Journal of Taibah University Medical Sciences (2022) 17(2), 271–282

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Formulation, characteristics and anti-bacterial effects of *Euphorbia hirta L.* mouthwash

Benni Iskandar, M.Sc [a,b,*], Anita Lukman, M.Pharm [b], Sandika Syaputra, B.Pharm [b], Ucy N.H. Al-Abrori, BDS [c], Meircurius D.C. Surboyo, MDS [d] and Ching-Kuo Lee, Professor [a]

[a] *School of Pharmacy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan*
[b] *Department of Pharmaceutical Technology, Sekolah Tinggi Ilmu Farmasi Riau, Pekanbaru, Riau, Indonesia*
[c] *Bachelor of Dental Science Degree, Faculty of Dental Medicine, Universitas Airlangga, Indonesia*
[d] *Department of Oral Medicine, Faculty of Dental Medicine, Universitas Airlangga, Indonesia*

Received 14 June 2021; revised 18 August 2021; accepted 23 August 2021; Available online 17 September 2021

الملخص

أهداف البحث: غسول الفم عبارة عن محلول سائل يستخدم لتحسين صحة الفم ونضارة التنفس وتقليل بكتيريا الفم. تهدف الدراسة إلى صياغة مستخلص الإيثانول من نبات الفربيون كغسول للفم وتقييم خصائصه الفيزيائية وتحديد تأثيره المضاد للبكتيريا ضد البكتيريا العقدية الطافرة.

طرق البحث: تم صنع كل تركيبة غسول للفم باستخدام تقنية الذوبان. تم صنع ثلاث صيغ لغسول الفم من تركيزات مختلفة من مستخلص الإيثانول من نبات الفربيون (٠.٥٪، و١٪ و٢٪)، ويشار إليها باسم ف١ وف٢ وف٣. وتم تقييم الخواص الحسية، ومستويات الأس الهيدروجيني، والجاذبية النوعية، واللزوجة، وخصائص التدفق، والثبات، ومستوى التهيج، ووقت التلامس والتأثيرات المضادة للبكتيريا لكل تركيز.

النتائج: كان لكل من الصيغ رائحة زيت النعناع المميزة ولها طعم حلو وحار. لم تتأثر هذه الخصائص أثناء تخزينها. وتراوحت مستويات الحموضة من ٤.٥٩-٦.٠، وتراوحت كثل الوزن من ٠.٩٦٩٣ جم/مل – ١.٠٧١٠ جم/مل، وتراوحت اللزوجة من ١.٥٠سنتي بواز – ٣.٠ سنتي بواز. كما كان تركيز ف٣ غير مزعج مع إنتاج المخاط عند ١١.٣٢٥٪، وكان وقت ملامسته أقل مع تحييد بنسبة ٥٧.١٤٪ من مستعمرات العقدية الطافرة في ٣٠ ثانية وأظهر تحكما ثابتا مضادا للبكتيريا العقدية الطافرة خلال الأسبوع الأول من استخدامها.

الاستنتاجات: تقدم هذه الدراسة أول دليل على فائدة نبات الفربيون لصحة الإنسان. تحتوي التركيبة على الخصائص الفيزيائية وخصائص الثبات المثالية لغسول الفم، ولها خصائص مضادة للبكتيريا يمكن أن تحارب البكتيريا العقدية الطافرة. ويحتاج غسول الفم المُركب إلى مزيد من الاستكشاف لاستخدامه السريري.

الكلمات المفتاحية: نبات الفربيون؛ صحة الإنسان؛ الطب؛ غسول الفم؛ البكتيريا العقدية الطافرة

Abstract

Objective: Mouthwash is a liquid solution used to improve oral health and breath freshness as well as reduce oral bacteria. This study aims to formulate a *Euphorbia hirta L.* ethanol extract for mouthwash, evaluate the physical properties, and determine its anti-bacterial effects against *Streptococcus mutans*.

Methods: Each mouthwash formula was created by utilising a solubilisation technique. Three mouthwash formulas were created from different concentrations of *Euphorbia hirta L.* ethanol extract (0.5%, 1%, and 2%), and referred to as F1, F2, and F3. The organoleptic properties, pH levels, specific gravity, viscosity, flow properties, stability, irritation level, contact time, and anti-bacterial effects were evaluated for each concentration.

Result: The resulting formulas featured a distinctive smell of *oleum menthae piperitae* and had a sweet and spicy taste. These characteristics remained unaffected during storage. The acidity levels ranged from 4.59 to 6.0,

* Corresponding address: School of Pharmacy, College of Pharmacy, Taipei Medical University, No. 250 Wu-Xing Street, Taipei, 11031, Taiwan.
E-mail: benniiskandar@stifar-riau.ac.id (B. Iskandar)
Peer review under responsibility of Taibah University.

Production and hosting by Elsevier

 https://doi.org/10.1016/j.jtumed.2021.08.009

the weight masses ranged from 0.9693 to 1.0710 g/ml, and the viscosity ranged from 1.50 to 3.00 cP. F3 concentration was non-irritating with mucus production at 11.325%, had lower contact time with a neutralisation of 57.14% of *Streptococcus mutans* colonies in 30 seconds, and showed stable anti-bacterial control against *Streptococcus mutans* ($p = 0.000$) within the first week of use.

Conclusion: The study results offer the first proof of *Euphorbia hirta L.* used for improving human health. The formulation features the ideal physical and stability characteristics of mouthwash, and possesses anti-bacterial properties that can potentially combat *Streptococcus mutans*. Clinical use of the formulated mouthwash must be explored in future research.

Keywords: *Euphorbia hirta L.*; Human health; Medicine; Mouthwash; *Streptococcus mutans*

Introduction

Bacteria found on the surface of teeth, especially in plaque, can cause several types of dental caries, such as *Streptococcus mutans, Streptococcus sobrinus*,[1] *Streptococcus downei*,[2] and *Staphylococcus aureus*.[3] *Streptococcus mutans*[4] is a common type of flora found in the oral cavity and dominates the composition of bacteria in plaque. Toothbrushes, interdental cleaners, mouthwash, and antimicrobial agents can be used to prevent plaque formation. Chemical substances used in mouthwash have antiseptic or anti-bacterial properties to inhibit plaque formation.[5] Mouthwash is used to improve oral health and aesthetics, reduce bacteria, remove bad odours and prevent dental caries.[6] Moreover, mouthwash can reach areas in the mouth that may be difficult to reach with a toothbrush.[7]

The use of herbal products as medicine is increasing in popularity, and a growing body of research is focused on the use of medicinal plants in Indonesia, particularly medicinal plants used to improve oral health. Herbal mouthwash products can be easily found in the Indonesian market. Indonesia is particularly known for its indigenous medicinal plants and has a long history of producing natural products. *Euphorbia hirta L.* (also known as Patikan Kebo) is a traditional medicinal plant widespread in Indonesia and used to treat conjunctivitis and skin, gastrointestinal, bronchial and respiratory diseases.[8] The leaves of *Euphorbia hirta L.* are traditionally ground and used in rural areas of Indonesia as an antiseptic solution and as medicine to reduce itching. These leaves are used to prepare tea for the treatment of asthma. *Euphorbia hirta L.* is a wild plant that typically grows on the surface of semi-dry soil and can be found throughout Indonesia. Currently, this plant is not receiving sufficient attention from the public, even though it has considerable potential for medicinal uses.[9] *Euphorbia hirta L.* can be used to treat various diseases because it contains alkaloid chemical compounds, tannins, polyphenol compounds, and flavonoids.[10]

Euphorbia hirta L. can be used to inhibit the formation of biofilms, such as *Pseudomonas aeruginosa,*[11] *Escherichia coli, Staphylococcus aureus*,[12] *Salmonella typhi*, *Proteus vulgaris*, *Proteus mirabilis*, and *Streptococcus pyogenes*.[13] Research has noted that *Euphorbia hirta L.* can inhibit the growth of specific types of Gram-positive bacteria, such as *Staphylococcus aureus*, *Micrococcus* sp., *Bacillus subtilis*, and *Bacillus thuringensis*. It also inhibits the growth of certain types of Gram-negative bacteria, such as *Escherichia coli, Klebsiella pneumonia, Salmonella typhi*, and *Proteus mirabilis,* and inhibits fungal growths, such as *Candida albicans*, with inhibition zones ranging from 16 to 29 mm.[14]

Therefore, determining the effectiveness of *Euphorbia hirta L.* for anti-bacterial preparation for oral mucosa treatment is necessary. The purpose of this study is to evaluate the physical properties and characteristics of *Euphorbia hirta L.* extract and determine its anti-bacterial effects against *Streptococcus mutans*.

Materials and Methods

Euphorbia hirta L. sample

Euphorbia hirta L. samples were collected from the Panam area in the subdistrict of Tampan, in the district of Simpang Baru, in Pekanbaru, Indonesia. Mature plants were collected in polythene bags, and the samples were identified in a botanical laboratory (Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Riau). The samples were assigned the registration number 66/UN/19.5.1.1.3/Bio/Botani/2019 (see Figure 1).

Euphorbia hirta L. extraction

A total of 3500 grams of *Euphorbia hirta L.* was used to create an ethanol extract. It was cleaned, dried at room temperature for 24 hours, and processed in a blender until its texture became smooth.

To obtain the extract from the processed *Euphorbia hirta L.*, 2000 ml of a 96% ethanol solution was used, and this process was repeated thrice. The initial extract was filtered and evaporated at temperatures ranging from 50 °C to 60 °C to obtain a 100% pure *Euphorbia hirta L.* extract. The pure extract was weighed, stored in a sealed glass container, and subsequently placed in a desiccator before being used as mouthwash.

Euphorbia hirta L. mouthwash formulation

As much as 100 ml of mouthwash was produced for each formulation with *Euphorbia hirta L.* extract as the active substance. The formulations of *Euphorbia hirta L.* mouthwash (referred to as F1, F2, and F3) are presented in Table 1.

Propylene glycol was included in the *Euphorbia hirta L.* extract and placed in a glass beaker. It was then raised to 60 °C, stirred with a magnetic stirrer at 300 rpm and Tween 80, and sorbitol and aquadest were added.

Benzoic acid and sodium benzoate were dissolved in aquadest and added to the solution and stirred with a magnetic stirrer until homogeneous. Subsequently, 100 ml of the sorbitol *qs* and aquadest *ad* was stirred until the solution became clear, and *Oleum menthae piperitae* was added.

Organoleptic testing

Three *Euphorbia hirta L.* mouthwash formulations were tested by random respondents. A questionnaire was administered to record observations on colour, smell, and taste and denote significant differences in preparation methods. Organoleptic testing was performed on the same respondents once a week for a duration of eight weeks.

Acidity (pH)

The pH testing was conducted with a Hanna pH meter once a week for eight weeks, and the pH meter was calibrated using a buffer solution (pH 4 and pH 7). The electrodes were rinsed with distilled water and dried. Measurements were noted while preparing each *Euphorbia hirta L.* mouthwash in a beaker.

Stability test

Stability testing was conducted with the freeze-thaw test and carried out on the *Euphorbia hirta L.* mouthwash formulations in six cycles. Each cycle was observed after 48 hours of storage at 4 °C and again after 48 hours at 40 °C, and this process continued for 24 days.

Weight mass

The weight mass of each *Euphorbia hirta L.* mouthwash preparation was measured once per week for eight weeks using a Pyrex pycnometer. The pycnometer was cleaned with distilled water and dried before each use.

Viscosity test

Viscosity was measured using a Brookfield viscometer. Before measuring, the tool was adjusted by levelling the spindle boundary found on the surface of the tool. Thereafter, 100 ml of *Euphorbia hirta L.* mouthwash was immersed to the specified spindle limit mark. The viscometer was turned on for ±10 seconds, the size was set, and the instrument was turned off. Viscosity was calculated by the viscosity value on the specified spindle scale.

Irritation test

A slug mucosal irritation test was conducted on each of the three *Euphorbia hirta L.* mouthwash formulations and a control solution containing no *Euphorbia hirta L.* extract. The snails were weighed, placed on a 1 g sample of mouthwash preparation and left for 60 minutes. The snails were then removed from the preparation and weighed once again. The percentage of mucus production indicates the rate of irritation and is demonstrated by the following formula:

$$\text{Mucus production} = \frac{\text{Mucus weight (g)}}{\text{slug weight (g)}} \times 100\%$$

Contact time

Contact times of the three *Euphorbia hirta L.* mouthwash formulations were measured in sterile test tubes by adding 100 µL of *Streptococcus mutans* suspension to 10 ml of each formulation. After 30 seconds of contact, the solution mixtures absorbed as much as 50 µL, and they were placed in a natrium agar medium. They were each rotated three times to the left and three times to the right. The plates were incubated at 37 °C for 18–24 hours, and the results were observed. Each area on the medium that showed the least amount of colony growth with the shortest contact time was determined the most effective. Decrease in bacteria colonies is demonstrated by the following formula:

$$\text{Colony decreasing} = \frac{\text{colony in the medi} - \text{colony in the formula}}{\text{control colony bacteris}} \times 100\%$$

Anti-bacterial activity

A standard diffusion test was conducted to determine the anti-bacterial effects of each of the *Euphorbia hirta L.* mouthwash formulations. Anti-bacterial properties of each formulation were measured before and after one week of storage.

Additionally, 300 µL of the isolated *Streptococcus mutans (Oxoid, Thermo Scientific)* colony was selected and suspended in a natrium agar medium. A total of 15 ml of each of the three *Euphorbia hirta L.* formulations were added to the medium. Enkasari mouthwash was used as a control group and did not contain the *Euphorbia hirta L.* extract. After an incubation period of 24 hours at 37 °C, the size of the inhibition zone, which appears as the clearing zone, formed around the paper disc was measured. The test was repeated thrice.

Data analysis

The anti-bacterial effects of *Euphorbia hirta L.* mouthwash were analysed using one-way ANOVA and post-hoc Tukey HSD tests with $p < 0.05$, thus showing significant differences in each group with SPSS software.

Results

Organoleptic testing

Organoleptic testing results are shown in Table 2. The F1 solution was yellowish brown, had a distinctive smell of

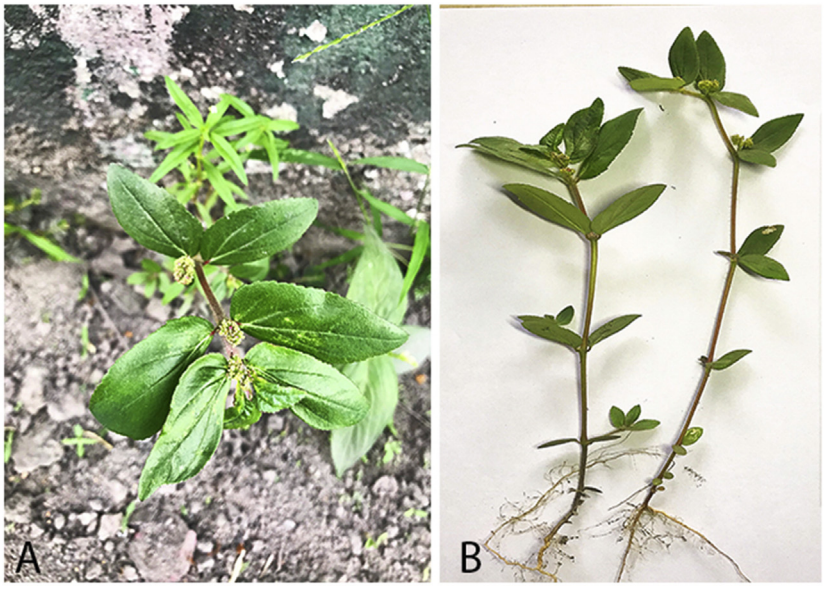

Figure 1: *Euphorbia hirta L* or Patikan kebo (A) and sample specimen (B).

Table 1: *Euphorbia hirta* L mouthwash formulation.

Component	Formulation (%)		
	FI	F2	F3
Ethanolic extract of *Euphorbia hirta L*	0.5	1	2
Propylene glycol *(CV.Clorogreen)*	25	25	25
Tween 80 *(CV.Clorogreen)*	5	5	5
Oleum menthe piperitae *(CV.Clorogreen)*	0.25	0.25	0.25
Benzoate acid *(CV.Clorogreen)*	0,1	0,1	0,1
Sodium benzoate *(CV.Clorogreen)*	1	1	1
Sorbitol 70% *(CV.Clorogreen)*	15	15	15
Aquadest	ad 100	ad 100	ad 100

Table 2: Organoleptics testing of *Euphorbia hirta L* mouthwash for 8 weeks.

		Week							
		1	2	3	4	5	6	7	8
FI	Form	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	Smell	OMP	OMP	OMP	OMP	OMP	OMP	OMP	OMP
	Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
		Spicy	Spicy	Spicy	Spicy	Spicy	Spicy	Spicy	Spicy
	Color	yellowish	yellowish	yellowish	yellowish	yellowish	yellowish	yellowish	yellowish
		brown	brown	brown	brown	brown	brown	brown	brown
F2	Form	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	Smell	OMP	OMP	OMP	OMP	OMP	OMP	OMP	OMP
	Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
		Spicy	Spicy	Spicy	Spicy	Spicy	Spicy	Spicy	Spicy
	Color	brown	brown	brown	Brown	brown	brown	brown	brown
F3	Form	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
	Smell	OMP	OMP	OMP	OMP	OMP	OMP	OMP	OMP
	Taste	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
		Spicy	Spicy	Spicy	Spicy	Spicy	Spicy	Spicy	Spicy
	Color	dark	dark	dark	dark	dark	dark	dark	dark
		brown	brown	brown	brown	brown	brown	brown	brown

OMP = Oleum menthe piperitae

Table 3: pH of *Euphorbia hirta L* mouthwash formulation for 8 weeks.

	Week							
	1	2	3	4	5	6	7	8
FI	6.05	5.22	5.06	5.04	5.01	5.00	4.88	4.79
F2	5.87	5.16	4.96	4.95	4.93	4.91	4.75	4.70
F3	5.79	4.98	4.78	4.75	4.72	4.72	4.68	4.59

Table 4: Weight mass of *Euphorbia hirta L* mouthwash for 8 weeks.

	Week							
	1	2	3	4	5	6	7	8
FI	0.9689	1.0274	1.0498	1.0551	1.0576	1.0589	1.0598	1.0641
F2	0.9693	1.0312	1.0504	1.0555	1.0581	1.0593	1.0613	1.0655
F3	0.9699	1.0399	1.0532	1.0569	1.0585	1.0598	1.0623	1.0710

Table 5: The viscosity of *Euphorbia hirta L*. mouthwash.

	Rpm	Storage	
		before (cP)	after (cP)
F1	20	1,50	3,00
F2	20	1,80	3,00
F3	20	1,80	3,00
control	20	2,10	3,06

oleum menthae piperitae, as well as a sweet and spicy taste. The F2 solution was brown, had a distinctive smell of *oleum menthae piperitae*, and a sweet and spicy taste. The F3 solution was dark brown and had a distinctive smell of *oleum menthae piperitae*.

The form, smell, taste, and colour of each solution remained consistent, with no changes after eight weeks of storage.

Table 6: The irritation test of *Euphorbia hirta L*. mouthwash.

	Replication	Mucous weight (g)	Snail weight (g)	Mucous (%)	Mean (%)
F1	1	1.173	14.330	12.219	11.325
	2	1.243	12.934	10.430	
F2	1	0.925	12.080	13.063	12.515
	2	1.512	18.097	11.967	
F3	1	0.934	15.755	16.865	14.965
	2	1.164	15.210	13.064	
control (−)	1	2.494	15.481	6.208	4.514
NaCl Fisiologis	2	5.499	15.501	2.819	
control (+)	1	1.989	15.748	7.918	8.229
Enkasari	2	1.610	13.747	8.540	

Table 7: Contact time evaluation.

	Colonies (CFU/ml)			mean ± SD (CFU/ml)	Colonies decreasing (%)
	1	2	3		
Control media	0	0	0	–	–
Control bacteria	105	97	112	105 ± 7.51	–
F1	74	71	79	75 ± 4.04	28.57
F2	60	54	61	58 ± 3.79	44.76
F3	49	41	46	45 ± 4.04	57.14

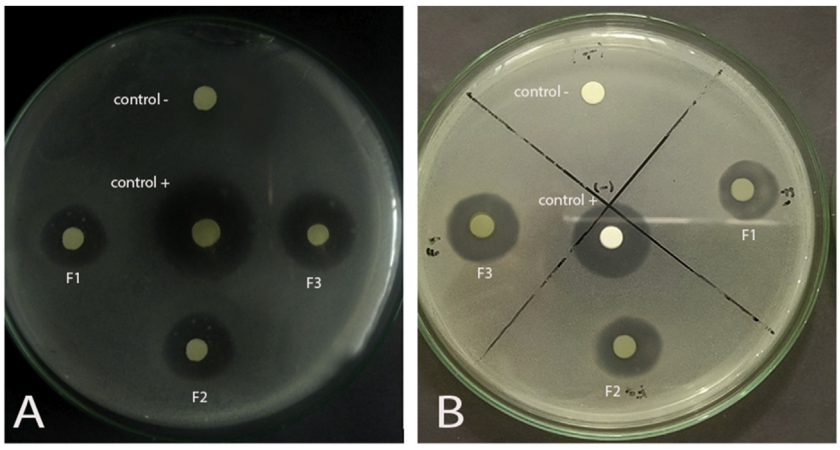

Figure 2: Anti-bacterial activity of *Euphorbia hirta L* mouthwash before storage (A) and after storage (B).

Table 8: Anti-bacterial activity of *Euphorbia hirta L* mouthwash.

	before storage				1 week after storage			
	Zone inhibition (mm)			mean ± SD	Zone inhibition (mm)			mean ± SD
	1	2	3		1	2	3	
F1	18.6	20.7	16.4	18.6 ± 2.15	17.5	16.8	17.2	17.2 ± 0.35
F2	20.4	19.3	20.7	20.2 ± 0.72	18.5	17.9	17.2	17.9 ± 0.66
F3	21.4	20.3	20.7	20.8 ± 0.56	21.3	19.9	217	20.9 ± 0.95
Control (−)	8.2	8.9	6.1	7.7 ± 1.46	6.1	7.1	6.7	6.6 ± 0.50
Control (+)	24.6	23.9	23.6	24.1 ± 0.51	23.6	23.2	24.5	23.7 ± 0.67

Table 9: Antibacteria activity of *Euphorbia hirta L*.

Part of *Euphorbia hirta L*	Extraction methods	Methods	Antibacterial activity	Reference
Leaves	Methanolic	disc diffusion method	- Inhibit *Staphylococcus aureus* at concentration 12.50 mg/ml - Inhibit *Micrococcus* sp at concentration 100 mg/ml - Inhibit *Bacillus subtilis* at concentration 100 mg/ml - Inhibit *Bacillus thuringensis* at concentration 100 mg/ml - Inhibit *Escherichia coli* at concentration 3.13 mg/ml - Inhibit *Klebsiella pneumonia* at concentration 100 mg/ml - Inhibit *Salmonella typhii* at concentration 100 mg/ml - Inhibit *Proteus mirabilis* at concentration 50 mg/ml	[14]
Leaves	Methanolic	disc diffusion method	Inhibit *Pseudomonas aeruginosa*	[26]
	Ethanolic	disc diffusion method		
	Aqueous	disc diffusion method		
Leaves	Methanolic	agar well diffusion method	- Inhibit *Bacillus subtilis* at concentration 220 µg/ml - Inhibit *Staphylococcus aureus* at concentration 220 µg/ml - Inhibit *Escherichia coli* at concentration 250 µg/ml	[27]
	Aqueous		Inhibit *Escherichia coli* at concentration 160 µg/ml	
Leaves	Methanolic		- Inhibit *Proteus mirabilis*	[28]

Table 9 (*continued*)

Part of *Euphorbia hirta L*	Extraction methods	Methods	Antibacterial activity	Reference
		Broth microdilution method	- Inhibit *Staphylococcus aureus*	
Young branches with leaves and inflorescence	Methanolic	Inhibitory Concentrations in Diffusion (ICD) method	- Inhibit *Escherichia coli* at concentration 25 µg/ml - Inhibit *Pseudomonas aeruginosa* at concentration 12.5 µg/ml - Inhibit *Proteus vulgaris* at concentration 50 µg/ml - Inhibit *Klebsiella pneumoniae* at concentration 12.5 µg/ml	[29]
	Aqueous		- Inhibit *Escherichia coli* at concentration 50 µg/ml - Inhibit *Pseudomonas aeruginosa* at concentration 25 µg/ml - Inhibit *Proteus vulgaris at concentration* 100 µg/ml - Inhibit *Klebsiella pneumoniae* at concentration 12.5 µg/ml	
Aerial	Methanolic	tube dilution method	- Inhibit *Escherichia coli* at concentration 0.189 mg/ml - Inhibit *Pseudomonas vulgaris* at concentration 1.2 mg/ml - Inhibit *Pseudomonas aeruginosa* at concentration 0.166 mg/ml - Inhibit *Bacillus subtilis* at concentration 0.296 mg/ml - Inhibit *Bacillus pumilus* at concentration 0.269 mg/ml - Inhibit *Streptococcus aureus* at concentration 0.216 mg/ml - Inhibit *Streptococcus faecalis* at concentration 0.214 mg/ml	[30]
Aerial	Methanolic	Broth dilution method	- Inhibit *Escherichia coli* at concentration 100 mg/ml - Inhibit *Klebsiella pneumoniae* at concentration 50 mg/ml - Inhibit *Shigella dysentriae* at concentration 100 mg/ml - Inhibit *Salmonella typhi* at concentration 50 mg/ml - Inhibit *Proteus mirabilis* at concentration 50 mg/ml	[31]
	Aqueous		- Inhibit *Escherichia coli* at concentration 50 mg/ml - Inhibit *Klebsiella pneumoniae* at concentration 25 mg/ml - Inhibit *Shigella dysentriae* at concentration 50 mg/ml - Inhibit *Salmonella typhi* at concentration 25 mg/ml - Inhibit *Proteus mirabilis* at concentration 25 mg/ml	
Aerial	Ethanolic	tetrazolium microplate assay	- Inhibit *Enterobacter aerogens* at concentration 1 mg/ml - Inhibit *Escherichia coli* at concentration 0.5 mg/ml - Inhibit *Klebsiella pneumonia* at concentration 1 mg/ml - Inhibit *Proteus mirabilis* at concentration 0.5 mg/ml - Inhibit *Proteus vulgaris* at concentration 0.25 mg/ml	[32]

(*continued on next page*)

Table 9 (*continued*)

Part of *Euphorbia hirta L*	Extraction methods	Methods	Antibacterial activity	Reference
			- Inhibit *Pseudomonas aeruginosa* at concentration 0.125 mg/ml - Inhibit *Salmonella typhi* at concentration 0.031 mg/ml - Inhibit *Shigella dysenteriae* at concentration 0.5 mg/ml - Inhibit *Staphylococcus aureus* at concentration 0.5 mg/ml - Inhibit *Bacillus subtilis* at concentration 0.25 mg/ml	
Aerial	Methanolic	tetrazolium microplate assay	Inhibit *Pseudomonas aeruginosa* at concentration 0.062 mg/ml	[11]
Aerial	Methanolic	Cup plate method	- Inhibit *Streptococcus pneumoniae* at concentration 60 mg/ml - Inhibit *Proteus vulgaris* at concentration 800 mg/ml	[33]
	Ethanolic		- Inhibit *Streptococcus pneumoniae* at concentration 60 mg/ml - Inhibit *Proteus vulgaris* at concentration 60 mg/ml	
	Aqueous		- Inhibit *Streptococcus pneumoniae* at concentration 80 mg/ml - Inhibit *Proteus vulgaris* at concentration 100 mg/ml	
Aerial	Ethanolic	broth microdilution method	Inhibit *Staphylococcus aureus* at concentration 63 µg/ml	[34]
Aerial	Methanolic	disk diffusion method	- Inhibit *Vibrio cholearea* at concentration 400 mg/ml	[35]

Acidity (pH)

The results of pH testing are presented in Table 3. The pH levels decreased in each formulation during eight weeks of storage. The pH levels of F1 ranged from 4.79 to 6.05, those of F2 ranged from 4.70 to 5.87, and those of F3 ranged from 4.59 to 5.79.

Stability

The freeze-thaw test was conducted on each of the three *Euphorbia hirta. L.* mouthwash formulations at 4 °C and 40 °C for six cycles and showed no separation.

Weight mass

The weight masses of each the three *Euphorbia hirta. L.* formulations over an eight-week period are presented in Table 4. F1 ranged from 0.9689 to 1.0641 g/ml, F2 ranged from 0.9693 to 1.0655 g/ml, and F3 ranged from 0.9699 to 1.0710 g/ml.

Viscosity test

A viscosity test was conducted on each of the three *Euphorbia hirta L.* formulations after eight weeks of storage, and findings ranged from 1.50 to 3.00 cP (see Table 5).

Irritation test

F3 had the highest mucus production at 14.965%, followed by F2 at 12.515% and F1 at 11.325%. Mucus production in the positive control was measured at 8.229%, higher than that of the negative control at 5.514%. These results show that *Euphorbia hirta L.* mouthwash is non-irritating (see Table 6).

Contact time test

Contact testing of *Euphorbia hirta L.* mouthwash at the lowest dilution (10^{-5}) showed that it can inhibit the growth of *Streptococcus mutans* colonies in 30 seconds, which is less than the total number of bacterial colonies. Decrease in the number of bacterial colonies in F1, F2 and F3 was 28.57%, 44.76%, and 57.14%, respectively (see Table 7).

Anti-bacterial activity

Anti-bacterial effects of *Euphorbia hirta L.* against *Streptococcus mutans* before storage showed a larger inhibition zone in F3 (20.8 ± 0.56), compared with the control negative (7.7 ± 1.46) ($p = 0.000$). The control positive (24.1 ± 0.51) showed a larger inhibition zone, compared with the control negative (7.7 ± 1.46), F1 (18.6 ± 2.15) and F2 (20.2 ± 0.72) ($p = 0.000$, $p = 0.002$ and $p = 0.023$) (see Figure 2A and Table 8).

Table 10: Antibacteria activity of other Euphorbia species.

Species	Part	Methods	Antibacterial activity	Reference
Euphorbia antiquorum L	crude latex	broth microdilution method	Inhibit *Streptococcus agalactiae* inhibit at concentration 250 µg/ml	36
Euphorbia helioscopia L.	Aerial	disc diffusion method	- Inhibit *Escherichia coli* - Inhibit *Pseudomonas aeruginosa* - Inhibit *Staphylococcus aureus* - Inhibit *Pseudomonas multocida* - Inhibit *Klebsiella pneumoniae*	12
Euphorbia helioscopia L	Aerial	disc diffusion method	Inhibit *Streptococcus aureus* inhibit at concentration 3.9 µg/ml	37
Euphorbia helioscopia L.	Aerial	agar well diffusion method	- Inhibit *Escherichia coli* - Inhibit *Staphylococcus aureus*	38
- *Euphorbia aleppica L* - *Euphorbia szovitsii Fisch& Mey. var. harputensis Aznav. ex M. S. Khan* - *Euphorbia falcata L. sub. falcata var. falcata,* - *Euphorbia denticulata Lam.,* - *Euphorbia macroclada Boiss.,* - *Euphorbia cheiradenia Boiss.&Hohen,* - *Euphorbia virgata Waldst.&Kit* - *Euphorbia petiolata*	Aerial	Broth microdilution method	- Inhibit *Streptococcus aureus* inhibition at 25 µg/ml - Inhibit *Streptococcus aureus* inhibition at 6.25 µg/ml - Inhibit *Streptococcus aureus* inhibition at 25 µg/ml - Inhibit *Streptococcus aureus* inhibition at 25 µg/ml - Inhibit *Streptococcus aureus* inhibition at 100 µg/ml - Inhibit *Streptococcus aureus* inhibition at 50 µg/ml - Inhibit *Streptococcus aureus* inhibition at 12.5 µg/ml - Inhibit *Streptococcus aureus* inhibition at 25 µg/ml - Inhibit *Streptococcus aureus* inhibition at 6.25 µg/ml - Inhibit *Streptococcus aureus* inhibition at 6.25 µg/ml - Inhibit *Streptococcus aureus* inhibition at 3.12 µg/ml	39

After a week of storage, the anti-bacterial effects of *Euphorbia hirta L.* against *Streptococcus mutans* showed the largest inhibition zone in F3 (20.9 ± 0.95), compared with F2 (17.9 ± 0.66), F1 (17.2 ± 0.35) and the control negative (6.6 ± 0.50) ($p = 0.000$ $p = 0.001$ and $p = 0.000$). The control positive (23.7 ± 0.67) showed the largest inhibition zone, compared with other formulations ($p < 0.05$) (see Figure 2B and Table 8).

Discussion

The importance of genus *Euphorbia* is not only represented by its growth forms but also the ability to treat cancer,[15] dengue fever,[16] vitiligo,[17] actinic keratosis, digestive system disorders, infections, respiratory system disorders, and pain.[10] The genus *Euphorbia* is also used as a catalyst in producing various nanoparticles for medical use.[18–25] Our results highlight the use of natural *Euphorbia hirta L.* for mouthwash.

This study shows that *Euphorbia hirta L.* mouthwash has anti-bacterial effects that fight *Streptococcus mutans*, which is the leading cause of dental caries. The leaves,[14,26–28] branches,[29] and aerial part[11,30–35] of *Euphorbia hirta L.* possess anti-bacterial properties. Studies have shown that *Euphorbia hirta L.* also has anti-bacterial effects against various types of *Streptococci*, including *Streptococcus aureus,*[30] *Streptococcus faecalis*, and *Streptococcus pneumoniae*.[33,35] In addition to *Streptococcus*, the anti-bacterial effects fight against various types of Gram-positive and Gram-negative bacteria (see Table 9).

Other species of *Euphorbia* are known to have anti-bacterial properties as well, such as *Euphorbia antiquorum L.*[36] and *Euphorbia helioscopia L.*[12,37,38] *Euphorbia antiquorum L.* has anti-bacterial effects against *Streptococcus agalactiae,*[36] and *Euphorbia helioscopia L.* has antibacterial effects against *Streptococcus aureus,*[37] *Escherichia coli, Staphylococcus aureus,*[38] *Pseudomonas aeruginosa*, *Pseudomonas multocida*, and *Klebsiella pneumoniae*[12,38] (see Table 10).

Other *Euphorbia* species, such as *Euphorbia aleppica L., Euphorbia szovitsii, Euphorbia falcata L. sub. falcata var. falcata, Euphorbia denticulata Lam, Euphorbia macroclada Boiss, Euphorbia cheiradenia, Euphorbia virgate*, and *Euphorbia petiolate,* have anti-bacterial effects against *Streptococcus aureus*.[39] The anti-bacterial properties fight not only *Streptococcus* species, but also other types of bacteria, such as *Pseudomonas aeruginosa, Pseudomonas multocida, Klebsiella pneumoniae,*[12] *Escherichia coli*, and *Staphylococcus aureus*.[38] (see Table 10).

The three mouthwash formulations used varying concentrations of *Euphorbia hirta L.*—0.5% in F1; 1% in F2 and 2% in F3. The purpose of this variation is to determine the concentration with the most effective anti-bacterial effects and proper consistency. Manufacturing mouthwash also requires the use of surfactants, such as propylene glycol and aquadest, to help increase solubility and reduce surface tension in oil and water solutions by stabilising the layers formed between the two phases and, thus, produce a clear and stable mouthwash.[40]

In this study, organoleptic, acidity (pH), specific gravity, viscosity, stability, irritation, and contact time tests were conducted on the three mouthwash formulations, and their anti-bacterial effects against *Streptococcus mutans* were determined. In general, a mouthwash with a concentration of 2% *Euphorbia hirta L.* had good acidity (pH), specific gravity, viscosity, and stability and tested well in irritation, preference, contact time and anti-bacterial effects against *Streptococcus mutans*.

The organoleptic test results showed that the three formulations maintained an acceptable and stable smell, taste, and colour during eight weeks of storage. These characteristics were used to determine preference for the formulations. The results showed that F3 was dark brown and had a distinctive smell of *oleum menthae piperitae*. Acidity (pH) tests aim to ensure stability during storage and avoid irritation to users. If it is too acidic, bacteria can develop easily and cause irritation to the oral mucosa. The results of this analysis showed no significant differences between the pH levels of each formulation of *Euphorbia hirta L.* mouthwash. The pH levels after storage ranged from 4.0 to 6.5. This increase may be caused by reduced concentrations of *Euphorbia hirta L.* from evaporation during storage or possibly due to temperature, humidity, or active substances or additives used during preparation.

Freeze-thaw examinations aimed to ensure the stability of mouthwash. This test was conducted on three *Euphorbia hirta. L.* mouthwash formulations at 4 °C and 40 °C for six cycles. Based on the freeze-thaw stability test results, each of the formulations showed stable preparation properties with no separation. The viscosity of a formulation was particularly important when gargled in the mouth. The viscosity of a formulation being closer to that of water ensured a better experience, and the results showed that formulation viscosity was directly related to weight.

Respondents reported that they preferred the F1 formulation of *Euphorbia hirta L.* mouthwash, compared with F2 and F3, and rated it 3.7, 3.7, and 3.9. Irritant tests were conducted to determine the irritation levels of *Euphorbia hirta L.* mouthwash. The highest mucus production was noted in F3 (14.965%), which contained 2% *Euphorbia hirta L.* extract. These results do not indicate irritation because mucus production was less than 15%. Therefore, this formulation can be assumed to contain only a small number of active substances and, thus, did not cause irritation.

Contact time tests were conducted to determine the time required for mouthwash formulations to inhibit bacteria in the oral cavity, and the best contact time was observed in F3. The anti-bacterial effects of *Euphorbia hirta L.* mouthwash was tested to determine effectiveness against *Streptococcus mutans* bacteria. Based on several examinations over an eight-week period, the best formulation is F3, with pH levels ranging from 4.79 to 6.05, the highest viscosity value, and a reduction of bacterial colonies by 57.14%. Higher concentrations of active substances can lead to increased antibacterial effects and reduced bacterial colonies.[41] The antibacterial effects of *Euphorbia hirta L.* inhibit the growth of *Streptococcus mutans* by deactivating certain enzymes in the bacteria, namely, *glucosyltransferase* (GTF) and *fructosyltransferase* (FTF). Deactivation of these two enzymes inhibits sucrose from producing glucans and fructose, thus altering the growth of *Streptococcus mutans* and eliminating the ability to adhere and colonise.

The study limitation is that the results only denote the characteristics and antibacterial activity of *Euphorbia hirta L.* mouthwash in vitro. However, the study results provide a fundamental basis for understanding the benefits of using *Euphorbia hirta L.* in a mouthwash. Future research may focus on verifying the benefits and clinical uses of *Euphorbia hirta L.* mouthwash.

Conclusion

The study results provide proof regarding the use of *Euphorbia hirta L.* for improving human health. Each of the formulations of *Euphorbia hirta L.* mouthwash showed no significant differences in either the activity or preference tests. The ethanol extract of *Euphorbia hirta L.* had ideal physical and stability characteristics for mouthwash and anti-bacterial effects that neutralise *Streptococcus mutans*. The F3 formulation containing 3% *Euphorbia hirta L.* showed enhanced characteristics, anti-bacterial effects, and stability, compared with the other two formulations, and the clinical use of this mouthwash must be explored further.

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Ethical approval is not required for an in-vitro study. This study was performed by strictly following standard protocols in Pharmaceutical Laboratory, Laboratory of Natural Material Pharmacy, Research and Biopharmaceutical Laboratory, and Sekolah Tinggi Ilmu Farmasi Riau.

Authors contributions

BI designed the study, and supervised, analysed, and interpreted the data, as well as partook in writing the original draft of the article. AL designed the study, and analysed and interpreted data, as well as partook in writing the original draft of the article. SS designed the study, conducted the research, interpreted data, and partook in writing the original draft of the article. UNHA, MDCS, and CKL individually revised the final article. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Acknowledgment

This research was conducted at the Pharmaceutical Laboratory, Laboratory of Natural Material Pharmacy, Research and Biopharmaceutical Laboratory, and Sekolah Tinggi Ilmu Farmasi Riau.

References

1. Veena R, Nagarathna C. Correlation of streptococcus mutans and streptococcus sobrinus colonization with and without caries experience in preschool children [Internet] **Indian J Dent Res 2020**; 31: 73. Available from: http://www.ijdr.in/text.asp?2020/31/1/73/281811.
2. Salman H, Senthilkumar R, Mahmood B, Imran K. Detection and characterization of Streptococcus downei, a rare bacterial

species of mutans streptococci from caries-active patients [Internet] **Indian J Dent Res 2019**; 30: 579. Available from: http://www.ijdr.in/text.asp?2019/30/4/579/271058.
3. Wang H, Ren D. Controlling Streptococcus mutans and Staphylococcus aureus biofilms with direct current and chlorhexidine [Internet] **AMB Express 2017**; 7: 204. Available from: https://amb-express.springeropen.com/articles/10.1186/s13568-017-0505-z.
4. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The Biology of Streptococcus mutans [Internet] **Microbiol Spectr 2019**; 7. Available from: http://www.asmscience.org/content/journal/microbiolspec/10.1128/microbiolspec.GPP3-0051-2018.
5. Parashar A. Mouthwashes and their use in different oral conditions [Internet] **Sch J Dent Sci 2015**; 2: 186–191. Available from: www.saspublisher.com.
6. Akande O, Alada A, Aderinokun G, Ige A. Efficacy of different brands of mouth rinses on oral bacterial load count in healthy adults [Internet] **Afr J Biomed Res 2010**; 7. Available from: http://www.ajol.info/index.php/ajbr/article/view/54160.
7. Claffey N. Essential oil mouthwashes: a key component in oral health management. **J Clin Periodontol 2003**; 30: 22–24.
8. Kumar S, Malhotra R, Kumar D. Euphorbia hirta: its chemistry, traditional and medicinal uses, and pharmacological activities [Internet] **Pharmacogn Rev 2010**; 4: 58. Available from: http://www.phcogrev.com/article/2010/4/7/1041030973-784765327.
9. Kausar J, Muthumani D, Hedina A, Sivasamy, Anand V. Review of the phytochemical and pharmacological activities of Euphorbia hirta Linn [Internet] **Pharmacogn J 2016**; 8: 310–313. Available from: http://phcogj.com/article/175.
10. Ernst M, Grace OM, Saslis-Lagoudakis CH, Nilsson N, Simonsen HT, Rønsted N. Global medicinal uses of Euphorbia L. (Euphorbiaceae) [Internet] **J Ethnopharmacol 2015**; 176: 90–101. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378874115301847.
11. Perumal S, Mahmud R. Chemical analysis, inhibition of biofilm formation and biofilm eradication potential of Euphorbia hirta L. against clinical isolates and standard strains [Internet] **BMC Complement Altern Med 2013**; 13: 346. Available from: https://bmccomplementalternmed.biomedcentral.com/articles/10.1186/1472-6882-13-346.
12. Lone BA, Bandh SA, Chishti MZ, Bhat FA, Tak H, Nisa H. Anthelmintic and antimicrobial activity of methanolic and aqueous extracts of Euphorbia helioscopia L [Internet] **Trop Anim Health Prod 2013**; 45: 743–749. Available from: http://link.springer.com/10.1007/s11250-012-0283-1.
13. Raja RDA, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V. Antibacterial activity of selected ethnomedicinal plants from South India [Internet] **Asian Pac J Trop Med 2011**; 4: 375–378. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1995764511601077.
14. Rajeh MAB, Zuraini Z, Sasidharan S, Latha LY, Amutha S. Assessment of Euphorbia hirta L. Leaf, flower, stem and root extracts for their antibacterial and antifungal activity and brine shrimp lethality [Internet] **Molecules 2010**; 15: 6008–6018. Available from: http://www.mdpi.com/1420-3049/15/9/6008.
15. Wang Y, Yu X, Wang L, Zhang F, Zhang Y. Research progress on chemical constituents and anticancer pharmacological activities of Euphorbia lunulata Bunge [Internet] **BioMed Res Int 2020**; 2020: 1–11. Available from: https://www.hindawi.com/journals/bmri/2020/3618941/.
16. Perera SD, Jayawardena UA, Jayasinghe CD. Potential use of Euphorbia hirta for dengue: a systematic review of scientific evidence. **J Trop Med 2018**; 2018.
17. An L, Liang Y, Yang X, Wang H, Zhang J, Tuerhong M, et al. NO inhibitory diterpenoids as potential anti-inflammatory agents from Euphorbia antiquorum [Internet] **Bioorg Chem 2019**; 92: 103237. Available from: https://doiorg/10.1016/j.bioorg.2019.103237.
18. Nasrollahzadeh M, Sajadi MM, Babaei F, Maham M. Euphorbia helioscopia Linn as a green source for synthesis of silver nanoparticles and their optical and catalytic properties [Internet] **J Colloid Interface Sci 2015**; 450: 374–380. Available from: https://doiorg/10.1016/j.jcis.2015.03.033.
19. Nasrollahzadeh M, Mohammad Sajadi S. Pd nanoparticles synthesized in situ with the use of Euphorbia granulate leaf extract: catalytic properties of the resulting particles [Internet] **J Colloid Interface Sci 2016**; 462: 243–251. Available from: https://doiorg/10.1016/j.jcis.2015.09.065.
20. Nasrollahzadeh M, Sajadi SM. Green synthesis of Pd nanoparticles mediated by Euphorbia thymifolia L. leaf extract: catalytic activity for cyanation of aryl iodides under ligand-free conditions [Internet] **J Colloid Interface Sci 2016**; 469: 191–195. Available from: https://doiorg/10.1016/j.jcis.2016.02.024.
21. Nasrollahzadeh M, Sajjadi M, Maham M, Sajadi SM, Barzinjy AA. Biosynthesis of the palladium/sodium borosilicate nanocomposite using Euphorbia milii extract and evaluation of its catalytic activity in the reduction of chromium(VI), nitro compounds and organic dyes [Internet] **Mater Res Bull 2018**; 102: 24–35. Available from: https://doi.org/10.1016/j.materresbull.2018.01.032.
22. Tajbakhsh M, Alinezhad H, Nasrollahzadeh M, Kamali TA. Green synthesis of the Ag/HZSM-5 nanocomposite by using Euphorbia heterophylla leaf extract: a recoverable catalyst for reduction of organic dyes [Internet] **J Alloys Compd 2016**; 685: 258–265. Available from: 10.1016/j.jallcom.2016.05.278.
23. Nasrollahzadeh M, Sajadi SM. Synthesis and characterization of titanium dioxide nanoparticles using Euphorbia heteradena Jaub root extract and evaluation of their stability [Internet] **Ceram Int 2015**; 41: 14435–14439. Available from: https://doi.org/10.1016/j.ceramint.2015.07.079.
24. Nasrollahzadeh M, Sajadi SM, Maham M, Salaryan P, Enayati A, Sajjadi A, et al. Optimal extraction method of phenolics from the root of Euphorbia condylocarpa. **Chem Nat Compd 2011**; 47: 434–435.
25. Nasrollahzadeh M, Sajadi SM. Preparation of Pd/Fe3O4 nanoparticles by use of Euphorbia stracheyi Boiss root extract: a magnetically recoverable catalyst for one-pot reductive amination of aldehydes at room temperature [Internet] **J Colloid Interface Sci 2016**; 464: 147–152. Available from: https://doi.org/10.1016/j.jcis.2015.11.020.
26. Kumari I, Pandey RK. Antibacterial activity of Euphorbia hirta L [Internet]. In: Mukhopadhyay K, Sachan A, Kumar M, editors. *Applications of biotechnology for sustainable development*. Singapore: Springer Singapore; 2017. pp. 1–5. Available from: http://link.springer.com/10.1007/978-981-10-5538-6.
27. Gupta R, Gupta J. Investigation of antimicrobial activity of Euphorbia hirta leaves. **Int J Pharma Bio Sci 2019**; 9: 32–37.
28. Singh G, Kumar P. Phytochemical study and screening for antimicrobial activity of flavonoids of Euphorbia hirta. **Int J Appl Basic Med Res 2013**; 3: 111–116.
29. Patel NB, Patel KC. Antibacterial activity of Euphorbia hirta L. Ethanomedicinal plant against gram positive UTI pathogens collection. **Int J Pharm Res Allied Sci 2014**; 3: 24–29.
30. Sudhakar M, Rao CV, Rao PM, Raju DB, Venkateswarlu Y. Antimicrobial activity of Caesalpinia pulcherrima, Euphorbia hirta and Asystasia gangeticum [Internet] **Fitoterapia 2006**; 77: 378–380. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0367326X06000888.
31. Abubakar EMM. Antibacterial activity of crude extracts of Euphorbia hirta against some bacteria associated with enteric infections. **J Med Plants Res 2009**; 3: 498–505.
32. Perumal S, Mahmud R, Pillai S, Lee WC, Ramanathan S. Antimicrobial activity and cytotoxicity evaluation of Euphorbia hirta (L.) extracts from Malaysia [Internet] **APCBEE Procedia**

2012; 2: 80–85. Available from: https://doiorg/10.1016/j.apcbee.2012.06.015.
33. Kuta F, Damisa D, Adamu A, Nwoha E, Bello I. Antibacterial activity of *Euphorbia hirta* against *Streptococcus pneumoniae, Klebsiella pneumoniae* and *Proteus vulgaris* [Internet] **Bayero J Pure Appl Sci 2014**; 6: 65. Available from: http://www.ajol.info/index.php/bajopas/article/view/100067.
34. Perumal S, Mahmud R, Ramanathan S. Anti-infective potential of caffeic acid and epicatechin 3-gallate isolated from methanol extract of Euphorbia hirta (L.) against Pseudomonas aeruginosa. **Nat Prod Res 2015**; 29: 1766–1769.
35. Tran N, Nguyen M, Le KP, Nguyen N, Tran Q, Le L. Screening of antibacterial activity, antioxidant activity, and anticancer activity of Euphorbia hirta Linn. Extracts [Internet] **Appl Sci 2020**; 10: 8408. Available from: https://www.mdpi.com/2076-3417/10/23/8408.
36. Siritapetawee J, Limphirat W, Wongviriya W, Maneesan J, Samosornsuk W. Isolation and characterization of a galactose-specific lectin (EantH) with antimicrobial activity from Euphorbia antiquorum L. latex [Internet] **Int J Biol Macromol 2018**; 120: 1846–1854. Available from: https://doi.org/10.1016/j.ijbiomac.2018.09.206.
37. Geng D, Yi LT, Shi Y, Min Z Da. Structure and antibacterial property of a new diterpenoid from Euphorbia helioscopia [Internet] **Chin J Nat Med 2015**; 13: 704–706. Available from: https://doiorg/10.1016/S1875-5364(15)30069-8.
38. Lin J, Dou J, Xu J, Aisa HA. Chemical composition, antimicrobial and antitumor activities of the essential oils and crude extracts of euphorbia macrorrhiza. **Molecules 2012**; 17: 5030–5039.
39. Kirbag S, Erecevit P, Zengin F, Guvenc A. Antimicrobial activities of some *Euphorbia* species [Internet] **Afr J Tradit, Complementary Altern Med 2013**; 10: 305–309. Available from: http://www.ajol.info/index.php/ajtcam/article/view/92346.
40. Vranić E, Lačević A, Mehmedagić A, Uzunović A. Formulation ingredients for toothpastes and mouthwashes [Internet] **Bosn J Basic Med Sci 2004**; 4: 51–58. Available from: https://www.bjbms.org/ojs/index.php/bjbms/article/view/3362.
41. Mukhopadhyay K, Sachan A, Kumar M. Applications of biotechnology for sustainable development [Internet]. In: Mukhopadhyay K, Sachan A, Kumar M, editors. *Applications of biotechnology for sustainable development*. Singapore: Springer Singapore; 2017. pp. 1–208. Available from: http://link.springer.com/10.1007/978-981-10-5538-6.

How to cite this article: Iskandar B, Lukman A, Syaputra S, Al-Abrori UNH, Surboyo MDC, Lee C-K. Formulation, characteristics and anti-bacterial effects of *Euphorbia hirta L.* mouthwash. J Taibah Univ Med Sc 2022;17(2):271–282.