

Potential Extract of Lerak Fruit (*Sapindus Rarak*), Pegagan (*Centella Asiatica*), Mimba Seed (*Azadirachta Indica* A.Juss) and Mixing of Third Extract to Motility and Viability of Spermatozoa Guinea Pigs (*Cavia Porcellus*)

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ABSTRACT

Background: Spermicides are substances that can kill spermatozoa. Spermicide circulating in the community contains Nonoxynol-9 which, if used for a long time, can irritate the vaginal mucosa and penis. Indonesia has a variety of plants that have the potential to act as anti-fertility, including *Sapindus rarak*, *Centella asiatica*, and *Azadirachta indica*. **Objective:** This study aim to evaluate extract *Sapindus rarak*, *Centella asiatica*, and *Azadirachta indica*. and mixing of third etract to motility and viability of spermatozoa Guinea pigs. **Methods:** The sample of this research was 15 guinea pig spermatozoa, 3-4 months old male guinea pigs taken from the cauda epididymis and vas deferens. Guinea pigs were divided into 5 group treatments with each group treatment incubated for 1, 10, and 30 minutes and repeated each for 5 times. The treatments in this study is control, 200 µl of spermatozoa suspension + 50 µl of *Sapindus rarak* fruit pulp extract 0.9%; 200 µl of spermatozoa suspension + 50 µl of gotu kola extract 0.9%; 200 µl of spermatozoa suspension + 50 µl of 0.9% neem seed extract; 200 µl of spermatozoa suspension + 50 µl of the third mixture extract 0.9%. This study used a completely randomized design (CRD). The observations in this study were the motility of guinea pig spermatozoa categories a + b and the viability of guinea pig spermatozoa using a microscope. The data obtained were analyzed statistically using One Way ANOVA. The results of this study indicated that the administration of extract *Sapindus rarak*, *Centella asiatica*, and *Azadirachta indica* and a mixture of the three extracts had a significant effect ($P < 0.05$) on the motility of guinea pig spermatozoa categories a + b and viability of guinea pig spermatozoa. **Conclusion:** This study concludes that the mixture of the three extracts has more potential in reducing the motility of spermatozoa categories a + b and the viability of guinea pig spermatozoa compared to single extracts. The mixture of the three extracts may contribute for reduce quality of sperm.

KEYWORDS: Lerak Extract (*Sapindus rarak*), Gotu Kola (*Centella asiatica*) Extract, Mimba Seed Extract (*Azadirachta indica* A.Juss), Spermatozoa guinea pig (*Cavia porcellus*), Motility, Viability

1. INTRODUCTION

Indonesia is a country with a high population density. According to Bappenas¹ the population in Indonesia by age and sex in 2019 reached 267 million. Indonesia is the fourth most populous country after China, India and the United States with a population growth rate of 1.49% annually. With this high rate of population growth, it will affect the level of welfare and life of the population. In this case the government makes efforts to suppress and control the population with the Family Planning (KB) program. one of the contraceptive methods is the use of spermicide.

Spermicides are substances that can immobilize and kill spermatozoa. Spermicide is said to be ideal, that is, it can quickly inhibit spermatozoa mobilization, is free from the effects of long-term use, does not cause irritation to the vaginal mucosa or penis, and is non-toxic². Nonoxynol-9 (N-9) is a spermicidal agent that is widely circulating in the community, but its use as a spermicide can cause inflammation and irritation of the vagina³, so it is necessary to look for the development of other alternative compounds, especially safe vaginal contraceptives. One way to develop ideal contraceptive drugs is to use alternative materials from natural ingredients because they are relatively safe and easy to obtain.

Indonesia has biodiversity with a number of plant species of 25,000-30,000⁴. Among them, apart from being a medicinal plant, there is also a potential for anti-fertility. Some of the plants that have the potential to act as anti-fertility are lerak, gotu kola, and neem seeds.⁵

Lerak is a plant that can grow in the lowlands and highlands which has the Latin name *Sapindus rarak*. Lerak contains, among others, saponins, alkaloids, steroids, anthraquinones, tannins, phenols, flavonoids and essential oils. The saponin content in

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lerak is a type of triterpenoid saponin. The saponin can reduce the motility and viability of spermatozoa⁶. Saponins work by binding to lipids and lipoproteins on the spermatozoa membrane, this can cause loosening of the spermatozoa cell membrane and disturbances in the cell nucleus resulting in a decrease in the integrity of the spermatozoa membrane, the integrity of the spermatozoa membrane interferes with the function of the cell membrane in regulating the transport of molecules into and to the outside of the cell⁶. Damage to the cell membrane results in inhibited molecular transport which results in disrupted cell metabolism. As a result, spermatozoa fertilization is inhibited⁷.

Gotu kola or with the Latin name *Centella asiatica* is a plant that is often used by the public as medicine. Gotu kola contains alkaloids, phytosterones, sesquiterpenes, alkaloids, sterols, and tannins. The use of gotu kola has been shown to work well as an anti-fertility in reducing the number of spermatozoa in male mice⁸. Tannin and alkaloid compounds have cytotoxic properties that can cause damage to spermatozoa. Damage to spermatozoa, among others, can occur in the tail, as a result, it can reduce spermatozoa motility. Tannin is an astringent, so it can affect the permeability of the sperm cell membrane. Tannin will cause shrinkage of the cell membrane, thereby disrupting the function of the cell membrane in transporting nutrients or nutrients. If the nutrients are reduced, the cell metabolism will be disrupted as a result, energy is also reduced. If the cell metabolism in producing energy is disturbed, it can cause the motility of spermatozoa to decrease, because the motility of spermatozoa requires energy.⁹

Alkaloids derived from plants are thought to interfere with the activity of the ATP-ase enzyme in the cell membrane in the middle of the spermatozoa tail. The internal homeostasis of sodium and potassium ions is maintained by the presence of the enzyme ATP-ase. The disruption of membrane permeability is caused by the disturbance of the homeostasis of sodium and potassium ions which is caused by the disturbed activity of the ATP-ase enzyme. If the permeability of the sperm membrane is disturbed, it will disrupt nutrient transport. As a result, cell metabolism in producing energy is also disrupted. If the energy required for spermatozoa movement is not fulfilled, there will be a decrease in spermatozoa motility¹⁰.

Neem or *Azadirachta indica* A. Juss is a tropical plant that has long been used as a traditional medicine. *Azadirachta indica* A.Juss (Neem) seeds have been used in traditional medicine for anti-diabetes, spermicide, anti-bacterial, and wound healing¹¹, besides the compounds in the neem plant have the potential to be anti-fertility, both in male and female animals¹². The effect of extracts from some parts of the neem plant is also known to affect reproductive function in male animals¹³. Neem leaf extract and neem seeds has an effect on decreasing the quality of spermatozoa and significant testicular damage¹⁴. The compound content in neem seeds as anti-fertility is almost the same as gotu kola or lerak. However gotu kola does not contain saponins. Saponins have long been known to function as natural spermicides and the highest saponin content is found in lerak fruit.

Spermicides are said to be successful, that is, apart from preventing fertilization, they must also meet the requirements of being safe, non-toxic, and non-irritating if used for a long time. Based on the literature search, there is still little information about the mixed extract of lerak, gotu kola and neem seeds regarding its effect on the motility and viability of guinea pig spermatozoa. Based on this background, it is necessary to research the effectiveness of extracts of lerak (*Sapindus rarak*), gotu kola (*Centella asiatica*), and neem seeds (*Azadirachta indica* A. Juss) and a mixture of the three extracts on the quality of marmot sperm in vitro as seen from the and spermatozoa viability.

2. METHODS

The research design used in this study was a completely randomized design (CRD). The population used in this study was 3-4 months old male guinea pigs, obtained from marmot farms in the Jambangan area of Surabaya. The sample used was 15 guinea pigs, consisting of 5 treatments with observation time of 1 minute, 10 minutes and 30 minutes and each time the observation was repeated 5 times. Simplicia lerak fruit, gotu kola and neem seeds are purchased from simplicia shops. The parameters observed were the motility category a+b and viability of spermatozoa. Eosin is used for staining the viability of spermatozoa.

2.1.Extract Making

The pulp of lerak, gotu kola, and dried neem seeds is mashed in a blender. After all the ingredients have been blended and smooth, then the simplicia powder is wrapped in a cloth and macerated. The lerak pulp is weighed as much as 100 grams, then macerated using 1000 ml of 80% methanol as a solvent and stirred until homogeneous, then let stand for 3 days and stir frequently. Preparation of gotu kola extract and neem seeds is by weighing 100 grams of gotu kola extract and neem seeds then macerated using 1000 ml 80% ethanol solvent and stirred until homogeneous, then let stand for 3 days and often stirred. After 3 days, each extract water was separated from the extract and each filtrate was concentrated using a rotary evaporator.

2.2.Preparation of Experimental Animals

Before preparing the experimental animals, the place for keeping the experimental animals was first prepared like a rectangular cage, covered with husks, equipped with a gauze cover and a drinking bottle filled with clean water, a place for guinea pigs to eat, guinea pig feed, then the guinea pigs were acclimatized at room temperature. This period is carried out for 7 days with the aim that marmots can adapt to environmental conditions.

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2.3.Spermatozoa Collection

Male guinea pigs were sedated using chloroform, then operated on and both testes were taken. Furthermore, the epididymal cauda and the vas deferens are carefully sorted and accommodated in a watch glass containing washing sperm solution then put into a microtube.

2.4. Division of Treatment Groups

There were 5 treatments in this study, namely; (1) 200 µl suspension spermatozoa without being given any extract, (2) Suspension of spermatozoa 200 µl + 50 µl of lerak fruit extract 0.9%, (3) Suspension of 200 µl spermatozoa + 50 µl of gotu kola extract 0.9%, (4) Suspension of spermatozoa 200µl + 50 µl of 0.9% neem seed extract, and (5) 200 µl spermatozoa suspension + 50 µl of the third mixture extract 0.9% extract. Each treatment was incubated in a 1.5 ml microtube and observed for a time span of 1, 10, and 30 minutes

2.5. Research Treatment

Observation of spermatozoa motility was carried out by dropping each treatment onto a glass object and then covering it with a glass cover and observed under a microscope with a magnification of 400 times with a count of 100 spermatozoa. Spermatozoa motility is calculated using the assessment criteria (A) moving fast forward straight (B) moving slowly forward straight (C) moving where (D) is not moving. Observation of the viability of spermatozoa was carried out by dropping each treatment onto a glass object and given 1 drop of Eosin then mixed and covered with a glass cover. Observations were made using a microscope with a magnification of 400 times. The percentage of live spermatozoa is known by counting the number of live spermatozoa from 100 spermatozoa. Living spermatozoa are those that are not colored by Eosin.

3. RESULT

3.1. Spermatozoa Motility Category a + b

Anova test results showed that there was a significant effect ($P < 0.05$) of the extract of gotu kola, lerak fruit, neem seeds and the third mixture.

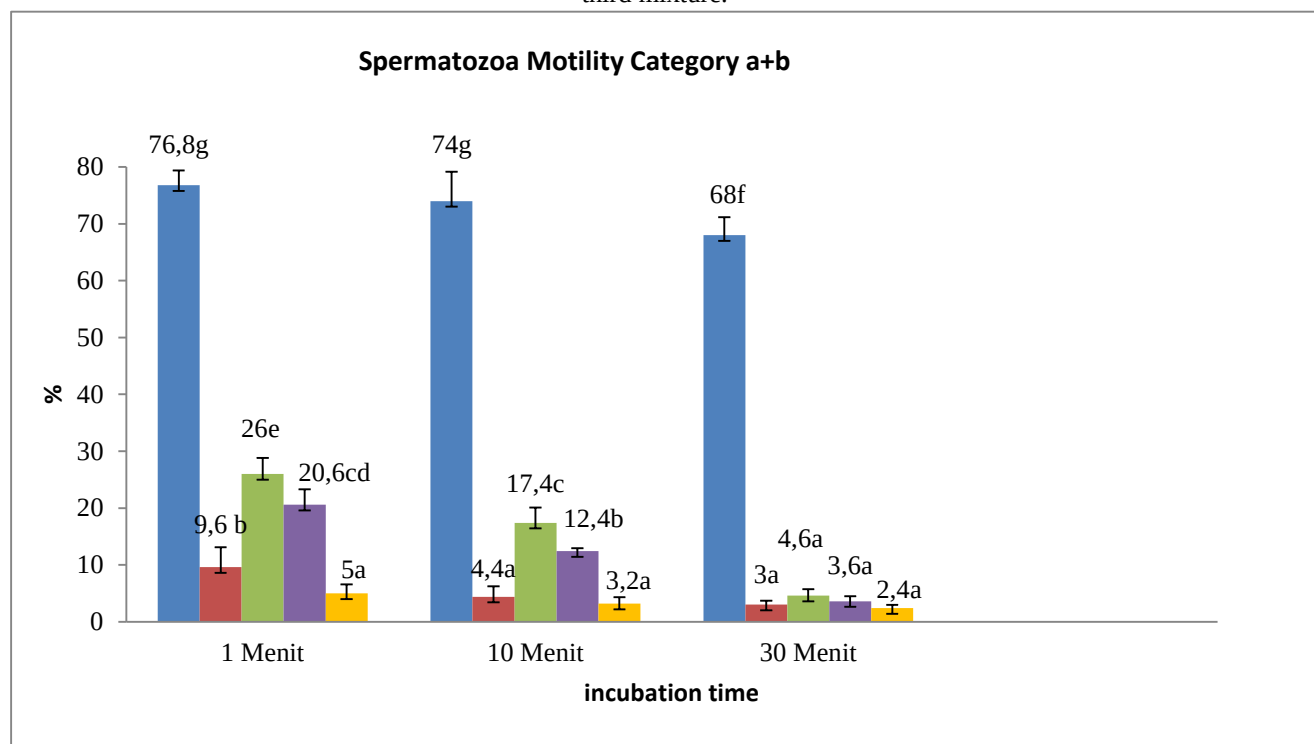


Figure 1: Effect of Lerak (*Sapindus rarak*), Gotu Kola (*Centella asiatica*), Neem Seeds (*Azadirachta Indica* A. Juss) and a mixture of the three extracts on Motility (%) Spermatozoa Guinea pigs (*Cavia porcellus*) Category a + b

3.2. Viability of Spermatozoa

The Anova test results showed that there was a significant effect ($P < 0.05$) of the extract of gotu kola lerak fruit, neem seeds and the third mixture

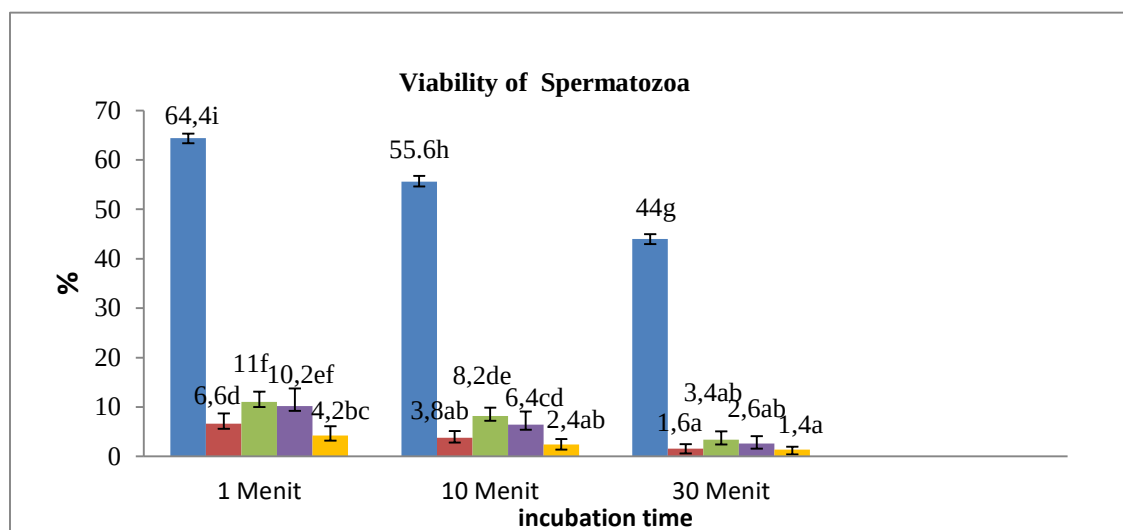


Figure 2: Effect of Lerak (*Sapindus rarak*), Gotu Kola (*Centella asiatica*), Neem Seeds (*Azadirachta Indica A. Juss*) and a mixture of the three extracts on the viability (%) of Spermatozoa Guinea pig (*Cavia porcellus*)

The results of this study indicate that the administration of lerak pulp (*Sapindus rarak*), gotu kola extract (*Centalla asiatica*), neem seed extract (*Azadirachta indica A. Juss*) and a mixture of the three extracts have an effect on spermatozoa motility in both category a + b and viability. guinea pig spermatozoa (*Cavia porsellus*). The decrease of spermatozoa motility in both category a + b and the highest viability of guinea pig spermatozoa occurred in the treatment of the mixture of the three extracts, either within 1 minute, 10 minutes or 30 minutes.

The decrease in spermatozoa motility is thought to be because the lerak extract contains saponin compounds.⁶ The decrease in spermatozoa motility occurs because triterpenoid saponins can cause damage to the spermatozoa membrane. Saponins work by binding to lipids and lipoproteins on the spermatozoa membrane, this can cause loosening of the spermatozoa cell membrane and disturbances in the cell nucleus resulting in a decrease in the integrity of the spermatozoa membrane, the integrity of the spermatozoa membrane disrupts the function of the cell membrane in regulating the transport of molecules into and out cells, this causes sperm motility to be inhibited¹⁵.

Spermatozoa motility is influenced by two factors, namely exogenous factors and endogenous factors. One of the most important endogenous factors is the availability of energy sources. Adenosine Tri Phosphate (ATP) is a source of energy used in the motility of spermatozoa. The active compounds contained in the extract of lerak pulp, gotu kola and neem seeds such as tannins can reduce the motility of marmot spermatozoa because these compounds have cytotoxic properties that can cause damaged spermatozoa¹⁶. Damage to spermatozoa occurs in the tail, which can reduce the motility of spermatozoa. Tannin is an astringent, so it can affect the permeability of the sperm cell membrane. Tannins reduce spermatozoa by causing shrinkage of the cell membrane, thereby disrupting the function of the cell membrane in transporting nutrients or nutrients so that cell metabolism will be disrupted and consequently energy is also reduced. The disturbance of cell metabolism in producing energy can cause the motility of spermatozoa to decrease, because the motility of spermatozoa requires energy¹⁷.

Alkaloid compounds can decrease the motility of spermatozoa¹⁸, also stated that the alkaloid content can interfere with the activity of the ATP-ase enzyme in the cell membrane in the middle of the spermatozoa tail¹⁹. The internal homeostasis of sodium and potassium ions is maintained by the presence of the enzyme ATP-ase. The disruption of membrane permeability is caused by disturbed homeostasis of sodium and potassium ions which is caused by disturbed activity of the ATP-ase enzyme. If the permeability of the sperm membrane is disturbed, it will disrupt nutrient transport. Disrupted nutrient transport will cause cell metabolism in producing energy to be disturbed, as a result the energy needed for spermatozoa movement is not fulfilled, so there will be a decrease in spermatozoa motility²⁰.

ATP is used by spermatozoa in addition to being a source of energy in the process of movement so that it can remain motile. ATP is also useful for maintaining its viability²¹. Spermatozoa viability is the vitality of spermatozoa. Living spermatozoa have a lipid bilayer layer on the cell membrane that can protect the entry of dye into the cell, so that the living spermatozoa cells are not

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stained with eosin, while the dead spermatozoa cells have damage to the cell membrane so that they can absorb the dye²². According to Susilawati (2000), the membrane functions as a cell protector, if there is structural damage to the membrane it can result in damaged organelles in the cell, such as mitochondria. Mitochondria serve as a place for cellular respiration that produces adenosine triphosphate (ATP)²³.

The condition for the survival of the spermatozoa is the integrity of the plasma membrane. If the spermatozoa metabolism is damaged, the spermatozoa metabolism will be disrupted²⁴. Chemical compounds such as saponins, alkaloids and tannins in extracts of lerak, gotu kola, and neem seeds can reduce motility, but also reduce the viability of spermatozoa. Saponin have an effect on decreasing the viability of spermatozoa because saponins can decrease the integrity of the spermatozoa membrane²⁵. The factor that can cause a decrease in the integrity of the spermatozoa membrane is thought to be because the triterpenoid saponin compounds which act as surfactants can dissolve membrane constituents such as lipids, proteins and carbohydrates directly so that the composition of the cell membrane is not intact.

The cytotoxic properties of tannins and alkaloids also result in decreased viability. Research by Ashfahani et al (2010) states that alkaloid compounds can result in impaired spermatozoa membrane permeability and decreased living spermatozoa which can interfere with nutrient transport needed by spermatozoa for survival²⁶. Research by²⁷ states that tannin compounds can bind to proteins and ions contained in the spermatozoa membrane which can cause the phosphorylation process in the spermatozoa membrane to be disturbed so that it can cause a decrease in the viability of living spermatozoa.

4. CONCLUSION

Based on the results of this study, the following conclusions can be drawn:

1. The administration of lerak pulp (*Sapindus rarak*), gotu kola extract (*Centalla asiatica*), neem seed extract (*Azadirachta indica* A. Juss) and a mixture of the three extracts had a significant effect on the motility of category a + b and viability of guinea pig spermatozoa (*Cavia porcellus*).
2. Mixed extract of lerak (*Sapindus rarak*), gotu kola extract (*Centalla asiatica*), extract of neem seeds (*Azadirachta indica* A. Juss) has the potential to reduce the motility of category a + b and viability of guinea pig spermatozoa (*Cavia porcellus*).

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