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EVALUATION OF ADULTERATION OF HERBAL MEDICINE USED FOR TREATMENT OF ERECTILE DYSFUNCTION IN NAIROBI COUNTY, KENYA:

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ABSTRACT: In the recent past, adulteration of herbal products has increased rapidly, and today it is a global problem. Synthetic Phosphodiesterase type 5 inhibitors are the main adulterants in herbal medicine used for erectile dysfunction. The present study evaluated the herbal medicines used as sex enhancers in Nairobi County, Kenya, for adulteration with Phosphodiesterase type 5 inhibitors (sildenafil, tadalafil, and vardenafil). The products were purchased from health stores in Nairobi County. Samples were screened for adulteration with sildenafil, tadalafil, and vardenafil using HPTLC. Quantification was done by integration of sample chromatograms against standard chromatograms. The samples analyzed, 33.5% were adulterated. All the adulterated samples were imported, and none of the locally manufactured samples were found adulterated. The study revealed that samples from China/Hong Kong had the largest number 14 (53.85%) of the adulterated samples. These findings were in agreement with previous studies. A number of samples (32.5%) samples studied were adulterated. Sixty percent (60%) of adulterated samples contained sildenafil, while a few samples had either tadalafil, vardenafil or the combination.

INTRODUCTION: In the herbal medicine industry, adulteration is more often than not intentional. Synthetic prescription medicines are the major adulterants. Adulteration poses health risks to patients and the general public. Synthetic Phosphodiesterase type 5 (PDE-5) inhibitors singly or in combination are the most common adulterants in herbal medicines used to treat erectile dysfunction.

Herbal medicine for sex enhancement has been very popular due to claims of being safe with no adverse effects ^{1, 2}. However, adverse effects have been documented in part due to adulterants in the products ³. In herbal medicine for sex enhancement, adulteration with PDE- 5 inhibitors is done mainly to enhance therapeutic effect ⁴.

There are several reported cases of adulteration of herbal medicine ^{5, 6}. In Saudi Arabia, adulteration of herbal medicine with tadalafil and sildenafil has been reported ⁷. Dural E investigated the presence of sildenafil in herbal dietary supplements by validated HPLC method, which indicated that samples had sildenafil ⁸. Assessment of common adulterants in herbal medicine for sexual enhancement in Malaysia indicated presence of

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Phospho-diesterase type 5 inhibitors⁹. In Australia, the Therapeutics Goods Authority conducted post-market surveillance on herbal products used for erectile dysfunction (ED) treatment found them to contain tadalafil¹⁰. Search in databases in western countries, Japan, China, and Taiwan, reveal that most herbal products contained conventional drugs, and side effects were associated with adulterants contained therein^{11,12}.

Furthermore, the analogs of PDE-5 inhibitors have also been reported in herbal products used as sex enhancers^{13,14}. A preliminary study suggests the presence of adulteration of herbal medicine used to treat erectile dysfunction in Nairobi County, Kenya, with Phosphodiesterase type 5 inhibitors. One study has reported adulterating herbal medicine used to treat erectile dysfunction in Nairobi County, Kenya, with Phosphodiesterase type 5 inhibitors¹⁵.

Adulteration with Phosphodiesterase type 5 inhibitors adulterants presents serious health risks to users. Sildenafil and other PDE-5 inhibitors are known to cause adverse drug reactions such as headaches, facial flushing, dyspepsia, visual disturbances, and muscle aches. They can cause potentially severe hypotension, which can be fatal in patients using Nitrates to treat hypertension¹⁶. They can also interact with other drugs (drug-drug interactions and herbal-drug interactions), posing serious health risks to consumers¹⁶. In some case the amount of adulterant is far above the recommended dose leading to death, while in other cases the adulterant was an analogue of PDE-5 inhibitor, which poses regulatory challenge¹⁷. Ran *et al* documented a case of hepatotoxicity induced by adulterated "Tiger King", a Chinese herbal medicine containing sildenafil¹⁸. In Hong Kong, one person presented with ataxia following ingestion of sexual enhancement herbal remedy adulterated with sildenafil analogue¹⁹. There have been no detailed studies in Kenya to evaluate the trend of adulterants in herbal medicine used to treat erectile dysfunction. The present work reports on the adulteration of herbal medicines used to treat erectile dysfunction in Nairobi County, Kenya.

MATERIALS AND METHOD:

Sample Collection: The study was carried out in Nairobi County, Kenya. Target products in the

study area were purchased from the identified healthcare stores (Pharmacies, Supermarkets, health stores, herbal kiosks, and open-air hawkers). Identification of the products was done by asking the attendants and checking the indications on the label. The samples purchased included tablets, capsules, liquids, gels, and powders. Only samples that had intact packs were purchased between July 2017 and August 2018.

Materials, Reagents, and Instrumentation: Standards of sildenafil, tadalafil, and vardenafil were obtained from TLC PharmaChem (Ontario, Canada). The methanol used was of LC grade obtained from Fisher Scientific, Loughborough, United Kingdom. Ethyl acetate was sourced from Carlo Erba, Val de Reuil, France. KSCN (98%) used was from Merck (Darmstadt, Germany). Formic acid was LC grade sourced from Fluka, St Louis, MO. Ammonia (35%) was sourced from Fisher Scientific instruments.

Screening for Adulterants and Instrumentation: Screening was done by the HPTLC method adapted from Tien *et al.*²⁰.

HPTLC Method, with the following Conditions: A Camag HPTLC Linomat 5 apparatus (ID QCE015 (A), with an automatic spotter and Camag TLC scanner 3 S/N 170308 (ID QCE015) (Camag, Muttenz, Switzerland), silica gel 60 glass plates, 20x20 cm were used for screening and quantification. Analysis was done at 254nm using a deuterium lamp (Lamp D2) with emission and absorption modes. Samples and standards were dissolved in methanol and methanol: ethyl acetate 40:80 was used as the mobile phase.

Standard Preparation: Five mg of standard (sildenafil, tadalafil, and vardenafil) were each separately dissolved in 5 ml of methanol. From the solutions, 1ml was taken and diluted to 10ml with methanol. 2 μ l and 5 μ l respectively (duplicate application) were used for spotting on the TLC plates. The plates were developed in the chambers and ran in the Linomat scanner to produce the chromatogram. Chromatograms of samples and standards were compared/merged by HPTLC software.

Quantification of Adulterants: The sample and standard preparations were used to generate the

chromatograms for the quantification of adulterants. The chromatograms were stored in TLC video scan software and used to quantify adulterants using TLC software's peak area and/or peak height integration.

RESULTS: 80 samples were screened for adulteration with either sildenafil, tadalafil, vardenafil, or a combination thereof. Twenty-six (32.5%) samples were found to be adulterated **Table 1.**

TABLE 1: QUANTITY OF ADULTERANTS IN SAMPLES

S. no	Country of origin	Sample No.	Quantity of adulterant
1	China	Mis-05303/2019	Sildenafil 148.7mg/Tab, Vardenafil 0.5mg/Tab
2	USA	Mis-05304/2019	Vardenafil 3.3mg/Tab
3	China	Mis-05305/2019	Sildenafil 67mg/Tab
4	USA	Mis-05306/2019	Sildenafil 11.62mg/Sachet, Vardenafil 36.6mg/Sachet
5	USA	Mis-05308/2019	Sildenafil 71.4mg/Tab
6	China	Mis-05310/2019	Sildenafil 88.5mg/Tab
7	India	Mis-05319/2019	Vardenafil 2.4mg/capsule
8	China	Mis-05330/2019	Sildenafil 124.3mg/Tab
9	China	Mis-02537/2019	Sildenafil 127.62mg/Tab
10	China	Mis-02538/2019	Sildenafil 19.86mg/Tab, Tadalafil 24.40mg/Tab
11	China	Mis-02540/2019	Sildenafil present (Sample inadequate for quantification)
12	China	Mis-02541/2019	Sildenafil 51.90mg/Tab
13	South Africa	Mis-02544/2019	Sildenafil present (Sample inadequate for quantification)
14	China	Mis-02555/2019	Sildenafil 7.73mg/Cap
15	China	Mis-02556/2019	Sildenafil 47.26mg/Cap, Tadalafil 13.38mg/Cap
16	China	Mis-02559/2019	Sildenafil present (Sample inadequate for quantification)
17	USA	Mis-02563/2019	Tadalafil 115.52mg/Cap
18	USA	Mis-02564/2019	Sildenafil 122.01mg/Cap
19	India	Mis-02571/2019	Vardenafil 1.63mg/Cap
20	France	Mis-02578/2019	Sildenafil present (Sample inadequate for quantification) Sample inadequate
21	China	Mis-02579/2019	Sildenafil & Vardenafil present (Sample inadequate)
22	Tanzania	Mis-02582/2019	Sildenafil present (Sample inadequate for quantification) Sample inadequate
23	USA	MIS-03629/2020	Sildenafil 0.9mg/Tab
24	USA	MIS-03630/2020	Sildenafil 37.56mg/Tab
25	China	MIS-03628/2020	Sildenafil 42.91mg/Tab
26	China	MIS-03631/2020	Sildenafil 1.07mg/Tab

Only imported products comprising 32.5% of the samples were found to be adulterated. Each sample (3.85%) manufactured in Tanzania, South Africa, and France was adulterated. Two samples (7.69%) were manufactured in India, and 7 (7.69%) from the USA were adulterated. China/Hong Kong had the largest number 14 (53.85%) of the adulterated samples.

Most of the adulterated samples contained sildenafil (17 samples (65.38%). Only one sample (3.85%) was adulterated with tadalafil while 3 samples (11.54%) were adulterated with vardenafil. There were combinations of sildenafil and tadalafil (1 sample, 3.85%) and sildenafil and vardenafil (3 samples, 11.54%). Overall, sildenafil was the

commonest adulterant and was found in 22 (84.62%) of the adulterated samples. Tadalafil was the least common adulterant detected in a total of 3 (11.54%) of the adulterated samples. The total number of samples from China/Hong Kong was 14, and all of them were adulterated.

There were 23 samples from the USA, out of which 7 (30.43%) were adulterated. The number of samples from India was 8; 2 (25%) were adulterated. From Tanzania, there were 6 samples, of which 1 (16.67%) was adulterated. The total number of samples from South Africa was 3, out of which 1 (33.33%) was adulterated. There were 7 samples from Europe, out of which 1 (14.29%) was adulterated.

Some samples had combinations of adulterants. The maximum daily allowable dose of sildenafil is 100mg²¹, while that of tadalafil and vardenafil is 20mg²².

DISCUSSION: This study revealed that samples from China/Hong Kong had the largest number 14 (53.85%) of adulterated samples. These findings were in agreement with previous studies. The presence of prescription medicine adulterants in Chinese herbal medicine (TCM) has been reported worldwide^{23, 24, 25}. In one study, Santillo *et al.* investigated adulteration of herbal and natural sex performance enhancement dietary supplements with synthetic phosphodiesterase type 5 inhibitors. The study showed that the products were adulterated with PDE 5 inhibitors, including sildenafil and/or tadalafil. The study also showed that some samples contained more than the maximum daily allowable amount of the drug adulterant²⁶.

In another study, Chinese authorities collected 200 products from health market labelled as natural enhancers of sexual performance and analyzed for adulteration with synthetic phosphodiesterase type 5 inhibitors. 35% of samples were found to contain sildenafil, and the amount greatly exceeded the recommended daily allowable dose of sildenafil²⁷. A Hong Kong study conducted in 2007 collected 26 herbal remedies from convenience stores revealed that 15(58%) samples were adulterated with sildenafil and its analogs²⁸. In the Czech Republic, dietary supplements intended for sex enhancers were collected in a Czech market between 2009-2015 and analyzed for adulteration with sildenafil, tadalafil, vardenafil, and analogs. This study demonstrated the presence of PDE-5 inhibitors in samples⁴.

There is the probable effort of manufacturers to mask the presence of PDE-5 inhibitors in sex enhancement dietary supplements⁴. In a study in Ghana, selected herbal supplements from Ghanaian market for better erectile function were evaluated for the presence of phosphodiesterase type 5 inhibitors by bioassay method. 90% of the samples showed activity indicating the presence of phosphodiesterase type 5 inhibitors. Some samples contained an amount higher than allowable daily dose of sildenafil (more than 100mg sildenafil)²⁹.

A study done on 80 dietary supplements intended to be introduced to herbal shops in Bushehr city, Iran, revealed that samples were adulterated with phosphodiesterase type 5 inhibitors, especially sildenafil³⁰.

A study conducted in the Lebanese market indicated that dietary supplements marked for sexual enhancement contained phosphodiesterase type 5 inhibitors, especially sildenafil and tadalafil. The amount of the adulterants also exceeded the maximal recommended daily allowable dose exposing the consumers to health risks³¹. Studies conducted by Tama *et al.* indicated that 48% contained Phosphodiesterase type 5 inhibitors adulterants, out of which 20% contained sildenafil and 22.5% contained tadalafil as adulterants³². Researchers purchased six products from the internet and health store in a Canadian study. The samples were evaluated for adulteration with undeclared Phosphodiesterase type 5 inhibitors.

Some samples were found to contain sildenafil, while others contained tadalafil³³. One French study analyzed products from China, Taiwan, Estonia/ Spain; the study indicated that 47% of samples were adulterated, 29.41% with sildenafil, 11.76% with tadalafil, and 5.88% with vardenafil³⁴. Wang *et al.* conducted an analysis of 23 illegal adulterated aphrodisiac chemical ingredients in health foods and Chinese traditional patent medicines by ultrahigh performance Liquid Chromatography coupled with quadrupole time-of-flight Mass Spectrophotometry³⁵. In Kenya, preliminary studies indicated that herbal products used as sex enhancers contained sildenafil¹⁴. Previous reports indicate that adulterants in the form of analogs of sildenafil have been detected in herbal products marketed for erectile dysfunction^{36, 37}.

The maximum daily allowable dose of sildenafil is 100mg³⁸, while that of tadalafil and vardenafil is 20mg respectively^{39, 40}; therefore a number of samples contained way above the daily allowable dose of phosphodiesterase type 5 inhibitors (highest for Sildenafil 148.7 mg/Tab) with high attendant risk to the users. These findings are in agreement with the findings in the current study. In the present study, three adulterants (sildenafil, tadalafil, and vardenafil) were investigated in the samples. The

study revealed that the samples were adulterated with sildenafil, tadalafil, and vardenafil. There is a need to study further the presence of analogs in the herbal medicine products used to treat erectile dysfunction available in Nairobi County, Kenya.

CONCLUSION: A number of samples (32.5%) of herbal medicine used to treat erectile dysfunction available in Nairobi County, Kenya, were adulterated. Sixty percent of the adulterated samples contained sildenafil, while a few contained either tadalafil, vardenafil, or the combination. Only imported samples were adulterated. The highest percentage of adulterated samples was imported from China/Hong Kong (53.9%) followed by the USA (26.9%). Two samples manufactured in India (7.7%) were adulterated. One sample each manufactured in Tanzania, France, and South A. All the adulterants in the samples were not declared on the label, posing health risks to the users. There is a need to put a policy in place to curb cases of adulteration of herbal medicinal products for the treatment of erectile dysfunction in the Kenyan market.

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Declarations:

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Ethical Approval and Consent to Participate:

Ethical approval was obtained from the Institutional Review Committee of the University of Nairobi/ Kenyatta National Hospital.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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