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Formulation of chewable lozenges of som jawa (*Talinum paniculatum* (Jacq.) Gaertn) leaves extract applied for *Candida albicans* topical infection

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ABSTRACT

Background: The bioactive compounds of Som Jawa (Talinum paniculatum (Jacq.) Gaertn) have many variations;one of them is flavonoid. This compound has antifungal activity towards Candida albicans, which the leading cause of topical infection. Chewable lozenges are one of the novel methods of delivering drugs for local action, especially in the mouth.
Objective: To formulate chewable lozenges from Som Jawa leaves extract and evaluate their active substances.
Methods: Extraction was done by rmaceration method with ethanol. The extract was evaporated using a rotary evaporator, then followed by a phytochemical investigation to express the active ingredients inside extract. Colour test was used to identify active ingredients. Som Jawa leaves extract was then formulated into chewable lozenges. Testing of physical properties of the chewable lozenges includes organoleptic test, weigh uniformity, hardness, elasticity, friability, chewiness and moisture content. Antifungal activity was tested by using a diffusion method.
Result: Som Jawa leaves extract with the concentration of 50% can inhibit the growth of Candida albicans with inhibition zone diameter of 22.69 mm. The organoleptic test resulted that the dosage is odourless, brown, and chewy. Weigh uniformity resulted that all lozenges were meet the British Pharmacopoeia range with the CV < 5%. The hardness test resulted for approximately of 0.2732 Kgf. The average of elasticity, friability, and chewiness respectively was 0.0826 Kgf, 6.046 mm, and 0.4998 Kgf.mm.
Conclusion: This formula chewable lozenge was potential as alternative herbal medicine for topical infection.

Latar Belakang: *Kandungan senyawa aktif daun Som Jawa (Talinum paniculatum (Jacq.) Gaertn) sangat beragam, salah satunya flavonoid. Senyawa flavonoid memiliki aktivitas antijamur terhadap Candida albicans, yang merupakan penyebab utama infeksi topikal. Sediaan chewable lozenges adalah salah satu metode penghantaran senyawa aktif yang bekerja secara lokal, terutama di dalam mulut.*
Tujuan: *Penelitian ini bertujuan untuk membuat chewable lozenges dari ekstrak daun Som Jawa dan menelusuri aktivitas senyawanya.*
Metode: *Ekstraksi dilakukan dengan metode remaserasi dengan etanol. Ekstrak tersebut diuapkan dengan rotary evaporator, kemudian diuji secara fitokimia untuk mengetahui kandungan senyawa aktif di dalam ekstrak. Identifikasi warna digunakan sebagai penanda adanya kandungan senyawa tertentu. Ekstrak daun Som Jawa kemudian diformulasikan dalam sediaan chewable lozenges. Pengujian karakteristik fisik dari chewable lozenges, meliputi organoleptik, keseragaman bobot, kekerasan, elastisitas, kekenyalan, dan kandungan air. Pengujian aktivitas antijamur dilakukan dengan metode difusi.*

Hasil: *Ekstrak daun Som Jawa dengan konsentrasi 50% dapat menghambat pertumbuhan jamur Candida albicans dengan diameter zona hambat 22,69 mm. Uji organoleptik menghasilkan data bahwa sediaan tidak berbau, bewarna coklat, dan kenyal. Keseragaman bobot memenuhi persyaratan British Pharmacopoeia dengan nilai CV < 5%. Uji kekerasan menunjukkan nilai rerata 0,2732 Kgf. Hasil rerata uji elastisitas, kerapuhan, dan kekenyalan berturut-turut yakni 0,0826 Kgf, 6,046 mm, dan 0,4998 Kgf.mm.*

Kesimpulan: *Formulasi chewable lozenges sangat potensial sebagai alternatif obat herbal untuk pengobatan infeksi topikal.*

INTRODUCTION

The numbers of infections secondary to pathogenic fungi have steadily increased over the past 30 years. Poor hygiene and sanitary are two factors that contribute to the increasing incidence of fungal infections. The most common cause of fungal infections worldwide is Candida, a genus of yeasts.[1]

Candida species commonly reside as commensal organisms, being part of the normal microbiome in the gut, oral cavity, or vagina in approximately 50% of the population. Although usually, these fungi cause no pathology, if there are changes in the local environment, such as alterations in normal microbiota or compromised local immune defences, then these fungi can become pathogenic. As such, they cause mucosal disease in a significant proportion of immunosuppressed patients and women of fertile age with the majority of these individuals experiencing superficial mucosal candidiasis such as scorbut.[2]

Medicinal plants represent a rich source of antimicrobial agents. Many of the plant materials used in traditional medicine are readily available in rural areas cheaper than modern medicine. Plants generally produce many secondary metabolites which constitute an essential source of micro biocides, pesticides and many pharmaceutical drugs. Plant products remain the principal source of pharmaceutical agents used in traditional medicine. The effects of plant extracts on bacteria and fungi have been studied by a vast number of researchers in different parts of the world.[3]

Talinum paniculatum (Jacq.) Gaertn, a herbal plant from Indonesia, revealed traditional remedies, such as for diarrhoea, menstruation, promotes breast milk, and scorbut.[4] One study which was done by Kumari et al. showed an antimicrobial activity of Som Jawa leaves against *Staphylococcus aureus* and *E.coli*.[5] Patel et al.(2018) also proved that leaf extracts from *Talinum paniculatum* (Jacq.) has inhibitory effects against broad spectrum of microorganism, including *Staphylococcus aureus*, *Serratia marcescens*, *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *Candida albicans*.[6]

Phytochemical investigation Som Jawa leaves extract showed flavonoids, saponins, tannin, and other polyphenol compounds.[7] Reis et al.(2015) found phytosterol compounds from Som Jawa leaves, consisting of β-sitosterol (10.60%), stigmastanol (2.76%), stigmasterol (0.85%), campesterol (0.80%), phytols (69.32%), α-tocopherol (0.99%), poly-saturated acid (0.43-3.41%) and high percentage of flavonoids.[8]

Flavonoids are bioactive compounds found in large quantities in certain plants. These compounds represent one of the essential phenolic groups (polyphenols) have various functions, including (1) the protection against ultraviolet and visible light; (2) for pollination; (3) plant hormones and (4) enzyme inhibitors.[9]

Herbal dosage form, especially for topical anti-infectiveformulation has been evaluated recently. Santos et al. showed the effectiveness of pomegranate gel to prevent and control oral candidiasis in patients undergoing anticancer treatment.[10] Palmieri et al.(2017) also developed herbal gel for vaginal treatment. The combination of basis gel (HPMC), *Calendula*, *Melaleuca*, and *Salvia officinalis* take control of the vaginal surface integrity, and mucosa restoration after mechanical or chemical or microbiological damage, primarily through the well-known *Calendula* contributes to body defences against external agents, has anti-inflammatory, antioxidant and healing properties; *Salvia* expresses an extensive and selective control altogether, supports the natural mechanism

for body´s purification, promotes the toxin elimination; *Melaleuca* expresses enteriley an extensive and selective control of pathogenic microflora enclosing the often relapsing *Candida albicans* superinfections.[11]

The focus of this research is to study the antifungal activity of Som Jawa leaves extract chewable lozenges formula. Chewable lozenges are various-shaped, solid dosage forms usually containing a medicinal agent and a flavouring substance, intended to be dissolved slowly in the oral cavity for localised effects, especially in the mouth.[12] Therefore, this formulation was potential to be developed as topical infection therapy towards *Candida albicans*.

MATERIALS

The Som Jawa leaves (*Talinum paniculatum* (Jacq.) Gaertn) was collected and determined by the Indonesian Institute of Science, Bogor, Indonesia (January, 2018). All reagents and solvents for extraction and phytochemical screening are analytical grades (Merck). All reagents are used without further purification. Reagents for chewable lozenges production are carrageenan, benzoic acid, high fructose syrup, citric acid, and essence. The antifungal activity used *Candida albicans* ATCC 10231, Dimethylsulfoxide (DMSO), and agar media (Sabouraud Dextrose Agar / SDA) and Nutrient Broth (NB).The instruments used are buchner funnel, separating funnel, water bath, incubator (China), mould apparatus (China), UV-Vis spectrophotometer Shimadzu (China), Moisture Balance OHAUS MB 45 (Germany), and Texture Analyzer TA-XT plus (China).

Extraction

The fresh Som Jawa leaves were washed, covered with black fabric and dried up under sun's exposure. The dried material was pulverized in 177 microns (mesh no 80) of particle size and then macerated with ethanol 95% at room temperature for 24 hours. The macerate was filtered, and the solid phase was re-macerated using the new same solvent. The re-maceration was quadruplicated,and the filtrates were combined to be concentrated under reduced pressure (rotary evaporator). The ethanol residue contamination was tested by adding a mixture of sulphanilic acid and sodium nitrite to the little amount of the concentrated extract. The extract was indicated to be free of ethanol residue contamination when there was no pink colour after adding the reagent. The second test for the same purpose was carried out by heating a little amount of the extract after adding a mixture of acetic acid and sulphuric acid. The extract was confirmed to be free of ethanol when there was no banana-like smell.[13]

Phytochemical Screening

Flavonoid identification was performed by dissolving 100 mg extract into ethanol, added with magnesium powder and amyl alcohol. All this content was mixed up together. The mixture was agitated, and the flavonoid content was identified when the amyl alcohol phase showed a red colour.[14] Saponin identification was performed by dissolving 100 mg into 20 mL of water and vigorously shaken. The presence of saponin was detected when the solution formed a foam phase.[15] Polyphenol identification was performed by dropping 100 mg extract with iron (III) chloride. The tannin (polyphenol) was present when the solution showed a dark-green colour.[16]

Formulation of Chewable Lozenges

The formula of chewable lozenges consists of Som Jawa leaves extract as an active compound, and the proportion of basic ingredients were high fructose syrup 40%w/w, sucrose 20% w/w, gelatin 10% w/w, citric acid 0.3% w/w, apple essence 1% w/w, benzoic acid 0.05% w/w, and water 28.65% w/w.

Gelatin was moistened by dissolving in water, soaked for 10 minutes, next boiled using water bath at 60°C. Carrageenan was prepared by diluting with boiled water. Another side, the sugar mixtures were made from sucrose and high fructose syrup which heated at 80°C. All components, such as Som Jawa leaves extract, gelatin, carrageenan, sugar mixture, essence,

benzoid acid, and citric acid were mixed on the water bath (60°C) until homogeny. The temperature was decreased and maintained at 40-50°C until it became thick. This mixture was poured into the mould apparatus, then stored in the refrigerator (2-8°C) until solidify. Lozenges were removed from the mould and were kept for air drying. The physical test evaluation was an organoleptic test, weight uniformity, hardness, elasticity, friability, and moisture content.[17]

Isolate of Fungi and Inoculum Preparation

The standard strain used for the study is *Candida albicans* ATCC 10231. Fungi were grown on SDA (Sabouraud Dextrose Agar (Merck)) overnight at 37°C for 24 hours and 48 hours. The 3-5 colonies of standard strain *Candida albicans* ATCC 10231 was suspended in 2mL of Nutrient Broth. The turbidity of the homogenous suspension was adjusted to approximately 0.5 Mc Farland standards.[18]

Antifungal efficacy study

The antifungal activity was conducted by the agar diffusion method, employing 'a cup plate technique'. A5 µL of suspension were pipetted and mixed with 20 mL of Sabouraud Dextrose Agar, then poured into the plates. These contents swirled gently to produce uniform mixtures. The agar plates were allowed to cool and solidify. The different concentration of extracts (30%; 40%; 50%) was introduced into the respective cups using the sterile pipette. The agar plates were incubated at room temperature for 48 h. The zone of inhibition (ZOI) was measured. The entire operation was carried out in aseptic condition throughout the study. Each solution was tested in triplicate.[19]

The zone of inhibition was identified by measuring the clear zone around the extracts. This result was undoubtedly accurate if the plate was inoculated correctly and all other conditions were correct (Figure 1.).[20]

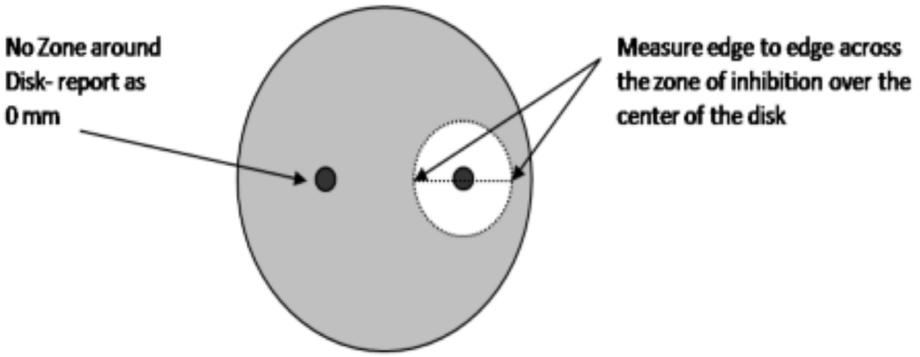


Figure 1. Measuring zones of inhibition. Gray shading represents a confluent lawn of bacterial growth. The white circle represents no growth of the test organism. [20]

RESULTS

Physical test evaluation

Re-maceration was selected as the method of extraction to avoid the chemical decomposition upon method utilising heat such as soxhlet. The product was characterised as a sticky dark brown extract (Figure 2). The extract was also free from ethanol residue contamination (Table 1). The phytochemical screening indicated the presence of flavonoid, saponin, and polyphenol (tannin) upon its phytochemical identification (Table 2 and Figure 3).

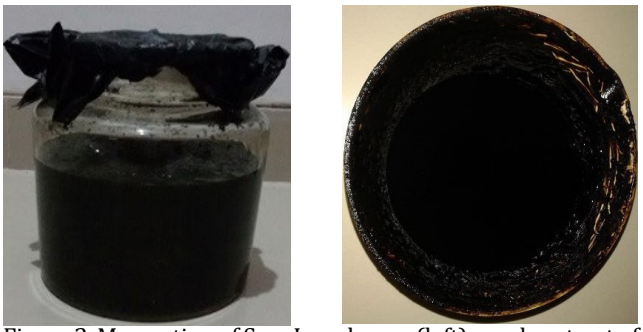
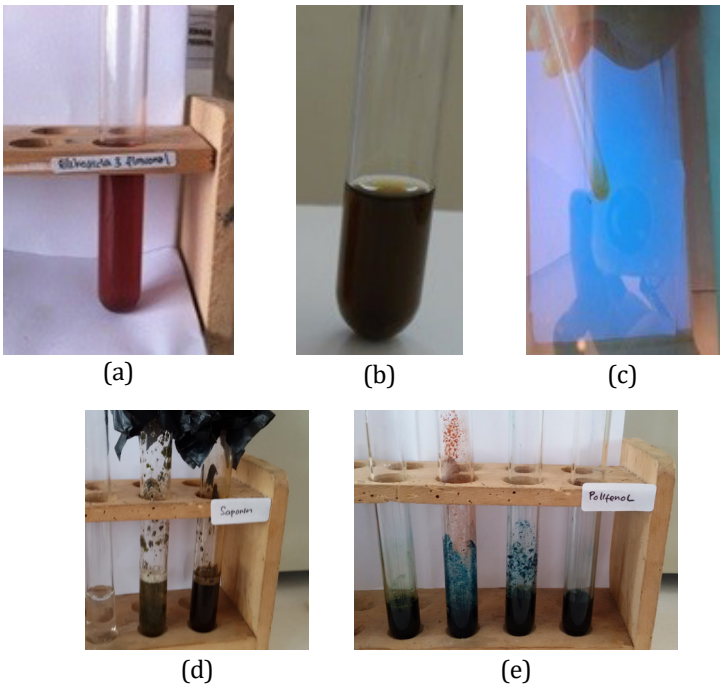


Figure 2. Maceration of Som Jawa leaves (left); crude extract of Som Jawa leaves (right)

Table 1. Result of ethanol residue contamination

Procedure	Identification	Result
Sulphanillic acid + NaNO2 + NaOH	No pink colour	Negative
Acetic acid + H2SO4	No banana-like smell	Negative



(a) (b) (c)

(d) (e)

Figure 3: (a) Glicoside flavonoid; (b) Shinode flavonoid; (c) Taubeck flavonoid; (d) Saponin; (e) Polyphenol

Table 2. Phytochemical screening of Som Jawa leaves extract

Parameters	Identification	Result
Glicoside Flavonoid (Fig 3.a)	Red	Positive
Shinode Flavonoid (Fig 3.b)	Yellow blackish	Positive
Taubeck Flavonoid (Fig 3.c)	Yellow greenish	Positive
Saponin (Fig 3.d)	Foam phase	Positive
Polyphenol (Fig 3.e)	Dark-green	Positive

Physicochemical characterization of chewable lozenges

The prepared chewable lozenges were evaluated for organoleptic parameters (odor, color, and taste), weigh uniformity, hardness, friability, and moisture content. All data analyzed by determining the mean ± standard deviation.

Organoleptic test

The organoleptic test resulted that the dosage is odorless, brown, and chewy. This color was obtained from the extract, thus the chewy properties based on their gelatin base.

Weigh uniformity

The twenty of randomly selected lozenges possessed acceptable uniformity of weight as per the pharmacopoeial limit, within the mean±5% British Pharmacopoeia range (Table 3). Weigh uniformity was the important parameter of solid dosage form.

Table 3. Weigh uniformity of chewable lozenges

Batch	Results	
	Weigh (grams)	CV (%)
1	2.7023	
2	2.7462	0.81
3	2.7208	

Hardness

Hardness is generally measured as the force needed to break the tablet in a specific plane. Tablet hardness was measured in the same unit of kilogram-force (Kgf). [21] The results showed a low value (approximately of 0.2732 kgf) (Table 4).

Table 4. Results of hardness of chewable lozenges

Batch	Hardness (kgf)
1	0.2675956
2	0.2865535
3	0.2655182
Mean±SD	0.2732224±11.59

Elasticity and Friability

The elasticity and friability was done by Texture Analyzer TA-XT plus. These parameters expressed with Kgf and mm, respectively (Table 5.)

Moisture Content

At Table 8, the moisture content of chewable lozenges was 1.48% (Table 6). This parameter was observed by analytical moisture balance.

Table 5. Result of elasticity, friability, and chewiness of chewable lozenges

Batch	Elasticity (Kgf)	Friability (mm)	Chewiness (Kgf.mm)
1	0.0837	6.0720	0.5082
2	0.0849	6.0258	0.5116
3	0.0793	6.0408	0.4795
Mean±SD	0.08263±0.003	6.0462±0.23	0.4998±0.01

Table 6. Result of moisture content of chewable lozenges

Batch	Moisture content (%)
1	1.38
2	1.61
3	1.45
Mean±SD	1.48 ±0.12

Antifungal activity

The antifungal activity was shown by different concentrations of Som Jawa leaves extract (Figure 4). The diameter of the inhibition zone was found maximum with 50% w/w of Som Jawa leaves extract (Figure 5). The results showed that the high concentration of extract provided the best inhibition.

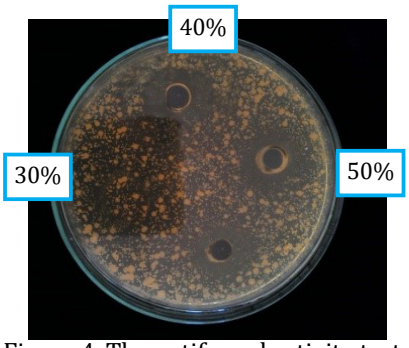


Figure 4. The antifungal activity test

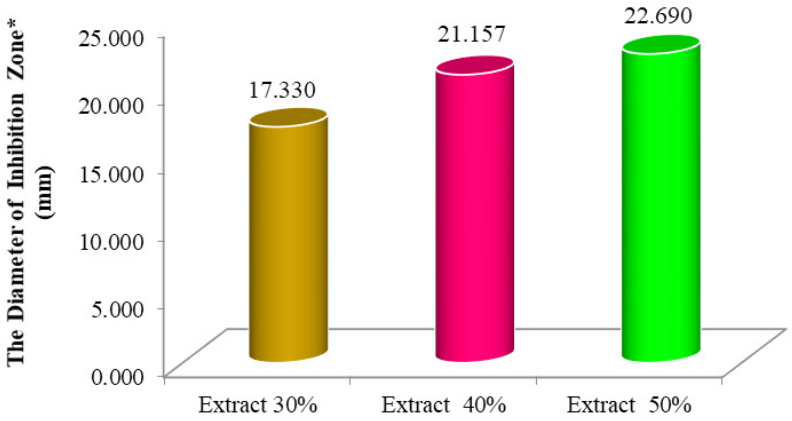


Figure 5. The result of antifungal activity

DISCUSSIONS

Phytochemical screening of secondary metabolites in Som Jawa leaves was conducted using a colour test. This test was used to determine the class of secondary metabolites, such as alkaloids, flavonoids, polyphenols, terpenoids/ steroid, saponins and tannins of the leaves.[22] The active compound from Som

Jawa leaves extract were flavonoids, saponins, and polyphenol, which have synergistic action decreasing the growth of Candida albicans.[23] The antifungal activity is based on the inhibition of spore development and mycelium hyphae elongation. Flavonoid anti-pathogenic activity can also be more specific. It is suggested that the mechanism of flavonoid antifungal activity is based on their ability to inactivate fungal adhesion and cell envelope transport proteins.

Several mechanisms of action of a phenolic compound such as tannin and saponin, involving cross-linking of microbial enzymes, inhibition of pathogen cellulases, xylanases and pectinases, chelation of metal ions relevant for enzymatic activities and tightening of cell walls, leading to the formation of a physical barrier against pathogen attack.[24]

The formulation into chewable lozenges was used to increase the acceptance, provide easy administration, convenience to a patient, large patient compliance, immediate onset of action, efficient treatment of low drug dosing, reduce dosage regimen, and cost-effectiveness.[25] Chewable lozenges are prepared by moulding a mixture of various carbohydrates to form candies, by moulding a matrix to form a soft lozenge, or by moulding a gelatin base into a chewable mass.[26] These gelatin-based were poured into the mould apparatus or out onto a sheet of uniform thickness. The dosage forms were then "punched out" using variously shaped punches.[27] The 50% w/w concentration of extract was chosen as an optimal concentration to be formulated into chewable lozenges. The humectants like fructose and sucrose can improve the mouth feels, especially for children. This dosage form also has a slightly acidic taste to mask the acrid taste of extract.

Weight uniformity test for tablets is required to ensure that the drug content in each tablet is distributed in a narrow range around the label strength because a slight variation in weight of tablet reflects variation in the content of active ingredient. According to the British Pharmacopoeia, drug products whose strength is >250mg, permissible limit of ±5% of the CV (coefficient of variation) is required to pass the test for weight uniformity.[28] These chewable tables were met the British Pharmacopeia requirements.

The hardness of chewable tablets should be such that they withstand the rigours of manufacturing, packaging, shipping, and distribution, as well as be easily chewed by the intended patient population. Applications submitted to FDA should use the same unit of measure in reporting results and specifications, including: kilopond (kp), kilogram-force (kgf), Newton (N), and Strong-Cobb Units (scu-1 kp = 1 kgf = 9.8 N = 1.4 scu).

FDA recommends that hardness for chewable tablets be kept low (e.g., < 12 Kgf). A higher hardness value (e.g., >12 Kgf) may be considered if brief (approximately 30 seconds) exposure to saliva before chewing results in significant disintegration and reduction in hardness of these tablets. The chewable lozenges were passed the following test (0.2732 kgf).

The elasticity and friability were the primary parameters which can be combined to obtain the secondary parameter, chewiness. This secondary parameter was important to get the acceptance feeling.

There is a correlation between moisture content and chewiness characteristics. The low moisture content proved the low chewiness. But on the other side, the high moisture content can stimulate the growth of microbial, like bacteria, yeast, and mould. Therefore, the moisture content of chewable lozenges must in the range of 1-3 %. [29]

CONCLUSION

The Som Jawa leaves extract formulated into chewable lozenges has the antifungal activity for Candida albicans. Physical characteristics of chewable lozenges have fulfilled the requirements, like the organoleptic test, weight uniformity, hardness, elasticity, friability, chewiness, and moisture content. Thus, this dosage form can be applicated as topical antifungal infection.

CONFLICT OF INTEREST
None declare

Acknowledgement
None declare

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