

Anti-microbial Activity of Onion and Shallot on *E. coli* and *S. aureus*

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Abstract

Herbal medicines have been used to cure ailments since ancient times. In this paper, two herbs, Allium cepa (onion) and A. ascalonicum (shallot), were used to study their anti-microbial activity on E. coli and S. aureus (TISTR 97). These two herbs are traditionally used to relieve cold and cure digestive-system ailments. The process has been done by blending onion and shallot to get extract employing solvent extraction method, using 95% alcohol and hexane, and the two-fold concentration of onion and shallot extracts. Then the herbal extract was tested for its ability to inhibit bacterial growth using Disc Diffusion Method. The results showed that S. aureus was more sensitive to onion and shallot concentrates than E. coli. The clear zones observed from using onion and shallot concentrates were both 2.7 mm for S. aureus, and 2.9 and 2.8 mm, respectively, for E. coli. The use of fresh onion, onion (alcohol), onion (hexane), fresh shallot, shallot (alcohol), shallot (hexane) gave effective results at a certain level. Concentrated extracts of onion and shallot had more anti-microbial effect than the other treatments. Result from this study can be used as a guidance to gain benefits from herbal medicines which are widely available at a low price.

Keywords: Anti-microbial activity, herbal medicines, herbal preservative, shallot, onion, bactericide, digestive-system diseases, solvent extraction, *S. aureus*, *E. coli*.

Introduction

The climate of Thailand is tropical, and usually fast-changing. This kind of climate causes various kind of ailments, for example, allergic to the air or flu. It also causes sickness to the digestive system. Modern medication can be used for the treatment of the flu symptom, but it is unnecessary unless there is also the sign of other ailments, for example the sinus. However, having a good rest will be sufficient and no medication will be needed. The disadvantages of using medicines are that they may cause side effects, and other symptoms may follow. From the literature, it was believed that onion and shallot can help soothe cold and digestive system sickness. Villagers crush shallot into bits and mix with alcohol then put them on children's head to soothe cold;

however there is no scientific proof to this yet. But there were some scientists who tried to prove this anti-microbial activity by using the squeezed juice from the onions to treat the patients. The results turn out that the juice from onions was able to stop many strains of microorganisms, including *Escherichia coli*, *Pseudomonas pyo-cyaneus*, *Salmonella typhi*, and *Bacillus subtilis*.

Objectives

The objectives of the present study are:

1. To study the anti-microbial activity of onion and shallot against *E. coli* and *S. aureus*.
2. To compare the anti-microbial activity of fresh, concentrated, and solvent extract of onion and shallot.

Literature Review

Herbal Medicinal Usage

Onion (Allium cepa Linn.)

- ◆ Onion juice is an anti-microbial agent *in vitro*, and experiments in animals demonstrate its hypoglycemic activity.
- ◆ A large number of minor and major disorders, including worm infection, diarrhoea, other infectious and inflammatory diseases can be cured by this plant.

Shallot (Allium ascalonicum Linn.)

- ◆ Relief cold and fever.
- ◆ The ripe bulb of shallot is taken for good digestion and cold sore.
- ◆ As ear-drop to anti inflammatory.
- ◆ Help in urination and menstruation.
- ◆ When crushed until the odor is sensed, it is used in place of ammonia.

Targeted Microorganisms

Escherichia coli: *E. coli* is so well known primarily because it is easy to work with in the laboratory. *E. coli* grows rapidly and has simple nutritional requirements. Enteropathogenic strains of *E. coli* are characterized by the ability to colonize the small intestine and to produce a toxin that causes symptoms of diarrhoea.

Staphylococcus aureus: *S. aureus* is gram-positive coccus bacteria, usually arranged in cluster, and is the main pathogen in Staphylococcal group. *S. aureus* also produces both exotoxin and enterotoxin (Baker and Breach 1967, cited in Techaprawakul and Ampansaeng 1996). The most common food poisoning is caused by *S. aureus* (Brock and Madigan 1991).

Antibiotic-Sensitive Test

One of the common tests is the Disc Diffusion Test. A plate of suitable agar medium is inoculated from a suspension of a pure culture of the pathogen; the entire surface of the medium is inoculated (often with a swab) so that near-confluent growth will develop on incubation (Singleton 1997). Before

incubation, several small absorbent paper discs, each impregnated with a different antibiotic, are placed at different locations on the inoculated medium. A zone of growth-inhibition develops around that particular disc if the organism is sensitive to a given antibiotic. The methods must be standardized: the presence of a no-growth zone can be interpreted correctly only when the whole procedure has been standardized in terms of type of medium, density of inoculum on the plate, etc.

Effect of Anti-Microbial Substances on Microorganisms

- ◆ Ampicillin could inhibit gram-negative bacteria better than gram-positive bacteria (Gupta and Viswanathan 1967).
- ◆ Onion and shallot could inhibit gram-positive bacteria (Chutiyasantayanon *et al.* 1985).
- ◆ Onion oil was highly active against gram-positive bacteria (Zohri *et al.* 1995).

Material and Method

Material

Herbal plants: Onion and shallot.

Reagents: 95% alcohol, hexane.

Media: Nutrient broth (NB), nutrient agar (NA), Mueller Hinton broth (MHB), Mueller Hinton agar (MHA).

Microorganisms: *E. coli* and *S. aureus*.

Method

1. Prepare the herb by cutting and blending the onion and shallot into small pieces.
2. The extraction of the herbal plants are accomplished in four conditions, viz.:

2.1 *Condition 1:* Extract the herbal plants by using the Bucher funnel.

2.2 *Condition 2:* Extract the herbal plants by using the Bucher funnel, then add 95% alcohol or hexane. The ratio of herbal extracts to solvent is 1:1 (w/v). The mixtures were shaken for 24 hrs and then filtered with Whatman No. 4

filter paper. Evaporate the solution by using the water bath shaker for 24 hrs.

2.3 *Condition 3*: Extract the herbal plants by using the Buncher funnel, then shake the herbal extracts for 24 hrs, and filter with Whatman No. 4 filter paper. Evaporate the solution by using the water bath shaker for 24 hrs.

2.4 *Condition 4*: Extract the herbal plants by using the Buncher funnel, then take the pulp from the onion and shallot and add 95% alcohol or hexane. The ratio of herbal pulp to solvent is 1:1 % (w/v). The mixtures were shaken for 24 hrs and then filtered with Whatman No 4 filter paper. Evaporate the solution by using water bath shaker for 24 hrs.

3. Prepare the disc by sterilizing it, and then slowly adding the concentrated herbal extracts.

4. Prepare the inoculum by inoculating the *E. coli* in NB and the *S. aureus* in MHB.

5. Do an anti-microbial test on the herbal extracts by using the disc diffusion method. Spread the *E. coli* on NA plate and the *S. aureus* on an MHA plate, then incubate them for 24 hrs. Measure the diameter of the inhibition zone.

Place one sterile disc each as control, containing herbal extract, and containing ampicillin.

Results

After incubation at 37°C for 18-24 hrs, the diameter of clear zone was measured. It was found that the onion and shallot concentrated extract gave better inhibitory action on both *E. coli* and *S. aureus* than fresh onion, onion (alcohol), onion (hexane), fresh shallot, shallot (alcohol), shallot (hexane).

Discussion

The extraction of onion and shallot shows the similar result for inhibiting *E. coli* and *S. aureus*. This is because the active substances in both onion and shallot may be composed of similar substances. The alcohol and hexane extracts gave inhibitory action lower than onion and shallot concentrate. These may be

because the active substances are not dissolved well by these solvents. Fresh onion and shallot have low anti-microbial activity because onion and shallot extract may contain low amount of active substances.

Pulps of onion and shallot do not have any activity to inhibit *E. coli* and *S. aureus*.

The results support the idea of using fresh onion to cure cold sore. To relieve cold, volatile substances in onion will activate the respiration system causing fast and strong breath. It also shrinks the capillaries to ease breathing. One possible assumption is the volatile substances and other extracts prohibit bacterial growth, which result in protection of secondary infection for cold (Poonsup 1985).

Table 1. The effect of onion extract on *E. coli*

Herbal medicine	Clear zone (mm)
Onion	1.2
Onion (alcohol)	1.9
Onion (hexane)	2.1
Onion (concentrate)	2.7

Clear zone of ampicillin = 16.8 mm

Clear zone of alcohol = 0 mm

Clear zone of hexane = 0 mm

Table 2. The effect of shallot extract on *E. coli*

Herbal medicine	Clear zone (mm)
Shallot	1.4
Shallot (alcohol)	2.2
Shallot (hexane)	2.2
Shallot (concentrate)	2.7

Clear zone of ampicillin = 16.8 mm

Clear zone of alcohol = 0 mm

Clear zone of hexane = 0 mm

Table 3. The effect of onion extract on *S. aureus*

Herbal medicine	Clear zone (mm)
Onion	1.9
Onion (alcohol)	2.6
Onion (hexane)	2.6
Onion (concentrate)	2.9

Clear zone of ampicillin = 4.6 mm

Clear zone of alcohol = 0 mm

Clear zone of hexane = 0 mm

Table 4. The effect of shallot extract on *S. aureus*

Herbal medicine	Clear zone (mm)
Shallot	2.1
Shallot (alcohol)	2.0
Shallot (hexane)	2.3
Shallot (concentrate)	2.8

Clear zone of ampicillin = 4.6 mm
Clear zone of alcohol = 0 mm
Clear zone of hexane = 0 mm

Table 5. The effect of pulp of onion and shallot on *E. coli*

Pulp of herbal medicine	Clear zone (mm)
Pulp of onion (alcohol)	-
Pulp of onion (hexane)	-
Pulp of shallot (alcohol)	-
Pulp of shallot (hexane)	-

Clear zone of ampicillin = 16.8 mm
Clear zone of alcohol = 0 mm
Clear zone of hexane = 0 mm

Table 6. The effect of pulp of onion and shallot on *S. aureus*

Pulp of herbal medicine	Clear zone (mm)
Pulp of onion (alcohol)	-
Pulp of onion (hexane)	-
Pulp of shallot (alcohol)	-
Pulp of shallot (hexane)	-

Clear zone of ampicillin = 4.6 mm
Clear zone of alcohol = 0 mm
Clear zone of hexane = 0 mm

Conclusion

From this study it was found that onion and shallot concentrates both gave high inhibitory action on both *E. coli* and *S. aureus*. For *E. coli*, the clear zone values of onion and shallot concentrates were both 2.7 mm. For *S. aureus*, the clear zone values of onion and shallot concentrates were 2.9 and 2.8 mm, respectively.

Onion and shallot also gave similar results for inhibiting *E. coli* (so as *S. aureus*). They may have active substances that can inhibit both gram-negative bacteria and gram-positive bacteria. Some active substances may have effect on anti-microbial activity of gram-negative bacteria and some active substances may have effect on gram-positive bacteria.

Suggestion

This project was conducted in order to prove that onion and shallot give effective results to anti-microbial at a certain level. Some other extraction methods with Soxhlet machine, which can obtain better yield and higher concentration, should also be tried. The active substances in onion and shallot have not been defined yet. Therefore, the study, to find out what active substances are, will also be very useful.

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