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Evaluation of Non-Pressurized Topical Spray Formulation of Miconazole and Neomycin

Neelam Pawar[1*], Pawan Jalwal[2]

[1,2]Department of Pharmaceutical Sciences, Baba Mastnath University, Asthal Bohar, Rohtak, Haryana, India, 124021

[*]neelampawar5555@gmail.com

ABSTRACT

Background: The prepared formulation of Non-Pressurized Topical Spray of Miconazole and Neomycin was analyzed in GC-MS depicting various components present and their biological activity. Antimicrobial and antifungal activity depicts that the maximum inhibitory zone was observed for non-pressurized topical spray in comparison to drug controls, furthermore, performed the cytotoxicity study of the formulation.

Material and Method: Gas Chromatography Mass Spectrophotometry, antibacterial, antifungal, and cytotoxicity experiments were performed on a non-pressurized topical spray formulation of Miconazole and Neomycin.

Results: The results of the Gas Chromatography Mass Spectrophotometry (GCMS-TQ 8050 NX with quadrupole) analysis show that Miconazole (9.42%) functions as an antifungal agent, Dodecanoic acid (10.6%) is as a disinfectant, Nonaneaol (0.63%) acts as an antibacterial agent, and 1H-Indole 3-acetic acid (5.90%) acts as a cell growth and organ development agent, Propylester (14%) as an antioxidant and cancer preventative, tetradecanoic acid Oleic acid (8.31%) acts as a 5-reductase inhibitor, Allergenic, reductase inhibitor, anti-inflammatory, antiandrogenic, cancer preventative, antialopecic, antileukotrience D4, 9-octadecenoic acid (Z)-2-hydroxy (4.05%) and Neomycin (4.71%) having antibacterial activity. *Candida parapsilosis*, *Candida tropicalis*, *Candida albicans*, and *Candida krusei* showed broad antifungal activity, with inhibition zones of 18 mm, 23 mm, 19 mm, and 22 mm for non pressurised topical spray, respectively. Inhibition zones for miconazole drug solution was 16mm, 21mm, 17mm, and 20 mm. Antibacterial activity was reported on *Escherichia coli*, *Staphylococcus aureus*, and *Micrococcus luteus*, with zones of inhibition of 15mm, 17mm, and 25mm for nonpresurized topical spray and 13mm, 14mm, and 22mm for Neomycin solution, respectively.

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Cytotoxicity tests were performed on HACAT (Human Epidermal Keratinocyte Line) at concentrations of 6.25, 12.5, 25, 50, 100, and 200 micrograms per millilitre to determine percent inhibition after 24 hours.

Conclusiuon: The non-pressurized topical spray's chemical constituents act as antifungal agent, disinfectant, antibacterial agent, cell growth development agent, cancer preventative agent. Spray showed broad antifungal and antibacterial activity against *Candida parapsilosis, Candida tropicalis, Candida albicans, Candida krusei, Escherichia coli*, *Staphylococcus aureus*, and *Micrococcus luteus.* Because the inhibition was less than 50%, non-pressurized topical sprays of miconazole and neomycin were determined to be non-toxic or had no appreciable cytotoxicity.

Keywords :-Miconazole**,** Neomycin**,** Non-pressurized Topical Spray**,** Microbiological Evaluation, Gas Chromatography Mass Spectrophotometer Analysis, Cytotoxicity Activity.

Introduction

As sprayed on the skin, a non-pressurized topical spray formulation comprising a polymeric drug solution will release the drug present in saturated form from the matrix, when the solvent evaporates and the medications diffuse through the skin barrier into the systemic circulation[1,2]**.** Gas Chromatography Mass Spectrophotometry is a technique for determining the purity of a substance or separating the constituents of a combination. In preparative chromatography, the gas chromatography can be used to prepare pure compound from a mixture. The mobile phase in gas chromatography is a carrier gas, which is usually an inert gas like Helium or a reactive gas like nitrogen. In nearly 90% of devices, helium is still the most often employed carrier gas. Inside a piece of glass or metal tubing known as the column, the stationary phase is a tiny layer of liquid or polymer on an inert solid support. The gaseous compound being studied interacts with the column's wall. Miconazole is an antifungal medicine whose azole derivatives inhibit the fungus cytochrome P450 enzyme, inhibiting ergosterol synthesis and triggering fungal cell death leading to irregularities in the plasma membrane. Miconazole is typically used to treat tinea pityriasis, cutaneous diseases, and otomycosis. The most common application of neomycin is for the treatment of skin infections. Neomycin is the best option for treating superficial *Staphylococcal* and gram-negative *Bacilli* infection [3-7].

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In vitro testing was used to determine the toxicity of a non-pressurized topical spray by measuring stained (vital dye) live cells. When MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium, a tetrazolium salt that does not require phenol red dye, was produced in water, yellowish colour solutions were created, and insoluble purple formazan was formed by cleaving the tetrazolium ring with the help of mitochondrial dehydrogenase enzyme from cultivated human keratinocyte cells (HACAT). The formazan is a water-insoluble chemical that can be dissolved in DMSO and acidified with ethanol or isopropanol. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). This purple formazan solution could be spectrophotometrically examined at 590 nm lambda max. The degree of toxicity caused by non-prescribed topical spray is indicated by a simultaneous change (increase or decrease) in cell quantity and amount of formazan produced (Figure 1). The antimicrobial activity against *Candida parapsilosis*, *Candida tropicalis*, *Candida albicans*, *Candida krusei*, *Escherichia coli*, *Staphylococcus aureus*, and *Micrococcus luteus* was calculated by measuring minimum inhibitory count (MIC) and Zone of inhibition [8-11].

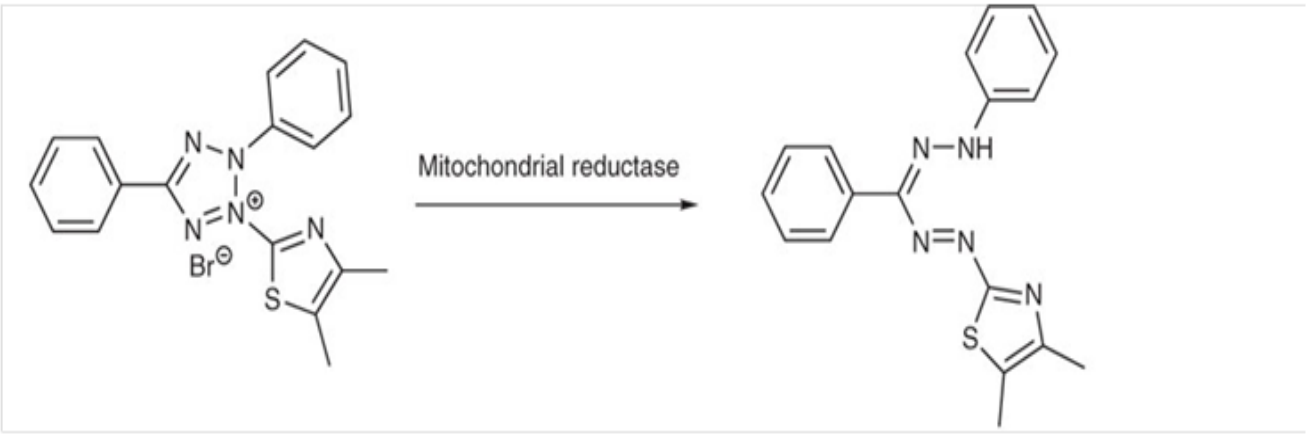

Figure 1: Mitochondrial Dehydrogenase reductase of MTT.

Methods

Gas Chromatography Mass Spectrophotometer

GCMS (Thermo Scientific, U.S., Model: Trace 1300 G.C. Coupled with Thermo TSQ 8000 Triple Quadrupole MS) was used to examine the composition of a non-pressurized topical spray

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of miconazole and neomycin. A total of 50 compounds were examined, with the dominant component constituting. GCMS-TQ 8050NX with quadrupole mass spectrophotometer has been used to evaluate non-pressurized topical sprays of miconazole and neomycin. All components were separated on a Rtx-5MS column (30mX0.25 mm inner diameter X 0.25 micrometre film thickness), and the operation was carried out under the following conditions: Split injection mode, 250°C oven temperature, Flow Control Mode: 9.0 mL/min, Pressure:53.5 kPa, Column Flow:1.00 mL/min, Linear Velocity :36.3 cm/sec, 3.0 mL/min purge flow, 5.0 split ratio High Barometric Pressure , Ion Source Temperature:200°C, Interface Temperature:260°C, Solvent Cut Time:4.50 min, Injection Pressure:250 kPa, Time:1min, 0.95 kV +0.20 kV Detector Gain, Mode: Relative to the Tuning Result, Mode:Q3 Scan, Event Time:0.2sec, Scan Speed:5000, Start Time:5min, End Time:62min, Acq. Mode:Q3 Scan, Event Time:0.2sec, Scan Speed:5000 Start with a m/z of 50 and end with a m/z of 800 **[12-14].**

Microbiological Evaluation of Non-Pressurized Topical Formulation

MTCC (Microbial Type Culture Collection and Gene Bank), Chandigarh, India, provided the fungal (*Candida parapsilosis*, *Candida tropicalis*, *Candida albicans*, *Candida krusei*) and bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus*) strains in lyophilized form. The strains were seeded on nutrient agar plates under ideal environmental conditions for fungal and bacterium growth, according to MTCC **[15-16]**.

Agar Plate Method

Preparation of Nutrient Agar Plate

The nutritional agar plates were made by aseptically pouring sterile culture media into sanitised petri dishes. Dissolving the MTCC growth medium elements in distilled water and combining them according to the protocol created the culture media (Table 1). Before being heated at 40°C, the nutritious agar plates were solidified at room temperature **[17-18].**

Table 1: Composition of Nutrient Agar Media

S. No.	Ingredients for Culture Media	Quantity
1	Yeast Extract	2 g
2	Peptone	5 g

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3	Sodium Chloride	5 g
4	Agar	15 g
5	Distilled Water	1 l ml

Inoculation of Bactria and Fungi

Inoculation of fungal (*Candida parapsilosis, Candida tropicalis, Candida albicans, Candida krusei*) and bacterial (*Escherichia coli, Staphylococcus aureus, Micrococcus luteus*) strains using 0.5 ml liquefied nutritional broth medium. Under aseptic circumstances, the agar plates were streaked with a sanitized loop (Streaking procedure) and stored in an incubator at 37°C in an aerobic atmosphere until growth had been seen. The bacteria were then subcultured in nutrient agar medium and saline solution. To seed fungal strains (*Candida parapsilosis, Candida tropicalis, Candida albicans, Candida krusei*) and bacterial strains (*Escherichia coli, Staphylococcus aureus, Micrococcus luteus*) in plates, a sterilised cotton swab was dipped in liquefied inoculum (saline containing bacteria) and rubbed lightly and evenly on the surface of solid. The disc diffusion method was used to perform microbiological and antifungal assays on the formulation, medicines solution, and empty spray formulation **[19-21].**

Preparation of Antibacterial and Antifungal Solution

Whatsman filter paper was used to make antibiotic discs for non-pressurized topical formulations, drug solutions, and empty spray formulations. To make little discs, the whatsman filter paper was cut into small circular pieces with a diameter of 6mm. These discs were dipped separately in drug-loaded and empty non-pressurized topical formulations and drug solution, and the volume of solution absorbed by each disc was quantified to determine the amount of antibiotic load on each disc. These discs were then dried at room temperature to make antibiotic-loaded discs, which were tested for antimicrobial activity against fungal (*Candida parapsilosis, Candida tropicalis, Candida albicans, Candida krusei*) and bacterial (*Escherichia coli, Staphylococcus aureus, Micrococcus luteus*) strains as antibacterial and antifungal models, respectively, using drug spray as a positive control and empty spray (blank) **[22].**

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Cytotoxicity Study

Sample Preparation

For cytotoxicity studies, Samples were considered 20mg/mL and serial two fold dilutions were prepared from 200µg/mL to 10µg/mL using DMEM plain media for treatment (Figure 2)[23-24]. List of material Used listed in Table 2.

Table 2: List of material used in Cytotoxicity Studies.

S. No.	Particulars
1	T25 flask
2	DMEM
3	RPMI media
4	Trypsin EDTA 0.05%
5	FBS
6	Penstrep
7	Sterile 96 well plate
8	MTT reagent
9	DMSO
10	Potassium chloride
11	Sodium chloride
12	Disodium phosphate
13	Monopotassium phosphate
14	1000µl tips
15	200µl tips
16	Microcentrifuge tubes
17	Serological pipettes
18	Filtration unit

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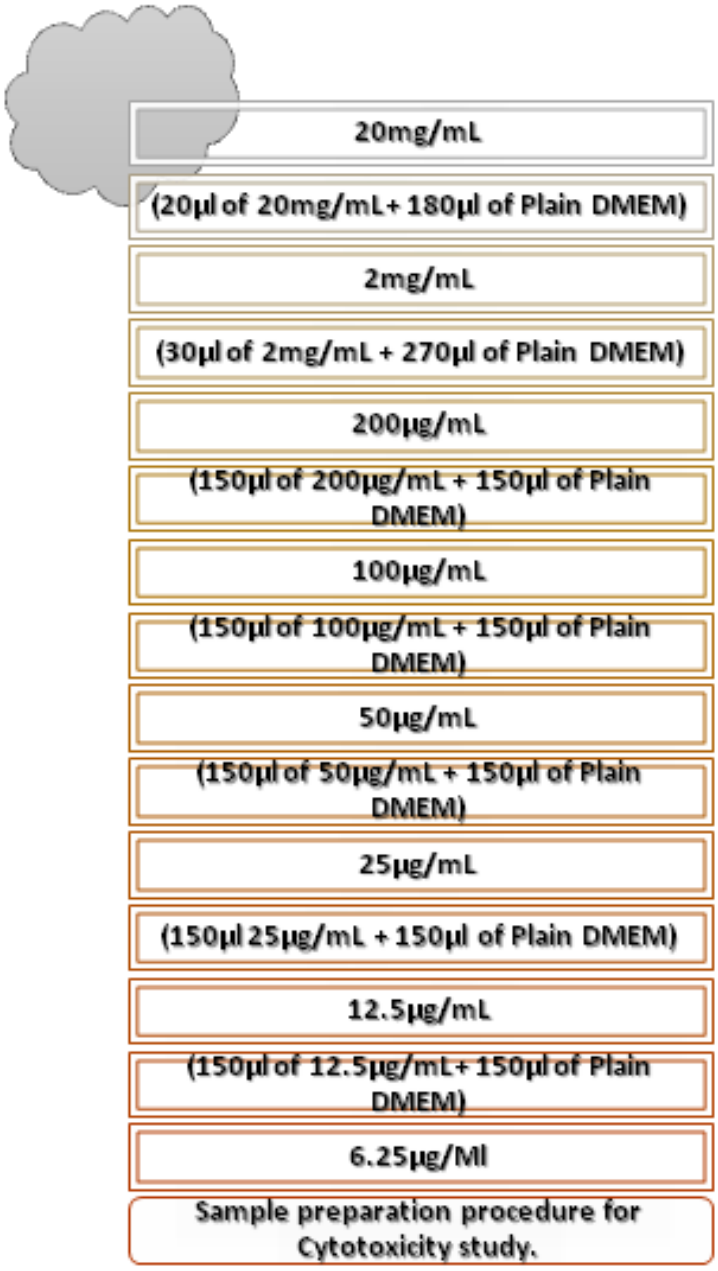

Figure 2: Procedure indicating sample preparation.

Culture Media and Cell Lines

HACAT cells were grown with 10 percent Fetal Bovine Serum (FBS) supplementation in Dulbecco's Modified Eagle's Medium (DMEM), Streptomycin (100 microgram per ml), and penicillin (100 IU/ml) in a 5 percent CO_2 environment at 37 degrees Celsius. The dissociating solution contained 0.05 percent glucose, 0.2 percent trypsin, and 0.02 percent Ethylenediamine

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Tetraacetic Acid in (Phosphate Buffered Saline) PBS. The HACAT cells were then attached to it, and the viability of the cells was checked. After that, 96 well plates were made, with 50000 cells seeded each well, and incubated at 37° Celsius for 24 hours with 5% carbon dioxide [25].

Procedure

Adjust the cell concentration with the help of medium in a trypsinized monolayer cell culture. The diluted cell suspension of $5x10^5$ microlitre was added to 96-well microlitre plate. The supernatant was removing when partial monolayer was formed after 24 hours then monolayer was washed and drug solution of 100 microlitre solution was added to this plate, which was then incubated for 24 hours at 37 degree celsius in 5% carbon dioxide atmosphere. After the supernatant was removed, 100 microlitres of DMSO (Dimethyl Sulfoxide) was added, where formazan was generated and detected at 590 nm using a microplate reader. The percentage of inhibition was determined using how much concentration test of solution was required for growth inhibition. The dose response curve revealed that 50% of the cells were viable [26].

Calculating Inhibition

% Inhibition = [(OD (Optical Density) of sample-OD of Control) / OD of Control X 100]

Result and Discussion

Gas Chromatography Mass Spectroscopy (GC-MS)

The components of the essential oil were identified using gas chromatography mass spectrometry (GC-MS). According to the principal elements, Miconazole (9.42%) is an antifungal drug, whereas Dodecanoic acid (10.6%) acts as a disinfectant. Nonaneaol (0.63 percent) is an antibacterial agent, and 1H-Indole 3-acetic acid (5.90 percent) is a cell growth and organ development agent, Propylester (14 percent) is an antioxidant and cancer preventative, and tetradecanoic acid oleic acid (8.31%) is a 5-reductase inhibitor, reductase inhibitor, anti-inflammatory, antiandrogenic, cancer-preventative, antialopecic, and antileukotrience agent. Along with certain significant elements, some minor constituents were also presented, including D4, 9-octadecenoic acid (Z)-2-hydroxy (4.05%) and Neomycin (4.71%) (Table 3, 4).

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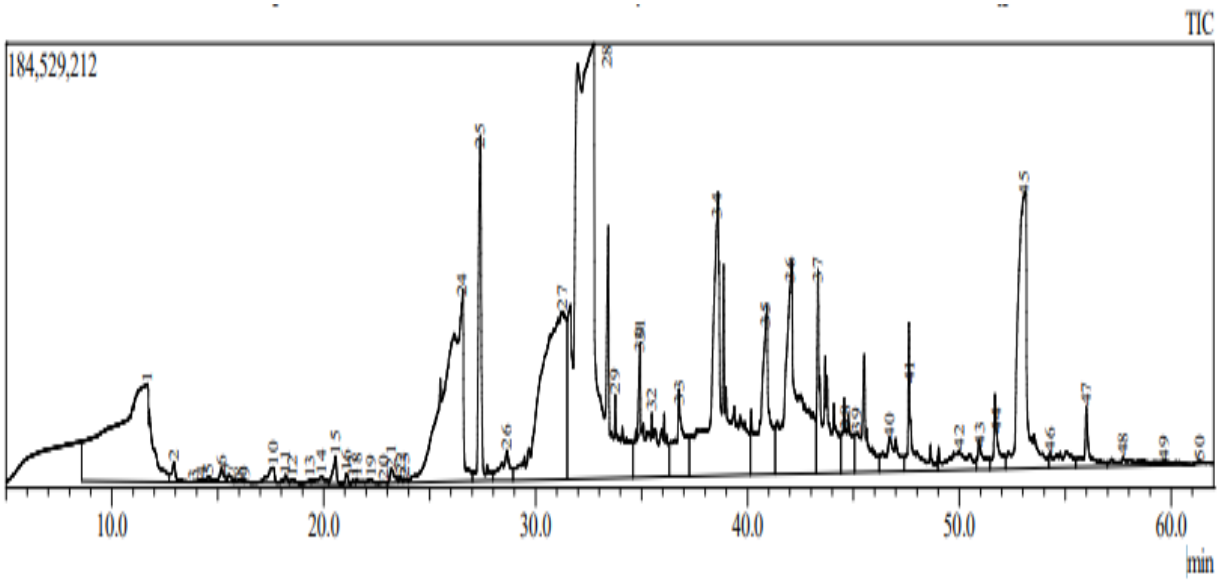

Figure 3: GCMS Chromatogram of Nonpressurized Topical Spray of Miconazole and Neomycin.

Table 3: Showing Retention Time, I. Time, F. Time, Area%, Height% and name of components found in Non-pressurized Topical Spray of Miconazole and Neomycin.

R. Time	I. Time	F. Time	Area%	Height%	Name
11.664	8.580	12.700	9.34	3.19	Trimethylphosphine
12.928	12.700	13.740	0.19	0.63	Nonanal
13.847	13.740	14.007	0.01	0.02	Octanoic acid
14.220	14.007	14.353	0.03	0.07	1,3-Cyclopentanediol
14.567	14.353	14.860	0.07	0.16	9,12-Octadecadienoic acid
15.178	14.860	15.420	0.15	0.44	1,3-Dioxane
15.526	15.420	15.767	0.07	0.23	Dodecanamide
16.008	15.767	16.247	0.04	0.10	Propane, 1,2,3-trimethoxy
16.300	16.247	16.673	0.01	0.07	5-Methoxy-1-pentanol, TMS derivative
17.616	16.833	17.793	0.24	0.49	2-Decenal
18.190	17.927	18.380	0.06	0.24	3-Hexanol, 2,3-dimethyl
18.521	18.380	18.887	0.04	0.12	4-Heptanol, 2,4-dimethyl
19.359	18.940	19.420	0.03	0.13	Hexadecane
19.896	19.420	20.247	0.14	0.23	Ethanone, 1-(2,4-dichlorophenyl)
20.551	20.247	20.860	0.23	0.88	2-Decenal
21.075	20.860	21.260	0.06	0.31	1,3-Dioxane
21.377	21.260	21.500	0.02	0.09	1,3-Dioxane
21.565	21.500	21.953	0.04	0.15	Tetradecane

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S. No.	Name	Biological Activity	Structure

22.238	21.953	22.593	0.05	0.14	2,4-Dichloro-N-methylbenzamide
22.818	22.593	23.020	0.01	0.04	2-Nonenoic acid, methyl ester
23.194	23.020	23.527	0.15	0.44	Ethanol, 2-[2-(2-methoxyethoxy)ethoxy
23.627	23.527	23.740	0.04	0.17	Benzoic acid, 2,6-dichloro
23.820	23.740	24.007	0.04	0.14	1,3-Dioxane
26.543	24.007	27.020	8.28	5.90	Diethyl Phthalate
27.373	27.020	27.980	2.45	10.61	Dodecanoic acid, 1-methylethyl ester
28.647	27.980	28.940	0.61	0.95	2-Naphthalenesulfonyl chloride
31.268	28.940	31.500	9.86	5.44	1H-Indole-3-acetic acid
32.605	31.500	34.593	23.66	14.01	Tetradecanoic acid, propyl ester
33.761	33.673	33.813	0.09	1.33	3,4-Dichlorobenzyl mercaptan
34.913	34.593	36.300	3.44	2.80	n-Hexadecanoic acid
34.931	34.773	34.983	0.36	2.94	n-Hexadecanoic acid
35.482	35.447	35.517	0.04	0.75	Hexadecanoic acid, ethyl ester
36.793	36.300	37.260	1.79	2.37	2-Methoxyethanol, TMS derivative
38.565	37.260	40.140	9.13	8.31	Oleic Acid
40.857	40.140	41.313	3.48	4.56	Benzene, 2-(bromomethyl)-1,3-dichlor
42.022	41.313	43.233	6.77	6.05	Benzene, 2-(bromomethyl)-1,3-dichlor
43.315	43.233	44.407	3.25	4.05	9-Octadecenoic acid (Z)
44.620	44.407	45.047	1.21	1.87	(2S,2'S)-2,2'-Bis[1,4,7,10-tetraoxacycle
45.157	45.047	46.220	1.77	1.24	Miconazole
47.654	47.393	48.993	1.39	2.86	9-Octadecenoic acid (Z)-, 2,3-dihydro
49.992	48.993	50.807	0.97	0.58	4-Nonanol, 2,6,8-trimethyl
50.966	50.807	51.447	0.39	0.75	2-Methoxyethanol, TMS derivative
51.767	51.447	52.193	0.72	1.80	9-Octadecenoic acid (Z)
53.053	52.193	54.220	6.25	8.99	Miconazole
54.278	54.220	55.500	0.64	0.43	Miconazole
59.667	59.020	60.940	0.20	0.11	10-Undecenoic acid
61.339	61.047	61.793	0.04	0.07	18,18'-Bi-1,4,7,10,13,16-hexaoxacyclo
48.934	48.024	49.479	2.11	2.19	Neomycin

Table 4: Component Name their biological activity and structure of component analyzed in GC-MS of Nonpressurized Topical Spray of Miconazole and Neomycin.

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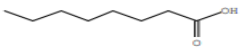
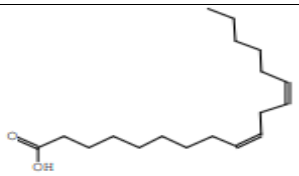
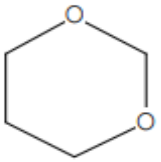
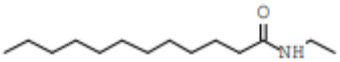
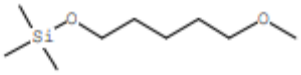
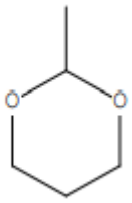
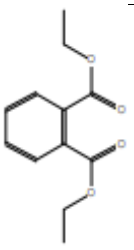
1	Nonanal	Antimicrobial activity	
2	Octanoic acid	Antioxidant	
3	9,12-Octadecadienoic acid (Z,Z)-	Skin Protectant	
4	1,3-Dioxane	Solvent	
5	Dodecanamide, N-ethyl-	Disinfectant	
6	5-Methoxy-1-pentanol, TMS derivative Silane	Antioxidant	
7	2-Decenal	Flavoring Agent	
8	1,3-Dioxane, 2-methyl	Solvent	
9	Diethyl Phthalate	Plasticizer	
10	Methoxytriethylene glycol	Solvent and plasticizer	

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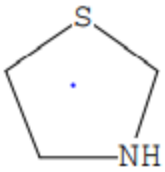
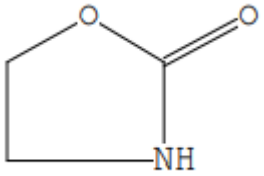
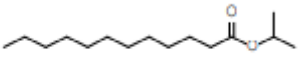
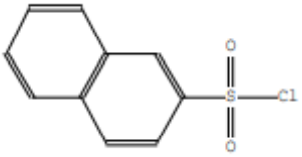
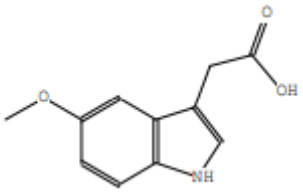
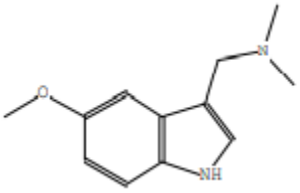
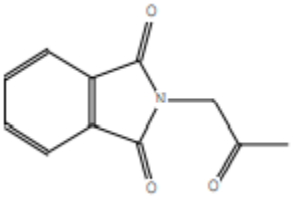
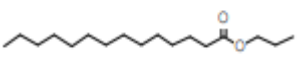
11	Thiazolidine	Antimicrobial, Anticancer, Anti-inflammatory	
12	Carbamic acid	Anticholinesterase	
13	Dodecanoic acid, 1-methylethyl ester	Antimicrobial, Viral infection treatment	
14	2-Naphthalenesulfonyl chloride	Histamine H3 receptor antagonist	
15	1H-Indole-3-acetic acid, 5-methoxy	Cell elongation, Organ development	
16	1H-Indole-3-methanamine	5-HT1B/1D receptor agonists	
17	Benzo[c]pyrrolidin-2,5-dione, N-acetylmethyl	Antitumor agents	
18	Tetradecanoic acid, propyl ester	NTCP inhibitors	

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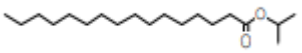
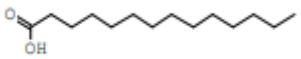
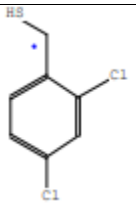
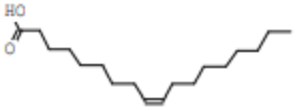
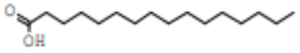
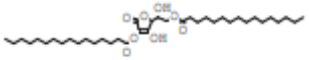
19	Isopropyl palmitate	Emollient and emulsifier, Anti-inflammatory	
20	Tetra decanoic acid $$ myristic acid	Anti oxidant, cancer preventive, hypercholesterolemic, nematicide, lubricant, cosmetic.	
21	2,4-Dichlorobenzyl mercaptan	NTCP inhibitors	
22	Oleic Acid $$ 9-Octadecenoic acid (Z)	5-α reductase inhibitor, allergenic, α-reductase inhibitor, anti inflammatory, anti androgenic, cancer preventive, anemiagenic, anti alopecic, anti leukotriene-D4, choleretic, dermatitigenic, hypocholestrolemic, insectifuge, perfumery, propecic, flavour.	
23	Palmitic acid	Emollient	
24	(+)-Ascorbic acid 2,6-dihexadecanoate	Anti oxidant, anti scorbutic, anti inflammatory, anti nociceptive, anti mutagenic, wound healing property.	

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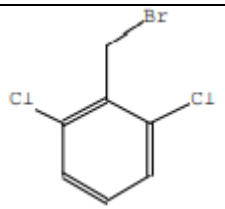
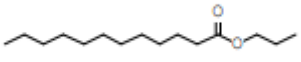
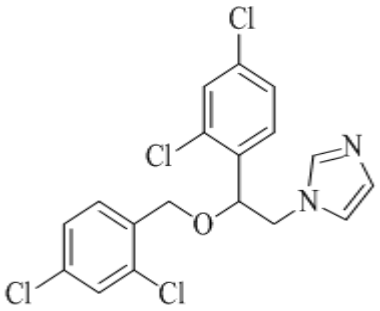
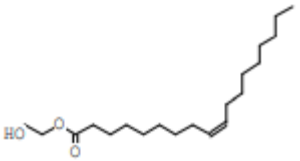
25	Benzene, 2-(bromomethyl)-1,3-dichlor	Bactericides	
26	9-Octadecenoic acid (Z	Antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge	
27	Miconazole	Antifungal	
28	:9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester $	5-α reductase inhibitor, allergenic, α-reductase inhibitor, anti inflammatory, anti androgenic, cancer preventive, anemiagenic, anti alopecic, anti leukotriene-D4, choleretic, dermatitigenic, hypocholestrolemic, insectifuge, perfumery, propecic, flavour.	

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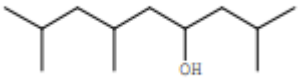
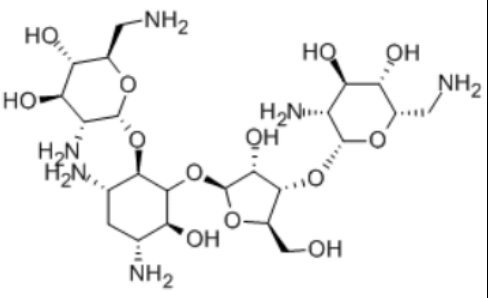
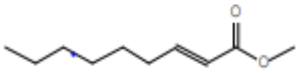
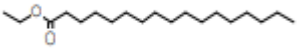
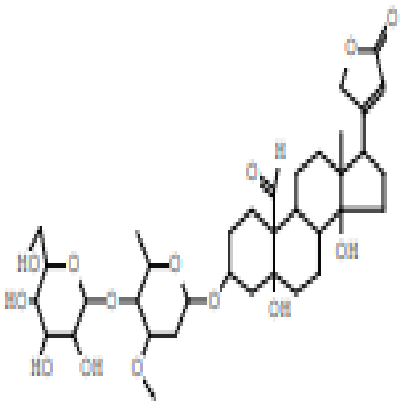
29	4-Nonanol, 2,6,8-trimethyl-	Antimicrobial	
30	Neomycin	Antimicrobial	
31	Nonanedioic acid ,dibutyl ester $$ Azelaic acid	Anti microbial, anti inflammatory, anti tumor, anti hyperpigmentative, anti proliferative, anti acne, cyto toxic, Anti leukemic, oxy radical scavenging activity.	
32	Heptadecanoic acid	Antioxidant, anti fungal, surfactant	
33	Card-20(22)-enolide, 3-[(2,6-dideoxy-4-O-.beta.	Used to treat skin disease, leprosy and ulceration.	

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Antimicrobial and Antifungal Activity

The *in vitro* antibacterial activity of non-pressurized topical formulation of Miconazole and Neomycin and blank was assessed using disc diffusion method in terms of zone of inhibition. The results obtained for diameter of zone of inhibition of formulation, drug solution and blank discs are summarized and compared as shown in Table 5.

Table 5: Diameter of Zone of Inhibition of non-pressurized topical formulation of Miconazole and Neomycin, Miconazole drug solution, Neomycin drug solution and Blank.

S. No.	**Samples**	**Coded Samples**	**Conc. used (µg/disc)**	**Diameter of Zone of Inhibition after 24 hrs ± S.D (mm)**						
				Candida parapsilosis	***Candida tropicalis***	***Candida krusei***	***Candida Albicans***	***E. coli***	***S. aureus***	***M. luteus***
1.	Non-pressurized topical formulation	F	30	19± 1.9	18±1.3	22±2.1	23± 2.3	15±1.3	17±1.5	25±2.0
2.	Miconazole drug solution as control, (+) ve control	MD	30	17 ± 1.5	16±1.7	20±1.9	21± 1.5			
3.	Neomycin drug solution	ND	30					13±1.5	14±1.1	22±1.3

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	as control, (+) ve control									

Results have shown a significant difference between zone of inhibition of samples. The maximum inhibitory zone was observed for non-pressurized topical spray possess zone of inhibition (18 mm for *Candida tropicalis*, 23 mm *Candida albicans*, 19 mm *for Candida parapsilosis*, 22 mm for *Candida krusei*) and 15 mm for *Escherichia coli* , 17 mm for *Staphylococcus aureus*, 25 mm for *Micrococcus luteus*) at a concentration of 30 µg/disc whereas miconazole drug solution showed zone of inhibition results 16 mm *Candida tropicalis*, 21 mm *Candida albicans*, 17 mm for *Candida parapsilosis*, 20 mm for *Candida krusei* at the same concentration, neomycin drug solution showed zone of inhibition 13 mm for *Escherichia coli*, 14 mm for *Staphylococcus aureus*, 22 mm for *Micrococcus luteus*. The significant difference between inhibitory zones of non-pressurized topical formulation and drugs samples or attributed that the non-pressurized topical formulation also possess significant antimicrobial activity and antifungal activity.

In-vitro Cytotoxicity Activity

Non-pressurized topical spray of Miconazole and Neomycin was treated with different concentrations in HACAT cells for 24 hours. There was no significant cytotoxicity found as the inhibition did not exceed 50%. Cell viability was above 50% and hence samples can be considered non-toxic (Table 6, Figure 4).

Table 6: % Inhibition of Control and Non-pressurized topical spray of Miconazole and Neomycin on HACAT cells.

HACAT										
Compound name	**Conc. µg/**	**OD at 590nm**	**% Inhibition**	**OD at 590nm**	**% Inhibition**	**OD at 590nm**	**% Inhibition**	**Mean % Inhib**	**% Cell Viab**	**Standard Devia**

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	ml							**ition**	**ility**	**tion**
Control	0	0.559	0.00	0.570	0.00	0.582	0.00	0.00	100.00	0.00
Sample	6.25	0.549	1.79	0.555	2.63	0.568	2.41	2.28	97.72	0.44
	12.5	0.512	8.41	0.503	11.75	0.516	11.34	10.50	89.50	1.82
	25	0.465	16.82	0.472	17.19	0.478	17.87	17.29	82.71	0.53
	50	0.411	26.48	0.416	27.02	0.405	30.41	27.97	72.03	2.13
	100	0.364	34.88	0.371	34.91	0.361	37.97	35.92	64.08	1.78
	200	0.342	38.82	0.345	39.47	0.368	36.77	38.35	61.65	1.41

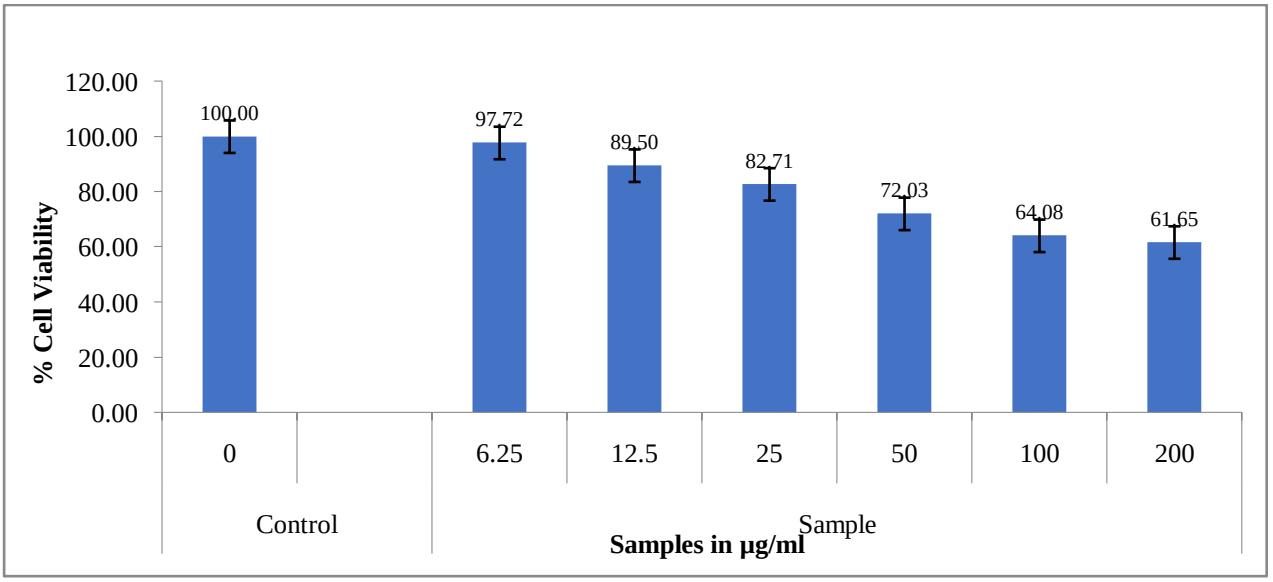

Figure 4: MTT Assay Using HACAT cell

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Conclusion

Non pressurized topical spray of Miconazole and Neomycin were analyzed using GCMS TQ 8050 to provide different peak which shows Miconazole (9.42%) acts as antifungal agent, Dodecanoic acid (10.6%) as disinfectant, Nonaneaol (0.63%) as antimicrobial activity, 1H-Indole 3-acetic acid (5.90%) as cell elongation and organ development, Tetradecanoic acid, propylester (14%) as antioxidant and Cancer preservative, Nematicide, Oleic acid (8.31%) act as 5-α reductase inhibitor, Allergenic, α reductase inhibitor, anti-inflammatory, antiandrogenic, cancer preventive, antialopecic, antileukotrience D4, 9-octadecenoic acid (Z)-2-hydroxy (4.05%) as Neomycin (4.71%) with antibacterial activity and many other compounds were analyzed.

Non pressurized topical spray of Miconazole and Neomycin was evaluated for antifungal and antibacterial activity on *Candida parapsilosis*, *Candida tropicalis*, *Candida albicans*, *Candida krusei*, *Escherichia coli*, *Staphylococcus aureus*, *Micrococcus luteus* was found significant zone of inhibition 18 mm for *Candida tropicalis*, 23 mm *Candida albicans*, 19 mm *for Candida parapsilosis*, 22 mm for *Candida krusei*) and (15 mm for *Escherichia coli*), 17 mm for *Staphylococcus aureus*, 25 mm for *Micrococcus luteus*) at a concentration of 30 µg/disc which shows significant zone of inhibition and poses antifungal and antibacterial activities. Antifungal and antibacterial activity of miconazole and neomycin drug solution was also assessed and found that zone of inhibition of spray was more than that of individual drugs. The Cytotoxicity study was assessed on HACAT cells for 24 hours at a concentration of 6.25 microlitre per ml, 12.5 microlitre per ml, 0.465 microlitre per ml, 0.411 microlitre per ml, 0.364 microlitre per ml and 0.34 to microlitre per ml. Percentage of inhibition was found to be less than 50% which shows the concentration of non prescribed topical Nano spray was non toxic and showed no significant Cytotoxicity.

Author Contributions

N.P.: investigation, formal analysis, data correction, writing, original draft; P.J., investigation and validation.

Conflicts of Interest

The authors declare no conflict of interest.

Abbreviation

HACAT: Human Epidermal Keratinocyte

GCMS: *Gas Chromatography-Mass Spectrometry*

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EDTA: Ethylenediamine Tetraacetic Acid

DMEM: Dulbecco's Modified Eagle's Medium

MTCC: Microbial Type Culture Collection and Gene Bank

µg: Microgram

µg/ml: Microgram per Milliliter

nm: Nanometer

OD: Optical Density

°C: Degree Celsius

MIC: Minimum Inhibitory Concentration

FBS: Fetal Bovine Serum

%: Percentage

mm: Millimetre

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