



Physicochemical, Proximate, Heavy Metals and Antimicrobial Assessment of Few Selected Herbal Medicinal Products Consumed in Awka, Anambra State, Southeastern, Nigeria

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Abstract. Traditional medicine remains the principal approach to healthcare in the third world, but knowledge about preparation methods, toxicological effects, and side effects is lacking. This study evaluates the quality of local herbal medicines in Awka, Anambra State, Southeastern, Nigeria by assessing the proximate composition, physicochemical, heavy metals, and antimicrobial efficacy of a few selected herbal medicines. The physicochemical analysis of herbal drugs revealed high acidity, except for the herbal medicine called 'Evacuation'. The evaluated Herbal medicines are rich in alkaloids, flavonoids, tannins, phenol, and saponins but poor in protein, resin, steroid, and terpenoid. The concentrations of iron, chromium, and lead exceeded WHO/FAO limits. The Herbal medicines have lower zones of inhibition as compared to the 99 % efficacy inscription on the label. In the case of proximate composition, the herbal medicine 'Super 7' contains higher protein, fat, and ash content, while 'Deep root' has the highest fiber, ADC has the highest moisture, and Evacuation has the highest carbohydrate. Therefore, from a toxicological point of view, some of these herbal medicines may not be safe for consumption and they may pose health risks ranging from kidney failure, gastrointestinal disorder, dysentery, and cancer of the vital organs of the consumers due to the presence of toxic metals, high acidity, and imbalance in concentrations of the bioactive constituents.

Keywords: Herbal medicine, physicochemical, phytochemicals, proximate compositions

1. Introduction

The growing population of sub-Saharan Africa and other developing civilizations depends mostly on medicinal herbs for healthcare and treatment of ailments. This stems from the fact that most herbs available in these regions present potential healing ability, hence they are being explored by natives for medicinal purposes. For instance, in Nigeria, about 1000 species (approximately 12 %) of the plant's diversity have been reported to show medicinal potential and are used by marginal communities to cure various diseases (Latif et al., 2004; Shinwari and Shoukat, 2003). History has proven that man in the consumption of herbs as a source of medicine and food dated up to 60,000 years ago in most cases resulting in side effects such as vomiting, diarrhea, coma, or even death (Shi et al., 2010; Fabricant & Farnsworth 2001; Goa et al., 2007). However, in this way, edible food and medically active herbs were discovered in the early days. The use of medicinal herbs by locals in today's civilization has

received a geometric increase (Jia and Zhang, 2005), and when not put under control may result in adverse health challenges due to side effects, composition imbalance, toxicity, and the presence of harmful heavy metals causing damage to vital organs like the liver and kidneys. In some growing economies, attempts are being made in other to bridge the gap between traditional medicine and the modern healthcare system to curb the impending disaster posed by traditional medicines. For example, India and China over the years have successfully transformed their traditional medicine into modern pharmacology leading to the discovery of new drugs for the treatment of ailments (Sivakrishnan and Pharm, 2018). In countries like Nigeria and other African economies, the use of traditional medicine constitutes a major part of healthcare due to the inability of individuals to access modern-day medicine and the high cost of modern medical healthcare, hence people consume traditional medications as an alternative way to stay alive. Abdullahi in 2021 described the role of culture, beliefs, and religion in the perspective of traditional medication and healing, which further recognized Nigerian regionality in the kinds of herbal medications being offered to locals within these regions.

The rising demand for herbal medicine has significantly driven the rapid growth of the herbal industry (Shinwari and Shoukat, 2003; Shinwari et al., 2006). Since most herbal remedies are consumed orally, it is essential to evaluate the nutritional content and proximate composition of the raw materials used (Kochhar, 2006; Pandey, 2006; Taiga, 2008). The World Health Organization (WHO) has also emphasized the importance of standardizing herbal drugs by analyzing their nutrient and micronutrient composition to ensure they meet quality standards (Niranjan and Kanaki, 2008; Ojokoh, 2008). Herbal formulations are typically derived from plant parts such as roots, stems, leaves, and pods (Abdullahi, 2021), which are rich in bioactive compounds like alkaloids, terpenes, flavonoids, and saponins that contribute to their therapeutic effects (Afolabi and Afolabi, 2013; Motaleb et al., 2011). These bioactive substances are responsible for the antimicrobial, pharmacological, and anti-inflammatory properties of medicinal herbs (Motaleb et al., 2011).

The bioactive components of herbal medicines have therapeutic efficacy and are referred to as active ingredients. If sufficient analytical techniques are available, the production of herbal medications whose active constituents have been identified should be standardized to contain a predetermined quantity of bioactive substances. Although the active compounds in herbal medicines are derived from plant parts such as leaves, roots, or flowers (Hertog et al., 1993), the term "natural" does not guarantee safety, as herbal medicines can still have harmful effects on the body. A good number of bioactive constituents have been analyzed in herbal medicines including flavonoids (Kuhnau, 2016; Cesarone et al., 1992) exhibiting anti-inflammatory, anti-hepatotoxic, and anti-ulcer activities (Robak et al., 2008), Alkaloids (Babalola, 2009), Phenolics, (Puupponen-Pimiä et al., 2008; Nwokocha and Peter 2011), Glycosides (Kar, 2007), Saponins (Batan, et al., 2006), (Griebel et al., 1995; Harborne, 2013), and Steroids (Haristoy et al., 2015).

In response to the persistent threat of diseases caused by pathogenic organisms, various antimicrobial agents—such as antibiotics derived from microorganisms, complex organisms, and medicinal plants—have been developed as therapeutic interventions (Alarcon et al., 1994). However, growing scientific concern surrounds the presence of contaminants and phytotoxicity in herbal medicines, often resulting from improper botanical identification, unsafe phytotherapeutic combinations, and the presence of bioactive compounds at toxic

levels. Therefore, this study aims to scientifically evaluate the proximate composition, physicochemical characteristics, phytochemical constituents, heavy metal contamination, and antimicrobial efficacy of selected herbal medicinal products commonly consumed in Awka, Anambra State, Nigeria.

2. Materials and Methods

2.1. Collection of Herbal Samples

A total number of six (6) herbal products namely: Ghana herbal drug, Super 7, Deep root herbal, Asheetu, ADC, and Evacuation herbal medicine were purchased from different hawkers in Awka metropolis. Some of the products had batch numbers, date of production, expiry dates and National Agency for Food, Drug Administration and Control (NAFDAC) registration numbers on their product labels. None of the products will expire before the conduct of the analysis. All the herbal drugs were stored in a refrigerator (28 – 30 °C) before its use.

2.2. Preparation of the Herbal Product Samples

Before analysis, the herbal medicines were transferred into clean and sterilized test bottles where their physical attributes like color, and turbidity were observed. Some initial investigations, such as the filtration of herbal medicines, were carried out to eliminate any impurities or suspended particles that may have resulted from the traditional methods of preparation.

2.3. Proximate Composition

- **Moisture Content:** Moisture content was determined by drying 2 g of the sample in a petri dish at 100°C for 2 hours until constant weight was achieved (AOAC, 2010).
- **Ash Content:** Ash content was determined by heating 2 g of sample in a platinum crucible at 500°C for 3 hours (AOAC, 2010).
- **Fiber Content:** Fiber content was determined by boiling 2 g of sample with H₂SO₄ and NaOH solutions, filtering, drying, and incinerating the residue (AOAC, 2010).
- **Protein Content:** Protein content was determined by Kjeldahl method, followed by titration with hydrochloric acid (AOAC, 2010).
- **Fat Content:** Fat content was determined by extracting 5 g of dried sample using hexane in a Soxhlet apparatus for 4-6 hours (AOAC, 2010).
- **Carbohydrate Content:** Carbohydrate content was calculated as 100 minus the sum of moisture, protein, ash, fat, and fiber percentages (AOAC, 2010).

2.4. Elemental Analysis of Heavy Metals

Heavy metal analysis was conducted by digesting samples with HNO₃, HCl, and H₂O₂, followed by analysis using Atomic Absorption Spectrophotometer (PG 990 AAS) (WHO, 2007).

2.5. pH and Temperature Determination

The pH of samples was measured using a digital pH meter, and the temperature was measured using a mercury thermometer (WJEUIP, model PHS-25).

2.6. Preliminary Phytochemical Screening

Phytochemical screening was conducted to detect bio-active compounds, including alkaloids, steroids, flavonoids, saponins, and others, following standard AOAC methods (2010).

2.7. Antimicrobial Activity Determination

Antimicrobial activity was tested using the Agar well diffusion method, with plant extracts reconstituted to 10 mg/ml and tested in different concentrations. Zones of inhibition were recorded after incubation at 37°C for 24 hours.

3. Result and Discussion

3.1. Proximate analysis of herbal products

Table 1. Proximate composition of herbal drugs consumed in Awka Anambra State

Herbal drug	Moisture (%)	Ash (%)	Fiber (%)	Fats (%)	Protein (%)	Carbohydrates. (%)
Evacuation	57.55 ^d ± 1.96	3.17 ^d ± 1.23	7.75 ^b ± 0.91	0.37 ^c ± 1.57	0.70 ^d ± 0.99	30.51 ^a ± 2.17
Ghana herbal	57.60 ^c ± 0.88	3.66 ^c ± 2.58	7.53 ^c ± 1.50	0.35 ^d ± 1.47	0.70 ^d ± 0.89	30.14 ^b ± 1.11
Deep root	59.00 ^b ± 1.28	5.00 ^b ± 2.27	8.70 ^a ± 1.00	0.39 ^b ± 1.79	0.75 ^c ± 1.17	26.14 ^e ± 1.24
Asheetu	59.00 ^b ± 1.28	5.00 ^b ± 1.49	5.50 ^d ± 0.50	0.36 ^c ± 0.10	1.10 ^b ± 0.88	29.27 ^c ± 0.83
ADC	59.95 ^a ± 1.30	5.10 ^a ± 2.58	5.33 ^e ± 0.00	0.37 ^c ± 0.70	1.11 ^b ± 1.17	28.48 ^d ± 1.28
Super 7	53.31 ^e ± 1.34	5.18 ^a ± 1.05	3.60 ^f ± 1.10	0.65 ^a ± 2.10	1.19 ^a ± 1.02	26.10 ^e ± 1.23

Keys: Mean values ± Standard deviation of triplicate ($p < 0.05$).

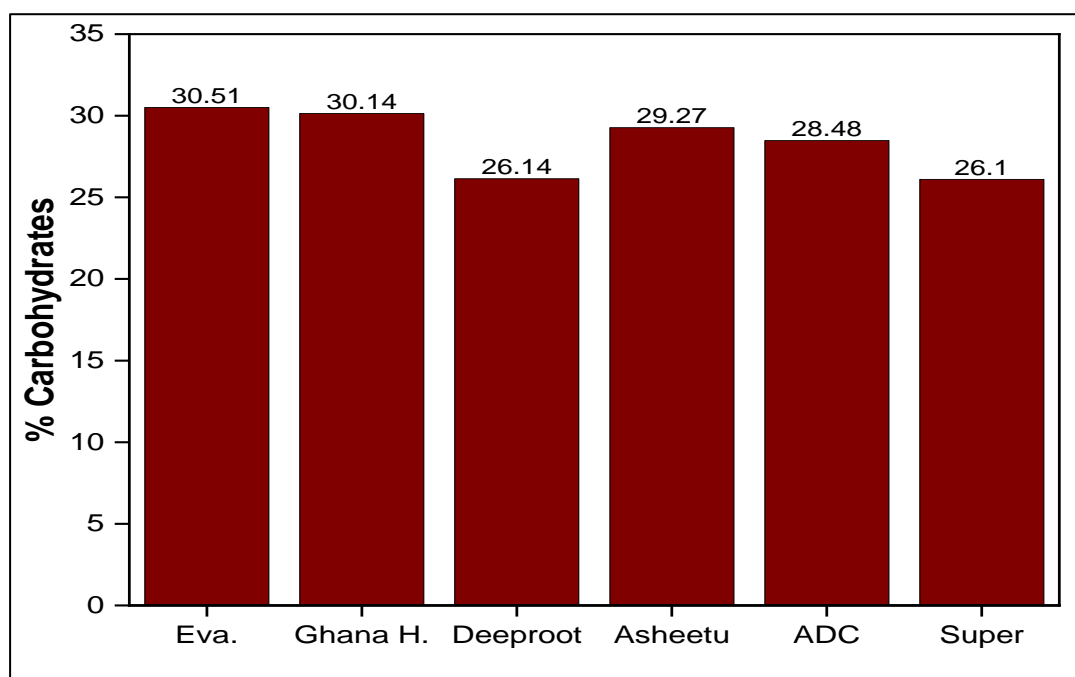


Figure 1. Percentage carbohydrate of the herbal medicines

Table 1 presents the results of the proximate analysis of the seven herbal medicines investigated within Awka metropolis in Anambra State, South eastern, Nigeria. Looking at the result, the herbal medicine called Super 7 presents high levels of protein (1.19%), fat (0.65%), and ash (5.18%) contents compared to the other products investigated in this research.

This result falls short compared to the values reported by Michael et al., (2023) for unprocessed medicinal plants like *Allium sativum*, *Vernonia amygdalina*, *Garcinia kola*, *Curcuma longa*, *Zingiber officinale*. The medicinal herb called Deeproot has the highest fiber (8.60%) while ADC has the highest moisture (59.95%). Finally, Evacuation has the highest carbohydrate (30.51%). In overall comparison, the Super 7 has the highest concentrations in fat, ash, and protein as compared to the other five herbal drugs used in the study. The high moisture content of this medicinal herb can impact the shelf life of the product in that it can facilitate activities of micro-organisms that are not inhibited by the products to either initiate fermentation as the carbohydrate level is appreciably high or even destroy the potency of the herbal medicine. With low fiber, fats, proteins, and even minerals as observed by the ash content, these products are of very limited value in terms of nutritional values and perhaps short of recommendations by WHO and even UNESCO. The percentage carbohydrate of each herbal medicine is presented in **Figure 1** showing Evacuation, Ghana herbal, and Asheetu to be the first, second, and third highest in carbohydrate content respectively even though these values are low compared to the 76 and 72 % reported by Michael et al., (2023) for unprocessed bitter cola and turmeric respectively.

3.2. Physical analysis

Table 2. Physical analysis of some of the herbal drug consumed in Awka, Anambra State

Herbal drug	pH	Temperature (°C)
Evacuation	10.30	28.00
Ghana herbal	5.33	30.00
Deproot	4.50	28.00
Asheetu	5.00	29.00
ADC	4.10	30.00
Super 7	4.00	28.00

The result of the physical parameters of the herbal medicine consumed in Awka, Anambra State is presented in **Table 2** showing the normal temperatures and pH of each of the medicinal herbs. All the analyzed herbal medicines had almost constant room temperatures of 28 - 30 °C which is normal for aqueous solutions at room temperature. The herbal medicines had pH values between the ranges of 4.10 - 10.30. The pH of the various herbal drugs shows the alkalinity and acidity of the herbal drugs with a pH of 1.0 - 6.9 considered acidic and a pH of 7.1 - 14 considered alkaline. The pH values obtained from the herbal medicine under investigation are either almost alkaline or almost acidic falling short of neutrality and thus when ingested may either increase the acidic content of the stomach causing gastrointestinal and immune system complications or even increase the alkalinity of the stomach. The result from the physiochemical analysis of the herbal drugs sold in Awka showed that the herbal medicines analyzed were acidic except for the Evacuation sample which was highly basic.

For optimal operation, all body fluids, organs, and cells require a particular pH. Since enzymes take on specific forms based on the pH of the medium they are in, they are extremely sensitive to acidity levels and will not work at their optimal levels unless they are in a medium that has a specified pH. When these extremely acidic herbal remedies are taken orally, the direct result is an increase in stomach acidity over what is required for metabolism, which

prevents enzymes from working. Additionally, the body has buffer systems to neutralize acids that build up; but, when these systems become overwhelmed, the body turns to alkaline minerals found in bones and essential organs to aid in the neutralization and removal of the acidic substances. Over time, this process may weaken bones and other organs, leading to osteoporosis in the latter situation. Additional side effects that have been documented by scientific research include foul-smelling, dark urine, poor digestion, exhaustion, soreness in the muscles and joints, excessive sweating, migraines, and bad breath (Shi et al., 2010; Goa et al., 2007).

3.3. Phytochemical Analysis

The results of the phytochemical analysis of all traditional medicinal herbs are presented in **Table 3** which shows the presence of phenols, alkaloids, and tannins in all samples while oil resins and proteins were all undetected qualitatively.

Table 3. Qualitative phytochemical constituents of herbal drugs hawked in Awka

Phytochemicals	Evacuation	Super 7	Ghana	Deproot	Asheetu	ADC
Saponin	+++	++	+++	-	+	++
Flavonoid	-	-	+++	-	-	+++
Alkaloid	++	++	+	+++	+++	+++
Tannin	+	+++	++	++	+++	+++
Steroids	-	-	+	-	++	+
Terpenoids	++	++	-	-	+	+
Glycosides	+	+	-	-	-	-
Carbohydrates	+	+	+	-	-	-
Protein	-	-	-	-	-	-
Anthrocynin	+++	+++	-	-	+	-
Phenol	+++	+++	+	+++	+++	+
Oil and resin	-	-	-	-	-	-
Reducing sugar	-	+	-	-	+	-

Keys: +++ = Present in high concentration, ++ = Present in moderate concentration
- = Not detected

The results of the phytochemical screening and quantitative estimation of the percentage crude yields of chemical constituents of the six (6) herbal drugs under study indicate that they are low in protein, resin, steroid, and terpenoid and high in alkaloids, flavonoids, tannins, phenol, and saponins, which may be the basis for their physiological and medicinal activity, as suggested by Sofowara (2014). The absence of oil/resin and protein in all six drug samples in the present study is in contrast with the opinion of Gill (1992) who noted that protein is one of the active constituents in herbal medicines. Steroids and flavonoids were also reported by Sofowara (2014) in two herbal medicines called Dr Aladin and Bitters which is the reason behind their efficacy compared to other herbal medicines as steroidal compounds are of hormonal importance in terms of sex hormones. Hence, Okwu (2001) reported that Dr. Aladin and Bitters are used by expectant mothers or breastfeeding

mothers to ensure hormonal balance since steroidal structure could serve as potent starting material in the synthesis of these hormones. The low steroid content in the understudied herbal medicines also disapproved of its potential hormonal capabilities.

3.4. Heavy Metal Content

Table 4. Mean heavy metal concentrations (ppm) in herbal drugs hawked in Awka

Samples	Cadmium	Chromium	Iron	Lead	Nickel	Arsenic
Evacuation	ND	0.84	77.80	2.89	ND	ND
Ghana harbal	ND	1.12	88.70	3.30	ND	ND
Deproot	ND	ND	92.80	ND	3.80	ND
Asheetu	ND	ND	113.00	6.90	ND	ND
ADC	ND	1.17	137.00	2.80	1.12	ND
Super 7	ND	0.69	227.00	7.50	1.88	ND

Keys: ND = Not Detected

The Mean Heavy Metal Concentrations in ppm for the Herbal drugs hawked in Awka are shown in **Table 4**. The results revealed that the herbal medicine called Super 7 has the highest concentrations of Fe, Pb, and Ni; Ghana herbal has the highest concentrations of Cr while Cadmium and Arsenic remain undetected in all herbal medicines. However, the levels of these concentrations were below the WHO recommendations for these metals. The mean concentration of Chromium in all the herbal medicine samples exceeded the WHO/FAO [2017] standard limit of 5.00 mg/Kg. Several papers have reported a lower concentration of chromium in herbal medicines which is below the standard limit of the WHO/FAO. All the herbal medicines exceeded the WHO/FAO permissible limits of 48.00 mg/ Kg for iron although below the Food and Nutrition Board (FNB, 2001) of the Institute of Medicine (IM) recommended dietary allowance of 7.00 - 10.00 mg/day for children, 8 mg/day for adults and 27 mg/day during pregnancy for mothers. Lead concentration in the herbal medicines ranged from 6.44 ± 0.07 mg/kg - 25.10 ± 0.13 mg/Kg (**Table 4**) and exceeded the Permissible limit of lead in herbal medicines 10.00 mg/kg.

3.5. Antimicrobial

Table 5. Mean Zones of inhibition (mm) of herbal drugs on some selected organisms

EXTRACT	<i>S. typhi</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>Aspergillus spp</i>	<i>Bacillus spp</i>
Evacuation	0.00± 0.00	15.17± 0.00	10.00± 0.00	0.00± 0.00	0.00± 0.00	13.87± 0.77
Ghana herbal	0.00± 0.00	11.30± 0.00	8.53± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00
Deproot	0.00± 0.00	9.00± 0.00	8.40± 0.00	0.00± 0.00	7.80± 0.00	18.03± 0.05
Asheetu	0.00± 0.00	7.20± 0.00	7.57± 0.00	0.00± 0.00	5.30± 0.00	14.37± 0.10
ADC	0.00± 0.00	0.00± 0.00	7.00± 0.00	0.00± 0.00	5.00± 0.00	10.51± 0.20
Super 7	0.00± 0.00	0.00± 0.00	4.67± 0.00	0.00± 0.00	4.00± 0.00	8.03± 0.50
CONTROL	34.83± 1.00	19.1± 1.15	22.33± 0.57	32.27± 1.36	12.00± 0.00	38.93± 1.00

The antimicrobial screening test demonstrated distinct inhibition zones around discs containing three concentrations of both herbal extracts and commercial antibiotics, which were tested on the two isolates. These inhibition zones provide clear evidence of the antimicrobial properties of the herbal extracts, with the mean values presented in **Table 5**.

Additionally, the Minimum Inhibitory Concentration (MIC) of the extracts was determined from the test results and is shown in **Table 6**, indicating the lowest concentration that effectively inhibits organism growth based on the inhibition zones. These antimicrobial activities in herbal medicines can be attributed to the presence of phytoconstituents which are potential antibacterial, antifungal, and antiviral agents. Several research has reported microbial activities in unprocessed extracts from plant parts ranging from the root's leaves, stems, and pods. Looking at the fact that most herbal medicines are extracts from these plant parts, it is no surprise that they could inhibit the growth of microbial colonies.

Table 6. Minimum Inhibitory Concentrations (MIC) of the herbal extracts on selected organisms

Microorganisms	Herbal extract	Antibiotic
<i>S. typhi</i>	9.50	1.30
<i>E. coli</i>	2.90	0.30
<i>S. aureus</i>	0.30	1.00
<i>B. subtilis</i>	0.50	1.00
<i>C. albicans</i>	5.00	10.00
<i>A. niger</i>	10.20	0.30

The claim on the label of the herbal medicine investigated in this research suggested that the products have antimicrobial activities with 99.9% efficacy which may not be completely true as suggested by the result of this investigation. The results show that the herbal products have lower zones of inhibition. In the Awka study, a higher concentration of the product (1g/ml) was required to observe a mild antibacterial effect. While bacterial isolates such as *Shigella spp.*, *Enterobacter spp.*, *Escherichia coli*, *Staphylococcus spp.*, and *Klebsiella spp.* showed sensitivity to amoxicillin and gentamicin, the current study found a significant resistance to amoxicillin. This resistance may stem from the overuse of amoxicillin in the area, creating selective pressure that favors the growth of resistant strains while inhibiting susceptible ones.

Conclusion

The study analyzed six herbal medicinal products hawked in Awka, Anambra State, South eastern Nigeria, revealing high acidic pH levels beyond WHO guidelines. The products contain phytochemicals like Alkaloids, Tannins, Terpenoids, Flavonoids, Reducing Sugars, Cardiac glycosides, and Saponins, which have therapeutic functions. Heavy metal analysis revealed Chromium, Iron, Lead, Nickel, and Arsenic levels exceeding WHO limits, but not Cadmium and Iron levels. According to the current investigation, the herbal medications sold in the city of Awka have less antibacterial activity when compared to the information on the medication's containers and labels. Because these herbal medicines were found to be toxic due to the presence of heavy metals, some of them may not be safe to consume and may even pose health risks to consumers. As a result, toxicological research and quality improvement may be necessary before these herbal medicines can be considered safe for consumption.

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Conflicts of Interest

The authors declare no conflict of interest.

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