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Fabrication and Characterisation of Honey Loaded Microsponges

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ABSTRACT

Ethylcellulose based microsponges loaded with honey were synthesized by Quasi-Emulsion solvent method. The honey loaded microsponges were characterised by Particle size distribution Analyser and High Resolution Scanning Electron Microscopy. The percentage drug content and Entrapment Efficiency of the loaded microsponges were determined. The antimicrobial and antioxidant activity of microsponges were evaluated.

Keywords: Microsponge, Honey, antimicrobial, antioxidant.

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1. INTRODUCTION

Benefits of honey are contributed by the composition of its elements such as glucose, fructose, glucose oxidase, vitamins and phenolic compounds. For health, honey can be used to treat wounds due to the antibacterial activity conferred by the hydrogen peroxide produced by glucose oxidase in honey. Anti-inflammatory, anti-oxidant, deodorizing and tissue regeneration activities in honey also help in the wound healing process[1]. Honey has a potential role in the field of tissue engineering and regeneration. Researchers have incorporated honey into tissue engineering templates, including electrospun meshes, cryogels, and hydrogels, with varying degrees of success[2]. Honey has been well proven antimicrobial and anti-bacterial since time immemorial[3,4].

Hence, the present study involves the synthesis of honey loaded microsponges by Quasi-Emulsion solvent method. The loaded microsponges were characterised by SEM, PSA and then evaluated for antimicrobial and antioxidant activity.

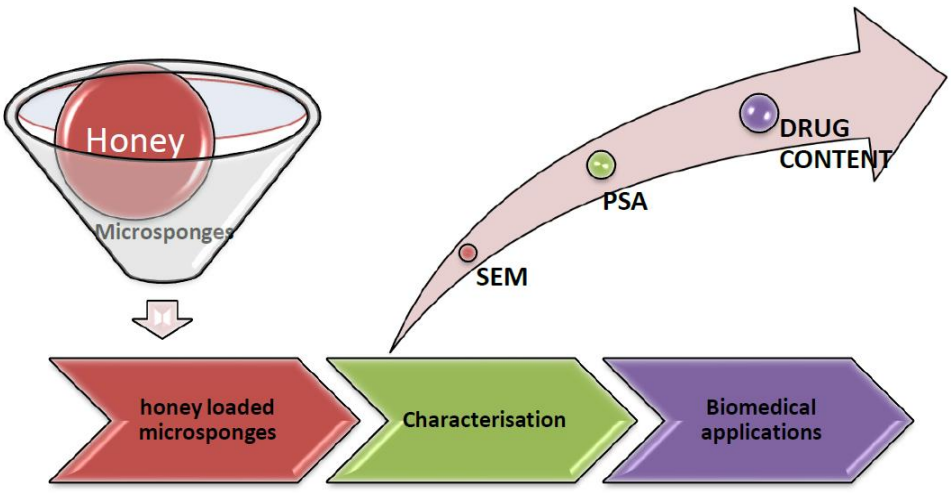

Figure 1: Scheme of the proposed work

2. MATERIALS AND METHODS

2.1 Materials

Ethyl Cellulose (EC), Polyvinylalcohol (PVA), Dichloromethane (DCM) of reagent grade were purchased and used without purification. Natural honey was selected for the study. Double Distilled water was used throughout the synthesis of microsponges.

2.2 Selection of Natural honey

Natural honey (H_A) was selected among four different types of honey by characterising them and checking its antimicrobial and antioxidant activity[5].

2.3 Synthesis of Honey loaded microsponges

Honey loaded Microsponges were formulated by Quasi-Emulsion Solvent Diffusion method. Five batches of microsponges (NS_0 – NS_4H_A) with varying proportions of Ethyl Cellulose (EC) and Polyvinyl alcohol (PVA) were taken. The Dispersed Phase consists of honey and required amount of EC dissolved in 20 mL of DCM. It was slowly added to PVA in 150 mL of aqueous continuous phase. Then the mixture was stirred at 1000 rpm under magnetic stirrer for 3 hours. The microsponges formed were filtered and dried in oven at 40 – 50 ºC for 24 hours. Then the dried microsponges were stored in vacuum dessicator to remove the residual solvent. The composition of the microsponge formulation was tabulated in Table 1. The prepared microsponges were characterised based upon the entrapment efficiency and particle size [2].

Table 1: Formulation of Microsponges with (H_A) Honey:

Sample Code	Honey (H_A) mL	PVA gm	EC gm	DCM mL	H_2O mL
NS0	-	2	2	20	150
NS_1H_A	1	2	2	20	150
NS_2H_A	1	2	3	20	150
NS_3H_A	1	3	2	20	150
NS_4H_A	1	3	3	20	150

2.4 Characterisation of Honey loaded microsponges

2.4.1 Microscopic studies

High Resolution Scanning Electron Microscopy (HRSEM) was used to study the morphology of the honey loaded microsponges and unloaded microsponges.

2.4.2 Particle size determination

The particle size of the honey loaded Microsponges was determined by using Laser Scattering Particle Size Distribution Analyser.

2.4.3 Percentage Yield

The percentage yield of honey loaded microsponges of various batches were calculated using the weight of final product after drying with respect to the initial total weight of drug and polymer used for the preparations[7].

2.4.4 Drug Content and Entrapment Efficiency

About 10 mg of microsponge from all batches were accurately weighed and dissolved in methanol in 50 mL standard flask and then made upto the volume of phosphate buffer pH 7.4. After appropriate dilution, the amount of drug was detected by a UV Spectrophotometric method at 210 nm using blank microsponges treated in the same manner[8]. The Entrapment Efficiency was calculated according to the following equation:

Entrapment Efficiency (%) = [Actual drug content in microparticles/Theoretical drug content]× 100

2.4.4.1 Preparation of Standard Calibration Curve

Preparation of Phosphate Buffer pH 7.4

Phosphate Buffer was prepared and pH was found to be 7.4 using digital pH meter[8].

Determination of λmax of honey(H_A)

The absorption maxima for honey(H_A) was found to be 210 nm[5].

Standard calibration curve of honey(H_A)

The absorbance of honey(H_A) standard solutions having a concentration range of 100-500 µg/mL in phosphate buffer pH 7.4 were plotted. The curve was found to be linear at λmax 210 nm. The calculation of the drug content and Entrapment efficiency were based on this calibration curve[8].

2.4.5 *In- vitro* antimicrobial study

Determination of Minimum inhibitory concentration (MIC) using Resazurin Microtitre Assay:

Preparation of resazurin solution

The resazurin solution was prepared by dissolving 270 mg in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Procedure

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 µL of sample was pipetted into the first well of the plate. To all other wells 50 µL of nutrient broth was added and serially diluted it. To each well 10 µL of resazurin indicator solution was added. 10 µL of bacterial suspension was added to each well. Similarly, the same set up was performed for antifungal activity in which 50 µL of potato dextrose broth was added and 10 µL of fungal suspension was added on each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 hrs. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value[9,5].

2.4.6 Antioxidant study

Determination of scavenging activity by DPPH assay

The percentage of antioxidant activity (AA %) of each substance was assessed by DPPH free radical scavenging assay. Different concentrations of sample were added to all the tubes except blank. Then 3 mL of ethanol and 0.3 mL of 0.5 mM DPPH radical solution in ethanol was added. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). Absorbance was read at 517 nm after 30 min of reaction[9,5]. The scavenging activity percentage (AA %) was calculated using the below formula

% Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

3. RESULTS & DISCUSSION

3.1 Microscopic studies

From SEM studies, it was found that the samples had porous and almost spherical sponge in nature. The pores were induced by the diffusion of the solvent[8]. The unloaded Microsponges (NS_0) shows shiny smooth surface morphology (Fig 2). The Honey loaded microsponge (NS_4H_A) shows porous smooth surface and spherical (Fig 3). SEM results revealed that surface morphology has been shown to be beneficial for topical usage under biomedical applications.

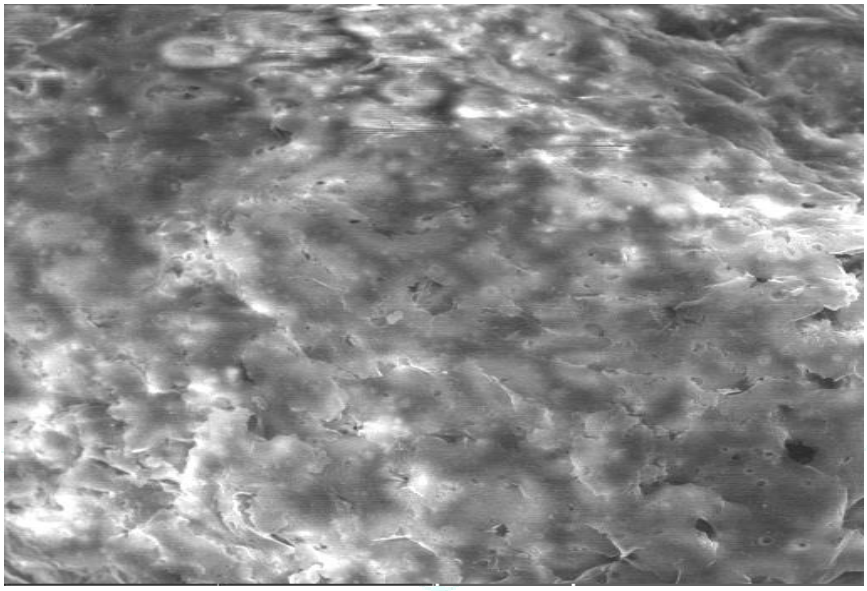

Fig :2 SEM photomicrograph image of unloaded microsponge NS_0

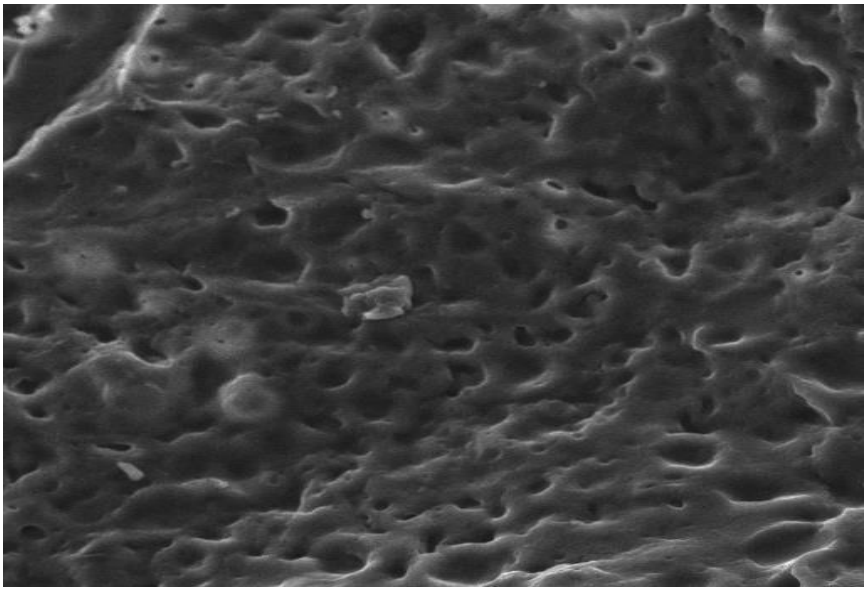

Fig :3 SEM photomicrograph image of loaded Microsponge NS_4H_A

3.2 Particle size:

The Particle size analysis of honey loaded and unloaded microsponges (Fig 4) revealed that the particle size was in the range of 59 – 152 µm.

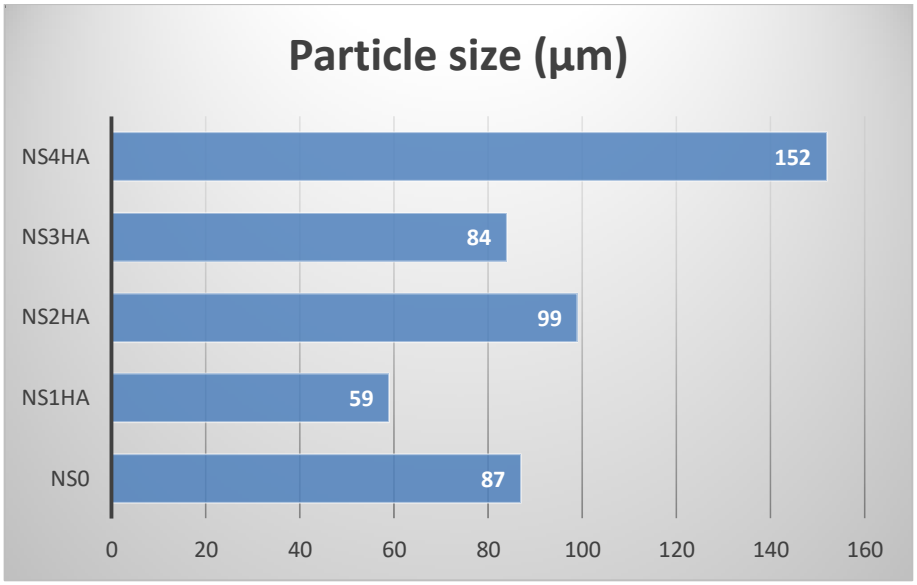

Fig :4 Particle size of Honey loaded and unloaded Microsponges

3.3 Production yield:

The production yield of all batches of microsponges were calculated and shown in the Fig 5.

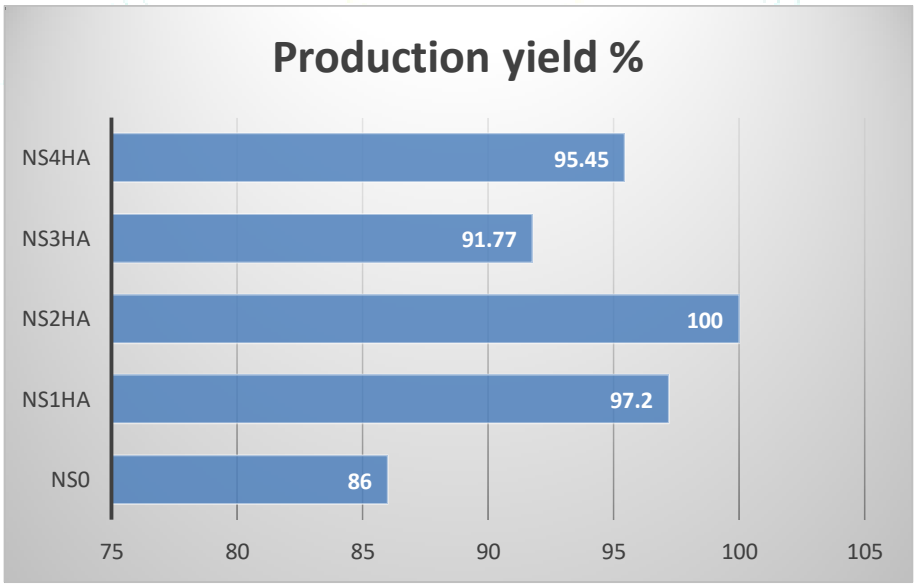

Fig :5 Production yield of Honey loaded and unloaded Microsponges

3.4 Drug Content and Entrapment Efficiency:

The Drug content and Entrapment Efficiency were calculated and displayed in the table 2.

Table 2. Drug Content and Entrapment Efficiency % of Honey loaded microsponge formulations

Sample Code	Absorbance	Concentration	Drug Content %	Theoretical Drug Content % in 10 mg	Entrapment Efficiency %
NS_1H_A	0.115	211	2.11	3.3333	63
NS_2H_A	0.121	206	2.06	2.5	82.4
NS_3H_A	0.105	219	2.19	3.3333	65.70
NS_4H_A	0.112	213	2.13	2.5	**85.2**

3.4.1 Standard Calibration Curve:

The standard calibration curve for honey in phosphate buffer pH 7.4 at 210 nm was shown in Fig 6.

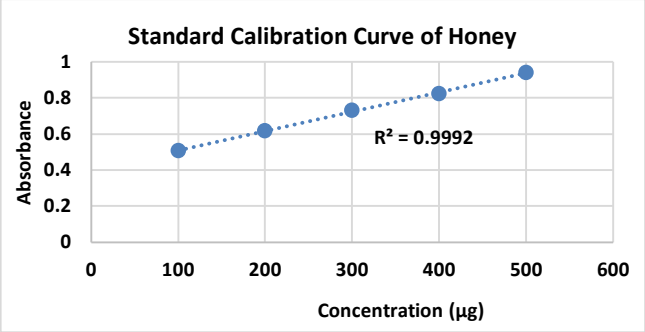

Fig :6 Standard calibration curve of Honey (H_A)

3.5 Antimicrobial activity by Resazurin microtitre assay

The synthesised honey loaded microsponge (NS_4H_A) was selected among other formulations due to its better Entrapment Efficiency. The Antimicrobial activity was evaluated by Resazurin microtitre assay(Table 3). It shows good antibacterial activity towards *E. coli* and *B. subtilis* whose MIC values are 500 µg and 125 µg respectively. Similarly, it shows good antifungal activity towards *C. albicans* whose MIC value is 125 µg. From the results, it shows more active towards *B. subtilis* and *C. albicans.* The MIC values of honey loaded microsponge NS_4H_A differs from that of the value of H_A[5].

Standard used for antibacterial: Streptomycin

Standard used for antifungal: Amphotericin B

Table 3. Antimicrobial activity of Honey loaded Microsponge

S.No	Microorganisms/sample	Growth of inhibition										
		1000 µg	500 µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	STD 10µg	Sterile water	Culture
	NS4HA											
1	*Escherichia coli*	-	-	+	+	+	+	+	+	-	+	+
2	*Bacillus subtilis*	-	-	-	-	+	+	+	+	-	+	+
3	*Candida albicans*	-	-	-	-	+	+	+	+	-	+	+

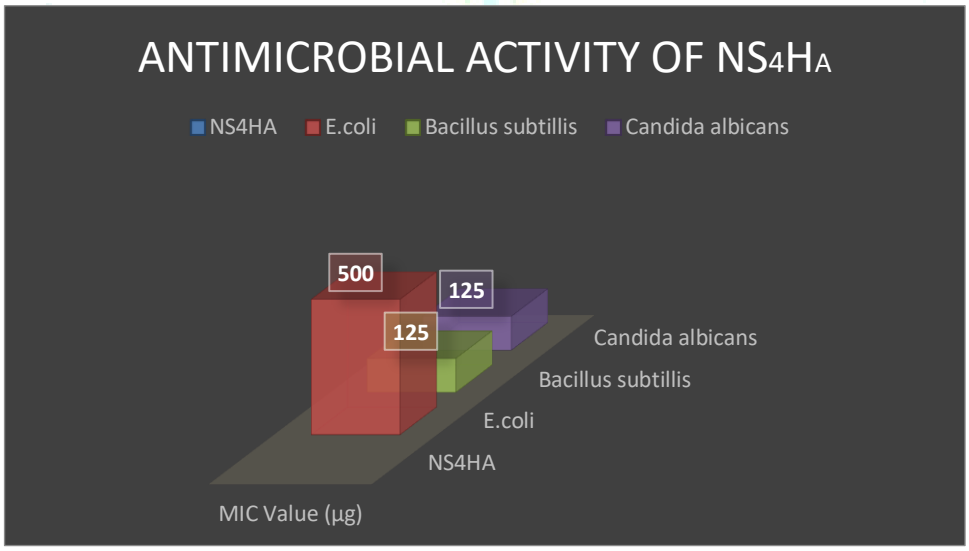

Fig :7 Antimicrobial activity representing MIC value of NS_4H_A

3.6 Antioxidant activity by DPPH assay

The honey loaded microsponge formulation NS_4H_A has an antioxidant potential of 34.0%(Fig 9). The percentage scavenging activity of honey loaded microsponge is slightly lower than the value of standard BHT (Fig 8). The H_A showed 77.1% of scavenging activity [5]. From the results, it shows that the antioxidant activity decreases in the honey loaded microsponge formulation (NS_4H_A).

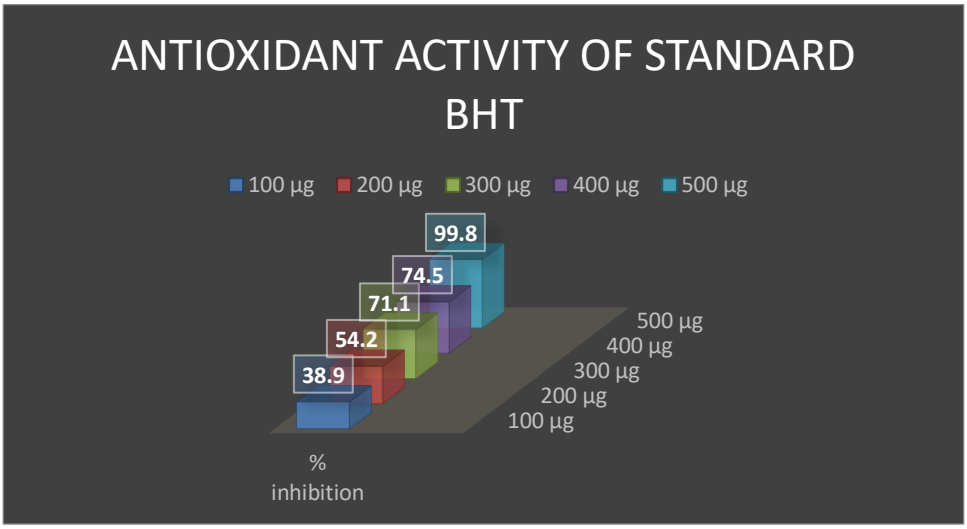

Fig :8 Antioxidant activity of standard BHT

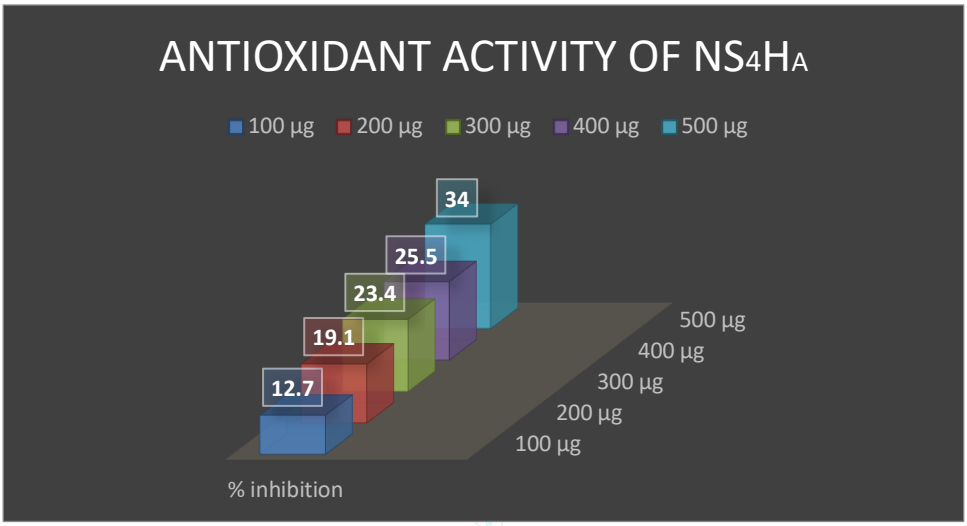

Fig :9 Antioxidant activity of honey loaded microsponge

4 CONCLUSION

In the present study, efforts were made to synthesis and evaluate the honey loaded microsponges. Of all the formulations, equal combination of EC and PVA (3:3) showed better results in terms of Entrapment Efficiency. This optimised formulation (NS_4H_A) showed good antimicrobial and antioxidant activity. Hence it can be concluded that the honey loaded microsponges would lead to a better choice for biomedical applications especially in wound healing and tissue regeneration. Further studies could utilise the honey incorporated microsponges in the field of cosmetics and dermatology.

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