



IN VITRO STUDY OF *BACOPA MONNIERI* AND *TAGETES ERECTA* EXTRACT-LOADED DOUBLE EMULSION FOR INHIBITORY ALZHEIMER'S DISEASE AND ANTI-CATARACTOGENIC

Supachai Chumchuen*, Somboon Tiranupan, Julalak Kantanalit and Yoskrai Sridhanasakulchai

Research and Development Department, Scigen Lab Co., Ltd 1401 21st Street, Sacramento, 95811, USA.

***Corresponding Author: Supachai Chumchuen**

Research and Development Department, Scigen Lab Co., Ltd 1401 21st Street, Sacramento, 95811, USA.

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ABSTRACT

Bacopa monnieri and *Tagetes erecta* extracts had properties to eliminate dementia and nourish eyes. Bacoside A and rutin are the major chemicals in both extracts, respectively. The study extracted bacoside A and rutin in a specific solvent. The preliminary control extraction by TLC method compares with the standard, study stability of extract-loaded double emulsion, and in vitro inhibition of acetylcholinesterase. The results showed 53.87 % and 46.98% entrapment efficacy of *Bacopa monnieri* and *Tagetes erecta*, respectively. This formulation contained an extract of 0.5-2 mg/mL that could be inhibitory acetylcholinesterase significant when compared with blank $p > 0.01$. However, 0.5 mg/mL of both extracts could inhibit acetylcholinesterase, which is the main reason for Alzheimer's disease. In the case of HLEB-3 cells, group No. 2 had higher efficacy cell available more than group No.1. Moreover, these formulations were stable at 45 °C for 3 months.

KEYWORD: Alzheimer's disease, *Bacopa monnieri*, *Tagetes erecta*.

INTRODUCTION

Double emulsions are instability systems^[1] on the thermodynamic property^[2] due to the abundance area of particles that affect the increment of surface free energy of systems.^[3] On the other point, in the field of the pharmaceutical industry, these systems be able to achieve dual encapsulation of active pharmaceutical ingredients.^[4] In this era, neurodegenerative diseases have promising increasing significance,^[5,6] it might be due to the accumulation of toxic in the body.^[7] Therefore, researchers are finding natural sources to overcome this dilemma.

Herbals seem to be efficacy in treatment of disorders of the brain. For proof of this data, Sundaramoorthy K et al. studied extraction leaf of *Evolvulus alsinoides* alleviating Alzheimer's disease *in vitro* study. Unfortunately, this extract is poorly stable that was applied on the products.^[8] Various herbal that are well stability when further used on the cosmetic or supplements like *Bacopa monnieri* and *Tagetes erecta* are widely used for improving memorization.^[9,10]

Tagetes erecta L. (Asteraceae) is known well by another name as marigold. Botanical characteristics: Marigolds are native in Mexico. Marigolds are 60-90 cm tall and single cluster at the tip. The flower petals are gutter-shaped. In the case of pharmacological, this herb is effective as antioxidant and to treat various diseases due to free radicals.^[11] Moreover, a lot of previous research

have been reported that marigold be able to eliminate optical disorder.^[12] Sing, Y et al., 2020 reported the extraction flower of marigold to prevent brain cancer by inhibiting the reaction autooxidation in cells prevent degeneration of cancer cells.^[13,14] In marigolds, chemical compounds are found such as phenolics, flavonoids, terpenoids, tocopherols, carotenoids, quinones, coumarins, rutin, and volatile oil.^[15,16]

Bacopa monnieri L. (Scrophulariaceae) has botanical characteristics that is a small plant and growing on damp, wet ground until waterlogged, so it is a plant that has amphibious properties. The stem looks succulent and smooth without hair. A width of about 5 mm. and a length of about 15 mm. The base of the leaf is narrow, the leaf tip is wide, and rounded, the edge of the leaf is smooth, and the flower is a single flower in the axillary.^[17-19] Brahmi has been used in Ayurvedic practice in India for the treatment of seizures, disorders of the brain, nourish the heart, neurological diseases, nourishing the brain, help enhance memory.^[20,21] Moreover, this herb can help to protect nerve cells anti-depression and antioxidant activity.^[22] The chemical composition is as follows such as alkaloids, nicotine, and herpestine. The saponins are hersaponin and the potassium salts of hersaponin, bacoside A, bacoside B, jujubogenin, pseudojujubogenin, ebelin lactone pseudojujubagenin, bacoside A, bacoside A, bacopasaponins A, B, C, D, G, bacopasides I, I, III, IV and V. Substances classified as phenylethanoid glycosides are

monnierasides I, II, III and plantainoside B; carbohydrates, including D-mannitol.^[23,24]

In the present study, the combination of both herbs in a double emulsion has not yet been reported. Therefore, this study aimed to develop the *Bacopa monnieri* and *Tagetes erecta* extract-loaded double emulsion for inhibitory Alzheimer's disease in vitro.

Reagents

Samples of Marigolds (*Tagetes erecta* L.) and Brahmi (*Bacopa monnieri* L.) was collected from the Samunpai Thaprachan Corporation Bang Chak, Mueang phasi charoen, Bangkok, Thailand., Bacoside A was purchased from Natural Remedies, Bangalore, (India)., Methanol, Ethyl acetate, acetone, and n-hexane were purchased from Qrec, (Newzealand)., Chloroform was purchased from RCI Labscan, Ireland., Sorbitane monooleate (Span 80) was purchased from Sigma-Aldrich (USA)., Polysorbate 80 (Tween 80) was purchased from Sigma-Aldrich (USA)., 5,5'dithiobis nitro benzoic acid, Ellman reagent and acetylthiocholine iodide were purchased from Sigma-Aldrich (USA)., TLC Aluminium sheet was purchased from MACHEREY-NAGEL Corporation, (Germany)., Ultrasonic Bath Model GT SONIC-D13 Made in (China)., and Rotary

Evaporator RV 10 digital V, IKA, (English), Black-Box Type UV Analyzer model BTU-6 Made in (China).

Preparation and characterization of plant extract

Briefly, 100 g of *Bacopa monnieri* and 200 g of *Tagetes erecta* as showed in figure 1 were separately soaked in 500 mL of deionized water at 25 °C for 3 weeks and then each extract was filtered. The mask of the herb was re-extracted by 500 mL of ethanol for 3 days. Afterward, the water and ethanol liquid were mixed, and then evaporation by a rotary evaporator. In the case of *Tagetes erecta*, 5 g of curd extract was extracted with 100 mL of coconut oil and continuously stirred at 500 rpm at 45 °C for 5 days. Then, 50 mL of extract was mixed with 100 mL of methanol. The solution was evaporated for further study. 4 mL of *Tagetes erecta* was mixed with 20 mL of chloroform and then the solution was centrifugated at 40,000 rpm for 20 min. The supernatant of the extract was evaporated and stored at 4 °C for further study. All of extraction process showed at figure 2. The crude of each herb was characterized by TLC compared with bacoside A in *Bacopa monnieri* and rutin in *Tagetes erecta*. Mobile phase of *Bacopa monnieri* Methanol : water : Ethyl acetate (1.5:1:7.5) and *Tagetes erecta* as Chloroform: Hexane: acetone eluent (6:2:2).



A

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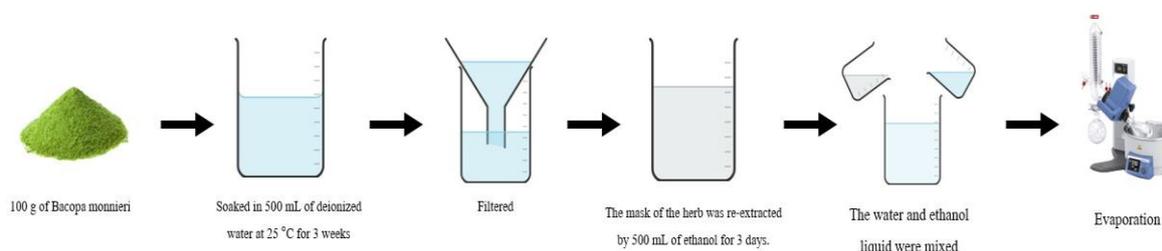
Figure 1: *Bacopa monnieri* (A.)



B

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Tagetes erecta (B.)



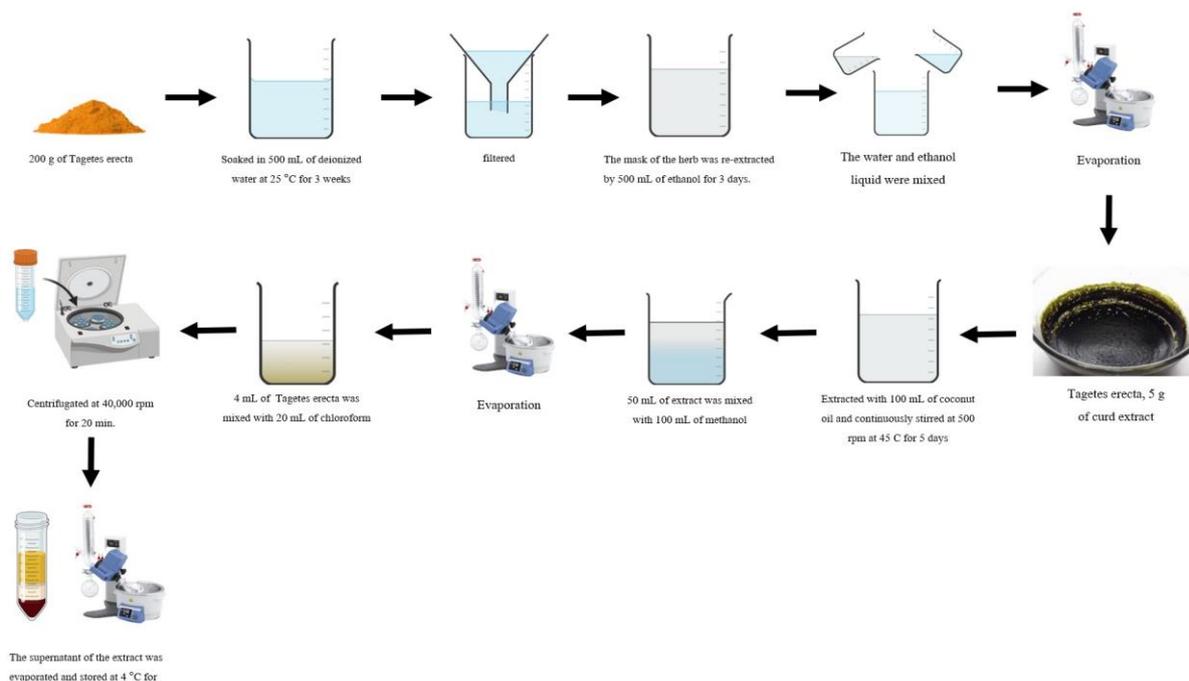


Figure 2: Process for extraction *Bacopa monnieri* and *Tagetes erecta*.

Preparation and characterization of *Bacopa monnieri* and *Tagetes erecta* extracted-loaded double emulsion

10 µg of *Tagetes erecta* extracted were mixed with 30 mL of olive oil and then 15 g of span 80 was added. The solution was stirred at 500 rpm at 50 °C for 10 min. Each of solutions were mixed with 10 mL of deionized water contained 5 g of tween 80 and 50 µg of *Bacopa monnieri* extracted. The solution in oil phase contained *Tagetes erecta* extracted was slowly added into water phase contained *Bacopa monnieri* extracted and added 1 mL of 0.1 M sodium chloride solution. Then, the solution was mixed with homogenizer at 50,000 rpm for 40 min. The double emulsions were analyzed particle size, zeta potential and PdI by zetazizer and stability at 45 °C for 3 months.

Entrapment efficacy study

10 mL of double emulsion was mixed with 20 mL of chloroform and then the solution was centrifuged at 90,000 rpm 4 °C for 60 min. The supernatant was used for analysis rutin entrapment. In the case of pellet, the sediment of double emulsion was dispersed with ethanol and re-centrifuged at 20,000 rpm for 60 min. The supernatant was transferred for analysis bacoside A. The equation for calculation entrapment efficacy is showed in this below.

Entrapment efficacy = $\frac{\text{the amount of residual drug X}}{\text{the amount of initial drug}} \times 100$

Acetylcholinesterase inhibition assay

This study was modified from Sundaramoorthy K et al. The Acetylcholinesterase inhibitory was determined by the Ellman method. Briefly, 80 µL of 3 mM 5,5'-dithiobis nitro benzoic acid, 80 µL AChE (1.5 mg/mL) and double emulsion which was contained plant extracts 10 µg/layer

that was mixed and incubated at 37 °C for 30 min in a micro-titre plate. Afterward, 35 µL acetylthiocholine iodide (15 mM) was added into solution and incubated for 4 hr. The blank solution contains all the reagents except the enzyme. Galantamine was used as positive control. The absorbance was analyzed at 412 nm.

Human lens epithelial cells study

This method was modified from Cao, J., Wang, T. and Wang, M.^[34] Briefly, the HLEB-3 cells were seeded at a density of 1×10^4 cells/cm² in a 96-well plate, grown overnight, and then administered treatment as appropriate. The cells were treated with 500 µM H₂O₂ (Group No. 1), 500 µM H₂O₂ *Bacopa monnieri* and *Tagetes erecta* extract-loaded double emulsion for 24 h (Group No. 2). Each of group was incubated at 37 °C for 4 h. At the end of this treatment, absorbance was measured at 450 nm.

Statistical analysis

Statistical analysis was performed ANOVA method, confidence level 99 % of the comparison was compared by individual pair Tukey's test.

RESULTS AND DISCUSSION

Extraction and formulation

Form the extraction processes, *Bacopa monnieri* and *Tagetes erecta* were able to achieve the amount of bacoside A and rutin, respectively. This assume showed in the preliminary scanning compared with standard that showed in figure 3. TLC method that is process for this study due to non-complication for preparation. The study was found the extraction process have R_f both of herbs closed with standard. Therefore, this extraction could be suitable using for chemical substances. In the case of

stability, initially of double emulsions are a slightly yellow color, turbidity texture and pH 7.2. When these emulsions were kept at 45 C for 3 months the serum of higher extract (2 mg/mL) that are increasing serum level whereas 0.1-1 mg/mL are slightly serum index. This study might be due to the lower interfacial tension of system leading to phase separation.^[25-27] Another assumption, it might be since the higher volume of extract attribute lower efficacy of anti-aggregation of systems^[28-30] that show in figure 4.

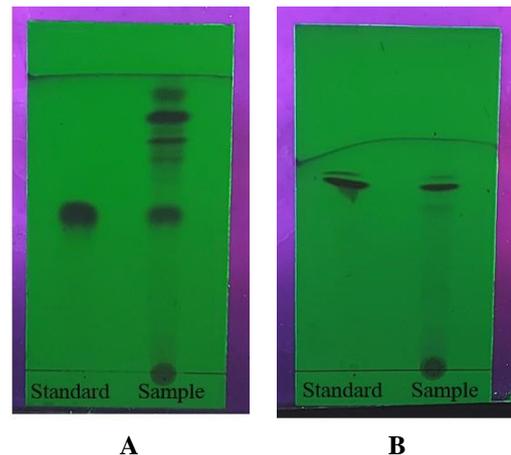


Figure 3: Characterization of *Bacopa monnieri* (A) and *Tagetes erecta* (B) extract compared with standards Bacosid A and rutin, respectively.



Figure 4: Stability of extracts at various concentrations loaded-double emulsion for 3 months.

Entrapment efficacy and cell study

The double emulsions were 53.87% and 46.98% entrapment efficacy of *Bacopa monnieri* and *Tagetes erecta*, respectively when compared with nanoemulsion (data not show) lower leaking rate. This finding is possibly the double layer of emulsion that could affect with drug release established inner core.^[31-33] For the acetylcholinesterase inhibitor study, the blank of double emulsion as negative control and galantamine as positive control, the extracts loaded-double emulsion in range 0.1-2 mg/mL had different significant inhibition enzyme when compared with blank ($p > 0.01$) as showed in figure

5. However, the double emulsions had lower efficacy inhibition compared with positive control. Therefore, both of extracts could be eliminated acetylcholinesterase that the factor is further occurring dementia or disorder of the brain in the future. In the case of HLEB-3 cells, group No. 2 had higher efficacy cell available more than group No.1. Moreover, this study will process study on clinical that are waiting approved by ethic on human at Thailand, the preliminary study on healthy 80 volunteers, this double emulsions are possibly reduced fatigued eyes (data non published).

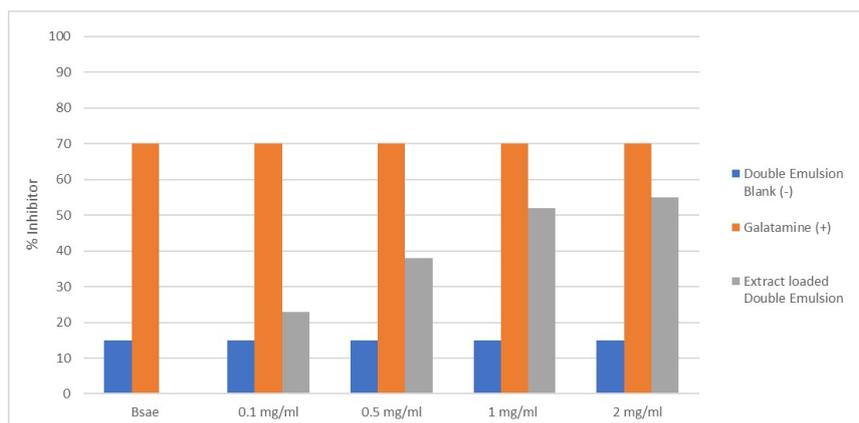


Figure 5: Percent inhibition of an acetyl cholinesterase by extracts loaded-double emulsions.

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