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TLC and IR detection of sildenafil as an undeclared adulterant in some herbal aphrodisiac products marketed in northwest Nigeria

Aminu T. Lawal[1], Aminu Musa[1], Musa A. Usman[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria.

Corresponding author: Aminu T. Lawal
Email: lawalaminu463@gmail.com; Phone: +2347035860436

ABSTRACT

Background: Sildenafil, a phosphodiestrate-5 (PDE-5) inhibitor is used in the treatment of erectile dysfunction. There have been reports of adulteration of herbal products with PDE-5 inhibitors without considering its deleterious consequences to public safety.

Objectives: To evaluate the presence of sildenafil as an undeclared adulterant in herbal products marketed in northwest Nigeria by TLC/IR Spectroscopy.

Methods: A survey of herbal aphrodisiac products marketed in four Northwestern Nigerian states was carried out. A sample size (50) representative of the total products surveyed was collected for this study. A simple, rapid thin layer chromatographic (TLC) method was employed for the identification of sildenafil adulterant in herbal aphrodisiac samples. Presence of sildenafil from the suspected products was confirmed by Fourier transform Infrared (FTIR) analysis.

Results: TLC method revealed 16 out of 50 herbal aphrodisiac samples to contain sildenafil which was affirmed by Infrared spectroscopy.

Conclusion: About 32% of the market products sampled were adulterated with sildenafil. The use of such products poses a serious threat to public safety.

Keywords: Herbal aphrodisiacs, sildenafil, adulteration, TLC, FTIR.

Détection TLC et IR du sildénafil comme adultérant non déclaré dans certains produits aphrodisiaques à base de plantes commercialisés dans le nord-ouest du Nigéria

Aminu T. Lawal[1], Aminu Musa[1], Musa A. Usman[1]

[1]Département de chimie pharmaceutique et médicinale, Université Ahmadu Bello, Zaria, Nigeria.

Auteur correspondant :Aminu T. Lawal
Email: lawalaminu463@gmail.com; Téléphone: +2347035860436

RESUME

Contexte: Le sildénafil, un inhibiteur du phosphodiestrate-5 (PDE-5) est utilisé dans le traitement de la dysfonction érectile. Il y a eu des rapports d'adultération de produits à base de plantes avec des inhibiteurs de PDE-5 sans tenir compte de ses conséquences délétères pour la sécurité publique.

Objectifs: Évaluer la présence de sildénafil comme adultérant non déclaré dans les produits à base de plantes commercialisés dans le nord-ouest du Nigéria par spectroscopie TLC/IR.

Méthodes: Une enquête sur les produits aphrodisiaques à base de plantes commercialisés dans quatre États du nord-ouest du Nigéria a été menée. Une taille d'échantillon (50) représentative du total des produits enquêtés a été recueilliepour cette étude. Une méthode de chromatographie en couche mince (TLC) simple et rapide a été utilisée pour identifier l'adultérant au sildénafil dans des échantillons aphrodisiaques à base de plantes. La présence de sildénafil dans les produits suspects a été confirmée par l'analyse infrarouge à transformée de Fourier (FTIR).

Résultats: La méthode TLC a révélé que 16 des 50 échantillons aphrodisiaques à base de plantes contiennent du sildénafil, ce qui a été confirmé par spectroscopie infrarouge.

Conclusion: Environ 32 % des produits du marché échantillonnés étaient frelatés au sildénafil. L'utilisation de ces produits constitue une grave menace pour la sécurité publique.

Mots-clés: Aphrodisiaques à base de plantes, sildénafil, adultération, TLC, FTIR.

INTRODUCTION

Sildenafil, a pyrazolopyrimidine derivative, the citrate salt is a 5-[2-ethoxy-5-(4-methylpiperazin-1-yl) sulfonylphenyl]-1-methyl-3-propyl-4H-pyrazolo[4,3-d]pyrimidin-7-one;2-hydroxypropane-1,2,3-tricarboxylic acid (Fig. 1). Is a phosphodiesterase-5 (PDE-5) inhibitor used in the treatment of erectile dysfunction (ED) and pulmonary arterial hypertension.[1] Despite the success of the three approved PDE-5 inhibitors (sildenafil, tadalafil and vardenafil) in the treatment of ED, their adverse effects are also of great clinical significance. The most commonly reported adverse effects are headache, flushing, visual disturbances, dizziness and dyspepsia. Also reported are palpitations, cerebrovascular hemorrhage, transient ischemic attack, epistaxis, muscle pain, diarrhea, vomiting, amongst numerous others.[2,3] As a result of these adverse effects, some patients resort to the use herbal remedies and/dietary supplements for treatment of ED due to the widespread believe that herbal medicines are safe because they are natural. Furthermore, the notion that "manliness" is gauged by how sexually virile a man is, explains the increase in the demand and proliferation of sex enhancing drugs.[4] Consequently, there has been an explosion in the usage of aphrodisiacs and other herbal remedies in both developed and developing countries in recent years.[5,6] As a result, some herbal practitioners and manufacturers of herbal products fraudulently incorporate active pharmaceutical substances and/or their structural analogues in preparations without corroborated efficacy to gain fast money.

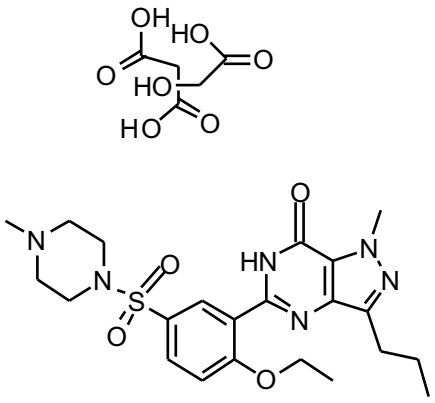

Figure 1: Structure of sildenafil citrate

Sildenafil has been detected as an undeclared substance in several herbal preparations meant for improving sexual performance in US, Indonesia and Sudan.[7,8,9] Such adulterated products may lead to hazardous herb-drug interactions or fatal side effect(s) of sildenafil in patients with preexisting cardiovascular risks.[10,11] Due to poor regulation and scarce data on the quality of herbal products in Nigeria, there is a need to assess herbal aphrodisiac products marketed in northwest Nigeria (where such products are very popular) for the presence of sildenafil.[6]

METHODS

Materials

Sildenafil citrate standard powder was purchased from Sigma-Aldrich. All the solvents and reagents used were of analytical grade; n-hexane (BPH Chemical, England), ethyl acetate (BPH Chemical, England), methanol (BPH Chemical, England). Samples were analyzed by Agilent Cary 630 Fourier-transform infrared spectroscopy (FTIR) Spectrometer.

Survey and sampling of herbal aphrodisiacs preparations

A survey of the herbal aphrodisiac products marketed in four (Zamfara, Kano, Kaduna, and Katsina) out of the seven Northwestern Nigerian states was carried out. A sample size (50) representative of the total products (596) surveyed was collected for this study. The sampling method also follows probability sampling, which means each sample in the population has an equal chance of being selected. The samples collected were assigned codes S1-S50 (Table 2).

Identification of sildenafil adulterant in herbal aphrodisiac samples

Preparation of the standard solution

Sildenafil citrate powder (5 mg) was accurately weighed and transferred into 10 ml volumetric flask, and dissolved in 5 ml of methanol, it was made up to the mark with methanol with a resulting concentration of 500 µg/ml. The solution was further diluted to 100 µg/ml stock solution.

Sample preparation

A quantity (300mg) of each aphrodisiac sample was weighed and transferred into 25 ml beaker containing 10 ml methanol. The mixture was filtered using whatman® filter paper. The resulting filtrate was used for TLC analysis.

TLC method analysis

Portions (2-20 µL) of solution of standard sildenafil powder and filtrate from each sample were spotted on a TLC coated on aluminium sheets (Silica gel 60 F_{254}, Merck Germany) with the aid of capillary tube, at least 2 mm

apart and 1 cm from the lower end of the plate. The TLC plate was introduced into the developing chamber containing a mobile phase of composition n-hexane: ethyl acetate: methanol (5:2:1 v/v). The plate was removed after the mobile phase reaches the solvent front and was allowed to dry and viewed under UV light at 254 nm.

The spots with the same Rf value as standard were suspect to be sildenafil.

FTIR analysis

Herbal samples whose TLC chromatograms have spots with Rf values similar to that of sildenafil were subjected to FTIR analysis. The spots were scrapped off the plate, dissolved in methanol and filtered. The filtrates were analyzed in the mid IR region (650-4000 cm^{-1}) at a resolution of 8 cm^{-1} with 16 scans using Agilent Cary 630 FTIR spectrometer. The FTIR spectra of the samples obtained were superimposed with that of standard sildenafil for identification. Similarity analysis (Correlation coefficient) of the adulterated products using wave numbers of 8 major FTIR peak values on Microsoft excel 2007 was also conducted.

RESULTS

Survey of Herbal Aphrodisiacs

The survey of herbal aphrodisiac products across four out of the seven states (Kano, Katsina, Kaduna, and Zamfara state) in the northwest Nigeria revealed a total of 596 products (Table 1).

Table 1: A Survey of herbal aphrodisiac products from some northwestern states

State	Number of products surveyed
Kano	357
Katsina	87
Kaduna	104
Zamfara	48
Total	596

A detailed description of all the products sampled is presented in table 2.

Table 2: Herbal aphrodisiacs products sampled

Product name	Code	Product name	Code	Products name	Code
Addyzoa	S1	Powder	S18	Da ManakusaLemunmata da maza (orange powder)	S34
Mai Ayu	S2	Mai Giwa	S19	Da manakusaLemunmata da maza (Brown powder)	S35
Big Man (Herbal tea)	S3	Mai Ganda	S20	Grey Powdered sample	S36
Great penis coffee	S4	WutaSallau Man power	S21	GarinmaiAyu Dan Zamfara	S37
Max Man coffee	S5	MskBusta powder	S22	Ak47 Dawa	S38
Wino fort	S6	Powdered sample	S23	Mai Doki	S39
Plant Viagra	S7	Powdered sample	S24	Mai Giwa	S40
Ebxyz (lady killer)	S8	Kara gudu	S25	Super Mona and Lisa For men and women	S41
Chew 9g	S9	Powdered sample	S26	TakadarinGari	S42
Powder	S10	GarinDadin Kowa	S27	Dan Agadas	S43
Powder	S11	Delay	S28	Na Gidan Mai Aku	S44
Powder	S12	Bulala	S29	Dan Agadas 2	S45
Powder	S13	Mai Bauna	S30	Powdered sample	S46
Powder	S14	MadaraKankana Ta maza da mata	S31	Na number Rimi	S47
Powder	S15	Powdered sample	S32	Powdered sample	S48
Powder	S16	Lamjib Traditional medicine	S33	Goko	S49
Powder	S17			Powdered sample	S50

Thin Layer Chromatographic Studies

The optimized TLC conditions for the elution of sildenafil at an Rf value of 0.56 are presented in Table 3, while the Chromatograms are shown in fig. 2.

Table 3: Optimized thin layer chromatographic conditions

Parameters	Description
TLC plate	Merck TLC silica gel 20 × 20
Mobile phase	Hexane: ethyl acetate: methanol (5:2:1v/v)
Mobile phase volume	8 mL
Band application	3 mm
Saturation	10 minutes
Development time	12 minutes
Drying time	2 minutes
Visualization	UV light (254 nm)
Rf value	0.56

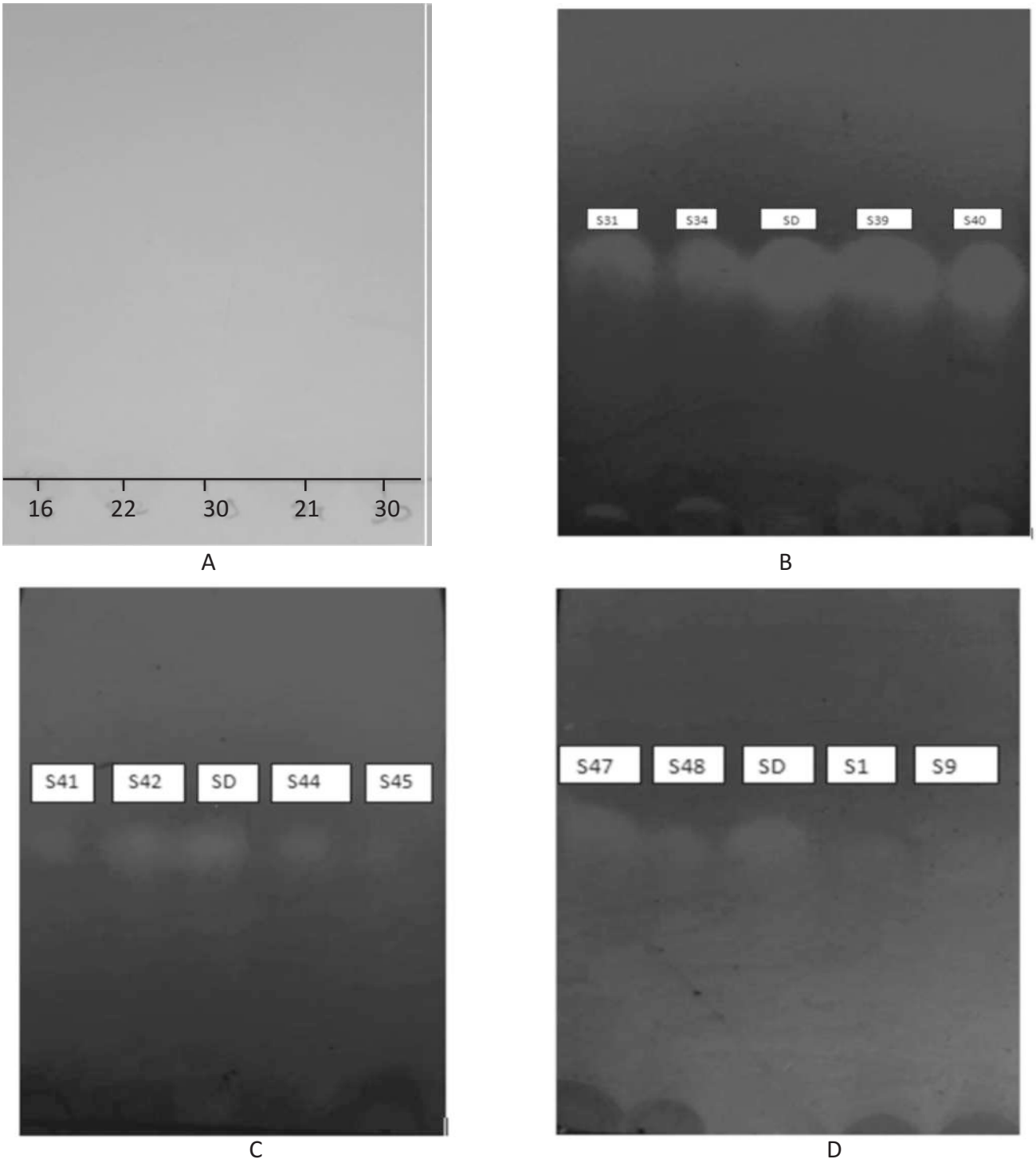

Figure 2: TLC chromatogram of standard sildenafil (SD) with adulterated samples of (A) S16, S22, S27 and S30. (B) S31, S34, S39 and S40 (C) S41, S42, S44 and S45 (D) S47, S48, S1 and S9.

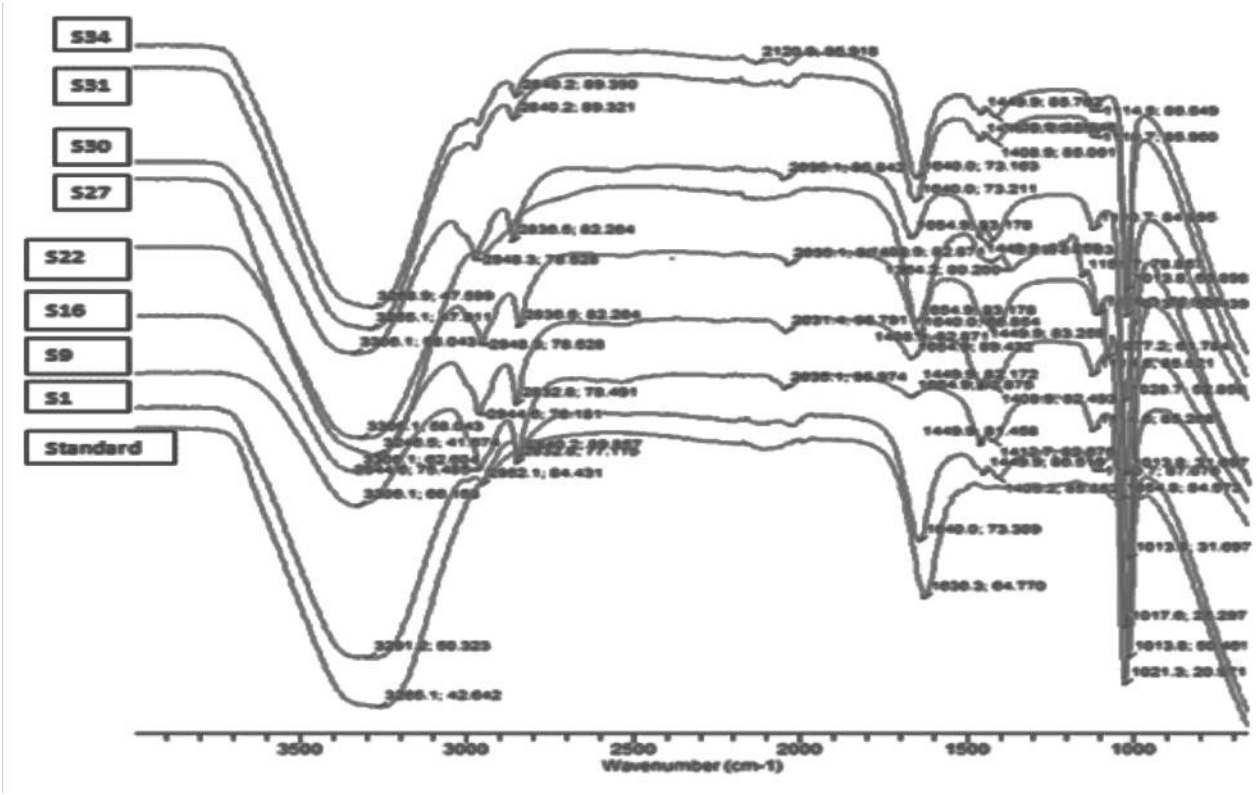

Figure 3: Superimposed FTIR spectrum of sildenafil standard solution and sample S1, S9, S16, S22, S27, S30, S31 and S34

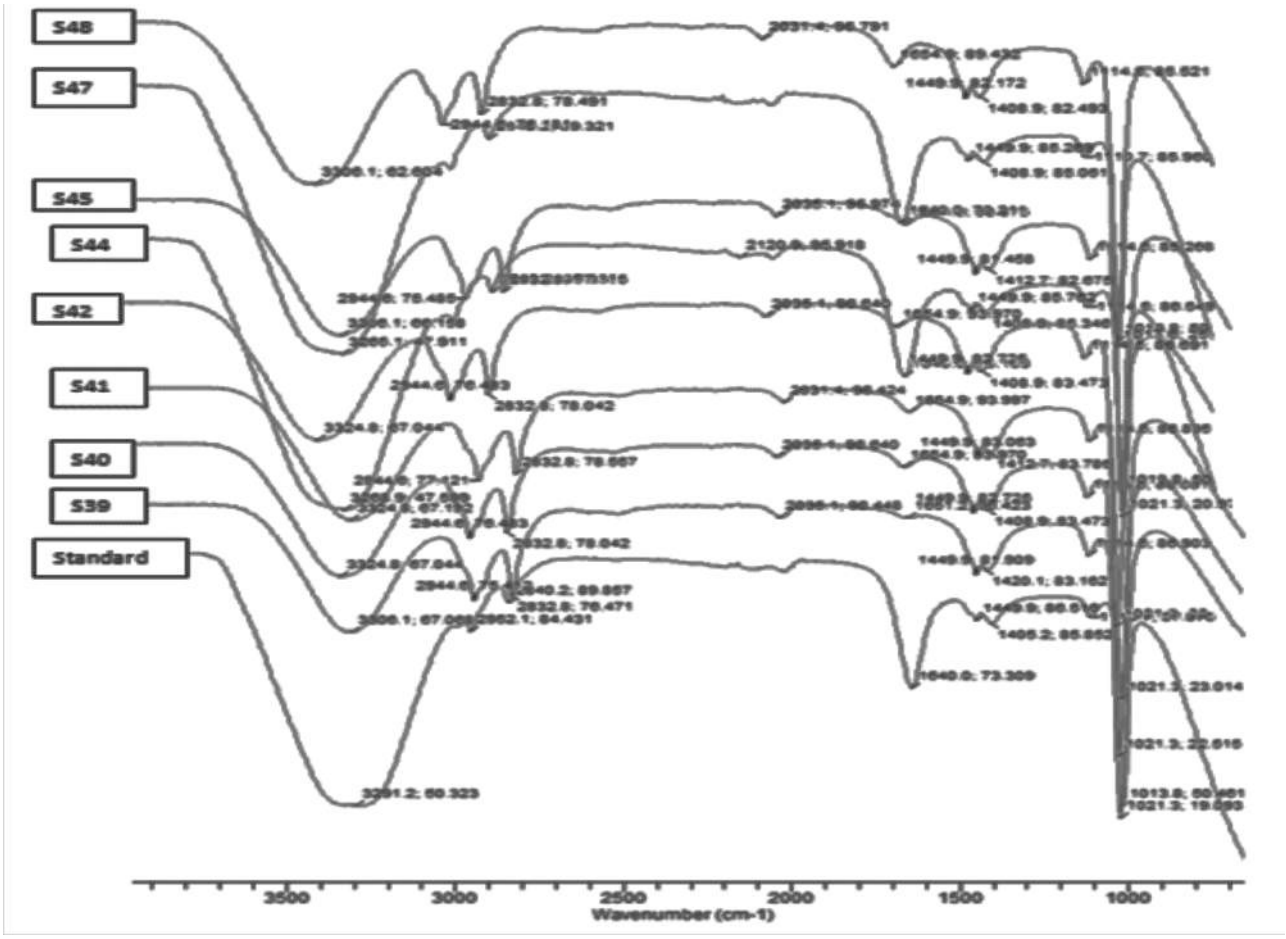

Figure 4: Superimposed FTIR spectrum of sildenafil standard solution and sample S39, S40, S41, S42, S44, S45, S47 and S48

Table 4: Diagnostic functional groups and their absorption bands in FTIR spectrum of standard sildenafil

Functional group	peak position (official ranges) [12] Cm^{-1}
N-H symmetric and asymmetric stretching	3291.2(3100-3500)
Aliphatic C-H stretching	2952.1 (2850-3000)
CH_3 asymmetric stretching	2840.2 (2850-3000)
C=O amide stretching	1640.2 (1640-1690)
C=C aromatic bond	1449.9 (1400-1600)
C-H bond in aromatic hydrocarbon	1405.2 (1350-1470)
SO_2 asymmetric stretching	1110.7 (1180-1360)
SO_2 symmetric stretching	1013 (1000-1180)

FTIR fingerprint analysis

The FTIR fingerprint spectra analyses of the adulterated herbal products are presented in Table 5 and 6.

Table 5: Similarity analysis of the adulterated herbal samples using wave numbers of eight major FTIR peaks

S/No.	Wave number (cm^{-1})							
	S1	S9	S16	S22	S27	S30	S31	S34	Peak
1.	1021.3	1017.6	1021.3	1028.7	1013.8	1013.8	1013.8	1021.3	SO_2 Symmetrical Stretching
2.	1114.5	1114.5	1114.5	1151.7	1110.7	1110.7	1114.5	1114.5	SO_2 asymmetrical Stretching
3.	1412.7	1408.9	1412.7	1364.2	1408.9	1408.9	1408.9	1420.1	C-H aromatic Stretching
4.	1449.9	1449.9	1449.9	1423.8	1449.9	1449.9	1449.9	1449.9	C=C aromatic Stretching
5.	1654.9	1654.9	1654.9	1640.0	1654.9	1640.0	1640.0	1651.2	C=O amide Stretching
6.	2832.8	2832.8	2832.8	2832.8	2836.6	2840.2	2840.2	2832.8	CH_3 asymmetrical Stretching
7.	2944.6	2944.6	2944.6	2944.6	2948.3	2944.6	2944.6	2944.6	C-H aliphatic Stretching
8.	3306.1	3306.1	3324.8	3306.1	3306.1	3265.1	3268.9	3306.1	N-H Stretching
	0.9998	0.9996	0.9999	0.9999	0.9997	0.9995	0.9991	0.9991	Correlation Coefficient

Table 6: Similarity analysis of the adulterated herbal samples using wave numbers of eight major FTIR peaks

S/No.	Wave number (cm^{-1})								
	S39	S40	S41	S42	S44	S45	S47	S48	Peak
	S1	S9	S16	S22	S27	S30	S31	S34	Peak
1.	1021.3	1017.6	1021.3	1028.7	1013.8	1013.8	1013.8	1021.3	SO_2 Symmetrical Stretching
2.	1114.5	1114.5	1114.5	1151.7	1110.7	1110.7	1114.5	1114.5	So_2 asymmetrical Stretching
3.	1412.7	1408.9	1412.7	1364.2	1408.9	1408.9	1408.9	1420.1	C-H aromatic Stretching
4.	1449.9	1449.9	1449.9	1423.8	1449.9	1449.9	1449.9	1449.9	C=C aromatic Stretching
5.	1654.9	1654.9	1654.9	1640.0	1654.9	1640.0	1640.0	1651.2	C=O amide Stretching
6.	2832.8	2832.8	2832.8	2832.8	2836.6	2840.2	2840.2	2832.8	CH_3 asymmetrical Stretching
7.	2944.6	2944.6	2944.6	2944.6	2948.3	2944.6	2944.6	2944.6	C-H aliphatic Stretching
8.	3306.1	3306.1	3324.8	3306.1	3306.1	3265.1	3268.9	3306.1	N-H Stretching
	0.9998	0.9996	0.9999	0.9999	0.9997	0.9995	0.9991	0.9991	Correlation Coefficient

DISCUSSION

A total of 596 samples surveyed across the four (Kano, Katsina, Kaduna, and Zamfara) states in the northwest Nigeria and of which 50 samples were selected which represent the sample population for the study (Table 1). A detailed description of all the products sampled was also presented in table 2. The sample size (50) selected provided high level of general capability in representing the general population with good statistical significance, thus allows for generalization of findings from the sample(s) to the population.

The optimized TLC conditions for the separation of sildenafil at an Rf value of 0.56 presented in table 3, while the Chromatograms were shown in fig. 2. A total of sixteen (32 %) of the herbal aphrodisiac samples analyzed had similar Rf value with sildenafil; suggesting that these samples might be adulterated with sildenafil. This was similar to previous studies conducted using HPTLC and TLC to detect adulteration of sildenafil in herbal preparations.[9,13]

The results of the TLC study were further confirmed by FTIR analysis; the spectra of the adulterated samples were found to be super imposable at the fingerprint region with the spectrum of standard sildenafil (Fig. 3 and 4). The FTIR spectrum of standard sildenafil showed eight (8) strong absorption bands due the presence of N-H, CH_3, aliphatic C-H, amide C=O, aromatic C=C, aromatic C-H and SO_2 groups (Table 4). However, both O-H and N-H stretching occur around 3500-3000 cm^{-1} region. N-H stretching can easily be identified because it occurs at the lower frequency of vibration than O-H group, and O-H has higher dipole moment, as such it is more intense and broader due to effect of hydrogen bonding,which is responsible for the enlargement of N-H stretching vibrations band(s) in the region of 3100 - 3600 cm^{-1} which are in abundance in the citrate complex; [14] these are in agreements with previously reported studies.[15,16,17,18,19]

FT-IR bands between 1200 and 950 cm^{-1} could also be due C-O stretching. However, sulphonamides also exhibit S-N stretching vibrational absorptions in the range of 924-906 $cm;^{-1}$ this tends to occlude any C-O vibrations that might occur in the region. These were similar to the findings reported by other studies.[16,17]

These eight absorption bands are diagnostic and their degree of similarity (as indicated by the correlation coefficients) between the FTIR values of the standard and the adulterated samples was confirmation of the presence of sildenafil in the samples analyzed (Table 5 and 6). Degree of similarity is said to be strongly positive ($p < 0.01$) as the value is close to unity.

The study could be carried out, using more sensitive and selective techniques like HPLC/UV or LC/MS with enhanced accuracy and precision.

CONCLUSION

Aphrodisiacs herbal preparations marketed in North

Western Nigeria were found to be adulterated with sildenafil, a synthetic PDE-5 inhibitor used primary for erectile dysfunction. The inherent serious adverse effects of sildenafil and its potentially harmful drug-drug interactions and/or drug-food interactions render consumption of these adulterated herbal aphrodisiac products very dangerous. Thus, the need for immediate intervention by authorities and public health practitioners.

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