2012), which included the following parameters:

a. Mount latency (ML): the time in seconds from the introduction of the female mouse to the first mounting by the male.
b. Intromission Latency (IL): the time in seconds from the recognition of the female mouse to the occurrence of the first intromission.

c. Ejaculatory Latency (EL): the time in minutes from the first intromission to the first ejaculation.
d. Mounting Frequency (MF): the total number of mounts observed within the 30-minute observation period.
e. Intromission Frequency (IF): the total number of intromissions observed within the 30-minute duration.
f. Ejaculation Frequency (EF): the total number of ejaculations observed within the 30-minute observation period.

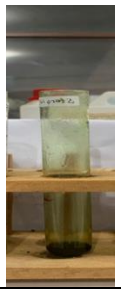
**Data Analysis**

The data are reported in the format of mean ± standard deviation (SD). All data underwent two initial tests: a normality test and a homogeneity test. When the data met the criteria of having both a normal distribution and homogeneity, data analysis proceeded with a one-way ANOVA, and followed by Least Significant Difference (LSD). However, if the data deviated from a normal distribution and lacked homogeneity, non-parametric statistical methods were utilized. In this case, the Kruskal-Wallis test was applied, followed by the Mann-Whitney test to evaluate the significanceof the difference among the groups. This analysis was conducted with the assistance of Rstudio software (Version 4.2.1, RStudio Inc. Boston, USA).

**RESULT AND DISCUSSION**

Thick EAFCL was obtained from fractionation using the liquid-liquid partition method. Phytochemical screening was carried out on the fraction to see the presence of compounds in the ethyl acetate fraction of clove leaves. Phytochemical screening test results for EAFCL contain compounds, steroids, and alkaloids (Table I).

**Table I. Phytochemical Screening Results of Clove Leaf Ethyl Acetate Fraction (*Syzygium aromaticum L.*)**

| Testing | Reagent | Observation | Result |
|---|---|---|---|
| Alkaloid | Dragendorf R | The formation of yellow-orange-red-brick deposits |  |
| Steroids | Concentrated sulfuric acid + anhydrous acetic acid | Formed blue hujau color |  |

Aphrodisiacs are described as substances that can increase sexual desire. The use of test animals is intended to determine the aphrodisiac activity of giving an ethyl acetate fraction of clove leaves to male white rats, which can be observed in several parameters, namely: MF, ML, IF,intromission lIL, EF, and ELas shown in Table II and Figure 1.

**Table II. Results of Aphrodisiac Effect Testing on Male White Rats.**

| Parameter | Control Group | | Treated group | *p*-value |
|---|---|---|---|---|
| | Healthy | X-gra | | |
| (y) ML | 26.20 ± 1.304[a] | 10.80 ± 2.683 | 11.4 ± 1.517 | 0.008 |
| (z) IL | 54.40 ± 2.881[a] | 14.80 ± 2.683 | 15.8 ± 3.834 | 0.000 |
| (y) EL | 4.55 ± 0.561 | 6.228 ± 0.131[b] | 6.228 ± 0.447[b] | 0.008 |
| (z) MF | 11.60 ± 3.362 | 24.80 ± 5.167[b] | 23.6 ± 4.336[b] | 0.000 |
| (z) IF | 9.20 ± 2.280 | 22.20 ± 5.020[bc] | 14.6 ± 2.074 | 0.000 |
| (y) EF | 1.00 ± 0.00 | 2.00 ± 0.00[b] | 1.6 ± 0.54[b] | 0.009 |

Note: (y) means the data was analyzed using Kruskal-Wallis followed by the Mann-Whitney test, and (z) means the data was analyzed using Least Significant Difference (LSD) test. ([a], $p<0.05$) shows a significant difference between group X-gra and treated group, ([b], $p<0.05$) shows significant a difference between the healthy group, and ([c], $p<0.05$) shows a significant difference between the treated group.

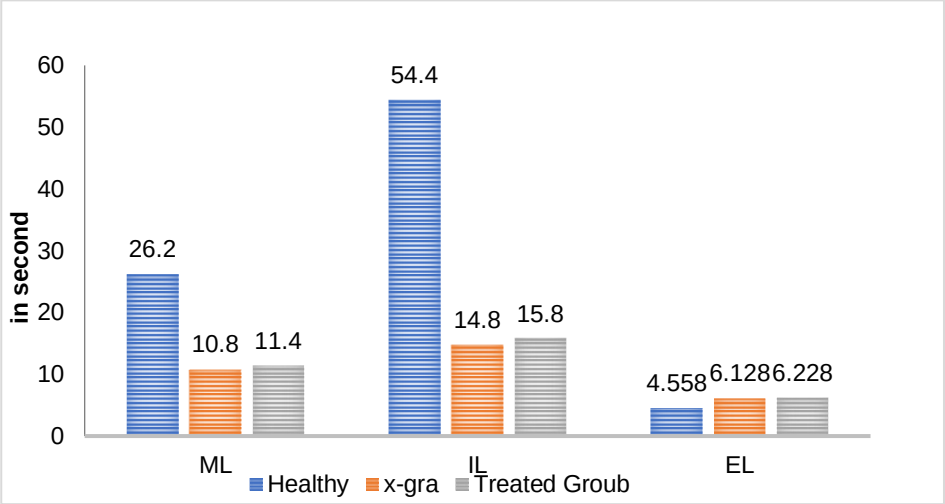



**Figure 1. Comparison of Several Parameters Among Different Treatments (ML, IL, EL)**

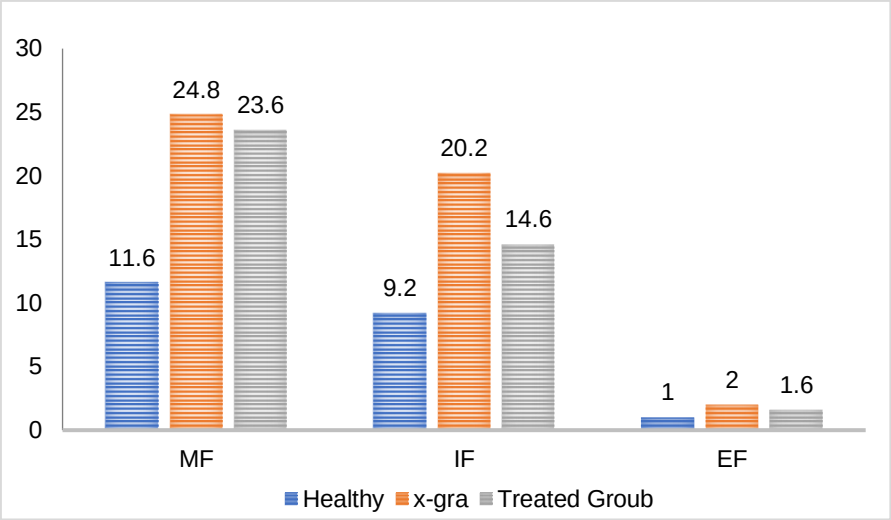



**Figure 2. Comparison of Several Parameters Among Different Treatments (MF=Mount frequency, IF, and EF).**

The healthy group (Na-CMC 0.5%), X-gra group, and EAFCL group were given to test animals for 30 days with the volume of administration of the given fraction adjusting to the body weight of the rats weighed daily before treatment. The preparation of the test material is made in the form of a suspension of EAFCL with a carrier material of 0.5% Na-CMC. The selection of Na-CMC as a carrier is based on its non-toxic nature and does not affect the reproductive system of mice (Bampidis et al., 2020; Chalah et al., 2022).

The normality and homogeneity test results showed that the IL, ML, and IFdata were normally distributed and homogeneous ($p>0.05$). Therefore, testing was carried out using the One-Way Anova test and continued with the LSD (Least Significant Difference*)* test to see the differences between the 3 treatment groups. Meanwhile, the parameters of EF, MF and EL were not normally distributed and homogeneous ($P<0.05$). Therefore, testing was carried out using the Kruskal Wallis test and continued with the Mann Whitney test to see the differences between the 3 treatment groups.

In the ML parameter, it can be seen in (Table II) that the lowest value is found in the positive group, with an average value of 10.80. In the treatment group, it gets an average value of 11.40, and in the healthy group it gets an average value of 26.2. The above results mean that the positive group has more effect than the ethyl acetate fraction and the healthy group. Then the results obtained on the ML parameter comparison between all control groups gave significantly different results between the ethyl acetate fraction and healthy group, while the positive group and the ethyl acetate fraction did not differ significantly. This means that the application of EAFCL influences the time of mounting in male rats. This is in line with the previous literature reporting that clove extract decreases the mounting latency significantly (Tajuddin et al., 2004).

In the MF parameter, it can be seen in (Table II) that the highest value is in the positive group with an average value of 24.80, while in the ethyl acetate fraction it gets an average value of 23.60 and in the healthy group with an average value of 11.60. The above results mean that the positive group has more effect than the ethyl acetate fraction and the healthy group. Then the results obtained in the MF parameter comparison between all control groups gave significantly different results between ethyl acetate fraction and healthy group. healthy group and positive group, while the positive group and ethyl acetate fraction did not differ significantly. This means that the administration of EAFCL at a dose of 250 mg/kg BW has an effect on the time of *mounting* in male rats and is in accordance with the previous study (Tajuddin et al., 2004).

In the intromission frequency parameter the group with the highest average intromission frequency was the positive group, with an average value of 22.20. while in the

ethyl acetate fraction it got an average value of 14.60, and healthy controls got a value of 9.20. The above results mean that giving the ethyl acetate fraction of clove leaves at a dose of 250 mg/kg BW influences the intromission of male white rats. Then the results obtained in the IF parameter comparison between all control groups gave significantly different results in each control group. This means that the administration of EAFCL at a dose of 250 mg/kg BW has an effect on the IF in male rats, and similar to the previous study result that stated that clove extract affects mating performance (Tajuddin et al., 2003).

IL parameter has the lowest value in the positive group with an average value of 14.80, while in the ethyl acetate fraction it gets an average value of 15.80, and in the healthy group it gets a value of 54.4. The above results mean that giving the ethyl acetate fraction of clove leaves at a dose of 250 mg/kg BW has an effect on the intromission of male white rats. Then the results obtained in the IL parameter comparison between all control groups gave significantly different results in the ethyl acetate fraction group and healthy groups, while the positive group and ethyl acetate fraction did not differ significantly. This means that the administration of EAFCL at a dose of 250 mg/kg BW has an effect on the IL in male rats, and in accordance with a previous study (Sharifi Olounabadi et al., 2021).

EL parameter, the highest value was found in the positive group with an average value of 6.228, while in the ethyl acetate fraction it had an average value of 6.228, and in the healthy group it had an average value of 4.558. The above results mean that EAFCL at a dose of 250 mg/kg BW influences the ejaculation of male white rats. Then the results obtained on the comparison parameters between all control groups showed significant differences between the healthy group and the positive group, healthy group, and the ethyl acetate fraction, while the positive group and ethyl acetate fraction were not significantly different. This means that the administration of EAFCL at a dose of 250 mg/kg BW is so influential on the time of ejaculation in male rats and is in accordance with the previous literature (El-Saber Batiha et al., 2020).

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 ethyl acetate fraction it got an average value of 1.60, and in the healthy group it got an average value of 1.00. The above results mean that giving EAFCL at a dose of 250 mg/kg BW has an effect on the ejaculation of male white rats. Then the results obtained in the ejaculation frequency (EF) parameter comparison between all treatment groups gave significantly different results in the healthy group and positive group, healthy group and ethyl acetate fraction, while in the positive group and ethyl acetate fraction were not significantly different. This means that the dose of 250 mg/kg BW had an effect on the timing of ejaculation in male rats, and it is in line with the previous study stating that the clove extract can prevent premature ejaculation (El-Saber Batiha et al., 2020).

Based on the results of research that has been obtained, aphrodisiac activity has an effect on the ethyl acetate fraction at a dose of 250 mg/kg BW. Due to the discovery of steroid chemicals and alkaloids that alter sexual behavior by acting as a substitute for cholesterol in the process of testosterone synthesis, this research has had an impact (Wahdaningsih et al., 2012). A cholesterol precursor called pregnolone is used to make testosterone. Progesterone, which is produced when pregnolone is converted, serves as a precursor to androgens such as testosterone (Mattison et al., 2020).

## CONCLUSION

EAFCL was found to contain secondary metabolite compounds, specifically steroids and alkaloids, while lacking saponins, tannins, and flavonoids. Moreover, the administration of EAFCL at a dose of 250 mg/kg body weight demonstrated aphrodisiac activity, as evidenced by significant effects on various parameters, including mount latency (ML) (11.40), intromission latency (IL) (15.80), ejaculation latency (EL) (6.228), mount frequency (MF) (23.60), intromission frequency (IF) (14.60), and ejaculation frequency (EF) (1.60).

These findings suggest a potential role for the ethyl acetate fraction in enhancing sexual behavior and performance.

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