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PHYSICOCHEMICAL ANALYSIS, HEAVY METAL ASSESSMENT, AND GC-MS PHYTOCHEMICAL PROFILING OF SELECTED HERBAL REMEDIES MARKETED FOR PROSTATE CANCER MANAGEMENT IN AWKA, ANAMBRA STATE, NIGERIA

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ABSTRACT

Herbal remedies are widely consumed in Nigeria as alternatives or complements to conventional treatments for prostate cancer, yet they are largely unregulated and lack standardized quality assurance. This study assessed the physicochemical parameters, heavy metal concentrations, proximate composition, and GC-MS phytochemical profiles of three commercially available herbal formulations, namely Onathec, Ganacin, and PROS-Q, marketed for prostate cancer management in Awka, Anambra State. Sample digestion was performed using aqua regia, followed by Atomic Absorption Spectrophotometry (AAS) for heavy metal quantification. Proximate analysis was conducted using standard AOAC methods, and GC-MS analysis was employed for phytochemical characterization. Heavy metal analysis revealed that cadmium (0.024–0.051 mg/kg), copper (0.044–0.130 mg/kg), nickel (0.109–0.149 mg/kg), and lead (≤0.033 mg/kg) were all within WHO/FAO permissible limits. Chromium was below detection limits in all samples. Proximate analysis showed high moisture content (95–99%), low carbohydrate (<1%), lipid (<0.1%), fiber (<1%), and moderate protein levels (~2.5%). GC-MS profiling identified fatty acid methyl esters (methyl oleate, methyl stearate, methyl linoleate) in Onathec and PROS-Q, while Ganacin demonstrated greater chemical diversity, including quinazolinone and heterocyclic derivatives. Notably, phthalate (dibutyl phthalate) and siloxane contaminants were detected in Onathec and Ganacin, respectively, raising concerns about packaging and manufacturing practices. The findings indicate these herbal preparations contain bioactive compounds of potential therapeutic relevance; however, the presence of synthetic contaminants necessitates stricter regulatory oversight and adherence to Good Manufacturing Practices (GMP) to ensure consumer safety.

Keywords: GC-MS; heavy metals; herbal remedies; physicochemical analysis; phytochemical profiling; prostate cancer.

1.0. Introduction

Cancer remains a leading cause of morbidity and mortality globally, with projections estimating over 21 million new cases by 2030 (Tiware et al., 2023). Prostate cancer is among the most prevalent malignancies affecting men worldwide, and its burden is rapidly rising in sub-Saharan Africa, including Nigeria. The limited access to conventional treatment modalities such as surgery, radiotherapy, and chemotherapy, owing to high costs, side effects, or inadequate healthcare infrastructure, has led many Nigerian patients to depend on herbal remedies as

affordable and readily available alternatives (Muhammad et al., 2022).

Herbal medicines have been used for centuries to manage diverse conditions and are estimated to provide primary healthcare to over 80% of the global population in developing countries (WHO, 2005). Several plant-derived bioactive compounds have been reported to exhibit anticancer properties through mechanisms such as induction of apoptosis, cell cycle arrest, inhibition of angiogenesis, and modulation of signaling pathways involved in tumor progression and metastasis (Adriana et al., 2021; Kure et al., 2019).

Despite their widespread use, herbal remedies marketed in Nigeria are largely unregulated and frequently sold without scientific validation of safety or efficacy. Herbal preparations are vulnerable to contamination by toxic heavy metals, including lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni), originating from soil, water, processing equipment, or packaging materials (Liu et al., 2018). The presence of synthetic adulterants, including phthalates and siloxanes, introduced through plastic packaging or industrial solvents, further jeopardizes product safety. Chronic exposure to these contaminants poses significant risks, particularly in cancer patients who may already have compromised physiological systems.

Physicochemical analysis and quality profiling of herbal medicines are essential for establishing safety benchmarks, authenticating composition, and guiding regulatory intervention (WHO, 2015). Despite the widespread consumption of herbal remedies for prostate cancer management in Awka and surrounding communities, scientific data on their heavy metal content, proximate composition, and phytochemical constituents remain scarce.

This study, therefore, aimed to bridge this knowledge gap by conducting a comprehensive physicochemical analysis, heavy metal assessment, proximate composition analysis, and GC-MS phytochemical profiling of three selected herbal formulations marketed for prostate cancer management in Awka, Anambra State, Nigeria.

2.0. Materials and Methods

2.1. Sample Collection

Three commercially available herbal preparations marketed for prostate cancer treatment were obtained from local pharmaceutical outlets and traditional medicine vendors in Awka, Anambra State. The selected formulations were: (i) Ganacin Herbal Super Cure, a polyherbal formulation marketed as an anticancer remedy; (ii) Onathec Herbal Mixture, a traditional herbal blend claimed to possess anticancer properties; and (iii) PROS-Q, an herbal preparation specifically formulated for prostate-related conditions. All samples were purchased in original packaging with batch numbers, manufacturing dates, and expiration dates recorded. Samples were stored at room temperature, protected from light and moisture, until analysis.

2.2. Sample Preparation and Digestion

Sample digestion was performed using the aqua regia method (Association of Official Analytical Chemists (AOAC), 2016). A precisely measured volume (10 mL) of each herbal sample was transferred to a 100 mL beaker and treated with freshly prepared aqua regia (3:1 ratio, HCl: HNO_3) at a sample-to-aqua regia volumetric ratio of 4:1 (i.e., 10 mL herbal sample to 2.5 mL aqua regia). The mixture was reacted at room temperature for 30 minutes before controlled heating at 80–90°C on a hot plate (under fume hood) until a clear, colorless solution was obtained. The digested solution was cooled, transferred quantitatively to a 50 mL volumetric flask, made to volume with distilled water, and stored in amber glass containers at 4°C pending analysis.

2.3. Heavy Metal Analysis

Heavy metal concentrations were determined using Atomic Absorption Spectrophotometry (AAS) with element-specific hollow cathode lamps. Standard calibration curves were prepared from certified reference standards for cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb). All measurements were performed in triplicate, and blank determinations were included. Results were compared against the WHO/FAO permissible limits for herbal medicines.

2.4. Solvent Extraction for Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

A 200 mL aliquot of each herbal sample was subjected to liquid-liquid extraction using 20 mL n-hexane in a separating funnel, as described by Luque de Castro and Priego-Capote (2010). The biphasic mixture was vigorously shaken for 5 minutes and then allowed to settle for 30 minutes. The hexane layer was separated and concentrated under reduced pressure using a rotary evaporator at 40°C. Concentrated extracts were stored in amber vials under nitrogen at −20°C until analysis.

2.5. GC-MS Analysis

GC-MS analysis was performed according to the method described by Adams (2017), by injecting 1 µL of the concentrated hexane extract in splitless mode into a capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with helium carrier gas at 1.0 mL/min. Oven temperature was programmed from 60°C (held 2 min) to 280°C at 10°C/min, held for 10 min. The mass spectrometer was operated in electron ionization mode (70 eV) with scan range m/z 50–500. Compound identification was achieved by comparison with NIST and Wiley spectral libraries. Results were expressed as the percentage total ion current.

2.6 Proximate Analysis

The liquid herbal samples were first evaporated to dryness on a water bath and ground into a fine powder. Proximate analysis was conducted following the standard methods of the Association of Official Analytical Chemists (AOAC, 2019). Moisture content was determined gravimetrically by drying 2.0 g at 105°C to constant weight (AOAC Method 925.10). Total ash was determined by incineration at 550°C in a muffle furnace (AOAC Method 942.05). Acid-insoluble ash was obtained after boiling the ash in 2 M HCl and re-incineration (AOAC Method 900.02). Crude protein was determined by the Kjeldahl method using a conversion factor of 6.25 (AOAC Method 984.13). Crude fat was determined by Soxhlet extraction with petroleum ether (40–60°C) for 6–8 hours (AOAC Method 920.39). Crude fiber was determined by sequential acid (1.25% H_2SO_4) and alkali (1.25% NaOH) digestion, followed by filtration, washing, drying, and incineration of the residue; crude fiber content was calculated as the difference between the dry weight of the residue before and after ashing (AOAC Method 978.10). Carbohydrate content was determined colorimetrically using the phenol-sulfuric acid method at 490 nm against glucose standards (Dubois et al., 2017).

3.0. Results and Discussion

3.1. Heavy Metal Concentrations

The concentrations of five heavy metals (Cd, Cr, Cu, Ni, and Pb) are presented in Table 1 alongside WHO/FAO permissible limits.

Table 1: Heavy Metal Concentrations (mg/kg) in Herbal Drug Formulations

Metal	Onathec	Ganacin	PROS-Q	WHO/FAO Limit
Cd (mg/kg)	0.051	0.22	0.024	0.3
Cr (mg/kg)	<0.001	<0.001	<0.001	2.0
Cu (mg/kg)	0.094	0.044	0.130	10.0
Ni (mg/kg)	0.149	0.109	0.145	1.5
Pb (mg/kg)	0.005	0.033	<0.001	10.0

Source: WHO (2019); FAO/WHO Codex Alimentarius (2020)

All five herbal formulations returned heavy metal concentrations below WHO/FAO permissible limits. Cadmium ranged from 0.024 mg/kg (PROS-Q) to 0.051 mg/kg (Onathec), well below the 0.3 mg/kg limit, suggesting minimal cadmium pollution in the cultivation or processing environments. Chromium was below the detection limit (<0.001 mg/kg) across all samples, indicating the absence of chromium contamination from industrial or anthropogenic sources such as tannery effluents and mining waste. Copper concentrations ranged from 0.044 mg/kg (Ganacin) to 0.130 mg/kg (PROS-Q), all substantially below the 10.0 mg/kg limit; copper is an essential trace element, and these levels pose negligible risk. Nickel ranged from 0.109 to 0.149 mg/kg across samples, well within the 1.5 mg/kg threshold. Lead was detected only in Ganacin (0.033 mg/kg) and Onathec (0.005 mg/kg); PROS-Q showed undetectable levels.

A direct comparison of the three formulations shows that PROS-Q had the highest copper concentration but the lowest cadmium and lead burden, while Ganacin recorded the highest detectable lead level among the three products. Although Ganacin's lead concentration is well below the WHO limit of 10.0 mg/kg, any detectable lead warrants monitoring, given its known cumulative neurotoxicity and the lack of a safe exposure threshold. These results compare favourably with reports from studies on herbal preparations in other Nigerian states (Liu et al., 2018), confirming that the sampled products do not pose acute heavy metal toxicity risks under normal use patterns.

3.2. Proximate Composition

The proximate composition results are presented in Table 2 below. All three formulations exhibited very high moisture content (95.51–98.84%), typical of liquid herbal preparations; the WHO ≤14% moisture standard applies specifically to dried plant materials and is therefore not directly applicable (WHO, 2007). Protein content was relatively higher in Ganacin (2.56%) and PROS-Q (2.55%) than in Onathec (1.64%), possibly reflecting differences in plant material composition.

Carbohydrate (<1%) and lipid (<0.1%) contents were uniformly low across all samples, consistent with the therapeutic rather than nutritional intent of these preparations. Ash content, a proxy for total mineral matter, ranged from 0.10% (Onathec) to 0.19% (Ganacin), all below the WHO maximum of 14%. Crude fiber ranged from 0.18% (Ganacin) to 0.98%

(PROS-Q), with PROS-Q showing the highest value, suggesting a relatively higher content of structural plant polysaccharides.

A comparison of the three formulations reveals that Ganacin and PROS-Q are broadly similar in protein and carbohydrate composition, whereas Onathec differs most markedly in its lower protein content and higher moisture content. These differences may reflect variations in botanical ingredients and processing conditions among the three products. The overall proximate data confirm that the therapeutic benefits of these formulations are attributable to phytochemical constituents rather than macronutrient content, consistent with WHO quality guidelines for liquid herbal preparations (WHO, 2007).

Table 2: Proximate Composition (%) of Herbal Preparations

Parameter	Onathec	Ganacin	PROS-Q	WHO Standard
Moisture Content (%)	98.84	95.57	95.51	≤14% (dried)
Protein (%)	1.64	2.56	2.55	-
Carbohydrate (%)	0.64	0.76	0.70	-
Lipid (%)	0.07	0.06	0.08	-
Ash Content (%)	0.10	0.19	0.18	≤14%
Crude Fiber (%)	0.68	0.18	0.98	-

Source: WHO Herbal Pharmacopoeia (2007)

3.3. GC-MS Phytochemical Profiling

The GC-MS analysis of hexane extracts from all three formulations identified 14 distinct compounds across the three samples. The identity, percentage total ion current (% TIC), and distribution of each compound are presented in Table 3. A comparative overview of the phytochemical profiles reveals both shared and unique constituents across the three herbal formulations, with notable implications for their claimed therapeutic activities and safety profiles.

Table 3: GC-MS Phytochemical Compounds Identified in Herbal Formulations

S/N	Compound	Class	Onathec (% TIC)	Ganacin (% TIC)	PROS-Q (% TIC)
1	Methyl oleate	Fatty acid methyl ester	28.4	ND	31.2
2	Methyl stearate	Fatty acid methyl ester	19.7	ND	22.5
3	Methyl linoleate	Fatty acid methyl ester	23.1	ND	18.6
4	Dibutyl phthalate	Phthalate plasticizer (contaminant)	12.6	ND	3.8
5	Quinazolinone derivative	Heterocyclic alkaloid	ND	18.3	ND
6	Diphenylketone (benzophenone)	Aromatic ketone	ND	14.9	ND
7	Nitro-aromatic compound	Aromatic nitro derivative	ND	11.2	ND

8	Acetaminophen-related derivative	Analgesic derivative	ND	9.4	ND
9	Siloxane (decamethylcyclopentasiloxane)	Organosilicon contaminant	ND	7.8	ND

ND = Not Detected. Values represent percentage of total ion current (% TIC) from GC-MS analysis of hexane extracts.

A comparative analysis of the three formulations reveals distinct phytochemical profiles that reflect differences in their botanical composition and manufacturing processes (Table 3). Onathec and PROS-Q share a fatty acid methyl ester-dominated profile, characterized by methyl oleate, methyl stearate, and methyl linoleate, compounds that have been reported to exhibit antiproliferative and anti-inflammatory activities in cancer cell lines (Fabian et al., 2015). In contrast, Ganacin presents a more chemically diverse profile, dominated by heterocyclic and aromatic structures, including quinazolinone and diphenylketone derivatives, which are absent from the other two formulations.

This compositional divergence suggests that Ganacin may act via distinct molecular mechanisms compared with Onathec and PROS-Q. Importantly, all three formulations contained contaminant compounds: dibutyl phthalate in Onathec (12.6% TIC) and at trace levels in PROS-Q (3.8% TIC), and siloxane exclusively in Ganacin (7.8% TIC). These contaminants were absent from their respective counterpart formulations, underscoring variability in manufacturing quality among products with similar therapeutic uses. Taken together, the comparative phytochemical data suggest that no single formulation is superior in all respects; Onathec and PROS-Q are relatively cleaner in terms of contaminant load and share beneficial fatty acid constituents, while Ganacin offers greater chemical diversity but carries a higher risk of contamination.

GC-MS analysis of the hexane extracts identified nine distinct compounds across the three formulations; their identities, chemical classifications, and percentage total ion current (% TIC) values are presented in Table 4.

Table 4: GC-MS Phytochemical Compounds Identified in Herbal Formulations Marketed for Prostate Cancer Management

S/N	Compound	Chemical Class	RT (min)	Onathec (% TIC)	Ganacin (% TIC)	PROS-Q (% TIC)
1	Methyl oleate	Fatty acid methyl ester (FAME)	18.42	28.4	ND	31.2
2	Methyl stearate	Fatty acid methyl ester (FAME)	20.17	19.7	ND	22.5
3	Methyl linoleate	Fatty acid methyl ester (FAME)	19.85	23.1	ND	18.6
4	Dibutyl phthalate	Phthalate plasticizer (contaminant)	22.56	12.6	ND	3.8
5	Quinazolinone derivative	Heterocyclic alkaloid	24.03	ND	18.3	ND
6	Diphenylketone (benzophenone)	Aromatic ketone	21.78	ND	14.9	ND

S/N	Compound	Chemical Class	RT (min)	Onathec (% TIC)	Ganacin (% TIC)	PROS-Q (% TIC)
7	Nitro-aromatic compound	Aromatic nitro derivative	23.44	ND	11.2	ND
8	Acetaminophen-related derivative	Analgesic aromatic derivative	16.91	ND	9.4	ND
9	Siloxane (decamethylcyclopentasiloxane)	Organosilicon contaminant	12.35	ND	7.8	ND

ND = Not Detected. Values represent percentage of total ion current (% TIC) from GC-MS analysis of n-hexane extracts. Shaded rows (pink) denote synthetic contaminants not expected in plant-derived herbal material. RT = retention time (approximate). % TIC values are rounded to one decimal place.

As shown in Table 4, the three herbal formulations exhibited markedly different phytochemical compositions, suggesting differences in their botanical sources, extraction conditions, and manufacturing processes. Onathec and PROS-Q shared a fatty acid methyl ester (FAME)-dominated profile, together accounting for 71.2% and 72.3% of total detected compounds, respectively, while Ganacin was characterized exclusively by aromatic, heterocyclic, and organosilicon compounds, with no FAME constituents detected.

The three FAMEs detected in Onathec, methyl oleate (28.4% TIC), methyl linoleate (23.1% TIC), and methyl stearate (19.7% TIC), collectively accounted for 71.2% of the total ion current, establishing this formulation as predominantly lipid-derived in its extractable fraction. Methyl oleate, the methyl ester of oleic acid (C18:1, omega-9), and methyl linoleate, the methyl ester of linoleic acid (C18:2, omega-6), have been reported to exert antiproliferative and pro-apoptotic effects in androgen-sensitive prostate cancer cell lines, with their activity attributed in part to modulation of the arachidonic acid cascade and inhibition of cyclooxygenase-2 (COX-2)-mediated inflammatory signalling relevant to prostate tumour progression (Fabian et al., 2015; Pender-Cudlip et al., 2017). Methyl stearate, though a saturated fatty acid ester, has been associated with antioxidant activity in herbal matrices. Of significant concern, however, is the detection of dibutyl phthalate (DBP) at a TIC of 12.6% in Onathec. DBP is a synthetic plasticizer with no natural occurrence in plant materials; its presence strongly implicates leaching from plastic packaging or contamination during processing. As an established endocrine-disrupting compound, DBP competitively inhibits androgen receptor signaling and has been shown to promote proliferation of hormone-sensitive cancer cells in vitro (Hauser and Calafat, 2016). Given the androgen-dependent pathophysiology of prostate cancer, chronic ingestion of a formulation containing DBP at detectable levels raises serious safety concerns that require urgent regulatory attention.

PROS-Q exhibited a phytochemical profile broadly similar to Onathec, with the same three FAMEs comprising 72.3% of total detected compounds: methyl oleate (31.2% TIC), methyl stearate (22.5% TIC), and methyl linoleate (18.6% TIC). The marginally higher FAME abundance in PROS-Q relative to Onathec suggests a comparatively richer lipophilic phytochemical fraction, which may be attributed to differences in raw material composition or extraction efficiency. Importantly, while dibutyl phthalate was also detected in PROS-Q, its concentration (3.8% TIC) was substantially lower than in Onathec (12.6% TIC), suggesting a lesser

but non-negligible contamination burden. The shared FAME profile between Onathec and PROS-Q, particularly the co-occurrence of omega-9 and omega-6 fatty acid esters, is consistent with preclinical evidence indicating that unsaturated fatty acid derivatives inhibit prostate cancer cell proliferation by suppressing lipid peroxidation and modulating inflammatory mediators (Pender-Cudlip et al., 2017). Nonetheless, the therapeutic relevance of these compounds in the context of the liquid formulation matrix and their bioavailability following oral ingestion remains to be established through pharmacokinetic studies.

Ganacin presented the most chemically complex and compositionally distinct profile among the three formulations, with all detected compounds belonging to aromatic, heterocyclic, or organosilicon chemical classes and no FAME constituents identified. The dominant compound was a quinazolinone derivative (18.3% TIC), followed by diphenylketone or benzophenone (14.9% TIC), a nitro-aromatic compound (11.2% TIC), an acetaminophen-related derivative (9.4% TIC), and decamethylcyclopentasiloxane (7.8% TIC). Quinazolinone scaffolds have attracted considerable attention as anticancer pharmacophores; preclinical studies have demonstrated that quinazolinone derivatives induce G1/S-phase cell-cycle arrest and caspase-dependent apoptosis in prostate cancer cell lines through inhibition of the epidermal growth factor receptor (EGFR) and phosphoinositide 3-kinase (PI3K)/Akt signaling pathways (Akbari et al., 2018).

The detection of a benzophenone derivative is also noteworthy, as several naturally occurring and synthetic benzophenones have been reported to exhibit cytotoxic activity against human cancer cell lines via topoisomerase inhibition (Fotie & Bohle, 2006). The nitro-aromatic compound and acetaminophen-related derivative detected at lower abundances raise questions regarding the botanical authenticity of Ganacin and may indicate the presence of synthetic pharmaceutical adulterants, a concern previously documented for polyherbal anticancer remedies marketed in West Africa. Of particular regulatory concern is the detection of decamethylcyclopentasiloxane (D5 siloxane) at a TIC of 7.8%. Cyclic siloxanes are industrial chemicals used in personal care product manufacturing and as processing solvents (Genualdi et al., 2011); their presence in an orally consumed herbal formulation indicates cross-contamination during manufacture and is inconsistent with Good Manufacturing Practice (GMP) standards. D5 siloxane has been classified as a substance of very high concern (SVHC) under REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), the European Union's primary chemicals regulatory framework, due to its persistence and potential endocrine-disrupting properties (ECHA, 2018).

4.0. Conclusion

The GC-MS data clearly show differences in the phytochemical makeup of the three formulations, affecting both their therapeutic potential and safety. Onathec and PROS-Q mainly derive their bioactivity from structurally similar FAME components, while Ganacin contains a wider variety of potentially active heterocyclic structures. However, all three contain contaminants, DBP in Onathec and PROS-Q, and siloxane in Ganacin, that pose safety risks, especially for vulnerable groups like cancer patients. The absence of these contaminants in the other formulations points to inconsistent manufacturing quality among products intended for the same treatment and emphasizes the urgent need for strict GMP regulation and routine chemical testing of herbal medicines in Nigeria.

4.1. Recommendations

Based on the findings of this study, the following recommendations are made:

(i) Regulatory Authorities: NAFDAC should strengthen surveillance and enforce mandatory registration, labeling, and quality control testing for all herbal products marketed in Nigeria.

(ii) Manufacturers: Adopt GMP and use food-grade or glass packaging to reduce phthalate and siloxane contamination. Source raw materials from certified, heavy metal-free environments.

(iii) Researchers: Extend studies to cover a broader range of herbal products; conduct in vitro and in vivo toxicological evaluations; develop GC-MS fingerprint databases to facilitate authenticity verification.

(iv) Consumers: Purchase only NAFDAC-registered herbal products, seek medical advice before combining herbal and conventional therapies, and report adverse events.

Conflict of Interest

The authors declare no conflict of interest.

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