Formulation And Evaluation Microcapsules Of Caesalpinia Sappan Linn. Using Emulsion Solvent Evaporation Method

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ABSTRACT: Caesalpinia extract has widely investigated as a potent source of antioxidants. Its antioxidant activity is majorly due to its high level of phenolic compounds. Microencapsulation is one of the stabilization methods existing to overcome the instability of polyphenol in order to maintain its stability prior to administration. The objective of this research is to encapsulate the caesalpinia extract using emulsion solvent removal method using ethyl cellulose as encapsulating agent and evaluate the physical characteristic and dissolution rate of encapsulated material. The heart wood of caesalpinia plant was extracted using three different solvent: 70% ethanol, 96% ethanol and destilled water. The three extracts obtained from maceration then measured its antioxidant activity and total phenolic compounds (TPC). The 70% ethanol extract shows the greatest TPC (48.36 %w/w) and IC_{50} in DPPH (3.572 ppm), thus, this extract was selected to be encapsulated. Microcapsules were prepared with three different drug-polymer ratio used in the formulas, 1 : 3, 1 : 4 and 1 : 5. These formulas were evaluated for drug encapsulation efficiency, microcapsule wall thickness and morphology, and also *in vitro* drug dissolution rate. According to the results obtained in this study, the microcapsule with the greatest physical characteristic and dissolution rate that is suitable for sustained release administration is formula III. From this study, it is suggested that caesalpinia extract is able to be formulated into microcapsules using emulsion solvent evaporation method and futher developed into sustained release oral preparation as a source of antioxidants.

Keywords: *C*aesalpinia sappan, Microcapsule, Solvent evaporation, Antioxidant

1 INTRODUCTION

Caesalpiniasappan Linn.belongs to family of Caesalpiniaceae and its cultivated in South-East Asia for the production of red dye, which is obtained from its heart wood [1]. The heartwood of the plant is widely used in traditional medicine. Chemical investigation resulted in the isolation of novel and interesting phytochemical possessing potent biological properties [2]. Many biological activity of C. sappan have been reported anticomplementary activity [3],antimicrobial [4], antioxidant [5], [6], [7] [8], anticarcinogenic[9], anticonvulsant compounds [10],antioxidant and hepatoprotective[11], antibacterial [12], hepatoprotective properties [13]. Flavonoids (Namikoshi and Saitoh, 1987; Namikoshi et al., 1987; Namikoshi, Nakata and Saitoh, 1987) and phenolic (Fuke et al., 1985; Saitoh et al., 1986) such as 4-O-methylsappanol, protosappanin A (Nagai et al., 1986), protosappanin B (Nagai and Nagumo, 1986), protosappanin E, brazilin (Kim et al., 1997), brazilein, caesalpin J (Miyahara et al., 1986), triterpenoid and steroid (such as campesterol, stigmasterol, β-sitosterol) were isolated from the wood. The hepatoprotection (Moon et al., 1992), immunomodulation (Choi et al., 1997), hypoglycemia (Kim et al., 1995; Moon et al., 1988), anticomplementary (Oh et al., 1998), anticonvulsant (Baek et al., 2000), anti-inflammatory, antibacterial (Nirajan Reddy et al., 2003), antioxidation (Badami et al., 2003; Yingming et al., 2004), and other biological activities of sappan have been reported [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26]. Nowadays, various microencapsulation techniques are available [27],[28], [29], [30], and the microencapsulated products are widely used in the food, pharmaceutical and cosmetic industries, but also in various other domains like personal care, agricultural products, veterinary medicine, industrial chemicals, biotechnology, biomedical and sensor industries. The particles obtained are called microcapsules or microspheres according to the internal structure, core-shell-like or matrix, respectively. The microcapsules may have size ranging from fractions of a micron to several millimeters, having different forms, all depending on the materials and methods used in their preparation. The outer 1777 material, which gives the microcapsule its form, is called encapsulating agent, while the inner ingredient constitutesthe active material [31]. The emulsion/evaporation techniques are traditionally recognized as unsuitable for water soluble drugs and all water soluble substances. Several methods and techniques are potentially useful to prepare polymeric microparticles in the broad field of microencapsulation. The preparation method determines the type and the size of microparticle and influences the interaction ability among the components used in microparticle formulations. Different encapsulation methods result, in most cases, in either a microcapsule or a microsphere. For example, interfacial polymerization and coacervation methods almost always produce a microcapsule, whereas solvent evaporation may result in a microsphere or a microcapsule, depending on the formulation and processing factors. Microencapsulation technique by emulsion solvent removal method has been applied extensively in pharmaceutical industries for various purposes such as controlled drug delivery, masking the taste and odor of drugs, protecting drugs from degradation, and protecting body from the toxic effects of the drugs [32], [33], [34], [35]. Thus, the objective of this study is to encapsulate the caesalpinia extract using emulsion solvent removal method with ethyl cellulose as encapsulating agents, and evaluate the physical charateristic and dissolution rate of the encapsulated material.

2 MATTERIALS END METHODS

Materials used in this study were 70% ethanol, 96% ethanol, acetone,destilled water, ethyl cellulose,Folin-Ciocalteau reagent, heart wood from caesalpinia plant,n-Hexane, liquid paraffin, sodium bicarbonate and tween 80®.

2.1 Preparation of Caesalpinia Extract

The heart wood from caesalpinia plant was shaved and dried. The dried heart wood was extracted using three different solvent: 70% ethanol, 96% ethanol and destilled water. 70% ethanol and 96% ethanol were used for maceration method, while the destilled water was used in infusion method. After macerated, the two ethanolic extracts were evaporated using rotary evaporator. Meanwhile, the water extract was lyophilized to obtain dry extract.

2.2 Determination of Antioxidant Acitivity

The antioxidant activity of the caesalpinia extract was determined according to Dudonne et al. (2009). The DPPH(2,2-diphenyl-2-picrylhydrazyl) solution in methanol ($6x10^{-5}$M) was mixed with 100 µL sample and after 30 min the decrease in absorbance at 515 nm was measured (AE). A blank sample contained 100 µL of methanol (AB). Percentage of cation inhibition was calculated using equation:

$$\% \text{ inhibition} = [(AB - AE)/A \text{x } 100\%$$

2.3 Determination of Total Phenolic Compound (TPC)

TPC was determined using the Folin-Ciocalteau reagent, according to a modified method of Lachman et al (1998). In brief, 70 µL of the sample was pipetted into a 15 mL volumetric flask containing 350 µL of Folin–Ciocalteu reagent, 4,2 mL of distilled water and 1,05 mL of 20% (w/v) Na-carbonate, and the volume was made up with distilled water. After 2h, the absorbance of was measured at 698 nm against a blank sample. Gallic acid was used as the standard and the results expressed as mg L^{-1} of gallic acid equivalents (GAE).

2.3 Preparation of Microcapsules

Microcapsules were prepared by the solvent evaporation method. Accurately weighed quantity of ethyl celluloseas a polymerwas dissolved in acetone, caesalpinia extract was dispersed slowly in polymer solution and this solution was added to heavy liquid paraffin and tween 80 with stirring (800 rpm). Microparticles were recovered by treating with hexan. Then filtered, dried in a desicator. Microcapsules of various drug polymer ratios prepared accordingly for further evaluations.
Three formulas were made with the following composition:

Table 1. *Compositions of caesalpinia microcapsule formulas*

Material	Formula		
	I	II	III
Caesalpinia extract	1 g	1 g	1 g
Ethyl cellulose	3 g	4 g	5 g
Acetone	60 ml	60 ml	60 ml
Liquid Paraffin	120 ml	120 ml	120 ml
Tween 80® (2% from 120 ml)	2,4 ml	2,4 ml	2,4 ml
n-Heksan	60 ml	60 ml	60 ml

2.4 Drug Encapsulation Efficiency (DEE)

Accurately weighed microcapsuleequivalent to 50 mg, were suspended in10ml of ethanol to dissolve thepolymer coat and analyzed by usingUV-Visible spectrophotometer (UV-1700, Shimadzu, Japan) after suitabledilution t 698 nm. Drug encapsulationefficiency was calculated using thefollowing equation:

DEE (%) = (Practical drugcontent/Theoretical drug content) × 100%,

Each sample was analyzed in triplicate (n=3).

2.5 Wall Thickness of Microcapsules

Wall thicknesses of the microcapsules were determined by the method as suggested by Luu et al. 9, using equation,

$$h = r (1\text{-}P) d1 / 3[Pd2 + (1\text{-}P) d1]$$

Where,h is wall thickness; r is mean radius of microcapsules from optical microscopic observations; d1 is density of the core material; d2 is density of coat material; P is proportion of medicament in the microcapsules. All the test sample was examined for three times (n=3).

2.6 Scanning electron microscopy (SEM)

A scanning electron microscope (JEOL, JSM- 6360) was used to characterize the surface topography of the microcapsules after gold coating.

2.7 In Vitro Drug Release

The *in vitro* dissolution studies were carried out in 500 ml of phosphate buffer, pH 7.4, maintained at 37 ± 0.5° and 100 rpm by using United States Pharmacopoeia basket type dissolution test apparatus under sink conditions. Accurately weighed samples of the microcapsule were added to the dissolution medium and at preset time intervals, 2 ml aliquots were withdrawn and replaced with anequal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed spectophotometrically at 698 nm. The concentration of extract in test samples was corrected and calculated using a regression equation of the calibration curve. The dissolution studies were carried out intriplicate and the mean values were plotted as percentage cumulative release versus time.

3 RESULTS AND DISCUSSION

Caesalpinia plant extract has been widely investigated as a potent source of antioxidants. Its antioxidant activity is majorly due to its high content of phenolic compounds [36]. However, the instability of polyphenols limits its usage. Microencapsulation is one of the existing stabilization methods for polyphenols and therefore becoming an interesting delivery system for maintaining the integrity of polyphenols until the administration [37]. Caesalpinia extract obtained from three various solvents: ethanol 70%, ethanol 90% and destilled water, were determined their total phenolic compounds (TPC) and antioxidant activities. The results of TPC and IC_{50} in DPPH free radical scavenging activity are shown in Table 2.

Table 2.*Total phenolic compounds and IC_{50}in DPPH scavenging activity of three different caesalpinia extracts.*

Caesalpinia Extract	TPC (%)	IC_{50} (ppm)
Ethanol 70%	48.4	3.5
Ethanol 96%	27.5	14.6
Destilled water	18.7	35.4

Correlation between total phenolic content and antioxidant activity has been studied in various plants and fruits. Some of the studies represented that the higher the total phenolic contents, the higher the antioxidant activity.This result shows that caesalpinia extract exhibit a great antioxidant activity for having IC_{50} value of less than 50 ppm [38]. Based on the determination, the ethanol 70% extract of caesalpinia plant exhibits the greatest total phenolic compounds and antioxidant activity. Therefore, it was selected to be formulated into

microcapsules in this research. Caesalpinia extract obtained in this research were prepared into three various microcapsule formulas, and then the microcapsules were evaluated its physical characteristics and dissolution rate. The results of particle size, drug encapsulation efficiency (DEE) and wall thickness evaluation are shown in Table 3.

Table 3. *Results of physical characteristic evaluation of caesalpinia extract microcapsules.*

Microcapsule Formula	Particle Size (µm)	DEE (%)	Wall Thickness (µm)
I	330.06	28.78	25.12 ± 0.21
II	314.55	42.59	34.15 ± 0.13
III	409.73	61.54	48.2 ± 0.09

The particle size of microcapsules is ranging from 2–2000 µm [39], thus all the microcapsules obtained are included in this particle size range.Various composition used in the microcapsule formulation has a great contribution to the release properties, encapsulation efficiency and wall thickness of microcapsules.The determination of drug encapsulation efficiencyshows that microcapsule III has the greatest result. The result shows that the higher polymer quantity used, the higher encapsulation efficiency.Various measurement of wall thickness obtained in different microcapsule formulas. This results show that there is a correlation between the quantity of polymer used in the formulation and the wall thickness of microcapsules. The morphology of microcapsules obtained was observed using scanning electron microscopy (Fig 1).Microcapsule I shows an aggregated microcapsule structure, while the microcapsule I and II show mononuclear structure. The higher quantity of polymer is more likely to form a mononuclear spherical capsule structure.

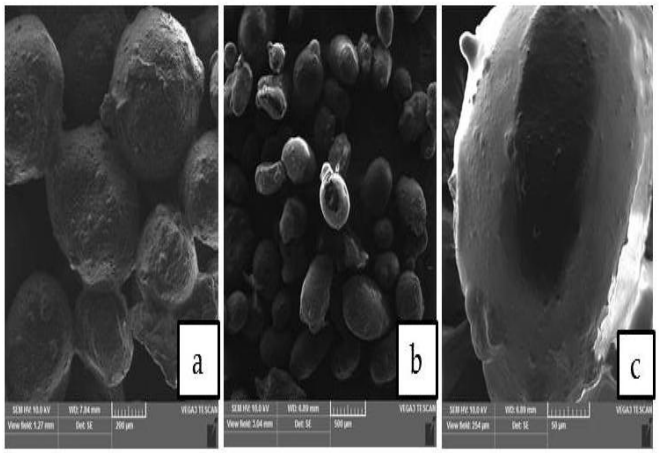

Fig. 1. *Morphology of caesalpiniaextract microcapsules.*

The evaluation of *in vitro* drug release was performed to determine the rate of phenolic substance dissoluted from the microcapsule into the media solution.Because this microcapsule is intended for sustained release oral administration, the lowest dissolution rate will be considered as the greatest formula as it releases the drug content in longer duration.Fig. 2 shows the *in vitro* drug release profile of caesalpinia microcapsules.

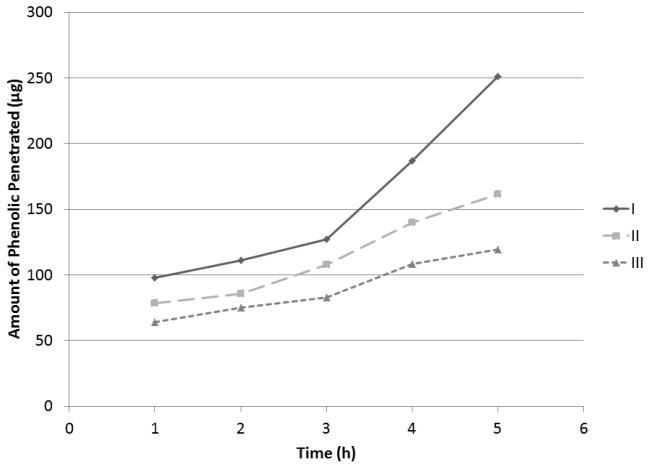

Fig. 2. *Release profile of phenolic contents from caesalpinia extract microcapsules.*

The result shows that the microcapsule IIIexhibits the greatest release profile. There is a linear correlation between microcapsule wall thickness and its dissolution rate. The microcapsule III with the thickest wall releases its content slower than other formulas. This formula is considered to have the longest duration of action in this study.

4. CONCLUSION

Caesalpinia extract has a great antioxidant activity because of its high content of phenolic compounds. This study suggests that it can be formulated into microcapsules to maintain its stability. From three various formulas using emulsion solvent evaporation method, formula III with drug-polymer ratio of 1 : 5, has the greatiest evaluation outcomes, thus, it can be further developed as oral sustained release preparation.

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