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Antibacterial Activity and Major Constituents of *Polyalthia cinnamomea* Basic Fraction

(Aktiviti Antibakteria dan Juzuk Utama Fraksi Bes *Polyalthia cinnamomea)*

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ABSTRACT

Polyalthia cinnamomea *Hook.f. & Thomson (Annonaceae) or locally known as 'Larak Batu Bukit', 'Pisang-pisang', 'Sugao' and 'Sigumpet Hutan' is a small woody plant found throughout Malaysia and Singapore. In this study, the basic chemical components from the leaves of the plant were fractionated and the antibacterial activity as well as the major constituents of the basic fraction was determined. To the best of our knowledge, this is the first study being carried out on the bioactivity and phytochemistry of* P. cinnamomea. *The basic fraction remarkably inhibited the growth of ten bacteria tested except one. The biggest inhibitory diameter and the lowest minimum inhibitory concentration were 19.0±4.6 mm and 0.125 mg/mL against respective* Bacillus subtilis *and* Bacillus thuringiensis. *Gas chromatography-mass spectrometry (GCMS) analysis of the basic fraction identified four major cyclosiloxanes of octamethylcyclotetrasiloxane (18.1%), decamethylcyclopentasiloxane (16.4%), dodecamethylcyclohexasiloxane (14.1%) and tetradecamethylcycloheptasiloxane (6.0%). The knowledge of the antibacterial potential of* P. cinnamomea *basic fraction and the major constituents present in the fraction can be utilized in the fields of natural products, pharmaceutical, cosmetic and personel care industries.*

Keywords: Antibacterial activity; basic fraction; cyclosiloxane; Polyalthia cinnamomea

ABSTRAK

Polyalthia cinnamomea *Hook.f. & Thomson (Annonaceae) atau dikenali tempatan sebagai Larak Batu Bukit, Pisang-pisang, Sugao dan Sigumpet Hutan merupakan tumbuhan berkayu kecil yang tumbuh di seluruh Malaysia dan Singapura. Dalam kajian ini, komponen kimia asas daripada daun tumbuhan tersebut telah difraksikan dan aktiviti antibakteria berserta juzuk utama fraksi bes tersebut telah ditentukan. Setahu kami, ini adalah kajian pertama yang dijalankan terhadap bioaktiviti dan fitokimia* P. cinnamomea. *Fraksi bes tersebut telah merencat secara luar biasa pertumbuhan sepuluh bakteria yang diuji kecuali satu. Diameter perencatan terbesar dan kepekatan perencatan minimum terendah adalah 19.0±4.6 mm dan 0.125 mg/mL masing-masing terhadap* Bacillus subtilis *dan* Bacillus thuringiensis. *Analisis kromatografi gas-spektroskopi jisim (KGSJ) fraksi bes tersebut telah mengenal pasti empat siklosiloksana utama, iaitu oktametilsiklotetrasiloksana (18.1%), dekametilsiklopentasiloksana (16.4%), dodekametilsikloheksasiloksana (14.1%) dan tetradekametilsikloheptasiloksana (6.0%). Pengetahuan potensi antibakteria fraksi bes* P. cinanamomea *dan komponen utama yang hadir dalam fraksi tersebut boleh dimanfaatkan dalam bidang hasilan alami, industri farmaseutik, kosmetik dan penjagaan peribadi.*

Kata kunci: Aktiviti antibakteria; fraksi bes; Polyalthia cinnamomea*; siklosiloksana*

INTRODUCTION

Polyalthia cinnamomea Hook.f. & Thomson that belongs to the family Annonaceae is widely found in lowland forest and widespread in Peninsula Malaysia and Singapore (Ridley 1967). Species of the Annonaceae are used all over the tropics in traditional medicine (Chkrabarti & Mukherjee 1968; FRIM 2016; Irausin et al. 2014; Manasa et al. 2004; Supaluk et al. 2009; Sumitra & Rathish 2010; Yamaguchi et al. 1964). The genus *Polyalthia* itself hold the meaning of 'many to cure' in Greek (poly-many *althea-* to cure). In many parts of India, *Polyalthia longifolia* is used in the Indian traditional system of medicine including Ayurveda (Bapuji & Ratnam 2009; Mahajan et al. 2010; Patel 2010; Poornima et al. 2012; Sinhababu & Baherjee 2013; Sugumaran et al. 2010; Sundaresan & Senthil 2013). The stem extract of *P. longifolia* was found to inhibit the growth of gram positive and gram negative bacteria (Faizi et al. 2003) while its methanol extract of leaves and green berries were found to possess promising antibacterial activity (Faizi et al. 2008). *Polyalthia cerasoides* on the other hand, was used as medicinal plant in Thailand. Its roots were made as tonic and febrifuge (Pharmaceutical Sciences 1996). As for Annonaceae plant species, it is well known that the species are active biologically and produced many promising phytocomponents (Faizi et al. 2003; Lertyot et al. 2011; Patoomratna et al. 2000).

Cyclomethicones are group of methyl siloxanes which is a class of liquid silicones that have the characteristics of low viscosity, high volatility and act as cosmetic solvents. They are particularly suited for use with other silicones and as delivery vehicles for a variety of active ingredients (Starch 2016). Cyclomethicone compounds can be found in plant that eventually influenced the potentiality of the plant antibacterial activity. From the previous study, the GC-MS analysis showed that the cyclomethicone components of the olive leaf extract caused the antimicrobial influence against thirteen bacteria whereby the first three major components of the extracts were cyclomethicones (hexamethylcyclotrisiloxane 36.98%, octamethylcyclotetrasiloxane 15.18%, decamethylcyclopentasiloxane 14.59%) (Dilek et al. 2012). Besides, the other previous study showed that *Toddalia asiatica* was used traditionally as medicine to treat various ailments, contained cyclomethicone phytocomponents (Arun & Varsha 2014).

All in all, many studies have been conducted to evaluate the bioactivity of the *Polyalthia* plant species as they are bioactively potent and consist of various bioactive compounds. Despite of all the studies, there were no study recorded on the specific plant of *Polyalthia cinnamomea* where its bioactivity and phytocomponents are still remain unknown. Hence, in this study, the leaf extract of the plant was obtained and the antibacterial activity against five gram positive bacteria and five gram negative bacteria were investigated and the major phytocomponents of the leaf extract were analyzed.

P. Cinnamomea

P. cinnamomea leaves

Materials and Methods

Plant material collection and identification

The part of Polyalthia cinnamomea studied was the leaf. It was collected from Hutan Simpan Universiti Kebangsaan Malaysia Bangi, Selangor. The plant species was identified by Mr. Sani Miran, a botanist of Universiti Kebangsaan Malaysia. For further reference, the plant voucher specimen of 115D was deposited at the Universiti Kebangsaan Malaysia Herbarium (UKMB).

Extraction of leaves extract

The plant leaves were detached and air dried. After the drying, the leaves were grounded into powder by using a mechanical grinder. In an empty, used and cleaned chloroform glass bottle, 300 g of the grounded *Polyalthia cinnamomea* leaves were soaked in 1.8 L methanol for three days with regular shaking. After that, the methanol extract was filtered using cotton wool and the filtrate was evaporated under vacuum using Buchi rotary evaporator at the temperature of less than 40°C and with 110 rpm rotation until 1/10 of its original volume (Neli et al. 2011). The concentrated extract was used for the extraction of organic base components, most probably of cyclomethicones, following the method of Hadi and Bremner (2001) with some minor modifications and yielded 0.1 g of leaf extract. It was stored under 4°C prior to use.

Preparation of extract for antibacterial assay

Stock solution of the test *Polyalthia cinnamomea* extract was prepared from concentration of 10 mg/mL for disc-diffusion method (methanol as solvent) and 30 mg/mL for minimum inhibitory concentration method (dimethyl sulfoxide, DMSO, as solvent). For disc-diffusion method, blank discs were prepared from the cut out of filter paper Whatman No. 2 and sterilized using autoclave machine at 120-124°C for 1-2 h before being loaded with 10 mg/mL concentration of the test extract and allowed to air dry at room temperature before use.

Bacterial strains

The five gram-positive bacterial strains of *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis*, *Bacillus subtilis* ATCC 11774, *B. thuringiensis* ATCC 10792, Methicilin resistant *S. aureus* (MRSA) and the five gram-negative bacteria strains of *Escherichia coli* ATCC 10536, *Salmonella thyphimurium* ATCC 51812, *Serratia marcescens* ATCC 13880, *Vibrio cholera* and *Vibrio fluvialis* were used. They were obtained from the Microbiology Laboratory culture collection, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

Preparation of culture media for antibacterial assay Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB)

To prepare MHA, 800 mL distilled water was added to a Schott bottle (1 L) containing 19 g of MHA powder. Meanwhile, to prepare MHB, 200 mL distilled water was added to a Schott bottle (500 mL) containing 4.4 g of MHB powder. The two mixtures were then shaken thoroughly to dissolve the powder. Then, 10 mL of the MHB solution was poured into 10 universal bottles (20 mL) and 5 mL of the same solution was poured into 10 universal bottles (10 mL). After that, the MHA and MHB solutions were sterilized for 1-2 h at 120-124°C using an autoclave machine. As for minimum inhibitory concentration

method, Tween 80 solution was used instead of distilled water to prepare the MHB. The sterilized MHB was kept at 4°C until use and the MHA solution was used to prepare agar plates.

PREPARATION OF AGAR PLATE

The laminar hood was first sterilized with 70% ethanol, and all the empty disposable commercial plastic Petri dishes (9 × 9 cm) were assembled in the laminar hood and sterilized using UV light for 15-30 min. Later, the sterilized MHA was poured into each of the disposable commercial plastic Petri dishes under the laminar hood; the Petri dishes were cooled under UV light for 15-30 min. Finally, the plates were covered and stored in the cool room to prevent contamination until use.

PREPARATION OF BACTERIAL INOCULUMS

The sterilized 10 universal bottles with 5 mL MHB solution were seeded with bacteria from the pure culture stock by a sterilized wire loop and incubated at 37°C for 24 h to obtain the culture. After 24 h incubation, 300 µL of the culture were taken and put into the 10 mL sterilized MHB solution that was prepared earlier. The turbidity of culture was measured and standardized against the McFarland 0.5 (optical density of 0.13 at 625 nm).

DISC-DIFFUSION ASSAY

The disc-diffusion assay according to CLSI (2009) and Scorzoni et al. (2007) was carried out to determine antibacterial activity of the leaf extract by measuring the inhibition diameter. The bacteria suspensions were loaded on a sterile cotton swabs and streaked over the dried surface of MHA plates for inoculation. The sterile filter paper discs with 6 mm in diameter were impregnated with the sample (10 mg/mL) and then placed on the inoculated agar. The plates were incubated at 37°C for 24 h. The antibacterial activity was determined by measuring the diameter of inhibition zone against the bacteria. The inhibition zone was measured in millimeter including the disc diameter. The test was carried out in triplicate. Streptomycine (10 µg), Vancomycine (30 µg), Peniciline (30 µg) and Kanamycine (30 µg) were used as the positive controls. For this assay, the extract was dissolved in methanol. A disc which was impregnated with DMSO was used as the negative control.

MINIMUM INHIBITORY CONCENTRATION (MIC) ASSAY

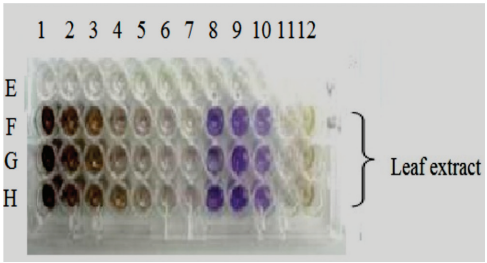
MIC is to determine the lowest concentration of the assayed antibacterial agent that, under defined test conditions, inhibits the visible growth of the bacterium (CLSI 2011). MIC was evaluated on bacteria strain that showed sensitivity to the extracts in the disc-diffusion assay according to CLSI (2011) and Irith et al. (2008). MIC was carried out using broth dilution assay following two-fold serial micro-dilution using 96-well microtiter plates. 100 µL of DMSO solution of the test extract was distributed into the first well and was serially two-fold diluted to obtain the extract concentration ranging from 16 to 0.06 mg/mL. Using a micropipette, 50 µL of the bacteria inoculums were then dispensed into each of the serially diluted extract. Each plate was only used for one bacterium. All tests were carried out in triplicate. For positive controls, Chloramphenicol (128 to 0.25 mg/mL) and Vancomycine (32 to 0.06 mg/mL) were used. The preparation of these antibiotics stock solutions and ranges determination for MIC were according to Andrews (2006). Meanwhile, the last three wells of the line were used as negative control by filling the mixture of 50 µL bacteria inoculums and 50 µL DMSO and as for the growth control, the well was filled with 100 µL of MHB and 50 µL sample. The covered plates were incubated at 37°C for 16-24 h in an incubator.

DETERMINING BACTERIAL GROWTH USING MTT ASSAY

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is a tetrazolium dye which can be reduced by the live bacteria into an insoluble purple formazan. Thus, the reagent therefore can be used to differentiate between the live and the dead bacteria (Stevens & Olsen 1993). MTT was prepared by dissolving in a phosphate-buffered saline (PBS) solution at 0.2 mg/mL. In this study, the MTT assay was incorporated into the MIC method to visually differentiate between the live and the dead bacteria. After 16-24 h incubation in the MIC method, 50 µL of MTT reagent was added into each well and the colour development was observed and recorded after 2 h incubation (Figures 1-9).

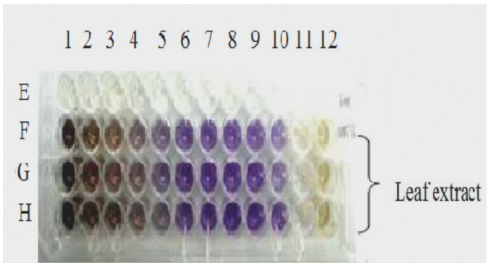
GCMS ANALYSIS OF MAJOR CONSTITUENTS

The leaf extract was subjected to Gas Chromatography and Mass Spectroscopy for identification of bioactive volatile major constituents. GC-MS analysis was performed on an Agilent 7890A gas chromatograph (GC) directly coupled to mass-spectrometer (MS) system of an Agilent 5975C inert MSD with triple-axis detector. The column used was polar



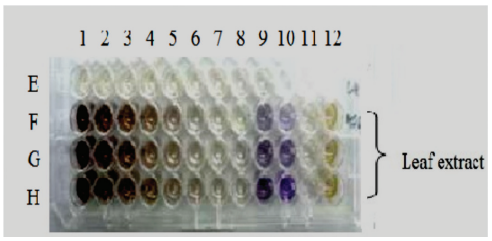
Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 1. MIC determination for leaf extract of *Polyalthia cinnamomea* against methicilin resistant *Staphylococcus aureus* (MRSA)



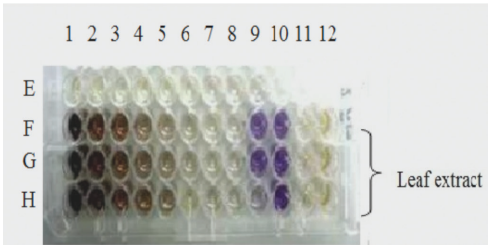
Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 2. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Staphylococcus aureus*



Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 3. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Bacillus subtilis*

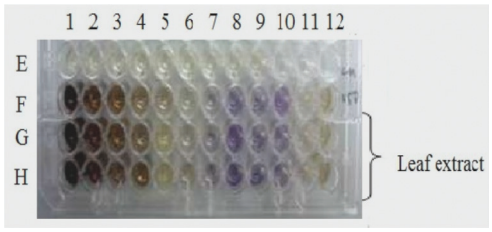


Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 4. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Bacillus thuringiensis*

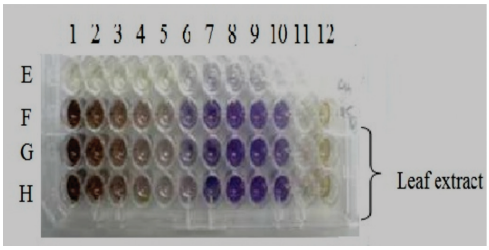
Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 5. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Salmonella thyphimurium*



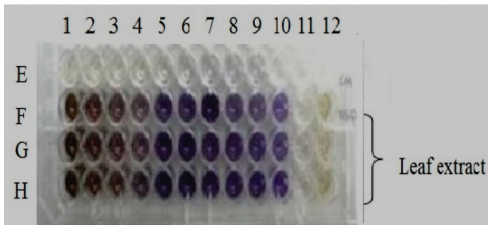
Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 6. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Escherichia coli*



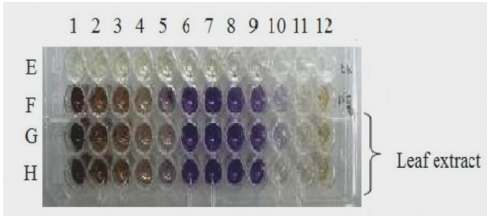
Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 7. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Vibrio fluvialis*



Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 8. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Vibrio cholera*



Microtiter plate wells F1-F9, G1-G9 and H1-H9 contain leaf extract ranges from 16 to 0.06 mg/mL in two-fold dilution. F10, G10 and H10 are negative control. F11, G11, H11 and F12, G12, H12 are growth control. E1-E9 is positive control

FIGURE 9. MIC determination for leaves extract of *Polyalthia cinnamomea* against *Serratia mercescens*

BP20(WAX) (30 m × 0.25 mm × 0.25 μm) with polyethylene glycol stationary phase. Helium gas was used as the carrier gas and the temperature programming was set with initial oven temperature at 50°C and held for 5 min and the final

temperature of the oven was 250°C with rate at 5°C/min. A sample of 1 µL was injected with split less mode. Total GC running time was 65 min. The relative % amount of each component was calculated by comparing its average peak area to the total area.

The MSD Chemstation was used to find all the peaks in the raw GC chromatogram. A library search was carried out for all the peaks using the NIST/EPA/NIH version 2.0 and the results were combined in a single peak table. Interpretation on mass spectrum GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The names, molecular weights, retention times and peak area percentages of the test material were deduced.

Results and Discussion

The leaf extract of *Polyalthia cinnamomea* showed a promising antibacterial activity against nine out of ten bacteria tested (Table 1). In disc-diffusion assay, the leaf extract inhibited four gram positive bacteria and five gram negative bacteria, of which the positive strain of *Bacillus subtilis* being the most inhibited one with the inhibition diameter of 19 ± 4.6 mm. There was no inhibition observed for *Staphylococcus epidermidis*. Meanwhile, the MIC results showed that the leaf extract gave the lowest minimum inhibitory concentration against the positive strain of *Bacillus thuringiensis* with the MIC value of 0.125 mg/mL.

From the data obtained, it showed that the antibacterial activity of the leaf extract was more pronounced towards the gram positive bacteria strains compared to the negative ones, where the zone of inhibitions of the former were more prominent than those of the latter. These findings were in agreement with the observation of the previous study conducted regarding the antibacterial activity of medicinal plants, where most of the active plants showed potent activity against gram positive bacteria strains (Ali et al. 2001; Herrera et al. 1996; Karou et al. 2005; Kelmanson et al. 2000; Masika & Folayane 2002; Nair et al. 2007; Puteri et al. 2017; Rabe & Van 1997).

It was reported that gram positive bacteria strains only have one peptidoglycan layer compared to gram negative bacteria strains which have two. Thus, gram positive bacteria strains did not have an effective barrier to the lipophilic solutes while the gram negative bacteria strains have outer phospholipidic membrane that makes the cell wall impermeable to liphophilic solutes, while the porines constitute a selective barrier to hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido & Vaara 1985; Scherrer & Gerhardt 1971). Hence, the observation of the antibacterial activity in this study showed potent antibacterial activity against gram positive bacteria strains.

The inhibition zone data was statistically calculated and analyzed through one way analysis of variance (ANOVA) to obtain the F value and P value. Values of $p<0.05$ were considered statistically significant and the data are presented in Table 2.

The *Polyalthia cinnamomea* leaf extract used in this study has never been evaluated for its phytochemical components. The major phytocomponents of the leaf extract of *P. cinnamomea* has been discovered by using the GC-MS. The GC-MS chromatogram of the leaf extract of *P. cinnamomea* showed four major peaks representing four major phytocomponents (Figure 10). They are cyclosiloxane mixtures of octamethylcyclotetrasiloxane (18.12%), decamethylcyclopentasiloxane (16.41%), dodecamethylcyclohexasiloxane (14.06%) and tetradecamethylcycloheptasiloxane (6.03%) (Table 3).

To the best of our knowledge, there is no report on the chemical composition nor any bioactivities of the *P. cinnamomea* plant extract. But, previous studies of other plant extracts showed that the isolated octamethylcyclotetrasiloxane, decamethylcyclopentasiloxane and other cyclomethicones are well known antimicrobial and antifungal compounds (Koç et al. 2007; Yusof et al. 2008; Zhen et al. 2008). Other previous study also showed that the antibacterial properties of olive leaf extract were suspected to be associated with the high content of hexamethylcyclotrisiloxane (Hajji et al. 2010). Besides,

TABLE 1. Inhibition diameters and MIC values of antibacterial activity for *Polyalthia cinnamomea* leaf extract

Bacteria/gram	Inhibition diameter (mm)	Positive control * (mm)	MIC (mg/mL)
Methicilin Resistant *S. aureus* (MRSA)/+	10.3±0.6	17±1.0	
Staphylococcus aureus/+	12.3±0.6	14.3±1.2	4.0
Staphylococcus epidermidis/+	N.I	15.7±0.6	4.0
Bacillus subtilis/+	19.0±4.6	34.0±11.0	0.25
Bacillus thuringiensis/+	6.7±1.1	23.3±1.2	0.125
Salmonella typhimurium/-	7.7±2.9	25.0±0.3	2.0
Serratia marcescens/-	6.7±1.2	27.3±1.5	4.0
Escherichia coli/-	7.0±1.7	15.3±2.9	1.0
Vibrio fluvialis/-	12.6±1.2	29.3±1.2	0.5
Vibrio cholera/-	7.6±0.6	10.7±1.2	16.0

NI - No inhibition, *Antibiotic Kanamycine for all bacteria except *B. subtilis* (Peniciline) and MRSA, *B. thuringiensis*, *S. epidermidis*, *S.typhimurium* (Vancomycine) *S. aureus* (Streptomycine)

TABLE 2. One way ANOVA for the antibacterial activity of *Polyalthia cinnamomea* leaf extract

Bacteria/gram	Inhibition diameter (mm)	Positive control* (mm)	F value	P value
Methicilin Resistant S.aureus (MRSA)/+	10.3±0.6	17±1.0	8.96	0.0005
Staphylococcus aureus/+	12.3±0.6	14.3±1.2	7.19	0.055
Staphylococcus epidermidis/+	N.I	15.7±0.6		
Bacillus subtilis/+	19.0±4.6	34.0±11.0	4.75	0.0947
Bacillus thuringiensis/+	6.7±1.1	23.3±1.2	312.10	0.0001
Salmonella typhimurium/-	7.7±2.9	25.0±0.3	108.12	0.0001
Serratia marcescens/-	6.7±1.2	27.3±1.5	314.57	0.0001
Escherichia coli/-	7.0±1.7	15.3±2.9	18.38	0.0128
Vibrio fluvialis/-	12.6±1.2	29.3±1.2	320.52	0.0001
Vibrio cholera/-	7.6±0.6	10.7±1.2	16.30	0.0156

*Antibiotic Kanamycine for all bacteria except *B. subtilis* (Peniciline) and MRSA, *B. thuringiensis*, *S. epidermidis*, *S.typhimurium* (Vancomycine) *S. aureus* (Streptomycine)

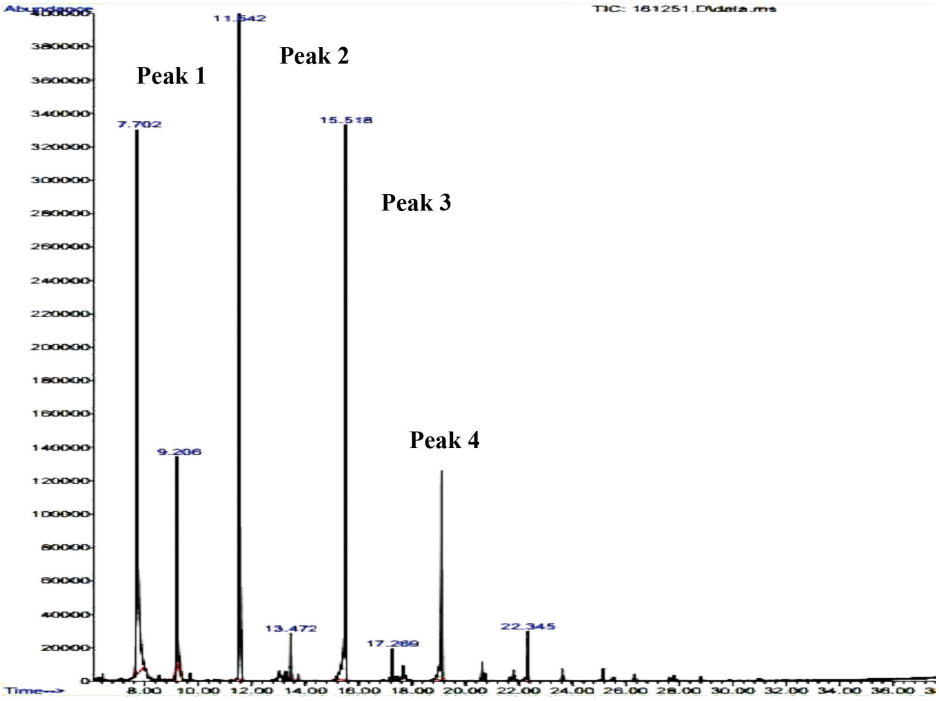


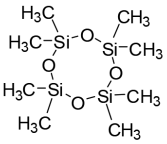
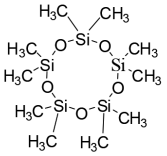
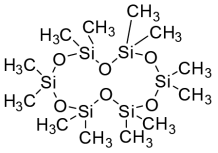
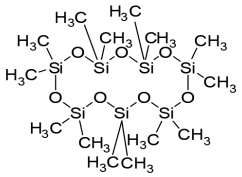
FIGURE 10. GC-MS chromatogram of the leaf extract of *Polyalthia cinnamomea*

in previous study, the oil derived from *Pterocephalus canus* that has dodecamethylcyclohexasiloxane showed significant antimicrobial activity against *Staphylococcus saprophyticus* and *Escherichia coli* (Moustafa et al. 2013).

Meanwhile, an aqueous extract from the leaves of *Casimiroa edulis* effectively controls *in-vitro* development of the post-harvest fungi *Alternaria* spp., *Fusarium* spp., *Pestalotiopsis* spp. and *Rhizopus* spp (Abou-elela et al. 2009) and GC-MS analysis showed it contains tetradecamethylcycloheptasiloxane (Vahedi et al. 2011). Furthermore, the phytocompounds of the methanolic extract of *in vitro* leaf in *Spermacoce articularis* showed the presence of cyclomethicone mixtures of dodecamethylcyclohexasiloxane (5.93%), tetradecamethylcycloheptasiloxane (4.04%) and hexadecamethylcyclooctasiloxane (2.29%) which indicates to be an antimicrobial agent and the plant was used traditionally to treat various ailments (Bautista et al. 2000).

The result presented in this study may suggest that the leaf extract of *Polyalthia cinnamomea* possesses antibacterial properties and it can be a potential source of antibacterial ingredients for the pharmaceutical industry. Furthermore, a recent research has indicated that many chemical compounds, which present in high amounts in several plants exhibited bioactive properties like antimicrobial, antifungal, anti-inflammatory and anti-oxidant (Barakat 2011; Shalini & Rachana 2009). Thus, it is justified that in this study, the major components of cyclosiloxanes in *P. cinnamomea* extract might be responsible for its potent antibacterial activity.

TABLE 3. Major phytocomponents identified in leaf extract of *Polyalthia cinnamomea*

RT	Name of compounds	Structure of compounds	MF	MW	Area %
7.702 (Peak 1)	Octamethylcyclotetrasiloxane		$C_8H_{24}O_4Si_4$	296.61	18.12
11.504 (Peak 2)	Decamethylcyclopentasiloxane		$C_{10}H_{30}O_5Si_5$	370.76	16.41
15.518 (Peak 3)	Dodecamethylcyclohexasiloxane		$C_{12}H_{36}O_6Si_6$	444.92	16.41
19.121 (Peak 4)	Tetradecamethylcycloheptasiloxane		$C_{14}H_{42}O_7Si_7$	519.07	6.03

CONCLUSION

In conclusion, the leaf extract showed sensitivity towards both gram positive and gram negative bacteria but more pronounced towards the gram positive bacteria strains. The phytochemical analysis of the extract showed the presence of four major components of cyclosiloxane mixtures of octamethylcyclotetrasiloxane (18.12%), decamethylcyclopentasiloxane (16.41%), dodecamethylcyclohexasiloxane (14.06%) and tetradecamethylcycloheptasiloxane (6.03%). Thus, from the findings it suggested that the major components of cyclosiloxanes in *P.cinnamomea* leaf extract might be responsible for its potent antibacterial activity as the data obtained from this study mostly showed a statistically significant value with the control (p<0.05). However further study need to be conducted to confirm the correlation between the major components and its antibacterial activity.

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