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QUALITY ASSESSMENT OF HERBAL APHRODISIAC PRODUCTS; ELEMENTAL ANALYSIS AND DETECTION OF SILDENAFIL AND TADALAFIL AS UNDECLARED ADULTERANTS

***Saidu, M., Awwalu, S. and Musa, A**

Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria

***Author for correspondence**: +2348038045602, muzammilsaidu01@gmail.com

ABSTRACT

The use of herbal preparations for the treatment of various ailments, including erectile dysfunction is on the increase globally. However, there is fear about the quality and purity of these products as most of them are not certified by the relevant agencies. The aim of this study is to conduct elemental analysis and detect the presence of sildenafil and/or tadalafil as undeclared adulterants in 22 herbal aphrodisiac products. Samples were processed and digested using nitric acid-hydrochloric acid (3:1). Digested samples were analyzed for the presence of lead, nickel, cobalt, manganese, zinc and iron using atomic absorption spectrophotometer. Also, samples were macerated using methanol filtered and the filtrate was analyzed for the presence of sildenafil and tadalafil as undeclared adulterants using thin-layer chromatography. Iron (Fe) (0.26 - 281.34 µg/g), Zinc (Zn) (1.10 - 24.44 µg/g), Manganese (Mn) (7.52 - 501.65 µg/g) and Lead (Pb) (0.00 - 10.55 µg/g) were detected in 91, 59, 73 and 27 % respectively of the samples. Only one sample has Pb above the permissible limit of 10 ppm. Sildenafil was detected in 14% of the herbal aphrodisiac samples screened, while tadalafil was not detected in the samples. The samples are of poor quality as some are adulterated with sildenafil

Keywords: Aphrodisiacs, phytochemical, physicochemical, erectile dysfunction, herbal preparations

INTRODUCTION

Herbal medicines are plant-derived substances with minimal or no industrial processing used for medicinal purposes. Plant-based traditional medicine continues to play an essential role in health care, with about 80% of the world's inhabitants relying on it for primary health care purposes (WHO, 2007).

Erectile dysfunction (ED), also called impotency, is defined as the inability to achieve or maintain penile erection sufficient for sexual intercourse (Pastuszak, 2014). ED is on the increase with prevalence rates at approximately 20% before the age of 30, 25% at the age of 30 to 39, 40% at the age of 40 to 49, 60% at the age of 50 to 59, 80% at the age of 60 to 69 years, and 90% in individuals above the age of 70 (WHO, 2019).

Herbal remedies are perceived to be safe and free of side effects (Corns and Metcalfe, 2002). However, problems can arise when drug interactions occur between commonly used pharmaceuticals and herbal remedies. This problem is compounded if herbals are adulterated with conventional medicines. Lack of adequate regulation of herbal remedies can lead to increased adulteration of the preparations. Numerous reports of adverse effects associated with herbal remedies are available (Zuckerman *et al*., 2002; Awwalu *et al*., 2017; Tama *et al*., 2020). The main adulterants in herbal remedies are metals or pharmaceuticals, these adulterants have been shown to induce hepatic, renal, and neurotoxic effects (Koh and Woo, 2000; Ernst, 2002).

Herbal products used for the treatment of erectile dysfunction are reported to be contaminated with pharmaceutical drugs such as sildenafil, tadalafil and their analogue (Bujang *et al*., 2017; Tama *et al*., 2020). Songlin *et al*., (2008) and Junhua *et al*., (2012) reported cases of inconsistent composition and toxicity due to adulteration and or toxic components of herbal medicine. Lead, cadmium, mercury and arsenic are the most toxic heavy metals (Lars, 2003). Some heavy metals e.g. Iron, Zinc and Copper are essential for body development but toxicity is ensured at high concentrations (Dzomba *et al*., 2012). Heavy metals accumulate in the body and destroy vital organs and glands such as brain, liver and kidney (Suranjana and Manas, 2009).

Reports have shown contamination of phytomedicines with heavy metals (Saeed *et al*., 2010; Dzomba *et al*., 2012). Furthermore, toxicity due to heavy metal contamination has been reported in Africa, India, Europe and United States (Denholm, 2010).The aim of this study is to detect the presence of some toxic and essential elements and sildenafil and tadalafil as undeclared synthetic adulterants.

METHODS

Sampling of Herbal Aphrodisiac Products

Twenty-two products were selected and coded S1 - S22. Their manufacturing and expiry dates, batch and NAFDAC registration numbers were then recorded.

Elemental Analysis

Sample preparation

Nitric acid-hydrochloric acid digestion was used following the procedure reported by the AOAC (1990). One gram of each solid sample and 5 ml each of liquid samples, as the case may be, were placed in a crucible (250 ml) and 7.5 ml of concentrated nitric acid was added. The mixture was boiled gently for 30 minutes to oxidize all easily oxidizing matter. After cooling, 2.5 ml of hydrochloric acid was added and the mixture was boiled gently until dense white fumes appeared, this was allowed to cool, 20 ml of distilled water was added and the mixture was boiled further to release any fumes. The solution was cooled, filtered, and made up to 30 ml with distilled water.

Sample analysis

The digested samples were analyzed for the presence of iron, zinc, manganese, cobalt, nickel and cadmium using atomic absorption spectrophotometer (AAS). Concentrations (µg/g) of the analytes were determined using the formula;

$$\text{Concentration } (\mu g/g) = \frac{(C) * (V) * (Df)}{W}$$

Where, C = concentration (µg/ mL), V = volume (mL), Df = dilution factor and W = sample weight (gram)

TLC analysis of herbal samples

Preparation of stock solutions and herbal samples

This was conducted using thin-layer chromatography. Stock solutions (1000 µg/ml) of sildenafil and tadalafil standard powders were separately prepared by weighing ten milligrams of sildenafil and tadalafil standard powders into two labelled volumetric flasks (10 ml) containing methanol (2 ml) and acetonitrile (2 ml) respectively and made up to mark with the same solvents.

Two grams or 2ml as the case may be, of the herbal aphrodisiac samples were weighed, transferred into beakers (25 ml) containing 10 ml methanol and the mixture was shaken and filtered.

TLC method development and analysis

The TLC method was developed by trying several solvent systems. After which acetonitrile and hexane, (3:1 v/v) as well as hexane, ethyl acetate and methanol (5: 2: 1 v/v) were chosen for tadalafil and sildenafil respectively. The mobile phases were transferred into the developing chamber, covered, and allowed to saturate for ten (10) minutes.

The prepared standard stock solutions and herbal samples were spotted on the TLC plate with the aid of a capillary tube, at least two (2) mm apart and one (1) cm from the lower end of the plate (baseline). The TLC plate was then introduced into the chamber and allowed to develop to the marked solvent front. The plate was then removed, allowed to dry and was subsequently viewed under UV light at 254 nm. The samples that were found to have the same Rf value as the standards were scrapped for FTIR analysis.

RESULTS

RESULTS AND DISCUSSION

Herbal medicines are reported to contain highly active pharmacological components including minerals and trace metals (Fabricant and Farnsworth, 2001). However, the intake of these trace metals above certain permissible limits can become harmful (Chrzan, 2016). Other non-essential metals such as nickel and lead are usually present in herbal medicines as contaminants (Luo *et al.,* 2021).

Iron was detected in 86% of the samples at a concentration range of 7.04 - 281.34 µg/g (Table 2). The WHO limit for iron in medicinal herbs has not been established. Iron is an essential component of haemoglobin in human beings and animals (Moses *et al.,* 2012). Usman *et al.,* (2021) analysed some herbal antihyperglycemic products and detected iron within the concentration range of 16.36 - 331.45 µg/g. Iron obtained in these samples is said to increase blood volume for optimal sexual performance (Rungby, 2010). Other functions of iron include oxygen supply, energy production, and immunity; however, toxicity occurs in high doses leading to dizziness, nausea, vomiting, diarrhoea, joint pain, shock, liver damage, impairment of metabolic functions and cardiovascular system (Abou-Arab and Abou Donia, 2000).

Zinc (1.10 – 24.44 µg/g) detected in 59% of the samples was significantly lower ($p < 0.05$) than the permissible limit of 50 ppm (WHO, 2005; WHO, 2006). Thus, the samples analyzed in this study may be considered safe for consumption for their Zn concentration. Zinc increases semen volume and testosterone levels for male sexual vitality. Zn was detected in some herbal antihyperglycemic preparations within the range of 0.34 - 36.64 µg/g (Usman *et al*., 2021). Zinc deficiency could lead to infertility, impotency, or poor reproductive system development (Rungby, 2010). Cobalt (Co) and nickel (Ni) were not detected (Table 2) in the analyzed samples which might be due to their concentration below the limits of detection of the metals in the developed methods.

Manganese was detected in 73% of the samples within a concentration range of 7.52 - 501.65 µg/g (Table 2). The WHO limit for Mn in medicinal herbs has not been established. Manganese (Mn) improves sexual activity by regulating cellular energy. Mn was found in some herbal antihyperglycemic preparations within the range of 0.01 - 4.00 µg/g (Usman *et al*., 2021). Mn is required for normal immune functions, regulation of blood sugar and cellular energy, and also in scavenging free radicals (Koh *et al.,* 2014). However,

exposure to elevated levels of manganese can result in neurotoxicity. Manganism is a chronic exposure to manganese that leads to neurological disorders (Koh *et al.,* 2014).

Table 1: Label Information of the Herbal Aphrodisiac Samples

CODE	COUNTRY OF ORIGIN	MANUFACTURING DATE	EXPIRY DATE	BATCH NUMBER	NAFDAC NUMBER
S1	Nigeria	Apr, 2023	Dec, 2026	-	1456804
S2	Nigeria	Jan, 2023	Dec, 2026	-	-
S3	Nigeria	Sep, 2021	Sep, 2024	-	-
S4	Nigeria	Jan, 2020	Dec, 2023	-	-
S5	Nigeria	Jan, 2020	Dec, 2023	-	-
S6	Nigeria	Dec, 2020	Dec, 2025	-	-
S7	Nigeria	Dec, 2020	Dec, 2025	BN:2577573	-
S8	Nigeria	Dec, 2022	Dec, 2026	-	-
S9	Nigeria	Nov, 2021	Nov, 2024	-	-
S10	Nigeria	Dec, 2022	Dec, 2026	-	-
S11	Nigeria	Aug, 2019	Aug, 2024	-	-
S12	Nigeria	Jun, 2020	Jun, 2024	-	-
S13	Nigeria	Dec, 2020	Dec, 2025	2577573	-
S14	Nigeria	-	-	0001	A7-5231L
S15	Nigeria	Feb, 2022	Feb, 2024	-	-
S16	Nigeria	Dec, 2022	Dec, 2026	-	-
S17	Nigeria	Jan, 2020	Dec, 2023	-	-
S18	Nigeria	Dec, 2021	Dec, 2024	-	-
S19	Nigeria	Dec, 2021	Dec, 2023	MS-00001	A7-4719L
S20	Nigeria	Jan, 2023	Jan, 2025	MS003	A7-4720L
S21	Nigeria	Jan, 2021	Oct, 2024	3292352	-
S22	Nigeria	Feb, 2023	Dec, 2023	JHML018	A7-2077L

Lead (Pb) was detected in 23% of the samples within the range of 1.09 - 10.55 µg/g with one sample (S15) having a concentration significantly ($p < 0.05$) higher than the permissible limit of 10 ppm (WHO, 2005; WHO, 2006). Thus, S15 is not considered safe for human consumption with respect to Pb. Lead is a non-essential trace element with no function in the human body or plant and they induce very toxic effects in human even at low doses. Usman and co-workers reported Pb concentration in the herbal antihyperglycemic preparations within the range of 0.35 - 14.23 µg/g (Usman *et al*., 2021). Some symptoms of lead poisoning include anaemia, colic, headache, convulsion, chronic nephritis, brain damage and central nervous system disorders (Khare *et al*., 2018).

Sildenafil was detected in 14% (S8, S15 and S19) of the herbal aphrodisiac samples (Plates 1 - 5). This was further confirmed by the superimposed FTIR of sildenafil standard with adulterated herbal products (Figure 1). Tama and co-workers reported the presence of sildenafil in the herbal drugs they analyzed (Tama *et al*., 2020). The availability of sildenafil in these Herbal samples could lead to severe side effects or fatal interactions with the herbal drugs or other orthodox medicines. Tadalafil was not detected (Plates 6 - 10) in all herbal aphrodisiac samples screened as none of the samples had a visible spot with a similar Rf value as that of tadalafil standard powder.

Table 2: Metal Concentration (µg/g) in the Herbal Aphrodisiac Samples

CODE	Fe	Zn	Co	Mn	Ni	Pb
S1	161.89 ± 0.00	24.44 ± 0.01	BLD	8.91 ± 0.00	BLD	4.50 ± 0.00
S2	176.21 ± 0.00	1.29 ± 0.00	BLD	12.48 ± 0.00	BLD	BLD
S3	20.10 ± 0.01	BLD	BLD	501.65 ± 0.00	BLD	BLD
S4	281.34 ± 0.00	9.38 ± 0.00	BLD	134.02 ± 0.00	BLD	BLD
S5	202.41 ± 0.00	BLD	BLD	263.98 ± 0.00	BLD	BLD
S6	252.89 ± 0.00	BLD	BLD	31.88 ± 0.00	BLD	BLD
S7	262.88 ± 0.00	5.04 ± 0.00	BLD	44.56 ± 0.00	BLD	BLD
S8	84.64 ± 0.00	9.88 ± 0.00	BLD	6.99 ± 0.00	BLD	BLD
S9	104.93 ± 0.00	BLD	BLD	7.52 ± 0.00	BLD	BLD
S10	53.32 ± 0.00	5.27 ± 0.00	BLD	43.46 ± 0.00	BLD	1.09 ± 0.00
S11	169.13 ± 0.00	BLD	BLD	21.76 ± 0.00	BLD	8.18 ± 0.00
S12	210.62 ± 0.00	19.93 ± 0.00	BLD	16.37 ± 0.00	BLD	2.89 ± 0.00
S13	228.18 ± 0.00	21.02 ± 0.00	BLD	26.68 ± 0.00	BLD	BLD
S14	33.34 ± 0.00	2.66 ± 0.00	BLD	BLD	BLD	BLD
S15	165.61 ± 0.00	2.46 ± 0.00	BLD	21.34 ± 0.00	BLD	10.55 ± 0.00*
S16	73.49 ± 0.00	BLD	BLD	20.23 ± 0.00	BLD	BLD
S17	136.51 ± 0.00	24.63 ± 0.00	BLD	146.25 ± 0.00	BLD	BLD
S18	BLD	1.10 ± 0.00	BLD	BLD	BLD	BLD
S19	BLD	BLD	BLD	BLD	BLD	BLD
S20	7.04 ± 0.00	BLD	BLD	BLD	BLD	BLD
S21	BLD	BLD	BLD	BLD	BLD	BLD
S22	27.30 ± 0.00	2.98 ± 0.00	BLD	BLD	BLD	BLD

FAO/WHO limits: Fe (Not established yet), Zn (50 ppm), Mn (Not established yet) and Pb (10 ppm)
BLD: Below the limit of detection
LOD: Limit of detection for Fe: 0.82, Zn: 0.08, Co: 1.06, Mn: 1.28, Ni: 2.97, Pb: 1.03
*Significantly higher ($p < 0.05$) than FAO/WHO limit

Plate 1: TLC profile of sildenafil standard powder using Hexane: Ethyl acetate: methanol (5:2:1)

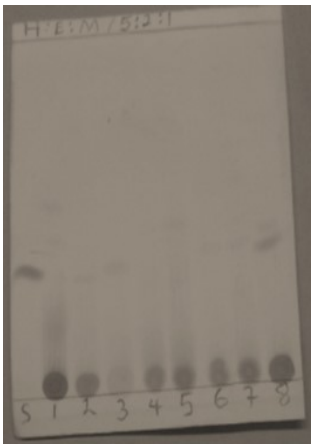

Plate 2: TLC profile of sildenafil with samples S1, S2, S3, S4, S5, S6, S7 and S8

Plate 3: TLC profile of sildenafil with samples S9, S10, S11, S12, S13 and S14

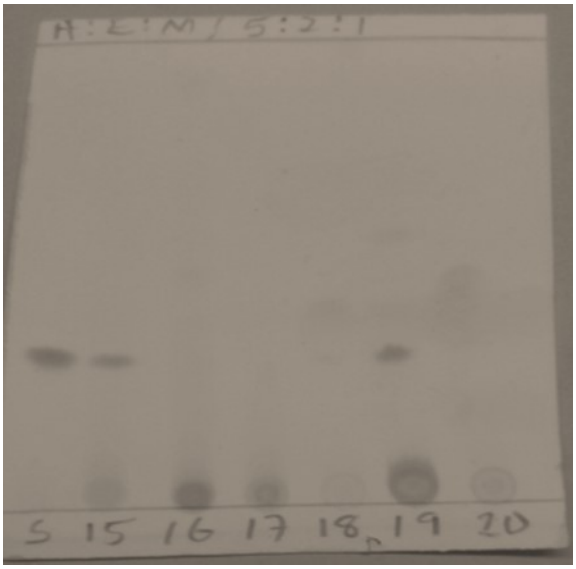

Plate 4: TLC profile of sildenafil with samples S15, S16, S17, S18, S19 and S20

Plate 5: TLC profile of sildenafil with samples S21 and S22

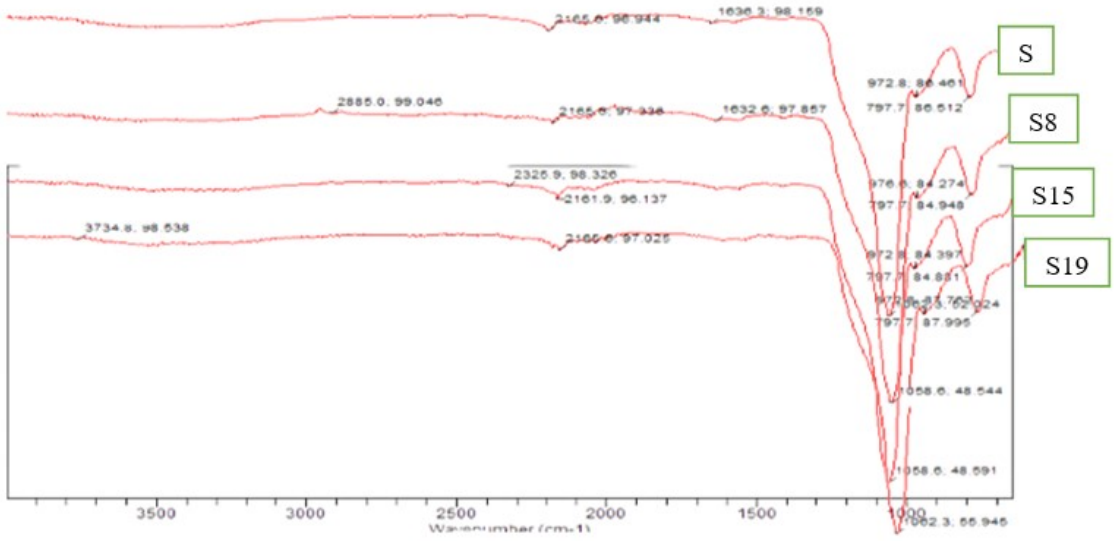

Figure 1: Superimposed FTIR spectra of sildenafil standard and sildenafil adulterated herbal samples; S8, S15, S19.

Plate 6: TLC profile of tadalafil standard using Acetonitrile: Hexane (3:1)

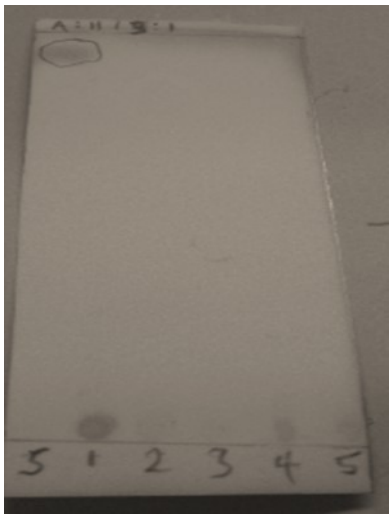

Plate 7: TLC profile of tadalafil with sample S1, S2, S3, S4 and S5

Plate 8: TLC profile of tadalafil with sample S6, S7, S8, S9 and S10

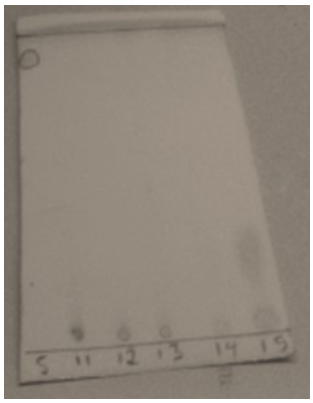

Plate 9: TLC profile of tadalafil with sample S11, S12, S13, S14 and S15

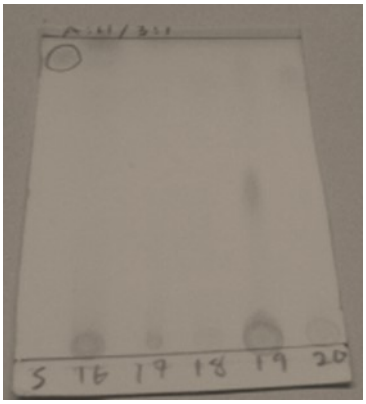

Plate 10: TLC profile of tadalafil with sample S16, S17, S18, S19 and S20

CONCLUSION

Despite the fact that the herbal samples contain minerals that could contribute to enhancement of sexual drive, they were however found to be of poor quality as some are adulterated with sildenafil and some contain some toxic metal. Adulteration of herbal drugs with synthetic drugs continues to be a growing problem as evidenced by the three samples in which sildenafil was detected as an undeclared adulterant.

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