

## Phytochemical Screening and Antibacterial Activity of *Azadirachta Indica* Leaves Extract on Common Skin Infection Bacteria

Mohamad Azhar Azman, \*Hamidah Jaafar Sidek, Muhamad Shafizul Md Sharudin, Noor Aisyah Nik Abdul Halim, Raja Muhammad Aliff Ariffin Raja Sahrom

Faculty of Applied Sciences, Universiti Teknologi MARA Perlis Campus, 02600 Arau, Perlis, Malaysia

e-mail: [hamidahjs@perlis.uitm.edu.my](mailto:hamidahjs@perlis.uitm.edu.my)

### Abstract

Skin infection is mainly caused by bacteria that are widely experienced by people around the world. The overuse of antibiotics has caused the development of resistant strains of bacteria. Thus, the need for a new natural antibiotics to provide treatment to the infection caused by these bacteria. *Azadirachta indica* is a very useful traditional medicinal plant and each part of the tree has various medicinal properties. This study was aimed at screening the active components and determining the antibacterial activity of the ethanolic crude extract of *Azadirachta indica* or commonly known as neem leaves on common bacteria that cause skin infection. The ethanolic crude extract of neem leaves was used for phytochemical screening and Kirby Bauer Disc Diffusion Assay. The result obtained from this study showed the presence of terpenoids, flavonoids, saponins, tannins, and alkaloids in the crude leaf extract. The antibacterial activity of the extract was tested on *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The zone of inhibition of the *Azadirachta indica* crude extract against *Escherichia coli* showed the largest zone inhibition (21mm) compared to *Staphylococcus aureus* (16.5mm) and *Pseudomonas aeruginosa* (10mm). The inhibitory effect of the neem crude extract towards *Escherichia coli* also outperformed the inhibitory effect of the positive control of the ampicillin disc. *Azadirachta indica* crude extract may contain pharmacologically active constituents that may be responsible for its activity against *S.aureus*, *E.coli*, and *P.aeruginosa* that need further study. Therefore, the use of neem plant seems promising to be a potential antibacterial agent in treating skin infection caused by these bacteria.

**Keywords:** *Azadirachta indica*, Antibacterial activity, Kirby Bauer Disc Diffusion Assay, Skin infection bacteria.

### Introduction

Nowadays, the use of plants as medicine is a useful alternative to cure many types of diseases such as skin diseases (Girish and Shankara Bhat, 2008). Skin diseases are the most common illness among people around the world. Most of the skin diseases are caused by bacterial infection. Common bacterial skin infections include cellulitis, erysipelas, impetigo, folliculitis, furuncles and carbuncles (Stulberg *et al.*, 2002). A bacterial infection occurs when bacteria successfully invade the soft tissues through small wounds on the skin surface or through existing condition. Late prevention of bacterial infection can cause to life-threatening situation. Bacterial infection may affect both the skin and the subcutaneous tissues beneath, and can spread to the lymph nodes and

bloodstream (Rowland, 2002). Cellulitis is one of the skin diseases that is caused by *Staphylococcus aureus*. *Staphylococcus aureus* is a Gram positive bacterium commonly found on skin and in nose. Besides this bacterium, cellulitis is also caused by large variety of organisms which are known to colonize chronic wounds, including *Streptococci*, *Staphylococci*, *Pseudomonas* spp., and *Bacteriodes* spp. (Cox and Lawrence, 1998).

Plants have the ability to synthesize many varieties of compounds that are used to achieve important biological purposes, and to protect against attack from predators such as insects, fungi and herbivorous mammals. The uses of plants as treatment exist before written human history. Traditional healers have long used plants to prevent or cure an infectious condition. Several pharmacological activities and medicinal

applications of various parts of neem are well known (Biswas *et al.*, 2002). Neem or its binomial scientific name, *Azadirachta indica* A. Juss., is an evergreen tree from the family Meliaceae, originated in India and can be found growing in tropical and semi-tropical regions. Locally in Malaysia it is known as “semambu” or “pokok mambu” (Samy *et al.*, 2005).

In the ayurvedic and homeopathic medicine as well as in Hindu traditional medicine, the neem extracts from different parts of the plant are used for treating many disorders like inflammation, infections, fever, dental disorders and skin diseases. The bark of the Neem also has some antiseptic properties that can heal wound (Girish and Shankara Bhat, 2008). Hundreds of diseases have been shown to respond favorably to neem. There are many uses of neem in medical field such that it can treat the chronic skin conditions that often fail to respond to medical drug. Ringworm, eczema, and scabies are among the condition that can be treated by using the dried neem leaf extract (Biswas *et al.*, 2002). Other biological activities of neem include antidiabetic (Halim, 2003) and anticarcogenic (Dasgupta *et al.*, 2004). Neem is now considered a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products (Imran *et al.*, 2010).

Skin infection is mainly caused by bacteria that is widely experienced by people around the world. The overuse of antibiotics has caused the development of resistant strains of bacteria (Davies and Davies, 2010). Thus, the need of a new natural antibiotics to provide treatment to the infection caused by these resistant strains of bacteria. So, our study was to determine the phytochemical constituents and the antibacterial activity of *Azadirachta indica* leaves against some of the human pathogenic bacteria strains that can cause skin infection which are *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*.

## Materials and Methods

### Collection of plant material

The leaves of *Azadirachta indica* were collected in the month of January, 2014 from the tree growing wildly in Bintong, Perlis, Malaysia.

### Extraction of ethanolic compounds from plant leaves

Ethanolic compounds were extracted according to the method used by Timothy *et al.* (2011). Initially, the fresh leaves were allowed to dry under shade for 14 days and ground into powder using a grinder. The powdered material was weighed using electronic

weighing balance and drying of the leaves was continued until a constant weight was obtained. Two hundred and fifty grams of the powder was placed in a container and was defatted using petroleum ether, following which it was subjected to maceration using 300 ml of 95% (v/v) ethanol in order to obtain the ethanolic extract of the plant. The mixture was stirred up and kept for 24 hours. The mixture was filtered and another 300 ml of the ethanol was added to the residue and kept for another 24 hours before filtration. This procedure was repeated 3 times and the combined filtrate was subjected to rotary evaporator to obtain the crude extract. The total weight of crude extract obtained was 30.5 g and thus, the percentage yield was 12.2%.

### Test organisms

The microorganisms used were *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* EC 11303 and *Staphylococcus aureus* S2014 and were obtained from Microbiology Laboratory 5 UiTM Perlis, Arau, Malaysia to represent skin infection bacteria. Cultures were prepared from stock cultures by streaking onto nutrient agar.

### Sterilization of the equipment and disinfection

All the equipments were disinfected with cotton wool soaked in 70% ethanol so as to maintain sterility throughout the process. Wire loop, conical flask and beaker were sterilized by hot air oven at 160°C for 45 minutes, whereas moisture insensitive materials were sterilized by autoclaving at 121°C for 15 minutes (Timothy *et al.*, 2011).

### Phytochemical analysis of plant extracts.

Phytochemical screening of the plant extracts was conducted following the procedure described by Nasrabadi *et al.* (2013) and Ayoola *et al.* (2008). The procedure consisted of several tests.

#### 1. Test for Terpenoids (Salkowski Test).

An amount of 0.2 g of the extract of the plant sample was mixed with 2ml of chloroform (CHCl<sub>3</sub>) and 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was carefully added to form a layer. A reddish brown coloration of the interface that was formed indicates positive results for the presence of terpenoids.

#### 2. Test for Alkaloids.

About 0.5 g of plant extract was diluted to 10 ml with 1% (w/v) aqueous hydrochloric acid, boiled and filtered. Then, 2 ml of dilute ammonia was added to 5 ml of the filtrate. Chloroform of 5 ml

volume was later added and was shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. Mayer's reagent was then added. The formation of a cream with Mayer's reagent indicates the presence of alkaloids.

3. Test for Flavonoids.  
A volume of 5 ml of dilute ammonia was added to a portion of crude extract. Then, 1 ml of concentrated sulphuric acid was added. A yellow coloration disappeared on standing indicating the presence of flavonoids.
4. Test for Saponins.  
Distilled water of 5 ml volume was added to 0.5 g of extract in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.
5. Test for Tannins.  
About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% (w/v) ferric chloride were added and observed for brownish-green or a blue-black coloration as indication for the presence of tannins.

*Preparation of media*

The nutrient agar that was used consists of (gm/liter): Agar 15.0 g, Reptic digest of Animal Tissue 5.0 g, Sodium chloride 5.0 g, Beef extract 1.5 g, and Yeast extract 1.5 g. Twenty-eight grams of nutrient agar was weighed and dissolved in 1000 ml of distilled water and adjusted to pH of 7.4 ± 0.2 at 25°C. This was sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure and was used for Kirby Bauer disc diffusion tests.

*Antibacterial activity assay of the plant extracts*

Kirby Bauer Disk Diffusion assay was carried out to get the zone of inhibition that showed the antimicrobial activity of *Azadirachta indica* towards the three microbes : *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. All the bacteria were subcultured into McCartney bottle that contain nutrient broth before being spread on the nutrient agar. First the agar was removed from the refrigerator, placed in incubator and let it to come down to the room temperature. In the meantime, the lab bench was first disinfected with 70% ethanol and the Bunsen burner

was lighted up to keep up with a sterile environment. Once the agar was warmed up, the agar was removed from the incubator for culturing. Three paper discs were used in the antibacterial assay. Paper disc of 10 µg ampicillin was used as the positive control, the second paper disc was soaked in ethanol as negative control and the third paper disc was soaked in *Azadirachta indica* crude extract to determine their inhibitory antibacterial effect. About 0.2 ml of the bacterium broth culture was transferred onto the nutrient agar medium, aseptically. Then, the broth culture of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was spread with a L-shaped glass spreader. For precaution the L-shaped glass spreader was dipped into ethanol first before being used in the next spreading. Then, paper discs that contained *Azadirachta indica* crude extract, ethanol, and 10 µg ampicillin were placed onto the nutrient agar surface. Each disc was slightly pressed down into the agar medium to ensure the complete contact with the Nutrient Agar. All plates were inverted and incubated at 37°C for 24 hours. The diameter of the clear zones of inhibition of the test organisms in response to the crude leaves extract of *Azadirachta indica*, ampicillin and ethanol was measured in millimeters.

**Results And Discussion**

The result showed that the ethanolic compounds present in crude leaves extract of *Azadirachta indica* contained terpenoids, alkaloids, flavonoids, saponins, and tannins (Table 1 and Table 2). These results were in agreement with the report of Timothy *et al.* (2011) and Biu *et al.* (2009) in the phytochemical constituents that were detected. The presence of these phytochemical compounds may be responsible for the observed antimicrobial activity of the plant leaf extract (Timothy *et al.*, 2011). However, in the study of phytochemical analysis of leaf extract of *Azadirachta indica* by Imran *et al.* (2010) using petroleum ether, chloroform and methanol as the solvents in the extraction, the only compounds present were glycosides, triterpenes and fatty acids but not alkaloids, saponins and tannins.

Table 1:Qualitative determination of phytochemical groups present in crude extract of *Azadirachta indica* leaves

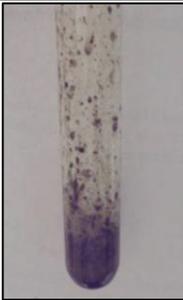
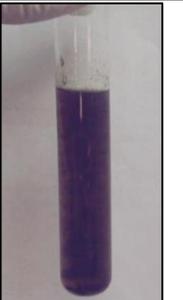
Phytochemical groups	Results
Terpenoids	+
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+

(+) = Present (-) =Absent

The zone of inhibition of the tested bacterial strains : *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were 21mm, 16.5mm and 10mm respectively (Table 3 and Figure 1). From the result, the zone of inhibition of *Escherichia coli* was the largest (21mm) and outperformed the standard zone of inhibition of the Gram negative bacteria made by the commercial antibiotic (10 µg ampicillin). *Escherichia coli* can be considered to be a good antibacterial agent

as it is susceptible to the neem leaves ethanolic extract. However, the bacteria response ranges of *Staphylococcus aureus* and *Pseudomonas aeruginosa* towards the neem leaves ethanolic extract are said to be resistant.

**Table 2:** Results from screening for phytochemical compounds in crude extract of *Azadirachta indica* leaves.

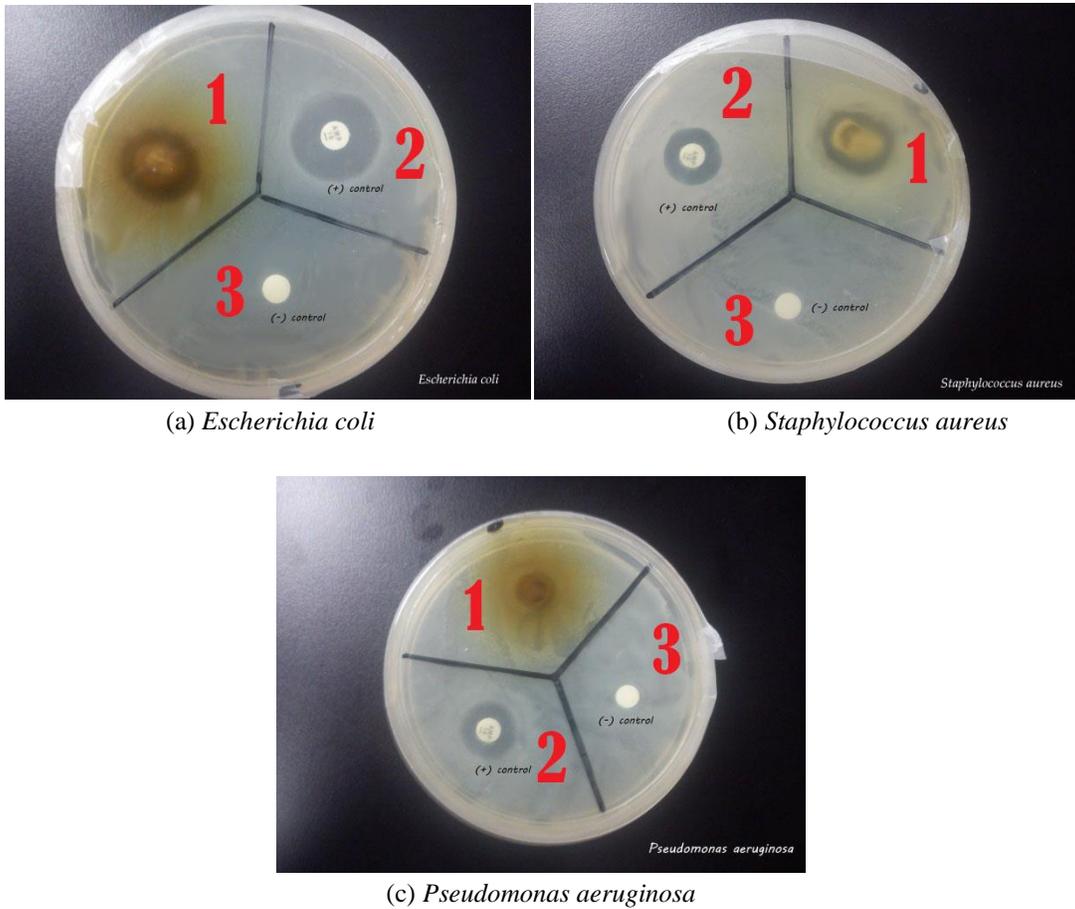
Compound	Terpenoids	Alkaloids	Flavonoids	Saponins	Tannins
Observation					
Description	A reddish brown coloration of the interface was formed	The formation of yellowish or cream coloured precipitate	The yellow coloration disappeared	An emulsion was formed	A brownish-green coloration of the interface was formed

Antibacterial activity of *Azadirachta indica* leaves ethanolic extract and ampicillin 10 µg against bacterial species tested by disc diffusion assay was displayed in Figure 2. The diffusion of the bioactive compounds from the extract into the media could be responsible for the observed effects. The result of this finding strongly agrees with several literature reported by Timothy *et al.*,(2011) and Imran *et al.* (2010) which found that *Azadirachta indica* possess significant antimicrobial activities against several pathogen. The study is also in accordance to the findings by Imran *et al.*, (2010) and Syarifah Masyitah and Izham, (2014) which showed that the neem leaf extract is less or not effective on Gram negative bacteria *Pseudomonas aeruginosa*. However, contradict finding by Joshi *et al.*, (2011) showed that the plant extract was found to be ineffective against *Escherichia coli*. From the result of this finding neem plant looks promising to become a

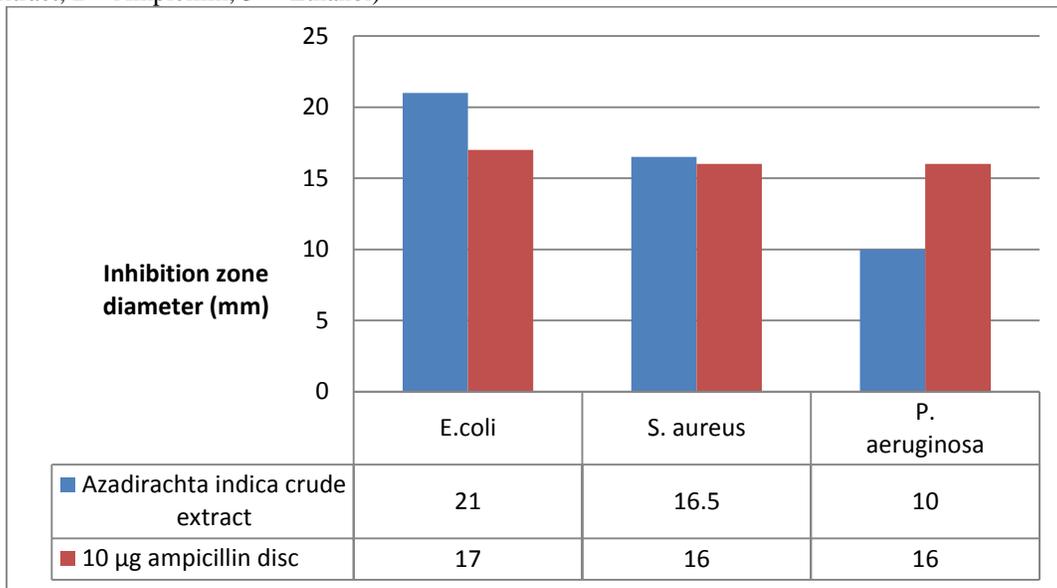
potential antibacterial agent in treating skin infection caused by these pathogens.

**Table 3:** Antibacterial activity of the crude extract of *Azadirachta indica* leaves and ampicillin against three bacterial strains tested by disc diffusion assay.

Sample	Zones of inhibition (mm)		
	<i>E.coli</i>	<i>S.aureus</i>	<i>P.aeruginosa</i>
<i>Azadirachta indica</i> leaves extract	21±0.00	16.5±1.66	10±0.00
10 µg Ampicillin	17	16	16
70% (v/v) Ethanol	0	0	0



**Figure 1:** Zone of Inhibition of a) *Escherichia coli* b) *Staphylococcus aureus* and c) *Pseudomonas aeruginosa* (1 = Neem extract, 2 = Ampicillin, 3 = Ethanol)



**Figure 2 :** Antibacterial activity of *Azadirachta indica* leaves ethanolic extract and ampicillin 10 µg against bacterial species tested by disc diffusion assay.

## Conclusion

From this study it can be concluded that *Azadirachta indica* leaf extract contains terpenoids, alkaloids, flavonoids, saponins, and tannins could be responsible for its activities against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The tested bacteria *Escherichia coli* was more susceptible to the neem leaves ethanolic extract compared to *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Furthermore, the inhibitory effect of the plant extract outperformed the inhibitory effect of the positive control of the 10 µg ampicillin disc, a commercialized antibiotic on *Escherichia coli*. Thus, the use of neem plant seems promising to be a potential antibacterial agent in treating skin infection caused by these bacteria. As this study was preliminary, further study need to be done by including minimum inhibitory concentration and using other parts of the neem plant and tested on clinical isolates of bacteria and resistant strains.

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