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Phytochemical, Cytotoxicity and Antioxidant Activities of Five Anti-malaria Plants

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ABSTRACT

The study investigated the phytochemical, cytotoxicity and antioxidant properties of *Allamanda cathartica* (AC), *Bixa orellana* (BO), *Cymbopogon citratus* (CC), *Ficus exasperata* (FE) and *Momordica charantia* (MC) used traditionally for the anti-malarial preparations “Agbo” in Nigeria. Phytochemical screening of the plants showed the presence of flavonoids, terpenoids, phenolics, cardiac glycosides and reducing sugars. Free radical scavenging activity of the plants with 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) recorded significant IC_{50} values for the inhibition of DPPH by ethanolic leaves extracts of AC (0.46), BO (0.45), CC (1.35) and FE (0.86), respectively and Vit. E (control), recorded higher activity at 0.5 mg mL^{-1} with an IC_{50} of $0.25 \text{ } \mu\text{g mL}^{-1}$. BO leaf extracts recorded the most potent effect (0.45) at low concentration of 0.5 mg mL^{-1} . The free radical scavenging activities of these plants doubtlessly contribute to their use in indigenous malaria therapy and may qualify them for anti-malarial drug screening.

Key words: Phytochemicals, cytotoxicity, antioxidants, anti-malaria, DPPH

INTRODUCTION

Several natural products are reported to contain large amounts of antioxidants other than vitamin C and E (Javanmardi *et al.*, 2003). Plants are rich in a wide variety of secondary metabolites such as alkaloids, flavonoids, terpenoids and saponins. Of these metabolites, phenolic compounds such as flavonoids (Pietta, 1998), phenolic acids and phenolic diterpenes (Shahidi *et al.*, 1992) have received increased attention as useful pharmaceuticals in managing diseases like malaria.

Free radicals, reactive oxygen species and reactive nitrogen species are associated with many pathological conditions such as inflammation, metabolic disorders, cell ageing and carcinogenesis (Robak *et al.*, 1998; Ames *et al.*, 1993). A study by Yildirim *et al.* (2001) implicated reactive oxygen species in several diseases including malaria. These antioxidants play a role in delaying, intercepting or preventing oxidative damages catalyzed by free radicals (Velioglu *et al.*, 1998).

Malaria infection is severe because anaemia develops alongside, other haematological changes like thrombocytopenia and increased erythrocytic adenosine deaminase activities (Mannel and Grau, 1997; Erel *et al.*, 1997). Oxidative stress appears to be one of the major factors responsible for anaemic conditions in malaria patients (Das and Nanda, 1999; Kreamsner *et al.*, 2000). Most infections, including malaria, instigate the release of reactive oxygen species by the immune system

of the body. Besides the host's immune system response, malaria parasite also stimulates certain cells to produce reactive oxygen species resulting in haemoglobin degradation (Das and Nanda, 1999; Loria *et al.*, 1999; Kremsner *et al.*, 2000).

Malaria is a global disease, with an estimated 1-2 million persons affected every year (Saxena *et al.*, 2003) and with high mortality rate in children and pregnant women, particularly in developing tropical countries like Nigeria, calls for increased attention. Traditional management of malaria in Nigeria and in most West African region involves the uses of a mixture of plants for anti-malarial formulations called "Agbo" among the locals (Conrad and Uche, 2013). Earlier, Sofowora (1994) identified *Azadirachta indica*, *Alstonia boonei*, *Carica papaya* and *Mangifera indica* has local plant resources for anti-malarial preparation in Nigeria. However, the list of potential anti-malarial plants is an ever-increasing one. The present study attempts to find out the relevance of adding five plants species to this list by indigenous practitioners, through the analyses of the phytochemical constituent, phytotoxicity and reactive species harvesting potentials of the plant species.

MATERIALS AND METHODS

Plant materials: *Allamanda cathartica* Linn. (AC), *Bixa orellana* L. (BO), *Cymbopogon citratus* (DC. ex Nees) Stapf. (CC), *Ficus exasperata* vahl. (FE) and *Momordica charantia* L. (MC) leaves were collected in Southern Nigeria from June-August 2011. The plant materials were air dried at room temperature (28-32°C) for two weeks and blended into uniform powder, using a Waring™ Laboratory (Two speed, Timer) Blender and stored in airtight containers.

Preparation of plant extracts: The powdered plant materials were extracted with ethanol at room temperature for 48 h. The extracts were filtered successively through Whatmann filter paper No. 42 and cotton wool, air dried and concentrated afterward, using a water-bath rotary evaporator at 40°C.

Phyto-constituents screening of plant extracts: The plant materials were screened for phyto-constituents using standard procedures as described by Trease and Evans (1989) and Sofowora (1994). Total phenolic content was determined as Gallic Acid Equivalent (GAE) according to Folin and Ciocalteu (1927).

***In vitro* antioxidant activity (1,1-Diphenyl-2- Picrylhydrazyl (DPPH) radical scavenging assay:** The radical scavenging activity of the plant extracts against 2, 2-Diphenyl-1-picrylhydrazyl radical (Sigma-Aldrich) was determined by measuring UV absorbance at 517 nm. The reaction mixture contained 1.0 mL of 1 mM DPPH-ethanol solution, 1.0 mL of methanol (absolute), Tris-HCl buffer (pH 7.4) 450 and 50 µL of the extracts.

A modified protocol by Naito *et al.* (1994) was used for the tests. Triplicates of tests solutions (extracts and standard-Vit. E) were prepared with vary concentrations of 0.5, 1.0, 2.0 and 5.0 mg mL⁻¹ in 1 mL of methanol (Sigma-Aldrich, Analar grade); followed by adding 1.0 mL of 1 mM DPPH solution. The test solutions (extracts and VE) were incubated for 30 min at 37°C. The blank solution contained equal amounts of methanol and DPPH. The absorbance of the tests and blank solutions were measured at UV-517 nm.

The DPPH free radical scavenging activity was calculated using the following formula:

$$\text{Scaavenging (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of tast sample}}{\text{Absorbance of control}} \times 100$$

The effective concentrations of sample required to scavenge DPPH radical by 50% (IC₅₀ value) were generated by linear regression analysis of the dose-response curve plotting between percentage inhibition and concentrations (Iranshahi *et al.*, 2009).

Statistical analysis: The data generated was analysed on Graphs and Standard Deviation (SD) and significance computed with Microsoft Excel (2007) for Windows and Cgi-bin (www.physics.csbsju.edu/cgi-bin/stats/anova).

RESULTS AND DISCUSSION

Phytochemical test: Plant phytochemicals such as phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers (Kahkonen *et al.*, 1999). The Phytochemical profiles of the plants investigated, showed the presence of alkaloids, saponins, flavanoids, terpenoids, phenolics and reducing sugar for all the plants (Table 1). The presence of phenolics and flavonoids demonstrates the plants as potential sources of antioxidants (Dawidowicz *et al.*, 2006).

Total phenolic content was obtained from the regression equation of the calibration curve of Gallic acid (Absorbance = 0.0011(Concentration)) and expressed as GAE. Total phenolic content was recorded as 1063.6±0.001, 1200.9±0.001, 11.8±0.001, 208.1±0.0005 and 568.1±0.0005 mg L⁻¹ for AC, BO, CC, FE and MC, respectively (Table 1) with BO recording higher phenolic concentrations.

Quantitative estimates of phytochemical content of leaves, root and stem bark of *Aegle marmelos* by Siddique *et al.* (2010) showed total phenolic content in the plant; varied from 21.39±0.927-46.28±0.543 mg g⁻¹, flavonoids content varied from 13.30±0.684-26.03±0.217 mg g⁻¹ and tannin content varied from 8.72±0.160-17.04±0.206 mg g⁻¹.

Ergastic active phytochemical constitution and antioxidant activity: There have been increasing advances in our understanding of the roles that flavonoids play in developmental processes of plants. The multiple cellular roles of flavonoids can reflect their chemical diversity, or might suggest that cellular targets shared between many of these disparate processes exist in cells (Taylor and Grotewold, 2005).

The AC, BO and FE recorded significant suppressive effect, similar to VE for the 0.5 mg mL⁻¹ treatment and for the 1.0 mg mL⁻¹ treatment (Fig. 1). As expected, VE (0.05-5.0 mg mL⁻¹), treatments also recorded significant suppressive effects (Fig. 1 and 2).

Table 1: Phytochemical constituents of ethanolic extracts of five plants

Phytochemical tests	AC	BO	CC	FE	MC
Alkaloids	+	+	-	-	+
Cardiac glycosides	-	-	-	+	+
Flavonoids	+	+	-	+	+
Phenols	+	+	+	+	+
Saponins	+	+	-	+	+
Sugars (reducing)	+	+	+	+	+
Tannins	-	-	-	+	-
Terpenoids	+	+	+	+	-
Total phenolic (concentration = mg mL ⁻¹)	1063.6	1200.9	711.8	208.1	568.1
Mean±SD	1.17±0.001	1.32±0.001	0.78±0.001	0.23±0.0005	0.63±0.0005

+: Positive, -: Negative, AC: *A. cathartica*, BO: *B. orellana*, CC: *C. citratus*, FE: *F. exasperata* and MC: *M. charantia*

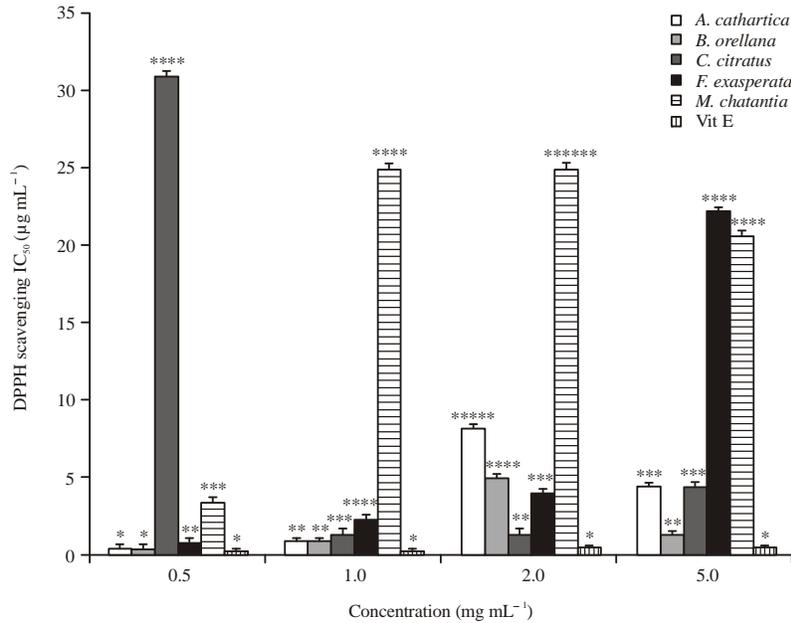


Fig. 1: Inhibition of DPPH by plant extracts and vit. E at 0.5, 1.0, 2.0 and 5.0 mg mL⁻¹ concentrations (AC, BO, CC and Vit E recorded significant values for 0.5 mg mL⁻¹; AC, BO and Vit E for 1.0 mg mL⁻¹; CC and Vit E for 2.0 mg mL⁻¹; BO and Vit E for 5.0 mg mL⁻¹ treatments)

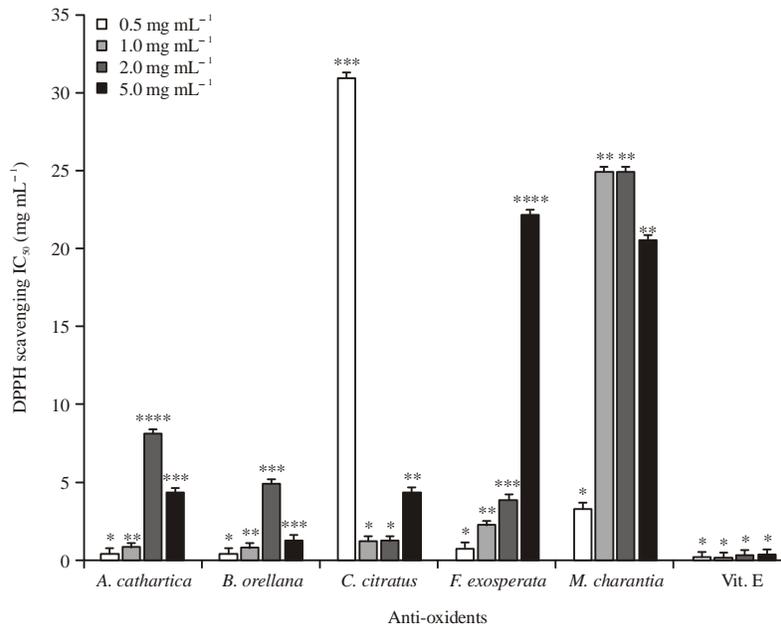


Fig. 2: Plant extract: *A. cathartica*, *B. orellana*, *C. citratus*, *F. exasperata*, *M. charantia* and vit. E inhibition effect at various concentration on DPPH. Vit. E treatments: 0.5 mg mL⁻¹ concentration of AC, BO, FE and 1.0 mg mL⁻¹ concentration of AC, BO, CC showed appreciable DPPH inhibition properties

The 0.5 mg mL⁻¹ pre-treatment recorded significant effects for AC, BO and FE (Fig. 2). However, the 0.5 mg mL⁻¹ pre-treatment for CC and MC were inconsistent with results reported for the others three plants under the same concentrations. The MC extracts recorded the least suppressive effect for any of the treatments. The 2.0 and 5.0 mg mL⁻¹ treatments recorded poor suppressive effects. This implies that administering high concentrations of the extracts were not effective.

In the study by Siddique *et al.* (2010), the free radical scavenging activity among the selected samples varied from 11.67±0.221-38.29±0.532 µg mL⁻¹. The correlation between the free radical scavenging activity with total phenol content (R² = 0.511), flavonoids (R² = 0.241) and tannins (R² = 0.690) showed tannins possess greater free radical scavenging strength in the selected plants.

Song *et al.* (2010) investigated the antioxidant activities of sulfated hetero-polysaccharides extracted from *Bryopsis plumosa* using various established *in vitro* systems. Data generated suggest that among the three samples, B3 (extraction with sodium hydroxide) showed significant inhibitory effects on superoxide radical and DPPH with IC₅₀ values of 9.2l and 1.7 g L⁻¹, its reducing power was also the strongest among the three samples. These *in vitro* results, establish the clear possibility that polysaccharides extracted from *B. plumosa* could be use as effective ingredient in health or functional food to reduce oxidative stress.

Tyrosinase inhibitory activities of *A. cathartica* extracts were examined with new compounds isolated and identified by series of chromatography, NMR and LC-MS. Among the extracts, glabridin recorded higher tyrosinase inhibitory activity (IC₅₀:2.93 µM), which is 15 times stronger than the positive control-Kojic acid (IC₅₀:43.7 µM) (Yamauchi *et al.*, 2011).

A wide range of compounds in plants has been shown to possess bioactivity potentials and can be employed for antioxidant purposes, where the phytotoxicities are within permissible levels. The prevalence of flavonoids and phenols in the plants species particularly for AC (1063.6 mg mL⁻¹) and BO (1200.9 mg mL⁻¹) may be responsible for the antioxidant activity levels recorded for the plant extracts even at low concentrations.

Dose-dependent anti-oxidant activity: The DPPH scavenging activity of the various ethanolic extracts (Fig. 1) recorded different concentrations in the IC₅₀ estimation. Optimum IC₅₀ values of 0.46±0.002 (0.5 mg mL⁻¹), 0.45±0.002 (0.5 mg mL⁻¹), 1.35±0.003 (1.0, 2.0 mg mL⁻¹), 0.86 (1.0 mg mL⁻¹) and 0.25 mg mL⁻¹ (0.5 mg mL⁻¹), were recorded for AC, BO, CC, FE and VE, respectively. The majority of the plants recorded low IC₅₀ values at 0.5 mg mL⁻¹ concentration; this shows the high antioxidant potency of the plants. The BO recorded higher phenolic content of 1200.9 mg L⁻¹ and this correlates with the DPPH scavenging IC₅₀ (µg mL⁻¹) activity of the plant. The DPPH assay analysis shows that AC (0.46±0.002 mg mL⁻¹), BO (0.45±0.002 mg mL⁻¹), CC (1.35±0.003 mg mL⁻¹) and FE (0.86±0.002 mg mL⁻¹) possess high antioxidant potentials. The AC, BO and FE recorded higher activities at 0.5 mg mL⁻¹ dose with VE (the standard) recording an IC₅₀ value at 0.25 µg mL⁻¹ concentrations. The antioxidant activities of the plant extracts are therefore, dose-dependent and this confirms the plants are good sources of dose-dependent phytochemicals for managing malaria.

Earlier studies on assessment of *in vivo* antioxidant properties of *Dacryodes edulis*, *Ficus exasperata*, *Allamanda cathartica* and *Bixa orellana* as anti-malaria plants (Conrad and Uche, 2013; Conrad *et al.*, 2013) showed a dose-dependent effects of the plant extracts on the enzymes; catalase, glutathione and the thiobarbituric acid reactive substances (TBARS).

In the study by Ayoola *et al.* (2008) to analyse four common malaria plants; the concentrations of the plant extracts required for 50% inhibition of DPPH radical scavenging effect (IC_{50}) were recorded as 0.04, 0.313, 0.58, and 0.054 mg mL⁻¹ for *P. guajava*, *M. indica*, *C. papaya*, *V. amygdalina* and vit. C, respectively. These plants showed potent inhibition of DPPH radical scavenging activity, *P. guajava* being the most potent. The antioxidant activities of the four plants are similar to those of the plants species investigated in the present study. The species; *M. indica*, *P. guajava*, *C. citratus* and *A. cathartica* have been cited in literature as common ingredients in folklore malaria medications in Nigeria and West Africa (Sofowora, 1994).

Similarly, in Western Cameroon, seven plants with anti-plasmodial activity and cytotoxicity were analysed using Lactate Dehydrogenase Assay (pLDH) and the cytotoxicity estimated on LLC-MK2 monkey kidney epithelial cells (Zofou *et al.*, 2011). *Dacryodes edulis* leaves recorded higher activity (IC_{50} of 6.45 μ g mL⁻¹ on 3D7 and 8.72 μ g mL⁻¹ on DD2) followed by *Vernonia amygdalina* leaves (IC_{50} of 8.72 and 11.27 μ g mL⁻¹ on 3D7 and DD2 resp.), *V. amygdalina* roots (IC_{50} of 8.72 μ g mL⁻¹ on 3D7), *Coula edulis* leaves (IC_{50} of 13.80 and 5.79 μ g mL⁻¹ on 3D7 and DD2 resp.), *Eucalyptus globulus* leaves (IC_{50} of 16.80 and 26.45 μ g mL⁻¹ on 3D7 and DD2) and *Cuviera longiflora* stem bark (IC_{50} of 20.24 and 13.91 μ g mL⁻¹ on 3D7 and DD2). These findings justify the use of the plants in malaria treatment by traditional healers of Western Cameroon.

An earlier report by Fennell *et al.* (2004) showed 49% of 134 South African plant species investigated using pLDH, had promising anti-plasmodial activity ($IC_{50} \leq 10$ μ g mL⁻¹) against *Plasmodium falciparum* strain D10. Bhaskar *et al.* (2012) recorded higher reducing power for methanolic and aqueous extracts of *Cassiaroburghii* (IC_{50} value of 1.01 and 0.55 at 1 mg mL⁻¹) than for ascorbic acid (0.91 at 1 mg mL⁻¹).

In the study by Khonkarn *et al.* (2010) to test for the antioxidant activity and the cytotoxicity against human cell lines of fruit peel extracts of rambutan (*Nephelium lappaceum*), mangosteen (*Garcinia mangostana*) and coconut (*Cocos nucifera*); they recorded varied activity levels. The rambutan peel crude extract via ABTS assay from its ethyl acetate fraction with a TEAC value of 23.0 mM mg⁻¹ and higher ferric ion reduction activity via FRAP assay from its methanol fraction with an EC value of 20.2 mM mg⁻¹ showed higher antioxidant activity. Using both assays, the fractions recorded higher antioxidant activity than the standard Butylated Hydroxyl Toluene (BHT) and vit. E.

Ficus sur and *Ficus sycomorus* (Moraceae), two medicinal species used in Burkinabe traditional medicine to treat sickle cell disease, were analysed (Ramde-Tiendrebeogo *et al.*, 2011) for the total phenolic and tannins contents. Using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), the *Ficus* extracts recorded radical scavenging activity with IC_{50} value of 9.60±0.02 and 31.83±0.55 μ g mL⁻¹ for *F. sur* against the IC_{50} value for standard-Quercetin (4.6±0.08 μ g mL⁻¹).

Methanol extracts from various parts of the plant *Warburgia ugandensis* showed high anti-plasmodial activity with IC_{50} values of less than 5 mg mL⁻¹ against both chloroquine-sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* and moderate activity against *P. berghei* (Wube *et al.*, 2008). Methanolic extract of *Oxalis corniculata* recorded potent antioxidant activity (with IC_{50} of 30 mg mL⁻¹) compare to the standard-Ascorbic acid (with IC_{50} of 30 and 37 mg mL⁻¹). This suggests that the MEOC possess higher antioxidant activity than ascorbic acid (Yalla *et al.*, 2010).

The discovery of plants with comparable or higher reactive species scavenging potential than standards like Vit. E, Vit. C and BHT; alongside appreciable phytotoxicity levels as with the plants in this study, is growing. This brings to the fore the need to speed up the search for more plant species with such properties before they are lost to deforestation and other human activities.

CONCLUSION

The study suggests that the ethanol leaf extract of AC, BO, CC and FE possess antioxidant properties that can counteract the oxidative damage induced by the malaria parasite at appreciable toxicity levels. The phenolic content of AC ($1063.6 \pm 0.001 \text{ mg mL}^{-1}$), BO ($1200.9 \pm 0.001 \text{ mg mL}^{-1}$), CC ($711.8 \pm 0.001 \text{ mg mL}^{-1}$) and FE ($208.1 \pm 0.0005 \text{ mg mL}^{-1}$); presents appreciable antioxidant potentials. This justifies the introduction and use of these plant species by indigenous, rural and semi-urban communities in treating malaria diseases in various parts of West Africa. The dwindling forest reserves that these communities depend, holds a rich diversity of plants with similar or better prospects. Increased scientific attention, albeit; screening for medicinal and related properties, is essential to capturing such prospects before they are lost.

The antioxidant prowess may be one mode of action through which the plants constituents interact in anti-malaria therapy. However, further investigations are required to ascertain the specific mechanisms of actions and the phytochemicals that confer these activities. Such efforts will help validate the species and speed up their acceptance in anti-malarial drug screening exercises.

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