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Research Article

Comparison of activity test of ethanol extract and palm root infusion (*Arenga pinnata*) as an aphrodisiac

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ABSTRACT

The palm tree (*Arenga pinnata*) contains secondary metabolites such as saponins, phenols, triterpenoids, alkaloids, and flavonoids that can be used as medicine, particularly as an aphrodisiac. Aphrodisiacs are natural substances, medications, or herbal supplements that have the potential to increase sexual arousal. The use of herbal aphrodisiac drugs has been increasing year by year in Indonesia. This study aims to determine the aphrodisiac activity of palm root water extract (*Arenga pinnata*) in test animals (Web Wiester mice). In this experimental study, the preparation of the extract begins with the processing of palm roots, which are then made into an infusion of palm root water (*Arenga pinnata*). The method of making the palm extract uses infusion and maceration techniques. The aphrodisiac potential test was conducted in vivo using 32 Web Wiester mice, which were divided into 5 groups; the dosage was determined by dividing the mice into five groups (positive control, negative control, 0.5 ml infusion, 1 ml infusion, and extract). The characterization of aphrodisiac properties in the extract included testing the libido of mice (*Mus musculus*). The results showed that the extract test group with a dose of 250 mg/kg had the highest mating retention among the other test groups.

1. Introduction

The general definition of sexuality refers to matters related to the reproductive organs or anything associated with intimate relations between men and women (Dewi et al., 2020). Sexuality is the manifestation of human erotic desires or lust, shaped and passed down from one generation to the next. It involves political, economic, cultural, and religious factors. The deepest essence of humanity is revealed through sexuality, which does not arise naturally but must be understood through a careful learning process. This learning involves

knowledge of body structure, ethics, human rights, reproductive health, and profound spiritual values (Fujiati, 2017).

A decline in sexual desire is more common in men than in women, and this symptom can occur across all age ranges (Zulkarnain et al., 2022). Among men aged 50-70, the prevalence of decreased sexual desire increases by more than 50% compared to younger ages (Akbar, 2020). This phenomenon is caused by the decline in several markers, including a reduction in Leydig cell numbers by around 40%, a decrease in pulsatile luteinizing hormone secretion, and a decline in free testosterone levels by about 1.2% annually (Anawalt et al., 2022). This sexual dysfunction can be influenced by two main factors: psychological and physical factors. Psychological factors involve feelings of anxiety, depression, stress, and fear of sexual failure, while physical factors include smoking habits, lack of physical activity, genetic factors, and certain medical conditions (Rahman et al., 2020).

In addressing various sexual disorders, traditional herbal medicine such as *jamu* has long been a popular alternative among the community (Kusumo et al., 2020). *Jamu*, as an ancestral heritage, has been passed down from generation to generation as a natural solution for various health issues, including decreased sexual desire. According to data from the Central Bureau of Statistics (2014), in 2012, around 28.12% of Indonesia's population relied on traditional medicine, while 66.95% preferred modern medicine, and 4.93% chose other methods of treatment. In East Java, the use of traditional medicine reached 28.12%, with 27.99% among men and 28.25% among women (Abdi et al., 2017).

The palm tree Arenga pinnata is known to contain secondary metabolites such as saponins, phenols, triterpenoids, alkaloids, and flavonoids, which have potential medicinal uses, including as aphrodisiacs. These compounds are part of a broader category of botanicals that have been traditionally used to enhance sexual function and treat sexual dysfunction. Saponins and flavonoids are known for their role in enhancing sexual desire and performance. Saponins, in particular, have been associated with increased libido and improved erectile function by influencing hormonal activity and blood circulation (Pallavi et al., 2011). Phenols and triterpenoids have antioxidant properties that can improve overall health, which indirectly supports sexual health. They may also have direct effects on sexual function by modulating stress and enhancing mood. Alkaloids known for their stimulating effects, alkaloids can enhance sexual arousal and performance by acting on the central nervous system (Ramandeep et al., 2013). The use of herbal aphrodisiac drugs has been increasing year by year in Indonesia. So, this study aims to determine the aphrodisiac activity of palm root water extract (*Arenga pinnata*) in test animals (Web Wiester mice).

2. Materials and Methods

Tools and Materials

The instruments used in the study include an infusion pan, feeding tube, mouse cages, mouse drinking containers, CCTV (EYESOC with FHD resolution and IR capability), animal scale, hydrochloric acid (HCl), Mayer's reagent, Dragendorff's reagent, FeCl3, Na CMC, and magnesium.

Sample Preparation

The sample used in this study was the root of the sugar palm (*Arenga pinnata* Merr). The sampling process involved collecting 2 kg of plant leaves. The harvested palm roots were then processed by dry sorting, separating the roots from the stems (Pertiwi et al., 2022). After the roots and stems were separated, the sample underwent wet sorting. Next, the sample was drained to reduce the moisture content during the wet sorting

process (Rachmatika et al., 2023). The separated and sorted palm roots were then chopped, followed by drying under direct sunlight. Afterward, the dried simplicia was ground and sieved using a 60-mesh sieve (Kusumawati et al., 2020).

Ethanol Extraction of Palm Roots

Five hundred grams of palm roots are finely ground and soaked in 96% ethanol solvent with a ratio of 1:5, meaning 500 grams of palm roots to 2500 ml of 96% ethanol, or one part palm root for every five parts of solvent, for three 24-hour periods. After that, the solution is filtered using filter paper, and the maceration process is repeated with a ratio of 1:2, or 500 grams of palm roots to 1000 ml of ethanol, for 3 days, In the maceration method, the palm roots are crushed and soaked in solvent with a ratio of 1:5 (1 part palm root to 5 parts solvent by volume). The maceration process involves manual stirring once every hour and is carried out at room temperature in a dark place for 5 days (Novriana, 2022). After this, the palm root extract is separated through filtering and pressing to obtain both the residue and the filtrate. The maceration process is then repeated for another 3 days. After the second maceration, the palm root extract is again separated through filtering and pressing to obtain the residue and the filtrate, The filtrate containing the palm root extract is then evaporated using a rotary vacuum evaporator at a temperature of 50-60ºC to separate the solvent from the palm root extract. This process is followed by heating in a water bath at 40ºC until it thickens (Sumarlan et al., 2020) .

Preparation of Arenga Root Infusion

The extraction process is carried out using infusion and maceration methods. Six grams of dried arenga root are soaked in 150 ml of distilled water at 90 degrees Celsius for 15 minutes, stirring periodically during the heating process (Hasanah et al., 2023). Afterward, the mixture is filtered using filter paper. The filtrate obtained is a water infusion of arenga root. In this study, 6 grams of arenga root were used because, in traditional use, 6 grams of either dried or fresh arenga root are brewed with 150 ml of water.

Preparation of Viagra or Sildenafil Suspension

The commonly tested dosage is 100 mg, which is then converted using the Laurence-Bacharach formula (Gumelar et al., 2017). The 100 mg dose of Viagra or Sildenafil, converted using the Laurence-Bacharach formula, amounts to 0.26 mg. Once the dosage is determined, the following calculation is performed:

$$Laurence - Bacharach = \frac{\text{Average normal mouse body weight}l}{\text{Mouse body weight to be tested}} \times \text{ Conversion result)}$$

For the preparation of the Viagra suspension, 2% Na CMC is mixed into 10 ml of suspension solution.

Animal Treatment

Each test animal is weighed individually and grouped into 4 groups, with each group consisting of 3 male and 3 female mice. Group K1 (Group 1) serves as the positive control, Group K2 (Group 2) serves as the negative control, Group K3 (Group 3) is given 0.5 ml of arenga root water infusion from the prepared infusion, Group K4 (Group 4) is given 1 ml of arenga root water infusion from the prepared infusion, and Group K5 (Group 5) is given the arenga root extract (*Arenga pinnata*) orally with varying doses.

3. Results and Discussions

Results of Phytochemical Screening of Arenga Root Extract Phytochemical screening tests on the arenga root extract have revealed that the extract contains various active compounds. These compounds belong to the groups of flavonoids, alkaloids, steroids, and terpenoids. Flavonoids are known for their strong antioxidant properties, which can protect body cells from damage caused by free radicals (Dellima et al., 2023). Alkaloids are commonly found in various medicinal plants and are known to have a range of pharmacological effects, including analgesic and antimalarial properties (Nindia et al., 2023). Terpenoids, the largest group of compounds found in plants, are known for their diverse biological activities, including antimicrobial and anticancer properties (Ramadhan et al., 2023). Saponins are known to possess antibacterial properties. When they interact with bacteria, saponins can increase the permeability of the bacterial cell membrane, leading to hemolysis of the bacterial cell (Masniawati et al., 2021). Tannins have various benefits, including stopping bleeding and treating burns. Tannins can form a protective layer on wounds and kidneys, helping to speed up healing. Additionally, tannins have long been used as a quick solution for diarrhea, dysentery, stopping bleeding, and reducing tumor size (Khasanah et al., 2021).

Table 1: Phytochemical Screening Results

Active compound class		Result
Flavonoids		+++
Alkaloids	Dragendorff	+++
	Meyer	+++
Saponins		+
Tannins		++
Terpenoids		+++
Steroids		-
Phenol		-

Keterangan :
+++ : There are many Compounds
++ : There are many compounds containing compounds
+ : There are many compounds with little compound content
- : Not many Compounds

The phenol content measurements of arenga root extract injected into mice yielded the following results: 56.60 mg GAE/gram, 68.34 mg GAE/gram, and 65.51 mg GAE/gram. The average phenol content of the extract was 63.48 mg GAE/gram. This value represents the phenol concentration in *Arenga pinnata* root extract, administered orally to mice in a volume of 1 ml.For the arenga root infusion, the measurements showed phenol content values of 0.75 mg GAE/gram, 0.79 mg GAE/gram, and 0.79 mg GAE/gram, with an average phenol content of 0.78 mg GAE/gram. This figure represents the phenol content in *Arenga pinnata* root infusion, also administered orally to mice in the same volume of 1 ml.From these two processing methods of arenga root, the infusion exhibited a higher phenol content compared to the extract. This indicates that the infusion method is more effective in extracting phenolic compounds from the arenga root than the extraction method. Below is the standard curve from the phenol quantitative testing.

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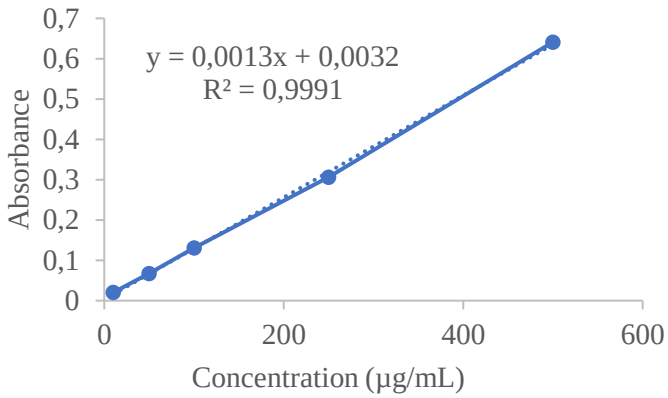

Figure 1. Phenol raw curve

The flavonoid content measurements of arenga root extract showed the following values: 8.27 mg QC/gram, 4.61 mg QC/gram, and 7.48 mg QC/gram, with an average of 6.78 mg QC/gram. This flavonoid content was calculated as the phenolic content in *Arenga pinnata* root, administered orally to mice in a volume of 1 ml.Meanwhile, the phenolic content in arenga root infusion was recorded at 0.083 mg QC/gram, 0.081 mg QC/gram, and 0.083 mg QC/gram, with an average of 0.082 mg QC/gram. This content was also calculated as the phenolic content in *Arenga pinnata* root, injected orally into mice with the same volume of 1 ml.Based on these measurements, the arenga root extract has a higher phenolic content compared to its infusion. Below is the standard curve from the quantitative flavonoid testing.

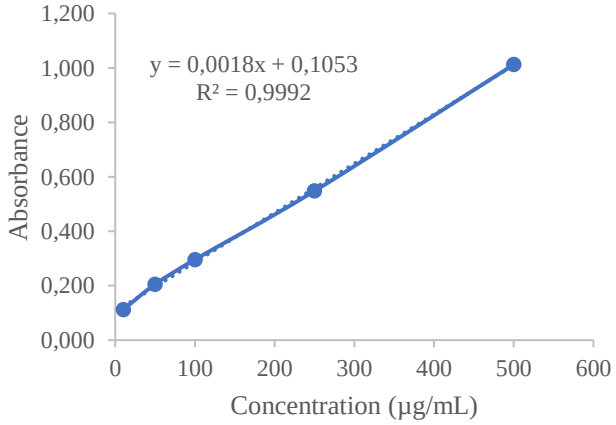

Figure 2. Flavonoid standard curve

The mating latency test in mice revealed that each treatment group showed differences in the number of mating events over a five-day period. The positive control group had a higher average number of mating events compared to the negative control group. The administration of a 0.5 ml dose of arenga root infusion and a 1 ml dose of extract given orally resulted in an average number of mating events almost equal to that of the positive control group. However, the 1 ml dose of infusion administered to the mice showed different results.The negative control group recorded the lowest average number of mating events among the four groups tested, indicating that the administration of distilled water did not have a significant aphrodisiac effect on male mice. These results highlight the importance of dosage and the type of treatment administered in influencing mating behavior in mice, with the positive control remaining the most effective treatment.This difference could be due

to the fact that the mice in the 0.5 ml infusion group were experiencing a peak in sexual arousal, which influenced the number of mating events. Therefore, the 0.5 ml infusion test group had a higher average number of mating events compared to the 1 ml infusion test group. Below is a graph illustrating the mating latency test results in mice over the five-day period.

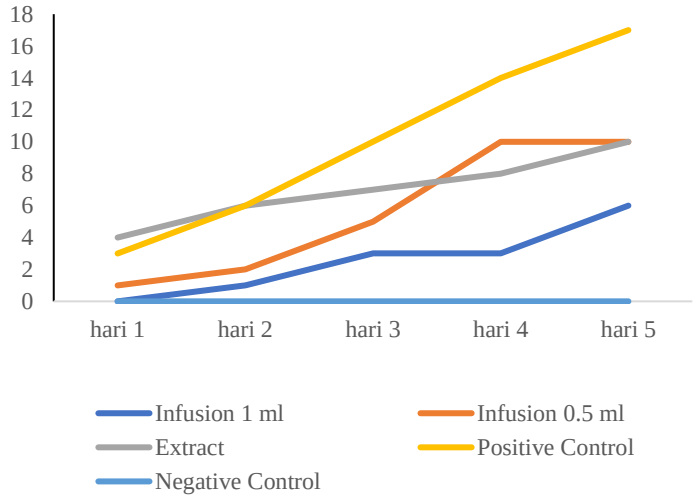

Figure 3. Graph of the number of matings in 5 days

A one-way ANOVA test was conducted to determine whether there were significant differences in aphrodisiac percentages among the various treatment groups. The results indicated that the aphrodisiac percentages among the treatment groups showed significant differences, with a p-value of 0.001. This suggests that each treatment had a significantly different aphrodisiac percentage, as the p-value obtained was less than 0.005 (Soraya, 2023).Thus, it can be concluded that the treatments given to each group affected the aphrodisiac percentage differently. Below is a table from the ANOVA test displaying the results..

Table 2. anova test results

Treatment groups	Significant value (p) of the ANOVA one-way test
Mating	0.001

In the Tukey test, the results showed a significant difference in aphrodisiac potency between the positive control and the negative control, as well as between the positive control and the 1 ml infusion. The significant values obtained were 10.000 and 7.400 ($p<0.005$), indicating a meaningful difference between the positive control and the negative control, as well as the test group receiving the 1 ml infusion. These results suggest a substantial effect of the treatment on aphrodisiac potency, where the positive control showed significantly different results compared to the negative control and the group given the 1 ml infusion. This is important to note because it demonstrates a clear difference in the effectiveness of the different treatments, which can be used as a reference in determining the dosage and effectiveness of the infusion as an aphrodisiac agent. This study shows that the treatment given had a measurable and significant effect, which is crucial for further research to confirm and understand the mechanism of action of the infusion. In conclusion, the significant differences found between the positive control and the negative control, as well as with the 1 ml infusion,

provide strong evidence of the effectiveness of the infusion in enhancing aphrodisiac potency, along with the Tukey test table.

Table 3. Tukey test results

Treatment Group (Mating Activity)	Comparison	Significance Value (P)
Positive Control	Negative Control	10.000*
	Extract	3.000
	Infusion 1 ml	7.400*
	Infusion 0.5 ml	3.800
Negative Control	Positive Control	-10.000*
	Extract	-7.000*
	Infusion 1 ml	-2.600
	Infusion 0.5 ml	-6.200
Extract	Negative Control	7.000*
	Positive Control	-3.000
	Infusion 1 ml	4.400
	Infusion 0.5 ml	0.800
Infusion 1 ml	Negative Control	2.600
	Positive Control	-7.400*
	Extract	-4.400
	Infusion 0.5 ml	-3.600
Infusion 0.5 ml	Negative Control	6.200
	Positive Control	-3.800
	Extract	-0.800
	Infusion 1 ml	3.600

* (*) : There was a significant difference because the p-value was <0.05.

4. Conclusions

Extract and infusion with a 1 ml dose of sugar palm root have potential as natural aphrodisiac agents.

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