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Phytochemical, antimicrobial and in-vitro antioxidant assessment of selected antimalaria plants in Nigeria

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ABSTRACT

Background: Harunganamadagascariensis, Khayasenegalensisand Enantiachlorantha are important Nigerian antimalaria plants which are rich in varieties of phytochemicals having antimicrobial and antioxidant properties. These herbal preparations could prevent the oxidative damage and microbial co-infection which accompanies some antimalaria treatment. Hence, a comparative phytochemical, antimicrobial and antioxidant study of the stem bark extracts of these plantshas been carried out.

Methods: The total phenolic (TPC) content was determined using Folinciocalteaumethod while the 1,1-diphenyl-2-picryl hydrazyl radical assay was employed in the comparative antioxidant study. Agar well diffusion technique was used to determine the antimicrobial activity of the extracts.

Results: Khayasenegalensisexhibited the highest TPC (92.15±0.283 mg/g GAE). Both Khayasenegalensis and Harunganamadagascariensis demonstrated comparable antioxidant potential while Enantiachloranthadisplayed the least DPPH scavenging potential and was the only extract which exhibited antimicrobial property against the tested bacteria.

Conclusion: The antioxidant and antimicrobial potential of these extracts promote them as valuable ingredients in antimalaria herbal formulation.

KEYWORDS: Antimalaria, antioxidant, antimicrobial**,**phytochemicals, Harunganamadagascariensis, Khayasenegalensis, Enantiachlorantha.

I. INTRODUCTION

Malaria is a life-threatening disease which has continued to affect millions of lives all over the globe. In 2015 alone, 215 million cases of malaria and 429,000 malaria related death were recorded worldwide(1)**,** with a large portion of the numbers from Nigeria. Particularly, 51 million cases and 207,000 deaths reportedly occur annually in Nigeria(2)**.**

Although there are current therapies which already procured notable cure for this disease, the accompanying side effects (3) and the emergence of drug resistant strains of Plasmodium falciparum have instigated several research into possible natural product alternatives (4). The poverty level in several developing countries also contribute to the quest for herbal antimalaria treatment since it is relatively cheap to procure (5).Particularly, Harunganamadagascariensis (Arunje), Khayasenegalensis(Aganwo) and Enantiachlorantha (Dokita Igbo)(Yoruba names in parenthesis) are among the plants which have been explored for their antimalaria potential in Nigeria(6).

A major side effect of malaria infection is that it induces excessive generation of reactive oxygen species which in turn leads to oxidative damage in cells(7). Antioxidants are therefore needed to prevent cell damage. However, some antimalaria therapies have been reported to effect treatment via mechanisms which induce oxidative stress and the use of antioxidants may interfere with their mode of action(7). Hence, the adoption of antioxidant herbs in anti-malarial treatment is a right step in the right direction. The antimicrobial potency of the extracts was also of interest since there is a possibility of microbial co-infection with malaria cases(8).

This study therefore aims at comparatively investigating the antioxidantpotential of selected antimalaria plants (Harunganamadagascariensis,Khayasenegalensisand Enantiachlorantha)which are currently employed

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by herbal practitioners in the south-western part of Nigeria.

II. MATERIALS AND METHOD

2.1. Plant material

Thestembarks of the selected antimalaria plants (Yoruba names in parenthesis): Harunganamadagascariensis (Arunje), Khayasenegalensis(Aganwo) and Enantiachlorantha (Dokita Igbo) were collected from a local herbal health care provider in Omu-AranKwara state. They were then identified at the herbarium in the Department of Plant Science, University of Ilorin, Nigeria. Voucher specimen numbers UIL-0031133, UIL-001852 and UIL-0021013 were obtained for Harunganamadagascariensis,Khayasenegalensisand Enantiachlorantharespectively.

2.2Preparation of extract

Dried samples of Harunganamadagascariensis, Khayasenegalensisand Enantiachloranthawere cut into pieces and macerated using 50% ethanol for 7 days followed by filtration using a filter paper. Eachextract solution was afterwards concentrated using a rotary evaporator at 60^{o}C to obtain the corresponding dry extracts which were subsequently subjectedto phytochemical and antioxidant analyses.

2.3Determination of total phenolics

Modified Folin-ciocalteu method (9)was used to determine the total phenolic contents in the extracts. In brief, 1mL of each plant extract was placed in a boiling tube plus 1 mL of Folin-Ciocalteu'sreagentand 1 mL of 7.5% sodium carbonate solution. The mixture was adjusted to 30 mL with deionized water after 3 minutes, shaken vigorously and allowed to stand for 90 minutes. The absorbance was taken at 765 nm using a JENWAY-6705UV-visible spectrophotometer and the concentration of phenolics was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

2.4 DPPH radical scavenging activity

The quantitative determination of the free radical scavenging activity (percentage antioxidant activity) of each of the extracts from selected plants was carried out spectrophotometrically using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical as described by Ayoolaet al. (10)with slight modification. 3mL of 0.1mM DPPH was placed in a test tube containing 1mL of each of the extract. The mixture was incubated in the dark for 30 minutes after which the absorbance of the resulting solution was recorded at 517 nm using methanol as blank. All determinations were done in duplicate and the free radical scavenging activity was calculated as follows:

$$\%\ \mathrm{DPPHscavengingactivity} = \frac{\mathrm{Ab} - \mathrm{Aa}}{\mathrm{Ab}} \times 100$$

Where: Ab= absorption of DPPH in methanol Aa = absorption of the solution containing the extract.

2.5 Antimicrobial activity

Antimicrobial activity of the three extracts from Harunganamadagascariensis, Khayasenegalensis and Enantiachlorantha against Aspergillus flavus(maintained on Potato Dextrose Agar), Escherichia coli, and Staphylococcus aureus(maintained on Nutrient Agar)was investigated. The Agar well diffusion method as described by Oluwaniyiet al. (11) was employed. Muller Hinton agar (for bacteria)and Potato Dextrose Agar (for the fungus) were prepared as prescribed by the manufacturer. 1 mL of bacteria inoculum was introduced into each Petri dish and 20 mL of Nutrient agar was added, swirled and allowed to set. After which a cork borer of about 6 mm was aseptically used to bore wells into the agar. 100µl of each extract (6mg/mL) was introduced into designated wells and the plates were incubated at 25^{o}C for 24 h in the case of E. coli and S. aureus while incubation was for 48 h in the case of the A. flavus. For the fungus, ketoconazole served as positive control while chloramphenicol was used in the case of bacteria. Distilled was used a negative control in both cases. The experiment was done in duplicate.

III. RESULTS AND DISCUSSION

The extraction process yielded2.01,3.26 and 2.33% extracts from Harunganamadagascariensis, Khayasenegalensis and Enantiachlorantha stem barks respectively.The total phenolic content of the extracts ranged from 54.890±0.141 to 92.15±0.283 mg/g GAE (Table 1).

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Table 1. Total phenolics and total flavonoid contents of selected antimalaria plant extracts

Plant extracts	Khayasenegalensis	Harunganamadagascariensis	Enantiachlorantha
TPC (mg/g GAE)	92.15±0.283	79.200±0.141	54.890±0.141

Values represent means ± standard deviation of duplicate determinations

Khayasenegalensisexhibited the highest TPCand was closely followed byHarunganamadagascariensis. Several reports have correlated high concentration of phenolicsin plant extracts with high antioxidant activity (12).Our results agree with this correlation as Khayasenegalensis with the highest TPC exhibited high ability to scavenge the DPPH radical (IC_{50} values in DPPH system: 0.025mg/mL). The comparable antioxidant potential ofHarunganamadagascariensis could be as a result of other antioxidant phytochemicals such as flavonoids.Enantiachloranthawhose stem bark methanolic extract is reportedly rich in antioxidant phytochemicals (13) however, presented the lowest antioxidant capacity (IC_{50} value: 0.6 mg/mL) in this study (figure 1).

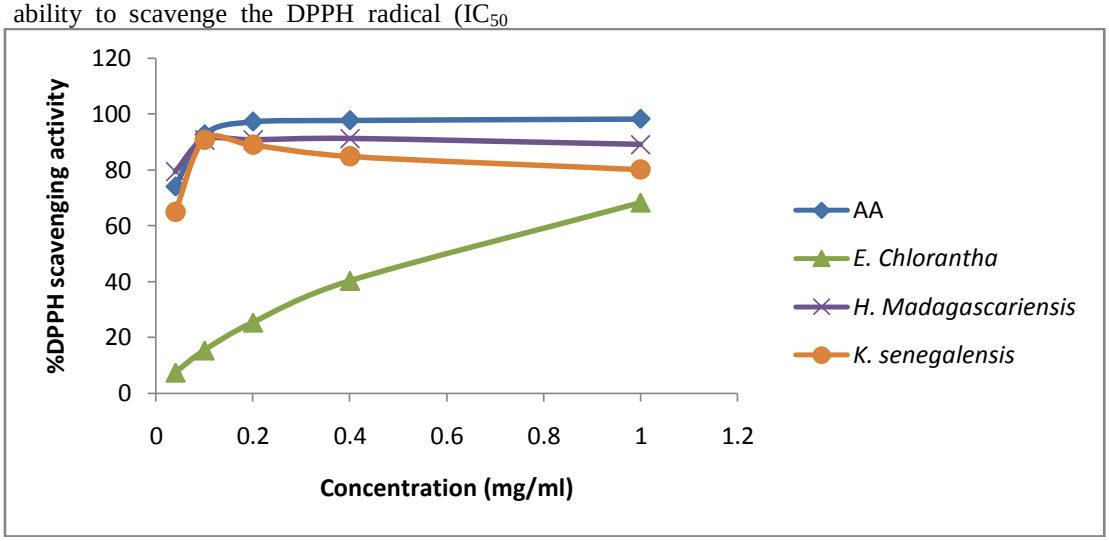

Figure 1: %DPPH radical scavenging activity of stem bark extracts from E. chlorantha, K. senegalensis and H. madagascariensis

Although the total phenolic contentofHarunganamadagascariensisextractherein reported is lower than 132.24±0.61 mgGAE/g which was earlier reported from the same plant byAntiaet al. (14), its antioxidant activitywas higher. This may also be as a result of the presence of other antioxidant phytochemicals in the extract. Furthermore, the IC_{50} value reported by Moussa et al.(15) for the leaves (0.0296 mg/mL) was almost the same as our determined IC_{50} value (0.025mg/mL) for the bark in DPPH system.

At the examined concentration, none of the extracts inhibited Aspergillusflavus. However, only Enantiachlorantha extract inhibited the growth of E. coli and S. aureus as shown in Table 2.

Table 2: Antimicrobial activity of E. chlorantha, K. senegalensis and H. madagascariensis against selected microorganisms

S/N	ZOI of extracts\Organism	Aspergillus flavus	Escherichia Coli	Staphylococcus aureus
1	Khayasenegalensis	-	-	-
2	Harunganamadagascariensis	-	-	-
3	Enantiachlorantha	-	13.000 ± 1.414	14.500 ± 0.707
4	Control	31.500 ± 2.121	31.500 ± 0.707	33.000 ± 2.828

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Values represent means ± standard deviation of duplicate experiments

The results revealed the ability of E. chlorantha extractto inhibit the growth of both Gram negative and Gram positive microorganisms as represented by E. coli and S. aureus. It however revealed that none of the extracts may have potential use as antifungal agents especially against A. flavus. This suggests that at increased concentrations the E. chlorantha stem bark extract will perform even better against the test isolates which enlists it a potential antibacterial agent. Having established its potency against these two organisms, further study is required to ascertain its effect against antibiotic resistant strains of bacteria. Based on the foregoing, these plant extracts could prevent unwanted oxidative damage and combat bacterial co-infectionwhile exerting their therapeutic effect duringantimalaria therapy thereby offering a more convenient way to combat malaria. Thesecomparative antioxidant and antimicrobial results provide useful information which may be applied during antimalaria herbal formulations.

IV. CONCLUSION

Extracts from the selected study plants (Harunganamadagascariensis,Khayasenegalensis, Enantiachlorantha) could be developed into wholesome antimalarial agents which will offer healing with little or no oxidative stress related side effects.

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Conflict of interest statement: The authors declare no conflict of interest

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