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DETERMINATION OF SYNTHETIC PHARMACEUTICAL ADULTERANTS AND PHYSICOCHEMICAL ANALYSIS OF SOME ANTIHYPERTENSIVE HERBAL PRODUCTS

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ABSTRACT

The use of herbal medicines and phytonutrients is expanding rapidly across the world with many people now resorting to these products for treatment of various health challenges. This is as a result of its cultural acceptability, affordability, and lack of access to modern medicine. The safety of these herbal remedies is however poorly understood**.** This study was aimed at determining the presence of synthetic pharmaceutical (bendroflumethiazide and furosemide) adulterants and evaluating the physicochemical parameters of some selected antihypertensive herbal products marketed within three Northwestern states (Kastina, Kebbi and Jigawa) of Nigeria. A total of twenty-three samples were collected for this study. Screening of the samples for the presence of bendroflumethiazide and furosemide as undeclared adulterants was carried out using thin layer chromatographic (TLC) and Fourier transform infra-red (FTIR) analyses. Physicochemical analyses of the antihypertensive herbal samples were conducted according to the standard methods. Four of the analysed samples (M7, M10, M11 and M14) were found to have spot corresponding to that of bendroflumethiazide standard powder. However, the spots were discovered not to be bendroflumethiazide by the FTIR analysis. Furosemide was not detected in any of the analysed samples. The physicochemical analyses of the antihypertensive herbal samples showed that 53 % of the sample failed the weight uniformity test as it was found to be above the permissible limit. Furthermore, 27 % of the samples had moisture content above recommended 8 % NAFDAC limit while all the samples were found to have extractives values of below the minimum recommended limits. The total ash values in 17 % of the samples were higher than the 14 % maximum acceptable limit recommended by European Pharmacopoeia. The analysed antihypertensive herbal products did not contain bendroflumethiazde and furosemide as undeclared adulterants; however, their quality may be considered substandard, since none complied with all quality tests conducted.

Keywords: Synthetic pharmaceutical adulterants, antihypertensive herbal product, physicochemical, bendroflumethiazide, furosemide, TLC, FTIR.

INTRODUCTION

Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain, as active ingredients, parts of plants, or other plant materials, or combinations thereof (WHO, 2005). It is the oldest form of healthcare known to humanity cutting across cultures throughout history and most people in the developing counties prefer the use of herbal medicines due to the widespread believe that they are safe (WHO, 2005; Barnes *et al*., 2007). Contrary to this belief, herbal remedies have been reported to have been adulterated with synthetic pharmaceuticals; which are marketed and consumed as natural supplements (Singh *et al.,* 2009; Rahimi

2015; Haider *et al*., 2017; Muhammad *et al*., 2020; Saidu *et al*., 2024; Oloyede *et al*., 2024). Herbal medicine is receiving much attention not only in the developing countries of the world like Nigeria, but also in the western world where it is estimated that about 75 – 80 % (WHO, 2005).

High blood pressure, also known as hypertension, is a chronic condition that if not properly managed can lead to heart disease and stroke. Hypertension is estimated to have affected about 16.2 % of the world's population (WHO, 2020). Several plants, e.g. *Hibiscus sabdariffa*, *Ocimum basilicum*, *Petroselimun crispum*, *Apium graveolens* have been reported to lower blood pressure **(**Sara *et al.,* 2019; Khafsa *et al.,* 2020). It has been reported that 25 – 65 % of adults with hypertension, living in northwestern Nigeria, use herbal medicinal product in the management and treatment of hypertension (Umar *et al.,* 2020).

Adulteration is the deliberate or accidental addition of inferior, spurious, or harmful substances, or the removal of valuable constituents from a product, thereby reducing its quality, safety, or efficacy (WHO, 2011a). Adulteration can be intentional including addition of orthodox or synthetic chemical drugs to enhance the effectiveness of the herb; or un-intentional which mostly occurs without the wish or knowledge of the producer mostly due to the lack of knowledge, and non-adherence to good manufacturing practices (GMP) by the manufacturer. Quality control of herbal medicines ensures the safety and efficacy of herbal medicinal product as it involves the identification and quantification of the complex and variable chemical constituents of the herbal products. Several studies have reported the presence of orthodox medicines, such as amlodipine, hydrochlorothiazide and captopril, in antihypertensive herbal products (NAFDAD, 2019; NAFDAC, 2021). The aim of this research is to screen for the presences of bendroflumethiazide and furosemide as undeclared adulterants and to carry out physicochemical analysis of selected antihypertensive herbal products marketed within three Northwestern states (Kastina, Kebbi and Jigawa) of Nigeria.

METHODS

Sampling of antihypertensive herbal products

A total of 23 antihypertensive herbal products were conveniently collected from pharmaceutical shops and super markets within the metropolitan areas of Katsina (10 samples, Kebbi (7 samples) and Jigawa (6 samples) States in Northwest Nigeria. These states were selected because of their active herbal markets and widespread reliance on traditional medicine. The use of convenience sampling was guided by accessibility, time, and resource limitation. The samples were coded (M1-M23) and their label information were examined and recorded.

Physicochemical analysis of the antihypertensive herbal samples

The label information of the antihypertensive herbal products were examined and recorded. Weight uniformity, moisture content, extractable substances and ash values were determined in triplicate using standard methods (BP, 2009; WHO, 2011b).

Detection of bendroflumethiazide and furosemide as an undeclared adulterant

Sample preparation

Stock solutions (1000 µg/ml) of furosemide and bendroflumethiazide standard powders

were separately prepared by weighing 10 mg of furosemide and bendroflumethiazide 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 of the antihypertensive samples were weighed and transferred into beakers (25 mL) containing 10 mL each of methanol and acetonitrile for the extraction of furosemide and bendroflumethiazide respectively. The solutions were filtered and then kept for TLC analysis.

TLC Method Development

The TLC method was developed by trying several solvent systems. After which acetic acid, ethyl acetate and toluene, (1:2:4 v/v/v) were chosen for TLC analysis of both furosemide and bendroflumethiazide. The mobile phase was transferred into the developing chamber, covered, and allowed to saturate for ten (10) minutes.

Co-TLC

The solutions of the standard powders and samples were spotted on the TLC plates with the aid of a capillary tubes at least two (2 mm) apart and one (1 cm) from the lower end of the plate (baseline). The TLC plates was then introduced into the TLC 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 found to have the same R_f value as the standards were scraped for FTIR analysis.

Fourier transformed Infrared spectroscopy (FTIR) analysis

The scraped spots were analyzed within the mid-IR region (650 - 4000 cm^{-1}) with 32 scans and at 8 cm resolution. The spectra obtained were superimposed in order to confirm their identity.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc test for multiple comparison at significance level of $p < 0.05$ using IBM SPSS.

RESULTS

RESULTS AND DISCUSSION

Majority (96%) of the sampled herbal antihypertensive products are indigenous and none of them (Table 1) is listed by National Agency for Food and Drug (NAFDAC). Hence, the need for regulatory bodies, particularly NAFDAC, to ensure that the herbal products marketed in Nigeria are well regulated. A study conducted on antihyperglycemic products marketed in Kaduna state, Nigeria reported that 75 % of the products do not have NAFDAC listing number (Usman *et al*., 2021). Saidu and his co-workers reported that 77 % of their analysed herbal aphrodisiac products were not listed by NAFDAC (Saidu *et al*., 2024). Research conducted on anti-asthmatic herbal products revealed that all the product has no NAFDAC listing number (Kassim *et al*., 2022). All the samples are in solid form, with majority (90 %) of them having brownish colour and were found to be palatable (Table 1).

Table 1: Label information of the herbal antihypertensive samples

Code	Dosage Form	Manufacturer's Address	NAFDAC Reg. No.	Batch Number	Expiry Date
M1	Powder	Kano State	NIL	NIL	2023
M2	Tea bag	Zhejang Yiwu	NIL	XH-096	2025
M3	Powder	Kano State	NIL	NIL	2024
M4	Tea bag	El-Maikano	NIL	004	2024
M5	Powder	Kano State	NIL	NIL	2024
M6	Powder	Nigeria	NIL	2738960	2024
M7	Powder	Kano State	NIL	NIL	2026
M8	Powder	Kano State	NIL	NIL	2025
M9	Powder	Kano State	NIL	NIL	2024
M10	Powder	Kano State	NIL	NIL	2024
M11	Tea bag	Kano State	NIL	NIL	2025
M12	Powder	Kano State	NIL	NIL	2025
M13	Powder	Kano State	NIL	NIL	2025
M14	Powder	Sokoto State	NIL	024	2023
M15	Powder	Katsina State	NIL	NIL	2024
M16	Powder	Kano State	NIL	NIL	2024
M17	Powder	Kano State	NIL	NIL	2026
M18	Powder	Kano State	NIL	NIL	2025
M19	Powder	Kano State	NIL	2444593	2024
M20	Powder	Kano State	NIL	NIL	2025
M21	Powder	Kano State	NIL	NIL	2023
M22	Tea bag	Lagos State	NIL	NIL	2023
M23	Powder	Kano State	NIL	NIL	2023

Physicochemical parameters of the herbal products

Table 2: Weight uniformity of herbal antihypertensive samples formulated as tea bags

S/No.	Samples	Weight (g ± SEM)	Mean deviation (%)	Number of samples that deviated by ≥ 7.5 %
1	M1	6.28 ± 0.14	0.02 - 19.44	3*
2	M2	4.09 ± 0.11	1.96 - 21.96	5*
3	M3	5.04 ± 0.08	0.51 - 16.60	2
4	M4	4.97 ± 0.13	3.96 - 28.81	6*
5	M5	5.13 ± 0.35	0.08 - 9.64	1
6	M6	5.53 ± 0.16	0.12 - 23.13	6*
7	M7	3.73 ± 0.10	0.56 - 24.46	4*
8	M8	4.25 ± 0.11	2.88 - 19.34	6*
9	M9	2.34 ± 0.03	0.42 - 14.10	2
10	M10	3.68 ± 0.07	1.62 - 15.23	1
11	M11	6.62 ± 0.19	0.40 -31.50	4*
12	M12	2.66 ± 0.02	0.09 - 9.48	1
13	M13	6.76 ± 0.08	0.02 - 13.32	1
14	M14	3.14 ± 0.02	0.16 - 7.15	0
15	M15	5.83 ± 0.21	2.05 - 29.69	5*
16	M16	8.05 ± 0.09	0.06 - 16.95	2
17	M17	6.75 ± 0.13	1.05 - 13.77	1
18	M18	5.12 ± 0.21	4.16 - 29.70	5*
19	M19	6.12 ± 0.11	0.14 - 15.03	6*
20	M20	5.35 ± 0.16	0.13 - 38.99	5*
21	M21	2.33 ± 0.02	0.00 - 9.44	1
22	M22	4.39 ± 0.13	0.55 - 38.35	3*
23	M23	6.47 ± 0.13	0.91 - 25.64	2

*failed the test as more than two of the products' weight deviated by ≥ 7.5%

Table 3: Total Ash and Moisture content values of the herbal antihypertensive samples

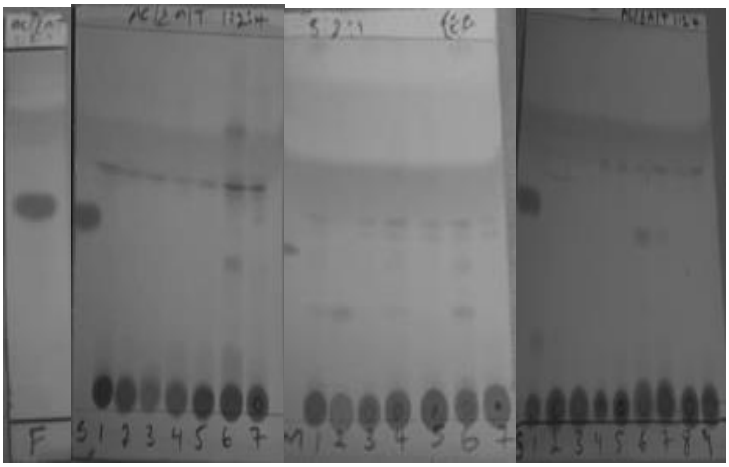
S/No.	Samples	Total Ash(%) ± SEM	Moisture Content (% ± SEM)
1	M1	16.66 ± 0.28	12.16 ± 0.28**
2	M2	14.83 ± 2.56	8.33 ± 0.28**
3	M3	14.50 ± 0.50	10.16 ± 2.46**
4	M4	8.00 ± 0.50*	6.16 ± 0.28
5	M5	10.66 ±0.28*	9.96 ± 0.46**
6	M6	9.16 ± 0.16*	7.33 ± 0.28
7	M7	11.50 ±0.28*	7.66 ± 0.28
8	M8	11.00 ±0.50*	6.33 ± 0.28
9	M9	5.16 ± 0.16*	6.66 ± 0.28
10	M10	11.50 ±0.00*	5.83 ± 0.16
11	M11	16.66 ± 0.28	7.16 ± 0.00
12	M12	11.66 ±0.76*	6.00 ± 0.50
13	M13	15.50 ±0.50	16.50 ± 0.50**
14	M14	11.00 ± 0.50	10.16 ± 0.28**
15	M15	14.00 ± 0.50	5.00 ± 0.00
16	M16	18.16 ± 0.16	6.66 ± 0.28
17	M17	6.00 ± 0.50*	4.83 ± 0.28
18	M18	14.83 ± 0.16	6.00 ± 0.00
19	M19	10.50 ±0.50*	6.50 ± 0.50
20	M20	6.16 ± 0.28*	7.66 ± 1.04
21	M21	9.33 ± 0.28*	4.83 ± 0.28
22	M22	14.00 ± 0.00	3.50 ± 0.16
23	M23	7.66 ± 0.28*	7.37 ± 0.00

*above the 14 % European Pharmacopoeia maximum limit
**above 8 % NAFDAC maximum permissible limit

Table 4: Water Extractive and Ethanol Extractive Values of the Herbal Antihypertensive Samples

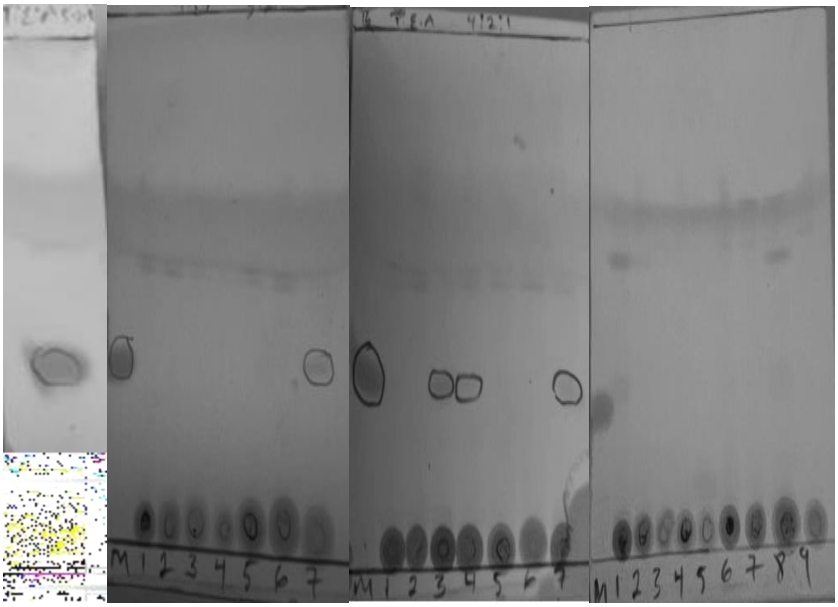
S/No.	Water extractive (% ± SEM)	Ethanol extractive (% ± SEM)
1	6.08 ± 0.14*	5.75 ± 0.25*
2	4.08 ± 0.00*	4.75 ± 0.25*
3	5.66 ± 0.14*	6.00 ± 0.81*
4	2.91 ± 0.28*	4.66 ± 1.60*
5	5.41 ± 0.46*	6.33 ± 0.38*
6	12.75 ± 0.89*	8.91 ± 0.52*
7	4.91 ± 0.28*	5.16 ± 0.38*
8	5.16 ± 0.08*	5.50 ± 0.43*
9	3.16 ± 0.29*	3.91 ± 0.62*
10	4.16 ± 0.14*	5.25 ± 0.50*
11	3.58 ± 0.25*	3.08 ± 0.38*
12	9.00 ± 0.11*	5.83 ± 0.28*
13	5.25 ± 0.25*	4.83 ± 0.14*
14	4.08 ± 0.14*	4.33 ± 0.28*
15	4.16 ± 0.14*	4.58 ± 0.28*
16	3.50 ± 0.25*	2.91 ± 0.38*
17	3.25 ± 0.00*	3.58 ± 0.38*
18	4.08 ± 0.01*	5.00 ± 0.25*
19	4.33 ± 0.14*	5.08 ± 0.14*
20	3.66 ± 0.14*	4.00 ± 0.25*
21	4.25 ± 0.25*	4.41 ± 0.38*
22	5.66 ± 0.28*	5.50 ± 0.25*
23	4.66 ± 0.14*	5.75 ± 0.25*

*lower than the 15 % European Pharmacopoeia minimum limit



a b c d

Plate I: TLC profile of furosemide standard powder (a) and co-TLC profiles (b, c, and d) of furosemide with samples M1- M23 in acetic acid: ethyl acetate: toluene (1:2:4)



e f g h

Plate II: TLC Profile of Bendroflumethiazide Standard Powder (E) And Co-TLC Profiles (F, G, And H) Of Bendroflumethiazide with Samples M1- M23 In Acetic Acid: Ethyl Acetate: Toluene (1:2:4)

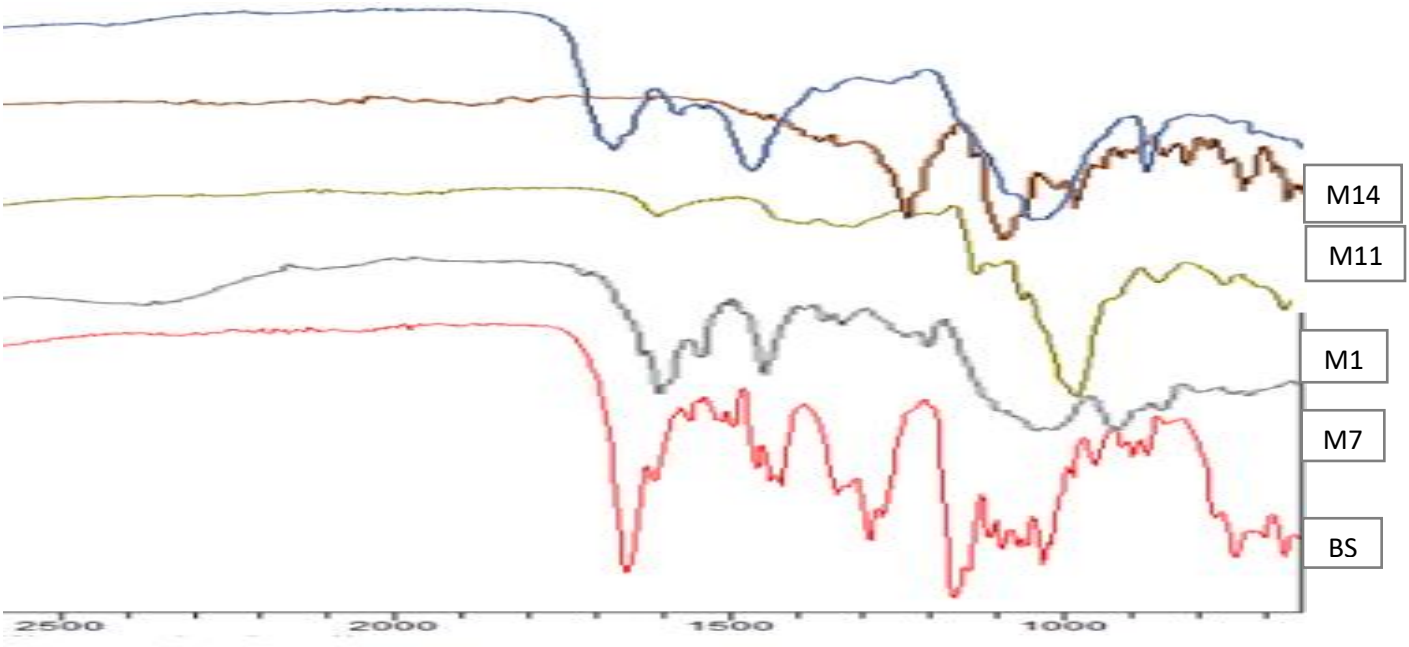


Figure 1: Super imposed FTIR spectrum of bendroflumethiazide standard (BS) powder with Sample M7, M10, M11 and M14

It was observed that most (82 %) of the sampled products were found to be in sachet form. More than half (53 %) of the products in sachet form failed weight uniformity test (Table 2) as the weight of more than two products deviated from the mean weight by more than 7.5 % (BP, 2009). This indicates that good manufacturing practice (GMP) was not properly adhered to during processing of the analyzed herbal antihypertensive products. This could lead to inconsistency in dosing during administration of these products thereby to leading therapeutic failure. Abdullahi and his co-workers reported that all the analysed local herbal samples failed weight uniformity test (Abdullahi *et al.,* 2018).

Furthermore, the moisture contents in most (74 %) of the analysed samples were above the recommended 8 % NAFDAC limit (Table 3). This could lead to microbial contamination and chemical instability of the products (Awwalu *et al*., 2018). Thus, individuals who use these herbal products might be exposed to microbial infection. Kassim *et al*., (2022) reported moisture contents above the NAFDAC limit in 50 % of their analyzed anti-asthmatic herbal samples. A study reported within moisture contents NAFDAC limit in all the analysed herbal products marketed within Zaria metropolis (Awwalu *et al*., 2018). The varying moisture content observed in the different products may be due to the intrinsic moisture holding capacities of the herbal materials and the efficiency of the packaging material which protect the herbal products from absorbing moisture from the atmosphere (Kambo *et al*., 2012).

The total ash values (Table 3) in 17 % of the samples were higher than the 14 % maximum acceptable limit by European Directorate for the Quality of Medicines and Healthcare (EDQM) (2007). Ash content indicates the mineral content of a sample however, it does not necessarily indicate

high quality (WHO, 2011b). Awwalu *et al*., (2018) and Kassim *et al*., (2022) reported that none and 30 % of their analysed herbal samples had total ash values higher than the maximum acceptable limit recommended by European Pharmacopoeia. A study conducted on local herbal remedies reported total ash value within the range of 3.90 to 43.92 % (Abdu *et al.,* 2015).

The Extractable values (water and ethanol) of all the analysed antihypertensive herbal products were lower than the 15 % minimum limit set by the EDQM (2007). Thus, neither solvent provides sufficient extraction efficiency to yield adequate amount of the phytochemicals. Kassim *et al*., (2022) reported that 40 and 10 % of their analysed anti-asthmatic herbal samples had water extractive values and alcohol extractive values respectively lower than the minimum limit recommended by the European Pharmacopeia.

TLC analysis revealed that none of the antihypertensive herbal products is adulterated with furosemide (Plate I). Four samples (M7, M10, M11 and M14) were found to have a spot with R_f value and color similar to that of standard bendroflumethiazide (Plate II). However, the FTIR spectra of these samples with R_f value corresponding to that of bendroflumethiazide was found not to superimpose, within the finger print region, against bendroflumethiazide spectrum (Figure 1). Thus, revealing that the spots are not bendroflumethiazide but could be analogues of bendroflumethiazide.

CONCLUSION

The analysed antihypertensive herbal products did not contain bendroflumethiazde and furosemide as undeclared adulterants; however, their quality may be considered substandard, since none complied with all quality tests conducted.

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