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TESTING OF GLYCERYL MONOESTERS FOR THEIR ANTI-MICROBIAL SUSCEPTIBILITY AND THEIR INFLUENCE IN EMULSIONS

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ABSTRACT

Natural anti-microbial agents have received great attention in the cosmetic preservation area due to their well-documented safety profile. The anti-microbial activities of palm-based glyceryl monoesters (monolaurin, monocaprylin and monocaprin) were compared with commercially available tea tree oil and potassium sorbate against Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus *and* Aspergillus niger, *using the anti-microbial susceptibility testing procedure. Monolaurin was found to exhibit excellent inhibitory activity against* S. aureus *and* Asp. niger, *whereas potassium sorbate and tea tree oil had no activity against* Asp. niger *and* S. aureus, *respectively. Monocaprylin was shown to have low inhibitory activity against* E. coli, *and no inhibitory activity towards* P. aeruginosa. *On the other hand, tea tree oil had a higher inhibitory activity than monolaurin at 2% against* E. coli *but showed no activity against* P. aeruginosa. *Similar trends were observed for monocaprin and monolaurin which showed no anti-microbial activity towards* P. aeruginosa *as well as* E. coli. *Interestingly, the presence of monolaurin was not only effective as a preservative, but was also found to induce the formation of liquid crystals at concentrations as low as 0.5%. The formation of liquid crystals is said to enhance the stability and functionality of cosmetic emulsions.*

Keywords: monolaurin, monocaprin, monocaprylin, anti-microbial activity, liquid crystals.

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INTRODUCTION

A major challenge in the cosmetic industry is in the prevention of microbial contamination in the products. The presence of bacteria and yeasts make these products unsafe and unacceptable for use. Contaminated products may cause microbial infections in human beings. There have been several reports related to infection caused by contaminated products. Contaminated hand lotions and creams were reported to be sources of nosocomial infections that resulted in septicemia due to gram-negative bacteria, particularly *Escherichia coli* (Morse and Schonbeck, 1968). The growth of microbes may result in the deterioration of the physical and chemical stability as well as in the degradation of the ingredients in the cosmetics (Paye *et al.*, 2006). Therefore, cosmetic products that are susceptible to microbial metabolism require effective preservatives.

An anti-microbial agent is a chemical compound added to cosmetic products that inhibits the growth of, or inactivate, pathogens or spoilage microorganisms (Davidson *et al.*, 2005). Parabens are anti-microbial agents, widely used in foods, cosmetics and pharmaceutical products. They are very effective anti-microbial agents with their activity increasing with the length of their alkyl chains, ranging from the methyl to the butyl groups (Pedersen *et al.*, 2007). However, an *ex vivo*

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experiment has proven that methyl paraben is a potential agent causing the UV-induced damage of skin keratinocytes (Handa *et al.,* 2007). Several studies found that paraben generated allergic contact dermatitis (Angelini *et al.,* 1997; Simpson, 1998; Mowad, 2000). Furthermore, it was proven to be the main cause for the disruption of the spermatogenic system which provoked spermatotoxic effects (Oishi, 2001), and it also possesses oestrogenic-like activity leading to breast cancer (Darbre, 2004). Hussein *et al.* (2007) proved that parabens in cream products could penetrate the skin. Therefore, these anti-microbial agents have become less popular in topical medicaments once they were found to induce contact hypersensitivity (Sasseville, 2004). Current concerns have resulted from toxicological studies suggesting that parabens in cosmetic products possess health risks to consumers.

Due to the safety issues associated with the use of chemical preservatives, there is a widespread trend towards 'natural' anti-microbials. Glyceryl monoesters such as monolaurin, monocaprin and monocaprylin are considered as 'natural' anti-microbial preservatives. Glyceryl monoesters have the added advantage in cosmetic products of acting as penetration enhancers for the delivery of the actives to the skin and as emulsifiers to stabilize the system. They also act as emollients which facilitate the spreading of cream on the skin. Monolaurin, monocaprylin and monocaprin are esters formed from glycerol and lauric acid, caprylic acid and capric acid, respectively. They have been proven to have strong anti-bacterial activity (Ryser and Marth, 1999; Fu *et al*., 2009). The testing of glyceryl monoesters against *E. coli* and *S. aureus* has been widely studied; however, the effect of glyceryl monoesters against other microorganisms such as *P. aeruginosa* and *Asp. niger* are not well studied. In this present study, the anti-microbial properties of glyceryl monoesters were compared with commercially available tea tree oil and potassium sorbate.

Sorbic acid is a widely used natural preservative which can be found in the berries of the mountain ash. It is well-known as an effective preservative against moulds and yeasts, but it has been shown to have lower activity against bacteria. It is commonly used to preserve food, animal feed, cosmetics and pharmaceutical products. It is sparingly soluble in water, and therefore its salt form, *e.g.,* potassium sorbate, is widely used due to its higher degree of solubility in water (Rietchel *et al.*, 2008). Potassium sorbate occurs as a white crystalline compound, and it is used in the same way as sorbic acid, especially when more water is required in the cosmetic formulations (Wilson *et al.*, 2004). Therefore, the anti-fungal activity of potassium sorbate against *Asp. niger* was also used in comparison with glyceryl monoesters in our study.

The oil of the Australian tea tree (*Melaleuca alternifolia*) is an essential oil which can be obtained by steam distillation of the leaves and terminal branches. It consists of terpinen-4-ol, cineol, pinens, terpinenes, cymene, sesquiterpenes and sesquiterpene alcohols. The most abundant compound in tea tree oil is terpinen-4-ol which makes up to 30% of its composition, and is responsible for its anti-microbial properties. The remaining compounds also synergistically contribute to this anti-microbial property (Salvador and Chisvert, 2007). Tea tree oil is well-known for its anti-bacterial properties, and it used widely in cosmetics like body hygiene formulations.

At present, the influence of glyceryl monoester on the physico-chemical properties of emulsions has not been well studied. Therefore, the objective of this study was to compare the anti-microbial properties of monocaprin, monocaprylin and monolaurin with tea tree oil and potassium sorbate against *E. coli*, *P. aeruginosa*, *S. aureus* and *Asp. niger* by the disc diffusion method (Lalitha, 2004). In addition, we also examined the effect of glyceryl monoesters on the structure of a typical emulsion.

MATERIALS AND METHODS

Span 40 (sorbitan palmitate) and Tween 80 (polysorbate 80) were purchased from Merck Sdn Bhd (Malaysia), whereas isopropyl palmitate was purchased from Intermed Sdn Bhd (Malaysia). Monolaurin, monocaprin and monocaprylin were purchased from Dr Straetmans Chemische Produkte GmbH (Germany), while *E. coli*, *P. aeruginosa*, *S. aureus* and *Asp. niger* were purchased from the Institute of Medical Research (Malaysia). Tea tree oil was supplied by Southern Cross Botanicals Pty Ltd (Australia), and potassium sorbate was purchased from Sigma Aldrich (M) Sdn Bhd (Malaysia). Tryptone soy agar, Müeller-Hinton broth and potato dextrose agar were purchased from Difco Laboratories (United States of America).

Anti-microbial Susceptibility Test (disc diffusion method)

E. coli, *P. aeruginosa* and *S. aureus* were grown for 24 hr on tryptone soy agar whereas *Asp. niger* was cultured for seven days on potato dextrose agar. Suspensions of these tested microbes were prepared in autoclaved water. The concentrations of the bacterial and fungal suspensions were adjusted to 1.5×10^8 cfu and 1.0×10^6 cfu, respectively.

A sterile swab was submerged in the suspensions containing bacteria or fungus and then swiped on the entire surface of the agar. Tryptone soy agar was inoculated by bacteria (*E. coli*, *P. aeruginosa*, *S. aureus*), while potato dextrose

agar (PDA) was inoculated by the fungus (*A. niger*). Six concentrations, *viz.* 2.0%, 1.0%, 0.50%, 0.25%, 0.13% and 0.060% (w/w) of the sample mixtures containing glyceryl monoesters and water, were prepared. Around 0.010% of Tween 80 was then added to all the mixtures in order to solubilize the glyceryl monoesters and tea tree oil in water. A sterile filter paper disc was dipped in the selected concentration and placed on the agar with sterilized forceps. The agar plates (in an inverted position) containing the filter paper discs of all the six concentrations were incubated in an oven at 35ºC for 24 hr for the bacteria, and at 29.9ºC for 48 hr for the fungus. After incubation, the diameter of each zone (in mm) was measured with a ruler.

Preparation of the Cosmetic Emulsion

The effect of the various concentrations of glyceryl monoesters in the formulations was investigated. The concentrations of glyceryl monoesters used were 0.50%, 1.0%, 2.0% and 4.0% (w/w). The compositions of the emulsions are shown in *Table 1*.

The required ingredients were weighed according to the stated composition in *Table 1*. The oil phase consisted of Span 40, hydrogenated palm kernel glycerides and isopropyl palmitate, whereas the aqueous phase contained water, glyceryl monoesters and Tween 80. Both phases were heated separately to 75ºC. The oil phase was then added slowly to the aqueous phase once the desired temperature was reached, and the mixture homogenized at 10 000 rpm, for 2 min using a Polytron PT 3100 homogenizer.

Optical Microscopy Measurement

Optical microscopy measurements were carried out using a Nikon Eclipse 80*i* microscope. Magnifications used in this study were X10 and X20. Microslides and coverslips were used in the sample preparation. The emulsion sample was carefully placed on a microslide to avoid destruction of the emulsion structure by shear stress. The sample was then covered with a coverslip and sealed using vacuum grease to prevent evaporation (Jiao and Burgress, 2003).

RESULTS AND DISCUSSION

Anti-microbial Susceptibility Test (disc diffusion method)

Monolaurin (C_{12}) showed anti-microbial activity at concentrations as low as 0.13%, whereas monocaprylin (C_8) did not show any anti-microbial activity against *S. aureus* at concentrations from 0.060% to 2.0% (*Figure 1*). Monocaprin (C_{10}) also showed anti-microbial activity against *S. aureus* but at a concentration higher than monolaurin, that was 1.0%, indicating that monolaurin has a higher resistance against the *S. aureus* than monocaprin. The result shows that monolaurin has outstanding anti-microbial activity compared to monocaprylin and monocaprin. The zone of inhibition by monolaurin at the various concentrations was: 0 mm at 0.060%, 10 mm at 0.13%, 15 mm at 0.25%, 20 mm at 0.50%, 28 mm at 1.0% and 34 mm at 2.0%. For monocaprin, no zone of inhibition was found at 0.060%, 0.13%, 0.25% and 0.50% concentrations. At 1.0% concentration of monocaprin, the zone of inhibition was 10 mm and at 2.0% it was 15 mm. No inhibition was observed for tea tree oil against *S. aureus*. This result is in agreement with published findings by Wilkinson and Cavanagh (2005) that tea tree oil is not effective in inhibiting the growth of *S. aureus* even at a concentration up to 10%. It is only able to inhibit the growth of *S. aureus* at a concentration of 100%.

Monolaurin is effective in inhibiting the growth of gram-positive bacteria like *S. aureus*. This is due to the ability of monolaurin to effectively block or delay the production of exotoxin by gram-positive bacteria (Projan *et al.*, 1994). Furthermore, it is also responsible for inhibiting the synthesis of most staphylococcal and other exoproteins at the level of

TABLE 1. COMPOSITION OF EMULSIONS WITH MONOLAURIN (ML), MONOCAPRIN (MC) AND MONOCAPRYLIN (MCL)

Sample	Ingredient (% w/w)					
	Span 40	Tween 80	Hydrogenated palm kernel glycerides	Isopropyl palmitate	Monolaurin	Water
No ML/MC/MCL	3.0	3.0	18	2.0	0	74
0.5% ML/MC/MCL	3.0	3.0	18	2.0	0.50	74
1% ML/MC/MCL	3.0	3.0	18	2.0	1.0	73
2% ML/MC/MCL	3.0	3.0	18	2.0	2.0	72
4% ML/MC/MCL	3.0	3.0	18	2.0	4.0	70

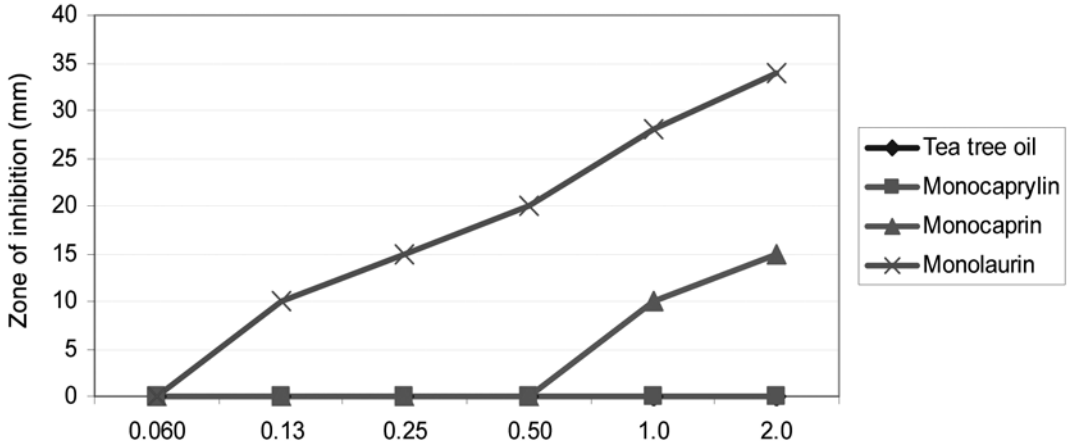

Figure 1. Zone of inhibition of Staphylococcus aureus.

transcription (Projan *et al.*, 1994). It is also known to inhibit the signal transduction pathways, that cause the expression of virulence factors including protein A, alpha-hemolysin, B-lactamase and toxic syndrome toxin 1 in *S. aureus* (Ruzin and Novick, 1998; 2000). This result is in agreement with the findings of Preuss *et al*. (2005) who suggested that monolaurin is effective in suppressing the growth of *S. aureus.* Kabara (1980) investigated the potential of certain lipids as anti-microbials and showed that the optimum anti-microbial activity of various types of fatty acids was found in those having chain lengths of 12 carbons.

There was no anti-microbial activity of monocaprin and monolaurin against *E. coli* from concentrations of 0.060% to 2.0%. Anti-microbial activity was shown by monocaprylin against *E. coli* at concentrations of 1.0% and 2.0%, and the zones of inhibition at these two concentrations were 6 mm and 7 mm, respectively. Tea tree oil showed inhibitory activity at 1.0% with a zone inhibition of 6 mm, but showed higher inhibition than monocaprylin at 2.0% with a zone inhibition of 12 mm (*Figure* 2). However, this concentration is not applicable in cosmetic products as the European Cosmetic, Toiletry and Perfumery Association (COLIPA) does not recommend the use of a concentration higher than 1.0% for tea tree oil in cosmetics (Salvador and Chisvert, 2007). Tea tree oil kept for prolonged periods has been reported to be allergenic due to the oxidation products formed which subsequently increases the peroxide value. The most important allergen could be terpinolene, α-terpinene, ascardiole and 1, 2, 4-trihydroxymethane (Hammer *et al.*, 2006).

Glyceryl monoesters were not as effective in suppressing the growth of gram-negative bacteria as compared to gram-positive bacteria. This result seems to agree with the findings by Altieri *et al.* (2009). The result is due to the complex organization of the gram-negative bacterial cell envelope which is associated with peptidoglycan, periplasmic space, outer membrane and lipopolysaccharide layer (Fischetti *et al.*, 2000). Such a structure provides more protection for the gram-negative bacteria than the gram-positive bacteria which has only a peptidoglycan cell envelope. The thick peptidoglycan layer of the gram-positive bacteria that surrounds the cytoplasmic membrane does not provide an effective barrier against the diffusion of small molecules, and therefore is unable to achieve resistance by denying access of anti-microbials into the cell (Fischetti *et al.*, 2000). Hence, anti-microbial agents can diffuse more easily into the cells of gram-positive bacteria than of gram-negative bacteria. Gram-negative bacteria have thus posed problems in the production of cosmetic products for centuries (Kabara and Orth, 1997). The inhibition exhibited by tea tree oil might be due to the presence of monoterpenes in the oil, which exert membrane-damaging effects (Sikkema *et al*., 1995). Sikkema *et al.*, examined *E. coli* cells (after exposure to tea tree oil) using electron microscopy, and they observed the loss of cellular electron dense material and the coagulation of cytoplasmic constituents. Tea tree oil has been said to stimulate the leakage of cellular potassium ions and inhibit respiration in *E. coli*, proving that the lethal action of tea tree oil against *E. coli* is related to cytoplasmic membrane damage (Cox *et al.*, 1998).

No inhibitory activity was exhibited by all the glyceryl monoesters from 0.060% to 2.0% against *P. aeruginosa*. A similar observation was made for tea tree oil. The outer membrane of *P. aeruginosa* consists of a strongly cross-linked surface layer of lipopolysaccharide. This outer membrane constitutes a barrier against the influx of lipophilic molecules. The higher intrinsic

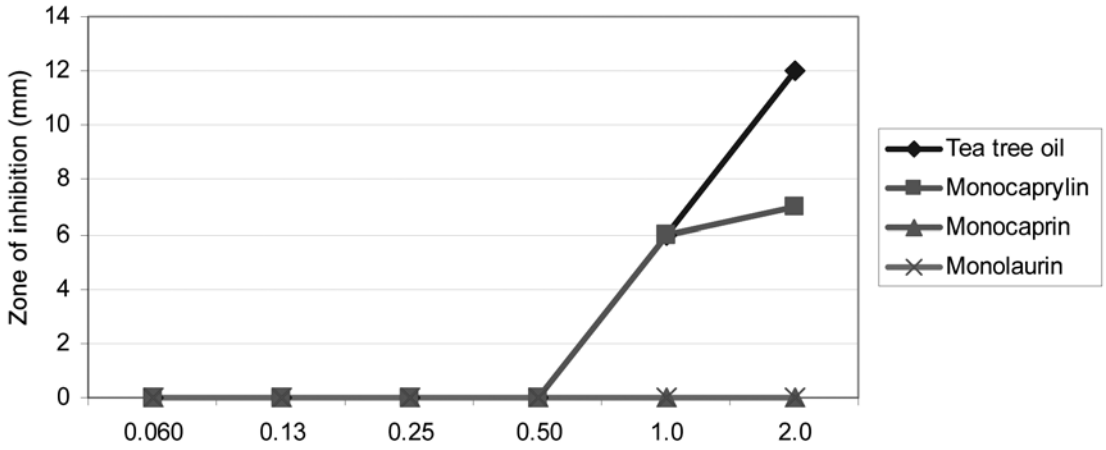

Figure 2. Zone of inhibition of Escherichia coli.

resistance of this microorganism to many anti-microbial agents as compared to *E. coli* is due to the relative permeability of its outer membrane to hydrophilic solutes which is about two orders of magnitude lower than that of *E. coli* (Russell *et al.*, 2004).

The high Mg^{2+} content of the outer membrane of *P. aeruginosa* aids in producing strong lipopolysaccharide-lipopolysaccharide links (Mara and Horan, 2003). The main porin of *P. aeruginosa* which is OprF, and is found abundantly in its outer membrane. It only allows very slow diffusion of small hydrophilic molecules, in this case, glyceryl monoesters. Most of the polypeptide chains of OprF porins are folded so that the central channel is closed. A multi-drug efflux system which has a similar structure to the AcrAB-TolC pump has been identified in *P. aeruginosa*. This multi-drug efflux system is constructed from an inner membrane transporter protein, a periplasmic protein which links the transporter to an outer membrane porin protein. In this way, they form channels spanning the complete cell envelope. This pump is able to pump out a wide range of structurally unrelated anti-microbial agents. The extraordinary resistance of *P. aeruginosa* to anti-microbial agents is due to a combination of active efflux pumps with a particularly low permeability of the outer membrane (Russell *et al.*, 2004).

Monolaurin also showed higher inhibitory activity against *Asp. niger* than monocaprin and monocaprylin. Monolaurin showed inhibitory activity at concentrations of 0.25% to 2.0%, whereas monocaprin showed inhibitory activity from 1.0% to 2.0%. The zone of inhibition for monolaurin at 0.25% concentration was 6 mm, 7 mm at 0.50%, 9 mm at 1.0% and 10 mm at 2.0%, whereas monocaprin showed an inhibition zone of 6 mm at 1.0%, and 7 mm at 2.0% concentrations (*Figure 3*). No inhibitory activity was shown by monocaprylin. This indicates that the longer the chain length of the glyceryl monoesters, the higher the inhibitory activity towards *Asp. niger*. Similar findings were reported that the fatty acids containing one to six carbons showing anti-fungal properties as they are able to inhibit the germination of spores, the formation of mycelium and the production of conidia in *Asp. niger* (Kato and Shibasaki, 1975). This inhibitory activity increases with increasing carbon chain length up to 12 (Kato and Shibasaki, 1975). Monolaurin and monocaprin were more effective than the commercial potassium sorbate, which did not show any inhibitory activity against *Asp. niger* at concentrations ranging from 0.060% to 2.0%.

Optical Microscopy Measurement

Interestingly, the sample which consisted of monolaurin and water seemed to 'shine' during the anti-microbial susceptibility test; thus, the presence of a liquid crystalline structure was suspected in this system. The presence of the liquid crystalline structures was then investigated by optical microscopy. The result is in agreement with the hypothesis. Onion-shaped birefringence was found under the polarized microscopy for all the formulations, except for those without monolaurin (*Figures 4* to *8*). This indicates that the presence of monolaurin could have induced the formation of liquid crystals. The onion-shaped birefringence indicates the presence of lamellar liquid crystals.

As reported by Rosen (2004), liquid crystals formed when there are a sufficient number of micelles in the emulsion to cause them to pack together in a number of geometric arrangements, depending upon the shape of the individual micelles. Liquid crystals are an intermediate state between a solid and a liquid, which indicates that they possess structural orders and mobility. Spherical micelles pack together to form cubic liquid crystals, cylindrical micelles pack together

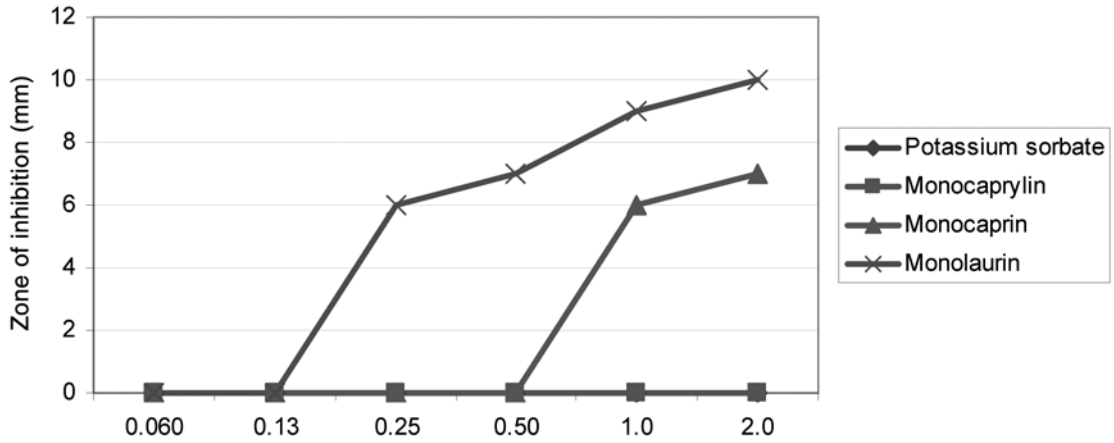

Figure 3. Zone of inhibition of Aspergillus niger.

Figure 4. Emulsion without monolaurin (20X magnification).

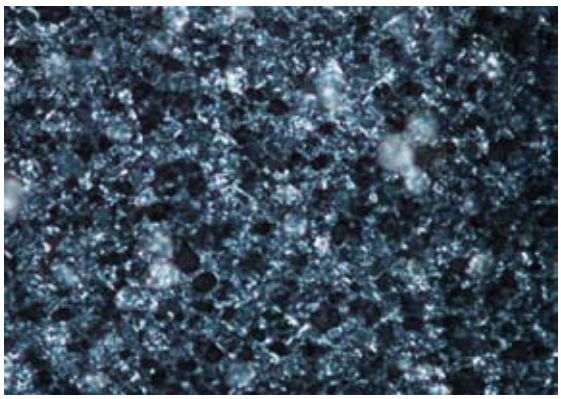

Figure 5. Emulsion with 0.50% monolaurin (20X magnification).

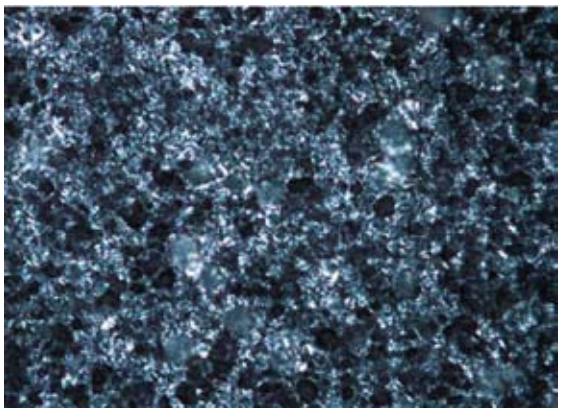

Figure 6. Emulsion with 1.0% monolaurin (20X magnification).

to form hexagonal liquid crystals, and lamellar micelles form lamellar liquid crystals. Hexagonal and lamellar phases are anisotropic and can be detected by their radiance under polarizing light microscopy (Rosen, 2004).

Hexagonal liquid crystals appear as fan-like structures or with a variety of non-geometrical structures, whereas lamellar liquid crystals appear as Maltese crosses or as oil streaks under polarizing microscopy. Hexagonal phases are more viscous than lamellar phases (Rosen, 2004). In general, the liquid crystalline lamellar phase is formed at the interface of water and oil droplets surrounding the oil droplets (Widlak, 1999). Lamellar liquid crystalline structures were found in the emulsions with monolaurin as Maltese crosses, and onion-shaped birefringence was detected under polarized microscopy (*Figures 5* to *8*).

Monolaurin is a non-ionic surfactant and is proven in this study to induce the formation of lamellar liquid crystalline structures. This is due to the structure of monolaurin which consists of molecules that are elongated and possess one polar head (*Figure 9*). Such molecules tend to arrange themselves parallel to each other due to the presence of attractive van der Waals forces. The weak interaction between water and the surfactant governs the formation of lamellar liquid crystals which are composed of bilayers of the surfactant and a layer of water (Atwood and Steed, 2004).

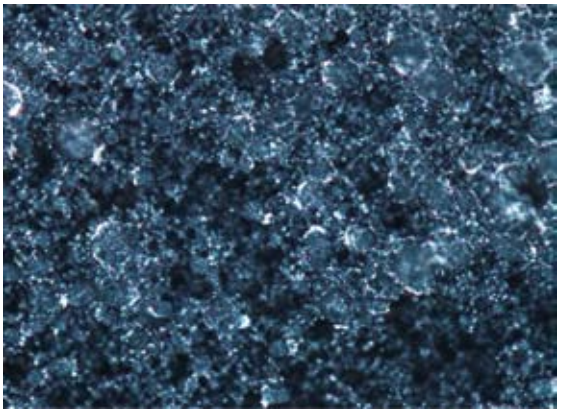

Figure 7. Emulsion with 2.0% monolaurin (20X magnification).

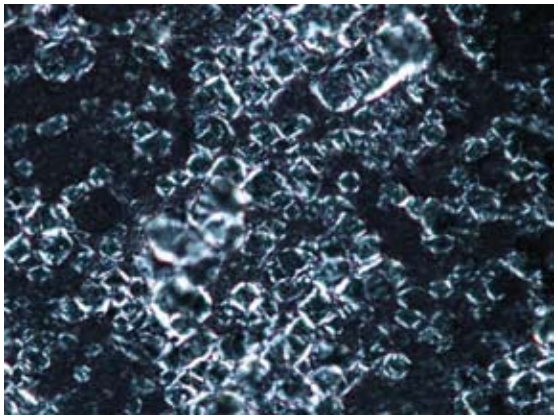

Figure 8. Emulsion with 4.0% monolaurin (20X magnification).

The onion-shaped birefringence became stronger with increasing monolaurin concentrations (0.50% to 4.0%). This indicates that the higher the concentration of monolaurin, the more stable is the emulsion. Friberg *et al.* (1976) proved that the lamellar liquid crystalline phases play a major role in the stabilization of emulsions. They found that the van der Waal's energy of droplets were reduced due to the presence of the liquid crystals. The liquid crystalline structure is important for cosmetic and pharmaceutical applications as it can control the moisture and the delivery of active ingredients to the skin (Thomas and Wolfgang, 1998).

There are two stabilizing actions of liquid crystals in emulsions. First, the lamellar crystal structure leads to a strong reduction of the van der Waals forces during the coalescence step. An emulsion with the presence of liquid crystals is highly viscous. A viscous film of liquid crystals in emulsion combined with a small compressive force enhances stability against coalescence. Secondly, the network of liquid crystals hinders the free mobility of emulsion droplets, and thus stabilizes the emulsion system. This is similar to the effect of increasing the viscosity of the continuous phase of a two-phase emulsion (Lieberman *et al.*, 1996).

Interestingly, the liquid crystalline structure was found in the emulsions with monolaurin at concentrations as low as 0.50%; however, this phenomenon was not observed in the emulsions of monocaprin and monocaprylin at concentrations ranging from 0.50% to 4.0%. This result agrees with published literature because monolaurin is proven to form a lamellar liquid crystalline structure in monolaurin-water systems. The phase behaviour of monoacylglycerol (MAG)-water systems has been reported. The lamellar-type liquid crystalline structure predominates for monoacylglycerol with 12:0 and 16:0 fatty acid side-chains, whereas the cubic-type liquid crystals are usually produced from MAG with longer fatty acid side-chains (Hui, 2006). This shows that monolaurin with 12 carbons is able to form lamellar liquid crystals in the presence of water.

A monocaprylin-water system is reported to exist as fluid isotropic at room temperature in the presence of 70% water (Schick, 1987). This observation is in agreement with the result in this study which suggests that the lamellar liquid crystalline structure is not present under room

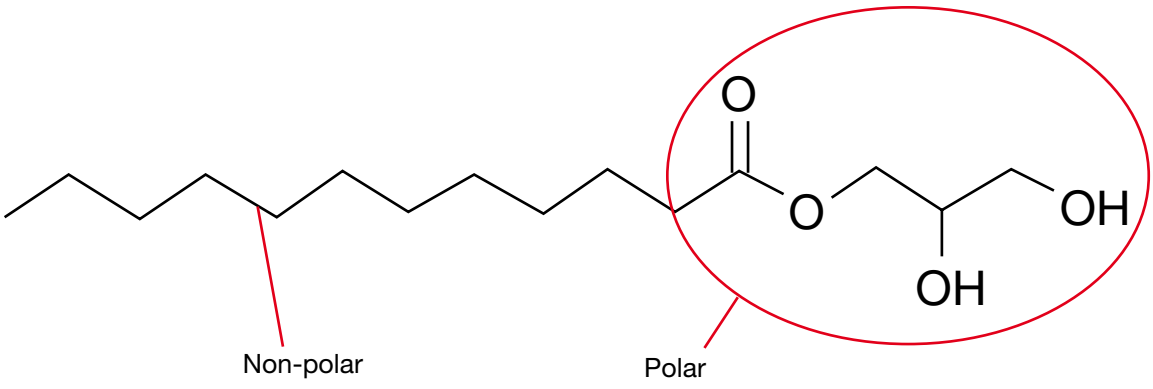

Figure 9. Molecular structure of monolaurin.

temperature conditions in the presence of 70%-75% water. For the monocaprin-water system, crystals (β_1) with water or dispersion exist at room temperature in the presence of 70%-75% water (Schick, 1987). This explains why a lamellar liquid crystalline structure was not present in the formulations in the presence of 70%-75% water in this study.

At room temperature, monolaurin remains in a non-hydrated crystalline phase (β phase) in equilibrium with a surplus of water. Monolaurin takes up water and a lamellar phase is formed at 40ºC. When the lamellar phase is cooled, a semi-crystalline phase known as the α phase is formed. The swelling of the lamellar and α phases indicates the presence of a strong repulsive hydration force (Hasenhuettl and Hartel, 2008). The packing parameter concept is used to predict the aggregate shape of the surfactant molecular structure. The 'packing parameter' is a geometric expression relating the hydrocarbon chain volume (*v*), length (*l*) and the interfacial area occupied by the surfactant's head group (*a*). The expression of the packing parameter is shown as:

$$\text{Packing parameter} = v/l_c\, a_0$$

where a_0 is the optimal surface area per head group, and l_c is the critical chain length (Tadros, 2005).

Lamellar liquid crystalline structures were found in emulsions with manolaurin, indicating that the surfactant mixture (Span 40 and Tween 80) and co-surfactant (*e.g*. monolaurin) had a packing parameter equal to 1. This parameter corresponds to the cylindrical packing shapes for the mixed surfactants and co-surfactants present in the emulsion. Surfactants and co-surfactants falling in this range often produce planar bilayers and lamellar mesophases (Moore and Spencer, 2001). Moore and Spencer (2001) reported that the lamellar liquid crystalline structure is predominant if the surfactant which forms a particular type of emulsifier is mixed with the surfactant which forms a lamellar liquid crystalline structure. They reported that the surfactant Tween 80 forms a hexagonal array of cylinders, and that this structure is modified into lamellar liquid crystals when it is mixed with a lamellar-type surfactant. In this case, the lamellar-type surfactant was monolaurin. The bulky head group of Tween 80 is accommodated within the lamellar structure by an increased thickness of the water layer (Lodén and Maibach, 2000).

The solution property of the surfactant is improved by the addition of a co-surfactant (*e.g*. glyceryl monoester) which is preferentially adsorbed at the oil-water interface. Due to the large differences in size between the mixed surfactants (*e.g*. Span 40 and Tween 80) and the co-surfactant, the co-surfactant is packed efficiently between the larger surfactant chains at the interface. A co-surfactant with a smaller size and lower hydrophilicity of the hydroxyl group functions by moderating the steric interactions among the primary surfactant head groups. This results in a more densely packed interfacial layer which makes possible the very low and transiently negative interfacial tensions (Meyer, 2006).

CONCLUSION

The anti-microbial potential of glyceryl monoesters can be of great importance as they have such a good safety profile. Most of the synthetic anti-microbial agents have been associated with serious side-effects that limit their long-term use. Natural products can be used for long period as they are less likely to produce negative side-effects. Results of this study suggest that monolaurin, monocaprylin and monocaprin have anti-microbial activity which can be used alone or in combination to preserve cosmetic products. Glyceryl monoesters were proven to suppress the growth of gram-positive bacteria and a fungus when compared with commercially available tea tree oil and potassium sorbate. Tea tree oil had anti-microbial activity towards *E. coli* at 2.0% concentration but no activity towards *S. aureus*, *P. aeruginosa* and *Asp. niger* at concentrations ranging from 0.060%-2.0%, whereas no anti-microbial activity was exhibited by potassium sorbate towards the tested microbes at all concentrations.

From this study, glyceryl monoesters seem to be less effective against gram-negative bacteria. However, the inhibitory effect of glyceryl monoesters can be increased by using citric acid or polyphosphoric acid as these compounds may remove the barrier of gram-negative bacteria. Thus, they can facilitate the penetration of glyceryl monoesters into the inner cell membranes of gram-negative bacteria, which are the primary sites for anti-microbial action. The use of Span and Tween surfactant series, and subjecting the surfactants to mild heating treatments, facilitate the removal of the barrier of gram-negative bacteria. This will enable the penetration of glyceryl monoesters across the bacteria cell wall and inhibit growth (Kabara, 1978). Furthermore, glyceryl monoesters were proven to improve the quality of the cosmetic products as their presence enabled the formation of liquid crystals which enhanced the stability of the emulsion. A combination of all the stated effects will enhance the delivery of the actives to the skin.

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