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Original Article

Encapsulation of babchi essential oil into microsponges: Physicochemical properties, cytotoxic evaluation and anti-microbial activity

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ABSTRACT

Babchi essential oil (BEO) is a valuable essential oil reported to possess a variety of biological activities such as antitumor, anti inflammatory, immunomodulatory, antioxidant, antifungal and antibacterial properties. Due to its anti-microbial properties, this oil possesses an immense potential for the treatment of dermatological disorders. Further, it has minimal tendency to develop resistance, a common issue with most of the antibiotics. However, its highly viscous nature and poor stability in the presence of light, air and high temperature, limits its practical applications. To surmount these issues, this research aims to encapsulate BEO in ethyl cellulose (EC) microsponges for enhanced stability, antibacterial effect and decreased dermal toxicity. The quasi emulsion solvent evaporation technique was used for fabrication of the BEO microsponges employing EC as polymer, polyvinyl alcohol (PVA) as stabilizer and dichloro methane (DCM) as solvent. The effect of formulation variables such as the amount of EC and PVA were also investigated. The prepared microformulations were evaluated for production yield, encapsulation efficiency, particle size and *in vitro* release. *In vitro* cytotoxicity was also checked to assess dermal safety of BEO microsponges. Results revealed that all the dispersions were in micro size range (20.44 ± 3.13 μm to 41.75 ± 3.65 μm), with good encapsulation efficiency (87.70 ± 1.20% of F2) and controlled release profile (cumulative drug release 73.34 ± 1.76%). Field emission scanning electron microscopy results showed that the microsponges possessed a spherical uniform shape with a spongy structure. Results of cytotoxicity study indicated that the prepared microsponges were safer on dermal cells in comparison to pure BEO. The optimized formulation was also evaluated for *in vitro* antimicrobial assay against dermal bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, which confirmed their enhanced antibacterial activity. Furthermore, the results of photostability and stability analysis indicated improved stability of BEO loaded microsponges. Hence, encapsulation of BEO in microsponges resulted in efficacious carrier system in terms of stability as well as safety of this essential oil alongwith handling benefits.

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1. Introduction

Community associated, foodborne and hospital acquired infectious disorders [1,2] are the major causes of mortality and morbidity worldwide, preferably due to the increased microbial resistance to synthetic drugs. Further, injudicious use of antibiotics is the single most important factor leading to antibiotic resistance around the world [3]. To handle this issue, there is strong need to investigate safe alternative options. But development of new drug moiety is time consuming and an expensive process and the genetic modifications in pathogens lead to development of resistance at a faster rate [4,5]. Nature being the main source of antiinfectives, might represent a source of alternative medicines, which can help in overcoming antibiotic resistance issues. Hence, essential oils, obtained from natural sources may possess strong possibilities as the new strategies on infectious diseases control in near future [6].

Essential oils are volatile and aromatic liquids derived from plants. These oils are rich source of bioactives such as terpenoids, phenols, coumarins, hydrocarbons and their derivative compounds. They possess wide applications in food/food packaging, cosmetics and pharmaceuticals, owing to their diverse therapeutic activities [7–9]. Although, the anti bacterial and antifungal potential of essential oils have long been utilized [9,10], but these have recently been paid more attention as natural antimicrobials for variety of applications [6,11]. An uncountable number of scientific publications have cited essential oils with clear inhibitory profile effective against variety of pathogenic fungi and bacteria. Within a great variety of essential oils (EOs), BEO extracted from *Psoralea coryfolia* L. (*P. coryfolia*) is well known for its antitumor, anti-inflammatory, immunomodulatory, antioxidant, antifungal and antibacterial properties [12]. Dry fruit of *P. coryfolia* is reported as a popular traditional Chinese medicine listed in Chinese pharmacopeia [13]. This plant is also reported for its wide use in variety of skin disorders such as leukoderma, leprosy and psoriasis [14]. The major components of BEO are bakuchiol, psoralen and isopsoralen which are known for their *in vitro* anti-microbial, anti-inflammatory, anti-tumor and anti-oxidant activity [15–18]. However, owing to its poor physical properties like hydrophobicity, light sensitivity, very high viscosity and susceptibility to degradation, practical use of BEO have been limited in pharmaceuticals. Formulations like PLGA (poly(lactic-co-glycolic acid)) microspheres [19], solid lipid nanoparticles, nanostructured lipid carriers [20], ethosome loaded gel [21], emulgel [22], ethosomes and liposomes [23] have been reported for BEO in literature. In order to overcome the limitations and enhance the stability of essential oils during storage, microencapsulation techniques have got wide acceptance. Among microcarrier systems, an advance delivery system composed of microsponges has been commercialized for wide variety of products. Microsponges are porous polymeric microparticles ranging from 5 to 300 μm in diameter. Microsponge based delivery system leads to reduction in side effects and dose, enhancement in stability and elegance, formulation flexibility and modification of the drug release rate, when compared to liposomes and microcapsules [24,25]. Further, these microcarriers possess the advantage of increased payload and cost effectiveness [26]. Additionally, using this delivery system, the dermatological potential of drug moieties can also be enhanced by further loading microsponges in suitable topical carriers like creams, lotions and gels. Hence, the present investigation was planned to encapsulate BEO in microsponges to beat the limitations of this oil, and to result a more useful, versatile and effective therapeutic product. BEO microsponges, in turn, can be incorporated in topical systems for strengthening the dermatological benefits of this essential oil.

Among several techniques available for microsponge fabrication, Quasi-emulsion solvent evaporation technique has been most commonly reported for encapsulation of hydrophobic moieties. Besides easy to perform at lab scale and scale up, this method is highly reproducible. Among the variety of polymers available, EC is a very popular cellulose derivative in which some hydroxyl groups present on the glucose moiety are modified into ethyl ether groups. This nonswellable hydrophobic polymer has been evidently reported for preparation of microcapsules [27]. It has been used by several research groups for fabrication of microsponges and nanosponges, as well.

The purpose of present investigation was to fabricate babchi essential oil microsponge using ethyl cellulose by Quasi-emulsion solvent evaporation method. We clarified the successful encapsulation of BEO by UV spectrophotometry, FTIR (Fourier transform infrared) spectroscopy, DSC (differential scanning colorimetry) and determined the shape, morphology and mean particle size of prepared microsponges by FE-SEM (field emission-scanning electron microscopy) and laser light scattering. The effects of polymer (EC) and emulsifier (PVA) on encapsulation efficacy and mean particle size of BEO microformulations were also investigated. In addition, the release profile of BEO from ethyl cellulose (EC) microsponges was investigated. In order to assess dermal safety of prepared BEO formulations, *in vitro* cytotoxicity study was also performed. Finally, BEO loaded microsponges were evaluated for *in vitro* anti-microbial assay. Photostability and stability analysis were also performed for selected BEO microsponge formulations.

2. Materials and methods

2.1. Materials

Babchi oil was acquired from Pukhraj Herbals, Mandsaur (India). Ethyl cellulose, ethanol and acetone were purchased from Central Drug House, New Delhi. Dichloromethane and polyvinyl alcohol were procured from SD Fine Chemicals Limited, Mumbai. Potassium dihydrogen phosphate and sodium hydroxide were supplied by HPLC, Mumbai. Nutrient agar was purchased from Hi-media, India. All the other chemicals and solvents used were of analytical grade. Distilled deionized water was used throughout this work.

2.2. Methods

2.2.1. Authentication of babchi essential oil

Gas chromatography-mass spectrometry (GC-MS) The essential oils were analyzed by GC-MS on a Shimadzu QP2010 Plus (Shimadzu Corporation, Kyoto, Japan) system equipped with a thermal desorption system TD20 (Shimadzu Corporation, Kyoto, Japan) with split injector. GC-MS was used under the following conditions: Restek Rtx-5 MS fused silica capillary column (30 m 90.25 mm i.d, 90.25 mm thickness film, composed of 5 %-diphenyl-95%-dimethyl polysiloxane). Helium (99.999%) was used as carrier gas at a constant flow of 1.2 ml/min. The injection volume was 0.2 μl (split ratio of 1:100), the injector temperature was 260 °C and the ion-source temperature was 230 °C and 270 °C. The oven temperature was programmed at 50 °C (isothermal for 2 min), with an increase in 3 °C/min to 280 °C, then 10 °C/min to 260 °C, ending with 300 °C (isothermal for 10 min). Mass spectra were taken at 70 eV with a scan interval of 0.5 s for fragments from 40 to 500 Da. The identification of active components was established on comparison of their mass spectra. The peak area measurement (expressed in area percentage) was used for quantitative analysis of constituents of babchi essential oil.

2.2.2. Fabrication and optimization of BEO loaded microsponges

Babchi essential oil microsponges were fabricated by quasi-emulsion solvent diffusion technique [28]. In this technique, BEO was added to organic internal phase comprising of ethyl cellulose dissolved in DCM (20 ml). Here, DCM acts as an effective solvent to dissolve both the essential oil and the polymer. This was followed by dropwise addition of internal phase into aqueous solution containing polyvinyl alcohol, placed in a vessel and stirred at 4000 rpm (propeller type agitator) for 3 h. Subsequently, due to removal of DCM from the reaction medium, microsponges were formed. At this stage, the formed microsponges were filtered, washed with distilled water and dried at room temperature [29].

Various parameters were known to affect the preparation of microsponges. Hence, optimization of babchi oil loaded microsponges was achieved by varying the concentration of ethyl cellulose (900 mg, 700 mg and 500 mg) and PVA (100 mg, 75 mg and 50 mg) keeping DCM constant (20 ml). Further, encapsulation efficiency, particle size and *in vitro* release were measured to choose the best babchi oil loaded microsponge formulation.

2.2.3. Physicochemical characterization of BEO loaded microsponges

2.2.3.1. *Fourier transform infrared spectroscopy*. The FTIR spectra of BEO, EC, PVA and BEO loaded microsponges were subjected to infrared analysis in order to observe any interaction. The samples were scanned from 400 cm^{-1} to 4000 cm^{-1} and were recorded with Perkin Elmer FTIR (Spectrum BX II, USA), using the KBr disk.

2.2.3.2. *Differential scanning calorimetry*. Differential scanning calorimetric analysis of BEO, PVA, EC, physical mixture and BEO loaded microsponges was carried out using differential scanning calorimeter (Q10, TA instruments, USA). Powder dried samples were crimped in standard aluminum pan and heated at constant rate of 10 °C/min over a temperature range of 25–500 °C. Analysis was performed by purging nitrogen gas at flow rate of 100 ml/min.

2.2.3.3. *Field emission-scanning electron microscopy*. For estimation of surface roughness and visualizing the surface texture and morphology, prepared microsponges were examined by field-emission scanning electron microscopy (QUANTA 200 FEG, Netherland) operated at 30 kV acceleration. By means of double-faced adhesive tape, samples were mounted on a metal stud and coated with gold-palladium under vacuum and finally detected using Everhart-Thornley detector. The obtained photographs were recorded at different magnifications (12X- 1000KX).

2.2.3.4. *Particle size determination*. The particle size analysis of microsponges was performed by using particle size analyzer, Microtrac (S3500-special, USA) with precise angular measurement of scattered light (full 180° angular range) through a full 180° angular range with three lasers and two detector arrays.

2.2.4. Entrapment efficiency

The BEO loaded microsponges were weighed (10 mg) and triturated with the help of motor and pestle. After trituration, particles were dispersed in ethanol solution (10 ml) to extract the entrapped essential oil. Then, the samples were filtered using a 0.45 μm membrane filter and the absorbance was measured at 262 nm in UV–visible spectrophotometer (Varian Cary -5000). Encapsulation efficiency was determined using this following equation:

$$\%\ \text{Encapsulation efficiency} = \frac{C_R \times V_R / M_{mp}}{M_D / M_D + M_P} \times 100$$

where C_R is the drug concentration of the release medium, V_R is the volume of release medium, M_{mp} is the mass of microparticles taken and M_D and M_P are, the mass of essential oil encapsulated and mass of polymer used initially, respectively [30].

2.2.5. In vitro *drug release*

To find the rate and extent of BEO release from microsponges, dissolution of different batches of BEO-loaded microsponges (as reported in Table 1) was carried out using USP dissolution test apparatus (USP II). An aliquot (2 ml) of microsponge

Table 1 – Evaluation data of babchi essential oil loaded microsponges.

Formulation Batches	Polymer (mg)	PVA (mg)	Particle size (μg)	Entrapment efficiency (%)	Cumulative Drug Release (%)
F1	900	100	41.75 ± 3.65	77.00 ± 1.00	58.35 ± 1.69
F2	700	100	30.31 ± 3.65	87.70 ± 1.20	73.34 ± 1.76
F3	500	100	28.02 ± 3.29	76.71 ± 0.68	74.11 ± 1.2
F4	900	75	30.56 ± 6.73	76.56 ± 1.54	60.53 ± 1.69
F5	700	75	31.49 ± 4.69	79.03 ± 0.71	75.43 ± 0.59
F6	500	75	27.72 ± 3.29	74.08 ± 0.47	80.33 ± 1.48
F7	900	50	37.15 ± 6.81	57.26 ± 1.96	68.84 ± 1.49
F8	700	50	26.11 ± 9.31	71.14 ± 1.01	77.51 ± 1.79
F9	500	50	20.44 ± 3.13	55.28 ± 1.54	86.21 ± 1.48

suspension consisting of BEO microsponges (equivalent weight 50 mg), 1.5 ml phosphate buffer solution (pH 5.4) and 0.5 ml ethanol was introduced in the dialysis membrane (pore size 14,000 Da, diameter 17.5 mm, HI-media). The dialysis sacks were tied to paddles and placed into dissolution vessels containing mixture of phosphate buffer (pH 5.4), DMSO and PEG 400 (80:10:10). Finally, vessels containing release media were stirred at a speed of 50 rpm under constant temperature (37 ± 1 °C). The drug release in the outer solution as a result of diffusion from membrane was assessed by sampling the solution at specific intervals of time. The drug content released from membrane was measured at 236 nm using UV visible spectrophotometer. The percentage of BEO released at various time intervals was calculated from the standard calibration curve (plotted and validated earlier) for the oil and plotted against time [21].

2.2.6. *Cytotoxicity studies*

The cytotoxicity of BEO and BEO loaded microsponges was checked using HaCaT (Human epidermal keratinocyte) cell lines. HaCaT is spontaneously transformed aneuploid immortal keratinocyte cell line from human skin [31]. Owing to their high tendency to antiproliferate and differentiate *in vitro*, these cell lines were chosen [32]. For cytotoxicity analysis, the samples were weighed and mixed to get the desired concentration. Further, the samples were dissolved in distilled DMSO and Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% inactivated Fetal Bovine Serum (FBS) was used to make up the volume. Stock solution (1 mg/ml) was prepared and sterilized by filtration. From the stock solution, serial two-fold dilutions (0–320 μg/ml) were prepared using FBS (for dilution) for cytotoxicity analysis.

For culturing HaCaT cells, DMEM medium supplemented in 10% FBS, streptomycin (100 μg/ml) and penicillin (100 IU/ml) in a humidified atmosphere (5% CO_2 at 37 °C), until confluent. For cell dissociation, TPVG solution (0.2% trypsin, 0.05% glucose and 0.2% EDTA in phosphate buffer saline solution) was used. Further, 50,000 cells were seeded in a 96 well plate and incubation was carried out for 24 h (37 °C, 5% CO_2 incubator).

The monolayer cell culture was trypsinized and using respective media containing 10% FBS, the cell count was adjusted to 1.0×10^5 cell per ml. To each well of the microfilter plate, 100 μl of the diluted cell suspension was added and the plates were incubated for 24 h (37 °C in 5% CO_2 atmosphere). After incubation, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide] (5 mg/10 ml of MTT in PBS) was added to each well. At this stage, DMSO (100 μl) was added to the supernatant after incubation. The plates were analyzed at 590 nm using a microplate reader and % growth inhibition was determined. Concentration of test sample needed to inhibit cell growth by 50% (IC 50) was also calculated.

2.2.7. In vitro *antibacterial studies*

The antibacterial activity of the BEO, blank microsponges and BEO loaded microsponges was determined by the agar well diffusion method in nutrient agar medium. Using a borer, wells (6 mm in diameter) were punched out in the agar medium and inoculums 1×10^6 cfu/ml of the test bacteria *i.e.* Gram-positive *Staphylococcus aureus (S. aureus)* and Gram-negative *Pseudomonas aeruginosa (P. aeruginosa)* and *Escherichia coli (E. coli)*, were spread on the surface of the medium with a sterile spreader. BEO, blank microsponges and BEO loaded microsponges (dissolved in ethanol-20 μl) were pipetted into the wells. The agar plates were incubated at 37 °C for 24 h and the diameter of the zone of inhibition surrounding the wells was measured. The diameters of zone of inhibition for different samples were compared with those produced by the control, commercial antibiotic streptomycin (10 μg/ml). Antibacterial tests were performed in triplicate and observed values (average values) of the zone of inhibition were expressed [33,34].

2.2.8. *Photostability analysis*

The photodegradation of babchi oil and babchi oil microporous colloidal particles was performed under UV lamp (Philips 40 W TL K05). The BEO and BEO loaded microsponges were kept 10 cm away from the lamp for 1 h and samples were withdrawn after 10 min. Withdrawn samples were dissolved in ethanol, and analyzed quantitatively by UV–visible spectrophotometer [35].

2.2.9. *Stability studies*

The prepared babchi essential oil microsponges were subjected for stability studies at 40 ± 2 °C and 75 ± 5% RH for three months. Samples were withdrawn at an interval of 30, 60, 90 days and evaluated for physical appearance and drug content. FTIR spectroscopy was also carried out in order to check stability of prepared formulations [36].

2.2.10. *Statistical analysis*

Data obtained from each experiment were presented as mean ± SD value. Statistical measurements were carried out

by GraphPad Prism version 5.01 software (GraphPad Software, Inc, USA). Statistical differences were tested by one-way analysis of variance (ANOVA) and the independent sample t-test. P value < 0.05 was considered significant in present work.

3. Results and discussion

3.1. *Authentication of babchi essential oil*

3.1.1. *Gas chromatography mass spectrometry*

Generally, the gas chromatography conjugated with mass spectrometry is used for qualitative analysis as well as for quantitative estimation. In the present study, GCMS was applied to identify the volatile bioactive components present in BEO. A huge number of compounds were ascertained in this essential oil. Among them bakuchiol (*Psoralea corylifolia*) was confirmed as major constituent with the highest amount (65.37%) followed by 2H-Furo (2,3-h)-1-benzopyran-2-one (2.59%), caryophyllene oxide (2.11%), oleoyl chloride (1.70%), 2-phenyl-4-anilino-6(1H)- pyrimidinone (1.47%), 9-octadecenoic acid (1.29%), 2-5-(2-methyl benzoxazole-7-yl)-(1H-pyrazol-3-yl)-phenol (1.15%), sigmast-5-en-3-ol (1.04%). The rest of the volatile constituents were found at concentrations lower than 1%. GC-MS may act as a useful tool for analysis of composition of essential oils, which may vary depending on the factors like growing environment, extraction technique, their plucking and collection time. Additionally, identification of major component of essential oil may help in proposing mechanism of action of that oil.

3.2. *Fabrication and optimization of BEO loaded microsponges*

Babchi essential oil loaded microsponges were fabricated by quasi-emulsion solvent evaporation method. Ethyl cellulose was used as a polymer to provide structural integrity to the microsponges. PVA was used as an emulsifier and DCM as a solvent. Preliminary trial batches were prepared to determine the concentration of ethyl cellulose and DCM to produce non-aggregating porous microsponges. Preliminary data suggested that the concentration of DCM required was 20 ml, which was kept constant in present investigation. Varying the concentration of ethyl cellulose and PVA showed remarkable effect on prepared microsponges.

As discussed above, the concentration of BEO and DCM was kept constant, however, the effects of ethyl cellulose and PVA on the encapsulation efficiency, size and *in vitro* release of microsponges were analyzed.

3.3. *Physicochemical characterization of BEO loaded microsponges*

3.3.1. *Fourier transform infrared spectroscopy*

FTIR spectra corresponding to BEO, PVA, EC, blank microsponges and BEO loaded microsponges with characteristic peaks were obtained. The spectrum of BEO showed characteristic peaks at 3479 cm^{-1}, 2929 cm^{-1}, 1740 cm^{-1},1612 cm^{-1}, 1514 cm^{-1}, 1377 cm^{-1}, 1169 cm^{-1} and 724 cm^{-1}, whereas blank microsponges showed bands at 2977 $cm^{-1}$and a slightly intense band at 637 cm^{-1} (Fig. 1). EC spectrum exhibited bands at 3483 cm^{-1}, 1376 cm^{-1}, 1110 cm^{-1} and 2978 cm^{-1}. The characteristic peaks detected in PVA spectrum were at 1442 cm^{-1} and 1144 cm^{-1}.

However, the spectrum of BEO microsponges depicted similar signal as in BEO at 3418 cm^{-1} but a slight increase in frequency of characteristic bands of 2929 cm^{-1} and 1612 cm^{-1} was also noted. Another peaks at 1456 cm^{-1}, 1169 cm^{-1} and 724 cm^{-1} present in BEO were observed at slightly lower frequency of 1412 cm^{-1}, 1111 cm^{-1} and 625 cm^{-1}, respectively, in BEO loaded microsponges (Fig. 1).

The FTIR spectrum of BEO was described by transmission peaks analogous to its main components such as bakuchiol, previously analyzed by GC-MS, confirming purity of BEO. EC spectrum showed band at 3438 cm^{-1} (alcohol stretching), 1376 cm^{-1} (carboxyl and hydroxyl group), 1110 cm^{-1} (very strong ether group stretching) and 2978 cm^{-1} (methyl stretching). Similar peaks for EC spectrum were reported by Kumar and Ghosh, 2015 with minor differences in frequencies [37]. The major peaks identified in PVA were at 1412 cm^{-1} related to presence of hydroxyl group and 1144 cm^{-1} corresponding to methyl group. The spectrum of blank microsponges showed peaks at 2977 cm^{-1} (–CH) and 1112 cm^{-1} (ether) related to characteristic bands of EC and a slightly intense band at 1110 cm^{-1} owing to deformation of ether moiety (Fig. 1). Additionally, FTIR spectrum analysis depicted appearance of no new peak, or disappearance of existing peaks, eliminating probability of any chemical interaction between BEO and EC. Hence, BEO, PVA and EC spectra showed that BEO was compatible with selected polymers and excipients. In the presence of BEO, no change was seen in peak at 2976 cm^{-1} (-methyl group) in the microsponges. However, the spectrum of BEO microsponges showed some similar bands as in BEO, but a slight increase in their frequency was observed, which may be due to contribution of two absorption peaks originated by BEO *e.g.* slight

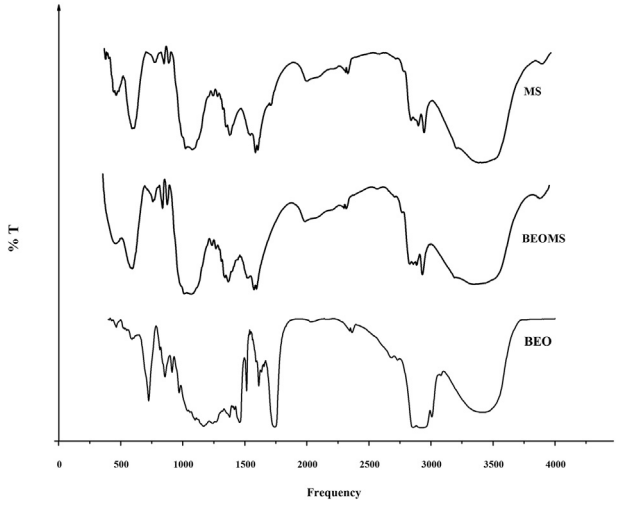

Fig. 1 – FTIR spectrum of blank microsponges, babchi essential oil loaded microsponges and babchi essential oil.

increase in characteristic bands at 2929 cm^{-1} and 1612 cm^{-1} was observed. Another peaks at 1456 cm^{-1} due to alcohol group, 1169 $cm^{-1}$and 724 cm^{-1} (-ether group) presented in BEO were represented at a slight lower frequency of 1412 cm^{-1}, 1111 cm^{-1} and 625 cm^{-1} in BEO loaded microsponges. Thus, the encapsulation and stability of BEO in the prepared porous microstructures can be confirmed from FTIR findings.

3.3.2. *Differential scanning calorimetry*

As documented in literature reports, differential scanning calorimetry can be used for recognition of microsponges when guest molecules were entrapped into their porous cavities [38]. In this process, boiling, melting or sublimating points of guest molecules are generally shifted to different temperatures or get disappeared. The thermal curves of BEO and BEO loaded microsponges are shown in Fig. 2. In a graph plotted between heat flow vs temperature, a sharp exothermic peak of BEO in thermogram appeared around 400 °C which corresponds to oil evaporation process. This peak was found reduced with loss of sharpness in BEO loaded microsponge formulations, confirming the successful entrapment of BEO in microsponges. Further, according to obtained thermograms, it can be inferred that the thermal properties of BEO were not modified during formulation of microsponges. The obtained results were in accordance with literature reports [28,38]. Further, DSC results corroborate FTIR findings.

3.3.3. *Field-emission scanning electron microscopy*

For surface topography and morphological investigations, prepared blank and babchi oil loaded microsponges were subjected to FE-SEM analysis. The FE-SEM images are illustrated in Fig. 3. FE-SEM results revealed that obtained microsponges were highly porous and predominately spherical. As explained by Kumar and Ghosh, the pores were formed as a result of diffusion of DCM from the surface of microsponges [37,39]. Further, it was proposed that the unique internal structure comprised of spherical cavities enclosing babchi oil in EC was present in prepared microsponges. The internal structure is composed of numerous annulled spaces and the formed particles appear in such a way that they were perfect to term as microsponges, as observed from FE-SEM images. Jelvehgari et al., have also reported porous and spherical ethyl cellulose microsponges containing benzoyl peroxide [40].

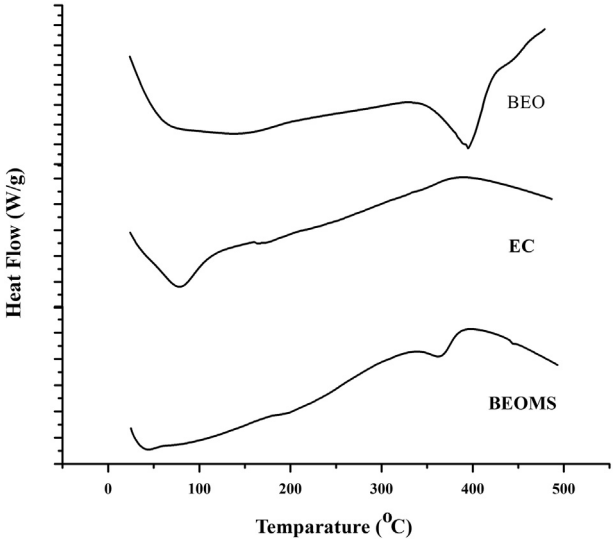

Fig. 2 – DSC thermograms of babchi essential oil, ethyl cellulose and babchi essential oil loaded microsponges.

3.3.4. *Particle size determination*

As per literature, the mean particle size of microsponges should lie in the range of 5–300 μm [25,41]. In present work, the particle size of the microsponges ranged from 20.44 ± 3.13 μm to 41.75 ± 3.65 μm (Table 1). Formulation F1 exhibited maximum particle size of 41.75 ± 3.65 μm, when both ethyl cellulose and PVA were at high levels and converse was also true, when both were at low levels (F9). At a given level of PVA, when amount of ethyl cellulose incorporated is increased, particle size was also found increased in the order F1>F2>F3. Same trend was also observed at other concentrations of PVA. The increase in particle size is attributable to viscous organic phase produced at higher concentrations of ethyl cellulose, which resulted in larger sized emulsion droplets and consequently, larger microporous colloidal particles. The influence of varying PVA at same level of EC was also observed. For this, particle size of microsponges from batches F3, F6 and F9 was measured, which was found 28.02 ± 3.29 μm, 27.72 ± 3.29 μm and 20.44 ± 3.13 μm (mean diameter), respectively. Similar trend was observed with other concentrations of PVA. This may be credited as the level of PVA increased, the emulsion droplets could not be easily divided into smaller droplets, and hence, leading to formation of larger microsponges [40]. Similar results were described to microsponges of curcumin, composed of PVA and ethyl cellulose [42].

3.4. *Encapsulation efficiency*

Formulation F2 showed maximum entrapment efficiency (87.70 ± 1.20%), which was followed by F5 (79.03 ± 0.71%). For a given concentration of PVA, when quantity of EC was varied, entrapment efficiency showed bell shaped pattern, high at mid-level (Table 1). On increasing the amount of polymer, the polymer available for each microsponge to encapsulate the oil was more. Increase in polymer concentration also increased the intramolecular forces and viscosity of internal phase [43,44]. As a result, bigger globules during emulsification were formed, resulting in bigger microsponges, and hence bigger particles can entrap more amount of essential oil. Pawar and his research group justified that with high polymer ratio, reduced diffusion of oil solution from concentrated EC solution into external phase occurs, which give more time for droplet formation leading to high entrapment efficiency [28]. On varying the concentration of EC, *in vitro* adsorption also varies and significant increase was observed due to resultant high polymer matrix wall thickness [35]. However, thereafter, adsorption of oil for entrapment in microsponges is hampered [42]. Batch F1, F4 and F7 formulated with same quantity of EC and varying PVA (100 mg, 75 mg and 50 mg) showed entrapment efficiencies of 77.00 ± 1.00%, 76.54 ± 1.54% and 57.26 ± 1.96%, respectively (Table 1), advocating adsorption as leading mode of babchi oil incorporation. Higher values of entrapment efficiency are also correlated to the porosity of microsponges [42]. According to Rizkalla et al., large numbers

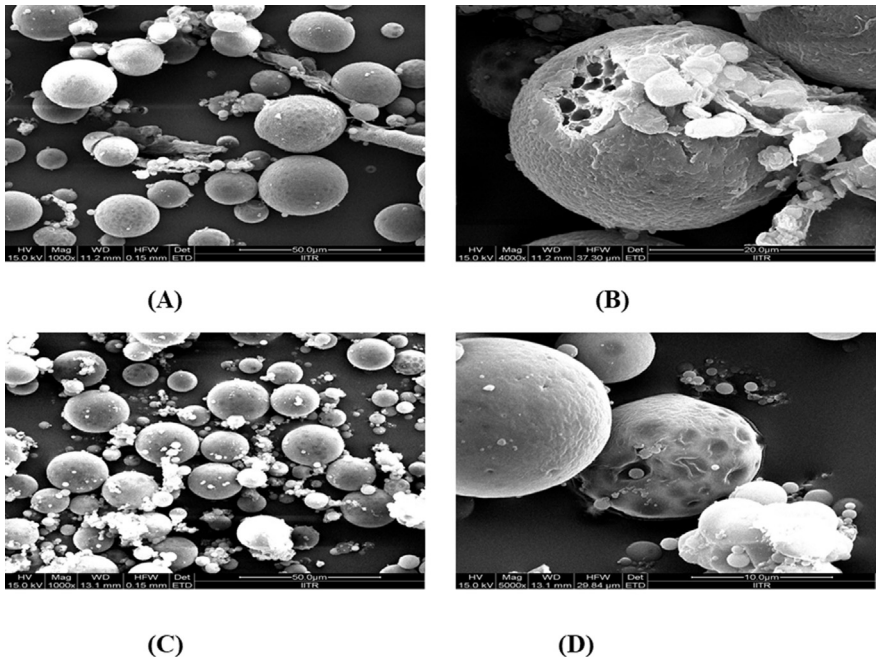

Fig. 3 – Field-emission scanning electron microscopy of blank microsponges (A, B) and babchi oil loaded microsponges (C, D).

of pores provided a large surface area for drug entrapment in microsponges [45]. Further, documented in literature, the pores present in micro colloidal system are homogeneously distributed throughout the whole carrier and are interconnected with each other, when amount of DCM kept constant. This close connection in pores favors essential oil loading in microsponges [25,46].

3.5. In vitro *drug release studies*

The *in vitro* release profiles of the formulated BEO microsponges in phosphate buffer (pH 5.4) are depicted in Fig. 4. Highest *in vitro* cumulative drug release (86.21 ± 1.45%) took place from F9, followed by F6 (80.33 ± 1.48%) at the end of 8 h (Table 1 and Fig. 4). Typically, for a given level of PVA, the formulations prepared with low level of EC showed high % CDR (cumulative drug release). Keeping the amount of PVA constant, an increase in the level of EC resulted in larger microporous particles and low % CDR (F1, F2 & F3). According to Chinna et al., on increasing the amount of ethyl cellulose, there occurs a decrease in the amount of drug close to microporous particle surface, with simultaneous increase in the amount of entrapped drug in the polymer matrix. This leads to retardation in the rate of drug release from the microsponges [47]. F3 designed with low amount of EC, produced small microsponges possessing higher surface area. This high specific surface area comes in contact with simulated fluid (phosphate buffer: pH 5.4) resulting in enhanced release rate [47]. Simulated fluid needs to traverse shorter path length owing to small size of microparticles resulting in improved release rate. Decrease in release rate was observed on increasing amount of PVA, if EC concentration was kept constant (F3, F6 and F9). Further, the extent of drug release decreases with increasing polymer as well as emulsifier amount [42]. The release of BEO from microsponges to the pores, facilitated the rapid penetration of the simulated fluid into the microsponges encouraging drug dissolution and diffusion from their polymeric matrix [48]. Hence, F9 formulation showed maximum release due to minimum EC concentration and low amount of PVA (5 mg). According to Abdelmalak and El-Menshawe, the higher amount of EC results in formation of thick layers which leads to controlled release of drug [49]. The present results correlated well with the earlier published literature in this field [42].

3.6. *Compatibility of the BEO microsponges with skin cells*

In spite of having good properties for dermal application, most of the essential oils have been known to produce skin irritation or toxicity. Hence, in order to explore the potential

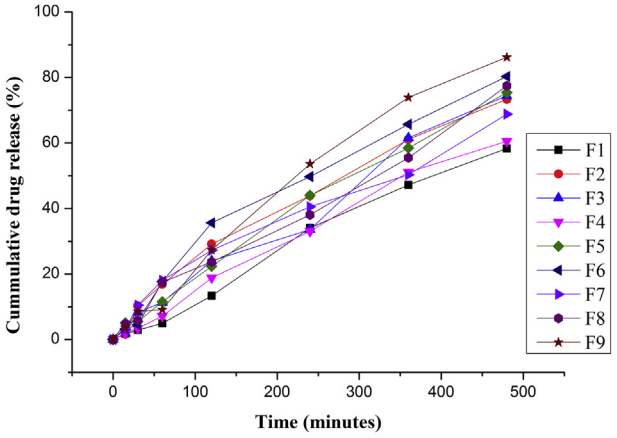

Fig. 4 – % Cumulative release of various batches of babchi essential oil loaded microsponges.

benefits of BEO loaded microsponges on human skin cells, the cytotoxicity of BEO and BEO loaded microsponges was carried out using MTT assay and HaCaT cell lines. The results of keratinocytes treated with their different concentrations (BEO and BEO loaded microsponges) are presented in Table 2. Both these samples induced dose dependent reductions in cellular viability. Further, the MTT assay exhibited that the treatment of these cells with BEO loaded microsponges at 320 μg/ml did not result in significant cytotoxic effect (Table 2) indicating HaCaT cells were much less affected at this concentration (IC50 value was not calculated as the % inhibition is less than 50%). Hence, the results of MTT assay indicated that the developed formulation can be safely applied on dermal cells in comparison to pure BEO (Table 2). Recently, Kumar and Ghosh carried out dermal toxicity studies for silver sulfadiazine microsponges using HaCaT and NIH-3T3 (mouse embryonic fibroblast) cell lines and reported its negligible cytotoxicity on dermal cells [37]. An another study conducted by Kumar and Ghosh, also illustrated similar results using metronidazole microsponges with HaCaT and NIH-3T3 cell lines [50].

3.7. In vitro *anti-microbial assay*

The BEO loaded microsponges represented good inhibitory action on growth of all the skin bacteria under test, and all the findings are summarized in Table 3 and Fig. 5. *S. aureus* was found most susceptible, among all the selected bacteria. The BEO treatment to *P. aeruginosa*, *S. aureus* and *E. coli* resulted in inhibition zones with diameter in excess of 11.67 ± 0.66, 12.67 ± 1.49 and 11.33 ± 0.98, respectively. The negative control (ethanol) did not exhibit an inhibitory effect on any of these bacteria. However, the positive control, streptomycin exhibited inhibition mode for all microorganisms with diameter 14.67 ± 1.22, 10 ± 1.35 and 16 ± 0.44, *P. aeruginosa*, *S. aureus* and *E. coli*, respectively. When the level of significance was checked, the BEO microsponges were found comparable to standard drug streptomycin. However, the prepared BEO microsponge formulations were observed remarkably significant when compared with pure essential oil. Dahiya and Purkayastha reported moderate to high susceptibility of BEO against multidrug resistant bacteria (clinical isolates) including *S. aureus*, *P. aeruginosa* and *E. coli* [51]. In the following year, Chopra et al. reported remarkable growth inhibitory action of ethanolic seed extract of *P. corylifolia* (*P. corylifolia*) against *S. aureus* and *Staphylococcus epidermis* due to the presence of bakuchiol (monoterpene phenol), an anti-bacterial agent [12]. In another study, Borate et al. evaluated anti-bacterial efficacy of *P. corylifolia* seed extract against variety of gram + ve and gram -ve skin pathogens including *P. aeruginosa*, *S. aureus* and *E. coli*. The methanol extract of *P. corylifolia* seeds exhibited zone of inhibition 20 mm, 18 mm and 16 mm for *E. coli*, *S. aureus* and *P. aeruginosa*, respectively. Efficacy of petroleum ether extract of *P. corylifolia* seeds was also checked and found satisfactory in this study [51].

3.8. *Photostability analysis*

Babchi essential oil gets absorbed in the UV region displaying a peak around 262 nm, whose intensity retarded upon UVA irradiation. The reduction in intensity indicated photolysis of essential oil. From the results of photodegradation studies, it was ascertained that BEO loaded microsponges were more photostable than pure oil under similar conditions as represented in Fig. 6. This might be due to encapsulation of BEO within microsponge system, which resulted in its enhanced photostability. Microsponges provided BEO with a physical barrier against UV- induced degradation. Moving to possible pharmaceutical applications of this investigation, it could be speculated that microsponges may protect the bioactives entrapped from degradation via UVA radiations.

3.9. *Stability studies*

BEO and BEO loaded microsponges were subjected for stability studies for a period of 3 months (with different time intervals). On physical observation of the stored samples, no change in color of microsponges was recorded and drug content did not change significantly ($p > 0.005$). Further, the FTIR spectra also revealed the stability of prepared micro formulations and no evidence of degradation of oil was observed, which proved microsponges more advantageous than pure babchi essential oil (Table 4).

Table 2 – *In vitro* cytotoxicity of babchi essential oil and babchi essential oil loaded microsponges.

Samples	Conc. (μg/ml)	% inhibition	IC50
Control	0.00	0.00	173.2 μg/ml
BEO	10	4.36	
	20	7.67	
	40	16.59	
	80	23.76	
	160	38.06	
	320	51.05	
BEO loaded microsponges	10	1.10	IC50 value was not calculated as the % inhibition is less than 50%
	20	2.74	
	40	5.92	
	80	10.03	
	160	20.96	
	320	26.34	

Table 3 – *In vitro* anti-microbial assay of babchi essential oil and babchi essential oil loaded microsponges.

Samples	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*
	Z.O.I (mm) ± SD	Z.O.I (mm) ± SD	Z.O.I (mm) ± SD
Streptomycin	14.67 ± 1.22*	16 ± 0.44*	10 ± 1.35
BEO	11.67 ± 0.66	11.33 ± 0.98	12.67 ± 1.49
BEO loaded microsponges	16.77 ± 1.14*	15.33 ± 0.75*	16.67 ± 0.82*

(*) Indicates a statistically significant difference compared to BEO ($p < 0.05$).

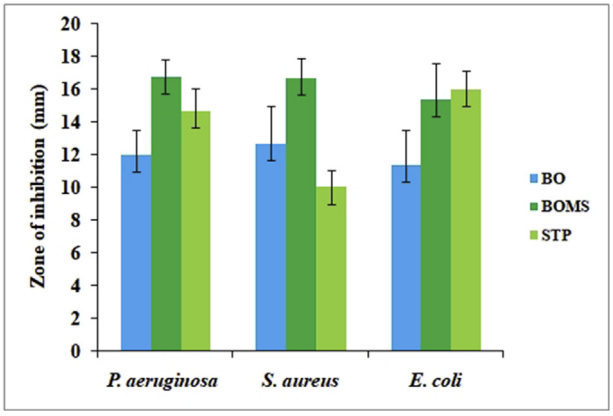

Fig. 5 – Comparable zone of inhibition for various samples (babchi essential oil and babchi essential oil loaded microsponges) against microorganisms.

4. Conclusion

Microsponges have not been explored for encapsulating essential oils till date, yet these may prove promising carriers for these bioactives. Hence, in the present study, BEO was successfully incorporated in EC microsponges using quasi emulsion solvent evaporation technique. The encapsulation of BEO resulted in good payload, retarded release, enhanced photostability and stability of this essential oil alongwith handling benefits. Surface investigation using field emission scanning electron microscopy revealed the spongy structure of microsponges with minute pores and controlled integrity.

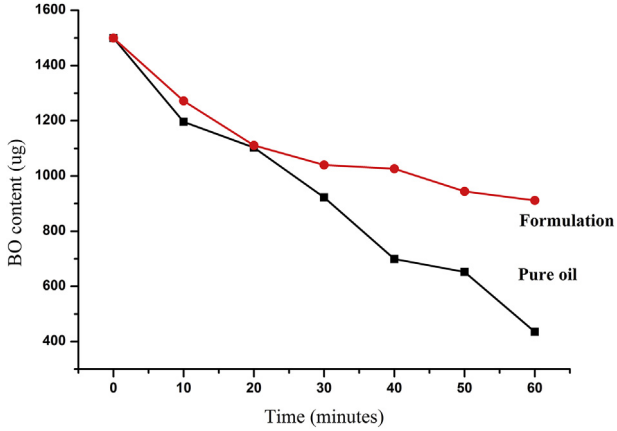

Fig. 6 – Photostability studies of babchi essential oil and babchi essential oil loaded microsponges.

Table 4 – Stability data of babchi essential oil loaded microsponges.

Sr. No.	Time (days)	Physical changes	% Drug content
1.	0	–	87.5 ± 1.20
2.	30	No change	87.1 ± 1.08
3.	60	No change	86.4 ± 1.12
4.	90	No change	85.9 ± 1.16

The selected BEO microformulation showed no cytotoxicity confirming their compatibility with skin cells. Good *in vitro* antimicrobial activity in skin pathogens like *S. aureus*, *P. aeruginosa* and *E. coli* was also observed. Hence, encapsulation of BEO in microsponges resulted in a stable efficacious delivery system. Further, in the light of dermatological potential of this oil and results of present investigation (*in vitro* release study at skin pH, antimicrobial activity on skin microbes and cytotoxicity studies on skin cell lines), this system can be optimized for skin problems in future, by loading prepared BEO microsponges in suitable topical carriers like cream, lotion or gel. This system can additionally help to overcome skin irritation problems by preventing direct contact of babchi oil and skin.

Conflicts of interest

All contributed authors declare no potential conflict of interest with respect to authorship, research and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jfda.2018.07.006.

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