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GC-MS based phytochemical profiling and assessment of antimicrobial activity of *Vitis vinifera* skin extract via Kirby-Bauer disk diffusion assay

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Abstract

The escalating crisis of antimicrobial resistance (AMR) necessitates the urgent exploration of novel, naturally derived antibacterial agents. Plant secondary metabolites represent a promising reservoir for such compounds. This study investigates the phytochemical composition and antimicrobial potential of *Vitis vinifera* (grape) skin, a significant agri-food by-product. An ethanolic extract was prepared from authenticated grape skins, yielding 22% w/w. Preliminary phytochemical screening confirmed a rich repertoire of bioactive constituents, including abundant flavonoids, anthocyanins, tannins, and phenolic compounds.

A comprehensive metabolic profile was elucidated using Gas Chromatography-Mass Spectrometry (GC-MS), which revealed a complex mixture of high-value lipophilic compounds. The extract was characterized by a high concentration of fatty acid esters (e.g., Ethyl Palmitate), phytosterols (e.g., γ -Sitosterol), and tocopherols (Vitamin E), underpinning its potent antioxidant and emollient properties. The antimicrobial efficacy was evaluated against clinically relevant Gram-positive (*Staphylococcus aureus* MTCC 7443) and Gram-negative (*Escherichia coli* MTCC 2665) pathogens via the standardized Kirby-Bauer disk diffusion assay.

The results demonstrated selective and significant antibacterial activity. The extract (100 mg/mL) produced a distinct zone of inhibition (7mm) against *S. aureus*, surpassing the activity of the standard antibiotic amoxicillin (13mm) under the tested conditions. Conversely, no inhibitory activity was observed against *E. coli*. This differential efficacy is attributed to the structural disparity in bacterial cell envelopes; the impermeable outer membrane of Gram-negative *E. coli* likely acts as a barrier to the extract's bioactive molecules, whereas the more permeable peptidoglycan layer of Gram-positive *S. aureus* is susceptible to disruption.

In conclusion, *Vitis vinifera* skin extract is established as a rich source of antioxidants and emollients with selective antibacterial activity. While its application as a broad-spectrum antimicrobial is limited, its pronounced effect against *S. aureus* and its rich phytochemical profile validate its potential as a valuable, sustainable ingredient for developing topical formulations in the cosmetic and nutraceutical industries, and warrant further investigation into its active principles for combating Gram-positive infections.

Keywords: *Vitis vinifera* skin, anthocyanin, phytochemical screening, GC-MS analysis, antimicrobial activity, *Staphylococcus aureus*, *E. coli*

1. Introduction

The growing global challenge of antimicrobial resistance (AMR) is a critical public health issue, diminishing the effectiveness of standard antibiotics against numerous infectious pathogens [1]. In response to this pressing threat, scientific exploration is increasingly directed towards discovering new and alternative antimicrobial compounds derived from natural sources [2]. In this context, plants are considered a highly promising reservoir due to their extensive diversity of bioactive secondary metabolites.

The grapevine (*Vitis vinifera* L.) is a globally significant fruit, and its skins, a major by-product of viticulture, are particularly noteworthy for their complex phytochemical profile [3]. These skins are abundant in various polyphenols, encompassing flavonoids like flavanols (e.g., quercetin) and anthocyanins, along with non-flavonoid compounds such as stilbenes (e.g., resveratrol) and phenolic acids [4, 5].

These metabolites are naturally produced by the plant for defence against microbial invasion and environmental stress, which implies a strong potential for antimicrobial activity^[6].

Although the antioxidant and other health-promoting properties of grape polyphenols are well-documented^[7]. Their specific role as antimicrobial agents requires more detailed and systematic evaluation. Evidence suggests that polyphenolic compounds can exert antimicrobial effects by damaging microbial cell membranes, suppressing key enzymes, and modulating pathogen virulence mechanisms^[8, 9]. To comprehensively characterize the chemical constituents of plant extracts, Gas Chromatography-Mass Spectrometry (GC-MS) is a widely used analytical tool^[10]. This technique is highly effective for separating and identifying volatile and semi-volatile components, as well as non-volatile compounds like specific phenolics and organic acids after chemical derivatization, providing a crucial metabolic fingerprint of the extract^[11].

For the initial screening of antimicrobial properties, the Kirby-Bauer disk diffusion method is a standard, efficient, and reproducible technique^[12]. It offers a quantitative measure of efficacy by producing a clear zone of inhibition, making it suitable for testing against a range of bacteria and fungi.

Consequently, this research seeks to connect the detailed phytochemistry of grape skin waste with its biological applications. The specific goals are: firstly, to perform a GC-MS-based phytochemical analysis of a *Vitis vinifera* skin extract to determine its composition, and secondly, to investigate its antimicrobial potential against selected clinically relevant pathogens using the Kirby-Bauer disk diffusion assay. This study could help establish grape pomace as a valuable, sustainable resource for developing new anti-infective agents to address the AMR crisis.

2. Materials and Methods

2.1 Collection and authentication of plant material

Fresh *Vitis vinifera* fruits (grapes) were locally sourced and taxonomically authenticated by Dr. N. M. Ganesh Babu, Associate Professor and Head of the Centre for Herbal Gardens at The University of Trans-Disciplinary Health Sciences and Technology, Bangalore, India.

2.2. Preparation of Grape Skin Extract^[13, 14, 15]

A total of 29 g of coarse powdered grape skin was obtained, of which 20 g was used for extraction. The sample was macerated in an acidified solvent (70% ethanol) for 72 hours in a covered laboratory flask. The final filtration was performed using Whatman No. 4 filter paper. The extract was then concentrated to a dry residue using a rotary evaporator, and the weight of the dry extract was recorded. The dehydrated extract was stored meticulously for the evaluation of its antimicrobial activity.

The percentage of extract yield was calculated by using the formula:

Percentage of extract yield (Weight in gm of extract obtained) / (Weight in gm of plant material taken) × 100.

2.3. Preliminary phytochemical screening of *Vitis vinifera* skin extract^[16, 17, 18]

The extract was analyzed for its phytochemical constituents.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

Analysis: The bioactive compounds in *Vitis vinifera* skin

extracts were profiled using Gas Chromatography-Mass Spectrometry (GC-MS). The analytical conditions were as follows:

- **Instrument:** GC-MS system (+EI TIC Scan GC-MS-03-6235.D) with an Elite 1 column.
- **Carrier Gas:** High-purity helium at a constant flow rate of 1.0 ml/min.
- **Injection:** 2 µL sample volume at an injector temperature of 280 °C.
- **Oven Program:** Initial temperature of 40 °C, ramped to a final temperature of 280 °C, followed by a 5-minute hold.

Compounds identification were based on the correlation of mass spectra and retention times with reference data. The relative quantity of each compound was determined from the normalized peak area^[19].

2.5. Assessment of Antimicrobial Activity by Kirby-Bauer Disc Diffusion Assay^[20, 21]

2.5.1. Microbial Strains and Culture Preparation

The antibacterial properties of *Vitis vinifera* skin extract (VVSE) were examined against Gram-positive *Staphylococcus aureus* (MTCC 7443) and Gram-negative *Escherichia coli* (MTCC 2665). Bacterial stocks were revitalized in nutrient broth and incubated at 37°C for 24 hours to achieve mid-logarithmic growth phase, ensuring optimal metabolic activity for antimicrobial testing.

2.5.2. Antimicrobial Susceptibility Testing

Antibacterial efficacy was evaluated using the standardized disc diffusion methodology according to established protocols. Mueller-Hinton Agar plates were prepared under aseptic conditions to achieve uniform surface consistency. Bacterial suspensions were adjusted to approximately 1.5×10^8 CFU/mL (0.5 McFarland standard) and evenly distributed across agar surfaces using sterile spreaders.

2.5.3. Experimental Setup and Disc Preparation

The ethanolic VVSE was reconstituted to generate a concentration gradient (50-400 µg/mL) in ethanol. Sterile cellulose discs (6 mm diameter) were saturated with 20 µL of each concentration, yielding final disc loads ranging from 1-8 µg. Control configurations included:

- **Positive control:** Amoxicillin (10 µg/disc)
- **Negative control:** Ethanol-impregnated discs
- **Blank control:** Untreated sterile discs

All discs were strategically positioned on inoculated plates with sufficient inter-disc spacing (≥ 24 mm) to prevent zone interaction, following established antimicrobial testing parameters.

2.5.4. Quantification and Statistical Analysis

Following 24-hour incubation at 37°C, inhibitory zones were measured metrically using digital calipers. Experimental replicates (n=3) provided data expressed as mean inhibition diameter $\pm$ standard deviation. The assay validation required:

- Distinct inhibition zones around positive controls
- No observable growth inhibition around solvent controls
- Confluent bacterial growth in negative control regions

3. Results

3.1 Extraction

Table 1: The percentage yield of the extract

Sl. No.	Weight of powdered plant	Weight of Extract	Extraction yield
01	20 g	4.4	22%

3.2 Phytochemical Screening

Table 2: Phytochemical screening of *Vitis vinifera* skin extract

Phytochemical constituent	Inference
Reducing sugars	++
Flavonoids	+++
Tannins	+
Cardiac glycosides	+
Proteins and amino acids	+
Phenolic compounds	+
Anthocyanins	++

3.3 Gas chromatography-mass spectrometry (GC-MS) analysis:

This study, therefore, aimed to identify the bioactive compounds in the ethanolic extract of *Vitis vinifera* utilizing Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The investigation revealed the presence of 63 distinct phytochemical compounds, details of which-including their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area%)-are compiled in Table 3. The corresponding chromatogram is displayed in Figure 1, while the mass spectra of the identified compounds are presented in Figure 2.

Table 3: Phytochemical constituents identified in skin extracts of *Vitis vinifera* by GC-MS analysis and Mass spectra of NIST database

Batch Path	D:\MassHunter\Data																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	</
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RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
11.7416	3-Hexadecanol	593-03-3	C16H34O	21395	66.1	0.07	0.27
11.8527	Isopropyl myristate	110-27-0	C17H34O2	39304	85.1	0.12	0.50
11.9860	1H-Purine-2,6-dithione, 3,7-dihydro-1,3,7-trimethyl-	32061-73-7	C8H10N4S2	81374	66.8	0.25	1.03
12.6637	cis-9-Hexadecenoic acid	1000333-19-5	C16H30O2	62385	79.4	0.19	0.79
12.8081	n-Hexadecanoic acid	57-10-3	C16H32O2	1655654	95.6	5.15	21.00
13.0081	Hexadecanoic acid, ethyl ester	628-97-7	C18H36O2	3050999	96.7	9.50	38.71
13.2414	1-Cyclohexene-1-carboxylic acid, 4-(1,5-dimethyl-3-oxohexyl)-, methyl ester, [R-(R*,R*)]-	17904-27-7	C16H26O3	93439	71.4	0.29	1.19
13.7524	Phytol	150-86-7	C20H40O	71349	77.7	0.22	0.91
13.9413	9,12-Octadecadienoic acid (Z,Z)-	60-33-3	C18H32O2	2493556	90.0	7.76	31.63
14.0635	Ethyl 9.cis.,11.trans.-octadecadienoate	1000336-69-8	C20H36O2	7882395	93.7	24.54	100.00
14.2412	Octadecanoic acid, ethyl ester	111-61-5	C20H40O2	1008233	95.5	3.14	12.79
15.2411	cis-11-Eicosenoic acid	5561-99-9	C20H38O2	133837	65.2	0.42	1.70
15.3744	Methyl 19-methyl-eicosanoate	1000336-23-8	C22H44O2	677105	89.0	2.11	8.59
15.8188	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	21061-10-9	C21H36O2	22099	56.7	0.07	0.28
15.9188	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	761-35-3	C35H68O5	86341	74.0	0.27	1.10
16.1410	Di-n-octyl phthalate	117-84-0	C24H38O4	34644	65.3	0.11	0.44
16.4187	Docosanoic acid, ethyl ester	5908-87-2	C24H48O2	604624	84.9	1.88	7.67
16.8298	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	17367-08-7	C20H38O2	76510	66.6	0.24	0.97
16.9520	Octatriacontyl pentafluoropropionate	1000351-89-1	C41H77F5O2	178209	65.4	0.55	2.26
17.4186	Ethyl tetracosanoate	24634-95-5	C26H52O2	148586	72.5	0.46	1.89
17.8741	Benzene, 1,2-bis(9-borabicyclo[3.3.1]non-9-yloxymethyl)-	1000159-64-2	C24H36B2O2	375193	66.4	1.17	4.76
17.9963	Dodecanoic acid, 2-phenylethyl ester	6309-54-2	C20H32O2	297914	74.2	0.93	3.78
18.1519	Hexadecanoic acid, 2-(octadecyloxy)ethyl ester	29899-13-6	C36H72O3	29095	53.0	0.09	0.37
18.3629	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	119-13-1	C27H46O2	98381	81.6	0.31	1.25
19.0851	.gamma.-Tocopherol	7616-22-0	C28H48O2	2224089	97.9	6.92	28.22
19.3961	Dodecanoic acid, 2-phenylethyl ester	6309-54-2	C20H32O2	327278	66.5	1.02	4.15
19.6961	Vitamin E	59-02-9	C29H50O2	1857401	96.1	5.78	23.56
20.7737	Campesterol	474-62-4	C28H48O	167455	77.9	0.52	2.12
21.0182	Stigmasterol	83-48-7	C29H48O	209012	79.3	0.65	2.65
21.6958	.gamma.-Sitosterol	83-47-6	C29H50O	3061013	86.5	9.53	38.83
22.3180	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	1000194-62-4	C30H48O	315728	68.2	0.98	4.01
22.5180	5-[(2-Hydroxyethyl)octadecylamino]pentanoic acid, ethyl ester	1000185-08-9	C27H55NO3	69280	50.1	0.22	0.88
22.7179	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-	1259-10-5	C32H52O2	75158	61.8	0.23	0.95
22.8290	9,19-Cyclolanostane-3,7-diol	1000186-58-9	C30H52O2	111398	67.1	0.35	1.41

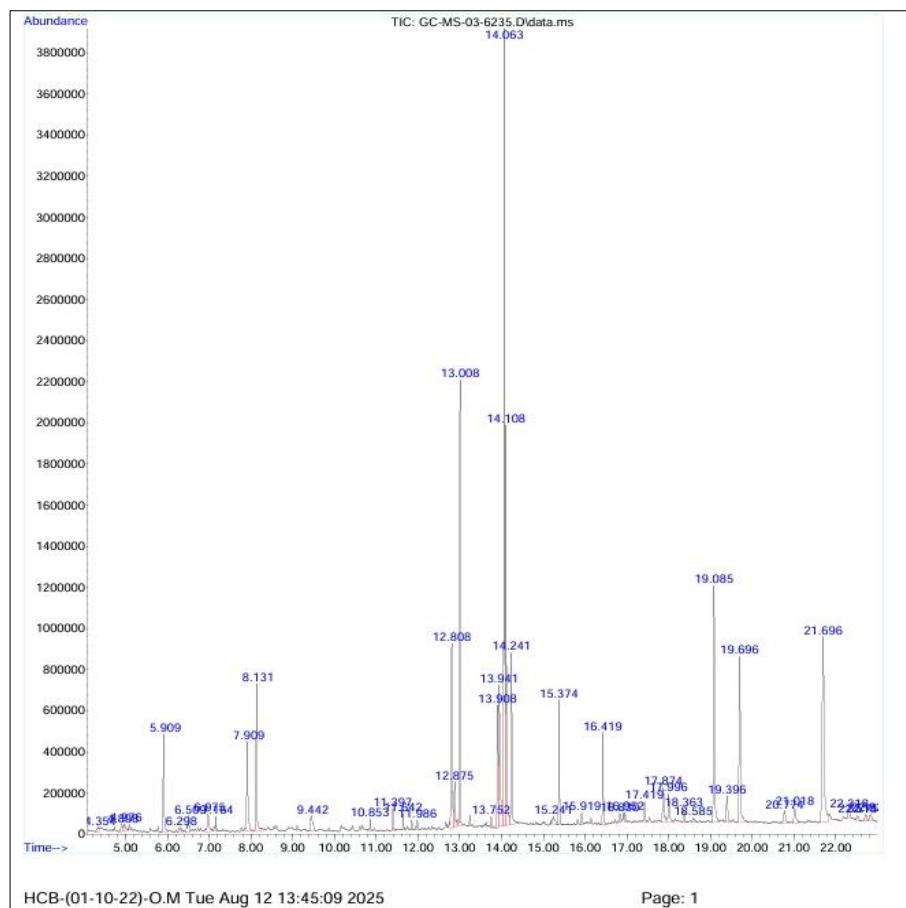


Fig 1: GC-MS Chromatogram of the ethanolic extract of *Vitis vinifera* skin

RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
5.9090	Phenylethyl Alcohol	60-12-8	C ₈ H ₁₀ O	796303	85.8	2.48	10.10
6.2978	4-Amino-1,5-pentadienoic acid	7766-85-0	C ₇ H ₁₁ NO ₄	62332	59.2	0.19	0.79
6.5089	Furan-2-carboxaldehyde, 5-(4-nitrophenoxy)methyl-	1000273-82-3	C ₁₂ H ₉ NO ₅	144570	73.8	0.45	1.83
6.7422	L- α -Terpineol	10482-56-1	C ₁₀ H ₁₈ O	19709	69.8	0.06	0.25
6.7977	2-Octanamine	693-16-3	C ₈ H ₁₉ N	15246	63.5	0.05	0.19

RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
4.3536	5,7-Dioxo-1-octene, 1-chloro-4-isopropyl-3-methyl-	1000157-79-2	C ₁₀ H ₁₉ ClO ₂	137706	52.7	0.43	1.75
4.7424	(R,R)-(-)-2,3-Dimethoxy-1,4-bis(dimethylamino)butane	26549-22-4	C ₁₀ H ₂₄ N ₂ O ₂	16670	61.7	0.05	0.21
4.8980	1-[p-Tolylsulfonyl]-2-methylaziridine	25856-77-3	C ₁₀ H ₁₃ NO ₂ S	77920	66.1	0.24	0.99
4.9757	Benzene, 1-ethyl-2,3-dimethyl-	933-98-2	C ₁₀ H ₁₄	96109	77.2	0.30	1.22
5.0868	Benzyl alcohol	100-51-6	C ₇ H ₈ O	69105	82.6	0.22	0.88
5.5868	Methyl 2-furoate	611-13-2	C ₆ H ₆ O ₃	10770	65.4	0.03	0.14

RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
8.5531	Diethyl .beta.-D-ribose	1000126-95-4	C ₇ H ₁₄ O ₅	31540	64.6	0.10	0.40
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
8.6086	Bisphenol C	79-97-0	C ₁₇ H ₁₂ O ₂	29954	69.0	0.09	0.38
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
8.8975	L-Lyxose	1949-78-6	C ₅ H ₁₀ O ₅	83760	63.7	0.26	1.06
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
9.0974	4H-Pyran-2,6-dicarboxylic acid, 4-oxo-	99-32-1	C ₇ H ₄ O ₆	29631	67.9	0.09	0.38
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
9.4418	.beta.-D-Glucopyranose, 1,6-anhydro-	498-07-7	C ₆ H ₁₀ O ₅	258302	83.6	0.80	3.28
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
9.8640	Dodecanoic acid	143-07-7	C ₁₂ H ₂₄ O ₂	13569	68.4	0.04	0.17
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
10.1640	Benzoic acid, 4-hydroxy-3-methoxy-, ethyl ester	617-05-0	C ₁₀ H ₁₂ O ₄	114960	77.1	0.36	1.46
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
10.4417	3,7,11,14,18-Pentaoxa-2,19-disilaicosane, 2,2,19,19-tetramethyl-	1000151-71-2	C ₁₇ H ₄₀ O ₅ Si ₂	65792	61.9	0.20	0.83
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
10.5973	Azelaic acid	123-99-9	C ₉ H ₁₆ O ₄	37717	65.0	0.12	0.48
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
10.6639	cis,cis-2-Methyl-1-thiadecalin	83349-26-2	C ₁₀ H ₁₈ S	86649	62.6	0.27	1.10

RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
10.8528	Cyclooctanecarboxylic acid, 4-methyl-, ethyl ester	1000149-78-5	C ₁₂ H ₂₂ O ₂	64774	72.9	0.20	0.82
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
11.3972	Tetradecanoic acid	544-63-8	C ₁₄ H ₂₈ O ₂	119516	92.2	0.37	1.52
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
11.6416	Tetradecanoic acid, ethyl ester	124-06-1	C ₁₆ H ₃₂ O ₂	93291	88.2	0.29	1.18
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
11.7416	3-Hexadecanol	593-03-3	C ₁₆ H ₃₄ O	21395	66.1	0.07	0.27
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
11.8527	Isopropyl myristate	110-27-0	C ₁₇ H ₃₄ O ₂	39304	85.1	0.12	0.50
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
11.9860	1H-Purine-2,6-dithione, 3,7-dihydro-1,3,7-trimethyl-	32061-73-7	C ₈ H ₁₀ N ₄ S ₂	81374	66.8	0.25	1.03
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
12.6637	cis-9-Hexadecenoic acid	1000333-19-5	C ₁₆ H ₃₀ O ₂	62385	79.4	0.19	0.79
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
12.8081	n-Hexadecanoic acid	57-10-3	C ₁₆ H ₃₂ O ₂	1655654	95.6	5.15	21.00
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
13.0081	Hexadecanoic acid, ethyl ester	628-97-7	C ₁₈ H ₃₆ O ₂	3050999	96.7	9.50	38.71
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M
13.2414	1-Cyclohexene-1-carboxylic acid, 4-(1,5-dimethyl-3-oxohexyl-, methyl ester, [R-(R*,R*)]	17904-27-7	C ₁₆ H ₂₆ O ₃	93439	71.4	0.29	1.19



Docosanoic acid, ethyl ester (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
16.8298	Ethanol, 2-(9,12-octadecadienyl-), (Z,Z)-	17367-08-7	C20H38O2	76510	66.6	0.24	0.97		
Ethanol, 2-(9,12-octadecadienyl-), (Z,Z)- (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
16.9520	Octatriacontyl pentafluoropropanoate	1000351-89-1	2	178209	65.4	0.55	2.26		
Octatriacontyl pentafluoropropanoate (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
17.4186	Ethyl tetracosanoate	24634-95-5	C26H52O2	148586	72.5	0.46	1.89		
Ethyl tetracosanoate (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
17.8741	Benzene, 1,2-bis(9-borabicyclo[3.3.1]non-9-yl-oxymethyl)-	1000159-64-2	2	375193	66.4	1.17	4.76		
Benzene, 1,2-bis(9-borabicyclo[3.3.1]non-9-yl-oxymethyl)- (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
17.9963	Dodecanoic acid, 2-phenylethyl ester	6309-54-2	C20H32O2	297914	74.2	0.93	3.78		

Dodecanoic acid, 2-phenylethyl ester (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
18.1519	Hexadecanoic acid, 2-(octadecyloxy)ethyl ester	29899-13-6	C36H72O3	29095	53.0	0.09	0.37		
Hexadecanoic acid, 2-(octadecyloxy)ethyl ester (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
18.3629	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-[(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]]-	119-13-1	C27H46O2	98381	81.6	0.31	1.25		
2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-[(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]]- (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
19.0851	gamma-Tocopherol	7616-22-0	C28H48O2	2224089	97.9	6.92	28.22		
gamma-Tocopherol (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
19.3961	Dodecanoic acid, 2-phenylethyl ester	6309-54-2	C20H32O2	327278	66.5	1.02	4.15		
Dodecanoic acid, 2-phenylethyl ester (NIST11.L)									
Counts									
RT	Compound Name	CAS#	Formula	Area	Match Score	Area%-T	Area%-M		
19.6961	Vitamin E	59-02-9	C29H50O2	1857401	96.1	5.78	23.56		

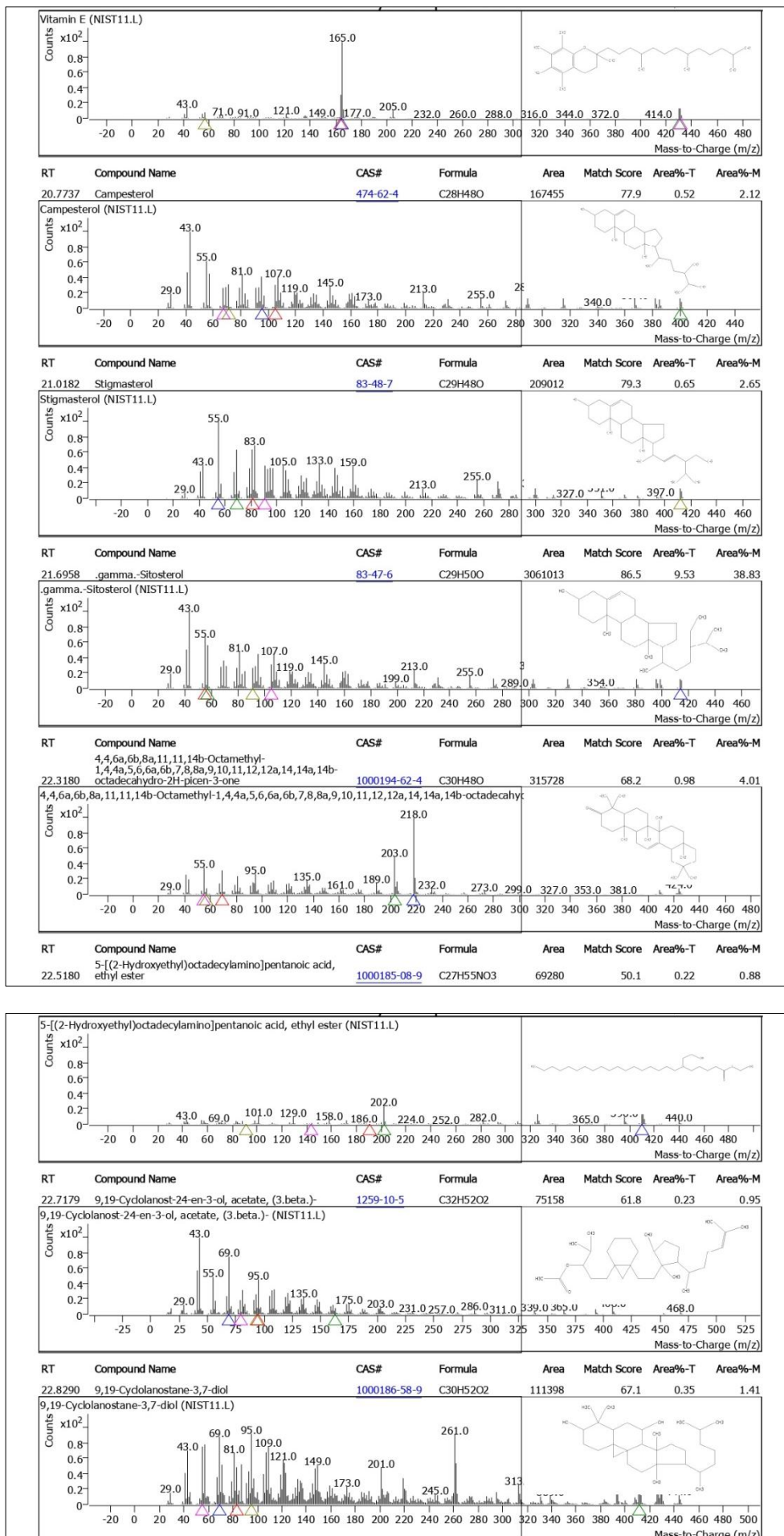


Fig 2: Mass spectra of the identified compound from the ethanolic extract of *Vitis vinifera* skin

3.4 Antimicrobial Activity

The antimicrobial activity of *Vitis vinifera* skin extract was tested against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the Kirby-Bauer method. The extract was tested at a concentration of 100 mg/mL. Amoxicillin served as a positive control.

Against *Staphylococcus aureus*

- *Vitis vinifera* skin extract created a zone of inhibition measuring 13 mm.
- The positive control (Amoxicillin) produced a clear zone of inhibition measuring 7 mm.

Against *Escherichia coli*

- No zones of inhibition were seen for the concentration of 100 mg/mL of *Vitis vinifera* skin extract.
- The positive control (Amoxicillin) showed no zone of inhibition.

Table 4: Measurement of Inhibition Zone

SL. No.	Test Organism	<i>Vitis vinifera</i> (ZOI in mm)	Standard (ZOI in mm)	Resistance
01	<i>S. aureus</i>	7 mm	13 mm	-
02	<i>E. coli</i>	-	-	+

(+) = detected, (-) = not detected



Fig 3: Antimicrobial activity of *Vitis vinifera* skin extract

4. Discussion

The findings of this study present a compelling narrative of significant chemical richness coupled with selective, albeit limited, biological activity. The preliminary phytochemical screening successfully identified a broad spectrum of secondary metabolites, with notably strong indications for flavonoids and anthocyanins. These compound classes are well-documented for their potent antioxidant and antimicrobial properties, providing a preliminary basis for the observed bioactivity.

The GC-MS analysis was instrumental in moving from a general class-based identification to a specific molecular-level profile. The chromatogram revealed a complex extract rich in high-value lipophilic compounds. The dominance of fatty acid esters (e.g., Ethyl 9-cis, 11-trans-octadecadienoate, Ethyl Palmitate) and phytosterols (e.g., γ -Sitosterol) explains the excellent yield of the ethanolic extract and points towards strong emollient and skin-barrier reinforcing properties, highly relevant for cosmetic applications. Furthermore, the significant quantities of tocopherols (Vitamin E and γ -Tocopherol) identified are powerful natural antioxidants, which contribute to the extract's ability to mitigate oxidative stress.

The antimicrobial results, however, highlight the context-dependent nature of this bioactivity. The extract's efficacy

against *S. aureus* but not *E. coli* is a classic demonstration of the differential susceptibility between Gram-positive and Gram-negative bacteria. The single, thick peptidoglycan layer of Gram-positive bacteria like *S. aureus* is more permeable to the disruptive action of phytochemicals, such as the tannins and flavonoids detected in the screening. In contrast, the impermeable outer lipopolysaccharide membrane of Gram-negative bacteria like *E. coli* acts as a formidable barrier, preventing many large or complex plant-derived molecules from reaching their target sites within the cell.

5. Conclusion

In conclusion, this research successfully characterizes *Vitis vinifera* skin extract as a chemically complex and valuable by-product. The GC-MS analysis reveals a profile exceptionally rich in fatty acid esters, phytosterols, and tocopherols, positioning the extract as an excellent ingredient for antioxidant, moisturizing, and skin-protective formulations in the cosmetic and nutraceutical industries.

The antimicrobial assessment confirms that the crude ethanolic extract possesses selective, mild antibacterial activity against the Gram-positive bacterium *Staphylococcus aureus*, but is ineffective against the Gram-negative *Escherichia coli* under the tested conditions. Therefore, the primary value of this extract lies in its antioxidant and emollient properties rather than its potency as a standalone, broad-spectrum antimicrobial agent.

6. List of abbreviations

AMR: Antimicrobial Resistance

CFU: Colony Forming Unit

GC-MS: Gas Chromatography-Mass Spectrometry

MTCC: Microbial Type Culture Collection

S. aureus: *Staphylococcus aureus*

TIC: Total Ion Chromatogram

VVSE: *Vitis vinifera* Skin Extract

ZOI: Zone of Inhibition

7. Conflict of interest

The authors declare that there is no conflict of interest

8. Acknowledgment

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