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Evaluation of Antibacterial Efficacy and Phytochemical Analysis of *Echinacea Purpurea* Toward MDR Strains with Clinical Origins

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Abstract

Microbes that are resistant to antibiotics are becoming a severe threat to the global health-care system. The best possible alternative to the possibility of medication resistance is the incorporation of natural remedies with considerable antimicrobial properties in the therapeutic approaches of bacterial illnesses. Multidrug-resistant bacteria (MDRB), methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis* (*E. faecalis*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter baumannii* (*A. baumannii*), *Proteus mirabilis* (*P. mirabilis*), *Stenotrophomonas maltophilia* (*S. maltophilia*) were isolated from 970 clinical specimens (nasal, groin, endotracheal secretions, tissues, blood, central line tips, wounds, and urine) taken from patients admitted to Al-Zahraa University Hospital and Cairo Specialized Hospital from January 2016 to June 2016. Antimicrobial impacts of various solvents including n-hexane, ethyl acetate, chloroform, and aqueous were screened against multidrug-resistant (MDR) strains isolated from clinical samples. *Echinacea Purpurea* ethyl acetate fraction showed the most promising antibacterial activity versus bacterial clinical isolates with an inhibition zone range of 16.8–22.7 mm, and minimal inhibitory concentration (MIC) range of 15.63–250 µg/ml, whereas other fractions were found to exhibit lower inhibition zones and higher MIC values than ethyl acetate fraction against MDR strains. None of the fractions have antibacterial action versus *S. maltophilia*. Transmission electron microscopic (TEM) investigation of MRSA treated by *Echinacea purpurea* ethyl acetate revealed its role in the lysis of pathogenic bacterial cells. The ethyl acetate fraction of the *E. purpurea* was analyzed using a Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric analysis system (L.C.-ESI- M.S.) to screen various molecules present in the fraction of *E. Purpurea* ethyl acetate fraction showed promising antioxidant activity with $IC_{50} = 14.24 \pm 0.58$ µg/m. *E. Purpurea* ethyl acetate fraction had a CC_{50} value of 1145.97 µg/ml upon testing on Vero cells highlighting its minimal toxicity. The study's findings will be used to further *in vivo* elucidate the *E. purpurea* ethyl acetate fraction for potential medicinal purposes.

Keywords: Antioxidant, Cytotoxicity, *Echinacea purpurea*, Multidrug-resistant bacteria, Transmission electron microscopy

1. Introduction

Antibiotic-resistant bacteria have emerged and are proliferating quickly, posing a severe threat to human health on a worldwide scale. Practically, the predominant antibiotic-resistant bacteria are those belonging to the ESKAPE species (*E. faecium*, *Staphylococcus aureus*, *Klebsiella*

pneumoniae, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*). Gram-positive *S. aureus* and Gram-negative *K. pneumoniae* show the most extreme antibiotic resistance and have emerged as prominent causes of death [1].

There is an immediate demand to develop novel antibacterial drugs to tackle bacterial resistance since the growth of multidrug-resistant (MDR)

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diseases has expanded at an astonishing level in recent years and caused massive issues that result in death [2]. To replace existing antimicrobial compounds, it is necessary to investigate novel antimicrobial molecules from all sources; the high cost of producing synthetic drugs and their negative side effects in comparison to naturally derived agents from plants encourage a return to nature [3]. Natural compounds from plant sources are one of the most effective ways to this issue because of their minimal toxicity, biodegradability, and environmental friendliness when compared with chemical or synthetic agents with antibacterial properties [4,5]. Many biologically active substances found in plant derivatives encouraged investigators to observe a broader range of potential medicinal applications for their generally safe substances [6].

Echinacea purpurea is a medicinal grass that is indigenous to North America and a member of the Asteraceae family. On the herbal and traditional market, it has grown to be one of the most well-liked herbal remedies [7]. Its medical history dates back to the Native American era in North America, and it has a reputation for treating eczema, tuberculosis, and bites from insects and snakes [8]. As it has been demonstrated to be beneficial for preventing and managing lung problems, several research has concentrated on its therapeutic properties, such as the immunomodulatory function [9,10]. *E. purpurea* has pleiotropic biomedical applications such as antiviral, antifungal, anti-inflammatory, and wound-healing roles [10–12]. To fight pathogen invasions, *E. purpurea*'s immune response boosts the activity of various immune cells [13]. Medical trials have shown that *E. purpurea* can be utilized to fight cancer or offer a replacement therapy for people with cancer [14]. *E. purpurea* has been shown to exhibit antibacterial action [11,12], but these investigations did not reach a conclusion regarding the bioactive components that have an anti-MDR effect. In the current investigation, an *in vitro* antimicrobial screening of ethyl acetate crude extract of *E. purpurea* and its fractions on seven MDR pathogenic bacteria from clinical samples.

2. Materials and methods

2.1. Materials used

Plastic wares, glasswares, broth solutions, and media were autoclaved for 15 min at 121 °C. Different agar mediums like nutrient agar, MacConkey agar, blood agar, chocolate agar, Cystine-Lactose-Electrolyte-Deficient (CLED) agar, and Mueller-Hinton agar

(Oxoid, Ltd, UK) and machines like Autoclave, Light Microscopy, TEM, VITEK-2 System, L.C.-ESI- M.S.

2.2. Processing of *E. purpurea*

E. purpurea flowers were obtained from the experimental farm of the Faculty of Agriculture, Cairo University. *E. purpurea* was briefly cleaned with tap water, let to air dry, and then cut into little pieces. *E. purpurea* was divided into slices and left to dry afterward for preparation. *E. purpurea* dried leaf material was ground into a coarse powder and then immersed in methanol for 7 days. The extracts were dried in an oven at 60 °C after methanol had been evaporated and filtered. The crude methanol extract underwent bioassay-guided fractionation, which started with water solubilization and progressed through n-hexane, chloroform, and ethyl acetate partitioning in order. Under reduced pressure, each gathered fraction was condensed to produce a black deposit [15].

2.3. Sampling

Random clinical specimens (nasal, groin, endotracheal secretions, tissues, blood, central line tips, wounds, and urine) collected from patients who were admitted to Al-Zahraa University Hospital and Cairo Specialized Hospital in Cairo city were cultured and streaked on a freshly prepared differential and selective culture media. The media is used to isolate bacteria (aerobic and facultative anaerobic bacteria) from samples under investigation. The streaked plates were incubated at 37 °C, overnight; the incubation was extended up to 48 h for slow-growing strains. After the completion of incubation, the plates were inspected for growth and colony characteristics. The colonies were picked up by a sterile loop and subjected to purification in the same isolation medium. The agar streak method was used for the purification process. A well-separated colony from each isolate was picked up on an isolation medium and incubated at 37 °C for 24 h. At the end of the incubation period, a single separate colony of distinct shapes and colors was picked up. Purity was checked by microscopic examination of the isolate using Gram stain. The purified isolates were subjected to a complete identification process and other studies [16].

2.4. Isolation and identification of pathogenic bacteria

Different biochemical tests like Gram's reaction, oxidase, and catalase were done with the bacterial

colonies differing in size, shape, and color for further characterization and identification of bacteria at the species level [17]. All biochemical reagents were obtained from (Oxoid, Ltd, UK). Blood agar, CLED agar, MacConkey agar, and Chocolate agar (Oxoid Ltd, UK) were used for culturing each collected sample. VITEK-2 was used to identify the isolated bacteria (Biomerieux, New Delhi, India) [18].

2.5. Antibiotic susceptibility test

The susceptibility to the commercial antibiotics of the bacterial isolates was evaluated using the disk diffusion method. Antibiotics used against Gram-positive bacteria included: cefoxitin, benzylpenicillin, oxacillin, imipenem, gentamicin, ciprofloxacin, moxifloxacin, inducible clindamycin resistance, erythromycin, clindamycin, vancomycin, tetracycline, fusidic acid, and trimethoprim/sulfamethoxazole. However, antibiotics used against Gram-negative bacteria included temocillin, ampicillin, amoxicillin/clavulanic acid, ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cephalothin, cefuroxime, cefotaxime, ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, tigecycline, fosfomycin, nitrofurantoin, pefloxacin, minocycline, colistin, and trimethoprim/sulfamethoxazole (Himedia Labs, Mumbai, India) [19]. The isolates were recorded as 'sensitive, intermediate, or resistant' based on the Clinical Laboratory Standards Institute (CLSI) criteria [20].

2.6. Phytochemical separation using chromatography

On an Agilent L.C.-ESI- M.S. (Agilent Technologies, Palo Alto, CA, U.S.A.) System-equipped 5-m C18 column (50 2.0-mm internal diameters; Bohus, Sweden), chromatographic separation was accomplished. The column was kept at a constant temperature of 40 °C. The film lasted 70 min in total. The mobile phase was given at a flow rate of 0.3 ml/min and was composed of acetonitrile water (50:50, v/v) containing 0.1% trifluoroacetic acid. Both of the mass spectrometer's quadrupoles were tuned to 0.7 full widths at half height, while it was operating in the positive ion mode (FWHM, unit resolution). In addition, the ion tube temperature was set at 210 °C with a spray voltage of 4500 V. The ancillary gases, nitrogen sheath, and ion sweep were set at 49, 2.0, and 14 arbitrary units, respectively. The ESI-MS/MS parameters were adjusted through infusion tests to produce the highest amount of deprotonated

molecules and the most efficient creation of distinctive fragment ions for all analytes. For the purpose of identifying and calculating the relative fraction of chemicals, MS-DIAL V. 3.70 software was utilized [21,22].

2.7. Antibacterial action

Agar diffusion was used to assess the extracts' antibacterial properties against microorganisms [23]. The culture media used was nutrient agar. Six-mm-diameter wells were punched into the solid agar. Using sterile swabs, the inoculums (1.5×10^8 CFU/ml) were distributed on Nutrient agar plates before being filled with 100 µl of extracts. The extracts used had a concentration of 10 mg/ml for all of them. Afterward, the plates were incubated for 24 h at 37 °C. Each extract's zone of growth inhibition was quantified following incubation. The active ethyl acetate fraction's MIC, as described by [24]. The extracts were serially diluted twice, in a nutshell. For the broth micro-dilution method, each inoculum was created in its appropriate medium; its density adjusted to 0.5 McFarland standards (10^8 CFU/ml), and its volume diluted to 1:100. After 24 h of 37 °C incubation, the MIC was measured on microtiter plates. The MIC is the lowest level of extract concentration at which the examined microorganism does not exhibit audible growth.

2.8. Ultrastructural changes in MRSA cells treated with the ethyl acetate fraction of *E. purpurea*

A total of 10^6 MRSA cells, both treated with 0.25 times the MIC concentration of *E. purpurea* ethyl acetate and control cells untreated, were incubated for 20 h without being disturbed to determine their ultrastructure. The samples were prepared by centrifuging the solution down to a pellet and washing it twice with phosphate buffer saline. These steps are conventional for fixing and embedding biological samples for TEM [25].

2.9. Testing for antioxidant activity

In a 96-well plate, the test samples were given 2.2 di (4-tretocetylphenyl)-1-picryl-hydrazyl stable-free radicals (DPPH) to react with the sample. The level of DPPH was maintained at 300 mM. At 37 °C, a reaction volume containing methanol, various extract concentrations, and DPPH was incubated for 30 min. Using a Tecan microplate reader, the absorbance decreased after incubation (USA). The test was run in triplicates, and Finney software was

used to compute the IC50 values, which were then expressed in g/ml [26].

2.10. Evaluation of cytotoxicity using viability assay

Vero cell lines were planted in 96-well plates with 1×10^4 cells per well and 100 μ l of the growth media. After 24 h of seeding, a new medium containing various quantities of the tested material was introduced. Confluent cell monolayers were placed into 96-well, flat-bottomed microtiter plates (Falcon, Jersey, NJ, USA) using a multichannel pipette, and successive two-fold dilutions of the tested substance were added. 48 h were spent incubating the microtiter plates at 37 °C in a humidified incubator with 5% CO₂. Absorbance was measured at 590 nm after staining with crystal violet [27].

2.11. Statistical analysis

All tests were done in triplets where the T-test was used by SPSS software for various analyses of experiments.

3. Results

3.1. Susceptibility of isolates toward antibiotics

The selected MDR isolates were two Gram-positive and six Gram-negative bacterial isolates tested for antibiotic susceptibility; 14 antibiotics were used against Gram-positive bacteria. *Enterococcus faecalis* was resistant to 13 of the 20 used antibiotics, whereas MRSA was resistant to 10 of the 14 used medicines. Regarding Gram-negative bacterial isolates, the ratios of the number of resistant antibiotics were as follows: *Escherichia coli* (18/23), *K. pneumoniae* (19/23), *P. aeruginosa* (16/16), *A. baumannii* (10/23), *Proteus mirabilis* (15/23), and *Stenotrophomonas maltophilia* (22/23) as shown in Table 1.

3.2. Antimicrobial activity of different fractions

Ethyl acetate fraction > crude methanol extract > chloroform fraction > n-hexane fraction were the four solvent fractions that had the lowest antibacterial activity in relation to *E. purpurea*. Besides, *P. mirabilis* had inhibition zones of 13.4 mm and 10.4 mm for the methanol extract and

Table 1. Resistance profile of multidrug-resistant isolates.

Microorganism	Resistance pattern of antibiotic agent (R)	Sensitivity pattern of antibiotic agent (S)	Ratio of the number of resistant antibiotics/total number of antibiotics
Methicillin-resistant <i>Staphylococcus aureus</i>	FOX, PG, OX, IMP, GN, CIP, E, CC, TET & FA.	MOX, LZD, TEC, VA, TET, TGC, RIF, SXT & FOS.	10/19
<i>Enterococcus faecalis</i>	PG, AMP, CXM, CXM/AXETIL, GN HL, STREP HL, LEV, MOX, E, CC, Q-D, TET & SXT.	AMP/S, IMP, LZD, TEC, VAN, TGC & NI	13/20
<i>Escherichia coli</i>	TEM, AMP, AMC, PIP, TZP, CF, CXM, CXM-Axetil, CTX, CAZ, CRO, FEP, ETP, MEM, CIP, NI & SXT.	AK, GN, TGC, FOS & CT.	18/23
<i>Klebsiella pneumoniae</i>	TEM, AMP, AMC, PIP, TZP, CF, CXM, CXM-Axetil, CTX, CAZ, CRO, FEP, ERT, AK, GN, CIP, IMP, MEM & NI.	TGC, FOS, CT & SXT.	19/23
<i>Pseudomonas aeruginosa</i>	TIC, TCC, PIP, TZP, CAZ, FEP, IMP, MEM, AK, GN, TM, CIP, PEF, MNO, CT, SXT	–	16/16
<i>Acinetobacter baumannii</i>	TEM, AMP, AMC, PIP, TZP, CF, CXM, CXM-Axetil, CTX, CAZ, FEP, IMP, MEM, GN, CIP, FOS, NI, CRO, ERT & SXT.	AK, TGC & CT.	20/23
<i>Proteus mirabilis</i>	AMP, PIP, CF, CXM, CXM-Axetil, CTX, CAZ, CRO, FEP, CIP, FOS, NI, SXT, TGC & CT.	TEM, AMC, TZP, ERT, IMP, MEM, AK & GN.	15/23
<i>Stenotrophomonas maltophilia</i>	TEM, AMP, AMC, PIP, TZP, CF, CXM, CXM-Axetil, CTX, CAZ, CRO, FEP, ETP, IMP, MEM, AK, GN, CIP, TGC, FOS, NI & SXT.	CL.	22/23

Used antibiotics.

A/S, Ampicillin/Sulbactam; AK, Amikacin; AMC, Amoxicillin/Clavulanic acid; AMP, Ampicillin; CAZ, Ceftazidime; CF, Cephalothin; CIP, Ciprofloxacin; CLN, Clindamycin; CRO, Ceftriaxone; CT, Colistin; CTX, Cefotaxime; CXM, Cefuroxime; CXM-Axetil, Cefuroxime Axetil; E, Erythromycin; ETP, Ertapenem; FD, Fusidic Acid; FEP, Cefepime; FOS, Fosfomycin; FOX, Cefoxitin; GN HL, Gentamicin High Level; GN, Gentamicin; ICR, Inducible Clindamycin Resistance; IMP, Imipenem; LEV, Levofloxacin; LZD, Linezolid; MEM, Meropenem; MNO, Minocycline; MOX, Moxifloxacin; NI, Nitrofurantoin; OX, Oxacillin; P, Benzylpenicillin; PEF, Pefloxacin; PIP, Piperacillin; Q-D, Quinupristin/Dalfopristin; RIF, Rifampicin; STREP HL, Streptomycin High Level; SXT, Trimethoprim/Sulfamethoxazole; TCC, Ticarcillin/Clavulanic acid; TEC, Teicoplanin; TEM, Temocillin; TET, Tetracycline; TGC, Tigecycline; TIC, Ticarcillin; TM, Tobramycin; TZP, Piperacillin/Tazobactam; VAN, Vancomycin.

chloroform, respectively, making it the least vulnerable pathogen to these chemicals. Following, *A. baumannii* (21.6 mm) and *E. faecalis* (22.7 mm), the ethyl acetate fraction of *E. purpurea* recorded the largest diameter of the size zone of inhibition against MRSA (21.4 mm). In comparison to the other solvent fractions, the hexane demonstrated low to moderate antibacterial activity with inhibition zone ranges (10.4–19.9 mm) and no antibacterial activity against *K. pneumoniae* and *P. mirabilis*. Furthermore, Table 2 contains records of the antibacterial activity. The ethyl acetate fraction's MIC values were established because it demonstrated the highest level of antibacterial activity. The ethyl acetate fraction had an MIC value of 3.9 µg/ml against MRSA. While *E. faecalis* and *A. baumannii* had an MIC value of 31.25 µg/ml, *K. pneumoniae* had an MIC value of 62.5 µg/ml. *P. mirabilis* was subjected to an ethyl acetate fraction MIC value of 125 µg/ml, while *P. aeruginosa* was subjected to a value of 250 µg/ml.

3.3. TEM examination

In the present study, MRSA was the most susceptible microorganism to *E. purpurea* ethyl acetate fraction. Accordingly, we aimed here to identify the ultrastructural alterations in *E. purpurea* ethyl acetate fraction-treated MRSA cells. TEM micrographs showed that untreated MRSA cells have uniform cytoplasmic density and intact cell membranes (Fig. 1a), while MRSA cells exposed to *E. purpurea* ethyl acetate fraction (at 0.25 × MIC) showed complete cell membrane detaches from the cell, translucent cytoplasm, shrunken, misshapen cells, and cell lysis (Fig. 1b).

3.4. Analysis of LC-MS chromatographic separation

The ethyl acetate extract of the *E. purpurea* was chromatographically separated using LC-MS resulting in the generation of characteristic fragment ions

that were identified by the system software and the relative percentages of the separated compounds were determined. However, it could be noticed that cichoric acid, caffeic acid, cynarine, undeca-2-ene-8,10-diynoic- acid isobutylamide, chlorogenic acid, glucopyranose, p-coumaric acid, and echinacoside were the most common compounds in *E. purpurea* extract as shown in Table 3. Moreover, in general, the separated compounds were included in eight phytochemical groups. However, 10 compounds were belonging to polyphenols and flavonoids that represented 52.6% of the total LC-MS chromatogram contents. Furthermore, the carbohydrate compounds (including glucans) were in second place representing about 14% of the total contents detected by eight compounds. The lipids were represented by fatty acids and represent 2.73% of the total contents. Alcohols (2.41%), alkylamides (6.32), and hydrocarbons (2.18%) were also presented as minor contents (Fig. 2 and Table 3).

3.5. Antioxidant impact

The outcomes demonstrated that the investigated metabolites' DPPH scavenging abilities were exhibited in a dose-dependent manner. In addition, the ethyl acetate extract from *E. purpurea* demonstrated good antioxidant activity similar to that of ascorbic acid, which was tested as the reference standard, with an IC₅₀ of 14.24 ± 0.58 µg/ml under these test conditions where ascorbic acid IC₅₀ = 11.21 ± 0.67 µg/ml.

(Fig. 3).

3.6. Cytotoxicity assay

It is interesting to note that the *E. purpurea* ethyl acetate extract had no detectable harmful effects even when examined at high doses (1000 µg/ml or less); under these screening circumstances, the calculated CC₅₀ value was 1145.97 µg/ml (Fig. 4).

Table 2. Susceptibility of MDR isolates to different *E. purpurea* fractions and MIC values for ethyl acetate fraction of *E. purpurea* against MDR strains.

Extracts Microorganisms	Methanol	Hexane	Ethyl acetate	Chloroform	Water	MIC of ethyl acetate extract (µg/ml)
	Mean of inhibition zones (mm)					
MRSA	21.2 ± 1.2	19.9 ± 2.1	22.7 ± 0.58	17.3 ± 2.1	NIZ	15.63
<i>E. faecalis</i>	20.3 ± 2.1	17.3 ± 1.2	21.4 ± 0.58	16.1 ± 0.52	NIZ	31.25
<i>E. coli</i>	16.9 ± 0.45	9.8 ± 1.8	19.3 ± 0.37	15.7 ± 0.19	NIZ	62.5
<i>K. pneumoniae</i>	18.3 ± 0.37	NIZ	20.2 ± 0.51	16.8 ± 0.36	NIZ	62.5
<i>P. aeruginosa</i>	14.2 ± 0.63	13.1 ± 1.2	15.3 ± 0.58	16.6 ± 0.52	NIZ	250
<i>A. baumannii</i>	18.2 ± 0.45	10.4 ± 1.8	21.6 ± 0.37	10.4 ± 1.3	NIZ	31.25
<i>P. mirabilis</i>	13.4 ± 2.2	NIZ	16.8 ± 1.9	NIZ	NIZ	125
<i>S. maltophilia</i>	NIZ	NIZ	NIZ	NIZ	NIZ	–

NIZ, no inhibition zones; MIC, minimum inhibitory concentration.

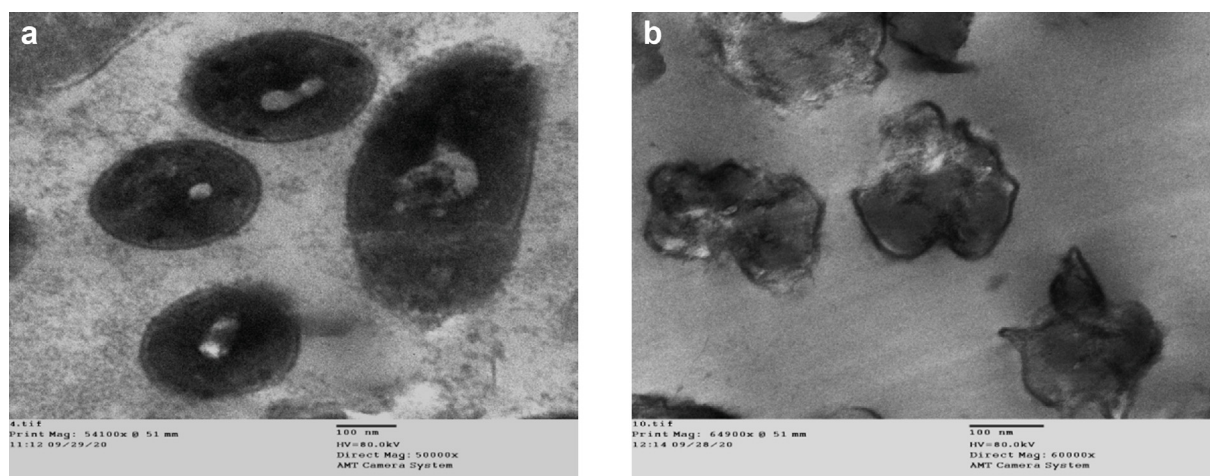


Fig. 1. Transmission electron micrograph: (a) Untreated MRSA cells and (b) MRSA cells treated with *E. purpurea* ethyl acetate fraction.

4. Discussion

One of the most popular medicinal herbs due to its immune-stimulant characteristics is *E. purpurea*, which is well known for its therapeutic benefits [28]. *E. purpurea*, in particular, is grown extensively over the world due to its beauty and purported medical benefits. *Echinacea* preparations have long been

utilized for breathing problems brought on by bacterial infections, immune system enhancement, and wound healing. *Echinacea* extracts are shown to have antioxidant and antibacterial properties, and they are also safe for use in both therapeutic settings and meals [29]. Because of its antimicrobial properties as well as its capacity to combat reactive oxygen species, *Echinacea* remedies are among the most well-

Table 3. Identification of the different molecules of ethyl acetate fraction of *E. purpurea* using LC/MS chromatographic separation technique.

Peak no.	Retention time (min)	Constituents identification	Relative content (%)	Chemical formula
1	8.26	Tridec-1-ene-3,5,7,9,11-pentayne	2.18	C ₁₃ H ₆
2	8.69	3-Octanol	0.59	C ₁₁ H ₂₆ O
3	9.81	4-hydroxybenzoic acid	1.27	C ₇ H ₆ O ₃
4	11.44	Caffeic acid	9.58	C ₉ H ₈ O ₄
5	11.99	Glycerol	1.82	C ₁₂ H ₃₂ O ₃
6	15.78	Pinacol,	1.97	C ₁₂ H ₃₀ O ₂
7	21.10	L-Arabitol,	0.35	C ₂₀ H ₅₂ O ₅
8	24.60	trans-caftaric acid	2.89	C ₁₃ H ₁₂ O ₉
9	24.96	Undeca-2-ene-8,10-diynoic- acid isobutylamide	6.32	C ₁₅ H ₂₁ NO
10	26.01	D-Galactofuranose	0.65	C ₂₁ H ₅₂ O ₆
11	26.19	1H-Indole, 6-methoxy-5-(phenylmethoxy)	2.65	C ₁₉ H ₂₃ NO ₂
12	26.63	L-Mannofuranose	1.48	C ₁₈ H ₄₄ O ₅
13	27.21	Glucopyranose	4.83	C ₂₁ H ₅₂ O ₆
14	27.63	Chlorogenic acid	5.58	C ₃₄ H ₆₆ O ₉
15	27.81	Shikimic acid	1.41	C ₁₉ H ₄₂ O ₅
16	28.08	D-fructofuranose	2.48	C ₂₁ H ₅₂ O ₆
17	29.20	cis-p-Coumaric acid	4.67	C ₉ H ₈ O ₃
18	29.51	D-Mannopyranose	2.13	C ₂₁ H ₅₂ O ₆
19	29.68	Myo-Inositol	0.89	C ₂₄ H ₆₀ O ₆
20	33.97	Palmitic Acid = Hexadecanoic acid	1.15	C ₁₉ H ₄₀ O ₂
21	38.37	Oleic acid	1.12	C ₂₁ H ₄₂ O ₂
22	38.67	9,12-Octadecadienoic acid	0.46	C ₂₁ H ₄₀ O ₂
23	41.34	Cynarine	6.94	C ₂₅ H ₂₄ O ₁₂
24	43.48	Cichoric acid	14.28	C ₂₂ H ₁₈ O ₁₂
25	43.72	Isorhamnetin	1.86	C ₁₆ H ₁₂ O ₇
26	44.53	Echinacoside	4.34	C ₃₅ H ₄₆ O ₂₀
27	45.25	Unknown	0.95	—
28	53.67	3-O-Coumaroyl-D-quinic acid	1.19	C ₃₁ H ₅₈ O ₈

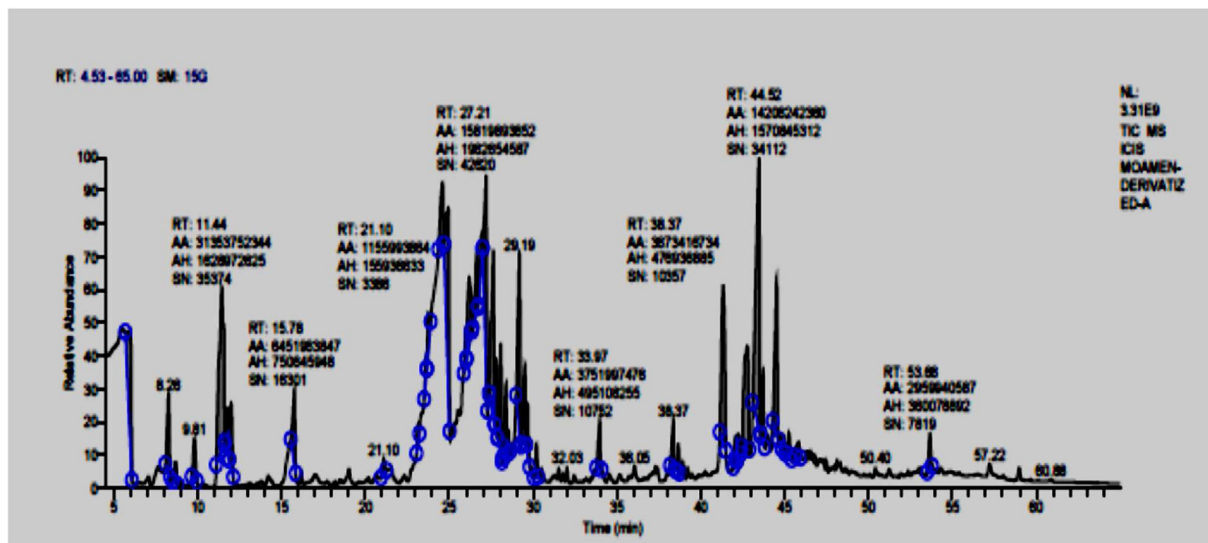


Fig. 2. LC-MS total ion chromatogram showing separated peaks of various compounds of *E. purpurea* ethyl acetate fraction.

liked [10,30,31]. Polysaccharides, flavonoids, caffeic acid derivatives, and other compounds can be found in the *Echinacea* species [32].

In the present study, the selected eight different MDRBs were obtained from clinical specimens of patients in two hospitals including Gram-positive and Gram-negative bacteria. The ethyl acetate fraction of *E. purpurea* had the highest inhibition

zone (22.7 ± 0.58) toward MRSA with MIC $15.63 \mu\text{g/ml}$. However, none of the reactions had activity toward *S. maltophilia*. In accordance with Rizzello et al. [33], *Yersinia enterocolitica*, *Lactobacillus plantarum*, and *Enterobacter aerogenes* were all inhibited by the crude extract of *E. purpurea*. Furthermore, Coelho et al. [28] assessed the antibacterial effect of *E. purpurea* extracts and concluded how several

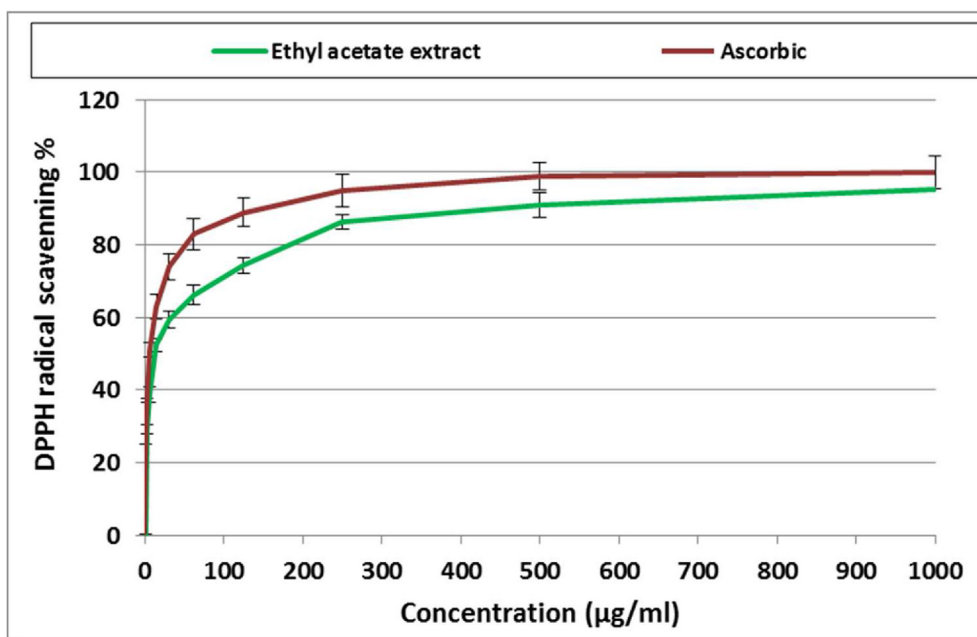


Fig. 3. DPPH radical scavenging efficiency proportions at various doses (g/ml) of the ethyl acetate fraction from *E. purpurea* are shown on the dosage response curve, which is then correlated to ascorbic acid as the reference standard, where $IC_{50} = 14.24 \pm 0.58 \mu\text{g/ml}$ for extract: ascorbic acid $IC_{50} = 11.21 \pm 0.67 \mu\text{g/ml}$.

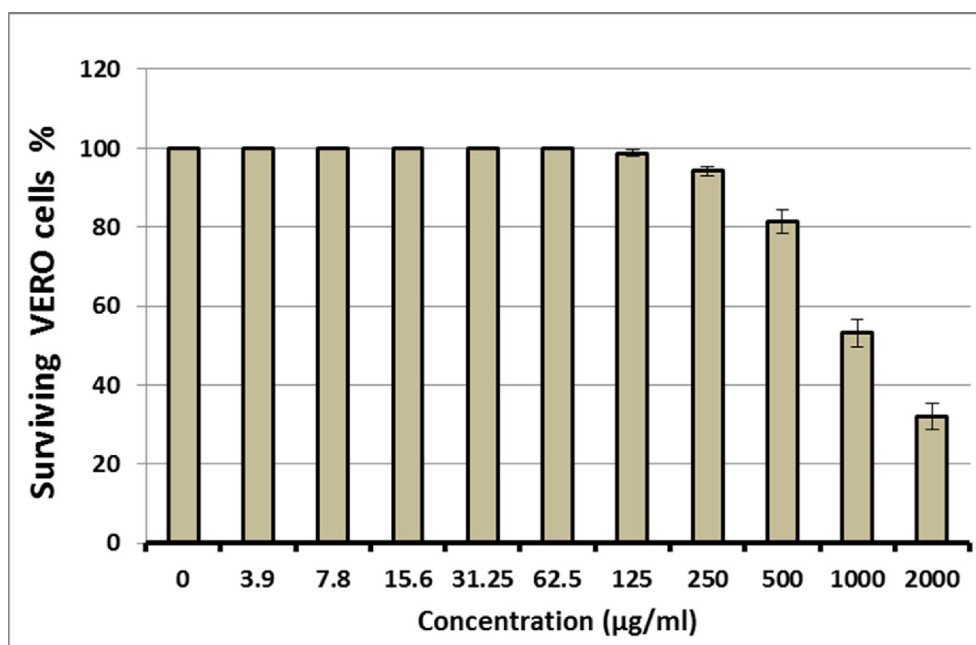


Fig. 4. *In vitro* cytotoxic effect of the *E. purpurea* ethyl acetate fraction on African Green Monkey kidney (VERO) cell line by MTT viability experiment to investigate the toxic effect at various doses. Data are presented as proportions of surviving cells at different concentrations (µg/ml) plus standard deviation over three replicates; where CC_{50} of the extract was 1145.97 µg/ml.

bacteria, such as *Morganella morganii* and *P. mirabilis* were more susceptible to ethyl acetate extract than to n-hexane.

The ethyl acetate fraction of *E. purpurea* exhibits anti-MRSA activity, which was originally demonstrated in this work. When used against clinical strains of *S. aureus*, fruit extracts from *Anadenanthera colubrina*, and *Pityrocarpa moniliformis* have shown similar effects in terms of cellular deformation, cell wall, and membrane breakdown, condensation of cellular components, and the existence of significant amounts of cytoplasmic material and membrane debris in the cell's external environment [34]. The observations, which are similar to ours, demonstrated that plant extracts caused pathologic injury to *S. aureus*, probably as a result of initial changes in metabolism that changed the cellular structures. In another study, various pathogens such as *Haemophilus influenzae*, and *Legionella pneumophila* were sensitive to the extract of *E. purpurea*; *E. faecalis* and *Klebsiella pneumonia* were relatively resistant to the drug, while *Candida albicans* and *Trichoderma viride* were essentially resistant to the prepared extract [35].

The polyphenols-caffeic acid derivatives caftaric acid and cichoric acid are the components of *E. purpurea* that are particularly active [36]. Additional substances have also been extracted and identified from the plant, including alkaloids, amides, and flavonoids and their free phenolic acids [21]. Echinacoside, an active component found in *Echinacea*

species, and cichoric acid, a caffeic acid derivative, are frequently utilized as indicators for classifying *Echinacea angustifolia* and *E. purpurea*, respectively [37,38]. One of the main active ingredients in *E. purpurea*, cichoric acid, is well known for its immunostimulatory effects both *in vitro* and *in vivo* as well as its capacity to block the enzyme hyaluronidase. This enzyme is implicated in bacterial infections and acts as an integrase inhibitor, which is necessary for the human immunodeficiency virus (HIV) to replicate and integrate its double-stranded DNA copy into host cells [31,36]. The primary lipophilic elements of *E. purpurea* that contribute to its pharmacological effects are already identified as alkylamides [21,39]. Undeca-2-ene-8,10-diyonic acid isobutylamide, one of the alkylamides, has a role in *E. purpurea*'s anti-inflammatory effects. This substance was discovered to significantly reduce TNF-alpha and nitric oxide production in mice macrophage cells when combined with other *Echinacea* alkylamides [40].

There are comparable antioxidant properties between this plant and the alkamides and cichoric acid that characterize *Echinacea*. The extracts' ability to neutralize free radicals was due to the presence of cichoric acid, whereas alkamides had no such ability [41,42]. Additional research on the plant's root extract indicated antioxidant activity, which may be related to the phenolic and cichoric acid content of the plant [21,43]. Comparable to flavonoids, cichoric

acid effectively scavenges free radicals from 2,2-diphenyl-1-picrylhydrazyl (DPPH). Although alkaloids have not demonstrated antioxidant action, they can boost cichoric acid activity through two different pathways. Initially, exterior action makes it easier for cichoric acid to penetrate the lipophilic droplets of an emulsion to limit lipid oxidation, and subsequently, generation of cichoric acid by giving allylic hydrogen to the one-electron oxidized cichoric acid [42,44].

The present investigation revealed the potency of *E. purpurea* as it has a minimal toxic effect upon testing on Vero cells. This is in the same line with Manayi et al. [21], who reported the possibility of *E. purpurea* to be applied in pharmaceutical products.

5. Conclusion

The results of the current investigation highlight the potential of the *E. purpurea* ethyl acetate fraction for application in the development of new antibiotics versus MDR bacterial strains. Therefore, more research may focus on identifying the cellular target(s) and the molecular basis behind the outcomes observed in this study.

Conflicts of interest

Authors declare no conflict of interest.

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