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ORIGINAL RESEARCH ARTICLE

Evaluation and *In vitro* Cell Line Studies of Phyto-Cosmeceutic Gel Based Hand Wash Formulation Using *Camellia sinensis* (Green Tea) And *Myristica fragrans* (Nutmeg)

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ABSTRACT

A phyto-cosmeceutic gel based hand wash was formulated using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg), and evaluated by physical parameters like, colour, odour, spreadability, pH and overall appearance of the formulation. Identification of unknown materials, determination of quality, consistency, amount of components and detection of functional groups and characterization of covalent bonding information of the formulation was qualitatively analyzed using Shimadzu FTIR-8400S Fourier Transform Infrared Spectrometer instrument and spectra obtained for each sample was interpreted with a chart for interpreted with a chart for Characteristics IR absorption frequencies of organic functional groups and carbonyl containing functional group. The best selected formulation was subjected to invitro cell line studies using 3T3 cells. The in vitro cell line toxicity and cell viability studies.of the formulation was assessed by MTT assay Thus, overall study showed that gel based hand wash containing mixture of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) is rich in phyto-chemicals and safe for utility

Key words: *Camellia sinensis* (Green tea), *Myristica fragrans* (Nutmeg), hand wash gel, FTIR qualitative analysis, *invitro* cell line studies

INTRODUCTION

Phyto-Cosmeceuticals are cosmetic products with biological active ingredients derived from medicinal plants that function as skin protectant, improving skin health. The presence of biological active constituents in plants with antioxidant, antimicrobial, anti-inflammatory, soothing and nourishing properties always contribute a best and effective botanical cosmeceutic product to public. Botanical cosmeceuticals contain botanical ingredients with traditional and folk medicine usage. These often include grape seed extracts, aloe vera, mushrooms, olive oil, green tea, coffee and nutmeg extracts [1]. The most important botanicals pertaining to dermatologic uses, such as cosmeceuticals, include teas, soy, pomegranate, date, grape seed, Pycnogenol, horse chestnut, German chamomile, curcumin, comfrey, allantoin, and aloe; only green and black tea, soy, pomegranate, and date have been studied to the extent that clinical trials for the treatment of parameters of extrinsic aging have been published [2].

Hand washings "remains the single most effective and cost-efficient method for preventing and reducing the transmission of nosocomial infections" [3]. Herbal companies all over the world produce a lot of cosmetics for one or the other purpose. The cosmetics are generally used externally like moisturizing lotion, fairness cream, and sunscreen lotions, anti ageing creams, face washes, hand and body washes etc. When an herbal cosmetic 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 one or the other regulations. There are several guidelines for the efficacy evaluation of cosmetics [4]. FTIR identifies chemical compounds in consumer products, paints, polymer, coatings, which provides information about the chemical bonds and molecular structure of a materials whether organic or inorganic [5].

In vitro cell line study was adopted in the study as an alternative for in vivo studies using human and animal models. 3T3 cells was obtained from Swiss mouse embryo tissue, established in 1962

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by George Todaro and Howard Green at the department of Pathology in the New York University. It has become the standard fibroblast cell line. The '3T3' designation refers to the abbreviation of "3-day transfer, inoculum 3 x 10^5 cells." This cell line was originally established from the primary mouse embryonic fibroblast cells that were cultured by the designated protocol, so-called '3T3 protocol'. The primary mouse embryonic fibroblast cells were transferred (the "T") every 3 days (the first "3"), and inoculated at the rigid density of 3 x 10^5 cells per 20-cm² dish (the second "3") continuously. The spontaneously immortalized cells with stable growth rate were established after 20-30 generations in culture, and then named '3T3' cells. 3T3 cells are often used in the cultivation of keratinocytes [6].

Camellia sinensis (Green tea) belongs to the family *Theaceae*, and is one of the most widely consumed beverages in the world, second only to water, and its medicinal properties have been widely explored. This plant has been traditionally useful in treating inflammations, asthma, heart diseases, lowering blood sugar and fights cancer. It is also useful in wound ulcers, coughs, bronchitis, burning sensation, diarrhea, dysentery, leprosy, fever, hair fall, greyness of hair and various skin diseases. Green tea is prepared by picking, lightly steaming and allowing the leaves to dry [7]. Green tea extracts are utilized either in liquids (infusions) form or as dry extracts for further purification of the extract for its active constituents [8]. Due to the high antioxidant activity and potent antimicrobial activity of green tea extracts, it is useful as phyto-cosmeceutic, neutraceutic, additive, preservative, antioxidant and a promising solution to prevent apple juice and other foods from microbial contamination [9].

Myristica fragrans (Nutmeg) is an aromatic tree, 8 m or more tall with a dense crown. Leaves alternate, oblong 13 cm x 6.5 cm, dark green above and pale waxy beneath; Flowers dioeciously, small, creamy yellow; Fruit pear-shaped to globosely drupe, 4-5 cm in diameter, yellowish, fleshy, splitting to reveal the seed (nutmeg) covered with a red, lacy, aril (mace) [10]. Its natural habitat is wet tropics and trees thrive with high, well-distributed annual rainfall, with little seasonal variation and temperatures over 25 – 35^0 C. [11] It is said to have stimulant, carminative and astringent properties. Its hallucinogenic properties are ascribed to the aromatic ethers myristicin, elemicin and safrole[12].

Hence the present study was aimed to qualitatively analyse by FTIR and evaluate the efficacy of gel based phyto-cosmeceutic hand wash formulation using 3T3 cell lines.

MATERIALS AND METHODS

Plant collection, extraction and formulation of gel based hand wash formulations using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) extracts in various concentrations.

Camellia sinensis (Green tea) was collected from Tea estates, Munnar, Kerala and *Myristica fragrans* (Nutmeg) was collected from the Changanacherry taluk of Kottayam District, Kerala and were authenticated in Tamil Nadu Agricultural University.

Preparation of hydro-ethanolic plant extracts: About 1.5 kg of fresh plants were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and powdered. The hydro-ethanolic extract was prepared using water by simple maceration technique.[13] About 50 g of the plant materials was extracted with 250 mL of hydro-ethanol (1:1) with occasional shaking for about 48 hours at room temperature 22-24 ºC, and filtered. The filtrate was evaporated to dryness.

Preparation of gel base [14, 15, 16]

S.No	Ingredients	Quantity taken
1	Carbopol-940	1g
2	Purified water	100 ml
3	Triethanolamine	q.s. to neutralize gel base

Procedure: Carbopol-940 was soaked in water overnight (12 hours). Then the swelled polymer was stirred using a mechanical stirrer to ensure the uniform dispersion of the polymer. The pH was adjusted to 7.0 using Triethanolamine. Then this base was used to incorporate medicaments or active ingredients *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg).

Formulation of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) based phyto-cosmeceutichand wash gel

Ingredients	Quantity
Gel base	30g
Camellia sinensis	1%
Myristica fragrans	1%
Sodium Lauryl sulphate	0.2%
Methyl paraben	0.1%

Determination of pH

pH of the prepared formulation was measured using digital pH meter

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Determination of Spreadability

The spreading ability of the formulations was evaluated at ambient temperatures with the following conditions. The spreading diameter of 0.01 g of the formulations, placed between two glass plates (16 x 16) was measured after 1 minute. The mass of the upper plate 125 g. the following classification was adopted for gel.

Determination of Consistency of the formulation

Fluid gel: > 70 mm,
Semi fluid gel: 70 mm≥ 55mm,
Semi stiff gel: 55mm≥ 47mm,
Stiff gel: 47mm≥ 40mm,
Very stiff gel: <40mm

Organoleptic evaluation: All three variations of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) based hand wash gel were exposed to different temperature conditions of 4^0 C 25^0 C and 37^0 C for a period of four weeks. A known amount of stored samples were taken out aseptically at different time intervals (24 hours, after seven days, after two weeks and after four weeks) and organolepically evaluated for its overall appearance (color, odor, gel consistency). Bleeding test was also performed to evaluate semisolid preparations by keeping them alternatively in different temperature zones, and observed for bleeding of liquid. Stability of the product for all climatic conditions is determined by the absence of liquid phase to omit out.[17]

Fourier transform infrared spectroscopy (FT-IR) Qualitative analysis of *Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) based hand wash gel

All three variations (F1, F2 and F3) of developed hand wash gel using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) were subjected to FTIR analysis using Shimadzu FTIR-8400S Fourier Transform Infrared Spectrometer instrument and obtained spectra for all 3 variations of the product was comparatively analyzed and interpreted with a chart for Characteristics IR absorption frequencies of organic functional groups and carbonyl containing functional group. The sample analysis Process with instrument specifications is

1. The source: IR energy is emitted from a glowing black-body source. The beam is passed through an aperture which controls the amount of energy predented to the sample (and, ultimately to the detector).
2. The Interferometer: The beam emitters the interferometer where the "spectral encoding" takes place. The resulting interferogram signal then exist the interferometer.
3. The sample: the beam enters the sample compartment where it is transmitted through or reflected off to the surface of the sample, depending on the type of analysis being accomplished. This is where specific frequencies of energy, which are uniquely characteristic of the sample, are absorbed.
4. The detector: the beam finally passes to the detector for final measurement. The detectors used are specially designed to measure the special intereferogram signal.
5. The computer: the measured signal is digitized and sent to the computer where the Fourier transformation takes place. The final infrared spectrum is then presented to the user for interpretation and any further manipulation
6. Advantages of FTIR over dispersive technique
7. Speed: because all the frequencies are measured simultaneously, most measuremen6ts by FTIR are made in a matter of seconds rather than several minutes. This is referred to as the Felgett advantage.
8. Sensitivity: is dramatically improved with FTIR for many reasons. The detectors employed are much more sensitive, the optical throughout is much higher which results in much lower noise levels, and the fast scans enable the co-addition of several scans in order to reduce the random measurement noise to any desired level (referred to as signal averaging).
9. Mechanical simplicity: the moving mirror in the intereferogram is the only continuously moving part in the instrument. Thus there is very little possibility of mechanical breakdown.
10. Internally calibrated: The instrument employs a He Ne laser as an internal wavelength. Calibration standards referred to as Connes Advantage. These instruments are self calibrating and never needs to be calibrated by the user.[18]

Cell viability and toxicity study of *Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) based phyto-cosmeceutic hand wash gel on 3T3 cell line

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Cell viability testing using in vitro cell lines to evaluate skin irritability was assessed by MTT assay [19].

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-cosmeceutic hand wash gel using 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 insoluble Formosan product measured at 570nm spectrophotometerically. Controls were maintained throughout the experiment (untreated wells as cell control. 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-cosmeceutic hand wash gel using *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) that was toxic to 3T3 cell line was recorded as the effective drug concentration.

RESULTS AND DISCUSSION

Mixture of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) based phyto-cosmeceutic hand wash gel exhibited good overall appearance, good spreadability and appropriate pH suitable for utility. FTIR spectral analysis exhibited that the formulated phyto-cosmeceutic hand wash gel contains more functional groups such as aromatic alkenes, alcoholic groups, amines and alkanes. This shows that the product is rich in many phytochemicals. The presence of carboxylic acid group might be due to the oxidation of primary alcohol and also due to oxidation of few of the aldehyde sites. Based on the results from the organoleptic evaluation and FTIR analysis, the phyto-cosmeceutic hand wash gel formulation with 2% concentration of *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) as active ingredients was subjected for in vitro cell line toxicity and cell viability studies.

Table 1: Organoleptic evaluation of *Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) based hand wash gel

S,No	Formulations	Parameters studied	Organoleptic evaluation (4^0 C, 25^0 C and 37^0 C for four weeks)
1	Formula 1	Overall appearance	Blackish green color, pungent odor, stiff gel
		Spreadability	47mm ≥ 40mm
		pH	4.1 – 6.7
2.	Formula 2	Overall appearance	Orangish green color, spicy odor, stiff gel
		Spreadability	47mm ≥ 40mm
		pH	4.1 – 6.7
3.	Formula 3	Overall appearance	Green color, pleasant odor, semi-fluid to semi-stiff gel, negative bleeding test
		Spreadability	Between 70 mm ≥ 55mm and 55mm ≥ 47mm,
		pH	4.1 – 6.7

Table 2: FTIR spectral analysis results of *Phyto-cosmeceutic hand wash gel formulation using Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg)

S.No	Wave number (absorptions) (cm^{-1})	Functional groups	Type of vibration	Intensity
1	715.59	=C-H (Alkene)	Bending	21.367
2	669.3	=C-H (Alkene)	Bending	21.891
3	767.67	=C-H (Alkene)	Bending	21.875
4	819.75	=C-H (Alkene)	Bending	22.411
5	1037.7	=C-H (Alkene)	Bending	13.026
6	1095.57	(C-N) amine,(C-O)Alcohol	Stretch	13.337
7	1423.47	(-C-H) alkane, (C=C) aromatic	Bending	11.879
8	1454.33	(C=C) aromatic (-C-H) alkane,	Stretch Bending	11.737
9	1508.33	(C=C) aromatic	Stretch	12.743
10	1525.69	(C=C) aromatic, (N-O) Nitro compounds	Stretch	12.327
11	1583.56	(C=C) aromatic	Stretch	10.036
12	1595.13	(C=C) aromatic (N-H) amide	Stretch Bending	9.942
13	1637.56	(C=C) Alkene	Stretch	9.796

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14	1728.22	(C=O) carbonyl	Stretch	12.246
15	2856.58	(C-H) alkane	stretch	8.009
16	2922.16	(C-H) alkane	stretch	6.261
17	3387	(N-H) Amine, (O-H) alcohol	Stretch	6.097
18	3402.43	(N-H) Amine, (O-H) alcohol	Stretch	6.094

Figure 1: FTIR spectra of *Phyto-cosmeceutic hand wash gel formulation using Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg)

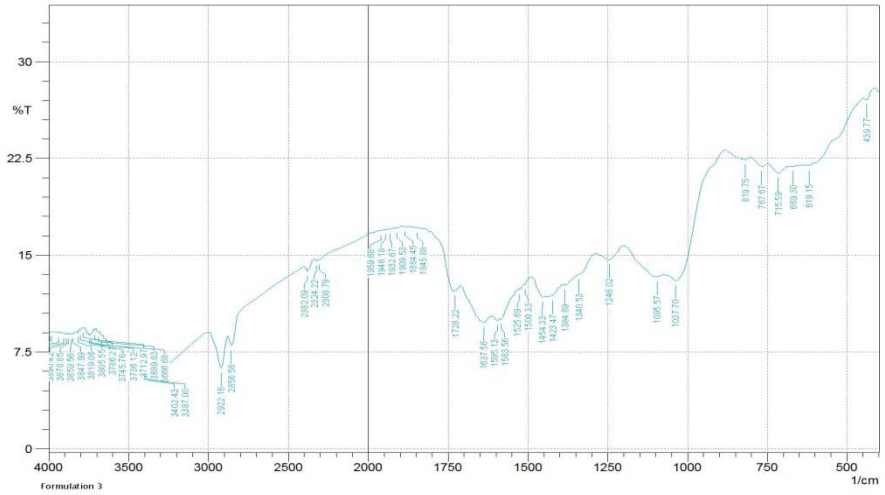
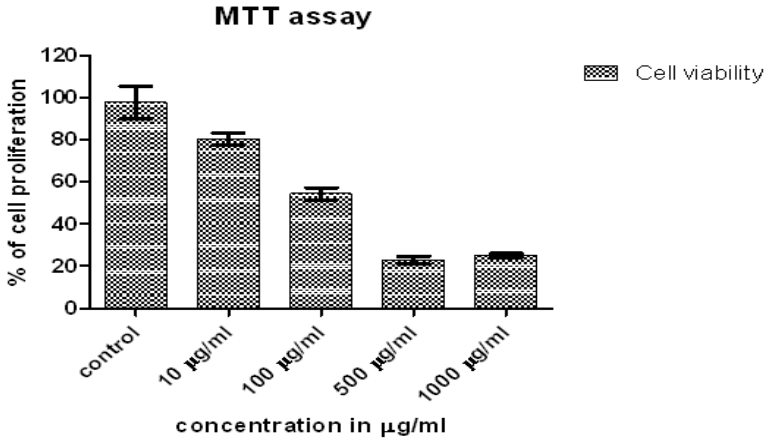


Fig 2. Cell viability and toxicity study of *phyto-cosmeceutic hand wash gel using Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) on 3T3 cell line



Viability of 3t3 in Co-culture assay

Control OD value 1.9 is equivalent to 100% viability. The bar diagrams plotted represents the percentage of cell viability by the treating phyto-cosmeceutic hand wash gel using *Camellia sinensis* (green tea) and *Myristica fragrans* (nutmeg) in different concentrations on 3T3 cell line. The Bar diagram of MTT assay [20] depicts that low and normal concentrations (control, 10µg/ml and100µg/ml) of the phyto-cosmeceutical hand wash gel administered on cell lines produced neligible to very less percentage (100%, 80% and 60%) of 3T3 cell proliferation. Higher or very increased concentrations (500µg/ml and 1000µg/ml) the phyto-cosmeceutical hand wash gel administered on cell lines produced negligible percentage (20% - 25%) of cell proliferation.

CONCLUSION

Organoleptic evaluation and FTIR analysis of Phyto-cosmeceutic hand wash gel formulation containing *Camellia sinensis* (Green tea) and *Myristica fragrans* (Nutmeg) exhibited its effective cleansing formula with therapeutic properties in a gel based carrier. The results of in vitro cell viability and toxicity clearly portray the efficacy and consumer utility prospects of the formulated phyto-cosmeceutical hand wash gel using *Camellia sinensis* and *Myristica fragrans*. *In vivo* studies can be further carried out for Phyto-cosmeceutic hand wash gel formulation containing Camellia sinensis (Green tea) and

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Myristica fragrans (Nutmeg) in animal and human models.

REFERENCES

1. Baumann LS (2007). Less-known botanical cosmeceuticals. Dermatol Ther. 20(5): 330-42.
2. Thornfeldt CR (2005). Cosmeceuticals: separating fact from voodoo science. Skinmed. 4(4): 14-220.
3. Antoniak J., (2004); Hand washing compliance, Candaian Nurse, [Electronic version] 100: 21-25.
4. Sneha and Swarnlatha., 2010. Bioengineering Techniques for the Efficacy Studies of Herbal Cosmetics, Research Journal of Topical and Cosmetic Science,; Volume 01, Issue 01.
5. 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., 2006. Predictions of CCR1 chemokine receptor structure and BX 471 antagonist binding followed by experimental validation..J. Biol. Chem,; 281, 27613–27620.
6. Todaro, G.J., and H. Green. 1963. J. Cell Biol. 17:299–313.
7. Jian L et al., 2004. Protective effect of green tea against prostate cancer: a case-control study in southeast China, Intl J Cancer,; 108(1):130-35.
8. H. Wang, G.J. Provan and K. Helliwell., 2000.Tea flavonoids: Their functions, utilization and analysis, J. Trends, Food. Sci. Tech ; 11: 152-160
9. Johnson I. T, 2004. New approaches to the role of diet in the prevention of cancers of the alimentary tract, Mutat Res,; 551(1-2), 9-28
10. Marcelle, GB., Murillos-Yepes, J. and de La Grenade, C., Personal communications from Grenada, 2005.
11. Weiss E.A, Spice Crops. 2002. CABI Publishing, CABI International, UK.
12. de Guzman C.C., and Siemonsma, J.S., (Editors).. Plant Resources of South-East Asia No.13. Spices. 1999 Backhuys Publishers, Leiden, The Netherlands.71
13. Ghouse, A.K.M.; Yunus, M.; Farooqui, F. & Sabir, D., A simple maceration technique for the separation of sieve elements from the barks of woody plants. Current Science, 1974; 43: 424-425.
14. 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
15. Pai M.R, Acharya L.D, Udupa N, Evaluation of antiplaque activity of Azadirachta indica leaf extract gel – a 6-week clinical study, J Ethanopharmacol. 2004; 90: 99-103.
16. Gupta G.D, Gaud R.S, Release rate of nimesulide from different gellants. Indian J. Pharma Sci. 1999; 61 (4): 227-30.
17. Hilda Butler.: Poucher's Perfumes, Cosmetics and soaps, 10 EDN, Springer, 2007
18. Demirdroven N, Cheatum H, S Chung, M Kalil, J Knoester, A Tokmakoff., 2004.Two dimensional infrared spectroscopy of anti parallel beta-sheet secondary structure, Journal of the American Chemical Society; 126 (25):7981.
19. Kang HS, Chung HY, Kim JY, Son BW, Jung HA, Choi JS. 2004. Inhibitory phlorotannins from the edible brown alga Ecklonia stolonifera on total reactive oxygen species (ROS) generation. Arch Pharm Res 27:194–198.
20. Nakagawa H, Wachi M, Woo JT, Kato M, Kasai S, Takahashi F, Lee IS, and Nagai K (2002) Fenton reaction is primarily involved in a mechanism of (–)-epigallocatechin-3-gallate to induce osteoclastic cell death. Biochem Biophys Res Commun 292**:** 94–101.

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