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Investigation of the Phytochemicals, Metals Content and Antibacterial Activities of Commercial Herbal Preparations Sampled from Lagos Market, Nigeria

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ABSTRACT

As the use of herbal remedies gains popularity as an alternative form of medicine, it is crucial to ensure their safety. These natural substances consist of a variety of chemical elements, some of which may have beneficial effects while others may not. This study aims to explore the phytochemical composition, levels of trace and heavy metals (including cadmium, lead, copper, nickel, zinc, iron, manganese, sodium, and potassium), as well as the antibacterial properties of fifteen distinct herbal preparations found in pharmacies throughout Lagos State, Nigeria. Standard qualitative techniques were utilized to assess the phytochemical content, while the aqua regia digestion method in conjunction with the atomic absorption spectrophotometric technique was used to determine the metal content. For measuring the zone of inhibition against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumonia*, the agar well diffusion method was utilized to determine the antibacterial activity. The results of this study showed that all the herbal products have the presence of reducing sugars in different degrees while triterpenoids were absent in all the products. Metal concentrations in samples A-O were within recommended limits. The range of mean concentrations of the metals, Cd, Pb, Zn, Fe, Mn, Cu, Ni, Na, K obtained were 0.0016-0.0153, 0.00005-0.0185, 0.0785-1.1395, 0.019-1.0380, 0.006-0.0915, 0.002-0.2900, 0.0017-0.0485, 0.4070-1.4795, 0.3195-2.334 µg/ml respectively. Only three samples B, D, and K were effective against *Bacillus subtilis*. While the other samples did not display antibacterial activities against the bacterial used. These herbal remedies can be considered safe, but long-term effects on health require further research.

Keywords: Metals, Antibacterial, Phytochemicals, Atomic Absorption Spectrometry

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Introduction

Since prehistoric times, medicinal plants have been identified and employed in traditional treatments. In traditional African medicine as well as other kinds of therapy from many cultures throughout the world, medicinal plants have served as the foundation for treating a variety of maladies. However, the usage of herbal products is widely available and poorly controlled in Nigeria and many other low-income nations. Uncertainty exists over the safety of certain natural medications.[1] Herbal medications are not examined with the same scientific rigour necessary for conventional drugs, and the majority of these product makers do not provide regulatory organizations with proof of safety and efficacy prior to marketing. This necessitates adequate oversight by regulatory agencies.[2]Among herbal preparations, the use of herbal bitters is growing, although there is little information available about the extent of usage within any particular population. These poly-herbal liquid formulations with bitter herbs are often used as aphrodisiacs, immunological boosters, anti-infective, antimicrobial, and digestive aids.

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On the one hand, some of these herbal remedies could have components that are essential for human metabolism, illness prevention, and healing, but on the other hand, they might also contain unhelpful components which could pose health hazards to the body system due to unascertained safety and efficacy.[3] Due to the high reliance on herbal preparations for therapeutic purposes, the significance of understanding the elemental contents of herbal preparations used for diverse medical purposes demanded more attention for screening than ever before.[4, 5] The pollution of the soil where plants were harvested before being transformed into various forms may be the cause of high metal concentrations in herbal treatments. In addition, plants can absorb these metals via contaminated soil and metal deposits on herb parts exposed to polluted air.[6, 7] It has been suggested that planting medicinal plants close to waste dumps and industrial areas be avoided as much as possible.[8,9] In Nigeria, where herbal preparations are widely used for therapeutic purposes, it is crucial to continually assess the safety and therapeutic claims of some these remedies due to inadequate quality monitoring by regulators. Hence, the current study investigates the phytochemical constituents, level of heavy and trace metals (cadmium, lead, copper and nickel, zinc, iron, manganese, sodium and potassium), and the antibacterial activities from fifteen brands of herbal bitter preparations sold in Pharmacies in Lagos state, Nigeria.

Materials and Methods

The analytical grade of all other chemicals and reagents was purchased from Sigma-Aldrich in Nigeria.

Parameters of samples used

A total of 15 brands of herbal products were purchased from pharmacies in Lagos state. Visual parameters such as the National

Agency for Food Drug Administration and Control (NAFDAC) registration number, batch numbers, manufacturing and expiry dates, pack size, indications, and ingredients were examined.

Phytochemical screening
The qualitative phytochemical examination of the herbal products was conducted by using standard methods. The samples were examined for the following phytochemicals; Saponins, Reducing Sugar, Phenolic Compounds, Tannins, Anthraquinones, Triterpenoids, Steroids, Flavonoids, Cardiac glycosides and alkaloids using appropriate reagents.[10]

Analysis for trace and heavy metals in the samples using Aqua regia digestion method
Samples digestion: The digestion of the samples was by wet (acid) and a hot plate method. A 50 mL of the liquid sample was transferred into a 250 mL beaker and 5 mL of concentrated nitric acid was added. The beaker was placed on a hot plate and evaporated to dryness. The beaker was then cooled and another 5 mL of nitric acid was added. Heating was continued until a light-coloured residue was observed. Then 1 ml of concentrated nitric acid was added and the beaker was warmed slightly to dissolve the residue and transferred into a 50 mL volumetric flask. The walls of the beaker were then washed with distilled water and also transferred into the volumetric flask. The volume in the volumetric flask was made up to the 50 mL mark. The concentrations of cadmium, lead, zinc, iron, manganese, copper, nickel, sodium and potassium were determined in the digested samples using the Buck Scientific AAS (model 210 VGP)
AAS analytical procedure: The analytical procedures are as previously reported in the literature[9]. Absorbances of the stock solutions and samples were determined using AAS. The nebulizer of AAS was rinsed by aspirating distilled deionized water while the trace metals in the samples were determined by aspiration into air acetylene flame. Absorption of metal in samples and the stock were then determined. The calibration curve of absorbance versus concentration for each metal were obtained using Microsoft excel software. The metal ion concentrations in samples were then determined by substitution of absorbance in the regression equation obtained.

Antibacterial assay of herbal preparation
Sample preparation: There were three working samples, 100%, 50%, and 25% which were achieved by diluting down the neat solution of herbal products with sterile distilled water
Neat solution of the herbal preparation, concentration = 100%
2 mL of A + 2 mL of sterile distilled water (diluent), concentration = 50%
2 mL of B + 2mL of sterile distilled water (diluent), concentration = 25%
Contents of bottles A, B, and C were the working samples of the herbal products.
Standard Preparation: Similarly, working standards of pure ciprofloxacin were of four values, 20µg/mL, 10µg/mL, 5µg/mL and 2.5µg/mL solutions of the standard solutions were achieved by having a decimal dilution of the stock standard and then double diluting further to have the working standard as follows;

Stock solution of ciprofloxacin, concentration = 200mg/100mL solution = 2mg/mL = 2000 µg/mL
1ml of A + 9ml of sterile distilled water (diluent),concentration = 200 µg/mL
1ml of B + 9ml of sterile distilled water (diluent),concentration = 20 µg/mL
2ml of C + 2ml of sterile distilled water (diluents),concentration = 10µg/mL
2ml of D+ 2ml of sterile distilled water (diluents),concentration = 5 µg/mL
2ml of D + 2ml of sterile distilled water (diluents),concentration = 2.5 µg/mL
Contents of bottles C, D, E and F were the working standards of ciprofloxacin. Ciprofloxacin was used as a reference due to its broad spectrum of antibacterial activity. It has been established to have bactericidal activity on the test organism.
Media preparation: The media were prepared following the instruction by the manufacturers. This was by weighing 38 g of the Mueller Hinton Agar powder media and dispersed in 1000 mL of distilled water. They were heated to melt in the water bath at 100^0C. The molten agar gels so formed were dispensed in 25mL portions into sample bottles and then sterilized by autoclaving at 121^0C for15 minutes. The 25mL portion of the agar gel in each of the sample bottles was the working volume of the agar for potency assay.
Assay organisms: The bacterial organisms were obtained from the Laboratory stock Culture in Pharmaceutical microbiology laboratory of the college of medicine, University of Lagos, Nigeria comprise *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia*. So, they were all clinical isolates that were primarily isolated on various diagnostic and selective media to suppress other contaminants. They were then subcultured onto Mueller Hinton Agar to remove the effects of indicators and suppressive chemical agents in primary isolation media[11.] They were then sub-cultured into sterile nutrient broth for optical density calibration. Incubation periods were 24 hours for all the bacteria at 37^0c.
Calibration of assay organisms: The assay organisms' bacterial load was adjusted using a reference standard suspension and sterile normal saline. Bacterial liquid cultures were added to the saline until the turbidity matched the densitometer's 0.5 McFarland standards, and the adjusted suspension was used in a 1mL portion.
Seeding of assay organisms: The assay medium which was prepared and measured was maintained at 45^0C so as to make it remain molten until it was needed. Organisms were vortexed to homogenize the assay organisms. A 1mL of calibrated organisms was seeded into the warm agar and was mixed thoroughly using the roll-palm method before pour-plating. After solidifying under sterile condition in a biological safety cabinet, they were prepared for cork boring.
Cork boring and seeding of standards and samples: After allowing all the seeded agars to set, a cork borer, size 10 mm cross-section was used for boring the wells. It was flamed and allowed to cool before using it to gently punch a hole in each of the sectors of the Petri dishes. All the cut portions were thrown into a dish of disinfectant. A 100 µL of various working concentrations was dispensed into the wells and allowed to stand for four hours before incubation.
Incubation of plates and readings: All the Petri dishes were incubated lid-up position. This was so in order to avoid spillage. After four hours on the Laboratory bench for the samples and standard concentration to diffuse, the plates for the antibacterial studies were incubated at 37^0C and observed after 24 hours and observed for zones of inhibition as a result of growth and responses of the bacteria to the samples. The zone reader was used to take triplicate readings, and the average zone values were determined and recorded.[12]

Statistical analysis
The data are presented as mean ± standard deviation.

Results and Discussion

The results of the visual inspection, phytochemical screening, trace and heavy metal analysis, antibacterial activities of both the standard and samples is shown in Tables (1, 2, 3, 4, 5) respectively.

Visual Inspection of the fifteen (15) herbal products:
Physical inspection of individual products showed no visible physical damage. Only samples J and L do not have batch number, sample F did not have list of ingredients. Manufacturing and expiry date is missing in samples L and M, while only expiry date is missing on samples G, I and O.

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Phytochemical screening

The results of this study showed that all the herbal products have the presence of reducing sugars in different degrees while triterpenoids were absent in all the products.

Atomic Absorption Spectrophotometry results

All the samples analyzed contained concentration of metals that were below the recommended permissible limits.

Zone of inhibition for standard antibacterial (Ciprofloxacin)

As a well-known antibiotic, ciprofloxacin demonstrated its effectiveness against the specific Gram + bacteria used for this study. The zone of inhibition range from 13.66 – 21.75 mm at 2.5 µg/mL, 19.17 – 24.17 mm at 5 µg/mL, 22.83 – 30.17 mm at 10µg/mL and 28.50 – 30.83 mm at 20 µg/ml.

Zone of Inhibition for the herbal products

The herbal products showed no antibacterial activity except in samples B, D and K that showed activity on *Bacillus subtilis* at all the concentrations tested.

Safety and Quality of medical herbal products

The quality and safety of medical herbal products are issues that worry health authorities, pharmaceutical firms, and the general public.[13] In Nigeria, the use of herbal medicine is widespread. The products control a large portion of the pharmaceutical industry.[14] When purchasing any goods, especially an orthodox or herbal treatment, the visual examination is crucial. It was noted that most of the herbal products sampled from various pharmacies in this study met the appropriate labeling standards by including all essential product information, nevertheless, samples L and M lack manufacturing and expiration dates, which is a notable deviation from the norm. Furthermore, Sample L absence of a batch number is a significant shortcoming and cause for concern.

Table 1: Description/characteristics of product samples analysed

Herbal preparations	Volume (ml)	Batch Number	Ingredients	Indications/Use	Manufacture date	Expiry date
A	200	EDMT 005	*Allium sativum 20%, Anogeissumleocarpus 20%, Vernonia amygdalina 15%, Eugenia aramatica 20%, Sorghum bicolor 25%*	It is used for Inhibition of platelet aggregation, Stomach cancer, Aphrodisiac, Bacterial infections, Fungal infections, Expel parasitic worms, Protozoal infections, Leishmaniasis, Prevent damage to the liver, Oxidative stress and other conditions.	Jan-20	Feb-23
B	200	22	*Cassia Alata, Citrus Medica Var Acida, Aloe Barbaris, Aloe Vera, Cassia Augustifolia*	It is trusted for ultimate, treatment of tummy problems, acute stomach ache, improves digestion, burn the fat, reduces pot belly, reduces excess weight, activate the flow of bile, prevent kidney and bladder infections, purifies blood normalize the operations of intestine, increases manpower, regulates blood sugar level and ease menstrual period.	May-19	May-24
C	200	018:5BHB	Purified water, Bark extracts of *Entandrophragma utile*, Bark extracts of *Anacardium occidentale*, Stem extracts of *Saccharum officinarum*	It has anti-microbial and blood (Red cell) stimulating properties. It may be used in chronic infections and anemic conditions. It has been found useful to the	Feb-22	Jan-25

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			Bark extracts of *Anacardium occidentale*	management of symptoms of combating such conditions as skin eruption, pimples, boils and minor form of acnes.		
D	200	17	*Vernonia amygdalina* 12%, *Saccharum Officinarum* 11.5%, *Allium sativum* 13%, *Cajanus cajan* 11.5%, *Zingiber Officinale* 0.5%, Caramel 1.5%, Water q.s	It eliminates internal heat, rumble stomach, indigestion of food, soften hard stool, reduces stomach sores and prevents piles. It purifies blood.	Mar-21	Feb-24
E	200	318	*Carica Papaya* 10.6%, *Citrus Aurantifolia* 10.5%, *Cajanus Cajan* 9.0%, *Saccharum Officinarum* 9.0%, Water q.s	It is used for the management of Chicken pox, Measles, and piles. It can also be used for Bedsores, Wound healing, Skin irritations, Jaundice and other conditions.	Mar-21	Mar-24
F	50	9	NA	It is used for Stomach Pain, Man Power, Menstruation Cure Rheumatism, Pile	Oct-2021	Oct-2023
G	100	2	Alcohol 30%, Water, Caramel, Herbal Extracts (*Angelica Archangelica*. *Cassia Cinnamomum* ,Rhizome)	It works to boost sexual performance in men	Feb-21	NA
H	200	2	Treated water, Ethanol, Sativum, *Zinigiber, Officinalis* Eugenia	It works for stamina, Its boost sexual performance, It is used for maximum sexual pleasure and It works for menstrual Cramps.	Dec-21	Nov-23
I	200	B1-2415	Herb Extracts (*Symphonia Globulifera, Garcina Kola, Tetrapleura Tetraptera, LanneaWelwitschii*), Demineralised water, Ethyl Alcohol, Colours E150(a) and brandy flavor	As energy booster which helps in increasing the libido and provides antioxidants that aids the digestive system of the body fight flu and common cold. This bitters aids in boosting the immune system and also help in the relief of stress.	Oct-21	NA
J	100	NA	Natural honey, US Moringa oil seed and leaf, popularly leaf	It is used for all kinds of pile, weak erection, Back Pain, Stomach Problem	Jan-21	Dec-23

K	200	701	*Cajanus cajan* leaves11.84%, *Citrus Aurantifolia* leaves 10.53%, *Saccharum officinarum* leaves 11.84%, *Carica papaya leaves*13.16%, Water 52.63%	It gives quick relief from Chickenpox, measles, piles and other anus troubles, convulsion, prickly heat and feverish conditions, diarrhoea, dysentery.	Mar-20	Feb-23
L	125	NA	Ethanol 38%, Water, Caramel, Sugar, Citric Acid, *RaphisLour* herbal extract. Sapo herbal extracts and *khaya Granifoliola* extract herbal bitters.	It is used to energize the body system.	NA	NA
M	100	B1-4103L	Water, Alcohol 40%, Caramel, Herbal flavor extracts, Angelic Root, *Cassia senna* leaf, Rhuherb root and Aloe.	It is used as an aphrodisiac to treat erectile dysfunction problems like weak erection, low libido, premature ejaculation, impotence, low sex drive etc..	NA	NA
N	100	7	Root &Leat of aloe 15%, Caramel Herbal Flavour Extract 10%, Ivorensis (Khaya) 10%, Soma Leaf 8%, Rhuherbs Root 7%, Water qs.	It is a traditional herbal mixture used as an energy booster, blood booster, in waist pain, pile, menstrual disorder	Sep-21	Aug-23
O	100	1	*Magnifera Indica*, *Allium Satinum*, *Croton Penduliflorus*, Natural Flavour, Water, Ethyl Alcohol	It is used in the management of pile. It is also used in alleviating lower back pain, general body pain, stomach aches and associated conditions.	Dec-20	NA

NA: Not Available

Table 2: Phytochemical screening of the herbal products

Phytochemicals	**Methods**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**
Saponins	Frothing Test	+	-	-	+	+	++	-	-	++	-	-	-	++	-	-
Reducing Sugar	Fehling's Test	++	++	+	+++	+	+++	+++	++	+	+++	+	+++	+++	+++	++
Phenolic Compounds	Lead Acetete test	+++	+	++	+	+	-	+	++	+++	+	+	+	+	+	+++
Tannins	Ferric chloride	-	++	-	+	-	-	-	-	+	-	-	-	-	-	-
Anthraquinones	Born Tragger's test	-	+++	-	++	-	+++	-	-	-	++++	-	-	+	-	-
Triterpenoids	Burchard Liebermann	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	Salkowski test	-	-	-	+	+	++	++	+	+	+++	+	+	++	+++	+
Flavonoids	Shinoda test	-	+++	-	+++	-	+++	+++	+++	+++	+	+	++	+++	+	+

Cardiac glycosides	Keller Killiani Test	-	+	-	+++	-	+++	+	+	++	+++	+	+	+++	+++	-
Alkaloids	Mayer's test	-	-	-	-	+	+	+	-	-	+	-	-	-	+	-
	Dragendorf's test	+	-	+	+	+	+	+	-	+	+	-	-	-	+	-
	Wagner's test	+	+	+	+	+	+	+	-	+	+	+	-	-	+	-

(-) Not detected, (+) slightly detected, (++) moderately detected, (+++) heavily detected

As a result, it is imperative that product manufacturers conduct thorough quality checks and adhere to current good manufacturing practice before releasing their products to the public, and regulatory agencies must rigorously monitor approved products to ensure compliance with labeling regulations.

The majority of herbal products includes undisclosed ingredients or hazardous levels of both heavy and trace metals. When taken in safe amounts, the majority of trace metals are helpful to human health, but when consumed in excess, they are hazardous[15].Upon conducting a thorough metal analysis, it was determined that the herbal samples contained trace and heavy metals within the safe levels for human consumption as shown in Table 3. As a result, we can assert that consuming the herbal sample will be advantageous for the human body. When present in appropriate amounts, trace metals such as sodium and potassium play a vital role in maintaining human health. Zinc is a crucial element that supports the immune system's ability to defend against harmful foreign germs and viruses. Furthermore, the body requires zinc for the production of proteins and the synthesis of DNA, the genetic material found in every cell. In cases where there is a shortage of iron, zinc supplementation may be recommended along with iron replacement. The most crucial physiological function of nickel, an essential micronutrient, is its participation in the metabolism of urea and urease. Plants need the enzyme urease to assimilate urea, and when Ni levels are low, it becomes less active. Heavy metal cadmium is recognized to have no physiological effect on humans or plants. Lead is a well-known heavy metal that, when exposed, has toxic consequences rather than any physiological or pharmacological significance. The body needs iron, an important element, in order to produce blood. Haemoglobin, a component of red blood cells that contain around 70% of the body's iron, is necessary for carrying oxygen from the lungs to the tissues in the blood. A trace metal that the human body needs is copper. In conjunction with iron, copper aids in the body's production of red blood cells by promoting iron absorption. Additionally, it promotes the health of the bones, neurons, immune system, and blood vessels. The presence of potentially harmful metals in herbal medicine may be attributed to contamination during processing/production, such as using metal equipment for grinding or other industrial utensils. Metal containers used for storage can also cause leaching of metals into the final product. [16, 17]However, this may not be the sole reason for the presence of metals in the herbal samples tested. According to Singh et al. (2011) and Prasad (2007), the varying capacities of herbal plants to absorb and accumulate harmful heavy metals from contaminated soil and metal deposits might also contribute to the variation in heavy metal concentrations observed across samples. [18, 19] A recent studies by Samali et al delved into the analysis of heavy metal concentration in Kano herbal preparations for major disease conditions. The findings revealed that of the six samples tested, 33% exceeded the permissible limit of 10 mg/kg set by the WHO for lead (Pb) concentration. Furthermore, all of the samples contained levels of Cadmium (Cd) that surpassed the permissible limit of 0.3 mg/kg.

The results of the antibacterial study indicated that Samples B, D, and K possess antibacterial activity against *Bacillus subtilis*. However, no zones of inhibition were observed for Samples G, H, L, and M, indicating a lack of antibacterial activity. While Samples A, C, E, F, I, K, N, and O suggested the potential for antibacterial use on their labels, however, the study did not provide sufficient evidence to support this claim as shown in table 1. This raises concerns regarding the accuracy and validity of the claims made on the labels of these commercially available products. Therefore, it is imperative for regulatory bodies to closely scrutinize the claims made by the manufacturers of these herbal medicines to ensure that they are truthful and reliable.

Table 3: Assay of trace and heavy metals of the herbal products

Herbal samples	**Cd**	**Pb**	**Zn**	**Fe**	**Mn**	**Cu**	**Ni**	**Na**	**K**
WHO/FAO Permissible Limit (mg/kg)	0.3	10	60	48	200	20	1.5	-	-
Concentation (µg/ml)									
A	N.D	0.0057±0.0005	0.0785±0.0035	0.019±0.003	0.006±0.002	0.1625±0.0575	N.D	0.7655±0.0405	0.9775±0.0425
B	0.0016±0.004	0.00005±0.00005	0.575±0.04	0.027±0.008	0.012±0.003	0.002±0.002	N.D	1.3053±0.2549	0.9838±0.0018
C	0.0800±0.0025	N.D	1.068±0.014	0.026±0.009	0.00785±0.00165	0.0185±0.0035	0.008±0.=0012	1.3195±0.3445	1.0741±0.0219
D	N.D	0.0058±0.0004	1.0135±0.0515	0.069±0.004	0.0081±0.0025	0.00645±0.00135	0.0056±0.0002	1.659±0.307	2.334±0.252
E	0.0020±0.002	0.011±0.004	1.0005±0.0255	0.0805±0.0045	0.028±0.004	0.0505±0.0345	0.0019±0.0001	0.2605±0.0545	0.3195±0.1005
F	0.0170±	0.0185±	1.0715±	1.0380±	0.0915±	0.1915±	0.0118±	1.4795±	1.5645±

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	0.0042	0.0050	0.0092	0.0226	0.0078	0.0191	0.0074	0.1775	0.0856
G	0.0153±	0.0031±	0.9135±	0.0670±	0.0255±	0.1210±	0.0017±	0.9135±	1.4405±
	0.0096	0.0043	0.0545	0.0028	0.0134	0.0212	0.0003	0.0870	0.1209
H	0.0093±	0.0027±	1.1395±	0.7050±	0.0305±	0.1595±	0.0115±	1.4440±	1.1685±
	0.0039	0.0016	0.1605	0.0665	0.0064	0.0106	0.0078	0.2942	0.2595
I	0.0019±	N.D	0.4195±	0.1805±	0.0405±	0.0186±	0.0035±	0.9610±	1.3080±
	0.0004		0.0926	0.0078	0.0064	0.0013	0.0050	0.0127	0.0622
J	0.0120±	0.0105±	0.9140±	0.6105±	0.0705±	0.2900±	N.D	1.0995±	1.3105±
	0.0042	0.0035	0.0537	0.7149	0.0078	0.0212		0.0912	0.0771
K	0.0046	0.0029±	0.9355±	0.4340±	0.0680±	0.2578±	0.0020±0.	1.0130±0.	1.1595
	± 0.008	0.007	0.0405	0.082	0.0040	0.0472	002	025	0.1055
L	0.0100±	0.0010±0.00	0.5090±0.	0.1875±0.	0.0900±0.00	0.2095±0.00	0.0485±0.	0.8900±0.	1.12±0.14
	0.002	1	083	0275	40	55	0385	064	5
M	N.D	0.0125±0.00	0.9630±0.	0.2800±0.	0.0245±0.00	0.0895±0.00	0.0025±0.	0.8750±0.	1.043±0.0
		35	043	026	75	45	0015	021	19
N	0.0065±	0.0185±0.00	0.8080±	0.2785±	0.0670±0.00	0.1065±0.00	0.0055±0.	1.2090±0.	1.192±0.0
	0.0015	25		0.0265	30	25	0015	055	14
O	0.0100±	0.0125±0.00	0.1110±0.	0.0910±0.	0.0570±0.00	0.0200±0.00	N.D	0.4070±0.	0.6585±0.
	0.01	35	043	006	30	5		055	0435

ND: Not detected, Cd: Cadmium, Pb: Lead, Zn: Zinc, Fe: Iron, Mn: Manganese, Cu: Copper, Ni: Nickel, Na: Sodium, K: Potassium.

Table 4: Antibacterial activity of the Ciprofloxacin standard

Organisms	**20 µg/mL**	**10 µg/mL**	**5 µg/mL**	**2.5 µg/mL**
	ZONE OF	**INHIBITION**	**(mm)**	
Staphylococcus aureus	30.83 ± 0.29	28.33 ± 0.29	24.17 ± 0.29	21.75 ± 0.35
Bacillus subtilis	28.50 ± 0.50	26.17 ± 0.29	23.17 ± 0.29	21.17 ± 0.28
Escherichia coli	35.17 ± 0.29	30.17 ± 0.29	23.50 ± 0.50	20.17 ± 0.29
Klebsiella pneumonia	29.17 ± 0.24	22.83 ± 0.24	19.17 ± 0.24	13.66 ± 0.24

Table 5: Antibacterial activity of the herbal products

	SAMPLE A (Zone of Inhibition mm)			
Concentration	***Staphylococcus aureus***	***Bacillus subtilis***	***Escherichia coli***	***Klebsiella pneumonia***
Solvent (water) 0%	0	0	0	0
25%	0	0	0	0
50%	0	0	0	20.33 ± 0.47
100%	0	0	0	0
	SAMPLE B (Zone of inhibition mm)			
25%	0	12.33 ± 0.47	0	0
50%	0	15.17 ± 0.24	0	0
100%	0	18.33 ± 0.47	0	0
	SAMPLE C (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0
	SAMPLE D (Zone of Inhibition mm)			
25%	0	15.33 ± 0.47	0	0
50%	0	18.33 ± 0.47	0	32.67 ± 0.47
100%	0	15.33 ± 0.47	0	0
	SAMPLE E (Zone of Inhibition mm)			
25%	0	0	0	0

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50%	0	0	0	0
100%	0	0	0	0
	SAMPLE F (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	15.17 ± 0.24	0	0
	SAMPLE G (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	13.17 ± 0.24	0	0
	SAMPLE H (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0
	SAMPLE I (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0
	SAMPLE J (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0
	SAMPLE K (Zone of Inhibition mm)			
25%	0	12.17 ± 0.24	0	0
50%	0	22.33 ± 0.47	17.50 ± 0.41	0
100%	0	15.33 ± 0.47	0	18.33 ± 0.47
	SAMPLE L (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0
	SAMPLE M (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0
	SAMPLE N (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0
	SAMPLE O (Zone of Inhibition mm)			
25%	0	0	0	0
50%	0	0	0	0
100%	0	0	0	0

Conclusion

The investigation found that the herbal products contain safe levels of trace and heavy metals. However, more research is needed to examine long-term effects, and regulatory bodies should increase efforts to protect consumers.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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