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Original Article

IDENTIFICATION AND QUANTIFICATION OF PHOSPHODIESTERASE-5 INHIBITOR AS FALSIFIED IN "NATURAL" MALAYSIAN HERBAL APHRODISIACS SOLD IN SOME BENINESE MARKETS

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ABSTRACT

Objective*:* Nowadays, there have been several reports of herbal products falsified with well-known synthetic molecules, leading to harmful health consequences for the consumer. The aim of this study was to assess the profile of 'natural' herbal aphrodisiacs in the local markets of the municipalities of Cotonou and Abomey-Calavi in Benin and to screen some of them for the presence of additives such as sildenafil and tadalafil.

Methods*:* A non-probability survey was conducted to identify the available aphrodisiacs and their characteristics. Some of them were then selected for analysis. Thin Layer Chromatography (TLC) was adopted for qualitative detection. The TLC positive extracts were then analyzed by HPLC on a C18 column with a mobile phase consisting of a mixture of 0.05M phosphate buffer (pH 5.8), acetonitrile and methanol (30:50:20). The Detection was performed at 290 nm.

Results*:* Seventy-seven aphrodisiacs were identified and from these, 18 were selected for analysis. Six of them were adulterated with tadalafil. The concentration of tadalafil in the samples was 1.7 to 4.6 times higher than the recommended dose of 20 mg.

Conclusion*:* This work opens the door to the need to control ''natural'' labeled products in order to ensure their quality.

Keywords: Herbal aphrodisiacs, Falsification, PDE-5 Inhibitors

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INTRODUCTION

The first evidence of the proven use of plants for aphrodisiac purposes goes back to antiquity in Roman, Chinese, Egyptian and Greek civilizations [1, 2]. Herbal products, whether traditional medicines, phytomedicines or dietary supplements, have always been popular due to the widely held belief that "natural" products are safer and healthier compared to synthetic product [3].

However, numerous cases of adulterated plant-based products have already been reported. One of the most frequent counterfeits is the addition of well-known synthetic substances. Thus, some so-called "completely natural" aphrodisiacs have shown the presence of PhosphoDiesterase 5 (PDE-5) inhibitors such as sildenafil, tadalafil or their analogues. The PDE-5 inhibitors are one of the pharmacological classes most found in aphrodisiac drugs in general [4] and in falsified aphrodisiac products in particular [5, 6]. These fully synthetic molecules were first discovered and marketed in the late 20th century and revolutionized the treatment of erectile dysfunction. Their addition to the products is supposed to be completely natural guarantees to their vendors the effectiveness of their products [7, 8].

According to a study conducted by the Biomedical Nuclear Magnetic Resonance group of the Laboratory of Synthesis and Physico-Chemistry of Molecules of Biological Interest (SPCMIB) at the University Paul-Sabatier in Toulouse, two-thirds of plant-based aphrodisiac products contain sildenafil or similar molecules, some of which have never obtained their approval to market [9]. In 2014, Podder *et al.,* found these molecules in more than a third of "natural" aphrodisiacs analyzed [10]. Although PDE-5 inhibitors usage is relatively safe, their addition to herbal products may cause multiple side effects due to interactions, poor product quality and overdose.

In Africa, the prevalence of sexual dysfunctions is at least 25% [11]. Benin is no less spared and the people who use herbal aphrodisiacs has increased.

In this study, pharmacies, supermarkets and traditional health practitioners in Cotonou and Abomey-Calavi were covered with a view to identify herbal aphrodisiacs labelled as 'totally natural'. Some of them were then analyzed to find the presence of well-known drugs such as sildenafil or tadalafil, in order to evaluate the effective falsification in our market.

MATERIALS AND METHODS

It was a descriptive and analytical cross-sectional study carried out in three phases: • a survey for the census of aphrodisiacs sold on the local market, as well as their characteristics • a selection and collection phase of samples to be analyzed• a qualitative and quantitative analysis phase, to search for sildenafil and tadalafil in the samples collected.

Target population, investigation and sampling for analysis

The target population consisted of herbal aphrodisiac products claiming to be "natural" sold in pharmacies, supermarkets and traditional health practitioners in Cotonou and Abomey Calavi. The list of traditional healers was supplied by the National Program of Pharmacopoeia and Traditional Medicine (PNPMT) of the Ministry of Health of Benin. Were included in the study the plant-based aphrodisiac products ready to use and sold in supermarkets, pharmacies and by traditional health practitioners in Cotonou and Abomey Calavi. Were excluded from the study aphrodisiac products in raw materials (stems, roots, bottled whole plants) which need preparation before use.

The two municipalities were covered using a non-probabilistic method. Pharmacies and supermarkets of the two municipalities were browsed using convenience sampling. The traditional health practitioners on the list were called by phone for an appointment. Data collection was performed using a questionnaire drafted for this purpose. The questions were a question of the indications and composition of the products and the informations on the package leaflet and the primary and secondary packaging.

Sample were selected for analysis if they had all the following characteristics of sildenafil and tadalafil: used by men for erectile dysfunction alone or associated, the onset of action ≤1h or not specified and presented in powder, liquid, tea bag or capsules

The selected products were purchased in a copy for the realization of the TLC. Those positive at this stage were then purchased in a copy for further analysis.

Qualitative and quantitative analysis

Standards and reagents

Reference standards of sildenafil citrate 98%, and tadalafil 98% were respectively purchased from USP by the National Agency for Quality Control of Health Products and Water of Benin (ANCQ) and were given to us as a gift; analytical grade methanol, chloroform, and acetonitrile were purchased from Honeywell, Merck and Central Drug House (CDH), respectively. Ultrapure quality water was obtained using a milli-Q device from Merck Millipore Laboratories (Manish, Germany)

Preparation of standards, sample extraction and preparation for TLC, TLC condition

For TLC, 0.25 mg of sildenafil and 0.125 mg of tadalafil were weighed, diluted in methanol and vortexed for one minute.

Twenty grams (20 g) of each sample was used regardless of its dosage form. Ten milliliters of methanol were used for the extraction with constant agitation overnight (12 h of time).

The standards and sample solutions were applied to 0.20 mm silica gel plate 60 with fluorescent indicator UV 254 nm (Merck, Germany) and developed with chloroform/ethanol (90: 1/2) as a mobile phase. Spots were located under UV radiation at 254 nm and 360 nm [12,13]. The sheets were then spread with sulfuric anisaldehyde and heated with a hair dryer.

Preparation of standards, sample extraction and preparation for HPLC, HPLC condition

For HPLC, 4 mg of tadalafil standard was diluted in 10 ml of methanol, sonicated for 10 min and filtered through a 0.45 micro membrane. Serial dilution was carried out to obtain five working standards (20 µg/ ml; 50 µg/ ml; 100 µg/ ml; 200 µg/ ml and 250 µg/ ml).

Five hundred milligrams (100 mg) of each sample positive to TLC were used regardless of its dosage form. Ten milliliters of methanol were used for the extraction with constant agitation overnight (12 h of time). The extracts were then sonicated for ten minutes and centrifuged for 30 min to facilitate the filtration through the micro-membrane (0.45 µm).

The assay was performed using a HITACHI HPLC with a Diode Array Detector (DAD) and a C18 analytical column (250 mm4 mm; 5 µm). The method used was adapted from a previous study with minor modifications [14]. Mobile phase consisted of a mixture of 0.05 M potassium dihydrogen phosphate buffer (pH adjusted to 5.8): acetonitrile: methanol (30: 50: 20) (v/v/v). The flow rate was 1 ml/min, the injection volume was 10 µL, and the detection wavelength was set at 290 nm.

RESULTS AND DISCUSSION

52 pharmacies, 20 supermarkets and 24 traditional health practitioners have been covered. A total of 77 herbal aphrodisiac products were listed. Given our limited means, our study only made it possible to survey a small number of pharmacies and supermarkets. The list of traditional healers provided by the National Pharmacopoeia and Traditional Medicine Program (PNPMT) of the Ministry of Health was perhaps not complete or updated, given a large number of existing traditional healers compared to the number present on the list.

Out of these 77 herbal aphrodisiac products identified, 63.64% were dietary supplements, 29.87% were herbal medicines and 6.49% were traditional recipes, and the most frequent indication was erectile dysfunction (72%). Eighty-two herbal species were inventoried in all the products. The most represented families are the Fabaceae, Apiaceae, Annonaceae, Apocynaceae, Zingiberaceae and Lauraceae. Batcho and al., also found the Fabaceae among the most represented family species in the treatment of sexual dysfunctions in Benin [12], but Panax ginseng CA Mey., was the most found species.

Fourty-four of the products listed came from Benin against 25.97% from Asian countries. Visual inspection revealed 67 cases of non-compliance out of 77, i.e., 87.01%. The non-compliance was about the absence on the packaging of information related to the safety of use or to the origin of some products. The leaflet was absent in most cases, but when present, spelling errors or lack of French translation were observed.

Among the 77 herbal products identified, a total of 18 samples were selected based on similar pharmacological characteristics with sildenafil and tadalafil and were collected for analysis.

Five were phytomedicines and 13 were dietary supplements. The samples included honey, capsules, solutions and powders. All the samples were claimed to contain only natural ingredients as per their respective formularies. They were randomly numbered from 1 to 18.

Sildenafil and tadalafil were searched in our products, but there are not the only possible PDE-5 adulterants. However, these two molecules are the most used in falsifications.

TLC remains an available and inexpensive, quick and easy method to perform for preliminary identification. This method has also been used by Moriyasu *et al.*, [13] and by Abourashed *et al.*, [14] for the identification of PDE-5 inhibitors in comfort products.

For our study, we tested several solvent systems. Among them, the chloroform ethanol mixture (9: 0.5; v/v) exhibited better separation and better migration of compounds compared to the mixture of Ammonia-Ethanol 96 °-Ethyl Acetate-Dichloromethane (1-20-30-50; v/v/v); and by the Hexane-Ethyl Acetate-Methanol (8-6-2; v/v/v) and Ethyl Acetate-Methanol (2-1; V/V) systems proposed by the European Pharmacopoeia.

Six out of 18 samples analyzed were found to have the same retention factor (Rf) as tadalafil 0.44±0.04, while none showed the same Rf as sildenafil (fig. 1). The compositions of all tested samples are rather complicated, explaining why some of them had a retention factor slightly superior or inferior (standard deviation of 0.04).

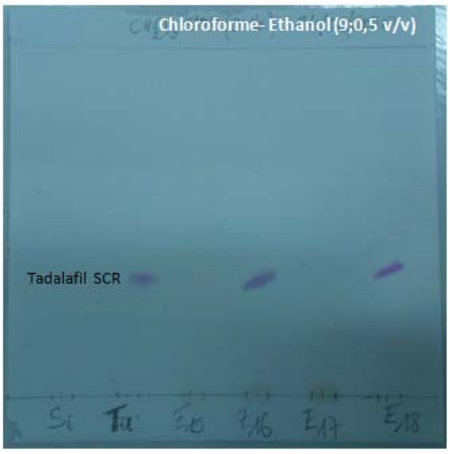

Fig. 1: TLC of the standards and samples after revelation to sulfuric anisaldehyde

The presence of tadalafil has been confirmed in the six tested samples by HPLC.

The identified samples not only showed a peak at the same retention time as tadalafil standard (4.087 min), (fig. 2) but also the same UV spectrum. The peaks were well-resolved with no overlapping. The quantity of tadalafil in the identified samples was determined as concentration from the calibration curve (r^2= 0.998), substituting the respective peak areas obtained for the identified sample preparations. The concentration of tadalafil found in the samples ranged from 35.63±0.02 mg to 92.52±0.27 mg. (table 1).

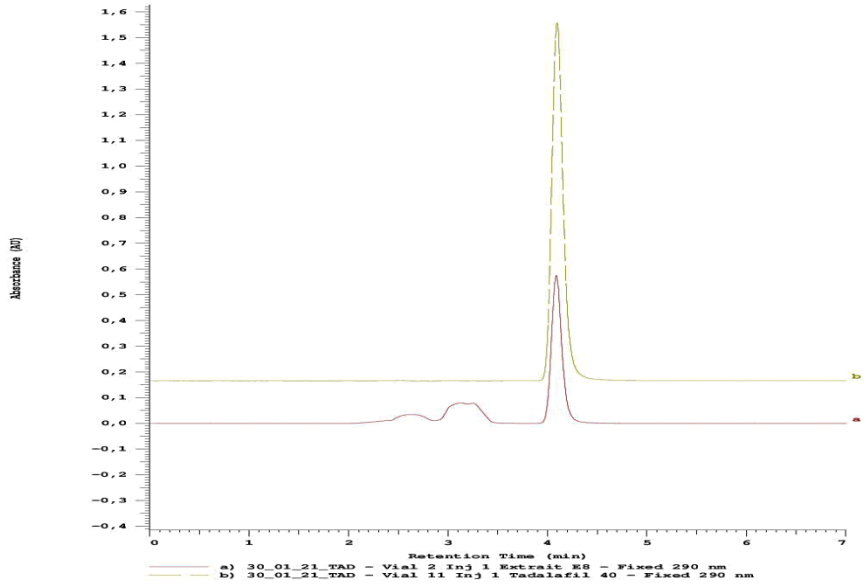

Fig. 2: HPLC chromatograms of a sample extract (b) and tadalafil standard (a)

The addition of active ingredients to so-called "natural" products is a common problem around the world and has been on the rise in recent years [15]. Numerous labels omit important ingredients that turn out to be drugs or experimental chemicals. On our tested products, none indicates tadalafil as the compound. According to the compositions displayed, only natural ingredients were obtained from a variety of herbs such as *Epimedium koreanum L.*, *Panax ginseng C. A. Mey*., *Eurycoma Longyfolia* or *Lepidium meyenii Walp*, were contained in these products. The samples analyzed contain a tadalafil quantity 1.7 to 4.6 times higher than the normal recommended dose of tadalafil of 20 mg.

Benin is one of the multiples countries where similar falsification of so-called ''natural'' herbal aphrodisiacs where found. In Bangladesh and Turkey, doses of PDE-5 inhibitors nearly 1.7 times and 4.6 times higher than the recommended doses were found in aphrodisiac products [10, 16].

Sildenafil and tadalafil may belong to the same class but are different from pharmacological characteristics. In fact, for 20 mg, tadalafil action is longer (35 h) than sildenafil's dosed at 100 mg (3 to 4 h). This can explain the preference of tadalafil over sildenafil in our samples. Tadalafil, therefore, guarantees a longer-lasting effect over time, as well as a lower manufacturing cost then greater benefits. These benefits are all more important as generic tadalafil is available at a lower cost, especially on the internet, where the price of one tablet can go down to 1.25 euros (800 XOF), while the prices of our falsified products vary from 2.6 to 19 euros (1,700 XOF to 13,000 XOF).

Table 1: Information and detected constituents of falsified products

N °	Product type	Form	Composition displayed	Collection place	Adulterant found	Quantity (mg)	Origin
E6	Dietary supplement	Powder	*-Eurycoma longifolia,-Panax ginseng, -Radix angelica sinensis, -Coffea sp*	Pharmacy	Tadalafil	41.56±0.59	Malaysia
E8	Dietary supplement	Honey	*-Eurycoma longifolia, -Coffea sp, -Lepidium meyeni*	Pharmacy	Tadalafil	58.63±0.07	Malaysia
E12	Dietary supplement	Honey	*-Eurycoma longifolia, -Panax ginseng, -Lepidium meyeni*	Pharmacy	Tadalafil	79.60±0.21	Malaysia
E14	Dietary supplement	Powder	*-Eurycoma longifolia, -Coffea s, -Lepidium meyeni, -Panax ginseng*	Pharmacy	Tadalafil	92.52±0.27	Malaysia
E16	Dietary supplement	Powder	*-Eurycoma longifolia, -Lepidium meyeni, -Panax ginseng*	Pharmacy	Tadalafil	55.75±0.34	Malaysia
E18	Dietary supplement	Candy	*-Panax ginseng, -Cynomorium songaricum,-Coffea sp*	Pharmacy	Tadalafil	35.63±0.02	Malaysia

CONCLUSION

Herbal aphrodisiacs are one of the most prone products to falsification involving the addition of PDE-5 inhibitors like sildenafil and tadalafil. In this study on herbal aphrodisiac products sold on the Beninese market, more than 80% were found to be non-compliant with good manufacturing practices, with the absence of essential elements on the labelling related to the safety or to the origin of the products. Six of them contained tadalafil, a substance not declared by the manufacturers, with doses above the recommended dose. However, none of them contained sildenafil.

It would be relevant to extend this study to other existing and frequently found PDE-5 inhibitors.

LIMITATIONS OF THE STUDY

The limitation of our work is the collection of the samples because the most falsified product are sold in the illicite matket.

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Nil

AUTHORS CONTRIBUTIONS

AGA, HG and CT designed the work. JEA, PD and LAY contributed for the analysis parts of the work. AGA, CT and KN contributed to the data collection and the, analysis and interpretation of the results. HG, LAY and AFG gave the facilities for the realization of this work. All authors reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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