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EVALUATION OF HEAVY METAL, PHYTOCHEMICAL, AND PROXIMATE COMPOSITION OF SELECTED HERBAL DRUG SAMPLES USED FOR THE TREATMENT OF PROSTATE CANCER IN NIGERIA

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ABSTRACT

The high cost and potential side effects of conventional treatments have necessitated a growing interest in complementary and alternative medicine, particularly herbal remedies. This study was carried out to evaluate the heavy metal, phytochemical, and proximate compositions of two (2) selected herbal drug samples commonly used to treat prostate cancer in Anambra and Plateau states. Proximate analysis showed a high moisture content of 85.0-89.0%, indicating that the samples are susceptible to microbial spoilage and that proper storage is advised. The ash, fat, fibre and protein are 2.0%, 0.5%, 1.5%, and 1.0% respectively for both samples. Phytochemical screening confirmed the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, saponins, and tannins, which are known for their antioxidant and anticancer properties. Heavy metal analysis revealed Cd (1.80 ± 0.02 mg/Kg) and Mn (11.02 ± 0.05 mg/kg) in sample S002 to be above the WHO permissive limit. Hence, proper testing and assessment for heavy metals in medicinal products are advised, as they can pose serious health hazards.

Keywords: Heavy metal; Herbal Drugs; Phytochemical; Prostate Cancer; Proximate.

1.0. Introduction

Prostate cancer (PCa) is the second most common cancer among men worldwide, with incidence and mortality rates showing significant variation between and within countries (Kensler and Rebbeck, 2020), and has contributed approximately 7% of all new cancer cases in 2020, highlighting a substantial and persistent global burden (Sung et al., 2021). Most cases occur in men over 65 years of age (Reinand et al., 2024). PCa risk factors include age, obesity, ethnicity, inherited risk, and other environmental factors (Sekhoacha et al., 2022), and patients often experience a wide spectrum of physical and psychological symptoms, including depression, anxiety, stress, fatigue, pain, and other psycho-social challenges. Common complications such as erectile dysfunction, impotence, sexual dysfunction, and urinary incontinence further impair quality of life (Sousa et al., 2012). The treatment landscape for prostate cancer (PCa) has evolved significantly over the years, yet it remains fraught with limitations and challenges.

In Nigeria, constraints in the health-care system, uneven access to screening, and late presentation contribute to poor quality of care for clinically localized disease, despite a high cure rate with

appropriate medication (Tolani et al., 2024). The increasing population and the incidence of side effects of synthetic medicines also accelerate the popularity of alternative medicines (Sen & Chakraborty 2015). In recent years, there has been growing interest in herbal and alternative medicine as people seek natural, holistic solutions to maintain and improve their health (Kahkashan et al., 2023).

Certain herbs have shown the potential to induce apoptosis in prostate cancer cells, offering alternative therapeutic avenues (Meng et al., 2017). Herbal drug formulations present a promising adjunctive strategy for the treatment of prostate cancer, mainly due to their potential to mitigate side effects and enhance the therapeutic efficacy of conventional treatments. Existing studies indicate that certain herbal compounds possess anti-cancer properties that might inhibit tumor growth, sensitize cancer cells to chemotherapy, or reduce the adverse effects associated with conventional treatments (Poonthananiwatkul et al., 2015; Jermini et al., 2019). These herbal formulations have garnered attention due to their perceived lower toxicity compared to synthetic drugs and their historical usage in various cultures. This study aims to evaluate the heavy metal, phytochemical, and proximate composition of selected herbal drugs used in the treatment of prostate cancer

2.0. Material and method

Two (2) herbal formulations used in the treatment of prostate cancer were purchased from Barki Ladi, Jos, Plateau State, and Ogbo Ogwu, the main market in Onitsha, Anambra State, Nigeria, and their information is presented in Table 1.0 below.

Table 1.0: Product Information on the Herbal formulation used in prostate cancer treatment

Name	Ingredients	Batch no	Production date	Expiration date	Company	Sample Code
Kambest Prostate and urine infection	Avocado, vegetables, bell peppers, garlic, olive oil, fresh lemon juice, celery root, pumpkin seed	0001	20/01/2024	19/01/2028	Kambest Tradomedical service	S001
Dr. Alpha Prostate & Urinary Infection total cure	Treated water, Prunus africana, Annona muricana	DAPCO4	08/24	12/27	Dr. Alpha	S002

2.1. Determination of Proximate composition of drug samples.

The moisture, ash, crude fat, and crude protein contents were determined according to the methods described by the Association of Official Analytical Chemists (AOAC, 2012) and Nkwocha et al. (2022). The proximate composition of the seeds was determined in triplicate. The total carbohydrate content was calculated by the difference from 100%.

2.2. Determination of the Phytochemical Components of the Drug Samples

Alkaloids, glycosides, flavonoids, saponins, tannins, steroids, phenols, and Terpenoids were analyzed in herbal

extracts using the methods described below (Abu et al., 2023).

2.2.1. Test for saponins

A specific mass of 0.5 grams of the powder was added to 5 milliliters of distilled water in a test tube. The mixture was subjected to vigorous agitation, and a steady, persistent froth was observed. Subsequently, three drops of olive oil were introduced into the frothing solution, and the mixture was shaken vigorously once more. The resulting solution was then assessed for the presence of an emulsion.

2.2.2. Test for tannins

A 0.5 g mass of the powder was heated to a boil in 10 milliliters of water in a test tube, after which the mixture was filtered. Following this process, several drops of 0.1% ferric chloride solution were added, and the resultant solution was analyzed for the emergence of a brownish-green or blue-black hue.

2.2.3. Test for anthraquinones

A 0.5 g mass of the powder was heated in 10 mL of sulphuric acid (H_2SO_4) and subsequently filtered while maintaining elevated temperatures. The resulting filtrate was combined with 5 mL of chloroform. Afterward, the chloroform layer was carefully transferred to a separate test tube using a pipette. To this solution, 1 ml of dilute ammonia was added and the resulting colorimetric changes were systematically recorded.

2.2.4. Test for steroids

A specific mass of 0.5 g of the powder was solubilized in 10 milliliters of chloroform, to which an equal volume of concentrated sulphuric acid (H_2SO_4) was carefully added along the walls of the test tubes. The emergence of a reddish upper layer, accompanied by a yellowish layer of sulphuric acid that exhibited green fluorescence, served as a qualitative indicator of the presence of steroids.

2.2.5. Test for cardiac glycosides (Keller-Killiani Test)

To a 0.5 g sample of the powder dissolved in 5 mL of water, 2 mL of a glacial acetic acid solution containing a single drop of ferric chloride solution was added. Subsequently, this mixture was layered with 1 ml of concentrated sulphuric acid (H_2SO_4). The emergence of a brown ring at the interface will indicate the presence of deoxysugars, which are characteristic of cardenolides. Furthermore, a violet ring may also manifest beneath the brown ring. In contrast, a greenish ring may form in the acetic acid layer just above the brown ring, gradually diffusing throughout this layer.

2.2.6. Test for flavonoids

A 0.5 g mass was heated in 10 mL of ethyl acetate in a steam bath for 3 minutes. Subsequently, the mixture was filtered, and 4 ml of the filtrate obtained was mixed with 1 ml of a dilute ammonia solution. The presence of a yellow colour indicates the presence of flavonoids.

2.2.7. Test for alkaloids

A mass of 0.5 grams from each sample was dissolved individually in dilute hydrochloric acid and then filtered. The resulting filtrates were treated with Mayer's reagent (potassium mercuric iodide), and the appearance of a yellow precipitate indicated the presence of alkaloids. Additionally, the filtrates were treated with Dragendorff's reagent, a solution of potassium bismuth iodide, and the formation of a red precipitate indicated the presence of alkaloids. Furthermore, the filtrates were analysed using Hager's reagent, a saturated solution of picric acid, where the development of a yellow precipitate confirmed the presence of alkaloids.

2.3. Heavy Metal Analysis

The herbal samples were digested following the protocol established by Zigau et al. (2024). Specifically, a solution consisting of 10 ml of nitric acid (HNO_3), hydrochloric acid (HCl), and hydrogen peroxide (H2O2) was

added to 1 g of each homogenized powdered plant material within a borosilicate flask. This mixture will be heated to 120°C on a hot plate for 3 hours. Upon completion of the digestion, the resulting colourless and transparent solution was transferred to a 50 ml volumetric flask. Each borosilicate flask was thoroughly rinsed with distilled water to remove any residual material before being combined with distilled water in the volumetric flask. Before analysis, diluted samples were stored in 100 ml high-density polyethylene plastic bottles. Each herbal sample was analyzed in triplicate to ensure the accuracy and reliability of the results. Standard solutions were prepared from stock solutions to address potential matrix effects. Concentration standards for each metal were prepared using the same reagents and solvents used in sample preparation. The absorbances of lead (Pb), copper (Cu), mercury (Hg), aluminum (Al), nickel (Ni), chromium (Cr), manganese (Mn), zinc (Zn), cobalt (Co), and cadmium (Cd) were measured using an atomic absorption spectrometer (AAS).

2.4. Statistical Analysis

The results of the assay were expressed as an average value ± standard deviation of three replicates.

3.0. Result and Discussion

3.1.Proximate composition of sample S001 and S002

Proximate analysis measures the dietary components of herbal products, including moisture, crude protein, total fat, carbohydrates, dietary fiber, and minerals (Ezeagha et al., 2024). Moisture content is critical as it affects the shelf life and microbial stability of herbal samples (Ibeabuchi et al., 2023). Samples S001 and S002 had high moisture content, suggesting they are prone to microbial spoilage and require strict preservation measures (Nkwocha et al., 2022). The ash content, an indicator of total mineral content, is the same for both samples, implying they contain the same level of minerals. The presence of fat is essential for the absorption of fat-soluble vitamins (Tukur et al., 2023); however, the samples' low fat content indicates they are not a significant source of fat in the diet. The carbohydrate content, an important energy source, is significantly higher in S002 than in S001.

Table 1: Proximate composition of S001 and S002 (%)

Parameters	Moisture	Ash	Fat	Fibre	Protein	Carbohydrate
S001	89.00	2.00	0.50	1.50	1.00	6.00
S002	87.00	2.00	0.50	1.50	1.00	8.00

3.2 Phytochemical screening of sample S001 and S002

Table 2: Qualitative analysis of phytochemicals

Parameters	Alkaloids	Terpenoids	Flavonoids	Steriods	C. glycoside	Phenols	Saponins	Tannins
S001	+++	+++	+++	+++	+++	+++	+++	+++
S002	+++	+++	+++	+	+++	+	++	+

Keys: (+) slightly present, (++) present, (+++) are highly present.

Qualitative analysis is crucial for identifying the phytochemical constituents in medicinal plants (Priyanka, 2020). The phytochemical analyses of these samples have revealed the presence of phytochemicals, including alkaloids, flavonoids, terpenoids, glycosides, phenols, tannins,

steroids, and saponins (Table 2). The phytochemical content of the samples is indicated by the symbols +, ++, and +++, where + represents a low content, ++ represents a medium content, and +++ represents a high content. Examining Table 2, it was observed that phenol, steroid, and tannin were found to be high in sample S001 compared to S002. Plants containing high concentrations of alkaloids tend to inhibit angiogenesis, a key factor in tumor growth and spread. This presents a promising prospect for cancer treatment; therefore, it holds potential for future advancement in drug development and clinical application (Mondal et al., 2019). C. glycosides have demonstrated promising anticancer properties and stimulate immune responses that specifically target tumors; therefore, they present new opportunities for immunotherapy and drug repurposing (Schneider et al., 2017).

3.3 Heavy Metal Analysis of Samples S001 and S002

Table 3: Heavy metal analysis of Sample S001 and S002 (mg/kg)

Parameters	S001	S002	WHO permissible limit (mg/kg)
Pb	1.84 ± 0.02	0.06 ± 0.02	10
Cd	0.12 ± 0.02	1.80 ± 0.02	0.3
Cu	0.28 ± 0.01	11.75 ± 0.01	20
Mn	0.79 ± 0.02	11.02 ± 0.05	5
Hg	_	_	0.1
Ni	0.19 ± 0.01	1.42 ± 0.03	1.5
Co	0.48 ± 0.002	0.202 ± 0.01	0.48
Zn	1.65 ± 0.02	16.49 ± 0.002	50
Al	2.04 ± 0.05	2.04 ± 0.05	15
Cr	0.83 ± 0.002	0.64 ± 0.001	2

The results of the heavy metal analysis in Table 3 provide important information about the safety and quality of the herbal samples. All the metals found in sample S001 were within the allowed WHO limit expected for herbal medicine, while some of S002 were above the limit. Cadmium (Cd) contamination was detected in the herbal sample S002, with a concentration of 1.80 ± 0.02 mg/Kg which exceeds the threshold. Cadmium toxicity in plants hinders nutrient absorption, increases oxidative damage, disrupts metabolic processes, and impairs plant structure and function (Haider et al., 2021). However, these plants tend to have a defence mechanism and a remediation strategy to counteract these effects (Haider et al., 2021) High levels of these heavy metals, if ingested, could cause serious health issues, therefore, there is a need for close monitoring of potential heavy metal concentrations in herbal drugs to ensure that they are safe for consumption before being administered to patients from herbal stores.

4.0. Conclusions

The herbal drug samples contain essential phytochemicals that are required for antioxidant and anticancer activities and are important in the treatment of prostate cancer. However, monitoring and controlling heavy metal contamination in these herbal products to ensure that they are safe for consumption is very important.

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