THE ATRACTYLODES LANCEA EXTRACT FOR BACTERIAL RESISTANT IN TEXTILES

Pichet Duangsri[1], Kantima Juntarapun[2], Chutimon Satirapipathkul[3]*

Chulalongkorn University, Faculty of Engineering, Department of Chemical Engineering,
Chemical Engineering Research Unit for Value Adding of Bioresources,
Bangkok 10330, Thailand
E-mail: chemeng54@gmail.com [1],kantima.j@student.chula.ac.th[2], Chutimon.s@chula.ac.th[3*]

Abstract: Different solvents of varying polarity were examined and compared their capabilities to extract essential active agents from dried *Atractylodes lancea*. Aqueous was found to be the best among interested solvents for extraction which yields 30.63% of dried herb. Methanol yields 21.3% whereas acetone and hexane can extract only 6.72 % and 4.17% of dried herb, respectively. Compared to all other extracts, the acetone extract was found to exhibit the most significant antibacterial activity against all types of bacteria while the aqueous extract showed no inhibition**.** Cotton fabrics were treated with the acetone extracts and evaluated for their antibacterial activities. The treated fabrics showed stronger activity against Gram-negative than Gram-positive.
Keyword: Atracylodes lancea, antibacterial activity, cotton fabric, textile

1. Introduction

Textile materials made from natural fibers can provide a good environment for microbial growth, because of their large surface area and ability to retain moisture. Many kinds of chemicals have been employed to impart antimicrobial activity to textile materials. These include organometallics, inorganic salts, phenols, antibiotics, heterocyclics with anionic groups, formaldehyde derivatives, and nitro compounds [1]. However, these chemical agents are toxic to humans and do not easily degrade in the environment [2]. The textile industry continues to look for eco-friendly processes that substitute for toxic textile chemicals. In this point of view, natural bioactive compounds from plants and herbs are excellent candidates for an eco-friendly textile chemical. Many natural products are reported to be candidates of new antimicrobial substances [3].

Atractylodes lancea, belonging to the Compositae Family, is widely distributed in Asia. It is known as Cangzhu in traditional Chinese Medicine [4] and has long been used as crude drugs to treat a lot of diseases such as rheumatic diseases, digestive disorders, night blindness and influenza [5]. In Korean and Japanese, *A. lancea* has been reported that it is used as diuretic and gastric drugs [6] against stomachic damage and dyspepsia [7]. In traditional Thai folklore, it has been shown to possess anticancer activities in various human cancerous cell lines [8]. A variety of polysaccharides purified from rhizomes of *A. lancea* have been found out to own intestinal immune system modulating activity [9] and intestinal mobility enhancing effect [10].

However, no study has ever been conducted to check the antimicrobial activity of *Atractylodes lancea* when applied on textile materials. The present study was undertaken to investigate the extraction efficiency of different solvents on *A. lancea* to obtain bioactive molecules. Additionally, the bactericidal properties of cotton textiles treated with the extracts against selected pathogenic bacteria were determined.

2. Materials and Methods

2.1. Plant materials

Rhizomes of *Atractylodes lancea* (Fig. 1) were purchased from Thai traditional herb shop located in Bangkok, Thailand. The rhizomes were washed by distilled water, sliced into small pieces, and incubated at 50 ^{o}C for 24 hour. All samples were physically ground into fine powder.

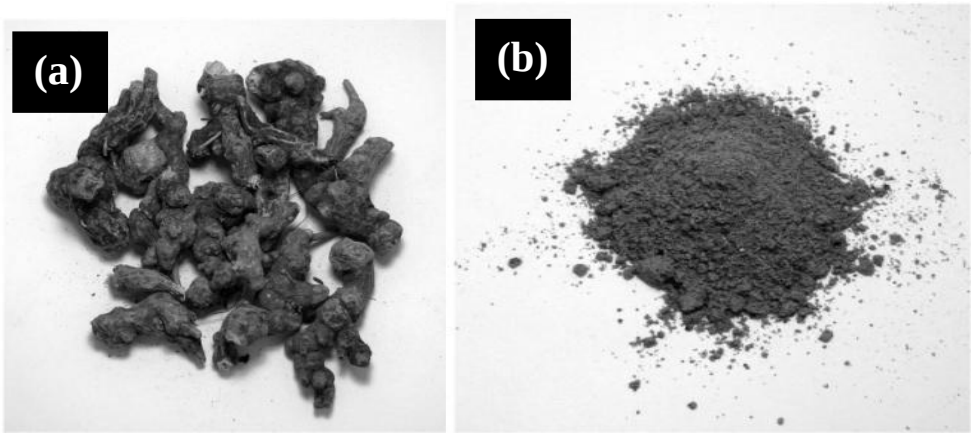

Figure 1. *Physical appearance of Atraclytodes lancea: dried rhizomes (a) and powder (b)*

2.2. Extraction of plant material

Four solvents varying in polarity, hexane, acetone, methanol and deionized water, were used in the extraction process. The herb powder was weighed 10 g and dissolved in 50 ml of different. All the mixtures were then carefully kept in a dark place at room temperature for 3 days. After the extraction period, the mixtures were filtered through Whatman filter paper IV to separate the treated powder and supernatants where the treated powder was going to be observed a change in morphology under scanning electron microscope later. In order to solely obtain the extracts, the solvent was evaporated under controlled pressure in a rotary evaporator. The weight of solid remains after evaporation completed was recorded to calculate a yield of extraction.

2.3 Microorganism strains

The microorganism stains used in this study were all purchased from Thailand Institute of Scientific Technology (TIST), Bangkok: E*scherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923).

2.4 Culture media

The nutrient agar was prepared by dissolving 5 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 5 g NaCl and 20 g agar in 1000 ml distilled water, boiling the mixture and adjusting its pH value to between 6.4–6.8. The nutrient mixture was then sterilized by autoclaving at 15 psi pressure (121 °C) for 20 min. Nutrient agar was prepared by pouring the nutrient mixture to the same thickness on sterilized petri plates. The test bacteria were then grown overnight at 37 ^{o}C, 120 rpm in 10 ml nutrient broth. This broth was used for seeding the bacteria onto the agar plates. These bacteria suspensions (approximately 10^5 cells/ml) were used for seeding the bacteria onto the agar plates.

2.5 Fabric treatment

The white cotton fabric was desized in a liquor containing 5 g of nonionic soap in a liter of water. The material to liquor ratio was taken as 1:40. The fabric was boiled at 95 °C for 1 h and dried under shade. The desized cotton fabric was pretreated with copper sulphate at 80 °C and then treated with the extracts of *Atractylodes lancea* for 45 min. The extracts (50 µg per ml) were applied to the cotton fabric by dipping in bath at material to extracted solution ratio of 1:10 at 60°C and neutral pH. Samples were treated with 10 % alum after treated with the extracts. The fabric was then dried at 80°C for ten minutes. Finally, the fabric samples were tested for antimicrobial activity.

2.6 Antimicrobial assay

Antimicrobial activity of the extracts was tested by the disc diffusion method. The treated and untreated fabrics were placed on top of the seeded media of the two bacterial strains. The antibacterial assay plates were incubated at 37°C for 24 h and the diameters of the zones of clearing were noted. For this study, the diameter of the zone of inhibition around each test fabric was taken as a measure of the antibacterial activity. Each experiment was carried out in triplicates and the mean diameter of the inhibition zones was recorded.

2.7. Morphological observations of herb powder

A. lancea powder before and after extracted with solvents were incubated at 50 °C to eliminate water content. After that, all the powder was observed under scanning electron microscope (SEM). The images of powder before and after extraction were compared to observe the changes in surface morphology, which implied the extraction efficiency of each solvent.

3. Result and Discussion

3.1 Effects of solvents type on yields of extracts

The extracting capability of five solvents varying in polarity on *A. lancea* was determined. The percentage yields of extracts from 10 gram of dried *A. lancea* were 30.63 % using aqueous, 21.3 % using methanol, 6.72 % using acetone and 4.17% using hexane as shown in Fig. 2. The results obviously indicated that increasing in polarity of solvents can yield higher extracts. From the results, aqueous was the best solvent for extraction on *A. lancea* followed by ethanol and methanol whereas the desired extracts were scarcely gained from both acetone and hexane.

3.2. Antibacterial activity of the *A. lancea* extracts

The antibacterial activity of *A. lancea* extracts from various solvents was studied on three bacterial strains, *E. coli*, *B. subtilis* and *S. aureus*, and the results were shown in Table 1. Acetone extract displayed excellent antibacterial activity against *E. coli* (20.23 mm), *B. subtilis* (15.42 mm) and *S. aureus* (13.06 mm). Hexane extract showed good activity against *E. coli* (14.97 mm), *B. subtilis* (13.33 mm) and *S. aureus* (11.84 mm). Methanol extract demonstrated moderate inhibitory activity against *E. coli* (12.94 mm), *B. subtilis* (10.77 mm) and *S. aureus* (11.44 mm). Aqueous extract had no capability to inhibit the growth of any strain. This implied that bioactive molecules showing an inhibitory effect against pathogenic bacteria should have low polar nature since they were generally dissolved in acetone and hexane. In addition, all the extracts were found to have higher antibacterial activity against gram-negative bacteria, which is *E. coli*, than gram-positive bacteria, which are *B. subtilis* and *S. aureus*.

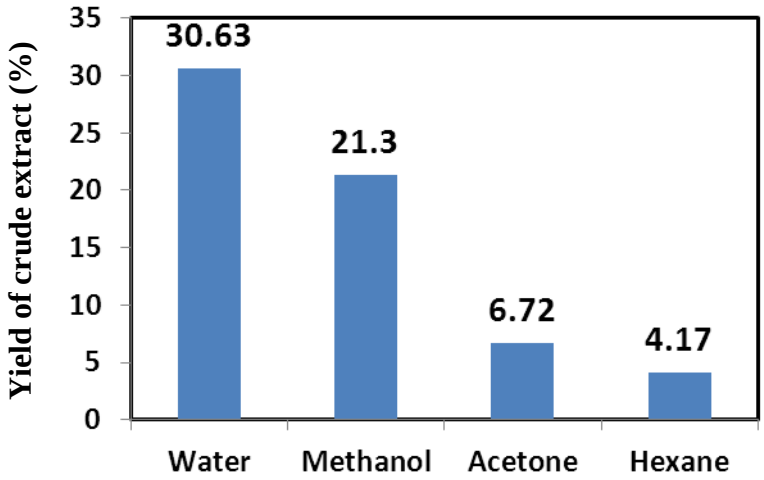

Figure 2*. Percentage yield of crude extract after extraction with various solvent*

Table 1. *Evaluation of antibacterial activity of the A. lancea extracts on pathogenic strains.*

Solvent	Diameter of Inhibition zone (mm) ± S.D.[a]		
	E. coli	***B. subtilis***	***S. aureus***
Hexane	14.97 ± 1.00	13.33 ± 1.26	11.84 ± 1.0
Acetone	20.23 ± 1.21	15.42 ± 1.10	13.06 ± 0.83
Methanol	12.33 ± 0.97	10.77 ± 1.22	11.44 ± 1.5
Water	no	no	no

[a] Mean value of three determinations, each from a different plate

3.3. Observation under scanning electron microscope (SEM)

The surface morphology of *A. lancea* powder before and after extraction by acetone was visualized. The SEM micrograph for untreated powder shows normal smooth surface with a small fraction of remnants scattering (Fig. 3a). For treated powder with acetone, the SEM micrographs indicate an augmentation in a degree of damage to the surfaces (Fig. 3b). The surface of treated powder is found to be midly destroyed and becomes irregular shape. A number of small holes are observed to appear on the surface, meaning that some of tissues were removed.

3.4 Antimicrobial activity of treated fabrics

The bioactive compounds from the acetone extracts of *A. lancea* bound to a textile fiber may be expected to show lower activity than in solution since some functional groups are modified by interaction with the fiber during the treatment process. In addition, this study also investigated the influence of common mordant, copper sulphate, on cotton fabric against three pathogenic bacteria. The results are summarized in Table 2. Untreated fabric (control) showed growth of all microorganisms and no zone of inhibition was observed. The antibacterial activity of the treated fabric with copper sulphate shows the zone of inhibition is 8.46 to 11.38 cm. The treated fabrics showed stronger activity against Gram-negative (*E.coli)* than Gram-positive (*S.aerus and B. subtilis)* in all case. The antibacterial activity of the treated fabric without copper sulphate shows the zone of inhibition is 7.22 to 9.16 cm. There was a reduction in activity when mordant was used. This could be the consequence of chelation of the bioactive compounds by the metal salt. It is well known that *A. lancea* contain high amount of phenolic compounds

and the functional groups present on these are hydroxy groups [3]. These hydroxy groups become involved in coordinate bond formation. It is therefore likely that the coordinate bond formation blocks the groupings responsible for antibacterial activity.

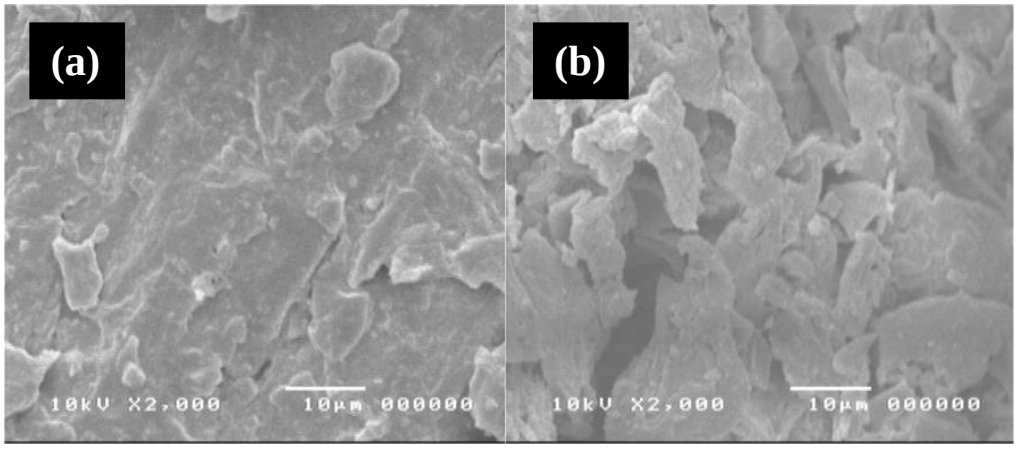

Figure 3. *SEM micrographs showing surface morphology of A. lancea powder before (a) and after extracted with acetone (b)*

Table 2. *Evaluation of antibacterial activity of fabrics samples*

Treatment	**Diameter of Inhibition zone (mm) ± S.D.**[a]		
	E. coli	***B. subtilis***	***S. aureus***
Control	ND[b]	ND[b]	ND[b]
the extract	11.38 ± 1.08	15.42 ± 1.40	8.46 ± 0.49
the extract + $CuSO_4$	9.16 ± 0.68	8.09 ± 1.03	7.22 ± 0.93

[a] Mean value of three determinations, each from a different plate

4. Conclusion

The present investigation convinces that the extracts from *Atractylodes lancea* rhizomes, showed activity against both gram-negative and gram-negative bacteria. Their degree of activity considerably depends on the type of solvents. The low polar solvents were observed to extract active molecules. Acetone extract exhibits the excellent growth inhibition of all strains. The acetone extracts of *A.lancea* improve antibacterial property of cotton fabrics. Treated fabrics show good antibacterial characteristics against tested pathogenic bacteria. Further work could focus on the antibacterial activity of the *A.lancea extracts* on a range of natural and synthetic fibers, leading to the identification of the functional groups responsible and the mechanism involved.

Acknowledgements

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