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Research Article

PHYTOCHEMICAL ANALYSIS AND DETERMINATION OF ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF *SUAEDA MONOICA*

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ABSTRACT

Objective: The aim of the study is to screen the whole plant extract of *Suaeda monoica* for its phytochemicals, determine its antibacterial and anticancer activities by sulforhodamine B (SRB) assay.

Methods: The powdered form of *S. monoica* with different solvents (ethanol, methanol, acetone, and diethyl ether) was used to screen its phytochemicals. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were performed using microdilution methods. Based on the results of MIC, the lowest concentration of antibacterial agent was subcultured on agar plates for MBC values. The zone of inhibition was determined using agar well diffusion method. The percentage of cell-growth inhibition for breast cancer cell line (MDAMB-231) was studied using SRB assay.

Results: The analysis revealed the presence of varied classes of phytochemicals except for acidic compounds with ethanol extract showing maximum compounds. All the extracts showed effective antibacterial activity against tested pathogens with MIC, MBC values ranging from 0.0156 to 0.625 mg/ml and 2.5 to 10 mg/ml, respectively. A significant zone of inhibition was observed with diethyl ether extract against *Staphylococcus epidermis*. The lowest concentration that showed 50% cell growth inhibition against MDAMB-231 was that of diethyl ether extract (60.18 µg/ml).

Conclusion: The present study depicts *S. monoica* as a natural source of bioactive compounds with significant antibacterial and anticancer activities. Further studies concerning the separation and isolation of the compounds are required.

Keywords: *Suaeda monoica*, Phytochemical analysis, Antibacterial activity, MDAMB-231, Anticancer activity.

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INTRODUCTION

Plants and the secondary metabolites produced by plants play key roles in today's drug development, particularly for antimicrobial and anticancer agents [1]. Infectious disease is a leading cause of an increase in morbidity and mortality in the developing world [2]. Although synthetic drugs are effective and economical, the usage of these is contentious due to the side effects of the drugs. Hence, the development of new drugs from plant resources has gained importance across the world. Tannins a group of secondary metabolites is reviewed in the year 1991 by Scalbert to show its antimicrobial activities [3]. Other essential biomolecules that were found to be inhibitory against the respiratory syncytial virus are flavone derivatives [4].

Breast cancer is the most common cancer that occurs in women, but it even targets men. It starts with DNA damage altering signaling pathways and the development of malignant cells in the ducts of breast or lining of mammary glands [5]. To overcome the side effects of synthetic chemoprotective agents, the use of plant-derived chemoprotective agents came into existence. One such natural product that is available on the commercial platform for treating cancer is Taxol, a terpenoid from the bark of Pacific yew tree. Ribose derivatives of benzoxazole were isolated from *Acanthus ilicifolius* Linn. (Acanthaceae) which were also active against cancers [6].

In comparison with the plants growing in normal conditions, the plants that grow in extreme conditions produce a diverse group of secondary metabolites to survive against unfavorable conditions. One such group of plants that have the potential to survive under adverse conditions is mangroves. Ethnobotanical reports by several authors revealed that the mangrove plants contain various compounds of medicinal value showing bioactive properties such as anti-antioxidant, anti-cancer, and phytotoxic activities [7].

Suaeda monoica (mangrove), Forssk. ex J. F. Gmel (Chenopodioideae), is a salt marsh halophyte that is confined to tidal or alkaline mudflats and salt marshes. It is distributed in the different regions of Africa, including Djibouti, Eritrea, Ethiopia, and Asia, including Syria, India, and Sri Lanka. In India, they are found in East South Coasts such as West Bengal, Orissa, Andhra Pradesh, and Tamil Nadu.

The leaves of this plant were traditionally used for wound treatment. The studies have also shown the antiviral activity of this plant due to the presence of sterols and triterpenoids. The hepatoprotective effect was performed on rats using leaves of *S. monoica* [8]. Researches proved that silver nanoparticles developed from this plant act as antitumor agents by reducing the development of tumor cells [9].

This work aims to evaluate bioactive compounds present in different solvent extracts of the plant, to examine the antibacterial activity of the plant against both Gram-positive and Gram-negative bacteria, and to determine the lowest concentration of plant extract that showed 50% cell growth inhibition in the MDAMB-231 cell line.

METHODS

Ethanol was procured from Merck (Bengaluru, India). Methanol, acetone, diethyl ether, and dimethyl sulfoxide were acquired from Fisher Scientific (Pittsburg, USA). Fetal bovine serum, trypsin-ethylenediaminetetraacetic acid, Roswell Park Memorial Institute (RPMI) – 1640 medium, potassium chloride, and sodium chloride were obtained from Himedia Laboratories (Mumbai, India). Potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate

dehydrate (Na_2HPO_4. $2H_2O$) were purchased from Sisco Research Laboratories (SRL, Maharashtra, India). Sulforhodamine B (SRB) dye and streptomycin sulfate were procured from Sigma-Aldrich (St. Louis, USA). Penicillin G was bought from PAN – Biotech GmbH (Aiden Bach, Germany).

Plant collection and processing

The whole plant of *S. monoica* was collected from Tirumala Hills, Andhra Pradesh. The plant was cleaned thoroughly under tap water and shade dried. After drying, the plant was pulverized to a fine powder and stored in an airtight container for further use.

Microbial cultures and cell lines

Staphylococcus epidermis (2044), *Bacillus cereus* (2128), *Klebsiella pneumoniae* (2451), and *Escherichia coli* (2412) were ordered from Microbial Culture Collection, Pune. Human breast cancer cell (MDAMB-231) was procured from National Center for Cell Science, Pune. Culturing of cells was done in tissue culture flasks with RPMI 1640 medium containing penicillin (100 IU/mL), streptomycin sulfate (100 g/mL), and fetal bovine serum 10% at 37°C in a humidified incubator with 5% CO_2.

Extraction

Ethanol, methanol, acetone, and diethyl ether were used for the extraction of the whole plant. The dried plant material was mixed with the desired solvent in the 1:10 (w/v) sample to the solvent ratio. The mixture was stored at room temperature for a period of 24 h and transferred to orbital shaker for a period of 48 h. Then, the solvent with extracted bioactive compounds is filtered using Whatman filter paper No. 1. The required volume of the resultant filtrate was used for phytochemical screening. The remaining filtrate was then concentrated using Rota evaporator under reduced pressure at different temperatures based on the boiling temperatures of the solvent. The obtained dried concentrated extract was weighed to know the yield percentage and was then preserved for further usage.

Phytochemical screening

The crude extract of the plant with different solvents was screened for their secondary metabolites using the below methodologies [10,11].

Test for alkaloids (Wagner's reagent)

It is done with Wagner's reagent. 3–5 drops of the reagent were added to 2 ml of extract and observed for the formation of a reddish-brown precipitate (or coloration), indicating the presence of alkaloids.

Test for carbohydrates (Molisch's test)

In this, few drops of Molisch's reagent were added to 2 ml of extract followed by the addition of 2 ml of concentrated sulfuric acid (H_2SO_4) along the walls of the test tube. The mixture was allowed to stand for 2–3 min and observed for the formation of a red or dull violet color at the interphase of the two layers indicating the presence of carbohydrates.

Test for cardiac glycosides (Keller–Kelliani's test)

In Keller–Kelliani's test, to 5 ml of extract, 2 ml of glacial acetic acid is added followed by adding a few drops of 5% aqueous ferric chloride solution. To this, 1 ml of concentrated H_2SO_4 was carefully under laid to the solution. The formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for flavonoids (alkaline reagent test)

In the alkaline reagent test, 2 ml of the extract was mixed with a few drops of 20% sodium hydroxide solution. The resulting yellow color solution becomes colorless on the addition of dilute hydrochloric acid indicating for the presence of flavonoids.

Test for phenols (ferric chloride test)

In the ferric chloride test, 2 ml of the extract was mixed with a few drops of 5% aqueous ferric chloride solution and observed for the formation of deep blue or black color indicating the presence of phenols.

Test for amino acids and proteins (1% ninhydrin solution in ethanol)

In this test, 2 ml of the extract was mixed with 2 ml of ninhydrin solution and the test tubes were covered with the foil and placed in a boiling water bath for 5 min. The formation of purple color indicates the presence of amino acids and proteins.

Test for saponins (foam test)

In the foam test, 2 ml of the extract was added to 5 ml of water. The mixture was shaken vigorously and observed for the formation of persistent foam indicating the presence of saponins.

Test for sterols (Liebermann–Burchard test)

In the Liebermann–Burchard test, 2 ml of the extract was mixed with few drops of chloroform and acetic anhydride was added. To this solution, concentrated H_2SO_4 was added carefully along the walls of the test tube and observed for the formation of reddish color indicating the presence of sterols.

Test for tannins (Braymer's test)

In Braymer's test, 2 ml of the extract was mixed with 2 ml of 10% alcoholic ferric chloride solution and observed for the formation of greenish color indicating the presence of tannins.

Test for terpenoids (Salkowski's test)

In Salkowski's test, 2 ml of the extract was mixed with 1 ml of chloroform followed by a few drops of concentrated H_2SO_4 and observed for the formation of reddish-brown precipitate indicating the presence of terpenoids.

Test for quinones

In this assay, 2 ml of the extract was mixed with few drops of concentrated HCl and observed for the formation of a yellow precipitate (or coloration) indicating the presence of quinones.

Test for diterpenes (copper acetate test)

In copper acetate test, 2 ml of the extract was mixed with 1 ml of aqueous copper acetate solution and observed for the formation of emerald green indicating the presence of diterpenes.

Test for resins

In this assay, 5 ml of distilled water was added to 2 ml of extract. The formation of turbidity indicates the presence of resins.

Antibacterial activity

Minimum inhibitory concentration (MIC)

The lowest antimicrobial agent (plant extract) concentration that will restrain the growth of microorganisms after incubating overnight is considered as MIC. It was determined by a widely accepted serial dilution microplate method in 96 well plates with some modifications [12]. To begin with, 100 µl of Mueller–Hinton (MH) broth was added to the well followed by 100 µl of a serial diluted plant extract with different concentrations. To this, 100 µl of 24 h microbial culture adjusted to 0.5% McFarland standards was added. The plates were then incubated for a period of 24 h at 37°C. After the incubation period, 20 µl–40 µl of 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent (2 mg/ml Phosphate- buffered saline [PBS]) was added and incubated for a period of 2 h. The plate was then observed for the development of color. The lowest concentration with purple color pigmentation in the well-indicating viability was considered as MIC.

Minimum bactericidal concentration (MBC)

The lowest antibacterial (plant extract) concentration that is needed for the death of bacteria is considered as MBC. It is obtained based on the microdilution tests of MIC by subculturing it on to the agar plates. The concentration with no bacterial growth on the plate is considered as MBC.

Inoculation preparation

Isolated colonies of each microbial culture were selected and transferred to a tube containing MH broth with the help of a loop. This suspension

was incubated for 24 h at 37°C and the turbidity was then adjusted to 0.5 McFarland standard (approx. 1.5×106 CFU/ml) [13].

Zone of inhibition

The zone of inhibition of *S. monoica* was determined using agar well diffusion method following the guidelines of clinical and laboratory standard institute [14]. Amikacin was used as positive control, while 50% of ethanol was used as the negative control. In this method, MH agar was poured in Petri plates and allowed to solidify. To these plates, microbial cultures in comparison with 0.5% McFarland standard turbidity were added, spread, and kept for overnight incubation at 37°C for 24 h (approx. 1.5×10^6 CFU/ml). Using 6 mm diameter borer, wells were dug in the agar plate to which different concentrations of 100 µl plant extracts were added and kept for incubation at 37°C, 24 h for a zone of inhibition development that was then measured.

SRB assay

In this assay, SRB binds to the protein content of cell lines in mild acidic conditions that can be then dissociated with basic conditions. The binding of the dye to the cell lines is stoichiometrically related and hence, the amount of dye released after washing is directly related to the amount of cell biomass that can be measured [15].

In this method, first, the 96 wells plate is seeded with the desired cell line and treated with the plant extract. After 24 h of incubation, the plate is treated with 10% trichloroacetic acid. The plate is then refrigerated for an hour at 4°C. Trichloroacetic acid is then discarded and the plate is washed with water. Then, the plate is allowed to dry completely. To the plate, 100 µl of SRB reagent is added and incubated for 30 min. SRB reagent is then discarded and 200 µl of 1% acetic acid is added. This step is repeated 4 times and each time acetic acid is added, mixed, and discarded. The plate is allowed to dry completely. To this, 100 mM tris at pH 4.0 is added and kept on the shaker for 10 min. The readings are then taken using multiscan at 565 nm [16].

% Cell growth= Absorbance of sample/Absorbance of negative control or untreated×100

% Growth inhibition=100−% cell growth

The effect of extracts was expressed by IC_{50} values (the lowest concentration of plant extract required for 50% cell growth inhibition) calculated by linear regression analysis.

Table 1: Percentage yield obtained using different solvent

Solvents	Yield percentage (%)
Ethanol	5.8
Methanol	12.2
Acetone	3.74
Diethyl ether	1.42

Statistical analysis

SRB assay for the plant extracts was performed in triplicates. Values for each extract are expressed as a mean ± standard deviation. The IC_{50} values obtained were used to determine statistical significance. Statistical analysis was conducted by one-way ANOVA using GraphPad Prism Software, and Version 8. $p\leq0.05$ was considered statistically significant.

RESULTS

Extraction yield

The ability of the solvent to extract specific components from the plant material is the extraction yield. The yield was measured by weighing 50 g of powdered plant material. The amount of crude extract obtained was 20 g. The yield percentage was found to be more in methanol, followed by ethanol, acetone, and diethyl ether (Table 1). The percentage yield was found to be less in non-polar solvents like diethyl ether when compared to the polar solvents. The yield percentage concludes that *S. monoica* has more polar bioactive compounds in comparison with non-polar compounds.

Qualitative phytochemical screening

Qualitative phytochemical screening of different extracts (ethanol, methanol, diethyl ether, and acetone) of whole plant *S. monoica* showed the presence of various bioactive compounds that might be responsible for its use in the medical field. A synopsis of the various compounds found in different extracts has been compiled and arranged (Table 2). Alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, sterols, terpenoids, quinones, and resins were universally found in all extracts. Phenols, tannins, and diterpenes were the compounds found in all extracts except diethyl ether extract. Amino acids and protein components were present only in ethanol and methanol extract. Acidic compounds when tested were not present in any of the extract. Methanol and ethanol extracts of the whole plant showed more number of phytoconstituents in comparison with the other extracts. The phytochemicals present in the different extracts justifies the use of *S. monoica* in traditional medicine.

Antibacterial activity

The antibacterial activity of plant extracts determined from MIC and MBC assays showed a satisfactory effect on the microorganisms. MIC and MBC values of all the extracts against different microorganisms have been organized (Table 3). Amikacin was used as a positive control. All the solvent extracts had an equal effect against Gram-negative *E. coli*. In *K. pneumoniae* highest MIC value was found in acetone extract (1.25 mg/ml) when compared to other extracts (0.625 mg/ml). In *S. epidermis* lowest MIC value was observed in diethyl ether extract (0.312 mg/ml) when compared to other extracts (0.625 mg/ml). In *B. cereus* lowest MIC value was observed in acetone extract (0.312 mg/ml) when compared to other solvent extracts (0.625 mg/ml). All extracts showed a comparative MIC effect on *E. coli* as that of positive control amikacin.

Table 2: Phytochemical compounds in different solvent extract

Phytochemical compound	Screening test	Ethanol	Methanol	Acetone	Diethyl ether
Alkaloids	Wagner's reagent test	+	+	+	+
Carbohydrates	Molisch's test	+	+	+	+
Cardiac glycosides	Keller–Kelliani's test	+	+	+	+
Flavonoids	Alkaline reagent test	+	+	+	+
Phenols	Ferric chloride test	+	+	+	−
Amino acids and protein	Ninhydrin test	+	−	−	−
Saponins	Foam test	+	+	+	+
Sterols	Liebermann-Burchard test	+	+	+	+
Tannins	Braymer's test	+	+	+	−
Terpenoids	Salkowski's test	+	+	+	+
Quinones	Conc. HCl test	+	+	+	+
Diterpenes	Copper acetate test	+	+	+	−
Resins	Turbidity test	+	+	+	+
Acidic compounds		−	−		

"+" indicates the presence of phytochemical compound. "−" indicates the absence of phytochemical compound

Table 3: MIC and MBC values of Suaeda monoica

S. No.	Microorganisms	Solvents	MIC (mg/ml)	MBC (mg/ml)
1.	*Staphylococcus epidermis* (2044)	Ethanol	0.625	5
		Methanol	0.625	5
		Acetone	0.625	5
		Dee	0.312	2.5
		Amikacin	0.015	0.312
2.	*Bacillus cereus* (2128)	Ethanol	0.625	5
		Methanol	0.625	5
		Acetone	0.312	5
		Dee	0.625	2.5
		Amikacin	0.015	0.0625
3.	*Escherichia coli* (2412)	Ethanol	0.156	5
		Methanol	0.156	5
		Acetone	0.156	10
		Dee	0.156	2.5
		Amikacin	0.062	0.25
4.	*Klebsiella pneumoniae* (2451)	Ethanol	0.625	2.5
		Methanol	0.625	2.5
		Acetone	1.250	2.5
		Dee	0.625	2.5
		Amikacin	0.039	0.0312

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

The MBC value of different extracts against *E. coli* ranged from 2.5 to 10 mg/ml; for *K. pneumoniae*, the value was 2.5 mg/ml for all extracts and for both *Staphylococcus aureus* and *B. cereus* it ranged from 2.5 to 5 mg/ml. Among all the extracts diethyl ether showed the lowest MBC value (2.5 mg/ml) against both Gram-positive and Gram-negative bacteria.

Zone of inhibition

Between all the Gram-positive and Gram-negative bacteria, diethyl ether extract showed a zone of inhibition against *S. epidermis* (Fig. 1). A small zone of inhibition was observed against *E. coli* (Fig. 2) with diethyl extract after 48 h of incubation. No zone of inhibition was observed against *Klebsiella pneumoniae* (Fig. 3) and *B. cereus* (Fig. 4). Amikacin was used as positive control and 50% ethanol was used as the negative control.

SRB assay

This assay is widely used for drug toxicity screening against both cancerous and non-cancerous cell lines. The dye released after washing is directly related to the amount of cell biomass which can be measured using Elisa reader at 565 nm. The cytotoxicity of the plant extract with different solvents was tested against the MDAMB-231 cell line with concentrations ranging from 100 µg to 500 µg/ml. The lowest concentration of plant extract required for 50% cell growth inhibition (IC_{50}) against MDAMB-231 with different solvents is tabulated (Table 4). The lowest IC_{50} value was obtained for diethyl ether extract (60.18 µg/ml) indicating its highest cytotoxic activity, followed by methanol (148.77 µg/ml), ethanol (172.38 µg/ml), and acetone (185.56 µg/ml).

There is a significant difference between IC_{50} values of only ethanol and diethyl ether extract. There is no significant difference between other extracts (Table 4).

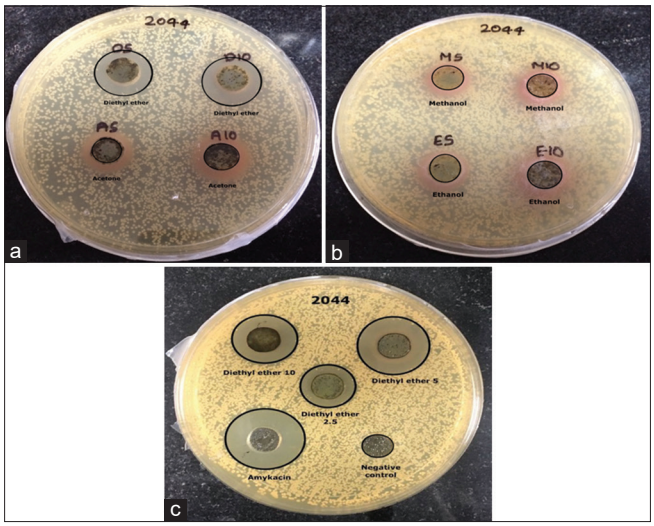

Fig. 1: *Staphylococcus epidermis*. (a) Zone of inhibition observed with different concentrations of diethyl extract (5 mg–2 mm; 10 mg–3 mm). (b) No zone of inhibition. (c) Zone of inhibition observed with different concentrations of diethyl extract and amikacin (10 mg–3 mm)

DISCUSSION

Varied plant types have been used for ages for the treatment of a wide range of diseases. This could be attributed to the presence of naturally occurring and biologically active secondary metabolites present in plants [17]. Mangroves are exceptional among the tropical forest types that exist under stressful conditions such as violent environments, high concentration of moisture, and high and low tides of water and thus are endowed with certain chemical compounds that can protect them from these biotic and abiotic factors [18]. The study on mangroves for its secondary metabolites has been emerging as one of the most important developments for use as biocontrol agents, cytotoxic agents, and drug discovery.

The purpose of the study was to determine the phytochemical constituents, antibacterial, and anticancer activities of whole plant *S. monoica*. The phytochemical screening of *S. monoica* revealed the presence of secondary metabolites proving it to be medicinally important. Different plants due to the presence of secondary metabolites are used as herbal drugs with the antidiabetic, antiulcer, antimalarial, anti-inflammatory, antimicrobial, and hepatoprotective activity [19].

Medicinal plants have different mechanisms against microorganisms. Some of these include inhibiting cell wall formation, depleting energy by accumulating in bacterial membranes, effecting permeability of cell membrane, thereby increasing permeability leading to loss of cellular components, disrupting cell membrane, and modifying cellular constituents, thereby causing mutations or damage to cell or cell death.

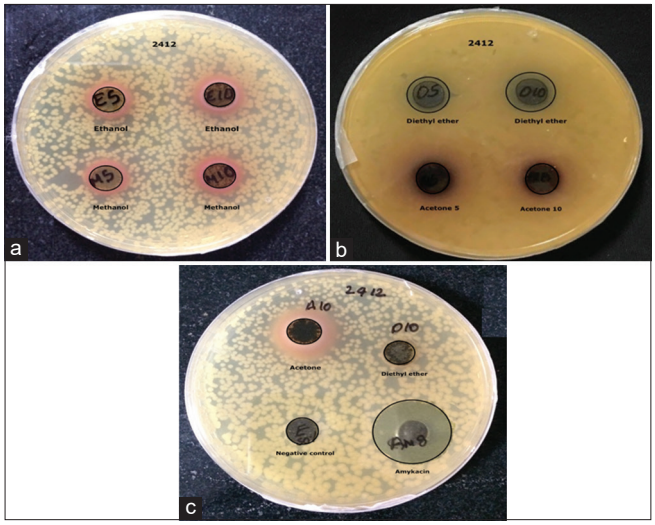

Fig. 2: *Escherichia coli*. (a) No zone of inhibition observed. (b) Zone of inhibition with diethyl extract after 48 h of incubation. (c) Zone of inhibition observed with amikacin

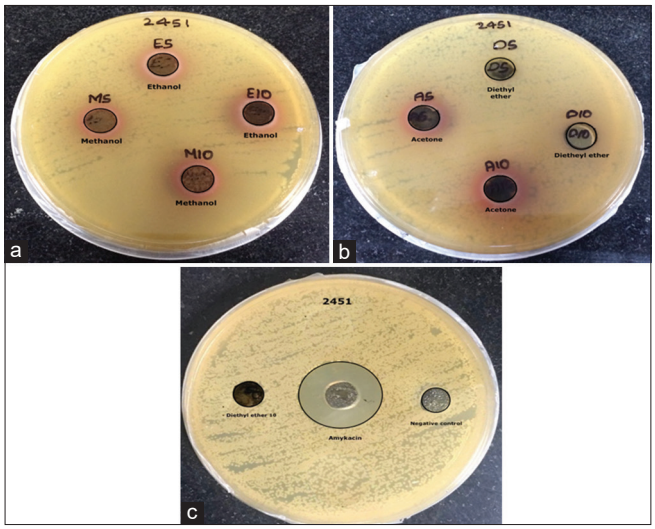

Fig. 3: *Klebsiella pneumonia*. (a and b) No zone of inhibition observed against *K. pneumonia*. (c) Zone of inhibition observed with amikacin (4 mm)

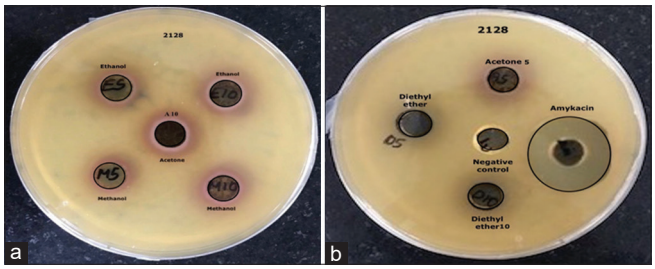

Fig. 4: *Bacillus cereus*. (a) No zone of inhibition observed against *B. cereus*. (b): Zone of inhibition observed with amikacin (6 mm)

The antibacterial activity of *S. monoica* with different plant extracts was determined using MIC and MBC, and the results were arranged (Table 3) indicating its effect against different microorganisms. Our findings revealed that the MIC values of the extracts were lower than their MBC values, indicating significant antibacterial activity.

Table 4: Comparison between IC_{50} values of different plant extracts against MDAMB-231

S. No.	*Suaeda monoica* extracts	IC_{50} values (µg/ml) against MDAMB-231
1.	Ethanol	172.38 ± 40.95^{a}
2.	Methanol	148.77 ± 10.57^{ab}
3.	Acetone	185.56 ± 1.312^{ab}
4.	Diethyl ether	60.18 ± 23.09^{b}

The data represents the mean±SD of three determinants and levels of significance. Rows with different letters (a-b) indicate significantly different $p\leq0.05$

Although the results show substantial MIC and MBC values for all the solvent extracts, the zone of inhibition was observed only with diethyl ether extract. There are various constraints involved in the zone of inhibition formation among which diffusivity of the drug is one of the vital conditions. The formation of a zone of inhibition with diethyl ether extract can be attributed to its successful diffusion through the MH agar medium in contrast with other solvent extracts [20].

SRB is a widely used cell cytotoxicity assay to detect the viability of cells and drug toxicity against different types of cancerous and non-cancerous cell lines. It possesses colorimetric endpoint and hence is stable and non-destructive in comparison with other assays. *In vitro* cytotoxicity of plant extracts of Euphorbiaceae family against human cancer cell lines was studied using SRB assay by determining its percent growth inhibition [21]. In the present study, the anticancer activity of plant extract with different solvents was studied by determining the percent of plant extract required for 50% cell growth inhibition (IC_{50}) using SRB assay. The results were compared and tabulated.

Before this study, no reports of antibacterial and anticancer activities of whole plant *S. monoica* were seen during literature survey. However, other species have been studied and documented.

CONCLUSION

The present study screens for the phytochemicals of *S. monoica* and determined that diethyl ether extract of *S. monoica* has higher antibacterial and cytotoxic activities against both Gram-positive and Gram-negative bacteria and MDAMB-231 cancer cell lines, respectively, indicating larger group of bio-control and cytotoxic compounds getting extracted from the plant using diethyl ether solvent in comparison with other solvents. Further studies are required for isolation and identification of biologically active substances from the extract that has antibacterial and cytotoxic activities and could be further used for curing of other stress-related diseases such as cardiovascular and other chronic diseases.

AUTHORS' CONTRIBUTION

Prof. Mangamoori and Ms. Swathi did the conception and design of the work. Ms. Swathi has done data acquiring, interpretation, and drafting of the article. Ms. Swathi and Ms. Chintalapani have done the statistical analysis. Prof. Mangamoori and Ms. Swathi did the critical revision of the article.

CONFLICTS OF INTEREST

We declare that there are no conflicts of interest.

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