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Phytochemical, Mineral and Proximate Analysis of Herbal Tea Formulated for Malaria Management

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of herbal tea preparations in the management of malaria infection has led to an increase in the production and research interest in teas. The readily available and cheap plant materials for production has made research efforts to be tailored towards development of different herbal tea preparations. In this study, the herbal tea, specially formulated for management of malaria infection, was subjected to various analysis. Qualitative and quantitative phytochemical determination indicated presence of saponin (9.71mg/100g), tannin (46.43mg/100g), phenols (904.01mg/100g), flavonoids (24.56mg/100g) and alkaloid (20.68mg/100g). The mineral component determination indicated that it was rich in Potassium (1.80 ppm), Magnesium (0.328 ppm) and Sodium (24.95ppm). Some heavy metals such as Cadmium (2.80ppm) and Iron (10.21ppm) were also detected. Proximate analysis carried out indicated moisture content (6.58%), Ash (9.23%),

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Carbohydrate (70.36%), Crude lipids (1.07%), Crude fibre (3.11%) and Crude protein (9.58%). Results shows that all the phytochemicals needed to fight malaria parasite are present in the herbal tea formulation. The mineral contents fall within World Health Organization acceptable level except for Cadmium and Iron.

Keywords: Herbal tea; conventional drugs; phytochemicals; malaria, proximate analysis.

1. INTRODUCTION

Globally, it is estimated that there are 249 million malaria cases and almost 1million deaths in 85 countries worldwide (WHO, 2022). It was reported that most of the malaria burden is carried by African countries (WHO, 2020). It was estimated that in 2021, Nigeria had 64 million cases of malaria and 194,000 deaths (WHO, 2022) which represents about 27% global incidence of malaria. Most of the deaths occurred in young children and pregnant women who have not developed resistance to the disease (Casella et al. 2024). In Nigeria, estimated malaria mortality rate for children under five is 729 per 100, 000 (Tolu et al. 2007). "The Federal Ministry of Health indicated that malaria was responsible for one out of ten deaths in pregnant women and has caused the Federal Government of Nigeria over one billion Naira annually in treating malaria" (Tolu et al. 2007). Malaria occurrence is mostly in poor tropical and subtropical parts of the world (Eligo et al. 2024). "The main reason for the high rate of malarial transmission in Africa is due to such vectors such as *Anopheles gambiae*, *Anopheles arabiensis*, and *Anopheles funestus"* (Sinka et al. 2010). Local weather conditions has also been identified to often allow transmission to occur year round (Mercado et al. 2019). Poor economic situation and scarce resources have been reported to hinder efficient malaria control activities (WHO, 2020, Srimokla et al. 2024).

Several studies have been carried out in determining the effective way to eliminate malaria as it was observed that it is necessary to use additional tools to control the vectors carrying the diseases (Sougoufara et al. 2020, Gatton et al. 2013). "The use of interventions such as larval source management to target the aquatic stages of malaria and other vectors was suggested" (Gu et al. 2006). Toxic bait traps is another intervention which was suggested could be used to control mosquitoes seeking hosts outdoor (Sougoufaraet al. 2020). In a study carried out by Franco et al. 2014 it was suggested that animal-based interventions could address the problem related to zoophagic mosquitoes. Unfortunately, advanced technology in the management of malaria infection is not available in Nigeria thus resort to the development of herbal medicines which have been used to treat the infection for thousands of years (Olorunnisola et al. 2014).

"The pharmacologic activities of medicinal plants in the management of malaria infection cannot be overemphasized" (Junaid 2020). "The presence of some important phytochemicals with diverse biological activities such as alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc contribute to these medicinal plants pharmacological activities" (Junaid 2020). "Across the world, over 1200 plant species from 160 families used in the management of malaria or fever have been documented" (Tolu et al. 2007, Ajibesin et al. 2008, Omoregie 2011, Dickson et al. 2011). "Plant extracts have been found to have good and moderate anti-malarial activity in mice with rodent *Plasmodium* species" (Nigussie 2022). "The most active species were *Ajuga remota* and *Capsicum frufescens*, which was observed to suppress parasitaemia by 77.34% and 72.65%, respectively, at an oral dose of 100 mg/kg and an LD_{50} of above 2000 mg/kg" (Nigussie 2022). "The compound Aloinoside reported from *Aloe macrocarpa* leave latex was the most potent; it suppressed parasitaemia by 100% at 400 mg/kg oral dose of *Plasmodium berghei* infected mice, and its LD_{50} was above 2000 mg/kg. Toxicity was shown to be safe in 84% of the plant extracts" (Nigussie 2022). "In a study, the in vitro combination of Lawsonia inermis and Tithonia. diversifolia (1:1) extracts against P. falciparum showed the highest synergy with IC50 of 0.43±0.02µg/mL and 2.55±0.19 µg/mL and it was found that the extracts were not toxic at the concentration tested" (Funmilayo et al. 2016).

"Tea, obtained from medicinal plants, is usually the most popular flavoured, functional drink worldwide and consist of prepared leaves of various plants" (Funmilayo et al. 2016). "The chief constituents of tea packaged in tea bags are moisture, tannin, nitrogenous matter

(including caffeine) inorganic matter (especially potassium salt) and crude fibre" (Akinyeye 2010). "It was reported that over 1200 plant species from 160 families are traditionally used to treat malaria and fever" (Tolu 2007, Ishola et al. 2014). "On average, a fifth of patients use traditional herbal remedies for malaria in endemic countries" (Willcox 2004). "Some herbal remedies have been reported to be safe and effective for the treatment of malaria, but better evidence from randomised clinical trials is needed before herbal remedies can be recommended on a large scale" (Willcox 2004). "The chemical composition of tea is complex. Some epidemiological studies have associated the consumption of tea with a lower risk of several types of cancers, malaria, oral cavity e.t.c" (Carmen et al. 2003). "In a study carried out on a tea preparation of *Artemisia annua* and *Artemisia afra* at small-scale clinical trials, it was observed that the tea was efficient in curing malaria infections but at a larger-scale trial, *they* were superior to artesunate-amodiaquine (ASAQ)" (Munyangi et al. 2019).

2. MATERIALS AND METHODS

Five medicinal plants locally used in Nigeria for the management of malaria, as claimed by the manufacturer, were used in the formulation of the herbal tea. The manufacturer claimed the plants were obtained from the herb sellers, thoroughly washed and dried in the shade. Thereafter, they were individually grounded and missed in the ratio used by the locals. The tea was then packaged and kept for analysis.

2.1 Tea Extract Preparation

In a 250 mL round bottom flask, 20 g of the sample was mixed with ethanol. The mixture was then subjected to refluxing using water baths set at temperatures of 50, 60, 70, 80, and 90 °C at varying times of 15, 30, and 60 min. Re-extraction was also performed bringing the total number of extracts collected to thirty-two. The residues were then filtered using gravitational filtration and then the solvent was evaporated under the fume hood. The percentage yield of the extract was determined, and the extracts were stored in a dry place. Same procedure was repeated using water as solvent of extraction.

2.2 Proximate Analysis

"These analyses were done using the method of Association of official analytical chemist (AOAC) and Pearson composition and analysis of food" (Pearson's composition and analysis of foods 1999).

Moisture content determination: Two grams (2 g) of the extract was weighed and placed in a crucible of constant weight. This was placed in an oven at 105^{o}C then dried; the weight was measured carefully to get a constant weight. The loss in weight indicates the moisture content.

Ash content determination: Crucible used for ash content determination were weighed and dried in a hot air oven at 110^{o}C to a constant weight for the two plants. Two grams (2 g) of the extract was weighed and placed in the crucible and weight of the crucible and extract was taken. This was placed in a furnace and ignited for 3 hrs at 55^{o}C till the samples had a cotton wool-like texture; it was cooled in a desiccator and weighed with a digital balance.

Crude protein determination: One gram(1g) sample was weighed into 2 kjeldahl flasks and 0.1g of Ca_2SO_4 was added into each flask with 20 ml Concentrated H_2SO_4. Each flask was slanted on kjeldahl heating mantle in the fume cup board. Digestion continued until there was a colour change from black to bluish green signifying completion of digestion. The blank was set up, digests removed and allowed to cool, diluted with water and made up to 200 ml on ice. After these, aliquot (50ml) of each digest was poured into a distillation flask, then, 30 ml of NaOH was carefully layered into solution in order to make it a strong alkaline. Following, $0.1NH_2SO_4$(50ml) and 2 drops of methyl red as an indicator, the distillate was titrated with 0.1M NaOH in the burette for each plant extract and blank and % of crude protein was calculated.

Lipid content determination:1 g of sample was weighed into a thimble of known weight and 150 ml of petroleum ether (60-80 ^{o}C) were poured into 250 ml conical flask using measuring cylinder. The soxhlet extractor where the sack and its content had been introduced was fitted and solvent boiled under reflux. The extraction process lasted for 8 hours, sack with its content were removed dried in an oven for 2 hours and then weighed with a digital balance.

Crude fibre determination: 5 g powder samples plus 200 mL (1.2%) H_2SO_4 was heated for 30 minutes and filtered via a buchner funnel after which the residue was washed with distilled H_2O until it was acid- free. Then, 200 mL (1.25%) was

used to boil the residue 30 minutes and also filtered and washed severely with distilled H_2O until it was alkaline-free and rinsed once with HCl (%), twice ethanol and finally petroleum ether trice. The residue was dried in a crucible, placed inside an oven at 105°C overnight. Following this, cooled in a desiccator and ignited in a muffle furnace at 550°C for 90 minutes to and finally weighed to obtain the crude fibre (AOAC 2000).

Total carbohydrate: The standard method of the AOAC (2000) was used. This was determined by the differences between the whole sample and the sum of the liquid, ash, protein, and crude fiber compositions of the sample: % $Carbohydrate$ = 100 – [% $protein$ + % fat + % Ash + % $Crude\ fiber$].

2.3 Determination of Mineral Content

"The mineral contents of the sample was determined using the method described by Onwuka (Onwuka 2005, Isiuku 2009). Half gram (0.5 g) of the dry milled sample was weighed into a pre – acid rinsed digest tube. 10 cm^3 of 6M HCl was added and heated to dryness in a water bath. The residue was dissolved in a mixture of 10 cm^3 of 6M HNO_3 acid, warmed on a water bath and filtered using a Whatman filter paper into 100 cm^3 calibrated flasks. The filter paper was washed with distilled water and the filtrate diluted with the distilled water and made up to the 100 cm^3 mark. The digest was for the determination of magnesium, sodium and potassium by the flame photometry method. The heavy metals such as Cadmium and Iron were determined using the atomic absorption spectrophotometer method" (Abbaspour et al. 2014).

2.4 Pythochemical Screening of Secondary Metabolites

Quantitative and qualitative phytochemical screening for alkaloids, flavonoids, glycosides, phenols, cardiac glycosides, saponins, sterols, tannins and anthraquinones were determined using various established methods.

2.5 Qualitative Determination of Chemical Constituents

"Test for alkaloids: A few drops of dilute iodine solution were added into 3 ml test solution added. Blue colour appeared; and disappeared on boiling and reappeared on cooling" (Khandelwal 2008).

"Test for flavonoids: 2-3 ml. of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids" (Szultka et al. 2013).

"Test for phenols: 0.5 ml of $FeCl_3$ (w/v) solution was added into 2 ml of test solution, formation of an intense colour indicates the presence of phenols" (Baciocchi et al. 2001).

"Test for saponin: 0.5g extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam, indicates the presence of saponins" (Aziz et al. 2019).

"Test for tannins: Few drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicates the presence of tannins" (Atanassova 2009).

2.6 Quantitative Determination of Chemical Constituents

2.6.1 Determination of alkaloid content

"Tea sample (5 g) was weighed into 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was removed and washed with 1% ammonium hydroxide and then filtered. The residue is the alkaloid and this was oven dried for 30 mins at 60°C and reweighed" (Adeniyi et al. 2009). The alkaloid content of the samples was determined by difference using the equation:

$$\text{Percentage alkaloid} = \frac{W2 - W1}{W} \times 100$$

Where,

W = weight of sample
W1 = weight of empty filter paper
W2 weight of paper + precipitate

2.6.2 Determination of saponin content

"Tea sample (20 g) each were put into conical flask and 100 mL of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^{o}C. The mixture was filtered and the re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 mL over water bath at about 90^{o}C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL n-butanol was then added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven to a constant weight" (Aziz et al. 2019). The saponins content was calculated thus:

% Saponin = Weight of Saponin/ Weight of Sample X 100

2.6.3 Determination of flavonoid content

Tea sample weighing 10 g were extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper no. 2. The filtrates was later transferred into a crucible and evaporated into dryness over a water bath and weighed to constant weight (Chirikova et al. 2010).

% Flavonoid = Weight of Flavonoid/ Weight of Sample X 100

Determination of tannin content Tea sample (500 mg) was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M Potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min. (Antonova et al. 2015).

2.7 Statistical Analysis

The result of the analyses, were subjected to statistical analysis of variance (ANOVA) to determine by difference in sample means and Duncans Multiple Range Test (DMRT) was used to separate means at $p \leq 0.05$ with statistical package for social science version 16.0 for windows (spss inc. illinious,USA)

3. RESULTS AND DISCUSSION

The sample tea was screened for the presence of some phytochemicals which include saponin, tannins, phenolics, flavonoids and alkaloids. Table 1 and Table 2 shows the results as presented. There was presence of saponins (9.714±0.0.35 mg/100g) which are known to decrease blood lipids. They also lower cancer risks and blood sugar thereby reducing the incidence of diabetes (Zhang 2022). The therapeutic role of saponins as hypoglycemic, anti-asmatic, hypolipidemic, antioxidant and antihypertensive is well documented (Yao et al. 2020). Tanin (46.435 ±0.417 mg/100g) was observed to be present (Table 2). The application of tannins as anticancer, antioxidant, antimicrobial and inflammatory has been reported by various researchers (Fraga-Corral et al. 2020, Suvanto et al. 2017). It was also reported to be useful in wound healing, cardiovascular protection, anti-diabetic and anti-diarrhoic (Tamokou et al. 2017). The presence of phenolics (904.011±1.432 mg/100g) was observed in the tea sample. Phenolics are known to relieve stress disorders and also provide protection against microbial infections. They have also be reported to be effective against cardiovascular disorders (Alotaibi et alo. 2021, Toma et al. 2020, Olas 2022). In addition, there is reported anti-aging, anti-oxidant and anti-inflammatory activities of phenolic compounds in medicinal plants. Flavonoid was present in the tea sample. They have been reported to be very beneficial against cancer and viruses (Ravishankar et al. 2013, Abotaleb et al. 2018, Maaliki et al. 2019). It was reported that flavonoids are also effective as antioxidants, anti-inflammatory and antihypertensive (Abotaleb et al. 2018). The amount of alkaloids present is 20.687±0.0444 mg/100g (Table 4). Alkaloid is known to have a range of pharmacological activities some of which are antibacterial (Wasihun et al. 2023, Zandavar 2023). antimalarial (Klonis et al. 2013). anticancer (Mandour et al. 2023, Tariq et al. 2021) antiashma (Dangi et al. 2015, Lv et al. 2020) antihistamic (Gupta et alo. 2021) Analgesic (Yimer et al. 2020, Ayanaw et al. 2023, Jiang et al. 2022) anti-hyperglycemic (Dizaye et al. 2019, Muhammad et al. 2021, Adeneye et al. 2015).

Table 1. Results for qualitative phytochemical constituents

Saponin	+
Tannin	+
Phenolics	+
Flavonoids	+
Alkaloid	+

Table 2. Results for quantitative phytochemical constituents

Phenolics mg/100g	Tannin mg/100g	Saponin mg/100g	Flavonoid mg/100g	Alkaloid mg/100g
904.011±1.432	46.435 ±0.417	9.714±0.0.35	240.821±0.257	20.687±0.0444

Values are means of triplicates with Standard Deviation

Table 3. Results for mineral contents determination

Mineral Elements	Concentration PPM
Potassium	1.8
Magnesium	0.328
Sodium	24.95
Cadmium	2.80
Iron	10.21

Table 4. Results for proximate analysis

Moisture %	Ash %	Carbohydrate%	Crude lipids%	Crude fibre %	Crude protein %
6.585±0.258	9.276 ±0.269	70.368±0.282	1.074±0.003	3.117±0.194	9.580±0.052

Values are means of triplicates with Standard Deviation

Table 3 shows the results for some mineral contents of the sample tea. Potassium, magnesium, sodium, cadmium and iron were present at various concentrations in the tea. To maintain good health, human body requires some minerals (Gharibzahedi 2017, Merrell 2017). The essential elements, potassium, magnesium and calcium were found at permissible levels while heavy metals such as cadmium and iron were found at concentrations higher than the permissible levels. Cadmium has been reported to be a cancer causing heavy metal (Nawrot et al. 2010). and its accumulation in the human body leads to demineralization and osteoporosis as it interferes with the metabolism of essential minerals such as calcium, magnesium and zinc (Bernard 2008). Iron has been reported to be very vital in the body system as it assists in the production of blood. Deficiency in iron leads to anaemia. The observed value of 10.21 ppm falls within the acceptable limits of 8-10 ppm. However, it should be noted this value falls short of the acceptable value for pregnant women which is about 27 ppm. When there is no sufficient amount of iron intake during pregnancy, this may lead to premature birth and the baby having very low weight (Abbaspour 2018). It was further emphasized that low iron in pregnant women makes them prone to infections as their immune system has been lowered (Dixit et al. 2021).

Results for proximate analysis is summarized in Table 4. The result for moisture content indicated 6.585% as shown in Table 4. Previous studies by scientists indicated that 70% of commercial tea sample have moisture content of 6.6% or less and 30% samples containing moisture content up to 8% will have a negative effect on the shelf life of the product (Li et al. 2010). Therefore, for better quality and stability of the product over a long period of time, the moisture content should be controlled at 2.5 - 6.5% (Duan et al. 2022). The ash content was determined to be 9.276% and falls within the acceptable limit. Though previous research on some teas indicated a range of 4.9 – 6.5% for black tea but higher values of 6.1 – 9.2% was reported for green tea. Higher ash content indicated presence of inferior or foreign inorganic matter which may comprise the quality of the tea (Ren et al. 2024). The value of 70.368±0.282 reported for carbohydrate in the

tea indicated that it will be a very rich source of carbohydrate when compared to previous studies carried out on the tea made from leaves of Terminalia cattappa by Mosii et al. (Mosii et al. 2023) who reported that they obtain the value of 18.44 ± 0.053% and (Packirisamy 2014) who reported 5.79 ± 0.62%. The tea sample when brewed indicated 1.074±0.003% for crude fat. This value falls within the acceptable limit given that brewed tea should have no or fewer crude fat (Wierzejska 2014) Crude fibre is described as the combustible and insoluble organic residue which remains after the sample has been subjected to certain conditions. The value obtained for crude fibre was 3.117±0.194. This value falls within the limit set by WHO which indicated that value of crude fibre for teas must be less than 16% (Jayawardhane 2016). Crushing and cutting processes of the leaves for the tea packaging and impurities such as stem might have contributed to the low crude fibre value (Jayawardhane 2016). Though it was reported that in most teas the percentage composition of protein is almost 0% (Poswal et al. 2019) but 9.580 ± 0.052% was obtained for the tea sample. This implies that the tea may likely be a good source of protein.

4. CONCLUSION

Plant-based products such as teas represent a source of bioactive natural substances and compounds, also it ranks next to water in terms of worldwide consumption. Teas have been known to have high beneficial contributions on health. This study highlight percentages of moisture content, ash, carbohydrate, crude lipids, crude fibre and crude protein fall within acceptable limits by WHO and ISO specifications. Human body requires essential elements for good health. Potassium, Sodium and Magnesium present in the tea sample indicated that the essential elements fall within the acceptable limits as specified by WHO while Cadmium and Iron were found to be above the acceptable limits.

DISCLAIMER

The materials used for this research are commonly and predominantly use materials in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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