Trop J Nat Prod Res, October 2021; 5(10):1847-1851 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Volatile Phytochemical Compositions of *Diodella sarmentosa* Leaf and its Total Dehydrogenase Inhibitory Potential

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ARTICLE INFO

Article history:
Received 04 February 2021
Revised 16 August 2021
Accepted 25 October 2021
Published online 02 November 2021

ABSTRACT

In southern Nigeria, *Diodella sarmentosa* leaf is traditionally used in the treatment of various microbial infections. This work, therefore, aimed to establish the science behind the traditional use of the ethanol leaf extract of the plant for the treatment of microbial infections. Volatile organic compounds in the ethanol leaf extract of the plant were assessed using gas chromatography equipped with mass spectrometry (GC-MS). The activity of the extract on selected microorganisms and, their total dehydrogenase enzyme were assayed. Various volatile compounds were revealed by the GC-MS analysis with the major constituents being squalene (29.50%), Phytol (24.68%), 3-Pentadecyl-phenol (18.58%), 3-Methyl-1-butanol Isopentyl alcohol (9.09%), and Hexadecanoic acid (7.78%). The antimicrobial activity of the extract was first determined on six selected gram-negative (*Salmonella typhi* and *Escherichia coli*), gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and fungi (*Candida albicans* and *Pennicilium spp*) isolates. *Bacillus subtilis, Candidas spp* and *Penicillium spp* recorded higher zones of inhibition than the others. As a result, further studies of the extract effect on the dehydrogenase activity of these three most sensitive microorganisms were studied. The activity of the enzyme from *Candidas spp*, *Penicillium spp*, and *Bacillus subtilis* was progressively inhibited at increasing extract concentrations from 0 to 2000 mg/mL; and the threshold extract inhibitory concentrations (IC_{50}) were 275 mg/mL, 322 mg/mL, and 411 mg/mL, respectively. From the findings, it can be concluded that the ethanol leaf extract of *Diodella samentosa* which is rich in antimicrobial volatile organic compounds inhibited microbial dehydrogenase activity.

***Keywords*:** *Diodella sarmentosa*, Volatile Phytochemicals, Microorganisms, Dehydrogenase enzyme.

Introduction

The widespread antimicrobial-resistant pathogens are a global threat, and of major concern is the rapid global spreading of "superbugs" which has been established as the causative agents of major untreatable infections using the existing antibiotics. Drug-resistant fungi are a source of major concern given the difficulties associated with the treatment of fungal infections.[1]

Given this, research efforts are now geared towards proffering solutions to this emerging global challenge. With the increasing ineffectiveness of the existing antibiotics amidst the rising global spread of these resistant pathogens,[1] plant extracts offer an alternative source of sustainable antimicrobial agents.[2]

Over the years, plant-based regimens have remained effective agents for combating microbial infections; and the continuous efficacy of these agents against bacteria, fungi and viruses may portent its reduced adaptability to microbes.[3] More so, the synergistic effects of the various phytochemical components of the plant-based regimen may enable it to act at different sites on the microbial targets. The antimicrobial property of plant-based regimens is widely reported [4-6] and, their mechanisms of action have also been recorded.[7,8]

D. sarmentosa is a perennial plant with alternating leaf arrangement on the stem. It is a common plant in the forest of tropical Africa.[9] It has been reported to poses anti-diabetic property[10, 11,] and; has also been found useful in the treatment of eczema, injury, and oedema[12,13] among the locals in southern Nigerian, and has been found to have antioxidant[14]and anti-inflammatory[15] properties.

However, it has been reported that the viability of microbial population depends on oxido-reduction metabolism[16] which in turn is catalyzed by oxido-reductases and includes dehydrogenase with Enzyme Commission number of 1.1.1 (EC 1.1.1).[16] Therefore, the assessment of the total dehydrogenase inhibitory property of the ethanol extract of *D. sarmentosa* is aimed at revealing the presence of volatile antimicrobial bioactive agents in the extract since the leaf extract of the plant is known to be used among the locals in southern Nigeria for the treatment of micobial infections.[12] The uniqueness of our research stems from the fact that this is the first time the ethanol extract of the plant is assessed for its volatile constituents, as well as, its viability against microbial dehydrogenase enzyme.

Materials and Methods

Plant collection and preparation

The leaves of *Diodella sarmentosa* were collected from the premises of Federal University of Technology Owerri (FUTO), Imo State, in April 2016, and the plant was identified by Mr. Farauwa Francis, a taxonomist in the department of Forestry and Wildlife, FUTO. A

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Citation: Iheme CI, Ukairo DI, Oguoma OI, Nwaogu LA, Ezirim AU, Nzebude CP, Ezerioha CC. Volatile Phytochemical Compositions of *Diodella sarmentosa* Leaf and its Total Dehydrogenase Inhibitory Potential. Trop J Nat Prod Res. 2021; 5(10):1847-1851. doi.org/10.26538/tjnpr/v5i10.23

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

voucher specimen (voucher number 001/FWT/FUTO/2016) was deposited in the herbarium. The fresh leaves were thoroughly washed with clean running tap water 2 – 3 times and then air-dried at room temperature for two weeks. The air-dried leaf sample was pulverized and stored in an air-tight container at room temperature. The pulverized sample (20 g) was mixed with 500 mL of 95% ethanol at the temperature range of 60 to 65 ^{0}C for 24 hrs using soxhlet extractor. The resultant mixture was concentrated *in-vacuo* and finally dried over a water bath at 39℃ to obtain a dark gummy mass extract (23% yields). The resulting viscous semi-solid extract was stored in an air-tight container wrapped with aluminum foils in a refrigerator at -4^{o}C for further analysis.

Identification of volatile constituents

The crude ethanol leaf extract of *D. sarmentosa* was analyzed for the quality and quantity of the volatile phytochemicals present in it using the GC-MS technique. The GC-MS was performed on a Thermo Scientific Co, Thermo GC-TRACE Ultra Ver. 5.0, thermo MS DSQ II. Experimental conditions of GC-MS include; BS-MS dimension: 30 Mts, ID: 0.25 mm, Film thickness: 0.25 µm. The flow rate of the mobile phase (carrier gas: helium) was set at 1.0 mL/min. In the gas chromatography part, the temperature program (oven temperature) was initially 40°C and was raised to 150°C at 10°C/min. The temperature was again raised to 230°C/min at the rate 5°C/min and the process continued till the temperature remained constant at 280°C at the rate of 20°C/min which was held for 8 minutes. The injector port temperature remained constant at 280°C and detector temperature was 250°C. The plant extract (1 g) was reconstituted in 1 mL of methanol. Then, 2 µL of the extract was injected into the port and vaporized down the column with helium as the carrier gas at the flow rate of 1 mL/min and the results were compared to Wiley Spectral Library Search Program. Interpretation of the mass spectrum of the constituents of the extract was carried out in comparison with the database of the National Institute of Standard and Technology (NIST). The mass spectrum of the known components in the NIST Library was compared to the extract spectrum. The relative % quantity of each component was calculated by comparing the average peak area to the total area.

Anti-microbial activity of the extract

The modified agar well diffusion method of Collins[17] was adopted to estimate the anti-microbial activity of the ethanol leaf extract. Various concentrations of the extract, ranging from 6.25 mg/mL to 100 mg/mL were prepared in aqueous solutions. Subsequently, 0.1 mL of the standard 24 hours stock cultures of the test organisms; *S. typhi*, *E. coli*, *B. subtilis, and S. aureus* in nutrient and *Candidas albicans* and *Pennicilium spp* in potato dextrose broths were spread unto sterile nutrient, and potato dextrose agar plates (Muller – Hinton Agar),[18] respectively. In addition, wells of 5 mm in diameter were bored on the plates using a sterile cork borer, to remove agar plugs. A 0.05 mL aliquot of the different concentrations of the extract was dispensed into the wells and each plate was allowed to stand for 15 minutes for the extract to diffuse into the agar. The bacterial plates were then incubated at 33^{o}C for 48 h, while the fungal plates were incubated at 24^{o}C for 72 h. Plates that showed higher zone of inhibitions were further subjected to dehydrogenase activity assay using the modified method of Nweke.[19]

Microbial dehydrogenase inhibitory activity of the ethanol extract

The activity of total dehydrogenase was assessed using the method of Nweke[19] as modified by Alisi.[20] Accordingly, 2,3,5 – triphenyltetrazolium chloride (TTC) was used as the artificial electron acceptor, which on acceptance of electrons, changes from colorless to red triphenylformazan (TPF). A total of twenty-four (24) test tubes grouped into A, B, and C of eight test tubes each were used for the experiment.

Group A contained 4 mL of nutrient broth – glucose-TTC-medium and was inoculated with *Bacillus subtilis*, groups B and C contained dextrose broth-glucose-TTC media and were inoculated with the *Pennicillium spp and Candidas spp* respectively. Then, various concentrations of the extracts ranging from 0, 50, 100, 200, 400, 800, 1600, to 2000 mg/mL were added to each test tube. The test tubes containing 0 mg/mL concentration of the extract in each group served as the control. The plates were incubated at room temperature (28 ± 2 ^{o}C) for 16 hrs. The concentration of the triphenylformazan formed, which is proportional to the dehydrogenase activity, was read with spectrophotometer at 500 nm. By using a dose-response curve (0-20 mg/mL TPF in pentan-1-ol), the quantity of formazan yielded was computed. Linear plots of the percentage inhibition for each test organism against the various concentrations of the extract were plotted at various inhibition rates based on linear regression.

Statistical Analysis

Values obtained in triplicates were placed on a two-way ANOVA and expressed as Mean ± standard deviation. The values at $P<0.05$ were considered statistically significant. Linear regression models were applied for the determination of the IC_{50} of the extract on the organisms.

Results and Discussion

The GC-MS profile of the volatile phytochemical constituents of the ethanol leaf extract of *D. sarmentosa* revealed various volatile phytochemical compounds (Table 1). The result indicated that the ethanol leaf extract was rich in bioactive compounds like squalene (29.50%), Phytol (24.68%), 3-Pentadecyl-phenol (18.58%), 3-Methyl-1-butanol Isopentyl alcohol (9.09%), and Hexanoic acid (7.78%). Other compounds found in relatively low quantities were trans-2,4-Dimethyloxetane (1.63%), 4,5-dibromo-decane (2.94%), *9-methyl-*(3β,5α)-*androstan-3-ol* (3.17%) and [4-[(-4-) (2-hydroxy benzoyl) amino (anilino)-4-oxo](1.67%). Other compounds found in trace quantities were; 7-Azabicyclo [(4.1.0)] heptane (0.56%) and Methyl 2,4-dimethyltetradecanoate (0.56%).

The inhibitory effect of the extract against the test organisms was observed to be dose-dependent. Lower zone of inhibitions were recorded for the plant extracts against the six studied microorganisms when compared to the 0.05 % ciprofit (standard drug) (Figure 1). The rich presence of volatile constituents such as hexadecanoic acid, phytol, and 3-Methyl-1-butanol Isopentyl alcohol may have contributed to the observed inhibitory property of the extract. These compounds have been reported to have antimicrobial activities by several authors[21,22,] (Table 2).

The highest dehydrogenase activity was recorded for *Penicillium spp*, followed by *Candidas spp*, and the least being *Bacillus substilis* (Table 3). Variations in activities among the microbial strains may be connected to the physiological variations of the dehydrogenase system among microbial populations.[22] At the inhibitory extract concentrations of 275 µg/mL, 322 µg/mL, and 411 µg/mL, 50 % activities of the enzyme from *Candidas spp*, *Penicillium spp*, and *Bacillus subtilis* respectively were inhibited (Table 4). This showed that at lower concentrations, the extract had more effect on fungi than on the bacterium strain. The inhibitory effect of the extract on the organisms might be due to the presence of the detected volatile compounds in the extract (Table 1); hexadecanoic acid which was detected in the extract might be responsible for the observed effects since the compound is reported to have reductase inhibitory potential[23] (Table 2). Other constituents such as Phytol, Hexadecanoic acid, and 3-Methyl-1-butanol Isopentyl alcohol have also been reported to have antimicrobial properties[21,24](Table 2). The effect of the extract on the dehydrogenase activity was concentration-dependent and differed markedly among the studied organisms (Figure 2). The activity of the enzyme strongly correlated with the extract concentrations (Figure 3), which is indicative of the dose-response relationship. Linearized plots of Log DHG Activity against Extract Concentration (µg/mL) for *B. subtilis*, *Candidas spp*, and *Penicillium spp* were presented in Figure 3A, Figure 3B, and Figure 3C respectively. The higher value R^2 0.981 ($0.948<R^2\leq0.981$) confirmed that the concentration of the extract is inversely related to the activities of the enzyme (Figure 3). This implies that increasing extract concentration may decrease the respiratory and carbon metabolism capacity of the organisms.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: GC- MS Analysis of Phytochemical Composition of Ethanol Extract of *Diodella sarmentosa (Sw*) *Bacigalupo El Cabral ex Borhidi* leaf

Peak#	R.Time	Area	Area%	Height	Height%	A/H	Name
1	3.641	253972	1.63	22584	0.79	11.25	trans-2,4-dimethyloxetane
2	4.966	1414451	9.09	318161	11.12	4.45	3-methyl-1-butanol isopentyl alcohol
3	33.649	59581	0.38	34292	1.2	1.74	7-azabicyclo[4.1.0]heptane
4	36.499	1211535	7.78	158951	5.55	7.62	Hexadecanoic acid
5	36.704	86930	0.56	39642	1.38	2.19	Methyl-2,4-dimethyltetradecanoate
6	39.054	3841156	24.68	704109	24.6	5.46	Phytol
7	39.641	457499	2.94	64896	2.27	7.05	4,5-dibromo-decane
8	45.125	2892152	18.58	489789	17.11	5.9	3-pentadecyl-phenol
9	45.174	492998	3.17	220043	7.69	224	*9-methyl-*(3β,5α)-*androstan-3-ol*
10	45.373	262824	1.69	69455	2.43	3.78	4-[4-[(2-hydroxybenzoyl)amino]amilino]-4-oxo-
11	50.222	4591353	29.5	740513	25.87	6.2	Squalene
Total		15564451	100	2862435	100		

*The Table showed the components of the extracts with squalene, phytol, and phenol, 3-pentadecyl- as the major components.

Table 2: Major Phytochemical composition of the ethanol extract of *Diodella sarmentosa (Sw*) *Bacigalupo El & Cabral ex Borhidi* leaf and their Biological Activities

S/N	R.Time	Peak Area (%)	Names	Biological Activities	References
1	4.966	9.09	3-Methyl-1-butanol Isopentyl alcohol	Antimicrobial activities	[22]
2	36.499	7.78	Hexadecanoic acid	Anti-inflammatory, nematicide, pesticide hemolytic, 5-Alpha reductase inhibitor, antifungal activities, and antibacterial activities	[23]
3	39.054	24.68	Phytol	Antimicrobial Activities; antioxidant, anticancer and antinociceptive activities.	[24; 25]
4	45.125	18.58	3-Pentadecyl-phenol	Antioxidant	[26]
5	50.222	29.50	Squalene	Antioxidant, Antitumor	[24]

*The Table showed the biological activities of the major components of the extract.

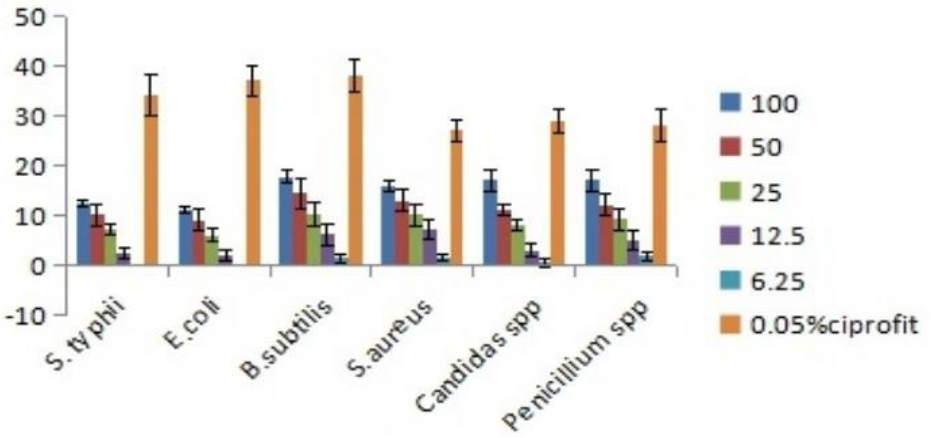

Figure 1: Diameter of Zone of Inhibition (mm) of ethanol extract of *Diodella sarmentosa* leaf against the test organisms

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Control Dehydrogenase Activity of the Microbial Isolates

Strains	Dehydrogenase Activities (mg formazan/mg cell dry weight)
Bacillus subtilis	1.064±0.14
Candidas spp	2.613 ± 0.10
Panicillium spp	2.869 ± 0.11

*At IC_{50} 275µg/mL, *Candidasspp*is the most sensitive to the extract

Table 4: Threshold Inhibitory Concentration of the extract

Strains	IC_{50} (µg/mL)	R^2
Bacillus subtilis	411	0.981
Candidas spp	275	0.954
Penicillium spp	322	0.948

* *Penicillium spp* recorded the highest dehydrogenase activity.

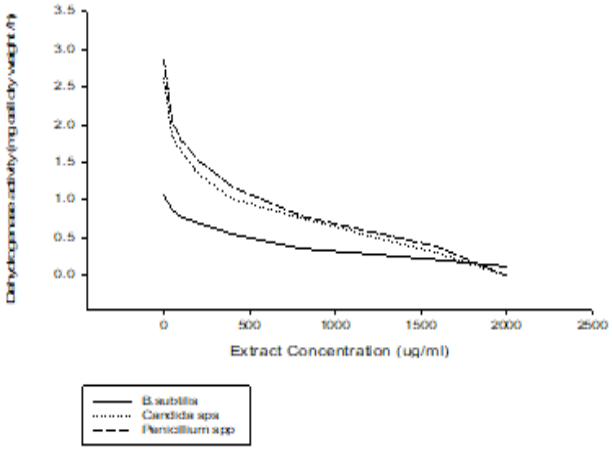

Figure. 2: Dehydrogenase activity of the organisms vs extract concentration (µg/mL).

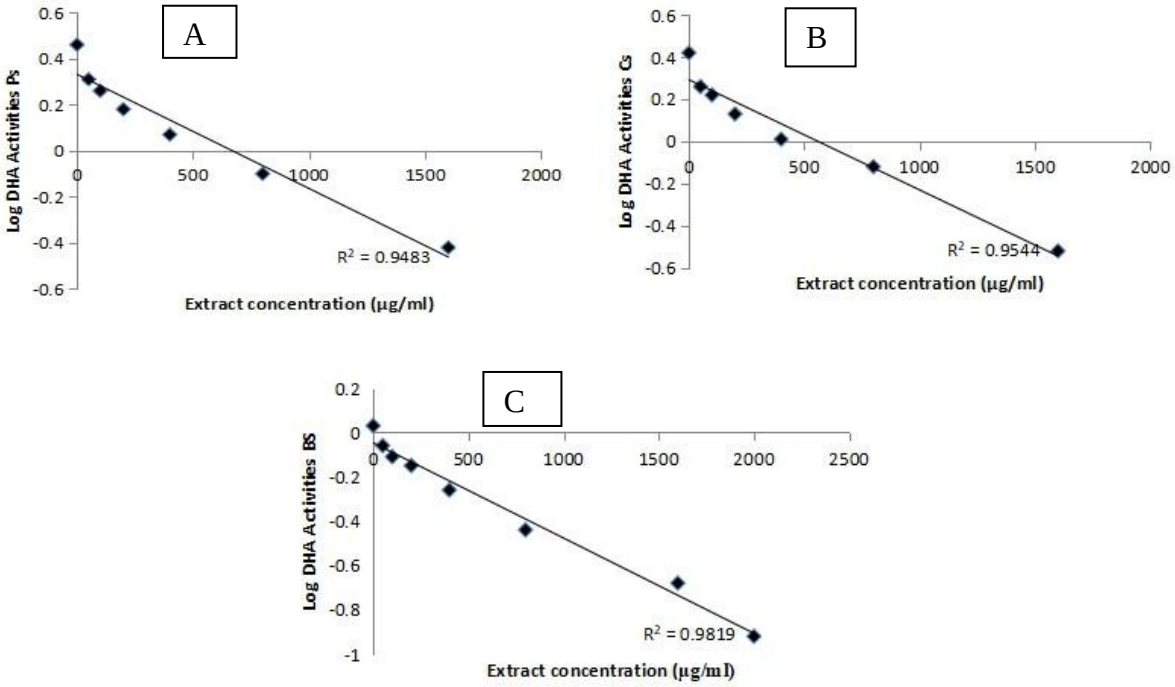

Figure 3: A, B and C**:** Linearized plot of Log dehydrogenase Activities vs Extract Concentration (µg/mL)
The R^2 values of 0.9483, 0.9544, and 0.9819 for *B.subtilis, Candidasspp, and Penicillum spp* respectively, is indicative of the strong correlation of the plant extract against the enzyme. The positive values further elucidate the inverse relationship between the extract and the enzyme.

Conclusion

Microbial dehydrogenase activity decreased with increased extract concentrations, with the activity from fungi isolates showing more sensitivity than the bacterial isolate. GC-MS result also revealed that the ethanol leaf extract of the plant is rich in squalene, phytol, hexadecanoic acid, and 3-methyl-1-butanol isopentyl alcohol. From the findings, it can be concluded that ethanol leaf extract of *Diodella sarmentosa (SW*) *Bacigalupo El & Cabral ex Borhidi* has inhibitory property against microbial dehydrogenase activity. Further study will be required to determine the action mechanism of the bioactive compounds against the microbial isolates.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors wish to acknowledge Mr. Francis, the Taxonomist that identified the plant.

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