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Research Article

ASSESSMENT OF *TRIANTHEMA PORTULACASTRUM* FOR ITS ANTIMICROBIAL POTENTIAL AND INVESTIGATION OF THEIR PHYTOCHEMICALS USING HPTLC, GC-MS, AND IR

KAVITHA D[a]*, PARVATHAM, R[a], AND PADMA, P. R[a]

[a]**Department of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore 641043, Tamil Nadu, India. Email: dkavithabiotech@gmail.com**

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ABSTRACT

Objective: To evaluate the leaves of *Trianthema portulacastrum* for its antibacterial activity, antifungal activity and also to identify the nature of the phytochemicals in the selected plant.

Materials and Methods: The leaves of the selected plant were extracted in varying solvents of different polarity and tested against the clinical isolates of bacteria and fungi. Qualitative tests, GC-MS, HPTLC and IR analysis were performed to identify the nature of the phytoconstituents.

Results: The methanolic extract was found to be effective against the bacterial and fungal strains than the aqueous and chloroform extracts and also the antimicrobial activity resided predominantly in flavonoid fractions. The leaves had a broad spectrum of active compounds. The spectral studies were suggestive of the presence of the flavonoids, leptorumol and C-methylflavone.

Conclusion: The leaves of *Trianthema portulacastrum* is an excellent source of various lead molecules that can be developed into a antimicrobial drug.

Keywords: Agar well diffusion, Dimethyl Sulphoxide, *Trianthema portulacastrum*, Zone of inhibition, Minimum Bactericidal Concentration(MBC), Minimum Fungicidal Concentration(MFC)

INTRODUCTION

Many microorganisms can cause several disease and inspite of tremendous advancement of medicinal science and technology, diseases are the leading health problem in the under privileged population in the remote rural areas in the developing countries. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. With the continuous use of antibiotics, microorganisms have become resistant. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectra of untreatable bacterial infections and adds urgency to search for new infection-fighting strategies [1].

Many classes of antibiotics discovered have helped tame many of the terrors of human health. The use of these 'wonder drugs' combined with improvements in sanitation, housing, nutrition and the advent of widespread immunization programs led to a dramatic drop in the death from fatal diseases [2]. Although antimicrobial drugs have saved the lives and eased the suffering of millions, we face growing resistance among gram positive and gram negative pathogens that cause infection in the hospital and in the community. The problems with drug resistance to microorganisms, side effects of modern drugs and emerging diseases where no medicines are available have stimulated renewed interest in plants as a significant source of new medicines. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on medicinal plants [3].

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Herbs are staging a comeback and herbal renaissance is happening all over the world. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity. The practices continue because of its biomedical benefits as well as place in cultural beliefs in many parts of the world and thus have made great contribution towards maintaining human health [4].

Pharmacological industries have produced a number of new antibiotics in the last three decades, in spite of that resistance to these drugs by microorganisms has also been increased. The world is now looking towards India for new drugs to manage various challenging diseases because of its rich biodiversity of medicinal plants and abundance of traditional knowledge such as siddha and ayurveda [5].

Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world's 12 biodiversity centers with the presence of over 40,000 different plant species. Of these about 15,000- 20,000 plants have good medicinal values [6]. Isolation of pure compounds from plants opened new era, for treating human ailments by using it alone or in combinations. For the last fifteen years, researchers have focused on drug development strategies to isolate and purify active principles from plant sources, since these drugs, in general, have lesser side effects in comparison with those synthesized chemically. The most significant bioactive compounds of plants include alkaloids, flavonoids, phenolics, terpenoids, tannins and saponins [7].

The classical examples of drug discovery were morphine, quinine, digoxin, etc. These natural products provide clues to synthesize new types of antimicrobial and antifungal chemicals that are relatively safe to man and it can help to meet expensive and limited supply of synthetic chemicals [8]. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. In particular the search for components with antimicrobial activity has gained increasing importance in recent times due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant microorganisms.

One such novel plant is the *Trianthema portulacastrum*. Linn. (Aizoaceae) which is a diffuse, prostrate, branched herb, upto 30-65 cm long weed. It is found mostly in tropical regions. The plant is used in the treatment of edema in the liver and spleen [9]. The plant is lithotropic for the kidney and bladder. In the Indian traditional medicine system, the plant is considered as a diuretic. Previously it was reported to have hepatoprotective activity [10]. It is attributed with analgesic, antipyretic, anti-inflammatory, CNS depressant and stomachic properties and used in asthma, bronchitis, jaundice and oedemas. The plant is reportedly used against throat troubles and as an anti-fungal agent. A decoction of the herb is used as a vermifuge and is useful in rheumatism; it is considered an antidote to alcoholic poisoning. The fleshy nature of leaves makes them suitable for use as a wound-dressing. Therefore, the need of hour is to investigate the plant and their isolated substances for new antimicrobial compounds as a source for new effective medicines. The objectives

of the present study were to evaluate the leaves of *Trianthema portulacastrum* for its antibacterial activity, antifungal activity and also to identify the nature of the phytochemicals in the selected plant.

MATERIALS AND METHODS

Collection of plant samples

Fresh, healthy and disease free leaves were collected from the areas of Coimbatore. They were identified and authenticated by a taxonomist at the Botanical Survey of India, Southern circle, Coimbatore and the voucher specimen was deposited (1629). They were washed thoroughly with running water and shade dried at room temperature for two weeks and grinded and sieved into fine powder.

Preparation of plant extracts

The plant extracts were prepared using the solvents of varying polarity namely water, methanol and chloroform. The leaf powder of 10g was taken and homogenized with 100ml of the solvents. The crude preparation was left overnight in the shaker and it was centrifuged at 4000rpm for 25 minutes. The supernatant was then transferred in a pre-weighed beaker and the extract was concentrated by evaporating the solvent. The plant residue was weighed and dissolved in known volume of Dimethyl Sulphoxide (DMSO).

Test microorganisms

The bacterial and fungal strains used in the present study were the clinical isolates obtained from P.S.G. Hospitals, Coimbatore. The bacterial strains used were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, *Proteus vulgaris* and *Klebsiella pneumoniae*. The fungal strains used were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Mucor* and *Rhizopus*.

Preparation of the test culture

Inoculums of the microorganism were prepared from overnight culture grown in nutrient broth and the suspension was adjusted with a turbidity equivalent to that of 0.5 MacFarland standard.

Antibacterial assay [11, 12]

Disc Diffusion Method

Mueller Hinton Agar plates were prepared and bacterial isolates of 6 hours incubation (0.5 MacFarland standard) were inoculated by spread plate method. Filter paper discs (6mm diameter) soaked with 20µl of the extract was kept in agar plates and incubated at 37°C for 24 hours. The diameter of zone of inhibition was measured in mm.

Agar Well Diffusion Method

Petriplates containing 20ml Mueller Hinton Agar medium were seeded, with the 100 µl of the inoculums prepared from a broth that has been incubated for 6 hours, when the growth was in logarithmic phase. Wells were cut in the agar and 25µl of the plant extracts were added in concentration of 100mg/ml. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by the diameter of zone of inhibition formed around the wells. Chloramphenicol was used as standard antibacterial agent.

Determination of Minimum Inhibitory Concentration

Minimum Inhibitory Concentration was determined by micro dilution method using serially diluted plant extracts and plant extracts were diluted in sterile nutrient broth. Microorganism in suspension was adjusted to turbidity to that of 0.5 MacFarland standards was added. These were incubated for 18 hours at 37°C. MIC of each extract was taken as the lowest concentration that did not give any visible growth [13].

Determination of Minimal Bactericidal Concentration

Minimum Bactericidal Concentration was determined for each set of wells from MIC determination. A loopful of broth was collected from those wells, which did not show any growth and inoculated on sterile Mueller Hinton agar. Plates inoculated were then incubated at 37°C for 24 hours. The concentration of the leaf extract at which no visible growth was taken as the minimum bactericidal concentration.

Antifungal assay [11,12]

Agar plug method

The fungicidal effect of the plant extracts can be assayed by the inhibition of mycelial growth of the fungus and is observed as a zone of inhibition near the disc or the wells. Petriplates with 20ml of Potato Dextrose Agar were prepared. A fungal plug was placed in the center of the plate. Sterile discs impregnated with the plant extracts were placed in the plates. Nystatin was kept as positive control. The growth was seen as a crescent shaped zone of inhibition.

Spore germination inhibition assay

All the three extracts were tested for spore germination of fungi. Aliquots of spore were prepared by mixing loopful of spore in distilled water. 25µl of spore was to added to 10µl of the extract and placed on separate glass slides. Slides were then incubated in moist chamber at 25°C ± 2°C for 24 hours. Each slide was fixed in lactophenol cotton blue stain and observed under microscope for spore germination or inhibition of spores by plant extracts.

Determination of Minimum Fungicidal Concentration

Potato Dextrose Broth having different concentrations of plant extracts were prepared. Tubes containing 10ml of above solution were inoculated with 0.1ml of different fungal spore suspensions separately and were incubated at 28°C ± 1°C for 5 - 7 days. The lowest concentration of the plant extracts that did not permit the growth of the inoculated test fungi in the broth medium was regarded as the MIC. Control was performed without the plant extract. The contents of the tubes showing no visible growth or turbidity were further sub cultured on freshly prepared potato dextrose agar plates to determine if the inhibition was reversible or permanent to assess the fungicidal efficacy. The lowest concentration of the extract that did not produce any fungal growth on the solid medium was regarded as MFC value.

Qualitative phytochemical analysis [14]

The extracts prepared were tested for the presence of alkaloids, phenolics, flavones or flavonoids, saponins, steroids and terpenoids using qualitative phytochemical analysis.

Extraction of alkaloids, phenolics and flavonoids [14]

The preliminary phytochemical analysis of the leaves indicated the presence of the secondary metabolites namely alkaloids, phenolics and flavonoids. These plant fractions were isolated and assessed for their bioactivity.

Extraction of Alkaloids

Fresh leaves of *Trianthema portulacastrum* (5g) were crushed in a mortar and pestle with 10% acetic acid in ethanol (200ml) and incubated for 4 hours in the dark. After incubation, the extract was filtered and the solution was concentrated to 1/4th volume in a boiling water bath. To the extract, 25% ammonium hydroxide or 25% ammonia was added until a precipitate was formed and then centrifuged at 2500 rpm for 5 minutes. The residue obtained was washed with 1% NH_4OH and filtered. The residue that contained alkaloids was then weighed, dissolved in ethanol and stored at 4°C.

Extraction of phenolics

Leaves (1g) were taken and crushed using a mortar and pestle. To the crushed sample, 20ml of 80% ethanol was added. The conical flask was plugged and placed in a boiling water bath for 15 minutes with occasional shaking. The content was then centrifuged and the supernatant thus collected was the phenolic extract.

Extraction of flavonoids

Approximately half the volume of the phenolic fraction was transferred to a 50ml separating funnel. The sample was then extracted with petroleum ether (40-60°C). The aqueous layer thus obtained was the flavonoid extract. These phytochemical fractions isolated were then assessed for their antimicrobial activity.

Antimicrobial activity of the isolated fractions

The isolated phytochemical fractions, namely the alkaloids, phenolics and flavonoids, were assessed for their antibacterial activity against the pathogenic bacteria namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhi* and *Proteus vulgaris.* The antifungal activity of the fractions was assayed against the fungal pathogenic strains namely *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Rhizopus oryzae* and *Mucor indicus*. All the procedures adopted for the antimicrobial assays were as described earlier. The minimum inhibitory concentration of the fractions against the test microorganisms were also determined.

HPTLC analysis

The methanolic residue (100mg) of the leaves of *Trianthema portulacastrum* was dissolved in 1ml methanol and centrifuged at 3000rpm for 5 minutes. The supernatant was collected and used as test solution for HPTLC analysis. 3µl of the test solution was loaded as a 8mm band in the 5 x 10 Silica gel 60 F254 TLC plate using a Hamilton syringe and CAMAG INOMAT 5 instrument. The leaf extract and reference loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapour) with the respective mobile phase and the plate was developed up to 90mm. The developed plate was dried in hot air to evaporate the solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and the images were captured in white light, UV 254nm and UV 366nm. After derivatization with the appropriate reagents, the plate was photo-documented at daylight for alkaloids and phenolics and at UV 366nm for flavonoids using the Photo-documentation chamber. Finally, the plate was fixed in the scanner stage and scanned at 500nm for alkaloids and phenolics and at UV 366nm for flavonoids. The peak table, peak display and peak densitogram of alkaloids, phenoilcs and flavonoids were noted.

Alkaloid profile

Nicotine was used as the reference standard for the analysis of alkaloids. The mobile phase used was ethylacetate:methanol:water (10:1.35:1). For derivatization of alkaloids, the developed plate was sprayed with Dragendroff's reagent, followed by 10% ethanolic sulfuric acid reagent and heated at 120ºC for 5 minutes in a hot air oven.

Phenolic profile

Quercetin was used as the reference standard for the analysis of phenolics. The mobile phase used was toluene:chloroform:acetone (4:2.5:3.5). For derivatization, the developed plate was sprayed with 25% aqueous Folin Ciocalteau reagent and heated at 120ºC for 5 minutes in a hot air oven.

Flavonoid profile

Rutin was used as the reference standard for flavonoid analysis. The mobile phase used for development of flavonoids was ethylacetate:butanone:formic acid:water (5:3:1:1). For derivatization, the developed plate was sprayed with 1% ethanolic aluminium chloride reagent and heated at 120ºC for 5 minutes in a hot air oven.

IR spectral analysis

The infra red spectrum of the methanolic extract of *Trianthema portulacastrum* was recorded in Shimadzu FT-IR spectrophotometer using KBr pellet method. The IR spectrum obtained was compared with the HPTLC and GC-MS spectra for interpretation.

GC-MS analysis

The powdered plant material was analysed using an Agilant-5 gas chromatography-MS spectrometer using a HP-5 column equipped with SEM detector with helium as a carrier gas at a flow rate of 1.5 psi. The compounds were identified using the database available in the light of the available literature in the journals and books.

RESULTS AND DISCUSSION

Antibacterial activity

The plant extracts were evaluated for their antibacterial activity against seven clinical bacterial isolates namely *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhi*, *Escherichia coli* and *Klebsiella pneumoniae.* Figures 1 and 2 shows the antibacterial activity of the aqueous, methanol and chloroform extracts of the leaves of *Trianthema portulacastrum*. The antibacterial activity by agar well and disc diffusion methods revealed that the zone of inhibition was found to be maximum in the methanolic extract followed by the chloroform extract. Aqueous extract showed the least antibacterial activity. The methanolic extract was found to be more effective against *Shigella flexneri* and *Escherichia coli*. It was moderatively potent against *Staphylococcus aureus*, *Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhi*. *Klebsiella pneumonia* was not susceptible to any of the plant extract. *Salmonella typhi* was equally susceptible to all the three leaf extracts.

There are several reports in the literature indicating the antibacterial and antifungal activity of the medicinal plants. The antibacterial and antifungal activities of aqueous, ethanol and ethyl acetate extract of *Torilis anthriscus* (L.) Gmel. (Apiaceae) were tested *in vitro* against ten species of bacteria and five species of fungi, wherein the ethanol extracts exerted the strong inhibition of microorganisms than the other extracts [15]. Among the n-hexane, ethyl acetate, n-butanol, methanol and water fractions of sorghum, the methanol extract elicited the maximum antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Candida albicans* and *Bacillus subtilis* [16]. An ethanol extract from the fruits of *Tribulus terrestris* L., was more active examined against both Gram-negative and Gram-positive bacteria than the leaf and root extracts, when tested on 11 species of pathogenic and non-pathogenic microorganisms [17]. The chloroform, ethyl acetate and methanolic extracts of *Leucas aspera,* which belongs to the family *Lamiaceae* has been investigated for its antibacterial activity against many phytopathogens and clinical pathogens. The methanolic extract had a stronger antibacterial activity than the other two extracts [18].

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

MIC was determined using the methanolic extract of *Trianthema portulacastrum*. Table 1 depicts the values of MIC and MBC. *Proteus vulgaris, Shigella flexneri, Escherichia coli* and *Pseudomonas aeruginosa* showed the appreciable MIC concentration of 1.25 mg/ml. MBC gives the dosage of the plant extracts on microorganisms.

Table 1: Minimum inhibitory concentration of *Trianthema portulacastrum*

Microorganisms	MIC (mg)	MBC (mg)
Staphylococcus aureus	1.25	1.25
Proteus vulgaris	2.5	2.5
Pseudomonas aeruginosa	1.25	1.25
Shigella flexneri	1.25	1.25
Salmonella typhi	2.5	2.5
Escherichia coli	1.25	1.25

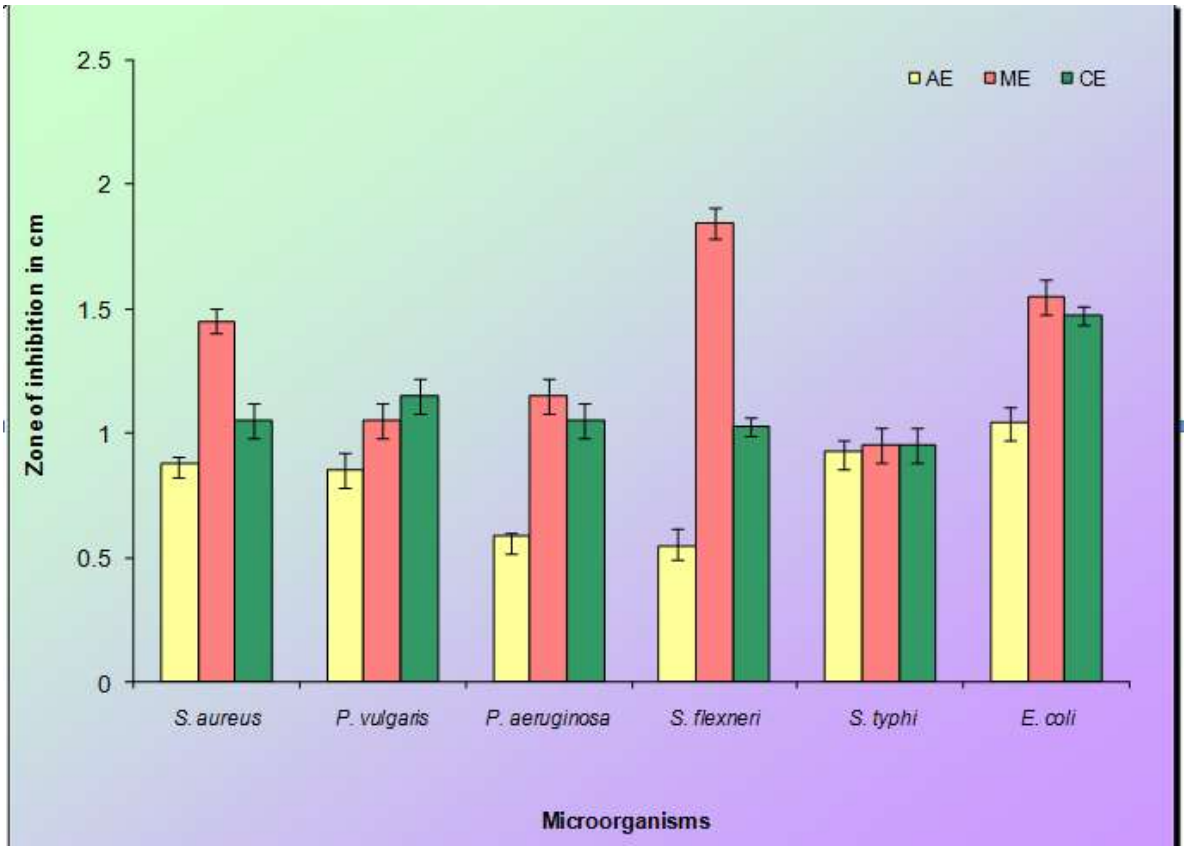
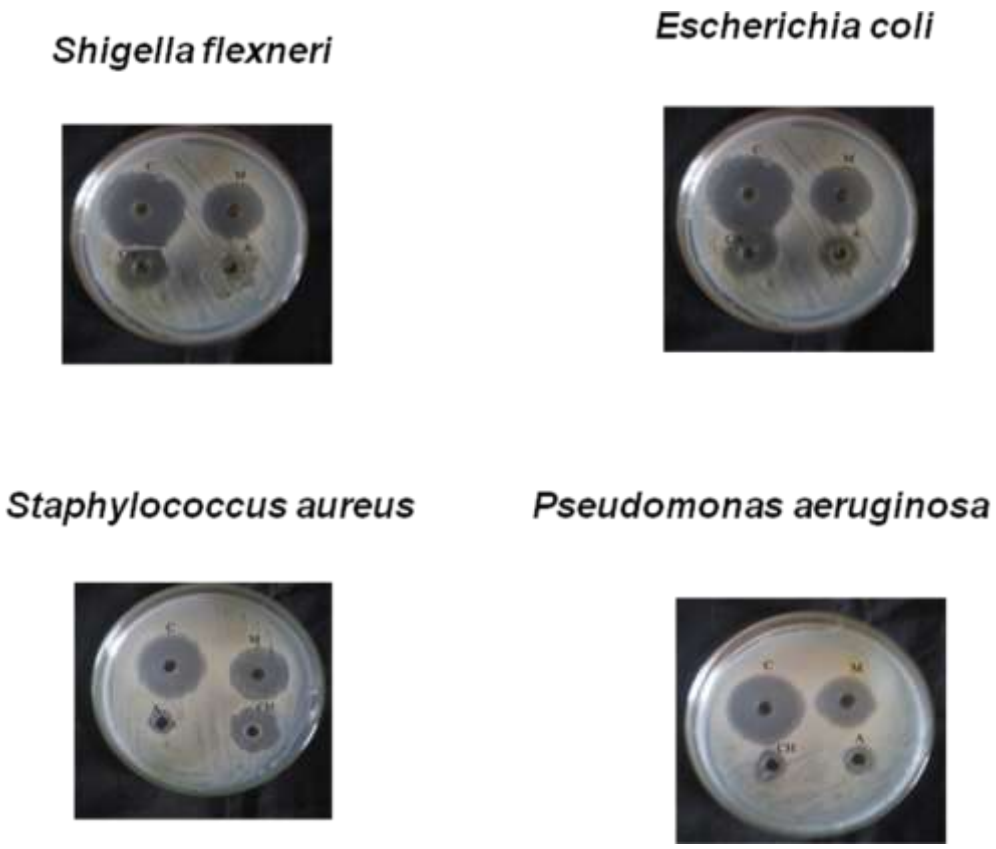


Fig. 1: Antibacterial activities of aqueous, methanolic and chloroform extracts of *Trianthema portulucastrum*



C-control, M-methanolic extract, A-aqueous extract, CH- chloroform extracts

Fig. 2: Antibacterial activity of *Trianthema portulucastrumn*

Antifungal activity of *Trianthema portulacastrum*

The antifungal activity was carried out against the clinical isolates of *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Rhizopus oryzae* and *Mucor indicus*. Table 2 and Figure 3 depicts the antifungal activity of *Trianthema portulacastrum.* The methanol and chloroform extracts showed less than 100% inhibition against *Aspergillus niger, Aspergillus fumigatus*, *Rhizopus* and *Candida albicans. Mucor* did not show any susceptibility to all the three leaf extracts. Minimum Fungicidal Concentration was determined and the values are represented in Table 3. MFC was found to be lower for *Candida albicans* and *Aspergillus niger*.

In an investigation on the antifungal activity of the leaf extracts of *Bauhinia variegate*, the methanolic extract showed the maximum activity against *A.fumigatus*, *Candida albicans* and *A.flavus* than the chloroform and ethanol extracts [19]. The antifungal activity on eight different medicinal plants (*Aloe vera, Ocimum sanctum, Cenetella asiatica, Piper betle, Calotropis gigantea, Vitex negundo, Ocimum basilicum* and *Azadirachta indica*) on aqueous, chloroform and methanol extracts was carried out where the methanol and chloroform extract of *Vitex negundo* showed the maximum activity and *Azadirachta indica* exhibited the least activity against *Colletotrichum falcatum* and *Rhizopus* spp [20]. The present study clearly proved the maximum activity was exhibited by the methanolic extract and the minimum activity was shown by the chloroform extract.

Spore germination inhibition assay

The methanolic extract was found to exhibit strong activity against the fungal species and only methanolic extract was taken for spore germination inhibition assay. The results of spore germination inhibition assay confirmed the results obtained by antifungal bioassay by agar plug method. The inhibition of spore germination are represented in Table 4. The spores of *Aspergillus niger, Aspergillus fumigatus*, *Rhizopus* and *Candida albicans* were found to be inhibited by the presence of the leaves of *Trianthema portulacastrum*.

Table 2: Antifungal assay of *Trianthema portulacastrum*

Microorganisms	Growth inhibition			
	AE	ME	CE	NYS
Aspergillus fumigatus	-	++	-	+++
Aspergillus flavus	-	-	-	+++
Aspergillus niger	++	++	++	+++
Candida albicans	-	++	++	+++
Rhizopus	-	+	-	+++
Mucor	-	-	-	+++

+ < 50% inhibition, ++ ≥ 50% inhibition, +++ 100% inhibition, – no inhibition.
AE→ Aqueous extract, CE→ Chloroform extract, ME→ Methanolic extract.
NYS – Nystatin

Table 3: Minimum fungicidal concentration of *Trianthema portulacastrum*

Microorganisms	MFC(mg)
Aspergillus fumigatus	5.0
Aspergillus flavus	5.0
Aspergillus niger	0.25
Candida albicans	0.125

Table 4: Spore germination inhibition assay of *Trianthema portulucastrum*

Microorganisms	Leaf extract	Control (Nystatin)
Aspergillus fumigatus	-	+
Aspergillus flavus	-	+
Aspergillus niger	+	+
Candida albicans	+	+

+no inhibition of spore germination
- No Inhibition of spore germination

Phytochemical analysis of the leaves of *Trianthema portulacastrum*

Phytochemical analysis was done in the leaves of *Trianthema portulacastrum* extract using standard procedures to determine the presence of natural bioactive compounds such as alkaloids, flavonoids, saponins, steroids, terpenoids and phenolic compounds. The phytochemical analysis of the leaves of *Trianthema portulacastrum* revealed the presence of alkaloids, flavonoids, phenols, steroids and terpenoids. Majority of the phytochemical compounds were separated in the methanolic extract of the leaves. Among the three extracts, the methanolic extract was to found to have the maximum number of phytochemical components, extracted from the leaves of *Trianthema portulacastrum.* The literature is rich with the reports indicating the presence of phytochemical constituents of the medicinal plants.

A study reported that the phytochemical screening of methanolic, N-hexane and aqueous extract of the *Senna italica* root and leaf extracts revealed the presence of alkaloids, steroids and flavonoids while saponins, glycosides and tannins were not detected. These compounds have been known to possess medicinal activities particularly antibacterial activity [21]. The preliminary phytochemical screening of the methanolic leaf extract of *Securinega virosa* revealed the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycol-sides, cyanogenic glycosides, resins, steroids or terpenoids and carbohydrates [22].

Antimicrobial activity of isolated phytochemical fractions

The antimicrobial activity of the isolated phytochemical fractions namely alkaloids, flavonoids and phenols have been validated for their antibacterial and antifungal activity. The antibacterial activity of the isolated phytochemical fractions are shown in Figure 4 and 5. The antibacterial activity by agar well and disc diffusion methods revealed that the zone of inhibition was found to be maximum in the flavonoid fractions followed by the alkaloid and phenolic fractions. *Shigella flexneri, Staphylococcus aureus* and *Escherichia coli* were found to be more susceptible to the flavonoid fraction, followed by

the phenolic and alkaloid fractions. The isolated phytochemical fractions were also found to be moderately effective against *Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhi.* MIC and MBC were found to be minimum for flavonoid fraction in *Shigella flexneri, Staphylococcus aureus* and *Salmonella typhi,* showing its maximum antibacterial activity.

C-control, ME-methanolic extract, AE-aqueous extract, CE- chloroform extract

Fig. 3: Antifungal activity of *Trianthema portulucastrum*

Table 5 represents the antifungal activity of *Trianthema portulacastrum*. Among the phytochemical fractions, flavonoid fractions showed maximum inhibition against *Aspergillus niger, Aspergillus fumigatus* and *Candida albicans,* while phenolic fraction showed a slight inhibition and alkaloid fraction did not show any antifungal activity. It was observed that the MFC of the flavonoid fraction was appreciable against *Aspergillus niger* and *Candida albicans*. The terpenoid fraction was ineffective against the bacterial and fungal organisms tested, while the alkaloid fraction showed antibacterial activity but not antifungal activity.

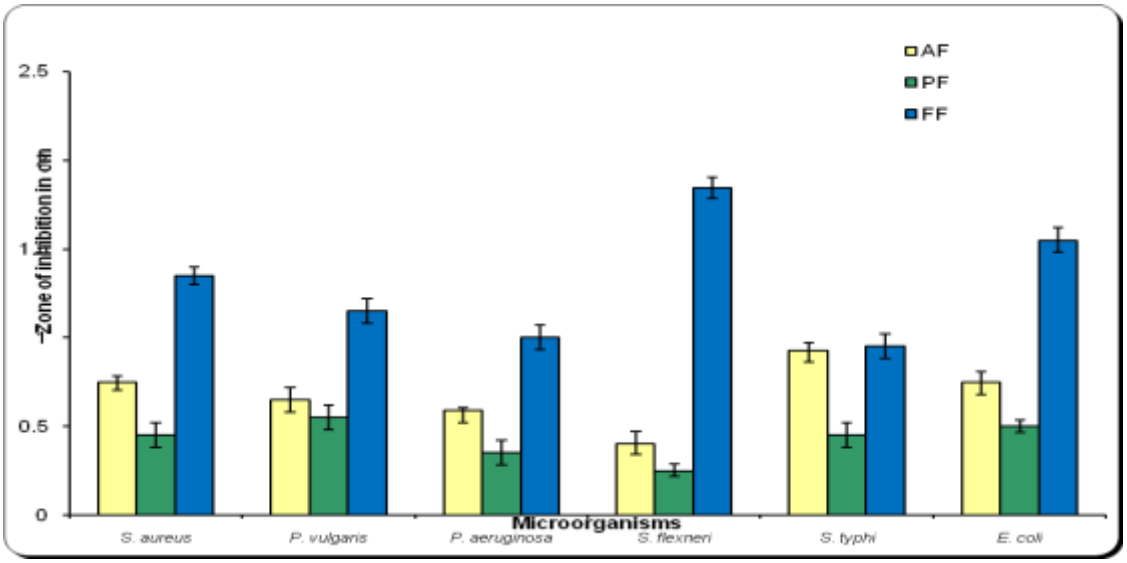
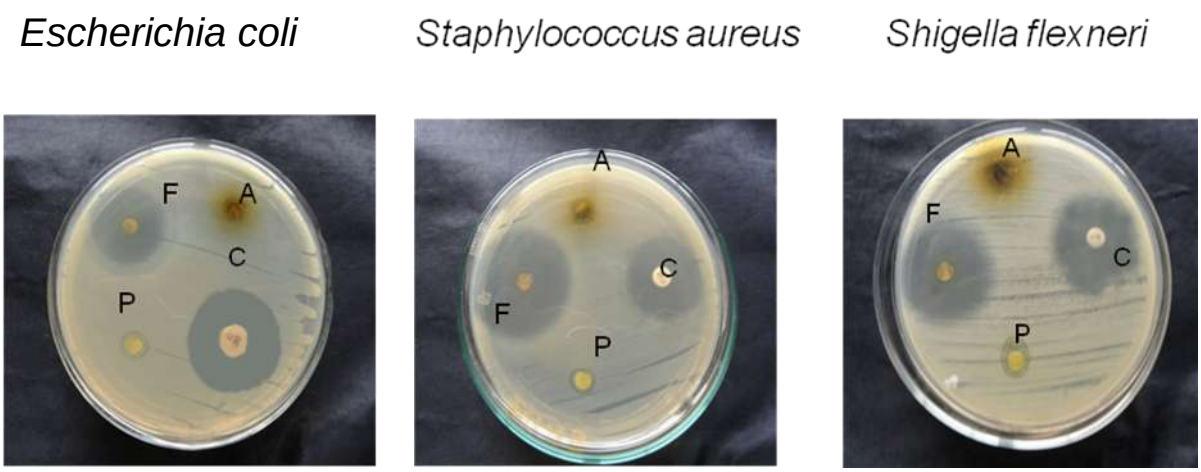


Fig. 4: Antibacterial activities of alkaloid fraction, phenolic fraction and flavonoid fractions of *Trianthema portulacastrum*

Table 5: Antifungal assay of alkaloid fraction, phenolic fraction and flavonoid fractions of *Trianthema portulacastrum*

Microorganisms	Growth inhibition			
	AF	FF	PF	NYS
Aspergillus fumigatus	-	+	-	+++
Aspergillus flavus	-	++	-	+++
Aspergillus niger	+	++	+	+++
Candida albicans	-	++	+	+++
Rhizopus oryzae	-	+	-	+++
Mucor indicus	-	-	-	+++

+ < 50% inhibition, ++ ≥ 50% inhibition, +++ 100% inhibition, – no inhibition.
AE→ Aqueous extract, CE→ Chloroform extract, ME→ Methanolic extract.
NYS – Nystatin



C- control, A- alkaloid fraction, P- Phenolic fraction, F- Flavonoid fraction

Fig. 5: Antibacterial assay of alkaloid fraction, phenolic fraction and flavonoid fractions of *Trianthema portulacastrum*

HPTLC

The leaves of *Trianthema portulacastrum* were subjected to HPTLC analysis for the presence of alkaloids, phenolics and flavonoids. The HPTLC fingerprint of alkaloids and its Peak densitogram are given in Figure 6. The alkaloid profile of *Trianthema portulacastrum* was done with the reference standard colchicine and the developed plate was sprayed with Dragendroff spray reagent (alkaloid-specific) and dried at 120°C. The plate was photodocumented at daylight using Photo-documentation chamber. Bright orange coloured zone in the Daylight mode was observed from the chromatogram, which confirmed the presence of alkaloid in the leaves of *Trianthema portulacastrum*. Finally, the plate was scanned at 500nm.

The HPTLC fingerprint of flavonoids and its Peak densitogram are given in Figure 7. The flavonoid profile of *Trianthema portulacastrum* was analysed using rutin as reference standard and 1% ethanolic aluminium chloride reagent as spray reagent. The plate was photo-documented at UV 366nm using Photo-documentation chamber. Yellow and yellow green fluorescence zone at UV 366nm was observed from the chromatogram, which confirmed the presence of flavonoids in the flowers of leaves of *Trianthema portulacastrum*.

The HPTLC fingerprint of flavonoids and its Peak densitogram are given in Figure 8. The phenolics present in the leaves of *Trianthema portulacastrum* were analysed using quercetin as reference standard and 25% aqueous Folin Ciocalteau reagent as spray reagent. The plate was kept in the Photo-documentation chamber and the images were captured at white light, UV 254nm and UV 366nm. Blue or blue-grey coloured zones were observed from the chromatogram in the daylight mode, which confirmed the presence of phenolic compounds in the sample.

GC-MS analysis

The GC-MS spectrum of *Trianthema portulacastrum* was obtained from Jeol GC mate, Mass spectrometer using an electron ionization (EI) source. Seven peaks at 21.21, 23.25, 25.23, 26.01, 26.68, 29.01 and 30.28 were observed in the GC and are shown in Figures 9, 10, and 11. In the MS spectrum of GC peak at 21.21, M+ ion was observed at m/z 215, and base peak was observed at m/z 59.1. This spectrum also shows two (m-28) peaks at m/z 131 and at m/z 116.1. This indicated the presence of 'CO' groups. In the MS spectrum of GC peak at 2325, M+ ion was observed at m/z 278, and base peak was observed at m/z 68.1. This spectrum also shows two (m-28) peaks at m/z 180 and at m/z 68. This indicated the presence of 'CO' groups. Also the presence of (m-14) peaks at m/z 123 and 95 indicated the presence of methyl group in the compound. The mass spectrum of the GC peak at 25.23 showed (m-28) peak indicating the 'CO' group. (M-CO2) peak at m/z147 in the mass spectrum of fraction at 29.01, indicated the presence of COOH group. (MCO2) (i.e.) (M-44) peaks were also observed at m/z 223 in the mass spectrum of fraction at 30.28 which are characteristic of the –COOH group. Also M-28 peak was observed in the spectrum at m/z 67 indicating the 'CO' group.

Infrared analysis

The IR spectrum of the methanol extract of *Trianthema portulacastrum* was recorded in a Shimadzu FT-IR spectrophotometer using KBr pellet method. The IR spectrum is shown in Figure 12. The spectrum showed strong bands at 1643cm^{-1}, 1383cm^{-1}, 1091cm^{-1} and 1016cm^{-1}. The strong intense band at 1643cm^{-1}is indicative of the presence of 'CO' group. The strong intense band at 1643cm^{-1}is indicative of the presence of 'CO'group. The peak at 1625 showed the presence of–C=O (carbonyl) group. The peak at 1091 due to the presence of -C-O linkage in –C-OH confirmed the presence of hydroxyl groups. The presence of above

functional groups indicated the presence of polyphenolics (-OH) and presence of flavonoid type compounds in the methanolic extract.

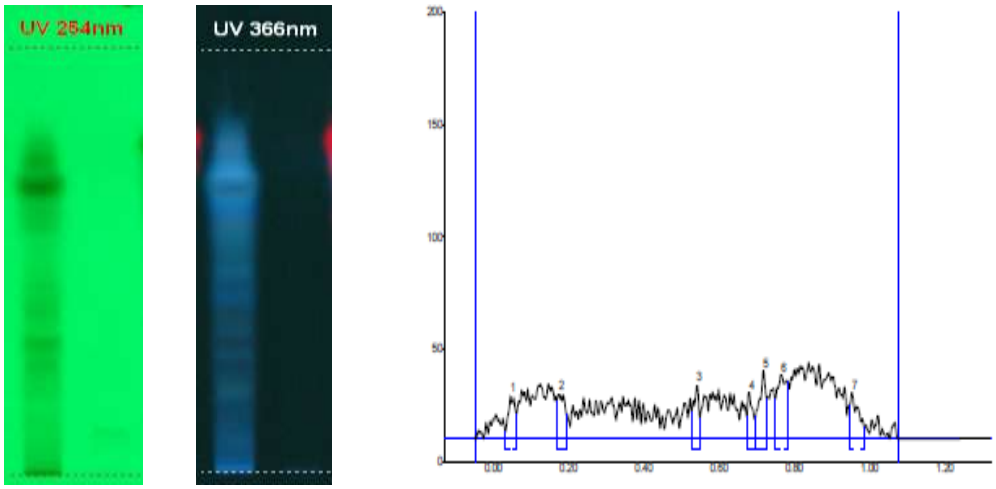


Fig. 6: HPTLC fingerprint of alkaloids of *Trianthema portulacastrum*

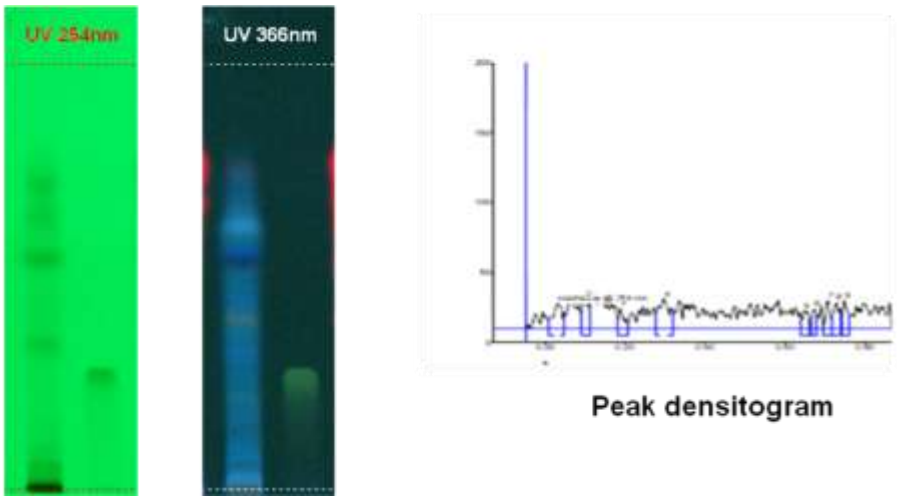


Fig. 7: HPTLC fingerprint of phenolics of *Trianthema portulacastrum*

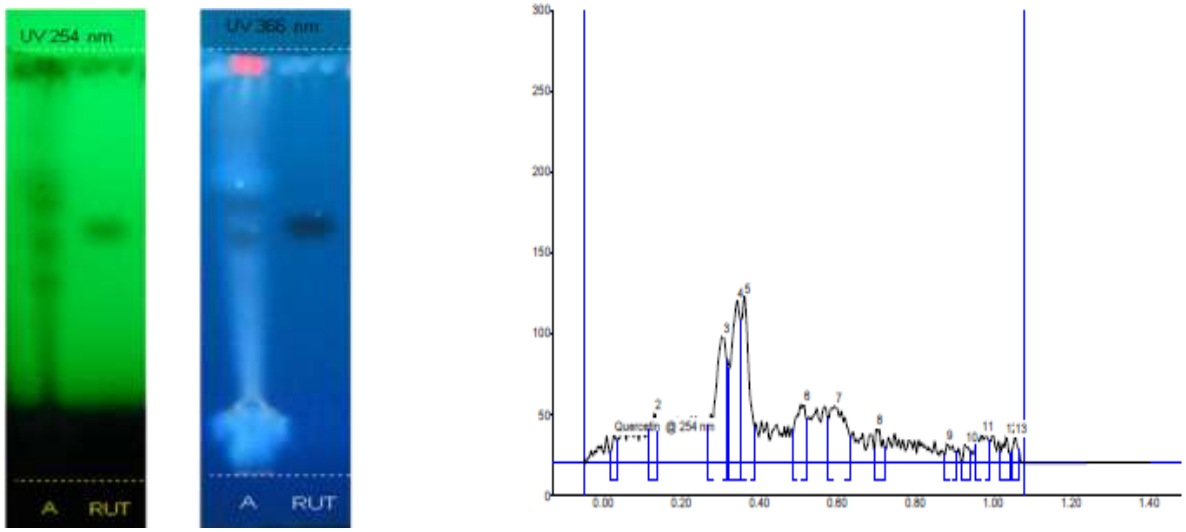


Fig. 8: HPTLC fingerprint of flavonoids of *Trianthema portulacastrum*

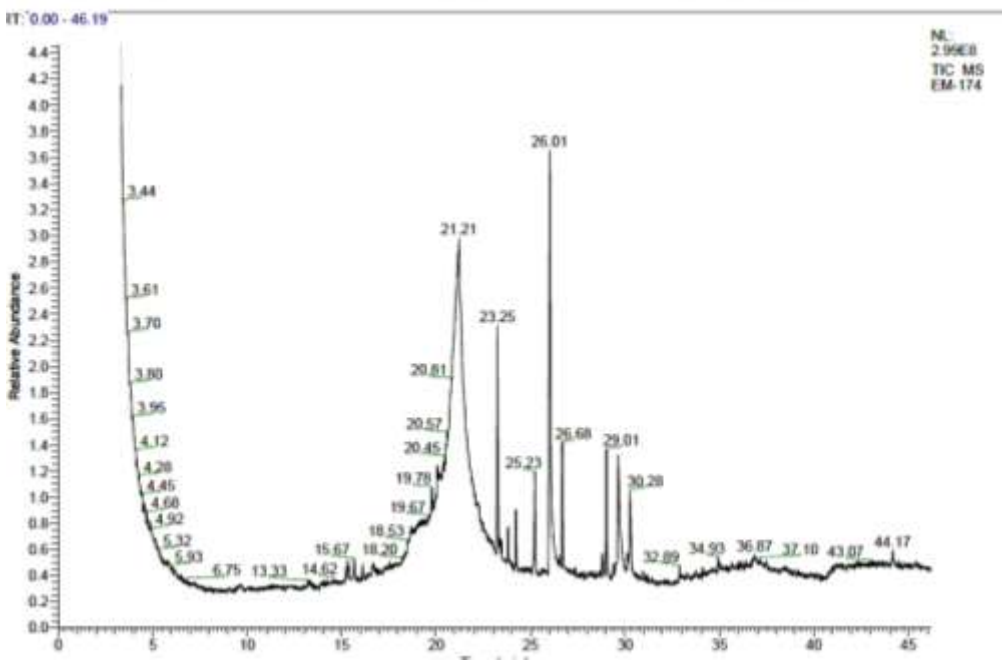
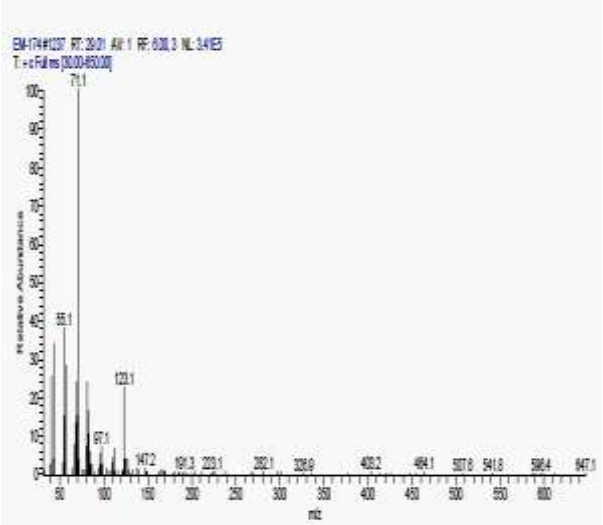
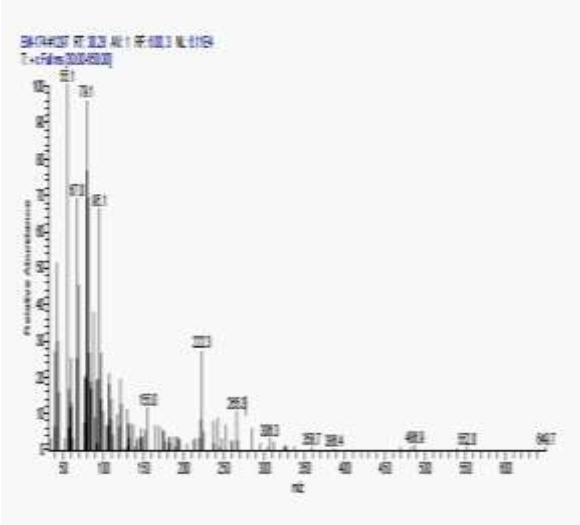
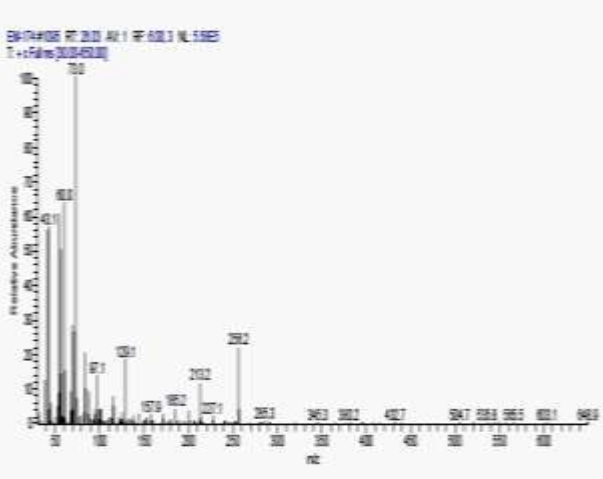
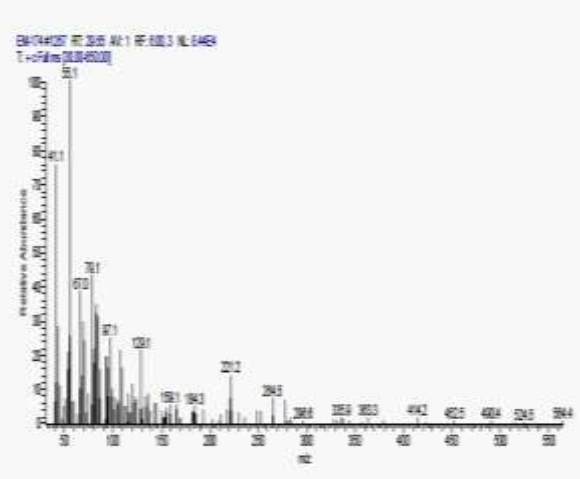


Fig. 9: GC-MS spectra of *Trianthema portulacastrum*









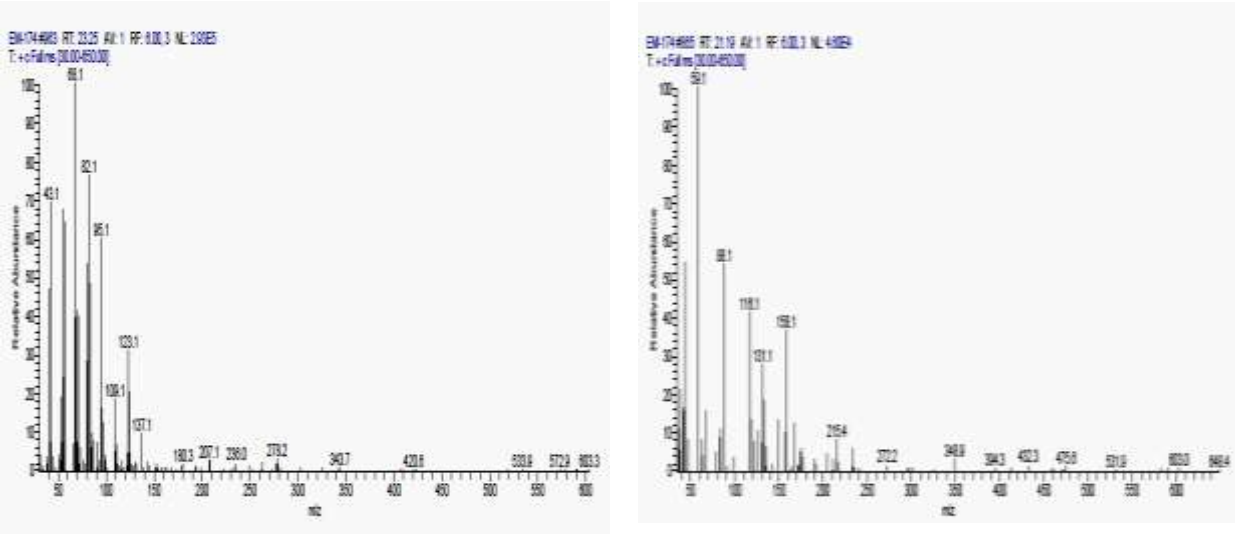
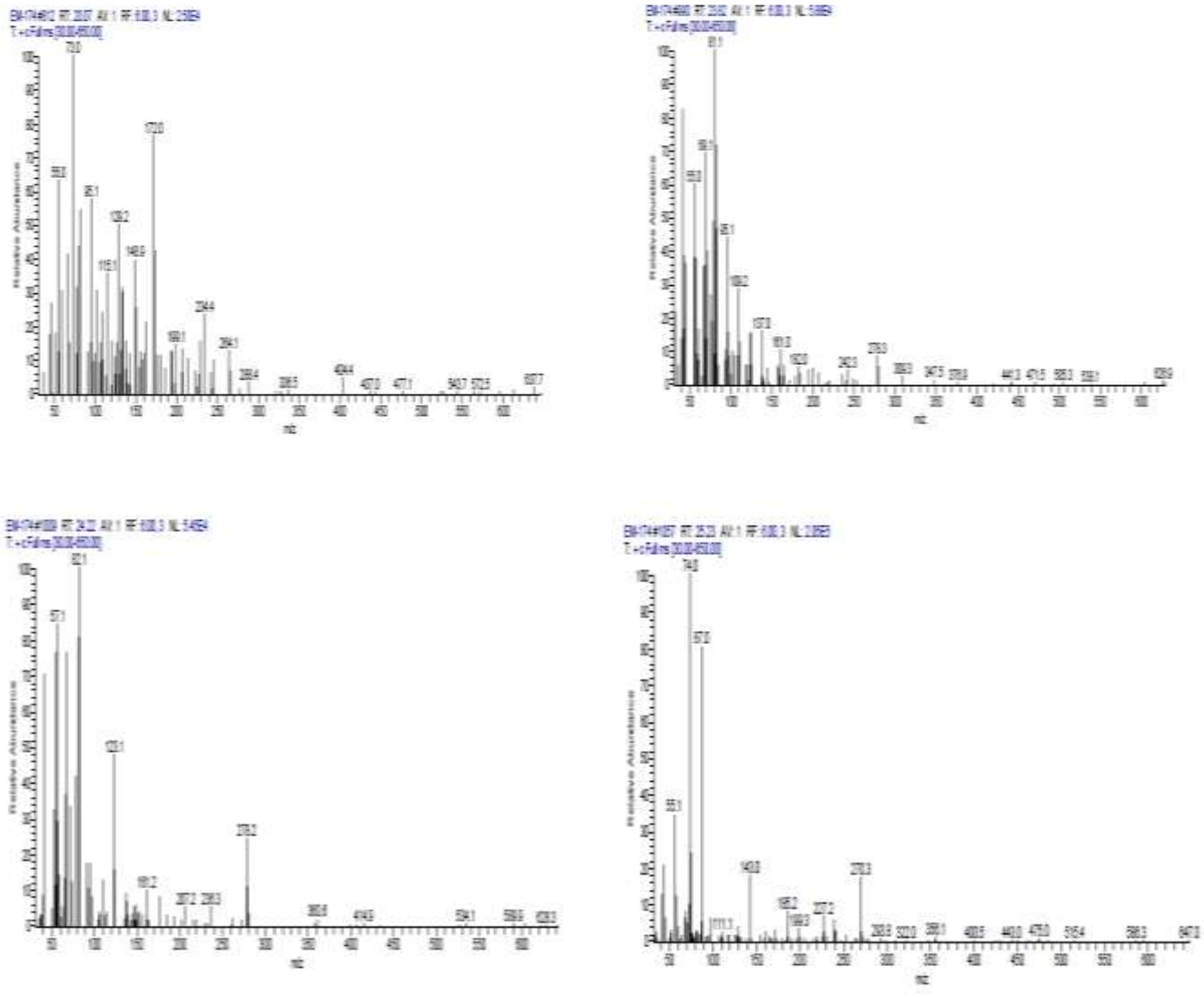


Fig. 10: GC-MS peak fragmentation of *Trianthema portulacastrum* at 30.28, 29.65, 29.01, 26.03, 23.25 and 21.19



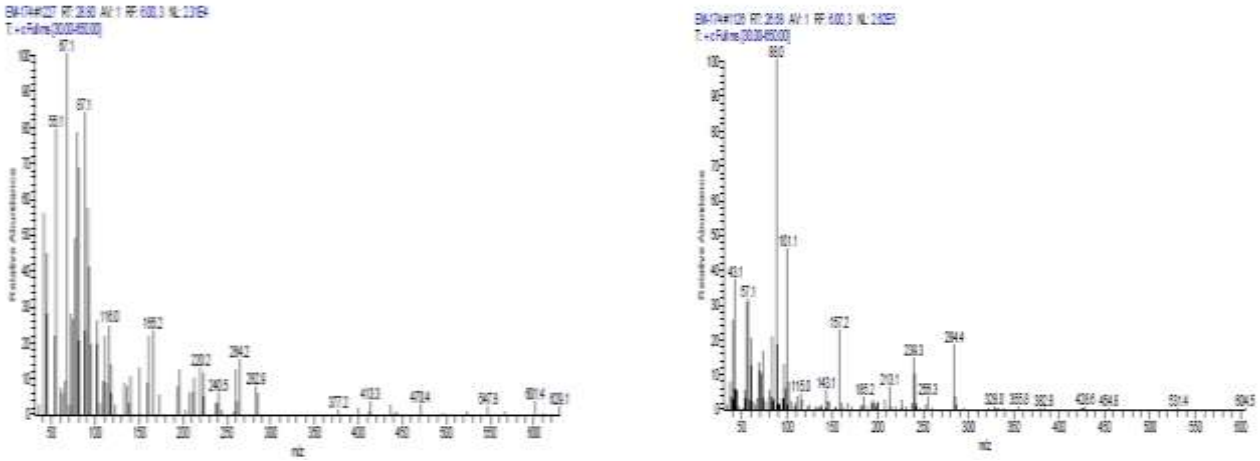


Fig. 11: GC-MS peak fragmentation of *Trianthema portulacastrum* at 28.80, 26.68, 25.23, 24.22, 23.82 and 20.07

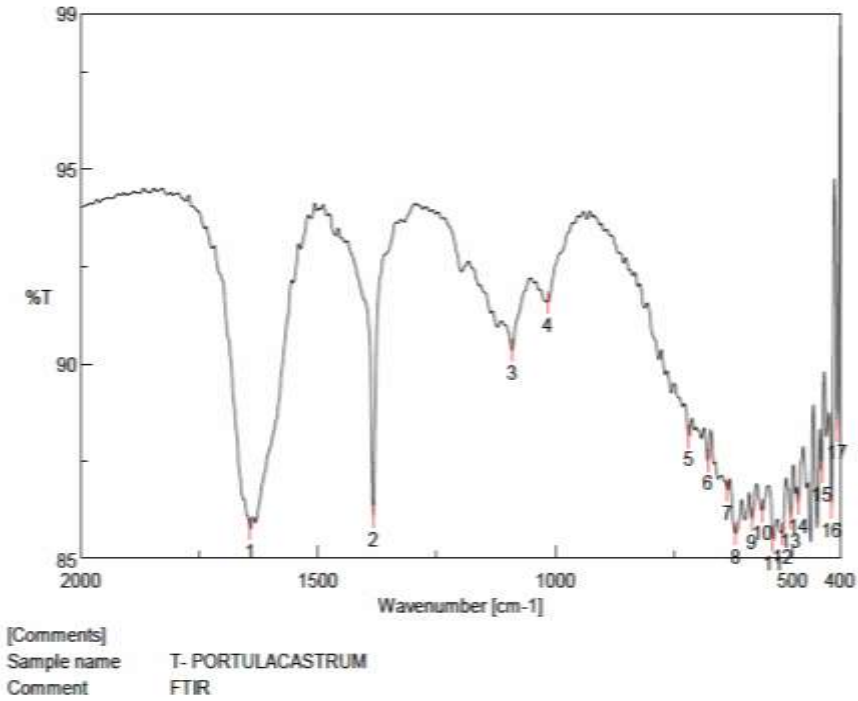


Fig. 12: Infra red spectra of *Trianthema portulacastrum*

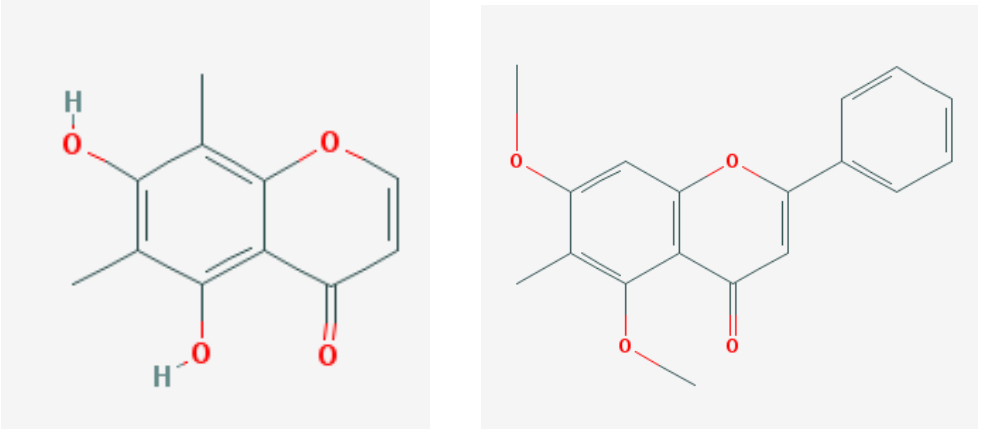


Fig. 13: Leptorumol (5,7- dihydroxy-6,8-dimethylchromone) and C-Methylflavone (5,7-Dimethoxy-6-C-methylflavone)

From the literature available on *Trianthema portulacastrum* and from our results of the combined analysis of spectra, there are many active compounds present in the leaves of *Trianthema portulacastrum*. They include trianthenol, C-methylflavone, leptorumol, trianthemine etc. [23]. Our results had proved that the antimicrobial potential resides in the flavonoid fraction. Among these active compounds, the spectra strongly indicated the structures of the active principle to match those of leptorumol (5,7-dihydroxy-6,8-dimethylchromone) and C-methylflavone (5,7-dimethoxy-6-C-methylflavone) (Figure 13) which are flavonoids now proved to be a good antimicrobial compounds.

With the support of these reports, our results clearly proved that the methanolic extract of the plant showed the presence of most of the phytochemical compounds. The phytochemical investigation of *Trianthema portulacastrum* confirmed the presence of wide range of active components, mainly the flavonoids to be the antimicrobial compounds.

CONCLUSION

The candidate plant of the present study, *Trianthema portulacastrum,* a novel weed plant, has been scientifically validated as an excellent source of antimicrobials. The leaves of *Trianthema portulacastrum* have been proven to contain the maximum phytochemical constituents and a source of lead molecule for the drug discovery that can be used for treating infectious diseases.

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