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Antioxidant Activity of Some Nigerian Medicinal Plants Used in Malaria Treatment

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ABSTRACT

Free radicals are reactive molecules associated with many diseases including malaria; causing complications. Thus, the need to explore compounds with free radical scavenging properties. Methanol extracts of the leaves of three medicinal plants (*A. barteri*, *O. basilicum* and *H. indicum*) used in Nigerian folkloric medicine for the treatment of malaria were evaluated for their antioxidant activty, total phenol and flavonoid contents. The antioxidant activity evaluation included various radicals or oxidation systems - ferric-reducing antioxidant power (FRAP), 2, 2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) radical (ABTS), and oxygen radical absorbance capacity (ORAC) assays. Total phenol and flavonoid contents were also evaluated. The leaves of *A. barteri* showed the highest levels of total phenol (222.30 ± 5.48 mg gallic acid equivalent/g), flavanol (22.90 ± 0.15 mg catechin equivalent/g) and flavonol contents (93.32 ± 2.80 mg quercetin equivalent/g) compared to *O. basilicum and H. indicum*. The antioxidant activity of these plants increased with increase in their total phenol and flavonoid contents. The order of the antioxidant activity of the plants was *A. barteri* > *O. basilicum* > *H. indicum*. These results suggest that the leaves of these plants contain polyphenols and could serve as potential sources of antioxidants which could be explored as therapeutic agents in the attenuation of free radical in malaria infection.

***Keywords*:** *Alafia barteri*, *Ocimum basilicum*, *Heliotropium indicum*, antioxidant activity, free radical, malaria.

Introduction

Free radicals such as reactive oxygen species (ROS) are continuously produced by an organism's normal use of oxygen.[1] There are ample evidence that an imbalance between formation and removal of these free radicals can lead to a pathological condition called oxidative stress resulting in many physiological processes like aging and chronic diseases, such as cancer, arthritis, cardiovascular diseases and liver injury. However, the human body employs molecules known as antioxidants to counteract these free radicals thus repairing free radical damage by initiating cell regeneration or cell repair.[2, 3] This has led to keen interest and widespread researches in the investigation of plant materials with antioxidant activity that can protect against these reactive oxygen species such as superoxide radical, hydroxyl radical, peroxyl radical, and nitric oxide radical and thus may play a role in disease prevention.[4, 5]

In recent times, studies have shown that free radicals associated with oxidative stress play crucial role in the development of complication caused by malaria.[6] Plants contain high concentrations of antioxidants, including polyphenols, tocopherols, tocotrienols, ascorbic acid, glutathione, carotenoids, which are believed to be the effective nutrients in the prevention of oxidative stress-related diseases.[7, 8] A number of traditional herbs including the leaves of *Mangifera indica*, *Artemisia annua*, *Carica papaya*, *Azadirachta indica*,, *Melissa officinalis*, *Alafia barteri*, *Ocimum basilicum* and *Heliotropium indicum* have been tested and used in the prevention and treatment of malaria.[9-11] It is assumed that these plants may have antioxidant activity that would scavenge free radicals associated with malaria thus attenuating possible complications that may occur in malaria.

Alafia barteri (*Olive*), a member of the Apocynaceae family, is a climbing shrub widely distributed in the tropics. It is commonly known as Guinea fowls crest, *agbari etu* (Yoruba), *otanza* (Igbo). In Nigerian traditional medicine, the stem and root decoctions are used to treat rheumatic pains, toothache and eye infections.[12] The infusion of the leaves of this plant is used to treat sickle cell anaemia and malaria. The fibre of the stem is used as binding material for roofs, the roots are used as chewing stick; latex obtained from the stem has been used to adulterate for better latex by tampering with the composition of latex.[11]

Heliotropium indicum (Linn) family Boraginaceae is an annual plant that can grow to a height of 15 to 50 cm. It is known as Indian Heliotrope, cocks-comb, *ogbe ori akuko* (Yoruba), *kalkashin korama* (Hausa), *azu uzo* (Igbo). It has small white flowers with a green calyx; it is usually associated with the moist rich soils of the lowland tropics near rivers and lakes, on the roadsides and in waste places. It is locally used for malaria fever, skin lesions, wounds, abscesses, gastric and varicose ulcerations, rashes and warts.[13]

Ocimum basilicum, a member of the Lamiaceae family, is known as an aromatic and medicinal plant and is widely cultivated as an ornamental and field crop throughout the year in many countries. It is popularly known as sweet basil and locally as *efirin wewe* (Yoruba), *ntong* (Efik), *Nchanwu* (Igbo) or *daidoya* (Hausa). It has several therapeutic potentials and serves as a basic component of the Mediterranean diet. The leaves of *O. basilicum* are used in folk medicine as a tonic and vermifuge and an infusion of its leaves is used for the treatment of nausea, flatulence and dysentery. The oil of the plant has been found to be beneficial for the alleviation of mental fatigue, cold, spasm and rhinitis and as a first aid treatment for wasp stings and snakebites.[14, 15]

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Following reports that free radicals are involved in malaria disease [16] and the knowledge that plants contain antioxidants which are known to scavenge free radicals; there is need to explore plant materials that are used in the treatment of malaria for their free radical scavenging and or antioxidant activity. The present study was therefore designed to evaluate the free radical scavenging activity of methanol extract of the leaves of *Alafia barteri, Ocimum basilicum* and *Heliotropium indicum* using various *in-vitro* analytical methods.

Materials and Methods

Chemicals

The chemicals and reagents used were of analytical grade obtained from Sigma Aldrich (Germany) unless otherwise stated. Potassium peroxodisulphate, Folin-Ciocalteu's reagent, dihydrogen sodium phosphate buffer and DMACA (4-Dimethylamino-cinnamaldehyde) were obtained from Merck (Germany).

Plant collection and extract preparation

The leaves of *A. barteri, H. indicum* and *O. basilicum* were harvested from Ifo, Ogun state, Nigeria in June 2014. They were authenticated in the herbarium unit of the Department of Botany, University of Lagos, Nigeria. Voucher specimens with voucher numbers LUH 6465, LUH 3672 and LUH 5562 for *A. barteri, H. indicum* and *O. basilicum*, respectively were deposited. The plant materials were dried at room temperature (25 ± 3˚C) for 7 days and powdered using a domestic blender. The dried powdered plant materials (500 g each) were extracted thrice by percolation in 1000 mL methanol for 24 h with constant stirring. Each extract was filtered and the combined filtrate was concentrated in vacuum at 40˚C using a rotary evaporator at reduced pressure. Thereafter, the crude extracts were air-dried. The extract residues were stored in impervious air-tight containers at -20˚C until used.

Phytochemical analysis

Phytochemical screening of the crude extracts of the plants was done using standard procedures.[17, 18]

Determination of total phenol content

Total phenolic contents were determined with Folin-Ciocalteu reagent (FCR) using the method of Shaver *et al*.,[19] with slight modifications. Briefly, 25 µL of the extracts (5 mg/mL in methanol) were mixed with 125 µL of Folin–Ciocalteu reagent and allowed to stand at room temp for 5 min; 100 µL of Na_2CO_3 (75 g/L) solution were then added to each micro plate. The mixtures were allowed to stand for 2 hr and the absorbance was measured at 725 nm. A calibration curve was prepared for gallic acid at concentrations 0 - 500 mg/L and total phenol content was extrapolated from the generated gallic acid calibration curve. Results were expressed as milligram gallic acid equivalent per gram extract (mg GAE/g extract).

Determination of flavonoid content

Determination of flavanols

The flavanol content in the extracts was determined according to the method described by Treutter, 1989 with slight modifications.[20] Briefly, 50 µL of extracts (5 mg/mL in methanol) were mixed with 250 µL of 4-Dimethylamino-cinnamaldehyde. The absorbance of each mixture was read at 640 nm after 30 min. A calibration curve was prepared for catechin at concentrations 0 - 27.2 mg/L and total flavanol content was extrapolated from the generated catechin calibration curve. Results were expressed as milligram catechin equivalent per gram extract (mg CE/g extract).

Determination of flavonols

Total flavonols in each extract was determined using the method of Srivastava *et al*.,[21] Briefly, 1.0 mL of 2% $AlCl_3$ in methanol and 1.5 mL of 5% sodium acetate solutions were added to 12.5 µl of each extract (5 mg/mL in methanol) solution. Each mixture was incubated in water bath at 37˚C for 30 min and absorbance taken at 440 nm. A calibration curve was prepared for quercetin at concentrations 0 - 100 mg/L and flavonol content was estimated by extrapolated from the standard curve. Results were expressed as milligram quercetin equivalent per gram extract (mg QE/g extract).

Antioxidant activity

ABTS Radical Scavenging activity

The relative abilities of antioxidants to scavenge ABTS radical cation ($ABTS^{\bullet+}$) were measured by comparison with the antioxidant potency of standard amounts of trolox (6-hydroxy-2, 5, 7, 8-tetramethyl chroman-2-carboxylic acid) following the method of Shirwaikar *et al*.,[22] $ABTS^{\bullet+}$ was prepared by a reaction between 5 mL of 0.0192 g of $ABTS^{\bullet+}$ in distilled water and 264 µL of the potassium persulphate solution and incubated in a dark room for 18 h at room temperature. Twenty-five micro liters of the extracts (5 mg/mL in methanol) were mixed with 300 µL of $ABTS^{\bullet+}$ solution. The micro plates were left for 30 min at room temperature and absorbance measured at 734 nm. A standard curve was obtained with trolox standard solution at various concentrations (0 - 1000 µM). The scavenging potential of the extracts was measured by extrapolation from trolox standard curve. Results were expressed in terms of Trolox Equivalent Antioxidant Capacity (TEAC), as micro molar trolox equivalents per gram extract (µM TE/g extract).

Oxygen radical absorbance capacity assay

The oxygen radical absorbance capacity (ORAC) assay measures the antioxidant inhibition of peroxyl radical (˙OOH) induced oxidations, thus reflects classical radical chain-breaking antioxidant activity by hydrogen atom transfer. The method followed the work of Alarcon *et al*.,[23] In the procedure, 12 µL of the extracts (5 mg/mL in methanol) were mixed with 138 µL of 10 µL fluorescein stock solution in 2 mL phosphate buffer, 240 µL of this solution diluted in 15 mL phosphate buffer and 50 µL of 2, 2˙-azobis-(2-methylpropionamidine) dihydrochloride: AAPH (150 mg AAPH and 6 mL phosphate buffer). The Area under the curve (AUC) was measured using a fluorescence microplate reader. A standard curve was generated using the AUC for trolox (0 - 1000 µM) and trolox equivalents of the sample was calculated using calibration function ($y = 15.272x + 21.396$; $R^2 = 0.985$; where y = trolox concentration (µM) and x = net area under the fluorescence decay curve). Results were expressed in µM TE/g extract.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay uses oxidation/reduction reaction to measure the ability of a sample to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). Procedurally, FRAP reagent was generated by mixing 25 mL of 0.3 M sodium acetate buffer (pH 3.6), 2.5 mL of 10 mM tripyridyltriazine (TPTZ) solution in HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$. Then 10 µL of the extracts (5 mg/mL in methanol) were mixed with 300 µL of the FRAP reagent. The test tubes were left for 30 min at room temperature and absorbance measured at 593 nm.[24] Ascorbic acid was used as standard (0 - 1000 µM) and results expressed in µM/g extract as extrapolated from the calibration function.

Statistical analysis

The results are reported as mean ± standard deviation (SD). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were considered significant at $P < 0.05$.

Results and Discussion

This study investigated the total phenol and flavonoid contents as well as the antioxidant activity of three Nigerian medicinal plants mostly used in the treatment of malaria among traditional healers. Methanol had been reported to be the most suitable solvent for extracting phenolic compounds from plant materials compared with chloroform, ethyl acetate and water, [25, 26] thus, this study employed methanol as solvent for extraction. Phytochemical tests of the plants showed the presence of various secondary metabolites (Table 1), the abundant presence of flavonoids in these plants may explain their possible effectiveness as antioxidants in malaria treatment and other ailments related to oxidative stress. Flavonoids are the most abundant polyphenols and are known for their role in ameliorating excess free radicals, which are involved in biological activities including anti-carcinogenic, anti-inflammatory, anti-atherosclerosis and antimalarial activities.[28]

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Table 1: Phytochemical tests of the crude extracts.

Plant materials	Phytochemical tests								
	Anthraquinone	Tannin	Flavanoid	Terpenoid	Cardiac glycoside	Saponin	Alkaloid		
							Wagner	**Mayer**	**Dragendoff**
O basilicum	-	+	+	+	-	+	+	+	+
A. barteri	+	+	+	+	+	+	-	-	-
H. Indicum	-	+	+	-	-	+	+	+	+

+: Indicates presence -: Indicates absence of phytochemical constituent.

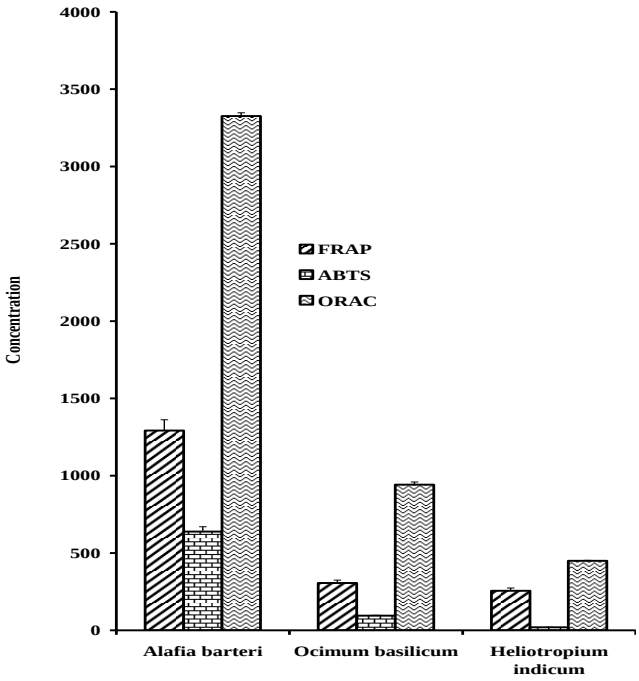

Figure 1: Antioxidant activity of methanol extract of *Alafia barteri*, *Ocimum basilicum* and *Heliotropium indicum.* Values are mean±SD, n = 3. FRAP is expressed as µmole/g extract; $ABTS^{\bullet+}$ and ORAC expressed as µmole TE/g extract.

The leaves of these plants showed rich polyphenolic contents (flavonoids and phenol), with the leaves of *A. barteri* showing significantly ($p < 0.05$) higher content of phenol, flavanols and flavonols (222.30 ± 5.48 mg, 22.90 ± 0.15 mg and 93.32 ± 2.80 mg, respectively) compared to the leaves of *O. basilicum (*44.59 ± 3.18 mg, 14.24 ± 0.48 mg and 88.17 ± 2.31 mg) and *H. indicum* (6.58 ± 4.80 mg, 14.0 ± 2.05 mg and 82.69 ± 4.29 mg) (Table 2). The high content of these phenolic secondary metabolites may be responsible for the high antioxidants potentials of these plants, thus their significant free radicals scavenging activity. Phenolic antioxidants are important plant constituents, efficient free-radical scavengers and help to relieve oxidative stress partly by their ability to act as hydrogen or electron donors, metal chelators, or singlet oxygen quenchers thus preventing free radicals from damaging biomolecules such as proteins, DNA, and lipids by breaking the chain reaction of lipid peroxidation at the initiation stage.[28, 29]

Antioxidant activity has been proposed to be related to reducing power. Therefore, the antioxidant activity of the crude extracts of *A. barteri, O. basilicum* and *H. indicum* were investigated for their ability to reduce ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) (Figure 1). FRAP assay has been used for the assessment of antioxidant activity of various food products. It is suggested to be essential and effective in the assay for antioxidant activity

Table 2: Total phenolic, flavanols and flavonols contents of three Nigerian medicinal plants used in folkloric medicine.

Plant materials	Total Phenolic (mg GAE/g extract)	Flavanols (mg CE/g extract)	Flavonols (mg QE/g extract)
O. basilicum	44.59 ± 3.18 [a, b]	14.24 ± 0.48 [a]	88.17 ± 2.31 [a]
A. barteri	222.30 ± 5.48 [b]	22.90 ± 0.15 [b]	93.32 ± 2.80 [b]
H. Indicum	6.58 ± 4.80 [c,b]	14.0 ± 2.05 [c]	82.69 ± 4.29 [c]

Values are expressed as mean ± standard deviation. Different superscript letters indicate significant different ($P < 0.05$). GAE = gallic acid equivalent, CE = catechin equivalent, QE = quercetin equivalent.

since most of the secondary metabolites are redox-active compounds that can be picked up in the FRAP assay.[30] The highest ferric reducing antioxidant power (FRAP) was evident in *A. barteri* (1292.10 ± 69.77 µM/g) followed by *O. basilicum* (305.68 ± 19.21 µM/g). The $ABTS^{\bullet+}$ scavenging activity also reflects hydrogen-donating ability. It is known that high molecular weight phenolics (tannins) have more ability to quench $ABTS^{\bullet+}$.[31] The low $ABTS^{\bullet+}$ scavenging activity of these plants especially of *O. basilicum* and *H. indicum* may be explained from the trace amount of tannins present in their leaves. However, the scavenging activity of *A. barteri* was significantly higher ($p < 0.05$) than *O. basilicum* and *H. indicum*. In the oxygen radical scavenging assay, *A. barteri* was shown to have a particularly high oxygen radical absorbance capacity (Figure 1). The order of scavenging activity was *A barteri* (3326.09 ± 20.87 µM TE/g extract) > *O. basilicum* (941.98 ± 47.75 µM TE/g extract) > *H. indicum* (449.80 ± 2.83 µM TE/g extract). In biological system, there is large production of peroxyl radical which would interfere with biological activities especially in malaria infection. The results of this study in the scavenging of oxygen radicals may also well explain the good use of *A. barteri* in scavenging free radical in malaria disease in folkloric medicine. The implications of free radicals through oxidative stress in the physiopathogenesis of malaria has been documented.[16,32,33] Malaria parasites release free radicals causing oxidative stress during the part of their life cycle when they inhabit erythrocytes. As the infection progresses, they also activate macrophages, one consequence of which is extracellular release of reactive oxygen species (ROS), with the propensity of inducing oxidative damage and cell destruction.[34-36] A study by Sohail *et al*.,[37] reported a significant decrease in the level of antioxidant enzyme, glutathione-S-transferase (GST), and increase in lipid peroxidation during pathology of *Plasmodium vivax* malaria. Treatment of malarial infection with chloroquine, has been shown to significantly increase the level and activities of superoxide dismutase (SOD) and catalase (CAT) in experimental animals,[38] however, some workers reported that chloroquine, primaquine and derivatives of artemisinin are inducers of free radical production during malarial treatment.[39-41] In all these, the body has a number of defense mechanisms including the production of antioxidants, glutathione peroxidase (GSH-Px), CAT, SOD, glutathione, glutathione reductase, glutathione S-transferase and glucose 6-phosphate dehydrogenase, to reduce the effects of free radicals.[42]

Conclusion

Finding from this study has shown that *A. barteri* leaves have good antioxidant activity and when properly harnessed could serve as a good source of antioxidant supplement against free radical-induced diseases.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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