Proceeding and Abstract Book of The International Conference of Pharmaceutical Sciences and Medicines 2018 (ICPAM 2018)

"Health Innovation for Aging Society"

3rd August 2018

Central Laboratory, Faculty of Sciences, Burapha University, Chonburi, Thailand
Organized by: Faculty of Pharmaceutical Sciences, Burapha University

Contents

Welcome address

Dear colleagues,

On behalf of Faculty of Pharmaceutical Sciences, Burapha University, it is our great pleasure to welcome you to the International Conference of Pharmaceutical Science and Medicines (ICPAM 2018) on August 3rd, 2018 in Bangsaen, Chonburi. The theme of the conference is "**Health Innovation for Aging Society**", which is relevant to the context of present situation in Thailand and ASEAN countries.

The conference covers guest lectures, oral presentation and poster presentation in various aspects of Pharmaceutical Sciences, Pharmacognosy, Pharmacology and Clinical Pharmacy. It is a great opportunity for scientists, researchers and students to share information among those with the same interest as well as to learn more from the experts. We do hope that the ICPAM could deliver key message that would be beneficial for all participants.

Chonburi is a city of beach, you can enjoy the natural beauties along with the great seafood to the fullest. We wish you a pleasant stay in Bangsaen and a fruitful and memorable ICPAM 2018.

Assoc. Prof. Dr. Mayuree H. Tantisira

Dean of Faculty of Pharmaceutical Sciences, Burapha University

Preface

Dear distinguished Participants,

On behalf of the Organizing Committee, we cordially welcome you to join the International Conference of Pharmaceutical Science and Medicines (ICPAM 2018). The aims of this conference are to provide an opportunity for academics, researchers, and graduate students in the field of Pharmaceutical Sciences as well as related fields from local and oversea institutions to present their research, exchange ideas and experiences leading to future research development and collaboration. This conference will address the role of pharmacy in a number of the major societal challenges such as health, science and technology, product development, packaging technology, biotechnology and applied sciences while searching for innovative opportunities at the multi facets level of scientific disciplines.

In addition, I wish to take this opportunity to express my sincere appreciation to the members of the Editorial Board / Scientific Committee, Distinguished Keynote Speakers, Chairpersons, and Co-chairpersons of the technical sessions and cooperating organizations for hosting the conference. Special thanks are due to all authors and reviewers without whom this conference would not have been possible. We also owe our gratitude to all of those individuals and numerous other people who in one way or the other contributed to the conference and the proceeding book.

And lastly, I wish the success of the conference and wish you all success and delight in the atmosphere of the ICPAM 2018 as well as of Bangsaen, Chonburi, the land of happiness.

With my warm wishes,

Asst. Prof. Dr. Boonyadist Vongsak,

Chair of the Organizing Committee of the ICPAM 2018

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Conference program

8.00-9.00	**Registration**
9.00-9.15	Welcome and opening by Dean of Faculty of Pharmaceutical Sciences, Burapha University, Assoc. Prof. Dr. Mayuree Tantisira
9.15-10.15	**Lecture 1:** *Prof. Dr. Pornsak Sriamornsak* ***Title :*** Design and Development of Pharmaceutical Dosage Forms for Elderly Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn university, Thailand
10.15-10.30	Coffee break
10.30-11.30	**Lecture 2:** *Assoc. Prof. Dr. Noppamas Soonthornchareonnon* ***Title*** Herbal Medicine Products for Elderly Medicinal Plant Information Center (MPIC), Faculty of pharmacy, Mahidol university, Thailand
11.30-12.30	Lunch
12.30-13.15	**Poster session**
13.15-14.00	**Lecture 3**: *Dr. Ponwanit Charoenputtakun* ***Title :*** Anti-Aging Supplement and Skin Care Trends Research and Product Development Manager of Anti-Aging Medicine Laboratory, Zen Innovation Co., Ltd., Thailand
14.00-14.20	Oral presentation 1
14.20-14.40	Oral presentation 2
14.40-15.00	Oral presentation 3
15.00-15.20	Coffee break
15.20-16.00	**Closing ceremony**

Invited speaker

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PhD in Pharmaceutics
Charles Sturt University, Australia, 1999-2002
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Design and development of pharmaceutical dosage forms for elderly

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Abstract

Aging of the population is a challenge for medicine because of the growing number of elderly patients. Moreover, most of elderly patients have limited physiological reserve, frail and deconditioned. Not only implications from a disease perspective but also implications for the number of medicines they may be taking (polypharmacy) for the control of diseases, which influence on compliance, adherence, efficacy, and safety of elderly patients. The selection and design of oral pharmaceutical dosage forms continues to be one of the most significant challenges in the development of pharmaceutical products for elderly populations due to the diverse needs and characteristics of these patients. In elderly patients, age or disease related swallowing difficulties affect their ability to take solid oral medicines that is the key formulation factor in designing oral dosage forms for elderly patients. Several technologies have been developed to aid the swallowing of solid oral dosage forms, for example, oral disintegrating tablets, disposable devices (Magic Jelly®, Medcoat®, and Pill Glide®). In addition, polypill is novel tablet design addresses one of the factors leading to poor adherence by combining appropriate medications in to a single tablet. Nevertheless, drug product design should consider and compare three factors: patient acceptability, safety, and access. These factors must be balanced each other, and in some situations, a compromise may need to be succeeded when selecting for design and development of an appropriate formulation for elderly patients.

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Herbal medicine products for elderly

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Abstract

Herbal medicine products (HMPs), vitamins and minerals (food supplements) are widely used and increasingly prevalent throughout the industrialized world. The use is associated with age, gender, and several sociodemographic and health behavioural factors. In comparison with men, females seem to be the more frequent users of food supplements, and among both men and women high use is seen among the elderly population. In the United States, the most commonly herbal products were gingko, garlic, ginseng, aloe vera, chamomile, spearmint, and ginger. Of these, gingko and garlic are the most commonly used among community-dwelling elderly. In Thailand, the commonly HMPs such as turmeric, *Andrographis paniculata* and Ya-hom preparation are used by elderly. The purpose of this article is to summarize current data regarding efficacy and safety of those HMPs, and to suggest how older patients should be counseled about the use of HMPs.

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Anti-Aging Supplement and SkinCare Trends

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Abstract

Age-related conditions are the leading causes of death and health-care costs. Reducing the rate of aging would have enormous medical and financial benefits. Aging can be defined as a progressive deterioration of physiological function accompanied by an increase in vulnerability and mortality with age. There are also new theories that explain the causes of aging and how to slow down the aging process, for example, telomere theory, mitochondria theory. Anti-aging based therapies are defined as those that delay the onset of multiple pathologies via core biological processes associated with age-related functional decline. The past few generations, perceptions, attitudes, and behaviors related to aging have changed. The desire to retain a younger look and feel isn't restricted to an older generation. People around the world are getting older and, thus, are more interested in products that make them healthier and appear younger, rejuvenated, and energetic. As the percentage of aging consumers increases, the demand for anti-aging products and services rises. Given its huge potential financial benefits, anti-aging science has tremendous commercial opportunities. We then discuss some of the challenges and pitfalls in business development based on anti-aging science and lastly provide a vision for how the field may progress in the future.

Anti-apoptotic effect of *Diplazium esculentum* extract in hippocampus rat after injection of 6-hydroxydopamine neurotoxin

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Abstract

Apoptotic neurodegeneration is observed following mitochondria dysfunction. Both increased caspase-3 and decreased Bcl-2 expression are reported to play crucial role on apoptosis pathway. Up-to-date, phenolic compounds can increase the endogenous antioxidant and lower the apoptotic responses. Therefore, anti-apoptotic effect of *Diplazium esculentum* extract [DEE] was examined. The objective of this study were to determine the effect of Survival neuron and neuron density both caspase-3 and Bcl-2 immunopositive neuron in hippocampus. Male Wistar rats were orally given DEE at doses of 10, 50 and 200 mg/kg at a period of 14 days before and 14 days after injection of 6-OHDA Neurotoxin. The results demonstrated that DEE at doses 50 and 200 mg/kg used in this study significantly attenuated the reduced neuron density in hippocampus. At the doses of 200 mg/kg DEE could decrease caspase-3 immunopositive neuron density but increased density of Bcl-2 immunopositive neurons in hippocampus. In conclusion, DEE is the potential natural substance to protect against apoptotic hippocampus neurodegeneration, in animal model for Parkinson's disease induced by 6-OHDA. However, further researches are essential to elucidate the other biological mechanism offered by DEE.

Keywords: *Diplazium esculentum* extract, antiapoptosis, Parkinson's disease

1. Introduction

Neuronal damage has been recognized of a leading cause of Parkinson's disease [PD]. Pathophysiology neurodegenerative disorder, characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta region, results in motor dysfunction and the hippocampus, results in memory impairment [1]. Oxidative stress is play the crucial role for etiology and progression of Parkinson's disease. So, the therapeutic agent is boost targeted against suppressing and alleviating the oxidative stress-induced cellular damage. However, accumulative lines of evidence during the past decade have revealed that after a PD, many neurons in the cascade will undergo apoptosis. It has been well established that Reactive Oxygen Species can be scavenged through utilizing natural antioxidant compounds present in foods and medicinal plants [2]. In addition, recent evidence demonstrated that naturally substance occurring phenolic compounds can successfully enhancing the endogenous antioxidant and lower the apoptotic responses. Based on evidenced-base, the anti-apoptotic effect of substance of diet which is rich in phenolic compounds has been focused.

Diplazium esculentum Retz. (Family: Athyriaceae) or is one of the commonly known as edible vegetable fern which is found mostly near river and swamp, especially in the east region part of Thailand. This plant is reported to contain phytochemical consisted of triterpenoids, phenols, flavones, flavonoids such as myrcetin and alpha-tocopherol[3],[4]. Pharmacological properties such as laxative, anti-inflammatory, antioxidant and antimicrobial [5]. Therefore, the hypothesis that, *Diplazium esculentum* extract [DEE] should be able to protect against neuronal in PD. This study was set up to determine the effect of *Diplazium esculentum* on density of survival neurons and densities of Bcl-2 and caspase-3 immunopositive neurons in various regions of hippocampus rat.

2. Materials and Methods

2.1 Animals

Adult male Wistar rats (250-280 g, 8 weeks old) were obtained from National Laboratory Animal Center, Nakorn Pathom and were housed in group of 4 per cage in standard polycarbonate cages at 22±2°C on 12:12 h light-dark cycle. All animals freely accessed to food and water. All the procedures carried out in this study were approved by the Institutional Animal Care and Use Committee.

2.2 Plants Praparation

Fresh leaf of *Diplazium esculatum* were collected from Sri Racha Distrct, Chon Buri Province, Thailand. It was grounded to powder and then subjected to Soxhlet extraction using ethanol. Phytochemical analysis of the extract was performed according to Harborne et al. [6]

2.3 Experimental protocol

Rats were given orally DEE at 3 different doses at 10, 50 and 200 mg/ kg while control group were given distilled water (n=8/group). All animals were treated with vehicle or DEE for 14 days before and 14 days after 6-Hydroxydopamine **(**6-OHDA**)** injection. The 6-OHDA infusion was performed using a Hamilton syringe**.** At the end of experiment, rats were sacrificed and their brains were removed to study the density of survival neurons, and the densities of Bcl-2 and caspase-3 immunopositive neurons in various regions of hippocampus.

2.4 Survival neurons of hippocampus (Cresyl Violet Staining for Nissl Substance)

The adjacent sections of hippocampus were stained with 0.5% cresyl violet. The neuron density in various regions of hippocampus was observed under light microscope (Olympus light microscope model CX-21; made in Japan) at 40X magnification by blinded observer.

2.5 Immunohistochemical staining of Bcl-2 and caspase-3 immunopositive neurons

A series of sections containing hippocampus were reacted in a mouse monoclonal antibodies directed against either Bcl-2 or Caspase-3 (Chemicon Internation, Inc., CA, USA) and a modification of a previously described protocol employing the DAKO Strept ABC Complex/HRP duet kit.

2.6 Statistical analysis

Data were presented as mean ± standard deviation (SD). The analysis was performed using one-way Analysis Of Variance (ANOVA), followed by Tukey's *post hoc* test. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

The effects of DEE on neuron density and densities of both Caspase-3 and BCl-2 immunopositive neuron were shown in Figure 1-3. It was found that rats received DEE at 50 mg/kg significantly enhanced neuron density in CA1, 2, 3 and Dentate gyrus (DG) (*p*-value<.05) However, only rats treated with DEE 10 mg/kg showed the enhanced neuron density in CA3 and DG

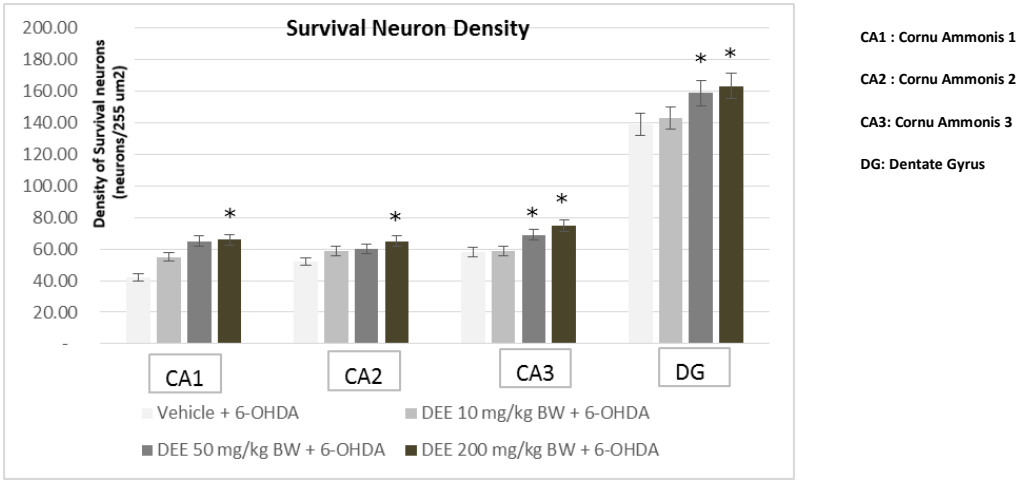

Fig.1 The effects of DEE on survival neuron by Cresyl violet stain (n=8/group)

Rats received to 50 mg/kg DEE showed the decreased density of caspase-3 immunopositive neurons in CA3 and DG (*p*-value<.05) whereas rats received 10 mg/kg DEE showed the decreased density of caspase-3 immunopositive neurons in CA3 In addition, the increased Bcl-2 immunopositive neurons density was also observed in CA1, CA3 and DG

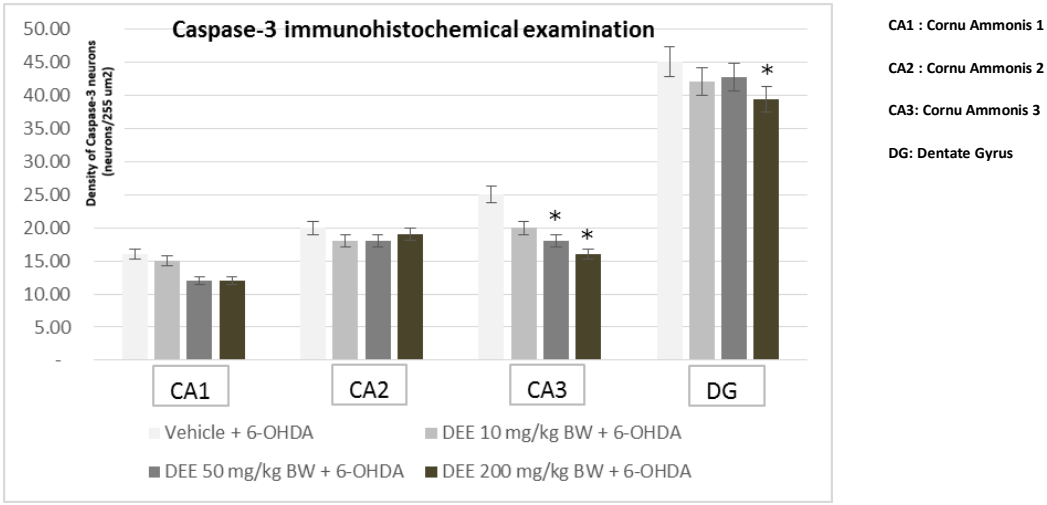

Fig. 2 The effects of DEE on Caspase-3 immunopositive neuron (n=8/group)

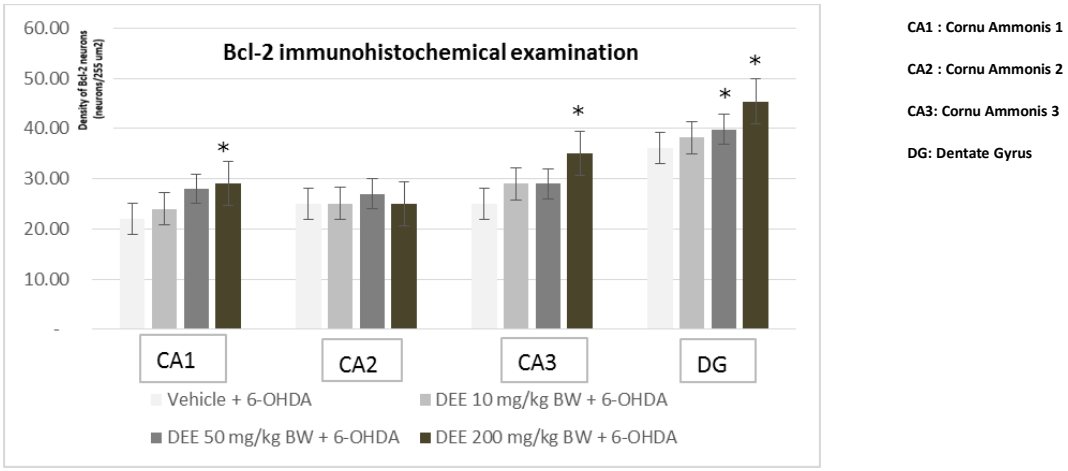

Fig. 3 The effects of DEE on BCl-2 immunopositive neuron **(**n=8/group**)**

In the present study, we demonstrated that DEE could enhance the densities of survival neuron in hippocampus. It enhanced density of Bcl-2- immunopositive neurons in hippocampus CA1 and CA3 and decrease caspase-3 immunopositive neurons in hippocampus CA2 and CA3.

Apoptotic cell death, has been show to play a crucial role on neurodegeneration following cerebral ischemia. Recent evidence showed that apoptosis-related cell death is linked to caspase activation. especially caspase-3, a key mediator of apoptosis. However, apoptotic neurodegeneration can be prevented by Bcl-2, anti-apoptotic protein localizing at the outer mitochondrial membrane. Recent findings also demonstrate that polyphenolic compounds in plants can attenuate brain damage in hippocampus following PD via up-regulation of Bcl-2 [8],[9]. Based on the evidence of study, we did suggest that *Diplazium esculentum* enhanced the density of Bcl-2 immunopositive neuron and decreased caspase-3 immunopositive neuron density in hippocampus resulting in the increased neuron density in the studied area.

4. Conclusions

Diplazium esculentum extract could exhibit the anti-apoptotic effect in the PD model, which is due to its promoting the survival neuron and caspase pathway. However, further investigations are still necessary.

Acknowledgments

The authors gratefully acknowledge the Research Grant of Burapha University through National Research Council of Thailand, under “Plant Genetic Conservation Project” (2560).

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Efficacy of *Moringa oleifera* Lam. leaf extract for protecting plasmid pET-15b DNA from gamma radiation

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Abstract

Moringa oleifera Lam. leaf extract has been reported to exhibit antioxidant, anti-cancer and possibly radioprotective activities. In this study, its ability in protecting pET-15b plasmid DNA *in vitro* against radiation-induced damage was investigated. pET-15b DNA was irradiated by gamma radiation at 100 Gy in the presence of 0-3 mg/mL *M. oleifera* leaf extract and DNA damage was measured using gel electrophoresis. Results showed that gamma radiation induced DNA strand breaks and led to alteration of plasmid DNA from a supercoiled to open circular form. *M. oleifera* leaf extract showed a potential for plasmid DNA damage protection against gamma radiation. DNA strand breaks were reduced with increasing the concentrations of *M. oleifera* leaf extract.

Keywords: *Moringa oleifera* Lam., leaf extract, radiation, protection, DNA

2. Introduction

Ionizing radiation, such as gamma and x-ray, can introduce adverse effects on living organisms, especially DNA. Ionizing radiation can break DNA strands directly by energy transfer process or indirectly by generation of free radicals [1]. Consequently, radiation is widely used in the treatment of cancer to destroy the DNA of cancer cells and kill them. However, radiation can also induce adverse effects on surrounding normal cells [2].

Moringa oleifera Lam. is a natural source of antioxidants that can inhibit the effects of free radicals responsible for diseases such as cancer and cardiovascular diseases [3]. Its protective effects against breast cancers and radiation have been reported. However, the mechanisms for radiation protection of DNA damage have rarely been described. This study aims to evaluate the mechanism of *M. oleifera* leaf extract by assaying its protective effect on plasmid DNA *in vitro*. The method was based on the decrease in the disappearance of the supercoiled form of the plasmid upon irradiation [4].

3. Materials and Methods

The powderized ***M. oleifera*** leaf sample was purchased from a local market in Thailand and mixed with distilled water in 1:10 (w/v) and boiled for 10 min. After cooling at room temperature for 1 h, the extract was filtered and lyophilized in a ScanVac freeze dryer (Labogene, Denmark) [7]. pET-15b plasmid DNA (Novagen, USA) was prepared by using competent JM109 *E.coli* cells (Promega, USA) for transformation and selection on LB agar plate with 100 µg/mL ampicillin. Plasmid were obtained from single ampicillin-resistant colonies following the miniprep kit manufacturer's procedure (GeneJET plasmid Miniprep Kit, ThermoFisher Scientific, USA).

The mixtures of 1.23 µg/mL pET15b plasmids with either 0, 1, 2 and 3 mg/mL of *M. oleifera* leaf extract or 0, 5 and 10 mM of gallic acid (Sigma-Aldrich, USA) (positive control) were prepared in polypropylene microtubes. Then, they were exposed to 100 Gy of gamma radiation at the dose rate of 2.98 kGy/h at Thailand Institute of Nuclear

Technology. Subsequently, the irradiated plasmid mixtures were electrophoresed on 0.8% agarose gels in 1xTBE in comparison with non-irradiated mixtures. The DNAs were stained with ethidium bromide to be photographed by a UV transilluminator.

4. Results and discussion

pET-15b is a 5,708-bp plasmid DNA. Its preparation yielded mostly the supercoiled form with some open or nicked circular form, while the linearized plasmid was not present. Supercoiled pET-15b ran at the apparent size of approximately 3,500 bp, and its open-circular form ran above 10,000 bp (Fig.1, lane 1). Exposure of pET-15b plasmid DNA to 100 Gy gamma radiation resulted in the disappearance of the supercoiled plasmid and the increase in the amount of the open circular form (Fig.1, lane 2). This was due to radiation-induced cleavages of phosphodiester bonds of supercoiled DNA converting it to an open circular form. The interaction between gamma radiation and the plasmid DNA was indirect and likely mediated by free radicals generated during irradiation. Addition of antioxidative gallic acid at 5 and 10 mM to the plasmid mixture could prevent radiation-induced conversion of the supercoiled plasmid to its open-circular form, thus preventing DNA damage (Fig.1, lanes 3-4). The report by Gandhi *et al*., 2005, similarly showed that the presence of gallic acid during irradiation could protect DNA from radiation-induced strand breaks [5].

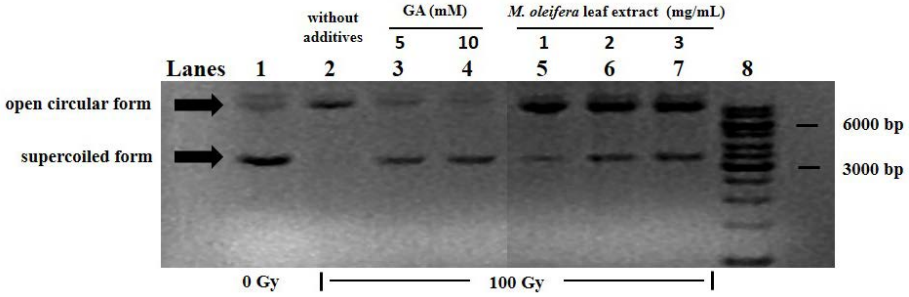

Fig.1 Effects of varying concentrations of *M. oleifera* leaf extract on gamma-radiation-induced strand breaks of plasmid pET-15b DNA at 100 Gy. Lane 1: control (0 Gy without additives); lane 2: 100 Gy without additives; lane 3-4: 100 Gy with 5-10 mM gallic acid; Lane 5-7: 100 Gy with 1-3 mg/mL *M. oleifera* leaf extract; lane 8: GeneRuler 1 kb DNA Ladder (ThermoFisher Scientific, USA).

When *M. oleifera* leaf extract was added to pET-15b plasmid mixture and exposed to 100 Gy gamma radiation, increased retention of the supercoiled form was observed comparing to radiation treatment of pET-15b without any additives (Fig.1, lanes 5-7). The retention of the supercoiled plasmid increased as the concentration of the extract increased from 1 to 3 mg/mL, indicating that the radioprotective effect depended upon the extract's concentrations. The ability of *M. oleifera* leaf extract to protect pET-15b from radiation-induced damage *in vitro* was likely due to the presence of antioxidative compounds in its composition. *M. oleifera* leaf extract contained high phenolic content such as gallic acid, chlorogenic acid, and ferulic acid that had potential antioxidant properties which could prevent the effects of free radicals, scavenging activities, and oxidative damage [6].

M. oleifera leaf extract could reduce the level of DNA double-strand breaks in MCF-7 breast cancer cells exposed to gamma radiation [7]. However, it was not known whether *M. oleifera* leaf extract exerted its radioprotective activity via a biological pathway or whether it could prevent DNA damage on its own. The current studied showed that *M. oleifera* leaf extract could protect plasmid DNA from radiation-induced damage to certain extent *in vitro*. Therefore, a mechanism that *M. oleifera* confers its radioprotective effect to a cell is by protecting the DNA from radiation-induced oxidative damage.

5. Conclusions

This study showed that the presence of gallic acid and *M. oleifera* leaf extract could protect pET-15b plasmid DNA from gamma-radiation-induced strand breaks. The protective effect is correlated to the concentrations of gallic acid and *M. oleifera* leaf extract. Increased retention of the supercoiled form together with decreased conversion to the open circular form was observed in their presence. Thus, *M. oleifera* leaf extract was confirmed to be a radioprotective agent for the DNA, the genetic material, from damage.

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Paclitaxel-loaded EGFR-targeted immunonanoparticles exhibit anti-cancer effects in triple negative breast cancer

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Abstract

The aim of this research was to investigate the targeting efficiency of c-KIT anchored immuno-nanoparticle (INP) bearing Paclitaxel (PTX) to the EGFR protein on the TNBC cells. The nanoparticle (NPs) was prepared by the nanoprecipitation method and the NPs were characterized for morphology, size, zeta potential, entrapment efficiency and drug release. EGFR mAb anchoring was confirmed by SDS-PAGE gel electrophoresis method. *In-vitro* anticancer activity was analyzed by MTT assay using MDA-MB-468 cell line and the NPs cellular uptake was confirmed by fluorescence microscopy using FITC as the marker. The *in-vivo* anti-tumor activity of INP was determined by using the Xenografts nude mice model and the targeting ability was confirmed by fluorescence microscopic imager. Prepared NPs was 335 nm in size and -3.48 mV zeta potential and release PTX up to 48 h. INP showed significant anticancer effects both in *in-vitro* and *in-vivo* models. Based on these studies, we recommend that EGFR antibody anchored PTX loaded NP may have the ability to target the TNBC cells and improve the therapeutic action and subsidize the side effects of PTX in the treatment of TNBC.

Keywords: Triple-negative breast cancer, EGFR protein, Paclitaxel

1. Introduction

Triple Negative Breast Cancer (TNBC) is an aggressive type of breast cancer, lacks of target receptor expressions such as Progesterone Receptor (PR), Estrogen Receptor (ER), and Human Epidermal Growth Factor Receptor 2 (HER2) [1] and thus there is no proper treatment available for these patients. About 15-20% of all breast cancer diagnoses and are clinically aggressive to TNBC, it’s contributing an undue high number of recurrences and breast-cancer related deaths [2]. Recent treatment options for the TNBC rely on toxic chemotherapy, surgery and radiation therapy [3]. PTX is the best microtubule stabilized drug which was approved by the USFDA for the treatment of various cancers like ovarian, breast, lung, Kaposi's sarcoma and also cervical, prostate, head and neck cancer [4].

Immuno-histochemical analysis evidence that TNBC is analogous of high expression of the proliferation bio-markers such as Ki-67, cyclin E, mutated p53, Epidermal Growth Factor Receptor (EGFR), P-cadherin, vimentin, and mutated BRCA1, CK5/6, c-KIT in all the populations [5,6]. Studies have been delineating that, 80% of the tumor that expresses EGFR basal marker in a western population [7]. The frequently

EGFR gene is mutated and over-expressed in the head, lung, neck, colon, pancreatic, brain and breast cancers especially in TNBC by stimulating the tumor progression. Consequently, EGFR is a fascinating drug target to inhibit EGFR expression by tyrosine kinase inhibitors (TKIs) and mAbs. We hypothesize that PTX-entrapped NPs anchored with an EGFR mAb to have a therapeutic action in TNBC.

2. Materials and Methods

PLGA-PEG polymer was purchased from Advanced Polymeric Materials lnc, Canada. Paclitaxel (PTX) was purchased from Chemtron Biotechnology Sdn Bhd, Malaysia EGFR mAb was purchased from Thermo Scientific, USA. Bovine Serum Albumin (BSA), fetal bovine serum (FBS), Ponceau red stain, TRIZOL was procured from Sigma Aldrich, USA. MDA-MB- 468 Cell lines and Leibovitz's L-15 medium were obtained from AddexBio (USA), Superscript 1V RT kit and Fluorescein isothiocyanate (FITC) were obtained from Thermo Fisher Scientific corporation (USA) and all other reagents & solvents used were analytical grade.

2. Methods

2.1 Preparation and evaluation of EGFR mAb anchored PTX loaded immunonanoparticle (INP)

PTX loaded nanoparticle was prepared by the nanoprecipitation method [8] using PLGA-PEG polymer and EGFR mAb was covalently cross-linked by m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) as a bonding agent. The prepared NPs were evaluated for morphology, particle size, zeta potential, entrapment efficiency and drug release. The particle size and zeta potential were evaluated by Malvern Zetasizer Nano ZS, EGFR mAb anchoring was confirmed by SDS-PAGE gel electrophoresis.

2.2 *In-vitro* anticancer activity

In-vitro anticancer activity was evaluated by MTT assay using MDA-MB-468 cell line. The NPs cellular uptake was confirmed by fluorescence microscopy using FITC as the marker [9].

2.3 *In-vivo* anti-cancer activity and targeting efficiency

Twenty athymic female mice (6 weeks matured) were injected about 0.1ml sample containing 2×10^5 MDA-MB-468 cells into the right flank of mice. Tumor volume was measured every day using an electronic caliper until the volume reached $120mm^3$ (7 days post injection). The tumor volume was estimated using the formula of an ellipsoid (length $\times$ width^2 $\times$ 0.5236) [10]. All the procedures were followed by accordance and guidelines approved by an animal ethical committee of AIMST University, Malaysia (Reference no: AUHACE/FOP/2015/11).

The animals were divided into four groups, each contains 5 mice. Each group was treated by the following injections. Group 1: PTX intravenous injection (2 mg/kg); Group 2: PLGA-PEG polymer solution (control); Group 3: PTX-NP (dose equivalent to 2 mg/kg) and Group 4: INP (dose equivalent to 2 mg/kg). Every 24 h, the tumor volume was measured for anti-tumor activity.

The tumor targeting efficiency of immunonanoparticle observed by *in-vivo* animal imager [10] and the image was compared with PTX-loaded nanoparticle and PTX. FITC labelled nanoparticles were injected into anesthetized tumor induced mice. After 24 hours

of administration, the mice were subjected to *in-vivo* fluorescence imaging system (Kodak In-Vivo FX Imaging Station, USA). The images were snapped at 1 second exposure.

3. Results and discussion

3.1 Preparation and characterization of INP

The NPs were formulated by nano-precipitation method utilizing the ouzo effect mechanism. TEM image (**Fig.1**) showed that spherical shaped, smooth surface nanoparticle with the particle size and zeta potential of 335±1.2 nm in size and -3.48±0.06 mV respectively. The entrapment efficiency of nanoparticle loaded PTX was evaluated by RP-HPLC method and showed the result of 85.58% . The NPs showed sustained release of the drug up to 48 h. When NPs are injected into the biological system or cellular fluid, the particles were quickly surrounded by biosystem macromolecules, which modify all the biological properties and gets eliminated from the human body by opsonin action. In order to avoid the elimination and sustain action, NPs were coated with an anti-opsonin polymer like polyethylene glycol (PEG). The EGFR mAb was covalently attached to the surface of the NP by using EDC as a cross-linking agent. The coupling reaction of EGFR mAb covalent bonding with PLGA carboxylic and amino functional groups [11]. The integrity of monoclonal antibody may be diminished by covalent bonding action. SDS-PAGE analysis proved that EGFR mAb integrity was not destroyed by coupling action.

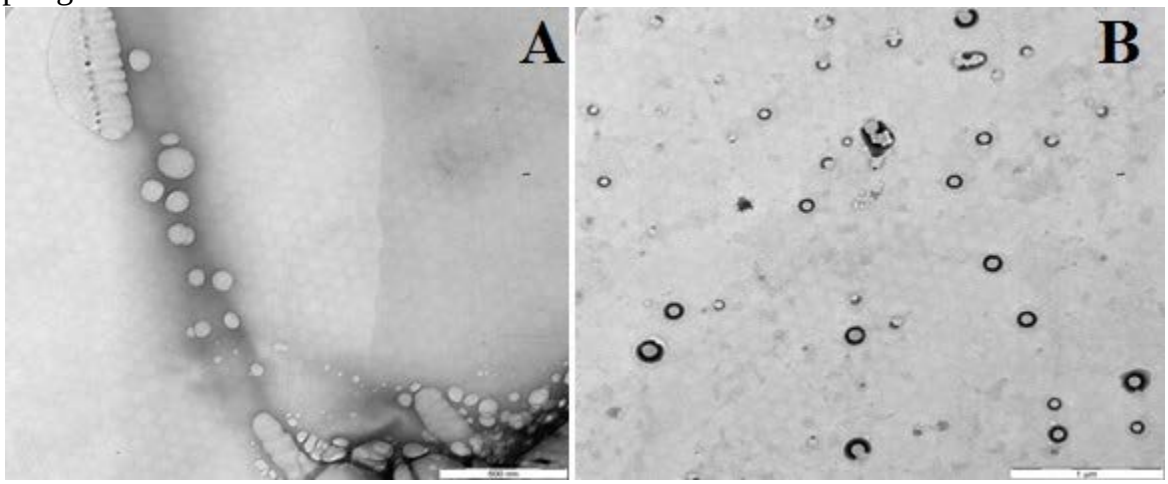

Fig. 1 Transmission electron microscopic (TEM) images of nanoparticles. A) PTX loaded nanoparticle, B) Immunonanoparticle.

3.2 *In-vitro* anticancer studies

The cytotoxicity of PTX, NP and INP, as well as plain NPs formulations were incubated with the MDA-MB-468 breast cancer cells at 24 h and 48 h. The plain NP showed cell viability in the range of 95.23 ± 2.4 to 96.2 ±1.8% in 24 to 48 h (**Fig.2**). The results show that PLGA and co-polymer NPs exhibit cytotoxicity on cancer cells, it could be equal to the 0.02 µg/ml concentration of PTX. NP showed the significant cytotoxic activity of 32.8± 1.8% and 18.2±1.6% at 24h and 48 h respectively. The INP showed

significant cytotoxicity. i.e, cell viability was remarkably reduced (32.4 ± 2.2% and 10.6 ± 3.4 % at 24 and 48 h respectively).

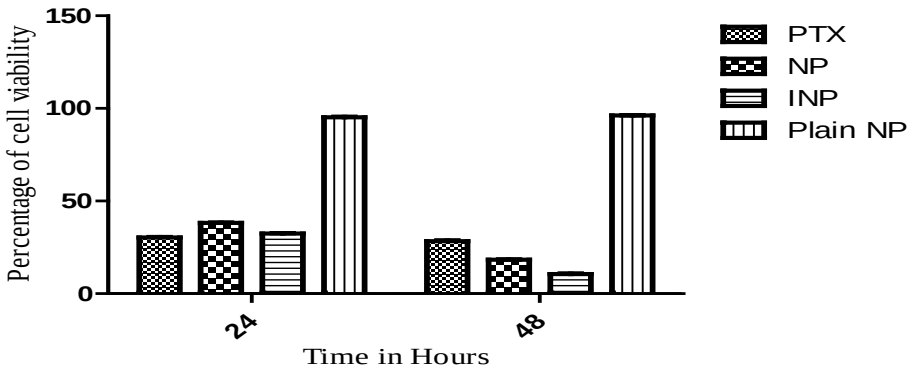

Fig. 2 Cell viability studies of NP and IN, PTX and Plain NP, incubated with MDA-MB-468 breast cancer cells for 24 and 48 hours.

The INP showed significant cellular uptake cancer cells by emitting the fluorescence light. The images show FITC tacked NPs (Green) bound by the cellular constituents. The pictures clearly showed that NPs penetrated within the cell membrane boundary. The anti-tumoral activity of NP depends on cytotoxicity and cellular uptake. Basically, NPs are non-specifically internalized into cells via endocytosis [12], broadly classified as phagocytosis and pinocytosis mechanisms. Pinocytosis is a favorable mechanism for small vesicle or particle uptake by cells. Pinocytosis occurs by any one of the following mechanisms:clathrin-mediated endocytosis, caveolin-mediated endocytosis, macro-pinocytosis, and caveolin independent endocytosis action. These mechanisms are favorable based on specification to cells and particles.

3.3 *In-vivo* anti-tumor activity and targeting efficiency

The *in-vivo* antitumor activity INP was evaluated by Xenografts model using nude mice induced cancer by MDA-MB-468 cells to reach the tumor volume of 120mm^3. Figure 2 showed that, PTX solution drastically reduced the tumor volume initially. Later, the tumor volume was increased, due to the high elimination rate of PTX from the body. NPs showed significant anti-tumor activity, due to the sustained release of PTX from the polymeric matrix of NPs. The immunonanoparticle showed significant tumor volume reduction than PTX loaded nanoparticle (**Fig. 3**).

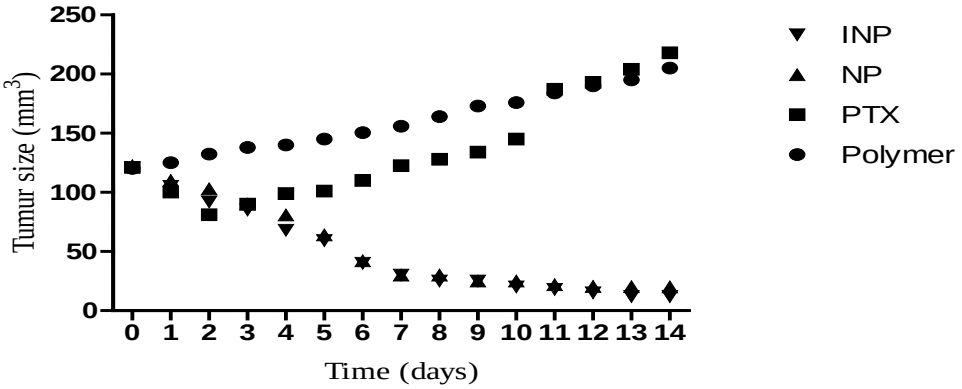

Fig. 3 *In-vivo* anti-tumor study in Xenografts athymic mice model

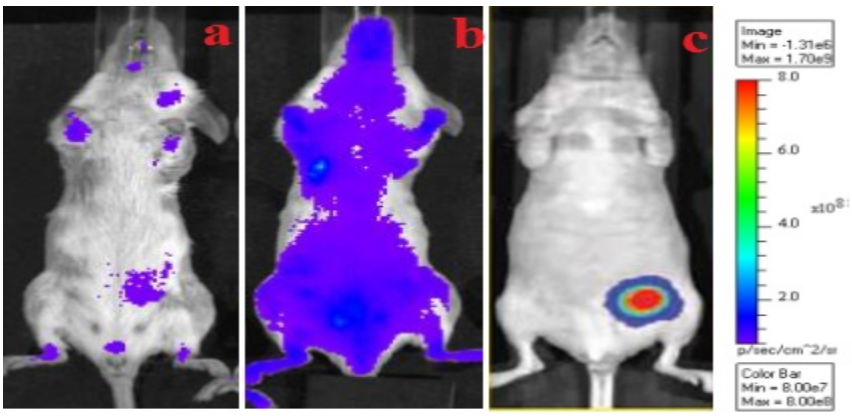

Fig. 4 *In-vivo* fluorescence imaging studies for tumor cells target. a) PTX treated mice b) PTX loaded nanoparticle treated mice c) EGFR anchored immunonanoparticle.

The targeting efficiency of immunonanoparticle was observed under the *in-vivo* fluorescence microscopic imager system. The immunonanoparticle showed significant specific targeting efficiency and high fluorescence intensity than other groups. Jing du et al., investigated that nanoparticle loaded anti-VEGFR2 and HER2 dual targeted showed significant targeting than single receptor [13]. Since the immunonanoparticle could specifically target strongly on TNBC vascular endothelial cell than PTX nanoparticle and PTX. The target efficiency is significantly less in case of PTX nanoparticle (**Fig.4**) shows that easily distributed to all the parts of the body including tumor endothelial cells. The imaging studies expressed that, PTX nanoparticles and PTX could reaches to normal cells and it could produce the toxicity. *In-vivo* imaging study, recommended that immunonanoparticle could be targeted specifically using EGFR protein on the TNBC cells.

4. Conclusions

TNBC is unable to treat with chemotherapy, due to lack of therapeutic receptors on the cell surface. We tried to target the EGFR protein on the TNBC cells. The

nanoparticle encapsulated PTX and anchored EGFR mAb, resulting in an average nanoparticle range that internalized by MDA-MB-468 cells. Treatment with these immunonanoparticle showed significant cytotoxicity compared with PTX nanoparticle and PTX. Moreover the immunonanoparticle significantly reduce the expression of EGFR protein and target specifically on TNBC cells than normal cell as compared with PTX nanoparticle and PTX. Based on the above studies, we concluded that TNBC could be successfully targeted and treated by using immunonanoparticle and this research could be readily translated into clinical trials.

Acknowledgments

This work was fully supported by the Fundamental Research Grant Scheme (FRGS) [Grant number: FRGS/1/2015/SKK09/AIMST/02/4] under the Ministry of Higher Education (MOHE), Malaysia.

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Effect of NaCl and urea on microemulsion systems containing water/IPM and tween80 versus water/IPM and tween80 plus PEG400

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Abstract

In this study, the objective is to explore the effect of common components in the cosmetic products to our designed microemulsion systems. Tween80, isopropyl myristate (IPM) and water were selected for preparing ternary phase diagrams due to their safety and widely used components. Polyethylene glycol 400 (PEG 400) was selected as a cosurfactant. Sodium chloride (NaCl) and urea were selected to study their effects on the mentioned microemulsions as an electrolyte and a humectant, respectively. The results of all phase diagrams showed that these microemulsion systems presented larger water in oil (W/O) region than oil in water (O/W) region. Addition of PEG400 did not provide the broader microemulsion region but provided a better homogenous clear gel. NaCl and urea had an effect on the microemulsion system containing tween80 as a surfactant alone by decreasing the O/W microemulsion region. NaCl and urea had less effect on the microemulsion system containing tween 80 and PEG 400 mixture.

Keywords: Microemulsion, Urea, NaCl, Tween 80, PEG 400

1. Introduction

Microemulsions (MEs) are thermodynamically stable and transparent liquid with nanosize droplets [1]. The systems typically consist of water phase, oil phase and surfactant/cosurfactant. MEs have been extensively studied during last decades in cosmetic fields because of their wide range solubility of components. In this study, IPM was selected as an oil phase due to its moisturizing effect and skin penetration enhancement [2]. Tween80, a non-ionic surfactant, was selected as a surfactant because of its low skin irritation and tolerance to charge disruption. PEG 400 was selected as a cosurfactant since it increases active ingredient solubility and permeability [3]. The ratio 3:1 of tween 80:PEG 400 was selected as a mixed surfactant and cosurfactant. Urea products are topically used for dry skin treatment. Salts are usually used to balance electrolyte in formulas. The aim of this study is to explore the effect of NaCl and urea on ME systems.

2. Materials and methods

IPM, PEG 400, tween 80, NaCl and urea were pharmaceutical grade supplied by S. Thong Chemicals Co., Ltd. Ternary phase diagrams were constructed by water titration method. IPM:tween 80 or tween80 mixed with PEG 400 ratio was varied from 0:10 to 10:0. Water was added drop by drop into each mixture and the mixture was mixed until became homogenous after each addition. Phase diagrams were obtained by visual inspection. ME and clear gel like regions were area of interest. Monophasic, clear, transparent and watery mixture was marked in the ME region. Monophasic, clear, transparent and viscous mixture was marked in the clear gel like region. The temperature was controlled at 25±1 °C throughout the experiment. Then water phase was replaced by

10%urea solution or 0.9%NaCl solution and ternary phase diagrams were constructed in the same fashion. All phase diagrams were depicted and compared.

3. Results and discussion

W/O ME regions, O/W ME regions and clear gel like regions are shown in figure1a-1f. The ME regions where the amount of water and oil were equal and high percentage of surfactant phase were expected bicontinuous ME region as labeled and the mixture became more viscous compared with another part of W/O ME region. The W/O ME region was wider than O/W ME region in all ME systems. These results might be explained by critical packing parameter (CPP) [4] which relates the ability of molecules aggregates based on their geometry of the molecules. The tween80-hydrocarbon tails had more favorable extension into a continuous IPM phase resulting in the lower interfacial tension of W/O ME than O/W ME. Tween80 alone was able to form ME without cosurfactant as shown in figure 1a, 1c and 1e. When PEG 400 was added, the O/W ME region was observed at lower concentration of surfactant mixture while W/O ME region became smaller as shown in figure 1b, 1d and 1f. Clear gel like region cannot be clearly identified as liquid crystalline without any further test.

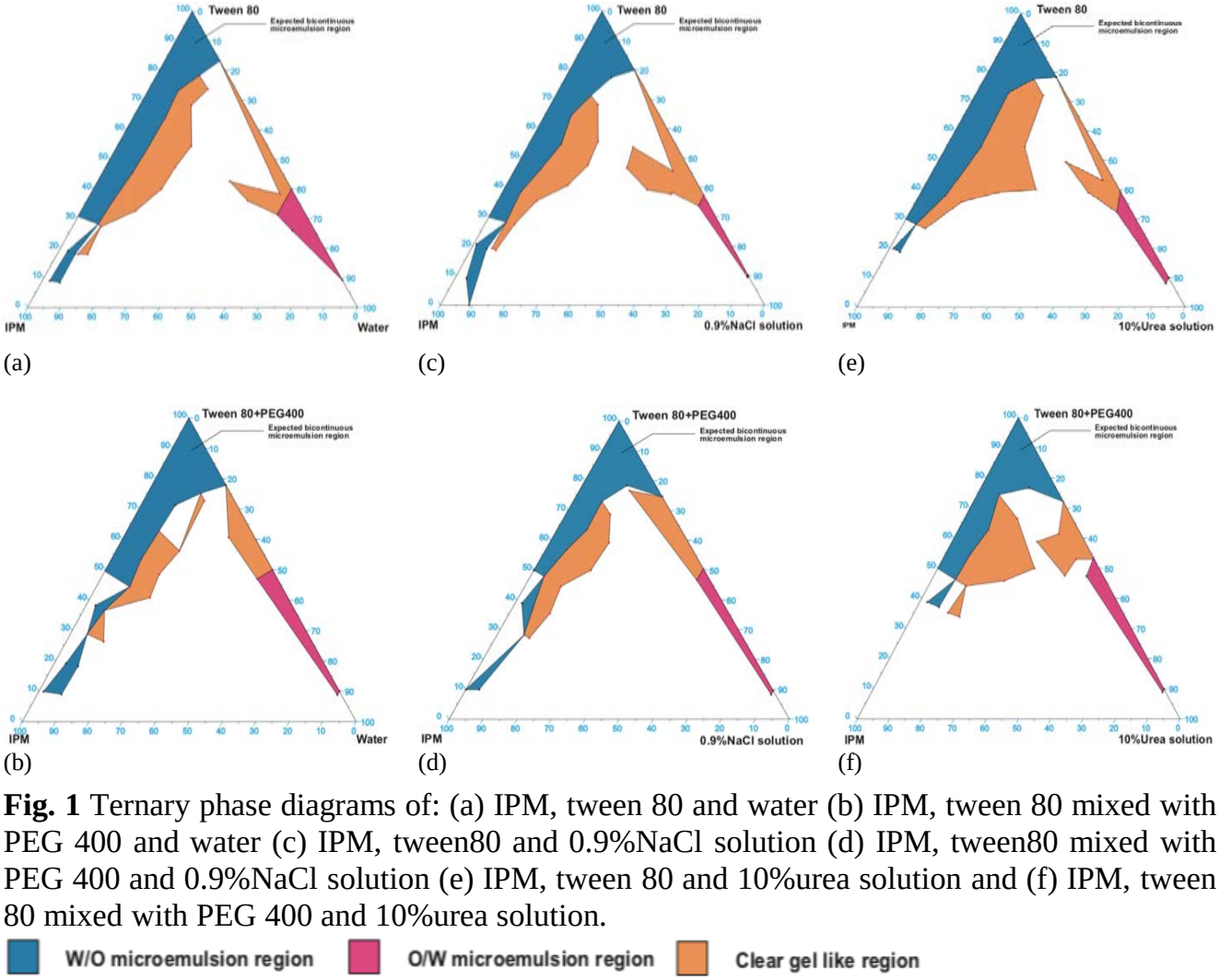

Fig. 1 Ternary phase diagrams of: (a) IPM, tween 80 and water (b) IPM, tween 80 mixed with PEG 400 and water (c) IPM, tween80 and 0.9%NaCl solution (d) IPM, tween80 mixed with PEG 400 and 0.9%NaCl solution (e) IPM, tween 80 and 10%urea solution and (f) IPM, tween 80 mixed with PEG 400 and 10%urea solution.

When both urea and NaCl, water soluble components, were added in the system without cosurfactant, slightly smaller O/W ME region was found and less effect was observed in the system with cosurfactant. This could be due to the interfacial tension and polarity changes in the water phase containing urea or NaCl by increasing of hydrophobicity of tween80, lowering cloud point of the surfactant [5] and increasing interfacial tension between oil and water which decreased the amount of oil being microemulsified [6]. The clear gel like region trended to be larger with this addition.

4. Conclusions

The ternary phase diagram of IPM, tween 80 and water were constructed. Adding PEG400 provided slightly broader region of O/W ME. NaCl and urea trended to decrease the O/W ME region containing tween 80 as a surfactant alone because NaCl and urea increased the hydrophobicity of tween 80 and affected its cloud point. Influence of NaCl and urea was less in the systems containing twee n80 plus PEG 400.

Acknowledgements

Financial support for this independent study was provided by Faculty of Pharmaceutical Sciences, Chulalongkorn University.

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Development and validation of a simple HPLC-UV method for the determination of rifampicin in rifampicin capsules

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Abstract

This study describes the development and validation of a HPLC method for the quantitation of Rifampicin in rifampicin capsules. The Hypersil C18 250x4.6 mm column was used. UV detection was performed at 360 nm. The mobile phase consisted of 0.01 M NaH2PO4: ACN ratio 50:50. Flow-rate was maintained at 1.0 mL/min in ambient temperature and injection volume of sample was 20 µl. The method proved to be linear (r^2 > 0.9998) and precise (RSD < 2.00%), with % recovery of Rifampicin in the capsule samples varying from 95.74% to 102.91%.

Keywords: Rifampicin, HPLC, Validation

1. Introduction

Rifampicin is an antibiotic used for the treatment of tuberculosis, leprosy and legionnaire's disease [1]. However, the United States Pharmacopeia (USP) gradient for Rifampicin analysis is difficult to apply in Thailand. The USP 41 condition [2] was determined by high performance liquid chromatography (HPLC). Mobile phase consisted of water, acetonitrile, phosphate buffer, 1.0 M citric acid, and 0.5 M sodium perchlorate at (510: 350: 100: 20: 20). In accordance with the law, sodium perchlorate is classified in the chemical warfare category (2.3), which makes it difficult to use for analytical methods like the USP at laboratory level or in the pharmaceutical industry as permission is required [3-5]. Here, a simple and easy-to-use method was developed as a guideline for monitoring Rifampicin both qualitatively and quantitatively for research into medicinal products.

2. Materials and methods

2.1 Materials

Rifampicin (purity≥98%) was purchased from Sigma-Aldrich. Acetonitrile is HPLC grade. sodium dihydrogenphosphate is AR grade. rifampicin 300mg/capsule (Rifam®, siam pharmaceutical and Rifadin®, Snofi) were purchase form Fascino drug store. Water for all applications was obtained from a Arium 611UV (Sartorius stedim biotech; resistivity 18.2 MΩcm).

2.2 HPLC

HPLC analysis was performed on a Agilent and column was a Hypersil C18 250x4.6 mm 5 um. All separations were carried out isocratically with a mobile phase comprised of 0.01 M NaH_2PO_4: ACN ratio 50:50. The flow-rate was maintained at 1.0 mL/min, and a 20 uL sample volume was injected for all experiments. The UV detection was performed at 360 nm

2.3 Method validation

The HPLC-UV method for the determination of rifampicin was validated for the linearity, accuracy (recovery), precision, LOD, and LOQ were determined according to AOAC guideline (2012).

3. Results and discussion

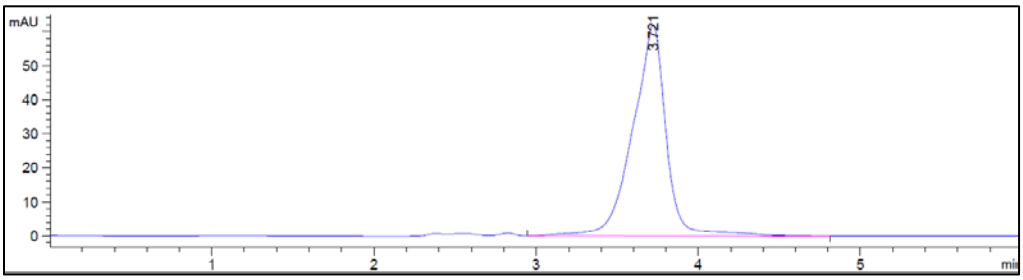

Fig. 1 Chromatrogram of Rifampicin

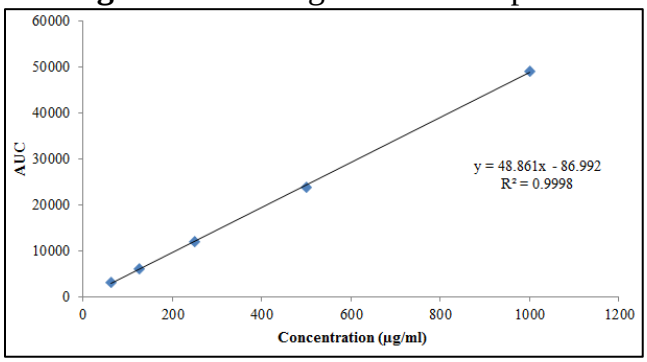

Fig. 2 Standard curve

3.1 Linearity

In the assay, a linear correlation was found between the peak areas and the Rifampicin concentrations range studied of 62.50-1,000 µg/ml. The regression analysis data are presented in Figure 2. The regression coefficients (r^2) were higher than 0.9998

3.2 Precision

Mean contents of rifampicin in the intra-day precision analysis (n=3) were 62.5 ug/ml (RSD = 0.26%), 125ug/ml (RSD = 0.14%) and 250 µg/ml (RSD = 0.34%) respectively. As the result, RSD values lower than 2% assure the precision of the method.

3.3 Accuracy

The rifampicin content (n=3) was found to be 102.91 ± 6.87 to 95.74 ± 3.53. The results are presented in Table 1.

Table 1 The retention time and % recovery of rifampicin

	Retention time (min)	% Recovery
Rifampicin standard	3.721±0.001	98.78±1.06
Rifadin®	3.721±0.000	102.91 ± 6.87
Rifam®	3.720±0.002	95.74 ± 3.53

3.4 Detection and quantitation limits

According to the determined signal-to-noise ratio rifampicin. The limit of detection and limit of quantitation were 3.00 ng/ml and 0.17 µg/ml, respectively.

4. Conclusions

The objective of this study was development and validation of rifampicin in pure form for rifampicin capsules. The developed method showed to be a simple, precise, accurate and suitable technique to quantify the rifampicin content. As well as in further studies might be employed for quality control analysis in other matrices such as plasma[6].

Acknowledgements

The authors express their gratitude to the National Research Council of Thailand for providing the research fund.

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Screening factors affecting on reducing sugar production by statistical design during solid state cultivation of cassava pulp

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Abstract

The fungal biopolymer chitin and chitosan can be applied for biomedical and pharmaceutical applications as wound dressing and carriers of drugs, and can be produced from agricultural by-product. For this reason, bioconversion of cassava pulp into fermentable sugar using starch-digesting fungi for further chitin and chitosan production was studied. In this study, the screening of ten factors affecting on reducing sugar production through solid state cultivation using *A. oryzae* was achieved using Plackett-Burman design. The result showed that moisture content, rice bran, inoculum size, yeast extract and peptone gave the negative effect on sugar yield. In contrast, incubation time $CaCl_2$, KH_2PO_4, NH_4Cl and urea gave the positive effect on sugar yield, which were selected for further optimization process.

Keywords: *Aspergillus oryzae*, Cassava pulp, Plackett-Burman design, Reducing sugar

1. Introduction

The biopolymer chitin and chitosan show excellent biological properties such as non-toxicity, biodegradation in the human body, anticancer, wound healing, haemostatic activity in cell culture, tissue engineering and drug delivery [1]. The chitin is in the fungal cell wall and septa of *Ascomycete, Zygomycete, Basidiomycete* and *Deuteromycatas* thus serving as a strengthening fibrous element responsible for cell wall rigidity [2]. Solid state fermentation (SSF) is an alternative technology for chitin and chitosan production. The SSF provides an environment to support growth of fungi on solid substrates, and is a cost-effective process for the bioconversion [3]. Cassava pulp (CP) is a global crop of economic importance containing an amount of starch and cellulose about 60%, which are biodegradable and can be a substrate to produce the value-added products [4]. Therefore, conversion of CP is the important step to produce the sugars and further ferment to the chitin and chitosan. There are a few of studies which were conducted to use fungi for degrading starch-based materials under SSF. There is need to develop a technology for conversion of these material into products, such as sugars, leading to sustainable waste management strategy. However, there are many variables for sugar production under SSF. Therefore, Plackett-Burman design (PBD) is used for rapid screening multifactor to find the most significant factors [5]. Therefore, this work aims to screen variables using PBD, including moisture content, time, rice bran, inoculum size, $CaCl_2$, KH_2PO_4, NH_4Cl, urea, yeast extract and peptone for sugar production during SSF.

2. Materials and Methods

The strain *Aspergillus oryzae* TISTR 3102 was propagated onto potato dextrose agar for 3 days at 30˚C. Cassava pulp (CP) was dried in hot air oven at 50˚C for 48 h. The experimental design used in this step was the matrix of Plackett-Burman design [5]. The variables were selected from the previous research studied [6-8]. Each variable was examined at two levels: -1 for a low level and +1 for a high level (Table 1). The medium were conducted in 250 ml Erlenmeyer flasks according to the statistical design as showed in Table 1. Then, the medium were autoclaved at 121˚C for 15 min, cooled at the room temperature. The medium were inoculated with spore suspension, soaked with distilled water to the desired moisture content, and incubated at room temperature. The samples were withdrawn after finish the fermentation. The crude extract was obtained by the addition of acetate buffer (50 mM, pH 5.0) for 1 h at 200 rpm. The solids were removed by filtration and the filtrate was measured the RS concentration by the dinitrosalicylic acid (DNS) method [9]. All experiments were performed in triplicates and the average of the observations was used. The main effects of each variable on RS yield were estimated as the difference between both averages of measurements made at the higher level and at the lower level. The software SPSS version 17 was used for statistical and linear regression analysis. The analysis of variance (ANOVA) was used to estimate the statistical parameters.

3. Results and discussion

The response listed in Table 1 indicated a variation in RS yield from 12.98-46.46 g/g CP. The analysis showed that standard run no. 11 gave the maximum yield of RS (46.46 g/g CP). The *t*-test was used to identify the effect of variables on RS yield. The effects of variables, coefficient, *t*-value and significance for the design are showed in Table 2.

Table 1 Plackett-Burman design for evaluation of factors affecting on RS production

Std.	Variable in actual value (code value)										RS yield (mg/g CP)
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	
1	75 (1)	3 (-1)	1 (1)	5 (-1)	0 (-1)	0 (-1)	0.2 (1)	0.2 (1)	0.2 (1)	0 (-1)	20.76 ± 0.67
2	75 (1)	5 (1)	0 (-1)	15 (1)	0 (-1)	0 (-1)	0 (-1)	0.2 (1)	0.2 (1)	0.2 (1)	13.82 ± 2.39
3	55 (-1)	5 (1)	1 (1)	5 (-1)	0.1 (1)	0 (-1)	0 (-1)	0 (-1)	0.2 (1)	0.2 (1)	34.97 ± 3.85
4	75 (1)	3 (-1)	1 (1)	15 (1)	0 (-1)	0.2 (1)	0 (-1)	0 (-1)	0 (-1)	0.2 (1)	12.98 ± 0.87
5	75 (1)	5 (1)	0 (-1)	15 (1)	0.1 (1)	0 (-1)	0.2 (1)	0 (-1)	0 (-1)	0 (-1)	23.15 ± 1.03
6	75 (1)	5 (1)	1 (1)	5 (-1)	0.1 (1)	0.2 (1)	0 (-1)	0.2 (1)	0 (-1)	0 (-1)	31.20 ± 0.57
7	55 (-1)	5 (1)	1 (1)	15 (1)	0 (-1)	0.2 (1)	0.2 (1)	0 (-1)	0.2 (1)	0 (-1)	32.88 ± 3.12
8	55 (-1)	3 (-1)	1 (1)	15 (1)	0.1 (1)	0 (-1)	0.2 (1)	0.2 (1)	0 (-1)	0.2 (1)	36.45 ± 0.92
9	55 (-1)	3 (-1)	0 (-1)	15 (1)	0.1 (1)	0.2 (1)	0 (-1)	0.2 (1)	0.2 (1)	0 (-1)	34.08 ± 1.74
10	75 (1)	3 (-1)	0 (-1)	5 (-1)	0.1 (1)	0.2 (1)	0.2 (1)	0 (-1)	0.2 (1)	0.2 (1)	22.92 ± 0.62
11	55 (-1)	5 (1)	0 (-1)	5 (-1)	0 (-1)	0.2 (1)	0.2 (1)	0.2 (1)	0 (-1)	0.2 (1)	46.46 ± 1.81
12	55 (-1)	3 (-1)	0 (-1)	5 (-1)	0 (-1)	0 (-1)	0 (-1)	0 (-1)	0 (-1)	0 (-1)	35.81 ± 0.70

**A*, moisture content (%); *B*, time (days); *C*, rice straw (g/g CP); *D*, inoculum size (%); *E*, $CaCl_2$ (g/g CP); *F*, KH_2PO_4 (g/g CP); *G*, NH_4Cl (g/g CP); *H*, urea (g/g CP); *I*, yeast extract (g/g CP); *J*, peptone (g/g CP)

Table 2 Analysis of variance for Plackett-Burman model with RS yield as response

Variables	Effects	Coefficients	*t*-value	*Sig*.	Confidence (%)
Constant		28.79	508.059	0.001	
Moisture content	-16.0	-7.98	-140.912	0.005*	99.5
Time	3.2	1.62	28.647	0.022*	97.8
Rice bran	-1.2	-0.58	-10.294	0.062	93.8
Inoculum size	-6.5	-3.23	-57.000	0.011*	98.9
$CaCl_2$	3.3	1.67	29.500	0.022*	97.8
KH_2PO_4	2.6	1.30	22.882	0.028*	97.2
NH_4Cl	3.3	1.64	29.059	0.022*	97.8
Urea	3.3	1.67	29.500	0.022*	97.8
Yeast extract	-4.4	-2.22	-39.147	0.016*	98.4
Peptone	-1.7	-0.86	-15.118	0.042*	95.8

*Statistically significant 95% of confidence level.

The positive sign of the effect (Ex_i) means that an increasing of the level of the variable causes an increase in RS yield; whereas, the negative sign means that an increasing of variable level causes decrease in RS yield. Reducing sugar production of cassava pulp for further chitin and chitosan production require maximum RS yield. Therefore, the positive effect of RS yield was selected. As showed in Table 2, the positive effects of time (3.2), $CaCl_2$ (3.3), KH_2PO_4 (2.6), NH_4Cl (3.3) and urea (3.3) were significant variables for increasing of RS yield. This result implied that an increase in these variables resulted in inducing the secretion of hydrolytic enzymes from fungi such as amylase, cellulase and raw starch degrading enzyme leading to increasing of RS yield [7, 10-12].

In contrast, the negative effects of moisture content (-16), inoculum size (-6.5), yeast extract (-4.4) and peptone (-1.7) were significant variables for decreasing of RS yield. This result indicated that higher moisture level may be decrease porosity, changes particle structure, promotes development of stickiness, reduces gas volume and exchange, and decreases diffusion which results in lowered oxygen transfer leading to reduce RS yield. Moreover, a small quantity of mycelium was enough to inoculate the substrate for RS production. Furthermore, organic nitrogen sources induced the formation of protease resulted in the proteolysis of hydrolytic enzyme [12].

4. Conclusions

From parameter screening by PBD during solid state cultivation of CP with *A. oryzae*, the results showed that, the important parameter affecting on RS production were incubation time, $CaCl_2$, KH_2PO_4, NH_4Cl and Urea. These parameters were selected for optimization of RS production and further chitin/chitosan production.

Acknowledgments

The authors gratefully acknowledge Burapha University (Grant No: 7/2560) for financial support.

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Isolation and identification of major compound from *Tetragonula fuscobalteata* propolis from mangosteen orchard

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Abstract

Today propolis is currently used as a popular remedy. The aim of this research was to isolate the major active compound of *Tetragonula fuscobalteata* Cameron propolis from mangosteen orchard in Thailand. The vacuum liquid chromatography and classical column chromatography were used, with the gradient elution of hexane:ethyl acetate, dichromethane:methanol and hexane:ethyl acetate, respectively. The major compound was elucidated using nuclear magnetic resonance spectroscopy technique and identified as alpha-mangostin.

Keywords: propolis, alpha-mangostin, stingless bee

1. Introduction

Propolis is widely used in folk medicine, and claimed to be useful in cosmetics and as a constituent of health food [1]. Numerous pharmacological activities of propolis were reported such as anti-bacterial [2], anti-inflammatory [3], and cytotoxicity on cancer cells [4]. In Thailand, 3-isomangostin, gamma-mangostin, beta-mangostin and alpha-mangostin were found in the extracts of *Tetragonula pagdeni* propolis [5], but it has not been previously reported in *T. fuscobalteata* propolis. Hence, in this study, we isolated major compound from *T. fuscobalteata* propolis in mangosteen orchard of Thailand.

2. Materials and methods

T. fuscobalteata propolis from mangosteen orchard in Chanthaburi province (50g) was cleaned and cut into small pieces and remove wax with hexane (500 mL) by sonication for 30 minute at 40℃(2 time). After that, the residual extracted with methanol (1L) by sonication for 30 minute at 40℃ (2 time) and evaporated using rotary evaporator. The crude extract 5 g was fractionated by vacuum liquid chromatography on silica gel with hexane:dichloromethane (1:1), dichloromethane, ethyl acetate and actone respectively. The fraction of hexane:dichloromethane (1:1) was subjected to silica gel with hexane:ethyl actate gradient system (100:0 to 0:100), provided 210 fractions, 40 ml each fraction. Next, the fractions 108-130 were combined and then separated using silica gel with dichloromethane:methanol (20:0, 20:0.5, 20:5 and 20:10) to give 51 fractions, 40 ml each fraction. The fraction 5-15 (61.3mg) were combined and purified using silica gel with hexane:dichloromethane (2:8, 1:9, 0:10) to give 32 fraction, 40 ml each fraction. The fraction 5-19 were combined and compared with standard (alpha-mangostin) by TLC and ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy technique [6].

3. Results and discussion

To confirm the structure of the isolated compound 400 MHz of ^{1}H and 100 MHz of ^{13}C NMR were utilized. The data was demonstrated and compared in Fig.1 and Table1 [6]. The isolated compound was identified as alpha-mangostin. The result revealed that

the major active compound of *T. fuscobalteata* propolis from mangoteen orchard was similar to *T. pagdeni* propolis from fruits garden in Chanthaburi province [5].

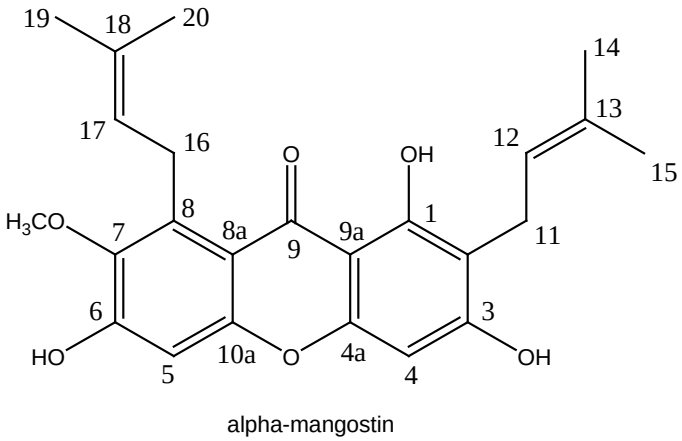

Fig.1 structure of alpha-mangostin

4. Conclusions

The major compound isolated from *T. fuscobalteata* propolis was alpha-mangostin.

Acknowledgements

This work was supported by the Research Grant of Burapha University through National Research Council of Thailand (Grant no.170/2560) and also supported by faculty of Pharmaceutical Sciences, Burapha University. The authors also would like to thank to Mr.Samart Khrueawan, Ms.Chanthon Khrueawan, Mr. Wisit Tanooard as well as Thai local bee keeper of Kon Chan Channarong (stingless bee) in Chantaburi province, Thailand for propolis collection.

Table 1 ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) assignments of alpha-mangostin and their references in $CDCl_3$ (δ, multiplicity (J in Hz))

Position	**^{1}H-NMR ($CDCl_3$)**	**^{1}H-NMR ($CDCl_3$) [1]**	**^{13}C-NMR ($CDCl_3$)**	**^{13}C-NMR ($CDCl_3$) [1]**
1 (-OH)	13.80, *brs*	13.78, *s*	160.6	160.6
2	-	-	108.5	108.4
3 (-OH)	6.26, *brs*	6.15, *s*	161.6	161.6
4	6.32, *s*	6.30, *s*	93.3	93.5
4a	-	-	154.5	154.5
5	6.86, *s*	6.83, *s*	101.6	101.6
6 (-OH)	6.40, *brs*	6.30, *s*	155.1	155.1
7	-	-	142.6	142.6
8	-	-	137.0	137.0
8a	-	-	112.2	112.2
9	-	-	182.0	182.0
9a	-	-	103.6	103.6
10a	-	-	155.8	155.8
11	3.47, *d* (7.2)	3.46, *d* (7.5)	21.4	21.5
12	5.30, *t* (7.6)	5.29, *t* (7.0)	121.5	121.4
13	-	-	132.1	132.2
14	1.76, *s*	1.77, *s*	25.8	25.8
15	1.80, *s*	1.78, *s*	18.2	18.2
16	4.11, *d* (6.0)	4.09, *d* (6.5)	26.6	26.6
17	5.30, *t* (7.6)	5.29, *t* (7.0)	123.1	123.1
18	-	-	135.8	135.1
19	1.88, *s*	1.84, *s*	17.9	17.9
20	1.72, *s*	1.69, *s*	25.9	25.9
7-OCH_3	3.83, *s*	3.80, *s*	62.1	62.1

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Preliminary of development and evaluation of wound dressing containing *Centella asiatica* extract (ECa233)

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Abstract

The herb *Centella asiatica* has historical used as a medicinal plant to treat various disorders and minor wounds but clinical efficacy has never been proven. A wound dressing containing *Centella asiatica* extract (ECa233) was developed and formulated by a film casting method using polyvinyl alcohol (PVA) 8% w/v as a polymer base combined with other hydrogel polymers. Each of the formulations was evaluated for chemical and physical characteristics. Results demonstrated that PVA + Sodium carboxymethylcellulose (SCMC) 3.0% w/v had the best swelling capacity. Combinations of PVA with other polymers at the ratio 8.0:1.0% w/v gave highest % elongation at breakage with the exception of combinations with gelatin. PVA combined with other hydrogel polymers showed higher levels of controlled release and swelling capacity than the control (PVA). This research is still in the preliminary stages and further studies are required to develop formulations with more suitable dosage forms.

Keywords: Wound dressing, *Centella asiatica* extract (ECa233)

1. Introduction

The herb Centella asiatica (Linn.) has long been used as a traditional medicine for wound healing, promoting anti-inflammatory, ant psoriatic, antiulcer, antibacterial and antifungal pharmacological benefits. Centella asiatica extract was developed as ECa233, containing major active compounds at more than 80% triterpenoid glycosides with a madecassoside to asiaticoside ratio of 1.5(±0.5):1.0. Preparations of ECa233 gel are used as a treatment for wound healing [1]. However, these preparations are limited with dosages which are hard to control.

ECa233 is a Centella asiatica extract which has a processed semipurification to high purity of the substance (asiaticoside and madecassoside). ECa233 has advantages over the crude extract because it is easy to control the quality of the drug.

Wound dressing applies topical dosage to control drug release, improve patient compliance, enhance wound exudate properties and decrease local irritation from antiseptic solution. Some reports suggest that wound dressing reduced wound healing rate [2]. Polyvinyl alcohol (PVA) is a synthetic biocompatible hydrophilic linear polymer. It is considered as toxicologically safe and normally used as an excipient for various pharmaceutical products. However, its controlled drug release, air permeability and absorption of wound exudate properties are limited [3]. improved these limitations by combining PVA with other hydrogel polymers. But, the use of ECa233 is not available in the commercial product. Especially for wound dressing patches. Therefore, this study

aims to discover the feasibility of preparing ECa233 to develop into a wound patch. By study the incompatibility, the ability to form sheets and physical stability.

2. Materials and Methods

Materials: Standardized extract of Centella asiatica (ECa233), sodium alginate, sodium carboxymethylcellulose (SCMC), hydroxypropyl methylcellulose E15 (HPMC E15) and gelatin, PVA (99% hydrolyzed), glycerin, methylene blue, ethanol, phosphate buffer pH 6.8 (monosodium phosphate and dibasic sodium phosphate), deionized (DI) water, and acetonitrile (HPLC grade) were used.

Methods: Wound dressings were prepared in triplicate lots by film casting method. Dissolved PVA in DI water and heated 110 °C 3 hrs add to PVA 8% w/v. Add hydrogel polymer (sodium alginate, SCMC, HPMC E15, gelatin) in various ratios (0.5, 1.0 and 3.0% w/v). Add ECa233 1.0% with glycerin 5% used as a plasticizer. A fixed volume (15 ml) of polymeric solution with ECa233 and plasticizer was poured into a glass Petri dish and then dried in an oven at 50 °C for 12 hrs.

Table 1 Formulation table of wound dressing containing ECa233

Formulations	FA00	FA01	FA02	FA03	FA04	FA05	FA06
PVA	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Sodium alginate	-	0.5	3.0	-	-	-	-
SCMC	-	-	-	0.5	3.0	-	-
HPMC E15	-	-	-	-	-	0.5	-
Gelatin	-	-	-	-	-	-	0.5
Glycerin	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Ethanol	5.0	5.0	5.0	5.0	5.0	5.0	5.0
ECa233	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Water qs to	100	100	100	100	100	100	100

Evaluation of wound dressing

- Compatibility: Detected by Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR).
- Physical appearance: Visually inspected for color, homogeneity and smoothness. Surface area morphology was measured and assessed by scanning electron microscope (SEM).
- Film thickness: Each formulation was measured at five different places on a single patch.
- Weight variation: Each patch with a diameter of 2x2 cm was weighed on a digital balance and mean values were calculated.
- Mechanical properties of tensile strength, elongation at break and Young's modulus were measured.
- Swelling properties: To determine the swelling index, preweighed patches (approximately 2x2 cm diameter) from each formulation were placed in Petri dishes containing phosphate buffer pH 6.8 to ensure complete immersion. At interval times, swollen patches were weighed accurately after removal of the excess fluid [4].

- *In vitro* drug release: Patches 2x2 cm diameter from each formulation were placed into 25 ml phosphate buffer pH 6.8 on an orbital shaker 100 rpm [5]. Samples of the solution were taken at different times (15, 30, 60, 180, 360, 720, 1440 minutes) for HPLC analysis at 220 nm.
- Stability study : To determine the physical properties of patches such as moisture content, appearance they were kept at room temperature for 45 days

Variable data are represented as mean ± standard deviation (n=3).

3. Results

All formulations demonstrated that physical appearance have homogeneity but when increase concentration ratio of hydrogel polymer, the smoothness decrease because more difficult film casting process. Determination of morphology surface area by SEM showed that 35X found surface of FA04 have less cracking than control. DSC thermograms indicated compatibility between PVA and the other polymers. Thermograms recorded Tm point and Td point in every formula but FA00, FA03, FA04, FA05 and FA06 showed increased Td point with increased stability. Some formulas had a shift down of Td point with decreased stability such as FA01 and FA02.

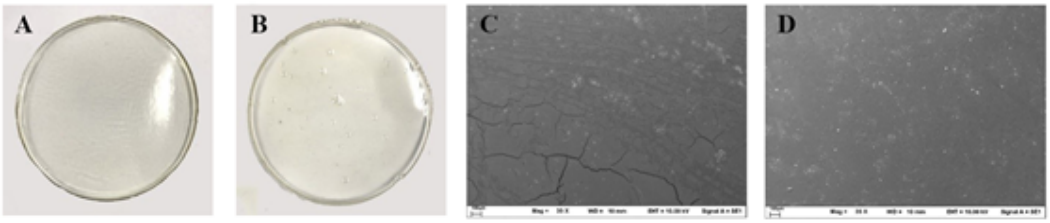

Figure 1 Representative photograph of (A) FA00, (B) FA04, (C) SEM FA00, (D) SEM FA04.

The swelling profile in Figure 2 (B) demonstrated an increase in % swelling index with increased concentration ratio of hydrogel polymer. Highest percentage swelling was recorded for PVA combined with SCMC with statistically significant differences between the other formulations. Swelling behavior can be explained as the hydroxyl group in the molecules plays an important role in the integrity of the swollen hydrophilic cellulose matrices [6]. This formulation showed less erosion properties than other hydroxyl group polymers such as sodium alginate. Study of the *in vitro* drug release of wound dressing containing ECa233 demonstrated release of the active compounds adecassoside and asiaticoside.

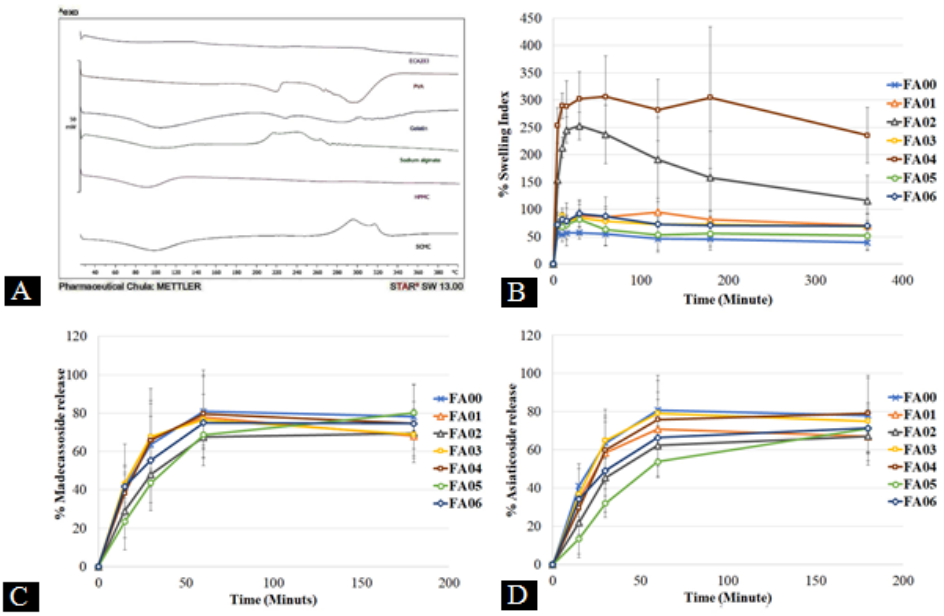

Figure 2 Representative photograph of (A) Thermogram of ECa233 and excipients, (B) Swelling Index of wound dressing contain ECa233, (C) Release profile of Madecassoside in wound dressing contain ECA233, (D) Release profile of Asiaticoside in wound dressing contain ECA233.

Formulation of PVA combined with 3.0% SCMC provided the highest cumulative madecassoside release about 80% at 60 minutes and cumulative asiaticoside release about 80% at 180 minutes. Stability studies of all formulations showed no significant change in physical characteristics after 45 days.

4. Conclusions

Results demonstrated that PVA combined with 3.0% SCMC gave the best swelling capacity (306.36 ± 74.85% swelling index, $p < 0.01$). Combinations of PVA with other polymers in the ratio 8.0 : 1.0% w/v gave highest % elongation at breakage, with the exception of the combination with gelatin. PVA combined with other hydrogel polymers showed higher levels of controlled release and swelling capacity than the control (PVA). However, this research is still in the preliminary stages. Therefore, further studies are necessary to develop formulations with more suitable dosage forms.

Acknowledgments

The authors would like to thank Assoc. Prof. Dr. Mayuree H. Tantisira for providing the *Centella asiatica* extract (ECA233) and Burapha University for their support throughout the study. The authors would also like to thank Mr. Watcharapong Chaemsawang, Dr. Nichakan Peerakam, Miss Putthiporn Khongkeaw and Miss Pattaravadee Srikoon for their constant encouragement and support.

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Antibacterial activity against *Propionibacterium acnes* of *Lysiphyllum strychnifolium* (Craib) A. Schmitz leaves extract products

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Abstract

The objective of this study was to study antibacterial activity against *Propionibacterium acnes* of *Lysiphyllum strychnifolium* (Craib) A. Schmitz leaves extract products. The *L. strychnifolium* (Craib) A. Schmitz leaves were extracted by maceration technique using 50% ethanol as a solvent. The antibacterial activity against *P. acnes* was determined by disc diffusion method. In addition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract were performed by broth microdilution method. The result showed that the extract inhibited *P. acnes* with the inhibition zone of 17.8 ± 2.1 mm. The MIC and MBC values of the extract against *P. acnes* were 6.25 and 50 mg/mL, respectively. Then, the extract was further developed as cosmeceutical products in two dosage forms consisting of creams and gels. After that, each product was tested for antibacterial activity against *P. acnes* using disc diffusion method. The result revealed that the cream showed a greater inhibition zone against *P. acnes* than the gel did. In conclusion, the *L. strychnifolium* (Craib) A. Schmitz leaves extract cream possessed ability to use as alternative anti-acne cosmeceutical product in the future.

Keywords: *Lysiphyllum strychnifolium* (Craib) A. Schmitz, *Propionibacterium acnes*, cosmeceutical product, antibacterial activity

1. Introduction

Acne is a skin disease which affecting personality and self-confidence especially in adolescence. Acne is caused by inflammation of pilosebaceous unit in the area of face, neck, back, and chest. In addition, several factors can cause acne such as hyperkeratinization, over production of fat and bacterial pathogen especially *Propionibacterium acnes***.** The standard treatment of *P.acnes* is using topical antibiotic and oral antibiotic in cases of mild to moderate acne and severe acne, respectively [1]. However, this regimen leads to antibiotics-resistance strain of *P.acnes*. The previous studies demonstrated the using of herbal plant extract for inhibition of *P. acnes* such as *Trachyspermum ammi* (L.) Sprague extract [2]. Therefore, herbal plants have the

potential to be developed as cosmeceutical product for treatment of *P. acnes* infection. The *Lysiphyllum strychnifolium* (Craib) A. Schmitz also known as Ya Nang Dang is a herb that generally found in Thailand. In Thai traditional medicine, the leaf and root can be used for detoxification and relief of constipation**.** Analysis of phytochemicals in this plant showed that it contained flavonoid compounds namely catechin and myricetin including phenolic compounds namely gallic acid, syringic acid and p-cumaric acid [3]. These phytochemicals possess the antibacterial activity. Therefore, this study was to study antibacterial activity against *P. acnes* of *Lysiphyllum strychnifolium* (Craib) A. Schmitz leaves extract products and propose an alternative product for treatment of acne caused by *P. acnes* infection.

2. Materials and Methods

2.1 Extraction of *Lysiphyllum strychnifolium* (Craib) A. Schmitz leaves

The *Lysiphyllum strychnifolium* (Craib) A. Schmitz leaves were washed and dried in the hot air oven at 50 °C for 10 hours. The dried leaves were cut into small pieces and macerated in 50% ethanol in the ratio of 1:10 (w/v) and shaking for 8-10 hours per day. After 3 days of maceration, the extract was vacuum-filtered and the residues of leaves were macerated in the same aforementioned methods in 3 times. The extract was pooled, evaporated using rotary evaporator and stored at 4 °C for further experiments. The percentage yield of the *L. strychnifolium* (Craib) A. Schmitz leaves extract was calculated as shown in equation 1.

The percentage yield = (weight of the extract / weight of the leaves) x100…equation 1

2.2 Determination the antibacterial activity of *L. strychnifolium* (Craib) A. Schmitz leaves extract against *P. acnes* by disc diffusion method

The *P. acnes* DMST 14916 was obtained from Department of Medical Science, Ministry of Public Health, Thailand. The pathogen was cultured on brain heart infusion (BHI) agar in anaerobic conditions at 37 °C for 72 hours. Eight hundred milligram extract was diluted with 1 ml of 95% ethanol. The 6 mm paper disc was impregnated with the extract and left for 30 minutes. The impregnated disc was placed onto the BHI agar containing *P. acnes*. The positive control was standard 10 µg of Norfloxacin and Ampicillin disc (Oxoid). The negative control was blank disc impregnated with 95% ethanol. After 72 hours of incubation in aforementioned conditions, the zone of inhibition around the disc was measured by using vernier caliper [**4, 5**]. The experiment was performed in triplicate.

2.3 Determination of *P. acnes* susceptibility to *L. strychnifolium* (Craib) A. Schmitz leaves extract by broth microdilution method

The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract against *P. acnes*. The thioglycollate fluid medium was supplemented with 10% dimethyl sulfoxide to dissolve the extract. The initial concentration of the extract before 2-folded dilution was 800 mg/ml. Fifty microliter of 2-folded serial dilutions of the extract were prepared in a 96-well plate. Fifty microliter of *P. acnes* culture was added into each well to make a final concentration of approximately 10^7 CFU/mL. The *P. acnes* cultured in broth and broth alone were used as positive and negative growth control, respectively.

The plate was incubated in anaerobic condition at 37 °C for 72 hours. The MIC value was defined as the lowest concentration of the extract that inhibited visible growth of the *P. acnes*. To determine the MBC value, 10 µL of broth was removed from each well and spotted onto BHI agar. After incubation at 37 °C for 72 hours, the number of surviving *P. acnes* was counted. The lowest concentration where less than 0.1% of the initial inoculum survived was defined as a MBC value. The experiment was performed at least triplicate.

2.4 Determination the antibacterial activity against *P. acnes* of *Lysiphyllum strychnifolium* (Craib) A. Schmitz leaves extract products

The suitable formulas of cream base and gel base were prepared. According to Table 1, the cream was performed using beaker method. The ingredients in oil phase (o) were heated to 70 degree Celsius in a beaker. On the other hand, the ingredients in water phase (w) were heated to 70 degree Celsius in the other beaker. The oil phase was poured into the water phase and continuing stirred. When the cream temperature was cooled to 45 degree Celsius, the extract and paraben concentrate were added and mixed well. For the gel, ethylene diamine tetra-acetic acid was dissolved in water. Carbopol 934® was dispersed in the solution of ethylene diamine tetra-acetic acid and stirred. Triethanolamine was added to neutralized until the dispersion was accomplished to pH 5.5. Finally, glycerin, paraben concentrate and the extract were added and mixed well. Both preparations were accelerated stability test by freeze thaw cycling method. The objective of freeze thaw cycling was to roughly determine the physical and biological stability of the preparations. One cycle of freeze thaw cycling consisted of 24 hours storage at 4 degree Celsius and switching to 24 hours storage at 45 degree Celsius. Antibacterial activity against *P. acnes* of both preparations was performed in triplicate by disc diffusion method before the first cycle and after the fifth cycle. Briefly**,** the 6 mm paper disc was impregnated with either the cream or the gel and left for 30 minutes**.** The procedure of disc diffusion method was performed as mention in 2.2 by using 95% ethanol as a negative control.

3. Results and discussion

3.1 Antibacterial activity of *L. strychnifolium* (Craib) A. Schmitz leaves extract against *P. acnes*

The percentage yield of the *L. strychnifolium* (Craib) A. Schmitz leaves extract was 28.87%. The concentration of the extract in the impregnated disc was approximately 800 mg/mL. The results in Table 2 showed that the extract could inhibit *P. acnes* with the inhibition zone of 17.8 ± 2.1 mm which larger than that of standard norfloxacin (10.5 ± 0.2 mm).The broth microdilution method was carried out to investigate further for MIC and MBC of the extract against *P. acnes* and the result revealed that MIC and MBC values were 6.25 mg/mL and 50 mg/mL, respectively. Kraithep *et al.* (2017) determined antibacterial activity of *L. strychnifolium* (Craib) A. Schmitz stem extract against *Streptococcus mutans* and found that the ethanol and the aqueous extract showed the MIC and MBC of 0.25 mg/mL and 0.50 mg/mL, respectively. Therefore, our results have

evidenced antibacterial activity of *L. strychnifolium* (Craib) A. Schmitz. extract against anaerobe Gram positive bacteria [6]

Table 1 Formulation of *L. strychnifolium* (Craib) A. Schmitz leaves extract cream and gel

Ingredients	*L. strychnifolium* (Craib) A. Schmitz leaves extract Cream (100 g)	*L. strychnifolium* (Craib) A. Schmitz leaves extract Gel (100 g)
Cremophor A25® (o)	2.0 g	-
Cremophor A6® (o)	2.0 g	-
Light liquid paraffin (o)	6.0 g	-
Stearyl alcohol (o)	2.0 g	-
Cetyl alcohol (o)	2.0 g	-
Paraffin wax (o)	2.5 g	-
Isopropyl myristate (o)	6.0 g	-
Propylene glycol (w)	8.0 g	-
Ethylene diamine tetra-acetic acid (w)	0.1 g	0.1 g
Paraben concentrate	1.0 g	1.0 g
Carbopol 934®	-	1.0 g
Glycerin	-	5.0 g
Triethanolamine	-	qs to pH 5.5
L. strychnifolium (Craib) A. Schmitz leaves extract	2.5 g	2.5 g
Purified water (w)	65.9	90.4

Table 2 Antibacterial activity of *L. strychnifolium* (Craib) A. Schmitz leaves extract against *P. acnes* by disc diffusion method

Pathogen	Inhibition zone (mm)			
	Lysiphyllum strychnifolium (Craib) A. Schmitz leaves extract	Norfloxacin	Ampicillin	Negative control (95% ethanol)
P. acnes	17.8 ± 2.1	10.5 ± 0.2	31.9 ± 2.3	No inhibition zone

3.2 Determination the antibacterial activity against *P. acnes* of *L. strychnifolium* (Craib) A. Schmitz leaves extract products

Antibacterial activity of *L. strychnifolium* (Craib) A. Schmitz leaves extract cream and gel against *P. acnes* before and after freeze thaw cycling was summarized in Table 3. The results showed that the cream demonstrated a higher inhibition zone than the gel did. It might cause by the formulation of the cream that consisting of 6% isopropyl myristate

as emollient. Because, Tsuji and Robertson (1973) reported that isopropyl myristate was able to reduce *P. aeruginosa* population [7]. Therefore, antibacterial activity of isopropyl myristate might increase the anti *P. acnes* activity of *L. strychnifolium* (Craib) A. Schmitz leaves extracts and hence leading to a large inhibition zone of the cream. In addition, the inhibition zone of each preparation after freeze thaw cycling was larger than that of before freeze thaw cycling. This phenomenon might cause by viscosity decreasing of the cream and gel that was observed after freeze thaw cycling. The less viscosity of the preparations might support the diffusion of the extract in the agar that led to a greater clear zones [8].

Table 3 Antibacterial activity of *L. strychnifolium* (Craib) A. Schmitz leaves extract cream and gel against *P. acnes* before and after freeze thaw cycling by disc diffusion method

Disc	Inhibition zone (mm)			
	L. strychnifolium (Craib) A. Schmitz leaves extract cream		*L. strychnifolium* (Craib) A. Schmitz leaves extract gel	
	Before freeze thaw cycling	After freeze thaw cycling	Before freeze thaw cycling	After freeze thaw cycling
The preparation	24.03 ± 3.83	34.27± 0.15	18.33 ± 1.11	26.43 ± 5.61
Negative control (95% ethanol)	No inhibition zone	No inhibition zone	No inhibition zone	No inhibition zone

4. Conclusions

The *Lysiphyllum strychnifolium* (Craib) A. Schmitz leaves extract could inhibit *P. acnes*. The cream containing the extract showed the better antibacterial activity against *P. acnes* than the gel. The results suggested that this developed cream could be used for alternative treatment of acne caused by *P. acnes* infection.

Acknowledgments

This work was supported by the research grant of Bansomdejchaopraya Rajabhat University through annual government statement of expenditure for fiscal year 2017.

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Radiolytic stabilizer for preparation and stabilization of radiolabeled ^{68}Ga-vascular adhesion protein 1

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Abstract

Vascular adhesion protein 1 (Vap-p1) is practically absent in normal endothelium and is induced upon inflammation. It is suitable for labeling to diagnose sterile inflammation. DOTA-PEG is the most suitable ligand conjugate structure when label with the radioisotope gallium-68. The structure is conjugated with ligands. Study on the optimum conditions for labeling DOTAVAP-P1 with gallium-68. The radiochemical purity was accepted when used sodium acetate or ammonium acetate as the buffer solution, at pH 4.4-5.0. A set of various concentrations ranged from 0 to 30 milligram w/w or 10% w/v of gentisic acid and ascorbic acid were added during the labeling in order to decrease impurities which caused by radiolytic oxidation by-products of ^{68}Ga-DOTAVAP-P1 that were observed in the chromatogram of the analytical radio-HPLC. The addition of those radiolytic stabilizer to the reaction mixture showed significant improvement on the radiochemical purity of ^{68}Ga- DOTAVAP-P1 and they were achieved in avoided the radiolysis and significantly increased the stability.

Keywords: Gallium-68, Vap-p1, DOTAVAP-P1, Radiolytic stabilizer

1. Introduction

Non-invasive imaging of inflammation would be a highly valuable. Nuclear imaging modalities, single photon emission computed tomography (SPECT) and positron emission tomography (PET), offer functional and molecular information with high sensitivity. Vascular adhesion protein 1 **(**Vap-p1**)** is a novel adhesion molecule for inflammatory disease; its membrane bound homodimer with the molecular weight of approximately 90 kDa/each monomer. The active site is large enough to accommodate an amino acid side chain and might interact with a larger molecule, such as a peptide or protein ligand. Vap-p1 contains several sites for glycosylation. The glycosylation is needed for Vap-p1 to function properly during leukocyte adhesion [1]. Vap-p1 is also found in other tissues in addition to the inflamed synovial. It is practically absent in normal endothelium and is induced upon inflammation. The early translocation of Vap-p1 onto the endothelial cell surface was within an hour after the stimulus. It's suggests that the function may be connected to the early recruitment of polymorphonuclear leukocytes.

Even though Vap-p1 seems to play an important role in the early events of inflammation, its expression on the cell surfaces stays constant for a longer time period, suggesting that its can still be targeted after the first phase of inflammation, and making it a promising target for anti-adhesive therapy. DOTA-PEG is the most suitable ligand conjugate structure of the conjugated Vap-p1 targeting peptides when label with the

radioisotope gallium-68. The structures were show in Figure 1. The aims of this study were to find the optimum condition of labeling ^{68}Ga- DOTAVAP-P1 and to evaluate novel PET imaging agents targeting vascular adhesion protein-1 in animal models of inflammation and cancer.

2. Materials and Methods

Vap-p1 was labeled with ^{68}Ga and DOTA ligand. Labeling conditions were optimized by adjusted the amount of peptide, pH and buffer.^{68}Ga was obtained from a ^{68}Ge/^{68}Ga generator by elution with 0.1 M HCl. [^{68}Ga]Cl_3, DOTAVAP-P1 was added, eluate was mixed with sodium acetate or ammonium acetate to give a pH of approximately 4.4–5.0. The mixture was incubated at 95°C for 20 min. A set of various concentrations of radiolytic stabilizer, ascorbic acid and gentisic acid, ranged from 0 to 30 milligram w/w or 10% w/v were added during the labeling. The effect of stabilizers on radiopeptides stability at room temperature was systematically categorized applying chromatography techniques. The radiochemical purity was determined by reversed-phase Radio-HPLC (Elysia-raytest) with C18 column (7.8×300 mm^2, 125 Å, 10 μm). The HPLC conditions were as follows: flow rate = 4 ml/min, λ = 215 nm, A = 2.5 mM trifluoroacetic acid, B = acetonitrile and C = 50 mM phosphoric acid. The linear A/B/C gradient was 100/0/0 for 0 to 3 min, 40/60/0 for 3 to 9 min, and 0/0/100 for 9 to 16 min.

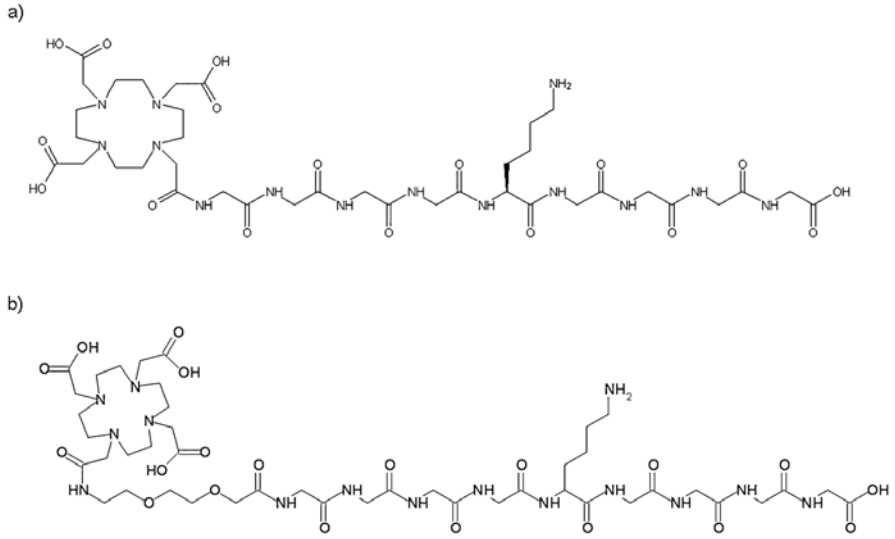

Fig. 1 Structures of VAP-1 binding peptides. a) DOTAVAP-P1 with the amino acid sequence: GGGGKGGGG and a molecular weight of 989.011 g/mol, b) DOTAVAP-PEG-P1 with the amino acid sequence: GGGGKGGGG and a PEG spacer (8-amino-3,6-diooxaoctanoyl). The molecular weight of the peptide is 1133.5 g/mol [2].

3. Results and discussion

The results as show in Figure 2 have been shown that 3 mg/ml and 5 mg/ml of gentisic acid increased the radiochemical purity of radiolabeled compound ^{68}Ga-DOTAVAP-P1 in sodium acetate and ammonium acetate pH adjustment solution, respectively. At pH 4.4 - 4.7, the 5 mg/ml and 3 mg/ml of gentisic acid increased the radiochemical purity of radiolabeled compound to 93.41±0.48 % in ammonium acetate and to 92.68±0.486 % in sodium acetate, respectively.

For ascorbic acid as show in Figure 3, 10, 15 mg/ml and 10 mg/ml of ascorbic acid increased the radiochemical purity of radiolabeled compound ^{68}Ga- DOTAVAP-P1 in sodium acetate and ammonium acetate pH adjustment solution, respectively. The 10 mg/ml of ascorbic acid increased the radiochemical purity of radiolabeled compound to 93.9±0.7% in ammonium acetate and to 81.4±0.4% in sodium acetate. Moreover, with 15 mg/ml of ascorbic acid can also increase the radiochemical purity of radiolabeled compound to 84.2±0.4%, but the shoulder peak still can be observed.

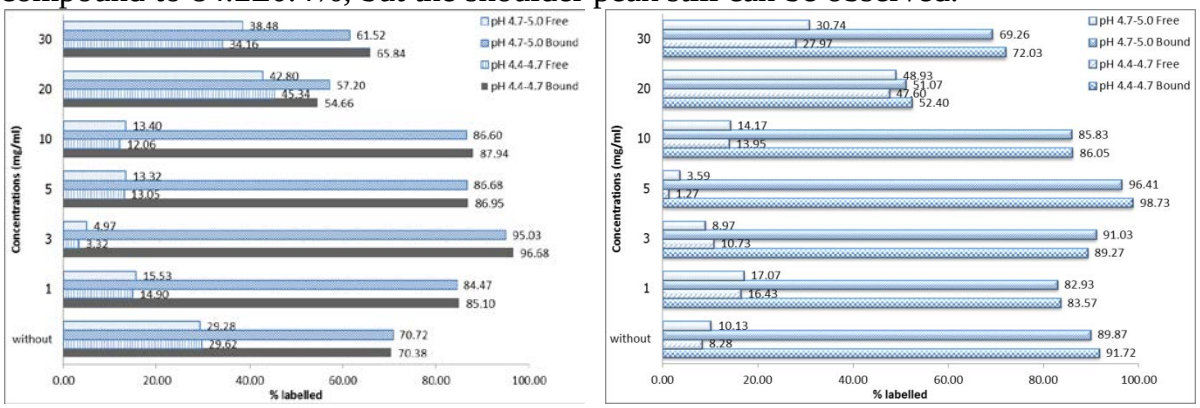

Fig. 2 The labeled percentage with concentration of gentisic acid

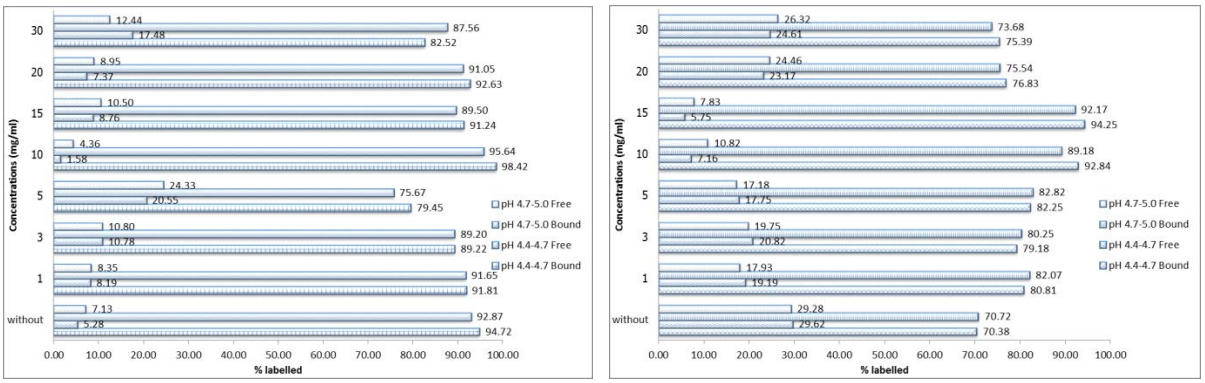

Fig. 3 The Labeled percentage with concentration of ascorbic acid

4. Conclusions

The results showed that as a radiolytic stabilizer, the suitable concentrations of both gentisic acid and ascorbic acid can significantly increase radiochemical purity of ^{68}Ga- DOTAVAP-P1 radiolabeled compound.

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Development of novel microemulsion for solubility enhancement of celecoxib

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Abstract

This study aimed to develop a novel microemulsion that contained Kolliphor EL and Transcutol P as a surfactant and a co-surfactant to enhance the solubility of celecoxib. The prepared microemulsion was evaluated for its celecoxib solubility, particle size, polydispersity index, zeta potential and electrical conductivity. The obtained microemulsion has a particle size of less than 600 nm and neutral surface charge. This microemulsion could dramatically improve the celecoxib solubility.

Keywords: microemulsion, celecoxib, solubility enhancement

1. Introduction

Rheumatoid arthritis, osteoarthritis and gout are common diseases which frequently found in older patients. These diseases always cause pain and disturb daily life of patients. To improve patients' quality of life, a drug providing high efficacy for pain relief with less side effects is needed.

Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) which has a specific cyclooxygenase (COX)-2 inhibitors. Celecoxib used for treatment of pain and inflammation of osteoarthritis, rheumatoid arthritis and gout without gastrointestinal tract (GI) irritation. However, celecoxib has very low water solubility (~5 μg/ml) [1] and very high log partition coefficient (log P= 3.9) [2]. Due to celecoxib's physicochemical properties, celecoxib exhibits low oral absorption and bioavailability. Microemulsions are transparent colloidal systems consisting of oil, surfactant, co-surfactant and water. The advantages of microemulsions are spontaneous formation, thermodynamic stability, the simplicity of manufacture and high solubilization capacity for both lipophilic and hydrophilic compounds. This study, therefore, selected microemulsion as a tool for solubility enhancement and to improve oral bioavailability of celecoxib.

2. Materials and Methods

Celecoxib was purchased from Power tech chemical Co. Ltd., Bangkok, Thailand. Kolliphor EL (PEG-35 castor oil) was purchased from BASF Corporation, Ludwigshafen, Germany. Transcutol P (diethylene glycol monoethyl ether) was purchased from Carlo Erba Reagents, Milan, Italy. All other reagents were of analytical grade and were commercially available.

2.1 Solubility study

The solubility of celcoxib in various oils and surfactants was determined by adding an excess amount of celcoxib into 2 g of each vehicle in a glass bottle, followed by mixing with a magnetic stirrer at 25 °C for 24 h. The suspension was centrifuged at 10,000 rpm for 10 minutes to remove the excess celcoxib, after which the concentration of celecoxib in the supernatant was measured using HPLC after an appropriate dilution

with isopropyl alcohol. The oil and surfactant that provided the highest solubility of celcoxib was chosen as oil and the surfactant for microemulsion preparation with the goal of increasing the solubility of celecoxib

2.2 Preparation of microemulsion

2.2.1 Construction of pseudo-ternary phase diagram of microemulsion

The surfactant and co-surfactant were weighed in each glass bottle and were vortexed vigorously for 20 s to produce the surfactant mixture (Sm). Afterward, the oil phase and the Sm were mixed to produce ratios of oil to Sm in the mixtures that varied from 9:1 to 1:9 (w/w). Distilled water was added dropwise to each oil/Sm mixture with gentle stirring to allow equilibration. After the addition of each aliquot of the water phase, the mixture was visually examined for transparency. The transparent mixtures were defined as the microemulsion region.

2.2.2 Solubility of celecoxib in microemulsion

An excess amount of celecoxib was added to the microemulsion formulations. The samples were continuously stirred for 24 h at 25 °C. Then, the supernatants were collected, and the celecoxib concentrations were determined using HPLC after an appropriate dilution with isopropyl alcohol.

2.2.3 Preparation of celecoxib-loaded microemulsion

Based on the microemulsion region in the pseudo-ternary phase diagrams, a microemulsion system that demonstrated good physicochemical properties was selected. The desirable criteria for choosing the formulations were having a particle size within 20-900 nm without signs of phase separation. Celecoxib was loaded into the microemulsions at 90 % of the respective solubility as determined from the experiments described in section 2.2.2. The blank microemulsion was prepared by mixing the oil, the surfactant/co-surfactant mixture and water by weight ratio using a magnetic stirrer at ambient temperature. Celecoxib was accurately weighed in a volumetric flask, and then the blank microemulsion was approximately added at 90 % of the flask volume. The volumetric flask containing celecoxib in microemulsion was placed in a sonicator bath at 25 °C until the clear solution was obtained. The blank microemulsion was added in volumetric flask to adjust the final volume.

2.3 Characterization of microemulsion (**Mean droplet size, surface charge, size distribution and electrical conductivity**)

The droplet size, surface charge (zeta potential), size distribution (polydispersity index) and electrical conductivity of the microemulsion formulations with and without celecoxib were measured using a Dynamic Light Scattering (DLS) particle size analyzer (Zetasizer Nano-ZS, Malvern Instrument, Worcestershire, UK) with a 4 mW He–Ne laser at a scattering angle of 173°. All the measurements were carried out under ambient conditions and in triplicate.

2.4 HPLC analysis

The celecoxib was assayed by HPLC (Agilent 1260 infinity II LC systems, Agilent Technology, Santa Clara, CA, USA) using a C18 reversed-phase column (VertiSep UPS C18, Vertical, Nonthaburi, Thailand) with dimensions of 5 µm, 4.6x250

mm. The mobile phase composed of an 75:25 % v:v mixture of acetonitrile:water. The flow rate was 1 ml/min, the injection volume was 20 µl, and the UV detection wavelength was 250 nm. The quantitative analysis of celecoxib was obtained from the calibration curve, which had good linearity ($r^2 \geq 0.999$) in the range of 0.1-500 µg/ml.

2.5 Statistical analysis

All data were statistically analyzed using paired t-test. Differences of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1 Solubility study

In case of oils, celecoxib exhibited the highest solubility in MCT oil (11.62±3.08 mg/ml) as shown in Table 1. MCT oil was selected as oil phase for microemulsion preparation. For surfactants and co-surfactants, celecoxib exhibited the highest solubility in Kolliphor EL (345.08±59.99 mg/ml) followed by Transcutol P (326.39±1.96 mg/ml). Kolliphor EL and Transcutol P were, therefore, selected as the surfactant and co-surfactant respectively for microemulsion preparation.

Table 1 Solubility of celecoxib in various oils and surfactants/co-surfactants

Oils	Solubility (mg/ml)	Surfactants/co-surfactants	Solubility (mg/ml)
Medium chain triglyceride (MCT oil)	11.62±3.08	Kolliphor EL	345.08±59.99
Isopropyl myristate	4.87±0.41	Transcutol P	326.39±1.96
Isononyl isononanoate	3.20±0.01	Tween 20	321.32±2.59
Octyldodecanol	0.99±0.02	Ethanol	79.15±10.25
Oleic acid	0.74±0.07	Labrafil M 1944cs	42.5±0.9

*Each value represents the mean±standard deviation (n=3).

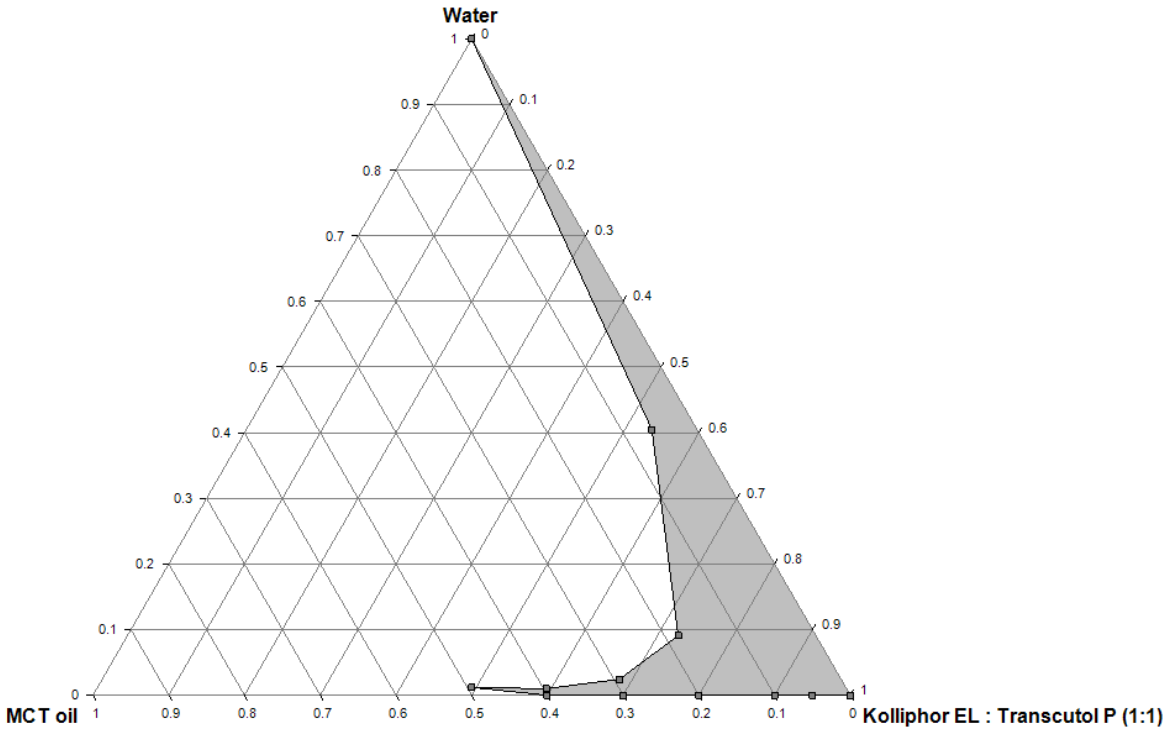
3.2 Preparation of the microemulsion (Construction of pseudo-ternary phase diagram of microemulsion)

To obtain the concentration range of the microemulsion components, pseudo-ternary phase diagram was constructed. The optimal microemulsion formulation could be acquired from the observed microemulsion region. The microemulsion systems composed of MCT oil as oil phase, water as the aqueous phase and various ratios of Kolliphor EL as surfactant and Transcutol P as co-surfactant were studied. The pseudo-ternary phase diagrams of microemulsions are shown in Figure 1. In thediagram, the gray area indicates the microemulsion region while the remaining area represents the coarse emulsion region. Six microemulsion formulations that had good stability (absence of phase separation) and a particle size of not more than 600 nm were prepared as shown in Table 2.

3.3 Solubility of celecoxib in the microemulsion

The celecoxib solubility was in the range of 77.32-282.9 mg/ml as shown in Table 2. The increase in surfactant/co-surfactant mixture and oil phase resulted in enhanced celecoxib solubility. Microemulsions can be used to fill soft gelatin capsules for orally convenient administration, but a high concentration of the drug is necessary to minimize the capsule size for ease in swallowing. Celecoxib was, therefore, loaded into the microemulsion formulations at 90 % of their solubility as shown in Table 2.

Fig. 1 Pseudo-ternary phase diagram composed of MCT oil as the oil phase with Kolliphor EL and Transcutol P (1:1) as surfactant and co-surfactant, respectively. The gray area represents the microemulsion region.



3.4 Characterization of microemulsion (Mean droplet size, surface charge, size distribution and electrical conductivity)

Both the microemulsion with celecoxib (celecoxib-loaded microemulsion) and without celecoxib (blank microemulsion) were evaluated for their particle size, zeta potential, polydispersity index (PDI) and conductivity as shown in Table 3. The particle size of the blank microemulsion was between 285.53 and 589.17 nm whereas the celecoxib-loaded microemulsion was between 225.23 and 538.87 nm. The average particle size of the celecoxib-loaded microemulsion was not significantly different from blank microemulsion indicating that the addition of celecoxib did not affect the particle size. The zeta potential of blank microemulsion was between -0.1072 and 0.1877 mV while the celecoxib-loaded microemulsion was between -0.2693 and 0.291 mV. Since

MCT oil, Kolliphor EL and Transcutol P are non-ionic molecules. The surface charge of blank microemulsion was neutral. Although celecoxib has an amino group in the structure, under neutral pH of microemulsion (pH~7), the protonation did not occur. Thus celecoxib-loaded microemulsion has neutral charge.

The PDI of the blank microemulsion was between 0.399 and 0.666 whereas that of the celecoxib-loaded microemulsion was between 0.439 and 0.701. The average PDI of the celecoxib-loaded microemulsion was not significantly different from blank microemulsion.

Three microemulsion types have been proposed which are oil-in-water microemulsion, bicontinuous microemulsion and water-in-oil microemulsion [3, 4]. The oil-in-water microemulsion is formed when the ratio of oil is lower than water. On the contrary, the water-in-oil microemulsion is occurred when the ratio of water is lower than oil. In system which the ratio of oil and water are equal, the bicontinuous microemulsion is formed [5]. The spherical nano-vesicles can be seen from oil-in-water and water-in-oil microemulsion while non-spherical nano-vesicles are the proposed structure of bicontinuous microemulsion [5]. An Electrical conductivity is a useful parameter for microemulsion type evaluation. According to the microemulsion type classification using electrical conductivity evaluation by Djordjevic et al. [6] The authors reported that microemulsion formulation which the ratio of oil phase was higher than water phase having conductivity between 2.9 and 3.8 µS/cm was water-in-oil microemulsion. For microemulsions which the ratio of water phase was higher than oil phase having conductivity ranged from 10.3 to 52.5 µS/cm were bicontinuous microemulsion whereas microemulsions having conductivity ranged from 80.5 to 94.3 µS/cm were oil-in-water microemulsion. Gundogdu et al. [7] revealed that the conductivity of water-in-oil microemulsion (the ratio of oil phase was higher than water phase) was 1.1 µS/cm. Xing et al. [8] evaluated three microemulsion formulations in which all formulations had the ratio of water phase more than oil phase. They discovered that their microemulsion had electrical conductivity between 65 and 71 µS/cm and reported that all microemulsion formulations were oil-in-water microemulsion. As there have been no reports about the cut-off value of electrical conductivity for microemulsion type classification. The criteria were, therefore, assumed based on the previously described literatures in order to identify microemulsion type using both the ratio of oil and water phase and electrical conductivity for this study. The microemulsion type identification was considered predominantly from the ratio of oil and water phase followed by electrical conductivity. If the ratio of oil phase is more than water phase, it will be water-in-oil microemulsion. If the ratio of water phase is more than oil phase with the conductivity over than 52.5 µS/cm, it will be oil-in-water microemulsion. It will be bicontinuous microemulsion, when the ratio of water phase equals to oil phase or the ratio of water phase is more than oil phase with the conductivity not more than 52.5µS/cm.

The conductivity of blank microemulsion ranged from 14.1 to 45.33 µS/cm whereas that of the celecoxib-loaded microemulsion ranged from 7.09 to 31.07 µS/cm. Regarding the mentioned criteria, this study suggested that blank ME 6 and celecoxib

loaded ME 6 were water-in-oil microemulsion. Blank ME 1, celecoxib loaded ME 1, blank ME 2, celecoxib loaded ME 2, blank ME 3, celecoxib loaded ME 3, blank ME 4, celecoxib loaded ME 4, blank ME 5 and celecoxib loaded ME 5 were bicontinuous microemulsion.

To investigate the effect of celecoxib addition on microemulsion conductivities, there were celecoxib loaded ME 2 and celecoxib loaded ME 5 in which the addition of celecoxib resulted in the significant decrease of the microemulsion conductivities.

Table 2 Formulations, component ratio, celecoxib solubility and celecoxib loaded in microemulsion

Formulations	Ratio			Solubility of celecoxib (mg/ml)	Celecoxib loaded in microemulsion (mg/ml)
	Oil	Surfactant mixture	Water		
ME 1	0.1	0.8	0.1	263.15±4.08	237
ME 2	0.1	0.7	0.2	139.21±7.11	125
ME 3	0.05	0.65	0.3	77.32±1.81	70
ME 4	0.05	0.8	0.15	205.22±7.09	185
ME 5	0.05	0.85	0.1	287.75±5.29	259
ME 6	0.15	0.8	0.05	282.9±3.4	255

*Each value represents the mean± standard deviation (n=3).

Table 3 Particle size, zeta potential, polydispersity index (PDI) and electrical conductivity of microemulsions without celecoxib (Blank ME) and celecoxib loaded microemulsions (Celecoxib loaded ME)

Formulations	Particle size (nm)		Zeta potential (mV)		PDI		Conductivity (µS/cm)	
	Blank ME	celecoxib loaded ME	Blank ME	celecoxib loaded ME	Blank ME	celecoxib loaded ME	Blank ME	Celecoxib loaded ME
ME 1	385.4±41.1	497.6±19.6	-0.1072±0.0724	0.0283±0.1487	0.399±0.047	0.534±0.012	18.33±10.62	7.13±1.01
ME 2	285.53±35.77	378.17±34.32	0.0266±0.2146	0.291±0.279	0.666±0.193	0.463±0.029	45.33±5.01	16.6±0.6
ME 3	328.4±112.2	225.23±23.87	0.1456±0.1951	-0.0157±0.0936	0.606±0.126	0.701±0.219	41.93±5.26	31.07±0.06
ME 4	438.87±26.9	303.57±33.18	0.1877±0.1079	0.2105±0.2125	0.429±0.027	0.46±0.13	18.97±1.51	17.23±11.75
ME 5	399.43±31.74	338.83±85.76	-0.0345±0.1461	-0.2693±0.3637	0.615±0.109	0.439±0.037	15.47±0.55	7.09±1.71
ME 6	589.17±36.62	538.87±98.88	-0.5514±0.4888	-0.1473±0.1944	0.53±0.05	0.574±0.075	14.1±5.2	11.97±14.67

Each value represents the mean± standard deviation (n=3).

3. Conclusions

A novel celecoxib-loaded microemulsion containing Kolliphor EL as the surfactant and Transcutol P as the co-surfactant was successfully developed. These microemulsion formulations could increase celecoxib solubility. These microemulsion formulations have nano-scale sized vesicles which will be a promising tool for oral absorption of celecoxib.

Acknowledgements

The authors are grateful to the research grant of Burapha University through National Research Council of Thailand (Grant no.127/2561) and also supported by faculty of Pharmaceutical Sciences, Burapha University.

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Development of *Zingiber cassumunar* Roxb. essential oil loaded solid lipid particles for use as antibacterials against wound causing bacteria

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Abstract

Zingiber cassumunar Roxb. (Plai) essential oil is one of essential oils possessing antibacterial activity against wound causing bacteria. Unfortunately, topical pharmaceutical dosage forms using an essential oil as active ingredient might be limited due to volatility and oxidation. Thus, this study aimed to solve the mentioned problems by encapsulation technique for developing Plai oil loaded solid lipid particles (SLP). Four formulas were prepared by melt dispersion technique. Entrapment efficiency (EE) of all formulas was determined and the formula possessing the highest percent EE was then selected. The selected SLP was measured the particle size and determined percentage of cumulative release using Franz diffusion cell. The results revealed that the Plai oil loaded SLP consisting of high quantity of Plai oil and low content of cetyl alcohol showed the highest percent EE and average particle size of 200 micrometer. In addition, the selected Plai oil loaded SLP revealed a sustained release profile in comparison with free Plai oil. In conclusion, the developed Plai oil loaded SLP possessed ability for use as a sustained antibacterial agent in topical preparation for wound treatment.

Keywords: *Zingiber cassumunar* Roxb, Plai, essential oil, solid lipid particles

1. Introduction

Zingiber cassumunar Roxb**. (**Plai**)** essential oil is known as anti-inflammatory agent**.** In our previous works, it was found that Plai oil showed a moderate antibacterial activity against wound causing bacteria such as *Staphylococcus aureus* ,*Staphylococcus epidermidis* ,*Staphylococcus pyogenes* ,Methicillin Resistance *Staphylococcus aureus* (MRSA) DMST 20645, DMST 20649, DMST 20654, DMST 20646, DMST 20651 and DMST 20652 ,*Escherichia coli* and *Pseudomonas aeruginosa* **[**1**].** However, topical pharmaceutical dosage forms using an essential oil including Plai oil as active ingredient might be limited due to volatility and oxidation of the oil**.** Therefore, essential oil encapsulation might be a suitable pharmaceutical technique for solving these problems**.** Solid Lipid Particles **(**SLP**)** are one encapsulation technique which is rather simple and easy to scale up**.** SLP is a solid lipid particle that can be incorporated of both hydrophilic and lipophilic drugs**.** In addition, SLP can increase drug stability and cause sustained drug release**.** SLP is different from emulsion**.** Emulsion is a dispersion system which

either contains oil droplets in external water phase or contains water droplets in external oil phase**.** But, emulsion cannot cause sustained drug release**.** In this study, four formulas of Plai oil loaded SLP were prepared by melt dispersion technique using either cetyl alcohol or stearyl alcohol as lipid shell while poloxamer 188 and sodium dodecyl sulfate were used as mixed surfactants**.** Entrapment efficiency **(**EE**)** of all formulas of Plai oil loaded SLP was determined by UV-visible spectrophotometry**.** SLP formula possessing the highest percent entrapment efficiency was then chosen to determine the average particle size by particle size analyzer and percentage of cumulative release using Franz diffusion cell**.** Hopefully, this finding would help to improve stability of Plai oil that might lead to a novel topical preparation possessing antibacterial activity against wound causing bacteria**.**

2. Materials and Methods

2.1 Preparation of Plai oil loaded SLP using a melt dispersion technique

To make Plai oil as a yellowish colour essential oil, it was supplemented with standard curcumin at concentration 1 mg/ml**.** Because, a yellow-color compound namely curcumin is found in Plai oil and can be detected by UV-visible spectrophotometer **[**2**].** The yellow Plai oil loaded SLP was prepared according to the method of Lertsatitthanakorn *et al.* **[**3**].** Briefly, amounts of either cetyl alcohol or stearyl alcohol, Plai oil, water and the mixed surfactants **(**poloxamer 188**:** sodium dodecyl sulfate at a 35**:**1 weight raito**)** were weighed **(**Table 1**).** Plai oil was dissolved in molten lipid at 65 ˚C and vortex-mixed **(**Vortex Genie 2, Scientific Industries, USA**)** for 1 minute**.** The mixed surfactants were dissolved in water, heated to 65 ˚C, added into the oil-lipid mixture and mixed for 1 minute**.** The occurring dispersion was cooled down in an ice bath **(**4 ˚C**)** for 30 minutes that hence giving an aqueous Plai oil loaded SLP dispersion**.**

Table 1 Ingredients of 4 formulas of Plai oil loaded SLP

Formula	Essential oil	cetyl alcohol	Stearyl alcohol	poloxamer	sodium dodecyl sulfate	water	total
C1	2.1	4.25	0	2.81	0.08	40.75	50.00
C2	3.6	2.75	0	2.81	0.08	40.75	50.00
S1	2.1	0	4.25	2.81	0.08	40.75	50.00
S2	3.6	0	2.75	2.81	0.08	40.75	50.00

2.2 Determination percentage of entrapment efficiency (percent EE) of Plai oil loaded SLP

The yellow Plai oil was diluted with absolute ethanol to make standard solution at 7 concentrations**.** Each standard solution was determined the absorbance at 420 nm using UV-visible spectrophotometer**.** Standard curve was constructed by plotting between the absorbance and concentration of standard solution**.** Each formula of Plai oil loaded SLP was weighed into volumetric flask, added with absolute ethanol, shaked well using ultrasonic bath and centrifuged**.** The supernatant was measured the absorbance and calculated percent EE of Plai oil loaded SLP using standard curve**.** Each formula of Plai oil loaded SLP was determined percent EE in triplicate**.**

2.3 Determination the average particle size of the selected Plai oil loaded SLP

Plai oil loaded SLP that possessing the highest percent EE was chosen to measure the average particle size by particle size analyzer**.** The selected Plai oil loaded SLP was diluted with distilled water to give a suitable concentration before measuring its particle size by Mastersizer 3000 **(**UK**)**. The experiment was performed in triplicate**.**

2.4 Determination percentage of cumulative release of Plai oil from the selected Plai oil loaded SLP

Three gram of the selected Plai oil loaded SLP that possessing the highest percent EE was placed in the donor cell of Franz diffusion cell**.** Cellophane was used as a membrane**.** Absolute ethanol was used as the receptor solution and collected for 4 ml at the sampling time until 24 hours**.** The fresh absolute ethanol was replaced at those time**.** The sampling receptor solution was measured the absorbance at 420 nm**.** Percent EE was calculated according to the method of Chandrasekaran *et al* [4]**.** On the other hand, free Plai oil mixed with lipid was performed in the same manners**.** Each experiment was done in triplicate**.**

3. Results and discussion

3.1 Entrapment efficiency determination

Table 2 Percent EE of 4 formulas of Plai oil loaded SLP

Formula	Mean	SD
C1	17.41	5.34
C2*	21.13	13.30
S1	12.98	11.52
S2	9.90	6.48

***** Statistical significant difference between C2 and S1, between C2 and S2 **(** p value < 0**.**05, Mann**-**Whitney U test**)**

According to Table 2, it was found that the formula C2 showed the highest percent EE **(**21**.**13**)** while the formula S2 revealed the lowest percent EE**.** This result was comparable with the finding of Lertsatitthanakorn *et al***.[**5**].** Because, a high percent essential oil entrapment might cause by using cetyl alcohol as a lipid shell**.** Therefore, the formula C2 was selected to study in the next experiment**.**

3.2 Particle size of the selected Plai oil loaded SLP determination

Table 3 The average particle size of the selected Plai oil loaded SLP

	Dx (10) (µm) (mean ± SD)	Dx (50) (µm) (mean ± SD)	Dx (90) (µm) (mean ± SD)
selected Plai oil loaded SLP **(**Formula C2)	22.2 ± 0.2	95.5 ± 0.3	200.0 ± 0.5

According to Table 3, the selected Plai oil loaded SLP showed the average size of 90 percent particles at 200 ± 0.5 micrometer**.** The particle size was large that might cause by insufficient force for mixing Plai oil and lipid with the mixed surfactants**.**

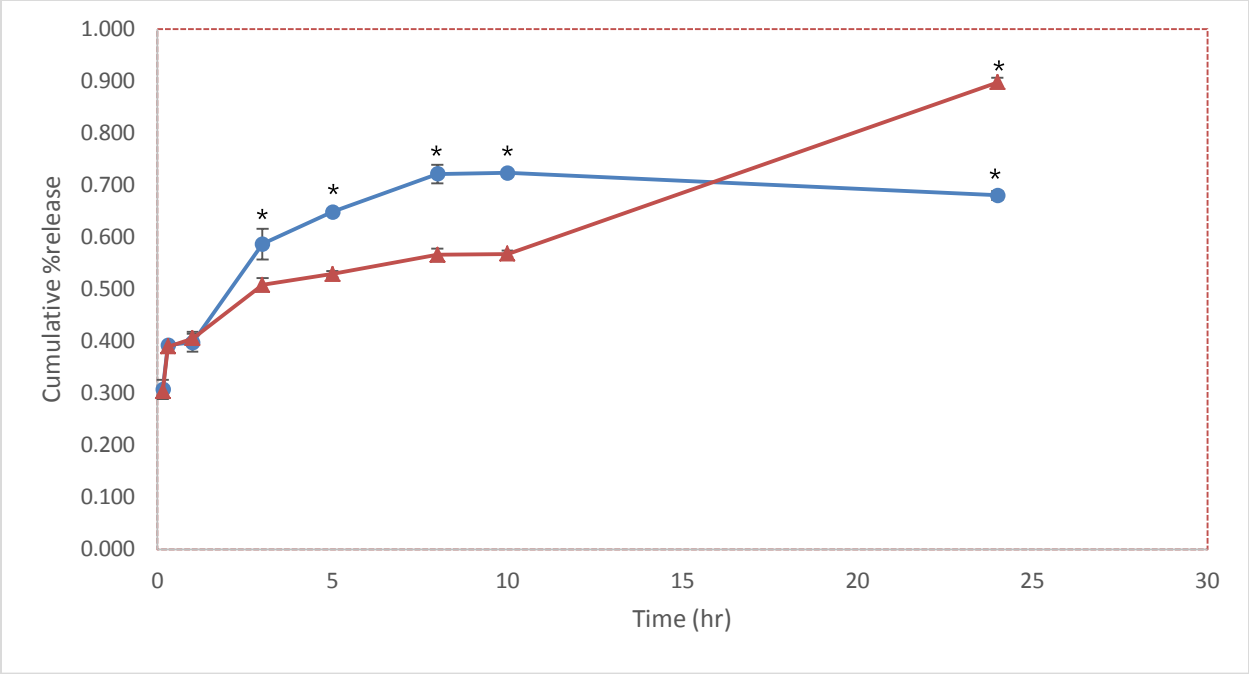

Fig. 1 Release profile of the selected Plai oil loaded SLP **(**Formula C2**)** in comparison with free Plai oil **(**circle symbol**:** Plai oil loaded SLP; triangle symbol**:** free Plai oil; ***:** statistical significant difference between Plai oil loaded SLP and free Plai oil with *p* value < 0**.**05 by Mann-Whitney U test**)**

Figure 1 demonstrated a sustained release profile of the selected Plai oil loaded SLP**.** This delivery system gradually released Plai oil within 10 hours**.** After that, Plai oil release was rather constant until 24 hours in comparison with free Plai oil**.** The sustained release profile of this SLP might cause by the large particle size**.** Moreover, the release kinetic of Plai oil from SLP seemed to be a zero-order**.** It might be suggested that a rate limiting step was Plai oil diffusion through solid lipid core **[**6**].** Therefore, SLP was one of encapsulation technique that affected the release profile of the entrapped drug and hence leading to a sustained release manner **[**7**].**

4. Conclusions

The results revealed that the Plai oil loaded SLP consisting of high quantity of Plai oil and low content of cetyl alcohol showed the highest percent EE**.** In addition, the selected Plai oil loaded SLP revealed a sustained release manner due to a rather large particle size**.** The developed Plai oil loaded SLP possessed ability for use as sustained antibacterial agent in a topical preparation for wound treatment**.**

Acknowledgments

This work was supported by the research grant of Thailand Research Fund **(**TRF**)**, fiscal year 2017 **-** 2018**.**

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Variation of alkaloids contents in *Stephania venosa* roots in Thailand

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Abstract

Stephania venosa (Blume) Spreng., vernacularly named in Thai as “Sa-Bu-Leud”, belongs to the Menispermaceae family. It has been traditionally used as a tonic drug, for the treatment of cancer, diabetes and as an aphrodisiac. In order to evaluate the quality and standardization of *S. venosa* roots, the HPLC analysis for quantification of the content of major constituents in *S. venosa* was performed. *S. venosa* samples collected from various locations in Thailand were quantitatively analyzed after extraction with methanol-water (70:30, v/v), which was determined as effective solvent for extraction. Dicentrine, tetrahydropalmatine, crebanine and stephanine were varied from not detected to 20.38, not detected to 9.19, 0.16 to 30.27, and not detected to 6.80 mg/g, respectively. A remarkable variation in the accumulation of alkaloids in each population and the between individual in the same population were observed, indicating the heterogeneity of *S. venosa* in Thailand. The present study provided a basis for quality assessment and standardization of this plant for the development of phytopharmaceutical products

Keywords: Alkaloids, standardization, Menispermaceae, HPLC

Quantitative analysis of phenolic compounds in *Pluchea indica* and their commercial products by HPTLC method

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Abstract

Pluchea indica has been widely used in Thai traditional medicine for the treatment of diabetes mellitus, cancer, hypertension, cystitis and wound-healing. Several biological activities of *P. indica* leaves has been reported such as anti-inflammatory, antioxidation, anti-tumor and anti-ulcer activities. The study was conducted to develop and validate a high performance thin-layer chromatography (HPTLC) method for the quantitative analysis of cholorogenic acid (CGA), 3,4 dicaffeoylquinic acid (3,4 DCQA) and 3,5 dicaffeoylquinic acid (3,5 DCQA) in *P. indica* leaf extract and their commercial products in Thailand. HPTLC analysis was performed on an aluminium sheet of silica gel 60 F_{254} using ethyl acetate:water:formic acid:toluene (20:2:2:1, v/v/v/v) as a mobile phase. The densitometric scanning was performed at the wavelength 326 nm. HPTLC method was validated according to ICH guideline. The proposed HPTLC method showed acceptable validation parameters. The correlation coefficient value was > 0.995. Intra-day and inter-day precisions with relative standard deviations of less than 5%. For quantitative analysis, it was found that the content of CGA, 3,4 DCQA and 3,5 DCQA were in the ranges of not detected - 2.17 ± 0.05 %w/w, 0.03 ± 0.16 %w/w - 0.71 ± 3.77 %w/w and 1.00 ± 0.01 %w/w – 4.72 ± 0.10 %w/w, respectively. HPTLC method showed several advantages such as rapid, reliable, less solvents used, and low cost analysis.

Keywords: caffeoylquinic acid, chlorogenic acid, *Pluchea indica*, HPTLC method

Standardized extract of *Centella asiatica* ECa233 decreased survival and sensitize colorectal cancer cells to 5-fluorouracil in vitro

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Abstract

Colorectal cancer (CRC) is a cancer type that develops in the colon and/or rectum. Surgical resection is a main curative approach to remove the tumor followed by chemotherapy administered after complete tumor resection, as an adjuvant in CRC patients. Currently, 5-Fluorouracil (5-FU) is a standard adjuvant regimen for CRC patients. As approximately 10-15% of long term use of 5-FU may develop resistance or become refractory to 5-FU therapy. Therefore, discovery of new agents that can increase the efficacy of 5-FU and decrease its side effects are importantly needed. ECa233 is a standardized extract of *Centella asiatica* containing madecassoside (46.3%) and asiaticoside (41.6%) displaying very good safety data appropriate for further development as herbal drug for human use. Recent studies demonstrated the pharmacological activity of ECa233 including anti-convulsant, wound healing activity, and ameliorating effects on memory impairment. However, the effects of ECa233 on colorectal cancer cells have not been reported. The purpose of the present study was to investigate the effect of ECa233 alone and in the presence of 5-FU on SW-620 colorectal cancer cells. MTT assay demonstrated that low doses of ECa233 inhibited cell survival of SW-620 cells in a time- and dose- dependent manners at 24, 48, 72, and 96 hr exposures. The median inhibitory concentration (IC_{50}) of ECa233 are 43.66, 29.40, 28.97, and 28.68 µg/ml, respectively. Co-treatment of ECa233 and 5-FU by drug combination method demonstrated that cell survival of SW-620 cells extremely decreased after being treated with 40 µg/ml of ECa233 upon a wide range of 5-FU concentrations. These results demonstrated that drug-drug interaction of ECa233 and 5-FU was synergism suggesting a potential treatment option for colorectal cancer. Preliminary data in the present study support the development of ECa233 as a novel adjuvant therapy for 5-FU in the management of colorectal cancer.

Keywords: ECa233, colorectal cancer, 5-Fluorouracil, cell survival, synergism

Psychometric properties of the SF-36 and health-related quality of life measurement in the general population of Thailand

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Abstract

Background: Health-related quality of life(HRQoL) is the most preferred patient-reported outcome measured in both clinical area and general population. The short form 36 health survey version 2 (SF-36v2) is one of the most commonly used questionnaires for measuring HRQoL level worldwide. To date, there have been several previous studies using the SF-36v2 to examine its psychometric properties and measure the HRQoL level in clinical area in Thailand. Nevertheless, evidence is lacking as concerns psychometric testing and HRQoL level among the general Thai population.

Objectives: This study aimed to investigate the psychometric performance and to evaluate the HRQoL level, and the factors associated with it in the general Thai population.

Methods: Cross sectional study using face-to-face interviews was conducted with 600 convenient Thai subjects living across five provinces in Thailand. Ceiling/floor effects, item-scale and scale levels validity using correlations, Principal component analysis (PCA) with Varimax rotation and internal consistency, were investigated. Multiple regression was used to determine demographic factors influencing HRQoL level measured by the SF-36v2 questionnaire.

Results: Cronbach's alpha of the SF-36 scales ranged from 0.703 to 0.858. A ceiling effect ranged from 3.7% to 78.3% while no floor effects were observed except for Bodily Pain and General Health. Scale levels and item-scale correlations supported the hypothesized correlations. PCA yielded a two-factor structure similar to a theoretical association. Advancing age and chronic disease were considered the two major factors deteriorating the HRQoL level.

Conclusions: These preliminary results confirmed that the Thai SF-36v2 is a valid and reliable instrument for the general Thai population. Multiple regression also revealed that advancing age and chronic disease diminished the HRQoL level. Future studies are recommended to investigate the HRQoL level among subjects of various levels of comorbidities.

Keywords: SF-36v2, health-related quality of life, psychometric properties

Combination effects of novel microemulsion and sonophoresis on dermal delivery of celecoxib

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Abstract

This study was aimed to develop a novel microemulsion that contained PEG-6 caprylic/capric glycerides as surfactant, to evaluate the optimum condition of sonophoresis for skin penetration enhancement of celecoxib and to study the combination effect of this novel microemulsion and sonophoresis on dermal delivery of celecoxib. The created microemulsion was evaluated for their size, zeta potential, polydispersity index, microemulsion types, *in vitro* release and *in vitro* skin penetration test. The celecoxib loaded microemulsion had a particle size in range of 48-269 nm with a neutral surface charge. The *in vitro* release of celecoxib loaded microemulsion was best fitted with the zero-order model. The microemulson significantly improved the skin penetration of celecoxib. The energy of sonophoresis for skin penetration enhancement of celecoxib was 30 watt/cm^2. The microemulsion formulation and sonophoresis condition which provided the highest amount of celecoxib in the skin were selected to investigate the combination effect on dermal delivery of celecoxib. However, the combination effect of microemulsion and sonophoresis resulted in the decrease of the celecoxib penetration into the skin comparing to microemulsion

Keywords: microemulsion, celecoxib, sonophoresis, dermal delivery

Effect of bile acid composition on the characteristics of curcumin-loaded liposomes

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Abstract

Curcumin (*Curcuma longa*) has a wide range of beneficial properties including anti-inflammatory, antioxidant, chemopreventive and chemotherapeutic activities. It can be extracted from the rhizomes of turmeric and is used in traditional herbal remedies and medical applications. Curcumin (Cur) has low water solubility (0.0004 mg/ml) which adversely affects its bioavailability and absorption. A new delivery system was investigated using liposomes to increase the solubility of curcumin. Studies have shown that the use of bile acids in delivery systems increases the effectiveness of intestinal absorption. A liposome delivery system containing three bile acids as cholic acid (CA), deoxycholic acid (DCA), sodium deoxycholate (NaDCA) and phosphatidylcholine (PC) was developed to test the effect of bile acid composition and efficacy on liposome properties. The ratio of liposome to curcumin and physical liposome properties such as particle size, zeta potential, and % entrapment efficiency were evaluated. The most suitable liposome formulation was PC: NaDCA: Cur (1: 8: 5) with an average particle size of 109.30 nm. This was smaller than the other formulae with average zeta potential of -54.22 mV indicating that the particles were stable and not clustered together. Curcumin retention within the liposome was not statistically significant ($P < 0.05$) based on the mechanism of curcumin binding to phosphatidylcholine. Only physical properties were investigated. Future studies should be conducted in other areas to investigate chemical stability and biological properties to further develop the formula.

Extraction and characterization of cellulose from fruit-hulls of durian (*Durio zibethinus* L.)

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Abstract

The objectives of the recent study were to extract and characterize cellulose from durian fruit rind of three different types such as Mhonthong, Chanee and Kradom. The various concentrations NaOH solution was used to extract durian fruit rinds. The exocarp-mesocarp part and the mesocarp part of durian fruit rinds was investigated. The cellulose powder from durian fruit rinds was observed under scanning electron microscope (SEM). The chemical structure and crystallinity of these cellulose powder were examined using Fourier transform infrared spectroscopy (FTIR), and powder x-ray diffractometer (XRD). The yield of cellulose powder was in the range of 13.43 - 25.49%. The SEM images show that the cellulose powder was spindle liked shape and had diameters in the range of 7 - 16 µm. The FTIR spectrum confirmed the removal of others component from the cellulose powder. From the XRD data, all of the cellulose powder showed more crystallinity index than untreated powder. The cellulose powder extracted from Chanee's mesocarp part showed the highest viscosity molecular weight (M_V). These results imply that the cellulose powder from the different type of durian fruit rinds, various NaOH concentration and different parts exhibited similarity in properties. In conclusion, these cellulose powders may have properties and potential to apply in pharmaceutical excipients

Keywords: Durian, Cellulose, Extration

Improvement dissolution of quercetin via self emulsifg drug delivery systems (SEDDS) and solid dispersions (ASDs)

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Abstract

Quercetin has been used for health promoting such as antioxidant, anti-inflammatory, anti-bacterial, anti-coagulative, anti-bacterial and antineoplastic. The poorly water soluble of quercetin has been a significant problem of bioavailability for oral administration. The purpose of this study is improvement the quercetin solubility by amorphous solid dispersion (ASDs) and Self-emulsifying drug delivery system (SEDDS). The hydroxypropyl cellulose polymer 3 types (SSL, SL and L) at the ratio 1:1, 1:2 and 1:3 were contained in ASDs. SEDDS was frabicated by dissolved quercetin in mixture of CCG: P35: DGE (at ratio 10:10:80). The dissolution profiles of SEDDS shown the higher solubility than unmodified quercetin and ASDs. Moreover, SEDDS can increase solubility of quercetin for 20.46 folds and 16.95 folds at 20 mins. and 120 mins., respectively.

Keywords: solid dispersions, self emulsifying, SEDDS, ASDs, quercetin, drug delivery systems

Effects of *Centella asiatica* (ECa233) on the inhibition of cell viability, colony formation, migration and invasion in SW-620 human colorectal cancer cells

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Abstract

Recently, colorectal cancer (CRC) is second leading cause of population death in the world. 5-Fluorouracil is the first-line therapy for CRC patients, although 5-FU have great-efficacy but it is affecting in normal cell and increasing many side effects. Therefore, developed and research pharmacological activity of new natural products for alternative-therapy using is importantly needed to treat CRC. So, essential extraction from natural products is the new destination for treating CRC. The objective of this study was to investigate the effect of standardized extract of *Centella asiatica* (ECa233) on the inhibition of cell viability, colony formation, migration and invasion in SW-620 human colorectal cancer cells and toxicity on fibroblasts cell to detect safety of ECa233. MTT assay, colony formation assay, wound healing (scratch assay) and Matrigel invasion assay to demonstrate pharmacological activities was used. Data in each group were calculated and expressed as mean and standard deviation (mean± SD). Data were analyzed using one-way ANOVA test in comparing data from more than two groups. The results shown that ECa233 can inhibit cell viability on CRC by dose and time-dependent manners relations after 24, 48, 72 and 96 h of treatment and ECa233 have small effect on normal cell, can inhibit colony formation, migration and invasion on CRC. Therefore, we suggest to study efficacy and safety of combination therapy with conventional medicine to develop ECa233 in the future.

Development and characterization of bilayer wound healing patch nanofiber fabricated by electrospinning

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Abstract

The present study aim to develop bilayered polymeric nanofiber patch (PNP) fabricated by electrospinning technique using for wound dressing. The nanofiber was prepared by various concentration of polyvinyl alcohol (PVA) and tamarind seed gum loaded with clindamycin HCL (CM) in first layer and Eudragit® S100 for a second layer. The polymer solutions were prepared and fabricated to nanofiber by electrospinning technique. The processing parameters of electrospinning were adjusted to obtain bilayered PNPs. From physical appearance using SEM for imaging, The PVA concentration increased diameter of fiber at 10% 12.5% 15% of PVA in polymer solution. The DSC and PXRD indicated drug in PNPs was in amorphous form in PVA. From biological test expressed that bilayered PNPs contained with PVA 10%,modified tamarind seed gum 5% and clindamycin 1% had an efficiency to inhibited *Staphylococcus aureus.* These results show the possibility of improving nanofiber patch strength by using Eudragit® S100 and modified tamarind seed gum as a natural material in nanofiber patch formulation.

Keywords: Electrospinning, Nanofiber, Bilayer, Carboxymethylated gum

The study of chemical constituents and free radical scavenging assay from the extracts of *Canavalia rosea* (Sw.) DC. and *Ipomoea pes-caprae* (L.) R.Br. leaves

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Abstract

The beach bean (*Canavalia rosea* (Sw.) DC.; CR) and the beach morning glory (*Ipomoea pes-caprae* (L.) R.Br.; IP) are the plants that often found together on the beach. By their similar characteristics of CR and IP can caused the confusion in jellyfish toxin treatment. This research project aimed to solve this problem by characterizes the morphology of both plants and comparison of their antioxidant activity and the chemical constituents by TLC, GC-MS and DPPH radical scavenging assay. The results found that the botanical morphologies of CR and IP are similar, but the free radical scavenging activity and chemical constituent of CR and IP methanol extracts are different. The methanol extract of IP show higher antioxidant activity than CR with IC_{50} 25.58 ± 0.35 µg/ml and 88.77 ± 3.18 µg/ml, respectively and the major chemical constituent from TLC (SiO_2, 9:1 CH_2Cl_2 : MeOH) of CR and IP are different by district Rf value. The further result from GC-MS revealed that the phenolic compounds in IP ethyl acetate might be the key component which exhibits the potent antioxidant. From this preliminary results of suggested that CR cannot use for treatment jellyfish toxin.

Keywords: beach bean, beach morning glory, free radical scavenging assay, GC-MS

Study of allopurinol prescription in gout patients at outpatient department in U-thong Hospital, Suphanburi Province

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Abstract

The present study evaluated appropriateness of allopurinol prescription in gout patients at The Department of Outpatient, U-thong hospital, Suphanburi Province. Data were retrospectively collected from medical record of 132 patients and being assessed in reference to the guideline of Rheumatism Society of Thailand.

About half of the study population were elderly. There were male (95 cases) more than female. Among them 62.9 were overweighed (BMI≥23). The most common pathology found in these patient was hyperuricemia (106 cases) followed by arthritis &joint swelling (68 cases) and tophus (3 cases). Based on the set criteria of gout that patients should have at least 2 out of 3 pathology mentioned above, appropriateness of allopurinol regarding indication was found in 51 cases. While the initial dose in the present study was 50-300 mg/day, the recommended initial dose of allopurinol is 50-100 mg/day according to kidney function, therefore, appropriateness regarding initial dose of allopurinol in the present study was found in 49 cases. Further assessment on co-prescription with other drugs that may result in adverse drug interaction revealed that there were 33 patients receiving other drugs in such manner causing drug interaction with allopurinol. When appropriateness in these 3 aspects were taken together, the number of patients receiving allopurinol appropriately regarding indication, initial dose and co-prescription was only 8 patients. Adverse drug reaction reported in these patients was exclusively skin-related which was found in 7 patients and moreover no relationship between the ADR reported and appropriateness of allopurinol use could be established.

Keywords: Allopurinol, appropriateness of allopurinol prescription, gout patients, hyperuricemia, arthritis, joint swelling, adverse drug reaction.

The advertisement circumstances of food supplements through social media in Thailand

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Abstract

This research aims to study on the advertising circumstances and to evaluate the advertising situations of food supplements that are usually illegal through Social media in Thailand. The methodology of this research is divided into three parts: (documentary research, qualitative research, quantitative research) Collection of the sampling group, which is focused only on Facebook ads that have been advertised during September 15 to October 15, 2017.All data was randomly selected by Microsoft Excel 2016 program (at 5% deviation) and the selected data from Microsoft Excel 2016 program, was analyzed using the descriptive statistics to get the results. The amount of samples in this study were 533 samples. The results of the evaluation of an advertising situations of food supplements that are illegal through Social media in Thailand, showed that the most illegal issue on the advertisement was “Do not show warning of food supplement on label” (99.4%)

Assessment oral glucose lowering medication adherence among diabetes mellitus type 2 patients (Self Report Questionnaire), Diabetes Clinic, Burapha University Hospital

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Abstract

This research is aimed to assess oral glucose lowering medication adherence among Diabetes Mellitus type 2 patients, Diabetes Clinic. Burapha University Hospital. There were 240 participants with the average age was 66.5 years old and 55.32% of the patients were female. Among those, 75.29% were non-alcoholic and 85.2% were non-smokers. Majority of them were non-employed (44.10%) and 53.25% were civil servants. About 95.68% visit Doctor by appointment and 65.73% had dietary control.

MMAS-8 self-reported questionnaires gave average value of 6.28 scores. The sensitivity, specificity, true positive value and true negative value are 31.67% respectively. Sensitivity, Positive Predictive Value The negative predictive value of the questionnaire was 78.00 56.00 61.67 and 38.33, respectively. The cut of point was 0.598 which mean 59.8 % is predicted correctly.

Keywords: Diabetes mellitus type 2, Adherence, Compliance, Self-report questionnaire

Development and stability study of ascorbic acid tablets

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Abstract

The purpose of this research is to study the correlation between the amount of ascorbic acid and the color level of tablets. The study was conducted by storing 5 different brands (A, B, C, D and E) of vitamin C tablets and the formulated vitamin C tablets under the accelerated condition (40±2°C, 75±5%RH) for 70 days. The result showed that the amount of ascorbic acid in tablet C and in tablet E were in the range of 90-110% of the label amount. Tablet A, B and D contained ascorbic acid less than 90% of the label amount. The study of the color level of the tablets showed that under the accelerated conditions color level reduced when storage time increased. The correlation (R^2) between the amount of ascorbic acid and the color level of tablet A was 0.9297 indicating high correlation. The result showed that under the accelerated condition for 7 days, the amount of ascorbic acid in the formulated tablets was not reduced, however the color level was slightly reduced. The correlation between the amount of ascorbic acid and the color level are under further investigation.

Keywords: ascorbic acid, vitamin C, tablet, stability, color level

Development and evaluation of astaxanthin oral dispersible tablet formulation for elderly using different types of co-pharmaceutical excipients

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Abstract

Oral dispersible tablets (ODTs) is an innovative dosage forms fabricated to overcome the problem of difficult in swallowing which is common problem among all age groups, especially elderly. This study was aimed to develop and evaluate ODTs loaded Astaxanthin prepared by direct compression technique using different types of co-excipients, including F-Melt Type C, F-Melt Type M and Pearlitol Flash. The physicochemical properties of the formulated tablets were evaluated such as hardness, thickness, diameter, friability, moisture content, *in-vitro* disintegration time and *in-vitro* dissolution. At the similar compression time and force, the tablets which were prepared by family of F-Melt proposed high compatibility and flow properties. Among all the formulation, the ODTs loaded Astaxanthin prepared by using 40 mg of F-Melt C or M as the co-processed excipient provided proper ODT properties. The optimized formulation offers the tablet hardness of 35.33 – 59.66 N, friability of 0.35-0.55%, moisture content of 0.20-0.80%, disintegration time of 20.33 – 84.33 seconds. Lastly, 80% of Astaxanthin is released within the first 3 minutes.

Keywords: Oral dispersible tablets, Astaxanthin, Co-processed pharmaceutical excipients

Chemical constituents of tunicate *Eudistoma* sp. in the East coast of Thailand

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Abstract

The tunicates *Eudistoma* sp. were collected from Aquaculture Cage at Kung Krabaen Bay Development Study Center initiated by His Majesty the King, Chanthaburi Province and were kept at -20 °C until extraction. The tunicates were freeze-dried and extracted with methanol:dichloromethane (1:1) 34.66 mg (18.63%). The crude extract was partitioned using various organic solvents to obtain hexane-(12.6492 mg, 34.62%), dichloromethane-(4.1052 mg, 11.84%), butanol-(2.3399 mg, 6.75%), and water extracts (14.7406 mg, 42.53%). The dichloromethane extract was selected according to ^{1}H-NMR signals and further isolated using chromatographic techniques including vacuum chromatography, Sephadex LH20, high performance liquid chromatography (HPLC), and thin layer chromatography (TLC) yielded 2 compounds including EUD2-4-3 (1.5 mg mixture, 0.04%) and EUD 2-2-2 (4.6 mg, 0.11%).

The mixture of EUD2-4-3 was identified as major 9- octadecenamide using GC-MS. Another EUD 2-2-2 was analyzed using ^{1}H-NMR spectrum. The spectrum indicated that EUD 2-2-2 is a pure compound which is on the process of structure elucidation.

The crude extracts were subjected to anti-oxidant activity using ABTS assay. The hexane, dichloromethane, butanol, and water extracts showed TEAC values 2.265, 22.308, 32.792, and 12.581 mM trolox/g extract, respectively whereas showed VEAC values 1.061, 10.394, 17.025, and 4.243 mM ascorbic acid/g extract, respectively.

Keywords: Eudistoma

Retrospective analysis the result of factors in use of factors associated to blood sugar controlling of type 2 diabetic patients at Burapha University Hospital

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Abstract

Type 2 diabetes mellitus (DM) is a chronic metabolic disorder in which prevalence has been increasing steadily and is a major public health problem in Thailand. Effective blood glucose control is essential for patients with diabetes as its affect health and quality of life. This study aims to study and analyze data on factors affecting glucose tolerance and ketoacidosis in 267 type 2 diabetic patients from the computerized database at Burapha University Hospital, retrospectively for a period of 3 years (1 January 2014 to 28 February 2017). Type 2 diabetes patients' information were analyzed by Pair t-test and MRA. The results showed that the association of fasting blood sugar in patients with normal blood sugar level at visit 1 with 2 and visit 1 with 3 were significantly different ($p < 0.05$ and, $p < 0.01$ respectively). Hyperglycemia patients' blood sugar level found that the first visit with the third visit was statistically significantly different ($p < 0.05$), indicating that the pattern of patients in these two conditions had better control of blood sugar level. The most lowing blood sugar level drug that have also been reported was a group of the SGLT-2 inhibitors. Male and older adolescents are more likely to have lowered glucose levels. Increased levels of creatinine and BUN resulted in a decrease in blood glucose levels due to decreased elimination. Ketoacidosis might not be a good factor in predicting blood sugar levels. In conclusion, this research can be used to predict the trend of blood glucose level of type 2 diabetic patients to better plan of treatment. Moreover, it is used as a guideline to recommend a combination of drugs to efficiently control the blood glucose level in type 2 diabetic patients with hyperglycemia such as the group of SGLT-2 inhibitors, Alpha glucosidase inhibitors and DPP-4 inhibitors.

Keywords: type 2 diabetes, ketoacidosis, blood sugar prediction

Assessment of anti-inflammatory activity of market-available herbal compress balls (Look-pra-kob)

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Abstract

Herbal compress ball (Look-pra-kob) in folk medicine was commonly used to relieve pain and reduce inflammatory but there had less academic evidence to support. We had recognized the importance of using Look-pra-kob. This study was conducted to assessment the anti-inflammatory activity (AIA) of Look-pra-kob on the market and composition of each Look-pra-kob in accordance with the effectiveness of anti-inflammatory. Study was carried out on two types of extract, aqueous and ethanol. Ethanolic extract obtained %yield (%w/w) more than aqueous extract. Proteinase inhibitory assay compared with Abhaiphubej extracts and study of anti-denaturation activity compared with diclofenac sodium. The study showed that all Look-pra-kob had AIA. In study of proteinase inhibitory assay found at 2.5 µg/ml ethanolic extract of Look-pra-kob from Prae had the most AIA but not statistically significant ($p \leq 0.05$).

At 5 µg/ml aqueous extract from Khon Kaen had the most AIA statistically significant ($p \leq 0.05$). In study of anti-denaturation activity found ethanolic extract from Sa Kaeo had the most AIA. Considering all 3 sources found the herbs was added to the formula: Khon Kaen added Croton roxburghi leaf, Blumea balsamifera leaf, Cryptolepis buchanani and Pogostemon cablin / Prae added Sida rhombifolia leaf, Croton roxburghi leaf, Cleoma viscosa and Pogostemon cablin / Sa Kaeo added Curcuma sp., Mentha cordifolia (Menthol) and Pogostemon cablin. Addition herbs that were most likely to have AIA were Cryptolepis buchanani, Croton roxburghi leaf and Pogostemon cablin.

Keywords: Herbal compress ball and anti-inflammatory

Fumigation effects of peppermint oil, lemon grass oil and kaffir lime oil against housefly, *Musca domestica* Linnaeus (Diptera: Muscidae)

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Abstract

Fumigation effects of peppermint oil, lemon grass oil, kaffir lime oil and their 1:1 mixture were evaluated against the housefly, (*Musca Domestica*). A total of 1,080 flies were used in the experiment; 540 of each sex. For each assay, 10 houseflies were in a small chamber at the center of 1-liter fumigation chamber. Distilled water (control), peppermint oil, lemon grass oil, kaffir lime oil and their 1:1 mixture were separately dropped on filter paper No. 1 size 1×1 inches locating at the bottom of each chamber. The chambers were then closed with tightly cap. The air inside the chamber was homogenized by a small electrical stirrer placing at the inner side of the cap. Houseflies were left within a chamber for 20 minutes while their movement was observed every 5 minutes. All assays were done in triplicate. After the fumigation periods, houseflies were transferred into the rearing cage, and their movement was observed again at 24 hours. The result showed that both of male and female houseflies were significantly anesthetized by peppermint oil and kaffir lime oil more than lemon grass oil. Peppermint oil and Kaffir lime oil (1:1) at concentration 200 and 600 µl/l air had more efficacy than the other groups. The 24 hours assessment maximum houseflies died from kaffir lime oil and the mixture of peppermint oil and kaffir lime oil (1:1) at concentration 200 and 600 µl/l air.

Keywords: Fumigation effect, peppermint oil, lemon grass oil, kaffir lime oil, housefly

Anxiety test predicted academic achievement in Pharmacy students at Burapha University 2017

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Abstract

Objectives: 1.To examine student's anxiety. 2. To identify relationship between anxiety and academic achievement. 3. To establish academic achievement prediction model by 1. Anxiety, 2. Social support and 3. Demographic data. Method: A cross-sectional survey study was performed at college of Pharmacy, Burapha University, Thailand, in 2017. All population (1st-5th year students), n=627, were selected as the census sample however, only 568 students participated, the return rate was 90.59%. A 2 page questionnaire consisted of Thai Spielberger's Form (TSF) and socioeconomic status. The TSF measured 2 dimensions namely: emotional and worry. It consisted of 14 measurement variables measured by visual analogue scale. Results: The TSF's internal consistency was detected by Cronbach's Alpha yield r=0.849. The second year student had the most anxiety score compared to all students (1st to 5th year). Anxiety had a negative correlation to GPAX (r = -0.084, p=0.047, Pearson's correlation). Hierarchical Stepwise Multiple Regression Analysis was employed to establish academic achievement prediction model by using anxiety, social support and gender as the predictors: GPAX = 0.061 boy/girl-friend + 0.109 friend support *+0.092 anxiety* -0.017 family support -0.008 parents -0.128 gender**. (p-value 0.010, 0.030 and 0.002 respectively) with R-square 0.410 Conclusion: Gender had the biggest impact on academic achievement and 3 significant predictors of Academic Achievement model were gender, friend support and anxiety.

Keywords: anxiety, academic achievement

Study of free radical scavenging activity and product development of Thai mushrooms

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Abstract

One of the biological effects of mushrooms is the free radical scavenging activity, which is beneficial in helping to restore the skin, reducing melanin stimulation and wrinkles. The purpose of this study was to investigate the free radical scavenging activity of seven species of mushroom extracts. The extracts of *Dictyophora indusiata* (Vent.) Desv, *Lentinula edodes* (Berk.) Pegler, *Pleurotussajor-caju* (Fr.) Singer, *Lilloa*. *Pleurotus eryngii* (Cand.Ex.Fr.), *Flammulina velutipes* (Curtis) Singer, *Volvariella volvacea* (Bul.ex Fr.) Singer, *Auricularia auricular* (Bull.) J.Schröt were determined by DPPH radical scavenging assay, ABTS cation radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and total phenolic content. The skin care products such as cream, lotion and gel from the best free radical scavenging activity of the mushroom extracts were also tested. Physical stability such as color, odor, pH, flow, viscosity, creaming and cracking were examined both in normal and accelerated conditions. According to the study, *V. volvacea* expressed the best free radical scavenging activity at IC_{50} 2.07 ± 0.65 mg/ml, 1.77 ± 0.66 mg/ml and 56.54 ± 7.43 g of $FeSO_4$ equivalent to 100 g extract from DPPH assay, ABTS assay and FRAP assay, respectively. In addition, the total phenolic content of the extract was 0.30 ± 0.12 g of gallic acid equivalent to 100 g extract. For skin care products testing, the results indicated that the most stable formulation was the gel preparation. These information may lead to the development of the better skin care products from the mushroom extracts in the further study.

Keywords: free radical scavenging activity, product development, mushrooms

The incidence of chronic kidney disease caused by untreated hyperuricemia at Laemchabang Community Hospital and Burapha University Hospital

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Abstract

Hyperuricemia is an excess of uric acid in the blood over 6.0 mg/dL (female) and 7.0 mg/dL (male). There have been reported hyperuricmia is the one cause of chronic kidney disease. Only patient with hyperuricemia and acute gout attack will be treated with antihyperuricemia. However, observational working by pharmacist, has mentioned that untreated hyperuricemia could reduce estimated glomerular filtration rate (eGFR). And there are study reported untreated hyperuricemia could lead to chronic kidney disease. This retrospective study aims to evaluate the incidence of chronic kidney disease caused by untreated hyperuricemia at Laemchabang Community Hospital and Burapha University Hospital. All data from an Electronic Medical Record by hosxp program were collected from October 1, 2013 to July 31, 2017. The result showed an untreated hyperuricemia patients were 118. The averages of eGFR among untreated hyperuricemia group increased from 80.76 to 93.76 ml/min/1.73m2 significantly p-value < 0.001). No incidence of chronic kidney disease occurred in 20 months of followed up. Moreover, 26 hyperuricemia patients treated with antihyperuicemia had eGFR increased significantly in 9 month and also increase continuing significant from 49.38 to 62.19 ml/min/1.73m2 (p-value = 0.004). Treating hyperuricemia with antihyperuricemia agent such as colchicine and allopurinol can decrease uric acid level and improve the renal function.

Health seeking behavior and medical pluralism and diabetes melitus

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Abstract

This study was an exploratory descriptive research aims to study health seeking behaviour which is using Medical pluralism among Diabetes Mellitus Type 2 patients in Diabetes Clinic at Burapha University's hospital. The population included Diabetes Mellitus patients type 2 not more than 10 years for 30 patients and HbA1C $\leq$7 who is treated in Diabetes Clinic at Burapha University's hospital. Duration of research studied was August-November, 2017. The data were collected by the in-depth interview methodology and face-to-face interview and were analysed by content analysis after interview. The data were collected from 30 patients and divided into 2 groups. For the first group, there were 14 (46.70%) patients using Medical pluralism such as diet control, doing physical activity, using Folk medicine or Thai Herbs. However, for the second group, 16 (53.30%) patients did not use the medical pluralism. More importantly, we found out that most of the Health seeking behavior of Diabetes Mellitus Type 2 patients is Professional health system -> Popular Health system + Professional health system for 7 patients (50%). The results show that the amount of Diabetes Mellitus Type 2 patients who used Medical pluralism as health seeking behavior, and the patients who used professional health system, are slightly different.

Association between Carbapenem Resistance Enterobacteriaceae (CRE) and death: a systematic review and meta-analysis

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Abstract

Carbapenem Resistance Enterobacteriaceae (CRE) has emerged in health care facilities around the world[1-3]. Several studies demonstrate data regarding clinical outcomes for CRE infections including death. The association between CRE infection and the risk of death is not conclusive. Some studies indicated that CRE infection was associated with a higher risk of mortality while several studies reported that CRE infection was not associate with death. This systematic review and meta-analysis summarized the existing literature concerning the association between CRE and mortality. A systematic literature review was performed by searching the EMBASE, PubMed, Scopus and International Pharmaceutical Abstract databases to identify studies assessing the association between CRE and death published from April 2012 to October 2017. A meta-analysis was performed using random effect model. Heterogeneity was assessed using the I^2-statistic. Eleven studies retrieved from literature search were included in this meta-analysis. The underlying populations were heterogeneous (I^2 = 64%). Pooled risk estimates from five studies demonstrated a significant association between CRE and death (pooled-adjusted OR [aOR] = 4.02; 95% CI = 4.42, 6.68). The unadjusted variable pooled from six studies demonstrated a significant association between CRE and death (pooled-unadjusted OR= 2.68; 95% CI = 1.68, 4.29). In conclusions, pooled risk estimates from this meta-analysis revealed that CRE was associated with increased mortality risk. Our analysis implies a need for strict infection control measures.

Keywords: Enterobacteriacae, carbapenem, resistance, death, mortality

The development and improvement process of One Tambon One Product. The case study: herbal green oil product from the community enterprises at Sattahip District, Chonburi Province

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Abstract

This research project aims to develop a process of product One Tambon One Product name “Herbal Green Oil”. The research was conducted in Sattahip district, Chonburi province. The methodology was a qualitative research. There were divided in 3 parts. The part I was a document research to prepare a basic data for the next part. The part II was the methodology which was In-depth interview (15 of key persons including the Herbal green oil owner, a marketing specialist, a marketing lecturer, the officer from the department of the community development and the product customers).The part III was integrated data of product information and problems were collected. The problem topics were divided in the production process and the marketing. The problems of the product are the mixing processing, product design, product variety and cost of raw materials. The marketing dimensions were brand awareness, promotion, distribution channels. In this step, the prioritize tools used Performance matrix and Root cause analysis. The final result from this research was the executive summary which was a guideline for the business owner. The suggestion were solved the problem that found before. In example order of mixing, product design, cost of raw material, marketing mix.

Keywords: herbal Products, marketing, one tambon one product, Chonburi

Molecular mechanisms of CK5/6 monoclonal antibody coupled nanoparticle for the treatment of Triple Negative Breast Cancer

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Abstract

Triple Negative Breast Cancer (TNBC) is extremely heterogeneous diseases due to the absence of receptors, such as estrogen receptor (ER), progesterone receptor (PR), as well as human epidermal growth factor receptor-2 (HER-2). The present study was aimed to develop a nanoparticle coupled with CK 5/6 monoclonal antibody (mAb) for potential targeting TNBC. The nanoparticle was formulated by emulsification method which was further evaluated. The monoclonal antibody was loaded with nanoparticle by using M-maleimidobenzoyl-N-hydroxysuccinimide (MBS) as cross linking agent. The nanoparticles were subjected to SDS PAGE and cytotoxicity was analyzed by MTT assay. Further, molecular mechanism was characterized by subjected to Western blot and RT-PCR studies. The SDS PAGE results showed that mAb integrity remained the same after conjugation with nanoparticle. The nanoparticle showed significant anticancer activity with MDA-MB-468 cell line and evidence that drastic reduction of CK 5/6 protein in MDA-MB-468 cell lines. These results showed that, reduction of CK5/6 protein expression of nanoparticle may be used to treat TNBC

Keywords: Triple negative breast cancer (TNBC), Nanoparticles, Paclitaxel, and CK5/6 mAb.

Comparative study of pharmacoinformatics database between Micromedex® and Mobile medical applications

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Abstract

Drug Information services (DISs) play an important role in providing pharmacy information and supporting pharmaceutical practice and care in hospitals and community pharmacy. Currently, mobile medical and drug information applications are increasingly being used, while access to reliable database data remains limited. Therefore, the purpose of our study is to study the pharmacoinformatics database with comparative analysis, data searching capabilities, and completeness in each mobile medical applications with gold standard Micromedex® database. The research questions were collected from the Chonburi Hospital Drug Information Center of 209 questionnaires, classified into 13 categories based on the type of questionnaire information. There were 194 randomly selected questionnaires for searching and comparing query capability, and query completeness in each Lexicomp®, Medscape®, and Epocrates® available mobile applications with Micromedex®. Using SPSS program, one-way analysis of variance (ONE WAY ANOVA) found that Epocrates® had the ability of searching and completeness of the query when comparing the Micromedex® database significantly ($p=0.017$) but Lexi-comp® and Medscape® did not differ significantly ($p = 0.178$) and ($p = 0.159$), respectively. Mean difference between Lexi-comp® and Medscape® was 0.007. It is concluded that Lexi-comp®'s capabilities and completeness over Medscape® and Epocrates® compared to the Micromedex® database. Therefore, for the purpose of considering mobile medical applications in answering the pharmacy question, Lexicomp® may consider as the first alternative to Micromedex® database due to its ability and completeness.

Keywords: Mobile medical applications, DIS, Micromedex

Safety monitoring in patients using dabigatran

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Abstract

Background and Methods Dabigatran is one of New Oral Anticoagulants (NOAC). It is just recently introduced for hospital use in Thailand, therefore this drug should be used with cautions. Previous studies have demonstrated Dabigatran-related undesirable effects. Our research group investigated incidence of side effects due to Dabigatran using 1 year retrospective descriptive study design in 42 outpatients who receive Dabigatran 110 mg twice daily. Data was collected from computerized database from Chonburi Hospital, Thailand. Primary endpoints are discontinuation of Dabigatran or The International Classification of Diseases, Tenth Edition (ICD10) related to Dabigatran's side effects.

Results Thirteen patients (30.95 %) discontinued Dabigatran and 7 patients (16.67 %) experienced side effects. Most common side effects found were gastritis and dyspepsia (2 patients each or 4.76 %). Other side effects found were major bleeding (gastrointestinal bleeding), tinnitus and minor bleeding (nose bleeding), in which 1 patient (2.38 %) experienced each side effect.

Summary Common adverse effects of Dabigatran are gastritis and dyspepsia. This information can help healthcare professionals to make rational decision of drug choice suitable for each patient.

Keywords: Dabigatran, Adverse effect, Monitoring

Evaluation of anti-inflammatory effects of the selected herbs from Eastern part of Thailand

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Abstract

Eastern part of Thailand is the region which have plenty of natural source and varieties of forest ecology. A lot of plants have been developed to be local Thai herb. However, only few herbs can be treated against inflammation. So this research is aim to evaluate the anti-inflammatory effect of herbs from Ethanol extract in the East of Thailand both Soxhlet extraction and Maceration from 5 herbs eg. *Calophyllum inophyllum*, *Clerodendrum disparifolium*, *Gelonium multiflorum A.*, *Gossypium arboreum* L., *Drosera Indica* L. The direct anti-inflammatory action will be studied by Proteinase inhibitory assay method while the indirect anti-inflammatory action will be studied by Anti-AGEs activity, FRAP and DPPH. *Gossypium arboreum* L. have the best direct anti-inflammatory action with Soxhlet extraction: IC_{50} 14.97 µg/ml. For the indirect anti-inflammatory action with FRAP method, *Calophyllum inophyllum* from Soxhlet extraction and *Suregada multiflora* from Maceration are the plants with best results with Trolox equivalent 33.0 ± 1.85 and 36.7 ± 3.93 respectively. Besides, DPPH and Anti-AGEs activity have the same direction which this information have possibility to adapt and use for pharmacology.

Keywords: anti-inflammatory, Anti-AGEs activity, FRAP, DPPH

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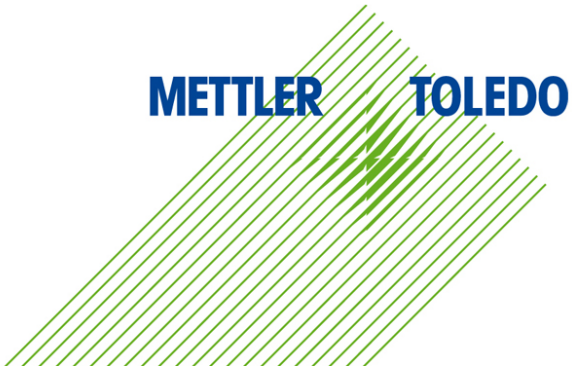


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