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ANTIMICROBIAL POTENTIALITY OF HERBAL NANOPARTICLES SYNTHESIZED FROM TRUE MANGROVE *AEGICERAS CORNICULATUM* AND MANGROVE ASSOCIATE *DERRIS TRIFOLIATA*

V.S.N. Lakshmi Yemineni and K. Babu and G. Rosaiah*

Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjunanagar-522 510, Guntur, Andhra Pradesh, India.

***Corresponding Author: G. Rosaiah**
Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjunanagar-522 510, Guntur, Andhra Pradesh, India.

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ABSTRACT
Mangrove and mangrove associate plants are important sources of potential bio active elements. Herbal nanopowders have an importance in newly emerging biomedical applications with less side effects. Herbal nanopowders are prepared from shade dried selected root, stem, leaves of true mangrove *Aegiceras corniculatum* and mangrove associate *Derris trifoliata* by ball milling technique. The XRD, SEM, UV-Vis analysis confirmed the size of herbal nanoparticles. FTIR analysis confirmed the presence of various functional groups. Nanoparticles synthesized from *A. corniculatum* root showed maximum zone of inhibition 29 mm against *S. aureus.* In case of *D. trifoliata* the nanoparticles synthesized from leaves reported highest zone of inhibition of 24 mm against *S. aureus.*

KEYWORDS: Mangrove Associates, Herbal nanoparticles, Phytochemicals, Methanol.

INTRODUCTION

Food and health are essential components of human existence. Through the implementation of various agricultural techniques, people are able to increase the amount of food they produce. However, the quality of health care needs to be enhanced in order to address the persistent health problems.

The role of plant based therapeutics in treating various ailments is inevitable (Padamaja *et al.*, 2022). Approximately three quarter of the global population relies on conventional medicines for their health care and 80% of the drugs used today are of plant origin (WHO, 2021). About 3.5 lakh vascular plants species are now used in drug manufacturing industry (Salmeron-Manzano *et al*., 2020). Phytochemicals synthesized by the plant body as a part of defence mechanism take part a critical role in plant-based drugs (Mahmood *et al.*, 2015; Changade *et al*., 2022). Plants hold a large number biologically potential phyto-constituents and it is quite difficult to use them without proper knowledge. By evaluating the potential substances we can synthesis new drugs. Various solvents such as chloroform, ethyl acetate, methanol, and n-hexane are commonly used for the extraction of bio-active compounds (Vijay kumar *et al*., 2019; Tirupathi swamy *et al*., 2019).

Role of silver nanoparticles in human health system is found to be significant and their accumulation in body may lead to major damage to the internal organs (Viajy Kumar *et al*., 2020; Padmaja *et al*., 2021). At present, trials have been raised in synthesizing human-friendly nanoparticles. Uses of herbal drugs proved to have no side effects and are with trust worthy therapeutic activities. The study of morphology and characterization of herbal nano particles is prime need to assess the formation of herbal nano particles and to determine their therapeutic potentiality (Zhang *et al*., 2016; Ranjitha *et al*., 2018).

Mangrove plants and their associates are specialized plant species confined to edges of sea and land, coastal regions of tropical and sub tropics of the world. Due to typical natural habitats mangroves and their associates are tend to produce secondary metabolites for their survival (Zaman *et al*., 2020; Mohammed*et al*., 2022). These natural products further used in synthesis of plant based medicines. The use of mangroves and their associates in traditional medicine is documented earlier for the treatment of numerous diseases (Sunila Rani, 2020).

True mangrove *Aegiceras corniculatum* and mangrove associate *Derris trifoliata* is growing commonly in Nizampatnam mangrove regions of Krishna Estuary. Green synthesis methods has been largely applied to different mangrove plants for production of nanoparticles (Vijay Kumar *et al*., 2018, 2019; Sunila Rani *et al*., 2019; Vijay Kumar, 2020) and very little is known about production of herbal nanoparticles from mangroves. Therefore the focus of the present study is to synthesize herbal plant powders at nano scale through ball milling and ascertain the influence of particle size on antimicrobial activity.

MATERIALS AND METHODS

Study area

The whole plant parts of true mangrove *Aegiceras corniculatum* and mangrove associate *Derris trifoliata* were collected from Nizampatnam (15° 53' 7" N latitudes and from 80° 38' 28" E longitudes) located on the south-east coast of Andhra Pradesh, India.

Collection of plant material

Healthy and fresh leaves, stems, and roots were collected from *A. corniculatum* (L.) and *Derris trifoliata* from the Nizampatnam mangrove regions of the Krishna Estuary. The collected plant samples were washed thoroughly under running tap water and subjected to shade drying with tap water and double-distilled water until dust was removed from the surface of the leaves. The leaves are shade-dried at room temperature. Dried leaf material was powdered with the help of a food processor and sieved.

Synthesis of herbal nanoparticles

The shade-dried plant parts were initially ground individually into a coarse powder using a food processor. The coarse powder is then further ground in a planetary ball mill (Fritzsch Pulverisette P6) using a steel vial with a capacity of 250 ml. In the planetary ball mill, particles were pulverized for roughly 10 hours at 300 rpm using zirconium balls of 20 mm. The obtained leaf powders were extracted with hexane, ethyl acetate, methanol, and water for 12 to 18 hours in a soxhlet extractor. The resultant crude extracts were concentrated using a vacuum rotary evaporator (Buchi Labortech Ag, model IR-215) at reduced pressure. The dried extracts were preserved at 4 °C until further use.

Characterization of herbal nanoparticles

Ultraviolet – Visible spectroscopy (UV-VIS)

The herbal nanopowder samples were dissolved in methanol and put in a quartz cuvette (1 cm^2) at room temperature for optical analysis. A UV-Vis spectrophotometer (LM-44; Perkin Elmer, Germany) operating from the UV to NIR (200–900 nm) spectral regions at a step size of 5A was used to evaluate the optical characteristics of the dispersed herbal nanoparticles.

X-Ray diffraction studies

The produced nanoparticles were characterized thorough X-ray diffraction (XRD). The leaf powder was deposited onto an XRD grid and subjected to a nanoparticle analysis (Schimadzu – 6100) at a voltage of 40 kV and a current of 30 mA with cu kal radiation. An array of 20 angles between 10° and 60° was used to measure the diffracted intensity. Nanoparticle sizes were determined for all of the studied plant powders using the Debye-Scherrer formula (Instrumental broadening) (Joerger*et al*., 2000).

$$D = 0.94 \lambda / \beta \cos \theta$$

Where D is the average crystallite domain size, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle.

Scanning electron microscopy (SEM)

The size and shape of herbal nanopowder were determined using scanning electron microscopy (SEM) (Carl Zeiss Japan), which was utilized to assess the grain size and surface morphology of nanopowders. A thin film of nanoparticle powder was produced by applying a small portion of the dried sample on the grid. Under a mercury lamp, the film covering the SEM grid was dried for five minutes. The AMT Camera System was employed at a magnification of 10,000X and an accelerating voltage of 100kV.

Fourier Transform Infrared Spectroscopic Analysis (FTIR)

The FTIR study was conducted with the use of the schimatzuIR affinity - 1s spectrophotometer. Approximately 500 mg of potassium bromide (SD Fine, IR Grade) was ground into fine powder with the aid of a mortar and pestle. The finely powdered potassium bromide was then heated to 400 °C. A sample of 10 mg plant powder was incorporated into the mortar and ground into fine powder. The sample was weighed and transferred to the KBr assembly, where the bottom and top components of the KBr were joined together. The sample was then compressed in the press assembly for approximately 60 seconds at an approximate pressure of 2000 kilograms per cubic millimeter (kg/cm2). The resulting disc was scanned at a resolution of 500 – 4000^{-1} cm.

Preliminary phytochemical screening

The existence of various phytochemicals such as phenolic compounds, coumarins, glycosides, saponins, tannins, flavonoids, terpenoids, and alkaloids was measured by using the methanolic extraction of shade-dried plant material following standard methods of Harborne (1973) and Gibbs (1974).

Antibacterial activity

The test microorganisms were grown on nutritional agar (NA) medium. A 100-ml container of nutritional agar medium was sterilized at 15 lbs of pressure for 15 minutes at 121 °C, refrigerated, and then inoculated with 0.1 ml of the test microbiological solution. The antibacterial activity of mangrove mangrove companions was tested against *Staphylococcus aureus* (MTCC 741), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 439), and *Candida albicans* (MTCC 3018). The antibacterial activity was tested using the agar-well diffusion method with three different concentrations of herbal nanopowders (50 g, 100 g, and 150 g). The antibiotic chloramphenicol (30 g/ml) was used as a positive control, while DMSO (30 l/ml) was utilized as a negative control. Bacterial cultures grown overnight at 37 °C were dispersed across the surface of agar plates. Nystatin (50 g/ml) was utilized as a positive control, and DMSO (30 l/ml) was employed as a negative control for antifungal activity. The antifungal activity was tested using three different concentrations of herbal nanopowders (50 g, 100 g, and 150 g).

RESULTS

XRD

X-ray diffraction studies were studied and tabulated in Table 1 and Figure 1. X-ray diffraction studies indicate that characteristic peaks of 2ϴ range from 10° to 60° were observed at 31.4117°, 31.4375° and 31.4243°in leaf, stem and root powders of *A. corniculatum*. In case of *D. trifoliata* significant peaks were examined at 31.5838°, 31.4108° and 31.5840°. The size of the nanoparticles was calculated by Debye-Scherer formula and the size was calculated as 59.04 nm, 65.44 nm and 48.61 nm in leaf, stem and root respectively in *A. corniculatum*. In case of *D. trifoliata* the particle size was recorded as 61.22 nm, 71.68 nm and 81.16 nm in leaf, stem and root samples. Among the two plants studied *A. corniculatum* root samples and in mangrove associate *D. trifoliata* particle size recorded as 61.22 nm in leaf samples.

Table 1: XRD analysis of *A. corniculatum* and *D. trifoliata*.

Plant part	2ϴ (degree)	d spacing (10^{-10} m)	FWHM (ß) (radians)	Particle size (D) nm
A. corniculatum				
Leaf	31.4117	2.84795	0.2362	59.04
Stem	31.4375	2.84567	0.3149	65.44
Root	31.4243	2.84684	0.2362	48.61
D. trifoliata				
Leaf	31.5838	2.83823	0.2362	61.22
Stem	31.4108	2.84567	0.3149	71.68
Root	31.5840	2.83281	0.2362	81.16

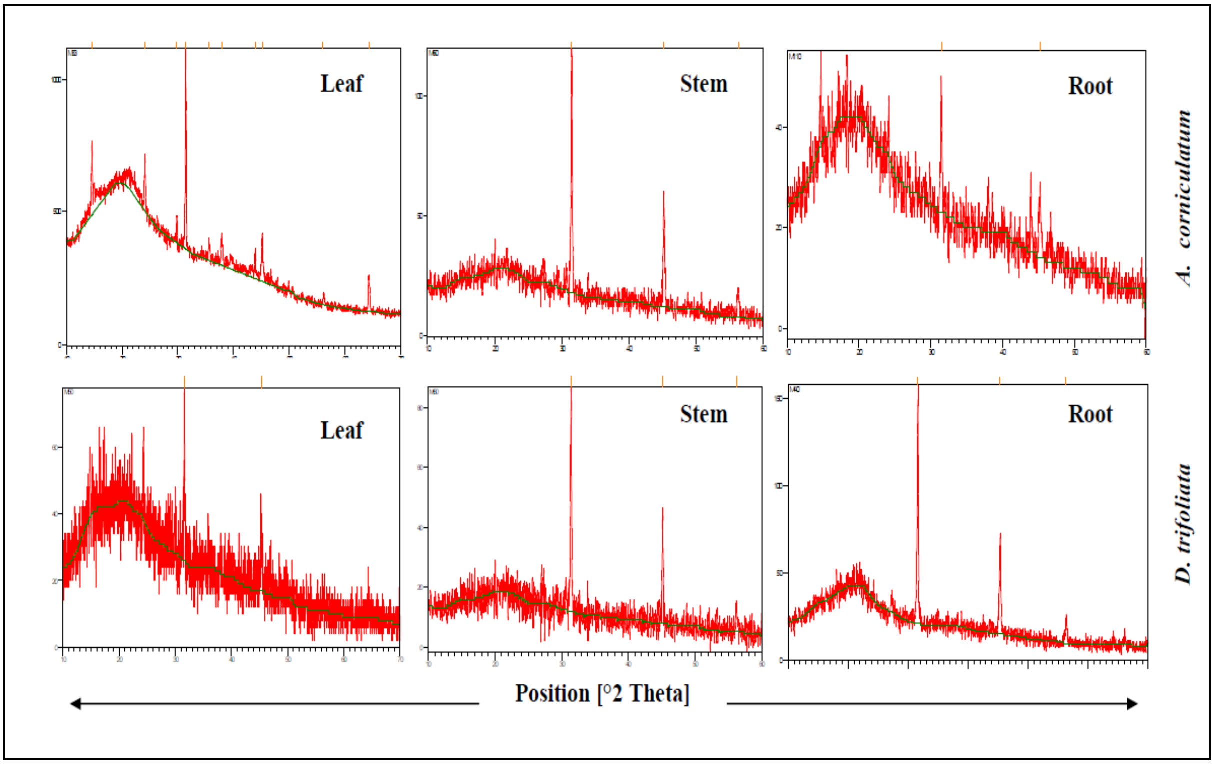

Figure 1: XRD spectrum of *A. corniculatum* and *D. trifoliata*.

SEM

The scanning electron microscopy analysis further confirms the morphology and the size of the synthesized herbal nanoparticles (Table 2 and Plate 1). Among the two plants studied powders of *A. corniculatum* showed less particle size (Leaf: 75.86 nm; Stem: 82.32 nm; Root:

90.34 nm) whereas in *D. trifoliata* the particle sizes recorded as 82.92 nm, 91.56 nm, 95.43 in leaf, stem and root respectively.

Table 2: SEM analysis of *A. corniculatum* and *D. trifoliata*.

Plant Name	Plant part	Particle size (nm)
Aegicerascorniculatum	Leaf	75.86
	Stem	82.32
	Root	90.34
Derris trifoliata	Leaf	82.92
	Stem	91.56
	Root	95.43

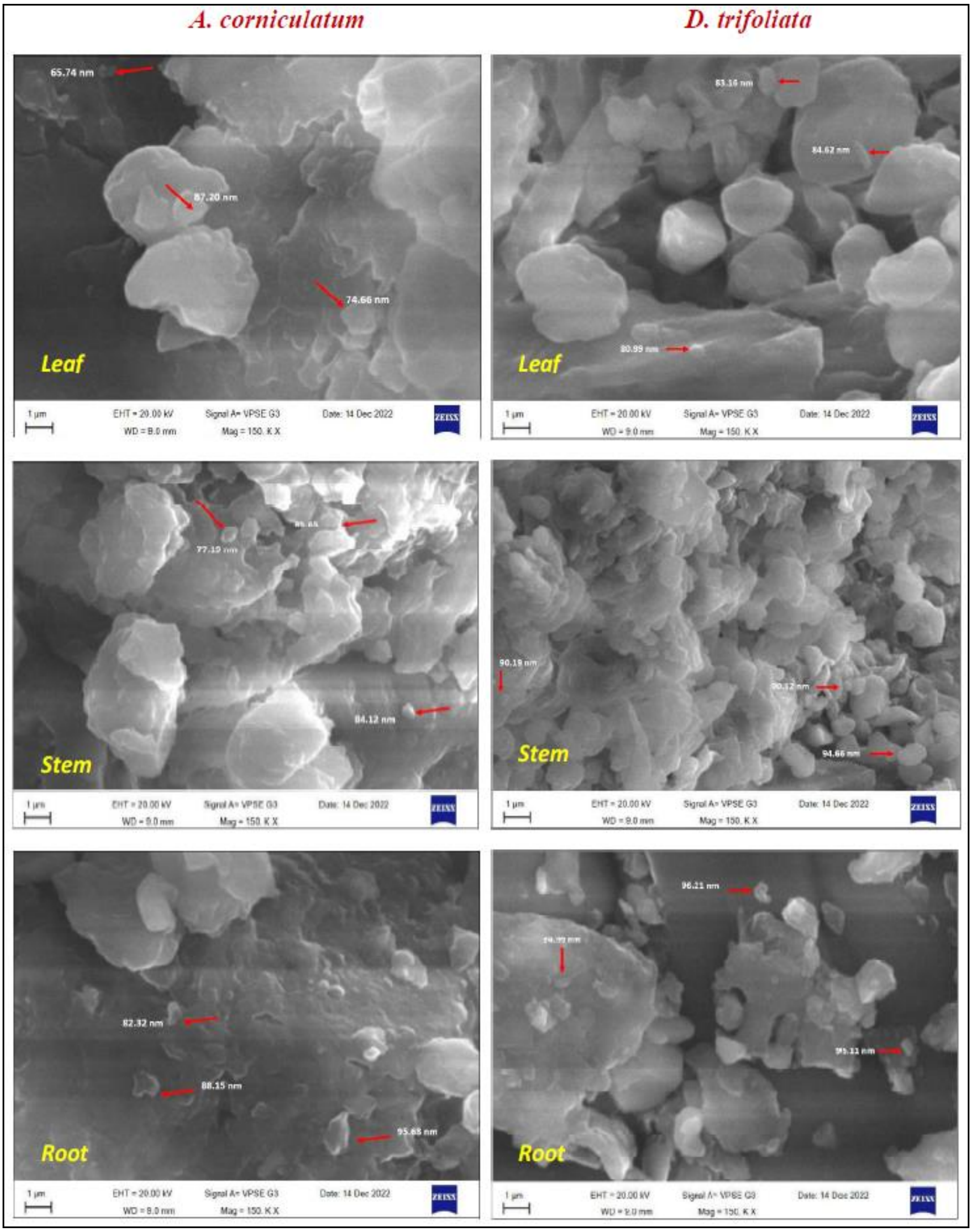

Plate 1: SEM analysis of *A. corniculatum* and *D. trifoliata*.

UV-Vis

The UV-Vis of methanolic extract was recorded in the present study to confirm the formation of herbal nanoparticles. The qualitative UV-Vis spectrum profile of all the plants was recorded between 200-900 nm. The leaf powder profile of *A. corniculatum* showed the peaks at 213.00 nm, 253.35 nm. The stem powder of *A. corniculatum* recorded the absorption peaks at 228.90 nm and 235.60 whereas in case of *A. corniculatum* root powder the absorption peaks recorded at 219.00 nm, 254.50 nm and 288.00 nm (Table 3 and Figure 2). The absorption peaks of *D. trifoliata* leaf powder recorded at 210.00 nm, 251.50 nm and 276.00 nm. The stem powders of *D. trifoliata* observed at 208.50 nm and 254.00 nm. In case of *D. trifoliata* root powder sample the absorption peaks are reported at 255.0 nm and 295.50 nm (Table 3 and Figure 2).

Table 3: UV-Vis studies of *A. corniculatum* and *D. trifoliata.*

Plant part	*A. corniculatum*		*D. trifoliata*	
	Wavelength (nm)	Absorption (OD)	Wavelength (nm)	Absorption (OD)
Leaf	213.00	3.10	210.00	2.51
	253.35	0.78	251.50	0.79
	-	-	276.00	0.84
Stem	228.90	3.89	208.50	1.92
	235.60	0.99	254.00	0.39
Root	219.00	3.57	255.50	0.52
	254.50	0.59	295.50	0.75
	288.00	0.61	-	-

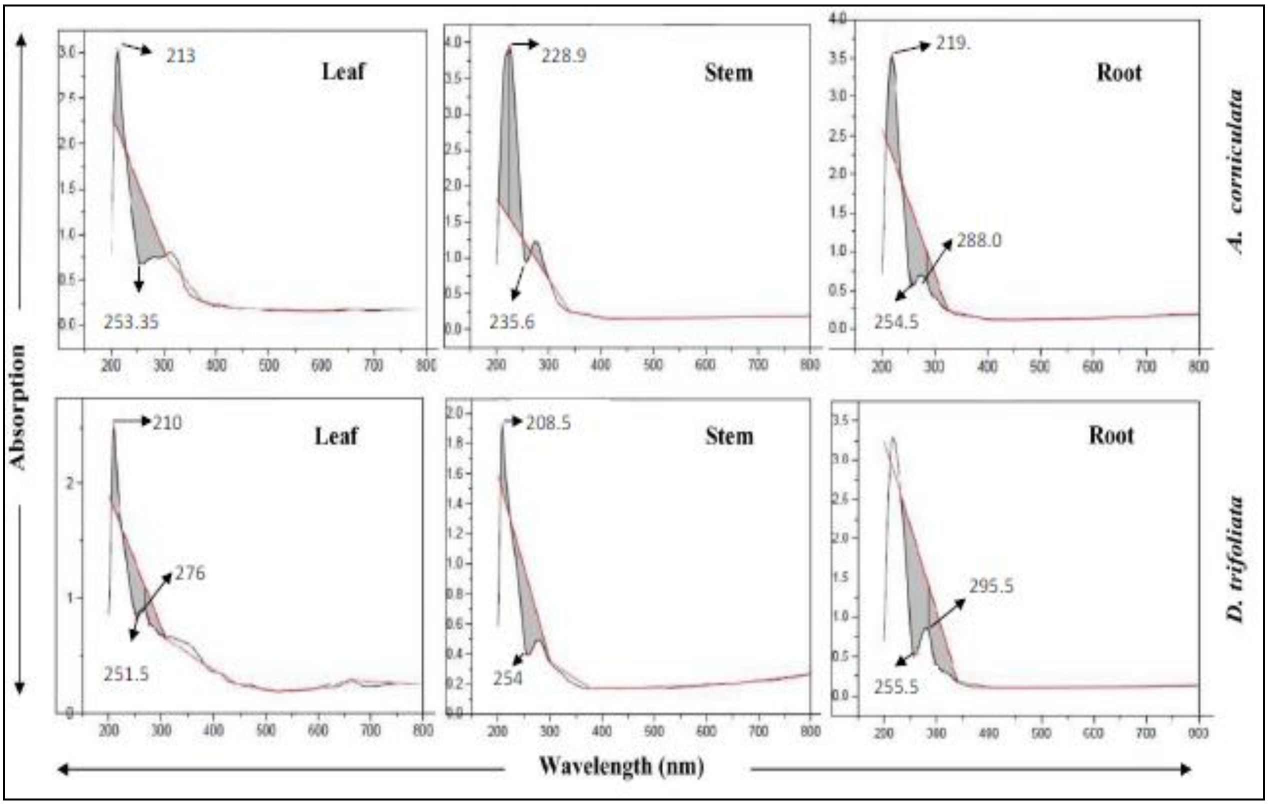

Figure 2: UV-Vis spectra of *A. corniculatum* and *D. trifoliate.*

FTIR

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR peak values and functional groups of *A. corniculatum* was illustrated in the Figure 3. The FTIR spectrum peaks of *A. corniculatum* leaf extract indentified between 1001 cm^{-1} - 3782 cm^{-1} confirmed the presence of various functional groups such as alcohol, aldehydes, aromatic and carboxylic compounds. The FTIR spectrum peaks of *A. corniculatum* stem extract indentified between 1007 cm^{-1} - 3777 cm^{-1} confirmed the presence of various functional groups such as alcohol, alkynes, aldehydes, carboxylic compounds, carbon dioxide, cyclic alkenes, alkens. The FTIR spectrum peaks of *A. corniculatum* root extract indentified between 1595 cm^{-1} - 3782 cm^{-1} confirmed the presence of various functional groups such as alcohol, alkynes, aldehydes, carboxylic compounds, carbon dioxide and cyclic alkenes. The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR peak values and functional groups of *D. trifoliata* were represented in Table 7 and the FTIR spectrum profile was illustrated in the Figure 4. The FTIR spectrum peaks of D. *trifoliata* leaf extract indentified between 1028 cm^{-1} - 3775 cm^{-1} confirmed the presence of various functional groups such as alcohol, carbondioxide, conjugated alkenes, vinyl ether and secondary alcohol. The FTIR spectrum peaks of *D. trifoliata* stem extract indentified between 1022 cm^{-1} - 3301 cm^{-1} confirmed the presence of various functional groups such as secondary amine, carboxylic compounds, conjugated acid and primary alcohol. The FTIR spectrum peaks of *D. trifoliata* root extract indentified between 1035 cm^{-1} - 3782 cm^{-1} confirmed the presence of various functional groups such as alcohol, carbon dioxide, conjugated alkenes and secondary alcohols.

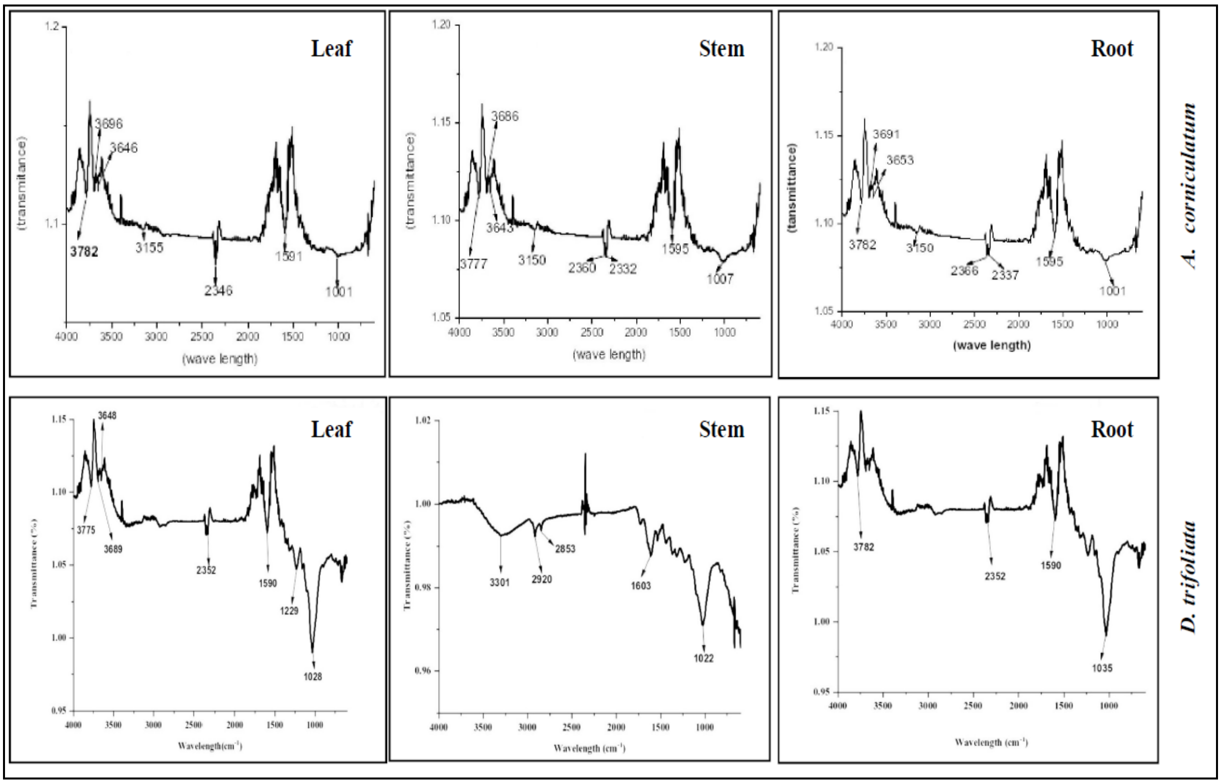

Figure 4: FTIR spectrum of *A. corniculatum* and *D. trifoliata.*

Microbial activity

Antimicrobial activity of leaf extracts of mangrove *A. corniculatum* and mangrove associate *D. trifoliata* was evaluated against selected bacteria and fungi at 50 µl, 100 µl and 150 µl (Table 4). And all the tested organisms found to be sensitive with increased concentration of leaf extract. A maximum of zone of inhibition was recorded at 150 µl of leaf extract. The antimicrobial activity of leaf extracts of *A. corniculatum* showed significant inhibitory activity against different bacteria and fungi was represented in Plate 2. The root methanol extracts (150 µl) of *A. corniculatum* showed maximum antimicrobial activity of 29 mm against *S. aureus*. In *D. trifoliata* highest antimicrobial activity of 24 mm was recorded against *S. aureus* on treatment with leaf hexane and methanolic extracts (150 µl) (Plate 3). After *S. aureus* the maximum antimicrobial recorded with root methanol extracts (150 µl) of *A. corniculatum* on *B. subtilis* (25 mm). The mangrove associate *D. trifoliata* hexane leaf extract (150 µl) reported significant antimicrobial activity of 20 mm against *B. subtilis*.

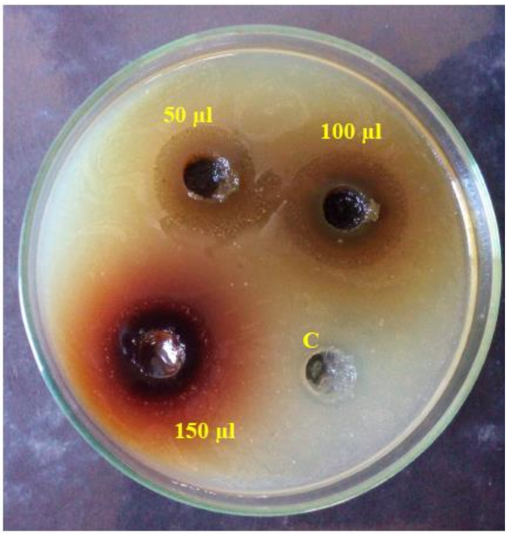

Plate 2: Antimicrobial activity of *A. corniculatum* root methanolic extract on *S. aureus.*

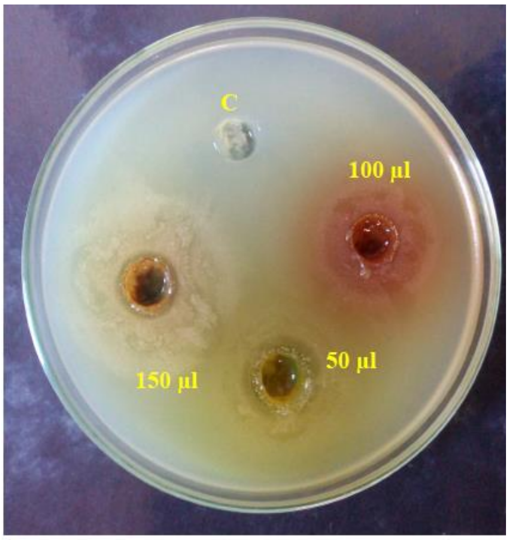

Plate 3: Antimicrobial activity of *D. trifoliata* leaf hexane extracts on *S. aureus.*

Table 4: Antimicrobial activity of true mangrove *A. corniculatum* and mangrove associate *D. trifoliata.*

Solvent ↓	Plant Name	Plant part	Zone of inhibition (mm/µL)											
			S. aureus			*B. subtilis*			*E. coli*			*C. albicans*		
			50 µl	100 µl	150 µl	50 µl	100 µl	150 µl	50 µl	100 µl	150 µl	50 µl	100 µl	150 µl
Hexane	*Aegiceras corniculatum*	Leaf	14	18	19	11	17	17	6	12	16	8	14	16
		Stem	13	16	16	12	14	15	7	10	14	7	12	14
		Root	16	22	20	15	20	21	7	15	22	15	19	23
	Derris trifoliata	Leaf	14	16	24	14	14	20	7	13	15	8	13	14
		Stem	12	13	19	7	10	12	7	8	10	6	9	11
		Root	14	16	20	12	14	19	8	10	12	13	14	21
Ethyl acetate	*Aegiceras corniculatum*	Leaf	10	14	21	8	11	12	3	7	10	4	8	10
		Stem	8	11	17	7	8	9	3	6	8	3	7	9
		Root	12	16	22	10	16	18	5	9	16	9	12	18
	Derris trifoliata	Leaf	9	11	19	8	9	14	4	7	11	4	8	9
		Stem	8	8	12	4	6	8	3	5	6	3	6	8
		Root	11	12	14	8	12	14	4	6	8	8	10	17
Methanol	*Aegiceras corniculatum*	Leaf	12	17	23	10	14	19	5	10	13	7	12	18
		Stem	11	15	20	10	12	16	5	9	12	6	10	12
		Root	15	20	29	14	18	25	6	13	19	13	16	24
	Derris trifoliata	Leaf	14	17	24	10	12	16	5	10	14	7	11	12
		Stem	10	11	17	6	8	11	4	6	8	5	7	9
		Root	13	14	18	10	17	18	5	8	10	11	13	19
Water	*Aegiceras corniculatum*	Leaf	10	12	14	7	11	13	3	8	10	4	10	12
		Stem	11	12	14	8	9	11	4	7	10	5	9	11
		Root	13	18	23	11	17	20	4	11	19	10	13	15
	Derris trifoliata	Leaf	8	11	16	12	10	12	4	9	11	5	9	11
		Stem	9	10	12	5	7	9	4	6	8	3	4	8
		Root	10	11	16	8	13	16	3	8	8	10	12	12

DISCUSSION

Mangrove plants are very important source of potentially bioactive constituents for the development of new chemotherapeutic agents. Mangroves and associated plants provide a wide domain for therapeutic application in recent years, most yet to be explored. Secondary metabolites of mangrove plants like flavonoids, tannins, polyphenols, etc. protect them from harsh environmental conditions like; low oxygen, high salinity, water logging, high wind and temperature, etc. (Banarjee *et al*., 2008; Okolie *et al*., 2014). These compounds also reduce the risk of developing chronic diseases like cancer, diabetes, cardiovascular diseases, etc. (Vijay Kumar *et al*., 2019; Tirupatiswamy, 2020; Sunila Rani, 2021). The XRD patterns in the present study confirm the crystalline nature of the nanoparticles prepared through physical method of ball milling (Vijay Kumar *et al.*, 2019; Padmaja, 2021). In present study the XRD spectra results of obtained herbal nanoparticle size is ranged between 48.61-65.44 nm in *A. corniculatum* and from 61.22 – 81.16 nm in *D. trifoliata* are tally with the results of different mangrove plant derived metallic nanoparticles synthesized through bioreduction (Ahmed *et al*., 2016; Yasir*et al*., 2018; Rautel *et al*., 2019; Kakakhel *et al*., 2021). On the other hand the obtained herbal nanoparticles size is lower than the silver nanoparticles produced through green synthesis from mangrove plants (Sunila Rani, 2020). Scanning Electron Microscopy (SEM) images of the herbal nanoparticles exhibit topographical nature of the obtained nanoparticles in studied mangrove plants (Viajy Kumar, 2020). The SEM images of the present study illustrate the discrete particles with flake like spherical structures more prominent in particles with small size. The shape and size of the nanoparticles are in accordance with the results of other studies in *Tridax procumbens* (Karthi *et al*., 2016), in *R. apiculata* (Vijay, 2020), in *A. scholaris* (Tiruapthiswamy, 2020). The UV absorption spectra data of all the herbal nanoparticles from selected mangrove plant *A. corniculatum* and mangrove associate *D. trifoliata* showed U.V absorption at 213.00, 253.35, 228.90, 235.60, 219.00, 254.50, 288.00 nm and at 210.00, 251.50, 276.00, 208.50, 254.00, 255.50, 295.50 which confirms the existence of nanoparticles (Karthi *et al*., 2016; Balakrishnan *et al*., 2016; Tirupathiswamy*et al*., 2019; Vijay Kumar *et al*., 2019). The possible phytoconstituents of nanoparticles is revealed by the FTIR studies, which can help in further functionalization with various molecules for various applications (Asmathunisha *et al*., 2010). The FTIR spectrum peaks of *A. corniculatum* leaf extract indentified between 1001 cm^{-1} - 3782 cm^{-1} confirmed the presence of various functional groups such as alcohol, aldehydes, aromatic, alkynes, cyclic alkenes, alkens and carboxylic compounds. The FTIR spectrum peaks of *D. trifoliata* leaf extract indentified between 1028 cm^{-1} - 3782 cm^{-1} confirmed the presence of various functional groups such as alcohol, carbon dioxide, conjugated alkenes, vinyl ether and secondary alcohol. The observed peaks are related to major functional groups in different chemical classes such as flavonoids, triterpenoids and polyphenols (Heneczkowski *et al*., 2001; Raghupathi *et al*., 2011). The results of the present study also confirm that the smaller particle size showed greater antimicrobial activity. Smaller size of the nanoparticles restricts the DNA replication easily when compared to the large sized nanoparticles as evident in other studies. Higher zone of inhibition was observed in *A. corniculatum* methanolic root extracts and *D. trifoliata* leaf hexane and methanol extracts relative to their smaller nano particle sizes in present study. Further smaller nanoparticles with large surface area facilitate easy penetration and thus denaturation of bacterial cell wall (Vijay Kumar *et al*., 2019; Tirupathiswamy *et al*., 2019; Padmaja *et al*., 2020).

CONCLUSION

In present study it was observed that among all the solvents methanol extracts found to be significant in extracting various bio active compounds. Root methanol extracts (150 µl) of A. *corniculatum* showed 29 mm zone of inhibition against *S. aureus.* In *D. trifoliata* (150 µl) highest antimicrobial activity of 24 mm zone of inhibition was recorded against *S. aureus* with leaf hexane and methanolic extracts. It concludes that both *A. corniculatum* and *D. trifoliata* showed maximum antimicrobial activity against *S. aureus*.

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