

**Original Article**

**IDENTIFICATION AND QUANTIFICATION OF PHOSPHODIESTERASE-5 INHIBITORS AS ADULTERANTS IN DIETARY SUPPLEMENTS MARKED FOR SEXUAL ENHANCEMENT IN THE LEBANESE MARKET**

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**ABSTRACT**

**Objective:** Ultraviolet Visible spectrophotometric was adopted to identify and quantify any adulteration with PDE-5 inhibitors (Sildenafil and Tadalafil) in selected dietary supplements used for sexual enhancement in the Lebanese market

**Methods:** Nine dietary supplements, randomly collected from Lebanese pharmacies, were screened for Sildenafil and Tadalafil using UV-spectrophotometry for both qualitative and quantitative detection.

**Results:** Tadalafil was detected in one sample at a dose of 59 mg/dosage unit, with the maximal recommended dose being 20 mg. Sildenafil was detected in five samples at doses ranging from 11.7 to 188.2 mg/dosage unit, with the maximal recommended dose being 100 mg.

**Conclusion:** This study demonstrates that regular analysis of supposed dietary supplements is needed for more effective quality control and health promotion. The method described for the extraction, identification and quantification of Tadalafil and Sildenafil would be useful for regulatory detection of adulterations.

**Keywords:** Adulteration, PDE-5 inhibitors, Sildenafil, Tadalafil, Herbal supplements, Spectrophotometry

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**INTRODUCTION**

The popularity of herbal supplements as effective substitutes for synthetic drugs has increased worldwide in the recent years [1]. The reason for this high popularity is the common belief that herbal drugs are safe and can be used without medical supervision [2]. Moreover, the majority of patients trust herbal supplements manufacturers and believe their claims [3]. Most of these herbal preparations are available in the market as over-the-counter (OTC) dietary supplements and thus, are subject to food legislation policies which does not obligate efficacy studies, safety assessment, or quality control [4].

Unfortunately, many studies have reported the adulteration of herbal products with undeclared pharmaceutical ingredients, even at higher levels than the maximum recommended dose, and consumers are unaware of these counterfeit products [5].

The Food and Drug Administration (FDA) reported 572 adulterated dietary supplements between 2007 and 2014 in the United States, of which the most common were sexual enhancers (41.6%) [1]. Most of these adulterants were phosphodiesterase type five (PDE-5) inhibitors (Sildenafil, Tadalafil, Vardenafil and their analogues). The majority of reports were from Asian countries (67%), followed by Europe (22%) and North America (11%), and the most frequently reported adulterants were sildenafil analogues (62%), followed by tadalafil (26%) and vardenafil (9%) [6].

Phosphodiesterase type 5 inhibitors are the leading oral drug for pharmacological treatment of erectile dysfunction (ED) [3]. They are recommended as the first line of treatment by both the European and American urological guidelines [7]. However, they must be used under medical supervision as they may predispose the consumer to potentially severe drug-drug interactions such as the development of life-threatening hypotension when used in combination with nitrates [4].

It is obvious that the unaware usage of such preparations is dangerous as the risk of adulterated drugs use overweighs their benefits, especially in patients with co-morbid conditions such as cardiovascular complications and diabetes and even polypharmacy

in older adults pursuing effective and safe alternatives to PDE-5 inhibitors [2].

Up to now, 80 illicit analogues of PDE-5 inhibitors have been discovered, and unfortunately, the number is rising [4]. Campbell *et al.* tested 91 herbal products that were mostly in the United States and Asia. None of the samples claimed to include synthetic drugs, however, PDE-5 inhibitors were detected in 74 of them (81%), most of which were Tadalafil and/or Sildenafil, often at amounts higher than the maximum approved dose [8].

In France, 61% of 150 herbal sexual enhancers marked as 100% natural preparations were found to contain PDE-5 inhibitors [9].

A study conducted in Malaysia has reported that 82% of 62 products that claimed to enhance man's sexual health, were adulterated with one or more PDE-5 inhibitors and analogues, and one of the samples was found to contain even 5 different PDE-5 inhibitors [10].

In Iran, an analysis of 80 herbal products sold as sexual enhancers showed that 22 samples (27.5%) contained at least one undeclared pharmaceutical ingredient. Sildenafil was found in 19 samples, and 3 samples were adulterated with a mixture of tramadol, diazepam, and sildenafil [5].

In a study conducted in Egypt, five of the most popular food supplements used for the treatment of erectile dysfunction were selected for analysis from over 50 products available in the Egyptian market (OTC and tele-advertised). Undeclared sildenafil was detected in all analyzed products in amounts reaching to 180 mg per single dose [11].

The success of PDE-5 inhibitors for the treatment of ED, the risks they propose, and the widespread adulteration, raises the question of whether dietary supplements used as sexual enhancers in Lebanon are adulterated by synthetic drugs. However, to our knowledge, there aren't any reported analysis of dietary supplements marked for sexual enhancement in the Lebanese market.

Upon screening the literature of the PDE-5 inhibitors (Tadalafil and Sildenafil), several analytical methods have been used for their

quantitative and qualitative detection. These include; nuclear magnetic resonance (NMR) spectroscopy [9], liquid chromatography (LC), high performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UHPLC) coupled to ultraviolet/diode array detector (UV/DAD) [6], mass spectrometry (MS), ultraviolet (UV) spectroscopy, and Fourier transform infrared spectroscopy associated with attenuated total reflection (FTIR-ATR) [12–14].

Spectroscopic methods have been suggested as an on-field screening tool to be used by customs that are frequently confronted with large numbers of products suspected to be adulterated and require analytical inspection. That is because these methods are known to be rapid, simple to use, have high throughput, and require little sample preparation [12]. For this reason, in our work, the analytical method used is UV spectrophotometry [15, 16].

The aim of the present study is to identify and quantify any adulteration with PDE-5 inhibitors (sildenafil and tadalafil) in

selected dietary supplements used for sexual enhancement in the Lebanese market using UV spectrophotometric method.

## MATERIALS AND METHODS

### Standards and reagents

Reference standards of Sildenafil citrate and Tadalafil were certified to contain 99.7% (PHARMADEX S. A. L, BEIRUT, LEBANON). Methanol of analytical grade (SIGMA-ALDRICH, CHROMASOLV®). Nine products randomly purchased from different local pharmacies in Beirut were selected for testing. Their dosage form, ingredients and manufacturing details are tabulated in table 1.

### Instrumentation

Jasco V-730 double beam UV-Vis Spectrophotometer interfaced to a computer programmed with Jasco Spectra Manager software was used for spectrophotometric measurements, spectral acquisition and elaboration. A pair of 1 cm quartz cells were used to measure the absorption spectra.

**Table 1: Specifications of the tested products**

Sample code	Batch number	Expiry date	Ingredients	Country origin	Dosage form
S01	01180121	01/2021	Epimedium, Ginkgo Biloba, Panax Ginseng, Royal Jelly	China	Hard gelatin capsule
S02	(none)	14/06/2020	Ginseng Alijao, root of membranous milk vetch	China	Hard gelatin capsule
S03	120617110622	11/06/2022	Raspberry, Solomonsal Rhizome, Chinese Yam Rhizome, Barbary Wolfberry Fruit, Cassia Bark	China	Hard gelatin capsule
S04	20085	11/2020	Epimedium, Tribulus Terrestris, Ginkgo biloba, Hypericum perforatum, Cistanche, Crataegus Oxycantha	Lebanon	Hard gelatin capsule
S05	18022	02/2021	Pomegranate Extract, Broccoli Extract, Curcumin, Bromelain, Quercetin, Lycopene, Resveratrol, BioPerine®, Zinc, Copper, Selenium	Lebanon	Hard gelatin capsule
S06	0203R	03/2021	Korean Ginseng Extract, Ginkgo Biloba Extract, Vitamin C, Vitamin E, Zinc, Selenium, L-Arginine, L-Carnitine, L-Methionine, L-Phenylalanine	England	Tablet
S07	(none)	30/12/2020	Ginkgo Biloba, Epimedium Sagittatum, Tongkat Ali, Panax Ginseng	Spain	Tablet
S08	(none)	30/12/2020	Angelica, Clove Dong Quai	Spain	Soft Gelatin capsule
S09	140111	04/2021	Pure honey 97%, Eurycoma Longifolia, Panax Ginseng, Bee Larva Powder	Malaysia	Syrup

### Standards and samples preparation

#### Preparation of standard stock solution

Standard stock solutions (1 mg/ml) were prepared by accurately weighing and transferring 50 mg Sildenafil citrate or Tadalafil into two separate 50ml volumetric flasks. To each flask, 20ml methanol were added, sonicated for 5 min, and diluted to the mark with the same solvent.

#### Preparation of standard working solutions

Accurate volumes from Sildenafil and Tadalafil standard stock solutions were transferred into 50ml volumetric flasks and diluted to the mark with methanol, to obtain two different standard working solutions of 100 µg/ml each.

#### Calibration curve

Aliquots of the standard working solutions were transferred into series of 10ml volumetric flasks, to obtain a calibration set in the range of 10–40 µg/ml, using methanol as a solvent.

#### Pharmaceutical application

Four dosage units (powdered tablets, capsule content, or syrup) of each product were weighed and mixed. Average weight equivalent to one dosage unit was transferred into a 50ml volumetric flask,

30ml methanol were added and the mixture was sonicated for 20 min. Another 10ml methanol was then added and sonicated for an additional 10 min. The prepared mixture was then filtered and made up to 50ml with methanol. From the prepared solution, a volume of 0.1ml was transferred into 10ml volumetric flask and made up the mark with methanol. The absorbance value of this solution was then measured in the range of 200–400 nm at 0.5 nm interval, using methanol as blank.

## RESULTS AND DISCUSSION

The UV absorption spectra of Tadalafil (fig. 1) and Sildenafil (fig. 2) showed maximum absorption at 284 nm and 291 nm, respectively. The UV absorption spectra obtained from the tested products showed spectrum corresponding to Tadalafil in sample S01 (fig. 3) and spectra corresponding to Sildenafil in samples S02, S03, S04, S07, and S09 (fig. 4, 5, 6, 9, and 11, respectively). Whereas samples S05, S06, and S08 spectra were not in correspondence to tadalafil or sildenafil (fig. 7, 8, and 10, respectively).

Calibration curves were constructed at a maximum wavelength of 284 nm and 291 nm for Tadalafil and Sildenafil, respectively (fig. 12 and 13). Calculations were carried out using absorbance of each sample at 284 or 291 nm (Tadalafil or Sildenafil, respectively) in correspondence with the calibration curve. The results obtained are presented in table 2.

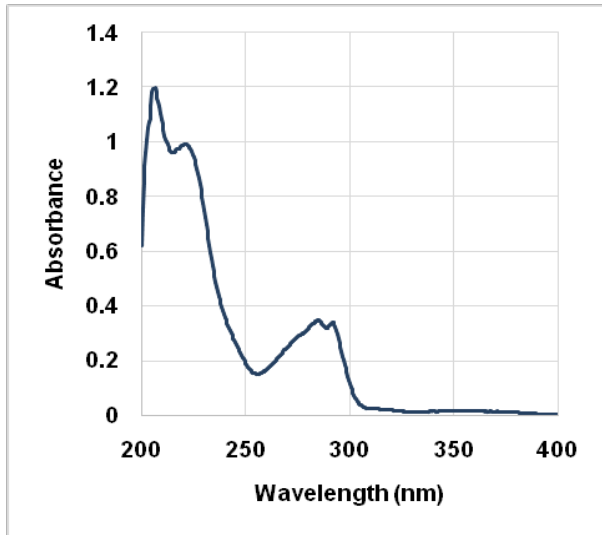


Fig. 1: Absorption spectrum of 10 µg/ml standard tadalafil in methanol

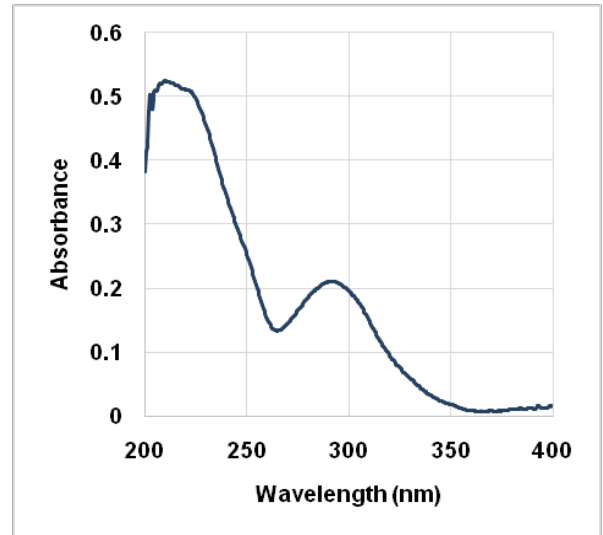


Fig. 2: Absorption spectrum of 10 µg/ml standard sildenafil in methanol

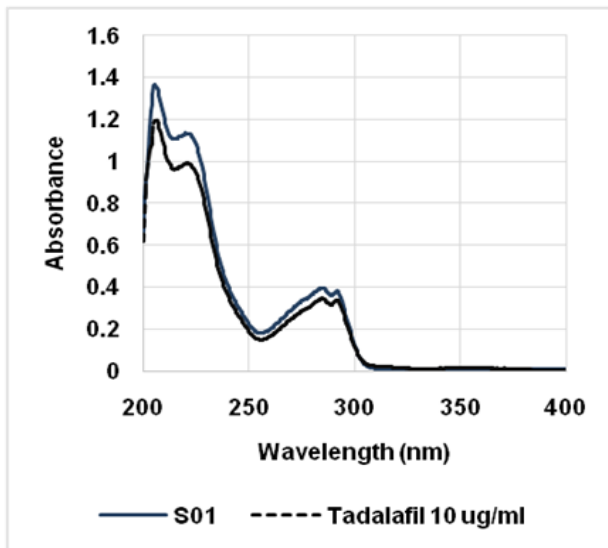


Fig. 3: Absorption spectrum of sample S01

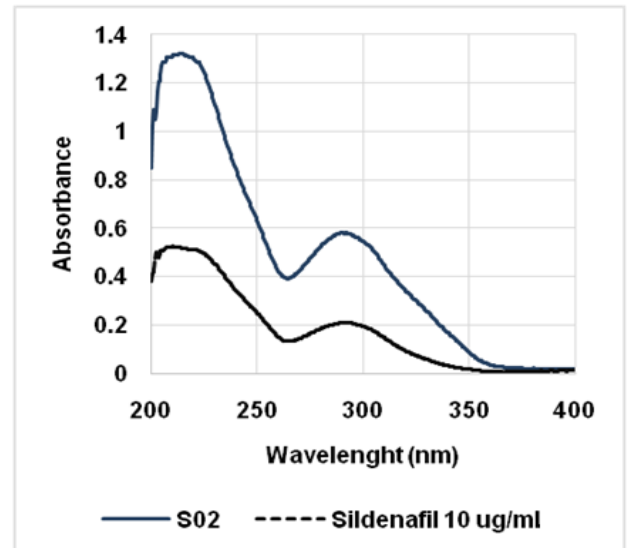


Fig. 4: Absorption spectrum of sample S02

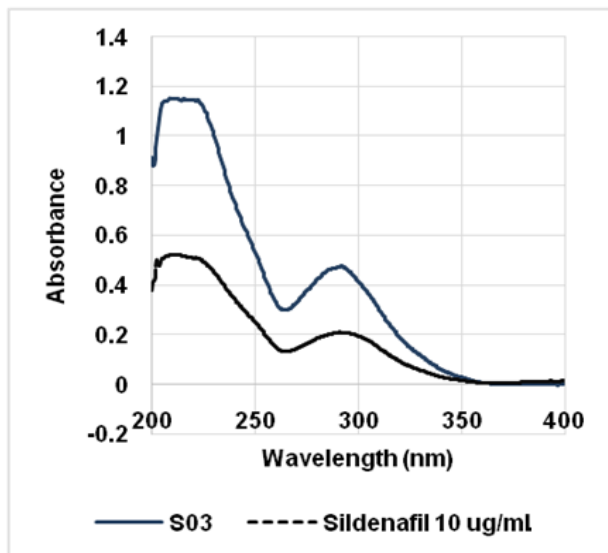


Fig. 5: UV Absorption spectrum of sample S03

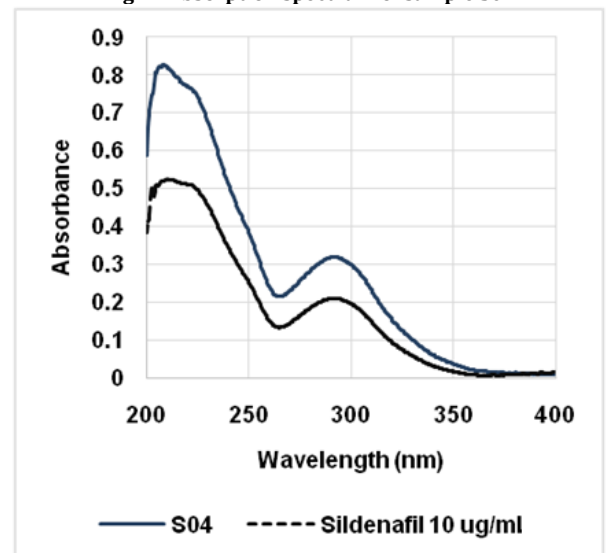


Fig. 6: Absorption spectrum of sample S04

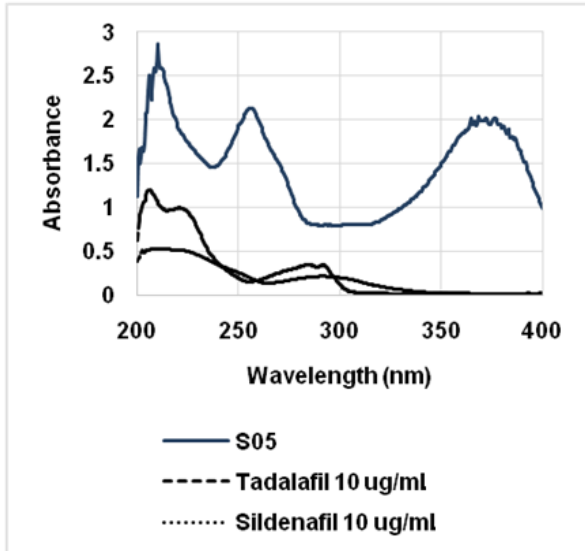


Fig. 7: Absorption spectrum of sample S05

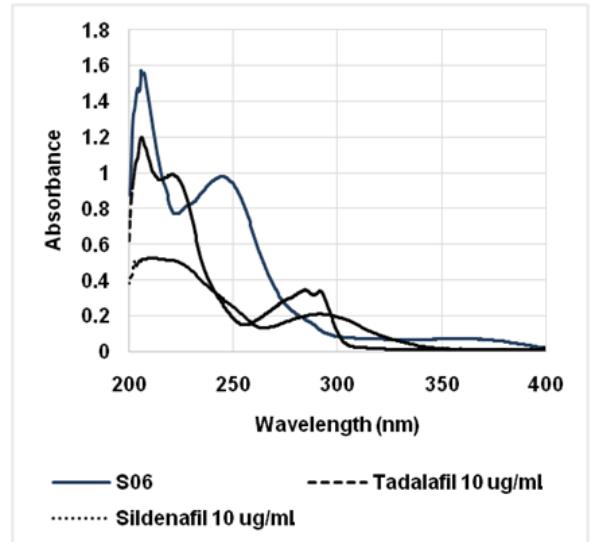


Fig. 8: Absorption spectrum of sample S06

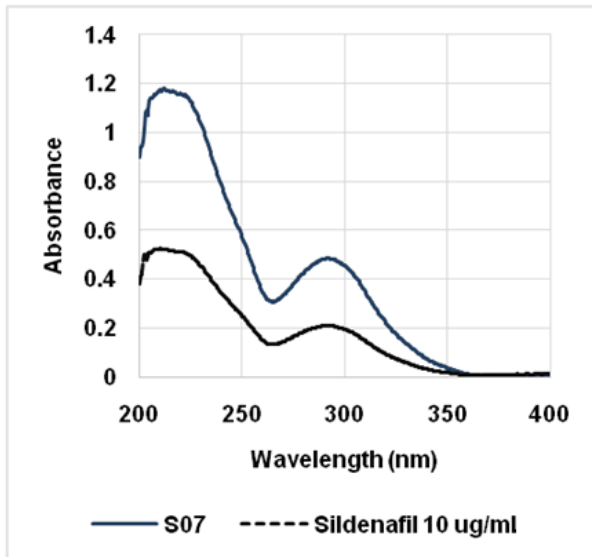


Fig. 9: Absorption spectrum of sample S07

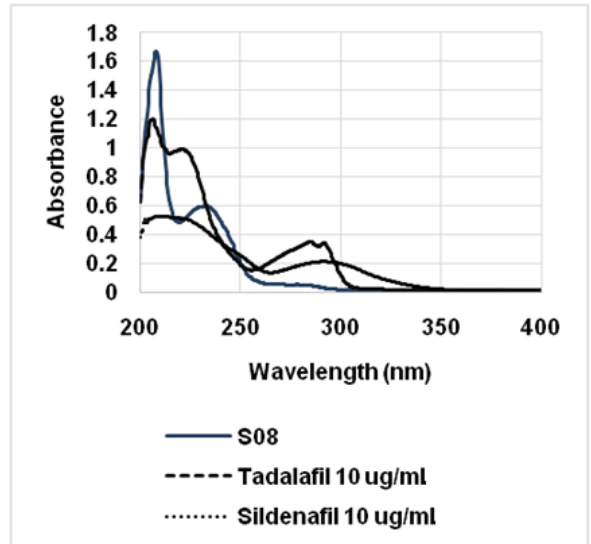


Fig. 10: Absorption spectrum of sample S08

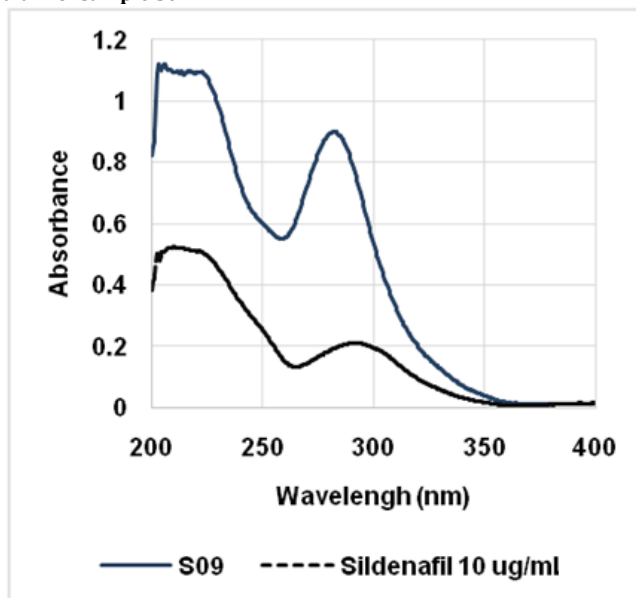


Fig. 11: Absorption spectrum of sample S09

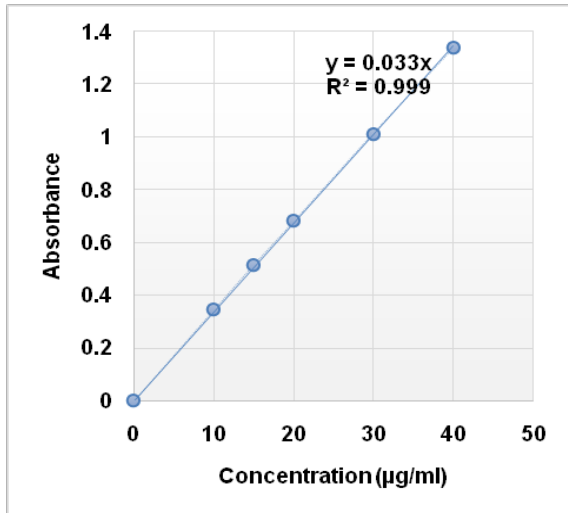
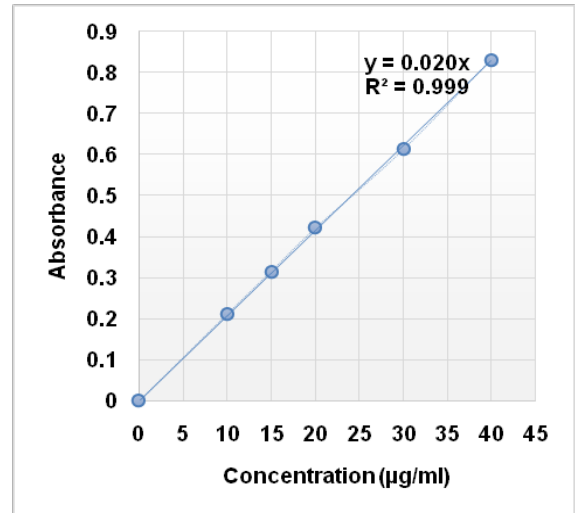
Fig. 12: Calibration curve of tadalafil at  $\lambda_{\max}=284$  nmFig. 13: Calibration curve of sildenafil at  $\lambda_{\max}=291$  nm

Table 2: Qualitative and quantitative results of the tested products

Sample code	Adulterant detected	Mean Amount per dosage unit (mg)* $\pm$ SD
S01	Tadalafil	59.0 $\pm$ 0.65
S02	Sildenafil	140.0 $\pm$ 0.33
S03	Sildenafil	115.0 $\pm$ 0.73
S04	Sildenafil	77.0 $\pm$ 0.44
S05	Negative	-
S06	Negative	-
S07	Sildenafil	11.70 $\pm$ 0.32
S08	Negative	-
S09	Sildenafil	188.20 $\pm$ 0.54

\* Mean of samples 5

Tadalafil was detected in one formulation at a dose higher than 20 mg/dosage unit, the maximal recommended dose. Sildenafil was detected in five formulations at doses ranging from 11.7 to 188.2 mg/dosage unit, with three containing more than 100 mg/dosage unit, the maximal recommended dose. These results are in accordance with other studies that reported adulteration with PDE-5 inhibitors at doses higher than the maximal recommended dose [9, 17, 18].

It is worthy to note that the labeled method of administration of most of the tested products was 1-2 tablets to be taken 2-3 h before sexual intercourse, while some were advised to be taken on daily basis. However, to our knowledge, there are no reported data about the effectiveness of herbal remedies in improving sexual performance when taken as a single dose before sexual intercourse.

One of the definitions of substandard and falsified (SF) medical products is "medical products that deliberately/fraudulently misrepresent their identity, composition or source". Therefore, adulterated herbal products are not determined as herbal medicine by the World Health Organization (WHO) [19].

Several studies showed that PDE-5 inhibitors were detected in adulterated herbal products present in the market and advertised on the internet to improve sexual performance [12]. In accordance with the results of the present study, 6 out of 9 products tested were adulterated with Tadalafil or Sildenafil, as shown in table 2.

The safety profile of such products is of dangerous health concern. Several guidelines that were set by the WHO provide practical guidance for monitoring the safety of herbal medicines, such as pharmacovigilance systems, 'Good manufacturing practice (GMP)' for dietary supplements and proper labelling of herbal products [20].

The European Union (EU) members consider dietary supplements as food, and they are subjected to the disposition of the General Food Law which set standards for maintaining food safety. Unfortunately, in many other countries, this area is not appropriately regulated [7].

In Lebanon, a committee affiliated to the Ministry of Public Health (MOPH) is responsible for the examination of all the submitted applications to permit the manufacturing or importing of herbal products and food supplements. Upon submission of applications, applicants must provide a certificate of analysis and methods of analysis issued by an officially recognized laboratory [21]. Moreover, the continuous control in dispensing adulterated herbal products inside pharmacies is subject to the inspection of the MOPH, while other points of sales remain under the control of the Consumer Protection Department of the Ministry of Economy and Trade [22].

The high prevalence of adulteration of herbal products, combined with the massive growing consumption of dietary supplements, necessitate the need to develop rapid and sensitive methods for detecting adulterants to protect the public from dangerous fraudulent practices.

## CONCLUSION

Among nine dietary supplements analyzed, six were adulterated with PDE-5 inhibitor. Some even contained doses higher than the maximal recommended dose. The method described for the extraction, identification and quantification of Tadalafil and Sildenafil would be useful for regulatory detection of adulterations.

It is recommended to implement the role of Ministry of Public Health in collaboration with the Ministry of Economy and Trade in inspecting random samples of the dietary supplement from the point of arrival (port), or point of domestic manufacturing, before being distributed in the Lebanese market. In addition to routine and random inspections as long as available in the Lebanese market.

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**AUTHORS CONTRIBUTIONS**

Razan Soubra performed the experiments and the measurements. Azza A. Gazy was involved in planning, design and supervised the work. May Saab, Marwa k. al Jamal processed the experimental data along with the calculations, designed the fig. and interpreted the results. Azza a. Gazy, May Saab, Marwa k. al Jamal revised the results and drafted the manuscript.

**CONFLICTS OF INTERESTS**

The authors declare that there are no conflicts of interest

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