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RESEARCH ARTICLE

Development of *Lactuca sativa* Extract Transdermal Patches Using Carboxymethylated Locust Bean Gum

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Abstract: ***Introduction*:** The objective of this study was to synthesize and characterize CMLBG and formulate matrix-type transdermal patches of *Lactuca sativa* extract, followed by *in-vitro* and *in-vivo* evaluation.

***Methods*:** CMLBG was prepared by alkaline carboxymethylation and confirmed by FTIR and $_{13}$C-NMR spectroscopy. Solvent casting was used to prepare transdermal patches and assess them based on physicochemical properties, drug content, moisture behavior, mechanical strength, and surface uniformity. The *in-vitro* permeation was characterized using an egg membrane, and release kinetics were modeled. Furosemide was used as a standard to determine *in-vivo* diuretic activity in albino rats.

***Results*:** Spectroscopic observation indicated that the (CH_2-COO) group was incorporated into LBG successfully. Formulations had a consistent thickness (0.3-0.5 mm), high folding endurance (>80), and a consistent drug concentration (96.7-99.8%). The moisture content and uptake values were satisfactory. Patches containing CMLBG had an improved *in-vitro* release, and formulation F6 had the maximum cumulative release of 47.36% at 300 min. The drug release followed Super Case-II transport kinetics. *In vivo* results showed that both *Lactuca sativa* extract patches and CMLBG-furosemide patches produced significant diuretic activity compared to the control. Urine output after 5 hours (6.25 ± 0.2 ml and 7.2 ± 0.23 ml) was slightly lower than oral furosemide (8.56 ± 0.25 ml), indicating good therapeutic potential of the transdermal patches.

***Discussion*:** The combined outcomes favor the possibility of transdermal patches of *Lactuca sativa* as a formulation of chemically treated natural polymers, aimed at controlled, long-term release and a diuretic effect. The data further indicate that when used in a transdermal form, herbal diuretics may offer a therapeutic advantage.

***Conclusion*:** *Lactuca sativa* extract was successfully delivered *via* CMLBG-based transdermal patches, which showed controlled release, increased permeation, and protracted diuretic activity.

Keywords: Transdermal patches, *Lactuca sativa*, carboxymethylated locust bean gum, locust bean gum, controlled release.

1. INTRODUCTION

Transdermal drug delivery systems (TDDS) have gained unprecedented prominence in pharmaceutical research, offering distinct therapeutic advantages over conventional oral and parenteral routes [1]. The transdermal route allows controlled, sustained drug release, prevents hepatic first-pass metabolism, and significantly reduces fluctuations in plasma concentrations, enhancing safety and compliance with the emblem of patient adherence [2]. Over the last 10 years, the number of drugs that can be delivered through the skin barrier has increased due to advances in polymer science and formulation engineering. Those developments include natural polymer-based films, chemically modified polysaccharides, nanostructured matrices, and new strategies to enhance permeation, which address the need for non-invasive, long-acting therapeutic systems [2, 3].

The ability to select a polymer matrix that can be used to create stable films and allow prolonged drug release is the key element of any transdermal formulation [4]. Natural gums and polysaccharides have received significant attention due to their biocompatibility and biodegradability, as well as their versatile functional properties. The most widely used of them was Locust Bean Gum (LBG), a galactomannan from

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Ceratonia siliqua, commonly used in controlled-release systems for its film-forming and stabilizing properties. Nevertheless, native LBG exhibits drawbacks, *e.g.*, partial insolubility and a limited swelling potential, which may limit its application as a transdermal material. To address these disadvantages, chemical modification of LBG, especially carboxymethylation, has been shown to be a powerful tool for overcoming this drawback. Carboxymethylation adds sodium carboxymethyl groups to the polymer backbone, thereby increasing its hydration, solubility, and rheological properties. The fact that modified gums (carboxymethylated guar and tamarind gums) exhibit better physicochemical characteristics and controlled-release properties than unmodified polysaccharides is confirmed by several studies [5-7].

The modified form of LBG, sodium carboxymethyl locust bean gum (CMLBG), is particularly promising for pharmaceutical applications. The addition of a carboxymethyl group largely improves its hydrophilicity, swelling capacity, and solubility, thereby facilitating drug incorporation and increasing matrix hydration [5, 6]. These alterations facilitate the continuous shaping of gels and release kinetics similarity- key factors in an effective transdermal drug delivery system. Furthermore, chemically modified polysaccharides exhibit greater flexibility in mechanical properties and have good film-forming properties, allowing them to be combined with auxiliary polymers, including hydroxypropyl methylcellulose (HPMC) and ethyl cellulose, which are popular controlled-release formulations [3, 7]. All of which, together with other qualities, place CMLBG and other modified natural polymers in an excellent position for use in the design of both stable and effective herbal transdermal patch systems [8].

Simultaneously with technological advances in polymer modification, recent research on herbal medicines as safer alternatives to synthetic drugs has been revived. The choice to use herbal diuretics is usually because of their lower-grade physiological action and lesser tendency to cause excessive electrolyte imbalance, a frequent adverse effect of the stronger loop diuretics like furosemide [9]. *Lactuca sativa* L. (lettuce) has been in the spotlight for its multiple pharmacological activities, among other medicinal plants. It has diuretic, sedative, anti-inflammatory, and detoxification effects, as traditionally attributed to it in Ayurvedic, Mediterranean, and European folk medicine [10, 11].

Nevertheless, herbal extracts with poor bioavailability, rapid metabolism, and inconsistent absorption have been reported to be highly ineffective *via* oral routes, which seriously restricts the therapeutic efficacy of most phytomedicines [12]. Novel methods and delivery systems can circumvent the numerous obstacles to achieving therapeutic levels in human patients, particularly transdermal systems, to bypass the gastrointestinal tract and first-pass hepatic metabolism, thereby enhancing systemic exposure, reducing doses, and achieving more consistent plasma concentrations [13, 14]. Moreover, the controlled-release properties of transdermal patches permit time-released delivery of drugs, reducing peaks and troughs in systemic levels and the risk of toxicity, such as sudden electrolyte imbalance typical of pharmacological diuresis [15, 16].

The combination of CMLBG with *Lactuca sativa* extract offers a new and cost-effective approach to creating a safe, effective, and long-acting herbal diuretic patch. The hydrophilicity of CMLBG facilitates continuous moisture absorption and swelling of the matrices, enabling phytoconstituents to permeate gradually through the skin [17, 18]. Combining HPMC and ethyl cellulose increases the flexibility, tensile strength, and moisture content of the films, creating an ideal environment for transdermal permeation. All these features enable the controlled-release system to achieve better treatment outcomes in patients with mild to moderate diuresis.

Considering such opportunities, the current research is devoted to the systematic preparation of transdermal patches containing *Lactuca sativa* extract, based on CMLBG as the dominant polymer. In the research, synthesis, and characterization of CMLBG are performed using both FTIR and NMR, and matrix-type patches are developed by the solvent-casting technique, along with in-depth physicochemical analysis including thickness, moisture content, drug content, and mechanical properties. It also entails *in vitro* permeation experiments by using Franz diffusion cells and *in vivo* evaluation of the diuretic activity in albino rats. Through this multidimensional exploration, the proposed research paper will introduce *Lactuca sativa*-loaded CMLBG patches as a potentially useful herbal transdermal diuretic, serving as an alternative to synthetic diuretics with better safety and patient tolerability.

2. MATERIAL AND METHODS

2.1. Drug and Plant Material

The drug frusemide was purchased from Sigma-Aldrich Chemicals Private Limited (Bangalore), and the leaves of *Lactuca sativa* Linn were collected from the local market in Moradabad (U.P.). Further identification and authentication were done by Dr. Ashok Kumar, Associate Professor, Department of Botany, School of Sciences, IFTM University, Moradabad (Uttar Pradesh). A specimen for the same was preserved in the herbarium of IFTM University, Moradabad (Uttar Pradesh), for future reference (Ref No. 2024/SOS/BOT/151).

2.2. Chemical & Regents

All materials used for the formulation were of analytical grade. Locust bean gum, polyethylene glycol (PEG 400), ethylene cellulose, di-butyl phthalate, chloroform, glacial acetic acid, mono chloroacetic acid, hydroxy propyl methyl cellulose, isopropyl myristate, propylene glycol, polyvinyl alcohol, and hydrochloric acid were purchased from Central Drug House (P) Ltd., New Delhi, India. Ethanol and Methanol were purchased from Qualigens Fine Chemicals, Mumbai, and the sodium hydroxide was purchased from Hi-media Laboratories, Mumbai.

2.3. Animals

This study employed 24 male albino Wistar rats, each weighing between 180 and 220 g and 2-3 months old, obtained from the animal house of the School of Pharmaceutical Sciences at IFTM University in Moradabad,

Uttar Pradesh. When the rats arrived, they were weighed and randomly placed in plastic polypropylene cages. At the animal house department of IFTM University, Moradabad, each rat was housed in a cage within the animal room under regulated conditions, including temperature (22 ± 2°C), humidity (55 ± 10%), and lighting (12 h light/dark). Water and commercial food were given to the rats ad libitum. Prior to the trial, the rats were acclimated for two weeks [19]. The experimental protocol was reviewed and authorized by the Institutional Animal Ethics Committee (IAEC) of IFTM University (Resolution No. 2023/837/04M.Ph./12). The IAEC is registered with the CPCSEA, New Delhi, Government of India (Registration No. 837/ac/04/CPCSEA). This study was assigned the reference number IAEC/2023/25/12.

2.4. Extraction of Plant Material

The extraction was performed using the Soxhlet apparatus with chloroform as a non-aqueous solvent. In this process, the powdered drug was placed into the extractor with chloroform as solvent. After extraction, the extract was collected and evaporated in a china dish / petri dish in a water bath [20]. It was then stored in a glass bottle with a tight cap and will be kept in the refrigerator for further investigation.

3. EXPERIMENTAL DESIGN

3.1. Carboxymethylation of Locust Bean Gum

The derivative of locust bean gum was sodium carboxymethyl locust bean gum. To eliminate the organic contaminants, 2.2 grams of precisely weighed locust bean gum was dissolved in 50 ml of methanol and magnetically agitated for two hours. After that, it was dried after passing through Whatman filter paper with an 11 µm pore width. Four milliliters of water heated to 80°C for 15 minutes and cooled was combined with the dried powder weighing 1 gram. Then, over the course of 45 minutes, 2.72 ml of a 55.59% (w/v) NaOH solution was added dropwise to ice-cold water. The aforesaid combination was kept at 15°C; then monochloroacetic acid (750 mg) was diluted in 1.66 ml of water and added gradually over the course of one hour. After gradually increasing the mixture's temperature to 65°C, it was agitated for at least an additional hour. Three separate volumes of a 20 ml 80% (v/v) methanol/water combination were used to wash the wetted bulk for 15 minutes. Glacial acetic acid was used to bring the suspension's pH down to neutral. After that, it was cleaned with methanol and allowed to dry at 50-60°C until three successive weights were equal, as shown in Fig. (**1**) [21-23].

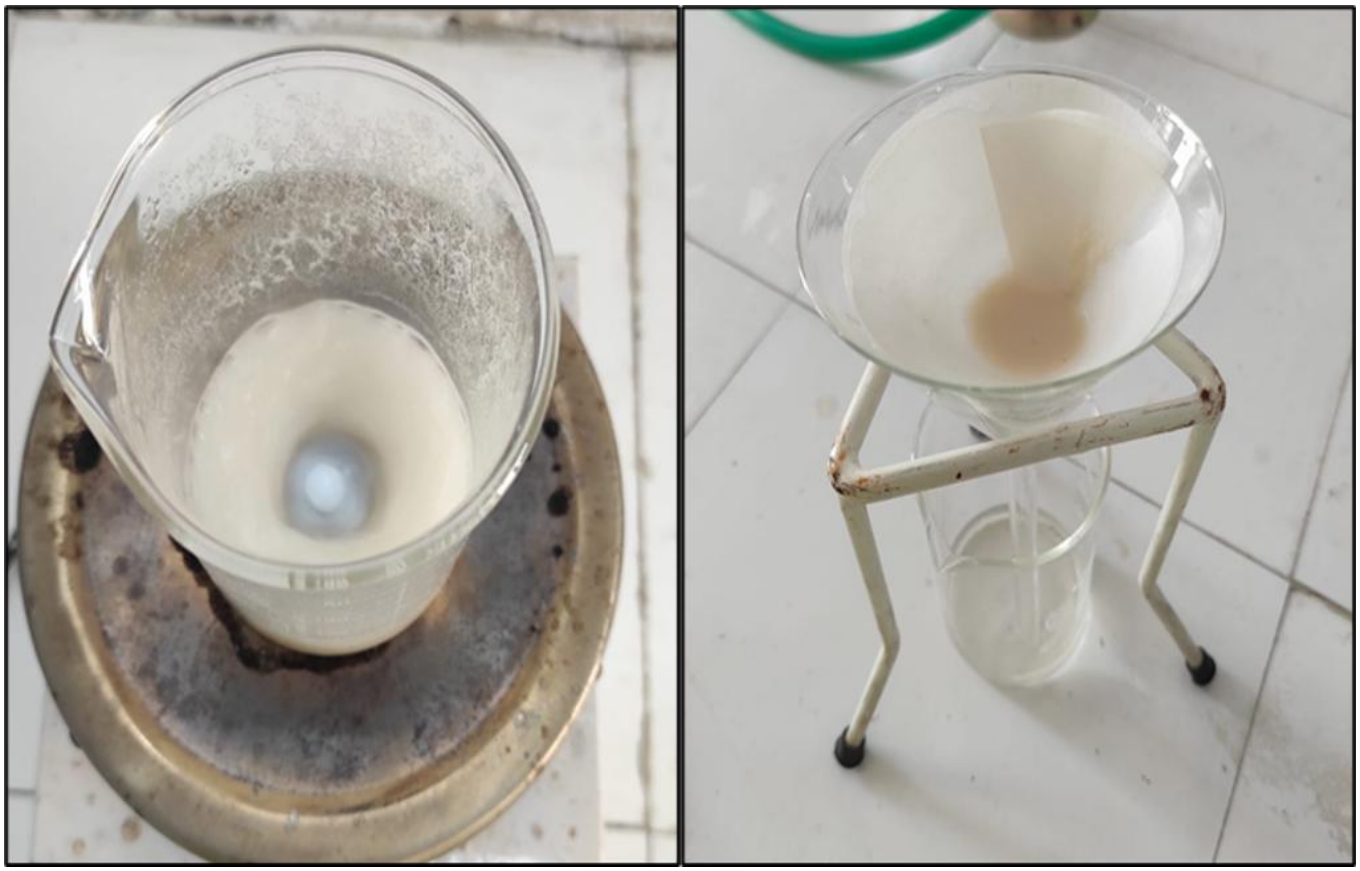


Fig. (1). Carboxymethylation method of locust bean gum. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.2. Fourier Transform Infrared (FTIR) and NMR Spectroscopy

Incorporation of the O-carboxymethyl functionalities in the chemical alteration of the native locust bean gum was evaluated utilizing a precise combination of Fourier transform infrared (FTIR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy. Both LBG and its compound, O-carboxymethylated derivative (CMLBG), were examined by a Shimadzu-1800 FTIR spectrometer (Kyoto, Japan) set at wavelengths of 4000-500 cm. It was carried out using analysis pellets with a 1:20 polymer-to-KBr ratio, prepared under 1000 kg/cm^2 for 10 minutes under hydraulic pressure [5, 7]. Parallel structural investigation was carried out in the solid state utilizing $_{13}$C and $_{1}$H NMR solid-state spectroscopy. About 50 mg of each polymer was also analyzed using a Bruker Advanced-400 MHz spectrometer (Switzerland), and its chemical shifts were reported between 25 and 200 ppm [22]. The pair of supporting spectroscopic approaches enabled thorough verification of the presence of functional groups and modification of molecular structure through derivatization.

3.3. Calibration Curve of Furosemide

Frusemide was determined to have a wavelength maximum of 277 nm using ultraviolet (UV)-visible spectroscopy (Shimadzu-1800, Kyoto, Japan) [24]. Using phosphate buffer (pH 7.4), a standard solution (10 µg/ml) was made from a stock solution (1 mg/ml). A 10-milliliter volumetric flask was filled with aliquots of standard drug solution, ranging from 1 to 10 milliliters, and diluted to the appropriate level with phosphate buffer, pH 7.4. Consequently, the final concentration falls between 1 and 10 µg/ml. At 277 nm, the absorbance of each solution was measured relative to phosphate buffer (pH 7.4) [25]. The drug's concentrations were plotted against absorbance. The linear regression technique was applied.

3.4. Formulation of Transdermal Patches

The solvent casting evaporation method was used in this investigation to create matrix-type transdermal patches of *Lactuca sativa* Linn (Fig. **2a** and Table **2**) and Frusemide (Fig. **2b** and Table **1**). Weighed amounts of HPMC (350, 400, and 450 mg), ethyl cellulose, and carboxymethylated locust bean gum (50, 100, and 150 mg) were dissolved in 10 milliliters of methanol and chloroform in a 1:1 ratio to create the casting solution. 10% w/w penetration enhancer and 0.5% w/w propylene glycol were added to the resultant solution as a plasticizer. To create a homogenous mixture, the medication (5 mg) was then added and properly combined. Next, a glass mold or Petri dish was filled with the properly prepared casting solution to capture the contents. The glass mold that held the casting solution was placed in a vacuum oven and allowed to air-dry at room temperature for a full day. The patch was peeled off and trimmed into a 1 cm^2 spherical shape [26]. After two days of further drying in desiccators, these patches were wrapped in aluminum foil and placed inside a self-sealing cover as shown in Fig. (**2**).

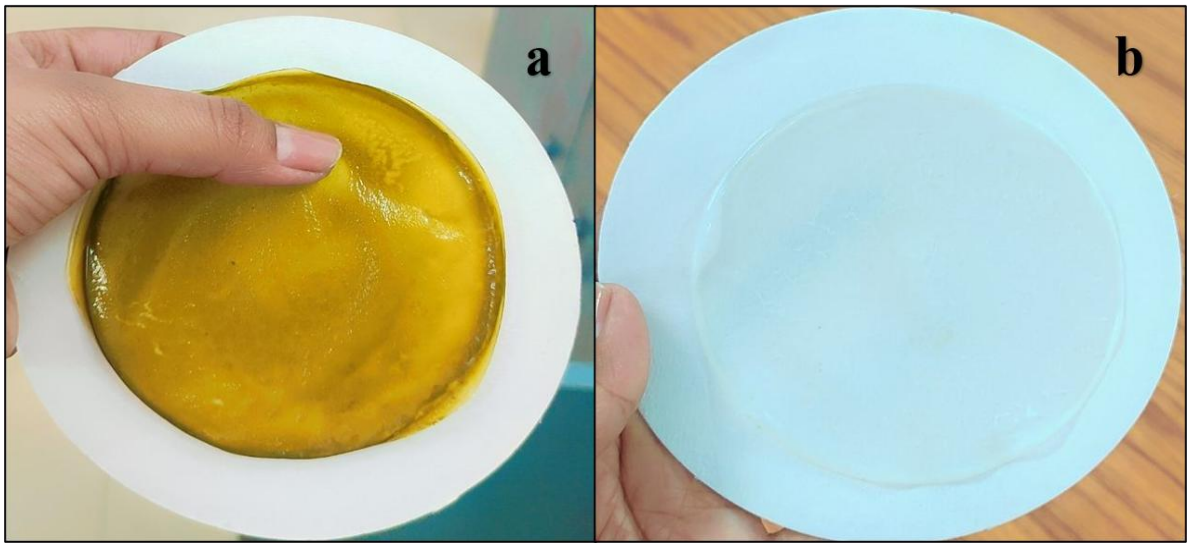


Fig. (2). Prepared transdermal patches of (**a**) Plant extract, (**b**) Standard drug frusemide. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 1. Formulation of transdermal patches of frusemide.

Formulation Code	Drug (mg)	HPMC (mg)	LBG (mg)	Polymer (CMLBG) (mg)	Ethyl Cellulose (mg)	Propylene Glycol (ml)	Permeation Enhancer (DMSO) (ml)	Methanol (ml)	Chloroform (ml)
F_1	5	450	50	-	100	0.5	0.5	5	5
F_2	5	400	100	-	100	0.5	0.5	5	5

(Table 1) Contd…

Formulation Code	Drug (mg)	HPMC (mg)	LBG (mg)	Polymer (CMLBG) (mg)	Ethyl Cellulose (mg)	Propylene Glycol (ml)	Permeation Enhancer (DMSO) (ml)	Methanol (ml)	Chloroform (ml)
F_3	5	350	150	-	100	0.5	0.5	5	5
F_4	5	450	-	50	100	0.5	0.5	5	5
F_5	5	400	-	100	100	0.5	0.5	5	5
F_6	5	350	-	150	100	0.5	0.5	5	5

Table 2. Formulation of transdermal patches of *Lactuca sativa.*

Formulation Code	Extract of *Lactuca sativa* Drug (mg)	HPMC (mg)	Polymer (CMLBG) (mg)	Ethyl Cellulose (mg)	Propylene Glycol (ml)	Permeation Enhancer (DMSO) (ml)	Methanol (ml)	Chloroform (ml)
F_7	5	450	50	100	0.5	0.5	5	5
F_8	5	400	100	100	0.5	0.5	5	5
F_9	5	350	150	100	0.5	0.5	5	5

3.5. Evaluation of Prepared Patches

3.5.1. Folding Endurance

A 1.5 x 1 cm strip was cut consistently, then folded at the same spot repeatedly until it snapped. The folding endurance rating was determined by counting the number of times the film was folded in the same spot without breaking [26].

3.5.2. Physical Appearance

The homogeneity, color, smoothness, and flexibility of each transdermal patch were visually assessed [26].

3.5.3. Film Thickness

Using a digital micrometer with a screw gauge, the thickness of each patch was measured five times on a single patch for each formulation, and the mean was computed [26].

3.5.4. Drug Content

For 24 hours, a predetermined patch area (1.5 × 1.5 cm) was dissolved in 100 mL of methanol and shaken constantly. After that, the entire solution was ultrasonically agitated for 15 minutes. Following filtering, the drug's content was determined by spectrophotometry at 277 nm and 650 nm [26].

3.5.5. Percentage Moisture Content

After being individually weighed, the produced films were stored for 24 hours in a desiccator with fused calcium chloride at room temperature. After a day, the films were weighed again, and the following formula was used to determine their moisture content [26].

$$\% \text{ Moisture Content} = \frac{\text{Initial weight} - \text{final Weight}}{\text{Initial weight}} \times 100$$

3.6. *In-vitro* Skin Permeation Investigation

Utilizing a Franz diffusion cell (Fig. **3**) (receptor compartment area: 2.5*2.5 cm; capacity: 80 ml; equal to 6.25 mg of medicine), the *in vitro* skin penetration investigation was conducted. After being detached, the egg membrane was used for an *in vitro* investigation. Forty milliliters of phosphate buffer with a pH of 7.4 were placed in the receiver compartment. The egg membrane was placed in the donor compartment after the transdermal patch was securely pressed onto its center. The donor compartment was then positioned so that the receptor fluid's surface just touched the membrane's surface. A magnetic stirrer and a thermostatic hotplate are used to provide heat. The receptor compartment's temperature was kept at 32 ± 0.5ºC [27]. After appropriate dilution with diluent, the samples were removed at different times and analyzed by UV-visible spectrophotometry for drug content (277 nm for frusemide and 650 nm for plant *Lactuca sativa).* The receptor phase was concurrently replaced with an equivalent amount of buffer solution at each time interval [26, 27].

Fig. (3). *In-vitro* skin permeation study using franz diffusion cell. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

3.6.1. Kinetic Study

To ascertain the first order (log percentage of drug to be released *v/s* time), Higuchi's (percentage of drug released *v/s* square root of time), zero-order (percentage of drug released *v/s* time) and Korsmeyer-Peppas model (log percentage of drug to be released *vs* log time) patterns were used to analyze the data in order to determine the mechanism of drug release from these formulations [26].

3.7. Statistical Analysis

The results of the investigation were subjected to multiple comparisons and to analysis of variance (one-way ANOVA).

3.8. *In Vivo* Animal Studies of Formulation of Furosemide/ Extract drug Patches using Locust Bean Gum

Albino rats weighing 150-200 g were kept overnight without food or water, after which saline (25 ml/kg, orally) was administered using an oral feeding cannula. The animals were further separated into four groups, each containing six rats. Group I received normal saline (control), while Groups II, III, and IV received saline supplemented with oral furosemide (5 mg/kg), test drug patches, and *Lactuca sativa* extract patches, respectively, as depicted in Table **3**. After drug administration, all animals were placed in separate metallic metabolic cages to collect urine samples individually. Urine was collected in graduated cylinders, and the latency of urination (the time elapsed until the first void) was recorded for each group. The cumulative urine volume was subsequently measured at 5- and 12-hour intervals post-treatment. Fig. (**4**) depicts the design of the metabolic cage adopted in the research [28, 29].

4. RESULTS

4.1. Synthesis of CMLBG

The synthesis of CMLBG was carried out at 75°C temperature using 29.76% w/v LBG, 45% w/v NaOH, and 45.05% w/v monochloroacetic acid solution. The synthesis of CMLBG involved a base-catalysed two-step reaction between LBG and monochloroacetic acid in the presence of sodium hydroxide. In the first stage, neutral LBG is converted to a polyelectrolyte by ionizing the hydroxyl groups in the presence of sodium hydroxide. CMLBG is synthesized in the second stage by introducing carboxymethyl groups *via* substitution. The carboxymethylation reaction of various polysaccharides depends on several factors, including reactant concentrations (NaOH and MCA), reaction time, and reaction temperature.

Table 3. Grouping and treatment of animals.

S. No.	Group	Treatment (n = 6)
1.	Group 1- Normal Control	Normal Saline
2.	Group 2- Standard Control	Furosemide (5 mg) orally
3.	Group 3- Test	CMLBG with Frusemide (5 mg) patches
4.	Group 4- Test	Non-aqueous Extract (5 mg) patches

Fig. (4). Metabolic cage with an albino rat for urine collection. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

4.2. Effect of Sodium Hydroxide Concentration

NaOH concentration plays a vital role in determining the success of the carboxymethylation reaction. Moreover, depending on the concentration of the base, the degree of substitution (DS) of the carboxymethylated product varies considerably. A lower NaOH concentration hinders MCA penetration into the interlocking polymer, thereby lowering the DS value. On the other hand, higher alkali concentration (>60%) increases DS to a certain level, after which DS declines rapidly. The prevalence of the competitive reaction of NaOH with MCA over the main carboxymethylation reaction can explain this particular phenomenon. It was found that both the solution viscosities and the DS of the carboxymethylated derivative were significantly affected as the base concentration was increased beyond a ratio of 1.0.

4.3. Effect of MCA (Monochloroacetic Acid) Concentration

MCA concentration also governs the success of the carboxymethylation reaction, thereby achieving a higher DS value for the final substituted product. Similar to NaOH, increasing the MCA concentration initially increases the DS, then decreases it. As the competitive side chain reaction between NaOH and MCA prevails over the carboxymethylation reaction at higher MCA concentration, the DS value declines rapidly.

4.4. Effect of Temperature

DS was found to increase with an increase in temperature in the range of 40-80°C. Above 80°C, the DS of the final product declined due to the hindrance of swellability of the polymer and the diffusion of the reactants.

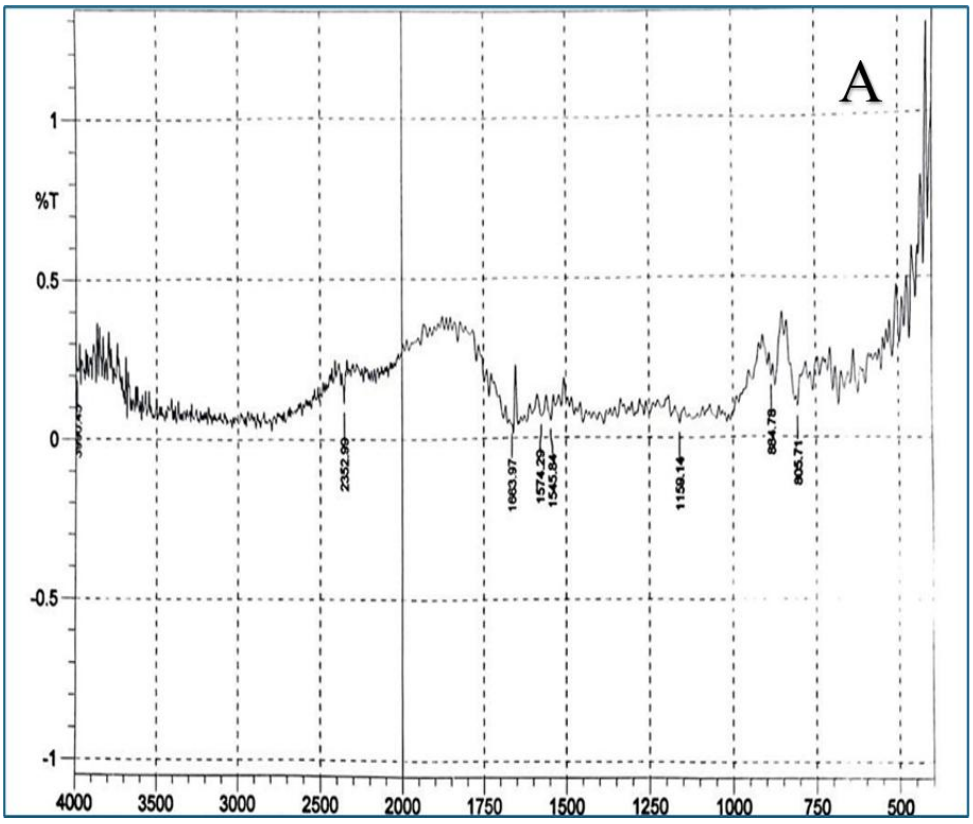
4.5. Effect of Reaction Time

Increased reaction time promotes polymer swelling and, in turn, the adsorption and diffusion of the reactants (NaOH and MCA), resulting in better contact between the reactants and the polymer. Thus, a 1-hour time period for the completion of the carboxymethylation reaction was found to be optimal. Considering the above factors influencing the reaction, the concentrations of LBG, MCA, and NaOH were fixed at 29.76% w/v, 45.05% w/v, and 45% w/v, respectively, and the reaction temperature and time were set to 75°C for 1 hour.

4.6. Fourier Transform Infrared (FTIR) Analysis

4.6.1. FTIR Interpretation of LBG & CMLBG

Fig. (**5**) displays the FTIR spectra of LBG and CMLBG, respectively. The N-H Stretching was found at 3990.45 cm^{-1} in LBG and 3990.93 cm^{-1} in CMLBG. It was discovered that the hydroxyl group in CMLBG has a very large O-H stretching (3747.91 cm^{-1}). The LBG and CMLBG spectra showed vibrations associated with -CH2 bending/wagging at frequencies of 2352.99 cm^{-1} and 2359.74 cm^{-1}, respectively. The carboxymethyl group in CMLBG was shown to be present at 840.42 cm^{-1}, indicating symmetric C-O-C stretching, but the band at 1000-1100 cm^{-1} was mostly ascribed to C-O-H stretching/bending and was similarly detected in the derivative without significant modifications.



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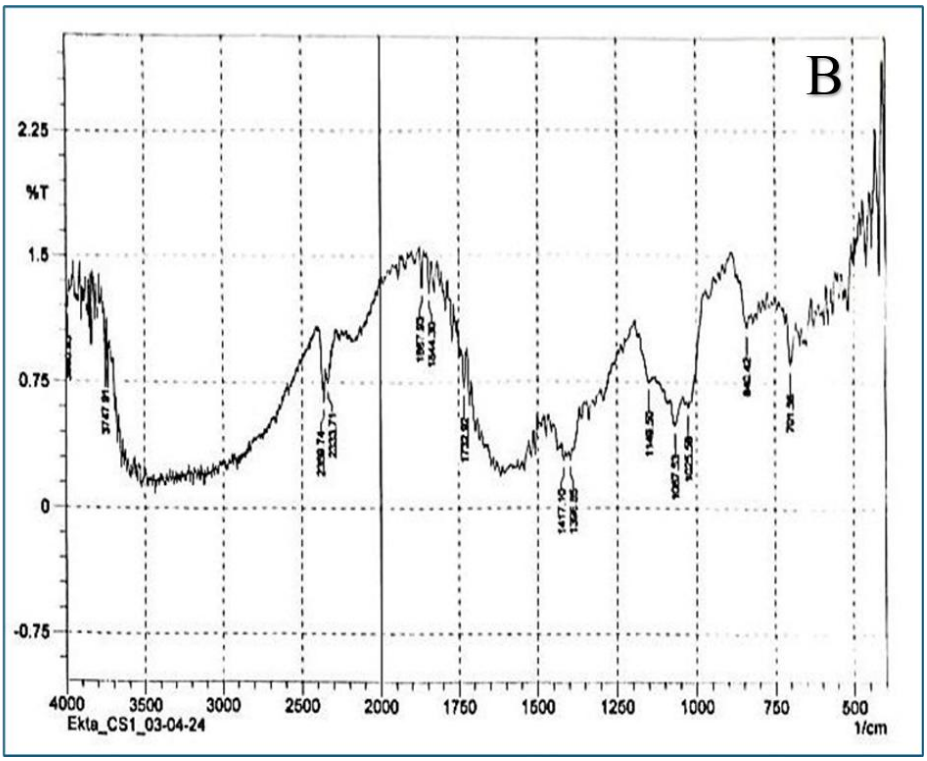

Fig. (5). FTIR of (**A**). LBG (**B**). CMLBG. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

The CMLBG has a higher CH2 stretching vibration (1396.85 cm^{-1}) than the LBG (1159.14 cm^{-1}). Additionally, derivatized gum displayed C=O stretching of acid at 1417.10 cm^{-1} and C-O stretching of ether at 17932.92 cm^{-1}. It wasn't the same for LBG. Thus, it may be said that o-carboxymethyl groups are now present in LBG.

4.7. $_{13}$C NMR Spectroscopy Analysis of CMLBG

Locust bean gum's $_{13}$C NMR spectrum showed three separate peaks at δ = 61.9 ppm (C-6) and a broad signal between δ ~ 69-81 ppm, aggregating the signals at δ = 69.7, 71.9, 75.5, and 80.2 ppm for (C-2, C-3, C-4, and C-5). At δ = 101.1 ppm, a few notches on the peak showed signs of signal merging. The carboxyl C of the carboxymethyl group was observed as extra signals at 77.542, 77.225, and 76.907 ppm in the $_{13}$C NMR spectrum of CMLBG. It is shown that LBG has undergone carboxymethylation, as evidenced by NMR data in Fig. (**6**).

4.7.1. Calibration Curve of Furosemide

The calibration curve of frusemide is provided in Table **4** and Fig. (**7**). The furosemide calibration curve was produced with phosphate buffer (pH 7.4). UV/visible spectroscopy was used to determine absorbance at 277 nm for various concentrations of furosemide reference standard solutions. With an equation of Y = 0.0598x - 0.0039, the calibration curve of furosemide in phosphate buffer demonstrated linearity in a concentration range of 0 to 50 µg/ml and a determination coefficient (R^2) of 0.9997 (Fig. **7**).

4.7.2. Evaluation of Prepared Patches

The developed patches were characterized based on their organoleptic and physical properties, including flexibility, folding endurance, and tackiness (Table **5**). Further quantitative analysis of thickness, drug content, and moisture kinetics (content and uptake) is detailed in Table **6**.

The transdermal patches developed with different concentrations of LBG and CMLBG were consistent, smooth, opaque, flexible, and non-sticky. Each of the nine patches had good folding endurance, ranging from ≥ 83 to ≥105 (Table **5**). The F5 formulation has high folding endurance. The transdermal patches developed with furosemide were white, while those made with the herbal extract were green (Table **5**). The prepared patches ranged in thickness from 0.3 to 0.5 mm; the maximum was 0.5 ± 0.03 mm for F4, while the minimum was 0.3 ± 0.01 mm for F_6 (Table **6**). Based on these data, it was determined that the polymer's concentration and solubility affect its thickness. The thickness of the patch would rise as the concentration rose and the solubility decreased. It implies that a suitable polymer must be used to create a patch with the ideal thickness, which might delay the drug's release from the patch. The drug content of each formulation ranged from 96.7±0.12 to 99.8± 0.12 (Table **6**). This suggested that the method used to make the patches in this investigation could produce patches with consistent drug content and minimal variation. Every formulation displays the amount of moisture. To provide the compositions' strength and flexibility, moisture must be present in the formed patches. The range of the moisture content is 3.84± 0.09 to 5.84± 0.15 (Table **6**). The moisture uptake in the formulations ranges from 1.42 ± 0.17 to 2.72 ± 0.15 (Table **6**). All the data revealed that the patches were uniform, as it was proven by the SD value.

4.8. *In vitro* Skin Permeation Study Results

The findings of *in-vitro* drug release examinations of Transdermal patches are displayed in the Table **6**. To predict

the reproducibility of the rate and duration of drug release, drug release studies are necessary. The significance of polymer dissolution in drug release from matrices has been recognized as essential for achieving sustained-release efficacy. The drug release of formulations F_1-F_9 ranged from 3.58% to 47.36%. The amount of release of drugs from a variety of formulations was found to differ. Among all formulations, Formulation F6 had the highest drug release percentage. The *in vitro* drug release findings (Table **7**) indicated that CMLBG had the maximum release rate, whereas LBG demonstrated the lowest among the herbal extract patches. The results indicated that the release of the drug from the patches increases with increasing CMLBG concentration. The percent of drug release in 300 min was noted. The drug release was found to increase with the increasing concentration of carboxymethylated locust bean gum in the polymer matrix. LBG exhibited better sustained drug release behaviour compared to LBG. This is because the dissolution of CMLBG in the polymer matrix leads to the formation of gelatinous pores. The formation of such pores leads to a decrease in the mean diffusion path length of drug molecules as they are released into the diffusion medium.

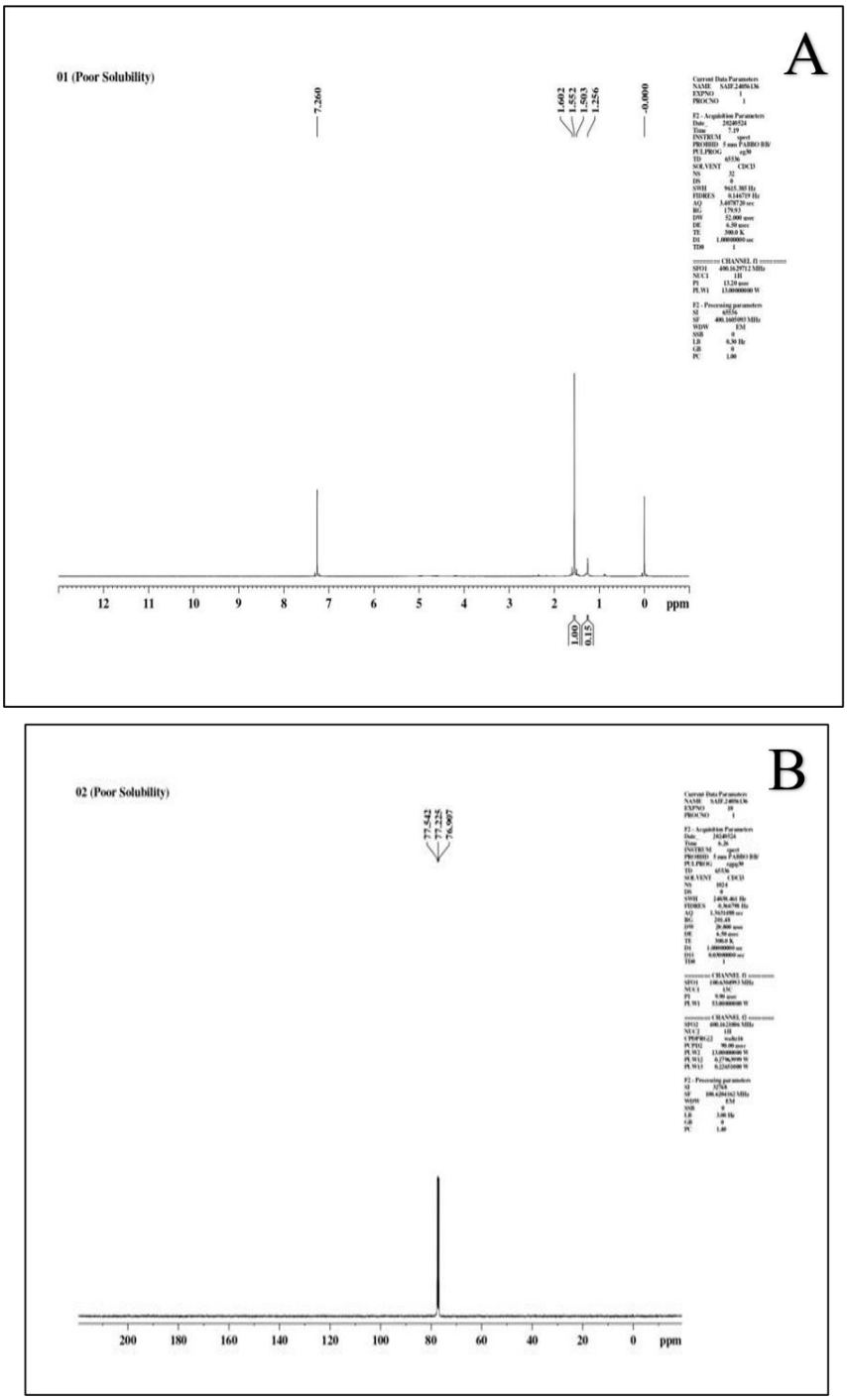

Fig. (6). $_{13}$C NMR study (**A**). LBG (**B**). CMLBG.

Table 4. Standard concentration with absorbance of frusemide.

S. No.	Concentration (µg/ml)	Absorbance
1.	0	0
2.	10	0.118
3.	20	0.234
4.	30	0.354
5.	40	0.465
6.	50	0.589

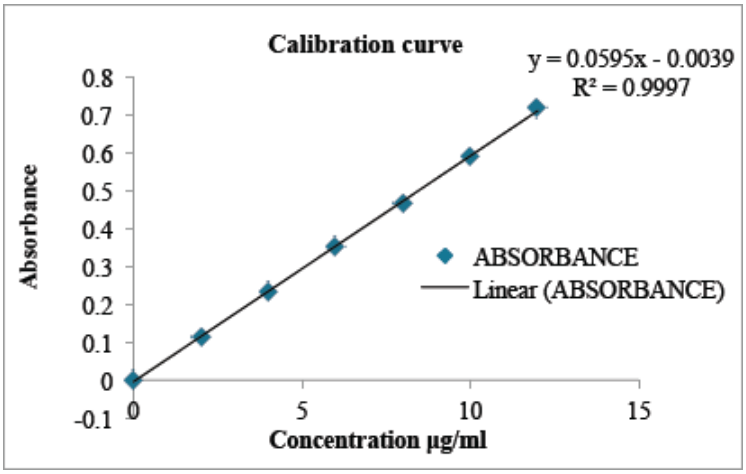

Fig. (7). Standard curve of furosemide. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 5. Physiochemical properties of prepared transdermal patches.

Formulation Code	Flexibility	Smoothness	Transparency	Stickiness	Folding Endurance	Colour
F_1	Flexible	Silky	Opaque	Not sticky	≥99	White
F_2	Flexible	Silky	Opaque	Not Sticky	≥90	White
F_3	Flexible	Silky	Opaque	Not Sticky	≥95	White
F_4	Flexible	Silky	Opaque	Not Sticky	≥86	White
F_5	Flexible	Silky	Opaque	Not Sticky	≥105	White
F_6	Flexible	Silky	Opaque	Not Sticky	≥96	White
F_7	Flexible	Silky	Opaque	Not Sticky	≥83	Green
F_8	Flexible	Silky	Opaque	Not Sticky	≥81	Green
F_9	Flexible	Silky	Opaque	Not Sticky	≥86	Green

Table 6. Thickness, drug content, moisture content and moisture uptake of prepared transdermal patches.

Formulation Code	Thickness ± SD, *n* = 3	Drug Content (%) ± SD, *n* = 3	Moisture Content (%) ± SD, *n* = 3	Moisture Uptake (%) ± SD, *n* = 3
F_1	0.4 ± 0.01	99.8 ± 0.12	5.89 ± 0.08	2.39 ± 0.12
F_2	0.3± 0.02	98.6± 0.15	3.84± 0.09	2.72± 0.15

(Table 6) Contd…

Formulation Code	Thickness ± SD, *n* = 3	Drug Content (%) ± SD, *n* = 3	Moisture Content (%) ± SD, *n* = 3	Moisture Uptake (%) ± SD, *n* = 3
F_3	0.3± 0.02	98.2± 0.16	5.66± 0.12	2.31 ± 0.14
F_4	0.5± 0.03	97.9± 0.14	5.25± 0.15	2.39± 0.15
F_5	0.4± 0.01	98.6± 0.14	5.74± 0.13	1.42± 0.17
F_6	0.3± 0.01	97.1± 0.15	5.84± 0.15	2.56± 0.12
F_7	0.5± 0.01	96.7± 0.12	3.93 ± 0.15	2.67± 0.13
F_8	0.4± 0.04	99.2± 0.15	3.41± 0.17	1.82± 0.15
F_9	0.4± 0.02	98.1± 0.14	4.91± 0.13	1.70 ± 0.12

Table 7. ***In-vitro*** **skin permeation study of prepared patches in franz diffusion cell.**

Time (min)	% Drug Release in Minutes								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
0 min	0	0	0	0	0	0	0	0	0
30 min	5.25	5.2	4.2	6.1	4.25	5.33	3.58	5.1	3.98
60 min	12.47	13.44	11.02	11.3	15.24	12.77	15.25	11.09	10.96
120 min	20.48	22.96	16.66	19.01	26.14	16.25	16.17	17.96	16.32
180 min	28.14	31.11	23.13	25.52	31.92	28.01	21.45	24.8	21.17
240 min	33.15	40.26	29.47	31.36	38.21	39.37	25.84	30.21	27.21
300 min	41.86	42.87	35.41	38.21	44.15	47.36	31.47	37.01	33.5

4.9. Zero Order Kinetics

In the formulation of herbal transdermal patches, the drug release kinetics were assessed by calculating the R2 value for each kinetic model, including zero-order, first-order, Higuchi's, and Korsmeyer-Peppas models, as shown in Table **8**.

The release characteristics of all prepared formulations were studied *in vitro* and compared. The results were given in Table **6**. Based on these results, F_1, F_2, F_5, and F_8 were selected as the optimized formulations. The *in vitro* release data for F_1, F_2, F_5, and F_8 formulations were well fitted to zero-order Fig. (**8**) and first-order equations, and the Korsmeyer-Peppas and Higuchi models were also applied to test the re-lease mechanism; results are shown in Table **7**. The drug transport of all the 9 formulation Super case-II transport.

4.10. Flux of *in-vitro* Study

Table **9** and Fig. (**9**) display the transdermal-patch fluxes per cm^2, normalized for the patch's application area. As anticipated, the SAS procedure's analysis of the equivalence shows that the two patches cannot be regarded as equivalent when the fluxes are determined using the surface unit ($g/cm^2/h$), but if the patch's entire surface is taken into consideration, the confidence interval falls within the range suggested by the EMA guideline.

4.11. *In-vivo* Activity Results

The urine volume, diuretic effect, and diuretic activity are given in Tables **10** and **11**, and in Figs. (**10** and **11**). During the 5 and 12-hour periods, the total urine volume was measured for the Normal Control, Standard Control (Frusemide 5 mg) administered orally, CMLBG with Frusemide patches, and non-aqueous extract patches. The results showed that non-aqueous extract patches had a modest diuretic effect compared with the standard control (Frusemide 5 mg) administered orally at 5 and 12 hours, and that the effect was dose-dependent. CMLBG with Frusemide patches also showed diuretic activity.

5. DISCUSSION

The current work demonstrates the effective development of transdermal patches containing *Lactuca sativa* extract and carboxymethylated locust bean gum (CMLBG), an optimized formulation, indicating the practicability of chemically altered galactomannans in controlled transdermal drug delivery. The overall results confirm that carboxymethylation can greatly improve the physicochemical properties of LBG, allowing it to design a stable, flexible, and sustained-release herbal transdermal system.

5.1. Carboxymethylation Effect on Polymer Structure/ Function

The FTIR and the $_{13}C$ -NMR tests identified the nature of introduction of (CH_2-COO) groups in the LBG backbone, as per the two-step process of alkaline substitution. The emergence of typical carboxylate bands at approximately 840 cm^{-1} (C-O-C bond), the intensified -CH^2 band at 1396 cm^{-1} and other NMR signals at 76-78 ppm all suggest the

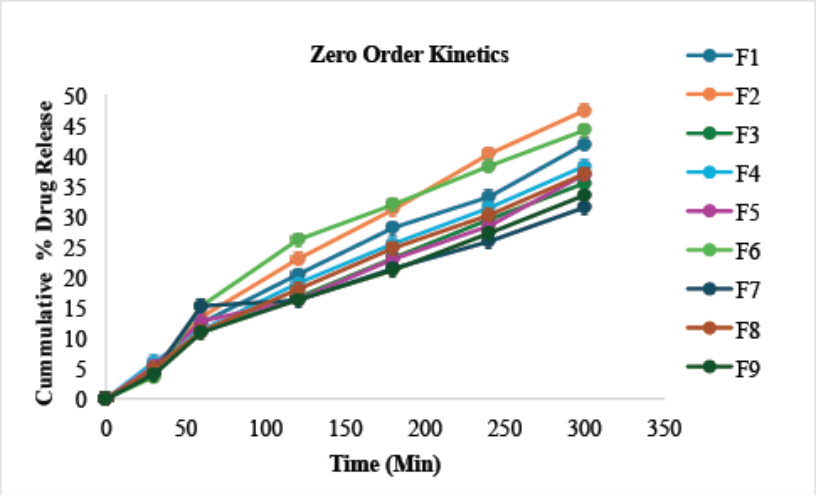

Fig. (8). Zero-order kinetics release of F_1-F_9 formulations. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 8. ***In-vitro*** **drug release kinetic of all formulations.**

Formulation Code	Zero Order r^2	Firstorder r^2	Higuchi r^2	Korsmeyer-peppas		Drug Transport Mechanism
				r^2	N	
F_1	0.999	0.9924	0.9531	0.9964	1.2407	Super case-II transport
F_2	0.9754	0.9984	0.9592	0.9956	1.1795	Super case-II transport
F_3	0.9896	0.9939	0.9592	0.996	1.1518	Super case-II transport
F_4	0.9539	0.9835	0.9586	0.9485	1.3731	Super case-II transport
F_5	0.9795	0.9968	0.966	0.9908	1.0402	Super case-II transport
F_6	0.9879	0.9793	0.9512	0.9969	1.0366	Super case-II transport
F_7	0.9881	0.991	0.9569	0.9907	1.1594	Super case-II transport
F_8	0.9834	0.9965	0.9617	0.999	1.104	Super case-II transport
F_9	0.9269	0.9463	0.953	0.9304	1.0358	Super case-II transport

Note: Where < 0.5 = Fickian diffusion, 0.5<n<1.0 Anomalous transport, 1.0 = Case-II transport and Higher than 1.0 Super case-II transport.

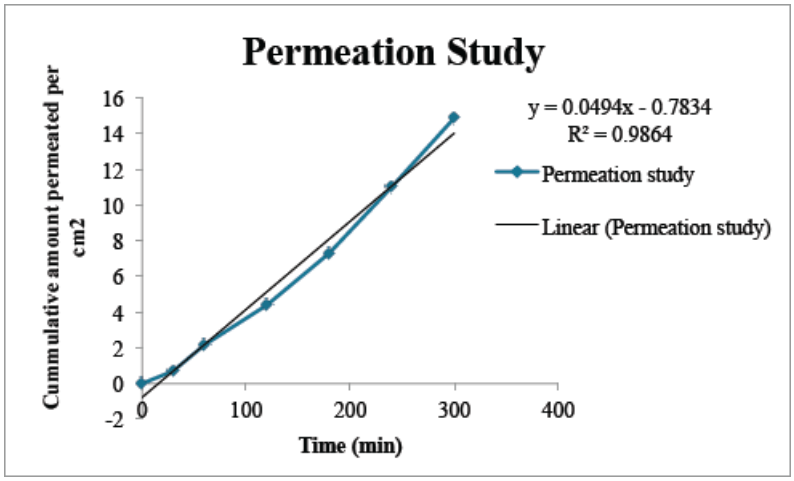

Fig. (9). Graph showing permeation study. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 9. Parameters of flux.

Area of diffusion cell	1.76 cm^2
Slope	0.0118
Flux	0.0494 µg/cm^2 /min
Amount of drug taken	5 mg
Permeability constant	0.0988 cm/min

Table 10. Effect of *Lactuca sativa* extract patches and Frusemide on urine excretion after 5 hours.

S. No.	Group	Urine Volume (ml) after 5 Hours
1.	Group 1- Normal Control	3.85 ± 0.1
2.	Group 2- Standard Control (Furosemide 5mg) orally	8.56 ± 0.25
3.	Group 3- CMLBG with Frusemide patches	7.2 ±0.23
4.	Group 4- Non-aqueous Extract patches	6.25 ± 0.2

Note: Value represented in SEM, where $n = 6$.

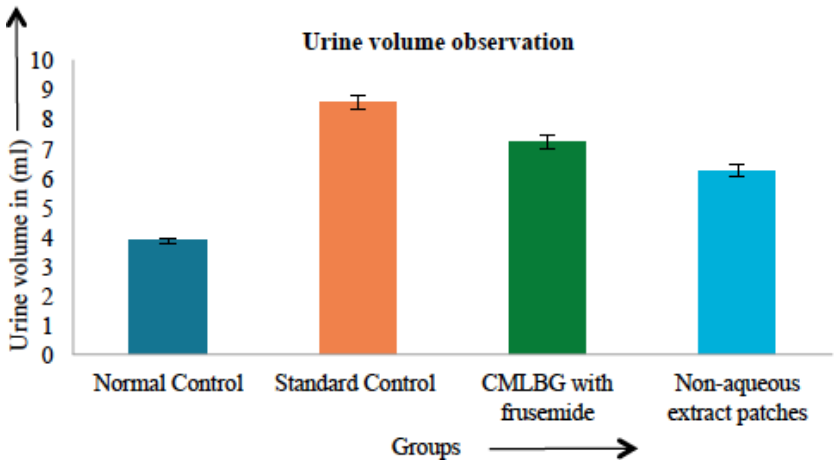

Fig. (10). Urine level observations after 5 hours. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

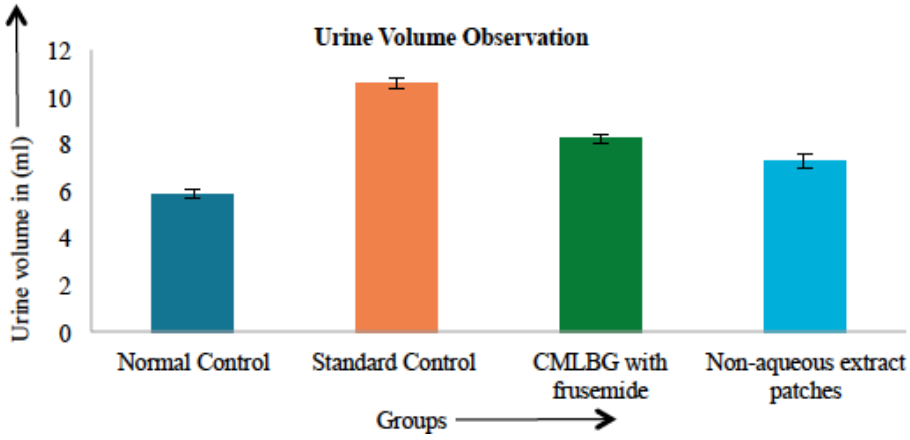

Fig. (11). Urine level observations after 12 hours. *(A higher resolution / colour version of this figure is available in the electronic copy of the article).*

Table 11. Effect of *Lactuca sativa* extract patches and Furosemide on urine excretion after 12 hours.

S. No.	Group	Urine Volume (ml) after 12 Hours
1.	Group 1- Normal Control	5.85 ±0.2
2.	Group 2- Standard Control (Furosemide 5mg) orally	10.56 ±0.34
3.	Group 3- CMLBG with Frusemide patches	8.2 ± 0.21
4.	Group 4- Non-aqueous Extract patches	7.25 ± 0.3

Note: Value represented in SEM, where $n = 6$.

achievement of structural alteration. These spectroscopic variations are consistent with other researchers who have studied carboxymethylated galactomannans and natural polysaccharides and have also observed similar changes in functional groups and reported improved hydrophilicity after modification. As an illustration, Gong *et al.* 2012 [5] reported almost the same FTIR and NMR shifts for carboxymethyl guar gum, allowing confirmation that the reaction mechanism and the structural results observed in the current study can be compared with the literature. Similarly, carbonyl signals and enhanced solubility in modified polysaccharides were observed by Silva *et al.* (2004) [7], which corroborate the structural implications in the present study.

Carboxymethylation had significant positive effects on the hydration, swelling, and solubility of the polymer- properties that are vital in transdermal activity. This agrees with previous reports that CMLBG derivatives have superior water-binding capacity and rheological behavior, resulting in stronger, more permeable gel matrices. These properties play a vital role in preserving the channels for drug diffusion and release during prolonged applications.

5.2. Physicochemical Sensitivity of Transdermal Patches

All formulations have desirable film properties, such as a smooth texture, sufficient strength, opacity, and non-stickiness. The folding endurance (81-105) indicates good mechanical integrity to be used in the application. The difference in thickness (0.3-0.5 mm) was mainly associated with changes in polymer concentration and demonstrates that CMLBG enhances film formation even at a relatively lower concentration.

The consistency of the drug content across all patches (96.7 to 99.8) indicates the accuracy of the solvent-casting method. The values of moisture content and moisture uptake were acceptable and supported the desirable flexibility, inhibited a brittle nature, and inhibited microbial growth. These findings are similar to earlier examples of natural-polymer-based transdermal systems that place low moisture stress as a sustainability criterion.

These outcomes align well with other studies that used natural or modified gums to fabricate transdermal films, showing similar ranges of thickness uniformity, moisture behavior, and mechanical strength. The consistency in drug content across formulations further supports the reliability of the solvent-casting method employed.

5.3. *In-vitro* Permeation Behavior and Release Mechanism

The results of the *in-vitro* permeation underscore the role played by CMLBG in enhancing the diffusion of drugs. Formulation F6, which had the highest concentration of CMLBG, showed the greatest cumulative release (47.36% at 300 min). Increasing the CMLBG concentration increased drug release, as hydrated porous channels formed within the polymer matrix. On the other hand, LBG-based patches demonstrated a slower release rate, which confirmed again that the limitations of solubility and swelling limit the drug transport of original gums.

Kinetic modeling showed a high level of linearity with zero-order, first-order, and Higuchi plots ($r^2 = 0.951099$), with all formulations based on Super Case-II transport ($n > 1$). This mechanism means that drug release is controlled by a combination of polymer relaxation, swelling, and erosion, rather than solely by diffusion. This is the behaviour of a chemically modified hydrophilic polymer in controlled-release matrices.

The flux value (0.0494 $\mu g/cm^2/min$) and permeability constant (0.0988 cm/min) indicate that the patches have sufficient driving force for transdermal permeation. The results can be compared with the published fluxes from previous literature on natural polymer-based transdermal systems, and hence, we can deduce that CMLBG is a good permeation-modulating polymer. The results of Ngwuluka *et al.* 2015 [30] with the native gum mix or the altered mix showed similar positive changes in permeation results, suggesting that CMLBG can exhibit the same or better behavior without synthetic polymer blends.

Similar results have been reported in the literature, showing that carboxymethylated gums continue to exhibit enhanced hydration and diffusion properties compared to their native counterparts. Both Pushpamalar *et al.* 2006 [31] and Gong *et al.* 2012 [5] reported enhanced water uptake rates and diffusion kinetics in carboxymethylated polysaccharides, confirming the release characteristics observed in the current study.

5.4. *In-vivo* Diuretic Activity

The *in-vivo* experiments supported that both CMLBG-furosemide patches and *Lactuca sativa* extract patches had notable effects of diuretic activity in comparison to the normal control. Extract patches (6.25 ± 0.2 ml) and CMLBG-furosemide patches (7.2 ± 0.23 ml) had significant urine output after 5 hours, but were still less than furosemide given

orally (8.56 ± 0.25 ml). At 12 hours, the same trend was noted, indicating a prolonged diuretic effect. Transdermal patches produce a continuous response, presumed to result from slow drug absorption and a longer exposure of the body to the drug. This trend corresponds to well-established pharmacokinetic concepts reported in transdermal drug delivery studies, in which steady dermal absorption reduces fluctuations in drug concentration and facilitates prolonged pharmacodynamic effects [32].

The diuretic effect of *Lactuca sativa* is supported by traditional medicine, which proposes that the plant has slight diuretic, detoxifying, and anti-inflammatory properties [33, 34]. The moderated, non-acute diuresis response is indicative of its potential as a safer drug for long-term fluid management, without the risk of electrolyte imbalance commonly associated with loop diuretics.

The increased efficacy of patches relative to control serves to confirm that transdermal delivery partially avoids gastrointestinal degradation and first-pass metabolism, thus improving bioavailability which is a commonly cited benefit of non-polar herbal drugs otherwise poorly absorbed orally.

5.5. Mechanistic Effect of *Lactuca sativa* Showing Diuretic Activity

Among the active compounds of *Lactuca sativa* are flavonoids, phenolic acids, and potassium salts, which can confer diuretic properties through different mechanisms. Flavonoids and phenolics also have antioxidant and vasodilatory action, which in turn could ameliorate microcirculation of the kidney, thus increasing GFR [35]. Sesquiterpene lactones and coumarins also mildly inhibit tubular sodium and chloride reabsorption, which leads to an increase in natriuresis followed by diuresis. The presence of natural potassium salts supports osmotic diuresis, increases tubular osmolarity, and decreases water reabsorption [9, 36]. Moreover, *Lactuca sativa* possesses anti-inflammatory as well as nephroprotective activity, decreasing tubular oxidative stress, thus contributing to the maintenance of urine flow [11, 37]. Both mechanisms account for the mild but significant diuretic response observed with the transdermal formulation in the current investigation.

6. STUDY LIMITATIONS

The current research has some limitations, which must be taken into account when interpreting the results. Even the *in-vitro* permeation experiment was based on an egg membrane, which is not a complete mimic of the permeability of the human skin. The *in-vivo* was done in a short period with a small group of samples, and the long-term safety, dermal irritation, and pharmacokinetic parameters were not studied. Also, the variability of the composition of the herbal extract and the lack of studies on its stability can affect the reproducibility of the formulation and shelf life. To validate the information on the broader scope of the developed transdermal system, further evidence, including a standardized extract, extensive pharmacokinetic analysis, long-term safety analysis, and clinical validation, is needed.

CONCLUSION

The present study determined the diuretic properties of transdermal patches prepared using carboxymethylated locust bean gum (CMLBG) as *Lactuca sativa* leaf extract. CMLBG was prepared through the optimization of reaction parameters. The functional presence of the O-carboxymethyl group in the locust bean gum structure was proven by FTIR and NMR spectroscopy. The solvent-casting technique was used to prepare matrix-type transdermal patches with different HPMC/ethyl cellulose/CMLBG compositions. The super case-II transport release kinetics were demonstrated by physicochemical characterization. *In vivo* testing in albino rats showed a significant improvement in the diuretic effects of *Lactuca sativa* extract patches compared to the control group. The findings suggest that chemically modified natural polymers used to develop *Lactuca sativa* transdermal patches have the potential to serve as controlled, long-term-release and diuretic delivery systems and offer greater benefits, with longer-acting, milder bioactivity compared with synthetic diuretics.

FUTURE DIRECTIONS

Future initiatives must characterize CMLBG-based matrices more thoroughly to determine the impact of hydration, swelling, and structural changes on controlled drug release. The *in-vivo* pharmacokinetic investigations should be expanded in order to reveal system absorption, controlled-release, and long-term safety. Additional improvements in carboxymethylation conditions and polymer mixtures can result in better mechanical strength and permeation efficiency. Analysis of herbal patches made from CMLBG regarding dermal safety, stability, and scalability will also be necessary to translate them into potentially clinically viable transdermal systems.

AUTHORS' CONTRIBUTIONS

EU, PU conceptualized the work. All authors participated in the laboratory work. EU, HV curated the data. EU, SU, and HV carried out the statistical analysis, and PU supervised the Research Work. SU Co- Supervise the Research Work. EU and HV wrote the original draft. The final draft was proofread, edited, and approved by all authors.

LIST OF ABBREVIATIONS

$_{13}$CNMR	=	Carbon-13 Nuclear Magnetic Resonance
CMLBG	=	Carboxymethylated Locust Bean Gum
CPCSEA	=	Committee for the Purpose of Control and Supervision of Experiments on Animals
FTIR	=	Fourier Transform Infrared Spectroscopy
HPMC	=	Hydroxypropyl Methylcellulose
LBG	=	Locust Bean Gum
MCA	=	Monochloroacetic Acid
NaOH	=	Sodium Hydroxide
NMR	=	Nuclear Magnetic Resonance Spectroscopy
PEG 400	=	Polyethylene Glycol

TDDS = Transdermal Drug Delivery Systems

UV = Ultraviolet Visible Spectroscopy

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The CPCSEA, New Delhi, India, Government of India, of IFTM University Registration no. 837/ac/04/2004/ CPCSEA, examined the application submitted to the Institutional Animal Ethical Committee (IAEC), authorized the experimental protocol (resolution number 2023/837/04M. Ph./12) for animal-based experiments, and provided the Approval number IAEC/2023/25/12.

HUMAN ANIMAL RIGHTS

All animal experimentation was performed in accordance with CPCSEA-approved guidelines.

This study adheres to internationally accepted standards for animal research and follows the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Data supporting the findings of this study are available from the authors on reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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