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Research Article

Aphrodisiac Activity of The n-Hexane Fraction of Clove Leaves (*Syzygium aromaticum* L.) on The Fertility of Male White Rats (*Rattus norvegicus*)

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ABSTRACT

Aphrodisiacs are stimulant that modulate the neuroendocrine system to enhance libido or sexual desire and affect reproductive activity. Hormonal or neurological imbalances, as well as disruptions in reproductive tissue, can affect male fertility. One plant known to stimulate sexual arousal is the clove (*Syzygium aromaticum* L.), with its active compound, primarily eugenol, presumed to enhance testicular function and thereby potentially address male sexual dysfunction. This study aims to examine the aphrodisiac activity of the n-hexane fraction of clove leaves (*Syzygium aromaticum* L.) on the fertility of male white rats by assessing parameters such as the mating index, fertility index, libido index, pregnancy index, and live fetus index. This research used an experimental method with a post-test modified randomized control group design, involving 20 male and 40 female white rats divided into four treatment groups in a 1:2 ratio. Each group comprised 5 male and 10 female rats. The groups included a normal control (Na-CMC), a positive control (X-Gra 51.37 mg/kg BW), a clove leaf extract group (250 mg/kg BW), and an n-hexane fraction of clove leaves group (250 mg/kg BW). The findings indicate that the n-hexane fraction of clove leaves (*Syzygium aromaticum* L.) at a dose of 250 mg/kg BW contains secondary metabolite steroids and exhibits significant aphrodisiac activity that affect fertility which is characterized by a significant differences in the percentages of pregnancy and fertility indices.

Keywords: Aphrodisiac; clove leaves; n-hexane fraction; fertility; male white rats

INTRODUCTION

Indonesia has a rich traditional of using various indigenous plants as natural aphrodisiacs. Among the diverse flora employed for this purpose, the clove plant stands out as one of the herbal remedies believed to possess libido-enhancing properties (Salaki et al., 2021). Aphrodisiacs are stimulants that can arouse sexual passion or desire (Wulandari et al., 2024). They assist in modulating the neuroendocrine system to enhance libido and sexual desire, disrupt the continuity of reproductive activities, restore damaged tissue function, and enhance psychoactive sexual desire. Hormonal or neurological imbalances and reproductive tissue disorders can impact male fertility (Dutta & Sengupta, 2018). Fertility, derived from the word fertile, refers to the ability to produce offspring. A man's reproductive potential is assessed by evaluating sperm characteristics. The key factors used to determine sperm health include the number of sperm cells produced, their shape, and their ability to move effectively. These aspects collectively define what is considered normal or high-quality sperm (Alfa et al., 2019).

The clove plant (*Syzygium aromaticum* L.) contains essential oils, sterols, tannins, flavonoids, and saponins, which serve as natural antioxidants (Salaki et al., 2021). Cloves are known for numerous health benefits, one of which is enhancing sexual arousal (libido) or acting as an aphrodisiac. Its active ingredient, primarily eugenol, is believed to enhance testicular function and thus potentially address male sexual dysfunction. Sexual activity is highly influenced by the regulation of hormones, including thyroid hormones. Previous studies have confirmed the involvement of thyroid hormones, triiodothyronine (T3) and thyroxine (T4), in the physiological changes in sexual activity resulting from aphrodisiac administration (Saras, 2023).

Previous research has shown that the n-hexane fraction of clove leaves exhibits aphrodisiac effects. n-hexane is used to extract sterol compounds, which are non-polar, and phenolic compounds, which are highly polar (Yanuarty et al., 2024). This study aims to examine the aphrodisiac activity of the n-hexane fraction of clove leaves (*Syzygium aromaticum* L.) on the fertility of male white rats by assessing parameters such as the mating index, fertility index, libido index, pregnancy index, and live fetus index.

METHODS

Equipments

Stirring rod, 40-mesh sieve, blender, maceration vessel, porcelain dish, glass funnel, Erlenmeyer flask, measuring glass, surgical scissors, watch glass, camera (recording device), animal testing cage, 40cm x 40cm observation cage, filter paper, electric stove, volumetric flask, round-bottom flask (500 mL), 75-watt infrared lamp, microscope, mortar and pestle, water bath, surgical board, dropper pipette, surgical tweezers, rotary vacuum evaporator, horn spoon, 3 mL oral probe, 3 mL injection syringe, test tube, analytical balance,water thermometer, gram scale, and containers (jars).

Materials

Concentrated HCl P, HCl 2 N, ammonia, aluminum foil, distilled water, surgical thread, cloth thread, scalpel blades (no.10, 23, and 15), Dragendorff's reagent LP, 96% ethanol, $FeCl_3$, gloves, HCl, concentrated H_2SO_4, cotton, label paper, n-hexane, magnesium P, masks, methylene blue, 0.5% Na CMC, 10% NaCl, plastic wrap, clove leaf dry powder, styrofoam, 20 male white rats and 40 female white rats, tissue, and X-gra.

Preparation of Materials and Preparation of Clove Leaf Ethanol Extract

Clove leaf samples were obtained from Sibado Village, Sirenja District, Donggala Regency. The leaves were wet-sorted, washed, air-dried, then ground into a powder, sieved 40, and weighed. Preparation of Clove Leaf Ethanol Extract The extraction was performed using the maceration method. A total of 800 grams of simplisia powder was placed in two maceration vessels, each containing 400 grams of powder, and macerated with 2 liters of 96% ethanol. The maceration was carried out for 3x24 hours in a room protected from sunlight and stirred occasionally to prevent saturation (Suteja, 2018). Total solvent used was 8 L and the extract obtained is concentrated using a rotary evaporator.

n-hexane Fractionation

The fractionation procedure of n-hexane refers to Nuari et al. (2017). The n-hexane component of clove leaves was isolated using a technique called liquid-liquid partitioning. The process began by dissolving 40 grams of clove leaf extract, which had been prepared using ethanol 96%, in 400 mL of purified water. This solution was then placed in a separatory funnel, and an equal volume (400 mL) of n-hexane was added. The mixture was carefully agitated for a quarter of an hour, then left undisturbed to allow the n-hexane and water layers to separate naturally. The n-hexane layer was then carefully removed from the water layer. This extraction process was three times repeated continuously until the filtrate appeared clear, indicates that all compounds have been dissolved. The n-hexane filtrate then was evaporated to obtain concentrated n-hexane extract.

Phytochemical Screening

Phytochemical analysis was performed to identify the presence of various classes of secondary metabolites. Phytochemical screening refers to Marjoni (2016).This screening process aims to provide initial insights into the groups of chemically active compounds that may contribute to the biological effects of a plant. Such analysis can be carried out on either the raw plant material or on plant extracts. Testing was performed on alkaloid, flavonoid, saponin, and tannin compounds through qualitative color reactions or precipitation tests.

Ethical Consideration for Test Animal

The ethical consideration was conduct to test animals prior to treatment. The ethical code was issued by the medical and health research ethics committee of the Faculty of Medicine, Tadulako University.

Fertility Test

The fertility test was conduct using 3-4 months old male and female white rats with a body weight of 250 mg. Twenty male and forty female white rats were acclimated separately for 7 days with standard feed and water, and their weights were measured The male rats were divided into four groups: Group 1 normal control (Na CMC 0.5%), Group 2 positive control (X-gra 51.37 mg/kg BW), Group 3 (clove leaf extract 250 mg/kg BW), and Group 4 (n-hexane fraction 250 mg/kg BW). Test and control preparations were administered once a day for 30 days. Administration of test and comparator preparations for 30 days, given to male white rats, orally by means of sonde. After 30 days of extract administration, the estrus cycle of the female rats was determined by vaginal smearing. Once the female rats entered the estrus phase, they were ready for mating. Each male rat was then moved to a separate cage, and female rats (ratio 1:2) were introduced every 3 hours per day for 14 days. The day when vaginal smears showed the presence of sperm was designated as the starting point (day 0) of the pregnancy. The female rats were then subjected to surgical examination 21 days after this confirmed conception date Male rat fertility was observed from several parameters (Wulandari et al., 2024) such as mating index (equation 1), fertility index (equation 2), libido index (equation 3), pregnancy index (equation 4), and live fetus index (equation 5).

$$\text{Mating Index} = \frac{\text{Number of male rats with positive sperm in females within 14 days}}{\text{Total number of male rats involved in mating}} \times 100\% \quad (1)$$

$$\text{Fertility Index} = \text{Number of days required for male rats to impregnate female rats} \quad (2)$$

$$\text{Libido Index} = \frac{\text{Number of female rats with positive sperm}}{\text{Total number of female rats involved in mating}} \times 100\% \quad (3)$$

$$\text{Pregnancy Index} = \frac{\text{Number of pregnant female rats}}{\text{Total number of female rats with positive sperm}} \times 100\% \quad (4)$$

$$\text{Live Fetus Index} = \frac{\text{Number of live fetuses}}{\text{Total number of fetuses during surgery}} \times 100\% \quad (5)$$

RESULTS AND DISCUSSION

The purpose of this study was to ascertain the aphrodisiac effect of the n-hexane fraction of clove leaves (S. aromaticum L.) at a dosage of 250 mg/kg BW on the fertility of male white rats. The leaves were extracted using the maceration method with 96% ethanol. A total of 98 grams of thick extract was obtained, yielding a 24.5% recovery rate. Then it was continued with the fractionation method and obtained a fraction n-hexane of 9.23 grams with a yield of 20.87%. The maceration method was chosen to prevent damage to the compounds contained within the clove leaves. Fractionation was conducted to separate the primary compounds from other groups based on the solvents' polarity (Yanuarty et al., 2024). Following this, phytochemical screening tests were carried out to identify the compounds present in the n-hexane fraction of clove leaves. The screening results revealed that the n-hexane fraction of *S. aromaticum* L. contains steroid compounds, which are associated with aphrodisiac properties, while no alkaloids, flavonoids, saponins, or tannins were detected. Table 1 shows the details of the phytochemical screening. These findings align with previous studies on the potential aphrodisiac effects of the n-hexane extract of clove leaves on rat sexual behavior (Wael, 2019).

Table 1. Phytochemical Test Results of the N-Hexane Fraction of Clove Leaves

Test	Reaction	Observation	Result
Alkaloid Test	Dragendorff LP	Formation of a yellow-orange-red precipitate	-
Flavonoid Test	Concentrated HCl and Mg	Formation of an orange-yellow color	-
Saponin Test	Boil + HCl N	Formation of foam	-
Tannin Test	10% NaCl solution + $FeCl_3$	Formation of a blue-black color	-
Steroid Test	Acetic anhydride-sulfuric acid (concentrated)	Formation of a green-black color	+

Note: (-) indicates a negative result; (+) indicates a positive result.

Table 2. Fertility Test Results

Parameter	Control Group		Treatment Group		p Value
	Normal	Positive	Clove Leaf Extract (250 mg/kg BW)	n-Hexane Fraction of Clove Leaves (250 mg/kg BW)	
	Na-CMC 0.5%	X-Gra (51.37 mg/kg BB)			
(y)Mating Index (%)	80	100	100	100	-
(z)Fertility Index (days)	1.7 ± 1.83	0.9 ± 0.99	1.3 ± 1.42	1.2 ± 0.92	0.013 < 0.05
(y)Libido Index (%)	40	60	60	70	-
(y)Pregnancy Index (%)	100	100	83.3	100	-
(y)Live Fetus Index (100%)	57	83	66	100	-

Note: (z) = Kruskal-Wallis test. (y) = Descriptive analysis. ± = Standard deviation. $p < 0.05$ = Significant difference.

This study used male and female white rats (*Rattus norvegicus*) as test subjects, a common choice for research due to their genetic similarities and biological characteristics akin to humans. Male white rats were chosen because they have a stable hormonal system and metabolize drugs more quickly than female white rats (Wirawan, 2018). Female rats were used to evaluate fertility parameters. The tests for normality and homogeneity showed that the fertility index was not normally distributed but was homogeneous, therefore the Kruskal-Wallis test was used. In contrast, the mating index, libido index, pregnancy index, and live fetus index were analyzed descriptively. The results indicated differences across all parameters. The mating index, which represents the number of male rats whose female partners tested positive for sperm within 14 days of the total male rats involved in mating, showed a variation Table 2 with the normal control given Na-CMC having an 80% rate. This result indicated the effect of extract on mating index.

The libido index is a metric for assessing increased sexual desire, demonstrated by the proportion of female rats that tested positive for sperm relative to the total number of females involved in mating. The results demonstrated that male white rats administered with a 250 mg/kg BW dose of the n-hexane fraction from clove leaves (*S. aromaticum* L.) exhibited a notable difference in response when compared to the normal control group. An increase in libidio index means more female mice are sperm positive, while a decrease means less. This suggests a distinct physiological effect attributable to the clove leaf extract at that dosage. In contrast, in the positive control group, the clove leaf extract group (250 mg/kg BW), and the n-hexane fraction group, the libido index was 50%, showing an enhancement in sexual desire (Ayu et al., 2023). Previous studies (Yanuarty et al., 2024) using the n-hexane fraction were positive for containing steroids. Testosterone is one of the steroid hormones. All mammalian steroid hormones are formed from cholesterol via pregnenolone through a series of reactions that occur within the mitochondria or endoplasmic reticulum of adrenal cells. Decreased cholesterol levels in the blood cause a decrease in steroid hormone-forming substances, one of which is testosterone. Testosterone is a hormone involved in sexual desire (libido) that provides sexual stimulation to encourage sexual activity, especially in male animals. According to Setyawati et al. (2017) that decreased testosterone can cause decreased libido, spermatogenesis, and diameter of seminiferous tubules.

The fertility index is a measure of the sexual capability of rats, defined by time period of male rats can cause female rats to test positive for sperm. A positive sperm result is determined by the number of days until sperm is detected in the vagina of female white rats. In this study, male white rats given the 250 mg/kg BW dose of the n-hexane fraction of clove leaves exhibited decreased performance compared to the normal control and the extract control. Statistically, this variation was significant, with a sign. value of 0.013 (< 0.05), indicating the presence of aphrodisiac activity that can influence the fertility of male white rats. This effect might be associated with the antioxidant properties of clove leaves, consistent with findings by Elisa et al., (2023) who noted that clove leaves contain potent antioxidants. It has strong antioxidant IC50 value of 31.19 %. Brinkhaus et al. (2000) explained that antioxidants can increase the process of spermatogenesis and the synthesis of steroid hormones such as glucocorticoids, androgens, and estrogens so that they can increase mating performance in male rats.

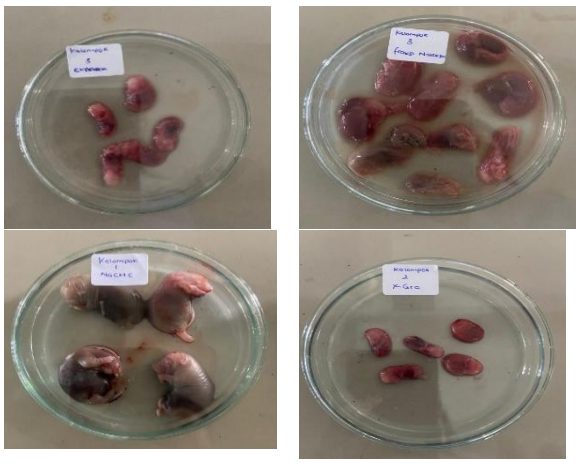

Figure 1. Live fetuses.

The pregnancy index is the ratio of female rats experiencing pregnancy compared to the total number of female rats that tested positive for sperm. The results showed that male white rats administered with the 250 mg/kg BW dose of the n-Hexane fraction of clove leaves exhibited similar outcomes when compared with the normal and positive controls. However, when compared to the clove leaf extract at a 250 mg/kg BW dose, there was a difference. The percentage values were 100% for the normal control, 100% for the positive control, 83% for the clove leaf extract, and 100% for the n-hexane fraction of clove leaves. This is because the clove leaf extract contains secondary metabolite compounds such as alkaloids, flavonoids, saponins, and tannins (Patala et al., 2024), while the n-hexane fraction contains steroids (Yanuarty et al, 2024).that influence sexual activity by replacing cholesterol in the synthesis of testosterone. Testosterone is synthesized from the cholesterol precursor pregnenolone. Pregnenolone is subsequently converted to progesterone, which serves as a precursor in inducing the formation of androgens such as testosterone (Hafez, 2000). One of the compounds found in cloves is eugenol. Eugenol acetate and β-caryophyllene have been used as attractants because these compounds are sex pheromones released by female animals to attract males (Sumala et al., 2010).

The live fetus index refers to the number of viable fetuses out of the total offspring counted during a surgical procedure on the 21st day of pregnancy. This index provides a measure of fetal survival at this specific gestational stage. The results showed that male white rats administered with the 250 mg/kg BW dose of the n-hexane fraction demonstrated differences across all groups (Figure 1) Only the n-hexane fraction at a 250 mg/kg BW dose achieved a live fetus index of 100%, indicating no aphrodisiac activity affecting fertility between the treatment groups and the live fetus index (Ayu et al., 2023).

In this study, the secondary metabolite compounds obtained are steroids. Steroids that are structured like cholesterol can be converted into pregnolone. Steroid compounds function to affect sexual activity through the mechanism of replacing cholesterol in synthesizing testosterone (Puruhita et al., 2020). Testosterone is synthesized from a cholesterol precursor known as pregnolone. Pregnolone will then be converted into progesterone which will act as a precursor in inducing the formation of androgens such as testosterone (Hafez, 2000).

Aphrodisiac activity against fertility can be affected by the hormone testosterone. The hormone testosterone functions to stimulate the development of reproductive organ activity in collaboration with FSH and LH in the process of spermatogenesis, sperm maturation and increasing fructose excretion by vesika seminalis as the main nutrient of spermatozoa. Several compounds that can affect testosterone hormones such as flavonoids, saponins and steroids (Putri, 2022). all three can stimulate the excretion of gonadotropins FSH (Folicle Stimulating Hormone) and LH (Luteinezing Hormone) and testosterone.

Fertility is also associated with antioxidant properties. Antioxidants can improve the process of spermatogenesis and synthesis of steroid hormones such as glucorthyroid, androgens, and estrogen so that they can improve mating performance in male rats. So it can be said that the n-hexane fraction of clove leaves has more effect than the use of Na-CMC, clove leaf extract and X-Gra. The disadvantage of using n-

hexane is that for now its use has been limited because it can cause toxicity.

CONCLUSION

In this research, the n-hexane fraction of clove leaves administered at a dose of 250 mg/kg BW was shown to contain steroid secondary metabolites. It also exhibited aphrodisiac activity that influenced fertility, as evidenced by significant differences in the pregnancy and fertility indices. This finding supports the potential of the n-hexane fraction as a natural fertility enhancer due to its steroid content and its impact on reproductive parameters in male white rats.

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