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Antibacterial, Antioxidant and Cytotoxicity Assessment of *Crassocephalum crepidioides* Leaf Extract

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Abstract

The goal of the present investigation was to demonstrate the antibacterial activity of different solvent extracts (methanol, ethanol, cold aqueous and hot aqueous) of *Crassocephalum crepidioides* against ATCC bacterial cultures of *Staphylococcus aureus, Escherichia coli, Methicillin resistant Staphylococcus aureus, Pseudomonas aeruginosa* and its antioxidant potential. Furthermore, the chemical constituents present in the extract was perused by Gas chromatography-mass spectroscopy (GC-MS), along with *in vitro* cytotoxicity assessment. All the extracts were shown to be sensitive against *S. aureus,* MRSA and *P. aeruginosa* except for the ethanolic extract which was resistant to *P. aeruginosa*. Of all the extracts, hot aqueous extract found to be the most effective. It was found that Minimum inhibitory concentration (MIC) value of hot aqueous extract against *S. aureus,* MRSA and *P. aeruginosa* were 5 mg/mL, 5 mg/mL and 40 mg/mL, respectively. DPPH results showed that *C. crepidioides* leaf extract has potent antioxidant activity with IC_{50} value of 57.9 µg/mL. 22 compounds were detected in hot aqueous extract through Gas chromatography-mass spectroscopy. The results of the cytotoxicity evaluation displayed that the IC_{50} value of the hot aqueous extract of *C. crepidioides* on Vero cell lines was 292 µg/mL. This study concludes that *C. crepidioides* leaf extract is non-toxic, has various bioactive components and strong antibacterial and antioxidant activities, thus making it a promising therapeutic agent for various biomedical applications.

Keywords: Antibacterial Activity, Antioxidant Potential, *Crassocephalum crepidioides*, Phytochemicals, GC-MS, Cytotoxicity, Vero Cell Lines

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INTRODUCTION

Antibiotics are widely used for treating bacterial infections in human as well as animal. However, there has been a steady increase in the antibiotic resistant bacteria and decrease in the potential antibacterial drugs to treat various infections.[1] Over-use, improper, unsystematic uses of antibacterial drugs and genetic mutations led to the development of drug resistance where the pathogens become ineffective to the available antibiotics.[2] Antibiotic-resistant bacterial infections typically result in significant disease and mortality as well as economic problem on medical management globally.[3] The Global Research on Antimicrobial Resistance study showed that almost 5 million human deaths were linked with drug-resistant bacterial infections.[4] The diminishing efficacy of antibiotics and the rise of antibiotic resistance in pathogenic bacteria have led to a sustained exploration of medicinal plants for their potential to combat resistant strains.[5]

A free radical is a molecule that possesses an unpaired electron in one of its atomic orbitals and is capable of existing independently. They can function as oxidants or reductants by donating or accepting electrons from other molecules.[6] In addition to the body's normal, crucial metabolic developments, external causes such as contact to X-rays, ozone, tobacco smoke, air pollution and manufacturing chemicals can also produce free radicals and other reactive oxygen species (ROS).[7] Overproduction of free radicals causes secondary damage through the cytotoxic and mutagenic effects of liberated metabolites, in addition to direct damage by the oxidation of DNA, proteins, lipids, and carbohydrates.[8] Antioxidants are crucial in preventing cancers, cardiovascular diseases and neurological illnesses as well as aging and inflammation. Natural plant products are believed to have antioxidant properties which protect cells from the harmful effects caused by free radicals.[9,10]

For millennia, plants have been used to cure a variety of bacterial illnesses. A potential strategy to combat antibiotic-resistant pathogens involves harnessing the therapeutic properties of medicinal plants, which offer a wide range of promising remedies through their secondary compounds.[11] Phytochemicals present in the plants include the phenolic compounds, alkaloids, flavonoids, saponin, tannin, quinones and coumarins, etc.[12] Phenolic compounds are considered as important phytochemicals because of its antioxidant and antimicrobial properties.[13] Flavonoids possess various medicinal benefits including antimicrobial, free radical scavenging, anti-inflammatory and antihistamines properties.[14] Tannins are a class of polyphenols which contain antioxidant and antibacterial properties.[15] Alkaloids has anticancer, antibacterial and anti-inflammatory properties.[16,17]

Crassocephalum crepidioides is a species belonging to Asteraceae Family which is an upright, annual and succulent herb growing in many tropical and subtropical regions. It is commonly known as Thickhead, Redflower rag leaf, Fireweed and locally as Terapaibi in Manipur, India.[18] It has been utilized as food constituents and traditional medicine.[19] The plant is found abundantly in the yards, roadsides, gardens and rice fields. It is traditionally used by local people of Manipur as a remedy for many ailments. The leaves are practised for the healing of stomach ulcer, hypertension and boosting the immune system.[20] Leafy paste has been employed to treat minor wounds by the local people of Manipur.[18] Researchers reported that it has antibacterial,[21] antioxidant,[22] anti-inflammatory,[23] antidiabetic,[22] antitumor[24] and wound healing activity.[25] Therefore, this study assesses the antibacterial, antioxidant potentials, along with the chemical constituents of *Crassocephalum crepidioides* and its toxicity nature was investigated on Vero cell lines to ensure the safe use of the plant.

MATERIALS AND METHODS

Collection and extraction of the plant leaves

The green leaves of *C. crepidioides* (Benth.) S. Moore were collected from different areas of Kakching District of Manipur, India and was authenticated by Dr. P. Palani, Centre for Advanced Studies in Botany, University of Madras, Chennai. After being shade-dried for 10 days, the leaves were powdered and extracted using various solvents including methanol, ethanol, and water. In each Erlenmeyer flask, 25 g of the powdered medicinal plant leaves were mixed with 250 mL of the corresponding solvent and incubated in a shaking incubator at 28°C for 3 days.[26] For the hot

aqueous extract, 25 g of the powder was mixed with water and heated at 60°C for 2 hrs, then it was cooled and left undisturbed for 24 hrs.[27] Subsequently, all the solutions were filtered, dried and stored for future use.

Qualitative screening of Phytochemicals

Each extract was screened for the investigation of six different phytochemicals such as tannin, saponin, phenols, flavonoids, terpenoids and alkaloids conferring to the following protocols.

Tannin test

The extract (0.5 g) was mixed with 10 mL of distilled water, then a few drops of 5% ferric chloride were added to the extract. Black or blue-green coloration was the indication of tannin presence.[28]

Saponin test

The foam formation that persists for 5min after shaking the extract (0.5 g) with the distilled water (10 mL) denotes the positive result for saponins.[29]

Flavonoids test

Few drops of 10% NaOH solution was added to 1 mL of the extract. Initially, bright yellow color formed but it gradually turned colorless after the addition of dilute hydrochloric acid which confirms the positive results of flavonoids.[29]

Phenol test

The change of the aqueous extract to dark green or bluish-black after adding 10% ferric chloride solution indicates the presence of phenol.[28]

Terpenoids test

Salkowski test: Conc. H_2SO_4 (3 mL) was added slowly along the side of the test tube containing the extract (5 mL) and chloroform (2 mL). Terpenoids presence was confirmed by reddish-brown coloration at the junction of two layers.[28]

Alkaloids test

Mayer's test: Cream colored precipitate formation after adding 1 mL of Mayer's reagent (Potassium mercuric iodide solution) to the extract in a test tube was the indication of the positive result for alkaloids.[29]

Bacterial cultures

Four ATCC cultures such as *Staphylococcus aureus* ATCC 29213*, Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were obtained from Centre for Drug Discovery and Development, Sathyabama University, Chennai and *Methicillin resistant Staphylococcus aureus* ATCC 43300 was purchased from HiMedia, Maharashtra, India. The obtained cultures were subculture onto the Nutrient agar medium and incubated for 24 hrs at 37°C. After the incubation, one colony was taken and inoculated into the Brain Heart Infusion broth, then, incubated for 3 hrs.

Antibacterial Activity Assay

The antibacterial potential of each extract was evaluated against the test organisms using the agar well diffusion method.[30] Lawn culture of the test organisms was made onto the sterile Muller Hinton agar plates. Wells of 8 mm diameter were made using sterile micropipette tip. A stock solution of 500 mg/mL concentration of various solvent extracts was prepared and 100 µl from the stock solution was loaded onto the wells and the final concentration is 50 mg/mL, incubated at 37°C for 24 hrs. Ciprofloxacin (5 µg), distilled water and 20% DMSO (Dimethyl sulfoxide) were used as controls. After the incubation, inhibition zone diameter was measured. The experiment was conducted in triplicate.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

By employing agar well diffusion method, MIC of the solvent extract with strong antibacterial activity was determined against the test organisms. Two-fold dilutions were made to make the concentrations ranging from 3.125 to 400 mg/mL. Wells were created on Muller Hinton agar plates and lawn culture was made using sterile cotton swab. Following the addition of 100 µl of various conc. to each well, the plates were incubated.[31] Distilled water and ciprofloxacin (5 µg) were utilized as negative and positive controls respectively. Zone of inhibition was noted after 24 hrs of incubation. The lowest concentration which produces zone of inhibition

was considered to be the MIC of the extract.

MBC was performed by touching inhibition zone of MIC plate of 4 lowest concentration of plant extract showing invisible growth and subculture onto the Nutrient agar plates. Following a 24 hrs incubation period at 37°C, the bacterial growth on these plates was monitored. The concentration of the plant extract that did not produce any bacterial growth on freshly inoculated plates was considered as the MBC.[32]

Antioxidant activity

The antioxidant capacity of hot aqueous extract of *C. crepidioides* was assessed by measuring its 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Hot aqueous extract of *C. crepidioides* at varying concentrations (20, 40, 60, 80 and 100 µg/mL) was combined with 0.004% (w/v) methanol containing DPPH (0.1 mM). Ascorbic acid was employed as a standard. The solution was shaken vigorously and placed in the dark condition at room temperature for 30 mins. At 517 nm, the absorbance of the reaction mixtures was measured. The negative control was used as the blank sample containing distilled water and DPPH solution. The percentage of DPPH scavenging effect was evaluated.[33]

$$\text{Inhibition \%} = \frac{\text{(Absorbance of control-Absorbance of test sample)}}{\text{(Absorbance of control)}} \times 100$$

GC-MS

The chemical components of hot aqueous extract of *C. crepidioides* were analyzed by GCMS QP 2010 Ultra (Shimadzu, Japan) which was equipped with a Rtx-5 MS fused silica column (5% diphenyl 95% dimethyl polysiloxane 30 m x 250 µm, film thickness of 0.25 µm. At 250°C, the injector was operated. With a split ratio of 1:50, the samples were injected in the split mode and the injection volume was 1 µl. Helium gas as a carrier gas was used at a steady flow rate of 1 mL/min. The oven temperature was as follows: 80°C was the starting temperature, which was maintained for 4 mins. The temperature then increased to 100°C at a rate of 2°C/min and then to 280°C at a rate of 5°C/min, which was maintained for 5 mins. The total elution was 54 mins. The compounds identification was based on matching the obtained mass spectra with the available mass spectral records (Willey 8, NIST 11 and 17 libraries).

***In vitro* Cytotoxicity analysis**

The toxicity of the hot aqueous extract of *C. crepidioides* was evaluated using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) reduction assay[34] on Vero cell lines. Vero cells were deposited onto a 96-well microtiter plate that contained DMEM medium supplemented with 10% FBS, penicillin G and streptomycin at a density of 1×10^4 cells/well and incubated for 24 hrs at 37°C with 5% CO_2. Following this, the medium was substituted with new medium, then, the various concentrations (1-500 µg/mL) of hot water extract of *C. crepidioides* were added and incubated for 24 hrs. After incubation, MTT reagent was added and incubated for 4 hrs. After removing the medium containing the MTT reagent, DMSO was added to dissolve the formazan crystals. The absorbance at 570 nm was measured to determine the cell viability (%) and calculated according to the formula[35]:

$$\text{Cell viability (\%)} = \frac{\text{(Absorbance of Sample)}}{\text{(Absorbance of Control)}} \times 100$$

Statistical study

The data were displayed as mean±SD. Using SPSS version 25.0 software, the obtained data of antibacterial activity were studied by one-way analysis of Variance (ANOVA) and Duncan test with p-value of <0.05 being considered statistically significant.

RESULTS AND DISCUSSION

Phytochemicals screening

Medicinal herbs have been discovered to be the rich source of phytochemicals with various pharmacological activities that can be utilized as a promising counteragent to treat a variety of illness. Phytochemicals present in the plant have been explored for their pharmacological action and potency including antibacterial activities.[21] Qualitative phytochemicals screening was performed for each extract for the presence and absence of tannin, saponin, phenol, alkaloids, flavonoids and terpenoids. The results (Table 1) revealed that Tannin and Phenols were detected

in methanolic, cold and hot aqueous except ethanolic extract. Saponin was present in cold and hot aqueous extract but absent in methanolic and ethanolic extracts. Methanolic and ethanolic extracts showed positive results for alkaloids. Flavonoids were present in all the extracts. Terpenoids was found only in hot aqueous extract. The presence of these phytochemicals in the plant extract can deliver a first description for their antibacterial properties. The variations in the antimicrobial properties of the extract may result from variations in both their chemical makeup and the way that their bioactive components work.[36]

The findings of our analysis of phytochemical screening of hot aqueous extract *C. crepidioides* were consistent with previously published research; yet, alkaloids were not present.[21] Adjatin *et al*. revealed that flavonoids, gallic tannin, and cathartic tannin were detected in the *C. crepidioides* aqueous extract.[37] Alkaloids and terpenoids were absent from the cold aqueous extract, while tannin, saponin, flavonoids, and phenol were detected. There were alkaloids and flavonoids in the ethanolic extract. In the methanolic extract, tannin, alkaloids, flavonoids, phenol were present whereas saponin, terpenoids were absent.

Antibacterial activity assay

The antibacterial potential of methanolic, ethanolic, cold and hot aqueous extracts of *C. crepidioides* were performed by agar well diffusion method against the test organisms. *S. aureus,* MRSA and *P. aeruginosa* were found to be sensitive to the methanolic extract of *C. crepidiodes*. The ethanolic extract was found to be sensitive to *S. aureus* and MRSA. Both the cold and hot water produced significant inhibition zone

Table 1. Phytochemicals screening of methanolic, ethanolic, cold and hot aqueous extracts

Phytochemicals	Methanol	Ethanol	Cold aqueous	Hot aqueous
Tannin	+	-	+	+
Saponin	-	-	+	+
Phenols	+	-	+	+
Alkaloids	+	+	-	-
Flavonoids	+	+	+	+
Terpenoids	-	-	-	+

'+' denotes the presence of the phytochemical and '-' denotes the absence of the phytochemical

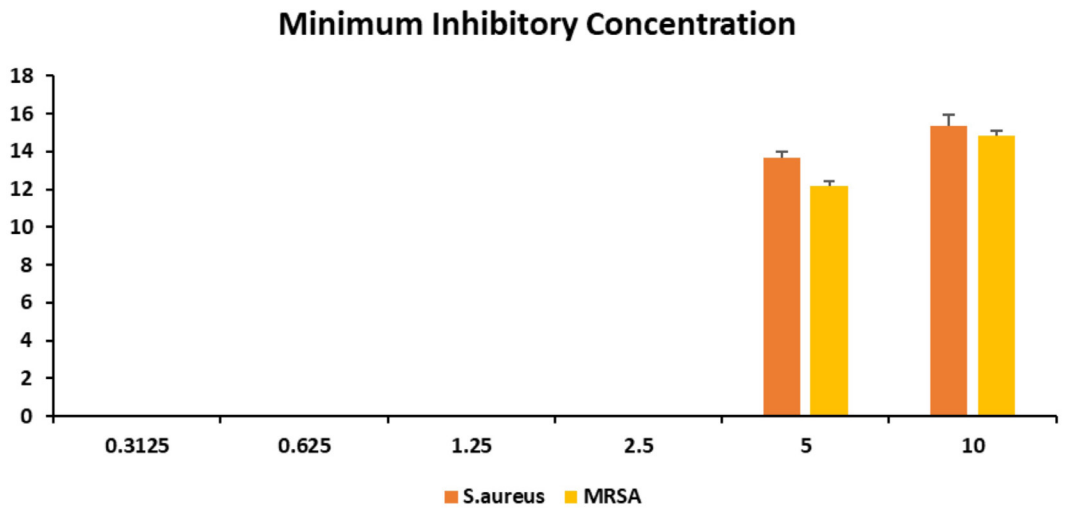

Figure 1. MIC of hot aqueous extract of *C. crepidiodes* against *S. aureus* and MRSA

against *S. aureus,* MRSA and *P. aeruginosa*. Among the extracts, hot aqueous extract was found to produce higher zone of inhibition when compared to other extracts which was shown in Table 2. *S. aureus* and MRSA was the most sensitive to all of the solvent extracts while *P. aeruginosa* was the least sensitive to methanol, cold and hot aqueous, but resistant to ethanolic extract. It was found that *E. coli* was resistant to all the extracts.

Among the extracts, hot aqueous extract was shown to be the most efficient with the zone of inhibition of 17.33 ± 0.57 mm and 12 ± 1 mm against *S. aureus* and *P. aeruginosa* respectively. Omotayo *et al*. revealed that the hot aqueous extract of *C. crepidioides* were sensitive to *Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli*.[21] The essentials oils of *C. crepidioides* was effective against pathogenic organisms.[38] The variation in these findings might be due to the plant extraction process, concentration and type of cultures used. As previously reported, the methanolic extract of *C. crepidioides* was found to be sensitive against *S. aureus* and *P. aeruginosa*, which is consistent with the present study.[39]

MIC and MBC

Since the hot aqueous extract of *C. crepidioides* revealed highest inhibition zone, the hot aqueous extract of *C. crepidioides* was used to check the MIC. The MIC value of hot aqueous extract was found to be 5 mg/mL against *S. aureus* (13.66 ± 0.28 mM), MRSA (12.16 ± 0.28 mM) and is not determined for *P. aeruginosa* (Figure 1). According to Omotayo *et al*., the hot aqueous extract of *C. crepidioides* was sensitive against *S. aureus* with MIC value of 45 mg/mL.[21]

Table 2. Inhibition zone in diameter of different solvent extracts of *C. crepidioides,* Ciprofloxacin, Distilled water and DMSO against the test organisms

Extracts	*S. aureus*	*P. aeruginosa*	*E. coli*	MRSA
Ethanol	10.83 ± 0.28^{e}	-	-	11.84 ± 0.76^{d}
Methanol	12.5 ± 0.5^{d}	11.16 ± 0.28^{b}	-	12 ± 0.5^{d}
Cold water	16 ± 0.5^{c}	11 ± 0.5^{b}	-	15.83 ± 0.29^{c}
Hot water	17.33 ± 0.57^{b}	12 ± 1^{b}	-	18.16 ± 0.76^{b}
Ciprofloxacin (Positive control)	22.16 ± 0.76^{a}	28 ± 0.5^{a}	31.16 ± 0.76^{a}	22.5 ± 0.5^{a}
Distilled water (Negative control)	-	-	-	-
DMSO (Negative control)	-	-	-	-

Results expressed as mean ± SD (mM). Different letters in the same column denotes significantly different ($p < 0.05$)

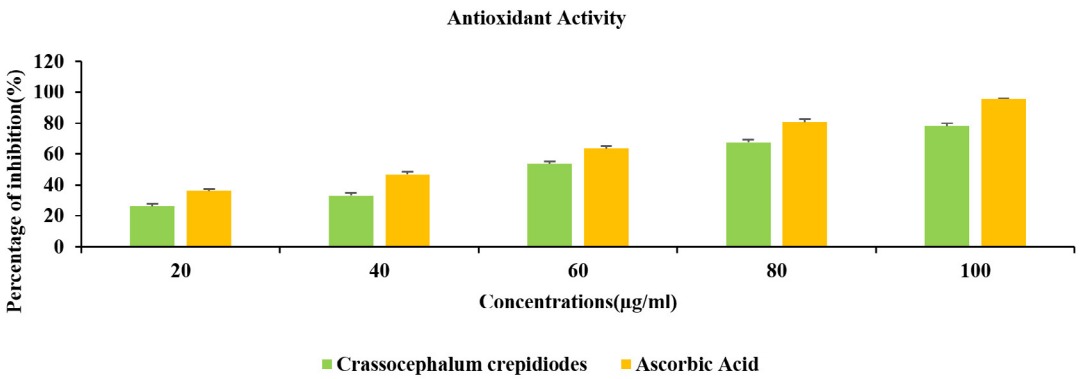

Figure 2. DPPH radical scavenging activity of hot aqueous extract of *C. crepidioides* and Ascorbic acid

The concentration showing no bacterial growth after subculturing the MIC onto the nutrient agar plate was considered as the MBC. Hot aqueous extract of *C. crepidioides* was able to kill *S. aureus* and MRSA having MBC of 10 mg/mL respectively.

Antioxidant activity

The DPPH radical scavenging activity of hot aqueous extract of *C. crepidioides* was tested to check its antioxidant potential by DPPH assay. The scavenging ability of the tested extract showed significance in a concentration-dependent manner at low concentration. Scavenging activity increased as concentration increased. It was found that the scavenging activity of hot aqueous extract of *C. crepidioides* was 78 ± 1.9% (100 µg/mL), 67.5 ± 1.7% (80 µg/mL), 53.6 ± 1.7% (60 µg/mL), 33 ± 1.8% (40 µg/mL) and 26 ± 1.7% (20 µg/mL) which is shown in Figure 2. The IC_{50} value

Table 3. Compounds detected through GC-MS analysis, along with their retention time, area % and chemical formula in the hot aqueous extract of *C. crepidioides*

Peak	Retention Time (RT)	Area (%)	Name	Formula
1	4.493	0.50	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$
2	5.466	2.19	Cyclotetrasiloxane, octamethyl-	$C^8H^{24}O^4Si_4$
3	6.258	1.01	Penta siloxane, dodecamethyl-	$C_{12}H_{36}O^4Si_5$
4	32.510	2.50	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
5	32.611	2.39	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$
6	34.380	0.84	Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl) -4-hydroxy-, ethyl	$C_{19}H_{30}O_3$
7	34.466	2.63	4-(3,5-Di-tert-butyl-4-hydroxyphenyl) butyl acrylate	$C_{21}H_{32}O_3$
8	34.550	1.78	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
9	34.620	2.88	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
10	36.126	14.89	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$
11	36.195	3.13	Bicyclo [8.2.0] dodecan-11-one, 12-chloro-	$C_{12}H_{19}C_{1O}$
12	37.018	5.30	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$
13	37.198	14.00	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$
14	37.830	5.17	Methyl stearate	$C_{19}H_{38}O_2$
15	39.189	1.21	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$
16	40.881	0.78	9-Octadecenoic acid, 12-hydroxy-, methyl ester, (Z)-	$C_{19}H_{36}O_3$
17	41.975	8.65	Ricinoleic acid	$C_{18}H_{34}O_3$
18	43.764	1.38	9-Octadecenoic acid (Z)-, oxiranylmethyl ester	$C_{21}H_{38}O_3$
19	45.765	1.53	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$
20	46.445	16.48	Glycidyl (Z)-9-nonadecenoate	$C_{22}H_{40}O_3$
21	49.077	1.34	Hydroxycitronellal, trimethylsilyl ether	$C_{13}H_{28}O_2Si$
22	49.892	9.44	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$

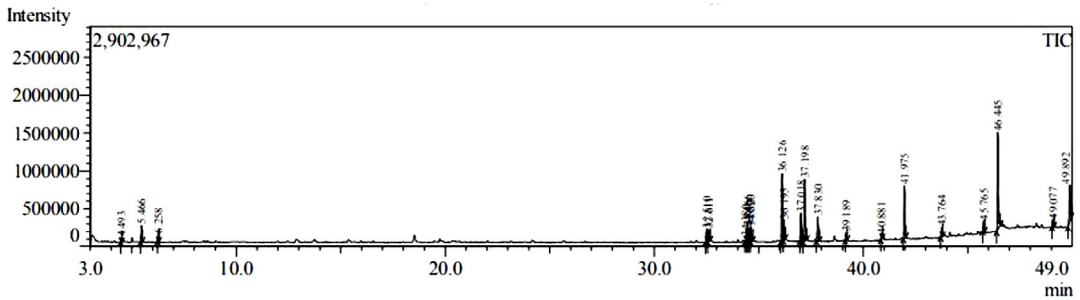

Figure 3. GC-MS chromatogram of *C. crepidioides* hot aqueous extract

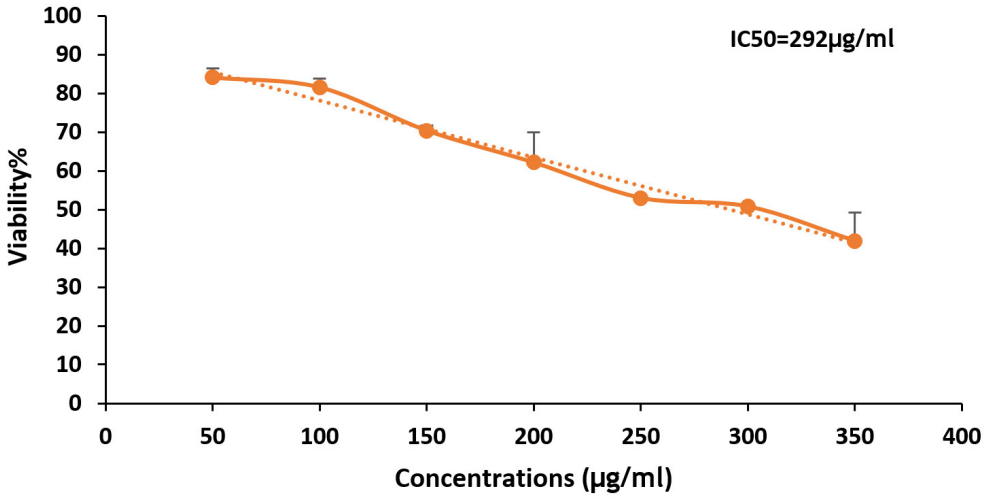

Figure 4. Cytotoxicity analysis of hot aqueous extract of *C. crepidioides* on Vero cell lines

of hot aqueous extract of *C. crepidioides* and ascorbic acid was found to be 57.9 µg/mL and 40.9 µg/mL, respectively. Antioxidant activity increases with decreasing IC_{50} value and vice versa. IC_{50} is the concentration needed in a system to scavenge 50% of DPPH radicals.[23] The current findings demonstrated that *C. crepidioides* is a potent antioxidant which may be helpful in the management of pathological harm caused by free radicals.

According to Akinpelu *et al*., hot water extract of *C. crepidioides* exhibits antioxidant activity with an IC_{50} value of 0.29 mg/mL.[23] The flavonoids and phenolics present in the extract may help reduce oxidative stress in cells and hinder the activities of α-amylase and α-glucosidase, acetylcholinesterase and butyrylcholinesterase.[40] Many phenolic compounds produced from plants such as flavonoids, anthocyanins, catechins, etc., have demonstrated strong antioxidant activity.[41]

GC-MS

The GC-MS chromatogram of hot aqueous extract of *C. crepidioides* was shown in Figure 3.

By comparing the observed spectra with the available standards, 22 compounds were found (Table 3) from the hot aqueous extract of *C. crepidioides*.

Some of the abundantly found compounds were Glycidyl (Z)-9-nonadecenoate (16.48%), 9-Octadecenoic acid, methyl ester, (E)- (14.00%), 13-Hexyloxacyclotridec-10-en-2-one (9.44%), Ricinoleic acid (8.65%), 9,12-Octadecadienoic acid (Z, Z)-, methyl ester (5.30%), Methyl stearate (5.17%), Hexadecanoic acid, ethyl ester (2.88), Hexadecanoic acid, methyl ester (2.50%), Cyclotetrasiloxane, octamethyl- (2.19%), etc. Among the identified compounds, Hexadecenoic acid, ethyl ester has antioxidant, pesticide and nematicide properties as previous literature reported.[42] 9-octadecenoic acid methyl ester has antioxidant activity,[43] antibacterial activity as previously reported.[44] Cyclotetrasiloxane, octamethyl- have been reported to possess antimicrobial property.[42] Hexadecenoic acid, methyl ester has antimicrobial, anti-inflammatory, anti-cancer, hepatoprotective, hypocholesterolemic, antihistaminic, antiarthritic properties.[45,46] Persson *et al*. reported that ricinoleic acid has antibacterial effect.[47] The majority of the obtained compounds have mainly antimicrobial, antioxidant and anti-inflammatory properties. These phytoconstituents also referred to as 'phytoprotectants' which are of ecological relevance in biomedical study.[48]

Cytotoxicity analysis

The cytotoxicity analysis was done on Vero cell lines to ensure the safe use of hot aqueous extract of *C. crepidioides*. IC_{50} value of less than 20 µg/mL was considered to be toxic as previously reported.[49,50] In the current study, IC_{50}

value of hot aqueous extract of *C. crepidioides* was 292 µg/mL, hence indicating the non-toxicity of the extract to Vero cell lines as shown in Figure 4.

Inhibitory concentration is the concentration that kills 50% of viable cells and is written as IC_{50}. Nguyen reported that *C. crepidioides* hydroethanolic leaf extract showed no toxicity to the cells at the concentrations of 125, 62.5, and 31.25 µg/mL.[25]

CONCLUSION

The results of the current study concludes that the hot aqueous extract of *C. crepidioides* leaf exhibits better antibacterial activity than the methanolic, ethanolic and cold aqueous extract. *S. aureus* was the most sensitive to hot water extract of *C. crepidioides*. The antioxidant potential of *C. crepidioides* was confirmed by DPPH assay. Phytochemicals such as tannin, saponin, flavonoids, phenol and terpenoids were found in the extract which are accountable for their antibacterial and antioxidant activities. GC-MS study gives an insight into bioactive compounds present in the extract which possess many biological properties. Hence, *C. crepidioides* leaf extract can be utilized as an alternative therapeutic agent for various biomedical applications. Further investigation on the isolation of bioactive compounds is needed.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analysed during this study are included in the manuscript.

ETHICS STATEMENT

Not Applicable.

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