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Phytochemical screening, evaluation free radical scavenging activity and toxicity study of three aphrodisiac products aphrodisiac products sold on the markets of Korhogo (Côte d'Ivoire)

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Abstract
This study is based on the phytochemical screening, antioxidant activity evaluation and toxicity study of three aphrodisiac medicinal products (HV, KA and KK) sold in the markets of Korhogo (Côte d'Ivoire), with a view to revealing to the population the advantages and disadvantages of their uses. Phytochemical sorting of secondary metabolites using color tube reactions and thin layer chromatography (TLC) revealed the presence of polyphenols, flavonoids, coumarins, tannins, phenolic acids, steroid-terpenoids, saponins and alkaloids in the HV medicinal extract. In contrast, only alkaloids and steroid-terpenoids were detected in KA and KK. The presence of these secondary metabolites is thought to underlie the antioxidant activities observed by both qualitative (tube and TLC) and quantitative (spectrophotometric) analytical methods towards the DPPH free radical. However, quantitative spectrophotometric analysis showed a better antioxidant activity of KA with IC_{50} = 0.113 mg/mL compared to that observed in HV and KK with IC_{50} of 0.602 mg/mL and 0.807 mg/mL respectively. The oral toxicity study using the OECD 423 method showed that the 03 drug products can be considered almost non-toxic with LD_{50} greater than 5000 mg/kg BW. The overall phytochemical and biological results highlighted the beneficial effects of these three medicinal products, whose use presents no major hazards for the organism.

Keywords: Aphrodisiac, phytochemical screening, antioxidant activity, toxicity

1. Introduction
Pleasure has always been sought by humans since their existence. Deprived of sexual pleasure, humans live with hidden frustration (Ondele *et al.*, 2015) [18]. Human sexual life and activity can be affected by various diseases. Such is the case of sexual dysfunction. This common pathology affecting both men and women manifests as persistent and recurrent pain or difficulty with sexual response (Lue *et al.*, 2004; Ajit *et al.*, 2017) [14, 1]. Factors implicated in the onset of sexual dysfunction include age, diabetes, cardiovascular disease, urogenital, psychiatric and all chronic conditions (Droupy, 2005) [7]. However, some so-called conventional treatments exist, such as drugs (Viagra®, Cialis®, Levita®), implants and surgery. (Giuliano *et al.*, 2001) [8]. However, the cost of these treatments is very high, and they have harmful side-effects for some patients, particularly those with heart failure and low blood pressure (Giuliano *et al.*, 2001) [8]. Faced with this situation, some people turn to traditional medicine, which provides them with treatments based on plant products presumed to be aphrodisiacs, at lower cost (Isaac, 2009; Virag, 1982) [10]. However, these mostly uncontrolled products are still not without toxicity risks (Owens *et al.*, 2014) [20]. With this in mind, this investigative study focused on three products supposedly made from plants sold on the markets of Korhogo (Côte d'Ivoire). Thus, the aim of this work is to know the content of these products, to enlighten the populations and manufacturers on their advantages and disadvantages.

2. Materials and Methods
2.1. Material
2.1.1. Medicated products
These are three manufactured, liquid products sold on Korhogo markets as tonic drugs and specifically considered as aphrodisiacs, whose names have been arbitrarily coded as « KA, KK and HV ».

These products are water-based. KA and KK products are manufactured and sold on a more or less industrial scale, while HV is traditionally manufactured and sold on a small scale.

2.1.2. Laboratory materials and equipment
The technical equipment consists of the usual laboratory glassware, an electronic balance (DENVER INSTRUMENT SI-234), a water bath (Neo-Tech SA), a hot plate (Rommelsbcher), a spectrophotometer (JENWAY) and a fume hood (Erlab and Trionyx).

2.1.3. Reagents and chemicals
Chemical equipment consists of analytical-grade solvents, developers and reagents. These products were purchased from Polychimie (Côte d'Ivoire). For thin-layer chromatography (TLC) tests, we used silica gel chromatoplates 60 F254, on an aluminum support (Merck, 1980) [16].

2.1.4. Animal material
The animals used in our experiments come from the animal house of the Pharmacology Department of the Pharmaceutical and Biological Sciences Faculty of the Université Félix Houphouët-Boigny (Côte d'Ivoire). These are white female rats of the Wistar strain, 8 to 9 weeks old and weighing between 160 and 200 g. The rats are kept in favorable rearing conditions. They are fed daily with a standard complete feed in pellet form. Each animal is identified by a number.

2.2. Methods
2.2.1. Obtaining the different extracts
2.2.1.1. Aqueous liquid extracts (drug products)
The three aqueous drug products were used directly as initially prepared by the manufacturers to.

- Prepare selective extracts and aqueous crude extracts;
- Evaluate phytochemical screening in tubes;
- Evaluate antioxidant activity in tubes.

2.2.1.2. Selectives extracts
A 15 mL volume of each aqueous extract was depleted by successive fractionations with (3 × 10 mL) hexane (C_6H_{14}), dichloromethane (CH_2Cl_2) and ethyl acetate (AcOEt). The various selective organic fractions were concentrated in an oven and then stored in a refrigerator at 4°C. They were used for phytochemical screening and evaluation of antioxidant activity on TLC plates (Ouattara *et al.*, 2016) [19].

2.2.1.3. Aqueous crude extracts
50 mL of each product were poured into a porcelain dish and placed in an oven at 50 °C for 3 days. After evaporation of the water, the crude aqueous extracts were powdered and used to assess the antioxidant activity of the various products by spectrophotometry, and to study their acute toxicity.

2.2.1.4. Phytochemical screening
Secondary metabolites (tannins, phenolic acids, alkaloids, polyphenols, flavonoids, coumarins, saponins, sterols and polyterpenes) were identified using TLC plate tests and tube tests using color and precipitation reactions.

2.2.2.1. Color reaction tests and tube precipitation
The detection of certain secondary metabolites (polyphenols, flavonoids, coumarins, saponins, sterols and polyterpenes) was carried out using color tests and test tube precipitation. Analytical techniques described in Békro *et al.*, (2007) [3]; Dohou *et al.*, (2003) [6] were used for these tests.

2.2.2.1.1. Polyphenols detection
A few drops of a 2% (w/v) aqueous iron (III) chloride solution ($FeCl_3$) were added to 2 mL of crude hydroethanolic extract. The appearance of a blue-black or green-black coloration indicates the presence of polyphenols (Békro *et al.*, 2007) [3].

2.2.2.1.2. Flavonoid detection: Shinoda test
5-7 drops of concentrated HCl and 2-5 Mg chips are added to 2 mL of crude hydroethanolic extract. In the presence of flavonoids, a pink-orange coloration is observed after 3 to 5 min. To accelerate the reaction and enhance color, the reaction mass is heated in a water bath for 2-3 min (Békro *et al.*, 2007) [3].

2.2.2.1.3. Coumarin detection: Potassium hydroxide (KOH) test
10 drops of 10% (w/v) alkaline methanolic KOH solution are added to 3-5 mL of plant extract. The mixture is heated in a water bath. Next, 5-10 mL of distilled H_2O are added and the reaction mass is vigorously stirred. The resulting solution is neutralized with 10% (v/v) HCl until an acidic solution is obtained. If cloudiness or precipitation is observed, the presence of coumarins is confirmed (Békro *et al.*, 2007) [3].

2.2.2.1.4. Sterol and polyterpene detection
An aliquot of hydroethanolic crude extract is dissolved in 1 mL acetic anhydride ($CH_3CO_3CCH_3$) in a test tube. Next, 0.5 mL of concentrated sulfuric acid (H_2SO_4) is slowly poured over the walls of the test tube. The appearance of a violet coloration, turning blue and then green, indicates a positive reaction (Békro *et al.*, 2007) [3].

2.2.2.1.5. Detection of saponins: Foam test
A 2 g mass of ground dry plant material is boiled in 100 mL distilled water for 30 min (or 20 min after initial boiling). After cooling and filtration, the solution is made up to 100 mL with distilled water. From this stock solution, 10 tubes (1.3 cm internal diameter) are prepared with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 mL; the volume in each tube is readjusted to 10 mL with distilled water. Each tube is shaken vigorously in the horizontal position for 15 sec. After 15 min rest in vertical position, the height (in cm) of the persistent foam is recorded. If it is less than 1 cm in all tubes, the foam index (Im) sought is less than 100. If it is 1 cm in one of the tubes, then the foam index is calculated by the following formula.

$$\mathrm{Im} = \frac{1\,000}{\mathrm{N°tube}}$$

The presence of saponins in the plant is confirmed with an index greater than or equal to 100 (Dohou *et al.*, 2003) [6].

2.2.2.2. Phytochemical screening on TLC plates
The detection of certain secondary metabolites (tannins, phenolics acids, and alkaloids) was carried out using TLC plate assays on the selective extracts following the methods described by Mamyrbékova-Békro *et al.*, (2008) [15] and Kabran (2011) [11].
Using capillaries, 2 µL of each selective extract is deposited as a dot 0.5 cm from both edges of the chromatographic plate. The TLC plates are then placed in the migration tank

containing the migration solvents (developing agents). After development, the chromatograms were visualized with specific developers in the visible range. Colorations appearing as spots are recorded and frontal ratios (Rf) calculated.

2.2.3. Antioxidant power estimation

2.2.3.1. Tube screening of medicated extracts for DPPH

The method used for this test is that proposed by Popovici *et al.* (2009) [21].

In a 0.5 mL volume of medicated extract solution of each organ, 1.5 mL of violet DPPH is added, and the positive reaction is reflected by the appearance of a yellow coloration in the medium after 15 min of incubation.

2.2.3.2. TLC screening of selective extracts for DPPH

The TLC antioxidant screening used is that developed by the method described by Takao *et al.*, (1994) [24].

A volume of 10 µL of each selective extract of the different drug products is deposited on a chromatoplate (silica gel 60 F_{254}, on aluminum support (Merck, 1980) [16], which is then placed in a chromatography tank saturated with migration solvent. After development, the chromatograms are dried and then developed with an ethanolic solution of DPPH (2 mg/mL). After 30 min of optimal time, extract constituents with potential free radical scavenging activity are revealed as pale-yellow spots on a violet background.

2.2.3.3. Assessment of antioxidant activity of crude drug extracts by DPPH spectrophotometry

The antioxidant potential of the extracts was assessed using the method of Blois (1958) [4].

DPPH was solubilized in absolute EtOH to obtain a solution with a concentration of 0.3 mg/mL. Different concentration ranges (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL) of the extract are prepared in absolute EtOH. 2.5 mL plant extract and 1 mL ethanolic DPPH solution are added to dry, sterile tubes. After shaking, the tubes are placed in a dark place for 30 min. The absorbance of the mixture is then measured at 517 nm against a blank consisting of 2.5 mL pure EtOH and 1 mL DPPH solution. The positive reference control is ascorbic acid (vitamin C). DPPH inhibition percentages are calculated according to the formula.

$$I(\%) = (A_b - A_e) / A_b \times 100$$

I: Inhibition percentage
A_b: Absorbance of blank
A_e: Absorbance of sample

The concentrations required to trap 50% (IC_{50}) of DPPH are determined from the graphs showing the percentage inhibition of DPPH as a function of extracts or vitamin C concentrations.

2.2.4. Toxicity test

The toxicity study was carried out in accordance with OECD (2001) guideline 423 for chemical testing. Tests were carried out on rats divided into batches of three according to mass. All rats used were female, as recommended by the guideline, due to their high sensitivity.

These healthy rats, weighing between 160 and 200 g, were kept in clean, well-ventilated cages. They were adapted to the laboratory's experimental environment for one week prior to experimentation. During this period, the animals were monitored daily to assess their mass and behavior.

The animals were fasted for 16 h and provided with water during the treatment.

Three dose levels were tested in ascending order on different batches of female rats: 50, 300 and 2000 mg/kg body weight. A volume of 1 mL of each aqueous crude drug extract was administered by gavage through a gastric tube to the different batches of rats. Mortality was regularly observed every day for 14 days, and the number of deaths used to determine the 50% lethal dose (LD_{50}).

2.2.5. Statistical analysis

Analyses of the measurements obtained during the various manipulations were carried out using EXCEL 2021 (version 16.0). It was used to plot the various diagrams and also to determine the IC_{50} parameter for each extract.

3. Results and Discussion

3.1. Results

3.1.1. Phytochemical screening

Phytochemical screening was carried out to get an idea of the presence of secondary metabolites in the three drug products studied.

3.1.1.1. Phytochemical screening in tube

Phytochemical screening of saponins, polyphenols, flavonoids, coumarins and sterol-terpenes was carried out using tube characterization reactions.

Saponins were screened using aqueous solutions of the various medicinal products. The foam heights used to calculate foam indices are all below 1 cm KA and KK, so these products contain no saponins, unlike HV which does, with a foam index of 500. The presence of polyphenols was confirmed by the appearance of a blue-black coloration after the addition of a 2% (m/v) $FeCl_3$ aqueous solution. Flavonoids were characterized by the Mg test in hydrochloric acid (Shinoda test), and the pink-orange coloration observed indicated a positive reaction. The presence of coumarins was observed by the appearance of cloudiness or precipitation in the test tubes. Finally, acetic anhydride ($CH_3CO_3CCH_3$) revealed sterols and terpenes by the appearance of a violet coloration turning blue and then green (Békro *et al.*, 2007) [3].

The different results of phytochemical screening in tubes are shown in Table 1.

Table 1: Detection of polyphenols, flavonoids, coumarins and sterol-terpenes

	Polyphenols	Flavonoids	Coumarins	Sterol-terpenes	Saponins
KA	-	-	-	+	-
KK	-	-	-	+	-
HV	+	+	+	+	+

(+): Présence; (-): Absence

3.1.1.2. Thin layer chromatography of selective extracts from KA, KK, and HV

This method was used in this study to search only for tannins, phenolic acids and alkaloids in the selective extracts. The colorations and frontal ratios (RF) of the spots observed are given in Tables 2 and 3.

Table 2: Detection of tannins and phenolics acids

Extrants	R_f (Color): Possible compound
KA	No tannins or phenolic acids identified
KK	No tannins or phenolic acids identified
HV	0.95 (gray): tannin; 0.00 (green): phenolic acid

Table 3: Detection of alkaloids

Extracts	R_f (Color): Possible compound
KA	0.27 (orange): alkaloid; 0.17 (orange): alkaloid; 0.05 (orange): alkaloid
KK	0.6 (orange): Alkaloid; 0.06 (orange): Alkaloid
HV	0.56 (orange): Alkaloid; 0.09 (orange): Alkaloid

3.1.1.3. Summary of phytochemical screening in tube and by TLC

Following qualitative detection tests using thin-layer chromatography (TLC) and tube color reactions for the secondary metabolites of the three drug products, all the results obtained are shown in Table 4.

Table 4: Summary table of phytochemical screening of secondary metabolites

	Polyph.	Flavonoids	Coumarins	Ste-ter.	Saponins	Alkaloids	Tannins	A. phe.
KA	-	-	-	+	-	+	-	-
KK	-	-	-	+	-	+	-	-
HV	+	+	+	+	+	+	+	+

(+); présence; (-): absence; Polyph: Polyphenols; A. PHE.: Phenolics acids

3.1.2. Antioxidant activities

3.1.2.1. Antioxidant activity in tube

After addition of the DPPH solution to the drug products studied, a yellow coloration was observed after a few minutes of incubation, indicating antioxidant activity in these drug products studied.

3.1.2.2. Antioxidant activity by TLC

Antioxidant compounds in the selective extracts of the three drug products appeared as pale yellow spots on a violet background. The results of this analysis are reported in Tables 5 and 6.

Table 5: Phytocompounds that trap DPPH In the developer CH_2Cl_2/AcOEt/ CH_3COOH (1:4:1) (V/V/V)

Extracts	R_f (Color): Possible compound
KA	0.95 (yellow): NI; 0.11 (yellow): NI; 0.00 (yellow): NI
KK	0.95 (yellow): NI; 0.11 (yellow): NI; 0.00 (yellow): NI
HV	0.95 (yellow): tannin; 0.82 (yellow): NI; 0.69 (yellow): NI 0.60 (yellow): NI; 0.52 (yellow): NI; 0.37 (yellow): NI 0.31 (yellow): NI; 0.25 (yellow): NI; 0.20 (yellow): NI 0.15 (yellow): NI; 0.00 (yellow): Phenolic Acid

NI: Unidentified Compound

Table 6: Phytocompounds that trap DPPH In the developer CH_2Cl_2/AcOEt /C_6H_{14}/ CH_3COOH (1:2:1:0.1)

Extracts	R_f (Color): Possible compound
KA	0.62 (yellow): NI; 0.56 (yellow): NI; 0.27 (yellow): alkaloid; 0.05 (yellow): alkaloid
KK	0.6 (yellow): alkaloid; 0.06 (yellow): alkaloid
HV	0.81 (yellow): NI; 0.09 (yellow): alkaloid

NI: Uni-dentified Compound

3.1.2.3. Evaluation of antioxidant power by spectrophotometry of extracts from KA, KK and HV recipes

In view of its scientific accuracy, spectrophotometric quantification of the antioxidant power of plant matrices was carried out to certify the manifestation of said activity, detected by TLC and tube color reactions.

3.1.2.3.1. Percentage inhibition of vitamin C and drug products

Figure 1 shows the different percentages of DPPH inhibition by the drug products KA, KK and HV compared with vitamin C.

These products show significant antioxidant potential at all extract concentrations. The inhibition percentages of the aqueous medicated extracts range from 31.236±0.175% at 0.25 mg/mL in KK to 69.485±0.874% at 2 mg/mL in HV. However, these are lower than the inhibition percentages for vitamin C, which range from 63.913±0.131% at 0.0625 mg/mL to 99.705±0.000% at 2 mg/mL.

3.1.2.3.2. Determination of IC_{50} for vitamin C and drug products

The IC_{50} of the drug products studied and of vitamin C were determined graphically using EXCELL software. Each IC_{50} is calculated by solving the equation y= ax+b of each trend curve with: Y=50. The mean IC_{50} values obtained are shown in Table 7.

Table 7: IC_{50} values for drugs and vitamin C.

	KA	KK	HV	Vitamin C
IC_{50} (mg/mL)	0.113	0.807	0.602	0.037

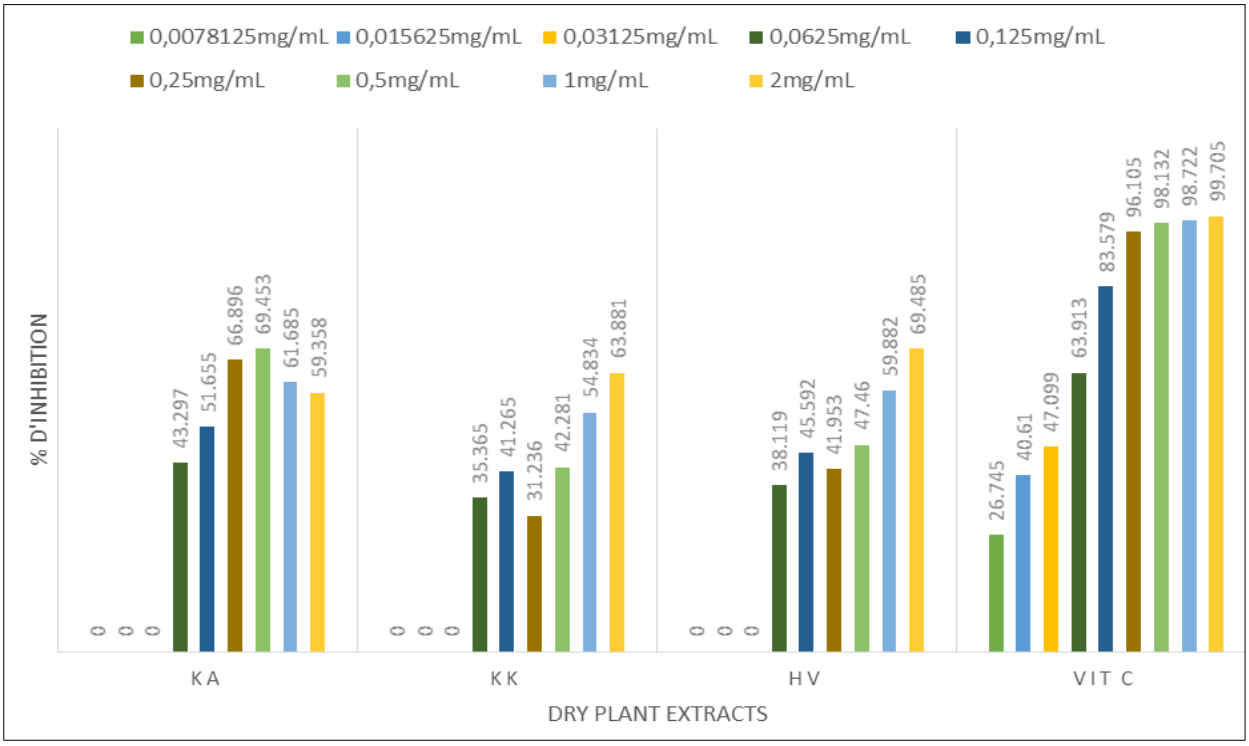

Fig 1: DPPH inhibition by aqueous drug extracts

3.1.3. Toxicity evaluation

After oral administration of drug extracts to female rats at various doses, a few clinical signs were observed (accelerated heart rate, respiratory difficulties and convulsions). However, the animals regained a normal appearance within minutes, and during the 14 days of observation, no abnormalities were observed in their general behavior. This behavior takes into account mood (grooming, crying, agitation, aggression and curiosity), the central nervous system (spontaneous activity; response to touch; response to pain) and vagus nerve stimulation (Defecation; Urination; Piloerection, sialorrhea or salivation). The number of deaths in female rats as a function of the doses administered, enabling the lethal dose (LD_{50}) to be determined, is shown in Table 8.

Table 8: Mortality of female rats as a function of doses of KA, KK and HV drug extracts.

Extracts		**KA**			**KK**			**HV**		
Batch of mice		**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 1**	**Batch 2**	**Batch 3**
Dose administered (mg /kg BW)		50	300	2000	50	300	2000	50	300	2000
Number of deaths per day	Day 1	0	0	0	0	0	0	0	0	0
	Day 2	0	0	0	0	0	0	0	0	0
	Day 3	0	0	0	0	0	0	0	0	0
	Day 4	0	0	0	0	0	0	0	0	0
	Day 5	0	0	0	0	0	0	0	0	0
	Day 6	0	0	0	0	0	0	0	0	0
	Day 7	0	0	0	0	0	0	0	0	0
	Day 8	0	0	0	0	0	0	0	0	0
	Day 9	0	0	0	0	0	0	0	0	0
	Day 10	0	0	0	0	0	0	0	0	0
	Day 11	0	0	0	0	0	0	0	0	0
	Day 12	0	0	0	0	0	0	0	0	0
	Day 13	0	0	0	0	0	0	0	0	0
	Day 14	0	0	0	0	0	0	0	0	0
Mortality		0	0	0	0	0	0	0	0	0
% of mortality		0	0	0	0	0	0	0	0	0

4. Discussion

This work is part of the phytochemical screening, antioxidant activity evaluation and toxicity study of three medicinal products KA, KK and HV sold in Korhogo markets.

Phytochemical screening for secondary metabolites in medicinal products using color tube tests and TLC chromatography showed the presence of polyphenols, flavonoids, coumarins, tannins, phenolic acids, alkaloids, saponins and sterol-polyterpenes in the HV product, while the KA and KK medicinal products contained only alkaloids and sterol-terpenes.

Evaluation of antioxidant activity by tube, TLC and spectrophotometry showed that the drug products KA, KK and HV have antioxidant power towards the DPPH radical. This observed activity is due to the synergistic action of the various secondary metabolites detected in the three organs (Bruneton, 1999; Sivapriya & Srinivas, 2007; Kolak *et al.*, 2009) [5, 23, 12]. Indeed, these secondary metabolites contain several pharmacological properties, including antioxidant,

anti-inflammatory, analgesic, platelet anti-aggregant, antidiabetic, anticancer and aphrodisiac properties (Singh *et al.*, 2004; Tona *et al.*, 2004; Kwan *et al.*, 2015; Basma *et al.*, 2011; Tuhin *et al.*, 2017) [22, 25, 13, 2, 26]. This information could justify the use of these three medicinal products by the people of Korhogo. However, evaluation of antioxidant activity by quantitative spectrophotometry showed that the reference molecule (vitamin C) is more active than the medicinal products, with an inhibitory concentration (IC_{50}) of 0.03664 mg/mL. Nevertheless, the antioxidant power of these medicinal products is not negligible compared with that of vitamin C. By comparing the various IC50 values obtained for the drug products, it can be established that IC_{50} (KA) < IC_{50} (HV) IC_{50} < IC_{50} (KK). Thus, the antioxidant power of KA is greater than that of HV, which in turn is greater than that of KK.

A study of toxicity by determining the lethal dose (LD50) showed that the three medicinal products KA, KK and HV are almost non-toxic according to the Hodge and Sterner (1980) [9] toxicity scale, with values above 5000 mg/kg PC. These products may therefore not present any danger to the human organism, justifying their current use in traditional medicine in Korhogo as aphrodisiac drugs.

5. Conclusion

The main aim of this master's thesis was to reveal the presence of families of chemical compounds, assess the antioxidant activity and verify the acute toxicity of three medicinal products sold in Korhogo markets, in order to enlighten the population and manufacturers about their advantages and disadvantages. To this end, aqueous extracts of these medicinal products were used to carry out phytochemical and biological investigations.

The results of phytochemical screening of the extracts by color reaction and TLC revealed the presence of polyphenols, flavonoids, coumarins, tannins, phenolic acids, steroid-terpenoids, saponins and alkaloids in the HV medicinal extract. In contrast, only alkaloids and steroid-terpenoids were detected in KA and KK.

The antioxidant activity of these products, measured against DPPH by qualitative and quantitative methods, shows that they contain antioxidant activity and could therefore be recommended as antioxidants capable of preventing or curbing damage caused by oxidative stress and associated diseases such as sexual dysfunction. The antioxidant properties observed in these three products are thought to be due to the synergistic action of the secondary metabolites detected in their breasts. Quantitative spectrophotometric analysis of these products showed that KA is more active than HV, which in turn is more active than KK.

The oral toxicity study showed that the three medicinal products can be considered almost non-toxic, with LD_{50} values of over 5000 mg/kg BW.

The overall phytochemical and biological results highlighted the beneficial effects of these three medicinal products, whose use would not present any major danger to the organism. However, it would be interesting to study the aphrodisiac activity of these three medicinal products and elucidate the active molecular structures within them, in order to determine whether they are typically plant-based products.

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