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Antimicrobial and In -Vitro Drug Release Studies of Microencapsulated Terminalia chebula extract finished Fabric

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Abstract: In this research, an attempt has been made to study the *in-vitro* drug releasing behaviour of *Terminalia chebula* loaded gelatin microcapsules. The microcapsules were prepared by spray drying technique and analysed with different core to wall ratio (50:50, 70:30 and 80:20). Scanning electron microscope and Optical microscope photographs of Samples revealed that all prepared microcapsules were almost spherical in shape and have a slightly smooth surface. The *in vitro* release profile of *Terminalia chebula* indicates that all the ratios of microcapsules showed controlled and prolonged drug release over an extended period of 7 hours. The release kinetics study reveals that the drug follows zero order kinetics and the mechanism of drug release was diffusion controlled type. From the *in vitro* drug release profiles, it was also observed that the drug release from microcapsules was decreased with an increase in coating material in the microcapsules.

The prepared microcapsules were finished on knitted cotton textile by simple pad-dry-cure method and charecterised with the help of Scanning electron microscope for wound dressing application. The finished textile were analysed for their drug releasing behavior in terms of antimicrobial property (SN 195920: 1992). The treated fabric shows promising bacterial protection up to 22-24 mm of zone of inhibition. Further the treated materials were evaluated for their comfort properties like water absorbency, water vapour permeability, air permeability and wicking, which is a requisite for a textile wound dressing.

Keywords: Terminalia chebula, microcapsulation, In-vitro analysis, antimicrobial study, wound dressing.

1.Introduction

Natural polymers provide great advantages in biomedical applications such as drug delivery and tissue engineering. They are biocompatible, non toxic and biodegradable materials. Also, they have good potential to incorporate drugs, enzymes, bioactive components etc.[1, 2]. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix, to form small capsules[3, 4] and build a barrier between the component in the particle and the environment. The core may be composed of just one or several ingredients and the wall may be single or double-layered. Taking into account food industry characteristics, microencapsulation should be defined as a technique by which liquid droplets, solid particle, or gas compounds are entrapped into thin films of a food grade microencapsulating agent[4].

Encapsulation techniques have been used extensively to entrap drugs and bioactive compounds and control their release into the gastrointestinal tract. Several techniques were developed to produce encapsulated microspheres. Drug delivery system developed with microsphere might increases the life span of the active ingredients encapsulated inside and control the release. Because of its small size, that have large surface to volume ratio, which is very much suitable for controlled delivery. Microencapsulation offers many advantages that include increased stability, prolonged *in vivo* half-life, reduction of possible adverse side effects, concentration of the

drug resulting in lower required doses, and ease of administration. Many techniques are available for micro encapsulation such as spray drying, spray cooling extrusion, freeze-drying, co-crystallization etc[5].
Gelatin is a protein substance derived from collagen, a natural protein present in the tendons, ligaments, and tissues of mammals. Gelatin's ability to form strong, transparent gels and flexible films that are easily digested, soluble in hot water, and capable of forming a positive binding action have made it a valuable commodity in food processing, pharmaceuticals. Gelatin is one of the most promising biomaterials due to its excellent biocompatibility and biodegradability. Hence the application of gelatin in wound dressing era increased. There are several researchers studied the gelatin directly as wound healing material or as a wall material in microencapsulation to encapsulate the particular drug.[6-11] Based on the biocompatible property in this research gelatin is selected as a wall material.

Terminalia chebula is an important medicinal plant in Indian traditional medicine and it is most frequently used herb in Ayurveda. *Terminalia chebula* is called the 'King of Medicine' in Tibet and is always listed at the top of the list in Ayurvedic Materia Medica due to its extraordinary power of healing. The dried ripe fruits of Chebulic myrobalan has traditionally been used in the treatment of asthma, sore throat, vomiting, hiccough, diarrhoea, bleeding piles,gout, heart and bladder diseases[12] . *Terminalia chebula* is a medium- to large-sized tree distributed throughout tropical and sub-tropical Asia, including China and Tibet. This tree is found in the forests of northern India, Uttar Pradesh and Bengal, and is common in Tamil Nadu, Karnataka and southern Maharastra. The traditional Indian systems of Ayurveda and Siddha medicines support the importance of medicinal plants to treat diseases[13]. The demand on plant-based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non -narcotic, easily biodegradable, pose minimum environmental hazards, have no adverse side-effects and are easily available at affordable prices.

Terminalia chebula is routinely used as traditional medicine by tribals of Tamil Nadu in india to cure several ailments such as fever, cough, diarrhea, gastroenteritis, skin diseases, candidiasis, urinary tract infection and wound infections[14]. Antibacterial activity of T. chebula extracts against several bacterial strains have been reported[15-18]. In this study an attempt has been made to firstly to charecterise and study the drug releasing properties of the *Terminalia chebula* loaded gelatin microcapsules through *in-vitro* analysis. The drug releasing profiles were also analysed for microcapsules with different core to wall ratio. In the second phase of the research the microencapsulated drugs were finished on knitted cotton fabric for wound dressing application. The fabric was characterized with SEM, antimicrobial studies and other essential comfort properties, which aids the wound healing property of the wound dressing.

2. Materials and methods

2.1 Raw material

The Gelatin in the powder form is obtained from Sigma-Aldrich, USA. The purchased gelatin were obtained from porcine skin. (Type A, Bio reagent, powder, suitable for cell culture). The Sodium sulphate was purchased from Merc chemicals, Mumbai, India. All the chemicals used were of analytical grade without any purification.

100% pure cotton, plain knitted fabric with 67 course per inch and 34 wales per inch were purchased in bleached state from the commercial outlets of Coimbatore, Tamilnadu, India. The fabrics were washed thoroughly until the temporary finishes wash off before the application.

2.2 Extraction of herbal material

Terminalia chebula fruits, chosen for this study were purchased from the commercial outlets of the Coimbatore District, Tamilnadu, India. The collected quantities of *Terminalia chebula* fruits were shade dried and powdered. The methanol and water extract of the powders were obtained. 10 gm of powder is soaked in water and methanol separately for 24 hours to obtain 10% concentrated solution, resulting in active substances being dissolved in methanol. The extracts were filtered and used for antimicrobial finishing.

2.3 Microcapsule preparation

2.3.1 The Microencapsulation process

The first stage to achieve microencapsulation was the formation of a fine and stable emulsion of the core material (herbal extract) in the wall solution (Gelatin). In practice, the process involves the preparation of gelatin-herbal extract, the different core to wall ratio (50:50, 70:30 and 80:20) is stirred using magnetic stirrer and the liquid active (sodium sulphate) ingredient is added. The stirrer speed is carefully controlled to obtain the correct droplet size for optimal diffusion and the vessel is heated to around 40˚C. It was agitated with a mechanical stirrer at 450 rpm. This agitation gently melts the gelatin membrane without destroying its structure, enabling preparation of the active ingredient at a practical rate.

2.3.2 Spray drying method

The emulsions prepared were spray dried and equipped with 0.5 mm diameter nozzle. The pressure of compressed air for the flow of the spray was adjusted to 5 bars. The inlet and outlet air temperatures were maintained at 165 ±5˚C and 80±58˚C, respectively and feed rate was adjusted to 360–540 mL/h. Emulsions were prepared at the moment of the spray drying process and the emulsions were kept under magnetic stirring during the whole process. The microcapsules were collected from the collecting chamber and transfered to double layer plastic bags and stored in darkness until analysis.

Figure 1 Spray dryer for the microencapsulation process

3. Characterization

3.1 Microscopical analysis of Microcapsules Shape

The microcapsule powder was weighted and examined with the help of a digital microscope (AnMo Electronics Corp.) to confirm the successful preparation of microcapsules. The shape of the individual spheres at the two possible magnifications, 5 x and 200 x was examined. Furthermore, the capsules of each size fraction were inspected by digital microscopy. Particle size analysis was carried out using bright feild microscopy[19]. About 100 microspheres were selected randomly and their size was determined using optical microscope fitted with a standard micrometer scale.

3.2 Scanning electron microscopy of microcapsule

3.2.1 Powder

The morphology was evaluated with a scanning electron microscope[20]. The scanning electron microscopy (SEM) technique was used to assess the morphology of microcapsules. Selected samples were analyzed by SEM for their surface morphology. The spray dried samples were sonicated for 15 min to re disperse the particles in aqueous media. Samples were then pipetted onto a Nucleapore filter membrane and vacuum deposited. The membrane was mounted on a 1/2 in. SEM stubs with double sticky carbon tape and secured with Ag paint around the perimeter of the membrane to the stub. The samples were sputter-coated and the SEM picture was taken on a scanning electron microscope.

3.2.2 Finished textile

The micro-encapsulated fabrics were given for testing the micro capsules deposits on the fabric surface using scanning electron microscope (SEM). The capsules were successfully formed on the surface of the fabric. Capsule size is indicated on the picture. This picture shows the micro capsules formed at magnification of 500X.The width of two yarns in a fabric is 50 micro meter. It clearly shows the capsules formed on and between the consecutive yarns

3.3 In vitro evaluation

To evaluate controlled release of drug from gelatin coated microencapsules, the different ratios of herbal extract loaded (50:50, 70:30 and 80:20) gelatin microcapsules were used. The *in vitro* release profile of *Terminalia chebula* from gelatin microcapsule was examined in phosphate buffer pH 7.4 using the colorimeter. Accurately weighed samples of microcapsules were added to dissolution medium kept at 37±0.5. At pre-set time intervals the sampes were withdrawn and replaced by an equal volume of dissolution medium to maintain constant volume. After suitable dilution the samples were analyzed spectrophotometrically at 274 nm.[21,22]

3.3.1 Drug release kinetics studies

In order to understand the kinetics and mechanism of drug release, the results of the *in vitro* drug release study were fitted with various kinetic equations[23,24] like zero order (cumulative percent drug released vs time), first order (log cumulative percent drug retained vs time), Higuchi (cumulative percent released vs √time), Peppas (log of cumulative percent drug released vs log time) and Hixson- Crowell's cube root model ((percentage retained)1/3 vs time). The kinetic model that best fits the dissolution data was evaluated by comparing the regression coefficient (r) values obtained in various models. Peppas model used 'n' (release exponent) value to characterize different release mechanisms[25-28].

3.4 Antimicrobial Test - (SN 195920: 1992)

The treated and untreated fabric samples were placed in the AATCC bacteriostasis agar, which has been previously inoculated (Mat culture) with test organisms (*Staphylococcus aureus (MTCC 737), Escherichia coli (MTCC 1687)* obtained from Microbial Type Culture Collection (MTCC) from IMTECH, Chandigarh*).* After incubation, a clear area of uninterrupted growth underneath and alongside of the test material indicates the antibacterial effectiveness of the fabric. The area of the inhibition is a qualitative measure of antibacterial activity[29-30].

3.5 Physical Property Assesment

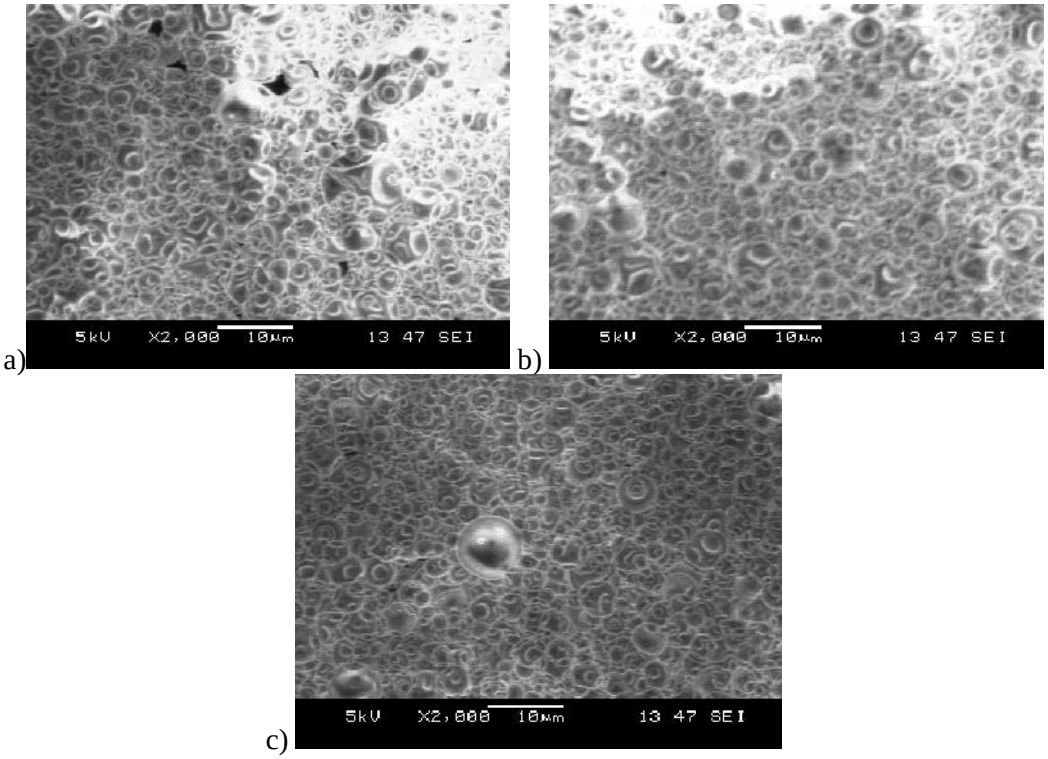
One of the basic requirements of wound dressing is their physical properties. The material should be able to allow water vapour and air to keep the moist environment but at the same time it should avoid the penetration of bacteria. Hence, the microencapsulated *Terminalia chebula* treated cotton fabric samples were tested to assess the following physical properties as per the given standard methods. Ten samples were tested for each test 5 times and the average value is given at the discussion.

- Water vapor permeability testing – BS 7209
- Wicking - AATCC TM 197-2011
- Air permeability - ASTM D 737-99
- Water absorbency - AATCC 39-1980.

4. Results And Discussion

4.1 Charecterisation Of Microcapsule

4.1.1 SEM analysis:

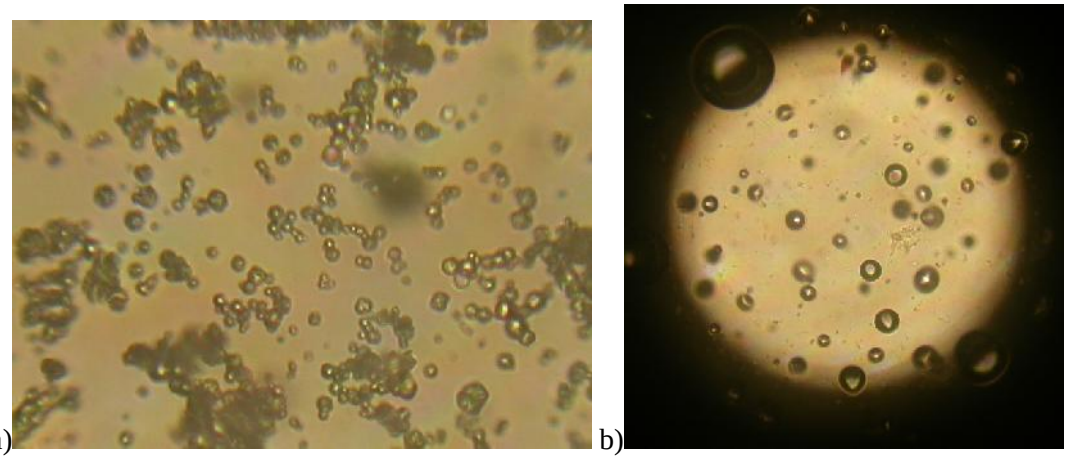


a) b) c)

Figure 2. SEM image for gelatin microcapsule of 50:50 , b) 70:30, c) 80:20 core to wall ratio.

The SEM image of microencapsulated herbal is shown in Figure 2. The microcapsules showed good spherical geometry as evidenced by the photographs of SEM analysis. From the Figure 2 it can be understood that the formed microcapsules were of uniform in their size and the average size is around 5 – 10 micrometer.

4.1.2 Microscopical Analysis:



a) b)

Figure 3. Microscopic view of gelatin microcapsule a) In normal state, b) in swelled state

Figure 3 represents the microscopical view of the prepared gelatin microcapsules. The microscopical analysis reveals that the formed capsules were of spherical shape with smooth outer surface. The capsules were distributed in evenly in size aspects. The sizes of the capsules were also measured with the help of microscope.

4.2 In vitro evaluation:

The *in-vitro* release studies were performed using PH 7.4 phosphate buffer, because the gelatin polymers are independent of the pH of the dissolution medium. The results represent that the release behavior of microcapsules. The encapsulated material releases the drug cintent by steady-state diffusion through a wall material. The rate of release remains constant as long as the internal and external concentrations of core material and the concentration gradient through the membrane are constant. The results show that the microcapsule with core to wall ratio 80:20 shows high amount of drug release than the other formulation. However the overall release time remains 390 to 400 min for all the formulation.

4.2.1 Effect of Wall concentration on the drug release:

The Figure 4 shows the core: wall ratio of microcapsule and their influences on the behavior of drug release. In 80:20 core: wall ratio, the amount of drug released was high when compared to the other 2 ratios and the duration of release is less. The results showed that the release from the microcapsules of 50:50 core:wall composition was significantly longer sustaining than the other formulations. This presumably was due to the higher concentration of the polymer. The higher concentration of the polymer resulted the extended release of drug. These results emphasized the fact that as the polymer thickness increases and the drug loading decreases, the release of the drug decreases from the microcapsules. In the case of core to wall ratio of 80:20, higher amount of drug release was observed then other formulation with respect to time. This is because the thicker the gelatin concentration, the thicker the microcapsules' wall membrane, and smaller the pore space between gelatin molecules, therefore causes difficulties for the *Terminalia chebula* extract to release from microcapsules. Thus we could have better control over the sustained release effect by changing microcapsule wall membrane thickness. Hence, it can be stated that less concentration of polymer can provide higher drug release.

4.2.2 Effect of capsule size

The sizes of different formulation of microcapsules were analysed with the help of microscope. The average particle diameter of 50:50 core to wall ratio is 3.9 µm. In the case of 70:30 and 80:20 the diameter is 3.5 µm and 2.5 µm respectively. Small particle size can increase the release rate of the herbal extract. This outcome is consistent with the research of the relation between particle size and sustained release rate by Yamamoto et al.[31]. The reason should be that smaller particle size microcapsules would have larger total specific surface area, therefore causes its release rate to be faster than that of larger particle size microcapsules. The release rate of the capsule with core to wall ratio is high mainly because of the particle size, This can be understood from the Figure 4. The other factor which influenced by the microcapsule size and core/wall ratio is the drug entrapment efficiency[32]. The uneven or non-uniform distribution of core and wall material in microcapsules decides the capsulation efficiency. The extent of drug loading influenced the particle size distribution of microcapsules.

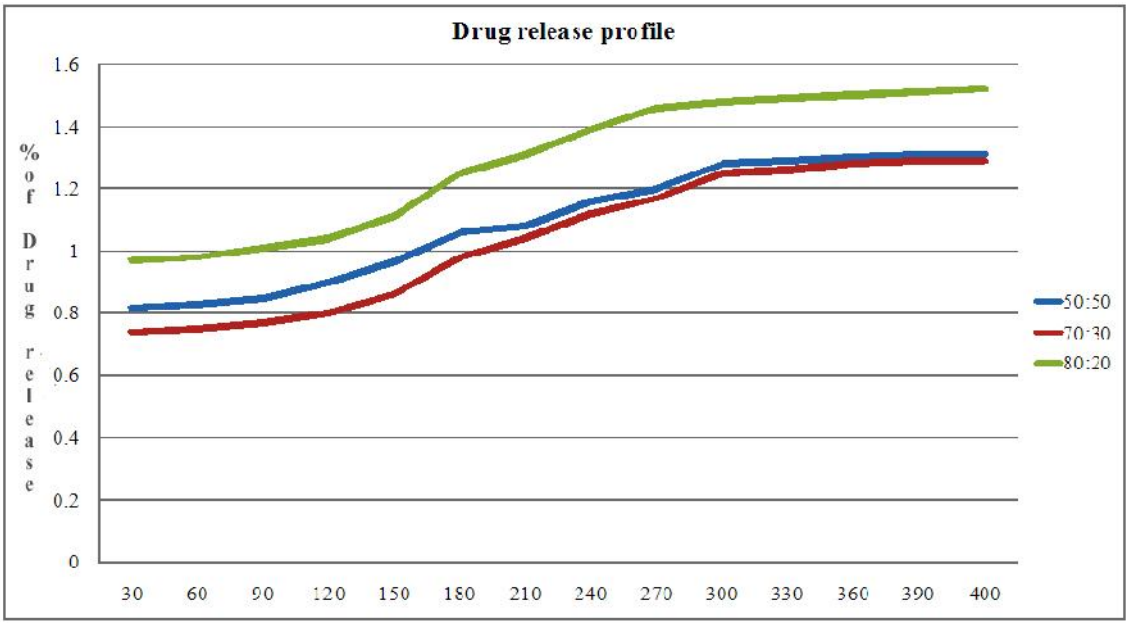


Figure 4. Invitro drug release profile for different core to wall ratios

4.2.3 Drug releasing mechanism

Release data of all the formulations were fitted into four different mathematical models namely zero order, first order, Higuchi model, Hixson Crowell and Korsmeyer-Peppas (power law) model to characterize the mechanism of drug release. The correlation coefficient (R2) from regressed plots of different kinetic models such as zero order, first order, Higuchi and the release exponent value (n) for all the formulations were noted. Considering the correlation coefficient (R2) values (0.985, 0.950), the drug release from most of the formulated microcapsule were found to follow zero order model rather than other models except 80:20 formulation. The 80:20 formulation has the highest r^2 value (0.934) for Higuchi model. The value of release exponent (n) obtained from Korsmeyer-Peppas model gives an indication of the drug release mechanism. The release exponent "n" value for the different formulation ranged from 0.01-0.05.

The release exponent "n" between 0.5 and 1.0 indicates the anomalous transport kinetics or non-fickian diffusion that means the drug was released by the combined mechanism of pure diffusion controlled and swelling controlled drug release. "n" value greater than 1 was to follow supercase II transport. The "n" value less than 0.5 were beyond the limits of Korsmeyer-Peppas model. Release kinetics plays a pivotal role in formulation development because if the kinetics of drug release is known, one can also advance for the establishment of *in vitro-in vivo* (IVIVC) correlation.[33]

Table 1 In vitro evaluation results

Time	50:50	70:30	80:20
30	0.82	0.74	0.97
60	0.83	0.75	0.98
90	0.85	0.77	1.01
120	0.90	0.80	1.04
150	0.97	0.86	1.11
180	1.06	0.98	1.25
210	1.08	1.04	1.31
240	1.15	1.12	1.39
270	1.22	1.17	1.46
300	1.27	1.25	1.48
330	1.32	1.26	1.49
360	1.36	1.28	1.50
390	1.38	1.29	1.51
420	1.38	1.29	1.52

Table 2. Correlation coefficient value (r^2) of different release kinetic model

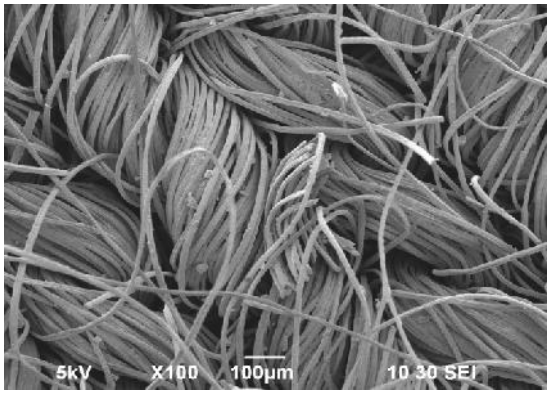
Model	Correlation coefficient (r^2)values		
	50:50	**70:30**	**80:20**
Zero order	0.985	0.950	0.925
First order	0.980	0.948	0.539
Higuchi model	0.957	0.939	0.934
Korsmeyer-Peppas model	0.891	0.876	0.883
Hixson Crowell	0.889	0.930	0.291

4.3 Charecterisation Of Treated Fabric

4.3.1 SEM analysis

The micro-encapsulated herbal treated fabrics were analysed for the presence of micro capsules on the fabric surface using scanning electron microscope (SEM). The pictures of the capsules viewed at different magnifications were analyzed. The capsules were successfully formed on the surface of the fabric. Capsule size is indicated on the picture. The magnification at 500X shows the clear indication of microcapsule deposition. It is also evident that the formed capsules were of micro size. However the treatment of the fabric with

microcapsules carried out in simple pad-dry-cure method, the uniformity level and the distribution of the microcapsule throughout the processing area need to be studied separately.

a) 

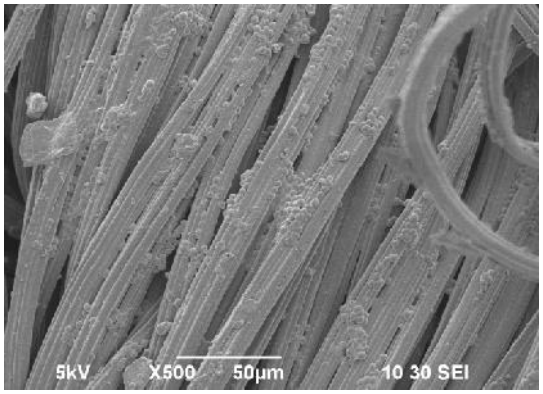
b) 

Figure 5. SEM photographs of Microencpsule treated textile material at a) 100x and b) 500x magnification

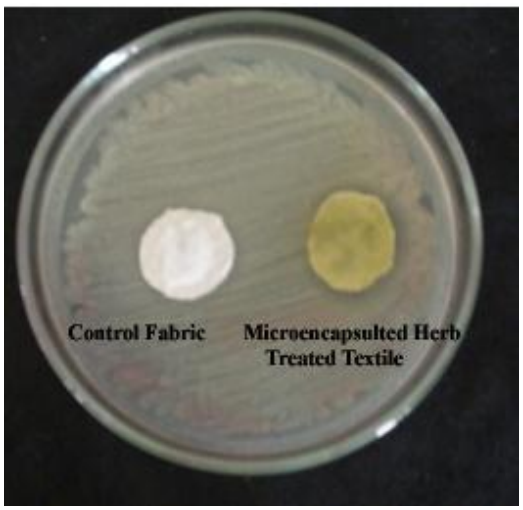
4.3.2 Antimicrobial test (Agar diffusion test):

Table 3 and Figure 6 show the antimicrobial efficacy of treated fabrics. The results indicate the presence of clear zone of inhibition of 22-24 mm diameter for methanol extract treated fabric against all the five selected microorganism namely *Staphylococcus aureus* and, *Escherichia coli*. The untreated fabric (control) shows bacterial growth under the test specimen. In Figure 1 the brown colour indicates the diffusion of antimicrobial agent into the agar. The zone of inhibition becomes apparent and its size provides some indication of the potency of the antimicrobial activity or the release rate of the active agent.[34]

Table 3. Zone of inhibition of treated textile material for different strains

S.No.	Bacterial strains	Zone of inhibition (in mm)	
		Control	*Terminalia chebula* fruit Methanol extract
1	*Staphylococcus aureus*	0	24
2	*Escherichia coli*	0	22

The agar diffusion test result revealed that the selected herbal material has the potential antimicrobial activity against both the gram positive and gram negative organism. This release rate of the drug from the microcapsule will be improved while in the contact with liquid medium.

a) 

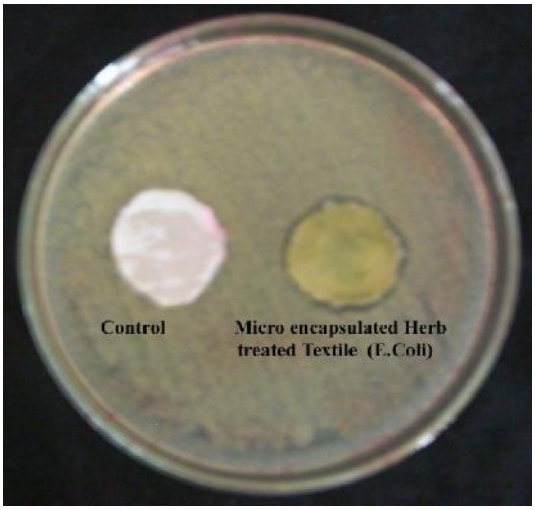
b) 

Figure 6. Agar diffusion test results for microencapsulated herb coated textile against a) *Staphylococcus aureus* and b) *Escherichia coli.*

4.3.3 Water absorption test

Table 4. water absorption rate of finished fabric (in seconds)

Sample	control	50:50	70:30	80:20
1.	0.9	1.6	1.7	1.8
2.	1.0	1.6	1.6	1.7
3.	1.0	1.5	1.8	1.7
4.	1.1	1.6	1.6	1.9
5.	1.0	1.7	1.7	1.8
Avg	**1.0**	**1.6**	**1.68**	**1.78**

The water absorbency test reveals that, the treatment of microcapsules on the fabric surface did not resist the water absorbency. Hence the capsules were in micro size, the absorbance resistance was very minimal. The average absorbency of the control sample was noted as 1 milli second. But for various ratio of microencapsulated herbal treated fabric shows 1.6, 1.68 and 1.78 sec respectively for 50:50, 70:30 and 80:20 core to wall ratio.

4.3.4 Air permeability test:

Table 5. Air permeabilityvalues of the finished fabric

Samples	Air permeability in cm^3cm^2/sec
Control	36.632
50:50	35.585
70:30	31.935
80:20	28.306

One of the comfort measures that greatly affect the wearer is air permeability. A material that is permeable to air is likely to be permeable to water, but very often may result in physical or psychological discomfort in the wearer. The result reveals that, the air permeability value of the microencapsulated herb coated textile material reduced when compared to the control material. The reduction in the air permeability may be due to the coating on the surface of the textile. This coating on the surface might have hindered the space between the yarn and the fiber. Hence the free movement of the air obstructed. However the reductions in the air permeability values are negligible.

4.3.5 Wicking test:

Table 6. Wicking ability of the finished fabric

Sample	Wickability in 1min	Wickability in 5min
Control	1.5cm	5cm
50:50	0.5cm	4.5cm
70:30	1cm	3.5cm
80:20	0.85cm	3cm

Wickability is also an important factor in determining the comfort of clothing for active wear. High wickability facilitates quick drying and fast cooling in hot environments. The wicking property of the microencapsulated herbal treated textiles was analyzed. The wicking of the 50:50 finished samples was better when compared to other wall to core ratio. As the core to wall ratio is low (80:20), the wicking was lower than other ratios. This may be because of the higher gelatin content in the microcapsule.

4.3.6 Water vapor permeability test:

Table 7. Water vapor permeability of the finished fabric

Sample	Initial weight	Final weight	Weight loss %	Wvpt g/m^2/day
Control	80.00	77.39	2.61	**2088**
50:50	79.00	77.00	2.59	**2072**
70:30	79.00	77.21	2.31	**1848**
80:20	79.00	77.68	1.69	**1352**

The water vapour transmission efficiency of a textile material is another critical factor that affects the comfort properties. The water vapour permeability of the textile is directly related with the inter fiber and intra fiber space in the structure. The finishing of the microencapsulation may block little amount of space between the yarn and within the yarn. Hence there is a reduction in permeability observed in the entire sample irrespective of the core to wall ratio. From the SEM image (Figure 1 and 2), it is evident that the pore in the fabric is modified because of the application of the microencapsulated herbs. However there is a reduction the water vapour permeability. However, the level of permeability of water vapour is still in the range of acceptable for a dressing material.

5. Conclusion

In this research, the *invitro* drug releasing studies of *Terminalia chebula* loaded gelatin microcapsules were studied. The microcapsules were charecterised with the help of SEM. The capsules were found to be releasing the drug for an extended time of 7 hours. The drug releasing behavior of the microcapsules follow the zero order releasing kinetics. Among the different formulations formed, the microcapsules with 80:20 core to wall ratio found to be with high release rate than other formulation. The capsules with 50:50 core to wall ratio are with slower release rate and sustained for long time comparatively with 80:20 core to wall ratio. The formulated microcapsules were applied in the textile fabric and the drug releasing ability was studied in terms of antimicrobial ability of the finished textile. Further the textile fabric was analysed for the essential comfort properties of a wound dressing. This research suggests that, the herbal material can be microencapsulated and finished on textile successfully and the drug releasing property of the capsules opens a new era in the health care and wound dressing sector. Further, the durability of the microcapsules on the textiles material and the drug releasing ability in real time situation are (*in-vivo*) need to be analysed for commercialization purpose.

6.Acknowledgement

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