ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Characterization, Antioxidant, and Antimicrobial Properties of Mulberry Lattices

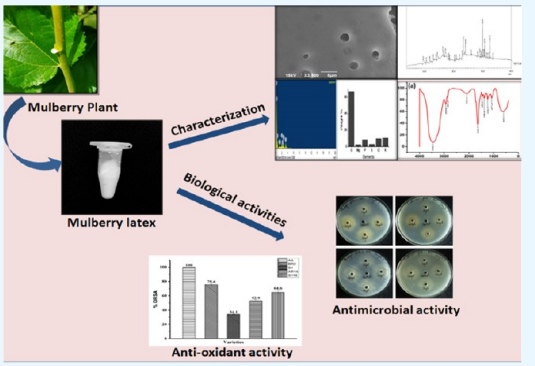
Venkatesh Kumar R,* Sunil Babu Gosipatala, Ram Kumar, Devika Srivastava, Vandana Singh, Kusumala Suman, Deepak Kumar Tripathi, Abhishek Verma, Akash Mishra, Karan Kumar Vishwakarma, Stuti Annapurna Singh, Tripti Pandey, Sanskrati Agarwal, Mohd Elyies, Ishani Singh, Pinky Kumari Sah, Chaya Sharma, Rishabh Parag, Pragya Saxena, Akanksha Raj, Anshika Tripathi, Poonam Devi, and Krishna Mohan Poluri

Cite This: *ACS Omega* 2023, 8, 47758−47772

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: In order to find the most advantageous bioactive compounds from mulberry latex for drug development in the near future, this study was conducted to characterize and evaluate antioxidant and antimicrobial properties from four different mulberry lattices (BR-2, S-1, AR-14, and S-146). The characterization of the lattices was performed by scanning electron microscopy with energy-dispersive X-ray spectroscopy, gas chromatography coupled to mass spectroscopy, and Fourier transform infrared spectroscopy. Further, screenings of the antioxidant and antimicrobial potential of selected lattices were performed in vitro using 2,2-diphenyl-1-picrylhydrazyl assay and agar well diffusion methods, respectively. Interestingly, the outcome of the current study revealed that tested mulberry lattices contain a considerable amount of bioactive phytoconstituents, particularly antimicrobial and antioxidant compounds, as revealed by chromatographic analysis. BR-2 latex was found to have significant antioxidant activity (75%) followed by S-146 (64.6%) and AR-14 (52.9%). The maximum antimicrobial activity was found in BR-2 latex compared to other tested latex varieties. The results of this investigation showed that mulberry latex from the BR-2 type may successfully control both bacterial and fungal infections, with the added benefit of having enhanced antioxidant capabilities.



1. INTRODUCTION

Mulberry is a rapidly spreading deciduous tree that grows in a wide range of soil, topographical, and climatic conditions from temperate to subtropical areas. The genus *Morus* has around 60 species, distributed over subtropical, tropical, and temperate zones in Asia, Africa, and North America.[1,2] Mulberry as a whole plant has long been used as a functional food because of its rich phytochemical composition. At the commercial and industrial scale, this adaptable medicinal plant still lacks a clear identity.[3] Mulberry (*Morus spp.*) with its unquestionable properties became appropriate for use not only in the sericulture sector, but also in the food chain, the pharmaceutical industry, and environmental safety.[1] Mulberry is also employed as a medical plant, with the biologically active pharmacokinetic components found in the leaf, stem, and root sections being used to improve and enhance human health.[4,5] Mulberry fruit, for example, includes a wide range of nutritive compounds, including fatty acids, amino acids, vitamins, minerals, and bioactive chemicals such as anthocyanins, rutin, quercetin, chlorogenic acid, melatonin, and polysaccharides.[6,7] As a result, mulberry fruits have documented antioxidant, anticancer, antidiabetic, hepatoprotective, neuroprotective, anti-inflammatory, antiobesity, hypolipidemic, and antibacterial properties.[8] Mulberry leaves contain minerals, vitamins, dietary fiber, amino acids, phytosterols, flavonoids, and other useful components, the most important of which are 1-DNJ (1-deoxynojirimycin), an antidiabetic medication. The extract from the leaves may aid in the reduction of blood sugar (diabetes), inflammation, cholesterol, and the prevention of heart disease.[9] Mulberry plant root barks contain a variety of compounds, including Diels−Alder adducts, flavonoids, benzofurans, stilbenes, polyhydroxylated alkaloids, and others. These compounds have demonstrated a wide range of bioactive properties such as anti-inflammatory, antioxidative, antimicrobial, and so on.[10] Further, Mulberry twig has been described as a promising antidiabetic

Received: August 18, 2023
Revised: October 31, 2023
Accepted: November 13, 2023
Published: December 6, 2023

Figure 1. Mulberry plant, a source of latex.

therapeutic candidate (Liu et al., 2023) and includes bioactive phytoconstituents such as terpenoids, alkaloids, chalcones and anthocyanins, phenolic acids, stilbenoids, and coumarins.[11]

In addition to this, the mulberry plant (Figure 1) is also recognized for producing a sizable amount of latex from various vegetative sections. Mulberry trees are also known to secrete latex when their stem, leaves, or roots become wounded.[12] Some ayurvedic curative medicines employ different parts of mulberry including latex to treat various human illnesses.[13] Conversely, modern studies are primarily focused on the defensive role of mulberry latex against herbivory.[14−17] Furthermore, mulberry latex is a valuable source of glycosidase inhibitors such as 1,4-dideoxy-1,4-imino-d-arabinitol, 1-deoxynojirimycin, and 1,4-dideoxy-1,4-imino-d-ribitol that have been reported to have antidiabetic properties.[17] Zhang et al.,[18] reported that deoxynojirimycin (DNJ)-rich mulberry latex significantly lowered the postprandial blood glucose and fasting blood glucose level in diabetic mice model. Similarly, there might be other useful therapeutic compounds in mulberry latex that could be screened for their synergistic effect in latex against various communicable as well as noncommunicable diseases. As a result, the current study was carried out for the first time to characterize and examine the antioxidant and antibacterial properties of latex from the BR-2, S1, AR-14, and S-146 varieties of mulberry plants.

2. MATERIALS AND METHODS

2.1. Chemicals. In the present study, the chemicals used were of analytical grades. Ascorbic acid (99.7%), methanol (99.9%), agar, tryptone, sodium chloride(99.9%), yeast extract, distilled water, tetracycline, and dimethyl sulfoxide (DMSO) (99.5%) were purchased from SRL Company, Bangalore, India. DPPH dye (>98%) was purchased from HiMedia.

2.2. Plant Identification and Sample Collection. Dr. G. Lokesh, Scientist-D, Central Sericultural Germplasm Resources Centre, Hosur, Tamil Nadu, India, precisely identified and confirmed each of the tested mulberry varieties. The cultivars BR-2 (ME-0256), S-1 (ME-0065), AR-14 (MI-0799), and S-146 (MI-0045) of mulberries were used to harvest fresh latex. The latex samples were collected at the start of the spring season during the early morning hours. Latex was physically obtained by physically slicing a slit in the internodal zone of the relevant mulberry plants. As soon as the lattices began to leak, they were collected in sterile Eppendorf tubes. On the collected latex samples, a subsequent examination was conducted.

2.3. Scanning Electron Microscopy−Energy-Dispersive X-ray Spectroscopy (SEM-EDX) Analysis. The microphotographs and values of the elemental analyses of tested mulberry lattices were recorded using a scanning electron microscope (JEOL, JSM 6490 LV, Japan) built with an equipped energy-dispersive X-ray analyzer (EDX)(EDS 133, EV Dry Detector (INCA x-act) of the OXFORD instruments, U.K.). For this procedure, selected lattices were coated on aluminum stubs, and the instrument was operated at the voltage of 15 kV followed by examining them under 1000 and 3500× magnifications and recording the elemental trends.[19]

2.4. Gas Chromatography−Mass Spectroscopy (GC−MS) Investigation. In order to analyze the mulberry lattices by GC−MS, the methanolic extracts of respective lattices were prepared by blending 0.5 mL latex samples with an equal amount of methanol (1:1 ratio), and the mixtures were thoroughly mixed for an hour using a vortex (DLAB). Subsequently, the samples were subjected to analysis using GC−MS (Shimadzu QP-2010 Plus with Thermal Desorption System TD 20, Japan), equipped with Rtx-5 silica MS column (30 m × 0.25 mm i.d. × 0.25 film thickness). In the presence of Helium as a carrier gas, the pressure and injection temperature were maintained at 81.9 kPa, and 260 °C respectively. At the total flow rate of 16.3 mL/min, the column flow rate was maintained at 1.21 mL/min Throughout the experiment run time of 46.98 min, the injection mode was split with a split ratio of 10.0 and the injection temperature was 260 °C. The column oven temperature was 60.0 °C.

To have control of the system and acquire the data, we engaged Shimadzu GC−MS solution software provided by the supplier. By comparing their mass spectra to data from NIST 11 (National Institute of Standards and Technology) and WILEY 8, the compounds present in mulberry lattices were identified.[20]

2.5. Fourier Transform Infrared (FTIR) Analysis. A Fourier transform infrared spectrometer (Thermo Nicolet 6700, Thermo Scientific) was used to determine the functional groups of the selected latex varieties. Initially, 10 μL latex samples were taken separately. The respective latex samples were mixed with 2% KBr, ground to make a fine powder, and formed into pellets using a hydraulic press. The spectral range 4000−500 cm^{-1} was investigated in the KBr background.[21]

2.6. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. The radical scavenging properties of latex samples were studied by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay with slight modification in the methods reported earlier.[22] A 0.004% methanolic solution of DPPH having an initial absorbance value of 0.9 was blended with 50 μL of latex samples. The mixture of compounds was mixed well in a vortex before being stored in the dark at room temperature for half an hour. Methanol was added to DPPH as a negative control, and an ascorbic acid solution (AA) was used as a positive control. The absorbance at 517 nm was measured by using an ultraviolet−visible (UV−vis) spectrophotometer (Eppendorf, Germany). To quantify the percentage inhibition of DPPH radicals as an indicator of latex's capability to neutralize reactive oxygen species (ROS), the following formula was used

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

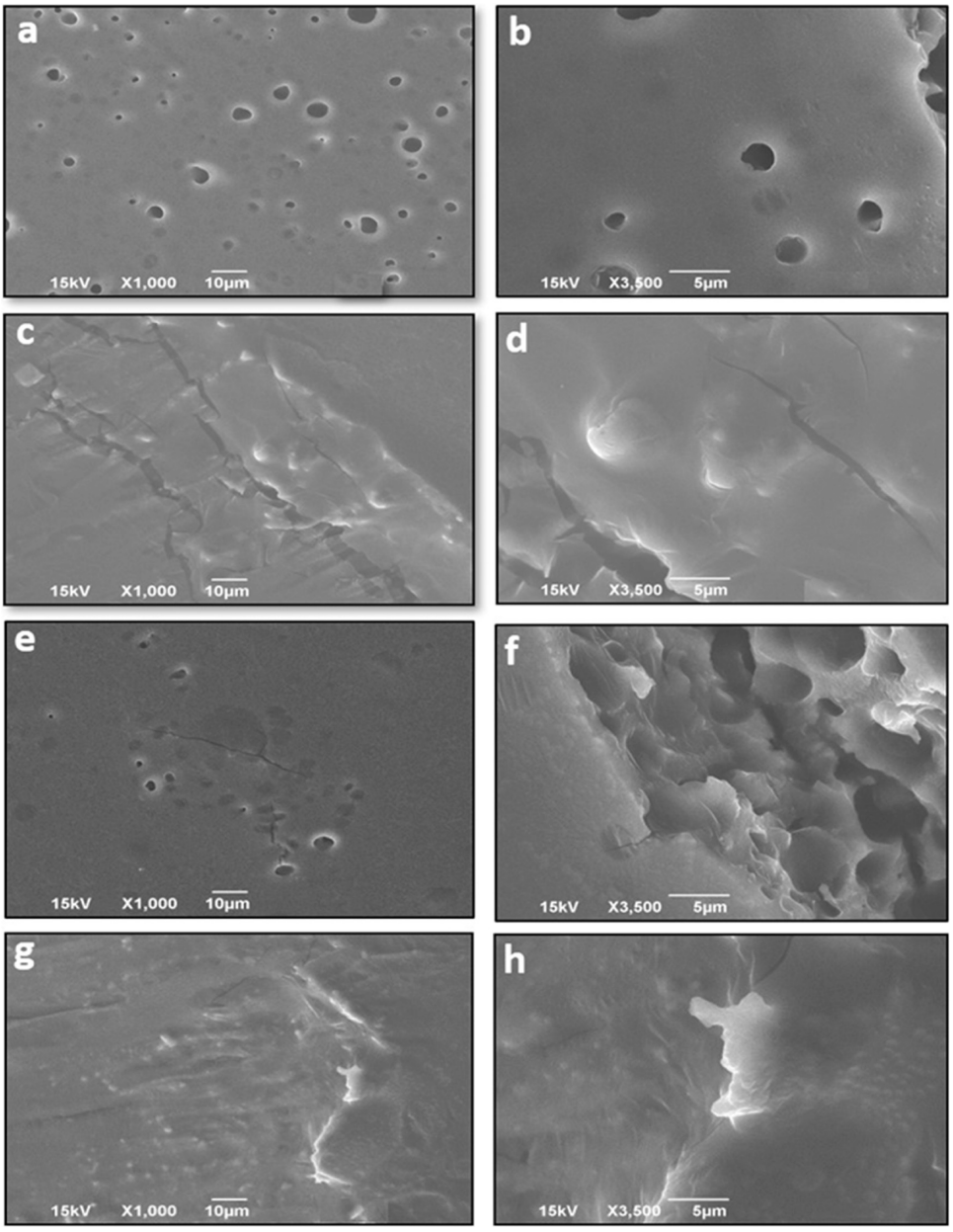


Figure 2. Surface morphology of mulberry latex of (a, b) BR-2 latex; (c, d) S-1 latex; (e, f) AR-14 latex; and (g, h) S-146 latex at different magnifications.

$$\%\text{radical scavenging activity} = \frac{OD_{blank} - OD_{sample}}{OD_{blank}} \times 100$$

where OD_{sample} denotes the absorbance of the lattices and OD_{blank} indicates the absorbance of the control reaction.[23]

2.7. Antimicrobial Analysis. The antibacterial abilities of the mulberry lattices (BR-2, S-1, AR-14, and S-146) against *Aspergillus niger* (isolated from natural source), *Candida albicans* (MTCC 3958), *Salmonella typhi* (MTCC 735), and *Staphylococcus aureus* (MTCC 96) were examined in duplicates.

2.7.1. Fungus Culture Media Processing. In two Erlenmeyer flasks, 30 mL of Potato Dextrose broth was prepared by boiling 6 g of potato in 30 mL of distilled water and filtering it, then adding 0.6 g of dextrose to the filtrate, and bringing the final volume up to 30 mL with distilled water. Then, the mixture was autoclaved for 15 min at 121 °C. The selected fungal strains were inoculated in the respective flask and incubated at 25 °C for 72 h.

2.7.2. Bacteria Culture Media Processing. In two Erlenmeyer flasks, 30 mL of Luria−Bertani broth was made by adding 0.3 g of tryptone, 0.3 g of sodium chloride, 0.18 g of yeast extract, and 30 mL of distilled water, and the mixture was autoclaved at 121 °C for 15 min. The selected bacterial strains were inoculated in 30 mL of sterilized LB broth flasks and incubated at 37 C for 24 h.

2.7.3. Latex Samples Preparation for MIC. Different aliquots of latex samples were prepared by pipetting 10, 20, 30, and 40 μL, and by adding DMSO, the final volume was increased to 50 μL.

2.7.4. Control Preparation. Tetracycline and fluconazole were used as standard antibacterial and antifungal drugs, respectively. In 1 mL of DMSO, 10 mg of tetracycline was dissolved followed by the preparation of aliquots of various dilutions: 10 (100), 20 (200), 30, (300), and 40 μL (400 μg); the final volume was raised to 50 μL by adding DMSO.

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

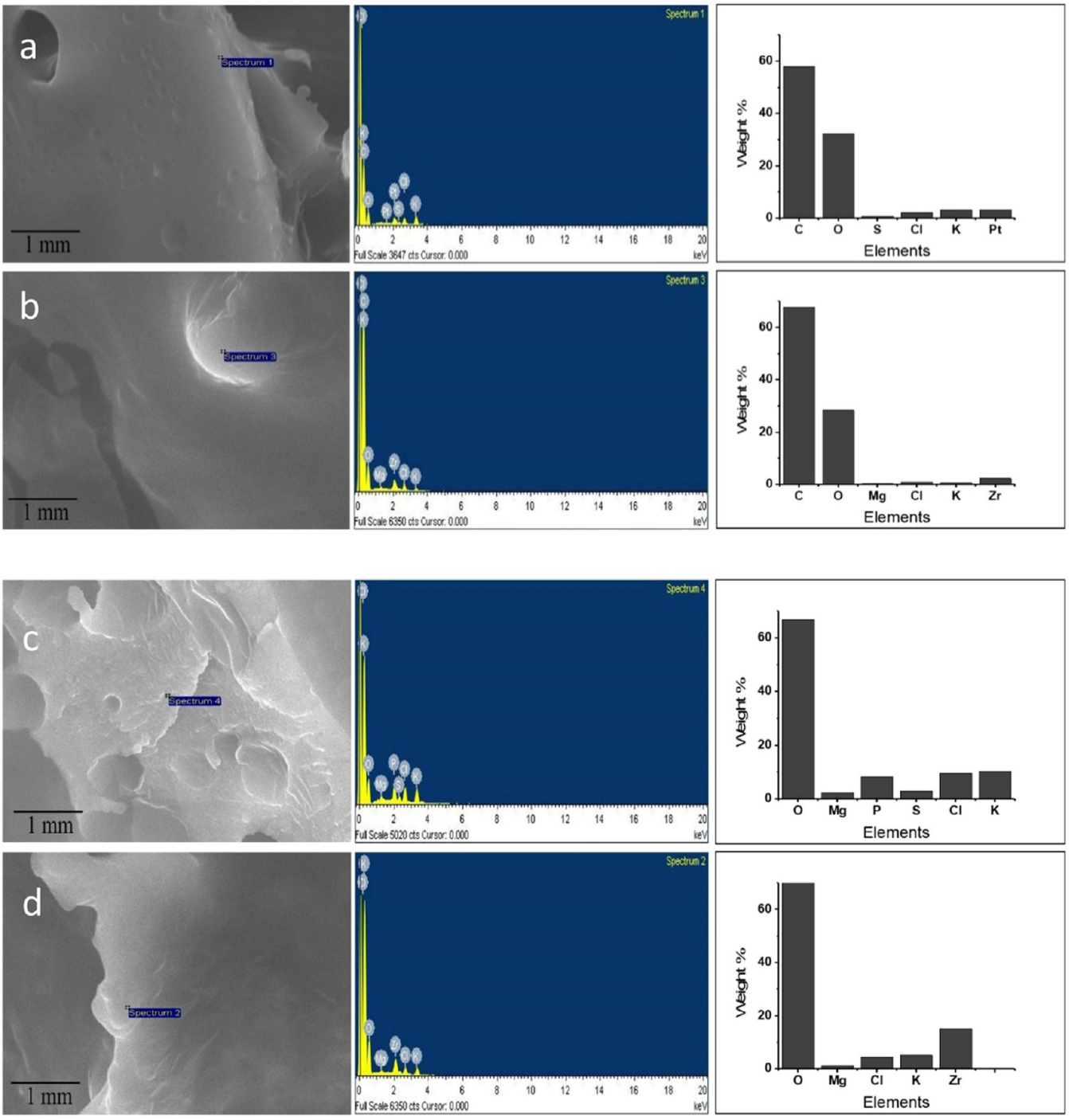


Figure 3. EDX spectrum of (a) BR-2, (b) S-1, (c) AR-14, and (d) S-146 mulberry lattices.

Furthermore, 100 mg of fluconazole was dissolved in 1 mL of DMSO, and different aliquots were prepared by pipetting 10(1), 20(2), 30 (3), and 40 μL (4 mg); the final volume was raised to 50 μL by adding DMSO.

2.7.5. Agar Plate Diffusion Assay. In the sterilized petriplates, approximately 25 mL of the media (PDA and LB agar) was poured and allowed to harden. A 200 μL inoculum of the selected microbes was placed onto agar plates and extensively dispersed with a plate spreader. A borer was used to make five 0.6 cm wells in each plate, and 50 μL of prepared latex samples and control medications were placed into the relevant plate wells, along with 50 μL of DMSO in the middle well as a control blank. The bacterial plates were incubated at 37 °C for 24 h, and the fungal plates at 25 °C for 72 h. Later, the zone of inhibition was measured in millimeters (mm).[24,25]

3. RESULTS

3.1. SEM-EDX Analysis. The SEM micrographs revealed notable variations among the tested mulberry lattices, namely, BR-2, S-1, AR-14, and S-146 (Figure 2) under 1000 and 3500× magnifications. The uniformity of the BR-2 latex (Figure 2a,b) surface was observed throughout the surface area with the presence of uniformly distributed minor pores. While the surface of S-146 latex (Figure 2g,h) seems to be uniform with occasional

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

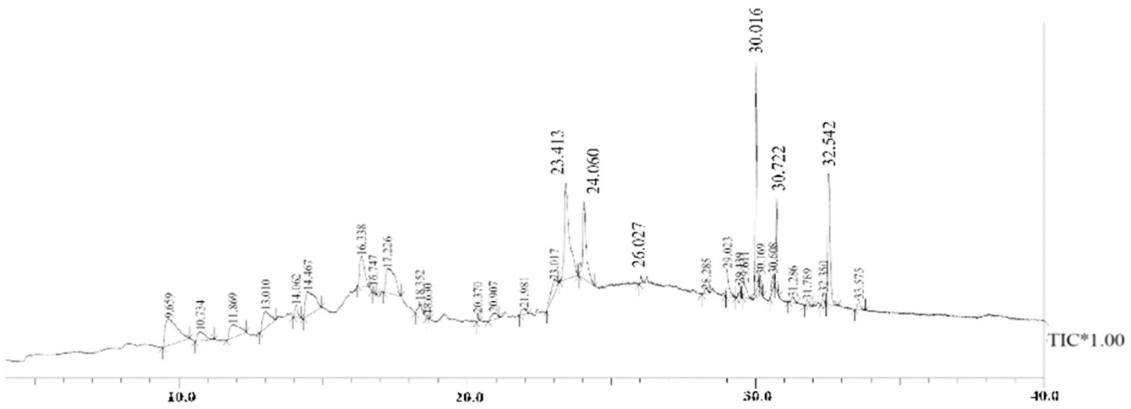


Figure 4. GC−MS analysis chromatogram of BR-2 mulberry latex showing peaks of bioactive phytoconstituents.

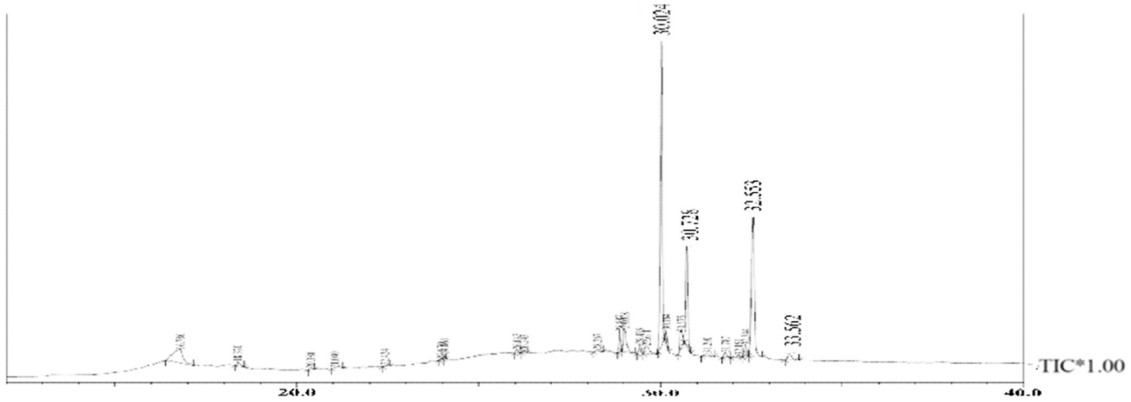


Figure 5. GC−MS analysis of chromatogram of S-1 mulberry latex showing peaks of bioactive phytoconstituents.

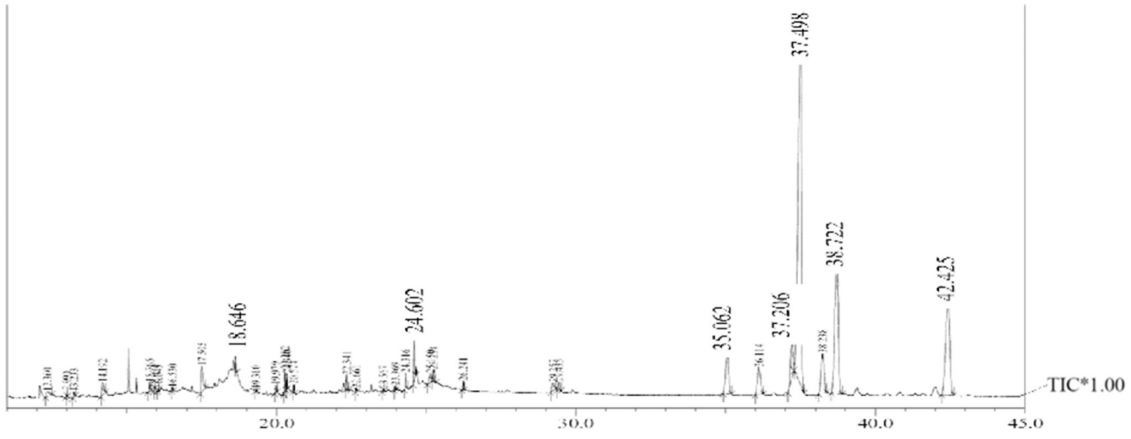


Figure 6. GC−MS analysis of chromatogram of AR-14 mulberry latex showing peaks of bioactive phytoconstituents.

flaky occurrences, the S-1(Figure 2c,d) and AR-14 (Figure 2e,f) lattice morphology showed irregularities due to the appearance of cracks, erosion, and warts, which were visible at 1000 and 3500× magnifications.

The EDX results for the latex varieties BR-2 (Figure 3a), S-1 (Figure 3b), AR-14 (Figure 3c), and S-146 (Figure 3d) showed the presence of diversified elements. Carbon (C) was the most abundant element found in BR-2 latex, followed by oxygen (O), platinum (Pt), potassium (K), chlorine (Cl), and sulfur (S). Similarly, C was the most abundant element in S-1 latex, followed by O, zirconium (Zr), Cl, K, and magnesium (Mg). In the case of AR-14 latex, the major element identified was O followed by K, Cl, phosphorus (P), S, and Mg. Similarly, S-146 latex was dominated by O, followed by Zr, K, Cl, and Mg.

3.2. Compositional Analysis of Mulberry Lattices. BR-2 (Figure 4), S-1 (Figure 5), AR-14 (Figure 6), and S-146 (Figure 7) latex extracts were analyzed using a GC−MS chromatogram, which revealed 30, 24, 33, and 29 compounds, respectively (Table 1). Table 1 displays the phytoconstituents in the latex varieties under study along with their corresponding retention times (RTs) and concentrations (%). The predominant compounds observed in the BR-2 variety were butanoic acid (14.49%) followed by acetic acid (11.06%), Lup-20(29)-en-3-ol, acetate (3.β.) (9.87%), 2,3-dihydro-benzofuran (9.32%), 2-methoxy-4-(1-propaneyl)-acetate (8.55%), and quinic acid

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

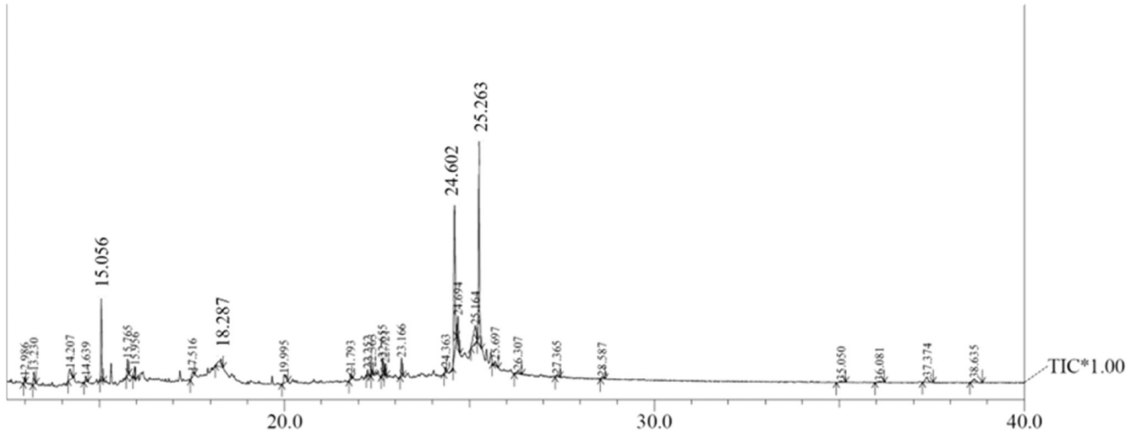


Figure 7. GC−MS analysis of chromatogram of S-146 varieties of mulberry latex showing peaks of bioactive phytoconstituents.

(6.64%). Further, the major compounds found in the S-1 variety were acetic acid, 17-(1,5-dimethyl-hex-4-enyl)-4,4,1 (36.12%) followed by lup-20(29)-en-3-ol, acetate (3.β.) (22.84%), and methyl-2,2-dimethyl-3-hydroxypropionate (7.51%). In the case of AR-14, the chromatogram observed three prominent peaks at retention times 37.49, 38.72, and 42.42, corresponding to compounds Lanosta-8,24-dien-3-ol (42.89%), Lup-20(29)-en-3-ol (17.26%), and Germanicol acetate (13.49%) respectively. Other major components found at Rt 35.06, 38.23, 37.20, and 36.11 were Lanosterol (4.40%), Glutinol (4.26%), Olean-12-en-3-ol (3.17%), and Lupeol (3.06%), respectively. Furthermore, the GC−MS chromatogram of the methanolic S-146 variety of mulberry latex extract detected 29 compounds as shown in Figure 7. Two elevated peaks of Phenol (28.16 and 23.75%) were observed at two different RTs (25.26 and 24.604). Further, other notable constituents at RTs 15.056, 25.16, 14.20, and 18.28, respectively, respond to diethyl phthalate (8.53%), salicin (6.02%), D-allose (3.42%), and benzyl (trimethylsilylmethyl) amine (18.28%). The remaining compounds present in these varieties are shown in Table 1, and the chemical structures of major compounds are shown in Figure 8.

3.3. FTIR Analysis. The presence of functional groups in the mulberry latex samples (BR-2, S-1, AR-14, and S-146) was confirmed by the FTIR analysis, as displayed in Figure 9. These spectra exhibit specific peaks that are suggestive of the well-known structural properties of latex. For instance, broad peaks at 3448.9, 3441.8, 3422.4, and 3420.2 cm^{-1} signify the stretching of the O−H group and the presence of alcohols and phenols. This peak indicates the presence of water molecules too, as the O−H stretching vibration is a characteristic of water molecule. Additionally, the intensity and shape of the peak indicate the concentration and environment of these functional groups.[26] Similarly, the peaks at 2934.7, 2933.3, 2929.9, 2929.8, 2858.3, 2857.3, and 2856.1 cm^{-1} connote the existence of C−H stretching vibrations in Amide B, indicating the presence of hydrogen atoms bonded to carbon atoms in the molecule. The peak at 2096 cm^{-1} is allied with the (OH) stretch of the carboxylic acid, and the peak at 2086.4 cm^{-1} is characteristic of the N−C−S isothiocyanate group. Furthermore, the peaks at 1643.6, 1641.9, 1641.5, and 1641.1 cm^{-1} exhibit C═C and C═O stretching, which suggest the presence of an alkene and carboxylic acids. The peaks at 1382.8, 1382.5, 1378.5, and 1368.0 cm^{-1} denote the O−H and −C−O bending which connotes the presence of alcohols, phenols, and amide III group. The peaks at 1243.8 and 1241 cm^{-1} represent the O−C stretching that indicates carboxylic acid and derivatives. The stretching vibration bands at 1077.6, 1076.6, 1076.4, and 1076.2 cm^{-1} indicate the C−OH and C═O group, which indicates the presence of phenols as well as primary and secondary alcohols. The stretching of the C−Br band at 647.0 and 636.0 cm^{-1} reflects halo compounds. The peak at 669.1 cm^{-1} indicates the presence of C−Br stretching in halogen compounds and the C−I stretching vibrations in halo compounds. The peak at 598.6 cm^{-1} represents aliphatic iodo compounds, C−l stretch.[27−31]

3.4. DPPH Assay. Antioxidants hinder the oxidation process by interacting with free radicals and act as a scavenger for reactive species. 2,2′-Diphenyl-1-picrylhydrazyl is a free radical that remains stable and accepts either an electron or a hydrogen radical, resulting in its transformation into a stable diamagnetic molecule. DPPH, characterized by an odd electron in its structure, is commonly employed to assess the radical scavenging activity. This assay hinges on the capacity of DPPH, a persistent free radical, to lose its coloration in the presence of antioxidants.[32,33] As an antioxidant donates an electron to DPPH, it loses its coloration, which can be quantified through changes in absorbance at 517 nm. The radical scavenging capability of various latex varieties was assessed by measuring their ability to counteract DPPH free radicals, with ascorbic acid serving as the standard. The result of the DPPH assay is presented in Figure 10. The antioxidant analysis of tested latex samples revealed that BR-2 latex was reported to highest antioxidant properties, with DPPH radical scavenging activity (DRSA) of 75 ± 1.2%, followed by S-146 and AR-14 varieties showing DRSA64.6 and 52.9 ± 1.0% respectively. However, S-1 latex showed low antioxidant activity, as the DRSA% of this latex was observed to be 34.1 ± 0.4%, which was less than 50%.

3.5. Antimicrobial Analysis. The antimicrobial properties of BR-2, S-1, AR-14, and S-146 mulberry lattices were assessed according to their zone of inhibition against selected micro-organisms (Table 2 and Figure 11). Tetracycline and fluconazole were used to compare the activity of the obtained results (zone of inhibition; Table S1). In the case of BR-2 latex, the highest inhibition zone diameter was found in *C. albicans* and *S. aureus*, with both strains having a diameter of 28 mm, followed by a 25.5 ± 0.70 mm zone of inhibition for *S. typhi*. Furthermore, when compared to common medications tetracycline and fluconazole, BR-2 latex performed better than these drugs except for *A. niger*. S-1 latex exhibited activity against *S. typhi* with a zone of inhibition of 15 ± 0.70 mm. Further, the rest of the tested strains were found to be ineffective by S-1 latex. AR-14

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

Table 1. Compositional Profile of Mulberry Latex Varieties through GC−MS

varieties	retention time	name of compounds	area %
BR-2	9.659	2,3-dihydrobenzofuran	9.32
	10.734	phenol,2-methoxy-4-(2-propenyl)-acetate	1.91
	11.869	benzene methanol, 4-hydroxy	3.68
	13.010	1,6-anhydro-β-D-glucopyranose	3.50
	14.062	cryptomeridiol	1.09
	14.467	1,3,4,5-tetrahydroxycyclohexanecarboxylic acid	6.64
	16.338	methyl-2,2-dimethyl-3-hydroxypropionate	4.00
	16.747	hexadecanoic acid, methyl ester	0.25
	17.226	DL-aspartic acid, -acetyl-, dimethyl ester	6.78
	18.352	2,7-nonadienoic acid, 3,8-dimethyl-, methyl ester, (Z)-	0.88
	18.630	triacontanoic acid, methyl ester	0.11
	20.370	eicosanoic acid, methyl ester	0.25
	20.907	myristic acid, 9-hexadecenyl ester, (Z)-	1.04
	21.981	pentacosanoic acid, methyl ester	0.80
	23.017	1,2-bis(4-methoxyphenyl)-*N*,*N*, *N*′,*N*′-tetramethylethane-1,2-diamine	2.52
	23.413	butanoic acid, 3-methyl-, 2-methoxy-4-(2-propenyl) phenyl ester	14.49
	24.060	acetyl eugenol	8.55
	26.027	2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	0.22
	28.285	spirost-5-en-3-ol, (3 β, 25r)-	0.48
	29.023	lanosterol	2.12
	29.439	lup-20(29)-en-3-yl acetate	0.68
	29.611	lupeol	1.41
	30.016	acetic acid 17-(1,5-dimethyl-hex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1h-cyclopenta	11.06
	30.169	13,27-cycloursan-3-ol, acetate, (3β,13β,14β)-	0.49
	30.608	lanosta-8,24-dien-3-ol, acetate, (3β)-	0.91
	31.286	6a,14a-methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy	0.49
	31.789	betulinaldehyde	0.41
	32.350	lup-20(29)-ene-3,28-diol, (3β)	0.75
	32.542	3-acetoxy-12-ursanol	9.87
	33.575	1,1,4,7-tetramethyldecahydro-1h-cyclopropa[*e*]azulen-4-ol	0.96
S-1	16.786	methyl-2,2-dimethyl-3-hydroxypropionate	7.51
	18.370	[1,1′-bicyclopropyl]-2-octanoic acid, 2′-hexyl-, methyl ester	0.69
	20.380	eicosanoic acid, methyl ester	0.21
	21.040	exo-2-hydroxycineole	0.15
	22.424	meadowlactone	0.24
	23.970	3-(2-methyl-5-nitro-2*H*-[1,2,4] triazol-3-Ylamino)-propionic acid methyl ester	0.11
	24.061	squalene	0.29
	26.043	solanesol	0.48
	26.243	24-norursa-3,9(11),12-t riene	0.24
	28.267	spirost-5-en-3-ol, (3β,25r)	0.45
	28.887	lanosta-8,24-dien-3-ol, acetate, (3β)	1.98
	29.018	lanosterol	3.74
	29.435	lup-20(29)-en-3-yl acetate	1.07
	29.611	lupeol	2.23
	30.024	acetic acid 17-(1,5-dimethyl-hex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1h-cyclopenta	36.12
	30.164	13,27-cycloursan-3-ol, acetate, (3β,13β,14β)	1.18
	30.575	isopropyl-3a,5a,8,8,11b,13a-hexamethyl-2,3,3a,4,5,5a,5b,6,8,9,10,11,11a,11b,12,13,13a,13b-octadecahydro-1*H*-cyclopenta[A] chrysen-9-ol	2.99
	30.728	lup-20(29)-en-3-ol, acetate, (3β)	12.27
	31.290	betulin	0.70
	31.787	lup-20(29)-ene-3,28-diol, (3β)	0.85
	32.151	A′-neogammacer-22(29)-en-3-ol, acetate, (3β,21β)-	0.61
	32.344	3-acetoxy-12-ursanol	1.65
	32.553	lupenyl acetate	22.84
	33.562	lupeol acetate	1.41
AR-14	12.360	benzene methanol, 4-hydroxy-	0.52
	12.990	*cis*-5,8,11,14,17-eicosapentaenoic	0.06
	13.233	1-piperidinecarboxaldehyde	0.21
	14.192	1,6-anhydro-β-D-glucopyranose	0.95

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

Table 1. continued

varieties	retention time	name of compounds	area %
	15.765	methyl (3-oxo-2-pentylcyclopentyl) acetate	0.25
	15.930	methyl 3-hydroxytetradecanoate	0.03
	16.045	1-oxaspiro [4.5] dec-3-en-2-one, 6-isopropyl-9-methyl-	0.08
	16.530	tridecanoic acid, methyl ester	0.08
	17.505	1,3-dimethyl-3-*n*-butyldiaziridine	2.03
	18.646	hexadecanoic acid, methyl ester	0.43
	19.310	tetradecanoic acid, ethyl ester	0.06
	19.979	2-dodecanone, 12-(5-hydroxy-1,6-dimethyl-2-piperidinyl)-	0.28
	20.282	9,12-octadecadienoic acid (*Z*,*Z*)-, methyl ester	0.53
	20.340	ethyl oleate	0.38
	20.574	methyl stearate	0.20
	22.341	eicosanoic acid, methyl ester	0.48
	22.661	hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (*E*)-	0.09
	23.567	10-nonadecanone	0.07
	23.969	docosanoic acid, methyl ester	0.11
	24.316	meadowlactone	0.52
	24.602	phenol,2-methoxy-4-(1-propenyl)-acetate	1.66
	25.150	salicin	0.67
	25.254	acetyl eugeno	0.37
	26.241	2,6,10,15,19,23-pentamethyl-2,6,18,22-tetracosatetraen-10,1	0.26
	29.252	adipic acid, monoamide, *N*-methyl-*N*-phenyl-, isobutyl ester	0.71
	29.433	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23	0.42
	35.062	lanosterol	4.40
	36.114	lupeol	3.06
	37.206	olean-12-en-3-ol, acetate, (3β)-	3.17
	37.498	lanosta-8,24-dien-3-ol, acetate, (3β)-	42.89
	38.238	glutinol	4.26
	38.722	lup-20(29)-en-3-ol, acetate, (3β)-	17.26
	42.425	germanicol acetate	13.49
S-146	12.986	10−12-pentacosadiynoic acid	0.61
	13.230	8-azabicyclo[3.2.1]octan-3-ol, 6-methoxy-8-methyl-	1.88
	14.207	D-allose	3.42
	14.639	2-buten-1-ol, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-	0.60
	15.056	diethyl phthalate	8.53
	15.765	methyl (3-oxo-2-pentylcyclopentyl)acetate	1.76
	15.956	1-(4isopropylphenyl)-2-methylpropyl acetate	1.58
	17.516	1,6-hexanediamine, 2,2,4-trimethyl-	1.11
	18.287	benzyl(trimethylsilylmethyl)amine	3.00
	19.995	DL-norleucine amide, *N*,*N*,*N*′*N*′-tetramethyl-	2.54
	21.793	succinic acid, di(neryl) ester	0.70
	22.252	2-((8*Z*,11*Z*)-heptadeca-8,11-dien-1-yl)-4,5-dihydrooxazole	0.63
	22.363	2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	0.86
	22.655	octanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	1.82
	22.721	geranyl oleate	1.21
	23.166	dotriacontyl propyl ether	2.22
	24.363	3-methyl-2oxaspiro[5.7]tridecane-1,7-dione	0.64
	24.604	phenol,2-methoxy-4-(1-propenyl)-acetate	23.75
	24.694	butanoic acid	2.87
	25.164	salicin	6.02
	25.263	acetyl eugeno	28.16
	100370	phenol,4-(1-piperidin-1-ylcyclohexylmethyl)	0.61
	26.307	β-dglucopyranoside, 2-(hydroxymethyl)phenyl,	0.76
	27.365	butanoic acid, 2-methyl-, 2-methoxy-4-(2-propenyl)phenyl	0.30
	28.587	but-2-enoicacid,amide, 3-methyl-N-(3,4-dimethoxyphenethyl)-	0.44
	35.050	9,19-cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, (3 β,4α,5α)	0.50
	36.081	cedran-diol, (8S,14)-	0.39
	37.374	lanosta-8,24-dien-3-ol, acetate, (3β)-	1.78
	38.635	lup-20(29)-en-3-ol, acetate, (3β)-	1.30

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

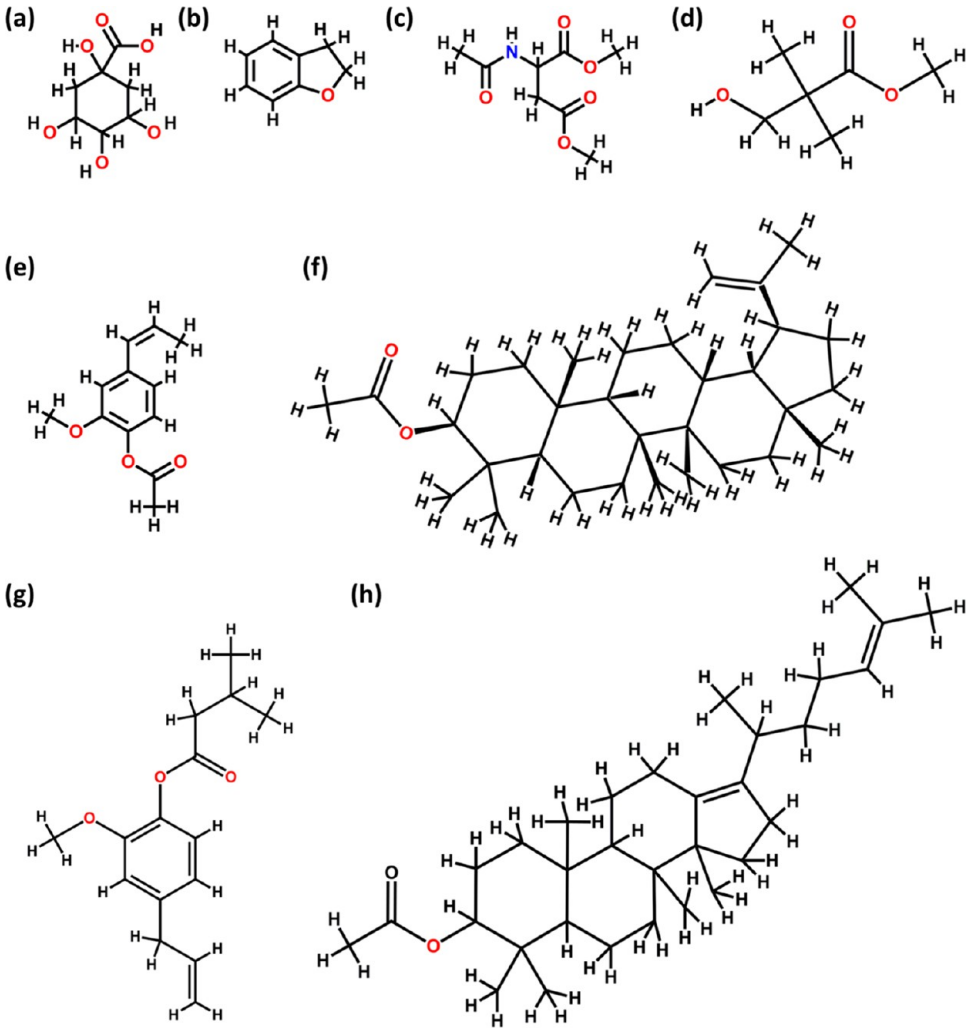


Figure 8. Chemical structure of major phytoconstituents reported in BR-2 and S-1, AR-14, and S-146 lattices. (a) 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid; (b) 2,3-dihydro-benzofuran; (c) dimethyl-2-(acetylamino)succinate; (d) methyl-2,2-dimethyl-3-hydroxypropionate; (e) phenol,2-methoxy-4-(1-propenyl)-acetate; (f) Dammara-13(17),24-dien- 3-yl acetate; (g) butanoic acid, 3-methyl-, 2-methoxy-4-(2-propenyl)phenyl ester; (h) Lup- 20(29)-en-3-ol, acetate, (3.*β*.)-.

and S-146 latexes did not show any inhibition against tested bacterial strains. However, both the varieties showed maximum zone of inhibition of 13.5 ± 0.7 and 13.5 ± 0 mm on *C. albicans* at 40 μL dilutions, respectively, and yet have failed to inhibit the growth of *A. niger* at any of the mentioned dilutions.

4. DISCUSSION

In the present study, we selected latex from four mulberry varieties to characterize and screen their activity to be used as potential herbal drug candidates. The surface morphology of lattices revealed a noticeable contrast among the surfaces of the BR-2, S-1, AR-14, and S-146 lattices. BR-2 latex had uniformity in its structure, with hardly any cracks and surface erosion visible. On the other hand, the surface of the other three lattices appeared cracked and eroded. That might be due to the low mechanical resistance of latex to small stresses that lead to cracks in the surface of latex.[34,35] Additionally, surface chlorination, microbial attack, and loss of oligomer have also been found to be associated with the erosion of the surface and the formation of voids on the latex surface.[36,37]

Elemental analysis of tested latex varieties revealed the presence of Cl, K, Mg, etc. These elements play a crucial role in overall growth and plant developmental physiology.[38−40] Interestingly, we reported the presence of zirconium (Zr) in S-1 and S-146 lattices; it is a transition metal having both stable and radioactive isotopes. Due in part to its prominence in the discussion about the mounting anthropogenic impact on the environment, this metal has drawn significant attention as a key contaminant of concern. Unlike other heavy metals (such as lead, cadmium, and nickel), the mechanism by which Zr enters plants is poorly understood. Soil-root transfer through ion channels might be the primary mechanism through which Zr is absorbed by plants.[41,42] This phenomenon might be the reason for the presence of Zr in the S-1 and S-146 latex.

The examined lattice compositional analysis revealed the presence of a variety of bioactive phytochemicals. These bioactive compounds could be responsible for the antimicrobial and antioxidant properties of the tested latex varieties of mulberry. The uses of these phytoconstituents have been well documented in previously reported studies. For example, butanoic acid, acetic acid, and phenols have been reported to be strong antibacterial agents.[43−46] Lanosterol, a tetracyclic triterpenoid, has immunomodulatory and cataract-preventing properties.[47,48] Quinic acid is well known for its therapeutic

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

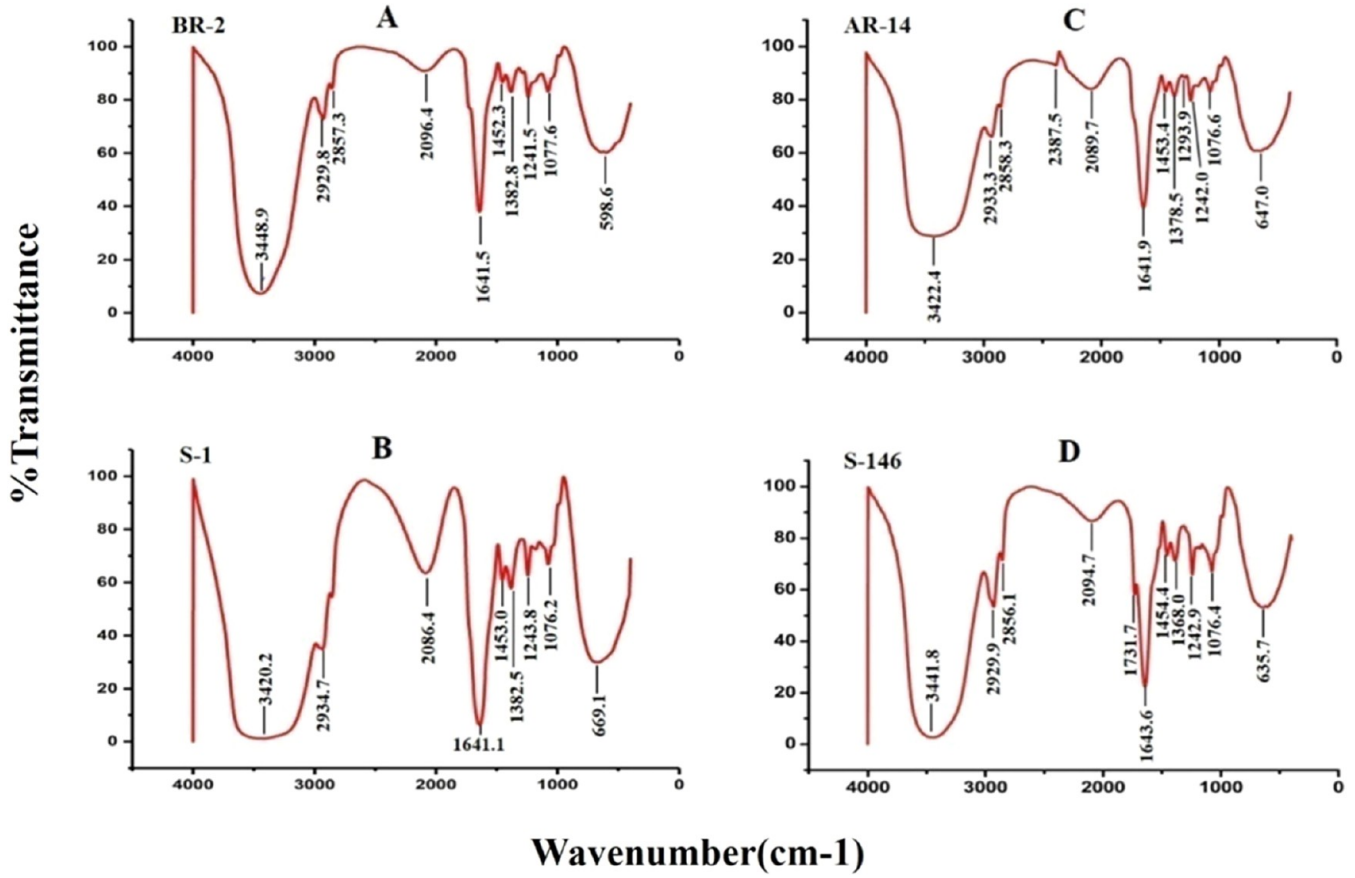


Figure 9. FTIR spectra of mulberry lattices (A) BR-2, (B) S-1, (C) AR-14, and (D) S-146.

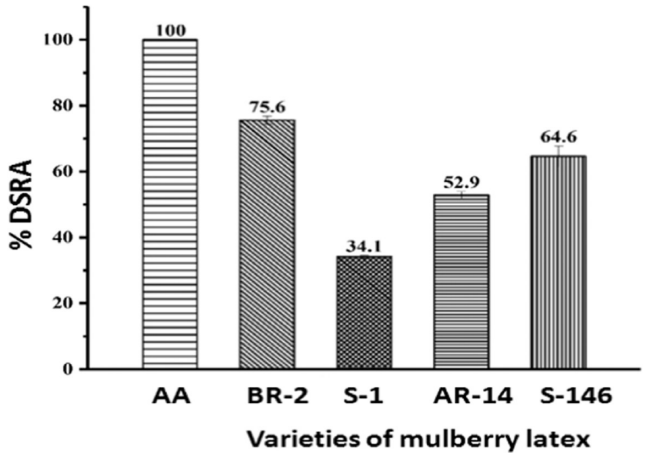


Figure 10. Comparative evaluation of DPPH radical scavenging activity (DRSA) of BR-2, S-1, S-146, and AR-14 latex samples with reference to ascorbic acid (AA) as a standard.

properties, including radical scavenging, antioxidant, antidiabetic, antibacterial, antiviral, and antiaging properties.[49] Lupeol, a major lupine-type triterpene, and its other derivatives including Lup-20(29)-en-3-yl acetate (Lupeol acetate) are reported to have anti-inflammatory, anticancer, antiprotozoal, antimicrobial, chemo-preventive, and neuroprotective activities.[50−53] Lanosta-8,24-dien-3-ols, a family of tetracyclic terpenoids, are medicinally used as anti-inflammatory, anticancer, and analgesic drugs.[54] Numerous natural products and synthetic compounds have the widespread structural motifs benzofuran and 2,3-dihydrobenzofuran, and many of these show a range of therapeutic properties, such as antiviral, antibacterial, and antiangiogenic.[55] Phenol,2-methoxy-4-(1-propenyl)-acetate is one of the synonyms of eugenol acetate. Eugenol acetate has been reported to exhibit significant antifungal, antibacterial, antiviral, and antioxidant properties.[56−58] D-Allose is a rare sugar that has strong anti-inflammatory and antioxidant properties, as well as an antihypertensive agent, and can protect against ischemia-reperfusion injury of the liver.[59] Salicin is a precursor for the synthesis of salicylic acid and shows valuable biological activities like anti-inflammatory, antitumor, and antiangiogenic.[60] In addition to being a plasticizer and softener, medicinal coating, cosmetic, additive, and pesticide, diethyl phthalate also has antibacterial properties.[61] The occurrence of these phytochemicals in the methanolic extracts of latex varieties may indicate a wide range of therapeutic benefits.

FTIR analysis revealed the presence of diversified functional groups in the tested mulberry lattices. The presence of carbonyl groups suggests the occurrence of oxidation processes in the latex samples. Additionally, the presence of hydroxyl and alkene groups indicates the potential for cross-linking reactions to occur within the latex structure. These groups are characteristic of latex and play a significant role in determining the properties and applications of latex materials. Many investigations showed that the existence of the stated functional groups in plant latex is caused by a complex mixture of substances, including proteins, alkaloids, sterols, fatty acids, starches, sugars, glycolipids, gums, and enzymes (Eid et al.; Jing et al.; Kain et al.; Nandiyanto et al.; Ranade et al.; Samrot et al.).[27−31,62] Therefore, the functional group analysis of mulberry lattices indicated the presence of

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

Table 2. Summary of the Diameter of Zone of Inhibition (mm) of Mulberry Latex Varieties

	zone of inhibition (mm) of latexes at various dilutions (10–40 μL) against microorganisms																			
	A. niger				*C. albicans*				*S. typhi*				*S. aureus*							
	10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
BR-2					20.5 ± 0.70	24.0 ± 0.0	26.0 ± 0.0	28.0 ± 0.0	16.0 ± 0.0	18.0 ± 0.0	22.5 ± 0.70	25.5 ± 0.70	20.5 ± 0.70	24.0 ± 0.0	26.0 ± 0.0	28.0 ± 0.0				
S-1											13.0 ± 0.0	15.5 ± 0.70								
AR-14								13.5 ± 0.70												
S-146					10.0 ± 0.0	11.0 ± 0.0	11.5 ± 0.0	13.5 ± 0.0												

bioactive secondary metabolites, which can be correlated with the results of GC−MS.

Different techniques are used to measure antioxidant activity, but the most popular ones are those that produce free radical species that are then neutralized by antioxidant agents. In this study, we investigated the radical scavenging activity of mulberry lattices using the DPPH assay, and significant radical scavenging abilities were observed in BR-2, S-146, and AR-14 latex varieties. Secondary metabolites found in these lattices may directly contribute to the antioxidative impact. Similar results were obtained from the latex of other tested plants such as *Ficus carica*,[63] *Thevetia peruviana*,[64] *Euphorbia neriifolia*,[65] and[65] *Cynanchum acutum*.[66]

The existing antimicrobial therapies have been seriously endangered by the growth and spread of drug-resistant bacterial and fungal infections. Having said this, screening for news sources of antimicrobial compounds has become necessary at present. Since plants produce a wide range of bioactive chemicals with proven medicinal capabilities, they can be a potential source of new molecules possessing antimicrobial properties.[67,68] Likewise, in our study, we have reported the strong antimicrobial activity of BR-2 mulberry latex against tested microorganisms except for *A. niger*. It is worth mentioning that the antimicrobial action of BR-2 latex was equivalent to that of commercial drugs (tetracycline and fluconazole). Moreover, in contradistinction to prior antimicrobial investigations on latex derived from diverse plant species, such as *T. peruviana* latex, which demonstrated inhibitory efficacy against *S. typhi* (20.67 mm) and *C. albicans* (21 mm), BR-2 exhibited superior outcomes against *S. typhi* (25 mm), *S. aureus* (28 mm), and *C. albicans* (28 mm).[69] Similarly, the latex of *Euphorbia antiquorum* showed inhibition against *C. albicans* (10 mm),[70] and latex from *Calotropis procera* has failed to inhibit the growth of *S. aureus* (Rajan et al., 2019). On the other hand, latex extracted from the leaf of *Aloe weloensis* showed antibacterial activity against *S. aureus* (25 mm); however, this trend is lower than our findings.[71]

The observed antimicrobial efficacy of the tested mulberry lattices is attributed to the presence of secondary metabolites that are reported to be present in the lattices, as demonstrated by the FTIR and GC−MS analysis. Previously, it has been reported that the structure of the compounds plays a significant role in the level of robustness of their antimicrobial activity.[72−74] As an example, it has been previously demonstrated that 2,3-dihydrobenzofuran and its derivatives exhibit noteworthy antimicrobial properties, a phenomenon associated with the substituent group positioned at the third position. Taking the derivatives into account, it was observed in one of the derivatives that the oxygen atom in the benzene ring establishes a hydrogen bond with the protein of the enzyme and binds to it as an antifungal drug.[75] Similarly, another secondary metabolite present in the latex is eugenol acetate or acetyl eugenolester whose carbonyl group was reported to be the active functional group that interacts and inhibits the activity of *Mt*PknE, anSer/Thr protein kinase of *Mycobacterium tuberculosis* which is one of the potential targets of antibiotic drugs. Further, the authors reported that acetyl eugenol demonstrated to have significant antimicrobial activity against *S. aureus* and *S. typhi*.[76] In a like manner, Cryptomeridiol,[77] a sesquiterpenoid, and Lanosterol along with its derivatives,[78,79] have previously been reported to exhibit significant antimicrobial activity against *S. aureus*. In addition to the aforementioned secondary metabolites, there exists a reservoir of antimicrobial secondary metabolites present in the latex, including but not restricted to 1,6-anhydro-β-D-

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

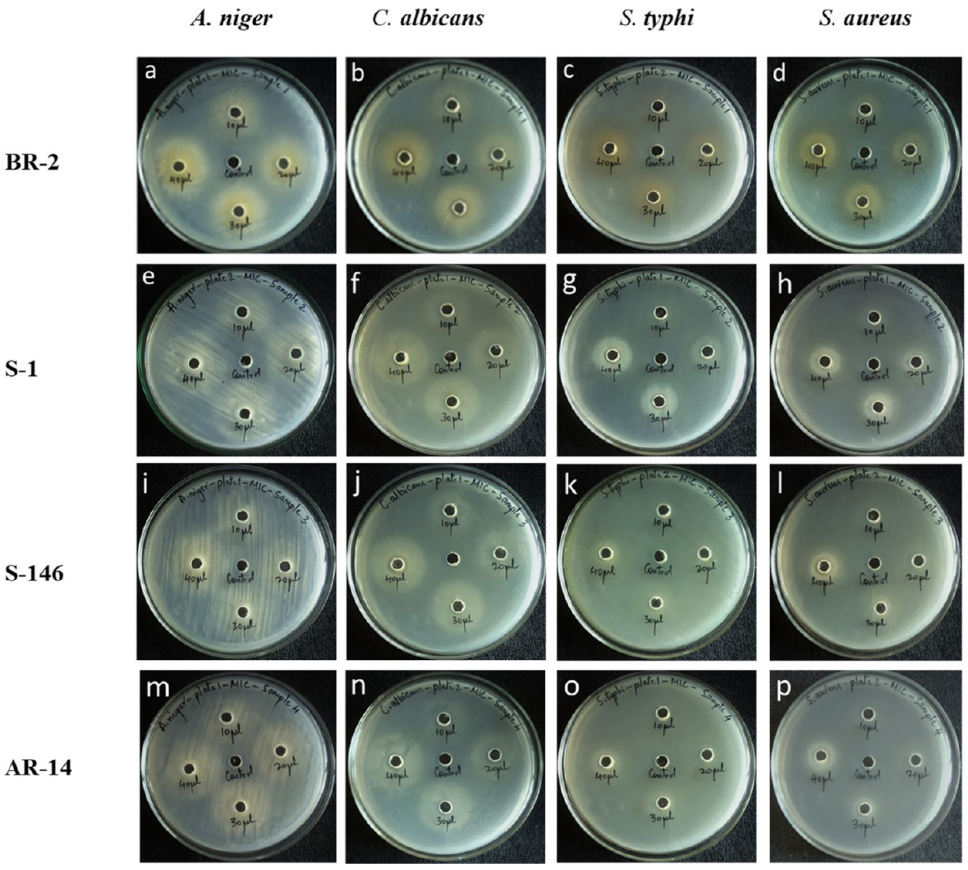


Figure 11. Antimicrobial evaluation using agar plate assay targeting *A. niger*, *C. albicans*, *S. typhi*, and *S. aureus* for (a–d) BR-2 Latex; (e–h) S-1 Latex; (i–l) S-146 Latex; and (m–p) AR-14 latex, respectively. The central disk in each plate represents the negative control.

glucopyranose, hexadecanoic acid methyl ester, lupeol, lupeol acetate, and glutinol, which have also been documented to exhibit antimicrobial properties, as outlined in Table 3.[80−84]

Table 3. List of the Phytochemicals of Mulberry Lattices with Antimicrobial Properties

s.no.	compounds	activities
1	acetic acid	antibacterial[45]
2	2,3-dihydrobenzofuran	antifungal[75]
3	acetyl eugenol	antimicrobial[76]
4	cryptomeridiol	antifungal[77]
5	lanosterol	antimicrobial[78,79]
6	hexadecanoic acid, methyl ester	antimicrobial[83]
7	lupeol	antimicrobial[84]
8	lupeol acetate	antimicrobial[80]
9	glutinol	antimicrobial[81]

5. CONCLUSIONS

In the current study, we have successfully extracted, characterized, and analyzed different mulberry lattices to know their antimicrobial and antioxidant properties. Among the tested mulberry lattices, BR-2 latex had the highest antimicrobial activity against tested strains *C. albicans* (28 mm), *S. typhi* (25.5 mm), and *S. aureus* (28 mm), along with robust radical scavenging activity (75%), when compared with other lattices. Such activities indicate the synergistic effect of the bioactive phytoconstituents present in mulberry latex. This study also indicates that several secondary metabolites present in these lattices could be applied to the development of novel therapeutic agents against various communicable as well as noncommunicable diseases. Hence, these mulberry lattices are considered to be an effective and alternative treatment for microbial as well as to treat oxidative stress. Further validation can be done after cytotoxicity and in vivo studies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06069.

Diameter of zone of inhibition (mm) of standard drug tetracycline and fluconazole (Table S1) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Venkatesh Kumar R − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*; Email: drvenkateshkumarr@gmail.com

Authors

Sunil Babu Gosipatala − *Departmentof Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*

Ram Kumar − *Department of Zoology, Shri Venkateshwara University, Gajraula, Uttar Pradesh 244236, India*

Devika Srivastava − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*; orcid.org/0000-0002-2309-2636

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

Vandana Singh − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Kusumala Suman − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Deepak Kumar Tripathi − *Department of Biosciences and Bioengineering, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand 247667, India;* orcid.org/0000-0002-6779-4234
Abhishek Verma − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Akash Mishra − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Karan Kumar Vishwakarma − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Stuti Annapurna Singh − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Tripti Pandey − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Sanskrati Agarwal − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Mohd Elyies − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Ishani Singh − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Pinky Kumari Sah − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Chaya Sharma − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Rishabh Parag − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Pragya Saxena − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Akanksha Raj − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Anshika Tripathi − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Poonam Devi − *Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226025, India*
Krishna Mohan Poluri − *Department of Biosciences and Bioengineering, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand 247667, India;* orcid.org/0000-0003-3801-7134

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c06069

Author Contributions

V.K.R. contributed to conceptualization, supervision, and correction of the manuscript; D.S., V.S., K.S., D.K.T., A.M., and A.V. conducted experiment, data analysis, and interpretation; K.K.V., S.A.S., T.P., S.A., M.E., I.S., P.K.S., C.S., R.P., P.S., A.R., A.T., P.D., O.E.A.-S., R.M.A., S.O.M., A.M.E.-D., and A.E.H. contributed to the investigation; all authors contributed to writing—original draft preparation; all authors contributed to writing—review and editing; SBG., R.K., and K.M.P. contributed to supervision. The final manuscript was read and approved by all authors.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the University Sophisticated Instrument Centre of BBAU and the Institute Instrumentation Centre, IIT Roorkee, for providing the instrumentation facilities.

■ REFERENCES

(1) Baciu, E.-D.; Baci, G.-M.; Moise, A. R.; Dezmirean, D. S. A Status Review on the Importance of Mulberry (Morus spp.) and Prospects towards Its Cultivation in a Controlled Environment. *Horticulturae* **2023**, *9*, No. 444.
(2) Acharya, R.; Bagchi, T.; Gangopadhyay, D.Mulberry as a Valuable Resource for Food and Pharmaceutical Industries: A Review. In *Medicinal Plants*; IntechOpen, 2022.
(3) Jan, B.; Parveen, R.; Zahiruddin, S.; Khan, M. U.; Mohapatra, S.; Ahmad, S. Nutritional constituents of mulberry and their potential applications in food and pharmaceuticals: A review. *Saudi J. Biol. Sci.* **2021**, *28* (7), 3909−3921.
(4) Rohela, G. K.; Shukla, P.; Muttanna, R.; Kumar, S.; Chowdhury, R. Mulberry (Morus spp.): An ideal plant for sustainable development. *Trees, Forests, People* **2020**, *2*, No. 100011.
(5) Maqsood, M. R.; Saeed, A.; Sahar, A.; Khan, M. I. Mulberry plant as a source of functional food with therapeutic and nutritional applications: A review. *J. Food Biochem.* **2022**, *46* (11), No. e14263.
(6) Yuan, Q.; Zhao, L. The Mulberry (Morus alba L.) Fruit—A Review of Characteristic Components and Health Benefits. *J. Agric. Food Chem.* **2017**, *65* (48), 10383−10394.
(7) Mandal, A. Nutraceutical and Medicinal Property of Mulberry Fruits: A Review on Its Pharmacological Potential. *Preprints* **2020**, No. 2020040105.
(8) Ramappa, V. K.; Srivastava, D.; Singh, P.; Kumar, U.; Kumar, D.; Gosipatala, S. B.; Saha, S.; Kumar, D.; Raj, R. Mulberries: A Promising Fruit for Phytochemicals, Nutraceuticals, and Biological Activities. *Int. J. Fruit Sci.* **2020**, *20* (3), S1254−S1279.
(9) Sarkhel, S.; Manvi, D.; Ramachandra, C. T. Nutrition importance and health benefits of mulberry leaf extract: A review. *J. Pharmacogn. Phytochem.* **2020**, *9* (5), 689−695.
(10) Hua, W.; Zhu, J.-J.; Liu, X.-Q.; Feng, W.-H.; Wang, Z.-M.; Yan, L.-H. Review of bioactive compounds from root barks of Morus plants (Sang-Bai-Pi) and their pharmacological effects. *Cogent Chem.* **2016**, *2* (1), No. 1212320.
(11) Shahana, S.; Pratima, A.; Nikalje, G. Phytochemistry and bioactivity of Morus alba (Mulberry) plant: A comprehensive review. *Asian J. Pharm. Pharmacol.* **2019**, *5* (2), 207−217.
(12) Zhang, H.; Ma, Z. F.; Luo, X.; Li, X. Effects of mulberry fruit (Morus alba L.) consumption on health outcomes: A mini-review. *Antioxidants* **2018**, *7*, No. 69.
(13) Bajirao, K. D.; Pallavi, J. The Properties and Nutritional Uses Of Mulberry (Morus Alba): A Review. *Int. J. Creat. Res. Thoughts* **2022**, *10*, d875−d886.
(14) Konno, K.; Ono, H.; Nakamura, M.; Tateishi, K.; Hirayama, C.; Tamura, Y.; Hattori, M.; Koyama, A.; Kohno, K. Mulberry latex rich in antidiabetic sugar-mimic alkaloids forces dieting on caterpillars. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1337−1341.
(15) Hirayama, C.; Konno, K.; Wasano, N.; Nakamura, M. Differential effects of sugar-mimic alkaloids in mulberry latex on sugar metabolism and disaccharidases of Eri and domesticated silkworms: Enzymatic adaptation of Bombyx mori to mulberry defense. *Insect Biochem. Mol. Biol.* **2007**, *37* (12), 1348−1358.
(16) Wasano, N.; Konno, K.; Nakamura, M.; Hirayama, C.; Hattori, M.; Tateishi, K. A unique latex protein, MLX56, defends mulberry trees from insects. *Phytochemistry* **2009**, *70* (7), 880−888.

(17) Kitajima, S.; Kamei, K.; Taketani, S.; et al. Two Chitinase-like proteins abundantly accumulated in latex of mulberry show insecticidal activity. *BMC Biochem.* **2010**, *11*, No. 6.
(18) Zhang, H. Y.; Chen, W.; Wang, T. C.; Liu, J. Z. Determination of DNJ of Mulberry Latex and Evaluation of the Hypoglycemic Effect on Mice. *Adv. Mater. Res.* **2011**, *175*−*176*, 36−40.
(19) Ramappa, V. K.; Singh, V.; Srivastava, D.; Kumar, D.; Verma, A.; Verma, D.; Fatima, E.; Chaudhary, P.; Kumar, U.; Kumar, D. Fabrication of mulberry leaf extract (MLE)-and tasar pupal oil (TPO)-loaded silk fibroin (SF) hydrogels and their antimicrobial properties. 3 *Biotech* **2023**, *13*, No. 37.
(20) Olivia, N. U.; Goodness, U. C.; Obinna, O. M. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. *Future J. Pharm. Sci.* **2021**, *7*, No. 59.
(21) Kiwaan, H. A.; El-Mowafy, A. S.; El-Bindary, A. A. Synthesis, spectral characterization, DNA binding, catalytic and in vitro cytotoxicity of some metal complexes. *J. Mol. Liq.* **2021**, *326*, No. 115381.
(22) Ko, E.-Y.; Kim, D.; Roh, S. W.; Yoonc, W.-J.; Jeon, Y.-J.; Ahn, G.; Kim, K.-N. Evaluation on antioxidant properties of sixteen plant species from Jeju Island in Korea. *EXCLI J.* **2015**, *14*, 133−145.
(23) Lapasam, A.; Shadap, L.; Tripathi, D. K.; Poluri, K. M.; Kaminsky, W.; Kollipara, M. R. Arene ruthenium, rhodium and iridium complexes containing N∩ O chelating ligands: synthesis, antibacterial and antioxidant studies. *J. Coord. Chem.* **2021**, *74*, 2365−2379.
(24) Nongpiur, C. G. L.; Ghate, M. M.; Tripathi, D. K.; Poluri, K. M.; Kaminsky, W.; Kollipara, M. R. Study of versatile coordination modes, antibacterial and radical scavenging activities of arene ruthenium, rhodium and iridium complexes containing fluorenone based thiosemicarbazones. *J. Organomet. Chem.* **2022**, *957*, No. 122148.
(25) El-Gammal, O. A.; Mohamed, F. S.; Rezk, G. N.; El-Bindary, A. A. Structural characterization and biological activity of a new metal complexes based of Schiff base. *J. Mol. Liq.* **2021**, *330*, No. 115522.
(26) Lahmar, I.; Nasri-Ayachi, M. B.; Belghith, K. Laticifer Identification, Rubber Characterization, Phenolic Content, and Antioxidant Activity of Pergularia tomentosa Latex Extract. *BioMed Res. Int.* **2022**, *2022*, No. 7158905.
(27) Ranade, A.; Acharya, R.; Shukla, V.; Roy, S.; Maji, J. Monitoring Of Seasonal Variation In Latex Of *Calotropis procera* AIT. AND Calotropis gigantea L.R.Br USING FTIR SPECTROSCOPY. *J. Res. Educ. Indian Med.* **2017**, *23*, 59−74.
(28) Samrot, A. V.; Sahiti, K.; Bhavya, K. S.; Suvedhaa, B. Correction to: Synthesis of Plant Latex Based Hybrid Nanocarriers Using Surfactants for Curcumin Delivery. *J. Cluster Sci.* **2019**, *30*, 297−298.
(29) Nandiyanto, A. B. D.; Oktiani, R.; Ragadhita, R. How to read and interpret FTIR spectroscope of organic material. *Indones. J. Sci. Technol.* **2019**, *4*, 97−118.
(30) Eid, A. M.; Fouda, A.; Niedbała, G.; Hassan, S. E.-D.; Salem, S. S.; Abdo, A. M.; F Hetta, H.; Shaheen, T. I. Endophytic Streptomyces laurentii mediated green synthesis of Ag-NPs with antibacterial and anticancer properties for developing functional textile fabric properties. *Antibiotics* **2020**, *9*, No. 641.
(31) Kain, D.; Kumar, S.; Suryavanshi, A.; Arya, A. FTIR and GCMS analysis of Euphorbia hirta L. and its In-vitro Antibacterial and Antioxidant Activities. *Indo Global J. Pharm. Sci.* **2022**, *12*, 104−109.
(32) Abou-Melha, K. S.; Al-Hazmi, G. A.; Althagafi, I.; Alharbi, A.; Shaaban, F.; El-Metwaly, N. M.; El-Bindary, A. A.; El-Bindary, M. A. Synthesis, characterization, DFT calculation, DNA binding and antimicrobial activities of metal complexes of dimedone arylhydrazone. *J. Mol. Liq.* **2021**, *334*, No. 116498.
(33) El-Gammal, O. A.; El-Bindary, A. A.; Mohamed, F. S.; Rezk, G. N.; El-Bindary, M. A. Synthesis, characterization, design, molecular docking, anti COVID-19 activity, DFT calculations of novel Schiff base with some transition metal complexes. *J. Mol. Liq.* **2022**, *346*, No. 117850.
(34) Bauer, G.; Gorb, S. N.; Klein, M.-C.; Nellesen, A.; von Tapavicza, M.; Speck, T. Comparative study on plant latex particles and latex coagulation in Ficus benjamina, Campanula glomerata and three Euphorbia species. *PLoS One* **2014**, *9*, No. e113336.
(35) Cornish, K.; Wood, D. F. Visualization of the Malleability of the Rubber Core of Rubber Particles from Parthenium argentatum Gray and Other Rubber-Producing Species Under Extremely Cold Temperatures. *J. Polym. Environ.* **2002**, *10*, 155−162.
(36) Muniandy, K.; Ismail, H.; Othman, N. Biodegradation, Morpholgical, And Ftir Study Of Rattan Powder-Filled Natural Rubber Composites As A Function Of Filler Loading And A Silane Coupling Agent. *BioResources* **2012**, *7*, 957−971.
(37) Shabinah, F. M. S.; Hashim, M. Y. A. Biodegradation of NR Latex-based Materials via a Carbon Dioxide Evolution Method. *ASEAN J. Sci. Technol. Dev.* **2013**, *30*, 50−62.
(38) Chen, W.; He, Z. L.; Yang, X. E.; Mishra, S.; Stoffella, P. J. Chlorine nutrition of higher plants: progress and perspectives. *J. Plant Nutr.* **2010**, *33*, 943−952.
(39) Sardans, J.; Peñuelas, J. Potassium Control of Plant Functions: Ecological and Agricultural Implications. *Plants* **2021**, *10*, No. 419.
(40) Xing, K.; Zhao, M.; Niinemets, Ü.; Niu, S.; Tian, J.; Jiang, Y.; Chen, H. Y. H.; White, P. J.; Guo, D.; Ma, Z. Relationships between leaf carbon and macronutrients across woody species and forest ecosystems highlight how carbon is allocated to leaf structural function. *Front. Plant Sci.* **2021**, *12*, No. 674932.
(41) Ferrand, E.; Dumat, C.; Leclerc-Cessac, E.; Benedetti, M. F. Phytoavailability of zirconium in relation to its initial added form and soil characteristics. *Plant Soil* **2006**, *287*, 313−325.
(42) Shahid, M.; Ferrand, E.; Schreck, E.; Dumat, C. Behavior and impact of zirconium in the soil−plant system: plant uptake and phytotoxicity. *Rev. Environ. Contam. Toxicol.* **2013**, *221*, 107−127.
(43) Du, K.; Bereswill, S.; Heimesaat, M. M. A literature survey on antimicrobial and immune-modulatory effects of butyrate revealing non-antibiotic approaches to tackle bacterial infections. *Eur. J. Microbiol. Immunol.* **2021**, *11*, 1−9.
(44) Kennedy, G. M.; Min, M. Y.; Fitzgerald, J. F.; Nguyen, M. T.; Schultz, S. L.; Crum, M. T.; Starke, J. A.; Butkus, M. A.; Bowman, D. D.; Labare, M. P. Inactivation of the bacterial pathogens Staphylococcus pseudintermedius and Acinetobacter baumannii by butanoic acid. *J. Appl. Microbiol.* **2019**, *126*, 752−763.
(45) Fraise, A. P.; Wilkinson, M. A. C.; Bradley, C. R.; Oppenheim, B.; Moiemen, N. The antibacterial activity and stability of acetic acid. *J. Hosp. Infect.* **2013**, *84*, 329−331.
(46) Sauceda, A. E. Q.; Sáyago-Ayerdi, S. G.; Ayala-Zavala, J. F.; Wall-Medrano, A.; de la Rosa, L. A.; González-Aguilar, G. A.; Álvarez-Parrilla, E.Biological Actions of Phenolic Compounds. In *Fruit and Vegetable Phytochemicals: Chemistry and Human Health*, 2nd ed.; Wiley, 2017; pp 125−138.
(47) Araldi, E.; Fernández-Fuertes, M.; Canfrán-Duque, A.; Tang, W.; Cline, G. W.; Madrigal-Matute, J.; Pober, J. S.; Lasunción, M. A.; Wu, D.; Fernández-Hernando, C.; Suárez, Y. Lanosterol modulates TLR4-mediated innate immune responses in macrophages. *Cell Rep.* **2017**, *19*, 2743−2755.
(48) Zhao, L.; Chen, X.-J.; Zhu, J.; Xi, Y.-B.; Yang, X.; Hu, L.-D.; Ouyang, H.; Patel, S. H.; Jin, X.; Lin, D.; et al. Lanosterol reverses protein aggregation in cataracts. *Nature* **2015**, *523*, 607−611.
(49) Benali, T.; Bakrim, S.; Ghchime, R.; Benkhaira, N.; El Omari, N.; Balahbib, A.; Taha, D.; Zengin, G.; Hasan, M. M.; Bibi, S.; Bouyahya, A. Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnol. Genet. Eng. Rev.* **2022**, 1−30.
(50) Gallo, M. B. C.; Sarachine, M. J. Biological activities of lupeol. *Int. J. Biomed. Pharm. Sci.* **2009**, *3*, 46−66.
(51) Wang, W.-H.; Chuang, H.-Y.; Chen, C.-H.; Chen, W.-K.; Hwang, J.-J. Lupeol acetate ameliorates collagen-induced arthritis and osteoclastogenesis of mice through improvement of microenvironment. *Biomed. Pharmacother.* **2016**, *79*, 231−240.
(52) Liu, K.; Zhang, X.; Xie, L.; Deng, M.; Chen, H.; Song, J.; Long, J.; Li, X.; Luo, J. Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy. *Pharmacol. Res.* **2021**, *164*, No. 105373.
(53) Sharma, N.; Palia, P.; Chaudhary, A.; Shalini; Verma, K.; Kumar, I. A review on pharmacological activities of lupeol and its triterpene derivatives. *J. Drug Delivery Ther.* **2020**, *10*, 325−332.

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772

(54) Pianowski, L. F.; Calixto, J. B.; Leal, P. C.; Chaves, C. P. Pharmaceutical Uses of lanosta-8, 24-dien-3-ols. U.S.Patent US20110136773A1, 2016.
(55) Qin, L.; Vo, D.-D.; Nakhai, A.; Andersson, C. D.; Elofsson, M. Diversity-oriented synthesis of libraries based on Benzofuran and 2, 3-Dihydrobenzofuran Scaffolds. *ACS Comb. Sci.* **2017**, *19*, 370−376.
(56) Lee, K.-G.; Shibamoto, T. Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. et Perry]. *Food Chem.* **2001**, *74*, 443−448.
(57) Vanin, A. B.; Orlando, T.; Piazza, S. P.; Puton, B. M. S.; Cansian, R. L.; Oliveira, D.; Paroul, N. Antimicrobial and antioxidant activities of clove essential oil and eugenyl acetate produced by enzymatic esterification. *Appl. Biochem. Biotechnol.* **2014**, *174*, 1286−1298.
(58) Musthafa, K. S.; Voravuthikunchai, S. P. Anti-virulence potential of eugenyl acetate against pathogenic bacteria of medical importance. *Antonie van Leeuwenhoek* **2015**, *107*, 703−710.
(59) Choi, M. N.; Shin, K.-C.; Kim, D. W.; Kim, B.-J.; Park, C.-S.; Yeom, S.-J.; Kim, Y.-S. Production of D-allose from D-allulose using commercial immobilized glucose isomerase. *Front. Bioeng. Biotechnol.* **2021**, *9*, No. 681253.
(60) Kong, C. S.; Kim, K. H.; Choi, J. S.; Kim, J. E.; Park, C.; Jeong, J. W. Salicin, an Extract from White Willow Bark, Inhibits Angiogenesis by Blocking the ROS-ERK Pathways. *Phytother. Res.* **2014**, *28*, 1246−1251.
(61) Premjanu, N.; Jaynthy, C. Antimicrobial activity of diethyl phthalate: An insilico approach. *Asian J. Pharm. Clin Res.* **2014**, *7*, 141−142.
(62) Jing, Y.; Wang, Z.; Chen, W.; Liang, Y.; Yang, Z.; Zheng, C.; Cao, D.; Zhang, Z. Preparation of Polyvinyl Alcohol/Graphene Oxide Composite Film with "Sandwich" Structure and High Toughness. *Poly. Sci., Series A* **2021**, *63*, 822−827.
(63) Shahinuzzaman, M.; Yaakob, Z.; Anuar, F. H.; Akhtar, P.; Kadir, N. H. A.; Hasan, A. K. M.; Sobayel, K.; Nour, M.; Sindi, H.; Amin, N.; et al. In vitro antioxidant activity of *Ficus carica* L. latex from 18 different cultivars. *Sci. Rep.* **2020**, *10*, No. 10852.
(64) Al-Rajhi, A. M. H.; Yahya, R.; Abdelghany, T. M.; Fareid, M. A.; Mohamed, A. M.; Amin, B. H.; Masrahi, A. S. Anticancer, anticoagulant, antioxidant and antimicrobial activities of *Thevetia peruviana* latex with molecular docking of antimicrobial and anticancer activities. *Molecules* **2022**, *27*, No. 3165.
(65) Chaudhary, P.; Janmeda, P. Quantification of phytochemicals and in vitro antioxidant activities from various parts of *Euphorbia neriifolia* Linn. *J. Appl. Biol. Biotechnol.* **2022**, *10*, 133−145.
(66) Soliman, M. I.; Mohammed, N. S.; EL-Sherbeny, G.; Safhi, F. A.; ALshamrani, S. M.; Alshamrani, S. M.; Alyamani, A. A.; Alharthi, B.; Qahl, S. H.; Al Kashgry, N. A. T.; Abd-Ellatif, S. Antibacterial, Antioxidant Activities, GC-Mass Characterization, and Cyto/Genotoxicity Effect of Green Synthesis of Silver Nanoparticles Using Latex of *Cynanchum acutum* L. *Plants.* **2023**, *12*, No. 172.
(67) Manandhar, S.; Luitel, S.; Dahal, R. K. In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J. Trop. Med.* **2019**, *2019*, No. 1895340.
(68) McDermott, A. Drug-resistant fungi on the rise. *Proc. Natl. Acad. Sci. U.S.A.* **2022**, *119*, No. e2217948119.
(69) Raghavendra, R.; Mahadevan, G. D. In vitro antimicrobial activity of various plant latex against resistant human pathogens. *Int. J. Pharm. Pharm. Sci.* **2011**, *3*, 70−72.
(70) Sumathi, S.; Malathy, N.; Dharani, B.; Sivaprabha, J.; Hamsa, D.; Radha, P.; Padma, P. R. Antibacterial and antifungal activity of latex of *Euphorbia antiquorum*. *Afr. J. Microbio. Res.* **2011**, *5* (27), 4753−4756.
(71) Emiru, Y. K.; Siraj, E. A.; Teklehaimanot, T. T.; Amare, G. G. "Antibacterial Potential of *Aloe weloensis* (Aloeacea) Leaf Latex against Gram-Positive and Gram-Negative Bacteria Strains". *Int. J. Microbiol.* **2019**, *2019*, No. 5328238.
(72) El-Bindary, A. A.; El-Sonbati, A. Z.; El-Dissouky, A.; Hilali, A. S. Stereochemistry, structural and models of novel 5-(4′-derivatives phenyldiazo)-3-phenyl-2-thioxo-4-thiazolidinone complexes. *Spectrochim. Acta, Part A* **2002**, *58* (7), 1365−1374.
(73) Al-Hazmi, G. A.; Abou-Melha, K. S.; Althagafi, I.; El-Metwaly, N. M.; Shaaban, F.; Galil, M. S. A.; El-Bindary, A. A. Synthesis and structural characterization of oxovanadium(IV) complexes of dimedone derivatives. *Appl. Organomet. Chem.* **2020**, *34*, No. e5672.
(74) El-Bindary, M. A.; El-Bindary, A. A. Synthesis, characterization, DNA binding, and biological action of dimedone arylhydrazone chelates. *Appl. Organomet. Chem.* **2022**, *36*, No. e6576.
(75) Nath, A.; Kumer, A.; Khan, M. W. Synthesis. computational, and molecular docking study of some 2,3-dihydrobenzofuran and its derivatives. *J. Mol. Struct.* **2021**, *1224*, No. 129225.
(76) Abdulhamid, A.; Awad, T. A.; Ahmed, A. E.; Koua, F. H. M.; Ismail, A. M. Acetyleugenol from Acacia nilotica (L.) Exhibits a Strong Antibacterial Activity and Its Phenyl and Indole Analogues Show a Promising Anti-TB Potential Targeting PknE/B Protein Kinases. *Microbiol. Res.* **2021**, *12*, 1−15.
(77) Liu, F.; Liu, C.; Liu, W.; Ding, Z.; Ma, H.; Seeram, P. N.; Xu, L.; Mu, Y.; Huang, X.; Liya, Li. New Sesquiterpenoids from Eugenia jambolana Seeds and Their Anti-microbial Activities. *J. Agri. Food Chem.* **2017**, *65* (47), 10214−10222.
(78) Araldi, E.; Fernández-Fuertes, M.; Canfrán-Duque, A.; Tang, W.; Cline, G. W.; Madrigal-Matute, J.; Pober, J. S.; Lasunción, M. A.; Wu, D.; Fernández-Hernando, C.; Suárez, Y. Lanosterol Modulates TLR4-Mediated Innate Immune Responses in Macrophages. *Cell Rep.* **2017**, *19* (13), 2743−2755.
(79) Shingate, B. B.; Hazra, B. G.; Salunke, D. B.; Pore, V. S.; Shirazic, F.; Deshpande, M. V. Synthesis and antimicrobial activity of novel oxysterols from lanosterol. *Tetrahedron* **2013**, *69*, 11155−11163.
(80) Awolola, G. V.; Koorbanally, N. A.; Chenia, H.; Shode, F. O.; Baijnath, H. Antibacterial and anti-biofilm activity of flavonoids and triterpenes isolated from the extracts of Ficus sansibaricaWarb. subsp. sansibarica (Moraceae) extracts. *Afr. J. Tradit., Complementary Altern. Med.* **2014**, *11* (3), 124−131.
(81) Adrielli, T.; Ariela, M. B.; Catarina, C. B.-C.; Alexandre, B.-C.; Caio, M. M. C.; Valdir, C. F. Chemical composition, antibacterial and antimycoplasma activities of four Eugenia species growing in Brazil. *J. Med. Plants Res.* **2017**, *11* (39), 596−602.
(82) Sánchez-Hernández, E.; Balduque-Gil, J.; González-García, V.; Barriuso-Vargas, J. J.; Casanova-Gascón, J.; Martín-Gil, J.; Martín-Ramos, P. Phytochemical Profiling of *Sambucus nigra* L. Flower and Leaf Extracts and Their Antimicrobial Potential against Almond Tree Pathogens. *Int. J. Mol. Sci.* **2023**, *24*, No. 1154.
(83) Shaaban, M. T.; Ghaly, M. F.; Fahmi, S. M. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. *J. Basic Microbiol.* **2021**, *61*, 557−568.
(84) Sharma, N.; Palia, P.; Chaudhary, A.; Verma, S. K.; Verma, K.; Kumar, I. A Review on Pharmacological Activities of Lupeol and its Triterpene Derivatives. *J. Drug Delivery Ther.* **2020**, *10* (5), 325−332.

https://doi.org/10.1021/acsomega.3c06069
ACS Omega 2023, 8, 47758−47772