

GC-MS ANALYSIS AND ANTIPLASMODIAL POTENTIALS OF BIOACTIVE COMPOUNDS PRESENT IN METHANOLIC AND ETHANOLIC LEAF EXTRACTS OF *DANIELLA OLIVERI*

ABSTRACT

Qualitative, quantitative and Gas Chromatography-Mass Spectrometry (GC-MS) analysis are useful for the determination of bioactive components necessary for accessing the antiplasmodial potentials of methanolic and ethanolic leaf extracts of *Daniella oliveri* (*D. oliveri*). The aim of the study was to screen *D. oliveri* for the detection of phytochemical components and determination of bioactive compounds using qualitative, quantitative and Gas Chromatography-Mass Spectroscopy (GC-MS) analytical techniques. The leaves were collected in Anyigba, from which methanolic and ethanolic extracts were prepared, phytochemical components detected and bioactive compounds determined using GC-MS. Results showed the presence of alkaloid, tannin, reducing sugar, saponin, terpenoid, phenol, cardiac glycosides and flavonoid in the extracts. Phenol showed the highest concentration (46.14 and 43.09 mg/100g) while terpenoid showed the lowest concentration (10.63 and 9.97 mg/100g) in methanolic and ethanolic extracts respectively. GC-MS analysis revealed the presence of higher components (57) in methanolic extract compared to ethanolic extract (27). This study provides scientific evidence that methanol may be a better extraction solvent for GC-MS analysis of *D. oliveri* leaves meant to be used for the determination of antiplasmodial activity than ethanol due to higher components detected in methanolic extract compared to ethanolic extract.

Keywords: GC-MS, Antiplasmodial, Bioactive, Leaf Extracts, *Daniella oliveri*

1.0 Introduction

Plants are sources of chemical compounds of biological and pharmacological importance that have also been shown historically to be a source of drugs. Plants are essentially important for screening of new compounds that could serve as lead to the discovery of new drugs [1].

An estimated 80% of the world's population, especially millions of dwellers in the rural areas and more than 65% of the global population uses traditional medicine for their basic health care needs [2].

Artemisinin was the most effective antimalarial preparation prescribed as first line antimalarial drugs obtained from a medicinal plant called *Artemisia annua* L., with greater antimalarial activities as discovered in China by You-You Tu in the early 1970s [3]. There have been documented cases of resistance to artemisinin derivatives and partner drugs [4]. Therefore, sourcing for more medicinal plants with folkloric evidence of antiplasmodial potential is important. The possible source of malaria medicine appears in traditional herbal medicine because, traditional herbal medicines have been the most available, affordable and cheap sources of malaria treatments for most communities [5].

The aim of this study is to determine the GC-MS and antiplasmodial potentials of bioactive compounds present in methanolic and ethanolic leaf extracts of *Daniella oliveri* (*D. oliveri*).

(D. oliveri) (Rolfe) (syn. *Paradaniellia oliveri* Rolfe) [6], is a medicinal plant of the genus *Daniella* that was first named in 1859 by W. F. Daniell. It belongs to family Fabaceae, sub-family Caesalpinioideae and commonly known as African copoiba balsam in English [7], Ilorin balsam (eepoia) or copaihu Africana [8]. In Nigeria, it is traditionally known by the three major languages in the country as 'Maje' in Hausa, 'iya/ozabwa/agba' in Igbo, 'Emi iya' in Yoruba, 'Agba' in Igala, 'Ukpilla' in Igede and Ubakwa in Idoma [7].

D. oliveri is found predominantly in both temperate and tropical regions of the world, the

Amazon region and other parts of South America and Africa with 630 genera and species with the being able to reach a height of 100 feet and trunk diameter of 4 feet [9]. *D. oliveri* is an indigenous African tree found extensively in Benin, Cameroon, Gambia and Nigeria [8]. For more than 400 years, studies have shown that the oleoresin chemical component consisting of sesquiterpene hydrocarbons with caryophyllene, a non-volatile resinous substances and small quantities of acid produced by *D. oliveri* have been used as medicine by indigenous people [9]. The oleoresin is traditionally used as an anti-inflammatory agent and in the treatment of a variety of genito-urinary tract diseases, scrotal elephantiasis, dysentery, ring worms, syphilis, typhoid fever, eye sore, ear ache and skin ailments with the leaves sometimes used singly or in combination with other plant parts for the traditional treatments of diabetes, fever, boil, back ache and yellow fever [8].

Studies have revealed that the root is a diuretic and a decoction is used to treat venereal diseases, absence of menses, anxiety, insanity, food poisoning, skin diseases and the leafy twigs are put in baths to treat fever, jaundice, as a tonic and a decoction of the leafy twigs with salt is used as a purgative, to treat constipation and stomach-ache [10]. The Igede people of Benue state, North-Central Nigeria uses a decoction of *D. oliveri* leaves and *Cassythia filiformis* to treat fever [11]. Based on the claimed folkloric evidence as obtained from traditional healers in some communities in Kogi state, *D. oliveri* leaf is used for the treatment of malaria fever, typhoid fever

and in the management of pains associated with pregnancy.

D. oliveri have been reported to contain 44.6% to 57% carbohydrates, 27% to 33.4% crude protein, 9% lipid, 4.17% ash and 0.60% crude fiber [12].

GC-MS experimental analysis by [13], detected the presence of twenty-two compounds, with δ -Cadinene (42.92%), copaene (11.36%) and cis-muurolo-4 (14%), 5-diene (9.56%) found to be the major constituents which constituted about 64% of the total compound present in the oleoresin of *D. oliveri*.

2.0 MATERIALS AND METHODS

2.1 Plant Leaf Collection and Authentication

D. oliveri leaves were harvested from the plant in the month of September, 2020 in Anyigba, a town in Dekina Local Government Area of Kogi State, (latitudes 7°15'N-7°29'N and longitudes 7°11'E – 7°32'E) having an average altitude of 385 meters above sea level and a land mass area of 420 M₂. Anyigba is in tropic with wet and dry climatic regions and the derived savanna associated with 1250mm and 25°C average rainfall and temperature per annum [14]. The voucher specimen number (Bio/FUTA/102) of the plant was deposited in the Department of Biology, Federal University of Technology, Akure, Nigeria after identification and authentication by the expert.

2.2 Preparation of Leaves and Extracts

The leaves were washed, air dried at room temperature for three weeks and pulverized using mortar and pestle. Five hundred grams (500g) of the pulverized leaf powder was macerated in 4,500ml of 75% ethanol and 75% methanol for 72 hours and then filtered using Millipore (pore size 0.7µm) filter paper respectively. Using rotary evaporator set at 40°C, the filtrates were concentrated to recover the ethanolic and methanolic extracts [15]. The weights of the dried extracts were measured and the percentage (%) yields were calculated as follows:

Extract yield (%) = weight of dried extract X 100

Weight of dried plant samples

2.3 Determination of Phytochemical Compounds

The qualitative Phytochemicals analysis of yielded methanolic and ethanolic leaf extracts of *D. oliveri* was determined using standard procedures adopted by [16], [17], [18], [19] and Trease and [20] for the determination of Alkaloid, Saponins, Flavonoids, Tannins, Anthraquinones, Cardiac glycosides, Steroids, Terpenoids and phlobatannins.

2.4 Quantitative Phytochemical Analysis

The quantitative analysis of phytochemicals which are constituents in the methanolic and ethanolic leaf extracts of *D. oliveri* were tested using standard quantitative techniques as adopted by [21], [18] and [22] for the detection of the total amount of Alkaloid, Saponins, Flavonoids, Tannins, Anthraquinones, Cardiac glycosides, Steroids, Terpenoids and phlobatannins

2.5 GC-MS Determination of Phytochemical Components

The MS was auto-tuned to perfluorotributylamine (PFTBA) applying a systematic approach to check the abundance of m/z 69, 219, 502 and ancilliary instrument are at their optimal and sensitivity conditions. The amounts of phytochemicals in the extract was determined using GC-MS by the operation of MSD set in Scan mode to ensure the detection of the target constituents at its peak level. Agilent 7820A gas chromatograph attached to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies, USA) was used. HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30 m length x 0.32 mm diameter x 0.25 μ m film thickness) was used in the stationary phase of separation of the compounds (Agilent Technologies, USA). Helium was the carrier gas utilized at constant flow of 1.4871 ml/min at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm/sec. The equipment was loaded with 1 μ L of the samples, injected in a splitless mode at an injection temperature of 300 °C. Purge flow to split vent was 15 ml/min at 0.75 min with a total flow of 16.654 ml/min; gas saver mode was switched off. Oven was initially programmed at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min) at a run time of 32.667 min with a programmed 5 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was via Scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate) [23; 24; 25].

3.0 RESULTS

3.1 Yield of Methanolic and Ethanolic Leaf Extracts of *Daniella oliveri*

From methanolic and ethanolic Leaf extracts prepared using same quantity of plant material (500 gm), the Percentage yield of ethanolic leaf extract (13.76 %) was higher than the yield obtained from methanolic leaf extract (10.62 %) (Table 3.1).

3.2 Phytochemical Components in Methanolic and Ethanolic Leaf Extracts of *Daniella oliveri*

The various phytochemicals components detected in methanolic and ethanolic Leaf extracts of *D. oliveri* are presented in Table 3.2. With the exception of steroids and phlobatanins that were not detected in both extracts, all other compounds were detected in both methanolic and ethanolic leaf extracts.

3.3 Concentrations of the Various Phytochemical Constituents Detected in Methanolic and Ethanolic Leaf Extracts

The concentrations of various phytochemical constituents detected in the methanolic and ethanolic leaf extracts of *D. oliveri* are presented in Table 3.3. Phenol (46.14 mg/100g) had the highest concentration while terpenoid (10.63 mg/100g) had the lowest concentration in methanolic leaf extract. Similarly, Phenol (43.09 mg/100g) had the highest concentration and terpenoid (9.97 mg/100g) had the lowest concentration in the ethanolic leaf extract.

3.4 Bioactive Components Identified in Methanolic Leaf Extract of *Daniella oliveri*

The quantity of various bioactive components identified in methanolic leaf extract showed the presence of 57 components. Some of the most abundant bioactive components obtained from the GC-MS analysis of methanolic leaf extract of *D. oliveri* are: 10-Octadecenoic acid (99%), methyl ester (99%), 9-Octadecenoic acid (99%), 9-Octadecenoic acid (99%), 9-Octadecenoic acid (Z)- (99%), 11-Octadecenoic acid (99%), cis-13-Octadecenoic acid (99%) while few of the least abundant bioactive components are Cyclohexasiloxane (12%) and dodecamethyl- (12%). The various components and their quantities are presented in Figure 3.1.

3.5 Bioactive Components Identified in Ethanolic Leaf Extract of *Daniella oliveri*

The quantity of various bioactive components identified in the ethanolic leaf extract is presented in Figure 3.2. Results showed the occurrence of 27 components obtained in ethanolic leaf extract. The most abundant bioactive components obtained are: 9-Octadecenoic acid (Z)- (98%), Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (99%), 1-Octadecene (99%) while some of the least abundant bioactive components are Phosphine, acetyldimethyl-, Propanethioic acid, 2-methyl-, S-ethyl ester (9%), 2-Phenylpyrido[3,4-d]-1,3-oxazin-4-one (9%) and Propane, 1-chloro-2-nitro- (9%).

Table 3.1: Percentage Yield of Methanolic and Ethanolic Leaf Extracts of *Daniella oliveri*

Extract	Medium	Plant part	Weight of powder (g)	Volume of Solvent (ml)	Yield (g) % Yield
Methanol	Ethanol	Leaf	500	500	4500
		Leaf	500	4500	53.1
			68.8	10.62	13.76

Table 3.2: Phytochemical Components in Methanolic and Ethanolic Leaf Extracts of

Daniella oliveri

Phytochemicals Result

Methanol Ethanol

Alkaloid
Steroid
Tannin
Phlobatannin
Reducing sugar
Saponin
Terpernoid
Phenol
Cardiac glycosides

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Flavonoid ++

Legend: Present = + and absent = -

Table 3.3: Concentrations of Phytochemical Constituents Detected in Methanolic and

Ethanolic Leaf Extracts of *Daniella oliveri*

**Phytochemicals Methanol Extract Ethanol Extract
(mg/100g) (mg/100g)**

Alkaloid
45.84
Flavonoid
32.79
Tannin
33.56
Cardiac glycosides
22.96
Reducing sugar
28.55
Saponin
28.38
Terpernoid 10.63
Phenol
46.14
41.86

34.97
31.35
24.18
29.49
41.66
9.97
43.09

Note: mg/100g- milligram per hundred gram

Figure 3.1: Bioactive Components Identified in Methanolic Leaf extract of *Daniella oliveri*

Figure 3.2: Bioactive Components Identified in Ethanolic Leaf extract of *Daniella oliveri*

4.0 DISCUSSION AND CONCLUSION

4.1 Discussion

In this study, high yield (13.76%) was obtained from ethanolic extract of *D. oliveri* leaves compared to methanolic extract (10.62%). The observed differences in yield might be due to the age of the plant, period of harvest of the leaf and nature of the soil [26]. This also could be due to the nature and concentration of the solvent and extraction technique used [27]. This finding is contrary to the lower yield obtained from methanolic (5.4%) and ethanolic (5.6%) extracts obtained by [28].

The phytochemical analysis of methanolic and ethanolic leaf extracts contained the phytochemicals reported. Phytochemicals are substances produced mainly by plants and have biological activity [29]. This agrees with [30] that leaves are rich in bioactive chemicals such as alkaloids, flavonoids, tannins and saponins.

Phenols and terpenoids being the highest and lowest phytochemicals obtained in both methanolic and ethanolic leaf extracts of *D. oliveri* respectively agrees with [31] that, phenol is the highest phytochemical component in *D. oliveri*. [32] also reported that Alkaloids, tannins, saponins, and phenolic compounds are present.

The greater number of chemical compounds identified in methanolic extract through GC-MS analysis compared to ethanolic extract suggests that methanol may be the better extraction solvent for GC-MS analysis of *D. oliveri* leaf extract meant for the determination of the actual chemical compound possessing the antiplasmodial property. The variation in the abundance is in agreement with [1] that, different extracts possess unique physicochemical characteristics which may be due to the molecular weight of the chemical entities as the compounds are naturally present in significant quantities. This is also in agreement with the work of [33], who identified *D. oliveri* from its ethnobotanical and industrial importance. In this study, the GC-MS analysis of methanolic leaf extract revealed the presence of Benzo[h]quinoline, 2,4-dimethyl-, similar to the work of [34] that, fused quinoline derivatives produce antimalarial effects. Similarly, [35] revealed that fused quinoline derivatives is antiplasmodium.

The qualitative, quantitative and Gas Chromatography-Mass Spectrometry (GC-MS) analysis reported by [36], for the determination of bioactive components used in accessing the antiplasmodial potentials of methanolic leaf extract of *D. oliveri* in mice resulted in a conclusion that, methanolic leaf extract of *D. oliveri* contained the bioactive secondary metabolites that could be implicated in a dose and time dependent antiplasmodial activities.

4.2 Summary

In this study, the phytochemical compounds detected in both methanolic and ethanolic leaf extracts of *D. oliveri* includes alkaloid, tannin, reducing sugar, saponin, terpenoid, phenol, cardiac glycosides and flavonoid while steroid and phlobatannin were not detected in both extracts. Phenol showed the highest concentration (46.14 and 43.09 mg/100g) while terpenoid showed the lowest concentration (10.63 and 9.97 mg/100g) in methanolic and ethanolic extracts respectively. Higher number of chemical compounds (57) were detected in methanolic extract of *D. oliveri* leaves compared to (27) in ethanolic extract. The abundant wealth of bioactive components available in *D. oliveri* plant showed that, the methanolic leaf extract of *D. oliveri* contained the bioactive secondary metabolites that could be used for determination of the antiplasmodial activities.

4.3 Conclusion

In conclusion, *D. oliveri* has been screened and found to possessed similar amount of phytochemicals in both ethanolic and methanolic leaf extracts. Higher bioactive components were recorded in methanolic extract compared to ethanolic extract. Some of these bioactive components may be implicated in the antimalarial activities of this important medicinal plant.

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4.5 Competing Interests

No competing interests exist

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