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Original article

Chemical Composition, Antioxidant and Cytotoxicity Potential of *Daniellia oliveri* (Rolfe) Hutch. & Dalz.

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The chemical composition of the oleoresin from *Daniellia oliveri* (Rolfe) Hutch. & Dalz. of the family Caesalpiniaceae was determined using GC-FID/GC-MS and its potentials as antioxidant and cytotoxity evaluated for the first time. The GC-MS analysis revealed the major constituents of the resin as volatile diterpenoids. The major compounds include δ-cadinene (42.92%), copaene (11.36%), cis-muurola-4(14),5-diene (9.56%), polyalthic acid (4.6%), β-calacorene (4.37%), 2(5H)-Furanone-5-(2,5-dimethylphenyl)-4-methyl- (4.35%) and aromadendrene (4.14%). A significant antioxidant activity (IC_{50}= 15.49 ± 0.39 mg/mL) and low cytotoxicity (IC_{50} > 30 µg/mL) on prostate cancer cell line was obtained for the oleoresin. The low cytotoxicity and moderate antioxidant potential of the oleoresin from *D. oliveri* is an indication of possible application in related *in vivo* studies for future therapeutic applications. The major compounds being sesquiterpenes could be used as a biomarker for the chemotaxonomical characterization of the species.

Key words: Cadinene, Copaene, *Daniellia oliveri,* Diterpenoids, Cytotoxicity, Antioxidant

Daniellia oliveri (Rolfe) Hutch. & Dalz. Bitkisinin Kimyasal Bileşimi, Antioksidan ve Sitotoksik Etkisi

Caesalpiniaceae familyasına ait *Daniellia oliveri* (Rolfe) Hutch. & Dalz. bitkisinin oleorezin içeriği GC-FID/GC-MS teknikleri kullanılarak belirlendi ve bitkinin antioksidan ve sitotoksik etkisi ilk defa araştırıldı. GC-MS analizlerinde, reçinenin ana bileşenleri olarak uçucu özellikteki diterpenoidler tespit edildi. Ana bileşiklerin, δ-kadinen (%42.92), kopaen (%11.36), cis-muurola-4(14),5-dien (%9.56), polialtik asit (%4.6), β-kalakoren (%4.37), 2(5H)-furanon-5-(2,5-dimetilfenil)-4-metil- (%4.35) ve aromadendren (%4.14) olduğu belirlendi. Oleoriezinin anlamlı derecede antioksidan aktivitesinin (IC_{50}= 15.49 ± 0.39 mg/mL) ve prostat kanser hücreleri üzerinde düşük derecede sitotoksik etksinin (IC_{50} > 30 µg/mL) bulunduğu tespit edildi. *D. oliveri* bitkisinden elde edilen oleorezinin düşük sitotoksik ve orta derecede antioksidan etkisi, ileri terapötik uygulamalar için *in vivo* çalışmalardaki uygulanabilirliğini gösterdi. Türlerin kemotaksonomik karakterizasyonu için ana bileşenler olan seskiterpenlerin işaretleyici bileşikler olarak kullanılabileceği öne sürüldü.

Anahtar kelimeler: Kadinen, Kopaen, *Daniellia oliveri,* Diterpenoidler, Sitotoksisite, Antioksidan

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INTRODUCTION

Daniellia oliveri (Rolfe) Hutch & Dalziel of the family Caesalpiniaceae is a well-known plant in Africa and the Amazon region (1). The plant is popularly known as "*emi ya*" in south west Nigeria. It is a grossly underutilized tropical tree with many potential economic and

health values. In Gambia, resins from aromatic trees, including *D. oliveri* are sold during the rainy season as mosquito repellent (2). The bark extract is used traditionally for control of gastro-intestinal parasite (3). Also, the bark, wood and very often the resin from the plant are burnt to act as mosquito repellant (4, 5). In some part of south eastern Nigeria, a decoction of the roots of *Sarcocephalus latifolius* Sm. (Rubiaceae) and *D. oliveri* (Caesalpiniaceae) is used in folk medicine as herbal remedy for hyperglycaemia (6). The plant is used as ornamental tree in many parts of south western Nigeria and it grows widely in the forest region.

The extracts of *Daniellia oliveri* leaves have been evaluated for antimicrobial activities (7, 8), *in vitro* antioxidant potential (9), *in vivo* anti typhoid potential (10), effect on skeletal muscle (11), anti-diarrheal activity (12) and cardiovascular indices in animal model (13). The stem bark is known to produce exudates which contain majorly oleoresin and terpenoids. Specifically, the plant exudate is used among a tribe in Nigeria for wound healing (14). The exudate has been applied as a component of cosmetics and its potential as antiwrinkle agent was recently patented (15). There are limited information in literature to the best of our knowledge about the chemical composition of the resin from the plant. However, a diterpene lactone (16), daniellic acid and daniellic alcohol (17) have been isolated from the wood. In this paper we report for the first time, the chemical composition of the oleoresin determined by GC-FID/GC-MS from *D. oliveri* and its *in vitro* antioxidant and cytotoxicity potentials.

MATERIALS AND METHODS

Materials

Daniellia oliveri exudate was obtained from the tree trunk within the premised of university of Ilorin, Ilorin, Nigeria. The plant was identified by a plant Taxonomist at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The Voucher specimen number UIH 964 was obtained.

GC-FID/GC-MS analyses

In order to determine the chemical composition of the exudate, about 0.5 g of the plant exudate was dissolved in approximately 4.0 mL of dichloromethane and filtered. It was further diluted to microgram unit and subjected to GC and GC-MS analysis using Shimadzu-GC-9A gas chromatograph, FID at 220, N_2 at1.0 mL/min, SPB-5 capillary column (30 m × 0.53 mm ID; 0.32 mm), split ratio 1:30, injector temperature 240°C, temperature of column maintained at 50°C for five minutes and then raised to 235°C (5°C/min) followed by five minutes at 235°C. GC-MS: Hewlett-Packard 6890 gas chromatograph, combined with a Jeol JMS-HX 110 mass spectrometer with source at 270°C at 70 eV. Injector was set at 270°C with splitting ratio 1:30. A mass spectral survey was performed using the NIST mass spectral program 2008.

Assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH), antioxidant activity

The DPPH spectrophotometric assay was carried out as previously descibed (18, 19). The DPPH free radical methanol solution (0.1 mM) was prepared and protected from light by keeping it in the cool and dark place. Stock solution of the oleoresin (1mg/mL) was diluted to final concentrations of 500, 250 and 200 µg/mL in methanol. 1 mL of 0.1 mM DPPH methanol solution was added to solutions of the resin or standard (α–tocopherol). The absorbance at 518 nm was monitored in presence of different concentrations of extracts. Blank experiment was also carried out to determine the absorbance of DPPH before interacting with the extract. Absorbance was recorded to check the stability of the radicals throughout the time of analysis. The antioxidant activity, AA was calculated using the following equations (20).

$$AA = 100 \times [(Abs_{control} - Abs_{sample})]/(Abs_{control}).$$

Where Ab_{sample} and Ab_{blank} is the absorbance value of sample and blank respectively.

Table 1. Chemical composition of compounds in *Daniellia oliveri* exudate

S/N	RT	Compounds	%Area
1	16.68	β-Cubebene	1.79
2	17.4	Copaene	11.36
3	17.75	Cis-muurola-4(14),5-diene	9.56
4	19.6	Aromadendrene	4.14
5	19.97	Murolene	1.08
6	21.12	δ-Cadinene	42.92
7	21.65	Bicyclo[3.3.1]nonane, 1-phenyl-	2.60
8	22.12	β-Calacorene	4.37
9	22.53	Spathulenol	1.65
10	22.62	1-Formyl-2,2-dimethyl-3-trans-(3-methyl-but-2-enyl)-6-methylidene-cyclohexane	1.95
11	23.23	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene	0.52
12	23.5	Mansonone C	0.34
13	24.38	Cadala-1(10),3,8-triene	1.06
14	24.57	2(5H)-Furanone, 5-(2,5-dimethylphenyl)-4-methyl-	4.35
15	25.18	14-heptadecenal	1.27
16	26.1	1.1-(4-Hydroxy-7-isopropyl-4-methyloctahydro-1H-inden-1-yl)ethanone	2.49
17	26.75	5,14-Dimethyl-12,17-dioxogonan-3-yl acetate	0.39
18	27.1	2,2,6,6-Tetramethylheptane	0.24
19	27.55	4-Hydroxyimino-but-2-enoic acid, ethyl ester	0.19
20	28.05	1,2-Dimethylcyclopentanol	1.24
21	30.65	n-hexadecanoic acid	1.78
22	39.28	polyalthic acid	4.60

RT: Retention time

Cytotoxicity evaluation – cell viability assay

Cytotoxic activity of the oleoresin was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazoliumbromide) colorimetric assay (21). PC-3 cells (Prostrate Cancer cell line), was cultured in Dulbecco's Modified Eagle's Medium, supplemented with 5% of foetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 25 cm^3 flask, and kept in 5% CO_2 incubator at 37 ^oC. Exponentially growing cells were harvested, counted with haemocytometer and diluted with the FBS medium. Cell culture with the concentration of $1x10^5$ cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of the oleoresin dissolved in the PBS medium (1-100 µM). After 72 h, 50 µL MTT (2 mg/mL) was added to each well and incubated further for 4 hrs. Subsequently, 100 µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microplate ELISA reader (Spectra Max plus, Molecular Devices, CA, USA). Doxorubucin was used as positive controls for Prostrate Cancer cell line. IC_{50} was recorded as concentration causing 50% growth inhibition (cytotoxicity) of the cells.

Data analysis

The 50% cytotoxic (IC_{50}) was calculated from dose-response- inhibition curves after nonlinear regression analysis. The results represent the mean ± standard error of the mean values of three different experiments.

RESULTS AND DISCUSSION

The GC-MS of the exudates (Table 1) revealed the presence of diterpenes as the major constituents and δ-cadinene (42.92%) is the most abundant. Others are copaene (11.36%), cis-muurola-4(14),5-diene (9.56%), polyalthic acid (4.6%), β-calacorene (4.37%), 2(5H)-Furanone, 5-(2,5-dimethylphenyl)-4-methyl- (4.35%) and aromadendrene (4.14%). The other compounds detected are less than 3% each.

Table 2. Cytotoxicity (on PC3 Cell line) and DPPH antioxidant capacity of *Daniellia oliveri* oleoresin

Samples	**Cytotoxicity IC_{50} (μg/mL)**	**Antioxidant capacity IC_{50} (μg/mL)**
Oleoresin	> 30	15.49 ± 0.39
Doxorubicin	2.8 ± 0.12	-
α - Tocopherol	-	0.25 ± 0.40

IC_{50}: 50% inhibition concentration. Results represent mean ± standard error of mean of replicate determinations.

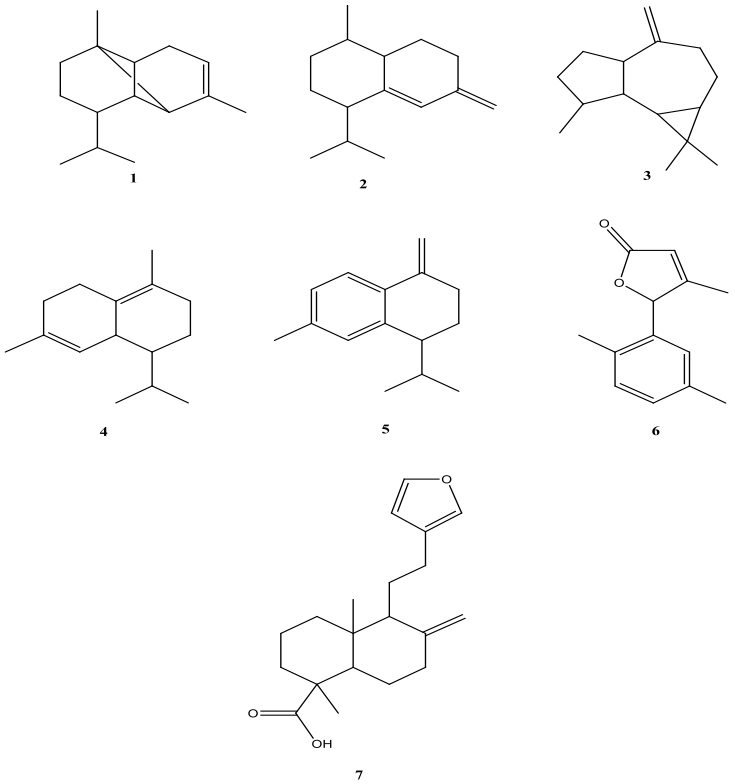


Figure 1. Major compounds identified in the *Daniellia oliveri* exudates
1- Copaene; **2**- Cis-muurola-4(14),5-diene; **3**- Aromadendrene; **4**- δ-Cadinene; **5**- β-Calacorene; **6** -2(5H)-Furanone, 5-(2,5-dimethylphenyl)-4-methyl; **7** - Polyalthic acid

These major compounds (Figure 1) certainly contribute to the overall odour as well as the antioxidant and cytotoxicity of the oleoresin. Cadinene has been detected in the volatile of strawberry guava (22), copaene in the volatile of *Michelia alba* flowers (23) while polyalthic acid has been isolated from *Daniellia oliveri*

and examined for its antiglycation potential (24).

The cytotoxicity of the plant exudates to PC3 cell line was low when compared to doxorubicin while the DPPH antioxidant potential was very significant (Table 2) though lower than the standard antioxidant, α-tocopherol.

CONCLUSION

The chemical composition of the oleoresin from *Daniellia oliveri* was investigated. The three major compounds being δ-cadinene (42.92%), copaene (11.36%), and cis-muurola-4(14),5-diene (9.56%) accounting for about 64% indicated that the plant could be a renewable source of natural sesquiterpenes. The identified sesquiterpenes may be a vital biomarker for the quick chemotaxonomical characterization of the plant species and family. The oleoresin indicated low activity against prostate cancer cell line and a moderate radical scavenging potential. The oleoresin may be a possible source of natural antioxidant for further investigations.

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