OPTIMIZATION OF EXTRACTION PARAMETERS OF SELECTED MALAYSIAN PLANTS TOWARDS ANTIUROLITHIATIC ACTIVITIES (IN-VITRO)

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I hereby declare that the work in this Master’s Thesis is my own except the quotations and summaries which have been duly acknowledged.

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ABSTRACT

Urolithiasis has become a worldwide problem and its management depends on surgical procedures that are costly and cause reoccurrence. *Anacardium occidentale* (*gajus*), *Ananas comosus* (*nanas*), *Aquilaria malaccensis* (*karas*), *Centella asiatica* (*pegaga*), *Ceiba petandra* (*kekabu*), *Euphorbia hirta* (*ara tanah*), *Ficus carica* (*ara*), *Melastoma malabathricum* (*senduduk*), *Piper sarmentosum* (*kaduk*) and *Tradescantia zebrina* (*kura-kura air*) have been used traditionally to treat urolithiasis. However, no scientific data has been recorded on the extraction parameters. Thus, the objective of this study is to assess for the best ethnobotanical plant extract with good antiurolithiatic properties, followed by optimization of extraction parameters of selected plant extracts. The antiurolithiatic activities conducted were turbidity and titrimetric assays (*in-vitro*). Ethnobotanical plant extracts assessment revealed *A. occidentale* and *A. malaccensis* had the best antiurolithiatic activities as both plants had no significant difference with standard drugs (*p* > 0.05). The extraction parameters studied were solvent concentration, extraction temperature and time that was screened using two-level factorial design and optimized by response surface methodology and central composite design. The results showed that optimum extraction condition for *A. occidentale* extract (0.4% ethanol, 31.5°C, 30 minutes) on both antiurolithiatic activities were exhibited 85.57±0.43% (turbidity) and 96.48±0.70% (titrimetric). Meanwhile, optimum condition of *A. malaccensis* extract (100% ethanol, 30.0°C, 30 minutes) demonstrated 83.58±0.75% (turbidity) and 86.57±0.80% (titrimetric). Phenols, alkaloids, saponins, flavonoids, tannins and terpenoids were identified in both optimized extracts and all have positive correlation on both antiurolithiatic assays except flavonoids. Toxicity testing using brine shrimp lethality assay presented non-toxic effect on optimized *A. occidentale* (LC50, 1412.50µg/mL) but *A. malaccensis* (LC50, 30.50µg/mL) revealed toxic effects. This study has given basic scientific evidence that optimum extraction condition is necessary to obtain optimum antiurolithiatic activity.
ABSTRAK

Urolithiasis telah menjadi permasalahan di seluruh dunia dan pengurusannya bergantung pada prosedur pembedahan yang mahal dan sering menyebabkan pembentukan semula. *Anacardium occidentale* (gajus), *Ananas comosus* (nanas), *Aquilaria malaccensis* (karas), *Centella asiatica* (pegaga), *Ceiba petandra* (kekabu), *Euphorbia hirta* (ara tanah), *Ficus carica* (ara), *Melastoma malabthricum* (senduduk), *Piper sarmentosum* (kaduk) dan *Tradescantia zebrina* (kura-kura air) telah digunakan secara tradisional untuk merawat urolithiatik. Bagaimanapun, tiada data saintifik yang direkodkan mengenai parameter pengekstrakan. Oleh itu, objektif kajian ini adalah untuk menyaring ekstrak herba etnobotanik terbaik dengan sifat antiurolithiatik yang baik, diikuti dengan pengoptimuman parameter pengekstrakan terhadap ekstrak terpilih. Kaedah antiurolithiatik yang dilakukan adalah kekeruhan dan titrimetrik (*in-vitro*). Analisis awal memaparkan aktiviti antiurolithiatik terbaik pada *A. occidentale* dan *A. malaccensis* kerana tiada perbezaan yang ketara dengan ubat standard, (p>0.05). Parameter pengekstrakan yang dikaji adalah kepekatan pelarut, suhu dan masa pengekstrakan yang disaring melalui kaedah faktorial dua peringkat dan dioptimumkan dengan kaedah permukaan tindak balas dan komposit pusat. Hasil kajian mendapati bahawa keadaan pengekstrakan optimum untuk aktiviti antiurolithiatik bagi ekstrak *A. occidentale* (0.4% etanol, 31.5°C, 30 minit) memperolehi 85.57±0.43% (keruhan) dan 96.48±0.70% (titrimetrik). Sementara itu, keadaan optimum ekstrak *A. malaccensis* (100% etanol, 30°C, 30 minit) menunjukkan 83.58±0.75% (keruhan) dan 86.57±0.80% (titrimetrik). Fenol, alkaloid, saponin, flavonoid, tanin dan terpernoid dikenal pasti dalam kedua-dua ekstrak yang dioptimumkan dan kesemuanya mempunyai hubungan positif dengan ujian antiurolithiatik kecuali flavonoid. Ujian ketoksikan mendapati kesan tidak toksik pada *A. occidentale* yang dioptimumkan (LC\(_{50}\), 1412.50μg/mL) tetapi *A. malaccensis* (LC\(_{50}\), 30.50μg/mL) menunjukkan kesan toksik. Kajian ini telah memberikan bukti saintifik asas bahawa keadaan pengekstrakan optimum diperluankan untuk mendapatkan aktiviti antiurolithiatik yang optimum.
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LIST OF SYMBOLS AND ABBREVIATIONS

% - Percent
°C - Degree celcius
cm - Centimeter
g - Gram
M - Molarity
mg - Milligram
mL - Milliliter
mm - Millimeter
N - Normality
μg - Microgram
μL - Microliter
ANOVA - Analysis of variance
BSLA - Brine shrimp lethality assay
CaOx - Calcium oxalate
CCD - Central composite design
CV - Coefficient of variation
ESWL - Extracorporial shock wave lithotripsy
GAE - Gallic acid equivalent
LC - Lethality concentration
PNL - Percutaneous nephrolithotomy
RE - Rutin equivalent
RSM - Response surface methodology
TAE - Tannic acid equivalent
TFC - Total flavonoid content
TPC - Total phenolic content
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Nur Fazira Abdul Rahim, Norhayati Muhammad, Norazlin Abdullah, Balkis A. Talip and Kay Huck Poh. 2020. The interaction effect and optimal formulation of selected polyherbal extracts towards antioxidant activity. Food Research, 4(6), 2042-2048. (Scopus)
CHAPTER 1

INTRODUCTION

1.1 Research background

In this century, medicinal plant therapies that have been acknowledged by traditional practitioners to cure various diseases has been well adopted and adapted by today communities all over the world. Globally, herbal medicines are growing fast nowadays might be because they are cost effective, eco-friendly, readily available and culturally acceptable as well as high margin of safety with minimal side effects as compared to synthetic drugs (Arya, Pandey, & Verma, 2017; Biglarkhani et al., 2017; Jain, 2003). Besides that, medicinal plants have a rich source of phytochemicals and allopathic constituents that have been conventionally used for treating ailments including urolithiasis which is characterized by the formation of stones in the urinary system (Ahmed, Hasan & Mahmood, 2016; Yadav et al., 2011; Ahmad & Ismail, 2003).

Malaysia is well-known to have wide variety of medicinal plant sources that may mediate urolithiasis. Diverse ethnicity in Malaysian communities such as Malay, Chinese, Indian and indigenous people was inherited with traditional knowledge on medicinal plants that could inhibit and disintegrate kidney stone formation (Adnan & Othman, 2012; Ong & Norzalina, 1999). Over the years, antiurolithiatic plants had been widely used in the form of decoction, infusion, or juice, to eliminate kidney stones and to prevent their reoccurrence (Ahmed et al., 2018). Previous pre-clinical and clinical studies have established valuable effects of many plants related with kidney functions and stones in experimental animals (Das, Vasudeva & Sharma, 2019).

Basically, the term used in urolithiasis is based on the location of the stone such as nephrolithiasis (renal calculi or kidney stones), ureterolithiasis (ureter calculi...
or ureter stone) and cystolithiasis (bladder calculi) (Mikawlrwng, Kumar & Vandana, 2014). It is a complex physicochemical process as its formation due to the super saturation of mineral salts in the urinary tract (Balaji, Banji & Banji, 2015). It is estimated to occur in approximately 12% of the global population and its occurrence rate in males is 70-81% and 47-60% in female (Shukla et al., 2017; Soundararajan et al., 2006). It can range from a minor health problem to a life threatening situation since it is a multifactorial disease with high prevalence and reoccurrence rate. There are four categories of urinary stones which are dominantly calcium oxalate (75-90%), followed by struvite (10-15%), uric acid (3-10%) and cysteine stone which only 0.5 to 1% (Aggarwal et al., 2010).

In this study, calcium oxalate (CaOx) stone is highlighted as it is the most common stone and it was prepared experimentally. The properties presence in the extracts of Anacardium occidentale (gajus), Ananus cosmosus (nanas), Aquilaria malaccensis (karas), Ceiba pentandra (kekabu), Centella asiatica (pegaga), Euphorbia hirta (ara tanah), Ficus carica (ara), Melastoma malabathricum (senduduk), Piper sarmentosum (kaduk), and Tradescantia zebrina (kura-kura air) were being determined for its antiurolithiatic effects as well as its phytochemical constituents. In evaluation of antiurolithiatic activity, polyherbal drug (cystone) and chemical drug (potassium citrate) were used as a reference standard. The effect of solvent concentration and also extraction time and temperature were evaluated to determine the optimum extraction condition for maximizing antiurolithiatic therapy against CaOx which induced urolithiasis (in-vitro) for the selected optimized extracts.

1.2 Problem statement

In recent years, urolithiasis has become a worldwide problem as it is long term ailment that give consequences throughout patient's lifetime (Al-yusofy et al., 2017; Patel et al., 2012). It is generally known as third common affliction of urinary system after urinary tract infection and prostrate disease with estimated occurrence in approximately more than 1/10th of population (Shukla et al., 2017; Bahmani et al., 2016; Hiatt & Friedman, 1982). The prevalence of this ailment has been increasing in the world ranging from 7% to 13% in North America, 5% to 9% in Europe and 1% to 5% in Asia (Liu et al., 2018). In Germany, it is reported that almost 750 000 kidney
stone cases per year with 25% experienced stone reoccurrence (Knoll, 2010; Hesse et al., 2003). This might be influenced by climate, dietary and lifestyle habits (Romero, Akpinar & Assimos, 2010).

Malaysia has shown the same pattern of incidence as it is growing for period of year in 1962 to 1981 per 100 000 populations (Liu et al., 2018; Alatab et al., 2016; Sreenevasan, 1990). Out of more than 4000 people subjected to this disease, the Chinese community lead with of 48%, followed by Malays (37.4%), Indians (13%) and mostly other races with 1.3% (Sreenevasan, 1990). However, another study done in teaching hospital in Kelantan, Malaysia reported that Malay ethnicity dominated with 91.1% (Nouri & Hassali, 2018). There were lack of documentation on this disease in Malaysia and last published was in 1990 (Nouri & Hassali, 2018; Sreenevasan, 1990). In addition, Malaysia has a subtropical climate that could contribute to the formation of kidney stones in humans. Such climate tends to accelerate body dehydration process caused by exposure to hot temperature. This situation causes urine concentration thus leading to stone formation and high frequency of urolithiasis (Hussein et al., 2013).

Nowadays, the management of urolithiasis includes surgical procedures such as extracorporial shock wave lithotripsy (ESWL), ureteroscopy (URS) and percutaneous nephrolithotomy (PNL). EWSL is the most widely used method as it involves noninvasive procedure that uses sound waves to fragment calculi (Silberstein, Lakin & Parsons, 2008). However, stones that larger than five (5) mm or stones failed to pass through the urinary tract required those interventional procedures (Mikawlrawng et al., 2014; Gilhotra, Mohan & Christina, 2013). In addition, this treatment has been proved to have renal side effects as well as quite expensive and high in reoccurrence rate (Tiwari et al., 2012).

Even though there are plentiful of progress in the study of the biological and physical manifestation of urolithiasis, there is truly no satisfactory drug available for the treatment of urolithiasis, particularly for the prevention of reappearance of the stones (Moe, Pearle & Sakhaee, 2011). The pharmaceutical drugs available nowadays are mostly might have adverse effects such as cause nausea, anxiety and also kidney damage that compromise their long-term use (Ankur et al., 2010; Atmani & Khan, 2000). In addition, it was reported that approximately 50% of patients with previous urinary or kidney stones have reappearance within 10 years (Ankur et al., 2010). Therefore, urolithiasis can be considered as a serious disease.
1.3 Research objectives

The main objective of this study is to optimize extraction parameters of selected Malaysian plants on *in-vitro* antiurolithiatic activity. Meanwhile, the specific objectives are as follows:

i. to assess the best ethnobotanical plant extract that had higher antiurolithiatic activities than standard drugs (positive controls) or no significant different with those drugs ($p>0.05$).

ii. to determine the optimum extraction parameters for maximum inhibitory and dissolution antiurolithiatic effect on selected plant extracts.

iii. to evaluate the phytochemical contents and toxicity effect of selected optimized plant extracts.

1.4 Scope of study

In order to achieve the research objective, the scopes of study that has been determined are:

i. The plant extracts used are *A. occidentale* (leaves), *A. cosmosus* (fruit), *A. malaccensis* (stem), *C. pentandra* (leaves), *C. asiatica* (whole), *E. hirta* (whole), *F. carica* (leaves), *M. malabathricum* (root), *P. sarmentosum* (leaves) and *T. zebrina* (whole).

ii. The extraction process on preliminary antiurolithiatic assessment extracts was done by decoction method.

iii. The best plant extracts on screening of antiurolithiatic properties were determined if the plant extracts demonstrate higher or no significant difference with standard drug, ($p>0.05$).

iv. The antiurolithiatic properties was analyzed by turbidity (nucleation) and titrimetric (calcium oxalate dissolution) assays.

v. The extraction parameters that were evaluated are solvent concentration, extraction temperature and extraction time.

vi. The screening of extraction parameters was designated using fractional factorial design (Resolution III, $2^{3-1}$ design).
vii. The optimum solvent concentration, extraction temperature and time were determined by using response surface methodology (RSM).

viii. The phytochemical contents of the optimized extract that were evaluated are phenols, alkaloids, saponins, flavonoids, tannins and terpenoids by qualitative and quantitative methods.

ix. The toxicity test of the optimized extract was done by using brine shrimp lethality assay.

1.5 Significance of study

The World Health Organization (WHO) reported that around 80% of the population in developing countries still depends on medicinal plants to treat various diseases (Bahmani et al., 2016; Ghatapanadi, Johnson & Rajasab, 2011). Recently, research on beneficial uses of medicinal plants have become mainstream globally including Malaysia. An estimate of 73% of households in Malaysia consume herbal products based on a survey conducted by the Forest Research Institute Malaysia (FRIM) (Ahmad et al., 2015). Malaysia have approximately 15000 species of vascular plants with about 8300 species in Peninsular Malaysia and 12000 species in Sabah and Sarawak (Saw et al., 2010). In addition, around 1300 species out of 14500 flowering plants are recorded to have medicinal use. Same goes to Sabah where about 1200 species out of 7411 species (excluding bryophyta, algae and fungi) found were used statewide for medicinal purposes including kidney stone problems (Kulip et al., 2010; Kulip, 2003).

Besides that, medicinal plants have been used traditionally to treat kidney stone even before the invention of modern treatments (Kumar, Latheef, & Remashree, 2014). There were studies reported that medicinal plants being effective and naturally safe remedies for kidney stone diseases (Alok et al., 2013; Butterweck & Khan, 2009). In the food industry, the formulated herbal supplement drinks from medicinal plants are becoming significant as consumer awareness is increasing in order to develop a healthy lifestyle for managing urolithiasis. Thus, this study will start by screening a few types of Malaysian medicinal plants that have been traditionally reported for its antiurolithiatic potential and determine the optimum conditions for evaluating the antiurolithiatic activities (in-vitro).
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