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ANALYSIS, CHARACTERIZATION AND MALARIA PARASITAEMIA SUPPRESSION POTENTIAL OF BIOACTIVE COMPOUNDS FROM AQUEOUS EXTRACTS OF A MEDICINAL PLANT USED FOR THE TRADITIONAL TREATMENT OF MALARIA IN AFRICAN COUNTRIES

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ABSTRACT

The properties exhibited by a plant, including the medicinal ones, is attributed to the type and nature of the bioactive compounds that are present in the plant. Thus the study of such bioactive compounds is very key in any medicinal studies of the plant. This study was carried out to analyze, characterize and evaluate the malaria parasitaemia suppression potential of the bioactive compounds of aqueous leaf, stem bark and root extracts of *Alstonia* boonei, a pan African medicinal plant, and position it for potential new antimalarial drug development. The extracts were subjected to Gas Chromatography – Mass Spectrometer analysis in order to determine their most active bioactive compounds. The most active bioactive compounds present in the extracts include 1,3,5-Triazine, 2-methylamino-4,6-bis(nonafluoro-tert-butyl) a methoxymethylbutyl class compound from aqueous leaf extract of *A. boonei***,** androsta--2,4,16-triene-3,6,17-triol, tri-TMS a lactone class compound from aqueous stem bark extract of *A. boonei* , 1-Oxo-forskolin a diterpenoid compound from aqueous root extract of *A. boonei*, respectively. These bioactive compounds were subjected to antimalarial tests in order to evaluate their antimalarial activity in mice infected with *Plasmodium berghei*. The tested bioactive compounds were found to exhibit significant dose – depent antimalarial activity as they suppressed the malaria parasitaemia in the treated infected mice. The results from this study revealed that the plant contains important medicinal bioactive compounds with significant antimalarial activity and should be further studied for the development of new drugs for the treatment of the increasing resistant malaria infection.

KEYWORDS: Analysis**,** Characterization**,** Bioactive compounds**,** Medicinal**,** Antimalarial drug

INTRODUCTION

The utilization of plants against diseases is believed to be derived from the chemicals available in them (Ajose *et al*., 2019). Many modern medicines are derived from plants that have been used by traditional medical practitioners (Anjali and Sheetal, 2013). The analysis and characterization of the bioactive constituents of a medicinal plant is a very important aspect of the process of studying the characteristics of the constituents of the plant in order to correctly position it for the development of drugs for the treatment of diseases (Asha *et* al., 2017; Imam *et al*., 2017).

Products from nature play important roles as leads for the discovery and development of new drugs **(**Madhiri and Vijayalakshini, 2018; Muhseen *et al.*, 2021*)*. Plants have proved to be sources of important new drugs (White, 2004). Drugs for treating malaria such as quinine and artemisinin came from plants (Obadoni and Ochuko, 2001). In many parts of the world the use of traditional medical products is very common and has been on the increase (Odugbemi *et al.*, 2007). These traditional medical products are obtained from local herbal plants and there is the need for them to be scientifically evaluated in order to position them for potential drug discoveries (Madhiri and Vijayalakshini, 2018; Muhseen *et al.*, 2021; Willcox and Bodeker, 2004).

Tropical rain forest plants have high potential as sources of new molecules. Thus, studies on plants from the African region is encouraged since the burden and impact of many diseases such as malaria, schistosomiasis and other tropical diseases (Adebayo and Krettli, 2011). *Alstonia boonei* is one of the many medicinal plants found in many African countries and it belongs to the Apocynaceae family. It is an herbal medicinal plant of West African origin and the parts of the plant have been traditionally used for their antimalarial, aphrodisiac, anti-diabetic antimicrobial, and antipyretic activities (Afolabi and Abejide, 2020; Uzor *et al.*, 2020).

MATERIALS AND METHODS

Preparation of plant materials

The leaves, stem bark and roots of *Alstonia boonei* earlier collected from Nsukka village in Nsukka Local Government Area in Enugu State, Nigeria were separately cut into small pieces, washed and air dried for two weeks under room

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temperature. The dry samples were then ground into powder with a mechanical blender (Afolabi and Abejide, 2020).

Extraction methods

Aqueous extraction

A quantity of 500 g of the ground fine powder obtained was percolated in 1600 mL of water for 72h after which it was filtered. This was followed by evaporating the filtrate collected to dryness using a temperature-regulated water bath pre-set at 40ºC to yield the extract concentrate which was stored in the refrigerator at 4ºC before use (Amole and Ilori, 2010).

Gas Chromatography-Mass Spectrometer (GC-MS) analysis of the bioactive compounds

The bioactive compounds present in the aqueous leaf, stem bark and root extracts of *Alstonia booei* were determined and identified by Gas Chromatography and Mass Spectrometer methods using (Angilent 6890 series) equipment following the procedures described by Eswaraiah *et al*, 2019. The equipment had a HP-5MS column mass spectrometer operated at initial column temperature of 30°C and heated up to 300°C at the rate of an increase of 10°C/min and maintained for 10 min. Injection port temperature was ensured at 250°C and Helium flow rate at 1.5 ml/min. The ionization voltage was 70 ev. The samples were injected in split mode of 10:1. Mass spectral scan range was set at 40-700 m/z. The ion source temperature was maintained at 230°C and Interface temperature was set at 240°C. The MS start time was 3 min and end time was 40 min with solvent cut time of 3 min. The identification of the compounds was done based on retention time, integral area of peaks and by using the database of National Institute Standard and Technology (NIST). The similarity of compounds matched with >70% age listed based on NIST 59 library search (Eswaraiah *et al*, 2019).

Animals used for the Study and Ethical Approval for their use

The animals used for this research are white albino mice of both sexes weighing between 30 and 35g. They were obtained from the animal house, Department of Animal and Environmental Biology, University of Nigeria, Enugu State, Nigeria. Animal tests were carried out according to the National Institute of health (NIH) guide for the care and use of laboratory animals. Approval was obtained for all animal experiments from the University of Nigeria Ethical Committee on the use of laboratory animals for research.

Malaria Suppression Tests

The bioactive compounds underwent antimalarial tests in *P. berghei*-infected white albino mice to determine their suppression effects on early malaria infection using methods described by Arise *et al*., 2012 and Babamale *et* al., 2017, which were modified to suit the aim of this study. On the first day a set of mice of both sexes were randomly selected and infected with 10^{7} *Plasmodium berghei*. Three hours later blood was taken from the tail of each infected mice and examined under the microscope to determine parasitaemia of early infection and then the infected mice were each treated orally with 100, 200 and 400 $mgkg^{-1}$ body weights of the bioactive compounds or $5mgkg^{-1}$ body weight of artesunate, the positive control, separately using corn meal as the vehicle. Another set of infected mice, the negative control, were given $5mlkg^{-1}$ distilled water. Treatment was continued for four consecutive days and on the fifth day, blood was again taken from the tail of each mice and examined for suppression of parasitaemia and the values were recorded accordingly.

Data analysis

The data obtained was analyzed using Statistical Package for the Social Science (SPSS) version 20. The mean values of the parameters studied were compared using one-way Analysis of Variance (ANOVA) at 95% confidence interval, and separated using Turkey-b post hoc comparison. Probability values of $p < 0.05$ were considered significant. Results were expressed as mean +/- standard error of mean (SEM).

RESULTS

The medicinal and other properties of a plant is as a result of the bioactive compounds that are present in the plant (Ajose *et al*., 2019). Hence, the evaluation of the bioactive compounds is very important in any medicinal studies of a plant (Afolabi and Abejide, 2020). If the bioactive constituents of a plant are found to have significant medicinal property, then the plant can be further investigated for drug development. The results of the analysis, characterization and malaria suppression tests of the most active bioactive compounds from aqueous extracts of *Alstonia boonei*, a medicinal plant used for the traditional treatment of malaria in African countries, are presented below.

The GC - MS analysis of the extracts produced active bioactive compounds with properties and chemical structures as shown below:

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AQUEOUS LEAF BIOACTIVE COMPOUND – NAME, FORMULA, MOLECULAR WEIGHT AND CHEMICAL STRUCTURE

Name: 1,3,5-Triazine, 2-methylamino-4,6-bis(nonafluoro-tert-butyl)
Formula: C12H4F18N4
MW: 546 Exact Mass: 546.014854

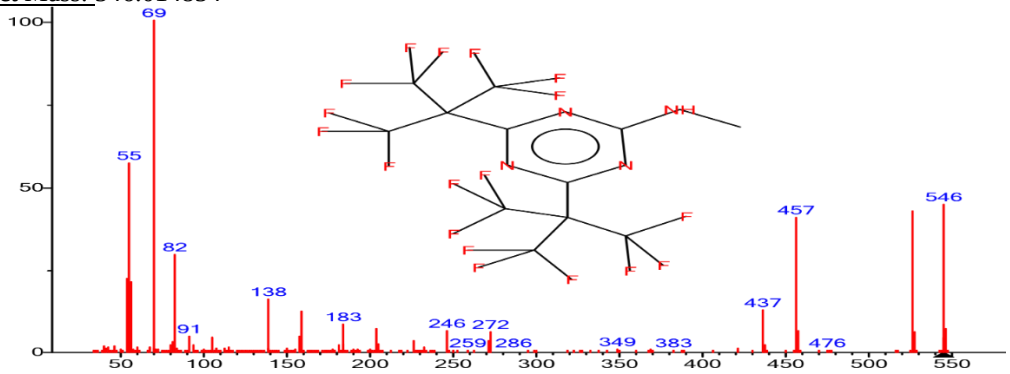

Figure 1. Chromatograph showing the peak of compounds and the structure of 1,3,5-Triazine, 2-methylamino-4,6-bis(nonafluoro-tert-butyl)

AQUEOUS STEM BARK BIOACTIVE COMPOUND – NAME, FORMULA AND MOLECULAR WEIGHT

Name: Androsta--2,4,16-triene-3,6,17-triol, tri-TMS
Formula: C28H50O3Si3
MW: 518 Exact Mass: 518.306774

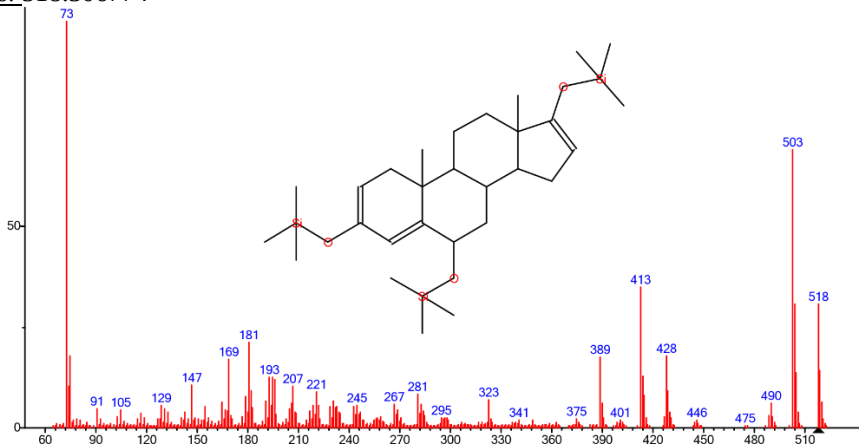

Figure 2. Chromatograph showing the peak of compounds and the structure of Androsta--2,4,16-triene-3,6,17-triol, tri-TMS

AQUEOUS ROOT BIOACTIVE COMPOUND – NAME, FORMULA, MOLECULAR WEIGHT AND CHEMICAL STRUCTURE

Name: 1-Oxo-forskolin
Formula: C22H32O7
MW: 408 Exact Mass: 408.214804

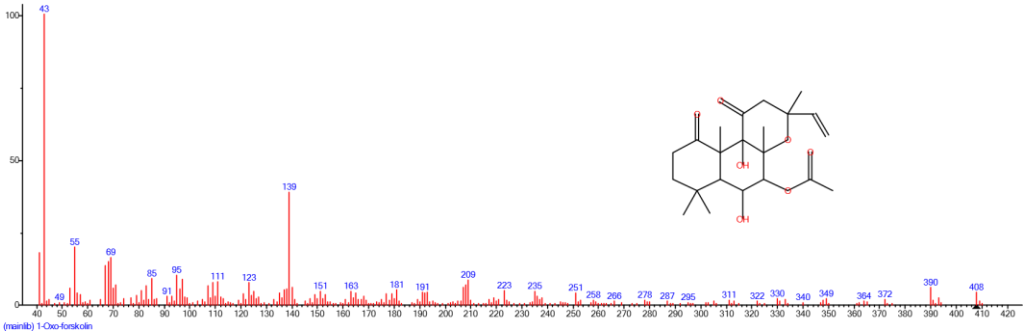

Figure 3. Chromatograph showing the peak of compounds and the structure of 1-Oxo-forskolin

The bioactive compounds were subjected to antimalaria tests in *Plasmodium berghei*-infected white albino mice in order to determine their malaria suppression potential. The tests revealed that the extracts have significant dose-dependent malaria suppression activity. The results of the malaria suppression tests are presented in Tables 1, 2 and 3 below

Table 1. Suppressive activity of 1,3,5-Triazine, 2-methylamino-4,6-bis(nonafluoro-tert-butyl), a compound from aqueous leaf extract of *A. boonei* and artesunate in mice infected with *Plasmodium berghei*

Treatments	**Suppression (%)**
Distilled water 5mlkg^{-1}	0.00 ± 0.00^{a}
Compound 100mgkg^{-1}	34.31 ± 0.64^{b}
Compound 200mgkg^{-1}	48.45 ± 0.63^{c}
Compound 400mgkg^{-1}	65.87 ± 0.64^{d}
Artesunate 5mgkg^{-1}	86.73 ± 0.63^{e}

[1]All values expressed as mean ± standard error (±SE).
[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison.

Table 2. Suppressive activity of androsta--2,4,16-triene-3,6,17-triol, tri-TMS, a compound from aqueous stem bark extract of *A. boonei* and artesunate in mice infected with *Plasmodium berghei*

Treatments	**Suppression (%)**
Distilled water 5mlkg^{-1}	0.00 ± 0.00^{a}
Compound 100mgkg^{-1}	48.71 ± 0.64^{b}
Compound 200mgkg^{-1}	59.64 ± 0.64^{c}
Compound 400mgkg^{-1}	68.31 ± 0.63^{d}
Artesunate 5mgkg^{-1}	94.52 ± 0.63^{e}

[1]All values expressed as mean ± standard error (±SE).
[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison.

Table 3. Suppressive activity of 1-Oxo-forskolin, a compound from aqueous root extract of *A. boonei* and artesunate in mice infected with *Plasmodium berghei*

Treatments	Suppression (%)
Distilled water 5mlkg^{-1}	0.00 ± 0.00^{a}
Compound 100mgkg^{-1}	44.15 ± 1.21^{b}
Compound 200mgkg^{-1}	50.63 ± 0.64^{c}
Compound 400mgkg^{-1}	62.31 ± 0.64^{d}
Artesunate 5mgkg^{-1}	94.22 ± 1.21^{e}

[1]All values expressed as mean ± standard error (±SE).
[2]Different superscript letters indicated significance difference ($p < 0.05$) in mean values among different treatments using Turkey's b *post hoc* comparison.

DISCUSSION

The bioactive compounds from the extracts belong to different classes including phenols, alkaloids, carboxylic acids, terpenes, acridones and lactones. Similar to the findings from this study, several bioactive compounds have been found in *Alstonia boonei* such as phenols, alkaloids (Adotey *et al.*, 2012; Babatunde, 2017), carboxylic acids (Balogun et *al.*, 2016; Batista *et al.*, 2009; Imam *et al.*, 2017), terpenes (Olanlokun *et al.*,2021; Rohloff, 2015; Ruikar, *et al.*, 2010) tannins, saponins (Uzor *et al.*, 2020; Ajayi *et al.*, 2019; Otuokere *et al.*, 2016; Saleh *et al.*, 2019; Saxena *et al.*, 2003), glycosides (Taiye and Pass 2014; Tarkang *et al.*, 2014), acridones and lactones (Uraku, 2015; Vasanth *et al.*, 1990). Other studies on bioactive compounds of the plant also revealed similar compounds as in this study comprising carboxylic acids (Wong *et al.*, 2021), diterpenoids (Saleh, 2019), acridones (Winter *et al.*, 2006), diosgenin (Omonirri *et al.*, 2021) and lactones (Chea *et al.*, 2006).

The suppressive activity of 1,3,5-Triazine, 2-methylamino-4,6-bis(nonafluoro-tert-butyl) a methoxymethylbutyl class compound from aqueous leaf extract of *A. boonei* showed that the lowest suppression effect was found in the lowest administered dose of 100mg/kg body weight while the highest suppression effect was in the highest administered dose of 400mg/kg body weight as shown in Table 1 above. In a similar study on methoxymethylbutyl class compound, Wong *et al.*, 2021 evaluated chemical constituents from the crude extract of *Dendrocalamus asper* using chromatographic methods for their antimalarial potential and of all the chemicals tested, dimethyl-15,16-dibutoxytriconta-11,13,17,19-tetraenedioate, methoxyl-4-hydroxybenzoate and 1-methoxy-4-(methoxymethyl)benzoate showed promising antimalarial activity.

In the suppressive activity of androsta--2,4,16-triene-3,6,17-triol, tri-TMS a lactone class compound from aqueous stem bark extract of *A. boonei* (Table 2), it showed that the minimal suppression effect was found in the lowest administered dose of 100mg/kg body weight while the optimal suppression effect was in the highest administered dose of 400mg/kg body weight as shown in Table 2 above. Other studies have reported the antimalarial effects of lactones from plants. Chea *et al.*, 2006 in their study found out that lactones possess antimalarial activity. They tested three lactones and compounds 1 8 alpha-tigloyloxy-hirsutinolide-13-*O*-acetate, 7 8 alpha-(4-hydroxymethacryloyloxy)-hirsutinolide-13-*O*-acetate and 8-alpha-(4-hydroxytigloyloxy)-hirsutinolide-13-*O*-ac exhibited significant antimalarial activity.

The suppressive test of 1-Oxo-forskolin a diterpenoid compound from aqueous root extract of *A. boonei* revealed that the lowest suppression effect was in the lowest administered dose of 100mg/kg body weight while the highest suppression effect was in the highest administered dose of 400mg/kg body weight as recorded in Table 3. Saleh *et al.*, 2019, evaluated the therapeutic potential of the Labdone Diterpenoid and also recorded a significant antimalarial activity by the compound which agrees the results from this study.

CONCLUSION

The results from this study revealed that the aqueous extracts of leaf, stem bark and root of *Alstonia boonei* contain important medicinal bioactive compounds that possess significant antimalarial activity and thus, they should be further investigated for new and novel antimalarial drug development.

Author's contributions: This work was carried out in collaboration among all authors. Authors OCA and ORNN designed the study and carried out antimalarial evaluation of the bioactive compounds and the analysis of the results. Authors NCG, UYH and EIC carried out the data analysis, wrote the protocol and the first draft of the manuscript. Authors OEU, ESS and OAQ carried out the GC-MS analysis, and managed the literature searches. All authors read and approved the final manuscript.

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