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Antibacterial, Antioxidant Activities and GC-MS Analysis of *Dichrostachys cinera* (L.) Ethanolic Leaves Extract

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Abstract

Traditional medicinal plants are one of the potential sources of antimicrobial drugs and there is a great concern in the use and development of herbal medicine for the treatment of various infections. This study aimed to evaluate the antibacterial, and antioxidant activities of *Dichrostachys cinera* ethanolic leaves extract and to determine the components of the crude extract. *D. cinera* extract was evaluated against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. The antibacterial, antioxidant activities and active constituents were determined using standard methods. Antibacterial activity of the crude extract findings showed that all bacterial candidates were susceptible where *S. aureus* represent MIC at 12.5 mg/ml and MBC at 25 mg/ml, *E. coli* and *P. aeruginosa* both showed MIC 25 mg/ml and MBC 50 mg/ml. In the free radical scavenging assay of the extract and the standard quercetin at concentrations of 250 µg/ml, 125 µg/ml, 50 µg/ml, 10 µg/ml, and 5 µg/ml. The radical scavenging activity for the extract was about 92%, 89.6%, 86.8%, 82.8% and 37.8% respectively, compared to quercetin which gave 89.7%, 85.8%, 62.1%, 55.5%, and 45% radical scavenging activity. The GC-Ms analysis of the total constituents demonstrated that 1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexo (21.26%) with different peaks,

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followed by Glycerin (11.56%), 1,2,3-Cyclopentanetriol (10.18%), 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-(6.18%), 1H-Pyrrole, 1-methyl-(6.08%), Phytol (5.91%) and 7-Bromo-6-(2-diethylaminoethoxy)-2,3-dihyd (5.44%) as major components in the extract. Finally, this study provided useful information on the therapeutic potential of *D. cinera* as an antibacterial agent and recommended to be evaluated against a wide range of Bacterial and fungal strains using different solvents and different parts from the plant.

Keywords

Dichrostachys cinera, Antibacterial Activity, Antioxidant, GC MS Analysis

1. Introduction

The infectious diseases are the second most common leading for death worldwide [1]. The emergence increase of multidrug-resistant (MDR) bacteria is of great concern in the development of new antimicrobial agents to fight the microbes [2]. In the last decades, Antibiotics discovery may look bright as the drug delivery and nanotechnologies used to manipulate the resistant gene and to discover new natural products with various bioactivities including antibacterial activity [3].

1.1. Antibacterial Resistance

The increase in the irrational use of Antimicrobial agents leads to exacerbation of infections [4] [5], with a high prevalence of MDR bacterial infections [6]. Especially the MDR Gram-negative bacteria which lead to significant health problems worldwide, since the wide use of broad-spectrum Antimicrobial agents including Penicillins, and other β-lactams, such as: Carbapenems, Monobactam, and Cephalosporins; which usually fail to combat the MDR bacteria [7], including *E. coli* and *P. aeruginosa* [4]. In addition, the treatment of severe infections is caused by Gram-positive bacteria and since the last decades, the threat of resistant strains has increased with life-threatening consequences [8].

1.2. Alternative Medicine

Traditional medicinal plants are one of the potential sources of antimicrobial drugs and there is a great concern in the use and development of herbal medicine for the treatment of a variety of infections [9] [10] [11] [12] [13]. Different studies were done for phytochemical screening, investigation of cytotoxic effect and antimicrobial activities of medicinal plants used in folk medicine [9] [14] [15] [16].

1.3. *Dichrostachys cinera*

D cinera is a plant of the family Fabaceae widely used in the Southern part of Africa including Tanzania, Zimbabwe and Zambia in the later the study carried out by Chinsembu *et al.* (2016), stated that *D. cinera* used traditionally for the management of skin, Oral, respiratory and sexually transmitted infections, di-

arrhea and malaria which need to be encouraged by further investigations for Antimicrobial activity and determination of the active components of this plant [17]. A previous study in Zimbabwe conducted by Medzengi *et al.*, (2017) approved that *D. cinera* roots extract showed good antibacterial activity against *S. aureus* ATCC33862 and *E. coli* ATCC25922, with methanol extracts compared to aqueous extracts and they were recommended for future study to determine the active ingredient of this plant [18]. Further study was done in Tanzania by Kweyamba and colleagues (2019) on malaria and other diseases [9].

The present study is designed to investigate the antibacterial and antioxidant activities and phytochemical analysis of *D. cinera* alcoholic leaf extract.

2. Materials and Methods

2.1. Plant Materials

2.1.1. Collection of the Plant Material and Preparation of the Extract

The leaves of *D. cinera* which locally known as Kadad were collected in February 2022 from Al-nihood city, Western Kordofan State, Sudan, authenticated in the Medicinal and Aromatic Plants Research Center (MAPRC) at the University of Gezira, Sudan (Herbarium voucher number: (D 1). After thoroughly washing, the leaves were air dried at room temperature. 50 g of the powdered *D. cinera* leaves were extracted by maceration using ethanol 96% (500 ml) at room temperature for 3 consecutive days with intermittent shaking. The plant extract was filtered through a Whatman No. 1 filter paper using a Bukhnur funnel vacuum (SUPERFIT^{TM,} India, Model No. R/212/14, Voltage: 220-230 V 50 Hz). The filtrate was collected and evaporated using a rotary evaporator at 60˚C to produce a dry extract [19].

Determination of the extract Yield

The evaporated dried extract on a dry weight basis (crude extract) was calculated by the following equation:

$$X = \frac{N}{N_0} \times 100\%$$

where N_0: the weight of the dry plant material loaded for extraction.

N: the weight of the extract after the solvent evaporated [19] [20].

2.1.2. Chemicals and Reagents

The Antibiotics used for this study include: Ceftriaxone 30MCG, Item No. SD065-5CT, Cat HIMEDIA*, Meropenem 10MCG, Item No. SD727-5CT, Cat HIMEDIA*, Vancomycin 30MCG, Item No SD045-5CT, Cat HIMEDIA*. All solvents used for this experiment had an Ethanol purity of 99.9%, Methanol purity of 99.9% (Research lab, India). The purified distilled water was prepared in the Quality control laboratory, Faculty of Pharmacy, University of Gezira, Sudan. All other chemicals were of analytical grade.

2.1.3. Equipment and Instruments

All glassware such as conical flasks, round bottom flasks, cylinders, beakers, test

tubes etc., were from SCHOTT, west Germany. The sensitive balance (BOECO, Germany), the water bath (Scott Science, U.K), the autoclave (Modle: YX-280A, Volume: 18L, Pressure: 0.14 - 0.16 MPa), Incubator (BACTERIOLOGICAL INCUBATOR i-therm Al-7741) and the Hot air oven (ELECTROTHERMAL Thermostat Dryer Model: G2X-DH-300 BS, Power: 1200 W, Voltage: 220, V: 50 Hz, Date: JUL 2011).

2.1.4. Bacterial Strains

Standard strains of Gram Positive Bacteria *S. aureus* ATCC 25923, Gram negative *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were kindly obtained by the donation from the Medical laboratory and blood transfusion safety services administration, General directorate of curative medicine, Ministry of Health, Khartoum, Sudan (March 2022). The bacterial pathogens were chosen based on the WHO recommendation for the priority pathogens according to their antibiotics resistance to encourage research and development of new antibiotics [21].

2.2. Preparation of Bacterial Inoculums

The 24 h old culture of Bacterial standard strains was emulsified in sterile nutrient broth media [22].

2.2.1. *In Vitro* Antimicrobials Screening

The antibacterial susceptibility performed by using the agar well diffusion method [23] and also the procedure mentioned by Manilal *et al.*, (2020) [24] with few modifications were considered, in which sterile Mueller Hinton's agar media (HIMEDIA) was aseptically dispensed into sterile Petri dishes and uniformly, seeded with 100 µl of a suspension containing 1.5×10^8 CFU/ml of appropriate standard strains of Gram Positive Bacteria *S. aureus* ATCC 25923, Gram negative *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 using sterile cotton swab [25]. The inoculums were previously refreshed from overnight cultures by the direct colony method. Where a single colony was picked up directly from the plate with a sterile wire loop and suspended into the sterile nutrient broth. The turbidity of the suspension to be inoculated was equivalent to 0.5 McFarland's standard solution [26]. After that, the tested organisms were uniformly streaked over the surface of Mueller-Hinton agar. Then punched with the back for sterile blue tips of a graduated pipette to form 7 mm diameter wells, which were filled with the 100 µl of the appropriate extract of concentration (50 mg/ml) that was prepared by dissolving (500 mg of the dried crude extract into 10 ml of 50% methanol in distilled water) and solvent blank (Methanol 50% in distilled water) used as a negative control where the positive control used standard Antibiotics disk placed on the surface of the medium [24] [26]. Vancomycin was used for *S. aureus* ATCC 25923, Ceftriaxone for *E. coli* ATCC 25922 and Meropenem for *P. aeruginosa* ATCC 27853. The plates were then incubated at 37˚C for an overnight. After incubation, the zone of inhibition was measured in millimeters (mm). Each experiment was done in triplicate to validate the findings statistically [24] [27].

2.2.2. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antimicrobial activities of *D. cinera* were further investigated by micro-dilution method to determine the minimum inhibitory concentration (MIC) where the method approved by Parvekar *et al.*, (2020), Cheng *et al.*, (2022), Hussain *et al.*, (2019), Carrol *et al.*, (2020) was followed with minor modifications [2] [6] [28] [29]. For this purpose, stock solutions of *D. cinera* extract 50 mg/ml, Mueller-Hinton broth and Bacterial strain suspension equivalent to 0.5 MacFarland's standard solution were prepared following standard methods [6]. Serial dilution of (50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml) was made in the tubes containing the broth media except for the last one which inoculated with 100 µl of the solvent to be considered as the negative control and the all different tubes were inoculated with 100 µl of the bacterial suspension. Subsequently, all concentrations that showed no change in color were transferred onto nutrient agar and incubated at 37˚C for overnight, the lowest concentration with no growth of bacteria was considered as MBC [30], which is known as the lowest concentration that eliminate 99.9% of the initial bacterial population [2].

2.3. Evaluation of Antioxidant Characteristics

Free Radical Scavenging Activity

This method was carried out according to that described by Shahinuzzaman *et al.*, (2021) and Ralte *et al.*, (2021) with few modifications [31] [32]. Sample stock solution (1 mg/ml) was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol. One ml of a 0.3 mM DPPH in methanol solution was added to a 2.5 ml solution of the different concentrations of the extracts and allowed to react at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm. The absorbance of control Methanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus methanol (2.5 ml) was used as a control. Stock solution (1 mg/ml) of quercetin was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol and used as a positive control. A freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance of 518 nm. The purple colour disappears when an antioxidant is present in the medium. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of the test compound to act as a free radical scavenger. Furthermore, the "efficient concentration" or EC50 value (the concentration of antioxidant that causes 50% loss of the DPPH activity (colour) was also used to assess the antioxidant activity of the plant extract compared to the standard drug. The higher the antioxidant activity, the lower is the value of EC50 [33]. The EC50 values were calculated by linear regression of plots where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percentage of antioxidant activity from three separate tests.

$$\text{Antioxidant Activity}\left(\text{inhibition }\%\right)=\frac{AC-AS}{AS}\times 100\%$$

where *AC*: The absorbance of a control solution,

AS: The absorbance of standard or sample solution.

Each sample and standard were measured in three replications.

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The ethanolic extract of *D. cinera* leaves was analyzed for its chemical composition using GC-MS systems. The GC-MS analysis was performed on Shimadzu (GC\MS 2010) Helium was used as carrier gas and the separation was achieved using a column (TG-SQG-15 ms × 0.25 mm × 0.25 μm). The starting oven temperature was programmed at 60˚C with an increasing temperature 10˚C until reached 280˚C. The crude extract was injected with split mode. Mass spectra were taken at 70 eV; fragments from 40 to 1000 Dalton. The final confirmation of constituents was made by computer matching of the mass spectra peaks with the Wily and National Institute of Standard and Technology (NIST) Libraries mass spectra database (Biomedical Research 2017).

2.5. Data Organization and Statistical Analysis

The Data were organized and tabulated by using Microsoft Word 2016 and the Microsoft Excel 2016. The experiments were carried out in triplicates and the average of the zone of inhibition and standard deviations (SD) were obtained as mean and standard deviation (M ± SD).

3. Results and Discussion

3.1. Extractive Alcoholic Value

The extractive value/yield from *D. cinera* leaves obtained by maceration in alcohol was found to score (16.67%). Ethanol was used as a solvent for its ability to extract a vast range of compounds of different polarities Saha *et al*., 2021 [34]. The high yield obtained indicated that alcohol (96%) is capable to extract compounds of different polarities in which *D. cinera* is rich including deoxy-sugars, glycerol, Cyclopentanetriol, pyrrole, phytol and fatty acids.

3.2. Antibacterial Activity

As shown in **Table 1**, the Antibacterial activity of *D. cinera* ethanolic extract at a concentration of 50 mg/ml was evaluated against three standard strains: *S. aureus* ATCC 25923*, E. coli* ATCC 25922 *and P. aeruginosa* ATCC 27853 by well diffusion method. The results revealed that all the mentioned strains were sensitive with diameter of 21 ± 1.00 mm, 30.66 ± 2.51 mm, and 29 ± 2.00 mm respectively [35]. The positive control for *S. aureus was (*Vancomycin 30mcg which showed 23.30 ± 1.52), for *E. coli (*Ceftriaxone 30mcg which showed 33.60 ± 1.15) and for *P. aeruginosa was (*Meropenem 10mcg which exposed 30.66 ± 2.51 mm). Nevertheless, the determination of MIC (50 mg/ml, 25 mg/ml, 12.5 mg/ml & 6.25 mg/ml) conducted by tube dilution technique and the MBC by agar diffusion, to present that the MIC of *D. cinera* extract against the standard strain of *S. aureus*

was 12.5 mg/ml, *E. coli* and *P. aeruginosa* were 25 mg/ml (Table 2). The above mentioned findings vitrified the uses of *D. cinera* for the treatment of wound and skin infections, Urinary tract infections (UTIs) and respiratory diseases. Also the results are in agreement with the study presented by Chinsembu *et al.*, (2016) who noticed that the root of *D. cinera* is used traditionally for pulmonary and respiratory problems [17] and with the study conducted by Mudzengi *et al.*, (2017), who reported the sensitivity of *S. aureus* and *E. coli* standard strains to *D. cinera* [18].

3.3. Evaluation of Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging assay of *D. cinera* leaves extract showed dose-dependent activity with EC50: of 6.63 µg/ml, compared to 12.00 µg/ml of quercetin standard (Table 3). These findings coincide with those reported by Pop *et al.*, (2022) and confirmed the good antioxidant and antibacterial potential of *D. cinera* extract.

3.4. Phytochemical Analysis

The chemical composition of *D. cinera* leaves extract was analyzed by using GC-MS systems. The separation of the total constituents revealed that 1,6-Anhydro-

Table 1. Antimicrobial susceptibility of *Dichrostachys cinera* ethanolic extract (50 mg/ml) dissolved in Methanol 50% against different Bacterial Strains vs suitable Antibiotics.

Bacterial strain	Mean Zone of inhibition (mm) ± SD		
	D. cinera Extract	Susceptibility (S/R)	Antibiotics (+ve control)
S. aureus ATCC 25923	21 ± 1.00	S	Vancomycin 30 mcg 23.30 ± 1.52
E. coli ATCC 25922	30.66 ± 2.51	S	Ceftriaxone 30 mcg 33.60 ± 1.15
P. aeruginosa ATCC 27853	29 ± 2.00	S	Meropenem 10 mcg 30.66 ± 2.51

S: Sensitive; R: Resistance; N.B: Mean Zone of Inhibition in CLSI (mm): *S. aureus* ATCC 25923 = 17 - 21, *E. coli* ATCC 25922 = 29 - 35, *P. aeruginosa* ATCC 27853 = 27 - 33.

Table 2. Determination of mininmum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for *Dichrostachys cinera* Ethanolic leaves extract against bacterial standard strains.

Bacterial strain	*Dichrostachys cinera* Ethanolic leaves	
	MIC	MBC
S. aureus ATCC 25923	12.5 mg/ml	25 mg/ml
E. coli ATCC 25922	25 mg/ml	50 mg/ml
P. aeruginosa ATCC 27853	25 mg/ml	50 mg/ml

2,4-dideoxy-.beta.-D-ribo-hexo of a sugar moiety (21.26%) with different peaks, followed by Glycerin (11.56%), 1,2,3-Cyclopentanetriol (10.18%) with different peaks, 8,11,14-Eicosatrienoic acid, (Z,Z,Z)- (6.18%), 1H-Pyrrole, 1-methyl- (6.08%), Phytol (5.91%) and 7-Bromo-6-(2-diethylaminoethoxy)-2,3-dihyd (5.44%), were the major constituents of 64.17% abundance (Table 4). These

Table 3. Radical scavenging activity of *Dichrostachys cinera* and Standard (Quercetin).

Concentration	Scavenging activity (%)	
	Dichrostachys cinera leaves Extract	Standard (Quercetin)
250 µg/ml	92%	89.7%
125 µg/ml	89.6%	85.8%
50 µg/ml	86.8%	62.1%
10 µg/ml	82.8%	55.5%
5 µg/ml	37.8%	45%

Table 4. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of *Dichrostachys cinera* leaves extract.

Peak	*Dichrostachys cinera* leaves extract			
	R. Time	Area	Area %	Name
1	2.296	37,6364	6.08	1H-Pyrrole, 1-methyl-
2	2.455	60,703	0.98	2,2-Dimethoxybutane
3	4.210	715,946	11.56	Glycerin
4	4.333	151,975	2.45	1-Butyl(dimethyl)silyloxypropane
5	5.714	145,891	2.36	4,5-Diamino-6-hydroxypyrimidine
6	6.104	174,902	2.82	Urea, 1-methylcyclopropyl-
7	6.747	203,369	3.28	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-
8	7.162	50,012	0.81	N-Methylpyrrole-2-carboxylic acid
9	7.843	143,046	2.31	Benzofuran, 2,3-dihydro-
10	7.887	64,564	1.04	Phenol, 4-ethenyl-, acetate
11	9.272	101,286	1.64	4-Hydroxy-3-methylacetophenone
12	11.613	166,193	2.68	3-Trifluoroacetoxydodecane
13	12.930	479,620	7.74	1,2,3-Cyclopentanetriol
14	13.573	238,556	3.85	4-Hydroxy-2-methylpyrrolidine-2-carboxylic
15	14.310	101,353	1.64	3-Trifluoroacetoxydodecane
16	14.570	75,413	1.22	1,2,3,5-Cyclohexanetetrol, (1.alpha.,2.beta.,3
17	14.593	151,077	2.44	1,2,4-Cyclopentanetriol
18	14.631	236,604	3.82	1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexo
19	14.733	345,976	5.59	1,6-Anhydro-2,4-dideoxy-.beta.-D-arabo-hexo

Continued

20	14.797	625,034	10.09	1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexo
21	14.843	140,384	2.27	2-Deoxy-D-galactose
22	14.898	108,716	1.76	1,6-Anhydro-2,4-dideoxy-.beta.-D-ribo-hexo
23	16.079	92,578	1.49	2-Pentadecanone, 6,10,14-trimethyl-
24	16.397	58,223	0.94	5-(Prop-2-enoyloxy)pentadecane
25	16.551	337,066	5.44	7-Bromo-6-(2-diethylaminoethoxy)-2,3-dihyd
26	17.241	8685	0.14	n-Hexadecanoic acid
27	17.431	63,099	1.02	8,9,9,10,10,11-Hexafluoro-4,4-dimethyl-3,5-
28	18.845	365,759	5.91	Phytol
29	18.958	382,891	6.18	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
30	20.037	27,752	0.45	[3,3'-Bi-1H-1,2,4-triazole]-5,5'-diamine
		6,193,037	100.00	

identified components in *D. cinera* leaves extract are known to possess high antioxidant activity [36], as well as antimicrobial effects [9] [17] [18]. The presence of Glycerin which known as bioactive with the ability of microbial suppression [37]. Eicosatrienoic acid which has antioxidant activity [38]. 1H-Pyrrole known as bioactive with antibacterial and antioxidant activities according to Özdemir *et al.*, (2017) [39]. Phytol has antibacterial activity proved by Ghaneian *et al.* (2015) [40] and diethylaminoethoxy derivatives are known to possess antimicrobial activity as stated by Collins *et al.*, (1975) [41].

4. Conclusions

- The study on *D. cinera* revealed that the ethanolic extract exhibits prominent antioxidant for the presence of (Eicosatrienoic and Pyrrole), and antibacterial activities for the presence of (Glycerin, Pyrrole, Phytol and diethylaminoethoxy) derivatives.
- This study provided useful information on the therapeutic potential of *D. cinera* as an antibacterial agent efficiently against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

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Author Contribution Statement

Sitelbanat Yassin: Conceived and designed the experiments; Performed the experiments; Contributed materials, Analyzed and interpreted the data; Wrote the paper.

Mohamed Abubker: Conceived and designed the experiments; Performed the experiments; Contributed materials, Analyzed and interpreted the data.

Anwar Mohamed: Contributed materials.

Salah Humeada: Contributed materials.

Selma Omer: Performed the experiments.

Elhadi M. M. Ahmed: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mirghain Abd Alrahman: Wrote the paper.

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Data Availability Statement

Data included in article/supplementary material/referenced in the article.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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