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ORIGINAL RESEARCH ARTICLE

In vitro Cell Viability and Microbial Challenge Testing of *Camellia sinensis* (Green Tea) and *Myristica fragrans* (Nutmeg) Based Phyto-Topical Applicant Against the Pyogens (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*)

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ABSTRACT

A Phyto-topical applicant was formulated using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) hydroethanolic extracts by incorporating into gel base containing Carbopol-940 and Triethanolamine. The evaluation of *in vitro* antimicrobial activity testing, *in vitro* cell viability testing using 3T3 cell line and Microbial challenge testing against the pyogenic microbes (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) to screen and authenticate the stability of the Phyto-Topical applicant. This provides detail about the efficacy and safety aspect of the formulation. Thus developed Phyto-Topical applicant formulations are of Ethano medicinal value, helps to treat skin infection without causing side effects and hypersensitivity. The multidrug resistance nature of the microbes can be overcome by using herbal formulation with the essence of traditional medical practices.

Key words: *Camellia sinensis*, *Myristica fragrans*, Hydroethanolic extraction, *in vitro* antimicrobial activity testing, *in vitro* cell viability testing, Microbial challenge testing, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

INTRODUCTION

Herbal companies all over the world produce a lot of formulation for various purposes. When a herbal formulation comes to market it is obvious that it had passed through several evaluation parameters direct from the crude drug to the finished product as per regulations. There are several guidelines for efficacy evaluation of formulation as co therapeuticals. The search and exploitation of natural products for its properties has been the mainstay of the herbal industries. [1]

Phyto-Topical applicant is a product with biological active ingredients derived from medicinal plants which posses the antimicrobial property to treat skin infection. The ability of these ingredients to protect skin against infection depends on an appropriate formulation that can maintain the integrity of the active ingredient and deliver it in a biological active form to the infection site. It reaches the target site in sufficient quantity to exert an effect and properly release bioactive substance from the carrier vehicle [2, 3].

Camellia sinensis (Green tea) belongs to the family *Theaceae*, and is one of the most widely consumed beverages in the world; its medicinal properties have been widely explored. This plant has been traditionally used in treating inflammations, asthma, heart diseases, lowering blood sugar and fights cancer, hair fall, greyness of hair and various skin diseases [4]. Due to the high antioxidant activity and potent antimicrobial activity of green tea extracts, it is used as phyto-cosmeceutic, neutraceutic additive, antioxidant and anti carcinogenic properties [5].

Myristica fragrans (Nutmeg) is an aromatic tree, 8 m or more tall with a dense crown. Its natural habitat is wet tropics and trees thrive with high, well-distributed annual rainfall, with little seasonal variation and temperatures. It is said to have stimulant, carminative and astringent properties. Its hallucinogenic properties are ascribed to the aromatic ethers myristicin, elemicin and safrole. The nutmeg was used to treat skin infection in ancient times. The powder form is used to improve the skin condition because of its nutritional and antimicrobial nature[6].

A pyogenic infection is an infection that is characterized by severe local inflammation, usually with pus formation, generally caused by the pyogenic microorganism. Skin diseases are

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often related with occupational diseases.[7] *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* are the commensals which may cause opportunistic infections and the pyogens dominate the incidences of Nosocomial infection. These microbes are used for the investigation of the ability and stability of the Phyto formulation.

Natural product search and discovery is synonymous with drug discovery. The examination of novel natural product, chemo types along with interesting structures and biological activities continue to be reported, this becomes the mainstay of drug discovery as it has been in the therapeutic areas [8].

MATERIALS AND METHODS

Hydroethanolic Extraction and Formulation of Phyto-Topical applicant using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg)

The hydroethanolic extracts of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) were used as active ingredients in the Phyto-Topical applicant formulation. It was prepared by simple maceration technique. About 50 g of the *Camellia sinensis* leaves and *Myristica fragrans* mace powdered plant materials was extracted with 200 mL of hydro-ethanol (1:1) separately with occasional shaking for about 48 hours, at room temperature 22-24 ^{0}C and filtered. The filtrate of both the plant material was evaporated to dryness [9].

Carbopol-940 was soaked in water for 12 hours. Then the swelled polymer was stirred using a mechanical stirrer to prepare a uniform dispersion of the polymer. Triethanolamine was used to adjust the pH to 7.0. The Hydroethanolic extracts of *Camellia sinensis* and *Myristica fragrans* was incorporated into this base [10].

Formulation of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) based Phyto-Topical applicant

Ingredients	Quantity
Gel base	30g
Camellia sinensis	10g
Myristica fragrans	10g
Methyl paraben	0.1g

In vitro Antimicrobial activity testing

The formulation of Phyto-Topical applicant using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) were subjected to *in vitro* antimicrobial activity testing using Agar well diffusion method. The sterile Muller Hinton Agar plates were prepared and with the help of a sterile well cutter, punctured 6mm diameter wells with uniform spacing for various concentrations of each extracts. The log phase culture broth of (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*) was swabbed over the plate using sterile cotton swab to obtain a uniform lawn of culture. The wells were filled with 0.1g of the Phyto-Topical applicant respectively. The plates were then incubated at 37^o C for 24 hours [11]. After incubation, the sizes of the zone of inhibition were measured to evaluate the degree of antimicrobial property and compared with the commercial antibiotics (Bacitracin, Neomycin and Ketoconazole**).**

In vitro cell viability testing using 3T3 cell line

In vitro Cell viability testing is a method to analyze the degree of skin irritability and sensitivity of the herbal formulations. This test was performed on 3T3 cell line which helps to evaluate skin irritability by MTT assay.

Methodology

The cells were grown in a 96-well plate in Delbucco's Minimum essential medium (DMEM) (HiMedia) supplemented with 10% fetal bovine serum (Gibco Laboratories) and antibiotics (streptomycin, penicillin-G, kanamycin, amphotericin B). About 1 mL cell suspension (10^5 cells/mL) was seeded in each well and incubated at 37^0 C for 48 hour in 5% CO_2 for the formation of confluent monolayer. The monolayer of cells in the plate was exposed to various dilutions of the Phyto-Topical applicant using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg). The cell viability was measured using MTT assay with MTT (5 mg/mL) and DMSO. This Tetrazolium salt is metabolically reduced by viable cells to yield a blue coloured insoluble Formosan product measured at 570 nm spectrophotometerically. Controls were maintained throughout the experiment. The assay was performed in triplicate for each of the extracts. The mean of the cell viability values was compared to the control to determine the effect of the extract on cells and % cell viability was plotted against concentration of the plant extract. The minimum concentration of Phyto-Topical applicant using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) that was non toxic to 3T3 cell line was recorded as the effective drug concentration [12].

In vitro Microbial challenge testing of Phyto-Topical applicant on Microorganisms treated 3T3 cell lines

The 3T3 cell lines were treated with predetermined bacterial load to contaminate the cells and further application of the formulated *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) based Phyto-Topical applicant

exposed to cells and reduction of microbial load was assessed after 15 minutes, 60 minutes and 180 minutes [13,14].

Methodology:

Three sets of Primary 3T3 cells at passage 10 were propagated at 37°C and in 5% CO2 to over 80% confluence in Duelbecco's minimal Eagle's media (DMEM; Life Technologies, Grand Island, N.Y.) containing 10% fetal bovine serum and transferred (after treatment with trypsin) a final count of 0.5 x 10^5 cells/well. After a 24-hours of incubation at 37°C, the medium was aspirated and a 100 µl suspension of microbial inoculums (10^6 cells/mL in 50% serum-free DMEM) was added to well. Prepare the formulation concentration (10 µg/mL, 100 µg/mL, 500 µg/mL, 1000 µg/mL) in 1x Phosphate buffer saline and add 100 µl to the corresponding well and the co culture was then incubated at 37°C. To determine bacterial survival, 100 µl of the co culture medium was collected and plated on to the selective media with respect to organisms. This procedure was repeated after 15 minutes, 60 minutes and 120 minutes.

RESULTS AND DISCUSSION

In vitro antimicrobial activity of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) based Phyto-Topical applicant

The Phyto-Topical applicant formulation was tested for the *In vitro* antimicrobial activity against pyogenic microbial flora to determine its Microbicidal efficacy. The formulation which contains the combination of both *Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) hydroethanolic extract shows high activity Results were tabulated in (**Table 1**).

Table 1: *In vitro* antimicrobial activity of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) based phyto-Topical applicant against pyogens

S.No	Microorganisms	Zone of Inhibition(mm)			
		Formulation	Bacitracin	Neomycin	Ketoconazole
1	*Staphylococcus aureus*	23	20	22	17
2	*Pseudomonas aeruginosa*	22	17	19	15
3	*Candida albicans*	28	13	15	21

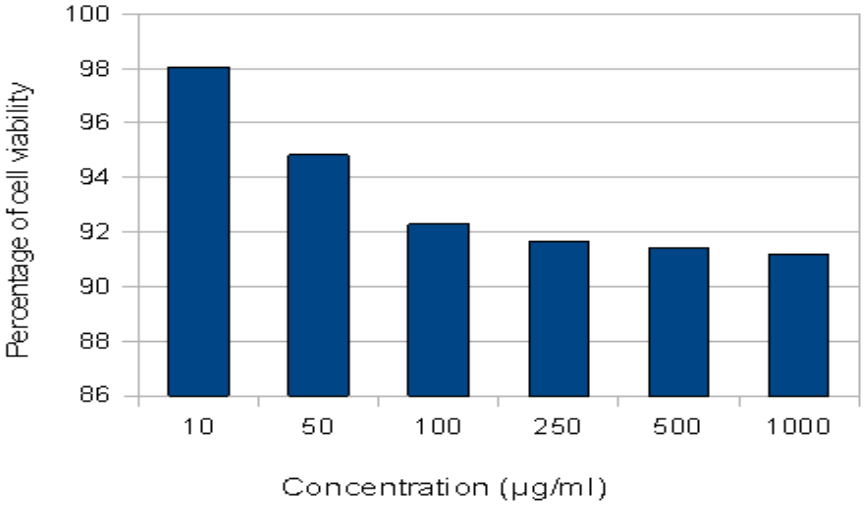
In vitro Cell viability testing

The result of cell viability study indicates that the formulation was non toxic to 3T3 cell lines. This indirectly reveals that the Phyto-Topical applicant can be applied on skin surface. A bar diagrams were plotted for cell viability and Phyto-Topical applicant on 3T3 cell line (**Fig 1**). The figure represents the Percentage of cell viability by treating with Phyto-Topical applicant in different concentrations.

Table 2: *In vitro* Cell viability and toxicity study of Phyto–Topical applicant using *Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) on 3T3 cell line

Sample	Concentration	Control OD	Sample OD	Non viable OD	% of Viability	Mean	SD
Formulation 3	10	0.875	0.874	0.001	99.886	98.06	2.69
		0.875	0.831	0.044	94.971		
		0.875	0.869	0.006	99.314		
	50	0.875	0.821	0.054	93.829	94.82	2.23
		0.875	0.852	0.023	97.371		
		0.875	0.816	0.059	93.257		
	100	0.875	0.801	0.074	91.543	92.27	5.92
		0.875	0.759	0.116	86.743		
		0.875	0.862	0.013	98.514		
	250	0.875	0.797	0.078	91.086	91.66	1.4
		0.875	0.816	0.059	93.257		
		0.875	0.793	0.082	90.629		
	500	0.875	0.828	0.047	94.629	91.39	2.83
		0.875	0.789	0.086	90.171		
		0.875	0.782	0.093	89.371		
	1000	0.875	0.801	0.074	91.543	91.2	0.59
		0.875	0.792	0.083	90.514		
		0.875	0.801	0.074	91.543		

Fig 1: *In vitro* Cell viability and toxicity study of Phyto–Topical applicant using *Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) on 3T3 cell line



***In vitro* Microbial challenge testing of Phyto-Topical applicant on bacterial contaminated 3T3 cell line:**

The reduction in microbial load with respect to increases in the concentration and time duration was observed, while treating the microbial contaminated 3T3 cell lines with different concentrations of Phyto-Topical applicant. Microbial log reduction occurred at each time interval (15 minutes, 60 minutes and 180 minutes) with increase in concentrations of the formulation. At 15 minutes plating on to the selective media showed 25 to 30 CFU, 60 minutes plating shows a reduction in the microbial colony forming unit and180 minutes plating showed no growth on the selective media. This result clearly denotes the efficacy and antimicrobial property of the developed Phyto-Topical applicant**.**

CONCLUSION

The formulated Phyto-Topical applicant has good antimicrobial property when compared with the commercial antibiotics. The cell viability and microbial challenge testing reveals that the Phyto-Topical applicant does not cause skin irritability and hypersensitivity. The reduction in the microbial count with respect to time duration supports the Ethanomedicinal value of the formulation. For further studies, the formulation was subjected to organoleptic evaluation and shelf life studies.

REFERENCES

1. Sneha and Swarnlatha., Bioengineering Techniques for the Efficacy Studies of Herbal Cosmetics, Research Journal of Topical and Cosmetic Science, 2010; Volume 01, Issue 01.
2. Baumann LS (2007). Less-known botanical cosmeceuticals. Dermatol Ther. 20(5): 330-42.
3. Reddy C. S, Rammohan T, Madhu N, Divakar M.C, Transdsermal diffusion studies of a poly herbal ointment and its topical therapy, Ancient Sci Life. 1998; 14(&2): 76-85
4. Werkhoven J.: Tea Processing, FAO, Rome (Italy), 1978: Ist Edtn Weiss E.A, *Spice Crops*. 2002. CABI Publishing, CABI International, UK.11
5. Jian L et al., Protective effect of green tea against prostate cancer: a case-control study in southeast China, *Intl J Cancer*, 2004; 108(1):130-35.7
6. de Guzman C.C., and Siemonsma, J.S., (Editors). *Plant Resources of South-East Asia No.13. Spices*. 1999Backhuys Publishers, Leiden, The Netherlands.71
7. Nair R., T Kalariya, Sumitra Chanda., Antibacterial Activity of Some Selected Indian Medicinal Flora, 2005. *Turk J Biol*, 29: 41-47.
8. Vaidehi, N., Schlyer, S., Trabanino, R.J., Floriano, W.B., Abrol, R., Sharma, S., Kochanny, M., Koovakat, S., Dunning, L., Liang, M., Fox, J.M., de Mendonca, F.L., Pease, J.E., Goddard III, W.A., Horuk, R., Predictions of CCR1 chemokine receptor structure and BX 471 antagonist binding followed by experimental validation..J. Biol. Chem, 2006; 281, 27613–27620.
9. Hostettmann, K., M.J, Pettei, I. Kubo and K. Nakanishi, 1977. Direct obtaining of crude plant extracts by preparative liquid chromatography Lloydia. Helv. Chim.

Acta, 60: 670-672. Make references like this style.

10. Carnali, J. O., Naser, M. S., 1992 "The Use of Dilute Solution Viscosity to Characterize the NetworkProperties of Carbopol® Microgels," Colloid & Polymer Science. 270(2), 183-193.
11. Nair R., T Kalariya, Sumitra Chanda., Antibacterial Activity of Some Selected Indian Medicinal Flora, 2005. *Turk J Biol*, 29: 41-47.
12. Todaro, G.J., and H. Green. 1963. *J. Cell Biol*. 17:299–313.
13. Zacchino SA, Rodriguez G, Pezzenati G, Orellana G. In vitro evaluation of antifungal properties of 8.O.4′-neolignans. J Nat Prod. 1997; 60:659-662.
14. Sridhar SR, Rajagopal RV, Rajavel R, Masilamani S, Narasimhan S. Antifungal activity of some essential oils. J Agric Food Chem . 2003;51:7596-7599.