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Phytochemical Composition And Adulteration Of Antimalarial Herbal Preparations Marketed In Enugu, South East Nigeria.

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Abstract
Background: *Herbal preparation is finished, labeled medicinal product that contains active ingredients from aerial or underground parts of plant or other plant materials or combinations thereof. Herbal products are locally available. However, it has been reported by researchers that some of the manufacturers of herbal products may add natural organic or synthetic active ingredients which may or may not be of plant origin. Antimalarial herbal preparations could be adulteration.*
Aim: *The aims of this study were to investigate possible adulteration and to evaluate the phytochemicals that might be responsible for the antimalarial effects of the herbal preparations marketed in Enugu, South East Nigeria.*
Methodology: *Ten most patronized antimalarial preparations marketed in Enugu Urban were identified. Qualitative and quantitative analyses of the phytochemicals were evaluated using standard methods. All analyses were carried out in triplicates. The Solomon-Saker's test was used to investigate if the herbal medicines were adulterated with chloroquine or similar compounds. Data generated were analysed using descriptive statistics with IBM SPSS computer software version 28. Results were presented as mean ±SE.*
Results: *The phytochemicals identified include reducing sugar, saponins, tannins, flavonoids, tepernoids, proteins, alkaloids, carbohydrate, steroids and glycosides. The mean percentage of the phytochemicals ranged from 0.34% of alkaloids to 13.33% of reducing sugar. Eighty percent of the herbal preparations tested positive to chloroquine and/or compounds with chemical properties similar to chloroquine.*
Conclusion: *The results of this study showed that the products had phytochemicals that may justify the claims being made by the manufacturers. However, the addition of chloroquine to the preparations was massive and this may cause adverse effects to the patients and antagonistic effects against the antimalarial phytochemicals.*
Recommendation: *Other herbal products might be similarly tainted; hence, it is recommended that adulteration of other herbal non-antimalarial preparations should be investigated.*
Keywords: *Phytochemicals, Adulteration, Chloroquine, Solomon-saker, Antimalarial*

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I. Introduction

From the time of creation of man, herbs and herbal remedies have been used in treatment of different types of diseases. The history of herbal preparations cannot be separated from prehistoric times till the introduction of the germ theory of disease in the 19th century [1]. Herbal remedies, which is regarded as a subset of traditional medicine is the use of extracts medicinal plants for treatment of disease [2]. The herbs are used for the manufacturing of raw or refined therapeutic concoctions otherwise known as herbal medicines or herbal preparations. Recently, human's way of life is getting techno-savvy, thereby moving away from nature. However, man cannot avoid nature because he is not just made from nature but he is also part of it. These herbal preparations are natural products which are comparatively safe, eco-friendly and locally available. The herbal products are said to be epitome of safety when compared to the synthetic drugs that are regarded as unsafe to human due to their adverse effects [3]. Traditional medicine is becoming indispensible in alleviating health challenges caused by malaria in Nigeria as they are affordable, culturally acceptable treatment option and have demonstrable efficacy against malaria [4].

Today, according to the World Health Organization (WHO), 80% of the human population depends on herbal preparations for their primary healthcare needs [5]. These practices have assumed an unexpected dimension as a result of gradual shift from allopathic to traditional medicines [3]. This is linked to recent awareness that indigenous herbal plants could have potential therapeutic effects in the management of more than one disease including malaria (Shu et al., 2019). However, World Health Organization (WHO) confirmed that between 2015 and 2017 no noticeable progress in reducing global malaria cases was made [6]. Though, this could be due to the fact that there are different species of malaria parasites, plasmodium with different sensitivities to the remedies. Generally, there are four different species of *Plasmodium* that cause malaria in humans: Plasmodium falciparum, Plasmodium vivax, *Plasmodium ovale* and Plasmodium malariae. *Plasmodium falciparum* is responsible for most of the mortality and morbidity cases of malaria disease [7]. These led scientists to focus their searchlight towards discovery of antimalarial drugs. The end result of this search is the discovery of antimalarial drugs like chloroquine, halofantrine, primaquine, atovaquone-proguanil, mefloquine, lumefantrine and artemisinin. The most effective orthodox antimalarial drug is artemisinin which is a natural alkaloid extracted from the sweet wormwood (*Artemisia annua)* plant. However, many individuals in the remote rural communities have little or no access to orthodox medical services partly because of poverty, illiteracy or complete absence of health facilities within their communities [3]. Nigeria faces highest malaria incidence globally and this led to the advocacy for increased funding for scientific investigations into the efficacy of herbal remedies [4]. Even though a lot of patients derive health improvement from these herbal preparations, some are still in doubt and most times seek advice from health professionals before administration of these herbal remedies. This means there could be health risks associated with the consumption of these herbal preparations for researchers have also shown that many local herbal preparations are contaminated with bacteria, fungi, heavy metals and other intentional addictives [3].

Again, these herbal preparations contain bioactive molecules produced by the plants for their own benefits. These molecules are called phytochemicals or phytonutrients interchangeably. Typical examples include Tannins, alkanloids, flavonoids, anthocyanins phytosterols, carotenoids, saponins, dietary fibers isoprenoids and polysaccharides [8]. These phytochemicals can be consumed for their antioxidant activities and exhibition of antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities [9]. Despite the presence of these phytochemicals, there have been some evidences that the antimalarial herbal preparations are tainted globally. Adulteration of herbal preparations has increased globally [10]. Sifumaet et al., (2022) uncovered that synthetic phosphodiesterase type 5 inhibitors such as sildenafil, tadalafil, and vardenafil are used to adulterate herbal medicines used for treatment of erectile dysfunction in Nairobi Kenya. In addition, there have been reports of intentional unlawful addition of synthetic substances to herbal preparations used to manage weight-loss and weight gain, aphrodisiacs, treat rheumatic and inflammatory diseases, antidiabetic medications, and antihypertensive preparations [11]. Herbal preparations tested in Ghana for the presence of chloroquine showed that two out of five were adulterated with chloroquine or metabolites of chloroquine [12]. It appears that the adulteration of herbal preparations occur across various categories of herbal medicines [12].

Adulteration of HPs with allopathic medicines should be of concern to global health organizations. And the herbal remedies marketed in Enugu, Enugu State, Nigeria have not been investigated for adulteration. Hence, this work was designed to investigate the possible adulteration of the antimalarial herbal preparations marketed in Enugu state, South East, Nigeria. The qualitative and quantitative analyses of phytochemicals in the herbal preparations were also conducted to ascertain the phytonutrients that could be responsible for their antimalarial actions.

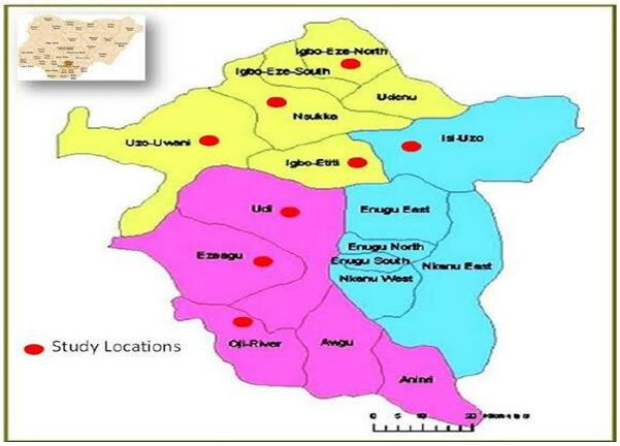

Fig 1 Map of Enugu State of Nigeria where the research was carried out

Enugu Urban includes Enugu North, Enugu South, Enugu East and parts of Nkanu west and Nkanu East Local Government Areas.

II. Materials And Methods

Identification of Antimalarial Herbal Preparations

Ten most patronized antimalarial herbal preparations marketed in Enugu Urban were identified using questionnaires answered by the major distributors of these herbal preparations.

Evaluation of the Adulteration of the Herbal Preparations with Chloroquine

The Solomon-Saker's test [13] was conducted on each of the selected samples to find out those that were adulterated with chloroquine or related compounds. 1 ml nitrogen phosphate buffer and 0.2 ml tetrabromophenol phtaline solution were mixed in a screw-cap conical centrifuge tubes. Then, 2 ml of herbal preparations was added to the mixture and the tubes were subsequently shaken vigorously for about 15 seconds and left to stand for about 15 min. Same was done to the positive control tubes which contain chloroquine at a concentrations of 2 µg/ml (low concentration control) and 4 µg/ml (high concentration control) respectively. A drug-free tube containing distilled water instead of herbal preparations was used as negative control. Yellowish-green colour of the organic layer indicated a negative test for chloroquine and/or its metabolites while red to purple colour of the organic layer indicated positive result with the shade of colour determined by the concentration of chloroquine and its metabolites present.

Qualitative and Quantitative Phyto-chemical Analysis of the Herbal Preparation

Phyto-chemical analysis was based on standard method outlined by Harbourne [14], and Trease and Evans [15].

Statistical analysis

Data analyses were done with SPSS Version 20 and some of the results presented in means.

III. Results

Table 1 shows the result of the Phytochemical analyses of the herbal preparations. All the phytochemicals tested were present except resins which were not found in any of the preparations. Flavonoids, tannins, carbohydrates, reducing sugar, alkaloids and terpenoids were found in 100% of the samples. Saponins were found in 90% of the samples, Steroids were tested positive in 70% of the samples while proteins and glycosides were found in 60% of the samples.

Table 1: Qualitative Analyses of Phyto-chemical Composition of the Herbal Preparations

S/N	Parameters	Sample ID (coded from their brand names)									
		BG	GS	EB	DR	BC	MF	AI	HB	GR	CH
1	Tannins	+	+	+++	++	+	+	++	+	++	++
2	Resin	-	-	-	-	-	-	-	-	-	-
3	Proteins	+	+	-	++	-	+	-	++	++	-
4	Flavonoid	+	++	+	+	+	+	+	+	+	++
5	Carbohydrate	+	+	+	+	+	+	+	+	+	+
6	Reducing Sugar	++	++	++	++	++	++	+++	+++	++	+++
7	Saponins	++	++	-	+	+	+++	+	++	++	++
8	Alkaloids	+	+	++	+	++	+	+	+	+	++
9	Glycosides	-	+	+	+	+	-	+	-	-	+
10	Steroids	+	-	-	+	+	+	+	+	-	+
11	Terpenoids	+	+	+	+	+	+	+	+	+	+
12	Ph	Acidic	Acidic	Acidic	Acidic	Acidic	Acidic	Acidic	Acidic	Acidic	Acidic

Note: + = slightly present, ++ = moderately present
+++ = abundantly present - = absent

Table 2 shows the results of the quantitative Analyses of Phyto-chemical Composition of the Herbal Preparations. The results which were presented in percentages showed that the phytochemicals were found in varying amounts in different herbal preparation. Tannins ranged from 7.27% in sample DR to 14.64% in sample GS with mean of 9.58%. Proteins ranged from 4.81% in sample BG to 7.81% in sample HB with mean of 6.8%. Flavonoids ranged from 0.4% in sample GR to 2.4% in sample GS with mean of 1.24%. Carbohydrates ranged from 3.34% in sample GR to 7.61% in sample AI with mean of 5.4%. Reducing sugar ranged from 11.2% in sample BC to 16.2% in sample GS with mean of 13.33%. Saponins ranged from 1.75%% in sample DR to 5.24% in sample MF with mean of 3.19%. Alkaloids ranged from 0.2% in samples AI and GR to 0.5% in

samples DR, EB and CH with mean of 0.34%. Glycosides ranged from 0.4% in samples GS, EB and BC to 0.6% in sample CH with mean of 0.52%. Steroids ranged from 0.3% in sample DR to 0.71% in sample CH with mean of 0.52%. Terpenoids ranged from 1.23% in sample BG to 2.95% in sample BC with mean of 1.9% while pH ranged from 5.7 in BG to 6.9 in sample GS with a mean of pH 6.26

Table 2: Quantitative Analyses of Phyto-chemical Composition of the Herbal Preparations

	Parameters	Sample ID										
		BG	GS	EB	DR	BC	MF	AI	HB	GR	CH	Mean($\bar{x}$) ±SE
1	Tannins (%)	10.88	14.64	9.35	7.27	9.77	7.31	9.60	7.63	9.66	9.72	9.58 ±0.62
2	Resin (%)	-	-	-	-	-	-	-	-	-	-	0.00 ±0.00
3	Proteins (%)	4.81	6.95	0.00	7.22	0.00	6.24	0.00	7.81	7.78	0.00	6.80 ±0.22
4	Flavonoid (%)	1.32	2.40	1.07	0.50	0.64	0.59	0.51	0.50	0.40	2.30	1.24 ±0.16
5	Carbohydrate (%)	4.90	4.62	6.13	5.67	5.16	7.33	7.61	3.40	3.34	5.79	5.40 ±0.38
6	Reducing Sugar (%)	11.47	16.20	11.42	13.43	11.20	12.56	15.25	14.60	11.28	15.88	13.33 ±0.51
7	Saponins (%)	2.20	2.55	0.00	1.75	2.00	5.24	3.52	3.66	4.52	3.25	3.19 ±0.25
8	Alkaloids (%)	0.30	0.30	0.50	0.50	0.30	0.30	0.20	0.30	0.20	0.50	0.34 ±0.01
9	Glycosides (%)	0.00	0.40	0.40	0.50	0.40	0.00	0.55	0.00	0.00	0.60	0.48 ±0.01
10	Steroids (%)	0.50	0.00	0.00	0.30	0.40	0.50	0.61	0.65	0.00	0.71	0.52 ±0.01
11	Terpenoids (%)	1.23	2.45	1.27	2.50	2.95	1.54	1.48	0.67	2.44	2.50	1.90 ±0.21
12	PH	5.7	6.1	6.3	6.5	6.4	5.9	6.0	6.4	5.9	6.1	6.26 ±0.00

The Mean Percentage of the Tested Phytochemicals are as shown in Fig 2 below. It shows that reducing sugars have the highest occurrence in these Herbal Preparations with 13.33% while Alkaloids and resins composition of these herbal preparation were 0.34% and 0.00% respectively. In between were tannins, protein, flavonoids, carbohydrates, saponins, glycosides, steroids and terpenoids had 9.58%, 6,80%, 1.24%, 5.4%, 3.19%, 0.48%, 0.52%, and 1.90% respectvely. The mean pH is 6.26

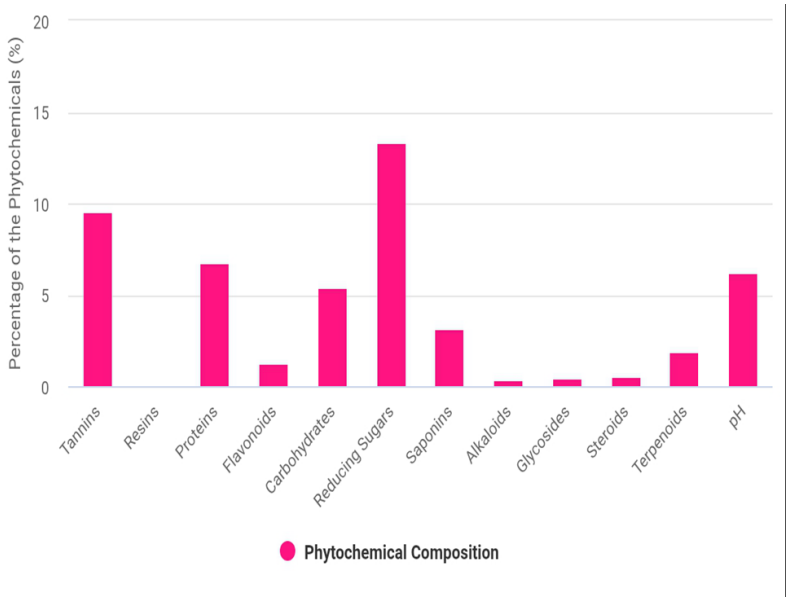

Fig 2: Percentage Presentation of the Mean of Phytochemical Composition of the Herbal Preparations

Table 3: The observations made in the process of testing for the presence of Chloroquine and its metabolites in the Herbal Preparations are as shown in the Table 3 bellow. It shows that nine (80%) out of the ten tested Antimalarial Herbal preparations was adulterated.

SAMPLES	COLOUR OBSERVED	INFERENCE	RESULT
BG	Purple colour	Chloroquine present	Adulterated
GS	Purple colour	Chloroquine present	Adulterated
EB	Purple colour	Chloroquine present	Adulterated
DR	Purple colour	Chloroquine present	Adulterated

BC	Purple colour	Chloroquine present	Adulterated
MFK	Yellow colour	Chloroquine absent	Unadulterated
AI	Purple colour	Chloroquine present	Adulterated
HB	Purple colour	Chloroquine present	Adulterated
GR	Yellow colour	Chloroquine absent	Unadulterated
CHU	Purple colour	Chloroquine present	Adulterated
Chloroquine 4 µg/ml*	Red colour	Chloroquine present	Chloroquine present
Chloroquine 2 µg/ml**	Purple colour	Chloroquine present	Chloroquine present
Distilled water***	Yellow colour	Chloroquine absent	Chloroquine absent

* = Positive control (high concentration) ** =Positive control (low concentration) *** = Negative control

Figure 3 below is a bar chart showing the percentage representation of the Adulteration of the herbal medicines. It showed that greater percentage of the herbal preparations was adulterated (80%) while only 20 % was unadulterated

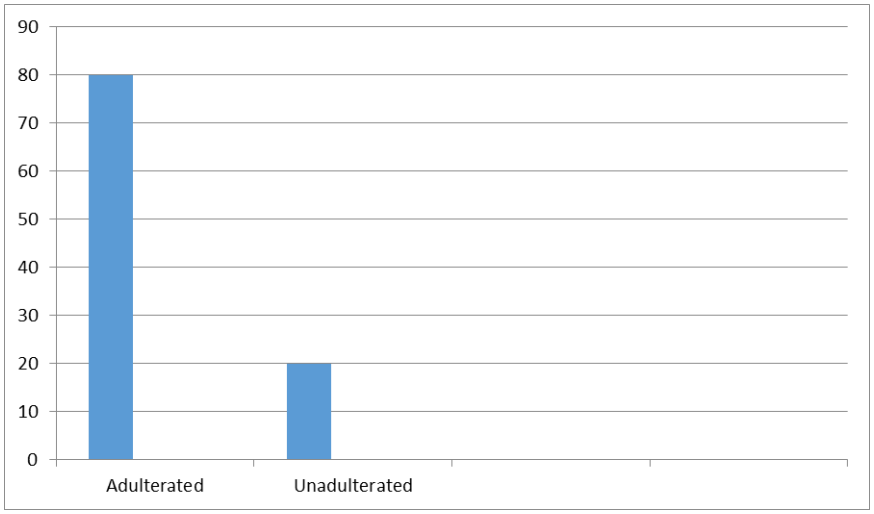

Fig 3 Percentage presentation of the result of the adulteration of the Herbal Preparations

IV. Discussion

Today, according to the World Health Organization (WHO), 80% of the human population depends on herbal preparations for their primary healthcare needs [5]. This is because of phyto-potency of indigenous herbal plants in the management of a cock-tail of diseases and the high economic burden associated with orthodox medical practices [3]. This work has shown that these herbal remedies tested in Enugu state South East Nigeria contain tannins, proteins, flavonoids, carbohydrates, saponins, glycosides, steroids and terpenoids as the bioactive ingredients. Their percentage composition are not negligible as they were discovered to be 9.58%, 6.80%, 1.24%, 5.4%, 3.19%, 0.48%, 0.52%, and 1.90% respectvely. Reducing sugars have the highest occurrence with 13.33% while alkaloids have the lowest composition of 0.34. This is a confirmation of the work done by Sharma et al,. [8] which showed that herbal preparations are enriched with phytochemicals including Tannins, alkaloids, flavonoids, anthocyanins phytosterols, carotenoids, saponins, dietary fibers, isoprenoids and polysaccharides [8]. The phytochemicals have a variety of bioactivities including antioxidant, antimicrobial activities, detoxification enzyme modulation, immune system stimulation and hormone metabolism modulation [16]. They can be consumed for their antioxidant activities, exhibition of antimicrobial, antidiarrheal, anthelmintic, antiallergic, antispasmodic, and antiviral activities [9]. Other roles of these phytochemicals are to regulate gene transcription, improve gap junction communication, improve immunity, and protect against lung and prostate cancers [17, 18, 19, 20, and 21]. Polyphenols, flavonoids, alkaloids, terpenoids, and saponins are the bioactive molecules with antimalarial effects [22]. Hence, the tested remedies could be used to treat a good number of diseases including malaria and cancer. This confirms the assertions by Abdullahi and Mainul, [23] who stated that the bioactive fractions, or compounds which are extracted from herbal plants are used for different purposes and the methods of extracting them are similar irrespective of the intended biological use. The believe system in the Western World that 'natural' is better than 'chemical' or 'synthetic' has resulted to the evolution of Neo-Western herbalism [24]. In fact, in the United States the herbal medicines are taken as food or food supplements, known as nutriceuticals, which are usually available for self-medication [24]. In Enugu state, Nigeria, these preparations are also available as over the counter remedies but the efficacy or safety of these extracts is yet to be verified, hence, the evaluation of the phytonutrient content of the herbal medicines.

One of the turn offs towards theses herbal preparations is the fact that there is no universal regulatory body in charge or control of the use of these herbal remedies that can confirm the therapeutic claims [24]. This body should have been involved in curbing or controlling adulteration, inappropriate formulation, or lack of understanding of plant and drug interactions which can be life-threatening or lethal [24]. This work has become an eye opener, as the result has shown that 80% of the herbal preparations tested were adulterated with chloroquine and/or its metabolites. The samples BG, GS, EB, DR, BC, AI, HB and CH were adulterated while only the sample MF and GR were not adulterated. Adulteration is not localized, it has increased globally [10] because as demand for therapeutic botanicals increases, so does the risk of adulteration [25]. This is confirmation of similar research work done in Ghana to test for the presence of chloroquine and/or its metabolites which showed that two out of five samples tested were adulterated with chloroquine or related compounds [12]. Again, in terms of adulteration of herbal preparations, synthetic Phosphodiesterase type 5 inhibitors such as sildenafil, tadalafil, and vardenafil are used to adulterate herbal medicines used for treatment of erectile dysfunction in Nairobi Kenya [10]. Again, recent work has shown that intentional unlawful addition of synthetic substances to herbal preparations used to manage weight-loss and weight gain, aphrodisiacs, treatments for rheumatic and inflammatory diseases, antidiabetic medications, and antihypertensive preparations and it appears that the substitution of plant species occurs across various categories of herbal medicines [11]. These adulterants of herbal preparations might not be toxic but they antagonise active phytochemicals thereby reducing their therapeutic effects [26]. Adulteration of herbal remedies with allopathic medicines should be of concern to global health organizations.

V. Conclusion

The tested antimalarial herbal preparations are enriched with polyphenols, flavonoids, alkaloids, terpenoids, tannins and saponins which are the bioactive molecules with antimalarial activities. These showed that the herbal medicines have potentials for management of plasmodium infection. However, the presence of chloroquine and other related molecules are worrisome as these adulterants reduce the antimalarial therapeutic effect of the phytochemical contents of the preparations. They do not just mask the antimalarial effects of the remedies but also sabotage the economic and quick recovery of patients in Enugu state, Nigeria.

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