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Effect of Different Extraction Techniques of *Persicaria odorata* Extracts Utilizing Anti-bacterial Bioassay

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Authors' contributions

This work was carried out in collaboration between all authors. Author RS designed the study, performed the statistical analysis, wrote the protocol. Author VK perform the experimental part, managed the analyses of the study. Authors JK, FA and EY managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Persicaria odorata is a common plant and well known in Malaysia as "*Daun kesum*" that is commonly used in cuisines and has various medicinal properties. This study was conducted to investigate the antimicrobial activity and the extraction technique that

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produce the most active plant extract. The leaves were extracted using decoction, maceration assisted by ultra-sonication and percolation with soxhlet extractor to produce the respective extracts. All extracts were tested against four bacterial strains which included gram positive and gram negative bacteria using disc diffusion method. In this research gentamicin 10 µg were used as the antibacterial standard. The antimicrobial activity of the active extract was evaluated quantitatively using three different concentrations. The result from this study shows that *Persicaria odorata* leaves have high potential to be used as natural antibacterial agent against some bacterial infections depending on the method used to extract the active ingredient. The results shows that the extract obtained with percolation with soxhlet technique shows the best antibacterial activity followed by maceration with ultrasonication. Decoction extracts shows the weakest antibacterial activity. The extract obtain from both maceration with ultra-sonication and percolation using soxhlet extractor show significant ($P=0.05$) antimicrobial activity against all four bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella spp.*).

Keywords: *Persicaria odorata*; anti-bacterial activity; *Staphylococcus aureus*; *Escherichia coli*; *Salmonella typhi*; *Bacillus subtilis*.

1. INTRODUCTION

Complementary and alternative medicine (CAM) has been used widely in general hospitals to overcome medical problems such as infections, complications as well as to maintain the patient's health [1]. Research on CAM has been established and the Food and Drug Administration (FDA) have approved several herbs for medical indications. Medicinal plants are considered an important element in various traditional systems of medication such as Traditional Chinese Medicine and Ayurvedic. In China about 30% to 50% of total medicinal consumption by the people is made up of traditional herbal medicine.

One of the potential local plants that can be looked into is *Persicaria odorata*. *Persicaria odorata* leaves are one of natural plant parts that have been traditionally used worldwide in medicine, cuisines, pharmacy and cosmetics [2]. *Persicaria odorata*, the Vietnamese coriander, is an herb whose leaves are used in Southeast Asian cooking. Other English names for the herb include Vietnamese mint, Vietnamese cilantro, Cambodian mint and hot mint. The Vietnamese name is rauräm, while in Indonesia, Malaysia and Singapore it is called *Daun kesum*, *daunkesom* or *Daun laksa* (laksa leaf) [3]. It is neither related to the mints, nor is it in the mint family *Lamiaceae* but the general appearance and odor are reminiscent. *Persicaria* is in the family *Polygonaceae*, collectively known as smartweeds or pinkweeds [4].

The Vietnamese coriander is a perennial plant that grows best in tropical and subtropical zones in warm and damp conditions [5]. In advantageous conditions, it can grow up to 15 to 30 cm. In the winter or when the temperature is too high, it can wither [6]. The top of its leaf is dark green, with chestnut-colored spots while the leaf's bottom is burgundy red. The stem is jointed at each leaf. In Vietnam it can be cultivated or found in the wild. It can grow very well outside in summer in non-tropical Europe [7]. Preferring full sun and well-drained soil and is ideal here for pots and tubs. It should be brought inside for winter and treated as a house plant. It rarely flowers outside the tropics, but it is the leaves that have strong culinary use [8].

Clinical studied showed that the *Persicaria Odorata* possess toxic oils; other constituents of the *Persicaria odorata* leaf have been shown to be toxic to bacteria, parasites and fungi [6,9]. These workers attempted to clone the substance defensins which are native to plants such as *Persicaria odorata* to test their effects in the petri dish [9]. The defensins extracted from plant tissues protect the plant from fungi. As an anti-parasitic agent. In vitro studies found that *Persicaria odorata* extracts were effective against resistant species of *Plasmodium* [10].

The aim of the study was to evaluate different extraction techniques in order to produce an extract of *Persicaria odorata* with the most potent anti-microbial property against pathogenic bacteria. The *Persicaria odorata* extracted using three different techniques, which are percolation using soxhlet, maceration with ultra-sonication and decoction [11]. The antimicrobial that might present in the extraction may be the key to the development of the newer sources of antibiotics [12].

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The fresh leaves of *Persicaria odorata* (Fig. 1), the Vietnamese coriander were collected locally in Jalan Banting Klang, Malaysia at 25th of August 2013. Identification and authentication of the plant was done at Forest Research Institute Malaysia (FRIM), Malaysia, for taxonomic identity of the plant.



Fig. 1. *Persicaria odorata* (L.)

2.2 Preparation of Extracts

The leaves were washed and air-dried for about 1 week. The dried leaves were ground in to fine powdery forms using electric grinder and stored in air-tight container [13]. The powder was then used to prepare the *Persicaria odorata* extracts. The *Persicaria odorata* extracts were obtain using three different techniques, in order to determine which extraction technique will give the *Persicaria odorata* extract with the most effective anti-microbial activity. The three different extraction techniques are; decoction, maceration and percolation techniques [14]. The extracts obtain from respective extraction technique was then filtered using a fine muslin cloth followed by filter paper [15]. Then the filtrates were evaporated under reduced pressure using rotary evaporator and kept in desiccators for further use in antimicrobial test.

2.3 Preparation of Different Concentration of the Extracts

For concentration of 1mg/mL, 100mg of the extracts was diluted with 100 μ L of DMSO solution and 100mL of distilled water [16]. The same method was followed to prepare concentration of 50mg/mL and 75mg/mL. For gentamicin 1mg/mL were used as the positive control.

2.4 Microorganisms Isolation

Four isolates of microorganisms which are *Staphylococcus aureus*, *Salmonella sp.*, *Bacillus subtilis*, and *Escherichia coli* were used in this study identified and obtained from the library of germs at MSU laboratory. All bacteria were isolated from each bacteria plate and were grown in nutrient broth where a loop full of each bacterial strain was inoculated in 5ml of nutrient broth in a test tube and incubated in an incubator for 24 hours at 37°C to get the active strains. These will be used as inoculums for subsequent studies [17].

2.5 Kirby-Bauer Disk Diffusion Susceptibility Test

Antimicrobial effects of the aqueous and methanol extracts of *Persicaria odorata* were determine by Kirby-Bauer disc diffusion method [18]. Experiment was performed under aseptic conditions. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and all four bacteria were spread separately on Mueller Hinton agar plates. 10µl of the plant extract at 3 different concentrations (100mg/ml, 75mg/ml, and 50mg/ml) were loaded onto each *whatman*[®] filter paper disks (6mm) and allowed to dry. Then, the discs were evenly placed on the agar surface. Standard disk of Gentamicin was used as a positive control and distilled water as negative control. The plates were incubated at 37°C for 24 hours (Rasha et al. [8]). The antimicrobial activity was assayed by measuring the diameter of clear inhibition zone formed around the discs. The diameter of zone of inhibition was measured in millimeters using a ruler. Test was repeated on three separate occasions for each microbial strain [19].

2.6 Data Analysis

Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented. The data collected was analyzed. The results of this experiment are presented as Mean+ SD triplicate experiments analyzed by using SPSS. Differences between mean is evaluated by one-way ANOVA at $p < 0.05$. Fig. 2 shows the overall summary of the work flow.

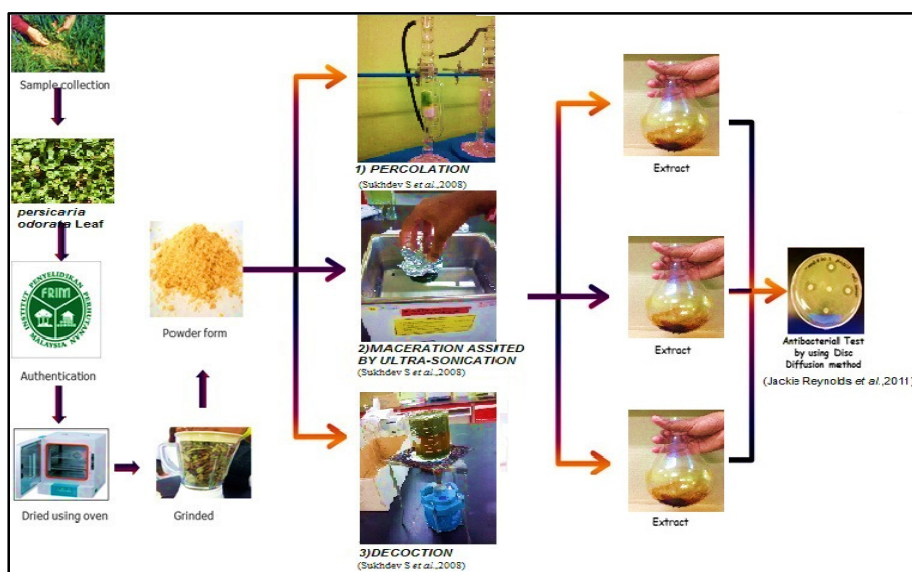


Fig. 2. Methodology flow chart

3. RESULTS

3.1 Antimicrobial Activity of Plants Extracts

Generally, the extract of *Persicaria odorata* obtained from percolation using Soxhlet extractor has higher antimicrobial activity than maceration extract obtained from maceration with ultra-sonication and decoction. The extract obtained from decoction only shows antimicrobial activity on *Escherichia coli* and *Staphylococcus aureus* at a concentration of 100mg/ml with zone of inhibition of 6mm respectively. Meanwhile the extract obtain from both maceration with ultra-sonication and percolation using soxhlet extractor show significant ($P=0.05$) antimicrobial activity against all four bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella spp*) with the zone of inhibition increasing with increasing concentration of the extract (Table 1).

Table 1. The mean diameter of zone of inhibition of *Persicaria odorata* leaves extracts against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonella typhi*

Organism	Leaf extract(mg/ml)/ Zone of inhibition (mm)									STD drug	Negative control
	Decoction			Maceration with ultra-sonication			Percolation using Soxhlet extractor				
	50	70	100	50	70	100	50	70	100		
<i>Escherichia coli</i>	0	0	6	10	10	11	14	14	15	25	0
<i>Staphylococcus aureus</i>	0	0	6	9	9	9	12	13	13	24	0
<i>Bacillus subtilis</i>	0	0	0	8	8	8	10	10	11	24	0
<i>Salmonella typhi</i>	0	0	0	8	9	9	8	9	9	22	0

GTM: Gentamicin; DW: distilled water

Escherichia coli and *Staphylococcus aureus* were the most susceptible microorganism towards the leaves extract obtained from both maceration and percolation technique. The highest zones of inhibition for *Escherichia coli* and *Staphylococcus aureus* were observed with the highest concentration of extract obtain from percolation technique with the diameter of 15mm and 13mm respectively. This indicates that antimicrobial testing at highest concentration of 100mg/mL for extract obtained from percolation technique exhibited the highest antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*.

The zones of inhibition against *Bacillus subtilis* and *Salmonella spp.* were lowest for both extracts obtained from maceration and percolation technique. The highest zone of inhibition for *Bacillus subtilis* and *Salmonella spp.* were observed on the highest concentration of extract obtain from percolation technique with the diameter of 11mm and 9mm respectively (Fig. 3).

The standard antibiotic, gentamicin inhibited the growth of all four test organism indicating that the organisms are not resistant to gentamicin. It demonstrated the highest mean of inhibition zone in *Escherichia coli* with diameter of 25mm followed by, *Staphylococcus aureus* and *Bacillus subtilis* with diameter of 24mm respectively and the lowest is in *Salmonella spp.* with diameter of 22mm. There was no zone of inhibition observed on the disk introduced with distilled water.

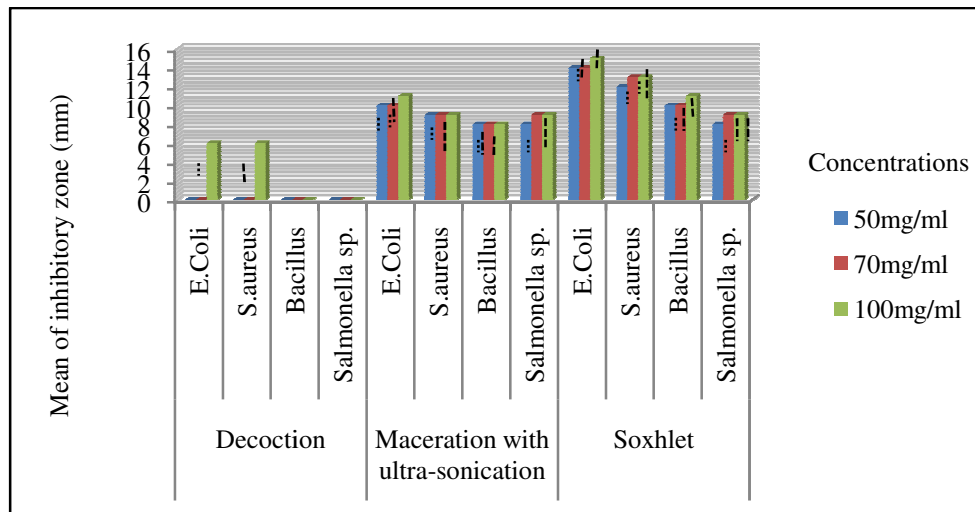


Fig. 3. Mean diameter of zone of inhibition of extract obtained by decoction, maceration with ultra-sonication and percolation using soxhlet

4. DISCUSSION

4.1 Effect of Using Different Concentration

Based on the results obtained from this study, it showed that in the extract obtained from different extraction technique, the mean of inhibition zone directly proportional with increasing concentration of plant extracts. This is likely due to the increasing amount of active compound present in higher extract concentration tested. However, the increase in the diameters of the inhibition zone is not significant statistically ($P > 0.05$). This indicate that the usage of different concentration of extract do not influence the antimicrobial activity of the *Persicaria odorata* significantly [20].

4.2 Effect of Using Different Extraction Techniques

The results obtain from the antimicrobial test has demonstrated that the extract obtain from percolation and maceration technique were more effective than decoction technique. This is because the type of solvent used in the extraction technique influence the solubility of the active component of the leaves [18,21]. Extracts obtained using organic solvent in the percolation and maceration techniques were more efficient than the extracts obtained using water as a solvent in the decoction technique in this study. The use of water as the only solvent yields to an extract with a high content of impurities (e.g. organic acids, sugars, soluble proteins) along with polar compounds which could interfere in the antimicrobial properties. On the other hand, the usage of 80% methanol leads to an increase in swelling of plant materials and the contact surface area between the plant matrix and the solvent finally improves the extraction yield of the volatile compounds properties [22].

4.3 Susceptibility of Bacteria Strains

It is found that the antimicrobial activity of extracts obtain from percolation using soxhlet more efficient than extract obtains from maceration assisted by ultrasound. This is because

even though the extract obtain from the maceration technique was filtered using the funnel filter they may still contain the undissolved materials [13,17]. Meanwhile, in the percolation technique using soxhlet, from solid impure products, required products were extracted with a suitable solvent in which it is dissolved, and once it is extracted in solvent, it will not come in contact with the mother impure solid product again. Only solvent vapour and solvent again and again extract the desired product from the mother impure products, so that the more pure product was obtained as other impurities or unwanted material will not come in contact with the extracted product again and again [14,20]. Thus the purity of the extract obtained was more compare to extract obtain from normal extraction technique.

The result also showed that antimicrobial activity between all four bacteria strains test exhibit different measurement of diameter in the inhibition zone. The various zone of inhibition suggest the varying degree of efficacy and different phytoconstituent of herb on the target organisms [21]. Among the four bacteria tested, *Escherichia coli* was the most susceptible to the plant extract obtained from percolation technique using soxhlet, followed by *Staphylococcus aureus* then *Bacillus subtilis* and *Salmonella spp.* Types of bacteria may contribute to the variation of antimicrobial activity.

5. CONCLUSION

This study indicated that the extracts of *Persicaria odorata* have potential natural antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*. and *Salmonella spp.* The extract obtained from percolation technique had the highest antimicrobial activity than the extracts obtained from the maceration with ultrasonication technique and decoction technique [22]. The demonstration of antimicrobial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in these plants for the development of newer antibacterial agents to combat various diseases.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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