

Identification and Characterization of Sildenafil Citrate in Energy Drinks Sold in Goma (Drcongo): A Case Study of Stronger, Libala Wine, Nkolo Mboka Wine, Nguvu Tangawisi 4×4 Wine, and Asili Tangawisi Wine

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ABSTRACT

The consumption of Stronger, Nguvu Tangawisi, Asili Tangawisi, Nkolo Mboka, and Libala wines is increasingly popular among the population of Kivu, particularly in Goma and the northern region, due to their effectiveness in enhancing male libido. Unscrupulously, producers incorporate aphrodisiac molecules such as sildenafil citrate, tadalafil, and others into these drinks without informing consumers and disregarding the potential side effects on adolescents and healthy individuals. Screening of the five samples revealed the presence of total polyphenols, flavonoids, leuco-anthocyanins, saponins, terpenes, steroids, and tannins in all five beverages, thus justifying their use. Their aphrodisiac activity. Faced with synthetic sildenafil citrate (vigoral 100 mg), our witness, the Asili Tangawisi and The Stronger beverages exhibited spots similar to those of the controls after thin-layer chromatography (TLC), with the same front ratio or values close to them. The quantification of synthetic sildenafil citrate was performed using high-performance liquid chromatography (HPLC), which revealed significant amounts of this compound in our samples. Asili Tangawisi wine showed a concentration of 13.6 mg/100 mL, while The Stronger wine had a concentration of 225.07 mg/100 mL.

Keywords: Aphrodisiac, energy drinks, sildenafil citrate, Viagra.

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1. INTRODUCTION

It is well known that pleasure, in general, and sexual pleasure have always been sought after by humans since their existence. Deprived of sexual pleasure, individuals experience hidden frustration. Sexual capabilities in men vary according to age, physiological, and psychological states. These capabilities reach their peak between the ages of twenty-five and thirty-five and gradually decline beyond this age range [1], [2]. However, human life and sexual activity can be affected by various diseases. This is the case with sexual dysfunction, characterised by disturbances that can alter the cycle of sexual response in one of its four phases: arousal, plateau, orgasm, and resolution.

It has been revealed that aphrodisiacs have variable effects on different dimensions of sexuality (desire, arousal, sexual response, bodily sensitivity, and genitality) depending on the context, dosage, and duration of use [3]. Indeed, several researchers have studied various plant or animal substances considered aphrodisiacs, with their usage and nomenclature varying from one society to another.





Fig. 1. Bottles of different studied drinks.

According to Müller-Ebeling [3], over 1000 plants have been identified in today's era that are used for sexual purposes. These plants come from different regions of the world and are used or consumed in various forms (infusions, decoctions, cooked dishes, ointments, powders, inhalations, etc.) to maintain and improve sexual functions [3].

However, the Congolese population in general, and specifically in the eastern part of the country, is not spared from this phenomenon. Due to the proliferation of indigenous products in the market, the consumption of aphrodisiacs has become a widespread practice in the eastern region of this large country in the heart of Africa (DR Congo), where these substances are used by the elderly, young people, and even adolescents.

As a result, numerous varieties of traditional and modern aphrodisiacs have emerged and are displayed on the sidewalks along the streets of cities and major urban areas in the DR Congo [4], [5]. In the pursuit of satisfying sexual desire, a wide range of plant-based aphrodisiacs and natural roots are increasingly consumed in the DRC. However, the potentially harmful health consequences associated with their consumption should never be overlooked.

Research has shown that excessive consumption of aphrodisiacs contributes to sexual dysfunctions by reducing desire, arousal, and orgasm; their effects could lead to sexual dysfunctions in 30% to 60% of consumers [3]. However, there is a lack of robust data for Africa in general and specifically for the DR Congo. It is within this context that our study aims to determine the quality of energy drinks sold in the city of Goma by identifying and quantifying sildenafil citrate.

2. MATERIALS AND METHODS

2.1. Materials

There are five different herbal-based beverages made with plants, sugar, honey, and preservative compounds, some of which include Nguvu tangausi, the stronger, vin asili tangausi, nkolo mboka, and libala (Fig. 1). The selection of these beverages was motivated by their availability, price, and consumer preferences in North Kivu.

2.2. Laboratory Equipment

During our analyses, we used the following equipment: an analytical balance, volumetric flasks, Erlenmeyer flasks, graduated pipettes, burettes, beakers, an ultrasonic bath, a stirrer, tanks, an oven, a hot plate, and a spectrophotometer. Added to this are the Agilent 1290 infinity II (CHEMETRIX) and simple VOTRIXS brand HPLC chromatographs, a UV-visible lamp with an iodine detector at a wavelength of 254 nm (in the Pharmacognosy Laboratory of the Faculty of Pharmacy and in the Analytical Chemistry Research and Application Laboratory of the Polytechnic Faculty at the University of Kinshasa).

2.2.1. Chemical Reagents

Methanol (CH_3OH) for liquid chromatography and hydrochloric acid (HCl) for analysis (37% of each) were supplied by Merck (Darmstadt, Germany). Ammonium formate (99% NH_4HCO_2) was purchased from Alfa Aesar (Karlsruhe, Germany). Ultrapure quality water (18.2 M Ω . cm) was obtained using a Milli-Q Plus 185 device from Millipore (Billerica, Massachusetts, USA) located in the LAPHAKI laboratory (Kinshasa, DR Congo). Sildenafil citrate was bought in pharmacies under the trade name "VIGORAL 100 mg".

TABLE I: DIFFERENT REAGENTS TO DETECT CHEMICAL GROUPS [6], [7]

| Chemical groups | Identification reagents | Identification reagents |
|--|---|---|
| Sterols and polyterpenes | Acetic anhydride-concentrated sulfuric acid Lieberman Burchadats reagents | Appearance at the interphase of a purple or violet ring, turning blue, then green |
| Polyphenols | Ferric chloride FeCl_3 (2%), $\text{K}_3\text{Fe}(\text{CN})_6$ 1% (1:1 v/v)-Burton's reagent | Appearance of a blackish blue or green color more or less dark |
| Flavonoids | Hydrochloric alcohol, Iso-amyl alcohol, mg chips-Shinoda's reagent | Release of heat, then pink-orange or purplish coloration |
| Anthocyanins | Hydrochloric acid and ammonia (NH_4OH) 50 | Purplish coloring |
| Catechic tannins and gallic tannins | CH_3COONa , FeCl_3 2%-Stiasny's reagent (30% formalin + concentrated HCl 2:1) Sodium acetate, Ferric chloride (CH_3COONa , FeCl_3) | Gelatinous precipitate in large flakes-pink. Intense blue-black coloring |
| Saponins | Foam index | Appearance of a persistent foam of at least 1 cm |
| Alkaloids | Dragendorf's reagent (Potassium iodo-bismuthate solution) or Burchard's reagent (Iodo-iodide reaction) | Reddish-brown colored precipitate |

2.3. Methods

2.3.1. Preparation of the Extracts

We have not made any preparations in the strict sense for our extracts, apart from the conservation and the time taken to settle the drinks and reduce the state of shock (agitation). The screening carried out was such that in the aqueous phase, we considered each drink as a preparation already ready for analysis.

2.3.2. Phytochemical Screening in Solution

In chemistry, the determination or analysis of the chemical composition of plant material, for example, is a series of tests and analyses based on chemical reactions (sometimes coloured) that, without being entirely specific and specialised, allow as far as possible to detect the presence of a certain number of phytochemical groups present in these plant materials (Table I). Chemical screening is, therefore, a method of qualitative analysis whose purpose is the determination of the phytochemical groups contained in each plant and their structures. This qualitative analysis can be done using several techniques [6].

2.3.3. Search for Synthetic Sildenafil Citrate

To confirm the results obtained for the screening in solution, we carried out the screening by chromatography. It should be noted here that only qualitative thin layer chromatography (TLC, silica gel 60F254 aluminium, Merck, Germany) has been tested. Dosage by HPLC using the following conditions: a stationary phase (XBridge C18, 250×4.6 mm (5 μm)), a column oven temperature of 30 °C, a wavelength of 290 nm, a mobile phase (Methanol/Water (70:30 v/v)), a flow rate of the mobile phase of 1.0 mL/minute, and an injection volume 10 μL . The HPLC is an Agilent 1290 infinity II (CHEMETRIX) and a simple VOTRIXS, with a UV-Visible lamp and an iodine detector.

2.3.4. Search for Synthetic Sildenafil Citrate

To confirm the results obtained for the screening in solution, we carried out the screening by chromatography. It should be noted here that only qualitative thin-layer chromatography (TLC, silica gel 60F254 aluminium, Merck, Germany) has been tested. Dosage by HPLC using the following conditions: a stationary phase (XBridge C18, 250×4.6 mm (5 μm)), a column oven temperature of 30 °C, a wavelength of 290 nm, a mobile phase (Methanol/Water (70:30 v/v)), a flow rate of the mobile phase of 1.0 mL/minute, and an injection volume 10 μL . The HPLC is an Agilent 1290 infinity II (CHEMETRIX) and a simple VOTRIXS, with a UV-Visible lamp and an iodine detector.

3. RESULTS AND DISCUSSION

It appears from the analysis of the chemical screening of our samples that the latter, like certain plant-based drugs, contain secondary metabolites (Table II). They all contain total polyphenols, saponins, alkaloids, terpenes, anthocyanins, tannins, and flavonoids (positive tests for all samples). Comparing these results with those of Ndombe *et al.* [1] and many other researchers [6], [8]–[10], there is reason to say that the aphrodisiac effect more expressed by our samples could be attributed to the existence of flavonoids and sterols revealed by phytochemical tests.

TABLE II: RESULT OF THE CHEMICAL SCREENING OF OUR SAMPLES

| Chemical groups | Nguvu tangawusi wine | The stronger wine | Asili tangawisi wine | Nkolo mboka wine | Libala wine |
|-----------------|----------------------|-------------------|----------------------|------------------|-------------|
| Saponines | + | + | + | + | + |
| Polyphenols | + | + | + | + | + |
| Flavonoids | + | + | + | + | + |
| Anthocyanins | + | + | + | + | + |
| Terpenes | + | + | + | + | + |
| Saponins | + | + | + | + | + |
| Alkaloids | + | + | + | + | + |
| Tanins | + | + | + | + | + |

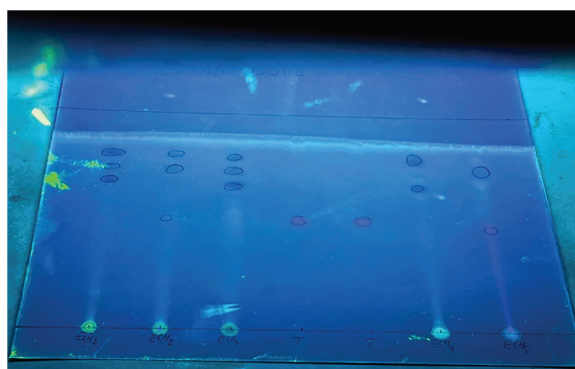


Fig. 2. TLC chromatogram for the identification of synthetic sildenafil citrate in the extracts of our 5 samples (The stronger, nkolo mboka, nguvu tangausi, vin libala, and vin asili tangausi). PS: Silicagel 60F254, PM/Methanol/Ethyl Acetate (1:2; v/v).

These bioactive substances would induce changes in the level of neurotransmitters involved in erectile function, modulate the action of these neurotransmitters at the level of their target cells, or raise the level of androgens. It is well established that in erectile function, androgens stimulate the expression of the neuronal nitric oxide synthase (nNOS) isoform and modulate the activity of phosphodiesterase type 5 [8].

However, several authors have reported that the secondary metabolites responsible for the aphrodisiac activity are more total alkaloids and polyphenols [3], [11]–[15]. For example, we recognise yohimbine, codeine, and morphine, which are alkaloids and behave in the body as selective antagonists of $\alpha 2$ -adrenergic receptors and which act through this on the autonomic nervous system, penile tissues, and muscle cells. vascular smoothness [16]. Saponins and tannins have been shown to be involved in the aphrodisiac activity, in the fortifying, stimulating, and stupefying characteristics of plants, and they inhibit a longer action than caffeine [17].

Identification of synthetic sildenafil citrate in samples by TLC In order to confirm or refute the use of synthetic aphrodisiacs in energy drinks sold in the city of Goma, thin layer chromatography was performed on our samples. Sildenafil citrate is used as a control in this work. Thin layer chromatography (TLC) was followed by HPLC for the quantification of our control in beverages. The result of the phytochemical screening by TLC showed the presence of sildenafil citrate in the samples of Asili tangawisi and the Stronger drinks. Fig. 2 below illustrates the results of the TLC analysis of all five (5) samples, including the two (2) controls used for the study.

In this image (Fig. 2), we can observe that all the samples show spots at different frontal ratios and different colorations. These spots are none other than secondary metabolites already characterised by phytochemical screening. At this stage, we were not interested in all these spots. Only the spots whose Rf are close to those of the controls. These are the spots on the samples of the Asili Tangausi wine at 0.46 and the Stronger wine at 0.43, which are close to or equal to 0.46 (Rf of the controls). However, we can say, in light of these spots, that these two samples find their stimulating effect through the use of sildenafil citrate. The other samples can be considered not to contain sildenafil citrate but other forms of the aphrodisiac compounds not studied in this work. The falsification of energy drinks and/or wines with aphrodisiac properties is a widespread practise throughout the world. It is also this practise that has thrown the quality assurance of these drinks into doubt and absolute uncertainty in the face of informed and proven minds [8], [11], [17], [18].

Indeed, sildenafil exerts an action on the mesopontine cholinergic neurons, which have a role in preventing the destruction of GMP, and thus maintains the concentration of nitric oxide (NO) responsible for erection. To understand the mechanism of action, it is important to know that the

TABLE III: DOSAGE OF SILDENAFIL CITRATE
IN THE SAMPLES ANALYZED

| Sample | Concentration in mg/100 mL |
|----------------------|----------------------------|
| Asili tangawisi wine | 13.60 ± 0.40 |
| The stronger wine | 225.07 ± 1.21 |

physiological mechanism responsible for penile erection involves the release of nitric oxide (NO) in the corpora cavernosa during sexual stimulation. Nitrogen oxide then activates the enzyme guanylate cyclase, which leads to increased concentrations of cyclic guanosine monophosphate (cGMP), inducing relaxation of the smooth muscles of the corpus cavernosum and promoting blood flow. Sildenafil (or its analogue) is a potent and selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE-5) in the corpora cavernosa [11], [14].

3.1. Dosage of Sildenafil Citrate in Drinks

Referring to the analysis of high-performance liquid chromatography (HPLC), we reinforce our position, that of the use of aphrodisiacs in drinks, the stronger and Asili Tangausi. Apart from containing secondary metabolites, these two types of drinks have been boosted by the addition of a few grammes of pure sildenafil citrate, like the one sold in pharmacies. These results allow us to say that so-called plant-based drinks with aphrodisiac properties do have an effect on sexual performance because of the presence of sildenafil citrate.

Indeed, sildenafil exerts an action on the mesopontine cholinergic neurons, which have a role in preventing the destruction of GMP; and thus maintains the concentration of nitric oxide (NO) responsible for erection. To understand the mechanism of action, it is important to know that the physiological mechanism responsible for penile erection involves the release of nitric oxide (NO) in the corpora cavernosa during sexual stimulation. Sildenafil (or its analogue) is a potent and selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE-5) in the corpora cavernosa [11], [14]. Since the manufacturers do not report the amount of these molecules in the samples, we assayed them by HPLC using sildenafil citrate as a control.

From Table III, it can be seen that Asili tangausi wine and the Stronger contain significant concentrations of sildenafil citrate. Asili Tangausi wine has a concentration of 13.6 mg/100 mL or 40.8 mg of sildenafil citrate for 300 mL of energy drinks contained in a bottle sold, and for the Stronger wine, we have a concentration of 225.07 mg/100 mL or 562.67 mg of sildenafil citrate. sildenafil for 250 mL energy drinks contained in a bottle sold in the market.

4. CONCLUSION

Indeed, although still taboo, erectile dysfunction has several origins: psychological, neurological, hormonal, or vascular. Erectile dysfunction is very common and affects men at any age, but mainly from 40 to 70 years old. It is, therefore, very important to improve the quality of life of men who are affected, as well as that of their sexual partner(s), by means of adequate treatment. But some men take these products for sexual performance without taking into account the short- and long-term effects on the body. This is the case for our five (5) drinks listed above. Thus, this study aimed to use chromatography (HPLC and TLC) to identify and characterise the sildenafil citrate, which would be the basis of the adulteration of these herbal drinks with aphrodisiac properties. The chemical screening upstream of these five samples revealed the presence of total polyphenols, saponins, flavonoids, alkaloids, terpenes, anthocyanins, steroids, and tannins, thus justifying their aphrodisiac activity. Compared to the synthetic sildenafil citrate (vigoral 50 mg) control, the Asili tangausi and the stronger wines revealed spots similar to those of the controls, with the same frontal ratio or similar values. The quantitative analysis carried out by HPLC revealed concentrations of the order of 13.600.40 mg/100 mL for the asili tangausi wine and 225.071.21 mg/100 mL for the stronger wine. This situation led us to conclude that the stronger and asili tangausi wines were doped with synthetic sildenafil citrate, a situation that justifies their effectiveness compared to other wines.

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CONFLICT OF INTEREST

The author states that there is no conflict of interest related to the material discussed in the manuscript.

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