OBSERVATION ON PRESERVATIVE ACTION OF BETEL LEAVES ESSENTIAL OIL IN PEEL-OFF FACIAL MASK*

Nay Yee Nyunt Oo[1], Shwe Zin Mon[2] , Soe Soe Than[3]

Abstract

Natural preservative effect of betel leaves essential oil on the shelf-life of the peel-off facial mask was studied in the present research. Different concentrations (0.1 %v/w, 0.15 %v/w, 0.2 %v/w, 0.25 %v/w, 0.3 %v/w) of betel leaves essential oil were added into previously formulated peel-off facial mask for consumer like cosmetic product. 0.25 % v/w betel leaves essential oil was found to be suitable for the consumer like peel-off facial mask based on sensory evaluation and skin irritation test. Its preservative effect on the shelf-life of the finished product was observed monthly by changes in pH and viscosity. Microbiological examination such as total plate count (TPC) and total count of yeasts and molds was conducted monthly. Slightly changes in pH and viscosity were observed after six months storage at the room temperature (27-30 ºC). Bacterial constituents in terms of total plate count in peel-off facial mask were under acceptable limit of up to 10^3 cfu/g until six-month storage. The growth of yeasts and molds was found in six- month aged peel-off facial mask.

Keywords: Essential oil, betel leaves, peel-off facial mask, shelf-life

Introduction

Preservatives are natural or synthetic substances that are added to increase their shelf-life without affecting the properties of the products itself. Preservative systems in cosmetic products contain various combinations of chemical compounds that can inhibit the growth of microorganisms and prevent contamination by consumers while in use (Siti Zuliakha *et al*., 2015). Most of the consumers have noticed the safety of chemical preservatives on their health such as allergy reactions (e.g. asthma attack and migraine), skin irritation, causing cancer, etc. Therefore, the cosmetic products with natural preservative or preservative free cosmetics are being greatly demanded to avoid the side effects of chemical preservatives. One of the ideas of this problem emerges the replacement of chemical preservatives with the essential oil possessing antimicrobial properties (Dreger & Wielgus, 2013).

Current interest for the development of facial mask formulation for skin care is attributed to their tightening, and cleansing effects. Peel-off facial masks are a viable alternative to promote the incorporation of active compounds into a plastic film-forming formulation that is designed to allow complete residue removal (Zague *et al*., 2007). The ingredients in skin care products are used to directly contact and penetrate the skin for the purpose of repairing the damage and inhibiting the ageing of it. This is why the use of preservatives in peel-off facial mask are taken as the prime factor into consideration (Emerald *et al*., 2016).

Essential oil from betel leaves consists of beneficial chemical compounds which shows antimicrobial activity. Betel vine (scientific name: *Piper betle* L.) is known as Kun in Myanmar (Dassanayake, 1996). The essence of betel leaf is owing to the presence of the essential oil which contain terpene and phenol compounds. The previous studies on betel leaves, root and whole

[1] Dr, Assistant Lecturer, Department of Industrial Chemistry, East Yangon University
[2] Associate Professor, Department of Industrial Chemistry, Dagon University
[3] Professor and Head, Department of Industrial Chemistry, University of Yangon
* Best Paper Award Winning Paper in Industrial Chemistry (2019)

extract (mixture of volatile and non-volatile) showed a very strong antimicrobial activity due to the presence of the large numbers of bioactive compounds (Sugumaran *et al*., 2011). Therefore, it has a great potential to develop commercial products with betel extract.

In the present study, the essential oil extracted from air-dried betel leaves by steam distillation were utilized as natural preservative in peel-off facial mask. The effects of various amounts of extracted essential oil on consumers' acceptance, skin irritation of the samples, the physicochemical properties and microbiological contamination in preserved peel-off facial mask were investigated.

Materials and Methods

Materials

For the extraction of essential oil from betel leaves, the sound and mature betel leaves (*Piper betle* L.) were collected from Kawhmu Township, Yangon Region. Air-dried leaves were used as raw materials in this research work. For the formulation of peel-off facial mask, cucumbers were purchased from Mingalardon Township, Yangon Region.

Extraction of Essential Oil from Betel Leaves by Steam Distillation

Before the extraction was carried out, the collected sample was washed with water to remove the dirt and the excess water on the surfaces was absorbed by paper towel or dry cloth. The sample was air-dried for 3 days in a clean and dry place. After that, the air-dried sample was pulverized into powder form for extraction. (100) g of betel leaves was used in each extraction. Each extraction was conducted for 4 hours. The resultant essential oil was collected from the Clevenger apparatus and was isolated using petroleum ether as the solvent. The extracted essential oils were dehydrated using sodium sulphate. The pure extracted essential oil was added in air tight amber glass bottle and stored in clean and dry place. The schematic diagram of the steam distillation apparatus is shown in Figure (1).

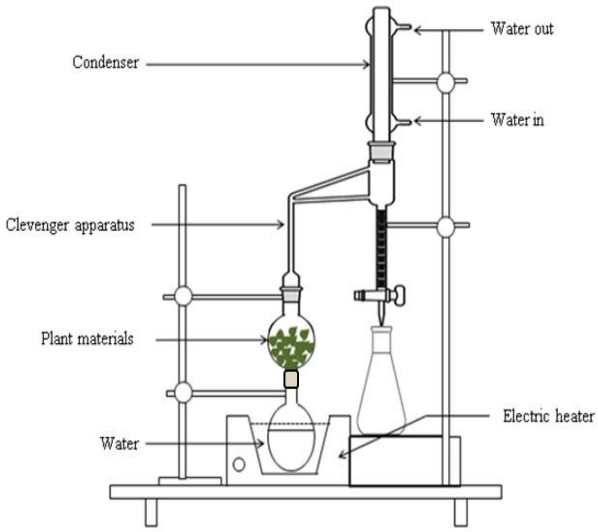


Figure 1 Schematic Diagram of Steam Distillation Apparatus

Determination of Physico-chemical Properties of Extracted Essential Oil

The physico-chemical characteristics such as specific gravity using density bottle (AOAC, 2000), refractive index using Abbe's refractometer and solubility of the extracted essential oil from betel leaves were analysed at room temperature.

Identification of Extracted Essential Oil by Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The compounds in extracted essential oil from betel leaves were identified by GC-MS (PerkinElmer, Clarus 680 GC coupled to PerkinElmer, Clarus 600 MS Detector equipped with an Elite 5MS capillary non polar column -30.0 m length x 0.25 mm ID x 0.25 μm film thickness). The components of essential oil were identified on their retention time and mass spectra, matching with National Institute of Standards and Technology (NIST05) libraries provided with computer controlling the GC-MS system. The GC-MS analysis of the essential oil extracted was carried out at the National Analytical Laboratory, Department of Research and Innovation, Ministry of Education.

Screening of Antimicrobial Activity of Extracted Essential Oil

Antimicrobial activity of extracted essential oil from betel leaves was determined by agar-well diffusion method (Cruichshank, 1975). Tested microorganisms were *Bacillus subtilis* (N.C.T.C–8236), *Staphylococcus aureus* (N.C.P.C–6371), *Pseudomonas aeruginosa* (6749), *Bacillus pumilus* (N.C.I.B – 8982), *Candida albicans,* and *E coli* (N.C.I.B – 8134). Screening test was carried out at the Microbiology Laboratory of Pharmaceutical Research Department, Ministry of Industry.

Formulation of Peel-off Facial Mask

The basic formulation of peel-off facial mask was carried out according to the previously literature study (Reveny *et al.*, 2016 and Beringhs *et al.*, 2013) and is described in Table (1). The composition of the main ingredients such as cucumber juice, PVA, ethanol and carbomer were varied according to the D-optimal mixture design. The experimental design of four main components systems was conducted by using Design Expert (version 7.0.0, State Ease Inc., Minneapolis, USA). In the preparation of peel-off facial mask, smooth and firm texture cucumber with no cut and no bruises or discoloration of cucumbers were selected. The selected cucumbers were peeled and chopped into 1 cm cubes. Then, cucumber juice was extracted by using a domestic juice extractor. Approximately, 100 g cucumber gave 90 g of cucumber juice. After that, polyvinyl alcohol (PVA) was dissolved in cucumber extract, heated over water bath at 80±2 °C and constantly stirred until PVA was dispersed in cucumber extract. And then, ethanol, carbomer, citric acid and disodium EDTA were added to the prepared mixture as the water phase. As an oil phase, olive oil and propylene glycol were mixed to the above prepared water phase. The mixture was agitated using ultraturrex at 2400 rpm for 5 minutes. Finally, the extracted essential oil was added as natural preservative into the mixture. The amount of essential oils for use in peel-off facial mask was determined by Hedonic Scale Test and Skin Irritation Test. The finished products were stored in a clean and dry place at room temperature (27-30°C) where direct sunlight could not reach.

Table 1 Basis Formulation of Peel-off Facial Mask

Ingredients	**Composition**
Cucumber juice (g)	94.07
Polyvinyl alcohol (g)	10.43
Ethanol (g)	15
Carbomer (g)	0.55
Olive oil (g)	2
Propylene glycol (g)	2
Disodium EDTA (g)	0.5
Citric acid (g)	0.2
Essential Oil (mL)	0.1-0.3

Evaluation of Consumers' Acceptance on the Added Amount of Extracted Essential Oil in Formulated Peel-off Mask

Nine point Hedonic Scale Test was used to evaluate the consumers' acceptance on the added amount of essential oil for the odour of the formulated peel-off facial mask. Ten panelists involved in the assessment of the overall acceptance of the samples.

Skin Irritation Test (Patch test)

Scores were given for skin irritation based on Patch test. Patch tests were performed on the forearms of each volunteer. The scores were recorded for the presence of erythema (skin redness) using a scale with 4 points from 0 to 3, where 0 stands for absence of skin irritation, 1 for mild skin irritation, 2 for moderate skin irritation while 3 stands for severe skin irritation. Each volunteer was asked to note their irritation/itching towards the patches and then assigned a score from the same scale (Frosch et al., 2006 and Akhtar *et al.,* 2011).

Observation on Shelf-life of Formulated Peel-off Facial Mask

The shelf-life of peel-off facial mask stored at room temperature (27-30°C) was observed monthly for 6 months by studying the parameters such as pH, viscosity and microbiological contamination (Yeasts and molds and Total Plate Counts). The pH of the sample was measured using a pH meter (Mi 150, pH/Temperature Bench Meter). The viscosity of the sample was measured using NDJ 8S Digital Rotational Viscometer. The microbiological examination of yeasts and molds, and total plate count (TPC) were conducted at Small Scale Industries Department, Ministry of Agriculture, Livestock and Irrigation. The growth of yeasts and molds, and total plate count (TPC) of the sample was determined by dry rehydratable film method (Petrifilm^{TM} Method).

Results and Discussion

Steam distillation was used to extract the essential oil from air-dried betel leaves. The yield percent was 1.41 ± 0.02 %v/w. Table (2) shows the physical properties of extracted essential oil. It was an assessment of the purity and quality of the essential oil as well as for identification. The specific gravity of the essential oil from betel leaves were found to be 0.91 - 0.99 that may be few containing oxygenated aromatic compounds in extracted essential oil (Barkatullah *et al.,* 2012). It was noted that the refractive index for betel leaves essential oil was

1.45±0.01. Caburian and Osi, (2010) and Sugumanran *et al*., (2011) stated that betel leaves essential oil was specific gravity for 0.9313 - 1.001 with 1.4526 - 1.529 as refractive index. The solubility of the essential oil was shown in Table (3). It was found that the essential oil with water (1:5) ratio was not separated as the layer and the oil dispersed in the water. However, the essential oil and water was not miscible in ratio of 1:1 but soluble in (1:1) ratio of ethanol and petroleum ether since it was the complex mixture of polar and non-polar compounds (Morsy, 2017). And also, it may be due to the specific gravity of betel leaves essential oil (0.91 -0.99).

The constituents of extracted essential oils was identified using Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS chromatogram of essential oil is shown in Figure (2) and Table (4) indicates the expected compounds of the essential oil with their retention time. Eugenol (69.63%) was the major component of essential oils from betel leaves and followed by eugenyl acetate (11.93%), caryophyllene (2.68%) and 4-allyl-1,2-diacetoxy benzene (1.38%). Six oxygenated compounds such as 2-allyl phenol, eugenol, methyl eugenol, eugenyl acetate, 4-allyl 1,2-diacetoxybenzene and phytol were found in the essential oil extracted by steam distillation. According to Joshi, (2013), Lee *et al*., (2015), PubChem CID:15624 and Vanin *et al*., (2014), the oxygenated compounds mainly provide the antimicrobial activity of the essential oils.

Screening of antimicrobial activities of essential oil is presented in Table (5) and Figure (3). From the results, the maximum inhibition zone of 21 mm against *Bacillus pumilus* followed by a zone of 20 mm against *Staphyloccocus aureus* and *Pseudomonas aeruginosa* were studied. It was observed that the essential oil showed against all six test microorganisms. It was due to the presence of the bioactive compounds in the extracted essential oil such as eugenol, caryophyllene and eugenyl acetate that provide the antimicrobial activities.

Table (6) shows the overall acceptance for the added amount of essential oil in peel-off facial mask. As shown in Table (6), 0.25 %v/w the essential oil from betel leaves cooperated in peel-off facial mask was the most preferable added amount in the facial mask. It was found that 0.1- 0.2 % v/w was not sufficient for the odour of the peel-off facial mask and 0.3 %v/w gave the slightly pungent odour and caused skin redness in some panelists. Seow *et al.*, (2013) stated that when the essential oils are added over threshold, undesirable organoleptic effects may prevail. Therefore, it is mandatory to equalize concentrations of essential oil for the usage as preservative in order to adjust the antimicrobial activities and organoleptic qualities of finished products. Dreger and Wielgus, (2013) also revealed that high concentration of essential oils leads to many problems like: phase separation, unfavorable viscosity of formulation and also strong undesirable odor. Skin irritation test was also conducted for sensitivity on the added amount of essential oil and is shown in Table (7). Skin irritation was found by the addition of above 0.25 %v/w. Sarkic and Stappen, (2018) stated that although essential oils are considered as safe and nontoxic when used at low concentration, skin sensitivity and irritations as well as other symptoms may arise after their application.

Changes in physico-chemical properties of peel-off facial mask formulated with 0.25 % v/w of essential oil for 6 months storage is shown Table (8)**.** It was observed that negligible change in pH of facial mask was found to be from 5.34-5.27 during 6-month storage. The pH of human skin typically ranges from 4.5 to 6.0 and that was considered to be average pH of the skin (Akhtar *et al.,* 2011). This negligible change in pH (±0.07) may be due to the oxidation of olive oil formulated in peel-off facial mask to aldehyde and organic acid (Raymond, Paul and Marian, 2009). In addition, very slightly change in viscosity was found to be increase in the storage time.

Raymond, Paul and Marian, (2009) also stated that the microorganisms grow well in unpreserved aqueous carbomer dispersion. Therefore, carbomer was only slightly reduced the viscosity at elevated storage temperatures if even antimicrobial agents is included in the formulation. It was noted that pH and viscosity of the samples with essential oil were not significantly changed until six-month storage time.

Table (9) shows the microbial count in peel-off facial mask formulated with 0.25 % v/w of essential oil . The count of yeasts and molds and total plate count were observed less than 10^3 cfu/g until five-month storage time but the growth of yeasts and molds and total plate count was found greater than 10^3 cfu/g at the sixth-month. Up to five months of storage time, CFU can be accepted under the guidelines of Cosmetic, Toiletry, and Fragrance Association (CTFA) and guidance of EU's Scientific Committee of Consumers Products (SCCP) (not more than 10^3 cfu/g for – bacteria, yeasts and molds) (Geis, 2006 and Detmer, Jørgensen and Nylén, 2007). It may be due to loss of activity in dilution and volatility of essential oil which can be highly undesirable for the products (Halla *et al*., 2018). Moreover, the container may allow for the diffusion of oxygen and carbon dioxide and the condensation of water can occur in the container and it can facilitate the microbial growth in the package. Therefore, the containers and bottles should be designed to the difficult entry of the microorganisms into the products for protection from all environmental insults. (Smart and Spooner, 1972 and Varvaresou *et al*., 2009).

Table 2 Physical Characteristics of Extracted Essential Oil

Sr No.	Characteristics	Extracted Essential Oil
1.	Specific Gravity	0.95±0.04
2.	Refractive Index	1.45±0.01

Table 3 Solubility of the Extracted Essential Oil

Sr No.	Solvent	Solvent Ratio (oil : solvent)	Solubility
1.	Water	1 : 1	Immiscible
		1 :5	Not separating as a layer and oil disperses in water
2.	Ethanol	1 :1	Miscible
3.	Petroleum ether	1 :1	Miscible

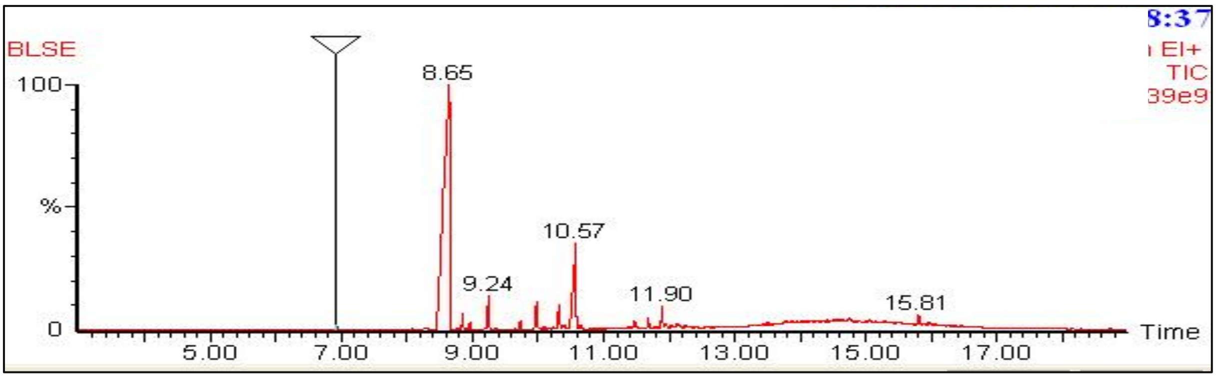


Figure 2 GC-MS Chromatogram of Extracted Essential Oil from Air-dried Betel Leaves by Steam Distillation

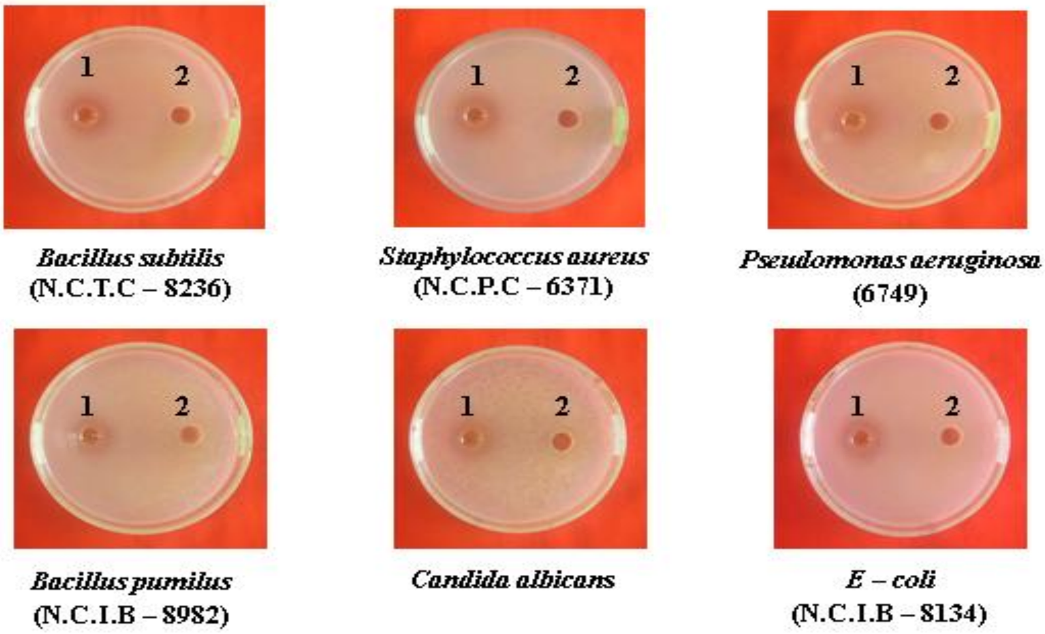
Table 4 Expected Compounds Present in Extracted Essential Oil from Air-dried Betel Leaves by Steam Distillation

Sr No.	Compounds	Retention Time (min)	Peak Area (%)	Formula	Compound Nature	Function	References
1	2-allyl phenol	6.92	0.38	$C_9H_{10}O$	Phenolic compound	Antifungal agent	PubChem CID:15624
2	Eugenol	8.65	69.63	$C_{10}H_{12}O_2$	Phenolic compound	Antimicrobial agent	Lee *et al.*,2015
3	Methyl eugenol	8.85	1.06	$C_{11}H_{14}O_2$	Phenolic compound	Antibacterial agent	Joshi, 2013
4	Caryophyllene	9.24	2.68	$C_{15}H_{24}$	Bicycilc sesquiterpene	Antimicrobial agent	Mohammed *et al.*, 2016
5	γ-muurolene	9.98	2.47	$C_{15}H_{24}$	Bicycilc sesquiterpene	Antibacterial agent	Mohan *et al.*, 2011
6	Eugenyl acetate	10.57	11.93	$C_{12}H_{14}O_3$	Aliphatic dicarboxylic ester	Antiseptic	Vanin *et al*,2014
7	4-allyl 1,2-diacetoxy benzene	11.9	1.38	$C_{13}H_{14}O_4$	Phenolic compound	Anti-platelet aggregation	Chen *et al.*, 2004
8	Phytol	15.81	0.73	$C_{20}H_{40}O$	Acyclic diterpene alcohol	Antimicrobial agent	Lee *et al.*,2015

Table 5 Antimicrobial Activities of Extracted Essential Oils from Air-dried Betel Leaves by Steam Distillation

Organisms	Essential Oil from Air-dried Betel leaves
Bacillus subtilis	18mm (++)
Staphylococcus aureus	20mm (+++)
Pseudomonas aeruginosa	20mm (+++)
Bacillus pumilus	21mm (+++)
Candida albicans	19mm (++)
E –coli	18mm (++)

Note : Agar well – 10mm, 10mm ~ 14 mm (+), 15mm ~ 19mm (++), 20 mm above(+++)



1 = Essential oil, 2 = Control

Figure 3 Antimicrobial Activities of Extracted Essential Oil of Air-dried Betel Leaves by Steam Distillation

Table 6 Overall Acceptance by Hedonic Scale Test for Added Amount of Extracted Essential Oil in Formulated Peel-off Facial Mask

Panelists	**Amounts of Betel Leaves Essential Oil (% v/w)**				
	0.1	**0.15**	**0.2**	**0.25**	**0.3**
1	8	8	9	9	3
2	7	7	8	9	4
3	7	8	8	8	4
4	7	8	8	8	4
5	7	7	7	7	4
6	7	7	7	9	4
7	6	7	7	8	4
8	8	8	8	9	3
9	8	8	8	8	3
10	7	7	7	7	4
Total Score	72	75	77	82	37
Average	7.2	7.5	7.7	8.2	3.7

Table 7 Score Given for the Added Amount of Essential Oil in the Formulated Peel-off Facial Mask Based on the Skin Irritation Test

Score	**Numbers of Panelists for Various Amounts of Betel Leaves Essential Oil (%v/w)**				
	0.1	**0.15**	**0.2**	**0.25**	**0.3**
0	10	10	10	10	5
1	-	-	-	-	3
2	-	-	-	-	2
3	-	-	-	-	-

Note : Number of Panelist = 10
0 - absence of skin irritation, 1 - mild skin irritation ,
2 – moderate skin irritation , 3 - severe irritation

Table 8 Changes in Physico-chemical Properties of Peel-off Facial Mask with 0.25 %v/w of Essential Oil during Storage

Sr No.	**Parameters**	**Storage time/Shelf-life (month)**						
		Fresh	**1**	**2**	**3**	**4**	**5**	**6**
1	pH	5.34 ±0.01	5.33 ±0.02	5.31 ±0.02	5.31 ±0.03	5.31 ±0.02	5.28 ±0.01	5.27 ±0.02
2	Viscosity (cP)	10853	10853	10850	10822	10791	10764	10725

Table 9 Microbial Count in Peel-off Facial Mask Formulated with 0.25 %v/w of Essential Oil from Betel Leaves

Sr No.	**Parameters**	**Type of Sample**	**Storage time/Shelf-life (month)**						
			Fresh	**1**	**2**	**3**	**4**	**5**	**6**
1	Yeasts & Molds (cfu/g)	BLEO	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	3×10^3
		Control	3×10^3	5×10^3	-	-	-	-	-
2	TPC (cfu/g)	BLEO	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	$<10^3$	1×10^3
		Control	40×10^3	59×10^3	-	-	-	-	-

Note : TPC – total plate count,
Control sample – no chemical preservatives and no essential oil sample

Conclusion

The added amount (0.1-0.3% v/w) of essential oil from betel leaves were used in formulated peel-off facial mask as natural preservative and the satisfactory amount of essential oil was determined based on the overall acceptance by Hedonic Scale Test. Above 0.25 %v/w essential oil from betel leaves were found to cause the irritation to skin. 0.25 %v/w of essential oil from betel leaves was found to be prolong the shelf-life of peel-off facial mask until 5-month storage under limitation. Therefore, it is recommended for conducting the synergistic action of the essential oil and other chemical components to prolong the shelf-life of cosmetic products. In addition to utilizing the essential oil as natural preservatives, adjusting pH of the formulation, lowering the water activity, proper packaging of the final products and good manufacturing practices (GMPs) are the alternative approaches to preserve the products for longer shelf-life.

Acknowledgements

I would like to express my gratitude to the Myanmar Academy of Arts and Science for allowing me to submit this article. I also gave special thanks to Dr. Thin Thin Naing, Professor and Head of Industrial Chemistry Department, East Yangon University for her kind permission to submit this article.

References

Akhtar N., Mehmood A., Khan B. A., Mahmood T., Khan. M. S. and Saeed T., (2011), Exploring cucumber extract for skin rejuvenation, African Journal of Biotechnology Vol. 10(7).

Barkatullah, Ibrar M, Rauf A. and Rahman I. U., (2012), Physico-chemical characterization of essential and fixed oils of *Skimmia laureola* and *Zanthoxylum armatum*, Middle-East Journal of Medicinal Plants Research 1(3): 51-58, ISSN 2309-8929.

Beringhs A. O. R., Rosa J. M., Stulzer H. K., Budal R. M., Sonaglio D., (2013), Green clay and aloe vera peel-off facial masks: response surface methodology applied to the formulation design, AAPS Pharm Sci Tech, Vol. 14.

Caburian, A. B. & Osi M. O., (2010), Characterization and evaluation of antimicrobial activity of the essential oil from the leaves of *Piper betle* L., E-International Scientific Research Journal.

Chen Y. C., Chen J. J., Chang Y. L., Teng C. M, Lin W. Y., Wu C. C., and Chen I. S., (2004) , A New aristolactam alkaloid and anti-platelet aggregation constituents from *Piper taiwanense*.

Cruickshank, R., (1975), Medicinal microbiology, Eleven Edition, E&S living stone, Landon.

Dassanayake, M.D.(1996), A revised hand book to the Flora of Ceylon, (Vol. V), University of Agriculture, Peradeniya : Sri Lanka.

Detmer A., Jørgensen C., Nylén D., (2007), A guidance document on microbiological control of cosmetic products, Environmental protection Agency, Danish Ministry of the Environment

Dreger M. and Wielgus K., (2013), Application of essential oils as natural cosmetic preservatives, Department of Biotechnology, Institute of Natural Fibers & Medicinal Plants, Poland.

Duarte M. C. T., Duarte1 R. M. T., Rodrigues R. A. F., and Rodrigues M. V. N., (2018) , Essential oils in food processing: chemistry, safety and applications - essential oils and their characteristics , First Edition.,. Published by John Wiley & Sons Ltd.

Emerald M., Emerald A., Emerald L., Kumar V., 2016, Perspective of natural products in skincare, Pharmacy & Pharmacology International Journal, Volume 4 Issue 3.

Frosch P.J., Menne T., and Lepoittevin J. P. (2006), Contact Dermtitis, 4th Edition, Chapter 22, pg 356-390.

Geis P. A. (Ed), (2006), Cosmetic microbiology – a practical approach, second edition, Taylor & Francis Group, New York.

Halla N., Fernandes I.P., Heleno S. A., Costa P., Boucherit-Otmani Z., Boucherit K, Rodrigues A.E., Ferreira., and Barreiro M.F., (2018), Cosmetics Preservation: A Review on Present Strategies.

Joshi, (2013), Chemical composition, In vitro antimicrobial and antioxidant activities of the essential oils of *Ocimum gratissimum, O. sanctum* and their major constituents, Indian J Pharm Sci.

Lee, W. F., Salleh, E. and Mamat, S. N. H., (2015), Extraction and qualitative analysis of *Piper betle* leaves for antimicrobial activities, International Journal of Engineering Technology Science and Research (IJETSR).

Mohammed G. J., Omran A. M. and Hussein H. M., (2016), Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography – Mass Spectrum and Fourier-Transform Infrared Spectroscopy, International Journal of Pharmacognosy and Phytochemical Research.

Mohan L., Negi A., Melkani A. B. , Dev V., (2011), Chemical composition and antibacterial activity of essential oil from *Salvia mukerjeei*, Natural Product Communications.

Morsy N, (2014), Phytochemical analysis of biologically active constituents of medicinal plants, Main Group Chemistry, 2014 7–21, DOI 10.3233/MGC-130117

Pubchem CID - 15624. Retrieved from https://pubchem.ncb.nlm.nih.gov/ compound / 2allylphenol/PubChem CID15624.

Raymond C. R., Paul J. S., Marian E.Q., (2009), Handbook of pharmaceutical excipients : carbomer, olive oil, 6th Ed, Landon, UK

Reveny J., Nazliniwaty, Umayah R., (2016), Formulation of peel-off mask from ethanol extract of water spinach leaves as anti-aging, International Journal of PharmTech Research.

Sarkic A. and Stappen I., (2018), Essential oils and their single compounds in cosmetics—a critical review.

Seow Y. X., Reo C. R., Chung H. L. and Yuk H. G., (2013), Plant essential oils as active antimicrobial agents, Critical Reviews in Food Science and Nutrition.p 625-644.

Siti Zulaikha R., Sharifah Norkhadijah S. I., and Praveena S. M., (2015), Hazardous Ingredients in Cosmetics and Personal Care Products and Health Concern: A Review, Public Health Research, Vol 5(1),): 7-15 DOI: 10.5923, Scientific & Academic Publishing.

Smart R. and Spooner R.S., (1972), Microbiological spoilage in pharmaceuticals and cosmetics, J. Soc. Socmet. Chem, Society of Cosmetic Chemists of Great Britain, vol 23, pg 721-731..

Sugumaran, M, Sureshgandhi M, Sankarnarayanan K, Yokesh M , Poornima M, Sree Rama rajasekhar, (2011), Chemical composition and antimicrobial activity of vellaikodi variety of *Piper betle* Linn Leaf oil against dental pathogens, International Journal of Pharm Tech Research, Vol.3, No.4, pp 2135-2139.

Vanin A. B., Orlando T., Piazza S. P., Puton B. M. S., Cansian R. L., Oliverira D., & Paroul N., (2014), Antimicrobial and antioxidant activities of clove essential oil and eugenyl acetate produced by enzymatic esterification, Appl Biochem Biotechnol, 1286-1298.

Varvaresou A., Papageorgiou S., Tsirivas E., Protopapa E., Kintziou H., Kefala V. and Demetzos C., (2009), Self-presrving cosmetics, Review article, International Journal of Cosmetic Science, 31, 163–175.

Zague, V., Silva D. D. M., Baby A. R., Kaneko T. M., and Velasco M. V. R., (2007), Clay facial masks: Physicochemical stability at different storage temperatures, Department of Pharmacy, University of Sao Paulo, Brazil.