Indonesian Journal of Pharmaceutical and Clinical Research

Development of Oral Thin Film Strip Contained Ethanol Extract of Clove Leaves (*Syzygium aromaticum* (L.) Merr. & Perry)

Bayu Eko Prasetyo*[1], Mariadi [1], Emil Salim[2], Rinny Argasari Sitanggang[3], Aranova Esovin Patricia ButarButar[3], Shafiyya Amali[3], Johanna Fransiska Lambue Siahaan[3]

[1]*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan-20155, Indonesia.*

[2]*Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan-20155, Indonesia*

[3]*Undergraduate Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan-20155, Indonesia*

Abstract. Halitosis or bad breath can occur due to poor oral hygiene care, dental caries or infections in the oral cavity. The use of mouth fresheners is one of the solution product to overcome the problem. Oral Thin Film Strip (OTFS) is an alternative mouth freshener. The preparation of OTFS was carried out using the solvent casting method. Five film formula were made with varying concentrations of gelatin and HPMC, namely F1 (0:3), F2 (1:3), F3 (1:1), F4 (3:1), and F5 (3:0). Evaluation of the preparation included organoleptic test, weight uniformity, thickness, disintegration time, pH, and folding resistance. Stability testing was carried out for 3 months at 25±2°C and 40±2°C. Antibacterial activity test against *Streptococcus mutans* (*S. mutans*) on the preparation was done using disc diffusion method. The OTFS preparation produced gave a homogeneous colour, aroma and taste of the extract. The resulting preparation had weight between 0.345-0.180 grams, thickness between 0.148-0.247 mm, disintegration time of 44-60 seconds, a pH between 6.06-6.33, and a film folding resistance between 339.83-673.67 times. The preparation was stable in storage for 3 months at a temperature of 25±2°C and 40±2°C. The preparation showed antibacterial activity against *S. mutans* with zone of inhibition between 8.0-9.1 mm. Clove leaf ethanol extract can be formulated into OTFS preparations with good physical characteristics, stable in 3 months storage and had antibacterial activity against *S. mutans* so that it has the potential to overcome bad breath.

Keyword: Halitosis, Clove, Syzygium aromaticum (L.) Merr. & Perry, Oral Thin Film Strip, Gelatin

Abstrak. *Halitosis atau bau mulut dapat terjadi karena buruknya perawatan kebersihan mulut, karies gigi atau infeksi pada rongga mulut. Penggunaan sedian penyegar mulut merupakan salah satu solusi untuk mengatasinya. Oral Thin Film Strip (OTFS) merupakan salah satu alternatif sediaan penyegar mulut. Pembuatan sediaan OTFS dilakukan menggunakan metode solvent casting. Lima formula film dibuat dengan variasi konsentrasi gelatin dan HPMC yaitu F1 (0:3), F2 (1:3), F3 (1:1), F4 (3:1), dan F5 (3:0). Evaluasi sediaan meliputi uji organoleptik, keseragaman bobot, ketebalan, waktu hancur, pH, dan daya tahan lipat. Pengujian stabilitas dilakukan selama 3 bulan pada suhu 25±2°C dan 40±2°C. Uji aktivitas antibakteri terhadap Streptococcus mutans (S. mutans) dilakukan pada sediaan dengan metode difusi cakram. Sediaan OTFS yang dihasilkan memberikan warna, aroma, dan rasa khas dari ekstrak yang homogen. Sediaan yang dihasilkan memiliki bobot antara 0,345-0,180 gram, ketebalan antara 0,148-0,247 mm, waktu hancur 44-60 detik, pH antara 6,06-6,33, dan daya tahan lipat film antara 339,83-673,67 kali. Sediaan film stabil pada penyimpanan selama 3 bulan pada suhu 25±2°C dan 40±2°C. Sediaan menunjukkan aktivitas antibakteri terhadap S. mutans dengan zona hamba tantara*

*Corresponding author at: Universitas Sumatera Utara, Medan, Indonesia

E-mail address: bayu@usu.ac.id

8.0-9.1 mm. Ekstrak etanol daun cengkeh dapat diformulasikan menjadi sediaan OTFS dengan karakteristik fisik yang baik, stabil dalam penyimpanan 3 bulan dan memiliki aktivitas antibakteri terhadap S. mutans sehingga berpotensi untuk mengatasi bau mulut.

Kata kunci: *Halitosis, Cengkeh, Syzygium aromaticum (L.) Merr. & Perry, strip film tipis oral, Gelatin*

Received 05 June 2022 | Revised 19 October 2022 | Accepted 12 November 2022

1. Introduction

Halitosis or bad breath is a common oral health problem that is often ignored in the community. The main symptom of halitosis is the appearance of an unpleasant odour from the mouth due to bacterial activity in the mouth, decay of food residues in the mouth or poor oral hygiene. Halitosis is an oral health problem that does not receive much attention from the public and government because it is rarely life-threatening. However, bad breath is very disruptive to comfort and communication with others [1].

Clove leaves are part of the clove plant that is rarely used compared to clove flowers which are more dominantly used. From the results of the study, it is known that clove leaves also contain antibacterial compounds such as flavonoids, phenolics, triterpenoids, tannins, phenolics, eucalyptol compounds, caryophyllene, limonene. Flavonoid compounds are active compounds that function as antibacterials [2]. Clove leaves function as antibacterials by inhibiting bacterial growth such as S. mutans bacteria that cause bad breath. In addition, another study showed that there was a significant decrease in hydrogen sulfide level after gargling with clove mouthwash, which means that clove mouthwash is effective in reducing hydrogen sulfide level in the mouth therefore it also helps reduce bad breath [3].

Oral thin film strip (OTFS) is a development of pharmaceutical preparations in the form of thin solid preparations made for oral use of drugs. This preparation is widely developed because it has various advantages such as easy to use and convenience compared to other preparations [4]. The development of pharmaceutical preparations using cloves has been widely developed with various therapeutic purposes, such as nanoemulsion [5] or oral gel [6]. However, clove leaves ethanol extract has not been investigated in the form of OTFS.

Based on the above explanation, researchers are interested in making OTFS preparations containing ethanol extract of clove leaves as a mouth freshening preparation using the solvent casting method with a combination of hydroxy propyl methyl celluloses (HPMC) and gelatin.

2. Materials and Methods

2.1. Materials

The clove leaves were obtained from Simalungun District, North Sumatera Province. Ethanol, glycerol, HCl, HPMC, gelatin and citric acid were obtained from Smart Lab Chemical, Medan, Indonesia. All chemicals were analytical grade without further purification.

2.2. Extract Preparation

Clove leaves were cleaned from impurities, then dried and grinded with a grinder until the sample became powder. Then, the sample was extracted using the maceration method with 96% ethanol until a thick extract was produced.

2.3. Phytochemical Screening Test

Phytochemical screening testing was carried out to determine the metabolite content contained in clove leaves extract, including alkaloids, tannins, glycosides, saponins, flavonoids, steroids / triterpenoids [7].

2.4. Formulation of OTFS

Five formulas were developed using 1.5% ethanol extract of clove leaves. The variation of HPMC and gelatin ratio were conducted based on Table 1. Corn starch 3% and glycerine 2% were added to all formula. Citric acid, menthol, sucralose and essential oil were added with same concentration for all formula.

2.5. OTFS preparation

Corn starch was dissolved with hot distilled water, then stirred at 500 rpm until a clear gel was formed. Gelatin dissolved with hot distilled water, and HPMC was developed with distilled water after being stirred and kept at 60^{o}C until a gel mass was formed. A 100 ml beaker glass was calibrated. Three polymer masses were put into the calibrated beaker glass and then mixed homogeneously. Then citric acid added with sodium benzoate, and mixed until dissolved. The ethanol extract of clove leaves was dissolved with ethanol gradually until it became diluted. Then, mixed using a magnetic stirrer at 500 rpm, mix the thick ethanol extract of clove leaves into the polymer solution. The essential oil, sucralose, and menthol were added into the beaker glass after combining, and then stirred until dissolved. To remove air bubbles, the mixture was allowed to stand at room temperature. After that, it is put into a mold and dried for twenty-four hours in a dehydrator at plus or minus 40^{o}C. After drying, remove the film from the mold and cut it into 2 x 3 cm pieces [8,9].

Table 1. The main polymer composition of OTFS

Materials	Formula (%)				
	F1	F2	F3	F4	F5
Ethanol extract of clove leaves	1.5	1.5	1.5	1.5	1.5
HPMC	3	2.25	1.5	0.75	0
Gelatin	0	0.75	1.5	2.25	3
Corn starch	3	3	3	3	3
Glycerin	2	2	2	2	2
Distilled water adds	100	100	100	100	100

2.6. Physical Evaluation

The physical evaluation was conducted to evaluate the physical characterization of the film. The evaluation such as organoleptic, weight uniformity, film thickness, disintegration time, pH, and folding endurance test. The weight uniformity of film was evaluated using 6 film to get the mean value. The thickness test was done using a calliper at 5 different side of a film. The disintegration test was done by embedding film into a petri dish containing 15 ml distilled water, and shaken until film started to break. The pH determination was checked using a calibrated pH meter [10,11].

2.7. Stability test

The stability test of OTFS preparation of ethanol extract of clove leaves (Syzygium aromaticum (L.) Merr. & Perry) was stored at room temperature (25±2ºC) and hot temperature (40±2℃). The preparation was stored in a tightly closed container and then wrapped in aluminium foil for 3 months. Observations were made every month by conducting examinations which included organoleptic, weight uniformity and pH [9].

2.8. Antibacterial Test

Put 0.1 ml of bacterial inoculum into a sterile petri dish, then poured 15 ml of Mueller Hinton agar media at 45℃. On the solid media, the ODTF preparation sheet of clove leaves ethanol extract was placed, then incubated for 24 hours at 35±2℃. Then, the growth of bacteria was observed and the diameter of inhibition was measured [12].

3. Results and Discussion

The results of the phytochemical screening test showed that the powder and ethanol extract of clove leaves contained alkaloids, flavonoids, glycosides, saponins, steroids and tannins (Table 2). These results are in accordance with Dahiru, 2021 who have examined the levels of metabolic compounds in clove leaves extract in various solvents. The content of these metabolite compounds was the compound responsible for the antibacterial effect of the extract [13].

Table 2. Screening phytochemical of powder and ethanol extract of clove leaves

Compound	Result	
	Powder	Extract
Alkaloid	+	+
Flavonoid	+	+
Glycoside	+	+
Saponin	+	+
Steroid	+	+

\+ = Contains compound
\- = Contains no compound

3.1. Physical Evaluation Test

Based on visual observation, the five-formula showed good homogeneity, reddish brown colour, extract aroma with elastic texture, but F3 and F4 produced oral thin films that were slightly stickier than the other. The weight uniformity of all formula tended to produce different weights depending on the polymer used. F1 with HPMC: gelatin (1:2) variation produced the largest weight and F3 with HPMC: gelatin (1:1) variation produced the smallest film weight. The use of HPMC and gelatin affects the density of the film formed resulting in differences in the character of the film produced [14]. The film thickness of F1 produced the thickest film compared to other formula, while the smallest film thickness was shown by F3 which was 0.148 mm. The thickness of the film was proportional to the weight of the resulting film where, F1 with the heaviest weight had the highest thickness. The disintegration time of the film showed that the resulting film was disintegrated after 44-60 seconds, where F3 and F4 showed the best results because they had a faster disintegration time than the others. The pH of the film was in the range of 6.06 to 6.40. The pH of this preparation was safe for oral use because it was still in accordance with the normal pH of saliva which was around 5. 9 to 7.1 [15]. Meanwhile, folding endurance testing for F1, F2 and F3 showed the highest results, which were above 600 times, while F3 and F4 were lower, but showed folding endurance above 300 times therefore they were also included in films with excellent durability and elasticity [16]. The physical test data of the resulting film preparations can be seen in Table 3.

Table 3. The physical evaluation of OTFS contained clove leaves ethanol extract

Formula	Weight uniformity (g)	Thickness (mm)	Disintegration time (s)	pH	Folding endurance(times)
F1	0.345±0.05	0.247±0.03	60±2	6.06±0.02	669.83±14.7
F2	0.303±0.02	0.235±0.01	59±1	6.33±0.02	647.33±14.7
F3	0.180±0.03	0.148±0.01	45±2	6.09±0.02	446.67±24.6
F4	0.262±0.02	0.215±0.01	44±1	6.16±0.04	339.83±19.1
F5	0.200±0.02	0.195±0.01	60±2	6.40±0.06	673.67±25.1

3.2. Stability Test

The stability test was done to evaluate the stability of OTFS during 3-month storage time. The test was carried out at two different temperatures (25±2℃ and 40±2℃). Based on the result, it can be seen that the film weight showed a good stability. The results of the examination of the weight of the film for 3 months at a temperature of 25±2℃ and 40±2℃ can be seen in Fig. 1 and 2.

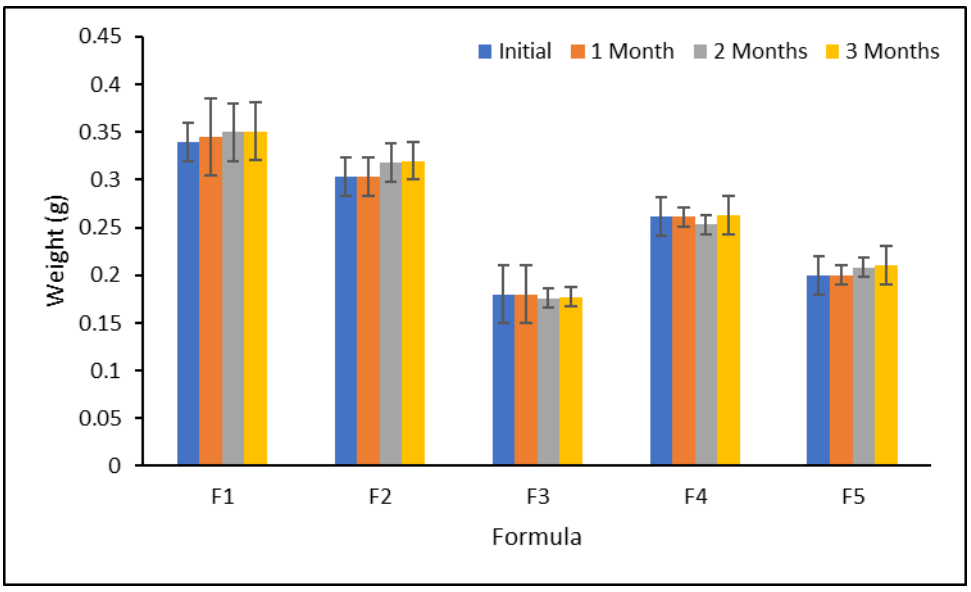

Figure 1. Weight uniformity data of OTFS for 3 months at 25±2°C

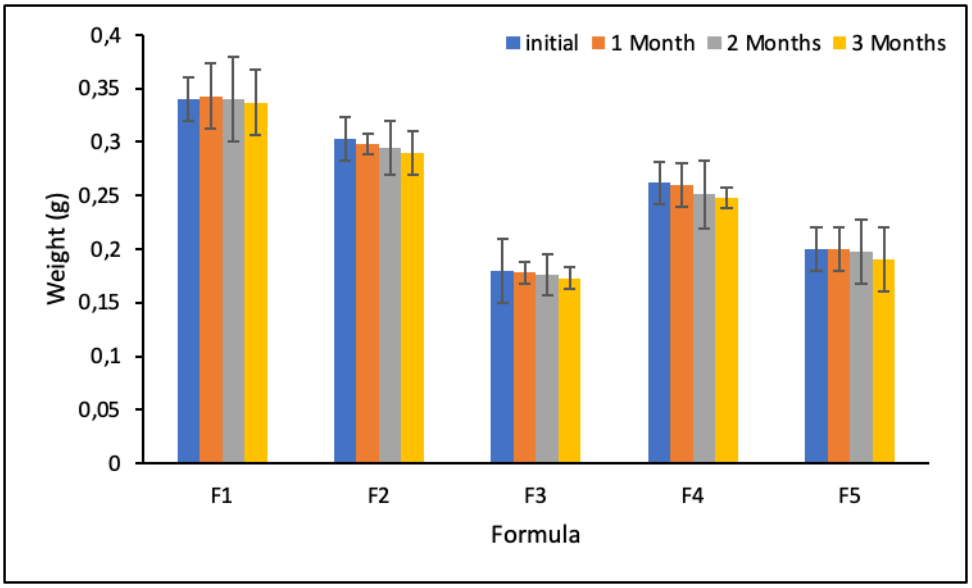

Figure 2. Weight uniformity data of OTFS for 3 months at 40±2°C

Based on the results of pH examination at 25±2℃, the pH value of stored OTFS preparations tends to be stable. The film containing HPMC (F1, F2, F3 and F4) tend to be more stable than film that do not contain HPMC because HPMC polymers are neutral and stable in various pH and the resulting preparations will appear clear and can form films well. The results of pH measurements of OTFS preparations at 25±2℃ and 40±2℃ for 3 months can be seen in Fig. 3 dan 4.

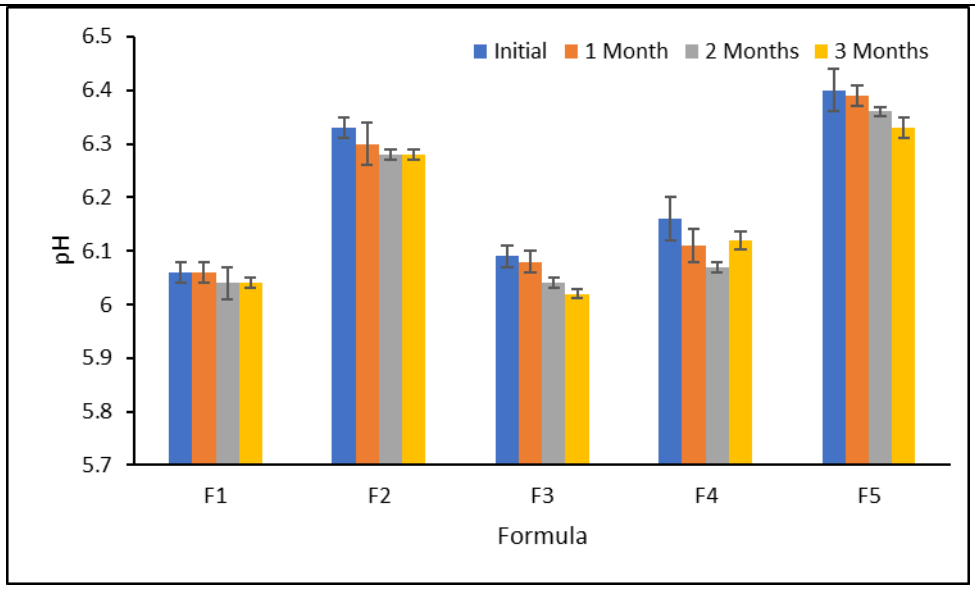

Figure 3. pH of OTFS for 3 months at 25±2ºC

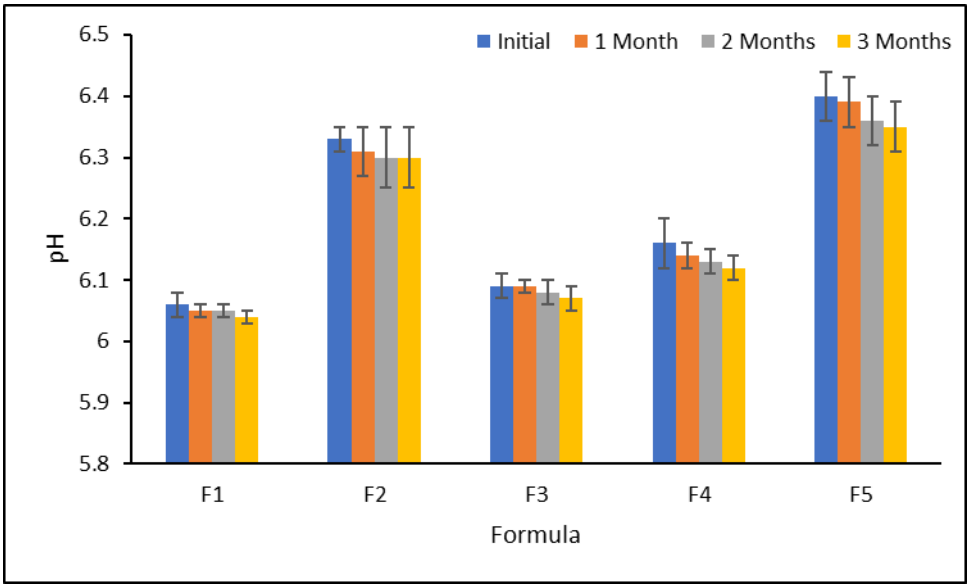

Figure 4. pH of OTFS for 3 months at 40±2ºC

3.3. Antibacterial activity of OTFS against *S. mutans*

Based on the antibacterial activity test of OTFS preparation of clove leaves ethanol extract against *S. mutans* bacteria, the inhibition zone diameter was 8.0 to 9.1 mm in the five formulas (Table 4). Based on this classification, the inhibition zone of OTFS preparation of clove leaves ethanol extract was categorised as moderate. The concentration used in the formulation was 1.5% for all formula. The inhibition zones of all formula showed differences because the film-forming polymers used were different therefore the physical characteristics and the release of active substances from the films were also different [17].

Table 4. The zone of inhibition of OTFS against *S.mutans*

Formula	Zone of inhibition (mm)
F1	8.6 ± 0.750
F2	9.1 ± 0.404
F3	8.0 ± 0.404
F4	9.1 ± 0.208
F5	8.4 ± 0.251

4. Conclusions

OTFS containing ethanol extract of clove leaves can be produced with good physical quality and stable during 3 months storage at 25±2ºC and 40±2ºC. The use of 1.5% clove leaves ethanol extract showed inhibitory activity against *S.mutans* with moderate category.

5. Acknowledgements

We appreciate to Universitas Sumatera Utara for funded this research in accordance with the contract of TALENTA research implementation with contract number 77/UN5.2.3.1/PPM/KP-TALENTA/2022.

REFERENCES

[1] Usra NF, Alimuddin H, Aisyah AR. Overview of knowledge about the causes of halitosis and its treatment in society (Literature review study). *DHeJA: Dental Health Journal of Aceh 2023*; *2*(1): 23-29.

[2] Suhendar, Usep, Sogandi. Identification of bioactive compounds in clove leaves (*Syzygium aromaticum*) extract as inhibitor *Streptococcus mutans. Jurnal Biologi*. 2019;12(2).

[3] Meramis JL, Ratuela JE. Gargling with clove leaves (*Syzygium aromaticum*) to improving oral and dental hygienen in elementary school children. *Journal of Dental Hygiene and Therapy*. 2022;3(1): 31-34.

[4] Ramadhani A, Saadah S, Sogandi. Antibacterial effect of clove leaf extract (*Syzygium aromaticum*) against *Escherichia coli* and *Staphylococcus aureus*. *Jurnal Bioteknologi & Biosains Indonesia*. 2020;7(2): 203-208.

[5] Laila L, Candra A, Permata YM, Prasetyo BE. The influence of *Catharanthus roseus* (L.) G. Don. ethanol extract in clove oil nanoemulsion: Physical characterization, antioxidant and antibacterial activities. *International Journal of Applied Pharmaceutics*. 2023; *15*(3):254–260.

[6] Abdellatif AO, Mohamed AAA, Seed-Ahmed SS, Said MM, Mohamed NA, Mohammed KA, Alqamar MO. Development of a pharmaceutical formulation containing clove (*Syzygium aromaticum*) extract for the management of oral candidiasis. *Saudi Journal of Biomedical Research*. 2023;*8*: 160-165.

[7] Reveny J, Maha HL, Laila L. A comparative study of phytochemical screening and DPPH radical scavenging activity of *Ficus carica* Linn. leaves extracts. *Tropical Journal of Natural Product Research*. 2023;*7*(2): 2337-2340.

[8] Fajria TR, Nuwarda F. Teknologi sediaan oral lapis tipis terlarut cepat (*Fast Dissolving Film*). *Majalah Farmasetika*. 2018;3(3): 58-64.

[9] Dewi WA, Mulya D. Formulasi dan evaluasi sifat fisik serta uji stabilitas sediaan *edible film* ekstrak etanol 96% seledri (*Apium graveolens* L) sebagai penyegar mulut. *Indonesia Natural Research Pharmaceutical Journal*. 2019;4(2): 32-40.

[10] Mahaparale S, Wagh BS. Formulation and evaluation of mouth dissolving film of glycopyrolate. *International Journal of Creative Research Thoughts*. 2021; 9(9): 511-514.

[11] Tamer MA, Hammid SNA, Ahmed B. Formulation and in vitro evaluation of bromocriptine mesylate as fast dissolving oral film. International Journal of Applied Pharmaceutics. 2018; *10*(1): 7-20.

[12]. Ningsih W. Formulasi dan uji efektivitas antibakteri *edible film* ekstrak biji pinang (*Areca catechu* Linn.). *Jurnal Ilmu Farmasi dan Farmasi Klinik*. 2018;15(2): 71-76

[13] Dahiru N, Paliwal R. Phytochemical analysis and antimicrobial properties of *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae). *Journal of Environmental Bioremediation and Toxicology*.2021; *4*(1): 4-7.

[14] Sari DI, Fitriana MI, Mulyadi RR, Hidayati L. Karakteristik dan uji stabilitas fisik sediaan *edible film* ekstrak etanol kulit batang kasturi (*Mangifera casturi kosterm*) berbasis gelatin. *Pros Semin Kefarmasian dan Present Ilm*. 2017; 8-17.

[15] Zubaydah WOS, Sahumena MH. Fast dissolving oral film salbutamol sulphate by using HPMC polymer. *Indonesian Journal of Chemometrics and Pharmaceuticals Analytics*. 2021;1(3): 134-136.

[16] Ozakar RSM, Ozakar E. Current overview of oral thin films. *Turkish Journal of Pharmaceutical Sciences*. 2021; 18(1): 111-121. doi: 10.4274/tjps.galenos.2020.76390

[17] Alfiah RR, Khotimah S, Turnip M. Efektivitas ekstrak metanol daun sembung rambat (*Mikania micrantha* Kunth) terhadap pertumbuhan jamur *Candida albicans*. *Jurnal Protobiont. Protobiont.* 2015; 4(1):52-57.