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(RESEARCH ARTICLE)



Phytochemical, antibacterial and free radical scavenging activities of a local antimalaria tea from Nigeria

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Abstract

Malaria is a reoccurring disease which affects about 296 million people globally. In countries where the people are overly attached to their culture like Nigeria, most of the population depends on local antimalaria remedies. One of such remedy is a tea of *Citrus aurantifolia* (leaves and peels), *Psidium guajava* (leaves) and *Ocimum gratissimum* (scent leaf) in alcohol. This study was aimed at determining the phytochemical, antibacterial and free radical scavenging activity of the methanol extract of this antimalaria tea, in order to determine the effect of its consumption on the human body. The phytochemical screening results showed the presence of compounds such as flavonoids, phenol, alkaloids and terpenoids amongst others. The extract also showed good antibacterial activity against *S. aureus*. The antioxidant activity results indicated that the lowest dose (0.25 mg/mL) of the tea had moderate antioxidant activity (62%) as compared with that of the standard antioxidant used. The highest dose level on the other hand had a very poor antioxidant activity (-7%). The results indicates that though consumption of the antimalaria tea may reduce the microbial load of *S aureus* in the body, excessive consumption of the antimalaria tea could lead to long term neurological or cell damage in the human body because of the ability of the tea to generate free radicals when taken in high concentration.

Keywords: Antimalaria; Antioxidant; *Ocimum gratissimum*; *Psidium guajava*; *Citrus aurantifolia*

1. Introduction

Malaria is a reoccurring disease which affects about 296 million people globally. It is distributed mostly through the tropics and subtropics [1]. In Nigeria *Plasmodium falciparum* is one of the parasites responsible for malaria infection [2]. In the past chloroquine was an effective drug for the treatment of malaria, but in recent years the development of resistance by the parasites has led to the development of drug combination therapies. Antimalaria combination therapy (ACT) is not only practiced in modern medicine but also practiced in traditional medicine, because it is believed that the combination produces rapid results and helps to slow down the development of drug resistance [3]. Various traditional medicinal plants have been reported to be used in combination for the treatment of malaria [4, 5].

In developing countries like Nigeria most of the population prefers using medicinal plants singly or in combination for the treatment of various diseases, this could be due to an attachment of the people to their culture or a lack of access to modern medicine which in most cases is associated with poverty. The preference for traditional medicine could also be born from the fact that the medicinal plants are easily accessible, efficient and safe [6]. Generally medicinal plants contain numerous compounds called secondary metabolites which can act as sources of potential drugs for the management of diseases [7].

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A combination of *Citrus aurantifolia* (key lime) leaves and peels, *Psidium guajava* leaves and *Ocimum gratissimum* (scent leaves) in either alcohol or water is a common local remedy consumed in southwestern Nigeria for the treatment of malaria [8]. *Citrus aurantifolia* is a popular species in the citrus family which is mostly used as an additive in food, drinks and deserts. It is an essential part of many herbal preparations in Nigeria [9]. Studies have shown that treatment of malaria with lime juice in addition with an appropriate antimalarial drug results in complete clearance of malarial parasites [10]. *Psidium guajava* is a plant native to Mexico, but has naturalized across West Africa [11]. It is mainly used as an anti-diarrheal agent; it is also used in reducing fever a major symptom of malaria [12]. *Ocimum gratissimum* is a plant of the *Lamiaceae* family that is cultivated worldwide for its medicinal and culinary uses [13]. The leaves of the plant are used to prevent mosquito bites; this could be probably due to the presence of thymol which is the major component of the leaves [14].

Various studies have established the fact that the individual plants have antimalaria activity and can prevent the generation of free radicals because of their antioxidants activities [15 – 19]. This present study is aimed at determining the chemical composition, antioxidant activity and antibacterial activity of a formulation combining the peels and leaves of *Citrus aurantifolia*, *Psidium guajava* leaves and *Ocimum gratissimum* leaves in order to determine the effect of its consumption on the human body.

2. Material and methods

Leaves of *Citrus aurantifolia*, *Psidium guajava* and *Ocimum gratissimum* were obtained from the Botanical garden of the University of Ibadan, and The Polytechnic, Ibadan, Nigeria, while the peels were gotten from fresh mature fruits bought in a local market in Ibadan, Oyo State. The fruits and leaves were washed free of sand and other impurities with distilled water. The rinds of healthy fruits of *Citrus aurantifolia* were peeled off with a knife. The peels and all other plant parts were air dried for 28 days and combined to make up 816g. The combination was extracted with methanol as solvent. The phytochemical investigation on the extract was done following the methods described by Harbone J. B. [20].

2.1. Antioxidant Activity

The antioxidant activity of the extracts was determined using the Diphenyl picryl hydrazine method (DPPH) method (2, 2-diphenyl-1-picryl hydrazine) as described by Mensor *et al.* [21], with some modifications [22]. Three different concentrations of the extracts were prepared (1.0, 0.5, 0.25 mg/ml). Butylated Hydroxyl Anisole and vitamin C were used as the reference standard antioxidant. Absorbance measurement was taken at 517 nm using spectrumbank S22PC visible spectrometer with model No 721S.

2.2. Antibacterial Activity

Environmental isolates of *Klebsiella pneumoniae* were obtained from the Department of Microbiology, University of Ibadan while clinical isolates of *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from Adeoyo State Hospital, Ibadan. Confirmatory test were carried out on them. Gram staining and biochemical test (Catalase, oxidase, coagulase, indole test) were done on each of the organisms. All organisms were sub-cultured to obtain a pure culture on Nutrient agar and maintained on agar slant and kept in a refrigerator until use. Analysis was carried out at the Department of Microbiology, University of Ibadan.

0.5 g of the extract was diluted with 10% DMSO (dimethyl sulfoxide) to concentrations of 50, 25, 12.5 and 6.25 mg/mL using a two-fold dilution method. Inoculums were prepared in 5 mL Normal saline with 3-5 colonies of each bacterial strain. The inoculums were adjusted to 0.5 McFarland standards for susceptibility testing [23]. The antibacterial activity of the extracts was determined in accordance with the standard agar-well diffusion method [24]. Muller-Hinton agar were prepared and poured into a Petri-dish, and allowed to solidify. Sterile swab sticks were used to swab the surface of the agar with the tested organisms. A sterile 6mm cork borer was used to punch holes (i.e. 5 wells) in the inoculated agar. Four wells were filled with different concentrations of the extract while the fifth well was filled with DMSO (negative control) which was labeled accordingly. Tetracycline was placed in the middle as the positive control. The Petri-dishes were left on the work bench for 40 minutes for adequate diffusion of the extract [25] and then incubated at 37 °C for 24 hours. Antibacterial activity of the extracts was determined by measurement of the zone of inhibition (mm) against each test bacteria using a ruler. The experiment was carried out in triplicates and the mean values of the result were taken as antibacterial activity [26 27].

3. Results and discussion

3.1. Phytochemical Screening

Preliminary phytochemical investigation of the methanol extract of the antimalarial tea of *C. aurantifolia* (leaves and peel), *P. guajava* (leaves) and *O. gratissimum* (leaves) revealed the antimalaria tea extract is rich in phytochemicals. The tea contained tannins, flavonoids, saponins, glycoside, steroids, anthraquinone, phlobatannins, and terpenoids. Some of these secondary metabolites were not detected in the individual plants, but the combination of these plants might have been the reason for the presence of these phytochemicals. A phytochemical study carried out by Pathan *et al.* [28] showed the absence of terpenoids in the leaves, tannins and flavonoids in the peels of *C. aurantifolia*. Also, Prasad *et al.* [29] also reported an absence of flavonoids, tannins, terpenoids, and saponins in the methanol extract of *C. aurantifolia* leaves. A study by Akinmoladun *et al.* [30] on the phytochemical constituents of methanol extract of *O. gratissimum* showed the absence of saponins and anthraquinones. Ayoola *et al.* [31] investigated the phytochemical constituents of *P. guajava*, anthraquinones and alkaloids were absent. Akinmoladun *et al.* [32] carried out a study on the phytochemical constituents of *P. guajava*, alkaloids and phlobatannins were not detected. These phytochemicals were all present in the combined tea extract. This may be as a result of some chemical reactions between compounds in the different plants. The result of the phytochemical screening shows that this combined mixture is rich in a wide variety of phytochemicals, hence, its use in herbal medicine.

3.2. Antioxidant assay

The percentage inhibition of DPPH radical by the methanol extracts of the antimalaria tea of *citrus aurantifolia*, *Psidium guajava* and *Ocimum gratissimum* is recorded in table 1. The extract showed a negative percentage inhibition (-7%) at the highest concentration of 1 mg/ml, this could be as a result of an antagonistic effect between the various compounds present in the extracts [33]. while at the lowest concentration of 0.25 mg/ml the extract had a percentage inhibition of 62% which was almost similar to that of the standard antioxidant used at same concentration. This result is peculiar because the different plants have been observed to have good DPPH radical scavenging abilities, though that of lime peel and juice varies depending on the type and concentration of the flavonoid present [17,19, 34]. A likely explanation for this observation could be that, at high concentration the other compounds present in the mixture mask the effect of the active components, but as the concentration reduces it is possible that the interaction between the various compounds becomes negligible allowing the active components to exert their antioxidant abilities. This may not necessary be a bad thing because studies have revealed that at low or moderate levels free radicals exert some beneficial effects [35]. But it still stands to reason that too much consumption of them can become a double edged sword, since the over generation of reactive oxygen species in the body results in oxidative stress which can lead to cell damage [36].

Table 1 Percentage inhibition of DPPH radical of methanol extract of antimalaria tea

Concentration mg/ml	AMT	BHA	VIT C
1.0	-7%	71%	94.4%
0.5	39%	71%	93.4%
0.25	62%	69%	92.4%

Key: AMT – Antimalaria Tea. BHA – Butylated hydroxyl anisole. VIT C – Vitamin C.

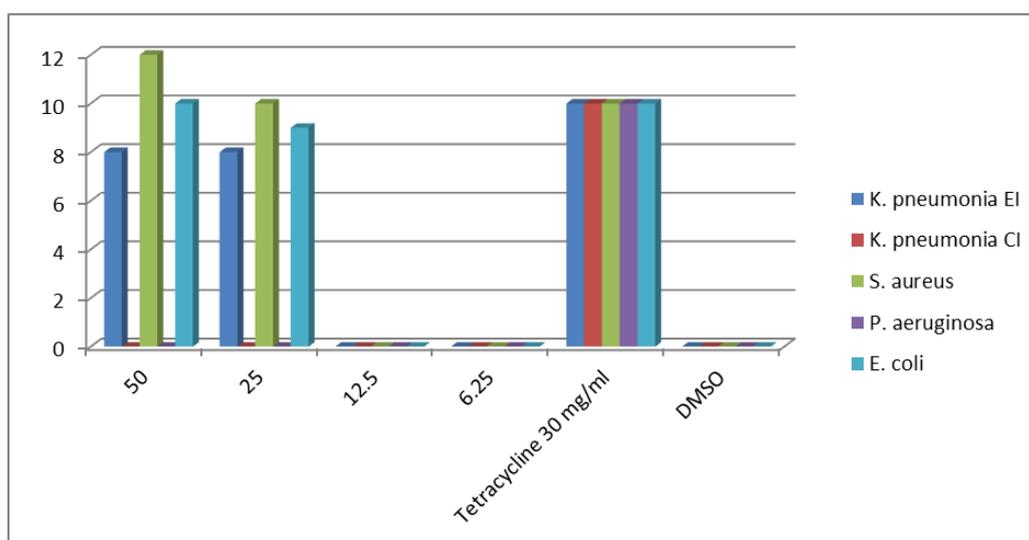
3.3. Antibacterial Activity

The antibacterial activities of the methanol extract of the antimalarial tea against a total of five bacterial are shown in Table 2. When the agar diffusion method was used, the extracts caused different inhibition zones, and had a varied antibacterial effect on the bacterial strains. *K pneumonia* and *P aeruginosa* were resistant to the extracts because at all concentration levels (50, 25, 12.5 and 6.25) they showed no zone of inhibition. All five bacterial showed no zone of inhibition at concentrations of 12.5 and 6.25 mg/ml. The extract at concentrations of 50 and 25 mg/ml was able to inhibit the growth of *S. aureus* to a level that was comparable to that of the standard tetracycline. Studies have shown that the individual plants extract of *Citrus aurantifolia*, *Psidium guajava* and *Ocimum gratissimum* has higher antibacterial activity as compared with that of the combined plants. Pathan *et al.* [28] reported the antibacterial effect of *C. aurantifolia* on the bacteria's studied in this work and they showed lower zones of inhibition. Qa'dan *et al.* [37] carried out a research of the antibacterial activity of *P. guajava* on *S. aureus* and the observed zones of inhibition were lower than that recorded in this study while the plant extract was not sensitive to *E. coli* and *P. aeruginosa*.

Table 2 Zones of inhibition (in mm) of antimalaria tea extract

Concentration of sample (mg/mL)	<i>Klebsiella pneumonia</i> (EI)	<i>Klebsiella pneumonia</i> (CI)	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
50	8	-	12	-	10
25	8	-	10	-	9
12.5	-	-	-	-	-
6.25	-	-	-	-	-
Positive control (30 mg/mL)	10	10	11	10	12
Negative control	-	-	-	-	-

Key: EI – Environmental isolate, CI – Clinical isolate, Positive control – Tetracycline, Negative control – Dimethyl sulphoxide (DMSO).

**Figure 1** Graphical representation of antibacterial activity of the antimalaria tea extract

4. Conclusion

The antimalaria tea of *citrus aurantifolia*, leaves and peels, *Psidium guajava* leaves and *Ocimum gratifolia* is a recipe that is rich in phytochemical, and could be useful in inhibiting the growth of *staphylococcus aureus* in the body. But at high concentration the antimalaria tea has no ability to scavenge free radicals in the body, so an excessive consumption of the tea is discouraged because at high concentration the tea could contribute to the generation of free radical in the body which could result in cancer, cardiovascular disease or premature ageing.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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