Bull. Chem. Soc. Ethiop. **2022**, 36(1), 85-94.
DOI: https://dx.doi.org/10.4314/bcse.v36i1.8

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

ANTIOXIDANT AND ANTIMICROBIAL EVALUATION OF THE METHANOL LEAF EXTRACT AND FRACTIONS OF *DACRYODES KLAINEANA* PIERRE (BURSERACEAE)

Ebere C. Ifejirika[1], Eze E. Ajaegbu[2*], Chioma I. Ibe[1], Felix A. Onyegbule[3] and Obidike C. Ezugwu[4]

[1]Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[2]Department of Applied Sciences, Faculty of Applied Sciences and General Studies, Federal College of Dental Technology and Therapy, Trans-Ekulu, Enugu State, Nigeria
[3]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[4]Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria

(Received November 12, 2020; Revised January 3, 2022; Accepted January 27, 2022)

ABSTRACT. *Dacryodes klaineana* is used in traditional medicine for the treatment of painful menstruation; tachycardia and cough. The study aims to evaluate the antioxidant and antimicrobial properties of the plant. The extract and fractions of *D. klaineana* were screened for their antioxidant effects using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and the total phenolic contents (TPC) assay methods. The antimicrobial activity of the plant was evaluated using the agar diffusion method against four different bacterialstrains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*) and two fungi strains (*Aspergillus niger* and *Candida albicans*). The crude methanol extract (MLE), hexane fraction (MLHF), ethyl acetate fraction (MLEF) and butanol fraction (MLBF) produced dose-dependent antioxidant activity using DPPH and FRAP assays. The antioxidant activity of MLEF was comparable to the standard drug, ascorbic acid. MLEF also gave the highest content of total phenol content with a percent gallic acid equivalent of 100.68 ± 1.17 mg GAE/g. The extract and fractions were active against the bacteria species, but inactive against the fungi species. The study demonstrated that the antioxidant and antimicrobial potentials of *D. klaineana* may offer a promising source of new antioxidants and antimicrobials in the ethnomedicinal management of diseases.

KEY WORDS: Medicinal plant, FRAP, TPC, DPPH, Antioxidant, *Dacryodes klaineana*

INTRODUCTION

Plants over the years have proven to be used medicinally and presently, numerous solutions to diseases or ailments have its origin from plants [1]. According to the World Health Organization, approximately 80% of the population utilizes traditional medicines globally. The majority of these medicines are gotten from plants as complementary or alternative medicine [2].

Antioxidant's crucial part is to protect against damages occasioned by oxidative stress. Oxidative stress has always been the primary contributor to the development of several diseases, such as cancer, diabetes and heart disease. Consumption of fruits and vegetables has been shown to reduce the risk of these diseases occasioned by oxidative stress [3]. Micro-organisms cause several diseases such as endovascular infection (*Staphylococcus aureus*), food poisoning (*Bacillus subtilis*), typhoid fever (*Salmonella typhi*), candidiasis (*Candida albicans*) and taenia infections (Dermatophytes). In previous years, the majority of these organisms have continued to show rapid resistance against current antimicrobial agents [4], hence, there is an urgent need for an alternative therapy for the treatment of the infections caused by these microbes.

*Corresponding author. E-mail: ajaegbuee@yahoo.com

Dacroydes klaineana Pierre commonly known as monkey plum or African cherry fruit, belonging to the family of Burseraceae [5]. It comprises of about 40 species, found in the American, Asian and African tropics. The ground leaf is effective when applied against painful menstruation. In Côte d'Ivoire, *D. klaineana* is used to treat tachycardia and cough [5]. The plants from such a genus and family are potential sources of antimicrobial and antioxidants agents [3]. Thus, the assessment of such properties remains an interesting and useful task, particularly to find new promising sources of natural antioxidants and potent antimicrobials for functional foods and /or nutraceuticals [3]. Therefore, in this study, we evaluated the antioxidant and antimicrobial activities of the methanol crude extract and fractions of *D. klaineana* leaves.

EXPERIMENTAL

Plant materials

The leaves of *D. klaineana* were collected from Orba, Nsukka, Enugu State Nigeria. Identification and authentication of the plant was done by a taxonomist, at the Department of Pharmacognosy and Environmental Science, University of Nigeria, Nsukka, on 18th August 2018.

Extraction and fractionation of the plant material

The extraction and fractionation processes were carried out as previously reported [6, 7]. Freshly harvested plant materials were cleaned and dried at room temperature and were mechanically pulverized into coarse powders. The powdered plant material (2 kg) was cold macerated with 10 L of methanol for 72 h with periodic shaking at 2 h interval. This was followed by decantation and the resulting solutions were collected and filtered using Whatman filter paper (No. 1). The filtrates were pooled and concentrated to dryness. The filtrate obtained was concentrated to dryness in *vacuo* using a rotary evaporator (RE300 - Stuart, Barloworld Scientific Ltd, Stone, Staffordshire, UK) at 30 ºC and the methanol extract (MLE) obtained. The MLE was adsorbed in silica gel (70–230 mesh size) and sequentially fractionated using hexane (MLHF), ethylacetate (MLEF), and butanol (MLBF) following the solvent polarities. The same rotary evaporator was used to concentrate the fractions at 40 ± 5 ºC. The crude methanol extract and fractions were stored in the refrigerator at -4 ºC before use.

2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay

A volume of 50 mL of 0.2 mM solution of DPPH in methanol was prepared by weighing 3.94 mg of DPPH to 50 mL of methanol. Also, 2 mL of 0.2 mM solution of DPPH was added to 2 mL of the sample and quercetin, respectively and dissolved in methanol (1 mg/mL, 1000 µg/mL). These final reaction mixtures resulted in a 2-fold dilution of both the extract and DPPH concentrations bringing them to a final concentration of 0.1 mM for the DPPH solution and 500 µg/mL for the samples. The absorbance readings were measured at 517 nm using a 6505 UV-VIS spectrophotometer (Jenway, UK) after thorough shaking and standing at room temperature for 30 min. The free radical scavenging activity was quantified using the formula [8]:

DPPH scavenging activity = 100 x [(AC - AS)/AC]. AC = Absorbance of control; AS = Absorbance of Sample. All the tests were performed in triplicates.

Ferric chloride reducing antioxidant power (FRAP) assay

The reducing concentration power of the extract and its fractions were carried out according to the procedures described [9]. Different concentrations of fungal extracts (10–50 µg/mL) were

added to 0.2 M sodium phosphate buffer and 2.5 mL of 1% potassium ferric cyanide solution. The mixture was shaken properly and incubated at 50 ˚C for 20 min with intermittent shaking. After the incubation, 2.5 mL of 10% trichloroethanoic acid was added to the mixture and centrifuged at 3000rpm for 10 min. 2.5 mL of the supernatant was collected and mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% iron (III) chloride ($FeCl_3$). The colored solution was read at 700 nm against blank using a UV spectrophotometer. The blank contained everything except the extract. Ascorbic acid was used as the standard. Tests were performed in triplicate.

Determination of total phenolic contents (TPC)

The total phenolic content was determined for individual extracts using the Folin-Ciocalteu method [10]. Briefly, 1 mL of the extract was mixed with 2.5 mLof 10% w/v Folin-Ciocalteu reagent. After 5min, 2 mL of Na_2CO_3 (75%) were subsequently added to the mixture and incubated at 50 ºC for 10 min with intermittent agitation, Afterwards, the sample was cooled and the absorbance was measured utilizing a UV Spectrophotometer at 756 nm against a blank without extract. The outcome data were expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of the dry extract.

Antimicrobial screening

Antimicrobial screening of the fungal extracts was carried out using the agar well diffusion assay method as previously described [8, 11]. A concentration of 1 mg/mL of the fungal extracts was prepared by dissolving the extracts in dimethyl sulphoxide (DMSO). A range of concentrations between 1 mg/mL and 12.5 µg/mL were obtained by two-fold serial dilutions. Standardized broth cultures (0.5 McFarland turbidity standard) suspension of each of the test isolates of test bacterial strains (*S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*, and fungal strains (*A. niger* and *C. albicans*) were spread aseptically onto the surface of Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates respectively by using sterile cotton swabs. All culture plates were allowed to dry for about 5 min and agar wells were made by using a sterile cork-borer (6 mm in diameter). These wells were respectively filled with 20 µL of the fungal extracts and controls. The plates were then kept at room temperature for 1 h to allow the agents to diffuse into the agar medium. Ciprofloxacin (5 µg/mL) and miconazole (50 µg/mL) were used as positive controls in the antibacterial and antifungal evaluations respectively; while DMSO was used as the negative control. The MHA plates were then incubated at 37 ºC for 24 h, and the SDA plates were incubated at 25-27 ºC for 2-3 days. The inhibition zones diameters (IZDs) were measured and recorded. The size of the cork borer (6 mm) was deducted from the values recorded for the IZDs to get the actual diameter. This procedure was conducted in triplicate and the mean IZDs calculated and recorded.

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out using SPSS version 16. Statistical analysis was also done using one-way analysis of variance (ANOVA). The obtained results were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Yields of the extract/fractions

Dacryodes klaineana is an evergreen tree with a low, spreading crown; usually growing up to 20 metres tall but with specimens up to 30 m being recorded [12]. Preliminary phytochemical

analysis indicates the appearance of terpenoids, alkaloids, saponins, cardiac glycoside, tannins, and flavonoids.

The yields of the extract/fractions are presented in Table 1. The methanol extract yielded 4.85% calculated from 2 kg of dried leaves. The butanol, ethyl acetate, and hexane fractions calculated from 96.93 g of the methanol extract yielded 41.07, 29.96 and 8.1 g, respectively. Butanol gave the highest followed by the ethyl acetate while hexane gave the lowest yield.

Table 1. Yields of the extract/fractions of the leaf of *D. klaineana.*

Extract/fractions	Yield (g)
MLE[a]	96.93
MLBF[b]	41.07
MLEF[b]	29.96
MLHF[b]	8.10

MLE = methanol crude extract, MLBF = methanol butanol fraction, MLEF = methanol ethyl acetate fraction, MLHF = methanol hexane fraction. a = yield calculated from 2 kg of plant material. b = yield calculated from 79.13 g of methanol leaf extract.

Antioxidant assay

The extract and fractions were screened for antioxidant activity using the DPPH and FRAP scavenging model. The results of antioxidant screening (DPPH and FRAP) are shown in Figures 1, 2 and Table 2. The ethyl acetate and butanol fractions have good activities against the scavenging effects on DPPH at a very low concentration of 31.25 µg/mL. This correlates with their EC_{50} values of 18.09 and 19.45 µg/mL, respectively. The ethyl acetate and butanol fractions were more potent than the standard drug, ascorbic acid, with an EC_{50} of 29.85 µg/mL.

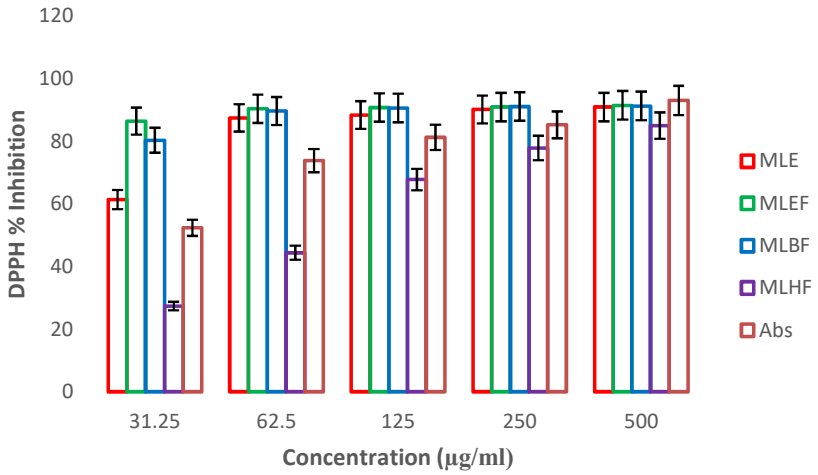

Figure 1. DPPH radical scavenging activities of extract/fractions from the leaves of *D. klaineana*. MLE = methanol crude extract, MLBF = methanol butanol fraction, MLEF = methanol ethyl acetate fraction, MLHF = methanol hexane fraction, Abs = ascorbic acid.

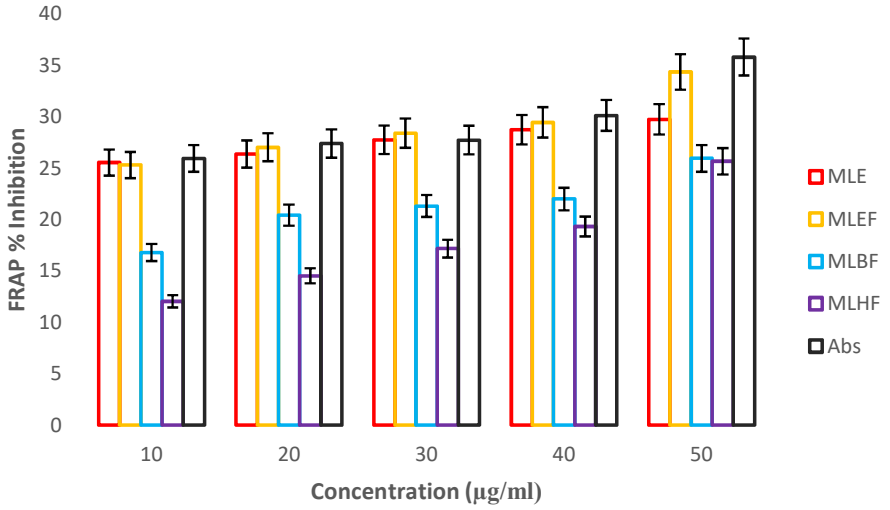

Figure 2. FRAP scavenging activities of extract/fractions from the leaves of *D. klaineana*. MLE = methanol crude extract, MLBF = methanol butanol fraction, MLEF = methanol ethyl acetate fraction, MLHF = methanol hexane fraction, Abs = ascorbic acid.

Table 2. EC_{50} of the extract and fractions of the leaves of *D. klaineana.*

Extract/fractions	EC_{50} (µg/mL)	
	DPPH	FRAP
MLE	25.46	84.01
MLEF	18.09	72.74
MLBF	19.45	96.30
MLHF	57.07	97.39
Abs	29.85	69.79

Free radicals are responsible for the oxidative harm to DNA by the causation of cancers and lipid oxidative harm in the formation of vascular diseases [13]. Excess of human exposure to these free radicals leads to degradation of biomolecules and cell damage, hence over-expression of oncogenes, mutagens formation, induction of atherogenic activity, or inflammation [14]. Phenolic compounds play a great role in protecting us against these diseases [15]. Medicinal plants have been reported to have antioxidant potentials in the treatment and control of these oxidative disorders [16].

Total phenolic contents of the extract and fractions of D. klaineana leaf

The total phenolic contents of the extract and fractions quantified by gallic acid equivalent are shown in Table 3. The methanol leaf extract showed high content of phenolic compounds with a gallic acid equivalent of 75.5 mg. Of all the fractions, ethyl acetate fraction showed the highest content of phenolic compounds (100.68 ± 1.17 mg gallic acid equivalent) while the hexane fraction showed the least content of phenolic compounds (2.05 ± 0.08 mg gallic acid equivalent). This correlates with the antioxidant screening result, where ethyl acetate fraction also had the highest activity. There was a correlation between the TPC with the antioxidant capacity of the

extract and fractions [17]. In this study, the high quantity of the phenolic compounds gave rise to high antioxidant properties. The antioxidant activity was attributed to the presence of phenolic compounds in the leaves [18]. Previous studies indicate that the total phenolic content in medicinal plants ranged from 6.80-32.10 mg gallic acid equivalents per g dry weight basis [19, 20]. This assertion aligns with the present study. Phenolic components in medicinal plants play a major role in flavor, aroma, taste and color modifications. It constitutes health advantageous impacts. It helps in defending medicinal plants against reactive oxygen components, microorganisms, herbivores and insects [20, 21].

Table 3. Total phenolic contents of the extract and fractions of *D. klaineana.*

Extract/fractions	TPC (mg GAE/g)
MLE	75.55 ± 0.58
MLEF	100.68 ± 1.17
MLBF	44.17 ± 0.49
MLHF	2.05 ± 0.08

Antimicrobial activity

Staphylococcus aureus, B. subtilis, E. coli, P. aeruginosa, A. niger and *C. albicans* are the most common pathogenic microorganisms with clinical significance to human [22].The outcome of the antimicrobial activities of the plant extract and fractions are shown in Figures 3-6 and Table 4. The fungal extract and fractions were tested against *S. aureus, B. subtilis, E. coli, P. aeruginosa, A. niger* and *C. albicans.* The antimicrobial activities of the extract/fractions were tested at different concentrations: 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.13 mg/mL. These were compared to the antimicrobial activity of the standard drugs, ciprofloxacin (5 µg/mL) and miconazole (50 µg/mL) for bacteria and fungi respectively. The crude methanol extract showed activity against *S. aureus* (MIC = 25 mg/mL), *B. subtilis* (MIC = 12.5 mg/mL), *E. coli* (MIC = 25 mg/mL), *P. aeruginosa* (MIC = 12.5 mg/mL). It gave no activity against *A. niger* and *C. albicans* even at 50 mg/mL.

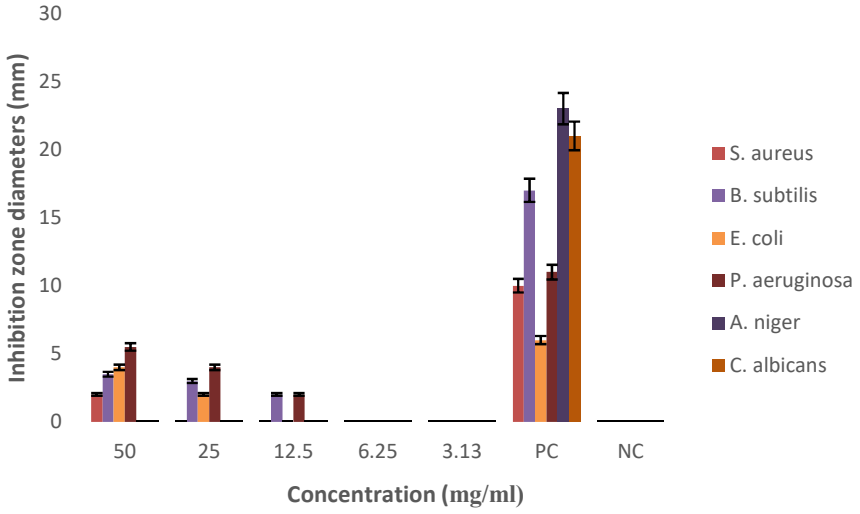

Figure 3. Mean inhibition zone diameters (mm) produced by different concentrations of methanol leaf extract of *D. klaineana*on test isolates. PC: positive control and NC: negative control.

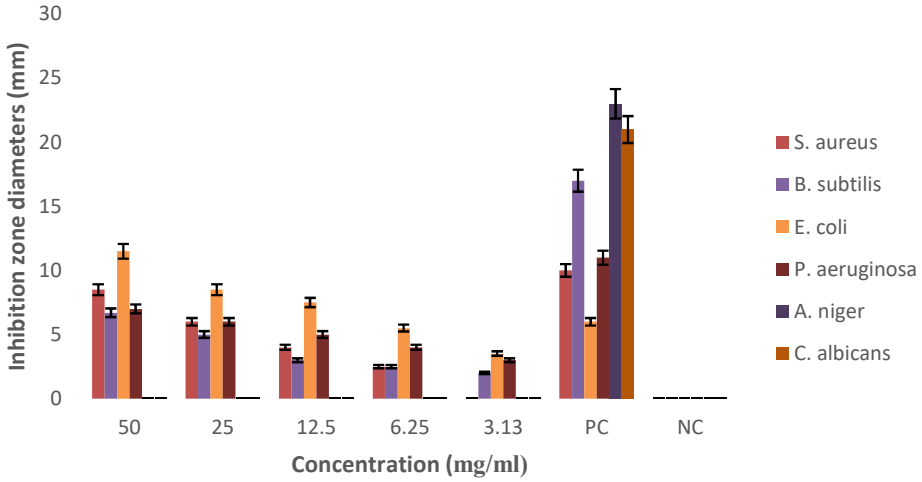

Figure 4. Mean inhibition zone diameters (mm) produced by different concentrations of methanol leaf ethyl acetate fraction of *D. klaineana*on test isolates. PC: positive control and NC: negative control.

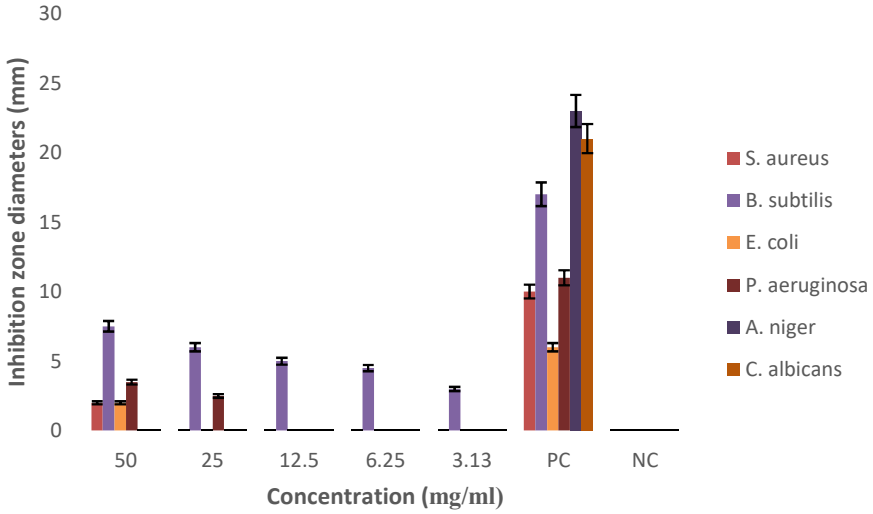

Figure 5. Mean inhibition zone diameters (mm) produced by different concentrations of methanol leaf hexane fraction of *D. klaineana*on test isolates. PC: positive control and NC: negative control.

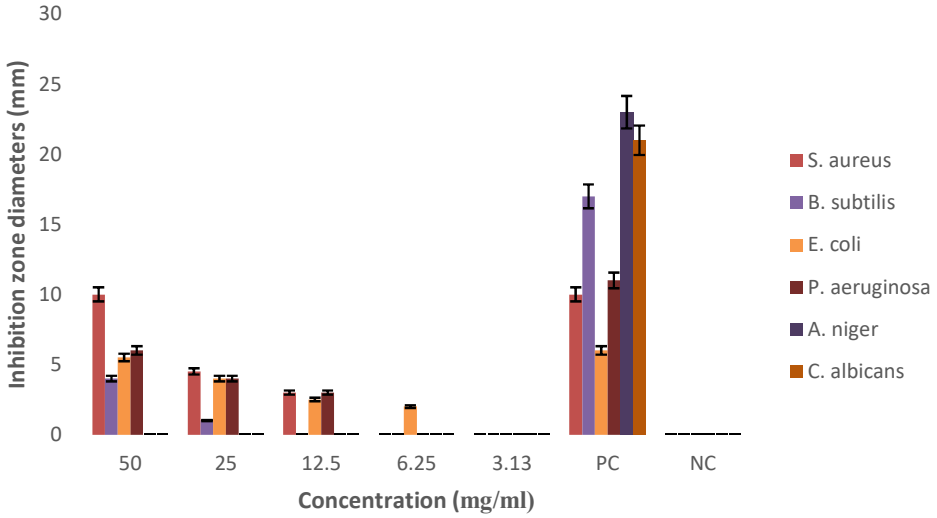

Figure 6. Mean inhibition zone diameters (mm) produced by different concentrations of methanol leaf butanol fraction of *D. klaineana*on test isolates. PC: positive control and NC: negative control.

Table 4. Minimum inhibitory concentrations of the extract/fractions of *D. klaineana.*

Test organisms	MICs (mg/mL)			
	MLE	MLEF	MLBF	MLHF
S. aureus	25	3.13	6.25	25
B. subtilis	12.5	3.13	25	3.13
E. coli	25	<3.13	12.5	25
P. aeruginosa	12.5	3.13	12.5	25
A. niger	ND	ND	ND	ND
C. albicans	ND	ND	ND	ND

MLE = methanol crude extract, MLBF = methanol butanol fraction, MLEF = methanol ethyl acetate fraction, MLHF = methanol hexane fraction. ND: not determined.

Among all the fractions, the ethyl acetate fraction of the plant had better antimicrobial activity against the bacteria species than other fractions, followed by butanol fraction. All the fractions had no antimicrobial activity against *A. niger* and *C. albicans*, even at the highest concentration (50 mg/mL) used for the study. The MICs of the ethyl acetate fraction ranged between < 3.13 and 3.13 mg/mL. The hexane fraction gave the lowest antimicrobial activity with its MIC range between 3.13 and 25 mg/mL. The results of this study showed that out of the four screened extract/fractions, all were active against bacteria and none against fungi. The inhibition zones diameters were between 2 and 11 mm. These extracts showed activities against more than two strains of bacteria with low MIC values.

Tchouya *et al*. [23] reported the antimicrobial screening of the aqueous alcohol extracts of the barks and leaves of *D. klaineana* against clinical isolates and ATCC strains involved in HIV/AIDS opportunistic diseases. At 10 mg/mL, the aqueous alcohol extract of the leaf was active against *P. aeruginosa*, *Shigella flexneri*, *Streptococcus agalactiae*, *S. aureus* and *E. coli* with MIC of 0.75,

1, 1.5, 1, 0.5 mg/mL, respectively. The aqueous alcohol extract of the bark was active against *P. aeruginosa*, *Shigella flexneri*, *Streptococcus agalactiae*, *S. aureus* and *C. albicans* (ATCC P37037) with MIC of > 1.5 > 4, 0.5 > 4 > 4 mg/mL, respectively [23]. The report by Tchouya *et al*. [23] is similar to the present study since the extracts of the leaves of *D. klaineana* from the previous and present study were active against *P. aeruginosa*, *S. aureus* and *E. coli*, but inactive against *C. albicans*.*Bacillus subtilis* and *P. aeruginosa* are the most sensitive microorganisms from the study; is strongly inhibited by all the extracts. The strong inhibition of *B. subtilis* and *P. aeruginosa*by all the extracts was in line with the previous study by Tchouya *et al*. [23]. According to Morales *et al*. [24], different parameters of antimicrobial activity were considered: MIC < 100 µg/mL, active; MIC 100 - 500 µg/mL, moderate; MIC 500 - 1000 µg/mL, weak antimicrobial activity; and inactive [24]. Based on the adopted criteria, the different plant extracts showed good activities against the test organisms. Therefore, a thorough examination of recent techniques for the growth of new antimicrobials is essential to improve the therapy of infections [25].

CONCLUSION

The present study has indicated that plant could serve as a potent source of antimicrobial and antioxidant substances. Since the extract and fractions were active against most of the pathogens used in this study, there is a need for more pharmacological and antimicrobial screenings to be carried out. The plant *D. klaineana* will be beneficial in the prevention of different diseases and complications and has great potentials for its utilization in pharmaceutical use.

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