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Physical evaluations of Haruan spray for wound dressing and wound healing

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Abstract

Haruan spray (aerosol) dosage form is a new dosage form formulated for use as wound dressing and wound healing. Spray can minimize pain and emotional trauma experience during the application and removal process of conventional dressing and bandages. Haruan extract is incorporated as an active ingredient of the aerosol concentrate for its ability to enhance the healing process. The aim of this study is to evaluate the physical properties of Haruan spray using different propellant. The propellants evaluated were 1,1,1,2 – tetrafluoroethane (HFA 134a), butane and propane. Concentrate of aerosol also contain hydroxypropyl methylcellulose (HPMC) as polymer and glycerine as plasticizer. All ingredients of the aerosol were packaged in an aluminium container fitted with continuous-spray valves. Evaluations for the Haruan spray included delivery rate, delivery amount, pressure, minimum fill, leakage, flammability, spray patterns and particles image. Glass containers were used to study incompatibility between the concentrate and propellant due to the ease of visible inspection. HFA 134a reacted with the concentrate and produced white aggregates, while propane had a very high vapour pressure. From safety and economics point of view, butane was chosen as the propellant because it met the requirements for aerosol and produced Haruan spray with the expected characteristics.

Keywords: Aerosols; Haruan; 1,1,1,2 – tetrafluoroethane (HFA 134a); propane; butane; aluminium canister

Introduction

There are many alternatives to conventional wound dressings in the market. Among these are sponges, films [1], sheets , hydrocolloid [2, 3], foam [4], ointment and hydrogel [5, 6]. These dressings could be with or without bioactive compound that promote the healing process. The newer dressings are designed to create a moist wound healing environment. The concept of moist wound healing has been around since the 1960s, but only recently being accepted widely [7-9]. These wound dressing forms, however, have some limitations in the application and removal processes. Ointment, hydrogel and foam have to be rubbed onto the wound, which may cause pain to the patient, while films, sheets and sponges have limited size to cover large wounds. In addition, it involves contact with the user's

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hand during the handling and application processes and is susceptible to contamination.

To surmount the limitations of the conventional wound dressings, spray or aerosol dosage form for topical application was formulated. The topical spray dosage form can deliver the bioactive compound directly to the injured area and produce a film that can cover wound of any size. The spray dosage form for oral and topical was developed in the early 1950s and used CFCs as propellant [10, 11, 12, 13]. However, the development of this dosage form using the CFCs as propellant was stopped due to the reported ozone-depleting properties of CFCs in 1974 [14]. Since hydro fluorocarbon, hydrocarbon and compressed gas were used as propellant in 1980s replacing the CFCs, production of the aerosol dosage form has increased [11, 15]. Topical products that have been formulated as an aerosol include first-aid products containing local anaesthetics and antiseptics, adhesive tape removers and bandage adherents, athletic and sports applications, burn remedies, foot preparations, germicidal and disinfectant products, spray-on bandages, protective, topical dermatologic, including antibiotics and steroids, body liniments and rubs, vaginal applications, including contraceptive foams, rectal foams, edible foams and saline solutions to cleanse contact lenses [16]. Topical pharmaceutical aerosols have been accepted by patients and physicians due to their aesthetic properties, ease of application, maintainability of sterility, tamperproof system and prevention of contamination of the unused contents. When applied to the body, aerosol could reduce pain that may result from the mechanical rubbing of ointments and creams onto the skin [11, 16].

Channa striatus (Haruan), a fresh water snakehead fish is indigenous to many tropical countries. It is consumed for its putative effects on wound healing [17, 18]. This fish is also used by the patients in the post-operative period in the belief that it promotes wound healing and reduces post operative pain and discomfort [19]. Haruan is known to contain polyunsaturated fatty acids that can regulate prostaglandin synthesis and hence induce wound healing. Certain amino acids like glycine, aspartic and glutamic acid are also known to play an important role in the process of wound healing [17, 19]. Mat Jais et al. [20] also reported that the fatty acid composition of Haruan may account for the promotion of wound healing process. Gam et al. [21] reported that there were no significant differences in the content of amino acid and fatty acid compositions of various sizes of snakehead fish obtained at different times of the year.

The objective of this study is to evaluate the physical properties of Haruan spray using different propellants. The Haruan spray formulated would offer many advantages. A spray-on bandage formulated in this manner would have many advantages including ease of application, prevention of contamination of the product by the user, no limit on the size of the wound to be covered and will replace the use of conventional plaster and bandage. The Haruan extract incorporated and form film together with HPMC and glycerine to cover the wound. This healing enhancer will be released from the film to promote wound healing process. Haruan spray is a combination between a good dressing for dermal wound and a source of nutrition to the wound site.

Materials and methods

Materials

Haruan extract was obtained from Major Interest Sdn. Bhd. (Malaysia). Hydroxypropyl methylcellulose (HPMC Viscosity 100 cps) and α-tocopherol (Vitamin E) were purchased from Sigma-Aldrich (St. Louis, USA). Glycerine, methyl paraben and propyl paraben were purchased from R&M Chemicals, Essex (U.K.). Ethanol was purchased from Systerm AR. Butane and propane was supplied by Cambrex – Henkel Sdn. Bhd. (Malaysia). HFA 134a was acquired from DuPont Mitsui Fluorochemicals Co. Ltd. All chemicals were used without further purification.

Methods

Preparation of Haruan spray

Methyl paraben (0.1 %), propyl paraben (0.02 %), and α-tocopherol (0.01%) were dissolved in 20 % ethanol. HPMC (2 %), glycerine (1 %) and Haruan extract (20 %) were added, mixed and dispersed into the solution. The weights of the dispersions were made up to 100 % with the addition of ethanol. The dispersions were homogenized by using ultra turrax homogenizer (IKA T18 Basic) at 10,000 rpm for 15 minutes as recommended by Febriyenti et al. [22]. Approximately 40 g of the dispersion was filled into each of 100 ml aluminium canister (container). The valve was placed and sealed to the canister before filling the propellant manually by using aerosol-filling machine. The evaluated propellants were HFA 134a, butane, propane and mixture of butane and propane (2:1). Weights of the propellants in this study were 15.3 g, 12.0 g, 5.5 g and 17.5 g for HFA 134a, butane, propane and butane-propane mixture respectively. The containers were shaken and the Haruan sprays were prepared for further evaluations. For compatibility study between the concentrate and the propellant, 20 g of the concentrate was filled into each of 100 ml glass container. The valves were placed and crimped to the container mouth and a half of each propellant was filled into the glass containers. Six glass containers were prepared for each formula except for propane, since the glass container could not withstand the vapour pressure of propane.

Physical compatibility of aerosol concentrates with propellants

Glass containers containing concentrate of aerosol and specified propellant were kept at room temperature on the laboratory benches but not protected from light. The contents were examined visually from time to time to detect any physical change indicating incompatibility.

Delivery rate

The delivery rate of Haruan spray was evaluated according to procedure stated in USP [23] and Sciarra and Cutie [24]. Six aerosol containers were used. Each valve was actuated for 5 seconds at a temperature of 25 ^{0}C. The test was repeated three times for each container. The average delivery rate was calculated, in g per second.

Delivery amount

The delivery amount of Haruan spray was determined by using six containers of aerosol according to procedure stated in USP [23]. The valves were pressed continuously for 5 seconds each time until no more spray emerged. The total weight loss was calculated from each container as the deliverable amount.

Pressure test

The pressure of six Haruan spray containers was determined according to the method in Sanders [25], Sciarra and Cutie [24] and USP [23], at a temperature of 25 ^{0}C. Each container was placed in an upright position. The actuator was pressed to remove liquid from the dip tube and valve. The actuator was removed and replaced with the pressure gauge. The gauge was pressed to actuate the valve and the pressure exerted by the propellant was noted for each aerosol container.

Minimum fill

Twelve filled containers were selected and weighed individually. The contents were removed from each container. The packages were opened or dismantled and any residue was removed by washing with suitable solvents and rinsed with a few portions of methanol. The container, the valve, and all associated parts were collected and heated to dryness at 100 ^{0}C for 5 minutes and cooled. The weight of each container together with their corresponding parts was determined. The difference between the weight of the filled container and the weight of the corresponding empty container was the net weight of the content [23].

Leakage test

The leakage test was conducted according to the method in USP [23]. Twelve aerosol containers were selected and the date and time were recorded to the nearest half hour. Each container was weighed to the nearest mg and recorded as W1. The containers were allowed to stand in an upright position at a temperature of 25.0 ± 2.0 ^{0}C for not less than 3 days, before the second weight was recorded as W2. The leakage rate, in mg per year, of each container was calculated using formula: (365)(24/T)(W1 – W2), where T is the test period, in hours.

Flammability

The flammability of Haruan spray was determined based on method in Sciarra and Stoller [26], Sanders [25] and Sciarra and Cutie [24]. The sample is flammable if the flame projects more than 18 inch beyond the ignition source with the valve fully opened or if the flame flashes back and burns towards the valve with any degree of valve opening.

Spray patterns

Products were sprayed onto absorbent paper for 2 seconds. The distance separating the container from the target was kept constant, at 15 cm. This will give a record of spray pattern which can then be used for comparison purposes [13, 24, 25].

Particles size and particles image

Haruan spray were sprayed onto separate slides and the images of particles were photographed by using Leica DMLB Microscope with Leica DC 300 Camera and Leica QWin Program, Leica Microsystems DI Cambridge CB 1 3xJ Type DC 300 V2.O equipped with an objective lens of 20 x magnification and 10 x magnification of eye piece lens that has been fitted with a standardized micrometer. The size of one thousand particles counted were determined to obtain the Martin's diameter [27].

Statistical analysis

The results were represented as mean ± SD and treated statistically by using SPSS software (version 15, USA). One-way analysis of variance was used to compare the results. Duncan's test was applied when there was a statistically significant difference (P<0.05) [28, 29].

Results

Compatibility of aerosol concentrates with propellants

Preparation and evaluation of the aerosol concentrate have been discussed in previous paper [22]. In this study, Haruan spray or aerosol were produced by filling the concentrate of aerosol into glass containers together with one of these propellants or mixture, namely HFA 134a, butane and butane-propane mixture. Propane could not be used in this study due to the glass container could not withstand the vapour pressure of propane (8 Bar). Compatibility study of the concentrate and the propellant was carried out; it is one of the physical properties determined and should be the first step in any rational formulation design [30, 31]. Glass containers were used in this study to observe any change taking place visually which reflect the compatibility of the formulated aerosol. Butane and butane-propane mixture were compatible with the concentrate because no change was observed in the formulated aerosols containing these propellants, while HFA 134a gas reacted with the concentrate to produced white aggregates, thus considered incompatible. The formulation that produced aggregates is not suitable because the aggregates could clog the valve.

Delivery rate, delivery amount and vapour pressure of the formulated Haruan spray

Delivery rate, delivery amount and vapour pressure of Haruan spray formulated are shown in Table 1. As expected, the delivery rate of Haruan spray was affected by vapour pressure. Propellants that produced high vapour pressure gave higher delivery rate. Propellant vapour pressure has no significant effect on the delivery amount of Haruan spray.

Leakage and minimum fill of the formulated Haruan spray

The leakage and minimum fill of the Haruan spray by using different propellant were significantly different, but the results still met the

Table 1: Delivery rate, delivery amount and vapour pressure of Haruan spray using different propellant, Mean ± SD, (n = 6)

Propellant	Delivery rate (g/sec)	Delivery amount (%)	Vapour Pressure (Bar)
HFA 134a	$1.10^{b} \pm 0.06$	$94.14^{a} \pm 1.13$	$7.03^{c} \pm 0.05$
Butane	$1.01^{a} \pm 0.02$	$94.08^{a} \pm 1.40$	$3.48^{a} \pm 0.04$
Mixture	$1.07^{a,b} \pm 0.02$	$94.12^{a} \pm 1.36$	$5.93^{b} \pm 0.10$
Propane	$1.13^{b} \pm 0.07$	$94.17^{a} \pm 2.94$	$8.00^{d} \pm 0.06$

Means within a column with a different letters are significantly different (P<0.05)

Table 2: Leakage and minimum fill of Haruan spray using different propellant, Mean ± SD, (n = 12)

Propellant	Leakage (%/year)	Minimum Fill (%)
HFA 134a	$0.58^{b} \pm 0.09$	$100.29^{a,b} \pm 0.28$
Butane	$0.34^{a} \pm 0.04$	$100.23^{a} \pm 0.23$
Mixture	$0.75^{c} \pm 0.08$	$100.51^{b} \pm 0.35$
Propane	$0.88^{d} \pm 0.11$	$100.02^{a} \pm 0.05$

Means within a column with a different letters are significantly different (P<0.05)

Table 3: Results of flammability test conducted on Haruan spray formulated using different propellant

Propellant	Flame Projection (Inch)	Flame Flash back (Inch)
HFA134a	19	2
Butane	19	4
Mixture	19	4
Propane	23	4

requirement of USP (Table 2). The product passed the average leakage test if the rate per year for the 12 containers is not more than 3.5 % of the net fill weight. The results showed all formula had leakage of less than 3.5 %. The product passed the minimum fill test if the net weight of the contents is not less than the labelled amount. All formula had a minimum fill of more than 100 %, which mean that the net weight of the contents was not less than the labelled amount and met the requirement.

Flammability of the formulated Haruan spray

The results of flammability test are shown in Table 3. Butane and propane are flammable while HFA 134a is not flammable. All Haruan sprays formulated with different propellant were flammable due to the presence of ethanol used as solvent in the formulation.

Spray pattern of the formulated Haruan spray

The results of spray pattern of Haruan spray are shown in Figure 1. The patterns and size were almost the same since all formula used the same type of valve. It means that spray patterns were not significantly affected by type of propellants and vapour pressure. The type of valve used might affect the spray pattern.

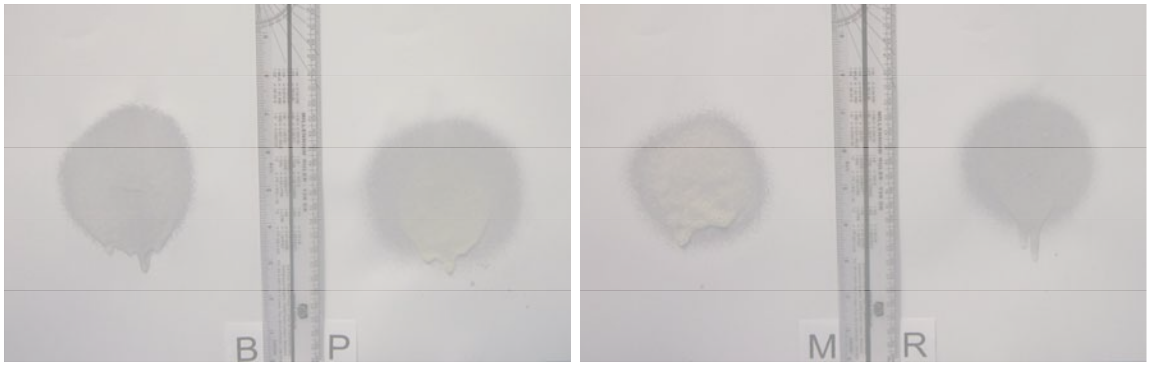

Fig 1. Spray pattern of Haruan spray that used (B) Butane, (P) Propane, (M) Butane-Propane mixture, (R) HFA 134a as propellants

Particle size and particle image of formulated Haruan spray

The particles size and particles image of Haruan spray using different propellant are shown in Figure 2 and Figure3, respectively. Particles size and particles image of Haruan spray using HFA 134a, as propellant was significantly different from other formula. This could be due to the interaction between HFA 134a and the aerosol concentrate.

Discussion

Aerosol containers are usually made of glass, plastic or metal. Suitable metals include tin-plated steel, aluminium and stainless steel. Glass is preferred, but its use is limited owing to its brittleness, danger of breakage and could explode, should the container containing high-pressure content accidentally be dropped. Glass is not safe when the total pressure is more than 25 psig (1.7 Bar) and amount of HC propellant is more than 50 % of the whole content of aerosol [32, 33]. Glass is not recommended for suspension aerosol due to the visibility of the suspended particles, which may present an aesthetic problem for the patients and consumers. In this study, glass containers were used for the ability to visibly inspect any change taking place when concentrate of aerosol is mixed with the propellant. The more suitable containers for the formulated Haruan spray are aluminium canisters because they can withstand high propellant's pressure, and thus, were used in this study.

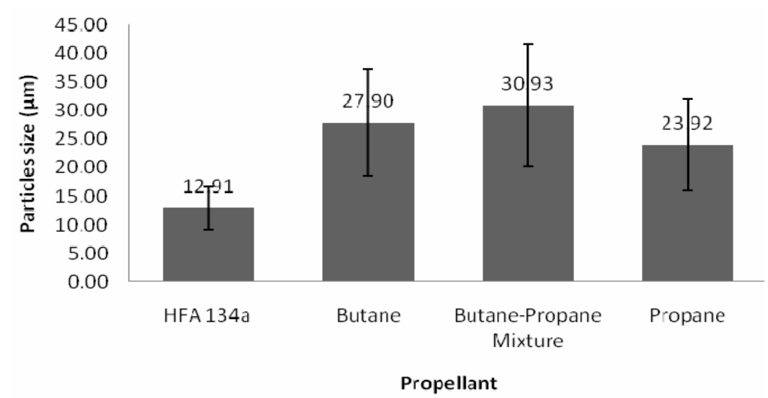

Fig 2. Particles size of Haruan spray by using different propellant

The four major groups of propellant are chlorofluorocarbon (CFC, i.e. Freon 11 and Freon 12), hydro fluorocarbon (HFC, i.e. HFA 134a), hydrocarbon (HC, i.e. propane and butane), compressed gas (i.e. carbon dioxide and nitrous oxide). Since CFCs were banned for use as propellants in 1978, hydro-fluorocarbon, hydrocarbons and compressed gases were used

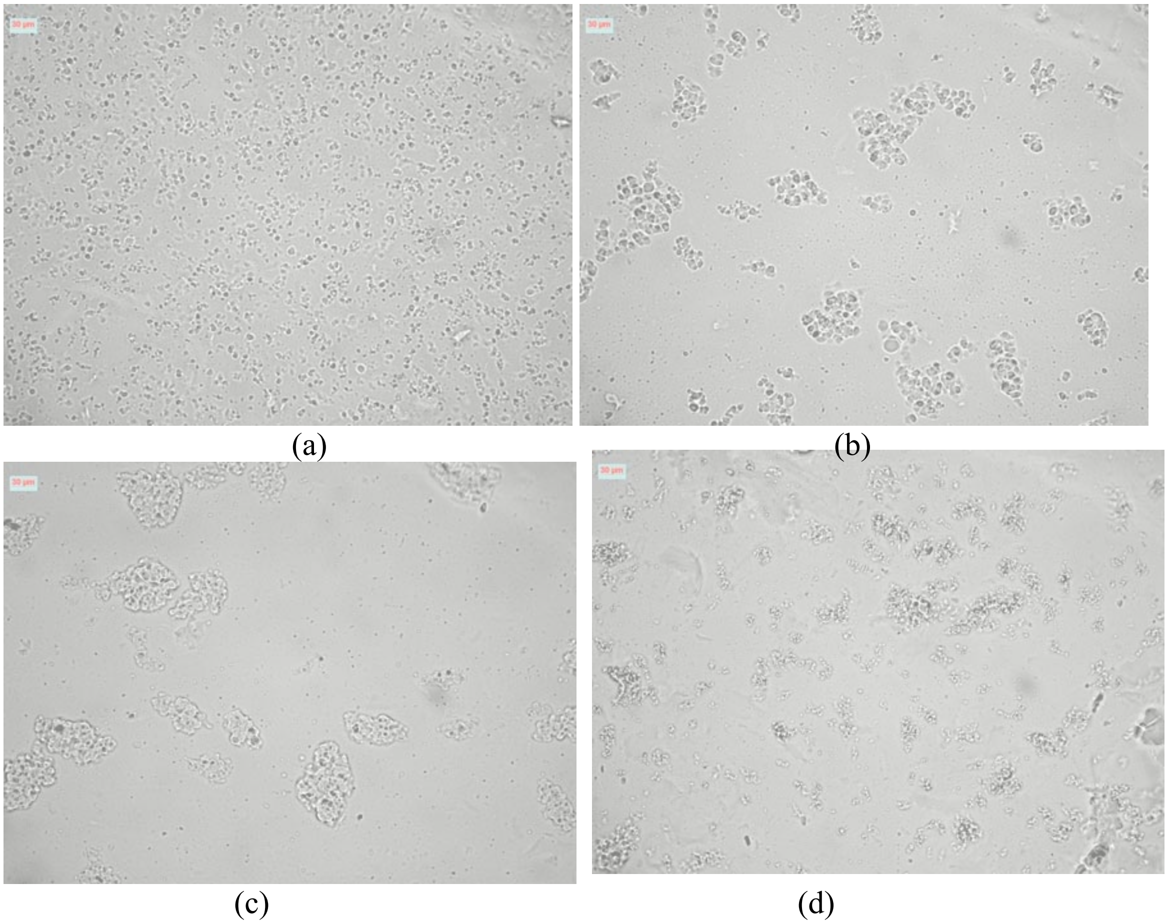

Figure 3. Particle image of Haruan spray using different propellant, (a) HFA 134a, (b) Butane, (c) Butane-Propane Mixture, (d) Propane

instead [15]. The compressed gases are odourless, colourless, and low in toxicity, non-flammable, inexpensive and environmentally acceptable. The disadvantages of a compressed gas are the reduction in the aerosol pressure of in every spray, which lead to the changes in spray characteristics during and after every discharge. Therefore, in this study, compressed gases were omitted and only HFC and HC were used. The selected HFC was HFA 134a while HC were represented by butane (low pressure) and propane (high pressure).

Delivery rate, delivery amount, leakage and minimum fill of the aerosol by using different propellant met the requirements of USP [23], as shown in Tables 1 and 2. Delivery amount should be close to 100 %. The results indicated that the type and the vapour pressure of propellant used in the formulations were able to deliver the entire aerosol concentrate used. This is expected when liquefied propellants are used. The pressure of the aerosols depends on the type of the propellant or mixture used. Propane has the highest vapour pressure while butane has the lowest. Mixture of propane and butane enable a propellant's vapour pressure in between propane's vapour pressure and butane's vapour pressure, according to their mol fraction. The vapour pressure of these liquefied propellant remain constant as long as there is still a drop of the propellant liquefied remain in the aerosol container. The hydrocarbons are stable noncorrosive and essentially odourless liquefied gases. Primary advantages of hydrocarbons as compared to fluorocarbons are their lower cost and they do not deplete the ozone. However, the main problem is their flammability [34].

Flammability test indicated the effect of an aerosol formulation on the extension of an open flame. The product was sprayed for about four seconds into a flame. Depending on the nature of the formulation, the flame was extended, the exact length being measured with a ruler. Butane and propane are flammable gases while HFA 134a is non-flammable. They should not have any effect on the flammability of the spray because they could not come out of the container until all aerosol concentrate has sprayed out. In this study all the formula including the one using the non-flammable HFA 134a were found to be flammable. This could be due to the presence of ethanol in the aerosol concentrate (Table 3).

Spray pattern are useful in determining the performance of a specific formulation and valve combination. In addition, the test can also serve as a check on the batch to batch uniformity of aerosols sprays [13, 25]. The spray pattern should remain the same when the same formulation and valve were used (Figure 1).

Particles size of Haruan spray was determined by using microscopic method. The use of this method is possible when particles size to be measured are in the range of 0.2 µm to 100 µm. Particles size of topical aerosol are usually less than 100 µm. Particles size is influenced by many factors. Among them are vapour pressure, the type and amount of solvents present in the formula, the type and amount of the propellant used, and the design of the valve system [35]. The image of particle of the spray sample could be photographed as a record because interaction of more than one component may produce agglomerates and can often be detected [27]. The results of the study showed that different type of hydrocarbon propellant used produced particle size and image of non significant difference. HFA 134a propellant of HFC group produced particles size and image that were significantly different from those produced by butane, propane or butane-propane mixture (Figure 2 and 3).

Conclusions

Haruan spray formulations, which met the requirements of USP, could be obtained by using butane, propane and butane-propane mixture as propellant. When considering of the safety and economics aspect, butane was chosen as the most appropriate propellant for Haruan spray. The results showed that when butane and aluminium were used as the propellant and the container respectively, they produced Haruan spray having the required characteristics. Propane and butane-propane mixture were not selected due to their higher pressures, which might create problem to the container upon storage at high temperatures. HFA 134a was not chosen due to the formation of aggregates with the concentrates.

Authors' contributions

Febriyenti carried out the formulation, evaluation studies, analysis and interpretation of data. Azmin Mohd. Noor participated in the design of the study and helped to draft the manuscript. Saringat bin Bai @ Baie participated in interpretation of data and have given final approval of the version to be published. All authors read and approved the final manuscript.

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