FORMULATION AND EVALUATION OF TOPICAL GEL CONTAINING PROPOLIS FOR ANTIFUNGAL ACTIVITY

Mukesh P. Ratnaparkhi1*, Swapnil R. Shinde[2], Prasad V. Kadam[3], Omkar V. Kamble[4], Dipti D. Kadam[5], Ashutosh B. Shewale[6], Vaishnavi A. Panhale[7], Arshad J. Shaikh[8]

Article History: **Received:** 30.05.2023 **Revised:** 02.07.2023 **Accepted:** 10.08.2023

Abstract

Background: The present research has been undertaken with the aim to develop a topical gel formulation of Propolis. Propolis is used for the treatment of local and systemic fungal infections. Commercially Propolis topical gel preparation is not available in the market, thus this formulation is made for better patient compliance and to reduce the dose of the drug, and to avoid negative effects such as liver and renal damage.

Methods: The gel was formulated by changing the polymer ratio. Various formulations (F1, F2, F3, F4, F5, F6) were developed by using a suitable polymer (carbopol 934p and HPMC K4M). The formulation was evaluated for Drug Content, spreadability, pH, extrudability, viscosity, and in vitro drug release study.

Results: Viscosity checks of various formulations revealed that formulation F4 outperformed the others. F4 performs better than all other formulations in terms of drug diffusion and Rheological characteristics. The pH of the F4 formulation is high enough to cure skin infections. The concentrations of Carbopol 934p and HPMC K4M in the gels were shown to have a substantial effect on drug release and rheological properties.

Conclusion: It was concluded that formulation F4 was the best formulation among this formulation. As a result, formulation F4 should be further improved for industrial scale-up. Viscosity studies of various formulations revealed that formulation F4 was better compared to others. F4 performs better than all other formulations in terms of drug diffusion and Rheological characteristics. The pH of the F4 formulation is high enough to cure skin infections. The concentrations of Carbopol-934p and HPMC K4M in the gels were found to have a substantial effect on drug release and rheological properties.

Keywords: Propolis, Carbopol 934p, HPMC K4M.

[1*]Dept. Of Pharmaceutics Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.
[2]Dept. Of Pharmaceutics Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.
[3]Dept. Of Pharmacognosy Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.
[4]Dept. Of Pharmaceutics Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.
[5]Dept. Of Pharmaceutics Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.
[6]Dept. Of Pharmaceutics Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.
[7]Dept. Of Pharmaceutics Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.

Email: [1*]mukeshparkhi@yahoo.co.in, [2]swapnil.shinde@mmcop.edu.in, [3]prasadkadamv@gmail.com, [4]salwankar@gmail.com,[5]omkar.kamble@mmcop.edu.in, [6]dipti.kadam@mmcop.edu.in, [7]arshad.shaikh@mmcop.edu.in

***Corresponding Author:**
Dr. Mukesh P Ratnaparkhi[1*]
[1*]Associate Professor, Dept. Of Pharmaceutics Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33, India.
Email: [1*]mukeshparkhi@yahoo.co.in

DOI: 10.31838/ecb/2023.12.6.270

1.

2. Introduction

Fungal infection of the skin is the most frequent dermatological issues today. The therapy options available ranging from solid dosage to semisolid dosage form to liquid dose formulation. Clear transparent gels have gained widespread acceptance among topical formulations in both the cosmetics and pharmaceutical industries. Clinicians and patients can choose from a number of vehicles, including solid, semisolid, and liquid formulations, for the topical treatment of dermatological diseases and skin care. Transparent gels have seen increased use in cosmetics and pharmaceutical preparations, two significant subgroups of semisolid preparations. For many decades treatment of an acute disease or a chronic illness has been largely performed by delivery of pharmaceuticals to patients utilizing various pharmaceutical dosage forms, including tablets, capsules, pills, suppositories, creams, gel, ointments, liquids, aerosols, and injections, as drug carriers. Local dermatological problems can be effectively treated with focused medication delivery. Because it eliminates oral administration's first-pass effects, gastrointestinal discomfort, and metabolic degradation, this method of drug delivery has grown in favor. Just 25–45% of the oral dose that is delivered reaches the blood circulation as a result of the first-pass effect. The gel formulations have been suggested as a topical treatment to get around these drawbacks. A high degree of physical and chemical cross-linking is put into a polymeric matrix to create a semi-solid system known as a gel. Apis melifera L. bees produce a powerfully adhesive resinous compound called propolis which has been utilized significantly to treat bacterial, fungal anti-inflammatory diseases. Propolis is a sticky resinous material, sometimes referred to as "bee glue." The bees collect the mushy material from plant exudates and mix the material with beeswax, pollen, and enzymes to produce a substance to toughen the hive while providing some antimicrobial properties. Propolis is rich in antioxidants, including total phenolics, flavonoids, organic acids, terpenes, beta-steroids, aromatic aldehydes, and alcohols. Additionally, propolis also has inhibitory activity against a wide variety of microorganisms, including bacteria, viruses, and fungi. Propolis is basically a complex resinous material that the honey bees produced from plant exudates, beeswax, and bee secretions. It is used to seal the hive and it has an antimicrobial role in the hive itself. Propolis has a very complex composition containing around 300 constituents. According to different areas, seasons, and vegetation of that particular area, the composition of propolis varies. These constituents included wax, resins, balsams, essential oils, amino acids, sugars, flavonoids, and derivatives of cinnamic acid. Since the composition of propolis varies from area to area and also from season to season so is the case with its different activities. Carbopol 934p, a hydrophilic polymer made of hydroxypropyl methylcellulose (HPMC), has been applied topically in gel drug delivery systems

3. Materials and Methods

Material:

Propolis was purchased from Hi-tech Natural Products Ltd., (U.P). HPMC, Carbopol934, Glycerine, Methylparaben and Propylparaben were purchased from Loba Chemicals Pvt Ltd., Mumbai. Triethanolamine was purchased from Research Lab., Mumbai. Water.

Methods:

Polymers like HPMC or Carbopol 934p and purified water were taken in a beaker and allowed to soak for 24 h. To this required amount of drug (2 gm) was dispersed in water and then Carbopol 934p or HPMC was then neutralized with a sufficient quantity of Triethanolamine. Glycerin was used as a moistening agent, and methylparaben and propylparaben were used as preservatives. The mixture was gently stirred until it formed a homogeneous gel.

Gel formulations of Propolis were prepared using different concentrations of carbopol934, HPMC. (Table no.1)

Table 1: Optimized formula of Propolis gel

Formulations	Ingredients								
	Drug (gm)	Carbopol (gm)	HPMC (gm)	Water (ml)	Alcohol (ml)	Methyl Paraben (gm)	Propyl Paraben (gm)	Glycerine (ml)	Tri-ethanol amine (ml)
F1	2	0.75	0.75	70	2	0.1	0.05	5	5
F2	2	0.5	0.5	80	5	0.1	0.05	15	3
F3	2	0.75	1	50	3	0.1	0.05	10	4
F4	**2**	**0.5**	**0.75**	**60**	**4**	**0.1**	**0.05**	**10**	**4**
F5	2	-	1	65	4	0.1	0.05	6	6
F6	2	1	-	60	3	0.1	0.05	10	2

Calibration Curve:
Calibration curve of the Propolis in ethanol was plotted by recording the absorbance of solutions at different concentrations (0.5-3µg/ml) & at a maximum wavelength of 260nm.

Table 2: Calibration Curve

Concentration	Absorbance
0.5	0.04647
1	0.05871
1.5	0.06927
2	0.07927
2.5	0.0934
3	0.1021

Evaluation of Propolis Gel
Physical appearance:
The gel preparations are tested visually for their colour, homogeneity, consistency, and phase separation.

Table 3: Physical Appearance

Organoleptic Characterization	Colour	Light Brown
	Odour	Characteristic
	Homogeneity	Good
	Clearity	Clear
	Texture	Semisolid

Drug content

Weighed 10 gm of each gel formulation were transferred in 250 ml of a volumetric flask containing 20 ml of alcohol and stirred for 30 min. The volume was made up to 100 ml and filtered. 1 ml of the above solution was further diluted to 10 ml with alcohol and again 1 ml of the above solution was further diluted to 10 ml with alcohol. The absorbance of the solution was measured spectrophotometrically at 260 nm. Drug content was calculated by the following formula.

$$\text{Drug Content} = \frac{\text{Absorbance}}{\text{Slope}} \times \text{Dilution Factor} \times \frac{1}{1000}$$

Determination of pH

The pH of the gel formulations was in the range of 5 to 9. There was no significant change in pH values as a function of time for all formulations considered acceptable to avoid the risk of irritation upon application to the skin because skin pH is 6.7 to 7.8.

Spreadability

A sample of 0.5 g of each formula was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected. The spreaded circles' diameter was measured in cm and taken as comparative values for spreadability. The results obtained are average of three determinations.

Viscosity estimation

The developed gel formulations were poured into the small adapter of Brook field viscometer and the angular velocity was increased gradually from 10 to 50 rpm. The optimum viscosity of the formulation should be in the range of 5 to 1000 m Pas before gelling and, 50 to 50,000 m Pas after the formation of a gel.

Extrudability

The gel formulations were filled into a collapsible metal tube or aluminum collapsible tube. The tube was pressed to extrude the material and the extrudability of the formulation was checked.

In vitro diffusion study

The experiment was carried out using a modified Franz diffusion cell with a dialysis membrane. Before carrying out the study, the membrane was kept in acetate buffer pH 5.5 for 24 hr and it was mounted carefully between the donor and receptor chamber. 200 mg of gel was weighed and homogeneity spread on the dialysis membrane. 12 ml of acetate buffer (pH 5.5) was placed in the receptor medium as dissolution media. Both donor and receptor compartments were kept in contact with each other and the whole assembly was maintained at a constant temperature of 37 ± 0.5°C. A magnetic bead was used to stir the solution of the receptor chamber. 1ml of the sample was withdrawn after specific time intervals and an equal amount was replaced with fresh dissolution media. Sample absorbance was calculated spectro-photometrically at 260nm and %cumulative drug permeation was calculated. In order to predict the drug release mechanism, the obtained values were fitted into various models like Zero order, First order, Higuchi matrix, and Korsmeyer-Peppas model. The best-fit model was selected according to the highest R2 values obtained.

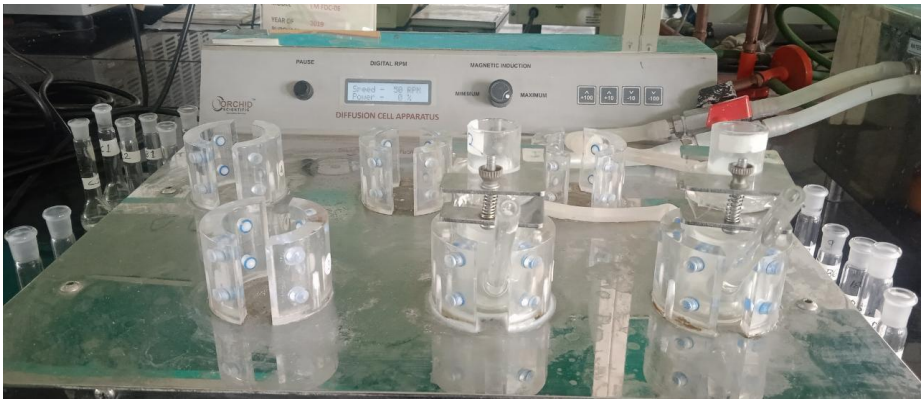

Figure No.1 In vitro diffusion study

Antifungal Activity

Fungal culture (Candida Albicans) was used in this study. Sabouraud dextrose agar (SDA) medium was used for the maintenance of fungal culture and stored at 4^0c before being used in the experiment. For fungal assessment, SDA medium was employed for the development of the fungus. The fungal strain was grown on SDA agar plates and stored at 40^0c. about 20 µl of drug formulation were placed on a petri plate. On SDA, the drug solution was used as a positive control. All of the plates are incubated at 28^0c for one week. Then the zone of inhibition of measured.

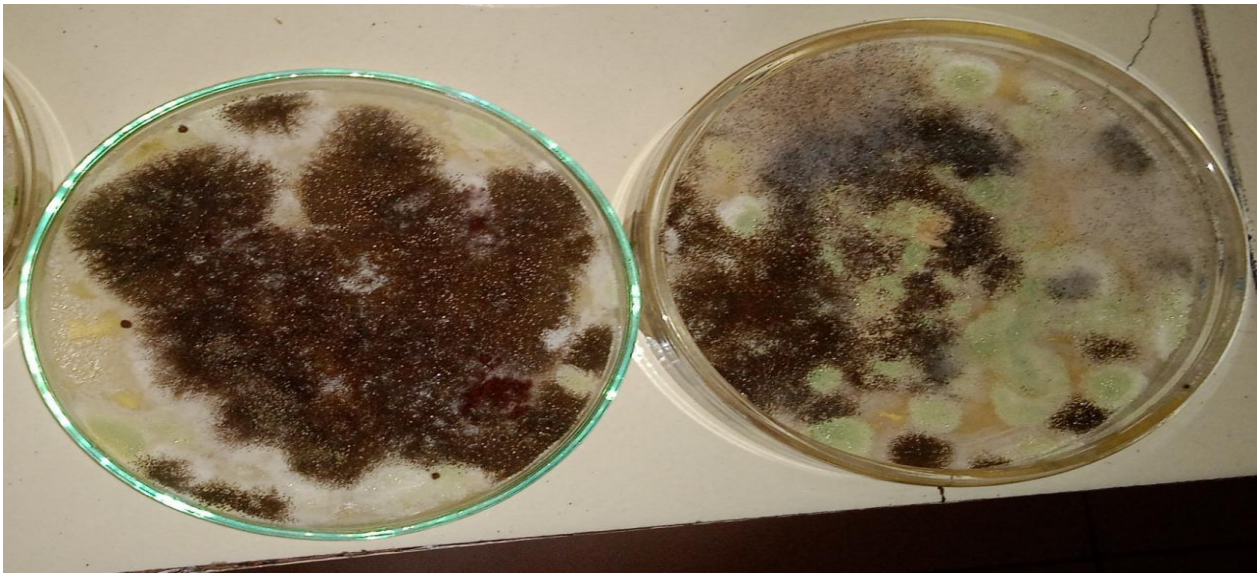

Figure No.2 Antifungal Activity

4. RESULTS

Table 4: Physical Chemical evaluation parameters and its obtained data for the prepared gel

Formulations	Drug Content	pH	Viscosity(cp)	Spreadability	Extrudability
F1	39.791	5.2	6000	4.7	-
F2	66.595	6.5	5200	4.5	+
F3	83.251	7.9	5800	4.3	++
F4	**91.171**	**6.95**	**4500**	**4.8**	**+++**
F5	72.967	5.8	3000	4.1	--
F6	38.945	8.5	3500	4.4	+

Extrudability: Excellent (+++), Good (++), Average (+), Poor (-)

Table 5: In Vitro Diffusion Chart

Time	%CDR					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0

30	1.64	2.27	2.27	2.27	2.27	2.27
60	4.06	4.77	4.33	4.24	3.59	4.12
90	7.18	8.14	6.48	6.87	5.51	6.62
120	10.86	12.49	9.11	10.17	8.23	8.57
150	14.77	14.99	12.29	14.10	11.43	11.21
180	35.33	17.72	15.04	24.82	13.39	31.13
210	60.30	21.33	27.46	44.01	23.06	40.80
240	63.47	46.60	43.64	68.34	48.34	55.20
270	66.89	80.81	62.34	**98.04**	75.42	82.46

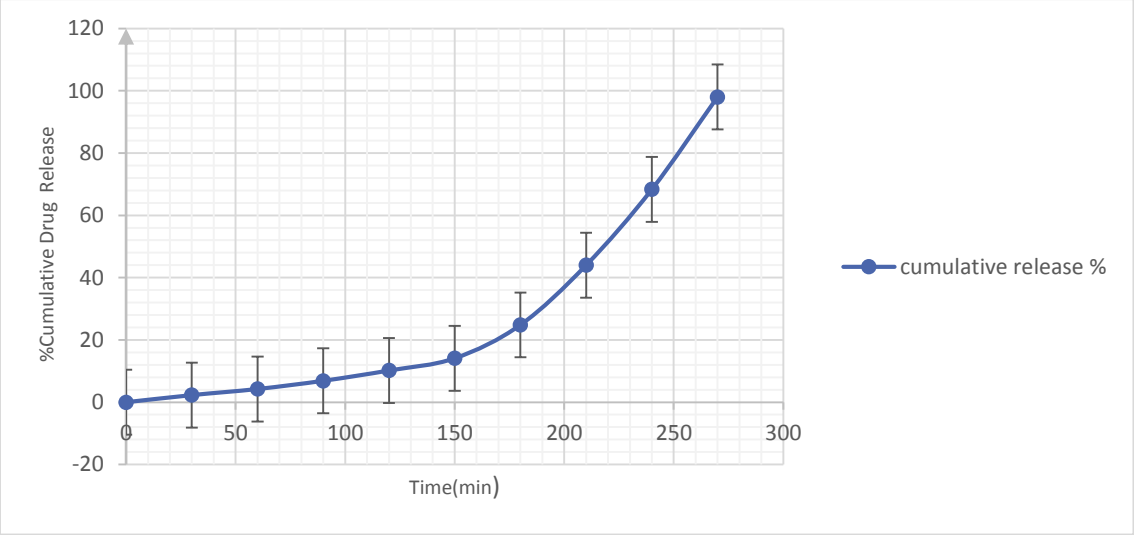

Figure.3 In vitro diffusion for F4 formulation

5. DISCUSSION

There are various advantages to using topical and transdermal drug delivery systems over oral administration systems. Patches, gels, creams, ointments, and lotions are examples of these delivery modalities. However, many adverse effects have been demonstrated by the oral delivery system of propolis, and this article will go over the side effects of oral dose form. The creation and evaluation of propolis gel changed the dosage form. Propolis is used to treat both local and systemic fungal infections. The current work attempted to synthesize propolis gel for efficient medication delivery to the skin. Propolis gel was created in this study utilizing carbopol 934p, alcohol, methyl paraben, propyl paraben, triethanol amine, and distilled water. Five different formulas were created. FT-IR was used to conduct a pre-formulation analysis of drug-excipient interactions, which revealed no interactions. Data from viscosity investigations, drug content, spreadability tests, in vitro drug diffusion, skin irritation, and antifungal studies all yielded positive findings.

6. CONCLUSION

Various formulations (F1, F2, F3, F4, F5, F6) were developed by using a suitable polymer (carbopol 934p and HPMC). Developed formulations of Propolis were evaluated for the physiochemical parameters such as drug content, pH, viscosity, spreadability, extrudability, in vitro drug diffusion, and antifungal study.

Viscosity studies of various formulations revealed that formulation F4 was better compared to others. F4 performs better than all other formulations in terms of drug diffusion and Rheological characteristics. The pH of the F4 formulation is high enough to cure skin infections. The concentrations of Carbopol-934p and HPMC K4M in the gels were found to have a substantial effect on drug release and rheological properties. The viscosity of carbopol-934p gels was very high as compared to HPMC K4M gels but both gels showed a decrease in drug release with an increase in polymer concentration. Thus, gels appropriate for topical administration can be successfully made utilizing Carbopol 934p and Hydroxypropyl methylcellulose as gelling agents in the ratio 1:3 (carbopol-934p and Hydroxypropyl methylcellulose). As a result, formulation F4 should be further improved for industrial scale-up.

Abbreviations

pH	Hydrogen ion concentration
cp	Centipoise
gm	Gram
CDR	Cumulative Drug Release
nm	Nanometer
^{0}C	Degree Celsius
SDA	Sabouraud dextrose agar
mm	Millimeter
ml	Milliliter
min	Minute
rpm	Revolution Per Minute

Authors Contributions

All the author has contributed equally.

Conflict of Interests

There is no Conflict of Interest.

REFERENCES

1. PROVOST C. Transparent oil-water gels. International Journal of Cosmetic Science. 1986;8(5):233–47. doi:10.1111/j.1467-2494. 1986.tb00453.x
2. Gupta A, Mishra AK, Singh AK, Gupta V, Bansal P. Formulation and evaluation of topical gel of diclofenac sodium using different polymers. Drug invention today. 2010 May 1;2(5):250-3
3. Sharma N, Sanadhya S, Nagarajappa R, Ramesh G, Naik D. Antifungal activity of Propolis, Fluconazole and Chlorhexidine against Oral Candida albicans–A Comparative in-vitro Study. Research Journal of Pharmacy and Technology. 2022;15(8):3589-94.
4. Przybyłek I, Karpiński TM. Antibacterial properties of propolis. Molecules. 2019 May 29;24(11):2047. doi:10.3390/molecules24112047.
5. Badria FA, Fathy HM, Fatehe AS, Ahmed MH, Ghazy MG. Chemical and biological diversity of propolis samples from Bulgaria, Libya and Egypt. J Apither. 2018;4(1):17.
6. de Groot AC. Propolis: a review of properties, applications, chemical composition, contact allergy, and other adverse effects. Dermatitis. 2013 Dec 1;24(6):263-82.
7. Kalia P, Kumar NR, Harjai K. Preventive effect of Honey bee propolis on Salmonella enterica serovar Typhimurium infected BALB/c mice: A Hematological Study. Research Journal of Pharmacy and Technology. 2020;13(7):3389-93.
8. Gerry Fink and the Fink lab. How antifungal drug kill fungi and cure disease; 2005. Available from: URL: http://www. medscape.com/viewprogram/296 3-pn.

9. Subramanian S, Prasanth B. sustained ophthalmic delivery of pH triggered Cromolyn sodium in situ gel. Research Journal of Pharmacy and Technological. 2021; 14(12):6211-5.
10. Golinkin HS, inventor; BP Corp North America Inc, assignee. Process for fracturing well formations using aqueous gels. United States patent US 4,137,182. 1979 Jan 30.
11. Sarkar U, Raha A, Mukherjee P, Paul M, Bagchi A. Development and evaluation of metronidazole containing topical gel using different gelling agents. Asian Journal of Pharmacy and Pharmacology. 2018;4(6):785–9. doi:10.31024/ajpp.2018.4.6.10
12. Wani M, Jagdale S, Khanna P, Gholap R, Baheti A. Formulation and Evaluation Opthalmic In-Situ Gel Moxifloxacin Coated Silver Nanoparticles. Research J. Pharm and Tech. 2020; 13(8):3623-3630
13. Hemalatha B, Priya TP, Manasa K, Greeshmika C, Kavya P, Shaik SS, Padmalatha K. Optimization of oxiconazole topical emulgel formulation for the treatment of skin infections. Asian Journal of Pharmacy and Technology. 2022;12(3):232-6.
14. Kasar PM, Kale KS, Phadtare DG. Formulation and evaluation of topical antifungal gel containing itraconazole. Research Journal of Topical and Cosmetic Sciences. 2018;9(2):49-52.
15. Hemalatha B, Priya TP, Manasa K, Greeshmika C, Kavya P, Shaik SS, Padmalatha K. Optimization of oxiconazole topical emulgel formulation for the treatment of skin infections. Asian Journal of Pharmacy and Technology. 2022;12(3):232-6.
16. Latha AV, Kumar JN, Sojana N, Mounika N, Priyanka G, Venkatesh A. Design and Optimization of Clotrimazole Emulgel by using various Polymers. Asian Journal of Pharmacy and Technology. 2021;11(1):41-7.
17. Subramanian S, Prasanth B. Sustained ophthalmic delivery of pH triggered Cromolyn sodium in situ gel. Research Journal of Pharmacy and Technology. 2021;14(12):6211-5.
18. Khanum M. Development and evaluation of nanoparticles based topical gel containing antifungal drug fluconazole. International Journal of Pharmaceutical and Bio-Medical Science. 2021;01(09). doi:10.47191/ijpbms/v1-i9-02.
19. Varma VN, Maheshwari PV, Navya M, Reddy SC, Shivakumar HG, Gowda DV. Calcipotriol delivery into the skin as emulgel for effective permeation. Saudi Pharmaceutical Journal. 2014 Dec 1;22(6):591-9.
20. Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi pharmaceutical journal. 2012 Jan 1;20(1):63-7.
21. Juniatik M, Hida K, Wulandari FP, Pangestuti N, Munawaroh NM, Martien R, Pratiwi SU. Formulation of nanoemulsion mouthwash combination of lemongrass oil (Cymbopogon citratus) and kaffir lime oil (Citrus Hystrix) for anticandidiasis against candida albicans ATCC 10231. Majalah Obat Tradisional. 2017;22(1):7-15.
22. Devkatte AN, Zore GB, Karuppayil SM. Potential of plant oils as inhibitors of Candida albicans growth. FEMS yeast research. 2005 Jun 1;5(9):867-73.
23. Saudagar RB, Samuel S. Formulation development and evaluation of topical film-forming lotion containing butenafine hydrochloride. Asian Journal of Pharmacy and Technology. 2016;6(4):238-48.