Short Communication:
Spermicidal properties of *Durio zibethinus* in the Mandiangin Forests, South Kalimantan, Indonesia

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²Department Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Lambung Mangkurat. Jl. A. Yani Km. 35.8 Banjarbaru 70714, South Kalimantan, Indonesia. Tel./Fax. +62-511-4773868

Abstract. Nurliani A, Kartinah N. 2017. Short Communication: Spermicidal properties of *Durio zibethinus* in the Mandiangin Forests, South Kalimantan, Indonesia. Biofarmasi (Rumphius J Nat Prod Biochem) 15: 26-28. Side effect caused by condom with synthetic spermicide encourages researcher to find alternative spermicide from plant which have fewer side effects. Bark of durian extract is potential candidate for herbal spermicide because it could decrease the percentage of human spermatozoa quality in vitro at the concentrations of 2%. This study was designed to evaluate its spermicidal activity by in vitro on motility, velocity of movement, viability and morphology of human spermatozoa. The formulation of gel with 2% of bark of durian extract were developed by using hydroxypropyl methylcellulose as gelling agent with 3 different concentrations, namely, 1.5; 2; and 2.5 %. Evaluation of gel preparations including physical appearance, viscosity, spreadability, and pH was done to obtain the best formula. Based on the evaluation, the best performance of gel was achieved by adding 2% of HPMC. Furthermore, the spermicidal activity of the gel with bark of durian extract was tested and compared to the bark of durian extract without gel, gel without bark of durian extract, and the fresh sperm as control. Formulation of gel with bark of durian extract significantly decreased all parameters of spermatozoa quality. Thus, formula containing 2% of bark of durian extract with HPMC 2% possesses appreciable spermicidal potential.

Keywords: Durian, gel, human sperm, Kalimantan, spermicide

INTRODUCTION

Since the 19th century, the condom has become one of the most popular contraceptive methods in the world and has been used for at least 400 years. To increase its effectiveness, some condoms are lubricated with spermicide chemicals such as nonoxynol-9 (Kestelman and Trussell 1991). However, recent studies indicate that nonoxynol-9 may potentially cause irritation and increase the risk of HIV (Asif 2013).

To resolve this problem, the efforts to develop safe and effective contraceptives must be done. Herbal contraceptive are in popular demand because they have fewer side effects. Previous investigation revealed that the barks of durian extract have spermicidal activity on human spermatozoa in vitro. The extract from bark of durian could decrease percentage of quality of human spermatozoa in vitro at the concentrations of 2% (Nurliani and Santoso 2010).

Gels are often used pharmaceutically as lubricants and as carriers for spermicidal agents (Esposito et al. 1996). Therefore, this study was designed to formulate and evaluate of spermicidal gel containing bark of durian extract for application in human as herbal spermicide in condom.

MATERIALS AND METHODS

Plant materials
The bark of durian was collected from the forest region of Mandiangin, South Kalimantan, Indonesia.

Chemicals
Ethanol (Merck Ltd), Hydroxypropyl Methyl Cellulose (HPMC), propylene glycol, glycerin, methyl paraben, propyl paraben, Hank’s Balanced Salt Solution (HBSS) Gibco®, eosin-Y, nigrosin, giemsa, and methanol.

Sample
Semen samples were donated by 6 healthy fertile men (25-30 years old). Semen samples were collected by masturbation in sterile glass cups after at least 3 days of sexual abstinence.

Preparation of bark powder
The bark was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts
Every 50 g of dried powder of bark was subjected to soxhlet apparatus. It was exhaustively extracted with 250...
mL of ethanol solvent in a soxhlet apparatus. The temperature was maintained at (60-70°C). The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.

**Preparation of gel formulation**

Formulation of spermicidal gel was obtained from mixture of HPMC, propylene glycol, glycerin, methyl paraben, propyl paraben, and 2 % of bark of durian extract. There are three formulas with various concentration of HPMC, i.e. 1.5% (F1); 2% (F2); and 2.5% (F3). HPMC was dispersed in distilled water with continuous stirring. Distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath. The solution was cooled, and then was added by glycerin and was mixed to fist solution. Further required quantity of durian bark extract was mixed to the above mixture and volume was made up to 100 mL by adding remaining distilled water. Finally, full mixed ingredients were mixed properly to the HPMC gel with continuous stirring and propylene glycol was added dropwise to the formulation for requiring consistency (Sudipta et al. 2011).

**Evaluation of gel formulation**

**Physical evaluation.** The color and odor of the prepared gels were checked.

**Measurement of pH.** pH of the gel was measured using pH meter.

**Spreadability.** Spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after one minute. The standard weight applied on the upper plate was 50 g.

**Viscosity.** Viscosity of gel was measured using Brookfield viscometer with spindle. The reading was taken at 1000 rpm using spindle no. 4.

**Sample preparation**

Semen samples were donated by 6 healthy fertile men (25-30 years old). Semen samples were collected by masturbation in sterile glass cups after at least 3 days of sexual abstinence. Spermatozoa which were free of seminal plasma were obtained by centrifugation at 1900 rpm for 20 minutes and adjusted in Hank’s Balanced Salt Solution (HBSS) media. Then it was made into pellet by microcentrifugation at 1000 rpm for 10 minutes (Purwaningsih 2000).

**Experimental procedure**

Semen samples were added to the gel of bark extract preparation and then assessed immediately for these parameters below.

**Sperm motility.** A drop of the evenly mixed sample was immediately placed on a clean and dry glass slide covered with cover slip. This slide was then examined under the binocular microscope (Olympus, Japan) at magnifications of x10, x40. At least five fields were rapidly examined and 100 spermatozoa were counted (Hyacinth et al. 2012).

**Movement velocity of sperm.** The velocity of spermatozoa was measured by calculating the time (seconds) needed by motile spermatozoa to reach 1 box microhaematocytometer. Velocity of spermatozoa is defined with micrometer per second unit.

**Sperm viability.** One drop of above treated sperm mixed with 2 drops of 1% EosinY. After 30 s, 3 drops of 10% Nigrosin solution was added. A drop of treated sperm-Eosin-Nigrosin mixture is placed on a clear microscope slide; allowed to dry and observed under microscope. Live spermatozoa had white heads and dead spermatozoa had heads that were stained red or dark pink. A total of 100 spermatozoa were evaluated manually on each slide at right-field optics at 400 x magnification (Ellasson 1977).

**Sperm morphology.** Sperm morphology of treated sperm was studied under the microscope using EosinY and Nigrosin staining method as described above. A drop of sperm-Eosin-Nigrosin mixture treated with gel of bark extract was examined separately at 400X under phase contrast microscope to record any change in morphology of the sperm (Jayendran et al. 1994).

**Statistical analysis**

Data were analyzed with SPSS system and presented as mean±standard deviation (SD). Statistical significance was evaluated with Non-parametric test (Kruskal-Wallis) and the difference was considered statistically significant at P<0.05.

**RESULTS AND DISCUSSION**

The result of physical evaluation of gel formulation can be seen in Table 1. The physical evaluation showed that all the formulations show similar organoleptic properties with brownish coloration due to the plant extract and a specific smell. The pH of the gel formulations was in the range of 4.61-4.96, which lies in the normal pH range of the skin and would not produce any skin irritation. The ideal value of pH for gel is 4.5-7 (Wasiatmadja 1997). Spreadability of gel was evaluated to test the ease of applicability of gels on skin. The spreadability of formulated gels was decreased as the concentration of polymer increased. Spreadabilities of formulated gels (F1, F2 and F3) were 5.26, 5.00, and 7.93 cm, respectively. The ideal value of spreadability for gel is 5-7 cm (Garg et al. 2002). Hence, spreadability of F2 and F3 formulation was better than F1 formulation. Viscosity is an important parameter for characterizing the gels as it affects the spreadability. The viscosity of gels was increase with the increase in polymer content which may be due to the increase in formation of three dimensional cross linking structure of gel, as expected. The ideal value of viscosity for gel is 2000-50.000 Cps (SNI 1996). By comparing the values of viscosity, the F3 formulation has an over value of viscosity. Viscosity of F1 and F2 formulation was more ideal than F3 formulation. According to the result of evaluation, the F2 formulation with 2% proportion of HPMC resulted in the best gel formulation.
The assessment of spermicidal activity from gel of bark durian extract can be seen in Table 2. The present study evaluated spermicidal properties of the gel with bark of durian extract and revealed a reduction (P < 0.05) in the percentage of motility, viability, normal morphology, and movement velocity of human spermatozoa. The results indicate that formulation of gel with bark of durian extract decreased percentage of motility, movement velocity, and viability of human spermatozoa significantly up to 0% and normal morphology of spermatozoa up to 20.17%. The percentage of motility, movement of velocity, viability, and normal morphology of human spermatozoa in gel preparation with bark of durian extract has significant difference with the bark of durian extract without gel; gel without extract and with control (Table 2). Gel with bark of durian extract has better spermicidal activity than the bark of durian extract without gel.

Phytochemistry screening of the extract of durian barks revealed the presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, and saponins. A large number of plants for spermicidal has been screened and a majority of plant-derived spermicides reported are triterpene saponins of several structural types, flavonoids and phenol compounds (Farnsworth and Waller 1982). The saponins of several plants have been reported to produce instant immobilization of human spermatozoa within 20 seconds (Primorac et al. 1985). Most plant-derived spermicides which caused inhibition of the sperm specific enzymes acrosin and hyaluronidase were confirmed to contain flavonoid. It is believed that the flavonoids and their derivatives, flavonones and flavonols, contain hyaluronidase inhibitory activity (Farnsworth and Waller 1982). The results indicate that the gel with bark of durian extract possesses strong spermicidal activity in vitro. However, mechanism of action from active components of bark of durian extract as spermicidal agents should be further evaluated.

Based on the spermicidal activity result, formulation of gel containing 2% bark of durian extract possesses appreciable spermicidal potential, which may be explored as an effective constituent of male contraceptive.

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