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Development of Polyherbal Buccal Films for Rapid Systemic Delivery of Therapeutic Agents

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ABSTRACT

Pain that originates from a temporary disruption of the peripheral or central nervous system is no longer classified as neuropathic pain. The present study was based on the research focuses on the formulation and evaluation of polyherbal buccal films consisting *Ocimum sanctum* and *Moringa oleifera*. Tulsi belongs to Lamiaceae family which is an upright, aromatic plant with many branches 30 to 60 cm tall, fragrant plant. *M. oleifera* (family- Moringaceae) is a deciduous tree with a trunk diameter of 45 cm (18 in) and a height of 10-12 m (33-39 ft). Fresh leaves of *Ocimum sanctum* and *Moringa oleifera* were collected from the Kanpur region, UP. Leaves were washed and dried under shade and authenticated by the Botanist. The dried leaves of *Ocimum sanctum* and *Moringa oleifera* were rendered into fine powder and extracted separately, using distilled water and ethanol (1:1) solvent system through cold maceration process. The polyherbal buccal films of *Ocimum sanctum* and *Moringa oleifera* were developed through solvent casting method and characterized the polyherbal buccal films using parameters i.e., weight variation, flatness, folding strength, moisture content, drug content determination, diffusion cell *in-vitro* model and stability studies. The results showed that the paclitaxel + polyherbal buccal film (F2) had an outstanding in-vitro release rate. After 48 hours, *in-vitro* drug release was estimated as 96.67±0.2, and 95.42±0.2 in F2 and F6, respectively which is a good indication of sustained drug release. In conclusion, the polyherbal buccal films exhibited an improved *in-vitro* release time in addition to improved looks and other characteristics, such as weight variation, flatness and spreadibility. The polyherbal buccal film is stable mucoadhesive film which is highly stable (shelf life) even for longer time of usage.

Keywords: Polyherbal buccal film, *Ocimum sanctum, Moringa oleifera,* systemic delivery.

INTRODUCTION

Ocimum sanctum (Tulsi)

Ocimum tenuiflorum belongs to Lamiaceae family of plants. It has contributed to science from ancient times to the present day in current practices because of its many restorative potentials (Cohen, 2014). Tulsi is an upright, aromatic plant with many branches. 30 to 60 cm tall, fragrant plant. The oval leaves have a basic green or purple colour, a gently serrated or dented edge, and a blade length of 5 cm. The blooms have a short, hairy stalk and are violet. The plant

produces reddish-yellow seeds and little fruit. It has a harsh and caustic taste (Prajapati et al. 2003).

Fig 1. *Ocimum tenuiflorum*

Taxonomy

Kingdom: Plantae
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species: *tenuiflorum*

Among the detected phenolic chemicals are isoeugenol, apigenin, rosameric acid, circimaritin, cirsilineol, and eugenol (in high concentrations). Orientin and vicenin were the two types of flavonoids extracted from the water-based leaf juice. Molludistin, orientin, luteolin, apigenin-7-O-glucuronide, ursolic acid, and luteolin-7-Oglucuronide are some of the additional compounds found in Tulsi leaf extract. This species of Ocimum also contains a number of monoterpenes and sesquiterpenes, such as bornyl acetate, β element, neural, pinenes (α and β), camphene, sitosterol, cholesterol, campesterol, and stigmasterol (Singh et al. 1996).

Moringa oleifera

Moringa oleifera Lam. (Family: Moringaceae), also known as the drumstick tree and tree, is indigenous to Northwest India, Pakistan, Bangladesh, and Afghanistan (Vijay and Kumar, 2012). Within the genus Moringa, this tree is the most commonly grown species. The leaves of Musa oleifera are an excellent source of protein, iron, potassium, β-carotene, vitamin C, and other nutrients (Pal et al. 1995).

M. oleifera is a deciduous tree with a trunk diameter of 45 cm (18 in) and a height of 10-12 m (33-39 ft). It grows quickly. There is thick cork surrounding the whitish-gray bark. The hairy bark of young shoots is purplish or greenish-white. The tree features an open crown with drooping, delicate limbs, and its foliage is composed of tripinnate leaves that grow fluffy. The fragrant, hermaphrodite flowers have five uneven, thinly veined, yellowish-white petals surrounding them. The blossoms measure approximately 1-4 cm (3⁄8⁄8 in) in length and 2 cm (3⁄4 in) in width. They grow in spreading or drooping flower clusters on slender, hairy stalks that are 10–25 cm (4-10 in) long (Parotta, 1993).

Fig 2. *Moringa oleifera* plant

1.7.2.1 Taxonomy

Kingdom:	Plantae
Order:	Brassicales
Family:	Moringaceae
Genus:	*Moringa*
Species:	*Oleifera*

The leaves of M. oleifera contain higher levels of the pigment lutein. The active ingredients found in plant leaves are what provide the plant its medicinal benefits. Gas chromatography–mass spectrometry identified about 35 chemicals in the plant's leaves. Among the 35 compounds that were extracted from the leaves, some of the more significant ones were tetra decanoic acid, palmitoyl chloride, cis-vaccenic acid, 5-O-acetyl-thio-octyl, pregna-7-dien-3-ol-20-one, γ-sitosterol, and β-l-rhamnofuranoside (Saini et al. 2014).

Based on literature survey, the research focuses on the formulation and evaluation of polyherbal buccal films consisting *Ocimum sanctum* and *Moringa oleifera* using suitable excipients and plasticizers and evaluation through standard parameters.

MATERIALS AND METHODS

Chemicals and Instruments

Leaves of *Ocimum sanctum* (g), *Moringa oleifera* (g), ethanol, propylene glycol, tween 80, HPMC, glycerol, sod. benzoate, and distilled water.

Digital weighing balance, digital pH meter, Franz diffusion cell and uv-spectrophotometer.

Collection, and authentication of plant materials

Fresh leaves of *Ocimum sanctum* and *Moringa oleifera* were collected from the Kanpur region, UP. Leaves were washed and dried under shade and authenticated by the Botanist.

Extraction process

The dried leaves of *Ocimum sanctum* and *Moringa oleifera* were rendered into fine powder and extracted separately, using distilled water and ethanol (1:1) solvent system through cold maceration process. The leaves of *Ocimum sanctum* and *Moringa oleifera* were soaked in a beaker containing distilled water and ethanol (1:1) solvent system for 15 days with gradual stirrings, separately. Each beaker was mounted with aluminium foil. After the due time, each beaker's aluminium foil was removed and filtered using the Whatman filter paper. The obtained slurry was made concentrated through rotatory evaporator. Thus, herbal extracts were

available in powder form and weighed to calculate the % yield. All the extracts were kept in desiccator to keep the extract moisture free (Khan et al. 2020).

Fig 3. Dried leaves extract of *Ocimum sanctum* and *Moringa oleifera*

Fig 4. Leaves extract *O. sanctum* and *Moringa oleifera*

Pre-formulation study

Organoleptic properties

The obtained herbal extracts of *Ocimum sanctum* and *Moringa oleifera* were observed for their physical characteristics like colour, odour, texture of drug and compared with as reported in official monograph.

Solubility

The solubility of herbal extracts of *Ocimum sanctum* and *Moringa oleifera* is determined by placing a small quantity of polymers (about 1-2 mg) individually in a test tube, adding 5ml of solvent (water, ethanol, methanol, phosphate 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 of herbal extracts

100mg of *Ocimum sanctum* leaves extract accurately weighed and dissolved in methanol (2ml) and volume was made up to 100ml with 0.1N HCl solution thus stock solution was prepared. The 10ml of stock solution was further diluted with 0.1 N HCl (pH 1.2) in 100ml to get 100µg/ml concentration solution. Then 0.2, 0.4, 0.6, 0.8, and 1ml of solution was taken in 10ml standard volumetric flask and made the volume up to 10 ml with 0.1N HCl to prepare 2µg, 4µg, 6µg, 8µg, and 10µg/ml solution. Then the absorbance was measured in a UV spectrophotometer at 270nm against 0.1 N HCl as blank. The procedure was repeated with phosphate buffer at pH 6.8 and absorbance was measured at wavelength 270 nm (Reddy et al. 2019). Same procedure was also performed for *Moringa oleifera* leaves extract.

Formulation of polyherbal buccal films

HPMC and PEG were weighed in specific ratios and they were dissolved in ethanol as solvent, using magnetic stirrer for proper solution. Leaves extract of *Ocimum sanctum* and *Moringa oleifera*, glycerol, sod. benzoate and tween 80 (plasticizer) were added to the above dispersion with continuous stirring. The uniform dispersion was poured in the petri plate. The rate of evaporation of solvent was controlled by inverting cut funnel over the patches. After 24h, the dried films were taken out and stored in desiccator to keep away from moisture (Kathar and Dumda, 2025).

Table 1. List of compositions for polyherbal buccal films

Composition	F1	F2	F3	F4	F5	F6
Ocimum sanctum (g)	1.0	1.0	1.0	1.0	1.0	1.0
Moringa oleifera (g)	1.0	1.0	1.0	1.0	1.0	1.0
HPMC (g)	0.5	1.0	1.5	-	-	-
PEG (g)	-	-	-	0.5	1.0	1.5
Tween 80 (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Glycerol (g)	0.5	0.5	0.5	0.5	0.5	0.5
Sod. benzoate (g)	0.01	0.01	0.01	0.01	0.01	0.01
Ethanol (ml)	5.0	5.0	5.0	5.0	5.0	5.0
Water (ml)	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.

Characterization parameters (Tirunagari et al. 2014; Reddy et al. 2019).

Weight variation

The all the buccal films were determined for their weights and to compare among to make sure that these are under limits of weight variation.

Flatness

Each buccal films were evaluated for its flatness by both sides. The length of each strip is measured and variation in the length because the uniformity in flatness represents constriction, considering 0% constriction equivalent to 100% flatness.

Folding strength

The folding strength is measured manually for the buccal patch. A strip of the films is cut evenly and repeatedly folded at the same place until it is broken-out.

Moisture content

The buccal films are weighed individually and kept in a desiccator for 24 h. The buccal films

are reweighed until a constant weight is obtained. Moisture content is calculated in percentage based on the difference between the initial and the constant final weights by using following formula-

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Drug content estimation

A small area of buccal film was cut and dissolved in PBS solution at pH 7.4. Then the solvent ethanol was added to make polymer soluble and the remaining volume was made up to 100 ml with PBS (pH 7.4). Then 1 ml was withdrawn from the solution and diluted again up to 10 ml. The absorbance of the solution was taken at wavelength 270 nm and drug concentration was calculated.

***In-vitro* drug release**

The membrane was mounted over a Franz diffusion cell and formulated buccal batch. The receiver compartment of the diffusion cell was filled with 15.0 ml of PBS pH 7.4 and the mixture is kept over a magnetic stirrer and temperature maintained at 37°C. Sample of 3 ml was withdrawn and replenished immediately from the receiver compartment at every 1, 2, 3, 4, 6 and 12h. They were stored in refrigerated condition until the analysis was performed. The content of herbal extracts in the samples was analyzed by UV-Visible spectrophotometer. The concentrations of drug are determined at wavelength 270 nm (Davies & Ingham, 2015).

Stability studies

Stability studies were carried out by keeping the optimized formulations in the butter paper and covered by aluminum foil and placed in the aluminum pouch. It was sealed by heat at the end for one month at room temperature. The films were taken at different time intervals like 0 to 4th week and are analyzed for its appearance, folding endurance, and drug content by following above mentioned protocols.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of the leaves of *Ocimum sanctum* and *Moringa oleifera* were calculated as 21.38% and 24.62%, respectively.

Pre-formulation studies

Organoleptic properties

The *O. sanctum* and *Moringa oleifera* were found as dark green powder with their characteristic odour. HPMC was observed as odorless and off-white powder. Polyethylene glycol was found as colourless liquid with its characteristic odour. Tween 80 was observed as yellow liquid. Moreover, Sod. Benzoate was found as odorless, white powder.

Table 2. Organoleptic properties of herbal extracts

Plant extract	Organoleptic characteristics		
	Appearance	Colour	Odor
Ocimum sanctum	Powder	Green	Characteristics

Moringa oleifera	Powder	Green	Characteristics
HPMC	Powder	Off-White	Odourless
PEG	Liquid	Colourless	Characteristics
Tween 80 (ml)	Liquid	Yellow	Characteristics
Sod. benzoate (g)	Powder	White	Odorless

Solubility estimation

In ethanol, *O. sanctum* and *M. oleifera* HPMC and Sod. Benzoate were found soluble. However, in phosphate buffer, herbal extracts, HPMC and Sod. Benzoate were found poorly soluble.

Table 3. Solubility studies

Solvent	***O. sanctum* extract**	***M. oleifera* extract**	**HPMC**	**Sod. Benzoate**	**Polyethylene Glycol**
Ethanol	Soluble	Soluble	Soluble	Soluble	Highly Soluble
Phosphate Buffer	Poorly soluble	Poorly soluble	Poorly soluble	Poorly soluble	Soluble
Tween 80	Readily soluble	Readily soluble	Readily soluble	Readily soluble	Readily soluble
Water	Insoluble	Insoluble	Soluble	Highly soluble	Soluble

Preparation of Std. Calibration curve of herbal extracts

The following table shows the conc. (µg/ml) of *O. sanctum* leaves extract and absorption (λmax 320 nm).

Table 4. Absorption of *O. sanctum* leaves extract

Conc. (µg/ml)	**Absorption (λmax 320 nm)**

2	0.18
4	0.39
6	0.57
8	0.78
10	0.94

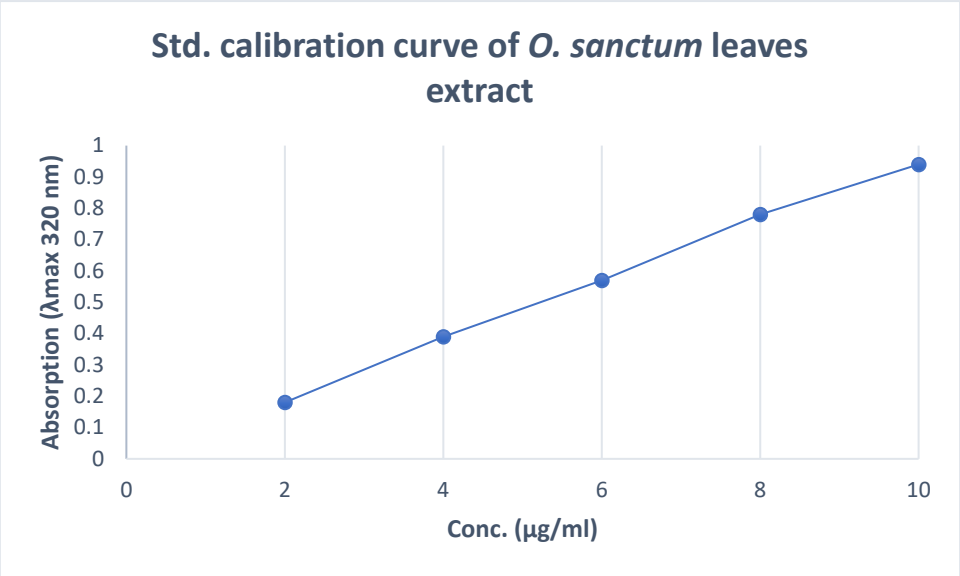

Fig 5. Std. Calibration Curve of *O. sanctum* leaves extract

The following table shows the conc. (µg/ml) of *Moringa oleifera* leaves extract and absorption (λmax 210 nm). At 10 µg/ml, absorption was found highest as 0.92, while at 2 µg/ml, it was found as 0.16.

Table 5. Std. Calibration Curve of *Moringa oleifera* leaves extract

Conc. (µg/ml)	Absorption (λmax 210 nm)
2	0.16
4	0.33
6	0.54
8	0.76
10	0.92

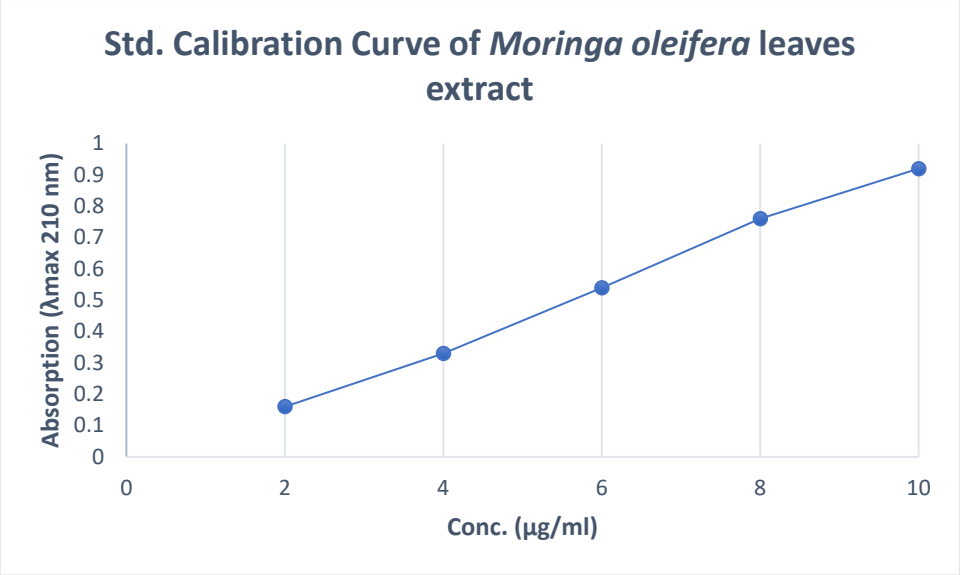

Fig 6. Std. Calibration Curve of *Moringa oleifera* leaves extract

Characterization parameters

Weight variations

All the buccal films showed a non-significant weight variations, ranging from 0.23±0.25 g to 0.38±0.34g. However, the minimum weight was observed for F2 as 0.23±0.33g and highest weight for F5 as 0.38±0.34. Non-significant weight variations exhibit the homogeneity and uniformity of the developed formulations.

Table 6. Weight variation of Buccal films

Formulation	Weight (g)
F1	0.29±0.25
F2	0.23±0.33
F3	0.32±0.18
F4	0.28±0.53
F5	0.38±0.34
F6	0.36±0.25

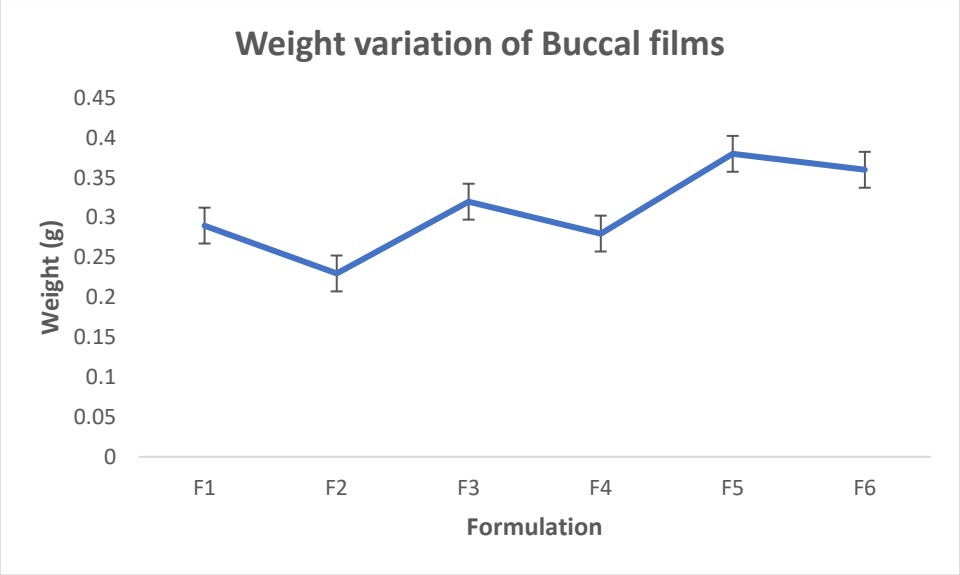

Fig. 7. Weight variation of Buccal films

Flatness

Flatness is a special feature of the buccal patches which assures the optimum drug release and bioavailability of the active API/herbal extracts. Among all the buccal films formulated, 0% constriction was found. Thus, 100% flatness was noted in all the formulations. Therefore, buccal films indicate the increased drug's solubility and absorption through buccal mucosa.

Table 7. Flatness of Buccal films

Formulation	Constriction (%)	Flatness (%)
F1	0.0	100
F2	0.0	100
F3	0.0	100
F4	0.0	100
F5	0.0	100
F6	0.0	100

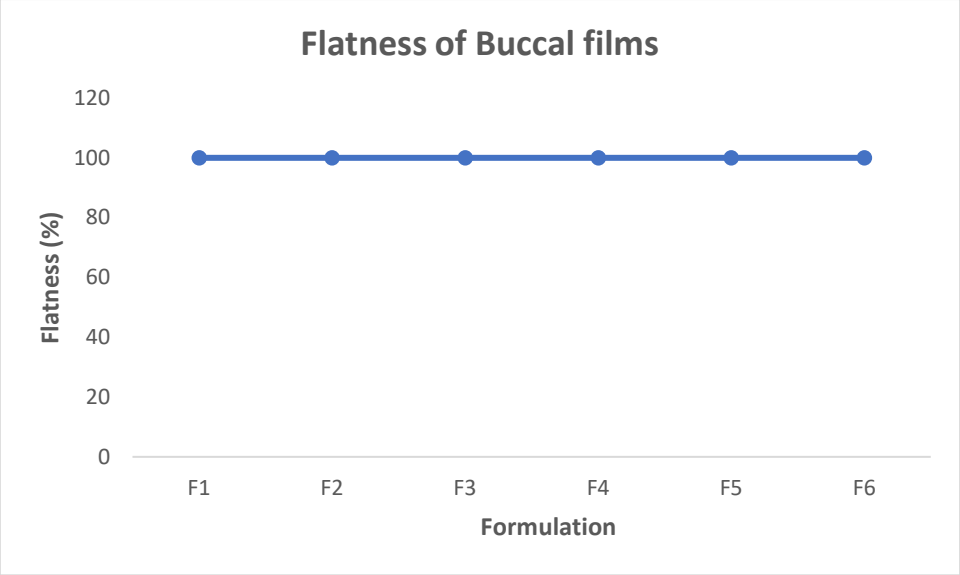

Fig 8. Flatness of Buccal films

Folding endurance

The developed buccal films were checked for folding endurance. Buccal films F1, F2, F3, F4, F5 and F6 showed the folding endurance as 0.21±0.42, 0.29±0.56, 0.26±0.47, 0.28±0.29, 0.23±0.38 and 0.27±0.11, respectively. Thus, all the buccal films had effective folding endurance. In terms of the film's capacity to fold, the buccal films mentioned above demonstrated a rigid folding endurance. It can withstand severe mechanical strain and a great deal of stress. Its enhanced folding endurance makes it more durable and stable. F2 showed the highest folding endurance.

Table 8. Folding endurance of Buccal films

Formulation	Folding endurance
F1	0.21±0.42
F2	0.29±0.56
F3	0.26±0.47
F4	0.28±0.29
F5	0.23±0.38
F6	0.27±0.11

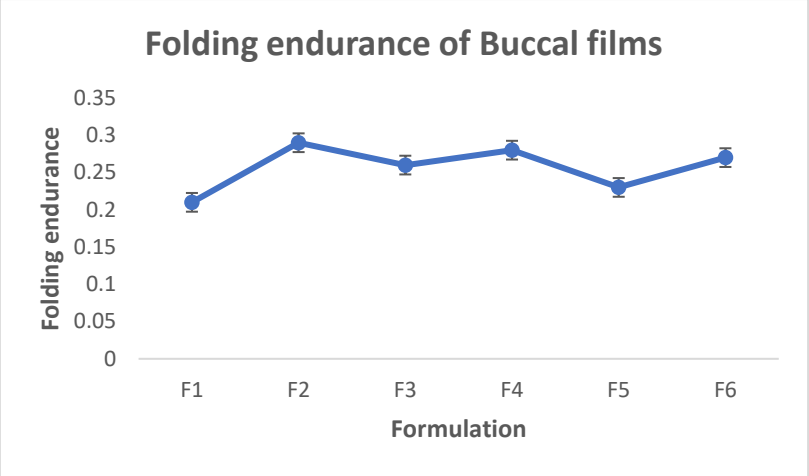

Fig 9. Folding endurance of Buccal films

Moisture content

Moisture content is directly proportional to the amount of the excipients and their hygroscopic nature. It was observed in the range of 1.12± 0.01 % to 1.58± 0.04 %. However, F5 demonstrated the minimum moisture content as 1.12± 0.01 %. Less moisture content preserves (makes stable) the formulations for longer period of time.

Table 9. Moisture content (%)

Formulation	Moisture content
F1	1.49±0.03
F2	1.16±0.04
F3	1.29±0.01
F4	1.34±0.03
F5	1.38±0.05
F6	1.19±0.06

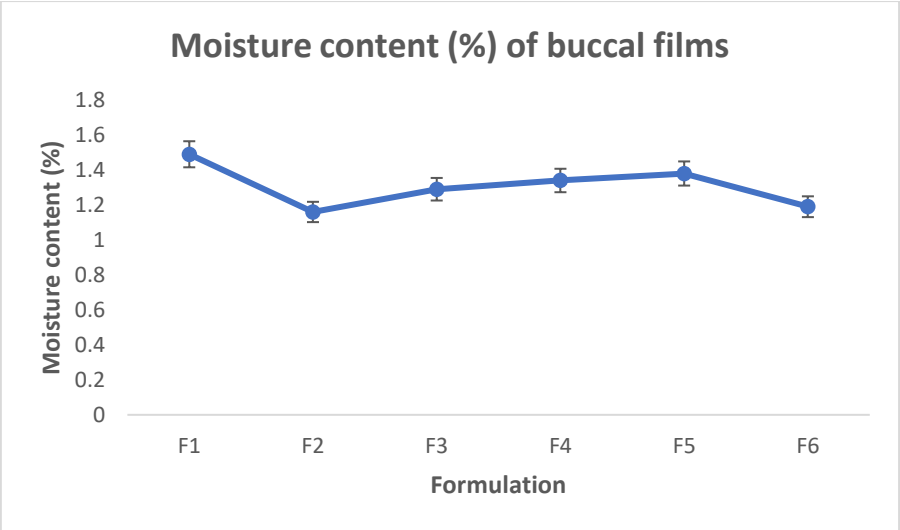

Fig 10. Moisture content (%)

Drug content estimation

Drug content confirms the significant release and bioavailability of drugs. It was determined for the confirmation of drug concentration which reaches into blood stream. From F1-F6, all the buccal films exhibited a significant drug content ranging from 93.20±0.28% to 98.31±0.17%. Among all, F2 exhibited the highest drug content as 98.31±0.17%.

Table 10. Drug content (%) of buccal films

Formulation	Drug content (%)
F1	94.45±0.12
F2	98.31±0.17
F3	93.20±0.28
F4	96.46±0.16
F5	95.72±0.39
F6	97.15±0.32

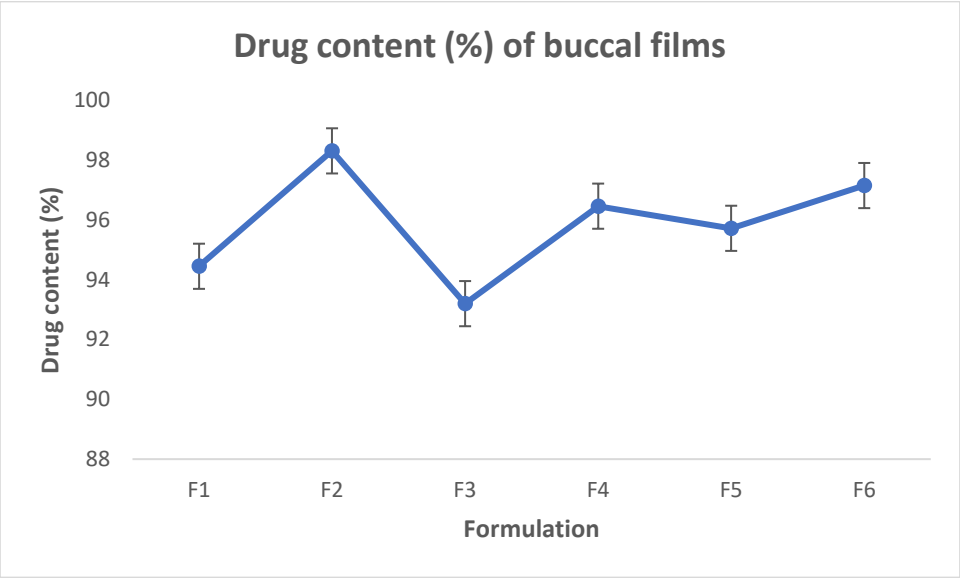

Fig 11. Drug content (%) of buccal films

***In-vitro* drug release**

It is an assumption to confirm the concentration of drugs that reaches into blood stream according to different time intervals including 0, 1, 2, 4, 6, 8,10, 12, 24, and 48 hours. After 48 hours, in-vitro drug release was estimated as 96.67±0.2, and 95.42±0.2 in F2 & F6, respectively. Herbal extracts might be effective in management of neuropathic pain for longer period through sustained release of drug.

Table 11. *In-vitro* drug release of buccal films

Time (hour)	*In-vitro* drug release					
	F1	F2	F3	F4	F5	F6
0	0.00	0.00	0.00	0.00	0.00	0.00
1	8.45±0.4	11.22±0.5	9.17±0.2	9.76±0.6	9.56±0.5	10.31±0.2
2	13.54±0.3	15.26±0.4	12.36±0.6	12.82±0.4	13.09±0.2	13.50±0.1
4	18.30±0.4	20.45±0.2	18.52±0.3	18.76±0.2	19.41±0.4	18.64±0.3
6	28.45±0.6	31.15±0.3	29.35±0.4	28.20±0.4	29.11±0.5	30.14±0.5
8	41.32±0.2	44.29±0.6	42.11±0.4	42.29±0.6	42.86±0.6	43.27±0.4
10	51.35±0.3	53.14±0.5	52.44±0.2	52.59±0.3	52.64±0.3	53.39±0.1
12	62.21±0.4	64.17±0.2	63.08±0.4	63.20±0.4	63.68±0.2	64.34±0.3
24	73.54±0.3	78.28±0.4	75.49±0.2	74.33±0.1	75.68±0.4	76.72±0.1
48	94.19±0.6	96.67±0.2	93.20±0.6	94.18±0.4	94.14±0.3	95.42±0.2

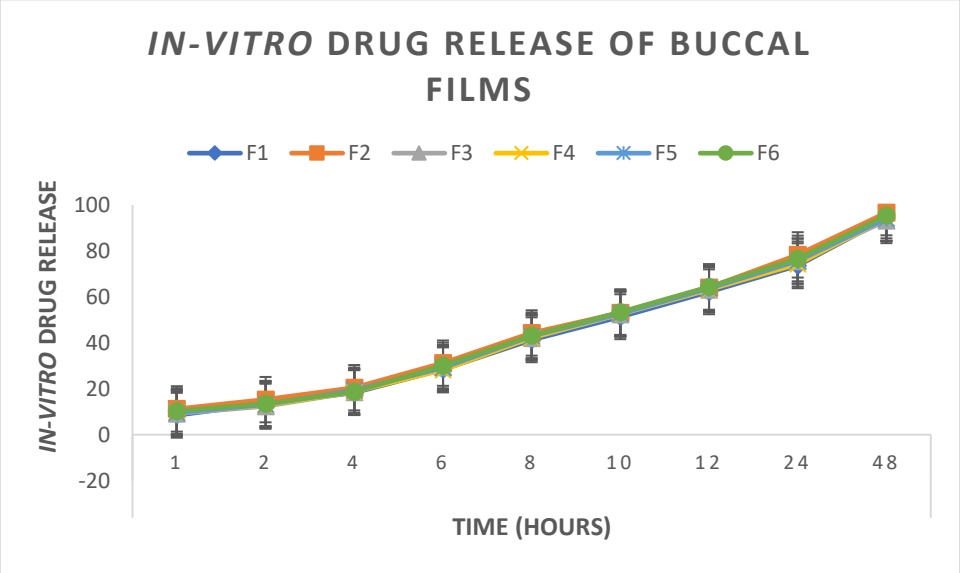

Fig 12. *In-vitro* drug release of buccal films

Stability Studies

The developed buccal films were determined for the stability after 1 month. The % drug content, folding endurance and physical appearance were estimated for all the formulations. After 4 weeks of storage, the buccal films were found stable because non-significant changes were observed in % drug content, and folding endurance. Physical appearance was also found unchanged.

In previous study, Iin contrast to the vehicle and paclitaxel-treated groups, the methylphenidate and atomoxetine buccal patches treated group significantly reduced paclitaxel-induced hyperalgesia during the first two weeks of observation, as shown by improved tail withdrawal

latency using the radiant heat technique. Furthermore, compared to mice treated with vehicle plus paclitaxel, there was an increase in tail withdrawal latency throughout the third and fourth weeks of observation. It was demonstrated that gabapentin alone has a strong anti-nociceptive impact when contrasted with other anti-epileptic drugs such as lamotrigine and topiramate. The same experiment showed that gabapentin combined with lamotrigine and topiramate had a high anti-nociceptive effect compared to monotherapy (Paudel et al. 2011). However, the effects of medication combinations were not examined in our study. Over the course of the four weeks of observation, the pregabalin-treated group likewise shown a significant increase in tail withdrawal latency. Furthermore, compared to the animal's receiving gabapentin during the research, the pregabalin-treated group exhibited a considerable reduction in thermal hyperalgesia.

During the four weeks of the tail immersion test, there was an anti-allodynic effect in both the gabapentin and pregabalin groups. Vinay Kumar and colleagues found in a previous study that gabapentin treatment had an anti-allodynic effect that was similar to but much stronger than that of lamotrigine treatment (Kumar et al. 2014). In contrast to gabapentin, pregabalin treatment produced anti-allodynia that lasted longer over the four-week observation period. In contrast to another study that showed the drug's anti-allodynic efficacy peaked after 12 days, ours showed pregabalin had a lasting effect of four weeks (Kumar et al. 2010). This was further supported by the fact that over the four weeks of the study, no rear paw rubbing, paw licking, foot withdrawal, or shaking took place during the one-minute observation time. During the first two weeks, gabapentin had a noticeable effect; however, by the fourth week, this effect had diminished, suggesting that the drug's anti-allodynic action, as measured by the acetone drop test, had considerably diminished. In contrast to intrathecal pregabalin administration, which was found to produce anti-allodynia in one study on oxaliplatin-induced neuropathy pain, our investigation showed that oral pregabalin administration using the acetone drop approach had a stronger anti-allodynic effect. Although studies have shown that gabapentin and pregabalin significantly reduce nociceptive and inflammatory pain, their effectiveness in reducing paclitaxel-induced neuropathic pain has not been demonstrated. Recurrent gabapentin and pregabalin medication considerably reduced paclitaxel-induced neuropathic pain during the course of the current study's four-week monitoring period.

The results showed that the paclitaxel + polyherbal buccal film (F2) had an outstanding in-vitro release rate. After 48 hours, *in-vitro* drug release was estimated as 96.67±0.2, and 95.42±0.2 in F2 and F6, respectively which is a good indication of sustained drug release. The physical appearance of the various films was determined to be the same after 30 days of storage.

CONCLUSION

This work advises other researchers to verify the mucoadhesive paclitaxel + polyherbal buccal film (F2) for improved pharmacokinetic profiles in vitro and subsequently in humans as well. Additionally, it must prioritize the design of the dosage form and assess its effectiveness in reducing neuropathic pain as well as the side effects that will validate the actual and permissible dose that must be maintained in the dosage form.

In conclusion, the polyherbal buccal films exhibited an improved *in-vitro* release time in addition to improved looks and other characteristics, such as weight variation, flatness and spreadibility. The polyherbal buccal film is stable mucoadhesive film which is highly stable (shelf life) even for longer time of usage.

CONFLICT OF INTEREST

Authors declare for none conflict of interest.

FUNDING

Nil.

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