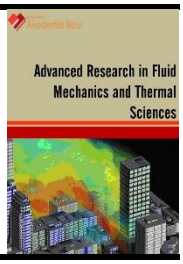
Penerbit Akademia Baru

Journal of Advanced Research in Fluid Mechanics and Thermal Sciences

Journal homepage: www.akademiabaru.com/arfmts.html
ISSN: 2289-7879



Phytochemical Analysis and *In-Vitro* Antiurolithiatic Properties of Selected Malaysian Herbs

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ARTICLE INFO

Article history:
Received 16 October 2019
Received in revised form 25 November 2019
Accepted 27 November 2019
Available online 15 December 2019

ABSTRACT

The aim of this study was to investigate the phytochemical content of selected Malaysian herbs and their potential antiurolithiatic effects using *in-vitro* method. The herbs involved are *Ceiba pentandra, Cymbopogon citratus, Euphorbia hirta, Melastoma malabathricum* and *Ortosiphon stamineus*. Aqueous extracts of each herbs were prepared through decoction while Standard drugs Cystone, was used as positive control in comparison. Qualitative analysis was carried out to detect phytochemical presence and nucleation assay to investigate their inhibition effects towards calcium oxalate crystallization urolithiasis in vitro. Based on results, the same trends were observed between phytochemical content and inhibition rate of calcium oxalate crystallization. *O. stamineus* extract (73.48%) which showed the highest inhibition rate hold the most phytochemical content. while the lowest inhibition rate was occupied by *C. citratus* extract (45.45%) with the least phytochemical content. The high amount of phytochemicals particularly saponin followed by steroid and terpenoid in *O. stamineus* extract might contributes to the high inhibition activities of calcium oxalate crystallization as compared to low amount of phytochemicals observed in *C. citratus* extract. It can be concluded that *O. stamineus* possesses highest inhibition percentage against calcium oxalate which could be attributed to its saponins, tannins, steroid and terpenoid content.

Keywords:
Malaysian; herbs; antiurolithiatic; in-vitro

1. Introduction

Urolithiasis is caused by uncontrolled pathological crystallization in the kidney, bladder or urethra. The disease occurs when solvent becomes supersaturated and individual's biological system is unable to maintain the calcium hemostasis then leading to the formation of precipitates in the body called as kidney stones [1, 2]. The stones are made up of tiny crystals from the minerals calcium, oxalate, calcium phosphate, uric acid, struvite and cysteine [3]. The development of kidney stone

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involved biological mechanism of supersaturation, nucleation, crystal growth, crystal aggregation, and crystal retention. Urolithiasis onset is due to multiple genetic and environmental factors, such as hot climate (fluid loss) and sun exposure (vitamin D) [3, 4]. A good start to hinder kidney stone disease is to drink an adequate amount of fluid while limiting sodium, protein and oxalate-rich food in daily diet. Another kidney stone diet is to consume calcium during meal time in order to avoid calcium oxalate formation [5].

The condition can be treated with several non-invasive treatments such as extracorporeal shock wave lithotripsy (ESWL) and percutaneous nephrolithotomy. However, the treatment is expensive and medical world have witnessed the high recurrence of stone formation after treatment [6]. Usage of herbs showed an increasing trend as market research's finding [7]. Therefore, indicates a significant increase in sales of herbal supplements. The increase is due to the popular use of herbs as preventive measure for numerous health problems. It is because high antioxidant herbs are well known of having high content of antioxidant compounds and have been recognized as having potential in reducing variety of diseases risk [8]. Apart from that, herbs can be said as more affordable, give lesser side effects and easily assessable. Hence, the study of biological activity and chemical composition of medicinal plant extracts as a potential source of natural antioxidants are becoming a trend in development of product [9, 10].

Malaysia has diversity of herbs that are not fully explored for their antiurolithiatic properties. From *C. pentandra* to *O. stamineus*, all of these herbs can be easily planted and grown due to Malaysia's climate, being hot and humid throughout the year. *Ceiba pentandra* (Bombacaceae) family is locally known as 'kekabu' in Malaysia and also as silk cotton tree [11] was used in many countries due to their demonstrating therapeutic nature such as diuretic and anti-inflammatory [12]. The stem bark also was scientifically proven with antiurolithiatic effect [13]. *Cymbopogon citratus* (C. citratus) in Malaysia is generally known as lemongrass in English and 'serai makan' in Malay [14]. With wide range of useful components in *C. citratus*, it usually employed as therapeutic treatment and also for nutritional purpose [15]. Decoction of water from *C. citratus*'s leaves and roots when consumed helps to ease urinary problems and stomach ache [16]. *Euphorbia hirta* (Euphorbiaceae) is known as 'Ara Tanah' in Malay. Amongst practitioners of traditional medicine, whole plant parts of E. hirta are usually made into decoction or infusion to treat kidney stones [17]. *M. malabathricum* (Melastomataceae) is commonly known as 'senduduk' in Malaysia [18]. The leaves and roots parts are used for wound treatment, diarrhea, and stomach ulcers and also for post-natal care in women by Malay people [19]. *Orthosiphon stamineus* (Lamiaceae) is a plant that resemble cat's whisker and commonly known in Australia and Southeast Asia as Java tea and Misai Kucing [20]. Due to its mild diuretic action properties, *O. stamineus* can used to treat kidney and bladder related problems [21].

2. Methodology

2.1 Chemicals and Instruments

Cystone tablet (Himalaya Drug Company, India) was purchased from Vycon Pharmacy, Kuala Lumpur, while chemicals used in this experiment which consist of calcium chloride (Bendosen, Germany), sodium oxalate (QReC, New Zealand), chloroform (HmbG, Germany), Mayer's reagent (QReC, New Zealand), sulphuric acid (QReC, New Zealand), hydrochloric acid (QReC, New Zealand) and sodium chloride (QReC, New Zealand) were analytical grade and commercially available.

2.2 Collection and Preparation of Plant Materials

The roots of *M. malabathricum* was collected from Department of Agriculture, Serdang, Selangor. The leaves of *C. pentandra* was obtained from Pontian, Johor, while the grinded pulps of *C. Citratus, E. hirta* and *O. stamineus* were purchased from Ethno Resources Sdn Bhd, Sungai Buloh, Selangor. All of the samples were collected during the month of July 2018. The plant extracts preparation was retrieved from Muryanto with slight adjustments [22]. The plant samples were thoroughly washed under tap water and cut into small pieces. Each sample were dried in an oven at 40°C for three days and was homogenized into coarse powdered form by using standard laboratory blender.

2.3 Preparation of Plant Extracts

Cystone was used as positive control in the evaluation of antiurolithiatic properties of plant extracts while distilled water was used as negative control. The plant samples were extracted by water decoction method in which 50 g of sample was weighed and slowly heated in 1000 mL of distilled until the volume reach one third of the mixture. Afterwards, the extract was cooled down and filtered using filter paper. The filtered extracts were concentrated in the oven at 40℃ untill solid form was visible. The extracts were weighed and placed in a universal vial. The crude extracts were stored in a freezer at -20℃ for future use [22].

2.4 Evaluation of Antiurolithiatic Properties by Nucleation Assay

The nucleation assay used was similar to the previous study with minor modifications [23]. Mixtures of calcium chloride and sodium oxalate were prepared to obtain final concentration of 10.0 mM and 1.0 mM, respectively; in a Tris buffer containing Tris 0.05 mM and NaCl 0.15 mM adjusted to pH 5.7. Briefly, 950 µL of calcium chloride solution were mixed with 100 µL of different plant extract. The addition of 950 µL of sodium oxalate solution into previous mixture initiated the crystallization process. The mixtures of both solution was magnetically stirred at 800 r.p.m. using a hot plate magnetic stirrer (Favorit Model HS0707V2, Malaysia). The temperature of mixture was maintained at 37°C. The optical density (OD) of the solution was monitored at 620 nm by using UV Vis spectrophotometer (Thermo Scientific, BioMATE 3S, USA) right after the addition of solution containing calcium within 10 min intervals. All experiments were done in a triplicate. The nucleation rate were determined by comparing the time of induction in the presence of both extract and control (no extract) using the following formula [24].

$$\text{Inhibition } (\%) = \left[1 - \frac{Tsi}{Tsc}\right] \times 100 \quad (1)$$

where
Tsc: slope of graph without inhibitor (negative control).
Tsi: slope of graph in the presence of inhibitor (positive control/ plant extracts).

2.5 Phytochemical Screening of Plant Extracts

Phytochemical screenings were tested on all extracts as stated in standard methods [25].

2.5.1 Detection of alkaloids

Plant extracts (2 mL) were diffused in dilute hydrochloric acid and was filtered. Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The presence of alkaloids was indicated by the formation of yellow colored precipitate.

2.5.2 Detection of saponin

Plant extracts (2 mL) were diluted in 20 mL distilled water and was vigorously shaken for 15 minutes in a centrifuges tube. The presence of saponin was determined by the formation of 1 cm layer of foam.

2.5.3 Detection of steroid

Plant extracts (2 mL) were flux in chloroform (2 mL) and later was added with concentrated sulphuric acid (2 mL). Red colour produced in the lower chloroform layer indicates the presences of steroid.

2.5.4 Detection of terpenoid

Plant extracts (2 mL) were flux in chloroform (2 mL) and left evaporated to dryness. Concentrated sulphuric acid (2 mL) was added later and heated for about 2 minutes. The appearance of a greyish colour demonstrates the existence of terpenoid.

2.5.5 Detection of tannin

A mixture containing 1% gelatin solution and sodium chloride was added into plant extracts (2 mL) test tube. The presences of tannins was indicated by the formation of white percipitates.

2.6 Stastistical Analysis

All experiments were done in triplicate and the data were presented as mean values and standard deviation. One way ANOVA were done in SPSS Statistics software with the level of significant (p<0.05).

3. Results and Discussion

The inhibition percentage on calcium oxalate crystal of each plant extracts and standard drug cystone was presented in Figure 1 while the presence of phytochemicals shown in Table 1. Phytochemicals produced by plants include compound such as tannins, flavonoids, glycosides, saponins, steroids, alkaloids and many more. The attractive colours and fragrance of the plants is due to specific phytochemicals present in them [26]. All of the selected plants showed presence of different types of phytochemical which could responsible to the antiurolithiatic activity of the plants. Higher inhibition percentage indicates more potency in dissolution of calcium oxalate crystals.

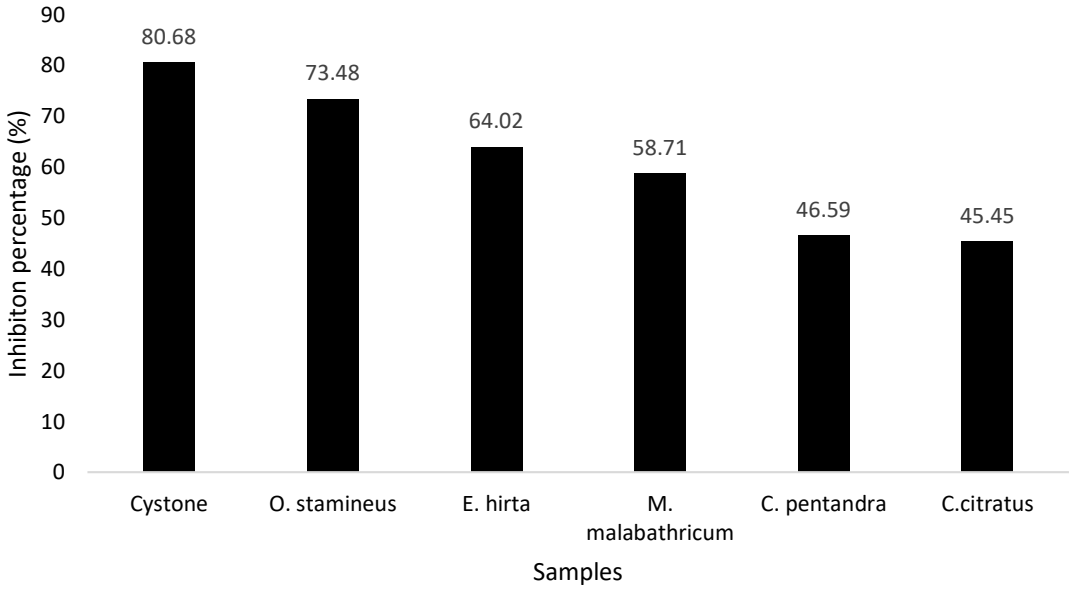

Fig. 1. Inhibition percentage of samples

Table 1
The phytochemicals screening for different types of plant extracts

Phytochemicals	Plant extract				
	O. stamineus	*E. hirta*	*M. malabathricum*	*C. pentandra*	*C. citratus*
Alkaloid	-	+	-	-	+
Tannin	+	-	++	-	+
Steroid	++	+++	++	+++	+
Terpenoid	++	+	++	++	+
Saponin	+++	+++	++	+++	++

Based on the results, extract of *O. stamineus* exhibited highest inhibition percentage. The high inhibition of *O. stamineus* extracts might due to the abundance amount of saponin, followed by stereoid and terpenoid. From previous research, it is identified that saponin rich fraction from fruits and plants gives therapeutic action and responsible for the beneficial effect in the treatment of kidney stone formation [27,28]. Although *E. hirta* and *C. pentandra* has the same amount of saponin, it has lower amount of other phytochemical tested.

The percentage inhibition of *E. hirta* and *M. malabathricum* extracts was almost similar which is (64.02 ± 1.74%) and (58.71 ± 1.32%) respectively. The value of inhibition percentage possessed by *E. hirta* is slightly higher compared to *M. malabathricum* mainly to the fact that it contains high amount of saponin and steroid. Saponin literally contributes significantly to the curing of renal problem with several positive characteristics. Moreover, saponins is one of the component existed in numerous medicinal herbs with high allegation of antiurolithiatic properties [29]. Thus proving its existences as a contributor to the high antiurolithiatic activity in *O. stamineus* and *E. hirta.*

Meanwhile, the inhibition activity in *M. malabathricum* might be influenced by the presence of tannin, steroid, terpenoid and saponin compound. Tannin inhibits the formation of calcium oxalate crystal by its complexation with calcium based on previous research reported on *S. grandiflora* leaf [30]. Similar to saponin, steroid existed in all plant extract and it has the potential to inhibit crystallization of kidney stone. *C. pentandra* (46.59 ± 1.14%) and *C. citratus* (45.45 ± 1.14%) have close inhibition percentage value to each other. However, *C. pentandra* plant extract showed better

inhibition percentage than *C. citratus* due to the presence of a slightly high amount of saponin, terpenoid and steroid. The presence of terpenoid also gives positive effect towards the inhibition activity of *C. pentandra*. This is associated with the ability of terpenoid to inhibit the area and reduce the size of calcium oxalate crystal [28]. The role of steroid as beneficial constituents which help to aid in the inhibition of calcium oxalate also can be found in several studies related to antiurolithiatic [31, 32].

Lastly, *C. citratus* plant extract showed inhibition percentage of 45.45 ± 1.14% due to the presence of tannin and a slightly high amount of saponin. Both tannin and saponin was proven as definite compound for calcium oxalate inhibitor as stated in previous studies [29, 33]. However, it has the lowest inhibition percentage among all of the plants even all phytochemical tested are presence. This might due to low amount of phytochemical which insufficient to ameliorate kidney stone.

4. Conclusions

The present investigations provide useful information of selected Malaysian herbs on phytochemical and inhibition towards the stone crystal formation. *O. stamineus* exhibited highest percentage of inhibition in comparison to the other plant extracts. High amount of phytochemicals in plant extract also contributed to the high inhibition percentage for calcium oxalate. This can be proven with several previous studies regarding the effect of phytochemicals towards urolithiasis. Eventhough cystone was found to be the most effective in the inhibition, all of the plant exract presented has the ability to inhibit the stone crystal formation.

Acknowledgement

This research is funded by the Ministry of Education Malaysia under Fundamental Research Grant Scheme (FRGS) Vot No. 1646. It is also partially sponsored by Universiti Tun Hussein Onn Malaysia under Research Management Centre Fund Research (RMC) Fund E15501and Postgraduate Research Grant Scheme (GPPS) Code H290 and H291.

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