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Full Paper

Preparation, Characterization, and *in vitro* Anti-arthritic Activity of Microcapsules Loaded with *Bridelia retusa* Aqueous Bark Extract for the Treatment of Rheumatoid Arthritis

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Abstract

In this study, aqueous bark extract (ABE) of *Bridelia retusa* was encapsulated into the calcium alginate microcapsules. Prepared microcapsules were characterized by scanning electron microscopic analysis and Fourier Transform-Infrared (FT-IR) spectroscopy. The encapsulation efficiency, the release profile and the *in vitro* anti-arthritic activity of the microcapsules were evaluated. Prepared microcapsules were in spherical appearance and the average size was found to be less than 1 μm. The FT-IR analysis revealed the presence of ABE within the microcapsules while confirming the cross-linking of the polymers. The maximum encapsulation efficiency of microcapsules was 8.34 ± 0.01% and the cumulative release profile of microcapsules showed a prolonged releasing behavior. The anti-arthritic activity of ABE-loaded microcapsules has shown a stronger anti-arthritic activity compared to the anti-arthritic activity of free ABE. The slow release of ABE from microcapsules could promote a prolonged and increased anti-arthritic activity for the treatment of Rheumatoid arthritis.

Keywords: aqueous bark extract, *Bridelia retusa*, microcapsules, rheumatoid arthritis, slow-release

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disorder with serious ramifications, often resulting in joint damage that can cause pain, swelling, and stiffness in the affected joints [1]. Currently, there is a high demand for the treatment and management of rheumatoid arthritis by using herbal plant extracts. In Sri Lankan traditional medicine, a number of herbal remedies are used for the treatment of rheumatoid arthritis. *Bridelia retusa* (Ketakela) is a plant widely used in the treatment of

rheumatoid arthritis in Sri Lankan traditional medicine. The presence of therapeutic activity, and anti-inflammatory phytochemicals in the aqueous bark extract (ABE) of *Bridelia retusa* is already proven through the previous studies [2]. Novel Drug Delivery Systems (NDDS) are now being used to improve the stability of the phytochemicals of plant extracts, to improve the bioavailability, and to increase patient compliance by accounting for a prolonged release in the treatment of many diseases [3].

Many herbal products demonstrate low therapeutic action because of their solubility problems and low bioavailability. Recent studies claimed these therapeutic issues can be addressed by using Novel Drug Delivery Systems [4]. A combination of novel drug delivery technology and herbal medicine has proven the safety and effectiveness of herbal therapies to the human [5]. In this regard, microencapsulation drug delivery has been proposed to be one of the best alternatives for the safe and successful delivery of non-steroidal anti-inflammatory drugs (NSAIDs) [6].

During this study, the possibility of using a single emulsion method for the formulation of alginate microcapsules containing aqueous bark extract (ABE) of *Bridelia retusa* was studied. Alginates are natural polysaccharides isolated from the cell walls of various species of brown algae. This biopolymer consists of a linear chain of (1–4)-linked residues of b-D-mannuronic acid (M) (M blocks) and a-L-guluronic acid (G) (G blocks) in different proportions and sequential arrangements. In the presence of calcium ions (divalent ions), the alginate macromolecules crosslink to form a three-dimensional network called hydrogel [7]. Through this approach, it is anticipated that the therapeutic agent will be encapsulated within the aqueous sodium alginate polymer, which is subsequently cross-linked by divalent cations through ionic interactions [8].

Previous studies have primarily focused on the phytochemical analysis of ABE from *Bridelia retusa* for therapeutic efficacy, examining compounds such as phytosterols, triterpenoids, and tannins [9], as well as investigating the *in vitro* anti-rheumatoid arthritic and anti-inflammatory properties of ABE from *Bridelia retusa* [2]. A study was done on the *in vitro* anti-rheumatoid arthritic and anti-inflammatory activities of aqueous bark extract (ABE) of *Bridelia retusa*. This was evaluated *in vitro* mode, using the inhibition of heat-induced denaturation of egg albumin protein, which is an index of anti-rheumatoid arthritic and anti-inflammatory activities. The results conclusively appear that ABE of *Bridelia retusa* possesses marked anti-rheumatoid arthritic and anti-inflammatory activities *in vitro*. However, the inhibition of heat-induced denaturation of egg albumin protein was concentration-dependent. The results also demonstrate that the bark of Sri Lankan-grown *Bridelia retusa* offers a high chance to create novel, safe, efficacious and cost effective anti-rheumatoid arthritic and anti-inflammatory agents. The results were obtained for the *in vitro* anti-rheumatoid arthritic and anti-inflammatory activities threefold higher than that of the reference drug, diclofenac sodium [2]. Another study discussed that phenolic compounds Gallic acid and Ellagic were identified as major contributors to the anti-inflammatory activities by analysis of the extract by High-Performance Liquid Chromatography (HPLC). Based on the results obtained from the study it can be concluded that *Bridelia retusa* possesses significant anti-inflammatory potential and is also effective in inflammation-associated pain (Kumar and Jain, 2014). The phytochemical study showed the presence of therapeutically effective phytoconstituents like phytosterols, triterpenoids and tannins [9].

Considering the above-mentioned facts, the main objectives of this study were to prepare, to characterize

and to evaluate *in vitro* anti-arthritic activity of ABE of *Bridelia retusa* loaded microcapsules for the treatment of RA which will be the first reported study for this purpose. The study involved the preparation of ABE-loaded microcapsules by a single emulsion method to improve the anti-inflammatory activity of the plant extract.

Materials and Methods

Materials

Matured, fresh stem bark of *Bridelia retusa* (Ketakela) was collected in the daytime from the Western province (Gampaha), Sri Lanka in August 2020. The collected plant material was air-dried well and pressed for the authentication process. The prepared specimen was identified by the National Herbarium, Royal Botanical Garden, Peradeniya, Sri Lanka.

Phosphate Buffered Solution (PBS), sodium alginate (Sigma Aldrich), calcium chloride (Sigma Aldrich), rosemary oil (Cliárá), Tween 80 (Sigma Aldrich), isopropyl alcohol (Sigma Aldrich), sodium hydroxide (NaOH), and conc. HCl were obtained from the laboratories of the Department of Pharmacy, Faculty of Allied Health Sciences, General Sir John Kotelawala Defence University.

Equipment used in the study were UV-visible spectrophotometer (Spectrum-SP-UV-5000DB), pH meter (Trans instrument, Model no BP 3001, S. No-Ti 18y0569), analytical balance (ACZET PVT LTD, CY 224C S. No 17409873), water bath (Equitron S. No: NR11HD-18611), optical light microscope (HumaScope PremiumLED), rotary vacuum evaporator (HAHNSHIN Scientific-model No: HS-2005, S. No V-00449), vortex mixer (HumaTwist S.No: VB192AH0011635), magnetic stirrer mixer (BIOLOGIX Model no: 01-310X), Fourier Transform Infrared analysis (FT-IR) instrument (PerkinElmer Spectrum 10.5.2 model) and scanning Electron Microscope (CARLZEISS, model No: EVO18 Research).

Methods

Preparation of Aqueous Bark Extract (ABE) of plant Bridelia retusa

Air-dried bark was cut into small pieces. An amount of 60.0 g of cut pieces was boiled in 1.92 L of distilled water approximately for 4 hours until the volume was reduced to 240.0 mL. Then the solution was filtered, and the filtrate was subjected to rotary evaporation to remove the solvent. The obtained dried aqueous bark extract (ABE) was labeled and stored in a refrigerator until use [2].

Preparation of Microcapsules

The microcapsulation process was carried out by adapting the work done by Dhamecha *et al*., 2019, with some modifications [8]. In this study, sodium alginate solution (2% w/v) was prepared by dissolving 0.5 g of sodium alginate powder in 25.0 mL of distilled water. Then 16.16 mg of ABE of *Bridelia retusa* was dissolved in the sodium alginate solution. A solution of calcium chloride (1.33% w/v) was prepared by dissolving 0.3325 g of calcium chloride in 25.0 mL of distilled water. Then 1 drop of Tween 80 was added

to the 15.0 mL of oil phase and mixed well by vortexing. While vortexing 15.0 mL of prepared sodium alginate solution was added dropwise using a 21-gauge syringe into oil containing Tween 80. Then the mixture was transferred into a beaker and stirred under 300 rpm for 5 minutes and 15.0 mL of prepared calcium chloride solution was added dropwise into the alginate system using a 21-gauge syringe while stirring. Finally, 3 drops of isopropyl alcohol were added and stirred for 15 minutes. Then it was centrifuged, washed, and air-dried.

Characterization of ABE of Bridelia retusa Loaded Microcapsules

Scanning Electron Microscope (SEM) Analysis

SEM analysis was performed on Aqueous Bark Extract (ABE) of *Bridelia retusa* loaded microcapsules, which were sputtered with gold and then analyzed by SEM (CARLZEISS, model No: EVO18 Research) at 10 kV accelerated voltage [10]. The accelerated voltage determines the energy of the electron beam used to scan the sample's surface. A higher voltage generally allows for greater penetration depth into the sample and improved imaging of its internal structure [11].

SEM images of the prepared microcapsules were then used to determine the size of the microcapsules formed with the use of ImageJ software which allows for precise measurements and analysis of features in digital images, making it suitable for tasks such as determining the size of particles, objects, or structures in SEM images [12].

Fourier Transform Infrared Analysis

Samples were subjected to Fourier Transform Infrared Spectroscopic analysis in the range of 550- 4000 cm^{-1}. Potassium bromide (KBr) Transmission mode was used where the test samples were mixed with KBr in order to get a pellet. FT-IR spectrum of alginate powder, blank sample, ABE loaded sample, rosemary oil and pure ABE of plant *Bridelia retusa* were recorded by using the PerkinElmer Spectrum 10.5.2 model. Alginate powder was the control sample used to establish a baseline for comparison with other samples [13]. By measuring the changes in infrared radiation as it passes through the sample, information about the chemical composition and structure of the sample was obtained [14].

Encapsulation Efficiency

The amount of ABE of plant *Bridelia retusa* encapsulated in alginate microcapsules was determined by UV/Visible spectrophotometer following the method described by Zhao *et al.,* (2020) with little modifications [15].

An amount of 100.0 mg of prepared microcapsules were crushed with glass mortar and pestle and placed into 100.0 mL water and stirred for 8 hours. The absorbance of the obtained solution was measured at 208 nm. Water, rosemary oil, sodium alginate solution and calcium chloride solution were used as the blank during the UV/Visible spectroscopic analysis. The encapsulated amount of the sample concentration

was calculated using the standard calibration curve generated by using different known concentrations of ABE of *Bridelia retusa.*

The EE% was calculated using the following expression,

$$\% \text{ of Encapsulation efficiency} = \frac{\text{Amount of ABE loaded into the microcapsules}}{\text{Initial amount of used ABE}} \text{ X } 100$$

In vitro Release Study

The releasing test was performed by using a UV/Visible spectrophotometer following the method described by li *et al*., (2013) with some modifications, and the method was replicated [16].

An amount of 1.0 g of ABE of *Bridelia retusa* loaded microcapsules was placed into a medium containing 100.0 mL of phosphate buffer (pH 5.0) and incubated at 37 °C and 50 rpm respectively. Aliquots were withdrawn at the desired time intervals and the medium was then replaced in order to maintain the constant amount of releasing medium. The absorbance was measured at 208 nm using a UV/Visible spectrophotometer. The releasing amount of ABE of *Bridelia retusa* was calculated using the standard calibration curve generated by using different known concentrations of ABE of *Bridelia retusa.*

In vitro Anti-arthritic Activity of ABE of Bridelia retusa Loaded Microcapsules

The anti-arthritic activity of ABE-loaded microcapsules was evaluated by a heat-induced protein denaturation method. [17]. The percentage of inhibition of protein denaturation was determined by using UV-visible spectroscopy [17]. The heat–induced protein denaturation method was replicated to evaluate the *in vitro* anti-arthritic activity.

Statistical Data Analysis

Statistical analysis was conducted by using SPSS software. Data were displayed as mean, SEM and statistical significance was set at $p<0.05$. In the *in vitro* anti-arthritic activity, the results of the protein denaturation test were statistically analyzed by using Graph Pad prism 8 software. The best fit non-linear dose-response curve was developed by using Graph Pad Prism 8 software.

Results and Discussion

Preparation of Microcapsules

Following Figure 1 shows the light microscopic image of ABE loaded microcapsules at a magnification of X100 which indicated that the prepared microcapsules were spherical in shape. Moreover, the Figure 2, depicts the physical appearance of the as formed microcapsules after the centrifugation (without drying) step.

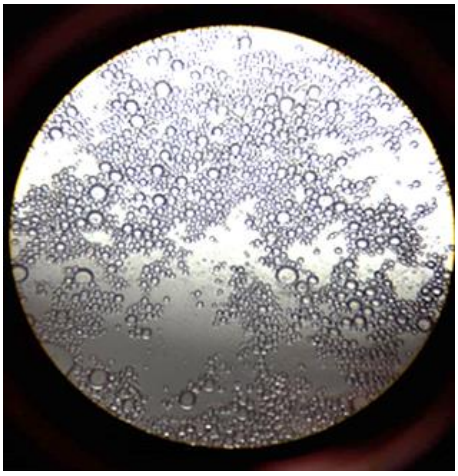


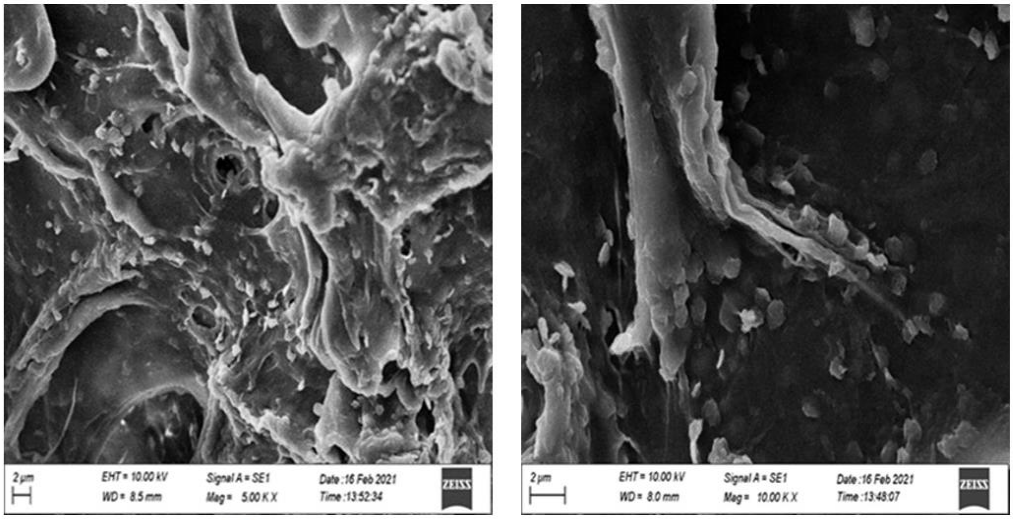
Figure 1. Light microscopic image of ABE of *Bridelia retusa* loaded microcapsules using single emulsion method (Mag = X100)

Figure 2. Physical appearance of ABE of *Bridelia retusa* loaded microcapsules using single emulsion method.

In the single emulsion method, water in oil (W/O) system initiates the formation of microcapsules. The oil phase produces micro-sized droplets (Dhamecha *et al*., 2019) which will act as the primary site of microcapsule formation initiation. In this study, Tween 80 was used as the emulsifying agent for the preparation of W/O emulsion to fulfill the intended purpose where it can be assumed that the oil droplet would work as the template for the preparation of microcapsule [18].

Characterization of the Prepared Microcapsules

The surface morphology of the ABE of *Bridelia retusa* loaded microcapsules as well as the size of the microcapsules were determined by SEM. Scanning electron microscopic images of microcapsules loaded with ABE of *Bridelia retusa* are shown in Figure 3(a) and Figure 3(b).



(a) (b)

Figure 3. Scanning electron microscope images of ABE of *Bridelia retusa* loaded microcapsules with (a) magnification 5.00X and (b) magnification 10.00X

All the samples showed nearly spherical structures without any crystals on the surface. The surface of the microcapsules is rough and scraggly. Also, the prepared microcapsules have deposited on some groves-like structures that could be the excess polymer. The average size of microcapsules lies within 0.8 ± 0.1 - 1 ± 0.1 µm size range.

Fourier Transform Infrared spectra of sodium alginate powder, ABE of *Bridelia retusa* (plant extract), blank sample (without loading ABE), and ABE-loaded microcapsule sample are shown in Figure 4.

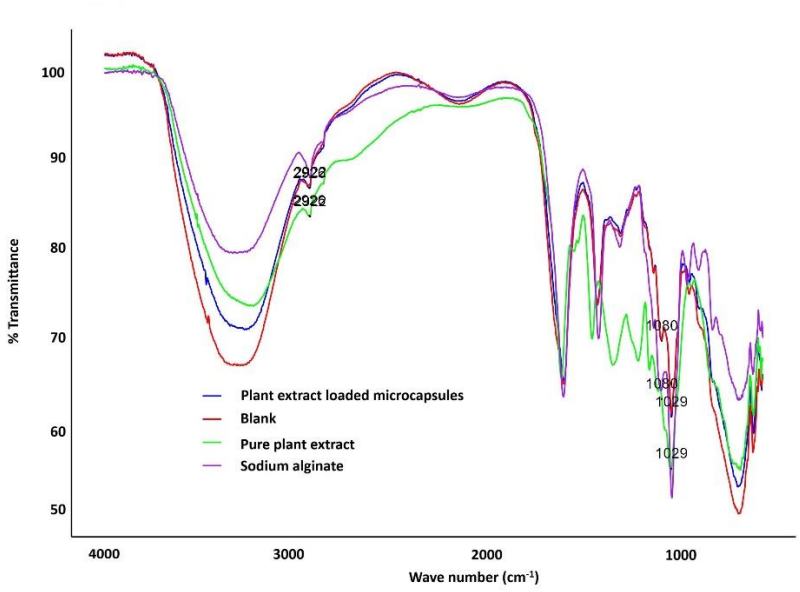


Figure 4. Fourier Transform Infrared spectra of sodium alginate powder, ABE of *Bridelia retusa* (plant extract), blank sample (without loading ABE) and ABE loaded microcapsule sample.

FT-IR results confirmed the presence of ABE of *Bridelia retusa* within the microcapsules and the cross-linking of the polymers leading to the formation of microcapsules. The peak at 1080.06 cm^{-1} relating to the C-O stretching can be also attributed to the presence of cross-linking of alginate COO^- groups with the Ca^{2+} ions [14]. The peak of C-C stretching at 1029.12 cm^{-1} shows higher intensity, suggesting either a stronger O-H binding stretching or stronger binding to the guluronic acid from sodium alginate [14]. Also, the small shift due to the changes in the ionic density of carboxyl groups indicates the replacement of Na^+ ions with Ca^{2+} ions [19]. Comparing the FT-IR spectrum of plant extract-loaded microcapsules and the FT-IR spectrum of the plant extract, well-distinguished peaks at the 3000-3500 cm^{-1} range can be identified owing to –O-H stretching [19]. Also, the plant extract spectra showed a peak at 2922.13 cm^{-1} and the plant extract loaded microcapsules spectra showed a peak of 2925.64 cm^{-1} corresponding to the C-H alkyl bending of a carbon-hydrogen bond [13]. Since the peaks corresponding to the ABE extract were overlapping with the peaks corresponding to the neat polymers, it was difficult to distinguish them separately as observed in similar studies in the past [20]. Also, significant peaks corresponding to the rosemary oil were absent in the FT-IR spectrum of the microcapsules that confirms the oil droplet works as the template for the microcapsule, and it would loss in the centrifugation and washing processes.

Encapsulation Efficiency of ABE Loaded Microcapsules

Percentage of Encapsulation Efficiency (EE %) within the ABE loaded microcapsules was obtained as 8.34 ± 0.01%.

According to the results of the encapsulation efficiency study, a relatively low concentration of ABE of *Bridelia retusa* has been encapsulated within the microcapsules formed. In here, a theoretical loading of ABE of *Bridelia retusa* 80% was expected, but the actual observation was 8.34 ± 0.01%. This low encapsulation efficiency might have been observed due to the loss of ABE of *Bridelia retusa* as a large fraction from the inner part of the microcapsules during the centrifugation and washing steps. However, this low encapsulation can be adjusted by varying the ratio of core material to shell polymer.

Releasing Profile of ABE Loaded Microcapsules

Figure 5 shows the cumulative release profile of the ABE of plant *Bridelia retusa* containing microcapsules. In here the preparation of microcapsules loaded with ABE of *Bridelia retusa* was targeted for the development of a topical formulation. Because of that, the releasing medium was selected with a pH 5 phosphate buffer solution to represent the pH of the skin. The release profile of ABE indicates that, at the very initial stage (first two hours) ABE of *Bridelia retusa* shows a burst release from the microcapsules and it becomes slow and stable after three hours. This burst release could be observed within the first release stage due to the release of surface-bound extract molecules while the next stage of release would remain relatively stable due to the release of extract molecules encapsulated inside the microcapsules [21].

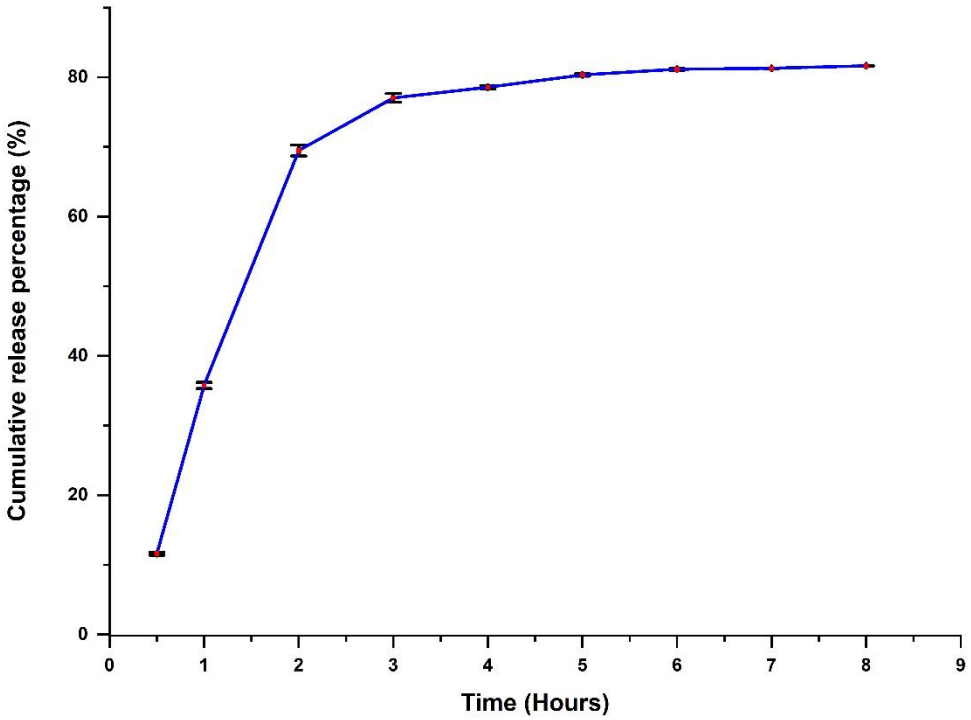


Figure 5. Cumulative release profile of ABE of *Bridelia retusa* from loaded microcapsules

Anti-arthritic Effect of the ABE Loaded Microcapsules

Figure 6 depicts the dose-response curves of free *Bridelia retusa* (a) and encapsulated *Bridelia retusa (b)*. From the results illustrated, it can be identified that the IC_{50} value obtained for encapsulated ABE of *Bridelia retusa* was significantly lower than that of the free ABE of *Bridelia retusa* (a). It further demonstrated that the ABE of *Bridelia retusa* containing microcapsules (b) significantly improved the anti-arthritic activity when compared with the free ABE of *Bridelia retusa*. The IC_{50} is the concentration of an active ingredient for 50% inhibition. Low IC_{50} values guarantee that the active compound is potent at low concentrations [22]. Therefore, the encapsulated ABE of *Bridelia retusa* (b) showed increased anti-arthritic activity at a lower concentration of ABE of *Bridelia retusa* than the unencapsulated ABE of *Bridelia retusa*(a). This might be due to the encapsulation process increasing the stability, bioavailability, and pharmacotherapeutic efficacy of the ABE of *Bridelia retusa* [23].

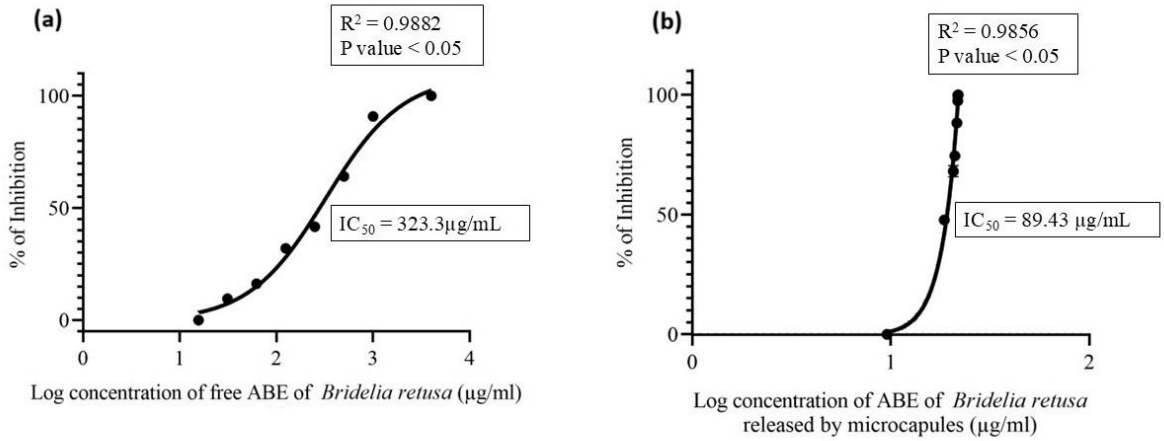


Figure 6. Dose response curves of *(a)* free ABE of *Bridelia retusa* and *(b)* encapsulated ABE of *Bridelia retusa.*

Also, this study will assess the effectiveness of the topical administration of medicinal substances for rheumatoid arthritis. Rheumatoid arthritis occurs with joint inflammation because topical application could be considered as an effective route of administration as well as creating a fast onset of action [24]. Also, micro-sized capsules as NDDSs have better permeability due to their size [25]. Other than oral painkillers, this kind of transdermal onset application could bypass the first pass metabolism. This would allow a prolonged duration of action. Additionally, the controlled-release formulation will increase patient compliance with the reduction of dosing frequency.

Conclusion

In conclusion, encapsulation of ABE of *Bridelia retusa* in calcium alginate microcapsules by a single emulsion method is an effective method to prepare a formulation with a controlled release ability of ABE of *Bridelia retusa* which could also promote a prolonged anti-arthritic activity for the treatment of rheumatoid arthritis. Furthermore, the encapsulated ABE of *Bridelia retusa* could lead to stronger anti-arthritic activity compared to the unencapsulated extract.

Conflicts of Interest

All authors declare no conflict of interest.

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