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Phytochemical screening and effects on spermatogenesis of extracts from leaves of *Flueggea virosa* (Roxb, ex Willd.) Royle and *Heliotropium indicum* L., two plants used against infertility in North of Ivory Cost

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ABSTRACT

Male infertility constitutes a public health problem today in developing countries. But populations are faced with very high costs and difficult access to specialized centers in order to benefit from quality care. These obstacles lead couples in distress to turn to medicinal plants to treat possible causes of infertility. The aims of this study is to evaluated effects of *Flueggea virosa* and *Heliotropium indicum*, two plants from northern Ivory Coast on spermatogenesis in Wistar rats male. Aqueous and hydro-ethanolic extracts of the two plants leaves were first analysed through phytochemical screening by the staining and precipitation methods. Then effects of their two aqueous extracts were evaluated on spermatogenesis in Wistar rats. Each extract was administrated at concentration of 100 mg/kg body weight orally to test Wistar rats. A reference standard fertilizer product, Fertilo Forte Denk (FFD) was used at 5 mg/kg body weight as positive control to treat Wistar rats. After 30 and 60 days of treatment, mobility, viability, density and morphology of rat's spermatozoa were evaluated by observation under an optical microscope. Phytochemical screening revealed the presence of polyphenols, flavonoids, gallic tannins, catechic tannins, alkaloids and saponosides in all the aqueous and hydroethanolic extracts of *F. virosa* and *H. indicum*. For spermatogenesis effects in Wistar rats, the study highlights that aqueous extracts from leaves of *F. virosa* and *H. indicum* induce an increase in the number, an improvement of the mobility and viability of spermatozoa after 60 days of treatment. But this increase is more significant ($P < 0.001$) in rats treated with *F. virosa* compared to control and group treated with FFD. According to these results, aqueous extracts from *F. virosa* and *H. indicum* leaves improve quality of sperm in rats. These data might justify the use of these two plants in treatment of certain cases of male infertility.

Keywords: *Flueggea virosa*, *Heliotropium indicum*, Phytochemical, Infertility, Wistar rat, Spermatogenesis.

INTRODUCTION

Couple infertility is a condition of male or female reproductive system. It is characterized by inability of couple to have a pregnancy after 12 months of unprotected sexual intercourse [1]. Today, Africa has the highest infertility rate in the world. Indeed, this rate is between 15% and 30%, compared to 5% to 10% in Europe and 10 to 15% of couples in the United States [2, 3]. The causes of this subfertility are multiple and can be anatomical, genetic, hormonal, immunological and infectious [4]. However, infertility in men is closely linked to deterioration in sperm quality. Lately in Côte d'Ivoire, approximately 15.6% of men seeking fatherhood had infertility [5]. Sperm abnormalities are numerous and the spermogram remains the key test to identify them [6]. These anomalies are linked to the drop in density, mobility, vitality, and typical shapes of the spermatozoa produced. To remedy this, several medical treatments are implemented by modern medicine [7]. But populations in developing countries are faced with very high costs and difficult access to specialized centers in order to benefit from quality care. These obstacles lead couples in distress to turn to medicinal plants to treat possible causes of infertility [8, 9]. To highlight the use of plants in couple infertility treatment, this study aims to evaluate effects of aqueous extracts of *F. virosa* and *H. indicum* on quality of sperm in albino male rats (Wistar). Previous studies have also revealed involvement of *F. virosa* and *H. indicum* in treatment of couple infertility in northern Ivory Coast [10, 11]. Therefore, phytochemical screening of aqueous and hydroethanolic extracts from leaves of *F. virosa* and *H. indicum* was carried out before evaluated effects of these aqueous extracts on quality of sperm in rats.

MATERIAL AND METHODS

Plant material

The plant material consisted of leaves of *Flueggea virosa* and *Heliotropuim indicum* harvested in Korhogo (northern Ivory Coast) in early morning using a knife. After harvest, these plants were authenticated at National Floristic Center of Félix HOUPHOUËT-BOIGNY University of Abidjan (Ivory Coast) where they are preserved respectively under herbarium numbers UCJ006375 and UCJ001745. Then, leaves were dried away from sun at room temperature for 30 days in a laboratory at Peleforo GON COULIBALY University in Korhogo (Ivory Coast). After drying, they were pulverized with an electric grinder (RETSCH, Type AS 200) to obtain a fine powder, used for preparation of extracts.

Animal material

The animal material consisted of 48 laboratory rats, *Rattus norvegicus* Wistar strain, with an average weight of 121±0.98 g and 98 weeks old. These rats are raised in cages made for this purpose at National Agricultural Development Support Laboratory (LANADA) in Korhogo with good ventilation and a photoperiod of 12 hours/12 hours. The animals were fed with a mixture composed of 70% corn and 30% growth food from company IVOGRAIN (Ivory Coast).

Preparation of plant extracts

The aqueous and hydro-ethanolic extracts were prepared according to method described by Ouattara et *al*. (2012) [12]. 100 g of powder from each plant (*F. virosa* or *H. indicum*) is macerated in 1 liter of distilled water for aqueous extract or in 1 liter of 70% ethanol for hydro-ethanolic extract. The whole is then homogenized in a Nasco brand blender (BL1008A-CB). The homogenate obtained is drained through white percale fabric and double filtered through hydrophilic cotton. The filtrate obtained was concentrated in an oven until solvent had completely evaporated in order to obtain dry aqueous and hydro-ethanolic extracts from leaves of *F. virosa* or *H. indicum*.

Phytochemical screening of plant extracts

The phytochemical screening was carried out with reference to techniques described in the work of Békro et *al*. (2007) and N'Guessan et *al*. (2009) [13, 14]. The chemical groups sought are: sterols and terpenes, total polyphenols, flavonoids, tannins, alkaloids and saponins. The tests carried out and the characteristic reactions of each chemical group are summarized in Table 1.

Table 1: Phytochemical test of different plant extracts

Researched chemical groups	Detection test	Positive reaction
Sterols and terpenes	Lieberman	Purple ring turning green
Total polyphenols	Perchlorure ferrique	Dark blue-blackish± color
Flavonoids	Cyanidine	Pink-orange or purplish color
Catechical tannins	Stiasny	Appearance of large flakes
Gallic tannins	Stiasny	Intense blue-black coloring
Alkaloids	Dragendorf	Orange coloring
Saponins	Agitation	Foam index > 100
Quinones	Borntrager	Red or purple coloring

Evaluation of effects of aqueous extracts of *F. virosa* or *H. indicum* on quality of rat sperm

Treatment of animals

The effect of aqueous extracts *F. virosa* or *H. indicum* on sperm quality was evaluated using four (04) batches of twelve (12) rats each. For each batche of rats, the different treatments were administered as follows:

- Lot 1 (control): receives pure tap water;
- Lot 2 (positive control): receives 1mL of "Fertilo forte Denk" (Germany) (FFD) at a concentration of 5 mg/Kg of body mass). This product is a concentrate of vitamin E, folic acid, coenzyme Q10, minerals and amino acids. It is available in pharmacies and is used to promote fertility as well as sperm production.
- Lot 3: receives 1mL of the aqueous extract of *F. virosa* (100 mg/Kg of body weight);
- Lot 5: receives 1mL of the aqueous extract of *H. indicum* (100 mg/Kg of body weight);

The animals received treatments daily by gavage for 60 days. Sperm samples were taken before any treatment (day zero), after 30 and 60 days of treatment.

For this, three (03) rats were selected at random from each batch then put to sleep after anesthesia with ether in order to collect the sperm by dissection of the scrotum. After dissection, the epididymis is removed and triturated in 10 mL of 9‰ NaCl in a water bath at 36°C [15].

Determination of sperm parameters after treatment with aqueous extracts of *F. virosa* or *H. indicum*

The different sperm parameters of rats such as mobility, vitality, density and morphology of spermatozoa were evaluated with the ground materials obtained, by observation under an optical microscope (Primo star) between slide and coverslip.

Sperm mobility

The mobility of spermatozoa is assessed by direct examination of ground solution of epididymis at 40 [16]:

$$\% \text{ live sperm} = \frac{\text{Live sperm count}}{\text{Total number of sperm}} \text{ X } 100$$

Sperm vitality

The vitality of the spermatozoa is assessed after staining with eosin. Dead sperm were colored pink while live ones appeared white. Finally, the number of live and dead spermatozoa is evaluated by counting randomly in five fields in order to calculate the vitality rate as follows [17]:

$$\% \text{ Mobile sperm} = \frac{\text{Mobile sperm}}{\text{Total number of sperm}} \text{ X } 100$$

Sperm density

Sperm density is determined using the Malassez cell. Thus, a drop of epididymis macerate was placed on the Malassez cell then covered with a coverslip. The sperm count was carried out at x40

magnification. The number of sperm per mm3 was estimated by formula of Sultan et *al*. 1982 [18].

$$N = X \times fd \times 10^6 / 4$$

X: Number of spermatozoa in 5 grids of 20 small squares of the Malassez cell; Fd: dilution factor (20); N: Number of sperm/mm3.

Typical shape of sperm

Typical sperm shapes are determined from a smear which has then been stained with eosin. The smear was examined using an optical microscope at X100 magnification [19]. The observation made it possible to assess the morphology of 200 spermatozoa, the number of atypical and typical forms was determined in order to calculate the rate of typical spermatozoa.

$$\% \text{ Typical sperm} = \frac{\text{Typical sperm count}}{\text{Total number of sperm}} \times 100$$

Statistical analysis

The statistical tests were carried out exclusively using SPSS software (statistics 26) and the data were entered using Word and Excel 2016 software. The significance of the differences observed between the different test groups is assessed by analysis of variance. (ANOVA) of Turkey's multiple comparison test.

RESULTS

Phytochemical screening of aqueous and hydro-ethanolic extracts from leaves of *F. virosa* and *H. indicum*

Results of phytochemical screening of aqueous and hydro-ethanolic extracts from leaves of *Flueggea virosa* and *Heliotropium indicum* are recorded in Table 2. These results shows that these plant extracts contain all chemical groups sought with the exception of sterols and terpenes which are absent.

Table 2: Phytochemical groups of hydo-ethanolic and aqueous extracts of *F. virosa* and *H. indicum*

Secondary metabolites	*F. virosa* Extracts		*H. indicum* Extracts	
	HE_{Fv}	AE_{Fv}	HE_{Hi}	AE_{Hi}
Polyphenols	+	+	+	+
Flavonoids	+	+	+	+
Catechical tannins	+	+	+	+
Gallic tannins	+	+	+	+
Alkaloids	+	+	+	+
Sterols and terpenes	-	-	-	-
Saponins	+	+	+	+

HE_{Hv}:Hydro-ethanolic extract of F. virosa; AE_{Hv}: Aqueous extract of F. virosa

HE_{Hi}:Hydro-ethanolic extract of H. indicum; AE_{Hi}: Aqueous extract of H. indicum; (+): Presence ; (-): Absence

Table 3: Variation in sperm parameters depending on the treatments on rats

Duration of the treatment	Rat batches	Spermatic parameters		
		% Motile sperm	% Live sperm	% Typical sperm
30 days	Witness	40.13±1.336	47.33±0.764	85.44±1.389
	FFD	60.44±1.338**	56.88±0.588**	88.66±3.214
	F. virosa	29.53±0.723** ++	37.58±0.549** ++	92.50±4.092
	H. indicum	36.34±1.336 ++	46.80±0.258 ++	92.00±6.557
60 days	Témoin	60.61±1.025	65.44±1.253	88.25±1.391
	D. fertilo	68.44±1.389**	70.44±2.142*	90.66±1.527
	F. virosa	87.92±1.127** ++	88.33±1.527** ++	98.00±1.00
	H. indicum	59.58±3.394 ++	68.27±0.750	85.65±6.806

FFD: "Fertilo forte Denk"; (*): Significant difference between the parameter values of the treated rats compared to the untreated control group; (+): Significant difference between the parameter values of the batches treated compared to the batch treated with FFD; (* or +) = p< 0.05: Significant difference; (**or ++) = p< 0.01: Very significant difference, (***or +++)=p< 0.001

Evaluation of effects of aqueous extracts of *F. virosa* and *H. indicum* on sperm quality

Aqueous and hydro-ethanolic extracts of *F. virosa* and *H. indicum* presented the same phytochemical composition, for this study only their aqueous extracts were used for tests on sperm parameters.

Effect of aqueous extracts of F. virosa and H. indicum on sperm vitality and mobility

The vitality and motility rates of spermatozoa collected from rats in the 4 batches for 30 and 60 days are presented in Table 3. Analysis of these results shows that rats treated with FFD presented the highest rate of vitality (56.88±0.58%) and mobility (60.44±1.33%). After 30 days of treatment. Furthermore, animals treated with *H. indicum* presented vitality and mobility rates similar to those of the untreated control group. However, these rates are significantly higher than those obtained with *F. virosa* (37.58±0.54% and 29.53±0.72%). After 60 days of treatment, high rates of vitality and mobility were observed

with *F. virosa* (88.33±1.52% and 87.92±1.12%) followed by FFD (70.44 ± 2.14% and 68.44±1.38%) compared to those of *H. indicum* and untreated control batch. There is no significant difference between the rates of the latter two.

Effect of aqueous extracts of F. virosa and H. indicum on sperm morphology

Analysis of spermatozoa after 30 and 60 days of treatment with different products revealed no variation in the rate of typical spermatozoa, therefore indicating no change in their morphology. There is no difference ($p > 0.05$) between the rate of typical spermatozoa in batches treated with *F. virosa* and *H. indicum* compared to control batches (Table 3).

Effect of aqueous extracts of F. virosa and H. indicum on sperm density

Figure 1 below highlights the sperm density in treated rats and in control rats for 30 and 60 days. After 30 days of treatment, the aqueous extracts of *F. virosa* and *H. indicum* showed an increase in sperm densities from $81.55.10^6$ /mL to $99.00.10^6$ /mL and from $84.73.10^6$ /mL to $106.00.10^6$, respectively. /mL. However, these increases were significantly lower than those of the control batch ($85.62.10^6$ /mL to $135.33.10^6$ /mL) and the batch treated with FFD ($86.72.10^6$ /mL to $234.33.10^6$ /mL). There was a significant difference on the one hand between the sperm levels of the treated rats and that of the untreated control rats and on the other hand between the levels of the rats treated with the aqueous extracts and that of the rats treated with FFD ($p < 0.001$).

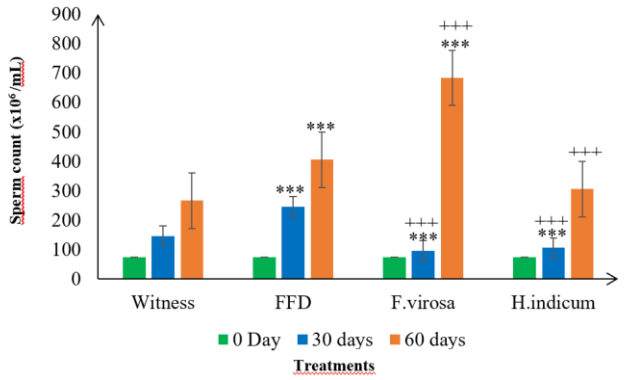

Figure 1: Rat sperm concentration according to the different treatments

(*): Significant difference between the parameter values of the treated rats compared to the untreated control group; (+): Significant difference between the parameter values of the batches treated compared to the batch treated with "Fertilo forte Denk"; (* or +) = $p < 0.05$: Significant difference; (**or ++) = $p < 0.01$: Very significant difference, (***or +++)=$p < 0.001$.

On the other hand, after 60 days of treatment, the aqueous extract of *F. virosa* recorded the highest sperm count ($683.67.10^6$/mL) followed by the reference standard product FFD ($405.69.10^6$/mL) compared to *H. indicum* and the untreated control batch whose levels were $268.023.10^6$ /mL and $262.43.10^6$ /mL respectively. The statistical analysis shows a significant difference on the one hand between the levels obtained with the two aqueous extracts and that of FFD ($p < 0.001$) and on the other hand between the levels of rats treated with *F. virosa* and those obtained with *H. indicum* and the untreated witness.

DISCUSSION

Phytochemical analysis of aqueous and hydro-ethanolic extracts from leaves of *F. virosa* and *H. indicum* revealed the presence of total polyphenols, flavonoids, gallic tannins, catechic tannins, alkaloids and saponins. Our results corroborate those of Zahoui et *al*. (2010) and Traoré et *al*. (2019) who respectively showed the predominance of the same secondary metabolites in the leaves of *H. indicum* and *F. virosa* [20,21]. The diversity of these phytochemical compounds could explain the use of these two plants in the traditional treatment of infertility in Korhogo region in Ivory Coast[11].

The analysis of sperm parameters after 30 and 60 days of treatment showed that the aqueous extracts from leaves of *F. virosa* and *H. indicum* increased the mobility, vitality and density of spermatozoa in rats compared to the reference product FFD (Fertilo forte Denk) and untreated controls. However, this increase is much more significant after 60 days of treatment with the aqueous extract of *F. virosa*. Indeed, the fertility or quality of sperm is closely linked to the mobility, vitality and density of ejaculated spermatozoa, therefore indicating that leaf extracts of the two plants improve male fertility [22]. The improvement in these reproductive parameters in male rats by aqueous extracts, particularly of *F. virosa*, could be explained by the presence of numerous compounds highlighted in the study of phytochemical screening of these extracts. Indeed, it has been shown that flavonoids and alkaloids possess estrogenic properties [23]. Flavonoids, alkaloids and saponosides also possess androgenic activities. Indeed, they are involved in the production of androstenedione which allows the biosynthesis of testosterone [24]. Previous studies have revealed that improvement in fertility or reproductive parameters is closely related to the rate of testosterone secretion. This hormone stimulates the male reproductive glands on the one hand and spermatogenesis on the other [25-27]. These beneficial results on the spermatogenesis of rats treated with *F. virosa* could also be linked to the antioxidant properties of alkaloids and phenolic compounds such as flavonoids and total polyphenols found in this plant according to Anel-Lopez et al. (2015); Ariyan *et al*. (2020) [28,29]. Indeed, they demonstrated that the antioxidant activity of phenolic compound by trapping free radicals protected the epididymis of red deer against oxidative stress. Antioxidant molecules, by preventing oxidative stress, improve the progression and speed of spermatozoa [30]. This helps to encourage fertility in men. Furthermore, several studies indicate the effectiveness of plant extracts in improving fertility. This is the case of the leaves of *Cnestis ferruginea* and the roots of *Hymenocardia acida* which made it possible to improve the quality of sperm in rats [27,31].

CONCLUSION

The objective of this study was to carry out a phytochemical screening of aqueous and hydro-ethanolic extracts 70% from leaves of *Flueggea virosa* and *Heliotropium indicum* and then to evaluate effects of their aqueous extracts on sperm parameters in rats. This work, explain that all the four extracts studied are rich in these phytochemical compound which are total polyphenols, flavonoids, tannins, alkaloids and saponins. Spermatic parameters were much improved with the aqueous extract of *F. virosa* compared to the standard reference product (Fertilo forte Denk) and the control. These results can serve as a basis for the implementation of traditionally improved medicines (TIMs) to treat male infertility.

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Conflicts of Interest

The author reports no conflicts of interest.

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