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Full Length Research Article

# PHYTOCHEMICAL, PHYSIOCOCHEMICAL AND ELEMENTAL ANALYSES OF SOME ANTIMALARIAL HERBAL CONCOCTIONS PREPARED IN KADUNA METROPOLIS

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**ABSTRACT**
Antimalarial herbal concoctions are mixtures of whole plants or parts of two or more plants species administered as oral decoctions, steam baths or infusions. The phytochemical studies of all the antimalarial herbal concoctions obtained from Gonin-gora (A), Ungwan-mu'azu (B), Central market (C), Tudun-wada (D) and Mando (E) revealed the presence of alkaloids, carbohydrates and cardiac glycosides but saponins were only found in Tudun-wada and Ungwan-mua'zu samples while tannins were present in Gonin-gora, Tudun-wada and Mando samples. However, anthraquinones were not detected in all samples. Thus the presence of flavonoids, saponins and tannins in all the samples could be the reason for the antimalarial activity of the concoctions. The elemental analyses of all the samples revealed the presence of Cd, Pb, Cr, Cu and Zn. The concentration of Cr in all the samples were found to be within the range of 0.26±0.01 to 5.68±0.07 which was above the W.H.O. maximum permissible limits (0.05mg/L). Pd was only found in Ungwan-mu'azu, Central market and Tudun-wada samples which was found to be within the range of 0.07±0.00 to 0.09±0.00 which was above the W.H.O. permissible limits (0.05mg/L). The pH of all the samples was found to be within the range of 6.23 to 6.77 which indicated them as weakly acidic. The total ash analyses of all the samples revealed that Mando (19.33±0.55%), Ungwan-mu'azu (55.83±0.35%) and Gonin-gora (48.57±0.65%) which indicated high level of contamination probably in the processing steps, while that of Central-market and Tudun-wada samples had the least ash contents (1.04±0.00%). The moisture content of all the samples were found to be within the range of 12.93±0.00% to 44.30±0.01% but that of Ungwan-mu'azu, Central-market and Tudun-wada samples had high moisture contents which indicated that these herbal concoctions can be easily attacked by micro-organisms while those from Gonin-gora (12.99±0.00%) and Mando (12.93±0.00%) were within the permissible limits (14%).

**Keywords:** Antimalarial herbal concoctions, Phytochemicals, W.H.O. permissible limit, infusions and Heavy metals.

## INTRODUCTION

Herbal preparations could be used in almost all disease states without proper dosage and toxic effects consideration and the practice has been on for over a long period of time (Piemen *et al*., 2006; Ogbonnia *et al*., 2010; Ali *et al*., 2012). Many of the herbal preparations were not well searched, their formulation and sales were unregulated and they may be associated with high risk of producing adverse effects (Oshikoya, 2008).

Malaria is a serious parasitic diseases transmitted through the bite of an infected female anopheles mosquito. There are five species of parasites which belong to plasmodium genus namely; *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* but the most deadly is *P. falciparum* which is the most virulent and responsible for severe clinical malaria (Sanni *et al*., 2002; WHO, 2014). The Malaria disease causes fever, fatigue, vomiting, headaches, yellow skin, seizures and death in severe situations (Caraballo, 2014). There were 198 million cases of malaria worldwide, where Africa took the highest percentage due to the fact that *Plasmodium falciparum* (the most deadly specie) exists more in communities in Africa which led to an increased mortality and morbidity rate annually (Fidock *et al.,* 2004;WHO, 2014; WHO 2016). Malaria remains a major public health problem world-wide and Africa is said to have the highest burden because of its high economic burden on the nation, high prevalence of mortality in children, pregnant women and non-immune individuals (Benjamin *et al*., 2004). The rapid increase of resistance to most of the available antimalarial drugs, as well as resistance of vectors to insecticides led to the re-emergence of malaria in many parts of the world. Drug resistant strains of *P. falciparum* have been reported in many endemic areas of the world, but chloroquine and antifolates (sulfadoxine-pyrime) have been associated with treatment failure (Zirihi, 2005). Plant-derived compounds played crucial roles in drug discovery and development for the treatment of malaria. The isolation of new bioactive compounds from medicinal plants based on traditional use appears to be a very promising approach (Newman *et al*., 2008).

Some medicinal plants used traditionally for the treatment of malaria include *Mangifera indica* (leaf and bark), *Psidium guajava* (leaf), *Artemisia maciverae* (whole plant), *Azadirachta indica* (leaf, stem, bark and root), *Anacardium occidentale* (leaf) and *Carica papaya* (leaf and root) (Ajaiyeoba *et al*., 2004) whose preparations are commonly sold in markets and public places or administered by traditional healers for many years (Abubakar *et al*., 2015). Nonetheless traditional medicine has proven to be the surest source of effective antimalarial. Afterall, the alkaloid quinine was extracted from the bark of Cinchona, and this was the first antimalarial drug used by the inhabitants of Peru to control malaria (Willcox *et al*., 2011; Michael, 2012; Mokuolu *et al*., 2007).Nigeria like many other developing countries, a large percentage of inhabitants cannot afford the cost of orthodox medicines and therefore source for herbal concoctions for treatment of most of their ailments without recourse to the potential risk of the herbs to human health ( Abubakar *et al*., 2015; Oluwatosin *et al*., 2015; Noedl, 2008).

Plants are the main link in the transfer of metals from the contaminated soil to humans. Heavy metals have a tendency to accumulate in the food chain and they have low excretion rates through the kidney which could result in damaging effects on human even at very low concentrations. The heavy metals of major concern to the human health include arsenic, cadmium, lead and mercury whereas, metals such as zinc, copper, iron, manganese, and chromium are essential nutrients and important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can become toxic (Elujoba *et al*., 2005; Blagojevic 2009). In Nigeria, most traditional-practitioners sell their products along busy traffic urban centers or display them outside their stores thus exposing them to air-borne metallic contamination (Hina *et al*., 2011). Many health problems have been linked to excessive uptake of dietary heavy metals including a decrease in immunological defense, cardiac dysfunction, and fetal malformation, impaired psychosocial and neurological behavior, gastrointestinal cancer, and many others (Singh *et al*., 2011). This research therefore is aimed at evaluating and assessing the phytocompounds responsible for the antimalarial activities of the herbal concoctions as well as their level of metallic contamination due to poor processing and handling.

## MATERIALS AND METHODS

### Sample Collection

The antimalarial herbal concoctions were sampled on 12th January, 2019 from Gonin-gora, Ungwan-mua'zu, Central market, Tudun-wada and Mando areas of Kaduna State which were labeled as A, B, C, D and E respectively, and they were stored in refrigerator until the time for the analysis.

### Evaporation of samples

The aqueous herbal concoctions were evaporated using hot water bath at multi-users laboratory, Ahmadu Bello University, Zaria. Each of the herbal concoctions was placed into an evaporating dish and placed on the water bath at $100^0$C to dryness.

### Phytochemical Analysis

All the herbal antimalarial concoctions were screened qualitatively for the presence of phytochemicals using the standard methods described by (Trease & Evans, 1983; Sofawora 1994; Silva *et al*., 1998 and Gafar *et al*., 2010).

### Saponins (Frothing Test)

Crude extract (0.5g) was shaken with water in a test tube. Frothing which persisted for 15 minutes indicates the presence of saponins.

### Tannins

Crude extract (0.5g) was boiled with water and filtered, 2 drops of ferric chloride was added to the filtrate. A blue-black or green precipitate indicates the presence of tannins.

### Flavonoid (Alkaline Test)

To the sample, 10 mL of ethyl acetate was added and heated over a steam bath for 3 minutes. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of diluted ammonia solution. A yellow colouration indicates the presence of flavonoids.

### Alkaloid (Dragendoff's Test)

To the sample, 1mL of Dragendoff's reagent was added. Appearance of orange brown precipitate indicates the presence of alkaloids.

### Anthraquinones

To the sample, 10 mL of benzene was added and filtered, 5 mL of 10% ammonia solution was added and on shaking, a pink colouration in the aqueous layer indicates a positive test.

### Carbohydrates (Molish Test)

To the sample, 1mL of concentrated $H_2SO_4$ and 3 drops of Molish reagent was added without mixing, the appearance of purple ring in the interface indicates a positive test.

### Cardiac Glycoside (Keller-Killani Test)

To the sample, 2 mL of acetic acid containing 1 drop of ferric chloride solution was added. A reddish brown ring at the interface indicates a positive test.

### Elemental Analysis

Crude sample (10 g) was placed in a platinum dish and transferred into the muffle furnace (L-031H1RN-120 Mabertherm) at 450 $^0$C for 3 h. It was removed and placed in a desiccator for cooling. $HNO_3$ and HCl (aqua regia) (1:4) was added to the ashed sample and heated on a heating mantle to dryness and later 25 mL of deionized water was added to the residue in the beaker. The solution was filtered using a filter paper Whatman No. 42 and rinsed up to 3 times into a volumetric flask of 50 mL and was made up to mark. Elemental analysis was carried out for the presence of any heavy metal such as Cd, Pd, Cu, Cr and Zn using Atomic Absorption Spectrophotometer model AAS 6800, Shimadzu.

### Physiochemical Constants

### Ash Values

### Total ash

Crude extract (5 g) was incinerated using a platinum crucible at a temperature range of 400-450$^0$C in a muffle furnace (L-031H1RN-120 Mabertherm) until they were free from carbon. They were further cooled and weighed. The percentage of the ash with reference to the air dried extract was calculated. The residue left after the incineration was the ash content of the crude extract.

### Calculations

Weight of the empty dish = X g, Weight of the extract = Y g, Weight of dish + ash = Z g
Weight of ash = (Z-X) g.
Therefore, the percentage of total ash value of the extract = (Z-X) 100/Y%

### Acid Insoluble Ash

The ash (2 g) obtained was boiled for 5 minutes in 25 mL of hydrochloric acid. The insoluble extract was collected on an ash-less filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash with reference to the air dried extract was calculated.

### Calculations

Weight of ash = X g
The percentage of acid insoluble ash value of the extract = X (100)/Y%

**Water Soluble Ash**
The ash (2 g) was boiled for 5 minutes in 25 mL of distilled water. The insoluble extract on an ash-less filter paper washed with hot water and ignited for 15 minutes at a temperature of about 450$^0$C. The weight of the insoluble extract was subtracted from the weight of ash (2 g). The difference in weight represents water soluble ash which was calculated with reference to the air dried extract.

**Calculations**
Weight of the water soluble ash = X g
Air dried extract – Y g
The percentage of water soluble ash value of the extract = X (100)/Y%.

**Moisture content**
The herbal antimalarial concoction (10 g) was placed into evaporating dish and dried in an oven at 105 $^0$C for 3 h. It was further cooled in a desiccator. The loss in weight was recorded until a constant weight of the extract was obtained.

**Calculations**
Moisture = $(W1-W_2)100/W_2$ %
Where W1 = Original weight of the sample before drying
W2 = weight of the sample after drying.

**pH**
The pH of the samples was determined using a simple glass electrode pH meter (7110pH meter InoLab).

**Statistical Analysis**
All the values were expressed as mean ± Standard deviation (SD) analysis for ANOVA and post hoc student's T-test. Differences between groups were considered significant at $p<0.05$ levels.

**RESULTS**
Table 1 is the result of phytochemical screening of the antimalarial concoctions sampled for analysis. Most phytocompounds associated with antimalarial activities have been detected. However, the absence of saponins, tannins and flavonoids in B & C in particular could be attributed to the use of dried materials and heavy heat during preparations. This could denature saponins and phenolics. Absence of anthraquinones in all the samples is an indication that they are not harmful to humans

**Table 1:** Results of Phytochemical Analysis of Herbal Antimalarial Concoctions

| S/N | CONSTITUENTS | TESTS | SAMPLES | | | | |
|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E |
| 1 | Saponins | Froth test | - | - | - | + | + |
| 2 | Tannins | Ferric chloride | + | - | - | + | + |
| 3 | Flavonoids | Lead acetate test | + | - | - | + | + |
| | | Sodium Hydroxide | + | - | - | - | - |
| | | Ferric Chloride | + | - | - | - | - |
| 4 | Alkaloids | Dragendroff's | + | + | + | + | + |
| 5 | Carbohydrates | Molisch's test | + | + | + | + | + |
| | | Barfoed's test- | + | + | + | + | + |
| | | Fehiling's test | + | + | + | + | + |
| 6 | Anathraquinones | Brontragers' test | - | - | - | - | - |
| 7 | Cardiac glycosides | Keller-Killani test | + | + | + | + | + |

**Key: A-** Gonin-Gora, B- Ungwan-Ma'azu, C- Central Market, D-Tudun Wada, E- Mando, (+) = Present; (-) = Absent

Table 2 is the result of elemental analysis of the antimalarial concoctions. Copper which is an essential element was not detected in any of the samples. The concentrations of some of the elements as indicated were higher than the permissible limits as such continuous consumption of such concoctions may be detrimental to health in the areas being sampled.

**Table 2:** Results of Elemental Analysis of Herbal Antimalarial Concoctions

| S/N | Samples | Cr (mg/L) | Pb (mg/L) | Zn (mg/L) | Cu (mg/L) | Cd (mg/L) |
|---|---|---|---|---|---|---|
| 1 | (A) | 0.26±0.01 | ND | 0.69±0.18 | ND | ND |
| 2 | (B) | 1.55±0.05 | 0.07±0.00 | 0.59±0.00 | ND | ND |
| 3 | (C) | 5.68±0.07 | 0.09±0.00 | 0.61±0.02 | ND | 0.01±0.00 |
| 4 | (D) | 2.34±0.00 | 0.09±0.00 | 0.60±0.00 | ND | ND |
| 5 | (E) | 2.35±0.00 | ND | 0.61±0.00 | 0.01±0.00 | 0.01±0.00 |

**Key: A-** Gonin-Gora, B- Ungwan-Ma'azu, C- Central Market, D-Tudun Wada, E- Mando, (ND) = Not detected

Table 3 is a result of physiochemical parameters of the herbal preparations. The ash value, total ash, acid insoluble ash values and water-soluble ash were found to be within the range of 1.04 - 55.83%. The results could serve as gateway to quality, authenticity and purity of the concoctions. The moisture contents of the range of 12.93 – 44,30 % could be due the preparation using water as traditional method of preparation. The values were quite higher than the acceptable limit as such the concoctions would not be stored for a longer period with higher chances of microbial attack and some other chemical reactions that may alter the nature of its active constituent.

**Table 3:** Results of Physiochemical constants of Herbal Antimalarial Concoctions

| S/N | Samples Location | [a]Total-ash Value (%) | [a]Acid-insoluble Ash (%) | [a]Water-soluble Ash (%) | [a]Moisture Content(%) | pH |
|---|---|---|---|---|---|---|
| 1 | (A) | 48.57±0.65 | 4.24±0.02 | 4.33±0.01 | 12.99±0.00 | 6.32 |
| 2 | (B) | 55.83±0.35 | 7.19±0.01 | 7.39±0.00 | 24.04±0.00 | 6.77 |
| 3 | (C) | 21.37±0.61 | 1.07±0.00 | 1.04±0.00 | 44.30±0.01 | 6.37 |
| 4 | (D) | 20.60±1.17 | 1.04±0.00 | 1.06±0.00 | 23.95±0.00 | 6.45 |
| 5 | (E) | 19.33±0.55 | 1.10±0.00 | 1.12±0.00 | 12.93±0.00 | 6.72 |

[a] = All the values were expressed as mean ± SD.
**Key: A**- Gonin-Gora, B- Ungwan-Ma'azu, C- Central Market, D-Tudun Wada, E- Mando,

**DISCUSSION**
Traditional medicinal plants contain therapeutic active substances, which could be precursors for the synthesis of herbal drugs thus important drugs have been derived from plant resources directly or indirectly (Morabad *et al*., 2017). The basis for the medicinal use of the plants is the presence of mixtures of different biologically active plant constituents or phytochemicals (secondary metabolites) such as alkaloids, glycosides, terpenoids, and so on that may act individually, additively or in synergy to demonstrate an effect which may be useful or harmful to health( Merlin *et al.,* 2019).
The phytochemical and elemental analyses of all the antimalarial herbal concoctions sampled from some areas of Kaduna metropolis have revealed the presence of some phytochemicals of medicinal values. The presence of some heavy metals which could be detrimental to human health as well as high moisture and ash

contents which could be due to processing.

The antimalarial activities of the samples could be due to the presence of flavonoids, saponins and tannins which were all indicated to have antimalarial property (Yadav *et al*., 2004). Medicinal plants owe their pharmacological properties from their phytochemical contents which were involved in both preventive and curative aspects of various diseases (Karunyadev *et al*., 2009). The polyphenols (flavonoids and tannins) in the samples were known to have possessed antimicrobial, antioxidant, anti-allergic, anti-inflammatory activities as well as cytotoxic effects.) while saponins possessed anti-microbial, anti-inflammatory activities and cytotoxic effects. Thus, the presence of these phytochemicals in the concoctions indicated their potential benefits in improving human health (Madziga *et al,* 2010; Matotoka & Masoko, 2018).

Anthraquinone was not detected in any of the concoctions which is an advantage because it have been indicated to cause abdominal cramps, gastrointestinal discomforts, vomiting, dermatitis, nausea, bloody diarrhea and dizziness (Chang, 1984; Nida, 2019).The overall results for the phytochemical screening of the herbal concoctions revealed other phytochemicals which include alkaloids, cardiac glycosides and carbohydrates which have various physiological activities as reported in other herbal preparations (Maton *et al* ., 1993; Oluwatosin *et al*., 2015).

The result of elemental analysis (Table 2) has shown the presence of Cr, Pb, Zn, Cu and Cd in all the antimalarial concoctions. The values ranged from 0.01 to 0.69 mg/L some of which are above the tolerant level, which could be detrimental to the health of the consumers. Thus, their presence in the concoctions may lead to toxicity that may improve one or more biological processes in the body system (Oluwatosin *et al*., 2015; Jigna and Sumitra, 2007). The wide variations in metal concentrations could be attributed to the differences in uptake and translocation capabilities of the herbs used in the preparation as well as the soil type and level of contamination during processing (Rania *et al*., 2015). The concentrations of Chromium in all the samples ranged from 1.55 to 5.68 mg/L and were above the WHO standard of 0.1 mg/L. in particular the sample from central market (5.68 mg/L) is alarming but not surprising since it is sold within large populace with series of human activities. Similar explanation can be attributed to the higher concentrations in other locations due to hawking and display at road sides. Similarly, the concentration of lead (0.07 to 0.09 mg/L) was found also to be higher than the WHO standard (0.05 mg/L) although not detected in Gonin-gora and Mando samples.

However, continuous consumptions of the concoctions containing high amount of lead may lead to adverse effects on blood, nervous, immune, renal, skeletal, muscular, reproductive and cardiovascular systems causing poor muscle coordination, gastrointestinal symptoms, brain and kidney damage, hearing and vision impairment as well as reproductive defects (Rania *et al*., 2015). Although the other metals in the concoctions are within the permissible limit. However, it should be noted that heavy metals have tendency to accumulate and have low excretion rates through the kidney which could result in damaging effects on humans even at very low concentrations. Although, Zn and Cu are essential nutrients for physiological function of the human body but they could be toxic when accumulated beyond the standard level (Korfali *et al*., 2013).

The result of the physiochemical analysis of the antimalarial concoctions has shown pH values that are indicating moderate acidity which means at least not much side effects in terms of heart burn or irritations. The total ash value which is an indication of amount of minerals and earthy materials in a plant was in a range of 19.33 to 55.83 %. This means they contain a lot of mineral contents as well as particles or impurities. It is possible that the herbs used in the preparation of the concoctions are more of fibrous materials. The amount of the acid insoluble siliceous material present in the concoctions was in the range of 1.04 to 7.19 % while the water-soluble extract value which indicates the amount of sugar, acids and inorganic compounds was in the range of 1.04-7.39 % and the moisture content was in the range of 12.93-44.30 % meaning that those above 14 % are liable to microbial attack and cannot be stored for quite a number of days (Swamy & Kamil, 2010; Regupathi & Chitra, 2015).

**Conclusion**

This research work revealed the presence of some phytochemicals in all the samples. The elemental analyses revealed not only the beneficial nutrients but the heavy metals which could be absorbed directly from the soil or due to handling and preparation of the samples. This study ensured the level of purity and quality of the concoctions which might be considered not very safe if allowed to stand for a long time without proper storage.

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