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The Effect of *Bajakah Kalalawit* (*Uncaria gambir)* Stem Extract on Spermatozoa Viability *In Vitro*

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Abstract

The prevalence process impairs sperm biochemistry and function by generating high levels of Reactive Oxygen species (ROS) and damaging sperm cells. In addition, ROS imbalance leads to decreased sperm viability, mitochondrial dysfunction, and DNA damage. The administration of *Bajakah Kalalawit* stem extract (*Uncaria gambir* (W. Hunter) Roxb.) is stated to maintain sperm quality. In vitro sperm viability tests have not been widely conducted. This study aims to determine the effect of *Bajakah Kalalawit* stem extract (*Uncaria gambir* (W. Hunter) Roxb.) on sperm viability in vitro. This research is experimental research, the type of design used is posttest only with control group design. The sample was determined by consecutive sampling and the samples taken were 15. The statistical test using LSD Post-Hoc test obtained between groups there were significant differences with $p < 0.05$. The compounds contained in the ethanol extract of *Bajakah Kalalawit* stem (*Uncaria gambir* (W. Hunter) Roxb.) are terpenoids, flavonoids, steroids, saponins and alkaloids. Viability of spermatozoa increased significantly from the concentration of 50ng/mL with a percentage of 74.13%, 100ng/mL by 83.00%, 500ng/mL by 87.60% and 1000ng/mL by 91.27%. The ethanol extract of *Bajakah Kalalawit* stem (*Uncaria gambir* (W. Hunter) Roxb.) can affect the viability of spermatozoa in vitro.

Keywords: Bajakah Kalalawit (*Uncaria gambir* (W. Hunter) Roxb.); Spermatozoa; Viability.

Abbreviations: Analisis One Way of Variance(ANOVA), Adenosine Triphospate(ATP), Cyclic Adenosine Monophosphate (cAMP), Cysteine Aspartate-Specific Proteases(Caspase), Biggers, Whitten, and Whittingham (BWW), Deoxyribonucleic Acid(DNA), Inhibition Concentration (IC), Intracytoplasmic Sperm Injection(ICSI), Intrauterine Insemination(IUI), In Vitro Fertilization(IVF), Reactive Oxygen species(ROS), Revolution per Minute(rpm).

INTRODUCTION

Fertilization is the ability of men and women to conceive offspring through sexual intercourse without the use of contraceptive methods. This process depends on the quality of both spermatozoa and oocytes. Fertilization failure may occur due to impaired sperm quality or oocyte dysfunction. According to data from the Indonesian National Population and Family Planning Board (2020), approximately two million couples in Indonesia experience infertility annually, with male factors contributing to about 50% of cases (Boitrelle et al., 2021; Puspitaningrum et al., 2022). Male infertility arises from various etiologies, including endocrine disorders related to hypogonadism (2–5%), sperm transport disorders (5%), primary testicular defects characterized by abnormal sperm parameters without a clear cause (approximately 65%), and idiopathic infertility in which sperm parameters appear normal (10–20%) (Leslie et al., 2024).

Advances in medical technology have led to the development of Assisted Reproductive Technologies (ART) to support infertile couples in achieving pregnancy. ART includes intrauterine insemination (IUI), intracytoplasmic sperm injection (ICSI), in vitro fertilization (IVF), and sperm cryopreservation (Assidi, 2022). The success of these techniques is highly dependent on sperm quality, particularly during processing and preservation. Cryopreservation, while essential in ART, can cause ultrastructural damage, oxidative stress, membrane disruption, and increased DNA fragmentation, leading to decreased sperm viability and fertilization capacity. Excessive production of reactive oxygen species (ROS) during freezing and thawing further contributes to mitochondrial dysfunction and lipid peroxidation, negatively affecting reproductive outcomes (Khan et al., 2021; Kumar et al., 2019). Therefore, strategies to reduce oxidative damage, such as antioxidant supplementation, are needed to improve post-thaw sperm quality. *Bajakah Kalalawit* (*Uncaria gambir* (W. Hunter) Roxb.) is a medicinal plant native to Central

Kalimantan, Indonesia, known to contain bioactive compounds including flavonoids, phenolics, tannins, terpenoids, saponins, and alkaloids (Rollando et al., 2022). Previous studies have demonstrated strong antioxidant activity of *Bajakah Kalalawit* extracts and their potential to enhance sperm motility (Permatasari et al., 2023) However, the effect of *Bajakah Kalalawit* stem extract on spermatozoa viability has not yet been investigated. Therefore, this study aims to evaluate the effect of *Bajakah Kalalawit* (*Uncaria gambir* (W. Hunter) Roxb.) stem extract on *in vitro* spermatozoa viability.

MATERIALS AND METHODS

Study area

The study entitled "The Effect of *Kalalawit Bajakah* Stem Extract (*Uncaria gambir* (W. Hunter) Roxb.) on *In Vitro* Spermatozoa Viability" was conducted at the Biomedical Laboratory, Faculty of Medicine, Palangka Raya University. This research was approved by the Health Research Ethics Committee of the Faculty of Medicine, Palangka Raya University (No. 44/UN24.9/LL/2024). It used an experimental design with a post-test-only control group design.

Procedures

Preparation of Bajakah Kalalawit Stem Extract

Kalalawit Bajakah (*Uncaria gambir* (W. Hunter) Roxb.) stems were randomly collected from Marang Subdistrict, Palangka Raya City, Central Kalimantan. The stems (2.5 kg) were washed, peeled, cut into 2–3 cm pieces, sun-dried, and ground into a fine powder. The powder was sieved and used as simplicia. Extraction was carried out by maceration using 96% ethanol for 3 × 24 hours, with solvent replacement every 24 hours. A rotary evaporator was used to concentrate the filtered extract. The resulting concentrated extract weighed 64.58 g.

Quantitative Phytochemical Screening of Bajakah Kalalawit Ethanol Extract

Quantitative phytochemical screening was performed on the ethanol extract of *Kalalawit Bajakah* (*Uncaria gambir* (W. Hunter) Roxb.) to identify and quantify its bioactive compounds. The analysis included flavonoids, terpenoids, alkaloids, steroids, and saponins. Total flavonoid content was determined using the aluminum chloride colorimetric method and measured by UV–Vis spectrophotometry at 510 nm, with quercetin used as the standard. Results were expressed as milligrams of quercetin equivalent per gram of extract, and measurements were performed in triplicate (Indra et al., 2019). Terpenoid content was analyzed by reacting the extract with chloroform and sulfuric acid, followed by incubation in the dark. Methanol was added, and absorbance was measured at 538 nm using a UV–Vis spectrophotometer (Hutasuhut et al., 2022). Alkaloid content was determined by acid extraction followed by complex formation with bromocresol green and chloroform extraction. The absorbance of the final solution was measured at 470 nm (Dewi, 2022). Steroid content was analyzed by reacting the extract with sulfuric acid, ferric chloride, and potassium hexacyanoferrate (III), followed by heating in a water bath at 70°C. Absorbance was measured at 780 nm (Hutasuhut et al., 2022).Saponin content was determined using thin-layer chromatography (TLC). The extract and standard saponin were spotted on a TLC plate, eluted using a chloroform–ethanol–ethyl acetate solvent system, and analyzed using a TLC scanner at 301 nm (Noer et al., 2018).

Sperm Preparation and Treatment

Semen samples from donors normozoospermia were collected in sterile containers and allowed to liquefy at room temperature for 30 minutes. The spermatozoa were then washed using a 50% Percoll gradient and centrifuged at 3000 rpm for 30 minutes. After centrifugation, the supernatant was discarded, and the pellet was washed with 3 mL of Biggers–Whitten–Whittingham (BWW) medium and centrifuged again at 3000 rpm for 20 minutes. The supernatant was removed, and the purified sperm pellet was resuspended in 1 mL of BWW medium and homogenized. Sperm concentration was determined by mixing 5 µL of washed spermatozoa with 95 µL of sperm diluting fluid. A 10 µL aliquot was placed in a Neubauer chamber, and sperm concentration was counted under a light microscope at 400× magnification following World Health Organization (WHO) standard semen analysis guidelines. The sperm samples were divided into five groups: a control group incubated with BWW medium only, and four treatment groups incubated with ethanol extract of *Bajakah Kalalawit* stem at concentrations of 50, 100, 500, and 1000 ng/mL. All groups were incubated at 37°C for 60 minutes (Permatasari et al., 2023; Permatasari & Pujianto, 2023).

Spermatozoa Viability Assessment

Sperm viability was evaluated using eosin Y staining. A 20 µL sperm sample was mixed with 20 µL eosin Y on a glass slide, covered, and air-dried for 1–2 minutes. Viability was assessed under a light microscope at 400× magnification by counting 200 spermatozoa. Live spermatozoa remained unstained, while non-viable spermatozoa showed red-stained heads. Results were expressed as percentages (Fransiskus et al., 2021).

Data analysis

Statistical analysis was performed using SPSS One-Way ANOVA test and the LSD post-hoc procedure on the collected data.

RESULTS AND DISCUSSION

Results of Bajakah Kalalawit Stem Extraction

In this study, the part of *Bajakah Kalalawit* used was the stem. The results of the *Bajakah Kalalawit* stem extraction are displayed in Table 1

Table 1. Results of Bajakah Kalalawit Stem Extraction.

Fresh Stem (Before Drying)	Dried Stem (After Drying)	Simplicia	Thick Extract
2.5 kg	2 kg	1 kg	64.58 g

Note: The ethanol extraction of Bajakah Kalalawit stem produced 64.58 g of extract with an extraction yield of 6.45%.

Results of Quantitative Phytochemical Screening of 96% Ethanol Extract of Bajakah Kalalawit Stem

Quantitative phytochemical screening was conducted to analyze the content of terpenoids, flavonoids, steroids, saponins, and alkaloids. The results of the quantitative phytochemical screening of the 96% ethanol extract of Bajakah Kalalawit stem (*Uncaria gambir* (W. Hunter) Roxb.) are presented in Table 2

Table 2. Results of Quantitative Phytochemical Screening of 96% Ethanol Extract of Bajakah Kalalawit.

Active Compound Analysis	Content in Bajakah Kalalawit Stem
Terpenoids (mg/mL)	192.800 ± 1.414
Flavonoids (mg/mL QE)	100.000 ± 0.354
Steroids (mg/mL)	54.121 ± 2.569
Saponins (%)	40.090 ± 0.665
Alkaloids (%)	28.075 ± 0.700

Results of the Effect of 96% Ethanol Extract of Bajakah Kalalawit Stem on *In Vitro* Spermatozoa Viability

The observation was conducted by assessing the viability of spermatozoa from a total of 200 spermatozoa per sample. Figure 1 showed that Spermatozoa that are alive are unable to absorb color, whereas those that are dead have red head.

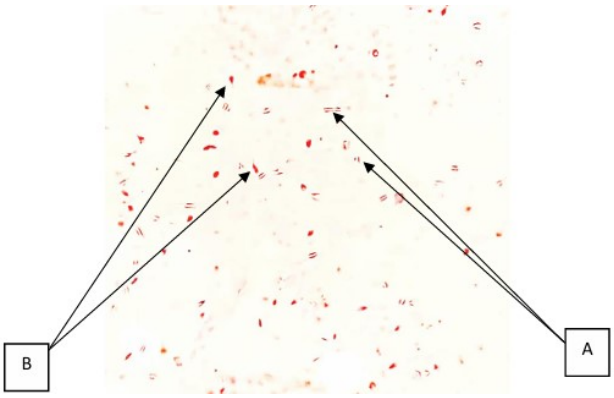

Figure 1. Microscopic images of live spermatozoa (A), which do not absorb eosin Y stain (unstained), and dead spermatozoa (B), which absorb eosin Y stain (red-stained head with a transparent tail). Magnification: 400×

In comparison to the control group, the one treated with a 96% ethanol extract of *Bajakah Kalalawit* stem had higher spermatozoa viability, as demonstrated in Table 3 and Figure 2.

Table 3. Effect of 96% Ethanol Extract of *Bajakah Kalalawit* Stem on *In Vitro* Spermatozoa Viability.

Concentration of extract of *Bajakah Kalalawit* stem	Samples (N)	Sperm Viability (Mean ± DS)
Control	15	62,93 ± 1,71
1000 ng/mL	15	74,13 ± 1,59 *
2000 ng/mL	15	83,00 ± 1,13 *
3000 ng/mL	15	87,60 ± 1,50 *
4000 ng/mL	15	91,27 ± 1,90 *

Notes: Superscript symbol (*) shows significant differences from the control (p<0.05) using LSD post-hoc test.

Based on the observations, administration of ethanol extract of *Bajakah Kalalawit* stem resulted in increased spermatozoa viability, as indicated by the absence of eosin Y uptake following incubation with extract concentrations of 50, 100, 500, and 1000 ng/mL, compared to the control group (BWW medium). An increase in viable (unstained) spermatozoa was evident starting at the 50 ng/mL concentration, with a mean viability of 74.13%, compared to 62.93% in the control group.

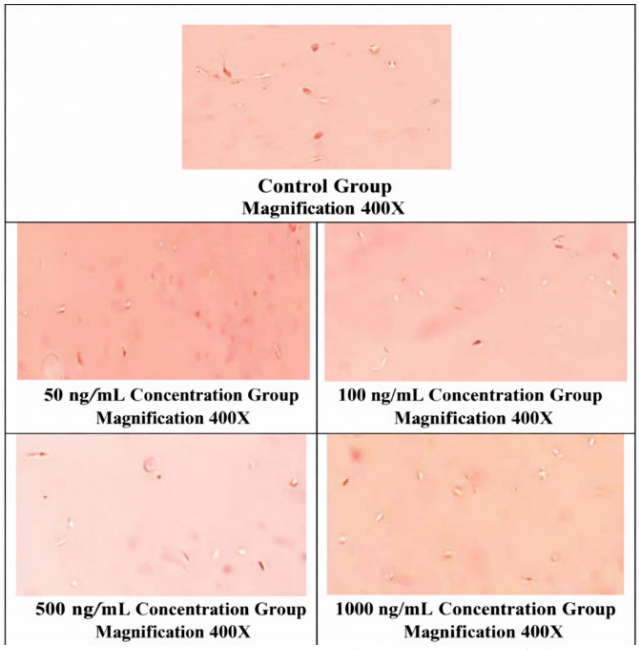

Figure 2. Microscopic appearance of spermatozoa viability among treatment groups.

Discussion

Bajakah Kalalawit (*Uncaria gambir* (W. Hunter) Roxb.) stems were collected from Marang Subdistrict, Palangka Raya, Central Kalimantan, and processed into simplicia before extraction using 96% ethanol via maceration. This method was selected due to its simplicity, efficiency, and ability to preserve thermolabile bioactive compounds (Kurniawati, 2019). Ethanol was chosen as the solvent because of its capacity to extract polar, semi-polar, and non-polar compounds.

Spermatozoa viability testing revealed that administering stem extract from *Bajakah Kalalawit* increased the proportion of viable spermatozoa when compared to a control group that did not receive extract. A higher concentration of the ethanol extract of the stem of the *Bajakah Kalalawit* plant results in an ever-increasing vitality. The vitality of spermatozoa can be affected dose-dependently by administering an ethanol extract of the stem of the Bajakah Kalalawit plant. This affects the efficacy and enhancement of the extract at different concentrations, which is marked by an upregulation of spermatozoa that are either colorless or do not absorb the eosin Y dye. In one ejaculation, the average proportion of viable spermatozoa is 54% according to WHO guidelines (Hajizah et al., 2014). In vitro fertilization (IVF) success rates may be improved with the inclusion of stem extract from *Bajakah Kalalawit*. The stem metabolite components of *Bajakah Kalalawit*, which include terpenoids, flavonoids, steroids, saponins, and alkaloids, might be responsible for this action. The antioxidant properties of these secondary metabolite molecules have a wide range of actions that can influence the viability of spermatozoa.

Quantitative phytochemical analysis revealed that the ethanol extract contained terpenoids, flavonoids, steroids, saponins, and alkaloids, all of which possess antioxidant properties. Flavonoids scavenge free radicals through hydrogen donation and may function as phytoestrogens that stimulate sperm function (Kartika et al., 2020). The components discovered in *Bajakah Kalalawit* are flavonoids quercetin and catechins, which function as phytoestrogens (Hasanah, 2019). They are both phytoestrogens that function as flavonoids. The estrogen receptors (ER)α and ERβ on capacitated and uncapacitated spermatozoa can be bound to by phytoestrogens, although their affinity is lower than that of estrogen. Spermatozoa functions like motility, acrosome response, and capacitation can be enhanced when phytoestrogens attach to the endoplasmic reticulum (ER) (Skibińska et al., 2023). Terpenoids act as primary antioxidants by inhibiting ROS chain reactions (Kartika et al., 2020). Wijayanti et al, found that antioxidant terpenoid chemicals found in the n-hexane fraction of parijoto can boost spermatozoa motility and shield their membranes from damage (Wijayanti et al., 2021).

Other phytochemical component of *Uncaria gambir* is steroid, saponins, and alkaloids. Steroid are believed to enhance the survivability of spermatozoa by raising testosterone levels, which are involved in spermatogenesis (Syarif et al., 2016). After 120 minutes of incubation at concentrations of 40 and 50 mg/mL in vitro, research by Khaleghi et al, shown that *Tribulus terrestris* can enhance sperm vitality (Khaleghi et al., 2017). A study conducted by Pujianto et al, demonstrated that the steroid hormone progesterone, when administered, reduced caspase 3, hence inhibiting apoptosis (Pujianto et al., 2019). The protease enzyme caspase is an essential regulator of the cell death cascade. As a prosurvival factor in spermatozoa, steroids activate the phosphatidylinositol-3-OH kinase pathway (PI3K)/Akt, which in turn demonstrate antiapoptotic effects with androgen receptors on the surface of spermatozoa cells (Schwartz et al., 2016). Steroids capacity to enhance transcription of anti-apoptotic genes including Bcl-2, Bcl-XL, and Mcl-1 is indicative of their antioxidant effect. This helps prevent the apoptotic process by inhibiting caspase activity. Furthermore, steroids can disrupt both intrinsic and extrinsic apoptotic pathways by suppressing the expression and activity of pro-apoptotic genes such as Fas, FasL, Bax, Bak, and Bok. Internal stress signals, like as oxidation, DNA damage, or starvation, set off the intrinsic apoptotic cascade. In response to these cues, cytochrome c is released by the mitochondria. The apoptosome complex is formed when this cytochrome c interacts to the Apaf-1 protein. Caspase-9 is further activated by this apoptosome complex, which further activates caspase-3 and caspase-(Akar et al., 2021).Saponins contribute to antioxidant defense and may enhance testosterone synthesis and as antioxidant can effectively decrease superoxide by producing hydroperoxide intermediates (Kumaradewi et al., 2021). According to Oyeyemi et al,

infertile male rats can experience a rise in fertility after being administered saponin, which is characterized by an increase in spermatozoa concentration and motility (Oyeyemi et al., 2015). Derbak et al, discovered that alkaloids protect ram spermatozoa from lipid peroxidation, keep their membranes intact, and promote sperm motility. Nitrogen atoms in alkaloids have one pair of electrons, which helps reduce ROS activity in the body (Derbak et al., 2021). Similar to flavonoids, alkaloids are main antioxidants; however, alkaloids have the ability to donate hydrogen atoms to free radicals (Kumaradewi et al., 2021).

CONCLUSIONS

Bajakah Kalalawit stem extract (*Uncaria gambir* (W. Hunter) Roxb.) contains terpenoids, flavonoids, steroids, saponins, and alkaloids, all of which slightly increased the viability of spermatozoa at dose dependent with 50 ng/mL identified as the effective concentration.

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