SCREENING OF SEVEN TYPES TERENGGANU HERBS FOR THEIR POTENTIAL ANTIBACTERIAL ACTIVITY AGAINST SELECTED FOOD spencer ROORGANISMS

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 nerrifola), 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 neriifola, 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 (Muñoz-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 micro-organisms 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 & Kaur, 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 taxolete (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 neriifola, 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 *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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta*. All herbs were extracted with different solvents in increasing order of polarity. The solvents used were methanol, choloroform 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^{o}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^{o}C and stored at -20^{o}C until they were used for the analysis. To screen the extracts of *Pogostemon cablin, Aphelandra squarrosa, Adhatoda vasica, Euphorbia neriifola, 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^{o}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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta* was evaluated against four food microorganisms (*Escherichia coli* (ATCC 85218), *Salmonella entrica 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^{o}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 neriifola, 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 µL 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 µm millipore filters. Sterile discs were impregnated with 30 µL of extract solutions (100 mg/mL) and placed on the inoculated agar. Negative controls were prepared using DMSO, while Penicillin G (10 µg/dics) and Gentamicin (10 µg/disc) were used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 37^{o}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.,* 2005).

Minimum inhibitory concentration

Minimum inhibition concentration (MIC) of the chloroform, EA and methanol extracts from *Pogostemon cablin, Aphelandra squarrosa, Adhatoda vasica, Euphorbia neriifola, 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.,* (2007). 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^{o}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.,* 2007). 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 neriifola, 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 µL of the bacterial suspension (10^5 CFU/mL) and incubated for 24 hours at 37^oC. The growth was observed both visually and by measuring optical density (O.D) at 600 nm at regular intervals. Then 20 µL 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^oC 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 ± 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 neriifola, 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 neriifola, Pedilanthu stithymaloides, Casia alata and Uraria picta* against *Escherichia coli, Salmonella typhi, Staphylococcus aureus and streptococcus pneumonia*are are shown in Table 1.

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

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

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).*
^{abc}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 pneumonia* (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 pneumonia* (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 pneumonia* (8.00 ± 0.05 mm) with the mean value of inhibition zones more than 6.00 mm.

The *Euphorbia neriifola* 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 pneumonia* (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 pneumonia* (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 pneumonia* (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 neriifola, 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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta* against *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Streptococcus pneumonia* 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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta.*

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

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).*
^{abc}*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 neriifola* 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 neriifola, 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 neriifola, 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 ± 0.03 mm) and *Streptococcus pneumonia* (7.50 ± 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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta.*

Extract/Organisms	*Escherichia coli*	*Salmonella typhi*	*Staphylococcus aureus*	*Streptococcus pneumonia*
Pogostemon cablin,	10.5 ± 0.02^{c}	11.5 ± 0.01^{c}	7.50 ± 0.09^{c}	11.0 ± 0.03^{c}
Alhelandra squarrosa,	9.00 ± 0.06^{c}	7.50 ± 0.04^{c}	7.50 ± 0.02^{c}	9.50 ± 0.01^{c}
Adhatoda vasica,	8.00 ± 0.09^{b}	10.5 ± 0.02^{b}	8.50 ± 0.03^{b}	7.50 ± 0.05^{b}
Euphorbia nerrifola,	11.5 ± 0.01^{a}	10.0 ± 0.07^{a}	9.50 ± 0.01^{a}	10.0 ± 0.08^{a}
Pedilanthus tithymaloides,	10.5 ± 0.05^{b}	6.00 ± 0.03^{b}	10.5 ± 0.08^{b}	9.50 ± 0.06^{b}
Casia alata	10.5 ± 0.09^{a}	7.00 ± 0.09^{a}	13.5 ± 0.03^{a}	9.50 ± 0.08^{a}
Uraria picta	9.50 ± 0.02^{a}	10.5 ± 0.05^{a}	14.5 ± 0.05^{a}	10.0 ± 0.02^{a}
Penicillin	8.00 ± 0.08^{a}	11.5 ± 0.01^{a}	14.5 ± 0.01^{a}	9.50 ± 0.03^{a}
Gentamicin	7.00 ± 0.01^{a}	8.50 ± 0.05^{a}	9.50 ± 0.07^{a}	9.50 ± 0.04^{a}

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).*
^{abc}*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 neriifola* and *Pedilanthus tithymaloides* extracts showed similar patterns of antibacterial activity, both extract samples showed a significant inhibitory effect against *Escherichia coli* (11.50 ± 0.01 mm; 10.50 ± 0.05 mm), *Salmonella typhi* (10.00 ± 0.07 mm; 6.00 ± 0.03 mm), *Staphylococcus aureus* (9.50 ± 0.01 mm; 10.50 ± 0.08 mm) and *Streptococcus pneumonia* (10.00 ± 0.08 mm; 9.50 ± 0.06 mm). The *Casia alata* extract also showed significant inhibitory effects on the growth of *Escherichia coli* (10.50 ± 0.09 mm), *Salmonella typhi* (7.00 ± 0.09 mm), *Staphylococcus aureus* (13.50 ± 0.03 mm) and *Streptococcus pneumonia* (9.50 ± 0.08 mm). The *Uraria picta* extract also showed significantly high inhibitory effects on the growth of *Escherichia coli* (9.50 ± 0.02 mm), *Salmonella typhi* (10.50 ± 0.05 mm), *Staphylococcus aureus* (14.50 ± 0.05 mm) and *Streptococcus pneumonia* (10.00 ± 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 neriifola, 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 neriifola, 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 nerrifola, Pedilanthus tithymaloides, Casia alata* and *Uraria picta* (100 mg/mL).

Samples	(mg/mL)	*E. Coli*	*S. Typhi*	*S. Aureus*	*S. Pneumonia*
Pogostemon cablin	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
Alhelandra squarrosa	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Adhatoda vasica	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Euphorbia nerrifola	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Pedilanthus tithymaloides	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Casia alata	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Uraria picta	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 neriifola* 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 neriifola, 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 nerrifola, Pedilanthus tithymaloides, Casia alata* and *Uraria picta* (100 mg/mL).

Samples	(mg/mL)	*E. Coli*	*S. Typhi*	*S. Aureus*	*S. Pneumonia*
Pogostemon cablin	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
Alhelandra squarrosa	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Adhatoda vasica	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Euphorbia nerrifola	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Pedilanthus tithymaloides	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Casia alata	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Uraria picta	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 neriifola* 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 neriifola, 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 nerrifola, Pedilanthus tithymaloides, Casia alata* and *Uraria picta* (100 mg/mL).

Samples	(mg/mL)	*E. Coli*	*S. Typhi*	*S. Aureus*	*S. Pneumonia*
Pogostemon cablin	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
Alhelandra squarrosa	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Adhatoda vasica	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Euphorbia nerrifola	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Pedilanthus tithymaloides	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Casia alata	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Uraria picta	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 neriifola* 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 neriifola, 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 nerrifola, Pedilanthus tithymaloides, Casia alata* and *Uraria picta* (100 mg/mL).

Samples	(mg/mL)	*E. Coli*	*S. Typhi*	*S. Aureus*	*S. Pneumonia*
Pogostemon cablin	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
Alhelandra squarrosa	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Adhatoda vasica	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Euphorbia nerrifola	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Pedilanthus tithymaloides	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Casia alata	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Uraria picta	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 neriifola* 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 neriifola, 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 nerrifola, Pedilanthus tithymaloides, Casia alata* and *Uraria picta* (100 mg/mL).

Samples	(mg/mL)	E. Coli	S. Typhi	S. Aureus	S. Pneumonia
Pogostemon cablin	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
Alhelandra squarrosa	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Adhatoda vasica	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Euphorbia nerrifola	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Pedilanthus tithymaloides	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Casia alata	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Uraria picta	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 neriifola, 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. Coli*	*S. Typhi*	*S. Aureus*	*S. Pneumonia*
Pogostemon cablin	100	-	-	-	-
	25	-	-	-	-
	6.25	-	-	-	-
	1.56	-	-	+	+
	0.39	+	+	+	+
Alhelandra squarrosa	100	-	-	-	+
	25	-	-	-	+
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Adhatoda vasica	100	-	-	-	+
	25	-	-	-	+
	6.25	-	-	-	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Euphorbia nerrifola	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Pedilanthus tithymaloides	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Casia alata	100	-	-	-	-
	25	-	-	-	-
	6.25	+	+	+	+
	1.56	+	+	+	+
	0.39	+	+	+	+
Uraria picta	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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta has* showed a broad spectrum of activity againt 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 abundancein 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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta.* The activity of the methanol extract of *Pogostemon cablin, Aphelandra squarrosa, Adhatoda vasica, Euphorbia neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta* againts *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.,* 1997). 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 neriifola, Pedilanthus tithymaloides, Casia alata and Uraria picta* against *Escherichia coli, Salmonella typhi, Staphylococcus aureus and Streptococcus pneumonia*is 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 neriifola, 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 neriifola, 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.

The results obtained may provide scientific data to support theuse 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 neriifola, 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.

REFERENCES

Abd Aziz, S. M., Low, C. N., Chai, L. C., Abd Razak, S. S. N., Selamat, J., Son, R., Sarker, M. Z. I., & Khatib, A. 2011. Screening of Selected Malaysian Plants against Several Food Borne Pathogen Bacteria. *International Food Research Journal*, **18 (3)**: 1195-1201.

Adel S. A., Ahmad Bustamam Abdul, Siddig Ibrahim Abdelwahab, Chew Yuan Peng, Syam Mohan, & Manal Mohamed Elhassan. 2009. Eleucineindica Possesses Antioxidant, Antibacterial and Cytotoxic Properties. *Evidence-Based Complementary and Alternative Medicine*, Vol. 2009.

Angulo, F. J., Johnson, K. R., Tauxe, R. V., & Cohen, M. L. 2000. Origins and Consequences of Antimicrobial-Resistant Non Typhoidal Salmonella: Implications for the Use of Fuoroquinolones in Food Animals. *Microbial Drug Resistance*, **6**: 77–83.

Arora, D. S., & Kaur, J. 1999. Antimicrobial Activity of Spices. *International Journal of Antimicrobial Agents*, **12**: 257-262.

Arya, R., Babu, V., Ilyas, M., & Nassim, K. T. 1989. Phytochemical Examination of the Leaves of *Anacardium occidentale. Journal of the Indian Chemical Society*, **66**: 67-68.

Aziz, Z., & Tey, N. P. 2009. Herbal Medicines: Prevalence and Predictors of Use among Malaysian Adults. *Complementary Therapies in Medicine*, **17**: 44-50.

Brooks, G. F., Butel, J. S., & Morse, S. A. 1998. *Jawetz, Melnick and Adelberg's Medical Microbiology*. 21st Ed., McGraw-Hill, New York, United States of America.

Chan, E., W. C., Lim, Y. Y., & Omar, M. 2007. Antioxidant and Antibacterial Activity of Leaves of *Etlingera*species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry*, **104**: 1586-1593.

Coelho de Souza, G., Haas, A. P. S., von Poser, G, L., Schapoval, E. E. S., & Elisabetsky, E. 2004. Ethnopharmacological Studies of Antimicrobial Remedies in the South of Brazil. *Journal of Ethnopharmacology*, **90**: 135-143.

Cohen, M. L. 1992. Epidemiology of Drug Resistance: Implications for a Postantimicrobialera. *Science*, **257**: 1050-1055.

Evans, C. E., Banso, A., & Samuel, O. A. 2002. Efficacy of Some Nupe Medicinal Plants Against *Salmonella Typhi:* An In-Vitro Study. *Journal Ethnopharmacology*, **80**: 21-24.

Geidam, Y. A., Ambali, A. G., & Onyeyili, P.A. 2007. Phytochemical Screening and Antibacterial Properties of Organic Solvent Fractions of Psidium guajava Aqueous Leaf Extracts. *International Journal of Pharmacology*, **3(1)**: 68-73.

Gutmann, L., Williamson, R., Collatz, E., & Acar, J. F. 1988. Mechanisms of Beta-lactam Resistance in *Haemophilus Influenzae*. *European Journal of Clinical Microbiology & Infectious Diseases*, **7**: 610-615.

Girish, H. V., & Satish, S. 2008. Antibacterial Activity of Important Medicinal Plants on Human Pathogenic Bacteria: A Comparative Analysis. *World Applied Sciences Journal*, **5 (3)**:267-271.

Ikram, E. H. K., Eng, K. H., Jalil, A. M. M., Ismail, A., Idris, S., Azlan, A., Nazri, H. S. M., Diton, N. A. M., & Mokhtar, R. A. M. 2009. Antioxidant Capacity and Total Phenolic

Content of Malaysia Underutilized Fruits. *Food Composition and Analysis Journal*, **22**: 388-393.

Kudi A. C., Umoh J. U., Eduvie L. O., & Gefu, J. 1999. Screening of Some Nigerian Medicinal Plants for Antibacterial Activity. *Journal of Ethnopharmacology*, **67**: 225-228.

López-Muñoz, F., Alamo, C., & García-García, P. 2006. The Herbs that have the Property of Healing: The Phytotherapy in Don Quixote. *Journal of Ethnopharmacology*, **106**: 429-441.

Low Dog, T. 2006. A Reason to Season: The Therapeutic Benefits of Spices and Culinary Herbs. *The Journal of Science and Healing*, **2**: 446-449.

Lu, H., Liang, Y., Yi, L., & Wu, X. 2006. Antiinflammatory Effect of Houttuyniacordata Injection. *Journal of Ethnopharmacology*, **104**: 245 – 249.

Livermore, D. M., Struelens, M., Amorim, J., *et al*. 2002. Multicentre Evaluation of the VITEK 2 Advanced Expert System for Interpretive Reading of Antimicrobial Resistance Tests. *Journal of Antimicrobial Chemotheraphy*, **49**: 289–300.

Mohanasundari, C., Natarajan, D., Srinivasan, K., Umamaheswari, S. A., Ramachandran, A. 2007. Antibacterial Properties of *Passiflorafoetida L.*: A Common Exotic Medicinal Plant. *African Journal of Biotechnology*, **6 (23)**: 2650-2653.

Muñoz-Mingarro, D., Acero, N., Llinares, F., Pozuelo, J. M., Galán de Merab, A., Vicenten, J. A., Morales, L., Alguacil, L. F., & Pérez, C. 2003. Biological Activity of Extracts from *Catalpa bignonioides* Walt. (Bignoniaceae). *Journal of Ethnopharmacology*, **87**: 163-167.

Ramzi, A. A, Mothana, R. A. A., Lindequist, U. 2005. Antimicrobial Activity of Some Medicinal Plants of the Island Soqotra. *Journal of Ethnopharmacology*, **96**: 177-181.

Salau, A. O., & Odeleye, O. M. 2007. Antimicrobial Activity of Mucunapruriens on Selected Bacteria. *African Journal of Biotechnology*, **6**: 2091-2092.

Smith-Palmer, A., Stewart, J., & Fyfe, L. 1998. Antimicrobial Properties of Plant Essential Oils and Essences against Five Important Food-Borne Pathogens. Lett. *Applied Microbiology*, **26**: 118-122.

Soedibyo, M. 1998. *Alam Sumber Kesehatan, Manfaat dan Kegunaan*. Balai Pustaka, Jakarta, Indonesia.

Spencer A. L. R., & Spencer, J. F. T. 2004. *Public Health Microbiology: Methods and Protocols*. Human Press Inc. New Jersey, pp. 325-327.

Thomson, C., Lutz, R. B., Ann, M. C., Cheryl, L. R., & Elaine, R. M. 2001. *Herbs and Botanical Supplements: Principles and Concepts, Nutrition in the Prevention and Treatment of Disease*. Academic Press, San Diego, pp. 261-275.

Tshibangu, J. N., Chifundera, K., Kaminsky, R., Wrightn, A. D., & Konig, G. M. 2002. Screening of African Medicinal Plants for Antimicrobial and Enzyme Inhibitory Activity. *Journal of Ethnopharmacology*, **80**: 25–35.

Tortora, G. J., Funke, B. R., & Case, C. L. 1997. *Microbiology: An Introduction*, 6th edition, Benjamin Cummings. p. 832.

Usman, H., Abdul Rahman, F. I., & Ladan, A. H. 2007. Phytochemical and Antimicrobial Evaluation of *Tribulusterrestris L.* (Zygophylaceae). Growing in Nigeria. *Research Journal of Biological Sciences Medwell Journals*, **2 (3)**: 244-247.

Vollekova, A. D., Kostalova, & Sochorova, R. 2001. Isoquinoline Alkaloids from *Mahonia aquifolium* Stem Bark is Active against *Malassezia Sp*. *Folia Microbiologica*, **46**: 107-111.

Vlietinck, A. J., Van Hoof, L., Totte, J., Lasure, A., Vanden Berghe, D., Rwangabo, P. C., & Mvukiyumwani, J. 1995. Screening of a Hundred Rwandese Medicinal Plants for Antimicrobial and Antiviral Properties. *Journal of Ethnopharmacology*, **46**: 31-47.

Werner, M. H., Shigesada, K., & Ito, Y. 1999. Runt Domains take the Lead in Hematopoiesis and Osteogenesis. *Nature Medicine*, **5**: 1356–1357.

Yang, H., Boersma, J. G., You, M., Buirchell, B. J., & Sweetingham, M. W. 2004. Development and Implementation of a Sequence-Specific PCR Marker Linked to a Gene Conferring Resistance to Anthracnose Disease in Narrow-Leafed Lupin (Lupinusangustifolius L.). *Molecular Breeding*, **14**: 145-151.