Spermicidal and Antibacterial Effects of *Oncoba welwitschii* Oliv. (Salicaceae)

Odette N. Kabena¹, Naomie E. Nyakembe¹, Jean Jacques D. Amogu¹, Lionel S. Asamboa¹, Emmanuel M. Lengbye¹, Justin E. Mboloko², Pius T. Mpiana³ and Félicien L. Lukoki¹

¹Department of Biology, Faculty of Sciences, University of Kinshasa, P.O.Box 190, Kinshasa XI, Democratic Republic of the Congo.
²Department of Gynecology, Faculty of Medicine, University of Kinshasa, P.O.Box 834, Kinshasa XI, Democratic Republic of the Congo.
³Department of Chemistry, Faculty of Sciences, University of Kinshasa, P.O.Box 190, Kinshasa XI, Democratic Republic of the Congo.

**Authors’ contributions**

This work was carried out in collaboration among all authors. Authors NEN, LSA and ONK designed the study and wrote the protocol. Authors PTM, FLL and JEM wrote the first draft of the manuscript. Authors JJDA and EML managed the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

**Aim:** The aim of this work is to evaluate the spermicidal, antimicrobial activity of *Oncoba welwitschii* Oliv. This will justify its use on reproductive health especially in the occurrence of infertility and the imbalance of vaginal flora. Carried out at the Department of Biology of the Faculty of Sciences of the University of Kinshasa, between January and May 2019, this study was initiated to determine the effect of the use of *O. welwitschii* Oliv. on human spermatozoa and vaginal microorganisms.

**Methodology:** The leaves of *O. welwitschii* Oliv. were collected and identified at University of Kinshasa. The spermicidal effect was performed by the Sander-Cramer method and antibacterial activity were evaluated by liquid dilution method.
However, these products are generally not directly or indirectly from these natural resources. Nowadays, many scientists are using medicinal plants to synthesize new molecules. More than 25% of the available medicines are derived from medicinal knowledge based on traditional plant and animal know-how [1]. Recent findings revealed that over 80% of the African population relies on medicinal plant species for their primary healthcare [2-4]. The Democratic Republic of the Congo (DRC), through its cultural diversity, the richness and diversity of its flora and its wildlife, constitutes a real reservoir of biodiversity as indicated by the results of recent works, which is why it has been allowed to occupy a privileged place among the countries of the Congo Basin as traditional plant and animal based medicinal knowledge with traditional plant-based know-how medicinal and/or animal [5]. Nowadays, many scientists are using medicinal plants to synthesize new molecules. More than 25% of the available medicines are derived directly or indirectly from these natural resources. However, these products are generally not always available, or when they are, they are expensive for the population [6].

Hence, the plants used for intimate hygiene would be a source for the development of new molecules. Kumar et al. (2012) reported in the literature a list of 577 plants with anti-fertility potential documented worldwide [7], most of which have demonstrated their action on the properties of semen. Oncoba welwitchii Oliv. is a plant used by women in the Democratic Republic of the Congo to wash their genitals [8], and in the treatment of abscesses [9]. It is within this framework that we have chosen this plant in order to scientifically determine its importance in the resolution of problems related to the functioning of the female reproductive system.

In the perspective of preserving the ethnomedical cultural heritage of the Democratic Republic of Congo, our study was undertaken to evaluate both the chemical composition of Oncoba welwitchii Oliv. leaves, determine the pH of the juice, assess the antibacterial activity of their juice, and use it for intimate hygiene.

Results: The results revealed that the juice of the leaves of O. welwitchii Oliv. have a spermicidal effect, as this juice reduce the initial mobility of the spermatozoids from 72±12% to 1±2% and their initial viability from 74±11% to 10±18%. This juice has shown bacteriostatic effects against *Escherichia coli* and *Staphylococcus aureus* (MBC/MIC > 4) and bactericidal effects against *Lactobacillus acidophilus* (MBC/MIC ≤ 4).

Conclusion: This study gives additional information on the phytochemistry composition and a spermicidal effects of *O. welwitchii* Oliv. The spermicidal effects of this plant could be associated to its acidity.

It should be noted that to the best of our knowledge, no study on the phytochemistry of *O. welwitchii* Oliv. is available in the literature.

Keywords: Intimate hygiene; medicinal plant; antibacterial activity; spermicidal activity; Oncoba welwitschii olive.

1. INTRODUCTION

Natural products have contributed extensively to the development of new drugs. Therefore, considerable importance has been placed on investigating medicinal plants for bioactive compounds [1]. Recent findings revealed that over 80% of the African population relies on medicinal plant species for their primary healthcare [2-4]. The Democratic Republic of the Congo (DRC), through its cultural diversity, the richness and diversity of its flora and its wildlife, constitutes a real reservoir of biodiversity as indicated by the results of recent works, which is why it has been allowed to occupy a privileged place among the countries of the Congo Basin as traditional plant and animal based medicinal knowledge with traditional plant-based know-how medicinal and/or animal [5]. Nowadays, many scientists are using medicinal plants to synthesize new molecules. More than 25% of the available medicines are derived directly or indirectly from these natural resources. However, these products are generally not always available, or when they are, they are expensive for the population [6].

Hence, the plants used for intimate hygiene would be a source for the development of new molecules. Kumar et al. (2012) reported in the literature a list of 577 plants with anti-fertility potential documented worldwide [7], most of which have demonstrated their action on the properties of semen. *Oncoba welwitchii* Oliv. is a plant used by women in the Democratic Republic of the Congo to wash their genitals [8], and in the treatment of abscesses [9]. It is within this framework that we have chosen this plant in order to scientifically determine its importance in the resolution of problems related to the functioning of the female reproductive system.

In the perspective of preserving the ethnomedical cultural heritage of the Democratic Republic of Congo, our study was undertaken to evaluate both the chemical composition of *Oncoba welwitchii* Oliv. leaves, determine the pH of the juice, assess the antibacterial activity of their juice, and use it for intimate hygiene.
and the spermicidal effects of the juice of this plant.

2. MATERIALS AND METHODS

2.1 Materials

Semen samples were provided by volunteer participants at the Reproductive Endocrinology and Physiology Laboratory (ENDOPHYRE) of Department of Gynecology, Faculty of Medicine, University of Kinshasa.

The bacterial strains were isolated from the vaginal secretions of women and preserved at the Laboratory of Microbiology Applied to Biological and Natural Resources of Department of Biology of the Faculty of Sciences at University of Kinshasa.

The activity of the plant samples was tested toward Staphylococcus aureus (S. aureus), Escherichia coli (E. coli) and Lactobacillus acidophilus (L. acidophilus) strains.

The young leaves of Oncoba welwitschii Oliv were harvested from the experimental garden of the faculty of the University of Kinshasa, located in the commune of Lumba, province of Kinshasa, DRC. This sample was identified at the Laboratory of Botany and Plant Systematics, Department of Biology, Faculty of Sciences, University of Kinshasa.

2.2 Methods

2.2.1 Extraction of the plant juice

The leaves of Oncoba welwitschii Oliv. were cleaned with water and the juice was extracted after grinding using a porcelain mortar and pestle. Whatman 1 filter paper of size Ø: 10 mm was used to collect the juice used for the experiments. The concentration (20 mg / mL) of this juice from the fresh leaves of O. welwitschii Oliv. was determined after evaporation of 100 mL of the filtrate in an oven at 40°C.

2.2.2 Determining the acidity of the juice

10 ml of the juice from the crushed Oncoba welwitschii Oliv. leaves were distributed to 3 test tubes. Using pH indicator paper, the acidity of the juice was taken.

2.2.3 Phytochemical screening

The phytochemical screening is a chemical screening that includes a number of qualitative analysis that allows the identification of secondary metabolites (alkaloids, saponins, total polyphenols, flavonoids, tannins, anthocyanins, leuco-anthocyanins, quinones, terpenes and steroids) present in a certain sample [10,11]. The detection of these chemical groups is performed through color and precipitation reactions occurring with the addition of specific reagents. This phytochemical screening was carried out according to the standard protocol as modified by Ngbolua et al. [12].

2.2.4 Evaluation of spermatozoa's proprieties

2.2.4.1 Selection of sperm samples to analyze

Semen samples were provided by the Endocrinology and Reproductive Physiology Laboratory of the University Clinics of Kinshasa (C.U.K.). These samples were examined on the basis of criteria meeting the standards for good semen quality according to the World Health Organization [13].

In particular, for motility, the spermatozoa are identified in class a (linear and progressive), b (nonlinear and progressive) and those of class (motility in place) and must represent at least 60% of the motility. Progressive motility is the percentage of sperm moving in a straight line under their own power, which is a visual estimate under a microscope. For viability, semen samples must display at least 70% of the sperm alive.

The evaluation of the sperm-immobilizing or spermicidal effect of the juice of the plant studied was made by following the evolution of motility and viability, as a function of time under the microscope, of the spermatozoa in different small squares of the Nebauer slide [14].

Microscopic observation and counting of sperm stained in the presence of eosin and nigrosin made it possible to monitor their viability. The count of non-viable (heads stained red or purple) and viable (unstained heads) was determined by the Williams test or the eosin and nigrosin methods.
2.2.4.2 Assessment of mobility and initial viability

A 0.5 ml amount of the juice was taken with a graduated pipette and mixed with 1 mL of semen in the test tube and allowed to stand at 37°C for 1 minute. Then a drop of this mixture was placed on the Nebauer slide. Microscopic observation was made at 40X magnification. Thus, this experiment was performed for 1 hour. It should be noted that the control consisted of 1 ml of sperm and 0.5 ml of physiological water.

The spermicidal effect was evaluated by the Sander-Cramer method and was observed by adding to the mixture made in the same way as the previous one, a drop of Eosin was taken with a Pasteur pipette and thirty seconds later a drop of nigrosine was added. The observation was made at 40X magnification.

This same process is repeated after thirty minutes and one hour considering as the starting time, the mixing time. For the control, Eosine and Nigrosine were added (Williams test). The mobility rate is obtained by calculating the average number of motile spermatozoids during microscopic observation.

The mean (n) of motile sperm counted in three microscopic fields is given by:

\[ n = \frac{S_1 + S_2 + S_3}{3} \]

The percentage of mobility is given by:

\[ \% M = \frac{S_1 + S_2 + S_3}{3} \times 100 \]

While viability was obtained by comparing the mean number of dead sperm observed during the experiment.

The mean (n) of dead sperm cells counted in three microscopic fields is given by:

\[ n = \frac{S_1 + S_2 + S_3}{3} \]

The percentage of dead sperm is given by:

\[ \% M = \frac{S_1 + S_2 + S_3}{3} \times 100 \]

2.2.5 Evaluation of the antibacterial effect of the juice

2.2.5.1 Determination of minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration (MIC) was determined by broth micro-dilution method as reported in our previously research work [15].

The inocula of used microorganisms were prepared from 24 hours old broth cultures.

The prepared microbial suspension was diluted (1/100) to achieve 10^5 CFU/ml. Stock solutions of the plant extracts were prepared in Tween 80 (Fisher chemicals) (3 mg/300 μl) and diluted to 2.7 ml with Mueller Hinton Broth (MHB) to achieve a Tween 80 final concentration of 0.1%. This solution was transferred in 96-wells plates (200 μl/well) and two-fold serially diluted with MHB to give final concentrations ranging from 1000 to 4 μg/ml.

2.2.5.2 Determination of the minimum bactericidal concentration (MBC)

The MBC was determined by streaking 10 μL of the contents of the microplate wells on nutrient agar with no visible bacterial growth from the MIC. The inoculated plates were incubated for 24 hours at 37°C. The lowest concentration at which there was no growth in the subculture was considered to be MBC.

2.2.5.3 Determination of bactericidal or bacteriostatic character

The liquid microdilution method allowed us to determine the minimum inhibitory concentration (MIC). Growth, in solid medium, of the cultures obtained with MICs made it possible to determine the minimum bactericidal concentration (CMB). These two variables were used to indicate whether the effect of the juice is bacteriostatic or bactericidal in nature, against bacteria. When this CMB / CIM ratio is greater than 4 but less than 16, the product is said to be bacteriostatic; if this ratio is less than or equal to 4, the drug is considered to be bactericidal.

On the other hand, when it is equal to 1, the juice is said to be absolute bactericidal [16]. However, when this ratio is equal to or greater than 32, the bacteria are tolerant to the extract [17].

3. RESULTS AND DISCUSSION

3.1 Phytochemistry

The phytochemical analyses performed on Oncoba welwitschii Oliv. leaves revealed the presence of alkaloids, total polyphenols, flavonoids, tanins, anthocyanins, leuco-anthocyanins, quinones. The presence of various secondary metabolites in the plant could justify its medical use. Indeed, Oncoba welwitschii Oliv. is reported to treat bacterial infections [18].
Compounds, which are significantly present in the plant, are well known for their large spectrum of pharmacological properties, including antimicrobial (alkaloids) and antioxidant (polyphenols) activities [11].

### 3.2 Acidity of *Onocoba welwitschii* Juice

The average pH of the leaf juice was 5. These data deviate from the range of pH values of the vaginal cavity. The literature reports vaginal acidity values ranging from pH 3.8 to 4.5; which could play a bacteriostatic role in preventing the proliferation of most microorganisms [19]. Analysis of our acidity results can prove that the use of the leaves of *Onocoba welwitschii* Oliv. in intimate hygiene does not contribute to the physiological maintenance of the balance of the vaginal pH.

According to Kabena (2016), *Lactobacillus* ssp are acid-tolerant microorganisms. The use of the juice of *O. welwitschii* Oliv. for intimate care would not damage these bacteria that protect women's vaginal health. Moreover, the acidity of this juice could have a selective inhibitory effect on the growth of certain bacterial strains [13].

It has been shown that the optimal pH for the growth of *S. aureus* is about 7, while *E. coli* grows best at pH 6-8 [20].

Based on these observations, we say that the acidity of this juice could disrupt the growth of *E. coli* and *S. aureus* species that could colonize the vagina and the destruction of spermatozoids due to its phytochemical composition. In addition, since the pH of the semen varies between 7.2 and 8.0 [12], the acidity of the juice of this plant species used has spermicidal or sperm-immobilizing activity on human spermatozoids. This shows that it does not contain saponins, a group of phytoarkers known for their spermicidal power, which would justify that the destruction of spermatozoids would be due to the acidity of the juice.

### 3.3 Immobilization and Spermicidal Effect

Spermatozoa motility and viability as function of time are shown in Figs. 2 and 3.

Those figures show that sperm motility and viability decreases with time. In fact, the longer the contact time increases, the lower the percentages of motility and viability decrease. It should be noted that after 60 minutes of exposure of the spermatozoa to the *Onocoba welwitschii* Oliv. leaves juice, their initial motility decreases from 72±12% to 1±2% and their initial viability from 74±11% to 10±18% respectively. Those parameters were observed in comparison with the control (physiological solution). However, we note that the immobilized spermatozoids did not necessarily die after 60 minutes. It should be noted that the spermatozoids lost their initial motility at 49% and their initial viability at 45% in the presence of this juice, compared to the physiological solution.

![Fig. 2. Evolution of sperm viability and motility in the presence of the *Onocoba welwitschii* Oliv. juice](image-url)
This figure shows that the rates of reduction in sperm motility and viability increase over time. We noticed that within 1 minute of exposure, there was a loss of motility in almost half (48%) of the cells present. This discovery teaches us the remarkable spermo-immobilizing effect of the juice. In contrast, within 60 minutes, 92% of the cells present became immobile while 82% were destroyed.

By combining the evolution of motility and viability, our results do not reveal an instantaneous immobilizing effect or total spermicidal activity. However, the rate of reduced mobility and the rate of reduced viability of spermatozoa change over time.

With the single concentration (20 mg/ml) of the leaves of *Oncoba welwitschii* Oliv., we did not notice a reduction rate of immobilization similar to the rate of sperm death. We believe that the properties of spermatozoa in the presence of juice are affected by the exposure time, the phytochemical composition of the leaves as well as their acidity.

According Zhou et al. [21] an acidic environment can reduce spermatozoid motility. This leads us to attribute the loss of properties of the samples analyzed to the acidity of the juice of *Oncoba welwitschii* Oliv. given that saponins are absent.

Dubois-Bunel et al. [22] reported that changes affecting mainly the sperm head were observed when comparing sperm groups treated with saponins from a plant (*Cestrum parqui*) with controls [20]. Morphological alterations are remarkable in sperm when treated with 200g/L of saponins [23].

For them, the spermicidal effect can probably be explained by the hypo-osmotic conditions created by the plant, resulting in an increase in the volume of the sperm cell due to the consequent entry of water. This in turn leads to membrane lysis of the spermatozoids and the loss of their fertilizing power.

### 3.4 Antibacterial Activity

The Table 1 gives the results of the antibacterial activity of the juice of *O. welwitschii* Oliv.

Analysis of this table shows that the strains of *Escherichia coli* and *Staphylococcus aureus* tested are sensitive to the plant extract and standard used at a minimal inhibitory concentration (MIC = 4 μg/mL). According to Bourgoin et al. (2016), the MBC/MIC ratio should be between 4 and 16, which is in agreement with our results which indicate that the *O. welwitschii* Oliv. juice has bacteriostatic activity against these two strains, notably *Escherichia coli* and *Staphylococcus aureus*. On the other hand, this extract showed bactericidal (MBC/MIC ≤4) effects against *Lactobacilus acidophilus* and bacteriostatic (MIC = 31.2 μg/ml) [17]. Although acid-tolerant at plant concentrations above 16 μg/mL, *L. acidophilus* was inhibited due to the chemical composition of the leaves of this plant species.
Fig. 4A. Live spermatozoa  
Fig. 4B. Die spermatozoa

Table 1. Ratio MBC/ MIC of the juice of O. welwitschii Oliv. against microorganism strains

| Microorganism strains | MIC (μg/ml) | MBC (μg/ml) | MBC/MIC (μg/ml) |
|-----------------------|------------|-------------|-----------------|
|                       | Juice of the O. welwitschii | Standard |
| Escherichia coli      | 4          | 4           | 20              | 5               |
| Staphylococcus aureus | 4          | 4           | 28              | 7               |
| Lactobacillus acidophilus | 31.2   | 4           | 4               | 0.12            |

Legend: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The in vitro sensitivity of Lactobacillus acidophilus to this juice was observed at a low dose, demonstrating that the use of Oncoba welwitschii Oliv. leaves as prepared by women as a juice for intimate care would damage the strain of L. acidophilus that contributes to the natural protection of the vagina. The action of this juice, for the well-being of the woman, should have been directed specifically towards pathogenic microorganisms.

4. CONCLUSION

The work focused on determining the qualitative chemical composition of the juice of the leaves of Oncoba welwitschii Oliv. on the one hand and on the other hand to evaluate the spermicidal or sperm-immobilizing and antibacterial activities of the juice of this plant used by women for their intimate hygiene in order to contribute to the valorization of this plant species used in traditional medicine in the Democratic Republic of the Congo.

This study showed that, Oncoba welwitschii Oliv. contains various secondary metabolites such as total polyphenols, flavonoids, quinones, tannins, anthocyanins, leucoanthocyanins; terpenoids; steroids; and alkaloids.

Against Escherichia coli and Staphylococcus aureus the juice showed a bacteriostatic effect, while the antibacterial activity is bactericidal in nature against Lactobacillus acidophilus (MBC/ MIC≤4). The extract of the leaves of this plant exhibited sperm-immobilizing and a spermicidal effect.

It is therefore necessary to carry out additional phytochemical studies in order to search for active anti-fertility compounds which could lead to the formulation of a contraceptive.

ETHICAL APPROVAL AND CONSENT

The samples were provided by volunteer participants at the Reproductive Endocrinology and Physiology Laboratory (ENDOPHYRE) of Department of Gynecology, Faculty of Medicine, University of Kinshasa, on the basis of their consent and the authorization of the Ethics Committee of the Department of Biology of the Faculty of Sciences.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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