World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

A COMPARATIVE STUDY OF THE MORPHOLOGY OF SPERMATOZOA STAINED WITH AQUEOUS AND HYDROETHANOLIC EXTRACTS OF *Hibiscus sabdariffa* IN COTE D'IVOIRE

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World Journal of Advanced Research and Reviews, 2026, 31(01), 191–201

Publication history: Received on 24 July 2026; revised on 01 September 2026; accepted on 03 September 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.31.3.2287

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ABSTRACT

The semen analysis is a first-line test in the evaluation and management of infertility in couples. This study focused on evaluating the use of hibiscus flowers (*Hibiscus sabdariffa*) as an alternative staining agent in the study of sperm morphology. This was a comparative experimental study conducted over a five (5) months period from January to May 2026 at the Laboratory of Histology, Embryology, and Cytogenetics of the Faculty of Medical Sciences in Abidjan at Felix HOUPHOUET-BOIGNY University. Staining extracts from *Hibiscus sabdariffa* were obtained from dried hibiscus flowers using two different methods: aqueous extraction and hydroethanolic extraction. These extracts were used to process semen samples collected from patients who had abstained for three(3) to five (5) days. A comparison was made with the routine dye. The experiments indicated that hydroethanolic extraction yielded results comparable to those obtained with conventional stains such as Papanicolaou. Significant economic and ecological benefits can be expected from the development of such natural plant-based stains, which are accessible to laboratories with limited resources.

Keywords: *Hibiscus sabdariffa*, Sperm Cell, Spermocytogram, Plant dye

1 INTRODUCTION

Reproductive biology is a medical specialty focused on identifying the causes of infertility in couples and developing methods for their management (1). In men, the World Health Organization recommends a semen analysis and sperm cytogram to assess both the quantity and quality of sperm. The sperm cytogram focuses more on evaluating sperm morphology (2).

The morphological study of spermatozoa requires staining followed by observation under an optical microscope. Routinely, the Papanicolaou staining technique is used (3). This technique requires the use of five (5) dyes divided into three (3) categories:

- nuclear staining solution (hematoxylin)
- cytoplasmic counterstain solution (orange G)
- polychrome solution (eosin Y, light green SF, Bismarck's brown Y) (4)(5).

The combination and order of application of these stains make it possible to obtain smears that can be analyzed under an optical microscope, revealing the morphological characteristics of spermatozoa and detecting any abnormalities (6).

However, in a context where health resources and investments are limited, many biomedical testing laboratories (LABMs) in low-income countries often face challenges related to the unavailability of reagents and supplies for certain diagnostic techniques (7).

Cote d'Ivoire possesses significant ethnobotanical resources that can provide locally-based solutions to various challenges, both in health research for the population and in the development of solutions and technical tools to improve biomedical laboratory analysis practices (8).

Hibiscus sabdariffa, also known as "bissap" in the Wolof language (West Africa) or "karkade" in Egyptian Arabic (North Africa), is a tropical plant whose bright red flowers owe their color to anthocyanins, natural pigments found in the plant that make it suitable for use as a dye in the textile industry (9). Its flowers are traditionally used to prepare refreshing and/or medicinal infusions. *Hibiscus sabdariffa* is readily available on the local market, and its purchase requires few resources (10). In addition, it has been described its blood-pressure-lowering and cholesterol-lowering effects, as well as its antioxidant properties which are linked to its high vitamin C content and are beneficial to the immune system (11).

The purpose of this study was to contribute to the search for substances from the Ivorian pharmacopoeia that possess dyeing properties as an alternative to synthetic dyes.

The overall objective of this study was to evaluate the performance of two (2) methods for extracting a staining solution from *Hibiscus sabdariffa* flowers for optimal cytological analysis of spermatozoa.

The specific objectives designed to achieve this were:

- 1/ To prepare a dye based on an aqueous extract and a dye based on a hydroalcoholic extract of *Hibiscus sabdariffa*
- 2/ To evaluate the staining indices of aqueous and hydroethanolic extracts of *Hibiscus sabdariffa*
- 3/ Compare the staining indicators of sperm stained with aqueous and hydroethanolic extracts of *Hibiscus sabdariffa* to those of reference stains

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 Study Site

The study was conducted at the Laboratory of Histology, Embryology, and Cytogenetics at the Abidjan Faculty of Medical Sciences of Felix Houphouet-Boigny University. The preparation of slides and their examination under the microscope took place there.

The preparation of the dye filtrate and the phytochemical analysis of the *Hibiscus sabdariffa* extracts were carried out in the Biology and Health Laboratories and the Pharmacognosy Laboratories, located in the Biosciences Division and the Pharmaceutical and Biological Sciences Division, respectively, of the aforementioned university.

2.1.2 Study Materials

2.1.2.1 Plant Material

Dried flowers of *Hibiscus sabdariffa* were used to prepare a natural dye (Figure 1).

2.1.2.2 Biological Materials

Sixty (60) semen samples were collected from patients who had abstained for 3 days. A total of 120 semen smears were prepared.

Figure 1: Dried calyxes of *Hibiscus sabdariffa*

2.1.2.3 Technical Equipment

The equipment included, among other things, a drying oven (Venticell, Germany), which is a controlled drying and heating device whose primary function is to maintain samples or materials at a uniform and constant temperature; it was used to remove the solvent and gases trapped in the mixture. A blender (Moulinex, Switzerland) was used to grind the *Hibiscus sabdariffa* calyxes and mix them with the solvent. Additionally, an electronic scale (FF1976, China) was used to weigh and measure precise quantities of *Hibiscus sabdariffa* for preparing the mixtures. The optical microscope (Motic, China) was used to observe smears at high magnification, which required the application of immersion oil. A vortex mixer (Drogon Lab, China) was used to homogenize the semen samples, along with laboratory glassware (microscope slides and cover slips for mounting, graduated cylinders, porcelain dishes, and funnels). In addition, a square of white cloth and cotton wool were used for successive filtration of the ground samples obtained after mixing. Finally, reagents (Papanicolaou, May-Grünwald, and Giemsa staining kits, toluene, and Eukitt) were used to process the smears according to routine cytological examination methods.

2.2 Methods

2.2.1 Study Design and Time Frame

This was a comparative experimental study conducted over a 5-month period from January to May 2026.

2.2.2 Inclusion Criteria

Semen samples with a sufficient sperm concentration as determined by semen analysis were included in this study (2).

2.2.3 Exclusion Criteria

Semen samples that revealed severe oligozoospermia or azoospermia on semen analysis were excluded from the study.

2.2.4 Parameters Studied

The parameters studied included staining indicators such as intensity, contrast, sharpness, and outline of the various parts of the spermatozoa observed under an optical microscope.

- Intensity: This measures the saturation or depth of the staining on the cellular components and verifies that the amount of dye absorbed is neither too low (pale) nor too high (saturated or masking).
- Contrast: This evaluates the difference in tone between different parts of the cell (such as the nucleus and the cytoplasm) or between the cell and the slide background. It allows for clear differentiation between acidic and basic components.

- Sharpness: This is determined by visual clarity and the absence of blurring or extraneous precipitation under the microscope, and it also ensures that fine intracellular structures remain clearly discernible.
- Contour: This analyzes the precision of the delineation of the cell membrane and the nuclear membrane. Furthermore, it confirms the sharpness of the transition between the cell's edge and its immediate surroundings (12)(13).

The indicators were evaluated by calculating the averages of the values assigned by two (02) independent observers who compared the smears stained with aqueous and hydroethanolic extracts of *Hibiscus sabdariffa* with those stained using routine dyes, whose indicators were set at 100% (reference)(13). Thus, criteria for judging the level of staining indicators were established. For an indicator value below 50%, the stain is classified as poor; when the value obtained is between 50 and 75%, the stain is classified as fair or acceptable. For an indicator value above 75%, the stain is judged to be optimal or excellent. In practice, Papanicolaou-stained smears served as a reference for determining the indicators of smears stained with aqueous and hydroethanolic extracts. Thus, a value of 100% was assigned to the indicators of the reference smears. Estimates were made as percentages based on visual observation.

2.2.5 Data Collection

Data collection was carried out by developing an evaluation grid for each parameter studied. This allowed for a comparative analysis of the observations made during optical microscopy of slides stained with aqueous and hydroethanolic extracts of *Hibiscus sabdariffa*.

2.2.6 Protocol for Extracting *Hibiscus sabdariffa*-Based Dye Filtrates

The extraction of the essence from *Hibiscus sabdariffa* flowers was performed according to the method described by an author (14).

After purchasing *Hibiscus sabdariffa* flowers at the "Forum" market in Adjame (Abidjan), 100 g of the sample were weighed and ground in a blender with either an aqueous solvent (1 liter of distilled water) or a hydroethanolic solvent (500 mL of distilled water and 500 mL of 95° ethanol). Next, the resulting mixtures were filtered to obtain "coloring filtrates" free of debris in order to reduce the risk of artifacts. After being placed in an oven at 50°C for 72 hours, the filtrates dried and solidified. The solidified filtrates were blended to produce powder according to the type of extraction performed. The extracts were divided into sterile jars and stored in a dry place.

2.2.7 Semen Analysis

Semen collected via masturbation was stored in sterile vials (2). After a liquefaction period lasting 30 minutes to 1 hour, 10 µl of semen was collected to prepare the smears. After drying at room temperature (approximately 10 minutes), the smears were fixed in absolute alcohol and stained. Once stained, the smears were mounted between a slide and a coverslip using a synthetic resin called Eukitt.

The aqueous and hydroethanolic stains based on *Hibiscus sabdariffa* were prepared by dilution, adding distilled water to the measured volume of dye powder. This resulted in the following dilution factors: 1/5, 1/10, and 1/20. For example, the 1/5 dilution was obtained by adding four (04) volumes of distilled water to one (01) volume of prepared dye powder.

The experimental staining times were 10 minutes, 30 minutes, and 1 hour.

After these steps, the prepared slides were evaluated by two independent observers to compare the performance levels of each staining method based on the average values assigned to each indicator.

2.2.8 Data Entry and Statistical Tools

Data entry was performed using Word and Excel 2016. Statistical calculations were performed using EpiInfo 7.2. The statistical tools used allowed for the expression of proportions as percentages, means, and standard deviations. Statistical differences were analyzed using the paired t-test and the calculation of the p-value. The threshold for statistical significance was set at 5%. Comparisons were made between the mean values obtained for each indicator using each of the two methods for extracting the prepared dye, as well as between these Hibiscus sabdariffa-based dyes and the indicators for the reference dyes.

2.2.9 Ethical Considerations

This study was conducted in accordance with the principles of anonymity and confidentiality required for clinical laboratory tests performed in the laboratory. Prior informed consent was obtained from the patients included in the study.

3 RESULTS

3.1 Phytochemical Screening Data

The phytochemical screening revealed the presence or absence of certain secondary metabolites, which are presented in Table 1. Among the molecular classes identified were polyphenols, polyterpenes, alkaloids, and organic acids, while flavonoids and tannins were absent.

Table 1: Summary of phytochemicals identified in extracts Of *Hibiscus sabdariffa*

Biomolecular Classes	Present	Absent
Sterols	x	
Polyterpenes	x	
Polyphenols	x	
Flavonoids		x
Catechin tannins		x
Gallic tannins		x
Quinones	x	
Bouchardât alkaloids	x	
Dragendorff alkaloids	x	
Saponins	x	
Organic acids	x	

Phytochemical analysis of the *Hibiscus sabdariffa* extracts used in this study reveals the presence of the major molecular classes typically found in this plant.

3.2 Properties of the Dye Derived from *Hibiscus sabdariffa* Extracts

The two (2) extraction methods used yielded dye solutions with pH values ranging from 1 to 2.5 depending on the dilution factors (1/5, 1/10, 1/20) and dyeing time (10 min, 30 min, and 1 h). The aqueous and hydroethanolic extracts obtained had identical appearances and pH values regardless of the dilution levels. These data are shown in Table 2. Regarding yield, from 100 g of dried calyxes, 11 g of powder (equivalent to a volume of 21 mL) was obtained for the aqueous extraction (R = 0.11), and 14 g of powder (equivalent to a volume of 27 mL) was obtained for the hydroethanolic extraction (R = 0.14).

Table 2: Characteristics of the color indicators based on the extraction method for the essential oil of Hibiscus sabdariffa

Dilution and dyeing time	Staining	pH
1/5th for 10 min	Dark red	1 – 1.5
1/5th for 30 min	Dark red	1 – 1.5
1/5th for 1 hour	Dark red	1 – 1,5
1/10th for 10 min	Medium-dark red	1.5 – 2
1/10th for 30 min	Medium-dark red	1.5 – 2
1/10th for 1 hour	Medium-dark red	1.5 – 2
1/20th for 10 min	Pure red—purplish	2 – 2.5
1/20th for 30 min	Purplish red	2 – 2.5
1/20th for 1 hour	Purplish red	2 – 2.5

At the same dilution and dyeing time, the appearance and characteristics of the reconstituted ready-to-use dyes were identical for both extraction methods.

3.2.1 Staining Properties of Aqueous Extracts of *Hibiscus sabdariffa*

Following staining tests on smears using aqueous extracts of *Hibiscus sabdariffa (*Table 3*)*, a 1/5 dilution with a staining time of 1 hour yielded the best staining results for spermatozoa. These results were quantified using the staining indices. In general, the values of the staining indices (intensity, contrast, sharpness, and outline) varied depending on the concentration of the extracts. For the 1/5 dilution, staining intensity ranged from 70% to 85% over a period of 10 minutes to 1 hour. The same was true for contrast, which ranged from 65% to 85%. For sharpness, the range was 65% to 80%. Finally, contour ranged from 70% to 75%.

For the 1/10 dilution, intensity and contrast ranged from 45 to 70% over a time span of 10 minutes to 1 hour. Sharpness ranged from 35 to 65%, while contrast showed little variation.

For the 1:20 dilution, intensity ranged from 35% to 65% over a time range of 10 minutes to 1 hour; contrast and outline then varied similarly, ranging from 45% to 55%. Finally, sharpness ranged from 30% to 50%.

Figure 2 showed spermatozoa stained with aqueous extracts of *Hibiscus sabdariffa*. The background is light, with spermatozoa exhibiting uniform staining of the nucleus and cytoplasm. The acrosome is also visible. The image also shows some morphological abnormalities in the spermatozoa, namely: at the head, macrocephaly, microcephaly, an elongated head, and an absence of the acrosome are observed. Next, at the midpiece, angulation is noted. Finally, in some spermatozoa, an absence of the flagellum was observed.

Table 3: Staining indices for smears stained with aqueous extracts of *Hibiscus sabdariffa*

Staining indicators (%)	**Smears staining dilution and dyeing time (n=60)**								
	Dilution 1/5	Dilution 1/10					Dilution 1/20		
	10 mn	30 mn	60 mn	10 mn	30 mn	60 mn	10 mn	30 mn	60 mn
Intensity	90 ±1.5	95±1.11	95±1.4	70±1.12	75±1.7	85±1.9	50±1.8	65±1.3	75±1.14
Contrast	90± 1.1	97±1.9	99±1.3	70±1.7	75±1.4	85±1.6	55±1.5	65±1.2	80±1.8
Sharpness	85±1.9	90±1.4	95±2.1	60±2.2	65±2	80±2.2	45±1.7	55±2.1	70±1.9
Contour	90±1.23	95±1.1	95±1.3	80±1,2	85±1.2	90±1.15	65±1.3	65±1.25	70±1.2

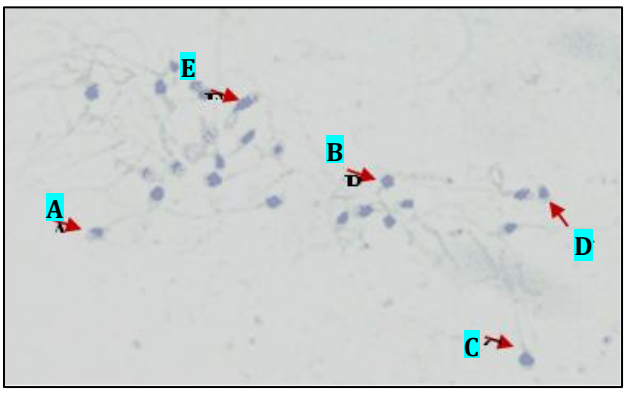

Figure 2: Spermatozoa stained with aqueous extracts of *Hibiscus sabdariffa* at a dilution factor of 1/5th for 1 hour (Magnification ×1000) A: macrocephaly, B: microcephaly and absence of acrosome, C: normal, D: absence of flagellum E: elongated and coiled

3.2.1.1 Staining Using Hydroethanolic Extracts of *Hibiscus sabdariffa*

Analysis of the staining indicators in smears prepared and then stained with hydroethanolic extracts of *Hibiscus sabdariffa* revealed that the best results were obtained with a 1:5 dilution and a staining time of 1 hour. The overall

pattern of the resulting diagrams showed that the levels reached by the staining indicators depended on the concentrations of the extracts used.

For the 1:5 dilution, color intensity ranged from 90% to 95% between 10 minutes and 1 hour; contrast ranged from 90% to 95%; sharpness ranged from 85% to 95%; and outline definition ranged from 90% to 97.5%. For the 1:10 dilution, intensity and contrast ranged from 70 to 85% over a period of 10 minutes to 1 hour. Sharpness ranged from 60 to 90%, and finally, contrast ranged from 80 to 90%. For the 1:20 dilution, intensity ranged from 50% to 75% over a period of 10 minutes to 1 hour, contrast from 55% to 80%, sharpness from 45% to 70%, and finally, outline from 65% to 70%.

Figure 3 showed spermatozoa stained with hydroethanolic extracts of *Hibiscus sabdariffa*. The background is light, with the spermatozoa exhibiting distinct staining. The various parts (head, acrosome, midpiece, and flagellum) are clearly visible. Some morphological abnormalities of the spermatozoa are also observed, namely: at the head, microcephaly and the absence of an acrosome; at the midpiece, angulation; and, in some cases, the absence of a flagellum.

Table 4: Staining indicators in smears stained with hydroethanolic extracts of *Hibiscus sabdariffa*

Staining indicators (%)	**Smears staining dilution and dyeing time (n=60)**								
	Dilution 1/5			Dilution 1/10			Dilution 1/20		
	10 mn	30 mn	60 mn	10 mn	30 mn	60 mn	10 mn	30 mn	60 mn
Intensity	70±1.3	80±1.7	95±1.6	45±1.66	60±1.3	70±1.93	35±1.23	45±2.3	65±1.63
Contrast	65±1.58	75±1.2	85±1.8	45±1.4	60±1.6	70±1.1	35±1.98	45±1.2	55±1,8
Sharpness	65±1.8	75±1.6	80±2	35±1.2	50±2.1	65±1.1	30±2.5	45±1.4	50±2.2
Contour	70±1.3	75±1.5	75±1	70±1.9	65±1.4	70±1.4	45±1.2	50±1.6	55±1.4±1.5

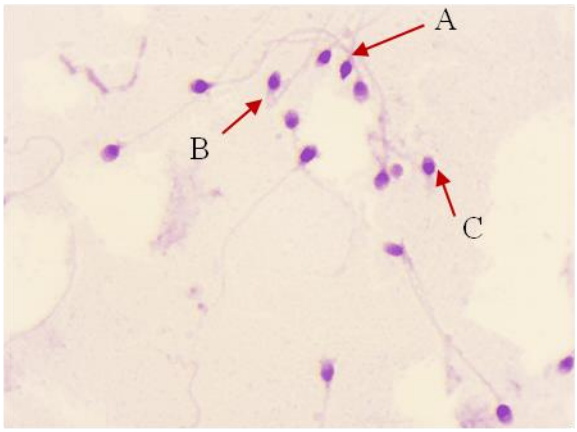

Figure 3: Spermatozoa stained with hydroethanolic extracts of *Hibiscus sabdariffa* at a dilution factor of 1/15th for 1 hour (Magnification × 1000) A: microcephaly, absence of acrosome, B: angulation, C: absence of intermediate piece and flagellum

3.3 Comparison of the Staining Properties of *Hibiscus sabdariffa*

A comparative evaluation of staining indicators in smears stained with aqueous and hydroethanolic extracts of Hibiscus sabdariffa reveals a statistically significant difference in favor of the hydroethanolic extracts, with a p-value < 0.001 at the set α = 5% significance level.

The calculated mean values for the indicators show an excellent staining level (value = 77.57%, i.e., > 75%) for smears stained with hydroethanolic extracts of *Hibiscus sabdariffa*, compared to an acceptable level (value = 58.05%, i.e., between 50% and 75%). These results are presented in Table 3.

Table 5: Characteristics of the color indicators based on the extraction method for the essential oil of *Hibiscus sabdariffa*

Dyes Indicator	Aqueous extracts of *Hibiscus sabdariffa*(n=60)	Hydroethanolic extracts of *Hibiscus sabdariffa* (n=60)	*t*-value	*p*-value
Intensity	61.67% ± 16.95	77.5% ± 14.68	-1.88	<0.001
Contrast	61.67% ± 13.93	79.16% ± 14.25	-2.08	<0.001
Sharpness	55% ± 17.31	71.67% ± 16.95	-1.89	<0.001
Contour	53.89% ± 11.11	81.94% ± 12.61	-3.32	<0.001
Mean	*58.05% ± 4.19*	*77.57% ± 4.33*	*-2.32*	<0.001

The average smear test results for smears stained with aqueous extracts of *Hibiscus sabdariffa* were considered acceptable (58.05%), while the average for hydroethanolic extracts was considered excellent (77.57%).

4 DISCUSSION

The search for alternatives to the use of synthetic dyes in clinical laboratories led us to conduct this study, which compared two (2) methods for extracting the coloring essence from the *Hibiscus sabdariffa* flower.

Hibiscus sabdariffa is a plant whose biochemical properties have been studied by various authors. These studies have revealed antioxidant, metabolic, and regulatory effects on the cardiovascular and immune systems (15)(16).

The extraction method for the Hibiscus sabdariffa-based dye, which combines water and alcohol as solvents, yielded the best results in this study. This could be explained, in part, by the fact that the ethanol used dissolves plant cell membranes and solubilizes the pigments contained within the cells; making the extraction of the essence more intense and efficient (17). It is also a polar organic solvent that acts as a preservative to extend the dye's shelf life and facilitate drying when applied to a cytology slide (12). These properties help overcome the limitations and shortcomings associated with using pure water alone as a solvent (18). The water-alcohol mixture preserves water's ability to extract anthocyanin, which is responsible for the reddish-purple coloration of the *Hibiscus sabdariffa* flower.

Certain molecular classes, such as flavonoids and tannins, were absent following phytochemical screening. These findings contradict the literature, which regularly reports the presence of these compounds (19)(20). This discrepancy could be due, in part, to the variety of extraction methods used. Indeed, it has been revealed, following a series of tests involving seven (7) different extraction methods (21). A higher concentration of flavonoids and, consequently, an increase in the effects of the angiotensin-converting enzyme (ACE) inhibitor when extraction was performed with 80% methanol. Furthermore, geographic origin and geoclimatic conditions can alter the composition of plants, as well as the plant part used in the study, which may be the root, bark, leaf, or flower (22).

The observed difference in the intensity of staining of sperm cell structures, depending on the dilution factor, suggests that it is necessary to use *Hibiscus sabdariffa* extracts at concentrations that ensure maximum binding. In addition to the dilution factor, the staining time for the smears appears to be a key parameter in the method for preparing spermocytogram slides stained with *Hibiscus sabdariffa*. Slides that were in contact with the stain for at least 30 minutes yielded better results when examined under a light microscope. These results are consistent with those of an author (23). However, the 1-hour staining time for the smears that showed the best staining results appears to be a limiting factor compared to the Papanicolaou staining method, where the average time is 30 minutes. The use of *Hibiscus sabdariffa* as a stain appears to have a limitation in terms of the long time the technique may take, which could impact the pace and speed of work, particularly when a large number of samples need to be processed (12)(5).

The pH of the prepared stains ranged from 1 to 2.5. The ability of *Hibiscus sabdariffa* to stain the nucleus and the cytoplasmic membrane can be explained by the fact that, on the one hand, at the nuclear level, there are basic proteins such as histones, which are octameric structures around which DNA molecules are wound (24). On the other hand, anthocyanins become positively charged in a strongly acidic environment and are neutral at higher pH levels. Thus, the high acidity of the reconstituted *Hibiscus sabdariffa* extracts used in our study (pH < 3) would favor the existence of anthocyanins in the form of a "flavylium cation" with an overall positive charge. This property could explain the ability of *Hibiscus sabdariffa* to intensely stain the sperm nucleus (25). Similarly, the visualization of cytoplasmic contours suggests an interaction between the cytoplasmic membrane and the dye. This can be explained by the fact that

membrane proteins are basic and carry positive charges that can interact with the negative charges carried by the highly acidic structures of the dye.

The level of distinction and assessment of sperm morphology was satisfactory, as it was possible to identify the four main components considered in the Krüger classification: the nucleus, the acrosome (which together form the head), the midpiece, and the flagellum (2).

The head abnormalities detected in the collected samples were similar to those identified by other staining methods commonly used in the laboratory to study sperm morphology (26). Cases of macrocephaly, microcephaly, and two heads were observed, as well as elongated or deformed heads. Some spermatozoa lacked an acrosome. This abnormality makes natural fertilization impossible because the acrosome contains a

a significant number of substances (e.g., acrosin and hyaluronidase) necessary for crossing the barriers surrounding the oocyte (27)(28).

The abnormalities detected also involved the neck and the flagellum. These were sometimes absent, coiled, or even duplicated. These are abnormalities that impair sperm motility and are clearly identified by the natural dye derived from *Hibiscus sabdariffa* as well as by conventional dyes (29)(30).

The economic and ecological benefits of a natural plant-based stain are significant. By way of comparison, the cost of producing one liter of *Hibiscus sabdariffa*-based stain is approximately five times less expensive than an equivalent quantity of MGG or Papanicolaou stain.

Making a natural plant-based dye available to laboratories with limited resources requires further research to confirm and specify safety and toxicity thresholds for users, as well as a longer-term follow-up to determine the maximum shelf life and optimal storage conditions.

The limitations encountered during this research were of a material and financial nature. To overcome the lack of equipment, a collaboration with the laboratories of the Biosciences and Pharmacy departments provided access to the materials and devices lacking at the HEC Laboratory of the Medical Sciences Department, which were necessary for extraction and phytochemical screening. Finally, the inability in this study to precisely determine the origin of the *Hibiscus sabdariffa* flowers obtained from vendors in Adjame, a commercial district in the city of Abidjan—posed a significant challenge. In fact, the origin of these flowers, which were sold openly on market stalls, could not be determined with certainty. Some speculations suggested production in northern Côte d'Ivoire, while other responses were more vague. The consequence of this situation is the inability to control the production and drying sites and processes. Since geoclimatic parameters can significantly affect the composition and concentration of active ingredients, it is necessary to ensure the traceability and reproducibility of the processes used to obtain these *Hibiscus sabdariffa* flowers if they are to be routinely used for the production of natural dyes. To achieve this, it might be feasible for the laboratory team to cultivate the flowers themselves in order to control the procedures and production conditions for these flowers (31)(32)(33).

5 CONCLUSION

The purpose of this study was to evaluate the contribution of a natural dye derived from the flower of *Hibiscus sabdariffa*—commonly known as "bissap" in West Africa—to the study of sperm morphology.

The experiments conducted determined the optimal preparation conditions for the dye, including the choice of solvent (water-alcohol), as well as the optimal dilution factor and staining time, which were $1/5^{th}$ and 1 hour, respectively.

Assessment of the morphological characteristics of spermatozoa was possible and revealed numerous abnormalities in the head (shape, nucleus, acrosome), neck, and flagellum.

Comparisons with conventional staining methods revealed superior image clarity following Papanicolaou staining and results similar to those obtained with Giemsa staining.

Significant economic and ecological benefits can be expected from the development of such natural plant-based dyes available on the Ivorian market, particularly for laboratories with limited resources.

Further studies will need to be conducted to clarify the current state of knowledge regarding the optimization of extraction processes, methods of use, storage, management of biosafety issues, and the dissemination of this project.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

We would like to thank the staff and researchers at the Ecology Research Center and the Laboratory of Histology, Embryology, and Cytogenetics in Abidjan for their generous cooperation.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest regarding this article.

Statement of ethical approval

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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