

## SCREENING OF SEVEN TYPES TERENGGANU HERBS FOR THEIR POTENTIAL ANTIBACTERIAL ACTIVITY AGAINST SELECTED FOOD MICROORGANISMS

Mohd Adzim Khalili R., Norhayati A. H., Emynur Shafekh S., Mohamad Fazari G, Azlina M., Tajul Zahili, M., & Ahmad Zubaidi A. L.

Faculty of Medicine and Health Sciences,  
Universiti Sultan Zainal Abidin (UniSZA),  
Kampus Kota, Jalan Sultan Mahmud, 20400 Kuala Terengganu, Terengganu, Malaysia.

**ABSTRACT.** *Recently more attention has been directed toward plant extracts and biologically active compounds isolated from popular plant species. Seven types of selected herbs available in Terengganu known as Nilam (*Pogostemon cablin*), Kuda Belang (*Aphelandra squarrosa*), Buah Keras (*Adhatoda vasica*), Sesudu (*Euphorbia nerrifolia*), Lelipan (*Pedilanthus tithymaloides*), Gelenggang (*Casia alata*) and Ekor Kucing (*Uraria picta*) was determined based on the widely used by local Terengganu people as traditional herbal remedies. Methanol, chloroform, and ethyl acetate extracts from these seven herbs were screened for their potential antibacterial activity against food microorganisms (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia*). The methanol, chloroform, and ethyl acetate fraction extracts from *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* were exhibited significant antibacterial activity with a range of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of 1.56 to 6.25 µg/mL.*

**KEYWORDS.** Herbs plant, antibacterial activity, fractionate extraction, food microorganisms

### INTRODUCTION

The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms (Mun~oz-Mingarro *et al.*, 2003; Coelho de Souza *et al.*, 2004; Girish & Satish, 2008). According to Lopez-Munoz *et al.* (2006), the uses of medicinal herbs to prevent and cure disease have been rising in recent years and their preventive use for the treatment of food borne pathogens is believed to be safer than synthetic antibiotics due to the long history of herbal usage. The use of herbs for treatment is very important but issues such as the dosage and frequency of consumption of these products still need to be considered (Thompson *et al.*, 2001). Low Dog (2006) mentioned the potential of herbs and spices to enhance digestion system, due to the salivary and gastric secretions induced by the pungent tastes and unique aromas. These effects increase the activity of digestive enzymes, bile volume and bile acid secretion and can also decrease the time food takes to travel through the gastrointestinal tract thus providing some protection against gastrointestinal disease.

Plant based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials is essential. Antimicrobials of plant origin have enormous therapeutic potential (Salau & Odeleye, 2007). Human infections, particularly those involving microorganisms i.e bacteria, fungi, viruses, can have serious effects in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic micro-organisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of diseases (Gutmann *et al.*, 1988; Mohanasundari *et al.*, 2007). Over the past twenty years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents (Cohen, 1992; Werner *et al.*, 1999; Girish & Satish, 2008). Different extracts from traditional medicinal plants have been tested and many reports have shown the effectiveness of traditional herbs against microorganisms. As a result, plants are among the bedrocks for modern medicine to attain new principles (Evans *et al.*, 2002).

In Malaysia and Indonesia several native herbs have been traditionally used to treat food borne pathogens. However, this use lacks scientific proof and validation (Arya *et al.*, 1989; Soedibyo, 1998; Arora *et al.*, 1999; Lu *et al.*, 2006; Chan *et al.*, 2007; Aziz & Tey, 2009; Ikram *et al.*, 2009; Abd Aziz *et al.*, 2011). The increasing interest in traditional ethno medicine may lead to the discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements. In fact, plants have given western pharmacopoeia about 7,000 different pharmaceutically important compounds and a number of top-selling modern drugs e.g. quinine and taxol (Tshibangu *et al.*, 2002).

The aim of this study was to investigate the potential antibacterial effect of methanol, chloroform and ethyl acetate from 7 medicinal plants (*Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta*) which are widely used by local people as traditional herbal remedies. The herbs were screened for their potential antibacterial activity against food micro-organisms (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia*), which have possible applications in the treatment of infectious diseases.

## MATERIALS AND METHODS

### **Extraction procedure**

The method of Adel S. Alzubairi *et al.*, (2009) was used for the extraction process. Seven types of herbs were obtained from Taman Herba, Sekayu, Terengganu, Malaysia. The plants were *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta*. All herbs were extracted with different solvents in increasing order of polarity. The solvents used were methanol, chloroform and ethyl acetate. After arriving at Food Analysis Laboratory, Faculty of Medicine and Health Sciences, UniSZA Kampus Kota, the herbs were carefully washed under running tap water, dried with a soft cloth, then dried in the oven at 50°C overnight and ground into small pieces and macerated in chloroform for 7 days with occasional shaking. This process was repeated three times.

The residue was air dried overnight and used for the next solvent extraction (ethyl acetate) as per the above procedure and the same procedure was repeated for the next solvent (methanol). Finally, all the extracts for each solvent were filtered through Whatman® No. 41 filter paper (pore size 20-25 µm) and then concentrated under reduced pressure at 40°C and stored at -20°C until they were used for the analysis. To screen the extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* (chloroform, EA and methanol extracts) compounds were dissolved in 1 mL of DMSO to give a stock solution of extract at 100 mg/mL. All extracts were kept at 4°C throughout the experiments.

### **Test micro-organisms**

The antimicrobial activity of the chloroform, EA and methanol extracts from *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* was evaluated against four food microorganisms (*Escherichia coli* (ATCC 85218), *Salmonella enterica typhi* (ATCC 10749), *Staphylococcus aureus* (ATCC 25179) and *streptococcus pneumonia* (ATCC 85218)). All the micro-organisms' strains were purchased from American Type Culture Collection (ATCC), Manassas, USA. All strains were carefully identified and grown using standard microbiological methods. The bacterial isolates were first sub-cultured in Mueller-Hinton broth (Merck, Germany) and incubated at 37°C for 18 hours.

### **Antimicrobial activity assay**

The antimicrobial activity of the chloroform, EA and methanol extracts from *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* was determined against four food micro-organisms (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *streptococcus pneumonia*) by the agar disc diffusion assay method as described by the National Committee of Clinical Laboratory Standards (1993). The bacterial strains prepared from overnight broth cultures were diluted using sterile normal saline to give an inoculum

size of about  $10^8$ cfu/mL. The density of bacterial suspension of the cultures was standardized turbidometrically to 500,000 – 1,000,000 colony forming units per millilitre (cfu/mL) at wavelength of 600 nm. A total of 100  $\mu$ L of suspension containing  $10^8$ cfu/mL of bacteria was spread on nutrient agar.

The crude and fractionated extracts were dissolved initially in dimethyl sulfoxide (DMSO), diluted to a concentration of 100 mg/mL and filtered using 0.45  $\mu$ m millipore filters. Sterile discs were impregnated with 30  $\mu$ L of extract solutions (100 mg/mL) and placed on the inoculated agar. Negative controls were prepared using DMSO, while Penicillin G (10  $\mu$ g/discs) and Gentamicin (10  $\mu$ g/disc) were used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. The diameter of inhibition zone was measured in millimeters (mm) by Vernier calipers. All tests were repeated ten times to minimize test error. An inhibition zone of 14 mm or greater (including diameter of the disc) was considered as high antibacterial activity (Ramzi *et al.*, 2004).

#### **Minimum inhibitory concentration**

Minimum inhibition concentration (MIC) of the chloroform, EA and methanol extracts from *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* was determined by combination methods introduced by Vollekova *et al.*, (2001) with slight modification by Usman *et al.*, (2005). MIC was defined as the lowest concentration where no visible turbidity was observed in the test tubes. The MIC was determined for the micro-organisms that showed reasonable sensitivity to the test extracts.

In this test, the bacteria were prepared using the broth dilution technique. The stock extract concentration of 100 mg/mL was made by dissolving 10 g of the extract in 100 mL of DMSO and the working concentrations were prepared by four-fold serial dilution technique ranging from 0.39 mg/mL to 25 mg/mL using Muller-Hinton broth (Merck, Germany) and later inoculated with 0.2 mL suspension of the test organisms. After 24 hours of incubation at 37°C, the tubes were observed for turbidity with the naked eye check and the optical density measured (O.D) at 600 nm. The lowest concentrations where no turbidity was observed were determined and noted (Usman *et al.*, 2005). Solvent blanks and positive controls were also included and all tests were performed in ten replicates and repeated five times to minimize test error.

#### **Minimum bactericidal concentration**

Minimum bactericidal concentration (MBC) of chloroform, EA and methanol extracts from *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata*, and *Uraria picta* was determined by a modification of the method of Spencer & Spencer (2004) and Smith-Palmer *et al.*, (1998). The tubes containing 5 mL nutrient broth with different concentrations of isolated samples inoculated with 50  $\mu$ L of the bacterial suspension ( $10^5$  CFU/mL) and incubated for 24 hours at 37°C. The growth was observed both visually and by measuring optical density (O.D) at 600 nm at regular intervals. Then 20  $\mu$ L was taken from broth with no visible growth in the MIC assay and sub-cultured on freshly prepared Muller Hinton agar plates and later incubated at 37°C for 48 hours. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plate. Solvent blanks and positive controls were also included and all tests were performed in ten replicates and repeated five times to minimize test error.

#### **Statistical Analysis**

Data were expressed as mean  $\pm$  standard deviation (SD) of ten replicates. Statistical analysis was performed with single factor and one way ANOVA to identify the significant differences ( $p < 0.05$ ) and Duncan test for coefficient variation on antibacterial effects of the chloroform, EA and methanol extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta*.

## RESULTS AND DISCUSSION

The results of the diffusion disc assay of methanol extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumoniae* are shown in Table 1.

**Table 1. Zones of inhibition (mm) of methanol extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta*.**

Extract/Organisms	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>
<i>Pogostemon cablin</i>	12.5 ± 0.03 <sup>c</sup>	10.0 ± 0.01 <sup>c</sup>	11.5 ± 0.01 <sup>c</sup>	8.50 ± 0.02 <sup>c</sup>
<i>Aphelandra squarrosa</i>	11.0 ± 0.06 <sup>c</sup>	12.5 ± 0.08 <sup>c</sup>	10.5 ± 0.06 <sup>c</sup>	9.50 ± 0.01 <sup>c</sup>
<i>Adhatoda vasica</i>	8.50 ± 0.07 <sup>b</sup>	11.5 ± 0.05 <sup>b</sup>	9.50 ± 0.05 <sup>b</sup>	8.00 ± 0.05 <sup>b</sup>
<i>Euphorbia neriifolia</i>	9.00 ± 0.10 <sup>b</sup>	7.50 ± 0.03 <sup>a</sup>	10.0 ± 0.01 <sup>a</sup>	7.50 ± 0.09 <sup>a</sup>
<i>Pedilanthus tithymaloides</i>	7.50 ± 0.08 <sup>a</sup>	7.50 ± 0.08 <sup>a</sup>	9.50 ± 0.02 <sup>a</sup>	11.50 ± 0.04 <sup>a</sup>
<i>Casia alata</i>	8.50 ± 0.20 <sup>b</sup>	6.50 ± 0.03 <sup>b</sup>	7.50 ± 0.08 <sup>b</sup>	10.0 ± 0.06 <sup>b</sup>
<i>Uraria picta</i>	10.5 ± 0.40 <sup>b</sup>	9.50 ± 0.05 <sup>b</sup>	8.50 ± 0.07 <sup>b</sup>	10.5 ± 0.04 <sup>b</sup>
Penicillin	15.0 ± 0.02 <sup>a</sup>	10.5 ± 0.06 <sup>a</sup>	11.5 ± 0.04 <sup>a</sup>	11.5 ± 0.08 <sup>a</sup>
Gentamicin	10.0 ± 0.03 <sup>a</sup>	11.5 ± 0.08 <sup>a</sup>	11.5 ± 0.09 <sup>a</sup>	11.5 ± 0.01 <sup>a</sup>

Results (100 mg/mL) are means ± standard deviation of tenth replicate repeated five times to minimize test error; na=no activity; there was no inhibition found in negative control (DMSO).

<sup>abc</sup>Variation in the following letters between samples indicates significance of difference by Duncan's test at 5% level ( $p < 0.05$ ). Inhibition zones are the mean including disc (6 mm) diameter.

The *Pogostemon cablin* extract showed very high activity of inhibition against tested bacteria; *Escherichia coli* (12.50 ± 0.03 mm), *Salmonella typhi* (10.00 ± 0.01 mm) and *Staphylococcus aureus* (11.50 ± 0.01 mm). Low antibacterial activity of *Pogostemon cablin* extract with inhibition zones less than 10.00 mm was observed against *Streptococcus pneumoniae* (8.50 ± 0.02 mm). The *Aphelandra squarrosa* methanol extract showed low antibacterial activity on *Escherichia coli* (11.00 ± 0.06 mm), *Salmonella typhi* (12.50 ± 0.08 mm), *Staphylococcus aureus* (10.50 ± 0.06 mm) and *Streptococcus pneumoniae* (9.50 ± 0.01 mm) as compared with the *Pogostemon cablin* extract. The *Adhatoda vasica* extract also showed significant inhibitory effects on the growth of *Escherichia coli* (8.50 ± 0.20 mm), *Salmonella typhi* (11.50 ± 0.05 mm), *Staphylococcus aureus* (9.50 ± 0.05 mm) and *Streptococcus pneumoniae* (8.00 ± 0.05 mm) with the mean value of inhibition zones more than 6.00 mm.

The *Euphorbia neriifolia* and *Pedilanthus tithymaloides* extracts showed a similar pattern of antibacterial activity, both extract samples showed a significant inhibitory effect against *Escherichia coli* (9.00 ± 0.10 mm; 7.50 ± 0.08 mm), *Salmonella typhi* (7.50 ± 0.03 mm; 7.50 ± 0.08 mm), *Staphylococcus aureus* (10.00 ± 0.01 mm; 9.50 ± 0.02 mm) and *Streptococcus pneumoniae* (7.50 ± 0.09 mm; 11.50 ± 0.04 mm). The *Casia alata* extract also showed significant inhibitory effects on the growth of *Escherichia coli* (8.50 ± 0.20 mm), *Salmonella typhi* (6.50 ± 0.03 mm), *Staphylococcus aureus* (7.50 ± 0.08 mm) and *Streptococcus pneumoniae* (10.0 ± 0.06 mm). The *Uraria picta* extract also showed significantly high inhibitory effects on the growth of *Escherichia coli* (10.50 ± 0.40 mm), *Salmonella typhi* (9.50 ± 0.05 mm), *Staphylococcus aureus* (8.50 ± 0.07 mm) and *Streptococcus pneumoniae* (10.5 ± 0.04 mm). Overall results showed a significant difference in inhibit activity between methanol extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* and standard antibiotic discs (penicillin and gentamicin).

The results of the diffusion discs assay of chloroform extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumoniae* are shown in Table 2. The *Pogostemon cablin* extract showed very high

inhibition against tested bacteria; *Escherichia coli* (11.00 ± 0.02 mm), *Salmonella typhi* (12.00 ± 0.03 mm) and *Staphylococcus aureus* (8.50 ± 0.01 mm). Low antibacterial activity of *Pogostemon cablin* extract with inhibition zones less than 8.00 mm was observed against *Streptococcus pneumonia* (7.00 ± 0.02 mm). The *Aphelandra squarrosa* extract showed low antibacterial activity on *Escherichia coli* (9.50 ± 0.04 mm), *Salmonella typhi* (10.00 ± 0.07 mm), *Staphylococcus aureus* (11.50 ± 0.07 mm) and *Streptococcus pneumonia* (7.50 ± 0.02 mm). The *Adhatoda vasica* extract also showed significant inhibitory effects on the growth of *Escherichia coli* (7.50 ± 0.02 mm), *Salmonella typhi* (8.00 ± 0.06 mm), *Staphylococcus aureus* (10.50 ± 0.01 mm) and *Streptococcus pneumonia* (8.50 ± 0.07 mm) with the mean value of inhibition zones more than 6.00 mm.

**Table 2. Zones of inhibition (mm) of chloroform extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta*.**

Extract/Organisms	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumonia</i>
<i>Pogostemon cablin</i> ,	11.0 ± 0.02 <sup>c</sup>	12.0 ± 0.03 <sup>f</sup>	8.50 ± 0.01 <sup>f</sup>	7.00 ± 0.02 <sup>f</sup>
<i>Aphelandra squarrosa</i> ,	9.50 ± 0.04 <sup>c</sup>	10.0 ± 0.07 <sup>b</sup>	11.5 ± 0.07 <sup>b</sup>	7.50 ± 0.02 <sup>b</sup>
<i>Adhatoda vasica</i> ,	7.50 ± 0.02 <sup>b</sup>	8.00 ± 0.06 <sup>a</sup>	10.5 ± 0.01 <sup>a</sup>	8.50 ± 0.07 <sup>a</sup>
<i>Euphorbia neriifolia</i> ,	10.0 ± 0.07 <sup>a</sup>	10.0 ± 0.07 <sup>b</sup>	11.5 ± 0.07 <sup>b</sup>	7.50 ± 0.04 <sup>b</sup>
<i>Pedilanthus tithymaloides</i> ,	9.50 ± 0.08 <sup>b</sup>	9.50 ± 0.05 <sup>a</sup>	10.5 ± 0.01 <sup>a</sup>	10.50 ± 0.06 <sup>a</sup>
<i>Casia alata</i>	9.50 ± 0.09 <sup>a</sup>	7.50 ± 0.08 <sup>a</sup>	8.50 ± 0.04 <sup>a</sup>	12.50 ± 0.08 <sup>a</sup>
<i>Uraria picta</i>	10.0 ± 0.05 <sup>a</sup>	11.5 ± 0.02 <sup>b</sup>	10.5 ± 0.01 <sup>b</sup>	11.50 ± 0.06 <sup>b</sup>
Penicillin	9.50 ± 0.01 <sup>a</sup>	12.5 ± 0.05 <sup>c</sup>	10.5 ± 0.01 <sup>c</sup>	15.5 ± 0.05 <sup>c</sup>
Gentamicin	9.50 ± 0.01 <sup>a</sup>	11.5 ± 0.02 <sup>b</sup>	15.0 ± 0.02 <sup>b</sup>	12.0 ± 0.01 <sup>b</sup>

Results (100 mg/mL) are means ± standard deviation of tenth replicate repeated five times to minimize test error; na=no activity; there was no inhibition found in negative control (DMSO).

<sup>abc</sup>Variation in the following letters between samples indicates significance of difference by Duncan's test at 5% level ( $p < 0.05$ ). Inhibition zones are the mean including disc (6 mm) diameter.

The *Euphorbia neriifolia* and *Pedilanthus tithymaloides* extracts showed similar patterns of antibacterial activity, both sample extracts showed a significant inhibitory effect against *Escherichia coli* (10.00 ± 0.07 mm; 9.50 ± 0.08 mm), *Salmonella typhi* (10.00 ± 0.07 mm; 9.50 ± 0.05 mm), *Staphylococcus aureus* (11.50 ± 0.07 mm; 10.50 ± 0.01 mm) and *Streptococcus pneumonia* (7.50 ± 0.04 mm; 10.50 ± 0.06 mm). The *Casia alata* extract also showed significant inhibitory effects on the growth of *Escherichia coli* (9.50 ± 0.09 mm), *Salmonella typhi* (7.50 ± 0.08 mm), *Staphylococcus aureus* (8.50 ± 0.04 mm) and *Streptococcus pneumonia* (12.50 ± 0.08 mm). The *Uraria picta* extract also showed significantly high inhibitory effects on the growth of *Escherichia coli* (10.00 ± 0.05 mm), *Salmonella typhi* (11.50 ± 0.02 mm), *Staphylococcus aureus* (10.50 ± 0.01 mm) and *Streptococcus pneumonia* (11.50 ± 0.06 mm). Overall results showed that there is a significant difference in inhibition activity between chloroform extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* compared to standard antibiotics discs (penicillin and gentamicin).

The results of the diffusion discs assay of ethyl acetate extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *streptococcus pneumonia* are shown in Table 3. The *Pogostemon cablin* extract showed very high activity of inhibition against tested bacteria; *Escherichia coli* (10.50 ± 0.02 mm), *Salmonella typhi* (11.50 ± 0.01 mm), *Staphylococcus aureus* (7.50 ± 0.09 mm) and *Streptococcus pneumonia* (11.00 ± 0.03 mm). The *Aphelandra squarrosa* extract showed low antibacterial activity on *Escherichia coli* (9.00 ± 0.06 mm), *Salmonella typhi* (7.50 ± 0.04 mm), *Staphylococcus aureus* (7.50 ± 0.02 mm) and *Streptococcus pneumonia* (9.50 ± 0.01 mm). The *Adhatoda vasica* extract also showed significant inhibitory effects on the growth of *Escherichia coli* (8.00 ± 0.09 mm), *Salmonella typhi* (10.50 ± 0.02

mm), *Staphylococcus aureus* ( $8.50 \pm 0.03$  mm) and *Streptococcus pneumonia* ( $7.50 \pm 0.05$  mm) with the mean value of inhibition zones more than 7.00 mm.

**Table 3. Zones of inhibition (mm) of ethyl acetate extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta*.**

Extract/Organisms	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumonia</i>
<i>Pogostemon cablin</i> ,	$10.5 \pm 0.02^c$	$11.5 \pm 0.01^c$	$7.50 \pm 0.09^c$	$11.0 \pm 0.03^c$
<i>Aphelandra squarrosa</i> ,	$9.00 \pm 0.06^c$	$7.50 \pm 0.04^c$	$7.50 \pm 0.02^c$	$9.50 \pm 0.01^c$
<i>Adhatoda vasica</i> ,	$8.00 \pm 0.09^b$	$10.5 \pm 0.02^b$	$8.50 \pm 0.03^b$	$7.50 \pm 0.05^b$
<i>Euphorbia neriifolia</i> ,	$11.5 \pm 0.01^a$	$10.0 \pm 0.07^a$	$9.50 \pm 0.01^a$	$10.0 \pm 0.08^a$
<i>Pedilanthus tithymaloides</i> ,	$10.5 \pm 0.05^b$	$6.00 \pm 0.03^b$	$10.5 \pm 0.08^b$	$9.50 \pm 0.06^b$
<i>Casia alata</i>	$10.5 \pm 0.09^b$	$7.00 \pm 0.09^b$	$13.5 \pm 0.03^b$	$9.50 \pm 0.08^b$
<i>Uraria picta</i>	$9.50 \pm 0.02^b$	$10.5 \pm 0.05^b$	$14.5 \pm 0.05^b$	$10.0 \pm 0.02^b$
Penicillin	$8.00 \pm 0.08^a$	$11.5 \pm 0.01^a$	$14.5 \pm 0.01^a$	$9.50 \pm 0.03^a$
Gentamicin	$7.00 \pm 0.01^a$	$8.50 \pm 0.05^a$	$9.50 \pm 0.07^a$	$9.50 \pm 0.04^a$

Results (100 mg/mL) are means  $\pm$  standard deviation of tenth replicate repeated five times to minimize test error; na=no activity; there was no inhibition found in negative control (DMSO).

<sup>abc</sup>Variation in the following letters between samples indicates significance of difference by Duncan's test at 5% level ( $p < 0.05$ ). Inhibition zones are the mean including disc (6 mm) diameter.

The *Euphorbia neriifolia* and *Pedilanthus tithymaloides* extracts showed similar patterns of antibacterial activity, both extract samples showed a significant inhibitory effect against *Escherichia coli* ( $11.50 \pm 0.01$  mm;  $10.50 \pm 0.05$  mm), *Salmonella typhi* ( $10.00 \pm 0.07$  mm;  $6.00 \pm 0.03$  mm), *Staphylococcus aureus* ( $9.50 \pm 0.01$  mm;  $10.50 \pm 0.08$  mm) and *Streptococcus pneumonia* ( $10.00 \pm 0.08$  mm;  $9.50 \pm 0.06$  mm). The *Casia alata* extract also showed significant inhibitory effects on the growth of *Escherichia coli* ( $10.50 \pm 0.09$  mm), *Salmonella typhi* ( $7.00 \pm 0.09$  mm), *Staphylococcus aureus* ( $13.50 \pm 0.03$  mm) and *Streptococcus pneumonia* ( $9.50 \pm 0.08$  mm). The *Uraria picta* extract also showed significantly high inhibitory effects on the growth of *Escherichia coli* ( $9.50 \pm 0.02$  mm), *Salmonella typhi* ( $10.50 \pm 0.05$  mm), *Staphylococcus aureus* ( $14.50 \pm 0.05$  mm) and *Streptococcus pneumonia* ( $10.00 \pm 0.02$  mm). Overall results showed that there is a significant difference in inhibition activity of ethyl acetate extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* on as compared to standard antibiotics discs (penicillin and gentamicin).

The minimum inhibitory concentration is expressed as the lowest extract concentration at which no visible growth in broth was observed. The MIC of methanol extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia* are shown in Table 4. The MIC values of methanol extract of *Pogostemon cablin* on *Escherichia coli* (1.56 mg/mL), *Salmonella typhi* (1.56 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and *Streptococcus pneumonia* (6.25 mg/mL). The MIC values of methanol extract of *Aphelandra squarrosa* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and there was no MIC value for methanol extract of *Aphelandra squarrosa* on *Streptococcus pneumonia*.

**Table 4. The minimum inhibitory concentrations of methanol extracts of *Pogostemon cablin*, *Alhelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* (100 mg/mL).**

Samples	(mg/mL)	E. Coli	S. Typhi	S. Aureus	S. Pneumonia
<i>Pogostemon cablin</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
<i>Alhelandra squarrosa</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Adhatoda vasica</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Euphorbia nerrifolia</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Pedilanthus tithymaloides</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Casia alata</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Uraria picta</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+

\*+= indicates bacterial growth; -= indicates no bacterial growth

The MIC values of methanol extract of *Adhatoda vasica* on *Escherichia coli* (6.25 mg/mL), *Salmonella typhi* (6.25 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and there was no MIC value for methanol extract of *Adhatoda vasica* on *Streptococcus pneumonia*. The MIC values of methanol extract of *Euphorbia neriifolia* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL). The MIC values of methanol extract of *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* has showed a similar pattern on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL).

The MIC values of chloroform extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia* are shown in Table 5. The MIC values of chloroform extract of *Pogostemon cablin* on *Escherichia coli* (1.56 mg/mL), *Salmonella typhi* (1.56 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and *Streptococcus pneumonia* (6.25 mg/mL). The MIC values of chloroform extract of *Aphelandra squarrosa* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and there was no MIC value for chloroform extract of *Aphelandra squarrosa* on *Streptococcus pneumonia*.

**Table 5. The minimum inhibitory concentrations of chloroform extracts of *Pogostemon cablin*, *Alhelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* (100 mg/mL).**

Samples	(mg/mL)	<i>E. Coll</i>	<i>S. Typhi</i>	<i>S. Aureus</i>	<i>S. Pneumonia</i>
<i>Pogostemon cablin</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
<i>Alhelandra squarrosa</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Adhatoda vasica</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Euphorbia nerrifolia</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Pedilanthus tithymaloides</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Casia alata</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Uraria picta</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+

\*+= indicates bacterial growth; -= indicates no bacterial growth

The MIC values of chloroform extract of *Adhatoda vasica* on *Escherichia coli* (6.25 mg/mL), *Salmonella typhi* (6.25 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and there was no MIC value for chloroform extract of *Adhatoda vasica* on *Streptococcus pneumonia*. The MIC values of chloroform extract of *Euphorbia nerrifolia* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL). The MIC values of chloroform extract of *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* has showed a similar pattern on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL).

The MIC values of ethyl acetate extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia* are shown in Table 6. The MIC values of ethyl acetate extract of *Pogostemon cablin* on *Escherichia coli* (1.56 mg/mL), *Salmonella typhi* (1.56 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and *Streptococcus pneumonia* (6.25 mg/mL). while the MIC values ethyl acetate of *Aphelandra squarrosa* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and there was no MIC value for ethyl acetate of *Aphelandra squarrosa* on *Streptococcus pneumonia*.



**Table 6. The minimum inhibitory concentrations of ethyl acetate extracts of *Pogostemon cablin*, *Alhelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* (100 mg/mL).**

Samples	(mg/mL)	<i>E. Coli</i>	<i>S. Typhi</i>	<i>S. Aureus</i>	<i>S. Pneumonia</i>
<i>Pogostemon cablin</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
<i>Alhelandra squarrosa</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Adhatoda vasica</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Euphorbia nerrifolia</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Pedilanthus tithymaloides</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Casia alata</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Uraria picta</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+

\*+= indicates bacterial growth; '-=' indicates no bacterial growth

The MIC values of ethyl acetate *Adhatoda vasica* on *Escherichia coli* (6.25 mg/mL), *Salmonella typhi* (6.25 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and there was no MIC value for ethyl acetate extract of *Adhatoda vasica* on *Streptococcus pneumonia*. The MIC values of ethyl acetate of *Euphorbia nerrifolia* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL). The MIC values of ethyl acetate of *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* has showed a similar pattern on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL).

The minimum bactericidal concentration recorded as the lowest extract concentration that exhibited capacity to kill 99.9 % of unicell inocula. *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia* are shown in Table 7. The MBC values of methanol extract of *Pogostemoncablin* on *Escherichia coli* (1.56 mg/mL), *Salmonella typhi* (1.56 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and *Streptococcus pneumonia* (6.25 mg/mL). The MBC values of methanol extract of *Aphelandra squarrosa* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and there was no MBC value for methanol extract of *Aphelandra squarrosa* on *Streptococcus pneumonia*.

**Table 7. The minimum inhibitory concentrations of methanol extracts of *Pogostemon cablin*, *Alhelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* (100 mg/mL).**

Samples	(mg/mL)	E. Coll	S. Typhi	S. Aureus	S. Pneumonia
<i>Pogostemon cablin</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
<i>Alhelandra squarrosa</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Adhatoda vasica</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Euphorbia nerrifolia</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Pedilanthus tithymaloides</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Casia alata</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Uraria picta</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+

\*+= indicates bacterial growth; \*-= indicates no bacterial growth

The MBC values of methanol extract of *Adhatoda vasica* on *Escherichia coli* (6.25 mg/mL), *Salmonella typhi* (6.25 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and there was no MBC value for methanol extract of *Adhatoda vasica* on *Streptococcus pneumonia*. The MBC values of methanol extract of *Euphorbia nerrifolia* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL). The MBC values of methanol extract of *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* has showed a similar pattern on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL).

The MBC values of chloroform extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia* are shown in Table 8. The MBC values of chloroform extract of *Pogostemon cablin* on *Escherichia coli* (1.56 mg/mL), *Salmonella typhi* (1.56 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and *Streptococcus pneumonia* (6.25 mg/mL). The MBC values chloroform of *Aphelandra squarrosa* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and there was no MBC value for chloroform extract of *Aphelandra squarrosa* on *Streptococcus pneumonia*. The MBC values of chloroform extract of *Adhatoda vasica* on *Escherichia coli* (6.25 mg/mL), *Salmonella typhi* (6.25 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and there was no MBC value for chloroform extract of *Adhatoda vasica* on *Streptococcus pneumonia*.

**Table 8. The minimum bactericidal concentrations of chloroform extracts of *Pogostemon cablin*, *Alhelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* (100 mg/mL).**

Samples	(mg/mL)	E. Coll	S. Typhi	S. Aureus	S. Pneumonia
<i>Pogostemon cablin</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
<i>Alhelandra squarrosa</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Adhatoda vasica</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Euphorbia nerrifolia</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Pedilanthus tithymaloides</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Casia alata</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Uraria picta</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+

\*+= indicates bacterial growth; -= indicates no bacterial growth

The MBC values of ethyl acetate extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia* are shown in Table 9. The MBC values of ethyl acetate extract of *Pogostemon cablin* on *Escherichia coli* (1.56 mg/mL), *Salmonella typhi* (1.56 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and *Streptococcus pneumonia* (6.25 mg/mL). The MBC values ethyl acetate of *Aphelandra squarrosa* on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and there was no MBC value for ethyl acetate extract of *Aphelandra squarrosa* on *Streptococcus pneumonia*.

The MBC values of ethyl acetate extract of *Adhatoda vasica* on *Escherichia coli* (6.25 mg/mL), *Salmonella typhi* (6.25 mg/mL), *Staphylococcus aureus* (6.25 mg/mL) and there was no MBC value for ethyl acetate extract of *Adhatoda vasica* on *Streptococcus pneumonia*. The MBC values of ethyl acetate of *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* has showed a similar pattern on *Escherichia coli* (25.0 mg/mL), *Salmonella typhi* (25.0 mg/mL), *Staphylococcus aureus* (25.0 mg/mL) and *Streptococcus pneumonia* (25.0 mg/mL).

**Table 9. The minimum bactericidal concentrations of ethyl acetate extracts of *Pogostemon cablin*, *Alhelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifola*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* (100 mg/mL).**

Samples	(mg/mL)	E. Coll	S. Typhi	S. Aureus	S. Pneumonia
<i>Pogostemon cablin</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
<i>Alhelandra squarrosa</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Adhatoda vasica</i>	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Euphorbia nerrifola</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Pedilanthus tithymaloides</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Casia alata</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
<i>Uraria picta</i>	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+

\*+ indicates bacterial growth; '- indicates no bacterial growth

Overall from these study, the methanol extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifola*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* has showed a broad spectrum of activity against all the bacteria used in the study. This indicates that methanol extract has better antibacterial properties than the narrow spectrum of activity of crude extracts similarly as reported by Geidam *et al.*, (2007) and contains a higher concentration of active antimicrobial agents. But it could also be attributable to the polarity nature of active antimicrobial agents. These may include alkaloids, glycosides, volatile oils or tannins, which are all found in more abundance in medicinal plants.

*Staphylococcus aureus* was found to be most susceptible to methanol, chloroform and ethyl acetate extracts of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifola*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta*. The activity of the methanol extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia nerrifola*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *E. coli* is interesting since *E. coli* strains have developed resistance to antimicrobial drugs commonly used in poultry production (Yang *et al.*, 2004) and even to frontline antimicrobials, such as the fluoroquinolones (Livermore *et al.*, 2002; Angulo *et al.*, 2000). The greater resistance of Gram negative bacteria to plant and fruit extracts has been documented previously by Kudi *et al.*, (1999) and Vlietinck *et al.*, (1995) and it is supported by the results of this study.

The observations are likely to be the result of the difference in cell wall structure between Gram-positive and Gram-negative bacteria, with the Gram-negative outer membrane acting as a

barrier to many environmental substances including antibiotics (Tortora *et al.*, 2001). The susceptibility of only Gram positive bacteria to the phenolics and anthocyanin extract was interesting as it may suggest that there could have been a possible interaction between these alkaloids and some constituents of the Gram negative cell wall composition. Therefore the phenolic extract could be explored as a narrow spectrum of phenolic antibacterial agent.

The MIC values are remarkable for a crude extract as well as the MBC values. The results obtained show that these *staphylococci* may be inhibited by a very low concentration of the extract while all studied concentrations had the capacity to trigger lysis of the cells by activation of autolysis enzymes in the cell wall. This situation may be referred to as tolerance, a resistance mechanism, due to the small difference between the MIC and MBC (Brooks *et al.*, 1998). Therefore these plant extracts may serve as bactericidal agents in the case of infections from non-MRSA.

## CONCLUSION

This study has demonstrated the antimicrobial activity of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumoniais* an indication that herbal extracts area potential source for production of drugs with a broad spectrum of activity. From the present study we can conclude that the crude compounds from *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* have great potential as antimicrobial compounds against micro-organisms and can significantly inhibit the growth of *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus pneumonia*.

The methanol extract of *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* has a broad spectrum of activity against bacteria and can be used in the treatment of infectious diseases caused by resistant micro-organisms. The results of the present study highlight the fact that the organic solvent extracts exhibit greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted through organic solvent media. It was suggested that organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and this was supported by many investigators previously (Mohanasundari *et al.*, 2007).

The results obtained may provide scientific data to support the use of the herbs in traditional medicine. Further chemical and pharmacological investigations isolate and identify minor chemical constituents in *Pogostemon cablin*, *Aphelandra squarrosa*, *Adhatoda vasica*, *Euphorbia neriifolia*, *Pedilanthus tithymaloides*, *Casia alata* and *Uraria picta* and to screen other potential bioactivities are recommended.

## ACKNOWLEDGEMENT

The authors would like to thank Faculty of Medicine and Health Sciences (FPSK), Universiti Sultan Zainal Abidin (UniSZA) for the financial aid and Faculty of Food Technology for providing the facilities and Taman Herba, Taman Pertanian Negeri Sekayu, Terengganu for supplying the plant materials. The authors also would like to acknowledge food microbiology dietetic students session 2011/2012 and all staff at Microbiology Laboratory, Faculty of Medicine and Health Sciences, UniSZA.

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