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Assessment of Antibacterial Activity of Various Solvent Extracts of Dictyota Dichotoma Against Multidrug Resistant Bacterial Strain

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Abstract

In this research, investigate the antibacterial properties of various crude extracts obtained from the brown alga Dictyota dichotoma (Hudson) J.V.Lamouroux. Specifically, we analyzed the hexane, chloroform, ethyl acetate, and methanol extracts in detail to assess their effectiveness against a wide range of multidrug-resistant bacterial strains. These strains encompassed Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Vibrio cholerae, Shigella flexneri, Proteus mirabilis, and P. vulgaris, representing a diverse array of challenging pathogens. The assessment of these extracts was conducted via the disc diffusion method, employing varying concentrations (250µg/disc, 500µg/disc, and 1000µg/disc). Notably, the resulting mean inhibition zones ranged significantly, spanning from 8.5 mm to 23.8 mm, indicative of varying degrees of antibacterial activity across the extracts. Complementing this, the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) further delineated the potency of these extracts, with MIC values spanning from 62.5µg/ml to 500µg/ml, and MBC values from 125µg/ml to 1000 µg/ml. Of particular interest was the ethyl acetate extract derived from D. dichotoma, which exhibited a remarkable mean inhibition zone of 26.5 mm, alongside the lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) values against S. aureus. This compelling finding underscores the potential of D. dichotoma as a reservoir for novel antibacterial compounds with efficacy against multidrug-resistant bacterial strains. Such insights illuminate promising avenues for the development of innovative therapeutic interventions targeting antibiotic-resistant pathogens.

Keywords: MRSA; Dictyota Dichotoma; MRSA; MIC; MBC

Abbreviations: MRSA: Methicillin Resistant Staphylococcus Aureus; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; DMSO: Dimethylsulfoxide; MHA: Muller Hinton Agar; MSSA: Methicillin-Sensitive S. Aureus; MHB: Muller Hinton Broth; MTCC: Microbial Type Culture Collections; IMTECH: Institute of Microbial Technology.

Introduction

Seaweeds, as renewable living sources, have undergone extensive global screening to isolate life-saving drugs or biologically active substances. The selective use of marine algae as a potential source of pharmaceutical agents has seen a recent uptick. Natural products serve as a primary reservoir for drug development, with a myriad of plants, microbes, and marine animals being scrutinized for bioactive secondary metabolites [1]. Marine sources are particularly noteworthy due to their high concentration of functional ingredients, including polyunsaturated acids, carotene, carotenoids, sulphated polysaccharides, and sterols. Antioxidants and antibacterials, among various compounds with functional properties, have received the most widespread attention. Nevertheless, despite this intensive research, only 2% of the known 150,000 seaweed species have been studied and identified [2]. Presently, seaweeds represent commercially significant marine renewable resources, offering valuable insights for the development of new drugs targeting cancer, microbial infections, and inflammations [3].

The most common organism causing joint infections is Staphylococcus aureus, and its colonisation in the anterior nares is 25 to 30% in the general population. Therefore, carriers of S. aureus are at an increased risk of developing SSI postoperatively. Postoperative infections are reported to be ten times greater in S. aureus carriers than in non carriers in developed countries, although recorded data is lacking for the developing world [4]. The two primary subtypes of S. aureus are Methicillin-Sensitive S. aureus (MSSA) and the more virulent MRSA. The risk of infection in MRSA-colonised patients is greater than in patients who are not colonised with MRSA [5]. Antibiotics resistant bacteria can be found in all different ecological niches. Selective pressure of misuse of antibiotics mainly in hospitals, agriculture and animal farming, goes in favor of bacteria by developing new genes responsible for the antibiotic resistance [6].

Dictyota is a genus of the family Dictyotaceae, which is known to bear a cosmopolitan nature. Dictyotales species (brown algae) produce a variety of bioactive secondary metabolites with broad antiherbivore effects in marine environments. Dictyota species are rich in phytoconstituents, mainly of the terpene class. Many compounds (about a third) identified from brown algae were reported from different Dictyota species. The most prevalent member of this family is one of the major seaweeds, Dictyota dichotoma. It has been extensively studied, though the studies have identified a wide variety of differences among its contents depending on the time and location of the collection. From the Dictyotaceae family, this species is responsible for the highest proportion of versatile bioactives, particularly diterpenes. Several bioactives from D. dichotoma have been previously reported, including two compounds, dictyohydroperoxide and hydroperoxyacetoxycrenulide, containing hydroperoxyl groups rarely found in algal terpenoids, and two diterpenoids, namely pachydictyol B and pachydictyol C [7]. Many researchers have recently given a great deal of attention to the genus, due to its economic significance as an animal feed and antibiofouling and medicinal agent, overthe past 10 years [8]. However, research exploring seaweed resources in Pakistan is poorly elicited although it has enormous potential.

Materials and Methods

Sample Collection

The marine brown alga Dictyota dichotoma (Hudson) J.V.Lamouroux. (Phaeophyceae) were collected on November to January, 2023 from Manappad (Lat. 8°30′N; Long. 78°8′E), Tuticorin district, the Gulf of Mannar Marine biosphere, Tamil Nadu, India. Collected algae were cleaned with seawater to remove from epiphytes, extraneous matter and necrotic. Samples were collected in sterilized polyethylene bags and put in an ice box, then transferred to the laboratory immediately until the experimental work was done at the same day. Samples were washed thoroughly with tap water then sterile distilled water. For drying, washed seaweeds were blotted on the blotting paper and spread out at room temperature in shade. Shade dried samples were cut into small pieces and then ground in a tissue grinder (IKA A 10, Germany) until reach fine powder shape The powdered samples were then stored in refrigerator for further use.

Preparation of Extracts

The algal samples were shade dried followed by oven drying at 50°C for an hour and crushed in an electrical blender. Five hundred grams of powdered samples were packed in Soxhlet apparatus and extracted with different solvents like viz., hexane, chloroform, ethyl acetate and methanol for 72 hours. The extracts were pooled and the solvent were evaporated under vacuum in rotary evaporator (Heidolph, Germany) at 40°C and the dried extracts were stored at 4°C in refrigerator for antibacterial assay.

Microorganisms Tested

The following Standard Bacterial strains used for assay viz., Staphylococcus aureus Bacillus subtilis (MTCC 441), Streptococcus pyogenes (MTCC 442), Escherichia coli (MTCC 443), Klebsiella pneumonia (MTCC 109), Pseudomonas aeruginosa (MTCC 741), Proteus mirabilis (MTCC 425), P. vulgaris (MTCC 426), Salmonella typhimurium (MTCC 98), Shigella flexneri (MTCC 1457) and Vibrio cholera (MTCC 3906) were procured from Microbial Type Culture

Collections (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The strains were maintained in the laboratory by regular sub-culturing onto nutrient agar slants. In vitro antibacterial activity was determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) was obtained from Himedia, Mumbai.

Antibiotic Sensitivity Test

Antibiotic sensitivity of the bacterial strains were determined by standard CLSI disc diffusion method [9]. Antibacterial agents from different classes of antibiotics viz., Amikacin (AK 30 µg/disc), Ampicillin (AMP 10 µg/disc), Cefixime (CFM 5 µg/disc), Ceftazidime (CAZ 30 µg/disc), Ciprofloxacin (CIP 5 µg/disc), Chloramphenicol (C 30 µg/disc), Erythromycin (E 15 µg/disc), Gentamycin (GEN 10 µg/disc), Methicillin (MET 10 µg/disc), Norfloxacin (NX 10 µg/disc), Nalidixic acid (NA 30 µg/disc), Ofloxacin (OF 5µg/disc), Streptomycin (S 10 µg/disc) and Tetracycline (TE 30 µg/disc) were obtained from Himedia, Mumbai.

Antibacterial Activity Assay

Disc Diffusion Method

The antibacterial activity of extracts of D. dichotoma was determined by disc diffusion method according to Bauer AW, et al. [10] with modifications. The Petri plates were prepared by pouring 20 ml of sterilized liquefy MHA after solidification doing work. All bacterial isolates were suspended in saline to a turbidity equivalent to 0.5 McFarland (1.5 x 108 CFU/ml) and 0.1ml standardized inoculum suspension was swab uniformly in MHA plates. The crude extracts were dissolved in 10% DMSO and under aseptic condition Sterile HiMedia paper disc (6mm) were impregnated with 20 µl of different concentrations (1000,500 and 250 µg/disc) of crude extracts. The discs with extract were placed on the surface on the medium with sterilized forceps and gently pressed to make certain contact with inoculated agar surface. Methicillin (10µg/disc) was used as positive control and a disc loaded with 10 % DMSO alone served as the C for 24 h. The antibacterial activity was evaluated by blind control in all the assays. Finally, the inoculated plates were incubated at measuring the inhibition zones (diameter of inhibition zone plus diameter of the disc). The assay in this experiment was repeated three times.

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) of the D. dichotoma crude extracts, a modified reaszurin microtitre plate assay was carried out according to methods of Sarker SD, et al. [11]. Sterile Mueller Hinton Broth 100 ml of respective broth was transferred in to each well of a sterile 96-well micro titer plate (Hi-Media TPG 96). The D. dichotoma extracts were dissolved in 10 per cent DMSO to obtain 1000 µg/ml stock solution. 100 ml of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth 100µl of the solution was transferred to the second well and in this way, the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 1000, 500, 250, 125, 62.5, 31.25, 15.625 µg/ml of the extract in each wells. To each well 10 µL of resazurin indicator solution was added. (The resazurin solution was prepared by dissolving a 270mg tablet in 40mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution). Finally, 10 µl of the standardized bacterial suspension were inoculated into all wells. Each plate was set up with a positive control (bacterial suspension adding 10 µl of Mueller Hinton broth and resazurin solution) and negative control (10% DMSO and reaszurin solution without bacterial suspension). The plates were incubated at 37°C for 24 h for all bacterial strains. The color change was then assessed visually. The growth was indicated by color changes from purple to pink (or colorless). In this study, the MIC was the lowest concentration of D. dichotoma extracts that exhibited the growth of the organisms in the values by visual reading.

Determination of the Minimum Bactericidal Concentration (MBC)

MBC of the D. dichotoma extracts were determined by plating a loop full of bacterial solution from each MIC assay well C for 24 h for all bacterial strains. The MBC with growth inhibition into freshly prepared MHA. The plates were incubated at 37 was recorded as the lowest concentration of the extract that did not permit any visible bacterial growth after the period of incubation.

Statistical Analysis

All statistical analyses were performed using SPSS version 16.0 statistical the results are expressed as the mean software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant difference between different extracts for in vitro antibacterial assays. Comparison of means for in vitro antibacterial assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. P value < 0.05 was considered statistically significant.

Results and Discussion

The multi-drug resistance profile of bacterial strains, encompassing both clinical and standard strains, was confirmed using the CLSI-M100-2012 method. Among the

standard strains tested, *S. aureus, B. subtilis, K. pneumoniae*, and *P. vulgaris* exhibited sensitivity to all antibiotics except CFM, AMP, MET, and CAZ. Conversely, *S. flexneri* and *P. mirabilis* were sensitive to all antibiotics except AMP, while *S. pyogenes* showed resistance to CFM, AMP, CAZ, NA, and E, yet remained sensitive to other antibiotics. *E. coli* was sensitive to all antibiotics except AMP and NA, while *P. aeruginosa* was resistant to CFM, AMP, and TE but susceptible to others. S. typhimurium was sensitive to all antibiotics except AMP and E, and V. cholerae exhibited resistance to AMP and intermediate resistance to S but sensitivity to other antibiotics.

In the current investigation, various solvents (hexane, chloroform, ethyl acetate, and methanol) were employed to extract D. dichotoma and evaluate its efficacy against multidrug-resistant standard bacterial strains. The ethyl acetate extract of D. dichotoma demonstrated the highest activity against S. aureus, with a mean zone of inhibition of 23.8 mm, followed by *B. subtilis* at 20.5 mm. Notably, all extracts of the marine macroalgae exhibited significant antibacterial activity against the tested bacterial strains compared to the available antibiotics. Mean values are detailed in Tables 1. Upon assay of the different extracts against the test bacteria via disc diffusion, the mean zone of inhibition ranged from 8.5 to 23.8 mm. Methicillin (10 µg/disc), utilized as a positive control, produced a mean zone of inhibition ranging from 10.0 to 13.8 mm. The blind control (10% DMSO) did not induce any zone of inhibition against the tested bacterial strains. MIC values for the different D. dichotoma extracts ranged from 31.2 to 500 µg/ml, while MBC values fell between 62.5 and 1000 µg/ml.

S. No.	Bacterial strains/ solvents	Mean zone of inhibitiona (mm)b				MIC (mg/mL)	MBC (mg/mL)
		Concentration of the extracts (mg/disc)					
		1000	500	250	Methicillin (10 mg/disc)		
1	***Staphylococcus aureus***						
	Hexane	17.0±0.50	13.0±0.38	10.0±0.37	10.0 ± 0.56	125	250
	Chloroform	18.5±0.45	14.5±0.25	11.1±0.10	11.3 ± 0.52	125	250
	Ethyl acetate	23.8±0.56	16.3±0.26	14.8±0.58	10.8 ± 0.76	31.2	62.5
	Methanol	14.0±0.50	12.3±0.43	10.5±0.05	11.0 ± 0.50	125	250
2	***Streptococcus pyogenes***						
	Petroleum ether	11.5±0.23	10.0±0.13	9.6±0.85	10.3 ± 0.57	250	500
	Chloroform	13.0±0.53	11.3±0.34	10.6±0.17	11.8 ± 0.28	250	500
	Ethyl acetate	15.5±0.80	13.1±0.50	10.5±0.25	11.5 ± 0.50	125	125
	Methanol	11.3±0.60	10.3±0.42	9.7±0.30	10.6 ± 0.76	250	500
3	***Bacillus substilis***						
	Hexane	13.0±0.48	11.5±0.30	9.5±0.28	12.3 ± 0.56	250	500
	Chloroform	15.8±0.38	12.3±0.40	10.0±0.30	12.0 ± 0.50	250	500
	Ethyl acetate	20.5±0.30	14.2±0.20	12.0±0.10	11.6 ± 0.76	31.2	62.5
	Methanol	16.1±0.82	13.5±0.08	11.3±0.59	10.8 ± 0.76	125	250
4	***Escherichia coli***						
	Hexane	12.0±0.50	10.0±0.40	9.1±0.30	12.2 ± 0.27	250	500
	Chloroform	13.1±0.20	11.3±0.10	10.0±0.50	12.3 ± 0.74	250	500
	Ethyl acetate	15.3±0.10	13.0±0.26	11.1±0.20	10.5 ± 0.55	125	125
	Methanol	13.3±0.30	11.5±0.20	10.0±0.10	10.8 ± 0.68	250	500

5	***Proteus vulgaris***						
	Hexane	12.3±0.10	11.6±0.5	9.0±0.50	10.3 ± 0.57	250	500
	Chloroform	13.3±0.30	12.2±0.20	10.1±0.10	11.8 ± 0.78	250	500
	Ethyl acetate	14.1±0.40	13.4±0.30	10.8±0.20	10.8 ± 0.53	125	125
	Methanol	13.8±0.57	12.5±0.40	10.5±0.30	11.3 ± 0.54	250	500
6	***Pseudomonas aeruginosa***						
	Petroleum ether	12.3±0.60	10.0±0.50	8.5±0.40	13.8 ± 0.76	250	500
	Chloroform	14.3 ±0.47	12.2±0.20	10.3±0.10	11.0 ± 0.16	250	500
	Ethyl acetate	15.3±0.63	13.1±0.10	10.5±0.53	11.6 ± 0.26	125	125
	Methanol	12.1±0.35	10.0±0.30	9.8±0.20	11.1 ± 0.18	250	500
7	***Vibrio cholerae***						
	Petroleum ether	12.3±0.20	11.1±0.12	9.1±0.42	11.5 ± 0.76	250	500
	Chloroform	13.8±0.43	11.6±0.17	10.3±0.27	12.1 ± 0.28	250	500
	Ethyl acetate	16.1±0.62	13.5±0.56	11.0±0.46	12.8 ± 0.57	125	125
	Methanol	13.2±0.18	12.1±0.23	10.5±0.19	11.6 ± 0.57	250	500
8	***Shigella flexneri***						
	Petroleum ether	11.5±0.30	10.0±0.23	9.5±0.25	13.1 ± 0.28	250	500
	Chloroform	12.3±0.41	11.0±0.50	9.8±0.28	11.8 ± 0.76	250	500
	Ethyl acetate	13.5±0.53	11.5±0.47	9.0±0.30	11.0 ± 0.50	250	500
	Methanol	10.5±0.20	10.8±0.32	8.8 ± 0.58	10.5 ± 0.57	500	1000
9	***Proteus mirabilis***						
	Petroleum ether	12.2±0.17	11.7±0.54	9.0±0.31	10.8 ± 0.28	250	500
	Chloroform	13.1±0.24	12.0±0.37	9.5±0.29	11.3 ± 0.57	250	500
	Ethyl acetate	14.8±0.28	13.5±0.48	10.0±0.32	10.6± 0.56	250	500
	Methanol	12.8 ±0.37	11.5 ±0.27	9.1±0.18	10.8 ± 0.76	250	500
10	***Salmonella typhimurium***						
	Petroleum ether	12.8 ±0.63	11.1±0.26	10.5±0.50	10.8 ± 0.46	250	500
	Chloroform	14.3±0.38	12.5±0.37	11.1 ±0.18	10.6 ± 0.63	250	500
	Ethyl acetate	15.1±0.72	12.8±0.82	11.3 ±0.36	10.3 ± 0.38	125	125
	Methanol	12.5±0.38	11.3 ±0.35	10.6 ±0.27	12.1 ± 0.58	250	500

[a]Diameter of zone of inhibition (mm) including the disc diameter of 6 mm
[b]Mean of three assays; ± - Standard deviation;*Significant at P<0.05

Table 1: Antimicrobial activity of various extracts of *Dictyota dichotoma*.

In present results indicated of the different solvents viz., hexane, chloroform, ethyl acetate, and methanol extracts of D. dichotoma possessed antibacterial activity against all the bacterial strains tested. The ethyl acetate extract of D. dichotoma showed the highest antibacterial activity than other extracts against *S. aureus, B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris, P. aeruginosa, S. typhimurium, S. flexneri*, and *V. cholerae*. The highest activity was displayed by ethyl acetate extract of *D. dichotoma* against S. aureus, the mean zone of inhibition (23.8 mm) followed by *B. subtilis* (20.5). The MIC values of the different extracts of D. dichotoma ranged between 31.2 and 500 µg/ml, while the MBC values were between 62.5 and 1000 µg/ml. Similar observation The ethyl acetate extracts of U. fasciata showed highest antibacterial activity against multi-drug resistant bacterial strains viz., *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, P. mirabilis* and *P. vulgaris* [12]. Chandrasekaran M, et al. [13] reported that the ethyl acetate extracts of *Sargassum wightii* showed the highest antibacterial activity

against multi-drug resistant bacterial strains viz., *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, V. cholerae, S. flexneri, S. dysentriae, P. mirabilis*, and *P. vulgaris*.

Salem WM, et al. [14] same reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *C. racemosa, Sargassum dentifolium, Padina gymnospora*; methanolic extracts of *Sargassum hystrix, C. racemosa, C. fragile, S. dentifolium* and *Cystoseria myrica* against *E. coli, S. aureus, E. feacalis, Salmonella sp., B. cereus* and *P. aeruginosa*. These results were in close agreement with those obtained by [15]. It was revealed that the chloroform and ethyl acetate extracts of Enteromorpha compressa, Chaetomorpha linum and Polysiphonia subtilissima were active against *B. subtilis, Bacillus brevis, E. coli, S. flexneri*, and *V. cholerae*.

In the present study, different solvents viz., hexane, chloroform, ethyl acetate, and methanol extracts of D. dichotoma possessed antibacterial activity against all the bacterial strains tested. Adaikala RG, et al. [16] reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *Stoechospermum marginatum* and *Caulerpa chemnitzia* against *B. subtilis, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa, S. typhimurium, Vibrio cholerae, Shigella flexneri, P. mirabilis* and *P. vulgaris*. In addition, these results confirmed the evidence in previous studies reported that the ethyl acetate is a better solvent for more consistent extraction of antimicrobial substances from marine plants compared to other extracts such as hexane, chloroform, acetone and methanol [17].

In the present work, the ethyl acetate extract of D. dichotoma possed highest antibacterial activity may due to the presence of phytochemicals, terpenoids, tannins, phenolic compound, and steroids. Polyphenols were reported to have microbicidal activity against many pathogenic bacteria [18]. Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [19]. Zapata O, et al. [20] reported that the role of phenolic compounds present in seagrasses could also enhance the antimicrobial activity. Steroid glycosides are a class of widespread natural products having either terrestrial or marine origins. Several cardiac glycosides are used therapeutically in the treatment of cardiac failure and atrial arrhytmias, and many glycoside compounds, belonging to other structural groups, show cytotoxic, antimicrobial, hypocholesterolemic and other biological activities [21].

In the present study, the different solvents viz., hexane, chloroform, ethyl acetate, and methanol extracts of D. dichotoma possessed antibacterial activity against all the standard bacterial strains tested. The ethyl acetate extract of P. tetrastromatica showed the highest antibacterial activity than other extracts against S. aureus and B. subtilis. The MIC values of the different extracts of D. dichotoma ranged between 62.5 and 500 µg/ml, while the MBC values were between 125 and 1000µg/ml. Tuney IBH, et al. [22] reported that the antibacterial activity of the methanol extracts of *Ulva rigida, Enteromorpha Linza, Padina pavonica, Cystoseria sniosa, Dictyopteris linearis, D. membranacea, C. mediterranea, E. siliculous, Ceramium rubrum, Gracilaria gracilis* and *Acanthophora nojadiformis* were screened for their antibacterial activity against *Escherichia coli*. Kayalvizhi KV, et al. [23] reported that the diethyl ether, chloroform acetone and methanol extracts of Sargassum wightii, Stoechospermum marginatum, Gracilaria foliifera and Padina boergesenii were investigated for their antimicrobial activity against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Enterococci sp. Proteus sp., Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella sp, Shewanella sp., Vibrio flurialis, Vibrio splendidus, Aspergillus niger, Candida albicans, Penicillium sp., Aspergillus flavus* and *A. tetreus*.

In this study, it was observed that gram-positive bacteria exhibited higher susceptibility compared to gram-negative bacteria to the plant extracts. This phenomenon aligns with previous findings on the seeds of Syzygium jambolanum [24] and the bark of *Cassia siamea* [25]. Taskin E, et al. [26] also noted similar trends, attributing the increased susceptibility of gram-positive bacteria to algal extracts to differences in cell wall structure and composition [27]. Gram-negative bacteria, on the other hand, possess an outer membrane that acts as a barrier against various environmental substances, including antibiotics. Additionally, the presence of a thick murine layer in the cell wall further impedes the entry of inhibitors [28]. The differing sensitivities between gram-positive and gram-negative bacteria may stem from their morphological distinctions [29].

In light of these observations, this study aimed to assess the antibacterial properties of the ethyl acetate extract of *D. dichotoma*. Such research could contribute to the development of a new alternative medicine system devoid of side effects. Despite the approval of numerous natural products as antibacterial drugs, there remains an urgent necessity to identify novel substances effective against highly resistant pathogens. The ethyl acetate extract of *D. dichotoma* shows promise as a potential natural antibacterial agent against the tested human pathogenic MDR bacterial strains.

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Author Contribution

All authors have read and agreed to the published version of the manuscript validation. AR: Conceptualization, Identification and Antioxidant activity, manuscript writing. MS: Plant collection and perform plant extraction, designed the research carried. S: Contributed to methodology and investigation, Original draft preparation. GS: Chemical evaluation of plant extracts and manuscript editing and data analysis. AR, MS, GS, S contributed to the articles and approved the submitted version.

Declaration

Consent to participate: Not applicable
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