THE EFFICIENCY OF NIOSOME IN ENTRAPMENT OF Andrographis Paniculata EXTRACT

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To my beloved mother and father

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ABSTRACT

A. Paniculata or also called bile of heart is herb always used in traditional Malaysian medicine for treatment of a wide range of diseases such as diabetes, diarrhea and snake bites. The main bioactive compound which is andrographolide act as an antioxidant, protecting the liver and digestive system, antimicrobial and others. This research is to study the efficiency of niosome in entrapment of bioactive compound (andrographolide). The aerial part of plant were taken and extracted in the solvent. The solvent used are ethanol and distilled water. Six of niosomal formulation for the delivery of andrographolide will be developing to achieve effectiveness in entrapment of andropholide. The primary metabolites of A. Paniculata were found and the result shows the water extract have the higher total protein (901.875 mg/mL \pm 0.0021) and ethanol extract have the higher glycosaponin in extract (40% \pm 0.0021). Zeta potential were in the range of -30mV to -40mV while particle size in the range of 90nm to 200nm. Zeta potential and particle size of niosome indicated all the formulation were stable. Drug entrapment efficiency in the niosomes will be determined spectrophotometrically. Formulation with ethanol solvent extract and surfactant: cholesterol ratio in 0.50: 0.50; F5 indicated the best formulation and give the optimum entrapment of A. Paniculata extract (7.431ppm of andrographolide). The result delivered that this niosomal formulation is determined greatly helpful to improve the encapsulation efficiency of drugs and reduce the release rate of the drug. This study provides a new insight into designing effective niosomal system.

ABSTRAK

A. Paniculata atau juga dipanggil "Hempedu Bumi" adalah tumbuhan herba yang sering digunakan dalam pengubatan tradisional di Malaysia untuk merawat pelbagai penyakit seperti diabetes, cirit-birit dan gigitan ular. Komponen bioaktif yang utama di dalam tumbuhan A. Paniculata adalah andrographolide yang bertindak sebagai antioksida, menjaga hati dan sistem penghadaman, antimikrob dan sebagainya. Kajian yang dijalankan ini adalah untuk mengenalpasti tahap kecekapan niosome dalam memerangkap komponen bioaktif (andrographolide). Seluruh bahagian tumbuhan dikeringkan dan diekstrak di dalam larutan ethanol dan air suling. Sebanyak enam formulasi niosome dikenalpasti untuk mengangkut andrographolide dan mendapatkan keberkesanan dan kecekapan niosome dalam memerangkap andrographolide. Metabolit pertama bagi A. Paniculata telah dikenalpasti dan hasil dari kajian menunjukkan bahawa ekstrak air mempunyai jumlah protein yang tinggi (901.875 mg/mL \pm 0.0021) dan ekstrak etanol pula mempunyai glycosaponin yang paling tinggi (40% \pm 0.0021). Zeta potential menunjukkan keputusan di antara skala -30mV hingga -40mV manakala saiz zarah niosome di antara 90nm hingga 200nm. Zeta potential dan saiz zarah niosome menunjukkan bahawa semua formula adalah stabil. Kadar kecekapan pemerangkapan niosome akan dikenalpasti secara spektrofotometrik. Formulasi dengan menggunakan ekstrak etanol dan nisbah surfakten: kolesterol, 0.50: 0.50; F5 menunjukkan formulasi yang terbaik dan ianya memberikan pemerangkap ekstrak A. Paniculata yang optimum (7.431ppm andrographolide). Dapatan dari keputusan bahawa formulasi niosome ini dapat membantu dalam meningkatkan tahap kecekapan dalam memerangkap andrographolide dan mengurangkan kadar pelepasan bioaktif komponen tersebut. Kajian ini diharap dapat menyediakan idea baru dalam merekacipta tahap sistem kecekapan ini.

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LIST OF ABBREVIATIONS

cm	-	centimeters
С	-	Carbon
Ν	-	Nitrogen
Н	-	Hidrogen
OH	-	Oxide
V	-	Voltan
L	-	Litre
Min	-	minutes
m	-	mili
g	-	gram
Nm	-	nanometer
UV	-	Ultraviolet
HIV	-	Human Immunodeficiency Virus
NaCO ₃	-	Sodium bicarbonate
РКС	-	Protein Kinase C
HPLC	-	High Performance Liquid Chromatography

LIST OF SYMBOLS

 $\beta \quad - \quad Beta \\ \gamma \quad - \quad Gamma \\ \mu \quad - \quad Mikro \\ ^{\circ}C \quad - \quad Degree celcius$

CHAPTER 1

INTRODUCTION

1.0 Background of the study

In traditional drug delivery system such as oral ingestion or intravascular injection, the drug is distributed throughout the body through the blood circulation. For therapeutic agents, only a small portion of medication contact with the organs are affected. Targeted drug delivery seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues.

The basic principle of drug delivery has been developed over previous decades. The aim of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue. Besides, drug delivery also provide many ways to increase absorption into the brain, lengthen a drug's action, carry it to a targeted part of the body or get other desired effects.

Drug delivery system has several types of drug carrier. Some of the most commonly used for drug carrier system such as liposomes, microsphere, soluble polymers, conjugated proteins, niosomes and nanoparticles. Each drug carriers have different structure and specific uses. Moreover, the best drug carrier must biodegradable, non-toxic, non-immunogenic, biocompatible and avoid recognition by the host's defense mechanisms.

1.1 Statement of the problem

The drug carrier bioactive compounds such as liposome or niosome have distinct advantages over conventional dosage forms. These nano-carriers as drug delivery system have been utilized to direct drug at the target organ or tissue. Many bioactive compounds have limited aqueous solubility, so there is a great need for delivery systems suitable for both hydrophobic and hydrophilic drugs. Liposomes and niosomes can carry hydrophilic drugs and hydrophobic drugs. Liposomes have been used to reduce toxicity, increase drug stability, enhance therapeutic effects, prolong circulation time and promote uptake of the entrapped drugs into the target site. However, liposome has some disadvantages which are poor stability due to hydrolysis of phospholipid molecules and high manufacturing costs of phospholipids.

Alternatively, non-ionic surfactant vesicles or niosome whose are similar to liposomes have been developed. This non-ionic surfactant do not produce ions in aqueous solution and exhibit a very low toxicity level. Technically, niosomes are promising drug carriers because they have greater stability and lower cost. Niosomes also have the advantages of simple method of production for the routine laboratory use and its biodegradable. (Shatalebi *et. al.* 2010).

For this reason, niosome is chosen in this present study focusing on efficiency in entrapment of *A. paniculata* extract as the nano-carrier to facilitate the active compound in delivery system.

1.2 Objective of the study

The objective from this research is to investigate the efficiency of niosome in entrapment of *A. paniculata* extract.

1.3 Scope of the study

The scopes from this research are:

- 1. Determination of *A. paniculata* extract primary metabolites.
- 2. Determination of particle size and stability of *A. paniculata* extract in niosome.
- 3. Determination and characterization of niosome in entrapment efficiency of *A*. *paniculata* extract.

1.4 Significant of the study

A. paniculata is widely used in traditional medicine among Asian community. This traditional herb medicine has been reported to have wound healing capacity because the main bioactive compound which is andrographolide is supposed to exhibit antiinflammatory effect. However, the application of topical delivery system contain in this plant material is yet has not been studied. The importance of this study is to overcome the limitation in topical application contained plant extract which mostly has the characteristic of hydrophobic compounds. Niosomes are non-ionic surfactant vesicles that have potential applications in the delivery of hydrophilic and hydrophobic drugs. Thus, by incorporating the *A. paniculata* extract into the niosome can overcome the limitation in topical wound healing product.

REFERENCES

Abida Latif, Khalid Husain, Nadeem I.B., *et. al.* (2013) Analytical and Biological Studies of Kanji and Extracts of its Ingredient Daucus Carota L. *Chem. Soc. Pak.* 35(6)

Arijit Gandhi, Suma, O.S., and Abhijit Paul. (2012). Current Trends In Niosome As Vesicular Drug Delivery System. *Asian Journal of Pharmacy and Life Science*. Vol. 2 (2)

Buckton, G., and Harwood. (1995). Interfacial phenomena in Drug Delivery and Targeting. *Academic Publishers*, *Switzerland*. p.154-155

Cheung HY, Cheung CS, Kong CK (2001). Determination of bioactive diterpenoids from *A. paniculata* by micellar electrokinetic chromatography. *J Chromatogr A*. 930(1-2):171-176

Kanokwan, J. and Nobuo, N. (2008). Pharmacological Aspects of A. paniculata on Health and Its Major Diterpenoid Constituent Andrographolide. Journal of Health Science. 54(4): 370-381

Koteswara R.Y., Vimalamma G., Rao C.V., and Tzeng Y.M. (2004). Flavonoids and andrographolide from *A. Paniculata*. *Pytochemistry*. 65(16): 2317-2321

Kumar, R.A., Sridevi, K., Kumar, N.V., and *et. al.* (2004). Anticancer and immunostimulatory compounds from *A. Paniculata. Journal of Ethnopharmacology*. Vol 92 (2-3): 291-295

Kumora A.C. and Hasan M. (2006) Extraction of Diterpenoid Lactones of Andrographis paniculata using Liquid Solvents: Effect of Solvent's Hildebrand Solubility Parameter on Extraction Efficiency. *4th National Technical Postgraduate Symposium*. 17 May 2006, Crystal Crown Hotel, Petaling Jaya, Selangor, Malaysia.

Lingan, M.A., Sathali, A. H., Vijaya, M. R. *et. al.* (2011). Formulation And Evaluation Of Topical Drug Delivery System Containing Clobetasol Propionate Niosomes. *Scientific Reviews and Chemical Communications, India.* 7-17

Malhotra, M. and Jain, N.K. Niosomes as Drug Carriers. (1994). *Indian Drugs*. 31 (3): 81-86

Navneet, K.V., Pushpendra, K.T., Chaudhari, S.K., and *et. al.* (2012). Niosomes: A Study on Novel Drug Delivery System-A Review. *International Journal of Pharmaceutical Research and Development*. Vol 3(12): 100-106

Niranjan, A., Tewari, S.K., and Alok Lehri. (2010) Biological Activities of Kalmegh and Its Active Principles- A Review. *Indian Journal of Natural Product and Resources*. Vol 1(2): 125-135

Pholphana N, Rangkadilok N, Thongnest S, Ruchirawat S, Ruchirawat M, Satayavivad J. (2004). Determination and variation of three active diterpenoids in *A. paniculata* (Burm.f.) Nees. *Phytochem Anal.* 15(6):365-371.

Poolsup N, Suthisisang C, Prathanturarug S, *et. al.* (2004). Andrographis paniculata in the symptomatic treatment of uncomplicated upper respiratory tract infection: systemic review of randomized controlled trials. *J Clin Pharm Ter.* 29:37-45.

Raja Naresh RA, Chandrashekhar G, Pillai GK and Udupa N. (1994). Antiinflammatory Activity of Niosome Encapsulated Diclofenac Sodium with Tween -85 in Arthitic rats. *Ind J Pharmacol*. 26:46-48. Sadegh H., Rashidi, A., *et. al.* (2013). Facile and economic method for preparation of nanocolloidal Silica with controlled size and stability. *International Journal Nano Dimension*. 2013. 5(2): 177-185.

Sharma Meenu, S. Aakanksha and T. Sandeep. (2011). Quantitative HPLC Analysis Of Andrographolide In *A. paniculata* At Two Different Stages Of Life Cycle Of Plant. *Acta Chimica & Pharmaceutica Indica*. India. 2(1): 1-7

Shatalebi, M.A., Mostafavi, S.A., and Moghaddas, A. (2010). Niosome as a drug carrier for topical delivery of N-acetyl glucosamine. *Res Pharm Sci.* 5(2): 107–117

Shen, Y.C., Chen, C.F., and Chiou, W.F. (2002). Andrographolide prevents oxygen radical production by human neutrophil: possible mechanism(s) involved in its anti inflammatory effect. *British Journal of Pharmacology*. Vol 135 (2): 399-406

Susmita M and Amar N.M. (2002). Nanoparticulate Carriers in drug delivery and targeting. *Proc. Indian Natn Sci. Acad (PINSA)*. 68(4): 349-360

Varma A, Padh H, Shrivastava N. (2009). Andrographolide: A New Plant-Derived Antineoplastic Entity on Horizon. *Evid Based Complement Alternat Med*.

Zhang. Z.F., and Tan B.K. (2000). Antihyperglycemic and antioxidant properties of *A. paniculata* in normal and diabetic rats. *Clin exp pharmacol physiol*. 27(5-6): 358-363