Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Gas Chromatography-High-Resolution Mass Spectrometry Elucidation and *in vitro* Cell Line Studies (Sulforhodamine B) on Niosomal Gel of *Benincasa hispida*

Gaurav Mahesh Doshi, Akanksha Dilip Badgujar[1], Ishan Sudhir Mathurvaishya[2]

Department of Pharmacology, SVKM'S Dr. Bhanuben Nanavati College of Pharmacy, Mithibai Campus, [1]Department of Quality Assurance, Vivekanand Education, Society's College of Pharmacy, Mumbai, [2]Department of Pharmaceutics, Dr. L.H. Hiranandhani College of Pharmacy, Thane, Maharashtra, India

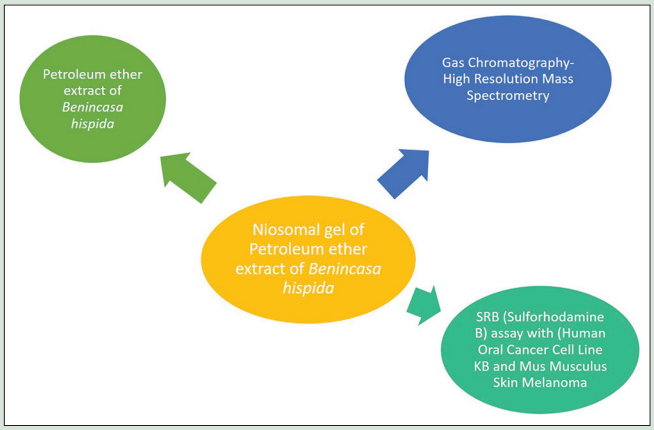
ABSTRACT

Background: The Cucurbitaceae family has been known for its presence of phytoconstituents, namely, lupeol, β-sitosterol, terpenoids, phenols, and proteins with therapeutic potential. **Objectives:** The research paper tries to focus on major therapeutic field of people demographic area of type of cancer. Soxhlet extraction of *Benincasa hispida* (BH) was carried out and crude extract was converted to powder. Niosomal gel was formulated after the optimization of drug concentration from the extract of Niosomal solution. Solution with maximum entrapment was selected for the preparation of gel. Gel was prepared by using different Carbopol grades and with comparison of different parameters. Gel was studied for gas chromatography-high-resolution mass spectrometry (GC-HRMS) evaluation for check toxicity. Biologic activity was analyzed by SRB (Sulforhodamine B) assay with *in vitro* cell lines (human oral cancer cell line KB and Mus Musculus skin melanoma B16-F10). **Results:** Solution which was clear and white was selected for gel development. Carbopol 934 (1%) as gelling agent showed good formulation properties. It was non-gritty, easily spreadable, and washable. The gel with maximum entrapment efficiency was evaluated further. GC-HRMS revealed 1.2-ethyl 2-Hexen-1-ol; 1,8-(3-Octyl-2-oxiranyl)-1-octanol; Dodecanoic acid, 2-penten-1-yl ester; Cholestane, 4, 5-epoxy-,(4α,5α); Cyclopentaneundecanoic acid; 17-octadecynoic acid; Octadecanoic acid, methyl ester; Methyl oleate; Tridecanoic acid, methyl ester; and Silane, dimethyl (2,3,6, tricholrophenoxy) heptadicycloxy. The selected cell lines did not show satisfactory activity at different concentrations in SRB assay. **Conclusion:** This research paper tried to witness the probable potential of BH for cancer with analytical base. Future studies may be undertaken at various stages and levels to further explore the potential of these families of plants.

Key words: *Benincasa hispida,* gas chromatography-high-resolution mass spectrometry, niosomal gel, oral cancer, sulforhodamine B

SUMMARY

- The authors have tried to screen the potential of *Benincasa hispida* gel formulation (PEBH) for using *vitro* cell lines (human oral cancer cell line KB and Mus Musculus skin melanoma B16-F10). Gel was studied for gas chromatography-high-resolution mass spectrometry in order to study the toxic degraded components in the formulation.



Abbreviations Used: GC-HRMS: Gas chromatography-high-resolution mass spectrometry, LC-MS: Liquid chromatography and mass spectroscopy, PEBH: Petroleum ether extract of *Benincasa hispida,* TEM: Transmission electron microscopy, BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma, SRB: Sulforhodamine B assay, Tz: Time zero, C: Control growth.

Correspondence:
Dr. Gaurav Mahesh Doshi,
Department of Pharmacology,
SVKM'S Dr. Bhanuben Nanavati
College of Pharmacy, Mithibai Campus,
Vile Parle (W), Mumbai - 400 056,
Maharashtra, India.
E-mail: gaurav.pharmacology@gmail.com
DOI: 10.4103/pr.pr_37_20

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Natural products research have known to witness upsurge in the 21st century.[1] The literature cited in herbal pharmacopoeia have proved to provide evidence-based research studies. These secondary metabolites have been gaining importance among the world population as "drug-likeness and biological friendliness than totally synthetic molecules." Photochemistry branch is evergreen bridge that always tries to fill the gap between botany and chemistry. The entire world has ample resources which needs to be explored.[2] There is co-existence of vast no. of species in various families of plants. Cucurbitaceae is one such family comprises of 965 species in 95 genera. *Benincasa hispida* (BH) is popularly known as vegetable crop which has medicinal and nutritional importance. Phytochemical analysis showed presence of vital constituents in fruits such as volatile oils, uronic acid, flavonoids, ß-sitosterin, glycosides, minerals, sacchrides, proteins, vitamins, and carotenes. The therapeutic potential includes central nervous

Cite this article as: Doshi GM, Badgujar AD, Mathurvaishya IS. Gas Chromatography-High-Resolution mass spectrometry elucidation and *in vitro* cell line studies (Sulforhodamine B) on niosomal gel of *Benincasa hispida*. Phcog Res 2020;12:342-51.

Submitted: 26-Apr-2020 **Revised:** 26-May-2020
Accepted: 18-Aug-2020 **Published:** 23-Jan-2021

effects (anxiolytic, muscle relaxant, antidepressant, Alzheimer's disease), antioxidant, antimicrobial, antidiabetic, nephroprotective, diuretic, antiasthmatic, anti-inflammatory effects.[3]

The skin mainly comprises of three layers, namely, epidermis (protective skin layer with external environment), dermis (mechanical resistance and elasticity), and hypodermis (deepest layer of the skin). There has been upinterest in the society for transdermal delivery. Nanocarriers have been as a powerful replacement for hydrophilic and lipophilic drugs. They are effective in various treatment remedies by their unique property of penetrating through the stratum corneum.[4] These nanocarriers (nanoparticles, liposomes, dentrimers, and ethosomes) can be made by the incorporation of various different materials, which are unique in their chemical and structural nature. Niosomes are spherical and consist of microscopic lamellar (unilamellar or multilamellar) structures. The bilayer is formed by nonionic surfactants, with or without cholesterol and a charge inducer. Examples of surfactants include sorbitan fatty acid esters and polyoxyethylene fatty acid esters.[5] They are too small to be detected by the immune system and effective in delivering the drug in the target organ using lower drug doses in order to reduce side effects. Carbomers have been used extensively in topical liquid preparations. Carbomers are considered to be non-toxic and non-irritant materials, no hypersensitivity reactions. Taking the above concepts idea, an attempt has been made to formulate Niosomal gel for studies.[6,7]

Melanoma comprises of 2% of skin cancer which is of malignant type and are fatal. More than 2 million cases of skin cancer have been reported in the USA in 2010.[8,9] These cancers include basal cell carcinoma (BCC) which is common slow growing, locally invasive and rarely metastasizes whereas squamous cell carcinoma (SCC) is non-melanomatous skin cancer (approx. 20% to 30%).[10,11] There are four major subtypes of invasive cutaneous melanoma that are grouped for their distinct histologic patterns: Superficial, nodular, lentigomaligna and acral lentiginous. Once a diagnosis has been made by biopsy of the lesion, pathologic staging must be completed to determine prognosis and treatment. India has been witnessing substantially arise in SCC cases.[12] B16 melanoma has been used as research model for human skin cancers which is considered as boon to chemotherapy regimens.[13-15]

Cucurbitaceae family of plants have revealed immense therapeutic potential in different ailments. Some plants have been fully explored to its utility in the major fields of the research, hence further studies needs to be undertaken. Our research paper is collective effort of plant based topical formulation followed by gas chromatography-high resolution mass spectrometry (GC-HRMS) studies of the components. The developed formulation was been exponentially studied at specific concentration using SRB (Sulforhodamine B) assay. The research paper tries to emphasize on the role of one such family of plant which would prove its evidence in cancer and explore future research.

MATERIALS AND METHODS

Collection, authentication and extraction

Dried seeds of BH were obtained from local market, Mumbai. They were previously authenticated by Agharkar Research Institute, Pune (authentication no. 13-083). The dried seeds were again sun dried at 30°C–35°C. The seeds powder was subjected to continuous Soxhlet extraction apparatus with petroleum ether (65°C) for 3–4 days till colorless liquid was obtained in the thimble (PEBH). The concentrate was then evaporated to complete dryness at 40°C using Rotary Evaporator. The crude extract was collected and stored in air-tight containers under refrigeration at 5°C.

Formulation development[16-20]

The niosomes were prepared by film hydration method using ROTAVAP (Rotary Evaporator) with following steps in details as follows:

- Step 1 – The surfactant and cholesterol was dissolved in chloroform and drug was added to it
- Step 2 – The homogenous solution was introduced in RBF and the film was formed under vacuum at 70°C ± 2
- Step 3 – It was then hydrated with phosphate buffer for 30 min
- Step 4 – The solution was ultrasonicated for 20 min
- Step 5 – It was then subjected to centrifugation on 10,000 rpm for 10 min for removal of unentrapped drug.

The Niosomal formulation was developed by using different excipient and excipient ratios. The cholesterol was added in the formulation to increase the efficiency of the drug entrapment. Various trials were conducted to optimize and obtain the high entrapment of the drug. Final Niosomal solution (F 14) was used for preparation of Niosomal gel (NG 1). The selected excipients for formulation of the gel were Carbopol 934, menthol, triethanolamine, methyl and propyl paraben, and distilled water. Carbopol 934 was added to small quantity of distilled water and soaked for a period of 24 h. After 24 h, the extract along with other excipients were added and stirred on a magnetic stirrer until a gel like consistency was obtained. Finally, triethanolamine was added to adjust the pH of the formulation.

Evaluation of the formulations[16-20]

Niosomal solution

The solution was physically checked for homogeneity and color (white). The pH was measured using a digital pH meter. The microscopy was performed to determine the size of niosomes formed using optical microscopy at ×45 and transmission electron microscopy (TEM). The entrapment efficiency of different solutions was evaluated and the optimized formulation was selected for preparation of Niosomal gel.

Niosomal gel

The gel was physically checked for homogeneity and color (white). The pH was measured using a digital pH meter. The viscosity of gel was determined using Brookfield viscometer with spindle S96 at 3 rpm. The microscopy was performed to determine the size of niosomes using optical microscopy at ×45. The spreadability of the gel formulations was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (10 cm × 25 cm). The standard weight used (200 g) was placed above it for 30 s. After 30 s, the upper tile was removed, and the formulations were found to spread on the tile. The total amount of formulations spread on the tile was measured with the help of a scale, by measuring the diameter. These measurements were taken thrice and the average values obtained by formula $S = M \times L/T$. The ease of removal of gel formulation applied was examined by washing the applied part with tap water. After the gel formulations have been set in container, testing for visual inspection, appearance, homogeneity, and presence of aggregates was done. The grittiness and uniformity of particles in the gels was checked microscopically. About 1 g of formulation was weighed and added to a 100 ml volumetric flask and volume was made up with phosphate buffer of 5.5 pH. The solution was filtered to remove any solid particles. Further dilutions were made and estimated spectrophotometrically using ultraviolet (UV)/visible spectrophotometer. The formulations were kept for a period of time, alternatively in the refrigerator, and at room temperature to observe the bleeding of liquid. It is tested in the form of a "patch test." Formulation is applied on 1 cm^2 patch of skin and observed for inflammation or rashes on skin.

In vitro drug release studies

The *in-vitro* drug release of gel was carried out by using Franz diffusion method. The diffusion of the drug from the semi-solid product throughout the membrane was regulated by assaying the receptor substrate with sequentially selected samples. UV spectroscopy was used to retrieve the aliquot of the sample from the receptor compartment for drug content analysis at determined time points. After each sampling, the receptor compartment was topped with a fresh medium.[16-20]

Stability studies

The stability studies were performed as per ICH guidelines at 30°C ± 2°C/65% relative humidity ±5% RH and 40°C ± 2°C/75% RH ± 5% RH.[20-24]

Gas chromatography-high-resolution mass spectrometry

Gel studies were undertaken at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Powai, Mumbai. The following are the details furnished with instrument specifications used during the experiment:

- Extracts: PEBH
- Chemical (solvent): Methanol

EI-MS spectrum was scanned at −70 eV with instrument details was:

- Model of MS: Joel
- Model: Accu time-of-flight analyzer (TOF) GCV
- Specification: Mass range – 10–2000 amu and resolution - 6000.
- Make of GC: Agilent 7890
- Detector: Flame ionization detector (FID)
- Run time: 40 min.

Gas chromatography-mass spectrometric (GC-MS) analysis was performed by splitless injection of 1.0 µL of the sample in methanol on a Hewlett Packard 6890 (USA) gas chromatograph fitted with a cross-linked 5% phenyl methyl siloxane HP-5 MS capillary column (30 m × 0.32 mm × 0.25 mm coating thickness), coupled with a mass detector. GC-MS operating conditions were as follows: injector temperature 215°C, transfer line 280°C, oven temperature program 80°C–280°C with ramping 5°C min,[1] carrier gas: helium at 1.5 mL min,[1] mass spectra: electron impact (EI+), individual components were identified by NIST MS 2.0 f structural library which was used for identification and interpretation of components.[21-24]

In vitro cell-line studies

The Niosomal gel was subjected to *in-vitro* cell-lines screening related to skin. Cell lines were developed in 1640 RPMI medium consisting 10% fetal bovine serum and 2 mM L-glutamine. In the current screening experiment, the cells were inoculated to 96-well microtiter plates in 100 µL at plating densities as described in the summary of the report above, based on the doubling time of individual cell lines. Upon cell inoculation, the microtiter plates were incubated for 24 h prior to the introduction of experimental drugs at 37°C, 5% CO_2, 95% air and 100% relative humidity. Gels were initially solubilized at 100 mg/ml in dimethyl sulfoxide and diluted with water to 1 mg/ml and preserved in frozen form before use. A frozen concentrate aliquot (1 mg/ml) with complete medium containing test article was thawed and diluted to 100, 200, 400, and 800 µg/mL at the time of drug introduction. Aliquots of 10 µl of these various drug dilutions were introduced to the suitable microtiter wells containing 90 µl of medium, resulting in the appropriate final concentrations of the drug i.e., 10, 20, 40, and 80 µg/ml. After the addition of gels, the plates were incubated for 48 h under standard conditions and the assay was completed by adding cold TCA. Cells were fixed *in situ* by adding 50 µl cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated at 4°C for 60 min. The supernatant was discarded; with tap water, the plates were washed five times and air dried. Sulforhodamine B (SRB) solution (50 µl) was introduced to each of the wells at 0.4 per % (w/v) in 1 per % acetic acid and plates were incubated at room temperature for 20 min. Unbound dye was retrieved after staining, and the residual dye was eliminated by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM Trizma base and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells ×100. Using the six absorbance measurements (time zero [Tz], control growth© and test growth in the presence of drug at the four concentration levels [Ti]), the percentage growth was calculated at each of the drug concentration levels.

Percentage growth inhibition was calculated as: (Ti/C) × 100%.[25-26]

RESULTS AND DISCUSSION

The Cucurbitaceae family of plants has been well known as gourd family. Some of them with well-known local names and useful for human consumption are Cucurbita (squash, pumpkin, zucchini etc.), Lagenaria (calabash), Citrullus-watermelon (*C. lanatus*, *C. colocynthis*), Cucumis – cucumber (*C. sativus*), and *Momordica* – bitter melon. Our selected PEBH yield was found to be 9.1%.

Natural products play an important role as nutraceuticals and treatment modules ranging from arthritis to multiple body supplements. The drug discovery process from natural products has its base in structural skeletons of carbon units. Due to the occurrence of secondary metabolites, the phytoconstituents elucidated from plants have gained interest.[1]

For targeting drugs, carriers such as serum proteins, synthetic polymers, immunoglobulin, microspheres, niosomes, and liposome microspheres erythrocytes have been used.[27] Out of all of these carriers, niosomes is regarded as the best among all. The probable mechanism is their concentration in the tissues with proper localization at the site.[28] They are capable of enabling maximum efficacy of the medication without affecting surrounding tissues.[29] As per the designed study protocol, different trials were undertaken with surfactant grades for Niosomal solution [Table 1]. The ratio of cholesterol was varied to obtain and evaluate maximum entrapment efficiency. The trial batches revealed F14 (Formulation 14) to be considered as optimized solution based on the evaluation results. The solution was homogenous and no drug leakage. The F 14 [Figure 1a] obtained after centrifugation with maximum entrapment efficacy [Figure 1b] was selected for the preparation of the Niosomal gel.

Gels are considered to be two component semisolid system consisting of dispersion made up of either small inorganic particles or large organic molecules enclosing and interpenetrated by liquids.[30] The application is done directly on the mucous membrane of the eye or on the skin. They are known as drugs which act for a long duration of time when injected intramuscularly. Carbomers have been used in liquid or semi-solid pharmaceutical formulations as rheology modifiers. Carbomer copolymers act as emulsifying agents in the preparation of oil-in-water emulsions for external administration. Carbomer formulations are

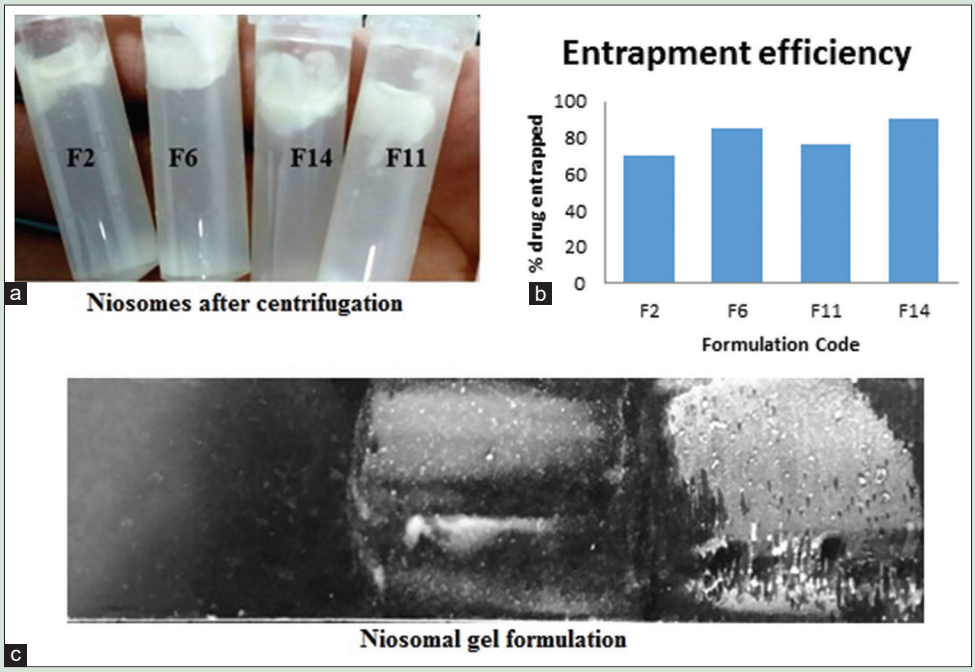


Figure 1: (a) Niosmal formulation after centrifugation, (b) niosomal gel formulation and (c) entrapment efficiency (niosomal solution)

Table 1: Trials for optimization of Niosome formulations of petroleum ether extract of *Benincasa hispida*

Formulation codes	Drug:cholesterol:surfactant	Surfactant grade
F1	1:0.5:1	Span 40
F2	1:1:1	Span 40
F3	1:1:0.5	Span 40
F4	1:1:1.5	Span 40
F5	1:0.5:1	Span 60
F6	1:1:1	Span 60
F7	1:1.5:1	Span 60
F8	1:1:0.5	Span 60
F9	1:1:1.5	Span 60
F10	1:0.5:1	Tween 80
F11	1:1:1	Tween 80
F12	1:0.5:(0.5:0.5)	Span 60:Tween 80
F13	1:1:(1:0.5)	Span 60:Tween 80
F14	1:1:(0.75:0.25)	Span 60:Tween 80
F15	1:1.5:(0.5:0.5)	Span 60:Tween 80

effective in improving moderately to severe symptoms of dry eye syndrome.[31]

In contrast to the conventional drug delivery like injection and oral drug administration, drug delivery by transdermal route proves to be an effective alternative. Not only the transdermal drug delivery provides convenience to the patient, but also is noninvasive and provides delivery of drug for a period of days. It offers multiple sites to avoid local irritation and toxicity, yet it can also offer the option of concentrating drugs at local areas to avoid undesirable systemic effects.[32] At present, drug delivery by transdermal route is limited as there are only few drugs which can be viable by transdermal route. The advantages of surfactant-based niosomes are:

- Non-immunogenic, biodegradable, and biocompatible
- They help to release drugs in a controlled manner through their closed bilayer structure
- They are found to be effective in providing improved response by mechanism of reduced clearance and specific targeting.
- They have studied for transdermal, parenteral, and ophthalmic route.[33]

NG1 was selected as the optimized batch [Figure 1c] as investigated under trial batches [Table 2]. It was found to have clear white appearance. NG1 was found to have appealing smooth texture and consistency. NG1 gel was evaluated by tanning electronic microscopy [Figure 2a]. The formulations were found to have pH in the range of 5.5–7.1 and had drug content ranging from 95% to 98% which suffices the specified limits as per ICH guidelines. Gel was observed to be non-gritty, easily spreadable, and washable [Figure 2b-e]. Physical mixtures of drug and excipients kept at specified conditions of temperature and humidity revealed the compatibility of excipients with PEBH extract [Table 3]. The Niosomal gel was prepared by using different Carbopol grades and with comparison of different parameters, and optimized Niosomal gel was determined based on evaluation results [Table 3]. It was observed that increasing the polymer concentration in gel directly increased the viscosity of the gel. *In vitro* drug release showed consistency with Carbopol 934 [Figure 2f]. Stability studies were performed of the optimized gel. There were no significant changes observed in the formulations. It was concluded obtained optimized gel was found to be stable over a period of 6 months [Table 4].

Chromatography is hyphenated technique which offers critical evaluation of phytoconstituents. The coupling of hyphenated technique has offered the entire world with interest in plant-related ailments. Analytical chemists have been able to best elucidate plant related constituents according to their area of interest related to therapeutic potential. The phytoconstituents elucidated has gained importance in to day-by-day affairs by offering their advantage over synthetic available treatments. Techniques such as liquid chromatography and mass spectroscopy (LC-MS) and GC-MS are considered important due as they are used in the identification of volatile and nonvolatile constituents.[1]

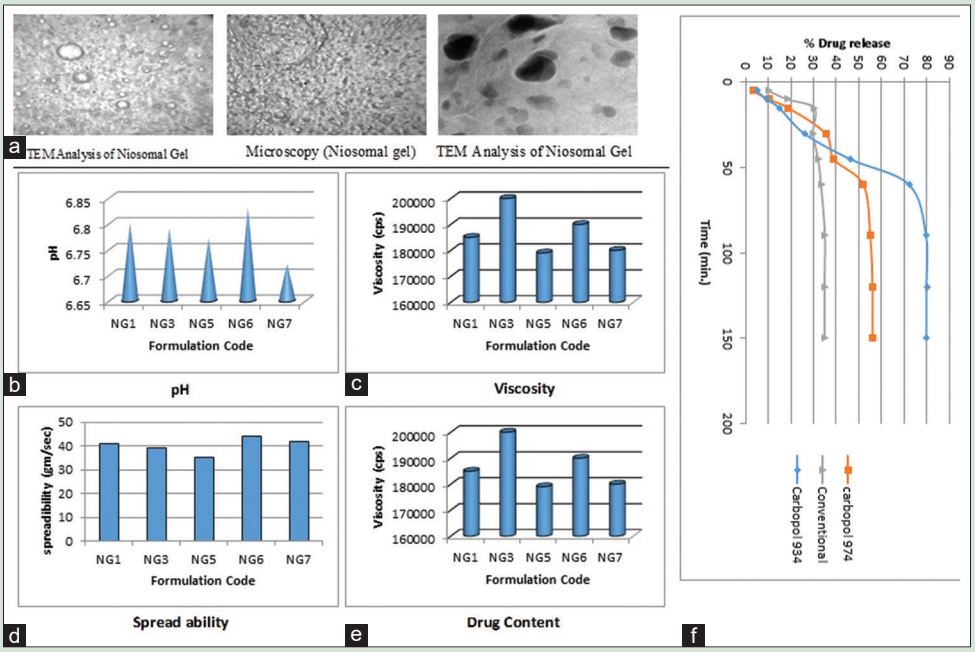


Figure 2: (a) Transmission electron microscopy analysis of niosomal gel, (b) niosomal gel (pH), (c) niosomal gel (viscosity), (d) niosomal gel (spreadibility), (e) niosomal gel (drug content), (f) *In vitro* drug release of niosomal gel

Table 2: Trials for optimization of Niosomal gel of petroleum ether extract of *Benincasa hispida*

Formulation code	Gelling agent	Formulation added	Methyl paraben	Propyl paraben	Triethanolamine	Distilled water
NG1	Carbopol 934 1%	F14	0.2%	0.02%	Q.S.	Q.S.
NG2	Carbopol 934 0.8%	F14	0.2%	0.02%	Q.S	Q.S
NG3	Carbopol 974 1%	F14	0.2%	0.02%	Q.S.	Q.S.
NG4	Carbopol 974 0.8%	F14	0.2%	0.02%	Q.S	Q.S
NG5	Carbopol 971 1%	F14	0.2%	0.02%	Q.S	Q.S
NG6	Carbopol 934 1%	Drug	0.2%	0.02%	Q.S	Q.S
NG7	Carbopol 974 1%	Drug	0.2%	0.02%	Q.S	Q.S

Table 3: Drug-excipient compatibility studies

Name of excipient	PEBH			
	30°C/60% RH		40°C/75% RH	
	Colour	Odour	Colour	Odour
Carbopol 934	NC	NC	NC	NC
Carbopol 974	NC	NC	NC	NC
Tween 80	NC	NC	Took up the colour of the extract	NC
Triethanolamine	NC	NC	Took up the colour of the extract	NC
Methyl paraben	NC	NC	NC	NC
Propyl paraben	NC	NC	NC	NC
Chloroform	NC	NC	NC	NC
Cholesterol	NC	NC	NC	NC
Span 60	NC	NC	NC	NC
Span 40	NC	NC	NC	NC

PEBH: Petroleum ether extract of *Benincasa hispida*, NC: No Change

Niosomal PEBH gel on GC-HRMS was found to elucidate ten components [Figure 3 and Table 5]. Component 1 [Figure 4a, 1.2-ethyl 2-Hexen-1-ol, 28%] with *m/z* 128 and fragment ions 55, 67, 85, 95, 110 containg hyroxy groups have been used as antibacterial agents.[34] Component 2 [Figure 4b, 1, Cis-9, 10-Epoxyoctadecan-1-ol, 35%] with *m/z* 171 and fragment ions 55, 69, 81, 95, 111, 123, 143, 155, 171 has been confirmed.[35] Component 3 [Figure 4c, Dodecanoic acid, 2-penten-1-yl ester, 33%] was found to be fatty acid with *m/z* 200 and fragment ions 57, 69, 85, 95, 109, 128, 141, 163, 183.[36] Component 4 [Figure 4d, Cholestane, 4, 5-epoxy-, [4α, 5α], 31%] with *m/z* 386 and fragment ions 55, 67, 81, 95, 107, 124, 137, 175, 203, 231, 246, 259, 273, 315, 371.[37] Component 5 [Figure 5a, cyclopentaneundecanoic acid, 36%] with m/z 268 and fragment ions 55, 74, 87, 97, 129, 143, 157, 158, 199, 225, 268.[38] Component 6 [Figure 5b, 17-octadecynoic acid, 34%] with *m/z* 265 and fragment ions 55, 67, 81, 95, 109, 123, 137, 177, 195, 223, 247.[39] Octadec-17-ynoic (monounsaturated fatty acid) acid has been discovered as acetylenic fatty acid which has been doubly dehydrogenated at positions 17 and 18 to give respective alkynoic acid. It has been well evaluated as a P450 inhibitor, leukotriene-B420-monooxygenase inhibitor and an alkane 1-monooxygenase inhibitor.[40,41] Component 7 [Figure 5c, Octadecanoic acid, methyl ester, 29%] with *m/z* 298 and fragment ions 55, 74, 87, 97,111, 129, 143, 157, 185, 199 has used as anti-foaming agent, nutrient additive and cosmetic industries.[42] Component 8 [Figure 5d, 9-Octadecadienoic acid (Z)-, methyl ester, 29%] with *m/z* 278 and fragment ions 55, 74, 81, 95, 123, 179, 180, 222, 264 has been known as hazardous but limitation puts its usage as functional additive.[43] Component 9 [Figure 6a, Tridecanoic acid, methyl ester, 29%] with *m/z* 228 and fragment ions 55, 74, 143, 185, 228 is a methyl ester of fatty acid.[44] It is known as metabolite of plant, flavoring agent and adds fragrance. Component 10 [Figure 6b, Silane, dimethyl [2, 3, 6 trichlorophenoxy] heptadecyloxy, 27%][45] with *m/z* 495 and fragment ions 55, 75, 97, 159, 219, 255, 313.The components present in the gel PEBH extract were predominantly found to be saturated and unsaturated fatty acids and their esters, diterpenes and methylated phenols.

Table 4: Stability studies of petroleum ether extract of *Benincasa hispida*

Parameter	Condition	Batch	0 day	15th day	30th day	60th day	90th day	180th day
Colour	30°C±2°C/65% RH±5% RH	NG1 (250 mg)	White	White	White	White	White	White
		NG1 (500 mg)	White	White	White	White	White	White
		NG1 (750 mg)	White	White	White	White	White	White
	40°C±2°C/75% RH±5% RH	NG1 (250 mg)	White	White	White	White	White	White
		NG1 (500 mg)	White	White	White	White	White	White
		NG1 (750 mg)	White	White	White	White	White	White
pH	30°C±2°C/65% RH±5% RH	NG1 (250 mg)	6.78	6.78	6.75	6.68	6.63	6.63
		NG1 (500 mg)	6.75	6.75	6.75	6.71	6.65	6.62
		NG1 (750 mg)	6.83	6.83	6.8	6.78	6.72	6.69
	40°C±2°C/75% RH±5% RH	NG1 (250 mg)	6.78	6.7	6.7	6.65	6.61	6.57
		NG1 (500 mg)	6.75	6.72	6.71	6.71	6.69	6.62
		NG1 (750 mg)	6.83	6.81	6.78	6.77	6.75	6.68
Viscosity (Cps)	30°C±2°C/65% RH±5% RH	NG1 (250 mg)	185000	185500	186000	186800	187200	187620
		NG1 (500 mg)	185500	185700	186200	187500	189000	189800
		NG1 (750 mg)	183000	185000	189700	192900	195700	197820
	40°C±2°C/75% RH±5% RH	NG1 (250 mg)	185000	182800	186500	179700	185400	186270
		NG1 (500 mg)	185400	196200	183500	187500	182700	188630
		NG1 (750 mg)	185500	185700	186200	187500	189000	189880
Spread-ability (g/sec)	30°C±2°C/65% RH±5% RH	NG1 (250 mg)	40	39.04	38.20	37.38	37.09	36.25
		NG1 (500 mg)	42.2	42	40.88	40.44	39.55	37.53
		NG1 (750 mg)	41.6	41.53	41.33	40.88	40.22	38.4
	40°C±2°C/75% RH±5% RH	NG1 (250 mg)	40	40.4	40.86	41.8	42.46	43.33
		NG1 (500 mg)	42.2	42.86	43.53	44.46	44.86	45.73
		NG1 (750 mg)	41.6	41.8	42.4	43.33	44.44	45.33
Drug content (%)	30°C±2°C/65% RH±5% RH	NG1 (250 mg)	96.04	95.6	94.71	93.81	93.37	93.16
		NG1 (500 mg)	97.14	96.48	95.14	94.26	93.81	93.37
		NG1 (750 mg)	96.24	95.44	94.9	94.1	93.3	93.04
	40°C±2°C/75% RH±5% RH	NG1 (250 mg)	96.04	95.82	95.37	94.5	93.38	93.16
		NG1 (500 mg)	97.14	96.7	96.04	94.17	94.04	93.37
		NG1 (750 mg)	96.24	94.9	94.64	93.57	92.78	92.24

Table 5: Gas chromatography-mass spectroscopy of niosomal gel formulation (petroleum ether extract of *Benincasa hispida*)

Name of the phytoconstituents, molecular formula	Synonyms	Obtained (%)
1.2-ethyl 2-Hexen-1-ol, $C_8H_{16}O$	3-methylhex-2-en-1-ol (2E)-3-methylhex-2-en-1-ol 3-methyl-2-hexen-1-ol (E) 2-Hexen-1-ol, 3-methyl-, (E)	28
cis-9,10-Epoxyoctadecan-1-ol, $C_{18}H_{36}O_2$	cis-9,10-epoxy-1-octadecanol cis-9,10-Epoxyoctadecan-1-ol Oleyl alcohol epoxide, cis- cis-9,10-Epoxystearyl alcohol	35
Dodecanoic acid, 2-penten-1-yl ester, $C_{17}H_{32}$	Oxiraneoctanol, 3-octyl-, 2E)-2-Penten-1-yl laurate (2E)-2-Penten-1-yllaurat Dodecanoic acid, (2E)-2-penten-1-yl ester Dodecanoic acid, 2-penten-1-yl ester Laurate de (2E)-2-pentén-1-yle (2E)-2-Pentenyl laurate	33
Cholestane, 4, 5-epoxy-,(4α,5α), $C_{27}H_{46}O$	4.alpha.,5.alpha.-Epoxycholestane 4.alpha.,5-Epoxy-5.alpha.-cholestane 5.alpha.-Cholestane, 4.alpha.,5-epoxy-	31
Cyclopentaneundecanoic acid, $C_{16}H_{30}O_2$	Cyclopentaneundecanoic acid Dihydrohydnocarpic acid	36
17- octadecynoic acid, $C_{18}H_{32}O_2$	17-octadecynoic acid 17-odya Octadec-17-ynoic acid Alkynyl Stearic Acid	34

Contd...

Table 5: Contd...

Name of the phytoconstituents, molecular formula	Synonyms	Obtained (%)
Octadecanoic acid, methyl ester, $C_{19}H_{38}O_2$	Methyl stearate Methyl octadecanoate Octadecanoic acid, methyl ester Stearic acid methyl ester	29
9- Octadecadienoic acid (z)-, methyl ester, $C_{19}H_{36}O_2$	Methyl oleate Methyl cis-9-octadecenoate Oleic acid methyl ester	29
Tridecanoic acid, methyl ester, $C_{15}H_{30}O_2$	Methyl tetradecanoate Methyl myristate	29
Silane, dimethyl (2,3,6, tricholrophenoxy) heptadicycloxy, $C_{12}H_{17}Cl_3O_2Si$	Silane, dimethyl (2-hexyloxy) propoxy-	27

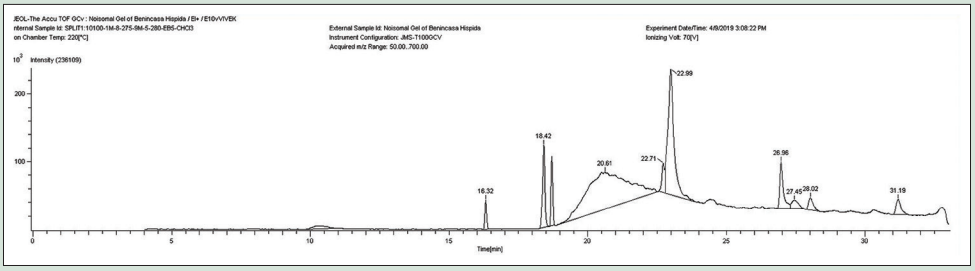


Figure 3: Gas chromatography-mass spectrometric spectrum of *Benincasa hispida* extract

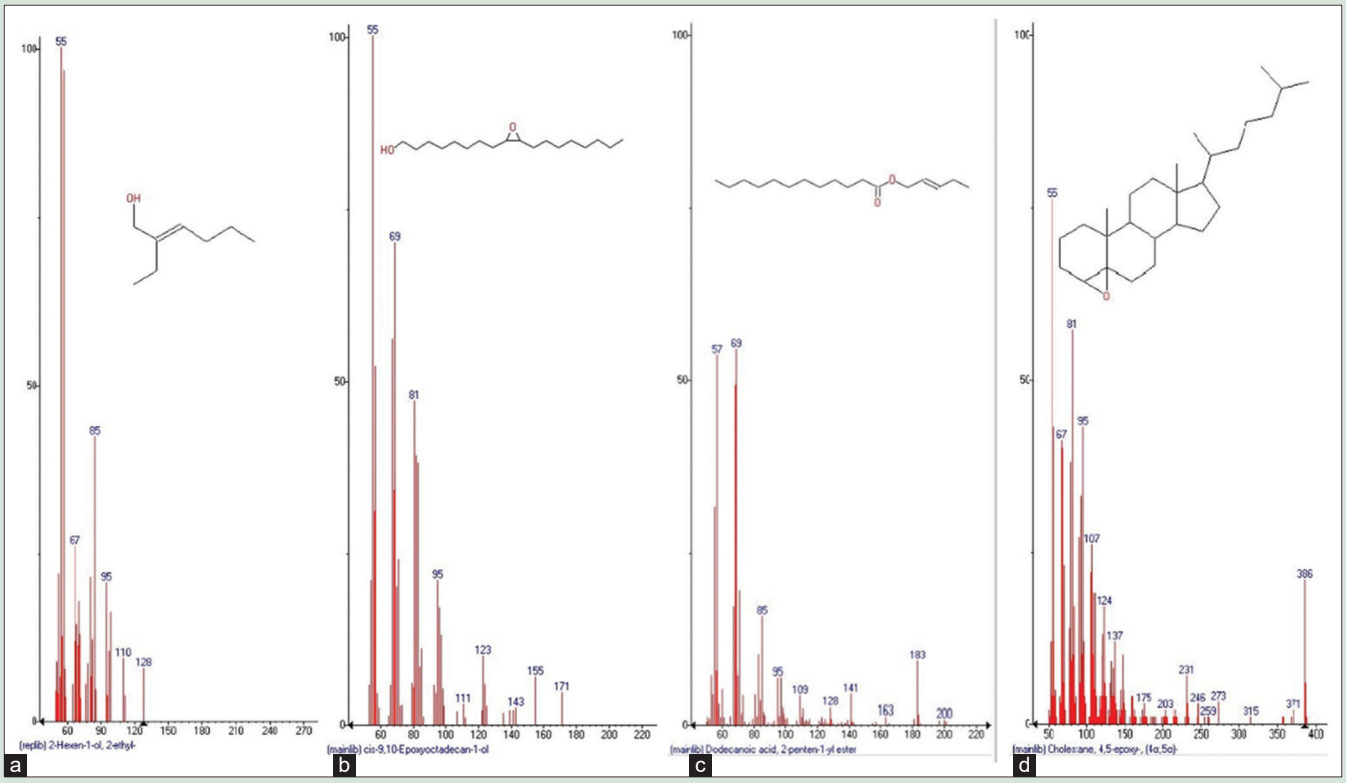


Figure 4: (a) 1.2-Ethyl 2-hexen-1-ol, (b) cis-9,10-epoxyoctadecan-1-ol, (c) dodecanoic acid, 2-penten-1-yl ester, (d) cholestane, 4, 5-epoxy-,(4α, 5α)

Melanoma is an aggressive malignant neoplasm derived from melanocytes. Malignant non-melanoma skin cancers originate from keratinized epithelial cells. BCC is caused by cell mutations induced by UV radiation and commonly noticed on sun-exposed areas (nose, ears, face and backs of hands). Cutaneous SCC arises from malignant, uncontrolled proliferation of epidermal keratinocytes.[46,47] Drug–delivery systems using niosomes as alternative therapy has been the focus of this research paper. There is recent interest among the researchers to search for complementary medicine for the treatment of cancer. Many of the existing drugs have not proved to be effective against different types of cancer. Some of the

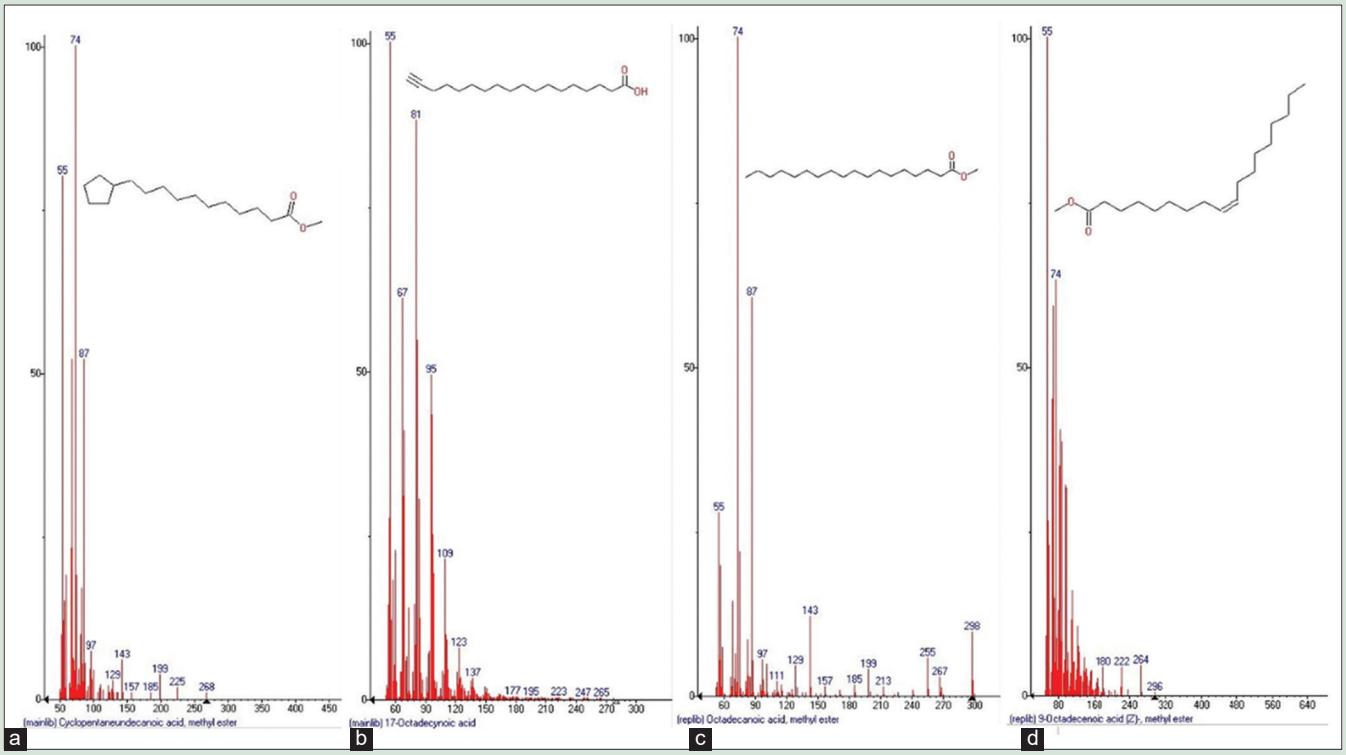


Figure 5: (a) Cyclopentaneundecanoic acid, (b) 17-octadecynoic acid, (c) octadecanoic acid, methyl ester, (d) 9-octadecadienoic acid (z)-, methyl ester

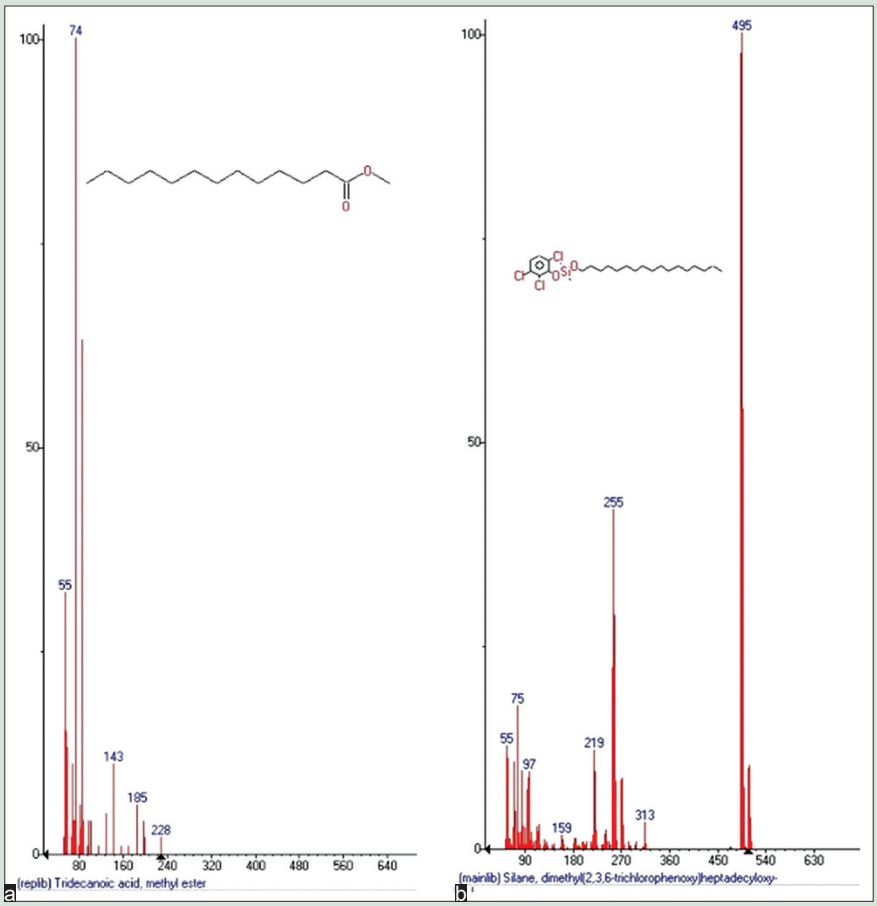


Figure 6: (a) Tridecanoic acid, methyl ester, (b) silane, dimethyl (2, 3, 6, tricholrophenoxy) heptadicycloxy

well-known plants used in chemotherapy of cancer *Rauwolfia serpentia*, *Panax ginseng* etc. India has been witnessing substantially arise in SCC cases.[13,14] B16 have been used for metastasis and solid tumor formation.[15] Our initial screening for human oral cancer cell line KB [Table 6] and Mus Musculus skin melanoma B16-F10 [Table 7] has shown unsatisfactory results at the selected concentrations as indicated by graphs [Figure 7a-h]. The article will help to further explore research endeavors in exploring plant-based treatment strategies. Nevertheless, the earth has ample resources dearth lies in the correct identification of the phytoconstituents. Proper phytoconstituent prospective studies help to explore many plants from various families for different therapeutic categories.

CONCLUSION

The researchers have attempted to evaluate potential of BH niosomal gel for *in vitro cell lines* of cancer with detailed physiochemical and analytical evaluation. Future studies may be undertaken at various stages and levels to further explore the potential of these family of plants in depth related to growth of multiple therapeutic areas.

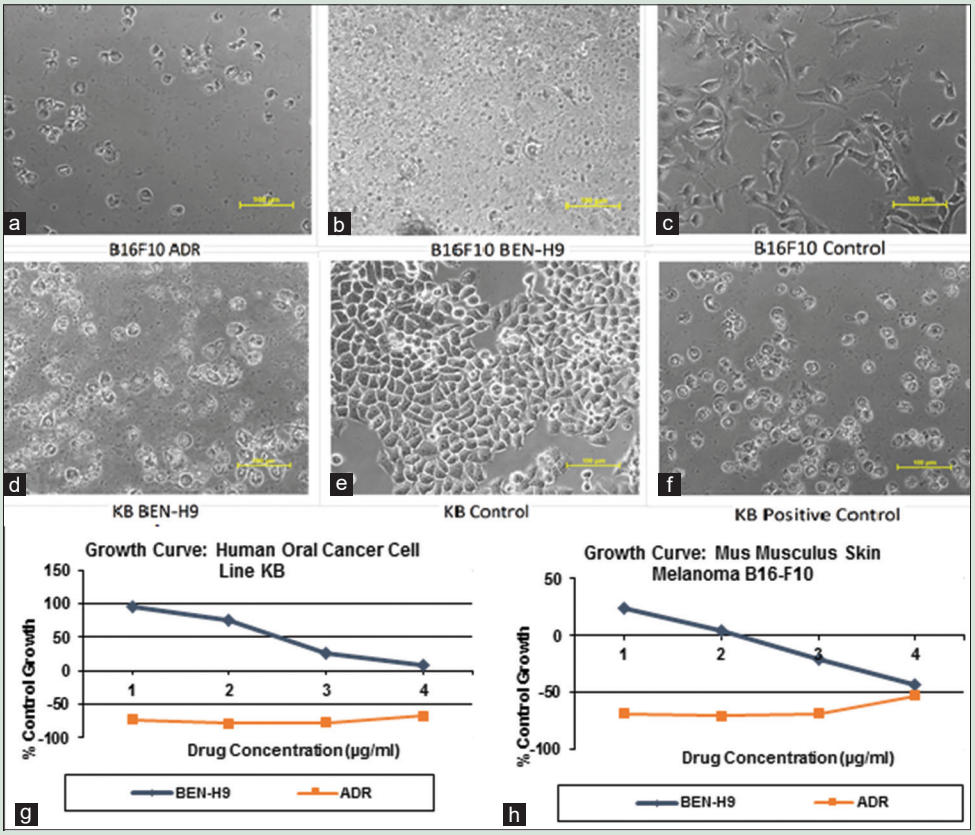


Figure 7: (a) BF 16 ADR, (b) BF 16 BEN-H9, (c) BF 16 control, (d) KB BEN-H9, (e) KB control, (f) KB positive control, (g) Growth curve: Human oral cancer cell line KB, (h) Growth curve: Mus musculus skin melanoma B16-F10

Table 6: Human oral cancer cell line KB of petroleum ether extract of *Benincasa hispida* Niosomal gel (% Control Growth)

Samples	Drug concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
BEN-H9	94.7	77.9	28.5	7.8	94.8	72.4	23.4	5.3	96.1	74.4	28.9	10.1	95.2	74.9	27.0	7.7
ADR	−71.1	−76.1	−75.5	−62.8	−75.7	−81.0	−77.5	−66.5	−72.8	−77.2	−78.3	−71.1	−73.2	−78.1	−77.1	−66.8

Table 7: Mus Musculus Skin Melanoma B16-F10 of petroleum ether extract of *Benincasa hispida* Niosomal gel (% control growth)

Samples	Drug concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
BEN-H9	25.1	5.0	−19.6	−42.3	23.1	2.8	−22.5	−45.2	24.5	4.8	−19.0	−42.6	24.3	4.2	−20.4	−43.4
ADR	−67.1	−69.2	−68.1	−53.7	−69.6	−71.2	−69.8	−49.8	−69.3	−70.4	−68.4	−54.0	−68.7	−70.3	−68.7	−52.5

Acknowledgements

We would like to acknowledge the help and guidance received from the SAIF Department, IIT, Mumbai. We also wish to acknowledge Hemen S Ved and Ami P Thakkar (Research Scholars, Department of Pharmacology, SVKM's Dr. Bhanuben Nanavati College of Pharmacy), for their help and support in the compilation of the article.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Harborne JB. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. Third edition, Springer (India) Private Limited; 2005.
2. Rana S, Suttee A. Phytochemical investigation and evaluation of free radical scavenging potential of *Benincasa hispida* peel extracts. Int J Curr Pharm Rev Res 2012;3:43-6.
3. Al-Snafi AE. The pharmacological importance of *Benincasa hispida*. A review. Int J Pharm Sci Res 2013;4:165-70.
4. Maroni A, Del Curto MD, Salmaso S, Zema L, Melocchi A, Caliceti P, *et al. In vitro* and *in vivo* evaluation of an oral multiple-unit formulation for colonic delivery of insulin. Eur J Pharm Biopharm 2016;108:76-82.
5. Escobar-Chávez JJ, Díaz-Torres R, Rodriguez-Cruz I, Dominguez-Delgado C, Morales R, Ángeles-Anguiano E, *et al.* Nanocarriers for transdermal drug delivery. Skin 2012;19:22.
6. Shirsand S, Para M, Nagendrakumar D, Kanani K, Keerthy D. Formulation and evaluation of ketoconazole niosomal gel drug delivery system. Int J Pharm Investig 2012;2:201-7.
7. Srinivas S, Kumar YA, Hemanth A, Anitha M. Preparation and evaluation of niosomes containing aceclofenac. Dig J Nanomater Bios 2010;5:249-54.
8. Shakeel F, Ramadan W. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. Colloids Surf B Biointerfaces 2010;75:356-62.
9. Sonneville-Aubrun O, Simonnet JT, L'Alloret F. Nanoemulsions: A new vehicle for skincare products. Adv Colloid Interface Sci 2004;108:145-9.
10. Symon Z, Peyser A, Tzemach D, Lyass O, Sucher E, Shezen E, *et al.* Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. Cancer 1999;86:72-8.
11. Gonçalves A, Braud AC, Viret F, Genre D, Gravis G, Tarpin C, *et al.* Phase I study of pegylated liposomal doxorubicin (caelyx) in combination with carboplatin in patients with advanced solid tumors. Anticancer Res 2003;23:3543-8.
12. Seiden MV, Muggia F, Astrow A, Matulonis U, Campos S, Roche M, *et al.* A phase II study of liposomal lurtotecan (OSI-211) in patients with topotecan resistant ovarian cancer. Gynecol Oncol 2004;93:229-32.
13. Sireesha D, Reddy BS, Reginald BA, Samatha M, Kamal F. Effect of amygdalin on oral cancer cell line: An *in vitro* study. J Oral Maxillofac Pathol 2019;23:104-7.
14. Teicher BA, editor. Tumor Models in Cancer Research. Humana Press: Springer Science & Business Media; 2011.
15. Yang CH, Yue J, Pfeffer SR, Handorf CR, Pfeffer LM. MicroRNA miR-21 regulates the metastatic behavior of B16 melanoma cells. J Biol Chem 2011;286:39172-8.
16. Gandhi AA, Chaskar S, Jadhav SP, Salunkhe KS. Transfersomes. In: International Medical Commission of Bhopal, editor. Transdermal Drug Delivery. Bhopal: Inventi Impact; 2011.
17. Patel R, Singh SK, Singh S, Sheth NR, Gendle R. Development and characterization of curcumin loaded transfersome for transdermal delivery. J Pharm Sci Res 2009;1:71.
18. Samala U, Pannala S. Ethosomes, a novel transdermal drug delivery system: A review. Drug Invent Today 2011;4:4628-30.
19. Keservani RK, Sharma AK, Ramteke S. Novel vesicular approach for topical delivery of baclofen via niosomes. Lat Am J Pharm 2010;29:1364-70.
20. Kumar GP, Rao PR. Ultra deformable niosomes for improved transdermal drug delivery: The future scenario. Asian J Pharm Sci 2012;7:96-109.
21. Aja PM, Okorie UC, Ozougwu VE, Onya-Mmaghiri EA, Agu KA, Nweke OL. Phytochemical and gas chromatography-mass spectrometric (GC-MS) analyses of whitfieldia lateritia leaf. Afr J Basic Appl Sci 2016;8:41.
22. Liu J, Liu Y, Pan YJ, Zu YG, Tang ZH. Determination of alkaloids in catharanthus roseus and vinca minor by high-performance liquid chromatography – Tandem mass spectrometry. Anal Lett 2016;49:1143-53.
23. Rency RC, Vasantha K, Maruthasalam A. Identification of bioactive compounds from ethanolic leaf extracts of premna serratifolia L. using GC-MS. Biosci Discov 2015;6:96-101.
24. Rajalakshmi K, Mohan VR. GC–MS analysis of bioactive components of *Myxopyrum serratulum* AW hill (Oleaceae). Int J Pharm Sci Rev Res 2016;38:30-5.
25. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc 2006;1:1112-6.
26. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82:1107-12.
27. Bates DM, Robinson RW. Biology and Utilization of the Cucurbitaceae. Cornell University Press, Ithaca, NY 14850; 1990.
28. Jeffrey C. A new system of cucurbitaceae. Bot Zhurn 2005;90:332-5.
29. Astruc D, Boisselier E, Ornelas C. Dendrimers designed for functions: From physical, photophysical, and supramolecular properties to applications in sensing, catalysis, molecular electronics, photonics, and nanomedicine. Chem Rev 2010;110:1857-959.
30. James-Smith MA, Hellner B, Annunziato N, Mitragotri S. Effect of surfactant mixtures on skin structure and barrier properties. Ann Biomed Eng 2011;39:1215-23.
31. Mihranyan A, Ferraz N, Strømme M. Current status and future prospects of nanotechnology in cosmetics. Prog Mater Sci 2012;57:875-910.
32. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, *et al.* Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev 2011;63:470-91.
33. Cappel MJ, Kreuter J. Effect of nanoparticles on transdermal drug delivery. J Microencapsul 1991;8:369-74.
34. National Center for Biotechnology Information. PubChem Database. 1-Hexen-1-ol, 2-ethyl-, CID=71317675. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/1-Hexen-1-ol_-2-ethyl. [Last accessed on 2020 Mar 27].
35. National Center for Biotechnology Information. PubChem Database. Cis-9, 10-Epoxyoctadecan-1-ol, CID=12550092. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/cis-9_10-Epoxyoctadecan-1-ol. [Last accessed on 2020 Apr 12].
36. National Center for Biotechnology Information. PubChem Database. CID=549343. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Dodecanoic-acid_-4-penten-1-yl-ester. [Last accessed on 2020 Mar 27].
37. National Center for Biotechnology Information. PubChem Database. 4alpha, 5alpha-Epoxycholestane, CID=537014. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/4alpha_5alpha-Epoxycholestane. [Last accessed on 2020 Mar 27].
38. National Center for Biotechnology Information. PubChem Database. Cyclopentaneundecanoic acid, CID=534549. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Cyclopentaneundecanoic-acid. [Last accessed on 2020 Mar 27].
39. National Center for Biotechnology Information. PubChem Database. 17-Octadecynoic acid, CID=1449. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/17-Octadecynoic-acid. [Last accessed on 2020 Mar 27].
40. Shmuel Y. Dictionary of Food Compounds with CD-ROM: Additives, Flavors and Ingredients. Boca Raton: Chapman & Hall/CRC; 2004.
41. Frank DG, Harwood JL, Dijkstra AJ. The Lipid Handbook with CD-ROM. Third edition: CRC Press Taylor and Francis Group; 2007.
42. National Center for Biotechnology Information. PubChem Database. Methyl stearate, CID=8201. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Methyl-stearate. [Last accessed on 2020 Mar 27].
43. National Center for Biotechnology Information. PubChem Database. CID=176473. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Octadecadienoic-acid_-methyl-ester. [Last accessed on 2020 Apr 12].
44. National Center for Biotechnology Information. 2004-. PubChem Compound Summary for CID 31284, Methyl tetradecanoate; . Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Methyl-tetradecanoate. [Last accessed on 2020 Sept. 13].
45. National Center for Biotechnology Information. PubChem Database. CID=91742136. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Silane_-dimethyl_2_3_6-trichlorophenoxy_butoxy. [Last accessed on 2020 Apr 12].
46. Fang YP, Huang YB, Wu PC, Tsai YH. Topical delivery of 5-aminolevulinic acid-encapsulated ethosomes in a hyperproliferative skin animal model using the CLSM technique to evaluate the penetration behavior. Eur J Pharm Biopharm 2009;73:391-8.
47. Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate: *In vitro* percutaneous permeation through human skin and *in vivo* anti-inflammatory activity on human volunteers. J Control Release 2005;106:99-110.