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FORMULATION AND *IN-VITRO* CHARACTERIZATION OF MUCOADHESIVE PATCHES OF ROOT EXTRACT OF *ADENIUM OBESUM*

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ABSTRACT

Patches are punched from a sheet that serves as the intermediate product. Oral mucosal medication delivery alternatives to tablets have been studied, including polymers like cellulose etc. can be used to create mucosal patches. The present research was based on the development and *in-vitro* characterization of mucoadhesive patches of root extract of *Adenium obesum* utilizing different polymers. *Adenium obesum* (fresh roots) was obtained from the Unnao region. Eudragit L-100, Propylene glycol, Tween 80, Methanol, Ethanol and HPMCK 4M & HPMCK 15M were purchased from the local market. The initial stages of processing involve grinding the roots into a coarse powder. The Soxhlet equipment was used to extract the powder and then weigh it. After preformulation study, the *Adenium obesum* mucoadhesive patches were created using the solvent casting technique. Patches were evaluated for physical appearances, thickness, pH, % drug content, in-vitro drug release, SEM and stability. In results, an excellent % drug release was demonstrated by the buccal patch. In order to estimate stability profile of mucoadhesive patches, it was evaluated for physical appearance. In conclusion, patches i.e., F2, F3 and F6 exhibited a significant estimation parameters like drug content, % drug release etc. The buccal drug of herbal *(A. obesum)* mucoadhesive patch has shown improved stability. It will ease to provide the long-lasting action by suppressing the symptoms of insomnia, agitations etc. Therefore, it may be produced commercially after its successful clinical trials.

Keywords: Mucoadhesive patch, *Adenium obesum*, solvent casting method, extraction and evaluation.

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INTRODUCTION

Patches are punched from a sheet that serves as the intermediate product. Backing membrane regulates the drug release and prevent the device from becoming deformed or disintegrating while it is in the mouth. Oral mucosal medication delivery alternatives to tablets have been studied, including polymers like cellulose etc. can be used to create mucosal patches (Verma et al., 2017). Delivery of drugs involves a process of providing a pharmacological chemical to have a beneficial effect in people/animal (Tiwari et al. 2012). A well-designed Novel Drug Delivery System can become a significant step forward in addressing challenges connected to drug delivery at a specific location and at a specific pace (Bhagwat and Vaidhya, 2012; Patel et al. 2013)-

Although Adenium obesum species first appeared in Africa, they have since spread throughout the rest of the tropics and subtropics. Several species of the preferred plant can be found in Oman. The Sultanate of Oman, for instance, is home to the desert rose. All parts of a given species are used to treat a wide range of illnesses. Several plant species have been singled out for commercial cultivation due to their medicinal value (Akhtar et al., 2017).

Taxonomy

Kingdom- Plantae
Subkingdom- Tracheobionta
Division- Magnoliophyta
Class- Magnoliopsida
Order- Gentianales
Family- Apocynaceae
Genus- *Adenium*
Species- *obesum*

Multiple chemical classes were identified in a locally cultivated AO whole plant, and the number of compounds increased with plant age (Malebo et al., 2009). Carbohydrates, flavonoids, cardiac glycosides, flavonoids, terpenoids, and pregnanes were all identified in the selected plant during the phytochemical examination (Amin et al. 2013). Stem and bark exhibited the chemical constituents as Betulin and Rosmarinic Acid. Stem showed 3,5,7,3,4,5-Hexahydroxy flavone and 5,7,3,4-Tetrahydroxy flavone whereas leaves confirmed for various chemical constituents i.e., Honghelin, Obeside-B & C (Hossain et al. 2013).

The present research was based on the development and *in-vitro* characterization of mucoadhesive patches of root extract of *Adenium obesum* utilizing different polymers.

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MATERIALS AND METHODS

Experimental Requirements

Adenium obesum (fresh roots), Eudragit L-100, Propylene glycol, Tween 80, Methanol, Ethanol and HPMCK 4M & HPMCK 15M and other types of hydroxypropyl methylcellulose, SEM, Weighing balance (digital), pH meter, Franz diffusion cell and UV-Spectrophotometer.

Collection, Authentication and Extraction of plant

A botanist was able to correctly identify and certify the fresh root of *A. obesum* that was sourced from the Unnao area. The roots are washed to remove any dust and dried in the shade or at room temperature. The initial stages of processing involve grinding the roots into a coarse powder. The Soxhlet equipment was used to extract the powder and then weigh it. Under a partial vacuum, a rotary evaporator or water bath dries the generated slurry of mixture. The yield of *Adenium obesum* extract can be determined using the formula shown below-

$$\%\,\text{Yield} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\%$$

Pre-formulation determination

To manufacture or construct stable, safe, and therapeutically effective and efficacious dosage forms, pre-formulation investigations are conducted prior to formulation development and focus primarily on characterizing the pharmacological substance.

Drug and excipients compatibility

To check for any changes in the drug's chemical composition following its combination with the excipients/polymers. The *A. obesum* extract mixed with potassium bromide was applied and pressed into the shape of a disc. The disc was examined using Shimadzu FTIR spectroscopy (4000-400cm-1).

Solubility

The solubility of the drug was determined by placing a small amount of it (about 1-2 mg) individually in a test tube, adding 5ml of solvent (water, ethanol, propylene gycol, 0.1N HCl, chloroform & 7.4 pH buffer), shaking vigorously, and holding for a while. Take note of the product's solubility in various solvents when it is at room temperature.

Preparation of Standard Calibration Curve

A stock solution is created by properly weighing 100 mg of *Adenium obesum* extract, dissolving it in 2 ml of methanol, and then adding 0.1 N HCl solution to bring the amount up to 100 ml. To create the 100 g/ml concentration solution, stock solution (10ml) is further diluted with 0.1 N HCl (pH 1.2) in 100 ml. Then, to prepare 2g, 4g, 6g, 8g, and 10g of drug/ml solution, 0.2, 0.4, 0.6, 0.8, and 1 ml of solution are taken in a 10 ml standard

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volumetric flask and the volume is increased to 10 ml with 0.1N HCl. The absorbance is then measured at 270 nm in a UV spectrophotometer using 0.1 N HCl as a blank. Repeating the process with phosphate buffer at pH 6.8, absorbance is measured at 271 nm (Reddy et al. 2019).

FORMULATION OF MUCOADHESIVE PATCHES

Adenium obesum mucoadhesive compositions were created using the solvent casting technique. The mucoadhesive polymers HPMC K-15M, PVA, and polyvinylpyrrolidone were included in the patch formulations (PVP K-30) and cellulose acetate. To prepare different polymer ratios, various stock solutions were employed. A stock solution of HPMC K-15M at 2% by weight was prepared in distilled water, and a 2 percent w/v PVA solution was also made concentration. 1% weight-to-volume (w/v) PVP K-30 and EC stock solution was also made in ethanol and water, respectively. Formulations included combinations like HPMC/PVA/PVP and HPMC/PVA/EC. The amount of plasticizer used to create patches, either 30 ml of propylene glycol (PG), or 2ml of PEG-400 was added to the mixture of polymers above. To the alcoholic extract solution in the amount (188mg/5ml) estimated above the polymer combination which results in the buccal patch's 50mg per unit (3cm diameter) concentration being included. Homogenized drug and polymer solution was put into Teflon. Place a covered Petri dish (9.2 cm in diameter) carefully on a flat surface. A glass funnel was placed over the Petri dish mould to guarantee even evaporation. The contents of the Petri dishes were first dried at ambient temperature for two hours and then dehydrated for 48 hours in a hot air dryer at 50ºC. The dried patches were arranged in a sphere taken from the mould and visually checked for any distortion. next buccal from the circular disc, patch units (3cm in diameter) were cut, resulting in one patch unit per disc possibly 100 mg of herbal extract. After that, each patch was sealed in aluminum foil and kept in a desiccator.

Table 1. Composition of *A. obesum* buccal patches

Formulation	PEG400 (ml)	HPMC K15M (ml)	PVA (2%) (ml)	EC (1%) (ml)	*A. obesum* (mg)
F1	2	10	10	10	100
F2	2	11	11	8	100
F3	2	12	12	7	100
F4	2	13	13	6	100
F5	2	14	6.5	5	100
F6	2	15	5	4	100

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EVALUATION

Physical appearance

All the formulated mucoadhesive patches were observed for physical appearances i.e., shape, thickness etc. (Tirunagari et al. 2014).

Weight variation

To ensure that the weight variations between patches fall within acceptable parameters, we calculated and compared their individual densities.

Swelling index

Patch was weighed (W), put in a 2% w/v agar gel plate, and allowed to sit for one hour at 37°C. The patch was taken from the petri plate at regular intervals of one hour (up to 3 hours) and any excess surface water was gently blotted away with tissue paper. The swelling index was then determined using the formula after the swelled patch was reweighed (W1)-

$$\% \text{ Swelling index} = W_1\text{-}W/W \times 100$$

Folding endurance

The buccal patch's folding strength is evaluated by eye. The films are split out by cutting a strip of them uniformly and repeatedly folding it in the same spot.

Thickness

Using a standard screw gauge, the thickness of three randomly chosen patches from each batch was measured.

Percentage moisture

Each patch is weighed before being stored for 24 hours in a desiccator. Up until a constant weight is attained, the patches are reweighed. A formula is used to determine the moisture content in percentage terms based on the difference between the beginning and constant end weights.

Drug content

Cut a small portion of the patch, and then dissolve it in a 7.4 pH PBS solution. After adding the solvent ethanol to make the polymer soluble (pH 7.4), the volume is adjusted to 100 ml with PBS. After that, 1 ml is removed from the solution and diluted to a final volume of 10 ml. The concentration of a solution can be calculated by measuring its absorbance at a wavelength of 270 nm. This allows for the calculation of the medication concentration (Nafee et al. 2003).

In-vitro drug release

A Franz diffusion cell was put on the membrane, and a buccal batch was created. 15.0 ml of PBS pH 7.4 are added to the receiver compartment of the diffusion cell, and the mixture is kept there over a magnetic stirrer with the temperature held at 37°C. Every 1, 2, 3, 4, 6 and 12 hours, a sample of 3ml is promptly removed and replenished from the receiver

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compartment. Before the analysis is done, they are kept in a refrigerator. The samples are subjected to UV-visible spectrophotometer analysis to determine their content. The medication concentrations are measured at 270nm wavelength (Davies & Ingham, 2020).

Surface pH

Patches were allowed to swell in agar plate for 3 hours while the agar is dissolved in simulated human saliva (NaCl, KCl, KSCN, KH_2PO_4, and urea) in 1 L of distilled water with a pH of 6.8 while being stirred. The solution is then poured into a Petri dish and allowed to gel at room temperature. A pH paper was placed on the surface of the swollen area to measure the surface pH.

SEM analysis

It was used to describe the morphology of films. Before being examined with a scanning electron microscope, samples were mounted on round brass stubs (12mm in width) using double-backed adhesive tape. Gold palladium was then sputter coated on the samples for 8 minutes at 1.1 LV under an argon atmosphere. The pictures were captured using a 35mm black and white Ilford PANF 50 film.

Stability

The optimal formulas are stored in butter paper, then wrapped with aluminum foil, and finally placed in an aluminum pouch for the duration of the stability study. For up to one month at room temperature, it is sealed at the conclusion using heat. Following the aforementioned processes, the appearance and drug content of the films taken at various intervals, such as 0–4 weeks, are examined (Khairner at al. 2009).

RESULTS AND DISCUSSION

Pre-formulation studies

Solubility

The extract of *A. obesum* was evaluated for solubility in the various solvents listed below. Both methanol and ethanol showed freely solubility of *A. obesum* extract. It was insoluble in distilled water. It was shown to be soluble in phosphate buffer, CHCl3, propylene glycol, and 0.1N HCl. Thus, it may be confirmed that *A. obesum* extract is more soluble in amphoteric solvent - ethanol compared to acidic and basic aqueous environments.

Table 2. Solubility of *A. obesum* extract

Solvent	*A. obesum* extract
Methanol	Freely soluble
0.1N HCl	Soluble
Ethanol	Freely soluble

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Distilled water	Insoluble
$CHCl_3$	Soluble
PEG	Soluble
Phosphate Buffer	Soluble

Drug excipients compatibility

Drug-excipient compatibility tests were also conducted on *A. obesum* extract, utilizing FT-IR spectroscopy both singly and in formulation.

The following is a log and demonstration of this compatibility:

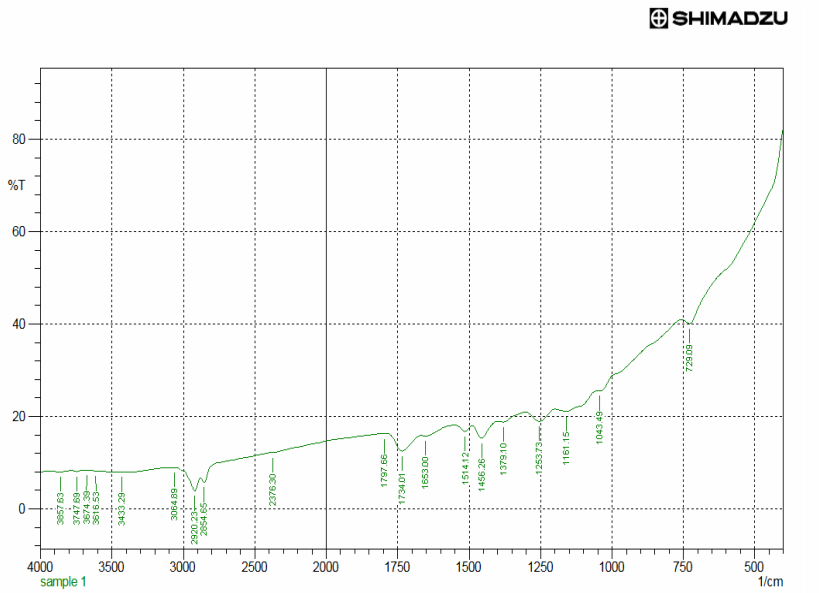

Fig 1. FTIR (spectra) of *Adenium obesum*

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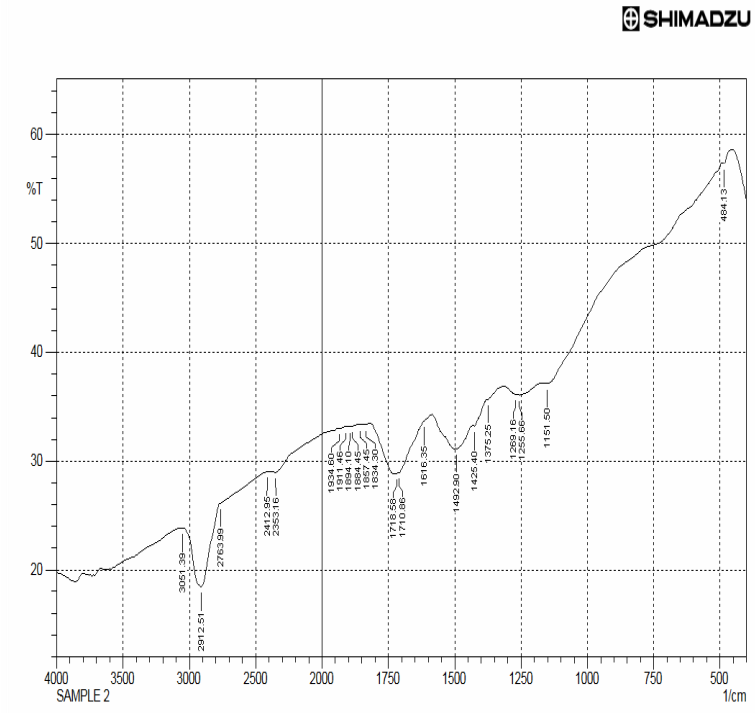

Fig 2. FTIR (spectra) of herbal extract + PEG 400

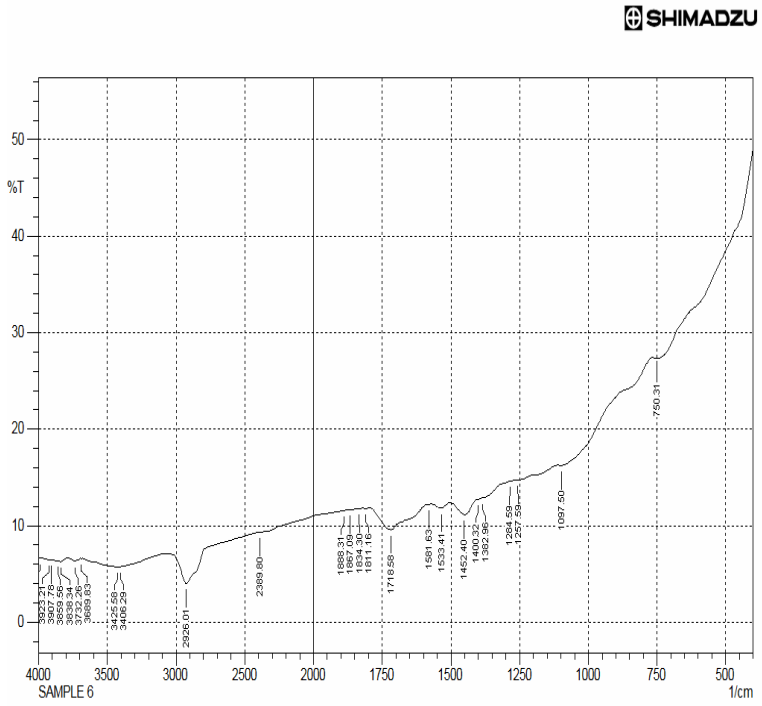

Fig 3. FTIR (spectra) of herbal extract + HPMC K-15M

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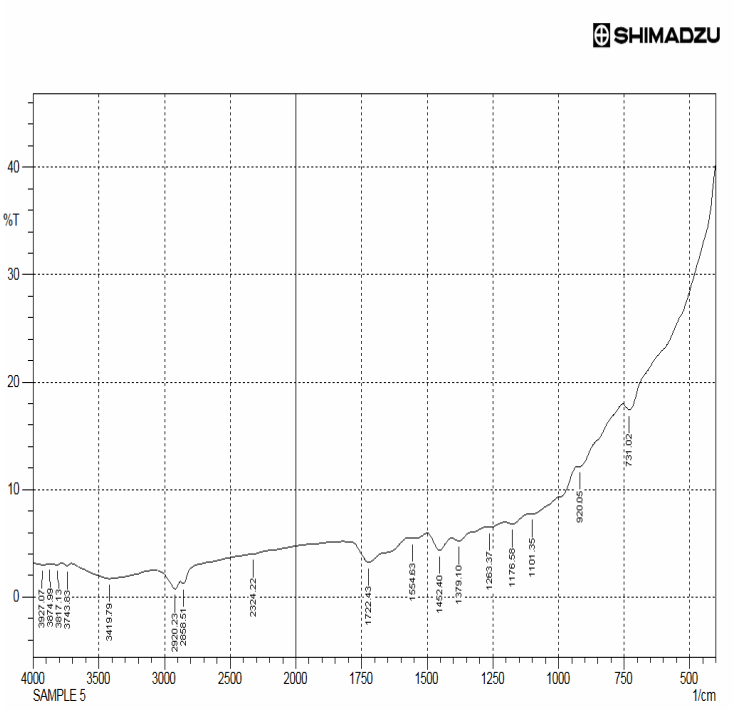

Fig 4. FTIR (spectra) of herbal extract + PVA

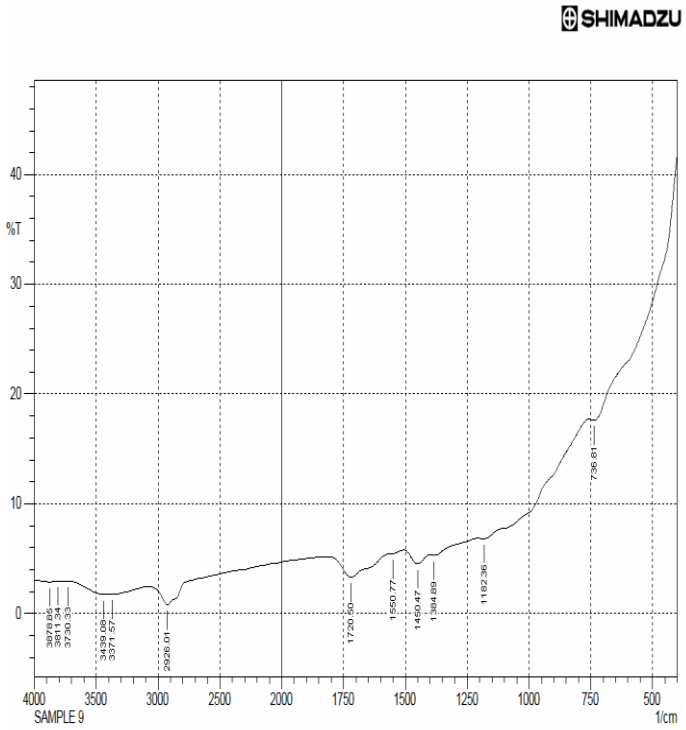

Fig 5. FTIR (spectra) of herbal extract + EC

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Standard calibration curve

The UV Spectrophotometric method was used to analyze A. obesum extract. The absorbance of the medication was measured at 274 nm in phosphate-buffered saline (pH 7.4) containing a trace quantity of methanol. The standard curve for the herbal extract in PBS at pH 7.4 was linear between 2 and 10 g/ml, starting at the origin. The curve follows Beer-Lambert's law.

Table 3. Standard calibration curve- *A. obesum*

Conc. (µg/ml)	Absorbance
2	0.13
4	0.27
6	0.45
8	0.59
10	0.76
12	0.90

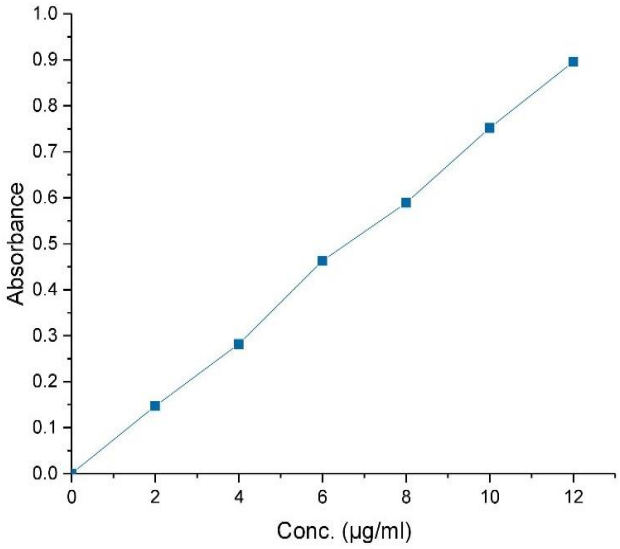

Fig 6. Standard calibration curve at pH 7.4 in PBS

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EVALUATION

Surface pH

The prepared patches (F1-F6) were studied for surface pH and demonstrated as slightly basic/acidic- near to neutral pH. Formulations in terms of patches- F2, F3 & F6 showed slightly acidic pH as 6.5± 0.27, 6.2± 0.14 & 76.8± 0.19, respectively. Whereas, F1, F4 & F5 showed slight alkaline pH when observed as 7.2± 0.15, 7.1± 0.35 & 7.2± 0.31, respectively. So, it may confirm that formulated patches were of optimum pH range for better tolerability and solubility in saliva.

Table 4. Surface pH (F1-F6)

Patch	Time (post formulation)	pH± S.D.
F1	1 hr	7.2± 0.15
F2	1 hr	6.5± 0.27
F3	1 hr	6.2± 0.14
F4	1 hr	7.1± 0.35
F5	1 hr	7.2± 0.31
F6	1 hr	6.8± 0.19

Folding endurance

Formulated mucoadhesive patches were evaluated for their folding capacity. Patches- F3 & F6 showed folding endurance as 27± 0.41 & 26± 0.36, respectively. Whereas, F4 & F5 exhibited folding endurance as 23± 0.49 & 24± 0.69, respectively. The aforementioned buccal patch formulation displayed a high degree of stiff folding endurance. Strong mechanical pressure and thrashing can be maintained. Because of its superior folding endurance, it is stable and reliable over time.

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Table 5. Folding endurance

Patch	Folding endurance± S.D.
F1	17± 0.29
F2	24± 0.49
F3	27± 0.41
F4	23± 0.49
F5	24± 0.69
F6	26± 0.36

Swelling index

It has demonstrated a remarkable swelling property when observed. Min. swelling index was seen in F2 whereas maximum swelling index was calculated in F2 (0.83± 0.27), F6 (0.84± 0.24) and F5 (0.79± 0.48). This power exhibits the concentration of polymers used for the development of herbal mucoadhesive patches.

Table 6. Swelling index study

Patch	Weight (g)	Weight after swelling (g)± S.D.
F1	0.91	0.73± 0.20
F2	0.78	0.83± 0.27
F3	0.65	0.69± 0.27
F4	0.74	0.78± 0.25
F5	0.73	0.79± 0.48
F6	0.78	0.84± 0.24

Drug content

The drug content was calculated as 74.23± 0.17, 85.13± 0.14, 78.23± 0.64 and 79.25± 0.4 in F1, F3, F4 & F5, respectively. Some patches showed remarkable and almost identical drug content: F2 (81.32± 0.23) and F3 (81.41± 0.38). It has efficient in vitro drug release that represents for better solubility, drug-excipient co-adhesion, and uniformity of drug content.

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Table 7. Drug content

Patch	Drug content± S.D.
F1	74.23± 0.17
F2	81.32± 0.23
F3	85.13± 0.14
F4	78.23± 0.64
F5	79.25± 0.4
F6	81.41± 0.38

Measurement of muco-adhesive strength

The patch formulation, which included several different types of mucoadhesive polymers, demonstrated outstanding adhesion to the mucosal surface. It aids in formulation stability development as well. The mucoadhesive patch containing the A. obesum extract is released in the appropriate solvent medium via a combination of polymers and excipients. The highest mucoadhesive strength was noted in formulations F3, F5 and F6 as 4.36± 0.18, 4.46± 0.39 and 4.19± 0.43, respectively.

Table 8. Measurement of mucoadhesive strength

Preparation	F1	F2	F3	F4	F5	F6
Muco-adhesive Strength	2.95± 0.23	3.67± 0.31	4.36± 0.18	3.19± 0.32	4.46± 0.39	4.19± 0.43

Determination of thickness

Thickness plays an important role in drug release and its action. Thickness was found as 0.43±0.27 and 0.49±0.30 in F1 & F6 respectively. Thickness of patches was estimated highest in patch- F2, F3, F4 & F5 as 0.51±0.23, 0.53±0.31, 0.52±0.26 & 0.56±0.62, respectively.

Table 9. Determination of thickness

Patch	Thickness± S.D.
F1	0.43± 0.27
F2	0.51± 0.23
F3	0.53± 0.31

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F4	0.52± 0.26
F5	0.56± 0.62
F6	0.49± 0.30

Physical appearance

The mucoadhesive patch F1- F6 were observed as colourless and smooth surfaces.

Determination of weight variation

Formulated patches were estimated for its weight variation. A negligible weight variation exhibits a better formulation. Weight was found as 0.37± 0.40g and 0.36±0.60g in F1 & F5, respectively. Weight of patches was estimated highest in patch- F2, F3, F4 & F6 as 0.34± 0.32, 0.31±0.26g, 0.38±0.27g &0.39±0.37g, respectively.

Thickness of patches were shown in below table-

Table 10. Determination of weight variation

Patch	Weight± S.D.
F1	0.37± 0.40
F2	0.34± 0.32
F3	0.31±0.26
F4	0.38±0.27
F5	0.36±0.60
F6	0.39±0.37

SEM Analysis

SEM was done of formulation F6 with 500X magnification power lens and observed as given figure as below. It showed that the formulated mucoadhesive patches were of significant quality in terms of surface's smoothness that indicates for drug content uniformity.

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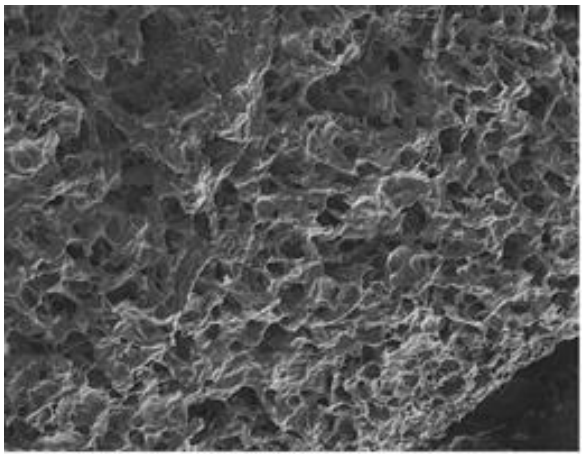

Fig 7. SEM analysis of F2

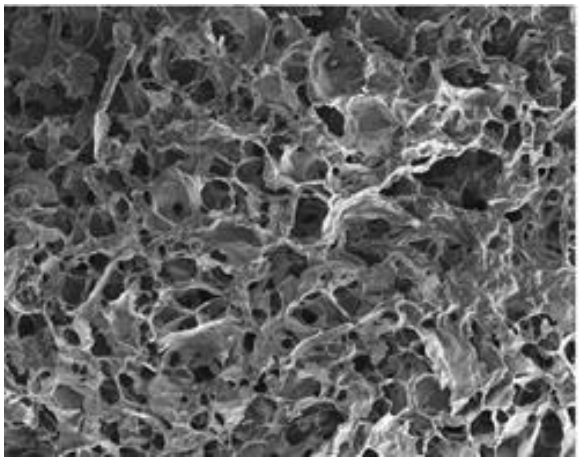

Fig 8. SEM analysis of F3

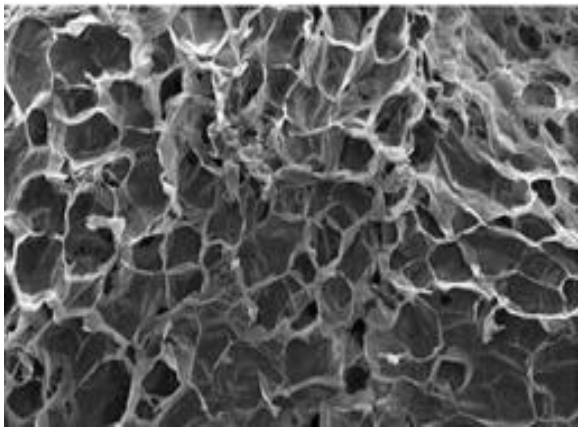

Fig 9. SEM analysis of F6

Stability

In order to determine the stability profile of mucoadhesive patches, it was evaluated for physical appearance. After 30 days of storage, physical appearance for different patches was

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found same. So, it may be concluded that mucoadhesive patch using these excipients are very much effective & stable too.

Estimation of % drug release

The in vitro drug release was estimated in the prepared mucoadhesive patches (F1-F6). It was found optimistic and near to 100% so it showed an excellent stability for API & different excipients used in.

After 6 hours, F1, F2, F3 showed % drug release as 93.1±0.2, 87.6±0.3 and 81.4±0.2, respectively. Whereas, F4 showed minimum % drug release as 74.2±0.2. While F5 and F6 showed moderate % drug release as 75.2±0.3 and 75.8±0.4, respectively that was lowest among all 6 patches.

Table 11. % drug release

Time (hr)	% Drug release± S D					
	F1	F2	F3	F4	F5	F6
1	35.1±0.2	29.3±0.2	25.3±0.4	22.5±0.3	18.2±0.4	19.1±0.2
2	44.2±0.3	35.7±0.3	33.2±0.5	35.2±0.3	29.3±0.3	30.2±0.3
3	55.3±0.2	43.4±0.7	41.1±0.2	46.2±0.4	36.1±0.2	44.1±0.4
4	66.8±0.3	65.7±0.2	57.3±0.5	56.6±0.6	54.2±0.3	52.2±0.2
5	82.3±0.5	72.4±0.3	69.2±0.4	62.5±0.3	65.1±0.2	64.5±0.5
6	93.1±0.2	87.6±0.3	81.4±0.2	74.2±0.2	75.2±0.3	75.8±0.4

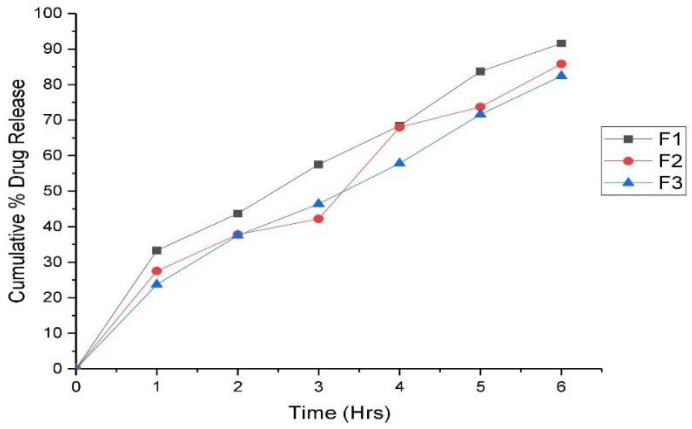

Fig 10. % drug release (F1, F2, F3)

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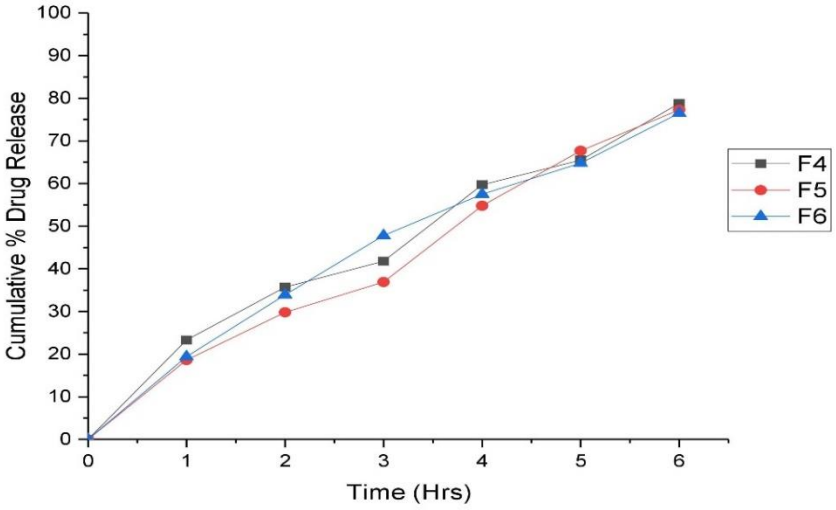

Fig 11. % drug release (F4, F5, F6)

Drug release kinetics

Formulated mucoadhesive patches were studies for their drug release kinetics. In this order, it showed Higuchi model in drug release kinetics with the slope- 28.49 & r^2- 0.963. The drug release was found maximum in F6 as 73.52%.

Table 12. Drug release kinetics

Time (hr)	Sq. root of time	Log time	Cum. % drug release	Log cum. % drug release
1	1.0	0.00	17.11	1.30
2	1.52	0.33	33.21	1.61
3	1.79	0.41	41.24	1.72
4	2.16	0.59	52.41	1.73
5	2.23	0.65	63.68	1.82
6	2.47	0.93	73.52	1.91

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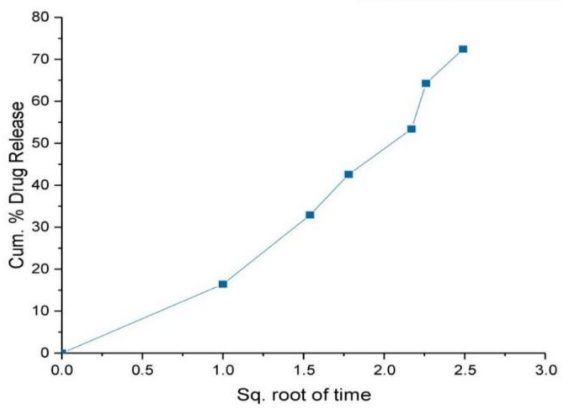

Fig 12. Cumulative % drug release (Higuchi Model)

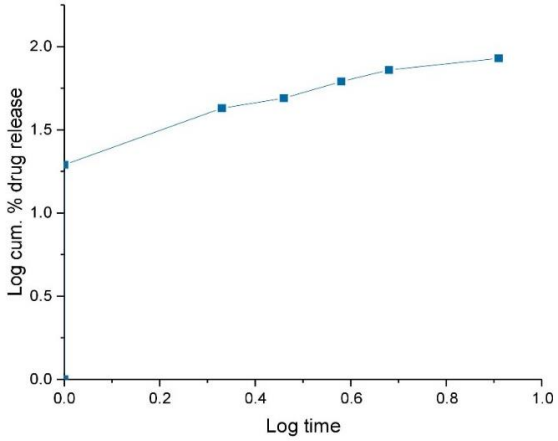

Fig 13. Log % drug release (Koresmeyer Peppas model)

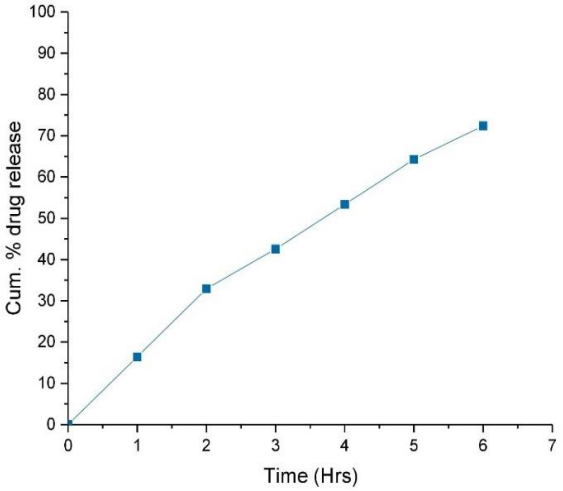

Fig 14. Cum. % drug release (Zero order kinetics)

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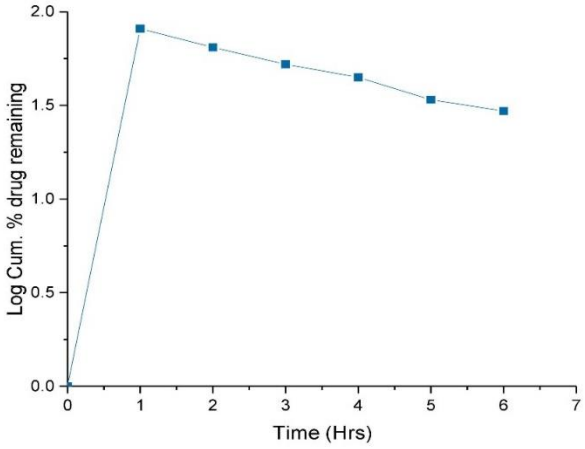

Fig 15. Log % drug release (First Order kinetics)

Swelling ability increased, but as time passed, swelling diminished, most likely due to erosion or another process. SFD permeability was boosted in in vitro drug release assays, and the medication was destroyed in higher proportions in the majority of cases. Its most Bioadhesive formulation is F6, according to ex vivo bio adhesion. Ex-vivo permeability experiments show that just a small amount of Sulfadiazine can pass through the epidermis layer, indicating that the film can be used for topical application safely. In vivo tests show that the Sulfadiazine film (F6), which was chosen owing to its physicochemical properties, promotes epidermis layer renewal and is more beneficial on epithelization, fibrous tissue thickness, and angiogenesis than the commercial entity. In terms of drug release, bioadhesive performance, physicochemical characteristics, and swelling index, mucoadhesive formulations in the form of erodible tablets have been produced to a suitable level. The HPMC polymer provided the best in-vitro drug release for up to 8 hours.

In results, an excellent % drug release was demonstrated by the buccal patch. In order to estimate stability profile of mucoadhesive patches, it was evaluated for physical appearance. After 30 days of storage, physical appearance for different patches was found same. The herbal (A. obesum) mucoadhesive patch incorporated in the mucoadhesive patches may be frequently employed in the modulation of numerous CNS activities with better absorption.

CONCLUSION

In conclusion, patches i.e., F2, F3 and F6 exhibited a significant estimation parameters like drug content, % drug release etc. It would be very beneficial for the for the management of neuropathic pain as stable mucoadhesive patches consisting herbal- *A. obesum* roots extract.

The buccal drug of herbal *(A. obesum)* mucoadhesive patch has shown improved stability. It will ease to provide the long-lasting action by suppressing the symptoms of insomnia, agitations etc. Therefore, it may be produced commercially after its successful clinical trials.

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CONFLICT OF INTEREST
None.

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