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Feminine intimate hygiene plants from Kinshasa / DR Congo: Potential source of contraceptive compounds

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Abstract

This study is part of the promotion of local medicinal plants by highlighting their spermicidal activity. An ethnobotanical survey carried out in Kinshasa made it possible to identify the plants used in female intimate hygiene. On the one hand, the aqueous extracts of the organs of ten (10) plants on the inventoried species were subjected to a phytochemical analysis. On the other hand, the recipes as applied by women as well as the total extracts of these plants have come into contact with human sperm. The analysis of the mobility and the evaluation of the viability of the sperm of the ejaculates, of good quality according to the WHO, were carried out according to the methods of Sander-Cramer and that of Williams, respectively. Thus, the concentration (EC_{100}) capable of killing 100% of sperm within 30 minutes of exposure was detected. Thirty-eight (38) plant species have been identified. From thirteen sought-after phytochemical groups, extracts of *Mangifera indica* and *Bridelia ferruginea* contain twelve and eleven respectively. Four (4) groups of metabolic compounds with a contraceptive effect are present in all ten extracts of feminine hygiene plants. Three (3) herbal recipes (*Abrus precatorius*, *Bridelia ferruginea* and *Ocimum basilicum*) out of the ten studied resulted in the cancellation of sperm mobility after 3 hours of exposure. And, the EC_{100} is defined at 200 mg/mL for *A. precatorius* and 25 mg / mL for *B. ferruginea.*

The results of this study show that feminine intimate hygiene plants contain compounds with spermicidal potential, which can be used for the development of vaginal contraceptives.

Keywords: Plants; Intimate Hygiene; Contraception; Spermicidal; Secondary Metabolites

1. Introduction

Every year, some 80 million women worldwide have an unintended pregnancy, and 60% of these are arboted [1]. Rapports indicate that the proportion of women reporting unintended pregnancies and unmet need for contraception remain high, while many women are dying annually from pregnancy related complications, of which 99% occur in developing countries [2].

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Efforts have been taken to tackle this serious problem by developing antifertility agents called contraceptive; those chemical substances that inhibit either the sperm production or sperm motility in males or prevent the formation of ovum and produce some changes in the endometrium, making it unsusceptible to a fertile ovum in female [3]. Although contraceptive drugs are today widely used, they still present a series of undesired side-effects. Safer contraceptives are becoming increasingly important in countries where there is a growing consciousness of the benefits of family planning, both individual and social contexts [4]. Synthetic hormonal contraceptives cannot be used continuously because of their health related negative effects, like increased blood levels of transaminases and cholesterol, dyspepsia, headache, depression, tiredness, weight gain, hyper menorrhea and intermenorrheal hemorrhage and also distrib the metabolism of lipid, protein, carbohydrate, enzyme and vitamins [5]. Therefore, scientists are on the hunt for newer alternative, with lesser side effects, self-administrable, less expansive and with complete reversibility. Much of these properties are observed in drugs of natural plant origin. Many plants are reported to have regulator activity [6].

Plants having such properties may have role in rapid decharge of the fertilized ova from the fallopian tube, inhibition of implantation due to interruption in estrogen-progesterone balance, fetal abortion due to lack supply of nutrients to the uterus and the embryo, and also on the male by affecting sperm count, motility, and viability. In recent years, many workers have reported a lot of traditional plant used for antifertility purpose [7-9]. Kumar et al., (2012) published a list of five hundred and seventy-seven (577) plants with anti-fertility potential documented around the world, most of which have been shown to act on the properties of spermatozoa [10].

Thus, in order to offer an alternative to traditional family planning methods, the present study contributes to the enhancement of local plants as a source of compounds capable of reducing the probability of conception or of preventing it. We have set ourselves the goal of determining the spermicidal effect of some of the women's intimate hygiene plants used in Kinshasa.

2. Material and methods

2.1. Survey of women and sellers of medicinal plants

We interviewed from January to December 2012 and on the basis of their consent, women we met across the city as well as sellers of medicinal plants in six main markets in Kinshasa. The names of the plants, their method of preparation and administration as well as the purpose of their use are mentioned. The specimens of various plants mentioned have allowed their botanical identification.

2.2. Phytochemical analysis of extracts from ten species

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 [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].

2.3. Research of the spermicidal effect of these plants

Human ejaculate samples were put into contact with recipe juices and total extracts of plants, at different concentrations. The mobility of spermatozoa in the presence of personal (intimate) hygiene plants was estimated based on the protocol of Saha et al. (2010), based on the Sander-Cramer technique. The viability was assessed by the eosin-nigrosine test or Williams test. The rate of killed sperm was evaluated based on the concentration of extracts and the exposure time. For each extract of the products, we detected the EC 100. This is the minimum concentration of spermicide capable of killing 100% of sperm within 30 minutes of contact [13-14].

3. Results and discussion

3.1. Phytochemical composition of some plant organs

Thirty-eight (38) identified species were divided into 22 botanical families. The chemical composition of secondary metabolites of ten plant organs analyzed varied from one plant species to another. Table 1 below shows the phytochemical groups of personal (intimate) hygiene plants.

Table 1 Phytochemical composition of some plant organs

Compounds sought	**Reagents used**	***Abrus precatorius***	***Alchornea cordifolia***	***Bridelia ferruginea***	***Citrus limon***	***Crossopteryx febrifuga***	***Hibiscus mechowii***	***Solanum esculentum***	***Mangifera indica***	***Ocimum basilicum***	***Zingiber officinale***	**Total**
Alkaloids	Draggennorff	+	+	+	+	+	+	+	+	+	+	10
Diterpenoids	H_2SO_4	+	+	+	+	+	+	+	+	+	+	10
Polyphenols	Burton	+	+	+	+	+	+	+	+	+	+	10
Triterpenoids	Liberman	+	+	+	+	+	+	+	+	+	+	10
Flavonoids	Shinoda+ Mg Isoamyl alcohol	+	+	+	+	+	-	+	+	+	-	8
Steroids	Liberman	+	+	-	+	+	+	+	-	+	+	8
Gallic tannin	Stiasny	+	+	+	+	-	+	-	+	+	+	8
Catechic Tannin	$FeCl_3$ 2%	-	+	+	-	+	-	+	+	+	+	7
Anthocyanins	HCl 20%	-	+	+	+	+	-	-	+	-	+	6
Leucoanthocyanins	Shinoda	+	+	+	-	+	-	-	+	-	+	6
Saponins	Distilled water	-	-	+	-	+	+	+	+	+	-	6
Free quinones	Borntrager NaOH 10%	-	-	+	-	-	-	-	+	-	-	2
Coumarins	Borntrager	-	-	-	-	-	-	-	+	-	-	1
Total number of substances per plant		8	10	11	8	10	7	8	12	9	9	

Legend; +: Presence of the desired compound; - : Absence of the desired compound

The data reported in this table show that alkaloids, diterpenoids, total polyphenols and triterpenoids are found in all ten extracts. Flavonoids, steroids and gall-type tannins are present in eight plant extracts. It should also be noted that the saponins, known for their spermicidal effect, are in six plants (*Bridelia ferruginea, Crossopteryx febrifuga, Hibiscus mechowii, Solanum esculentum, Mangifera indica* and *Ocimum basilicum*).

3.2. Evolution of sperm mobility

In light of our data, the reduction in mobility is gradual in the presence of recipes and witnesses. But, after three hours of contact, the sperm motility was canceled out against three recipes (*A. precatorius*, *B. ferruginea* and *O. basilicum*) only, but not against physiological solution. Thus, the spermicidal effect of these three plants was investigated using their total aqueous extracts (Figure 1).

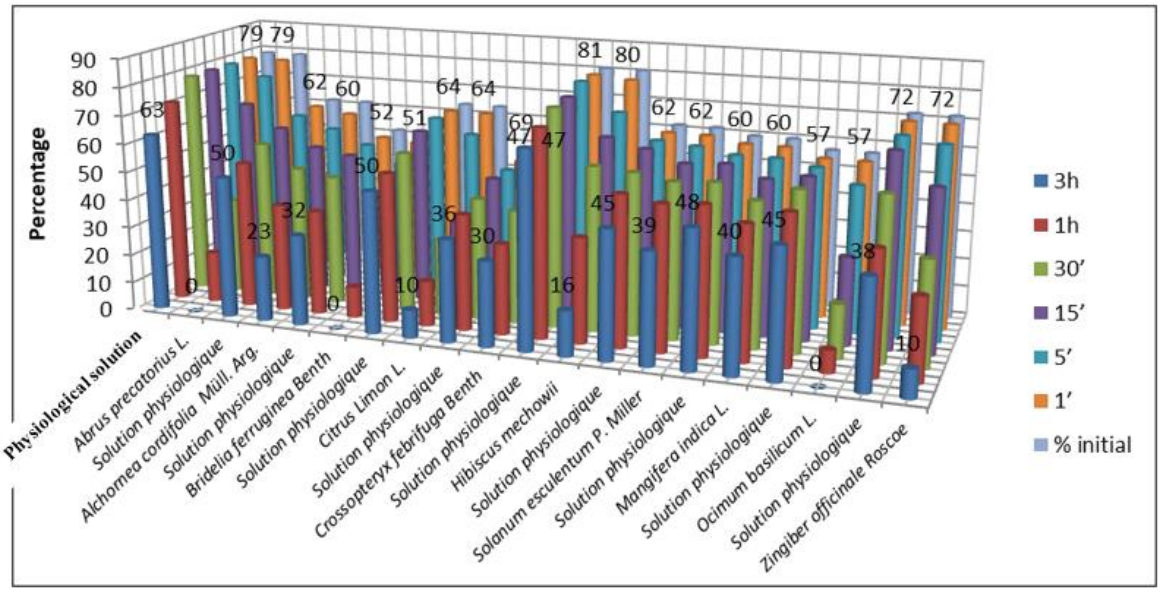

Figure 1 Evolution of sperm mobility over time and in the presence of recipes as used by women, compared to physiological solution

3.3. Percentages of spermatozoa mobility depending on the plant recipes and time

In contact with extracts of *A. precatorius*, immobilized spermatozoa do not necessarily die. The concentration capable of killing 100% of spermatozoa at 30 minutes of exposure (EC_{100}) is 200 mg / mL. The lowest spermo-immobilizing concentration is 6.25 mg / mL at 3 hours of exposure and 25 mg / mL at 30 minutes (Figure 2).

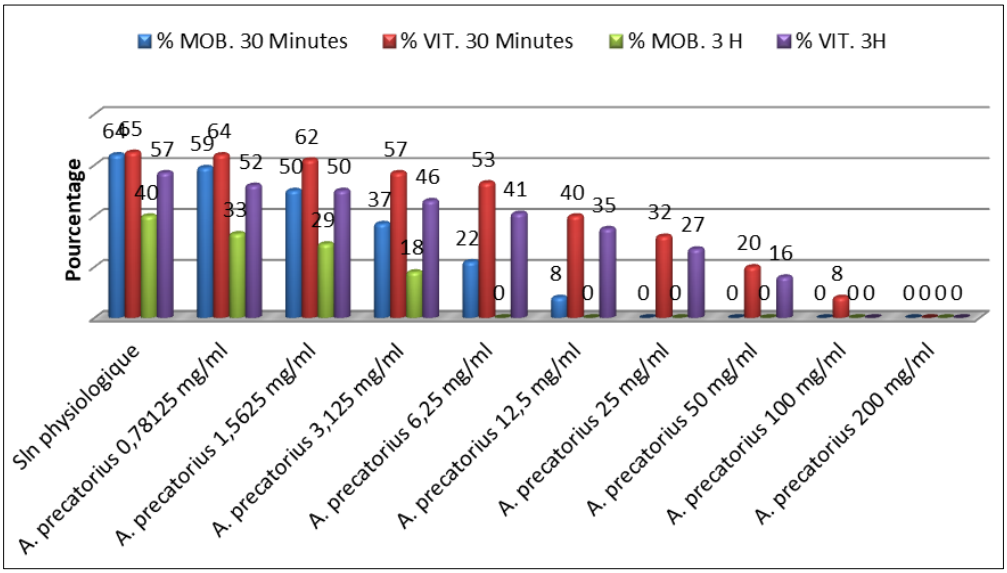

Figure 2 Mobility and viability (%) of spermatozoa in the presence of *A. precatorius*

3.4. Mobility and viability (%) of spermatozoa in the presence of *B. ferruginea*

B. ferruginea causes immobilization of sperm 30 minutes after contact at a concentration of 6.25 mg / mL, so the EC_{100} (Figure 3).

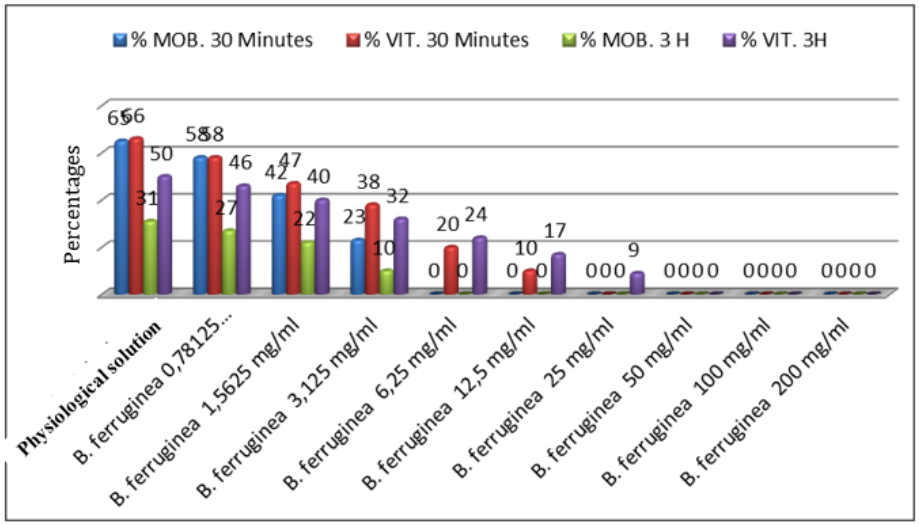

Figure 3 Mobility and viability (%) of spermatozoa in the presence of *B. ferruginea*

3.5. Mobility and viability (%) of spermatozoa in the presence of *O.basilicum*

No concentration of *O.basilicum* extracts is spermo-immobilizing or spermicidal. Exposure of spermatozoa to extracts of *O. basilicum* failed to detect a concentration causing death after 30 minutes (EC_{100}).

We observe that the rate of living spermatozoa is higher than that of those who were motile, after 30 minutes of contact in the control. However, at low concentrations of *O. Basilicum* (0.78 mg / mL and 1.56 mg / mL), it is found that the level of live spermatozoa is identical to that of motile spermatozoa. This finding is still noted at 100 mg / mL of concentration, after three hours of contact.

The averages of the percentages of spermatozoa mobility against the total aqueous extracts of *A. precatorius, B. ferruginea* and *O. basilicum*, during 30 and 180 minutes, were compared by ANOVA; and their evaluation is given in figure 4.

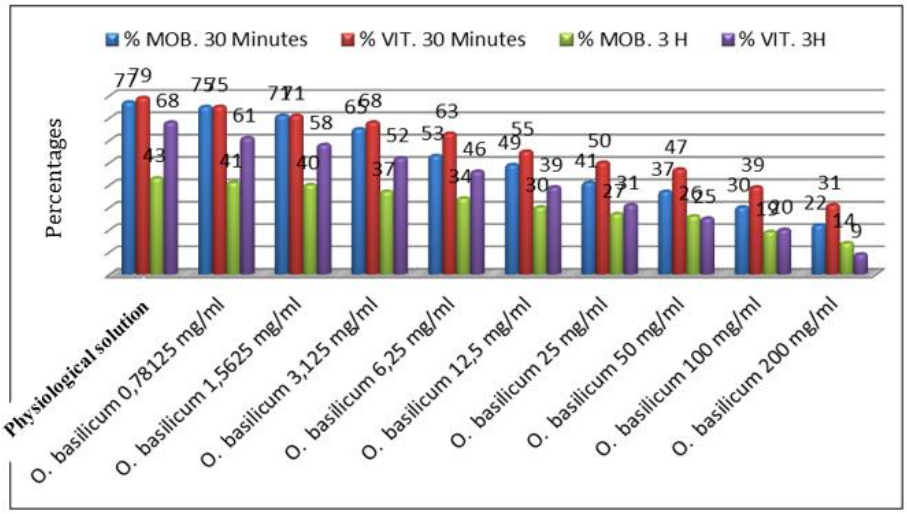

Figure 4 Mobility and viability (%) of spermatozoa in the presence of *O.basilicum*

3.6. Evolution of the mobility of spermatozoa against extracts

It appears that the spermo-immobilizing concentrations are respectively 6.25 mg / mL for *A. precatorius* and 1.56 mg / mL for *B. ferruginea* after 180 minutes of exposure. For O. *basilicum* extracts, the lowest concentration (0.78mg / mL) could not reduce spermatozoa motility; but ultimately it helped them move, after 180 minutes of exposure (Figure 5).

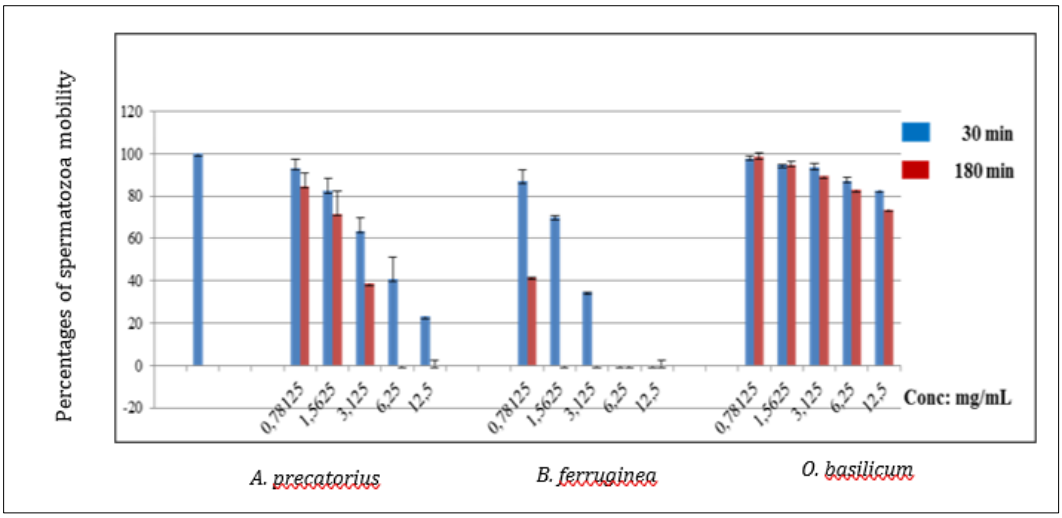

Figure 5 Evolution of the mobility of spermatozoa against extracts between 30 'and 180'

4. Discussion

Our results show that in the presence of total extracts of *A. precatorius*, *B. ferruginea* and *O. basilicum*; the more the concentration and the contact time increase, the more the percentage of mobility decreased. At the low concentration of extracts of *O. basilicum* (0.78 mg / mL), the spermatozoa exposed to them are re-energized compared to those in the presence of physiological solution. It would be defined as a spermo-energizing concentration for *O. basilicum*, unlike the spermo-immobilizing concentration observed with the extracts of *A. precatorius* and *B. ferruginea*, after 180 minutes. For the same exposure time, extracts of *B. ferruginea* (1.56 mg / mL) are more immobilizing than those of *A. precatorius* (6.25 mg / mL). The behavior of spermatozoa in the presence of plant extracts could be influenced by the phytochemical composition.

Examination of the phytochemical groups in the aqueous extracts of three *A. precatorius*, *B. ferruginea* and *O. basilicum* reveals that they share six types of secondary metabolites, namely: alkaloids, diterpenoids, total polyphenols, triterpenoids, flavonoids and gallic tannins.

Their composition differs in the absence of saponins in *A. precatorius* and steroids in *B. ferruginea*. The four phytochemicals found in extracts: alkaloids, diterpenoids, total polyphenols, and triterpenoids; are mentioned in the literature as sources of contraceptive compounds. Bibata (2018) indicates the presence of alkaloids in one of the medicinal plants used in contraception in Mali [15].

In addition, the works of Mannowetz et al. (2017) demonstrate the involvement of triterpenoids from some plants in blocking the catSper calcium channel activating receptor in sperm [16].

With regard to Polyphenols, it is reported that some saponosides are used by the pharmaceutical industry for the semisynthesis of estrogen-progestogens involved in the regulation of fertility. Plants rich in saponosides could act as proestroprogestogens, in favor of their use as traditional contraceptives [17].

Much research has focused on saponins for their spermicidal effects [18-19].

The presence of saponins in the extracts could explain the reduction in mobility and/or viability observed. Since the extracts of *A. precatorius* do not contain it, one would think that *B. ferruginea* and *O. basilicum* exhibit a spermicidal effect because of the saponins.

However, the aqueous extracts of *O. basilicum* did not prove to be spermicidal after 3 hours of exposure. In light of this observation, the effect on spermatozoa observed would be due to the acidity of the recipe. According to [20], the pH of *B. ferruginea* is 4.45 ± 0.05, an acidity which does not allow spermatozoa to survive.

On the other hand, for *A. precatorius* and *O. basilicum*, whose respective pH values (6.16 ± 0.14 and 6.61 ± 0.01), evolve towards neutrality, their acidity would promote the survival of spermatozoa. *B. ferruginea* is therefore presented as the

best intimate hygiene recipe that can be exploited as a source of contraceptive substances. It is commonly used in intimate care, its pH corresponding to the physiological pH of the vagina and it is capable of destroying spermatozoa in vitro. However, exploiting the bark of its trunk for recipe preparation limits its sustainability benefits than that of the leaves in the case of other plants. This natural extract (*Ocimum basilicum*) as an anti-oxidant can protect ovary tissue and follicles and it also able to reduce apoptosis in ovary tissue and can increase the mobility of spermatozoa [21].

The concentration and duration of exposure resulting in total loss of mobility do not correspond to those of loss of life in spermatozoa in contact with plant extracts. The presence of alkaloids in these plants justifies its spermcidal effect [22].

The EC 100, the minimum concentration of spermicide capable of killing 100% of spermatozoa after 30 minutes of exposure, was set at 200 mg / mL for *A. precatorius*. It was detected for *B. ferruginea* at 25 mg / mL. In the presence of extracts of *O. basilicum*, EC_{100} is not defined.

5. Conclusion

Since the practice of using plants in the genital area exists, the analysis of the effect of these intimate hygiene plants on spermatozoa constitutes a research direction in the field of plants for the regulation of births. The intimate hygiene plants constituting an inexhaustible source of drugs, they can be used for the formulation of vaginal contraceptive preparations.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

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.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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