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Spectroscopic analysis, Microscopic imaging and Antibacterial activities of *Syzygium cumini*, *Andrographis paniculata*, *Gymnema sylvestre* and *Mimosa pudica*

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The aim of this study is to evaluate the bioactivity of powdered medicinal plants. A collection of medicinal plant powders *Syzygium cumini* (*S. cumini*), *Andrographis paniculata* (*A. paniculata*), *Gymnema sylvestre* (*G. sylvestre*) and *Mimosa pudica* (*M. pudica*) was procured from Tamil Nadu, Virudhunagar Sarvodaya Sangam, India. Functional groups including hydroxyl, amines, ketone, and aldehydeof aromatic compounds have been characterized by Fourier transform infrared spectroscopy. The small energy gaps of compoundshave been found as 5.0866 eV, 5.1746 eV, 4.8831 eV and 5.1568 eV in *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica*, respectively, facilitated by UV-Visible spectroscopy helps to identify the chemical reactivity. SEM with EDX has been utilized to assess the size, shape and elemental compositions of the plant powders. SEM imaging has been used to identify the micrometre-range size of each plant powder. *Escherichia coli* and *Staphylococcus aureus* has been tested for antibacterial activity of plant powders. We identified that *S. cumini* (zone of inhibition of 27 mM) and *M. pudica* (zone of inhibition of 26 mM) showed the highest antibacterial activity against *Staphylococcus aureus*. Consequently, medicinal plant powders can be used as antibacterial medicines for the treatment of diseases caused by gram-positive and gram-negative bacteria.

Keywords: Antibacterial, FT-IR, Medicinal plant powders, SEM with EDX, UV-Vis

Research on medicinal plant powders goes well beyond just finding novel medications. Natural materials provide countless possibilities for the new medication, whether in the form of pure chemicals or standardized plant powders[1]. The area of medicinal plant knowledge has been growing and including a wide range of topics, such as power negotiation[2]. A variety of pharmacological actions, such as anti-tumorigenic, anti-apoptotic, anti-inflammatory, anti-hyperglycemic, anti-bacterial, anti-microbial and anti-emetic effects, have been demonstrated by medicinal powders[3]. As a result, the examination of these components might be useful in identifying the different biological activities of plant powders.

A novel treatment for the chronic illness may be provided by the area of phytomedicine, given that more than 800 plants have historically been used to treat diabetes and that the majority of plants have a range of therapeutic effects. However, only a small number of plants were backed by solid clinical data, according to a previous analysis of nutrients and herbs that were pushed for improving diabetes control[4,5]. The herbs *Syzygium cumini*, *Andrographis paniculata*, *Gymnema sylvestre*, *Mimosa pudica*, *Phyllanthus emblica*, *Trigonella foenum-graecum*, *Momordica charantia*, *Annona squamosa*, *Azadirachta indica* and *Nigella sativa* showed promising therapeutic results[6]. In this study, the four herbs *Syzygium cumini* (*S. cumini*), *Andrographis paniculata* (*A. paniculata*), *Gymnema sylvestre* (*G. sylvestre*) and *Mimosa pudica* (*M. pudica*) were of interest due to their long history in the treatment of diabetes with their unique and diverse effects. Hence, the aim of this research is to find out whether these four medicinal powders have any antibacterial effects other than diabetes. The antimicrobial activities of these plant powders have been studied independently in many literatures[7-11], but there is no single comparative study that links the four medicinal powders: *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. Pudica* by using spectroscopic, elemental analysis and antibacterial studies. Hence, in this study, the functional groups and band gaps of the four medicinal powders was investigated based on the findings of FTIR and UV-Visible spectroscopy analysis[12,13]. Also, the size, shape, and elemental

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content of the plant powders have all been ascertained by SEM and EDX mapping[14]. In this work, the antibacterial activity[15] of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. Pudica* plant powders against human pathogenic bacteria, including *S. aureus* and *E. coli* have been evaluated and are compared with the standard antibiotic Ampicillin.

Materials and Methods

Materials

Syzygium cumini

In the medical field, herbal remedies are important since they provide supplementary and alternative methods to traditional therapy. *Syzygium cumini* (*S. cumini*) is a member of the Myrtaceae family, which is also known by the names Saval naval, Black Plum, and Jamun in, Tamil, English and Hindi, respectively. This tree is native to India and may also be found dispersed over Madagascar, Eastern Africa, South America, and the Asian continent[16]. Historically, a variety of illnesses and ailments, such as diabetes, gastrointestinal issues, asthma, diarrhoea, coughs, and bronchitis, have been treated using *S. cumini* species. Different traditional medical systems[17] have made use of different plant components, including fruit, seeds, bark, and leaves. Leucorrhoea, stomachaches, fever, dermopathy, constipation, and radiation-induced DNA damage[18] are among the conditions for which the leaves are used as remedies. In India, traditional medicinal systems like as Siddha, Ayurveda, and Unani employ the fruits of the *S. Cumini* plant for treating splenomegaly, chronic diarrhoea, stomachic, astringent, antiscorbutic, diuretic, and antidiabetic symptoms.

Andrographis paniculata

The plant *Andrographis paniculata* (*A. paniculata*), a member of the Acanthaceae family, is mostly found in parts of America, China, and Southeast Asia. There are several names for *A. paniculata* in other languages, such as Nilavembu (Tamil), King of Bitters[19] (English), and Mahatita (Hindi). Traditional Asian medicine has historically used the whole plant, aerial portion, and roots of *A. paniculata* for treating a wide range of conditions, including colic, diabetes, inflammation, fever, intermittent fever, hypertension, malaria, cancer, respiratory infections, and hepatitis. A number of bioactive substances found in *A. paniculata* affect its pharmacological actions[20]. Recent clinical investigations have demonstrated the utility of *A. paniculata* in the management of mild to severe coronavirus illness 2019 symptoms[21-23] (COVID-19).

Gymnema sylvestre

The *Gymnema sylvestre* (*G. sylvestre*) belong to Apocynaceae family and its popular names are "Sirukurinja" in Tamil, "Cowplant" in English, and "Gurmar" in Hindi, which mean "sugar destroyer" or "destroyer of sweets." Its historic function in controlling diabetes and lessening sugar cravings is reflected from its properties. Due to its popular names, which include "Australian cowplant," *G. sylvestre*[24] is also known as the "Cowplant". The plant, which grows in tropical Asia, including India, Sri Lanka, and Indonesia, is known by these colloquial names that are frequently used in traditional medical systems to describe its characteristics. *G. Sylvestre* has a long history of use in traditional medical systems, especially in Ayurveda, where it has been prized for centuries for its therapeutic qualities. In Ayurvedic medicine, *G. Sylvestre* has been primarily recognized for its potential benefits in managing diabetes and promoting healthy blood sugar levels[25].

Mimosa pudica

Mimosa pudica (*M. pudica*) is a creeping annual or perennial plant that is commonly grown for its curious compound leaves, which droop and fold inward when touched, only to emerge again in a matter of minutes. Mimosa is a genus of over 400 species of shrubs and plants belonging to the Legume family. The plant is indigenous to tropical America and has spread over almost all of India's tropical and subtropical regions. It is typically found in open areas including natural woods, roadside ditches, agricultural land, and waste areas. Due to their potential benefits to society, phytomedicines are still used in all conventional therapeutic systems, such as Chinese, Ayurvedic, and Greco-Arab (Unani-Tibb) medicine[26]. Alkaloids, the non-protein amino acid mimosine, flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids have all been found in phytochemical investigations on *M. pudica*. The phytochemical examination of *M. pudica*'s root revealed the presence of D-glucuronic acid, linoleic and linolenic acids, ascorbic acid, crocetin, mimosine, b-sitosterols, palmitic and stearic acids. Among *M. pudica*'s identified secondary metabolites are bufadienolide,

Fig. 1 — Plants and Powder of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica*

D-pinitol, norepinephrine, P-coumaric acid, mimopudine, and mimosine[27].

Methods

Collection of plant materials

The medicinal plants *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* depicted in (Fig. 1) were procured from the Sarvodaya Sangh of Virudhunagar, Tamil Nadu, India. As a solvent for natural ingredients used in food and natural medicine, ethanol is safe for human ingestion. Therefore, ethanol is chosen as a solvent for phytochemical extraction. Weighing out 20 grams of each plant powder, 150 millilitres of solvent were added, and the mixture was left for three days. Whatman No. 1 filter paper was used to filter the extract, and the supernatant was then collected. Supernatants were recovered when the residue was extracted twice more, separated by three days between each extraction. With the water bath set at 50°C, the supernatants were combined and evaporated using a rotating vacuum evaporator. Ultimately, the residues were gathered and put to use in a later investigation.

FTIR instrumentation

FTIR characterization[28] was conducted using Perkin Elmer's FT-IR spectrometer. Conveyor discs were made by encapsulating 1 mg of the dry powder in 10 mg of KBr pellet. Using a resolution of 4 cm^{-1} and a scan range of 400 to 4000 cm^{-1}, the pellet sample in powder form was placed into an FTIR spectrometer and recorded.

UV-Vis instrumentation

To analysis the UV-Vis spectra[29], after centrifuging the extract for ten minutes at 3000 rpm, Whatman No. 1 filter paper was used to filter the samples. The raw materials are crushed into coarse powder for extraction. Extracts were collected and examined using a UV-Vis spectrometer. Using ethanol as solvent, the samples were diluted to a ratio of 1:4. The distinctive peaks were found when the extract was scanned with a Perkin Elmer Spectrophotometer at wavelengths between 200 and 600 nm.

SEM with EDX instrumentation

Scanning electron microscopy (SEM) is another method used to assess the biomass of medicinal powders that is both natural and rich in microelements. Ethanol was used to dehydrate every sample, ranging in concentration from 30% to 100%. The plant powders were taken into two planes so that its surface and cross-section could be seen. Researchers can swiftly produce information on the pharmaceutical powders of a sample, including the elements present and its concentration and distribution, by using Energy-Dispersive X-ray (EDX) mapping. The plant powders were placed on the proper stub, gold-sputtered, and then examined and captured on camera using a Leo Zeiss 435 VP SEM (Oberkochen, Germany) running at 20 kV. A RONTEC energy dispersive X-ray system was installed in the microscope to gather data on the elemental composition of the plant powders. Every plant powder filled with a specific microelement had its X-ray spectra acquired[30].

Antibacterial analysis

The medicinal plant powdersextract made of *S. cumini*, *A. paniculata*, *G. sylvestre* and *M. Pudica* have been evaluated for their ability to kill bacteria by an agar disc diffusion technique that causes sickness

in humans such as *Staphylococcus aureus* and *Escherichia coli*. A vast collection of over 12000 microbial strains can be found in the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The MTCC has a variety of strains that are useful for research and industry. For assessing the antimicrobial susceptibility of bacterial cultures in the bioassay, standard strains of *S. aureus* (MTCC 3160) and *E. coli* (MTCC 443) were employed in conjunction with streptomycin 10mcg discs from the HiMedia catalogue. The overnight fresh culture of each strain was swabbed uniformly onto its plate. The impregnated Extracted solution in volumes of 25, 50, and 75 µL was placed on the plates and heated to 37°C for 24 h. As a benchmark, commercial antibiotic discs were employed. The extent of zonation's

Table 1 — Vibrational assignment for *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica*

Wavelengths (cm^{-1})				Assignment
S. cumini	*A. paniculata*	*G. sylvestre*	*M. pudica*	
3746	3744	3748	3680	O–H stretch
3433	3426	3428	3425	O–H stretch
-	2924	2924	2920	C–H stretch
1736	1744	-	1741	C=O stretch
1620	1638	1647	1639	N–H bend
1522	1510	-	1514	N–O asymmetric stretch
1456	1425	1452	1454	C-H bending, C–C stretch (in–ring)
1356	1325	1319	1323	C-H, C-O stretch
1234	1246	1250	1248	C–N stretch
1157	-	-	-	C-O
1022	1040	1034	1039	C=C
860	897	-	-	C-H (aromatics)
768	-	777	-	C-H rock (alkanes)
575	610	419	-	C–Br stretch (alkyl halides)

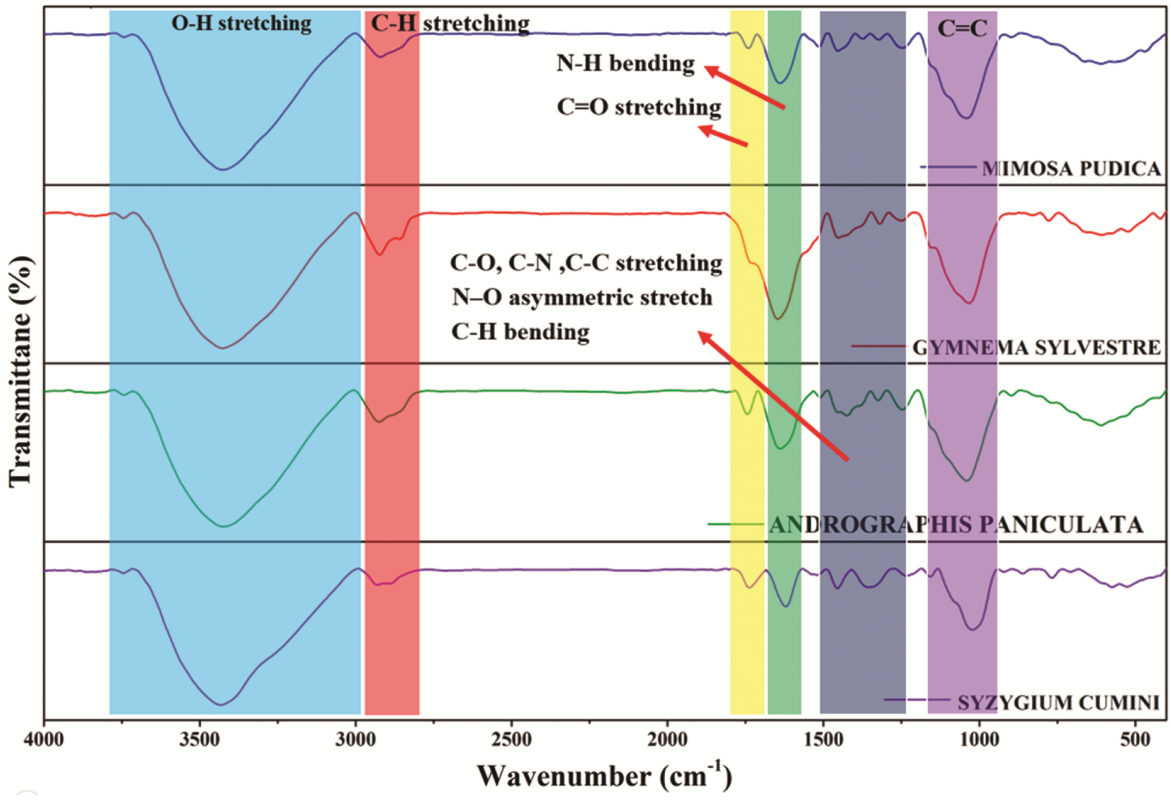

Fig. 2 — FTIR spectrum of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica*

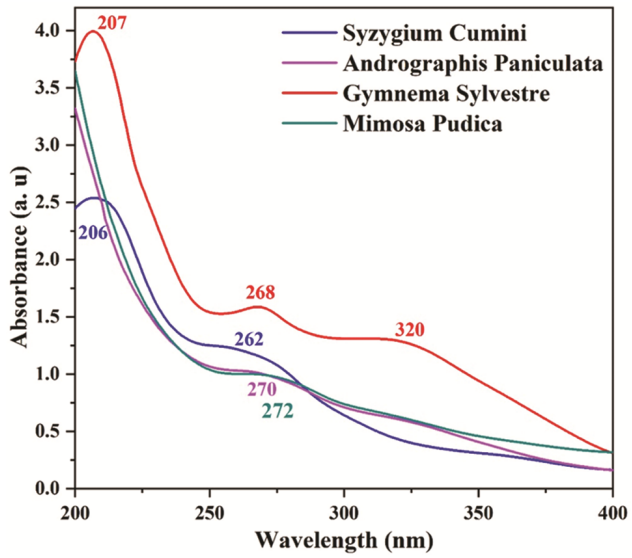

Fig. 3 — UV-Vis spectrum of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. Pudica*

development around the disc was evaluated after an appropriate incubation period[31].

Results and Discussion

FTIR spectral analysis

The FT-IR spectrum was used to recognize the functional groups of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. Pudica* were identified based on their peak's ratio[32]. The results of FTIR wavelengths and functional groups were shown in (Table 1). The FTIR spectra were represented in (Fig. 2). The FTIR of plant powders have revealed strong peaks at 1620, 1638, 1647, 1639 cm^{-1}, which are shown the presence in primary amines of N-H bending vibrations. The C=O ketone always lies between 1680 and 1750 cm^{-1}, hence in this study, the bands at 1736, 1744, 1741 cm^{-1} have been assigned to C=O vibrations. In this study, the broad peaks found at 3746, 3744, 3748, 3680, 3433, 3426, 3428 and 3425 cm^{-1} attributed to O-H stretching vibrations. These indicate the water content in the powder samples. Every plant powder has the capacity to move via the hydrogen bond network by proton hopping. The hydrogen bond's broad peak suggests more favourable circumstances for proton transfer. The peaks at 1022,1040, 1034, 1039 cm^{-1} are due to alkenyl C=C stretching, and the peaks obtained at 1234, 1246, 1250, 1248 cm^{-1}shows the presence of C≡N stretching in the powder plants. Hence, the FTIR spectra confirmed that *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* have the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds and amines[33]. There is no absorbance in the 2220-2260 cm^{-1} range, indicating that there are no cyanide groups in these extracts and hence none of the four plant powders contain any hazardous chemicals.

UV-Vis spectral analysis

The UV-Visible spectra are a beneficial tool to investigate the electronic properties of powders in their excited energy states. The profile showed the absorption wavelength of 206 and 262 nm for *S. cumini*, 270 nm for *A. paniculata*, 207, 268 and 320 nm for *G. sylvestre* and 272 nm for *M. pudica*, which indicates the charge transfer from lone pairs of oxygen, nitrogen, bromine to C-C and C-H bonds (Fig. 3). Tauc plot for the UV-Vis spectrum (Fig. 4) have been used to obtain the energy gaps of the plant powders[34]. In this study, the energy gap of 5.0866, 5.1746, 4.8831 and 5.1568 eV was calculated for *S. cumini*, *A. paniculata*, *G. sylvestre* and *M. pudica*, respectively. A large energy gap denotes the strong stability and low chemical reactivity, whereas a small energy gap shows softness, which correlates to high chemical reactivity and poor stability[35]. This indicates that *A. paniculata* (E_g = 5.1746 eV) has high stability than other plant powders.

SEM with EDX analysis

SEM indicates the microstructures of the powder plants at various magnification levels and the average particle size can be determined accurately[36]. Figures 5-8 illustrate SEM images of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica*at various magnifications. *S. cumini*, magnified to 10 µm, shows the structure of the rock and is mostly composed of oxygen (43%) and carbon (56%). In 10 µm, the *A. paniculata* depicts the rock structure, with carbon accumulating at a very high proportion (59.3%). The *G. sylvestre* demonstrates a structure similar to that of rock, with a high percentage of carbon (56.3%). The SEM picture of *M. pudica* reveals a structure resembling rock with a high percentage of carbon (56.6%) and oxygen (40.7%). Plant powders belonging to *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* have been discovered to have average practical sizes of 63, 61, 41, and 85 µm. Additionally, Mg, K, Ca, Al, Si, P, S, and Fe elements are present in these *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* samples in a modest amount. These also attest to the fact that the powder samples' structures were free of any dangerous components.

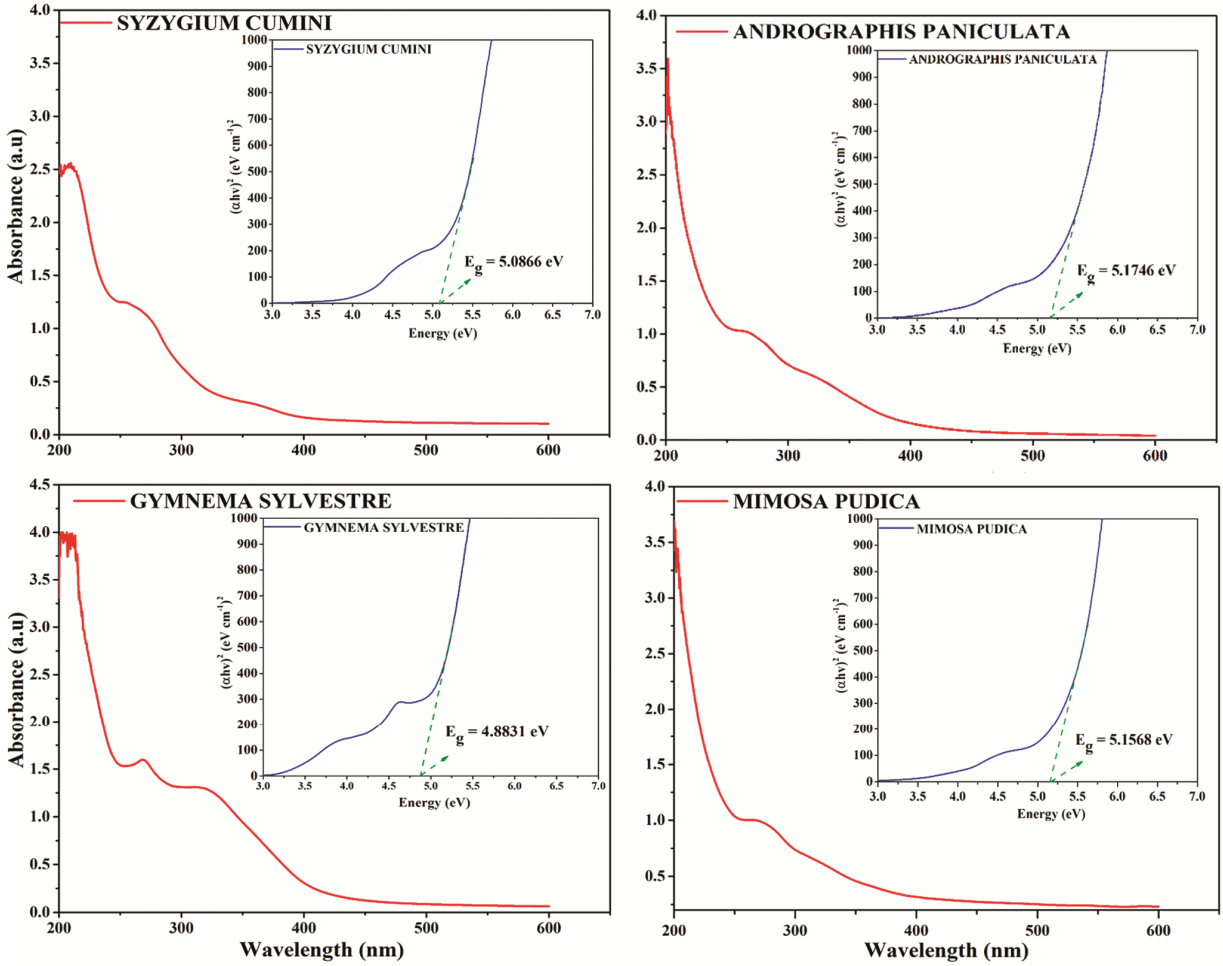

Fig. 4 — UV-Vis spectrum with Tauc plot for *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. Pudica*

Antibacterial activity analysis

The antibacterial activity of powder extracts from *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* against gram positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria[37-39] (*Escherichia coli*), have been shown to differ significantly from one another. Table 2 displays information on the antibacterial properties of four different kinds of medicinal plant powder extracts in terms of the inhibition zone against the bacterial species *E. coli* and *S. Aureus* at different concentrations (25, 50 and 75 µL). As seen from the Table 2 and Figure 9, the zone of inhibition increases with increasing the concentrations of the plant powder. In particular, the highest inhibition zone of *A. paniculata*'s antibacterial activity against *E. coli* was found to be 23±1.3 mm, whereas, *S. cumini*'s antibacterial activity against *S. aureus* was discovered to have a maximum inhibition zone of 27±1.5 mm. Nonetheless, out of the four plant powders, *M. pudica* exhibits a dominating zone of inhibition in gram-negative and gram-positive bacteria, measuring 23±1.5 and 26±1.2, respectively. This might be because *Mimosa pudica* contains alkaloids or flavonoids. Many of its phytoconstituents, including glycosides, alkaloids, terpenoids, and flavonoids, have been linked to antibacterial and strong antioxidant effects[40]. Further, these results are comparable with the standard antibiotic Ampicillin[41] which has the zone of inhibition ranges from 16-22 mm and 27-35 mm, for *E. coli* and *S. aureus*, respectively.

Statistical analysis

To find any significant differences between the plant powders of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. Pudica* against *E. coli* and *S. aureus* bacteria, the one-way Analysis of variance (ANOVA) statistical test was employed. Statistical significance is commonly defined as a p-value of 0.05 or less. The p-value calculates the likelihood of getting the observed outcomes on the assumption that the null hypothesis is correct. The statistical

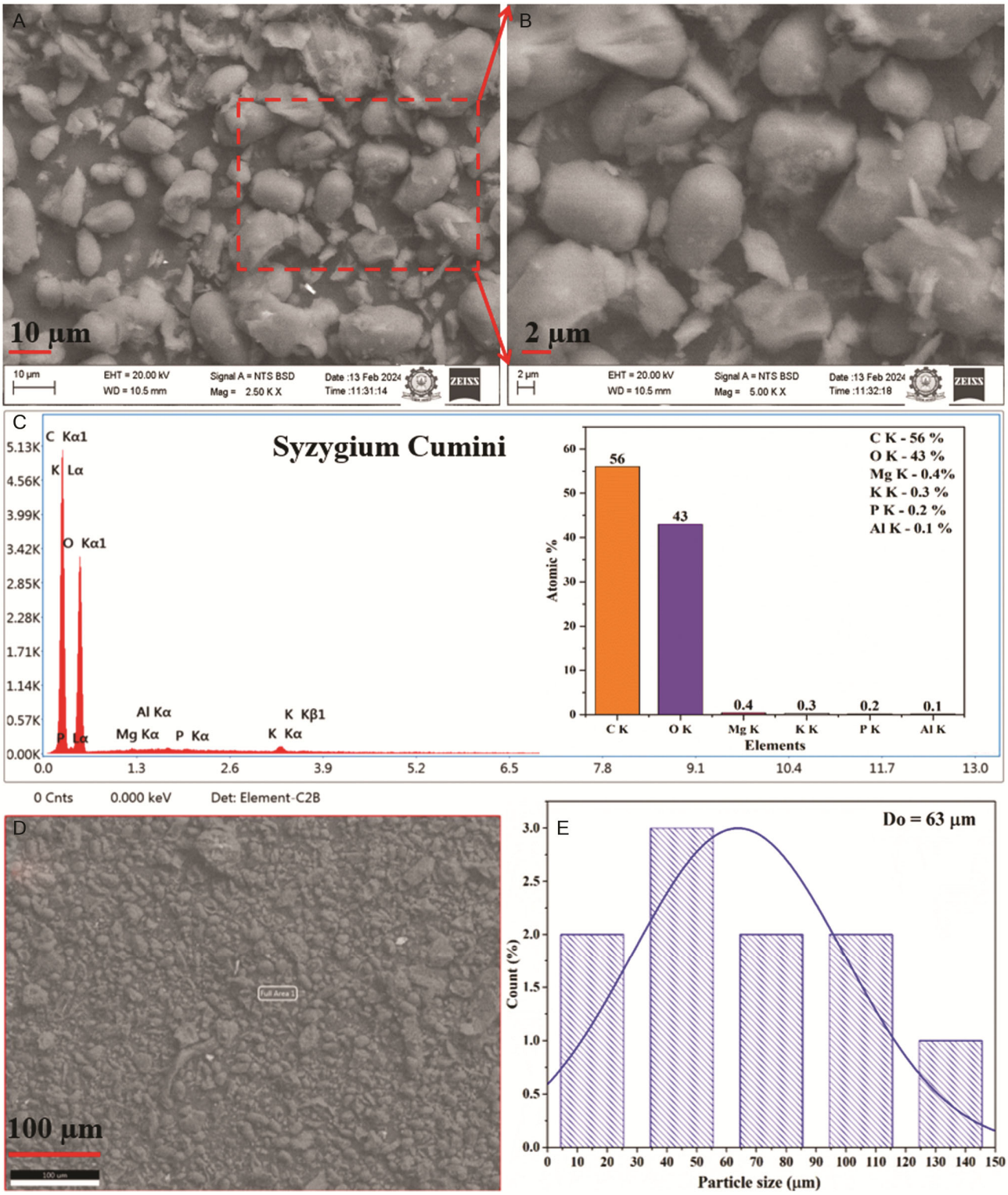

Fig. 5 — SEM with EDX and particle size of *S. cumini*

significance of the observed difference increases with a lower p-value. Table 3 displays the findings of the statistical data analysis performed with a one-way analysis of variance. In this investigation, the antibacterial activity of the plant powders *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* against

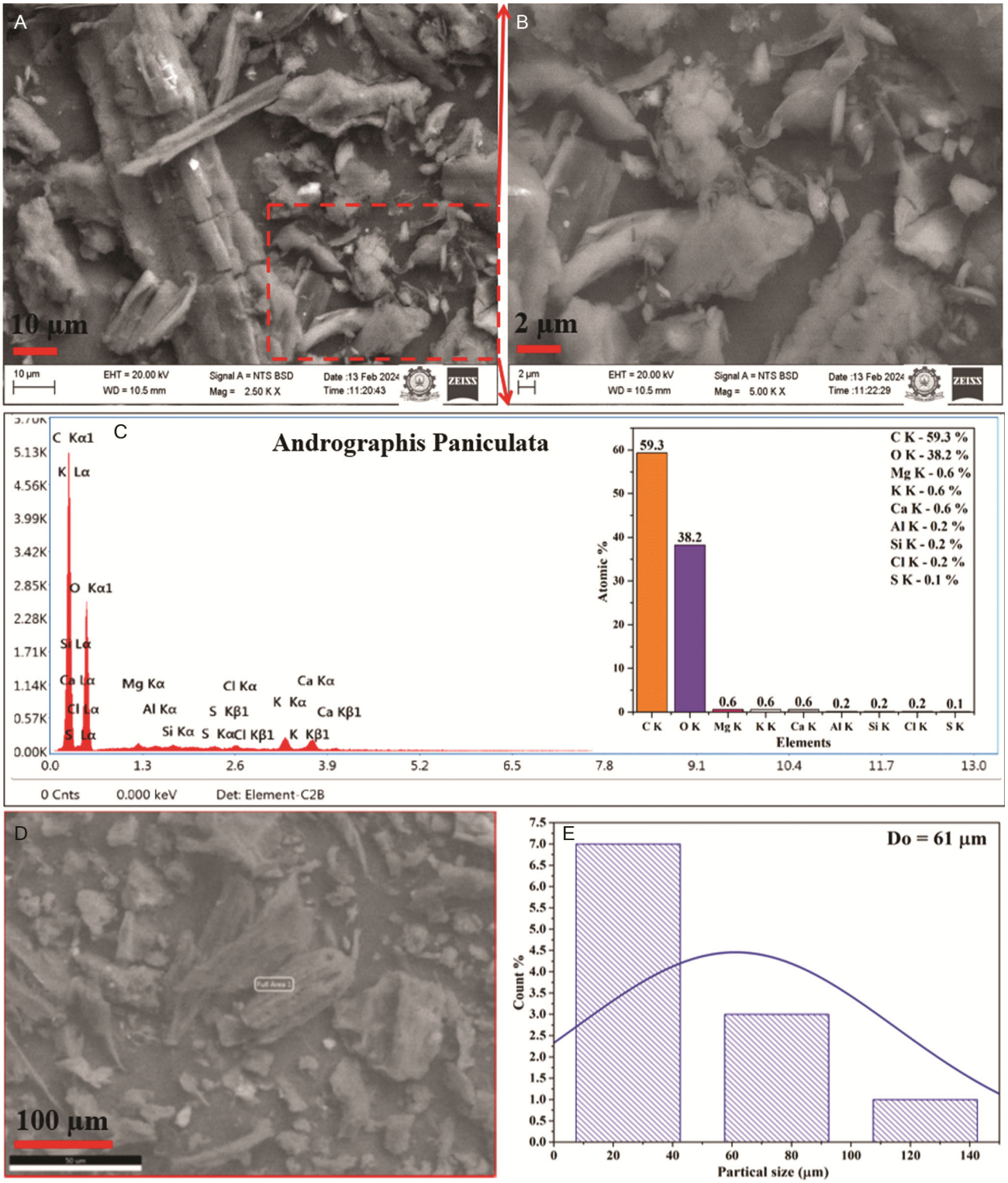

Fig. 6 — SEM with EDX and particle size of *A. paniculata*

E. coli was statistically significant (P = 0.0013), but the antibacterial activity of the plant powders against *S. aureus* was not statistically significant (P = 0.1549). Because the p-value is less than 0.05, the plant powders as a whole had higher statistical significance (P = 0.0123) against both *E. coli* and *S. aureus*.

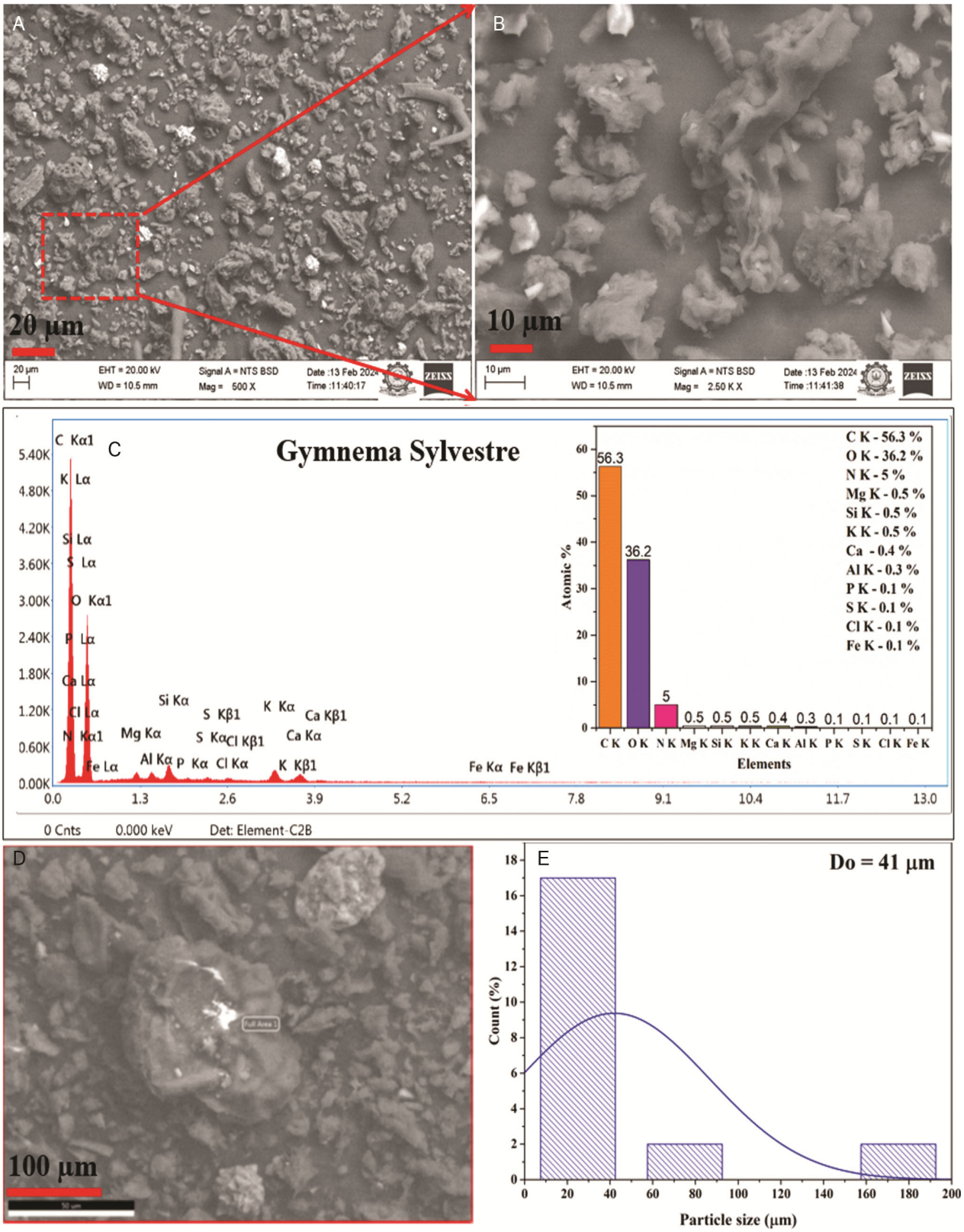

Fig. 7 — SEM with EDX and particle size of *G. Sylvestre*

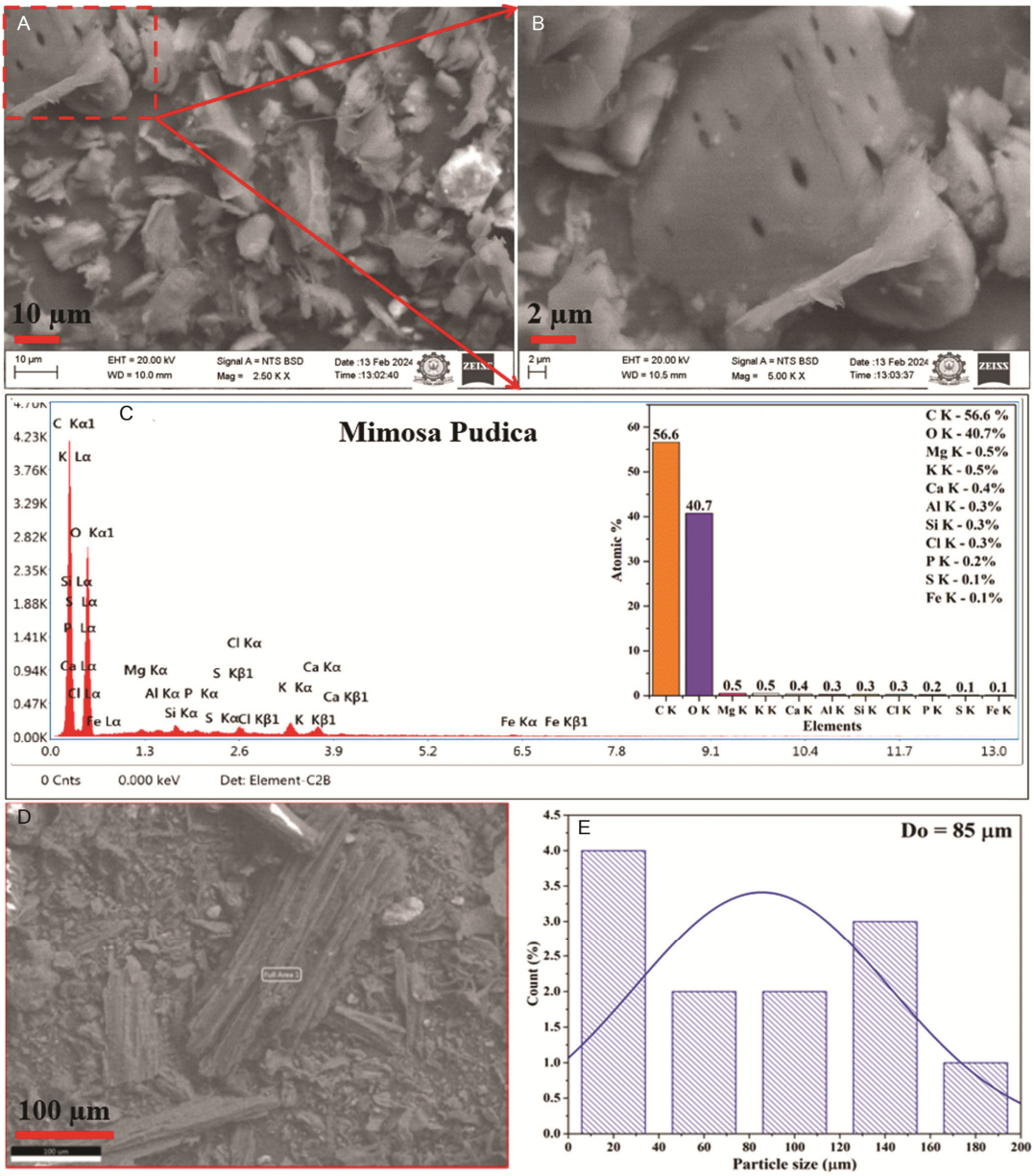

Fig. 8 — SEM with EDX and particle size of *M. pudica*

Table 2 — Antibacterial activity of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* with *E. coli* and *S. aureus*

Plant powders	Bacterial Organism	Zone of Inhibition (mm)			
		Positive control	25 µL	50 µL	75 µL
S. cumini	*E. coli*	20±1.3	13±0.9	17±1.6	19±1.3
A. paniculata	*E. coli*	19±1.4	17±1.3	18±1.8	23±1.3
G. sylvestre	*E. coli*	19±1.2	10±1.2	19±1.4	21±1.8
M. pudica	*E. coli*	17±1.0	10±1.4	16±1.6	23±1.5
S. cumini	*S. aureus*	29±1.5	19±1.1	21±1.3	27±1.5
A. paniculata	*S. aureus*	19±1.9	13±1.6	16±1.6	21±1.6
G. sylvestre	*S. aureus*	18±1.4	10±1.3	13±1.4	17±1.3
M. pudica	*S. aureus*	23±1.7	20±1.3	24±1.2	26±1.2
Ampicillin (10 µg)	*E. coli*		16–22		
Ampicillin (10 µg)	*S. aureus*		27–35		

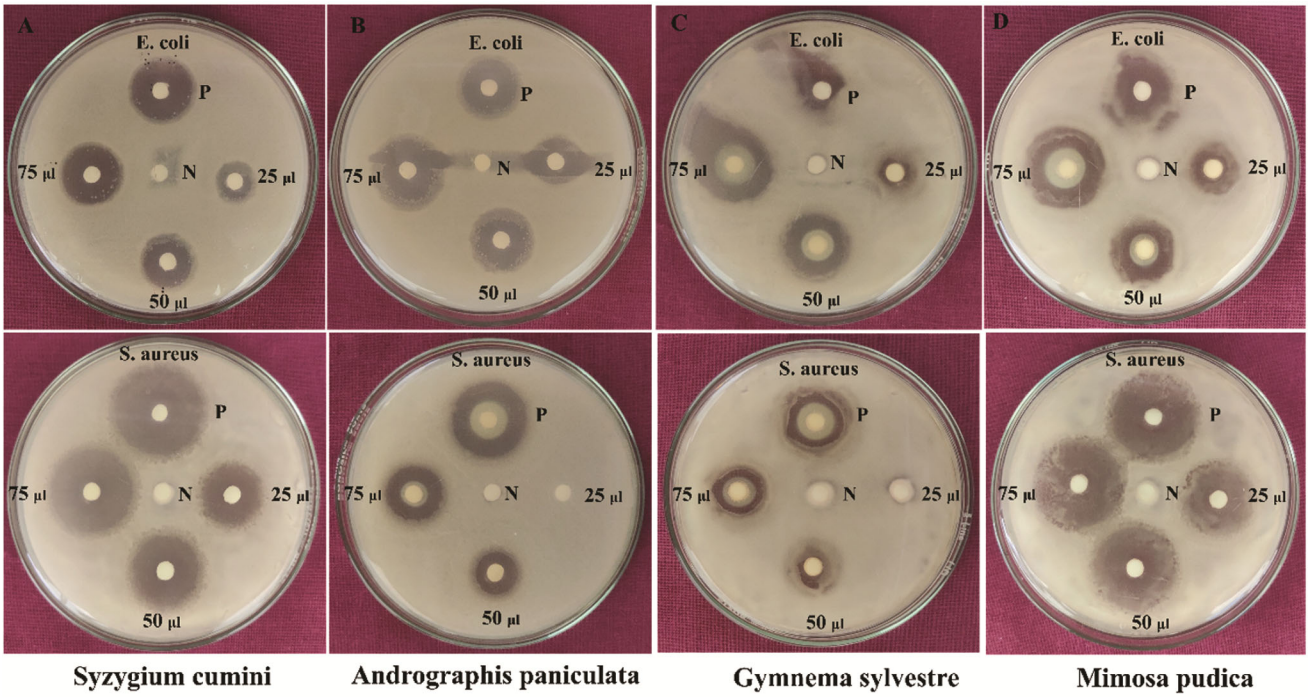

Fig. 9 — Antibacterial activities of *S. cumini*, *A. paniculata*, *G. sylvestre* and *M. pudica* with *E. coli* and *S. aureus*

Table 3 — The statistical data analysis using one-way analysis of variance

Variable	Degree of freedom	Sum of Square	Mean Square	F Statistic	*P*-value
		Escherichia coli			
Between groups	2	162.6667	81.3333	14.9388	0.0013
Within groups	9	49	5.4444		
Total	11	211.6667	19.2424		
		Staphylococcus aureus			
Between groups	2	106.1667	53.0833	2.3108	0.1549
Within groups	9	206.75	22.9722		
Total	11	312.9167	28.447		
		Both *Escherichia coli* and *Staphylococcus aureus*			
Between groups	5	287.2083	57.4417	4.0428	0.0123
Within groups	18	255.75	14.2083		
Total	23	542.9584	23.6069		

Conclusion

FTIR and UV-Vis spectroscopy have been used to analyze the vibrations and band gaps of the powdered medicinal plants *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica*. The plant powders' good soluble nature has been shown by the identification of their O–H, C–H, C=O, N–H, and C–Br functional groups. The charge transfer between lone pairs of oxygen, nitrogen, bromine, and other C-C and C-H bonds is displayed as absorption peaks. The high band gap of 5.0866, 5.1746, 4.8831, and 5.1568 eV indicates the powders' good stability and bioactivity. The SEM and EDX techniques have been examined to get insight into the samples' elemental compositions and structural properties. Plant powders with varying rock-like structures may be seen in SEM images. The typical practical sizes of *S. cumini*, *A. paniculata*, *G. sylvestre*, and *M. pudica* are 63, 61, 41, and 85 µm, respectively. We have determined that the plant powders include a high percentage of innocuous components like carbon and oxygen based on the EDX examination. *M. pudica* has the greatest zone of inhibition of any plant powder in this study, indicating superior bacterial resistance, according to the antibacterial analysis of plant powders against *S. aureus* and *E. coli*.

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Conflict of interest

All authors declare no conflict of interest.

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