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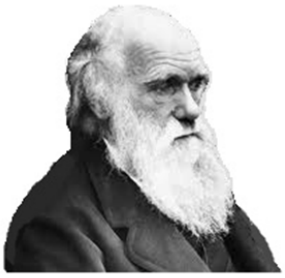

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Phytoconstitution and Antioxidant Activity of the Ethanolic Extract of an Antimalarial Herbal Mixture

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ABSTRACT

The phytoconstitution and antioxidant profiling of mixture of herbs used in the treatment of malaria has been carried out. Herbs used as alternative medicines in the local treatment of malaria were collected from a herbal vendor in Ibadan. The herbal materials were extracted using absolute ethanol and the crude extract, EEA subjected to qualitative phytochemical screening and 2,2-diphenyl-1-picrylhydrazyl (DPPH) standard procedures. The presence of alkaloids, saponins, tannins, terpeniods, flavonoids and phenols were identified. EEA was found to exhibit antioxidant activity in the dose range of 25-400 $\mu g ml^{-1}$. The significant presence of major series of phytochemicals has justified the associated antimalarial ethnomedicinal claim. Thus, the present study has established the phytoconstitution and antioxidant activity of an antimalaria herbal mixture.

Keywords: Phytoconstitution, antioxidant, herbal mixture

1. INTRODUCTION

The need to further establish medicinal data on alternative medicine is essential to the study and understanding of the phytoconstitution and antioxidant capacity of herbal mixture used in the treatment of malaria. This is vital as this will assist in signaling the therapeutic potential of herbal mixtures employed in the treatment of malaria.

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Herbal mixtures are often used in the treatment of various ailments in rural and sub-urban centres in developing countries like Nigeria. The herbal mixture is even being patronized due to affordability [1] with the global hike in the cost of drug substances and access to health facility in most remote rural communities especially in the Niger Delta region of Nigeria.

Furthermore, probing ethnomedicinal or the trado-graded herbal recipes for malaria is also vital in the discovery of new therapeutic agents. This will further contribute to existing pharmacological substances with antimalaria efficacy. The issue of multidrug resistance is another obvious reason to explore existing herbal recipes.

Antioxidant profiling of herbal mixtures is essential because oxidative stress induced malaria can be ameliorated via antioxidant substances. Ailments such as cataracts, mental dysfunction, diabetes, ageing , cancer and immune system compromise have been linked to oxidative stress [2-4]. The aim of the present study is to establish the phytoconstitution and evaluate the antioxidant activity of the ethanol extract of antimalaria herbal mixture.

2. MATERIALS AND METHODS

2. 1. Chemicals and Reagents

Chemicals and reagents used in the course of this work were products Sigma-Aldrich. They include: absolute ethanol, sulphuric acid, hydrochloric acid, wagner reagent, acetic acid anhydride, sodium hydroxide, aluminium chloride, iron (iii) chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), n-hexane and distilled water.

2. 2. Collection of Herbal Recipes

The antimalaria herbal recipe for the present work was sourced from local vendor with approved license for traditional medicine practice in the ancient city of Ibadan, Oyo state. The antimalaria recipe was a mixture of the leaves of eepodongoyaro (*Azadirachta indica*), ewe ejinrin (*Momordica charantia*), ewe efinrin (*Ocimum gratissimum*) and ewe owu (*Chromolaena odorata*).

2. 3. Extraction of Phytoconstituents

50 g of the dried herbal recipe consisting were macerated using 150 ml of absolute ethanol for 72 hrs. The ethanolic extract was concentrated with the aid of a rotator evaporator and labeled as EEA. The qualitative phytochemical screening and antioxidant profiling was then carried out on EEA.

2. 4. Qualitative Phytochemical Screening of EEA

Standard methods were used for the analytical qualitative phytochemical screening. The phytoconstitutions investigated include: alkaloids, saponins, tannins, steroids, flavonoids, cardiac glycosides and phenols.

2. 4. 1. Test for Alkaloids

1 mg EEA was stirred with 5 ml of 1% aqueous HCl on a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate was treated with a few drops

of Wagner's reagent (solution of iodine in Potassium iodide). The formation of a reddish-brown precipitate with Wagner's gives a positive test for alkaloids [5].

2. 4. 2 Test for Saponins

5 ml of aqueous EEA was transferred into test-tube. The mixture was vigorously shaken and few drops of olive oil were and further shaken. The formation of froth indicates the presence of saponins [6, 7].

2. 4 .3. Test for Tannins

2 ml of 5% ferric chloride was added to EEA. The appearance of a greenish black or dark blue coloration indicates the presence of tannins [8].

2. 4. 4. Test for Terpenoids (Salkowski Test)

2 ml EEA was mixed with 2 ml of $CHCl_3$ and 3 ml concentrated H_2SO_4 was carefully added. A reddish brown colouration at the interface was formed to show positive results for the presence of terpenoids [9].

2. 4. 5. Test for Flavonoids

Drops of dilute sodium hydroxide solution was added to ml of EEA in a 1:1, the presence of yellow colour which became colorless on adding dilute HCl indicates the presence of flavanoids [10].

2. 4. 6. Test for Glycosides (Keller – Killani Test)

10 ml EEA was treated with 2 ml of glacial acetic acid containing one drop of 2% ferric chloride solution, then few drops of concentrated sulphuric acid was added into the solution. A brown ring in between layers indicates the positive test for cardiac steriodal glycosides [7].

2. 4. 7. Test for phenols

1 ml of EEA was mixed with 2 ml of 5% iron (III) chloride solution. A dark blue coloration indicates the presence of phenols [10].

2. 5. Antioxidant Screening

The antioxidant activity of EEA was carried out using the 2,2-diphenyl-1-picrylhydrazyl, DPPH method with absorbance read at 517 nm in a concentration dose range from 25 – 400 $\mu g ml^{-1}$. The percentage inhibition was calculated using the relationship:

$$\% I = \frac{EEA_{BLANK} - EEA_H}{EEA_{BLANK}} \quad (1)$$

where:

% I – percentage inhibition,
EEA_{BLANK} – blank absorbance,
EEA_H – ethanolic extract absorbance [11]

2. 6. Statistical Analysis

Data obtained in the course of this work were in triplicates and expressed as mean±standard deviation. Statistical Package for Social Scientists (version 20.0) was used for data analysis.

3. RESULTS AND DISCUSSIONS

The result of the phytoconstitution of EEA is presented in Table 1. The qualitative phytochemical screening showed that the herbal mixture contains blend of alkaloids, saponins, tannins, terpeniods, flavonoids and phenols. The presence of these phytochemicals is a strong evidence of the antimalarial potential of the local herbal remedy. The alkaloids are series of phytochemicals whose chemistry is a function of the amino and other associated functional moieties. They have been implicated in nature to have therapeutic potentials [12]. Therapeutic characteristics of alkaloids include: cytotoxicity, analgesic, antisplamodic, antibacterial and anti-HIV [13-16].

A number of alkaloid series have been screened and reported to have shown promising antimalarial activity. These series include: terpenoidal, steroidal, indole, bisindole, quinoline and isoquinoline. Quinine, an alkaloid, was the first antimalaria agent isolated from the plant Cinchoma. Other examples of alkaloids with antimalaria potential include: caesalminines A (isolated from seeds of *Caesalpinia minax*), 8-α-polyveolinone, N-acetyl-8-α-polyveolinone, N-acetyl-8-α-polyveolinone (isolated from the stem bark of *Polyalthiaoliveri* [17-19].

Table 1. Phytochemical classes present in the herbal ethanol mixture

Secondary Metabolite	Observation
Alkaloids	+
Saponins	+
Tannins	+
Terpenoids	+
Flavanoids	+
Glycosides	-
Phenols	+

+ present, - absent

The antioxidant profiling (Table 2) reveals a direct relationship between the concentration of EEA and its percentage inhibition. The antioxidant activity increases as the concentration of the EEA increases. The presence of flavonoids is vital to the antioxidant characteristics of the

ethanolic extract. This is made possible by the lone pairs of electrons offered by the polyhydroxyl centres of flavoniods. Literature has shown that flavonoids have strong free radical scavenging mode. This is often achieved via inhibition of enzymes and chelation of trace elements involved in free radical generation, thus, terminating reactive oxygen species, ROS formation [20]. Literatures have also shown that flavonoids offer pharmacological activities such as: hepatoprotective, anticancer and anti-inflammatory activitivies [21, 22].

Table 2. Antioxidant screening of volatile extract

Conc. (µg/mL)	1	2	3	Mean	Sd	Mean±Sd
400.00	29.97	27.95	30.05	29.32	1.19	29.32±1.19
200.00	28.03	27.95	28.18	28.05	0.12	28.05±0.12
100.00	27.56	27.64	27.25	27.48	0.21	27.48±0.21
50.00	27.17	27.33	27.33	27.28	0.09	27.28±0.09
25.00	26.86	26.86	26.86	26.86	0.00	23.34±0.00

*SD-standard deviation

4. CONCLUSION

Herbal mixture being used in the treatment of malarial has been justified with the established series of phytochemicals. The presence of alkaloids amongst the phytochemicals is a strong precursor for such antimalarial activity. Polyhydroxyl units in identified phytochemicals such as the flavonoids, phenols and tannins are inherent factor responsible for the antioxidant activity of the herbal mixture.

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