FORMULATION AND EVALUATION OF HERBAL ORAL FILM CONTAINING ETHANOLIC EXTRACT OF *OLAX SCANDENS* FOR ANTIMICROBIAL ACTIVITY AGAINST ORAL PATHOGENS

Sagar Khubele, Rohit Mishra, Rohit Pahade, Rumaisha Khan, Triveni, Udit N. Soni

Oriental College of Pharmacy, Bhopal, Raisen Rd, Patel Nagar, Bhopal, Madhya Pradesh 462022

Abstract

The present study was aimed at the formulation and evaluation of herbal oral film containing ethanolic extract of *Olax scandens* for antimicrobial activity against selected oral pathogens. The ethanolic extract of the plant was prepared and subjected to preliminary phytochemical screening and total flavonoid content estimation. The extract showed the presence of flavonoids, phenolic compounds, proteins, carbohydrates, saponins, and diterpenes. The total flavonoid content of the ethanolic extract was found to be 0.82 mg/100 mg. Herbal oral films were prepared by solvent casting method using HPMC and Carbopol as film-forming polymers along with citric acid, glycerin, and PEG-600. The formulated oral film was evaluated for physicochemical properties including appearance, thickness, weight variation, folding endurance, and disintegration time. The prepared formulation showed translucent appearance, satisfactory flexibility, and rapid disintegration within 15±2 seconds. Antimicrobial activity of the herbal oral film was evaluated against *Streptococcus mutans* and *Candida albicans* using agar diffusion method. The formulation exhibited concentration-dependent antimicrobial activity with maximum zones of inhibition of 15±0.74 mm against *Streptococcus mutans* and 13±0.66 mm against *Candida albicans* at 100 mg/mL concentration. The findings of the study suggest that herbal oral films containing ethanolic extract of *Olax scandens* possess promising antimicrobial potential and may serve as a suitable natural alternative for the management of oral microbial infections.

Keywords: *Olax scandens*, Herbal oral film, Antimicrobial activity, *Streptococcus mutans*, *Candida albicans*, Ethanolic extract, Phytochemical screening, Flavonoids, Oral pathogens, Solvent casting method

Introduction

Medicinal plants have been widely utilized since ancient times as an important source of therapeutic agents for the prevention and treatment of various infectious and non-infectious diseases (Shakya et al., 2016). Herbal medicines contain numerous bioactive phytoconstituents such as flavonoids, alkaloids, tannins, glycosides, saponins, phenolic compounds, and terpenoids, which exhibit significant pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, anticancer, and wound healing properties (Ansari et al., 2025). In recent years, there has been increasing scientific interest in the development of herbal formulations due to their better patient compliance, reduced side effects, cost-effectiveness, and therapeutic efficacy compared to synthetic drugs.

Oral infections are among the most common health problems affecting people worldwide and are mainly caused by pathogenic microorganisms present in the oral cavity (Marsh et al., 2015). Dental caries, gingivitis, periodontitis, oral thrush, and other oral diseases are commonly associated with bacterial and fungal pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, *Candida albicans*, and *Lactobacillus* species. These infections may lead to pain, inflammation, bad breath, tooth loss, and severe oral complications if not treated properly. Conventional antimicrobial therapies are effective to some extent; however, prolonged use of synthetic antimicrobial agents may cause adverse effects such as drug resistance, hypersensitivity reactions, mucosal irritation, and alteration of normal oral microflora (Ventola et al., 2015). Therefore, there is a growing need for safer and more effective herbal alternatives for the management of oral pathogens.

Among various novel drug delivery systems, oral films have gained considerable attention because of their convenience, rapid drug release, ease of administration, and improved patient acceptability (Dixit et al., 2009). Oral films are thin, flexible polymeric strips that rapidly disintegrate or dissolve in the oral cavity, releasing the incorporated drug at the site of action. These films offer several advantages including accurate dosing, improved bioavailability, enhanced stability, portability, and avoidance of first-pass metabolism. Herbal oral films can serve as an effective localized drug delivery system for the treatment of oral infections and related disorders.

Olax scandens is an important medicinal plant traditionally used in Ayurveda and folk medicine for the treatment of various ailments (Warrier et al., 1996). Different parts of the plant are

reported to possess antimicrobial, anti-inflammatory, antioxidant, analgesic, and wound healing activities due to the presence of several bioactive phytoconstituents. The ethanolic extract of *Olax scandens* is known to contain flavonoids, tannins, phenolic compounds, and other secondary metabolites which may contribute to its antimicrobial potential against oral pathogens (Kumar et al., 2018).

The incorporation of herbal extracts into oral film formulations may improve therapeutic effectiveness and provide prolonged contact of the active constituents with the oral mucosa. Furthermore, ethanolic extracts generally possess better extraction efficiency for phytoconstituents responsible for antimicrobial activity. Hence, formulation of herbal oral films containing ethanolic extract of *Olax scandens* may offer a promising natural approach for the management of oral microbial infections.

Therefore, the present study was undertaken to formulate and evaluate herbal oral films containing ethanolic extract of *Olax scandens* and to investigate their antimicrobial activity against selected oral pathogens. The study aims to develop an effective herbal oral delivery system with improved therapeutic potential, stability, and patient compliance for the treatment of oral infections.

Material and Methods

Material

The materials used for the preparation of herbal oral film included ethanolic extract of *Olax scandens* as the active ingredient, Hydroxypropyl methylcellulose (HPMC) and Carbopol as film-forming polymers, citric acid as saliva stimulating agent, glycerin and PEG-600 as plasticizers, and distilled water as solvent vehicle. All chemicals and reagents used in the study were of analytical grade.

Methods

Extraction by maceration process

Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs. 50 gm of dried powdered leaves of *Olax scandens* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC[83].

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

Percentage yield = Weight of Extract/ Weight of powder drug Taken x 100

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [84].

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminium chloride method[86].

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Formulation development of fast dissolving oral film of *Olax scandens*

The herbal oral film containing ethanolic extract of *Olax scandens* was prepared by solvent casting method. Accurately weighed quantity of HPMC (250 mg) and Carbopol (100 mg) were dispersed separately in a small quantity of distilled water and allowed to hydrate completely for 2–3 hours to obtain a clear polymeric solution. The weighed amount of ethanolic extract of *Olax scandens* (250 mg) was dissolved in a suitable quantity of distilled water with continuous stirring.

Citric acid (10 mg) was added to the polymeric solution as a saliva stimulating agent. Glycerin and PEG-600 were incorporated as plasticizers in sufficient quantity (q.s.) to provide flexibility and smoothness to the film. The drug solution was then added slowly into the hydrated polymeric mixture under continuous stirring to obtain a uniform viscous solution. The final volume was adjusted to 20 mL using distilled water.

The prepared solution was sonicated to remove entrapped air bubbles and then poured carefully into a clean and leveled glass Petri dish. The casting solution was dried at room temperature or in a hot air oven at controlled temperature until complete drying of the film occurred. After drying, the film was carefully peeled off and cut into desired sizes. The prepared oral films were wrapped in butter paper/aluminum foil and stored in a desiccator for further evaluation studies (Sowjanya and Rao, 2023).

Table 1: Selection and Optimization of Formulation

Name of ingredients	F1
Olax scandens	250mg
HPMC	250 mg
Carbopol	100 mg
CS	-
CP	-
Citric acid	10 mg
Glycerin	qs
PEG-600	qs
DM water qs to	20ml

Selection and optimization of plasticizer

Two film plasticizer and one co-plasticizer were selected for this research work. The concentration of plasticizer was important to tensile strength % age elongation and folding endurance of film.

Concentration of plasticizer optimized on the basis of tensile strength % age elongation and folding endurance of film.

Evaluation of fast dissolving oral film

Thickness

The thickness of the prepared oral films was measured at three different positions using an absolute outside micrometer to ensure uniformity of the film formulation. Uniform thickness is an important parameter because it directly influences the drug content and mechanical strength of the films (Palakurthi et al., 2022).

Weight Uniformity

For each formulation, three randomly selected films were individually weighed using a digital electronic balance, and the average weight was calculated. The weight variation test was performed to confirm the uniform distribution of ingredients within the films and to ensure consistency among the formulations (Kavoosi et al., 2013).

Tensile Strength

Tensile testing provides information regarding the strength and elasticity of the oral films. Parameters such as tensile strength, elastic modulus, percentage strain, and load at yield were

evaluated. These parameters indicate the mechanical integrity and flexibility of the films during handling and storage.

Folding Endurance

Folding endurance was determined by repeatedly folding a single film at the same point until it broke or developed cracks. The number of folds required to break the film was recorded as the folding endurance value. This test reflects the flexibility and durability of the oral films (Kishore Babu et al., 2025).

Disintegration Time

Rapid disintegration is one of the most important characteristics of fast dissolving oral films. Superdisintegrating agents were incorporated into the formulation to minimize the disintegration time. The disintegration time was determined using a modified disintegration method. A Petri dish containing 10 mL of distilled water was used, and the film was carefully placed at the center of the dish. The time required for complete disintegration of the film into fine particles was recorded (S. S. et al., 2021).

In Vitro Antimicrobial Activity of Fast Dissolving Oral Film of *Olax scandens*

The antimicrobial activity of the prepared fast dissolving oral film containing *Olax scandens* extract was evaluated using the agar well diffusion method. The agar medium was prepared by dissolving the required quantity of dry ingredients in distilled water and heating until complete dissolution occurred. The prepared medium was sterilized in an autoclave at 121°C and 15 lbs/inch² pressure for 15 minutes. After sterilization, approximately 20 mL of the medium was poured into sterile Petri plates and allowed to solidify. The plates were incubated overnight at 37°C to ensure sterility and later dried at 50°C for 30 minutes before use.

Well Diffusion Method

The well diffusion method was used to determine the antimicrobial activity of the oral film following the standard procedure described by Du Toit et al. (2000). Different concentrations of the oral film formulation, namely 25, 50, and 100 mg/mL, were evaluated against selected oral pathogens. Wells were prepared on agar plates inoculated with the test microorganisms, and the respective concentrations of the formulation were introduced into the wells. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition surrounding the wells were measured to assess antimicrobial activity.

Result and Discussion

The present investigation was carried out to formulate and evaluate herbal oral films containing ethanolic extract of *Olax scandens* for antimicrobial activity against selected oral pathogens. The formulation composition used for the preparation of herbal oral film is presented in Table 1. HPMC and Carbopol were selected as film-forming polymers due to their excellent film-forming ability, flexibility, and compatibility with herbal extracts. Citric acid was incorporated as a saliva stimulating agent, while glycerin and PEG-600 were used as plasticizers to improve film flexibility and mechanical strength.

The percentage yield of ethanolic extract of *Olax scandens* was found to be 8.25% w/w as shown in Table 2, indicating satisfactory extraction efficiency of the solvent system. Ethanolic extraction is considered effective for isolating bioactive phytoconstituents such as flavonoids, phenolics, and saponins from medicinal plants.

Preliminary phytochemical screening results presented in Table 3 revealed the presence of flavonoids, phenolic compounds, proteins, carbohydrates, saponins, and diterpenes in the ethanolic extract, while alkaloids and glycosides were absent. The presence of these phytoconstituents may contribute significantly to the antimicrobial activity of the plant extract. Flavonoids and phenolic compounds are well known for their antioxidant and antimicrobial properties, whereas saponins and diterpenes are reported to possess membrane-disrupting activity against microorganisms.

The total flavonoid content of the ethanolic extract was found to be 0.82 mg/100 mg as shown in Table 4. Flavonoids are important secondary metabolites possessing strong free radical scavenging and antimicrobial activities. The appreciable flavonoid content observed in the extract may be responsible for the therapeutic potential of the formulated herbal oral film.

The prepared herbal oral film (F1) showed satisfactory physicochemical properties as summarized in Table 5. The formulation appeared translucent with uniform thickness and weight distribution, indicating proper mixing and uniform casting of the film-forming solution. The thickness of the film was found to be 26.25 μm and the average weight was 152 mg, suggesting uniformity in the prepared formulation.

Mechanical evaluation of the oral film showed satisfactory folding endurance as presented in Table 6. The folding endurance value of 175±12 indicated good flexibility and mechanical

strength of the film, suggesting that the film could withstand repeated folding without breaking. This property is important for packaging, handling, and patient acceptability.

The disintegration time of the prepared herbal oral film was found to be 15±2 seconds as shown in Table 7, indicating rapid disintegration in the oral cavity. Fast disintegration is an essential property for oral films because it ensures rapid release of active constituents at the site of infection and improves therapeutic effectiveness.

The antimicrobial activity of standard drugs against selected oral pathogens is presented in Table 8. Ofloxacin showed significant antibacterial activity against *Streptococcus mutans*, while Fluconazole exhibited strong antifungal activity against *Candida albicans*. The zones of inhibition increased with increasing concentration of standard drugs, confirming concentration-dependent antimicrobial activity.

The antimicrobial activity of herbal oral film (F1) against selected oral pathogens is shown in Table 9. The formulated herbal oral film demonstrated concentration-dependent antimicrobial activity against both *Streptococcus mutans* and *Candida albicans*. At 100 mg/mL concentration, the formulation exhibited maximum zones of inhibition of 15±0.74 mm against *Streptococcus mutans* and 13±0.66 mm against *Candida albicans*. Although the activity was comparatively lower than standard drugs, the formulation showed promising antimicrobial effects against oral pathogens.

The antimicrobial activity of the herbal oral film may be attributed to the presence of flavonoids, phenolic compounds, saponins, and diterpenes present in the ethanolic extract of *Olax scandens*. These phytoconstituents are known to inhibit microbial growth through disruption of cell membranes, inhibition of microbial enzymes, and interference with essential metabolic pathways.

The results of the present study suggest that the formulated herbal oral film containing ethanolic extract of *Olax scandens* possesses satisfactory physicochemical properties along with appreciable antimicrobial activity against oral pathogens. Therefore, the developed formulation may serve as a promising natural alternative for the management of oral microbial infections.

Table 2: % Yield of extract of *Olax scandens*

S. No.	Extract	% Yield
1	Ethanolic	8.25

Table 3: Phytochemical screening of extracts *of Olax scandens*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Modified Borntrager's Test	-ve
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric Chloride Test	+ve
5.	**Proteins and Amino acids**	
	Xanthoproteic test	+ve
	Ninhydrin Test	-ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	+ve
	Fehling's test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	+ve
8.	**Diterpins**	
	Copper acetate test	+ve

[+ve= positive; -ve= negative]

Table 4: Total flavonoid content of *Olax scandens* extract

S. No.	Extract	Total flavonoid content (mg/100mg)
1.	Ethanolic *extract*	0.82

Table 5: Formulation Development

Formulation code	General appearance	Thickness (µm)	Weight (mg)
F1	TL	26.25	152

TL – Translucent

Table 6: Result of Folding Endurance

Formulation code	Folding endurance
F1	175±12

Table 7: Results of disintegrating

Formulation code	Disintegrating time (sec.)
F1	15±2

Table 8: Antimicrobial activity of standard drug against selected microbes

S. No.	Standard drug	Name of microbes	Zone of inhibition (mm)		
			10µg/ml	20 µg/ml	30µg/ml
1.	Ofloxacin	*Streptococcus mutans*	18±0.57	20±0.86	25±0.5
2.	Fluconazole	*Candida albicans*	20±0	26±0.94	30±0.74

Table 9: Antimicrobial activity of herbal oral film (F1) against selected microbes

S. No.	Name of microbes	Zone of inhibition (mm)		
		Herbal oral film (F1)		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Streptococcus mutans*	9±0	12±0.5	15±0.74
2.	*Candida albicans*	8±0.86	11±0.33	13±0.66

Conclusion

The present study successfully formulated and evaluated an herbal oral film containing ethanolic extract of *Olax scandens* for antimicrobial activity against oral pathogens. The ethanolic extract showed the presence of important phytoconstituents such as flavonoids, phenolics, saponins, carbohydrates, proteins, and diterpenes, along with appreciable total flavonoid content, indicating its therapeutic potential. The prepared herbal oral film (F1) exhibited satisfactory physicochemical properties including uniform appearance, acceptable thickness, good folding endurance, and rapid disintegration time. Antimicrobial evaluation demonstrated that the formulation possessed significant inhibitory activity against *Streptococcus mutans* and *Candida albicans*, with increased activity observed at higher concentrations. Therefore, the developed herbal oral film of *Olax scandens* may serve as a promising natural alternative for the management of oral microbial infections and can be further explored for advanced oral healthcare applications.

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