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Design, development and evaluation of veterinary transdermal film of *Azadirachta indica* extract for treatment of mastitis

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ABSTRACT

Mastitis is an infectious disease condition resulting in inflammatory reaction which occurs when a large number of leukocytes migrate into the mammary gland. Mastitis causes significant financial losses for the global dairy industry. The present study aimed at development of transdermal film comprising of *Azadirachta indica* extract for treatment of mastitis, which is economical, safe and will not emerge multidrug resistance amongst pathogens. *Azadirachta indica* extract was prepared by sonication method. The extract was evaluated for minimum inhibitory concentration against gram-positive and gram-negative organisms. Transdermal films with *Azadirachta indica* extract were prepared with ethylene vinyl acetate and polyvinyl acetate then subjected for the physical characterization, drug content, *In vitro* dissolution, tensile strength and antibacterial activity. The minimum inhibitory concentration of extract displayed 5mg/ml. Based on quantification of azadirachtin by UV spectrophotometer 100 mg of extract was incorporated in each transdermal film. Transdermal films of *Azadirachta indica* extract possessed desirable physical properties, and exhibited the drug release of 91.54% in 8 hours. Transdermal film of *Azadirachta indica* showed sensitivity with a zone of inhibition of 19mm, 16mm, 19mm and 22mm against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively. Data obtained revealed that transdermal films of *Azadirachta indica* extract exhibited good drug release profile and desirable physical properties, with good antibacterial activity. The present work reveals that transdermal film of *Azadirachta indica* extract can be considered for the treatment of mastitis.

Introduction

One of the most common and enduring diseases of the mammary gland in high-yielding dairy animals is mastitis. Bovine mastitis is the most prevalent, expensive condition that affects dairy cattle's health and milk output. Mastitis is a multi-etiological complex disease that affects dairy production. Mastitis causes economic losses due to reduced milk production, wasted milk, early replacement costs, decreased cow sale value, premature culling, veterinary services, high veterinary treatment costs, labour costs, and negative effects on milk quality (Radostits *et al*., 2006). The word mastitis is derived from the two Greek words "mammae" or "mastos" meaning "mammary gland" and suffix "itis" denotes inflammation. Inflammation is characteristically defined as a response to microbial infection or cell/tissue injury. Microbial infection can cause acute/chronic and local/systemic inflammation on the different type of tissues (Bradley *et al*., 2002; Radostits *et al*., 2006). According to the international dairy federation, mastitis is an intra-mammary infection (IMI) of the parenchyma of mammary gland and that can be infectious, traumatic or toxic in nature. Causative agents for inflammations are microorganisms that enter through the teat canal and mammary tissue. More than 140 species of pathogens are responsible for mastitis such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp, *Streptococcus agalactiae*, *Corynebacterium bovis*, *Mycoplasm*, *Pseudomonas* spp, *Citrobacter* spp., Coagulase-negative staphylococci, *Streptococcus* spp., *Streptococcus uberis*, anaerobic bacterial species, fungi, and yeasts (Ceresia *et al*., 2009).

The majority of mastitis caused by bacteria are grouped into 3 different groups contagious pathogens, opportunistic teat skin or environmental mastitis (Bradley *et al*.,2002; Radostits *et al*., 2006; Zigo *et al*., 2021). Invading microorganisms grow inside the mammary gland, where they release toxic by-products that trigger inflammatory reactions. Mastitis is challenging to manage since a variety of microbe species can infect the udder. Swelling, heat, redness, soreness, a high somatic count, decreased milk supply and quality are the hallmarks of inflammation (Devi and Dutta, 2018). The most commonly used strategy for treatment of mastitis is the administration of antimicrobial agents. Increasing antibiotic resistance in pathogenic bacteria has led to a growing demand for alternative safe and natural antimicrobials (Adkins and Middleton, 2018; FDA, 2018).

Ethno-veterinary knowledge is acquired through practical experience and has traditionally been passed down orally from generation to generation. The aetiology of animal diseases and plant based ethno-veterinary curative techniques, clinical studies observed in farmers premises to assess an efficacy of traditional healing methods can be used to develop the herbal formulation for treatment of mastitis (Dilshad *et al*., 2010; Dutta *et al*., 2020).

Traditional herbal medicines are plant or plant-derived substances which are used to treat illness, as they possess less side effects. Now herbal medicine has been developed formulations such as tablets, topical formulations such as transdermal patches, films, topical gels etc which are of patient convenience and showing increased therapeutic effect. Herbal therapy holds its importance because its most efficient, safe, economical treatment and positive results witnessed by farmers when applying herbs. Herbal medicines comprise plant-based medicines can be used for therapeutic, prophylactic or diagnostic application in animal health care and disease prevention (Patel *et al*., 2013; Misal *et al*., 2012).

Azadirachta indica commonly known as neem is well known for its

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medicinal values and has been well accredited from ancient times for its role. Neem is widely used traditional medicinal plant in India. The majority of plants are endowed with medicinal properties and have been used as traditional medicine or household remedies against various human ailments and used as ethanoveterinary medicine by farmers like neem paste applied to cow mammary gland to treat mastitis. The most important active constituent is azadirachtin. Various pathogens such as viruses, bacteria, and fungi can be inhibited by constituents of *Azadirachta indica*, majorly act as antimicrobial agent by inhibiting microbial cell wall. Azadirachtin, a complex tetranortriterpenoid limonoid present in neem. Ethanolic extract of neem leaves shows *In vitro* antibacterial activity with greatest zones of inhibition and possess anti-inflammatory activity (Alzohairy, 2016; Kokate *et al*., 2005; Solanki *et al*., 2022).

In recent years various approaches in drug delivery systems has been innovated to increase the bioavailability and therapeutic efficacy of active pharmaceutical ingredients (APIs) through various routes of administration. Conventional drug delivery system such as oral administration is very difficult in case of veterinary animals, however other animals like dogs, cats it can be administered in food but this is impossible in case of cows. In cows for treatment of the mastitis intra-mammary infusion, intramuscular or intravenous injections of different antibiotics is preferred by veterinarians due to its convenience, economic factors, ease of administration and non-invasive drug delivery. But this systemic drug delivery is very painful for cows as it alters their life cycle they are kept for a dry period, culling of cows where their habited is disturbed and they are stopped from lactating calves as the residues of antibiotics in milk will affect them. To overcome these drawbacks, recent advances in developing novel drug delivery systems to administer the drug(s) through layers of skin which is typically known as the transdermal drug delivery system. The transdermal drug delivery system permeates the drug in the dermal region of the skin through the systemic circulation. Plant derived compounds are valuable so in traditional ethnoveterinary practices they were widely used. They have advantages in non-inducing resistance even after prolonged use. The wide variety of plants which possess very effective medicinal activity for treatment of many ailments. Transdermal film is prepared for the treatment of mastitis in cows (Ansari *et al*., 2011).

Study aimed at the development of herbal transdermal film for the treatment of mastitis in cows which is economical, safe and which will not emerge multidrug resistance amongst pathogens.

Materials and methods

The *Azadirachta indica* leaves were collected and authenticated from SK Arts & HSK Science Institute Vidyanagar, Hubballi campus Dharwad district in Karnataka, India. The following chemicals and reagents were bought from various sources, including S.D. Fine Chemicals Ltd. for the ethanol, methanol, chloroform, and dimethyl sulfoxide. Purchases were made of polyvinyl acetate from Alpha Chemika and ethylene vinyl acetate from Kemtech Impex. Hi-media Laboratories Pvt. Ltd. provided the Muller hinton broth and dibutyl phthalate. Standard strains of *Pseudomonas aeruginosa* NCIM 2200, *Escherichia coli* NCIM 2687, *Bacillus subtilis* NCIM 2063, and *Staphylococcus aureus* NCIM 2079 were used.

Preparation of Azadirachta indica ethanolic extract

Azadirachta indica leaves were washed and dried at 40°C in hot air oven. Dried leaves were grinded to coarse powder and stored in an air-tight container. Ethanolic extract was prepared by ultra-sonication and maceration method with 70% ethanol. Which was filtered through Whatman filter paper and excess of ethanol was evaporated on 105°C, kept in desiccator for removal of moisture and completely dried extract was scraped. (Ranjha *et al*., 2021).

Quantification of herbal extract of Azadirachta indica by Ultraviolet spectroscopy

Determination of absorption maxima of *Azadirachta indica* extract

Azadirachta indica extract (AIE) 100 mg was dissolved in 2-3 ml of methanol and volume was made up to 100ml in a volumetric flask using water and 7.4 pH phosphate buffer separately to obtain a concentration of 1000 µg/ml (SS-I). From the stock solution 100 µg/ml solution of *Azadirachta indica* extract was prepared by doing suitable dilutions with water and 7.4 pH phosphate buffer and scanned between 200-400 nm.

Development of standard calibration curve of *Azadirachta indica* extract

Azadirachta indica extract 100mg was dissolved in 2-3ml of methanol and volume was made up to 100ml in volumetric flask using water and 7.4 pH phosphate buffer separately. Further dilutions were made from the working standard *Azadirachta indica* extract solution with water and 7.4 pH phosphate buffer separately to obtain the concentration of 50, 75, 100, 125, 150, 175, 200, 225, 250 and 275µg/ml of *Azadirachta indica* extract and absorbance was measured against blank for water at 269nm and 7.4 pH phosphate buffer at 268nm using UV – visible spectrophotometer (Sharma *et al*., 2019).

Determination of Minimum Inhibitory Concentration (MIC) of Azadirachta indica extract

MIC is the least concentration of the *Azadirachta indica* extract showing no growth in the Mueller Hinton broth medium as detected by the naked eye after incubation. The 100mg of *Azadirachta indica* herbal extract was dissolved to 10ml of dimethyl sulfoxide and further diluted to obtain concentrations of 10, 5, 2.5, 1.25, 0.625 and 0.3125mg/ml. Standardized inoculums of test pathogens used were of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. In different test tube 4ml of Mueller Hinton broth, 10µl of dilutions prepared of different concentration and 10µl standardized inoculum of test pathogen were added. The procedure was carried out with four different standardized inoculums of test pathogen. All the test tubes were properly corked and incubated at 37°C for 24 hrs and then observed for growth of microorganisms to determine MIC (Faujdar *et al*., 2020).

Formulation of Azadirachta indica extract transdermal film

Transdermal film was formulated containing 100 mg of *Azadirachta indica* extract, which was casted on petri plate using 6 ml of each polymer, 12% ethylene vinyl acetate (EVA) and 4 % polyvinyl acetate (PVAc). 8% w/w of polymeric content dibutyl phthalate was added as plasticizer. The membrane of EVA and PVAc was optimized by Channammanavar *et al*. in 2019. Separately, 12% ethylene vinyl acetate and 4% polyvinyl acetate were soaked in chloroform for 10 minutes. After that, the polymers were ultrasonically dissolved to produce a transparent solution. Chloroform was used to dissolve 100 mg of *Azadirachta indica* herbal extract, which was then added to the polymeric solution along with dibutyl phthalate (as plasticizer). This solution was placed into the petri dishes, covered with inverted funnel and chloroform was allowed to evaporate in order to ensure that the films are dried evenly (Channammanavar *et al*., 2019).

Differential Scanning Calorimetry (DSC)

Drug and polymer interactions were analysed from DSC thermograms of the *Azadirachta indica* powder, *Azadirachta indica* extract and film (EVA + PVAc + *Azadirachta indica* extract) that are obtained using Differential Scanning Calorimeter (DSC 60 Plus, Shimadzu, Japan). Samples were weighed, crimped in aluminium pans, and were heated up to

300ºC at a flow rate of 10ºC/min (Mutalik and Udupa, 2005).

Characterization Study of Azadirachta indica extract transdermal film

Physical appearance

Films were visually inspected for colour, smoothness and flexibility (Pandit *et al*., 2009; Sarukh *et al*., 2019).

Thickness of films

The thickness of drug loaded films was determined by vernier calliper at three different points on the films. Average values and standard deviation values were then calculated (Pandit *et al*., 2009; Sarukh *et al*., 2019).

Uniformity of weight

The films were weighed on the digital weighing machine in triplicate, average weight and standard deviation was determined (Pandit *et al*., 2009; Sarukh *et al*., 2019).

Folding endurance

To evaluate the effectiveness of the plasticizer and the durability of films made from various polymers, folding endurance tests are conducted. The number of folds necessary to break any polymeric film is the folding endurance. Manual folding endurance testing involves repeatedly folding a tiny (2 x 2 cm) section of the film at same location until breaks. Value of folding endurance is determined by how many times the film could be folded in the same position without breaking or cracking. Films in triplicate were examined (Pandit *et al*., 2009; Sarukh *et al*., 2019).

Surface pH

Films were kept in contact with the 0.5 ml double distilled water for 1hr in a glass beaker and allowed to swell. Then the pH is analysed with pH meter (Singh and Bali, 2016).

Percentage moisture absorption

The percentage moisture absorption test was used to determine whether the films would remain stable and intact under highly humid conditions. The films moisture absorption was measured. Each of the formulated films were precisely weighed before being exposed to room temperature, stored in desiccator in 100 ml of saturated potassium chloride solution. The films were weighed on a regular basis every 24, 48, and 72 h. The formula below was used to calculate the percentage moisture absorption (Singh and Bali, 2016).

% Moisture uptake = (Final weight – Initial weight)/ Initial weight X 100

Percentage moisture content

Percent moisture content was carried out to check the integrity of films under dry conditions. The amount of moisture content in the films was analysed. The prepared films were individual weighed of specified area were placed in a desiccator containing fused anhydrous calcium chloride at room temperature. The films were weighed at regular time intervals of 24, 48, and 72 h. The percentage moisture content was determined by using the following formula (Singh and Bali, 2016).

% Moisture content = (Initial weight – Final weight) / Initial weight X 100

Swelling ratio and erosion studies

Film dimension of 1x 1cm was weighed and initial weight was determined (Wi) and it was soaked in 7.4 pH for 24hrs. The film was weighed (Ws) and dried at 60°C and again reweighed (Wd). Following procedures were used to calculate percent swelling ratio and percent erosion (Kriplani *et al*., 2021).

Percent swelling ratio = (Ws- Wi) / Wi x 100

Percent erosion = (Wi – Wd) /Wix 100

Drug content determination

Films were cut into 1.5x1.5cm size from three different films and put into phosphate buffer pH 7.4 solution and ultrasonicated for 1hr. The solution was then filtered through Whatman filter paper. The filtered solution was analysed at 268 nm and from the absorbance values the drug content was calculated (Ahire and Ahire, 2017).

Tensile Strength

Tensile strength is the maximum stress applied to a point at which the film breaks. It is calculated by the applied load at rupture divided by the cross-sectional area. It is also known as ultimate stress. Tinius Olsen consist of two load cell grips. The lower one was fixed and upper one was movable. Film strips with dimension 5cm (l) x 2.5cm (b) were placed between grips and force was gradually applied till the film broke (Ahire and Ahire, 2017).

In vitro drug release

Drug release of prepared transdermal film of *Azadirachta indica* was studied. 30ml phosphate buffer pH 7.4 was used as dissolution fluid. Samples of 2ml were withdrawn at time intervals of 1hr and replaced with fresh dissolution fluid up to 8hrs. Samples were suitably diluted to 10ml of phosphate buffer pH 7.4 and analysed at wavelength of 268 nm using ultraviolet spectrophotometer. Graph of cumulative percentage drug release v/s time has been plotted (Gönüllü and Saki,2017).

Kinetic modelling of dissolution data

The drug release profile of transdermal film was evaluated analysed in three kinetic models such as zero order, first order and Higuchi to ascertain the kinetic drug release of the dosage form. From these model r2-values are compared and the best fit model was selected (Singhvia and Singh,).

Zero order kinetics

It refers to the process of constant drug release from a drug delivery device independent of the concentration, can be represented by the following equation.

Qt = Q0+ K0t

Where,

Qt = amount of drug dissolved in time t

Q0= initial amount of drug dissolved in solution

K0t= zero order release constant

First order kinetics

The first order Equation describes the release from system where release rate is concentration dependent, expressed by the following equation: log Qt = log Qo + K1t / 2.303

Where,

Qt = Amount of drug released in time t

Qo = Initial amount of drug in the solution

K1 = First order release constant

Higuchi Model

Higuchi developed several theoretical models to study the release of water soluble and low-soluble drugs incorporated in semisolids and solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The equation is as following: Qt = KH x t ½
Where,
Qt = Amount of drug released in time t and
KH = Higuchi dissolution constant.

Antibacterial activity

The transdermal film of AIE was analysed for antibacterial activity which was compared with the standard disc of amoxicillin prepared with Whatman filter paper. The test organism used were *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. Mueller Hinton agar was prepared by mixing specified quantity of powder in distilled water that was sterilized by autoclaving at 15lbs temperature 121°C for 15 minutes. MHA was poured on petri plates which was inoculated with test organism and incubated at 37°C for 4-5 h. By disc diffusion method transdermal film of AIE of 5mm was punched, sterilized and aseptically placed on agar surface and incubated for 24hrs at 37°C (Nigussie *et al*., 2021)

Results

Prepared herbal extract

Percentage yield of AIE by ultra-sonication method was 15.06% and maceration method was 3.4%. Ultra-sonication method was chosen for preparation of herbal extract of *Azadirachta indica* as it was of higher yield. Maceration extraction takes 24 hours whereas ultra-sonication can be achieved within 2 hours hence it is less time consuming. Crystalline AIE was shown in Fig. 1.

Fig. 1. Crystalline *Azadirachta indica* extract.

Spectral analysis

Absorption spectrum of the pure drug was over the range of 200 to 800nm with (100mcg/ml) concentration prepared in distilled water and pH 7.4 phosphate buffer. The absorption spectra of *Azadirachta indica* extract showed one peak at 269nm in distilled water and 268nm pH 7.4 phosphate buffer which represents the maximum absorption (λmax) of the drug.

Standard Calibration Curve of Azadirachta indica extract

The Standard calibration curve of *Azadirachta indica* extract was carried out by a UV absorption spectrophotometer. The required concentrations were prepared in distilled water and 7.4 pH phosphate buffer. The calibration curve obtained for *Azadirachta indica* extract showed good linearity with a regression coefficient (R2) value of 0.9993 in distilled water and 0.998 in 7.4 pH phosphate buffer over the concentration range of 50– 275µg/ml. The standard calibration curve of *Azadirachta indica* extract is depicted in Fig. 2.

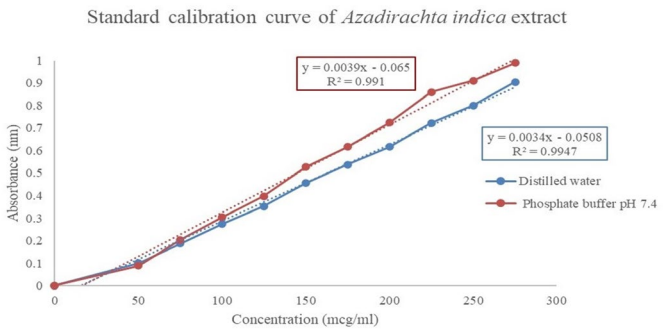

Fig. 2. Standard calibration curve of *Azadirachta indica* extract.

Minimum Inhibitory Concentration (MIC)

Table 1 shows the MIC of test pathogens on *Azadirachta indica* extract. The MIC of the extracts ranged from concentration of 0.625– 10mg/ml. *Staphylococcus aureus* (2.5mg/ml), *Escherichia coli* (5mg/ml), *Bacillus subtilis* (1.25mg/ml) and *Pseudomonas aeruginosa* (5mg/ml). Considering MIC of four different pathogen 5mg/ml was MIC value of *Azadirachta indica* herbal extract. Fig. 3a, 3b, 3c and 3d depicts the MIC of *Azadirachta indica* extract on *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* respectively.
Formulated *Azadirachta indica* extract transdermal film is shown in Fig. 4.

Physical appearance

Formulated herbal films of *Azadirachta indica* were green in colour due to the presence of prepared extract which was green in color. Film

Table 1. Minimum Inhibitory Concentration of *Azadirachta indica* extract

S. No	Concentration of *AIE* (mg/ml)	Bacterial Growth			
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
1	10	-	-	-	-
2	5	-	-	-	-
3	2.5	-	+	-	+
4	1.25	+	+	-	+
5	0.63	+	+	+	+
6	0.31	+	+	+	+
7	Positive control	+	+	+	+
8	Negative control	-	-	-	-

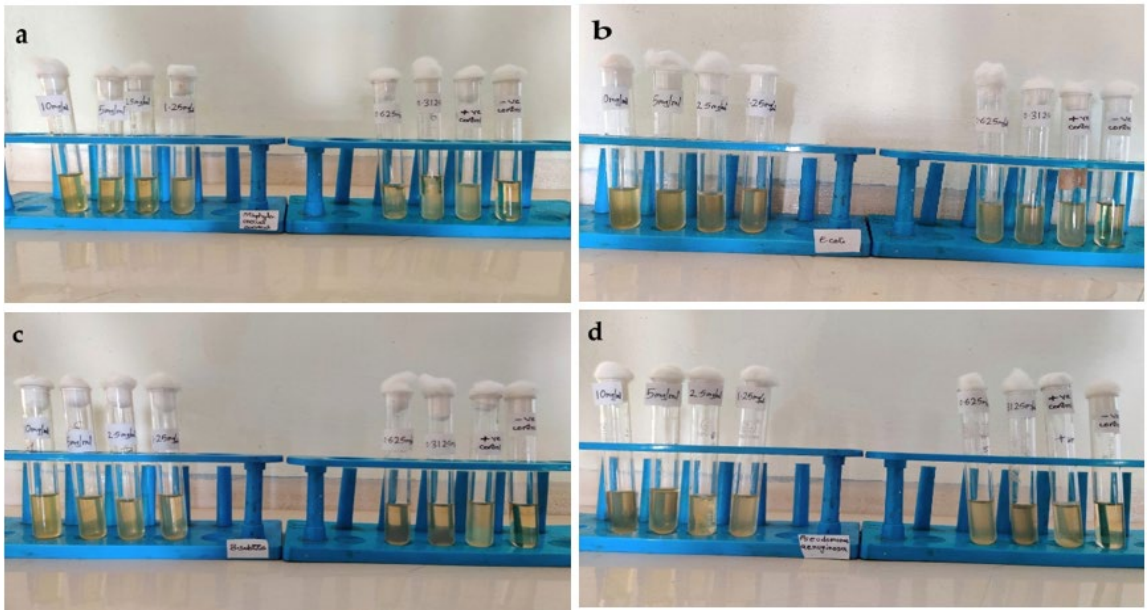

Fig. 3. MIC of *Azadirachta indica* extract against a. *Staphylococcus aureus*, b. *Escherichia coli*, c. *Bacillus subtilis* and d. *Pseudomonas aeruginosa*.

was flexible and smooth in nature.

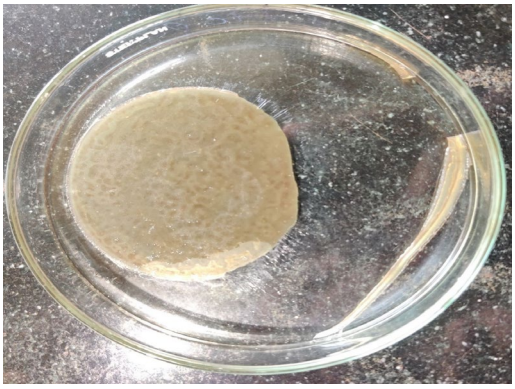

Fig. 4. *Azadirachta indica* extract transdermal film.

Differential Scanning Calorimetry

DSC thermogram of prepared *Azadirachta indica* leaves powder was depicted in Fig. 5a in which no sharp peak was observed hence it is amorphous in nature.

DSC thermogram of *Azadirachta indica* extract was depicted in Fig. 5b. where it is observed that *Azadirachta indica* leaves powder after the formulation of extract gives crystalline extract which is confirmed by obtaining the sharp peak at 117.09°C and 120.56°C in DSC thermogram.

DSC thermogram of formulated *Azadirachta indica* extract transdermal film was depicted in Fig. 5c. where it displayed a single peak at 60.54°C similar to polyvinyl acetate which is in the amorphous state and peak at 68.28°C which is similar to ethylene vinyl acetate copolymer, they are confirmed by literature value. The crystalline herbal extract of *Azadirachta indica* changes to amorphous state after formation of film and undergoes recrystallization at 280.34°C.

Characterization studies

Characterization parameters of *Azadirachta indica* extract transdermal film are mentioned in Table 2. n= 3 data was expressed in terms of Mean ± SD.

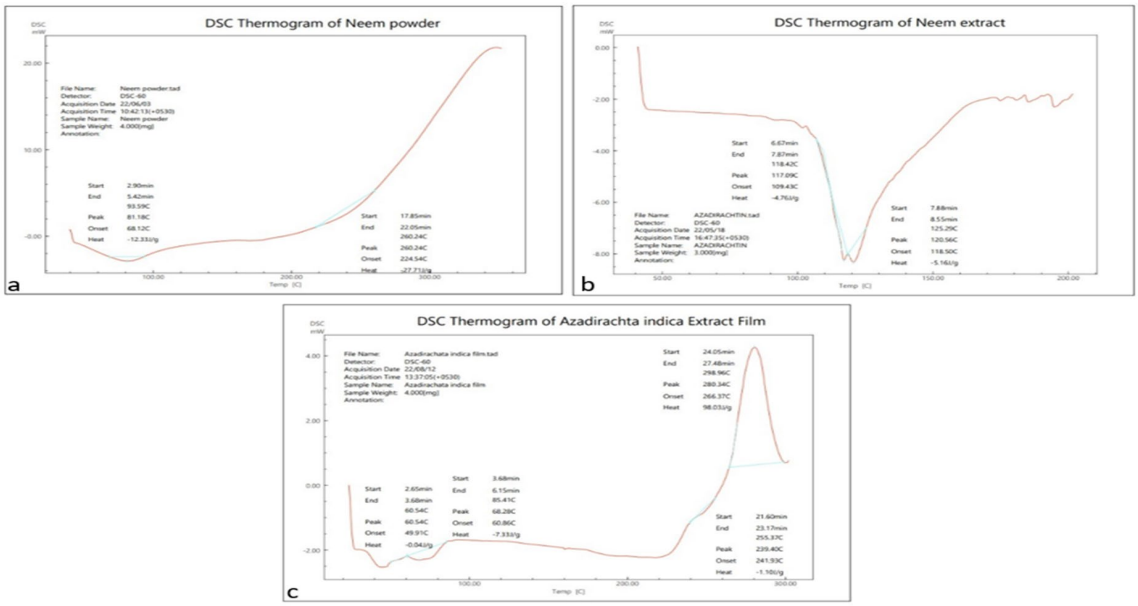

Fig. 5. DSC thermogram of a. Neem powder, b. Neem extract and c. *Azadirachta indica* extract transdermal film.

Thickness

Thickness of the film was 0.043 mm which was measured using vernier callipers of the least count of 0.005 mm. Films had the uniform thickness at three different points on the film and results are stated in Table 4.

Uniformity of weight

Three films were weighed on the digital weighing balance and weight is noted down. The Average weight of films was found to be 1.813 g.

Folding endurance

Folding endurance is performed manually 142 was the average count where the film is teared after folding at the same point. Hence the film exhibited good plasticity.

Surface pH

The surface pH determines the disturbances or irritation in the topical pH 7.4 of skin. Average surface pH 7 which revealed non-irritating nature to the skin.

Percentage moisture absorption

Moisture uptake was found to be 0.8 percentage, which indicates the hydrophobicity of formulation.

Percentage moisture content

The amount of moisture was present, which eventually looses from the film after keeping in desiccator with hygroscopic material. Percentage moisture content was found to be 0.4 percent.

Swelling ratio

Swelling ability of the film after keeping with water, 0.596 percentage was swelling of these herbal films of *Azadirachta indica*.

Erosion studied

Percent of erosion occurred in the film after exposing to water and again drying in heat. 1.093 percent was the percentage eroded of the film.

Drug content

The percentage drug content of the films was evaluated to be 93.14 percent.

Tensile strength

Tensile strength was performed to evaluate the mechanical properties of the film which is depicted in Fig. 6. Tensile strength is also known as the ultimate stress of films which was found to be 16.8 N/mm^2 with elastic modulus of 306 N/mm^2.

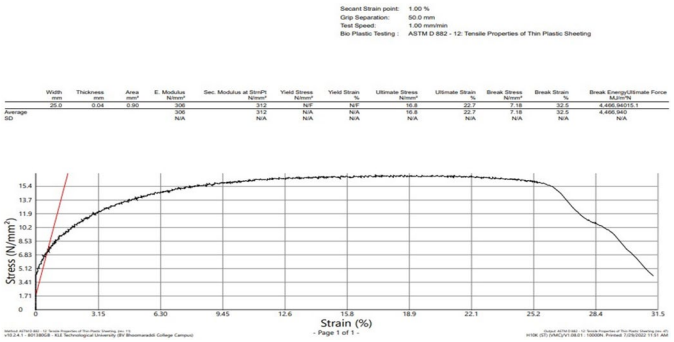

Fig. 6. Tensile strength graph of *Azadirachta indica* extract transdermal film.

In-vitro dissolution studies

In vitro release of azadirachtin from the transdermal film was achieved to be 91.54 percent after 8 hours in phosphate buffer pH 7.4 as the film was prepared to target topical skin of the mammary gland of the cow for treatment of mastitis. The In-vitro drug release profile of *Azadirachta indica* extract transdermal film was depicted in Fig. 7.

Table 2. Characterization parameters of *Azadirachta indica* extract transdermal film.

S. No	Parameter	Film 1	Film 2	Film 3	Mean± SD (n = 3)
1	Thickness of films	0.05	0.04	0.04	0.043 ± 0.0057
2	Uniformity of weight (g)	1.83	1.8	1.81	1.813 ± 0.0152
3	Folding endurance	143	142	143	142 ± 0.577
4	Surface pH	7.1	7	7	7.0 ± 0.057
5	Percentage moisture absorption	0.30	0.89	1.20	0.793 ± 0.453
6	Percentage moisture content	0.30	0.30	0.60	0.399 ± 0.17322
7	Swelling ratio	0.60	0.60	0.30	0.697 ± 0.174
8	Erosion studies	0.90	1.20	1.20	1.093 ± 0.168
9	Percentage Drug content	92.82	93.14	93.47	93.143 ± 0.325

Table 3. Antibacterial activity of *Azadirachta indica* extract transdermal film against standard amoxicillin.

S. No	Pathogens tested	Inhibitory diameter (mm)	
		Herbal Film of *Azadirachta indica*	Standard amoxicillin
1	*Staphylococcus aureus*	19	14
2	*Escherichia coli*	16	15
3	*Bacillus subtilis*	19	17
4	*Pseudomonas aeruginosa*	22	21

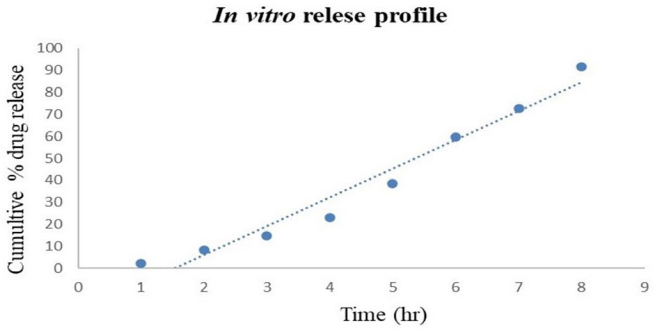

Fig. 7. *In vitro* drug release profile of *Azadirachta indica* extract transdermal film.

Kinetic modelling of dissolution data

For each kinetic model, graphs were plotted and are displayed in Fig. 8a, 8b, and 8c. The *Azadirachta indica* transdermal film's kinetic modeling dissolution data revealed that the zero order R2 value of 0.9604 is the highest of the three models indicating zero order is the best fit model.

Antibacterial activity

Azadirachta indica extract transdermal film was tested for antibacterial activity against four different bacterial strains and compared with amoxicillin as standard, and zone of inhibition diameter (mm) was mea-

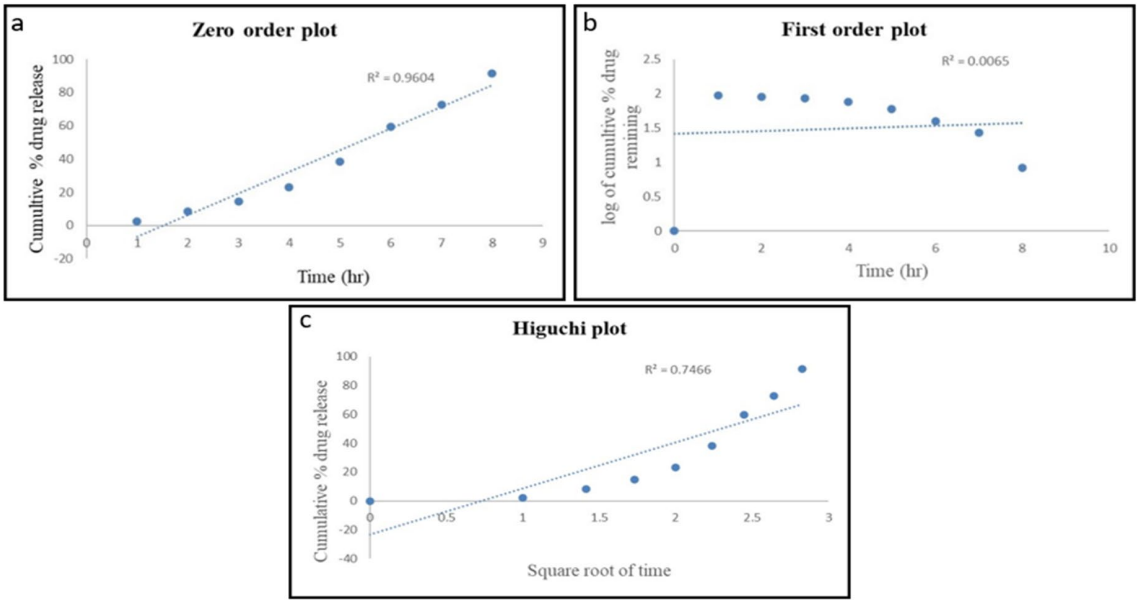

Fig. 8. Kinetic plots of *Azadirachta indica* extract transdermal film – a. Zero order plot, b. First order plot and c. Higuchi plot.

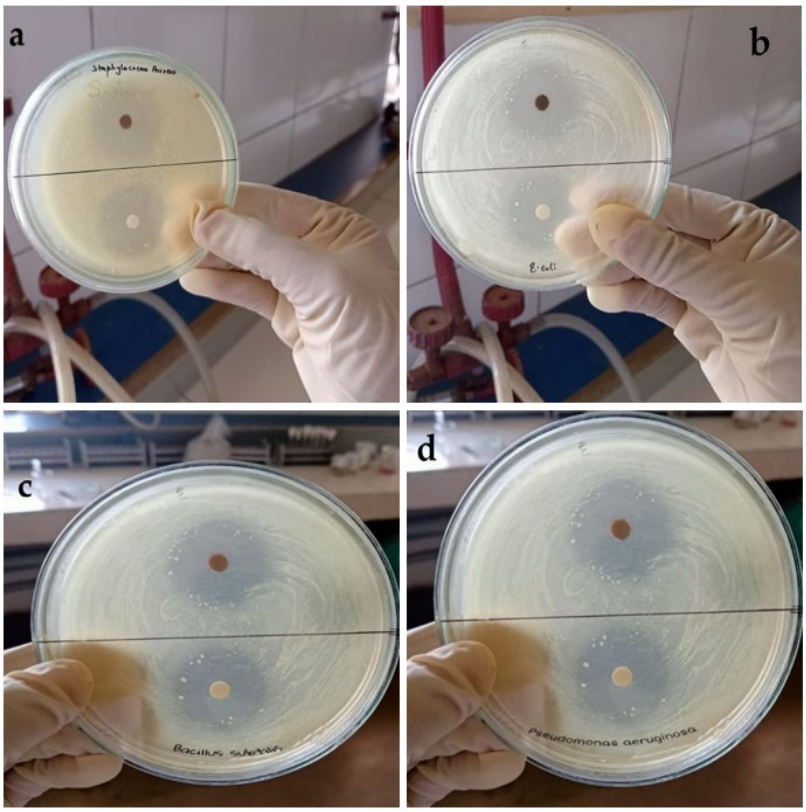

Fig. 9. Antibacterial activity of of *Azadirachta indica* extract against a. *Staphylococcus aureus*, b. *Escherichia coli*, c. *Bacillus subtilis* and d. *Pseudomonas aeruginosa*.

sured as indicated in Table 3. Fig. 9 displays the antibacterial activity of *Azadirachta indica* extract transdermal film against four distinct bacteria.

Discussion

Methods for the evaluation and characterization of transdermal film formation were developed and assessed. The novel transdermal drug delivery with herbal extract was investigated from the above experimental results.

The herbal extract of *Azadirachta indica* was analyzed and the amount of azadirachtin was quantified. Extract showed minimum inhibitory concentration which is the least concentration required to inhibit the growth of pathogens. Films prepared showed good, smooth appearance and green color of extract is visible as the polymeric solution is transparent in color. *Azadirachta indica* powder, extract and transdermal films were analyzed for differential scanning calorimetry various peaks were observed.

Various characterization parameters for *Azadirachta indica* extract transdermal film were carried out. Film possesses good physical properties the thickness of the film is based on the quantity of polymeric solution which was optimized in earlier thesis. It is also uniform in weight at different points on the same specimen. Good folding endurance with such thin film is achieved due to the plasticizer dibutyl phthalate. Surface pH is neutral as it mimics the transdermal drug delivery, so the standard calibration curve is also performed in pH 7.4. The properties such as water moisture absorption and content are very less in value than the polymers and the extract used are very poorly soluble in water. They take up and contain very little moisture to be loose. The swelling ratio slightly swells in water to some extent. The erosion studies showed its eroding nature after being exposed to water and heat but even the erosion is not extreme. Film has the best mechanical property of tensile strength, takes a lot stress of load and can bear with the force.

In-vitro drug release was achieved in pH 7.4 at 8 hours shows the proper drug delivery from the films for the treatment. Based on this kinetic modelling is done and for this formulation it shows that it flows zero order kinetics. The prepared film shows good antibacterial activity in case of some pathogenic bacteria shows considerable better sensitivity as compared to the amoxicillin which is usually given for treatment of mastitis.

Conclusion

A novel approach of transdermal drug delivery system with herbal drug is a very effective and less harmful. Herbal is gaining its importance widely because they are safe, effective, less or negligible side effects.

The approach of veterinary herbal formulation is for well-being of animals. Also directly related to humans as in case of mastitis due to use of antibiotics there are antibiotic residues in milk. Antibiotic resistance is major care and concern as nowadays there are use of synthetic antibiotics, prolong use effect veterinary animals as well as humans.

The herbal drug *Azadirachta indica* were selected based on traditional and ethnoveterinary medicinal treatment reported. Antibacterial activity is confirmed by MIC and zone of inhibition which shows positive resultant support for use of herbal extract. Formulation of film is also achieved properly with good physical properties, mechanical strength and drug release profile inactive as promising dosage form for treatment of mastitis. It can be concluded that prepared transdermal film of herbal extract of *Azadirachta indica* on based on further studies can be considered for the treatment of mastitis.

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Conflict of interest

The authors declare that they have no conflcit of interest.

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