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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2012, Vol. 28, No. (2):
Pg. 803-808

The Microemulsion of *Beakea frustecence* Stabilized by SDS and Span 80 and its Antibacterial Potential

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(Received: February 26, 2012; Accepted: March 25, 2012)

ABSTRACT

A study is conducted to examine the antibacterial activities of the emulsions containing essential oil of *Beakea Frustescence*. The emulsions were formulated according to the information obtained from the constructed phase equilibriums consisting water, SDS, Span 80 and hexane. Followed by the preparation was a stability test, which was carried out within the following three weeks to determine the emulsion's stability index. Lastly, several formulations were selected to examine their antibacterial activity.

Key words: *Beakea frutescence*, Surfactants, SDS, Span 80, Emulsion, Antibacterial activity.

INTRODUCTION

Beakea Frustecence or commonly known as *Cucur atap* is a common edible herb species of family Mrytaceae that can be found everywhere within South East Asia. Besides playing an important role in the South East Asian medicinal herb and also as flavour and fragrance agents, as well as in perfumery and medicinal preparation. The chemical constituents of the cucur atap essential oil were mostly monoterpene hydrocarbons, with limonene (30.73%) and β-pinene (18.76%) as the major components, whereas the minor components were terpinene-4-ol (10.63%), α-terpineol (8.35%), α-terpinene (6.18%), α-terpinene (5.09%) and terpinolene (4.33%)[1]

In general, essential oils are a rich source of biological active compounds. This is because essential oils have very complex natural mixtures which can contain 20 to 60 components. Therefore, they have been used widely as bactericidal, virucidal, fungicidal, anti-parasitical, and insecticidal in various applications, especially in the pharmaceutical, sanitary, cosmetic, food and agricultural industries[2]. While its applications are expanding in several industries, studies have been conducted to examine the biological activities exhibited by the essential oil of *Beackea Frutescence*. The studies showed that the essential oil of *Beackea Frutescence* has effective bactericidal effect on bacterial strains like *Propionibacterium acnes*, 20 serotypes of

Salmonella [4] as well as other bacteria causing skin disease like *Bacillus substilis, Staphylococcus epidermis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* [5].

Due to the *Beackea Frutescence* pleasant aroma and vast biological properties, it has become a great source of economic potential. It is a common component in perfumery and toiletries like shampoo, body wash, lotion and etc. These products fall in a colloidal category known as the emulsion[6]. An emulsion is a dispersion of two immiscible liquids, generally referred to oil and water. These two phases may form some sort of dispersion under intense agitation, whereby fine droplets of one phase were dispersed into the other phase. However, without any stabilizing agent, such dispersion is not stable. The droplets will coalesce and eventually the two phases will be separated, and such action normally starts immediately once the agitation stopped[7]. This is due to the differences of the polarity between the aqueous and non-aqueous solution as well as the high interfacial energy between the two phases. In order to enhance the dispersion process and to stabilize the emulsion, emulsifying agents are added. The role of emulsifiers in the system is to rectify this instability[8].

With much attention focusing on the essential oil of comeceutical products recently, it is thought that a study on formulating emulsions containing the essential oil of *Beackea Frutescence* with antibacterial properties will be interesting. In the early stage of the investigation, stability of oil-in-water (O/W) emulsions containing the essential oil of *Beackea Frutescence* were prepared from systems containing water, hexane, sodium dodecyl sulphate (SDS) and Span 80, was examined. After the stability test, the emulsions were tested for its antibacterial properties against three bacterial strains namely *E. coli*, *B. substilis* and *S. aureus* respectively.

MATERIALS AND METHODS

Materials

The anionic surfactants, sodium dodecyl sulphate (SDS) (98%) were purchased from Sigma; Sorbitan monooleate (Span 80) (>95%) were purchased from Sigma-Aldrich. n-hexane were purchased from PC Laboratory. Beeswax was purchased from Aldrich. Mueller-Hinton agar was purchased from Merck. All components were directly used without further purification. The essential oil of *Beackea Frutescence* was obtained from Malaysian Agricultural Research and Development Institute (MARDI). Doubly distilled water was used throughout the study.

Preparation of Emulsions

For the preparation of emulsions, inversion technique was applied. The surfactant and the beeswax were first heated to 70°C and mixed. The mixture of hexane and essential oil was added in as the solution's temperature has dropped to 50ÚC. When the mixture has reached homogeneity, water heated to 50°C was added. Then it was homogenized by the homogenizer and the mixture was stirred slowly with the stirrer until the temperature dropped to 35°C. Then the samples were placed in a dark and cool place for storage purpose. For the stability test, the samples were under daily observation in order to detect any changes from a period of 3 weeks.

Antibacterial Test

In the antibacterial test, disc diffusion method was applied and Mueller-Hinton agar is used as the nutrient agar. 21 g of agar powder was dissolved in 1L of distilled water. The mixture was mixed to homogeneity and followed by sterilization process in the sterilizer at 121°C for 15 minutes. After the solution has been sterilized, it was poured into several Petri dishes, each dishes contained approximately 20 mL nutrient agar. After the agar solidified and the temperature had dropped to room temperature, the agar was swabbed with culture bacteria. In each agar dish, approximately 100 µL of test bacteria was spread onto it. The agar was left to dry.

The disc with diameter of 6.0 mm was soaked with the test sample and placed gently onto the agar that contain the cultured bacteria. For positive control, ampicilin were used as standard. After loading the disc onto the agar, the Petri dishes were sealed and left in the incubator at 37°C for 24 hours.

At the following day, the inhibition zones that were produced by different discs were measured. The inhibition zone represented the test sample's ability in destroying the targeted bacteria since it was the area in which the bacteria were destroyed by the test sample. The classification of the strength of the antibacterial activity can be categorised into four levels with respect to the diameters of their inhibition zone namely strong (>16 mm), good (11-16 mm), weak (7-11 mm) and none (<7 mm).

RESULTS AND DISCUSSION

Preparation of emulsions

During the preparation of emulsions, the compositions of water, hexane, Tween 80 and Span 80 were based on the phase equilibriums constructed previously [9-10]. The percentage of beeswax was maintained at 10% where it acted as an effective thickening agent and the essential oil was at 2% by weight to obtain optimum antibacterial performance. From the phase equilibriums, composition points at 60% of water, 4% of hexane and 36% of surfactants were selected to formulate the O/W emulsion since it has the lowest possible content of surfactant and hexane. Furthermore, it was within the region that is in equilibrium with the liquid crystalline phase; consequently this would increase the stability of the emulsions since liquid crystalline phase was a very stable association structure[11]. The formulations of the emulsion system were shown in Table 1.

By substituting the surfactant from SDS to Span 80, and with the mixtures of both at the ratio of 90:10 and 75:25, it was found that the emulsions'

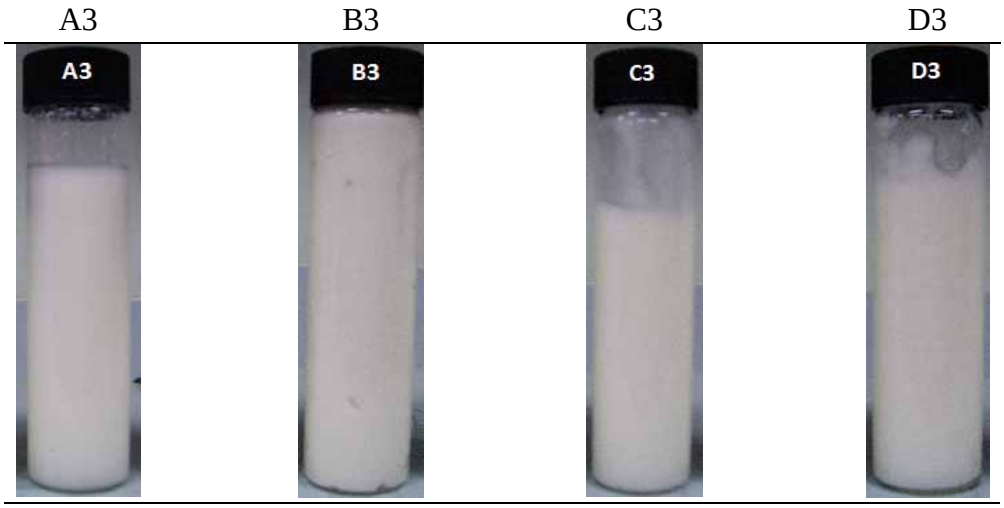

Fig. 1: The appearance of the emulsions after three weeks of storage

Table 1: The formulation of the emulsion samples

Ingredient	% (by weight)			
	A3	B3	C3	D3
Bees wax	10.0	10.0	10.0	10.0
Essential oil	2.0	2.0	2.0	2.0
Tween 80	31.7	-	28.5	23.8
Span 80	-	31.7	3.2	7.9
Hexane	3.5	3.5	3.5	3.5
Water	52.8	52.8	52.8	52.8

Table 2: Stability of the emulsions

	A3	B3	C3	D3
Day 1	√	√	√	√
Day 3	√	√	√	√
Day 5	√	√	√	√
Day 7	√	√	√	√
Week 2	√	√	√	√
Week 3	√	√	√	√
Appearance	Milky white	Milky white cream	Milky white	Milky white
Stability Index	1.00	1.00	1.00	1.00

Table 3: The inhibition zone produced by the pure essential oil at different concentration

[Essential oil] (% by weight)	Diameter of the inhibition zone (± 1.0 mm)		
	***E. coli*[a]**	***B. substilis*[b]**	***S. aureus*[c]**
0.5	-	-	-
1.0	7.0	11.0	-
1.5	7.0	12.0	6.0
2.0	16.0	15.0	9.0

[a] inhibition zone of ampicilin = 11.0 mm, [b] inhibition zone of ampicilin = 10.0 mm
[c] inhibition zone of ampicilin = 8.0 mm

Table 4: The inhibition zone produced by the emulsions

Emulsions	Diameter of the inhibition zone (± 1.0 mm)		
	***E. coli*[a]**	***B. substilis*[b]**	***S. aureus*[c]**
A3	17.0	8.0	9.0
B3	11.0	9.0	9.0
C3	18.0	8.0	11.0
D3	14.0	7.0	10.0

[a] inhibition zone of ampicilin = 13.0 mm, [b] inhibition zone of ampicilin = 8.0 mm
[c] inhibition zone of ampicilin = 10.0 mm

viscosity and texture had changed. As the content of Span 80 increased, the viscosity of the emulsion increased as well. Furthermore, when Span 80 alone was used, the emulsion appeared to be a cream while the rest appeared to be lotion like (Fig 1). In terms of stability (Table 2), the emulsions did not undergo separation after three weeks of storage regardless which surfactants or at which ratios were used.

Antibacterial susceptibility test against *Escherichia coli*, *Bacillus substilis* and *Staphylococcus aurues* were conducted after the emulsions had underwent stability test. These bacteria species were chosen as they were the most

common bacteria that cause skin infection 5. The method to run the susceptibility test in this study is by disc diffusion method or it is also known as the *Kirby-Beuer Method.* In the test, ampicilin is used as positive control.

The results on the inhibition zone caused by the essential oil and formulations against the three bacteria were tabulated in Table 3 and Table 4, respectively. The results showed that the effective percentage of essential oil is at 2.0% when it is tested against *E. coli* and *S. aureus*, and 1.5% against *B. substilis*. The results were consistent to the finding of another study in which the beackea frutescence essential oil was effective in inhibiting the growth of these bacteria strains[5]. However, the effective concentration in this study differed from the reported results. In Kongtun and Sarcherdkaiti's[5] study, the effective concentration to inhibit these bacteria strains were at 1% but the results in the study showed otherwise. In this study, the effective concentration was at 2% in order to destroy the bacterial strains effectively. This is probably due to the variability of the essential oil constituents, as it may differ according to their geographical origin[2].

In order to ensure that the emulsions produced had the susceptibility to inhibit bacterial growth, the percentage of the essential oil usage was maintained at 2% by weight while formulating the emulsions. The results showed that all emulsions were susceptible in destroying the bacteria. In the case of *E. coli*, formulation A3, C3 and D3 were able to destroy the bacteria effectively as the inhibition zones produced were larger than the control since their inhibition zone diameters were 17.0 mm, 18.0 mm and 14.0 mm, respectively whereas the control's was only 13.0 mm. Meanwhile, formulations A3, B3 and C3 had effectively inhibited the growth of *B. substilis* by creating inhibition zone diameter of 8.0 mm, 9.0 mm and 8.0 mm, respectively and only emulsions C3 and D3 were successful in preventing the growth of *S. aureus* effectively by producing inhibition zones with the diameter of 11.0 mm and 10.0 mm respectively. This showed that only the formulations C3 had the most effective antibacterial property as the inhibition zone created by the formulation are bigger or similar to that of the control in all three bacterial strains. It also showed that the antibacterial activities of the emulsion were not affected by the concentration the surfactant. This is consistent with a similar study conducted to examine the effect of surfactant type on the antibacterial activity of the emulsion. According to Rozaini and Hassan[12], non-ionic surfactant had no significant effect on the antibacterial activities.

CONCLUSION

From this study, it may be concluded that composition point at 60% of water, 4% of hexane and 36% of SDS and Span 80 was suitable to formulate stable O/W emulsion. The emulsions with at least 2% of the essential oil of *Beackea Frutescence* proved to be susceptible in inhibiting the growth of *E.coli*, *B. substilis* and *S. aureus*. However, only emulsion with the surfactant mixtures of SDS and Span 80 at the ratio of 90:10 had effective antibacterial properties.

ACKNOWLEDGEMENTS

The financial and encouragement support provided by ministry of higher education via fundamental research grant scheme (FRGS) 2010.

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