

## Evaluation of Antimicrobial Activity of *Emblica officinalis* against Skin Associated Microbial Strains

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### Abstract

The present study was undertaken to assess antimicrobial activity of fruit extracts of Amla (*Emblica officinalis*) against skin associated microorganisms. The antimicrobial activity of five different solvents viz. methanol, ethanol, distilled water, chloroform and petroleum ether against Gram positive, Gram negative bacteria and yeast namely *Propionibacterium acne*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* was assessed by using agar well diffusion method. The methanolic extract of *Emblica officinalis* showed maximum zone of inhibition, aqueous extract was most effective against *S. aureus* and ethanolic extract was most effective against *C. albicans*. Comparison of antimicrobial activity of *Emblica officinalis* extracts with antibiotics revealed that *Emblica officinalis* methanolic extract had maximum effective antimicrobial activity against all tested microorganisms. MIC and MBC of methanolic extract of *Emblica officinalis* against the microbial strains was ranged between 0.50 to 0.03125mg/ml. The synergistic interaction of *Emblica officinalis* with antibiotics (Gentamicin, Amikacin and Clotrimazole) indicated much better results as compared to antibiotics susceptibility pattern alone. Phytochemicals analyses showed the presence of Alkaloids, Saponins, Glycosides, Proteins, Phenols and Phytosterols. The compounds identified by GC-MS analysis had been useful as skin conditioning agent. The present study reflects a hope for the development of novel agents of biomedical importance.

**Keywords:** Antimicrobial activity, *Emblica officinalis* fruit, GC-MS analysis, Phytochemicals, Skin disease.

### Introduction

Human skin is one of the largest organ of the body. It is a very complex tissue consisting of several distinct layers and components. The most obvious function is to protect the body against external influences. The skin is a highly organized, stratified structure consisting of three main layers, called the epidermis, dermis and hypodermis (1). Normal microflora of skin is dominated by Gram-positive bacteria such as *Staphylococcus*, *Micrococcus*, *Corynebacteria* (*Corynebacterium*) and Diptheroids (*Propionibacterium acne*). In addition to resident skin flora, the dust particles may also carry Fungi and Bacilli. *Aspergillus*, *Penicillium*, *Cladosporium* and *Mucor* are the major types of fungi found under the nails (2). Multidrug resistant bacteria including nosocomial pathogens have become important cause for higher skin care costs. A novel compound, with difference in mode of activity of antibiotics against microbes, is an attractive alternative against multidrug resistant microorganisms. Plant kingdom is a gold mine for novel and affordable skin care acting through novel mechanisms against skin pathogen (3).

*Amla* which is known as *Emblica officinalis* is an Indian herb which is extensively used in ayurvedic system of medicine. *Amla* is a prestigious herb finds it mention in Charak Samhita

as a Rasayan. The herb is also aphrodisiac, haemostatic, nutritive tonic and rejuvenative. It increases red blood cell count. It improves complexion and removes wrinkles. *Amla* is also used to treat constipation and is used as a cooling agent to reduce the effects of sun strokes and sun burns. It is the main ingredient used in the shampoo (4). *Amla* fruit is widely used in the Indian system of medicine as diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, anti-pyretic, hair tonic and ulcer preventive and for common cold, fever; as alone or in combination with other plants. Phytochemical studies on amla disclosed major chemical constituents including tannins, alkaloids, polyphenols, vitamins and minerals. It is used as analgesic, anti-tussive, antiatherogenic, adaptogenic; cardio, gastro, nephro and neuro protective, chemopreventive, radio and chemomodulatory and anticancer properties. *Amla* is also reported to possess potent free radical scavenging, antioxidant, anti-inflammatory, anti-mutagenic, immune-modulatory activities, which are efficacious in the prevention and treatment of various diseases like cancer, atherosclerosis, diabetes, liver and heart diseases (5).

#### Materials and methods

**Collection of plant materials and solvent extraction:** The fruits of the *Amla* (*Emblica officinalis*) were washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade-dried, powdered and used for extraction. Five gram of shade dried powder of *Amla* was put in 50 ml each of chloroform, distilled water, ethanol, methanol and petroleum ether in separate conical flasks. The solutions were placed in shaker for 24hrs so as to shake them properly. All these five extracts were filtered through Whatman filter paper no. 44 and evaporated in the water bath at 65° C. The extracts were dissolved in 2% DMSO to make the final concentration (1 mg /ml), which kept in refrigerator till further use (6).

**Test Microorganisms used:** The total five test organisms were used in the present study, which included Gram positive bacteria (*Staphylococcus aureus* (isolate) and *Propionibacterium acne* (isolate)), Gram negative bacteria (*Pseudomonas*

*aeruginosa* MTCC 741 and *Escherichia coli*-MTCC 483) and yeast (*Candida albicans*- MTCC 183).

**Preparation of the microbial inoculums:** The density of test bacteria and yeast was adjusted equal to that of the 0.5 McFarland standards ( $1.5 \times 10^8$  CFU/ml) by adding sterile distilled water. McFarland standards were used as a reference to adjust the turbidity of microbial suspensions so that the number of microorganisms may be within a given range. For the preparation of the 0.5 McFarland standard, 0.05 ml of 0.17%w/v  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$  was added to 9.95 ml of 0.18M  $\text{H}_2\text{SO}_4$  (1.0% w/v) with constant stirring. The McFarland standard tube was tightly sealed to prevent loss by evaporation and stored for up to 6 months (7).

**Antimicrobial Activity:** The antimicrobial activity of five different extracts of *Amla* (*Emblica officinalis*) against five test microorganisms was evaluated by using Agar well diffusion method.

**Antibiotic susceptibility pattern of test microorganisms used:** Antibiotic susceptibility testing against test strains was done according to Kirby-Bauer Disc Diffusion assay. Autoclaved medium (Nutrient Agar for gram negative bacteria i.e. *E. coli* and *P. aeruginosa*, Brain Heart Infusion Agar (BHI) for *Propionibacterium acne*, Mannitol Salt Agar medium for *S. aureus* and Malt extract Agar medium for *C. albicans*) was poured in sterile Petri plates thereafter the antibiotic disk diffusion assay was carried out after 24 hours. 100 $\mu$ l standardized culture was spread on these agar plates. Antibiotic Hexa discs of Hi-Media were placed on the inoculums seeded plates. After incubation for 24 hrs at 37°C, the plates were observed. If antimicrobial activity was present on the plates; it was indicated by an inhibition zone surrounding the disc. The zone of inhibition was measured and expressed in millimeters (8).

**Determination of MIC, MBC and MFC of most potent plant extract:** For MIC (Minimum Inhibitory Concentration), the macro-dilution agar method, a two-fold serial dilution of the plant extract was prepared in sterile distilled water to achieve a decreasing concentration ranging from 1mg/ml to 0.03125mg/ml in different tubes. Sterile cork borer

of 6.0 mm diameter was used to bore well in pre-solidified medium agar plates and 50-100µl volume of each dilution was added aseptically into the wells made in agar plates in triplicate that had test bacteria and yeast seeded with the standardized inoculums ( $1.5 \times 10^8$  CFU/ml). 50-100µl solvent was introduced into the well used as control. All the test plates were incubated at 37°C for bacteria and 35°C -37°C for yeast and were observed for the growth after 24 hrs. The lowest concentration of an extract showed a clear zone of inhibition was considered as the MIC. The MIC plates were further incubated for 24-48hrs (9). The lowest concentration that yields no growth following this further incubation was the MBC (Minimum Bactericidal Concentration). The same method was used for antifungal activity. The standardized fungal inoculum was used. The inoculated plates were incubated at 35°C-37°C for the *Candida albicans* growth. The above mentioned procedure was done for the fungal strain. The lowest concentration that yields no growth following further incubation of MIC plates was considered as the MFC (Minimum Fungicidal Concentration).

**Synergistic activity of plant extract/s and commercially available antibiotic:** The bacterial cultures were grown in culture broth at 37°C. After growth, each bacterium was inoculated on the surface of MHA agar plates. Subsequently, the antibiotic disk of 6 mm diameter was placed on the surface of each inoculated plate and then added 20µl of plant extract (at a concentration of 1mg/ml), to identify synergistic effect between the plant extract and antibiotic used. The plates were incubated at 37°C for 24 hrs. The diameter of clearing zones was measured (9).

**Phytochemical analysis of most potent plant extract:** Freshly prepared extract was subjected to standard phytochemicals analysis to find the presence of the following phytoconstituents phenols, flavonoids, alkaloids, glycosides, tannins, saponins, carbohydrates, phytosterols, proteins and steroids by using Mayer's test, Molisch's test, Modified Borntrager's Test, Foam Test, Salkowski's Test, Ferric chloride test, Lead

acetate test, Ninhydrin test and copper acetate test (9).

**Partial characterization of most potent plant extract:** It was done by GC-MS (Gas Chromatography-Mass Spectroscopy). The plant extract was analyzed with the help of GC-MS analyzer (GC Clarius 500 Perkin Elmer).

- On Elite-1 column the data was generated. The carrier gas helium (99.99%) was used at flow rate of 1ml per min in split mode (10:1). Methanolic sample (2 µl) was injected to column at 250°C injector temperature.
- Temperature of oven starts at 110°C and hold for 2 min and then it was raised at rate of 10°C per min to 200°C without holding. Holding was allowed for 9 min at 280°C at program rate of 5°C per min. Temperature of ion source was maintained at 200°C.
- The injector temperature was set at 250°C and detector temperature was set at 280°C. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode from 45 to 450 Da atomic mass units. A 0.5 seconds of scan interval and fragments from 45 to 450 Da was maintained.
- Total running time was 36 minutes (10). The electron gun of mass detector liberated electrons having energy of about 70eV. The column employed there for the separation of components was Elite 1(100% dimethyl poly siloxane).
- The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and with published literatures. NIST08 LIB9, WILEY8 LIB10 library sources were also used for matching the identified components from the plant material (11).

**Result**

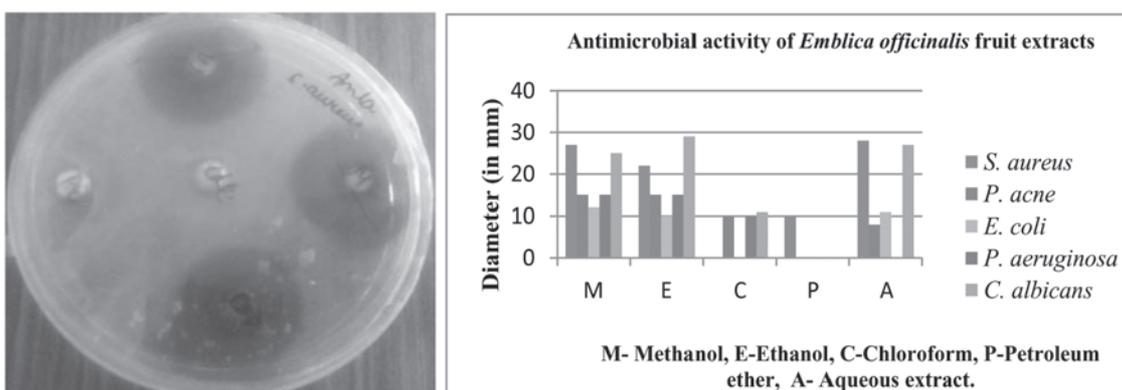
The present study revealed the scientific validation of natural products as an antibacterial and antifungal agent. The five solvents viz. Ethanol, methanol, chloroform, distilled water and petroleum ether were used for the extraction from Amla (*Emblica officinalis*) and used for antimicrobial activity against tested microbial strains. Methanolic extracts of *Emblica officinalis* showed maximum zone of inhibition i.e. 27 mm, while aqueous extract of *Emblica officinalis* was most effective against *S. aureus* (28 mm). Ethanol extract of *Emblica officinalis* was most effective against *C. albicans* (29 mm). Antimicrobial activity of *Emblica officinalis* extracts was compared with antibiotics and *Emblica officinalis* methanolic extract was the most promising plant having maximum effective antimicrobial activity against all tested microorganisms as shown in Table-1 and Fig.-1. For the antibiotic susceptibility pattern, Gentamicin was the most effective for Gram positive test bacteria, whereas Amikacin was the most effective antibiotic for Gram negative test bacteria and Clotrimazole for test fungal strain as shown in Table-2.

MIC and MBC of methanolic extract of *Emblica officinalis* against the microbial strains was ranged between 0.50 to 0.03125 mg/ml as shown in Table-3 and Fig.-2.

Synergistic effect of methanolic extracts of *Emblica officinalis* with antibiotics against tested microorganisms showed that the zone of inhibition was found to be greater when compared to zone of inhibition of different antibiotics used alone. As shown in Table-4, for Gram positive bacteria *S. aureus*, the methanolic extract of *Emblica officinalis* with Gentamicin showed the synergism of zone of inhibition of 29 mm. For *P. acne*, the synergism between *Emblica officinalis* with Gentamicin was observed with zone of inhibition of 30 mm. In case of Gram negative bacteria *E. coli*, the methanolic extract of *Emblica officinalis* with Amikacin showed the synergism of zone of inhibition of 30 mm. For *P. aeruginosa*, the synergism between *Emblica officinalis* with Amikacin was observed with zone of inhibition of 30 mm. In case of *C. albicans*, there was no synergism effect observed for *Emblica officinalis* to the Clotrimazole as shown in Table-4.

Phytochemicals analysis of *Emblica officinalis* included Alkaloids, Saponins, Glycosides, Proteins, Phenols and Phytosterols respectively as shown in Fig.-3.

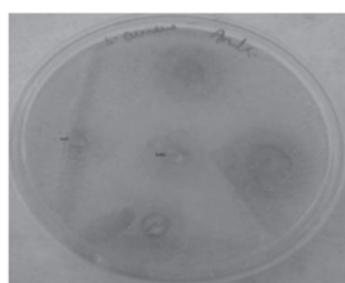
Major Phytocompounds along with their uses present in the methanol extract of the *Emblica officinalis* included Cyclopentasiloxane, decamethyl (deodorants, sunblocks and skin



**Fig. 1.** Antimicrobial activity of *Emblica officinalis* extracts against test microorganisms with Graphical representation.

**Table 1.** Antimicrobial activity of *Emblica officinalis* fruit extracts

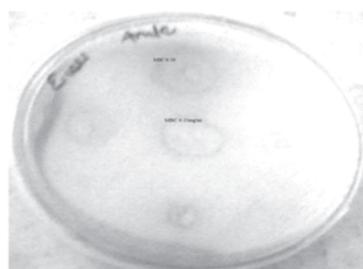
Microorganisms	Diameter of zone of inhibition(mm)				
	Methanolic	Ethanollic	Chloroform	Petroleum ether	Aqueous
<i>S. aureus</i>	27	22	NA	10	28
<i>P. acne</i>	15	15	10	NA	8
<i>E. coli</i>	12	10	NA	NA	11
<i>P. aeruginosa</i>	15	15	10	NA	NA
<i>C. albicans</i>	25	29	11	NA	27



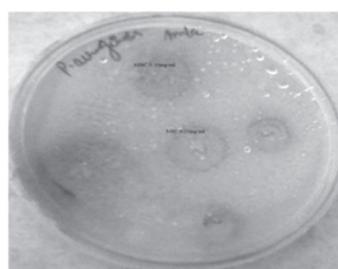
(a) *S. aureus*



(b) *P. acne*



(c) *E. coli*

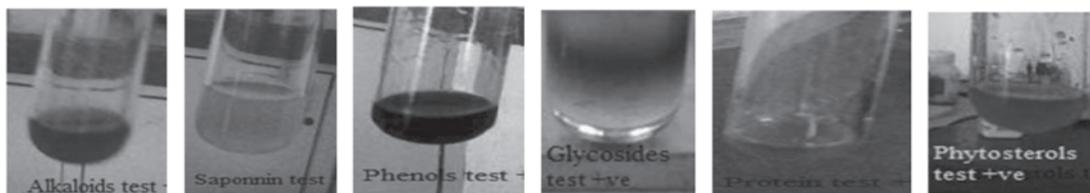


(d) *P. aeruginosa*

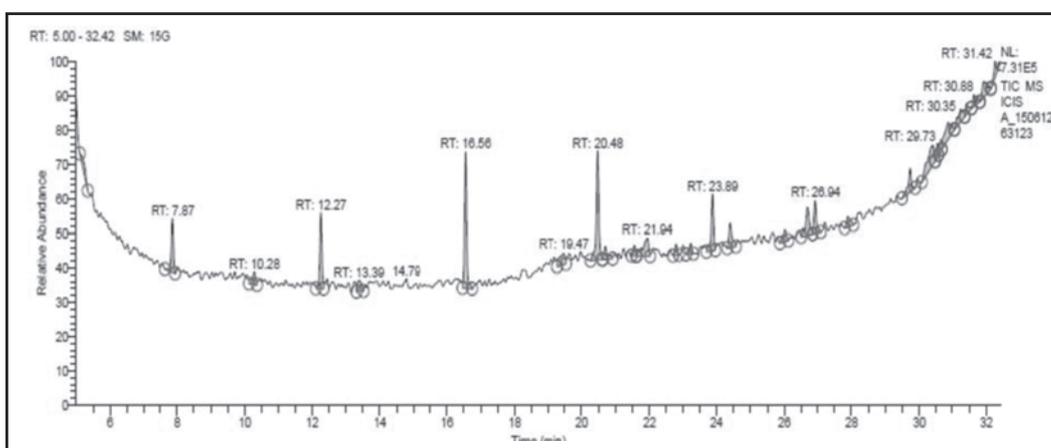


(e) *C. albicans*

**Fig. 2.** MIC, MBC and MFC of methanolic extract of *Emblica officinalis* against test microorganisms (a) *S. aureus* (b) *P. acne* (c) *E. coli* (d) *P. aeruginosa* and (e) *C. albicans*



**Fig. 3.** Phytochemical analysis of methanolic extracts of *Emblica officinalis* (a) Alkaloids test (b) Saponin Test (c) Phenol Test (d) Glycosides Test (e) Protein Test (f) Phytosterols test



**Fig. 4.** Total Ion Chromatogram (TIC) of methanol extract of *Emblica officinalis*

care), 3,4 Dihydroxy mandelic acid, ethyl ester, tri TMS(antioxidant), Rhodopin (Carotenoid), Hyocholic acid (precursor for steroid synthesis), Cyclohexasiloxane, dodecamethyl12 (Conditioning agent, emollient, defoaming agent and lubricant.), 6,9,12,15 Docosatetraenoic acid, methyl ester (essential fatty acid and abundant in retina and brain), Cycloheptasi-loxane, tetradecamethyl Anti-caking agent and Skin-Conditioning agent), Cycloocta-siloxane, hexadecamethyl (skin conditioning agent), Tetradecanoic acid, 9a (acetyloxy) 1a,1b,4,4a, 5,7a, 7b, 8,9,9a decahydro 4a, 7b dihydroxy3 (hydroxymethyl) 1,1,6,8tetramet Hyl5oxo1 Hcycloproa [3,4] benz [1,2e] azulen9yl ester (Cosmetic and topical medicinal preparations where good absorption through the skin is desired, Cyclonanosiloxane, octadecamethyl (Textile, polishes and waxes, electronics manufacture, monomer

in the production of polysiloxanes and laboratory reagent), Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13 tetradecamethyl (bioaccumulative), Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11 dodecamethyl (Lubricant and de-foaming agent), 9,12,15 Octadecatrienoic acid, 2,3bis [(trimethylsilyl)oxy] propyl ester, (Z,Z,Z) (tumor growth suppressor), Ethyl isoallocholate (Antimicrobial, Diuretic Anti-inflammatory, Antiasthma), 1Heptatriacotanol (Antimicrobial) and 2[4methyl6 (2,6,6 tri methyl cyclohex1enyl) hexa1,3,5trienyl] cyclohex 1en1carboxaldehyde (cosmetic products soaps, eaudetoilettes, after shaves and deodorants allergen) and 1Monolinoleoylglycerol trimethylsilyl ether (Ether compound antimicrobial, antioxidant, antiinflammatory). The compounds identified by GC-MS analysis of methanolic extract of *Emblica officinalis* are useful as the conditioning agent,

emollient, defoaming agent, lubricant, antimicrobial, diuretic anti-inflammatory, antiasthma, lubricant, de-foaming agent and skin conditioning agent (Fig-4).

the Soxhlet apparatus whereas 5g sample in 50 ml of ethanol using water bath apparatus was taken in this study and even then obtained better results.

**Discussion**

The result of the aqueous extract of *Emblica officinalis* in terms of zone of inhibition diameter against *S. aureus* were better than the findings of (12), perhaps due to the fact that they demonstrated the antimicrobial activity of *Emblica officinalis* on the basis of phytochemicals constituents of seeds extracts of *Emblica officinalis*. For *C. albicans*, the result was better for the ethanolic extracts of *Emblica officinalis* than the findings of (13). They demonstrated the antimicrobial and anticandidal activity of *Emblica officinalis* by taking 100 g of powered plant sample, extracted with 200 ml of ethanol using

The reason for different sensitivity between Gram positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms; Gram negative bacteria having an outer polysaccharide membrane carry the structural lipopolysaccharide components. As discussed by (14), this makes cell wall impermeable to lipophilic solutes, the Gram positive are more susceptible having only an outer peptidoglycan layer which is not effective permeability barrier.

The results of screening revealed that the most of the plant extracts were active against

**Table 2.** Antibiotic susceptibility pattern of test microorganisms

Antibiotic- Symbol (concentration)	Zone of inhibition (in mm)				
	Gram positive bacteria		Gram negative bacteria		Fungus
	<i>P. acne</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Ciprofloxacin- CIP(5mcg)	32mm	19 mm	—	—	—
Gentamicin-GEN(10 mcg)	28 mm	24 mm	19 mm	20mm	—
Vancomycin-VA(30 mcg)	26 mm	23 mm	—	—	—
Linezolid- LZ(30mcg)	11 mm	39 mm	—	—	—
Ampicillin- AMP 10 10 mcg	10 mm	8 mm	25 mm	22 mm	—
Streptomycin-S10(10 mcg)	15 mm	18 mm	—	—	—
Amikacin- AK(30 mcg)	26 mm	30 mm	—	—	—
Tetracycline-TE(30 mcg)	—	—	21 mm	25 mm	—
Chloramphenicol-C(30 mcg)	—	—	26 mm	16 mm	—
Cotrimoxazole-CO(25 mcg)	—	—	21 mm	22 mm	—
Clotrimazole- CC (10mcg)	—	—	—	—	15mm
Micanazole- MIC(30mcg)	—	—	—	—	No zone
Nystatin- NS(50mcg)	—	—	—	—	No zone

**Table 3.** Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values of methanol extract of *Emblica officinalis*

S. No.	Microorganisms	Plant Extract	MIC (mg/ml)	MBC (mg/ml)	MFC (mg/ml)
1.	<i>S. aureus</i>	<i>Embliica officinalis</i>	0.50	0.25	-
2.	<i>P. acne</i>	<i>Emblica officinalis</i>	0.0312	0.0312	-
3.	<i>E. coli</i>	<i>Emblica officinalis</i>	0.25	0.50	-
4.	<i>P. aeruginosa</i>	<i>Emblica officinalis</i>	0.25	0.50	-
5.	<i>C. albicans</i>	<i>Emblica officinalis</i>	1	-	1

**Table 4.** Synergistic activity of methanolic extract of *Emblica officinalis* with antibiotics

S. No.	Microorganisms	Plant extract + Antibiotic	Zone of inhibition (mm)	Comparative Effect on zone of inhibition
1.	<i>S. aureus</i>	<i>Emblica officinalis</i> + Gentamicin	29 mm	Activity increases
2.	<i>P. acne</i>	<i>Emblica officinalis</i> + Gentamicin	30 mm	Activity increases
3.	<i>E. coli</i>	<i>Emblica officinalis</i> + Amikacin	30 mm	Activity increases
4.	<i>P. aeruginosa</i>	<i>Emblica officinalis</i> + Amikacin	30 mm	Activity increases
5.	<i>C. albicans</i>	<i>Emblica officinalis</i> + Clotrimazole	NA	Activity decreases

Gram positive bacteria (*S. aureus* and *P. acne*) than Gram negative bacteria (*E. coli* and *P. aeruginosa*). In case of antibiotic susceptibility pattern, our results were in agreement with the findings of (15), for the susceptibility test of ciprofloxacin and ampicillin against *S. aureus*, (16), for the susceptibility test of amikacin against *E. coli*, (17), for the susceptibility test of amikacin against *P. aeruginosa* and (18) and for the susceptibility test of clotrimazole against *C. albicans*.

Gupta *et al.* studied the antimicrobial activity of *Emblica officinalis* and found that the MIC values of methanolic extract ranged between 0.025 mg/ml to 0.050 mg/ml against *P. aeruginosa*, *E. coli*

and *S. aureus* which might be due to change in methodology followed and concentration of *Emblica officinalis* extract considered by them (12).

Synergistic effects of extracts of *Emblica officinalis* with antibiotics against tested microorganisms showed that the Zone of inhibition found to be greater when compared to zone of inhibition of different antibiotics used alone i.e ranges from 0.25 mg/ml to 0.50 mg/ml (Table-4). Such synergistic effects could not be traced in the available literature.

Phytochemicals analysis was done by (9) and (19) for methanolic extract of *Emblica*

**Table 5:** Phytocompounds present in the methanolic extract of the *Emblica officinalis* GC-MS Peak Report TIC

Peak	Retention Time	Chemical formula	Compound	Uses	Cas#
1	7.87	C10H30O5S i5	Cyclopentasiloxane, decamethyl	<u>deodorants, sunblocks</u> and <u>skin care</u>	541026
1	7.87	C16H30O4S i3	Benzoicacid, 2,6- bis[(trimethylsilyl)oxy],trimet hysilyl ester	Not found	3782852
1	7.87	C19H36O5S i3	3,4-Dihydroxymandelic acid, ethyl ester, triTMS	antioxidant	NA
2	10.28	C40H58O	Rhodopin	Carotenoid	105920
2	10.28	C24H40O5	Hyocholic acid	<u>precursor</u> for steroid synthesis	547751
3	12.27	C12H36O6S i6	Cyclohexasiloxane, dodecamethyl12	Conditioning agent, emollient, defoaming agent and lubricant.	540976
4	13.39	C20H13N5 O2	2,7- Diphenyl1,6- dioxypyridazino[ 4,5:2',3'] pyrrolo[ 4',5'd] Pyridazine	Undergo direct nucleophilic addition and substitution reactions	91757061
4	13.39	C23H38O2	6,9,12,15-Docosatetraenoic acid, methyl ester	essential fatty acid and abundant in retina and brain.	17364340
5	16.56	C14H42O7S i7	Cycloheptasiloxane, tetradecamethyl	Antimicrobial Agents in Cosmetics	107506
6	19.47	C25H42N4 O4	2-Nonadecanone 2,4-dinitrophenylhydrazine	sensitive to <u>shock</u> and <u>friction</u>	28813618
7	20.48	C16H48O8S i8	Cyclooctasiloxane, hexadecamethyl	Antimicrobial Agents in Cosmetics	556683
7	20.48	C14H42O5S i6	Hexasiloxane, tetradecamethyl	Antioxidants	107528
8	21.94	C36H56O8	Tetradecanoic acid, 9a(acetyloxy)1a,1b,4,4a,5,7 a,7b,8,9,9adecahydro4a,7b dihydroxy3(hydroxymethyl) 1,1,6,8 tetramet Hyl 5-oxo,1- Hcyclopropa[3,4]benz[1,2e] azulen9yl ester.	Cosmetic and topical medicinal preparations where good absorption through the skin is desired	16561298

9	23.89	C18H54O9Si9	Cyclononasiloxane, octadecamethyl	Textile, polishes and waxes, electronics manufacture, monomer in the production of polysiloxanes and laboratory reagent.	556718
10	26.94	C14H44O6Si7	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl	bioaccumulative	19095239
10	26.94	C12H38O5Si6	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11 dodecamethyl	Lubricant and de-foaming agent.	995824
11	29.73	C27H52O4Si2	9,12,15Octadecatrienoic acid,2,3bis[(trimethylsilyl)oxy]propyl ester,(Z,Z,Z)	tumor growth suppressor	55521227
11	29.73	C20H13N5O2	2,7Diphenyl1,6dioxopyridazino[4,5:2',3']pyrrolo[4',5'd]Pyridazine	Pipeline	91757061
12	30.35	C20H13N5O2	2,7Diphenyl1,6dioxopyridazino[4,5:2',3']pyrrolo[4',5'd]Pyridazine	pipeline	91757061
12	30.35	C26H44O5	Ethyl isoallochololate	Antimicrobial, Diuretic Anti-inflammatory, Antiasthma	NA
13	30.88	C37H76O	1Heptatriacotanol	Antimicrobial	105794589
14	31.42	C23H32O	2[4methyl6(2,6,6trimethylcyclohex1enyl)hexa1,3,5trienyl]cyclohex1en1carboxaldehyde	cosmetic_products soaps, eaude toilettes, after_shaves and deodorants and allergen.	NA
14	31.42	C27H54O4Si2	1Monolinoleoylglycerol trimethylsilyl ether	Antimicrobial Antioxidant Antiinflammatory Antiarthritic Antiasthma, Diuretic	54284456

*officinalis*. All the prepared plant extract were subjected to preliminary screening for the presence of alkaloids, tannins, saponins, glycosides, proteins, phenols, carbohydrate, diterpenes, phytosterols and Flavonoides. The methanolic extract of *Embllica officinalis* includes alkaloids, saponins, glycosides, proteins, phenols and phytosterols, respectively. (20) also studied the phytochemicals present in *Embllica officinalis* and found the presence of quercetin, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C and also contain various polyphenolic compounds. The results may be varied due to the solvent selection and the methodology followed.

GC-MS analysis was used for the analysis and identification of the bioactive compounds present in the *Embllica officinalis*. GC-MS analysis for *Embllica sp.* revealed the presence of the octasiloxane, hexadecamethyl, cyclononasiloxane, octadecamethyl, heptasiloxane, tetradecamethyl, octadecatrienoic acid, ethyl isoallocholate, phthalic acid, 2-cyclohexylethyl butyl ester, rhodopin, benzoic acid and hyocholic acid. (21) also studied the GC-MS analysis of methanolic extract of *Embllica officinalis* and found the presence of flavonoids, carbohydrates and saponins. The results were slightly varied because of the GC-MS analyzing methodology.

### Conclusion

In this way, this study revealed the methodology and identification of the bioactive compounds present in the *Embllica officinalis* which were responsible for their antimicrobial activity against skin associated microorganisms. The use of crude drugs of *Embllica officinalis* as an agent to control microbial strains needs further extensive research for their better economic and therapeutic utilization. Thus, the present study reflects a hope for the development of novel agents of biomedical importance.

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