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# Study on the Phytochemical Constituents in Essential oil of *Pandanus amaryllifolious* Roxb. Leaves and their Anti-bacterial Efficacy

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## Abstract

The study was conducted to evaluate the phytochemicals and antibacterial efficacy of essential oil of *Pandanus amaryllifolius* leaves against various Gram-positive and Gram-negative bacterial. The phytochemicals of essential oil of *P. amaryllifolius* leaves were analyzed by using GC-MS, while the mass spectra of the compounds found in the essential oil was confirmed by comparison of their Kováts retention indices relative to $C_8$-$C_{22}$ *n*-alkanes. The gas chromatography–mass spectroscopy (GC-MS) analysis of *P. amaryllifolius* leaves essential oil showed the presence of 54 compounds were identified, representing 98.28 % of the total oil composition. It was observed that phytol in essential oils was the major component, with the highest concentration in oil (21.35%), followed by α-thujaplicin (18.64%), dodecanol (12.55%), *n*-tetradecanol (8.93%), benzyl acetate (8.08%). The finding of present study revealed that the essential oil of pandanus leaves demonstrate the presence of potential extractive active substances and their antibacterial potential. The essential oil had the greatest antibacterial activity against all Gram-negative bacteria. The essential oil had the largest inhibition zones against *E. coli* and *M. luteus*, measured at 15.3, 10.7, respectively. The lowest MIC value (31.2 µg/mL) was observed for the drugs, while the essential oil showed higher MIC values at concentrations of 31.25 µg/mL for *E. coli* and *M. luteus*, 62.5 µg/mL for *Ps. aeruginosa* and 125 µg/mL for *S. aureus*, respectively.


**Key words:** *Pandanus amaryllifolius*, essential oil, GC-MS, phytol, antibacterial

## Introduction

*Pandanus amaryllifolious* Roxb. (Pandanaceae family) is a tropical plant in a screw pine genus. It is an erect green plant with fan-shaded spray of long, narrow, blade like leaves and woody aerial roots which approximately 4 in (10cm) long. Pandan leaf is commonly used when preparing rice dishes as means of enhancing flavor. This plant is used to wrap foods like fish or shrimp; pandan leaf paste is a kind of desert with sweetness and bright green color. Moreover, pandan leaf is an effective and natural cockroach repellent (Buttery et al., 1983). The essential oil of the pandanus leaves is used in traditional medicines as earache, headache, arthritis, debility, giddiness, laxative, rheumatism, small pox, and spams (Kumar & Sanjeeva, 2011).

Essential Oils are low volume-high value products. Essential Oils are complex mixtures of volatile chemicals found in natural matrices of living organisms. They are responsible for the color and sometimes taste properties of aromatic materials which may be of plant, material, or microbial origin. Such materials which are generally odorous and are named also as volatile oils, essences, aetheroleum or ethereal oils due to their oil-like nature. Aromatic plants are the major source of essential oils which may be found in almost all parts of a plant such as leaves, flowers, bark, seeds, fruits, wood, rhizome, root, root bark, etc. (Baser, 2015).

Most essential oils are made up of different chemical compounds which are very complex; alcohol, aldehyde, ester, terpenes, sesquiterpenes, their oxygenated derivatives and other chemical compound. These chemical compounds are mostly responsible for the desired beneficial properties as plants are known as a source of secondary metabolites that come with


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a variety of structural arrangements and properties with interesting biological activities (De Fatima et al., 2002). Therefore, plant-based medicine has become more popular due to the increasing concern of consumers with regard to the use of synthetic chemical preparations and use of artificial antimicrobial preservatives, especially in modern food protection practices (Isao Kubo et al., 1993).

Essential oil form pandan leaves can be extracted by several methods which include hydrodistillation, carbon dioxide extraction, cold pressing as well as florasol/ phytol extraction. In this study, essential oil from pandan leaves was extracted by hydro distillation method. The extract of pandan leaves was used of the screening of differed phytochemical constituents by GC-MS analysis. Moreover, determination of minimum inhibitory concentration (MIC) of antimicrobial drugs for a large number of organisms was performed by paper diffusion method. After making the water of pandan leaves extract, a phytochemical test was done to determine bioactive compounds such as alkaloid, carbohydrate, cardic, flavonoids, phenol, phlobatannins, sponins, tannis, terpenoids and quinones.

## Materials and Methods

### Plant Materials

Fresh *Pandanus amaryllifolious* leaves were collected in July 2016 and in January 2017, from University of Yangon, Yangon, Myanmar. The voucher herbarium specimen for *P. amaryllifolious* was discriminated and deposited by Professor Dr. Soe Myint Aye, Department of Botany, University of Mandalay, Myanmar.

### Hydrodistillation

The isolation of fresh *P. amaryllifolious* leaves (150 g) was performed in a Clevenger-type apparatus for 2 h to obtain the leaves' oil. After isolation, the distillate was made collection in a conical flask which was then dried over anhydrous sodium sulfate and concentrated by the way of the vacuum rotary evaporator. The oil content (w/w%) was estimated on the fresh sample weight basis. The essential oils were then stored in sterile bottles, under refrigerated conditions, until further analysis.

### Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Essential Oils Analysis

The chemical compositions of the essential oil was investigated using a gas chromatograph-mass spectrometer (Agilent 6890 and HP5973 mass-selective detector, Agilent Technologies, USA) equipped with a fused-silica capillary column, HP-5MS (5% phenyl-polymethylsiloxane), with dimensions of 30 m × 0.25 mm i.d. × 0.25 mm film thickness (Agilent Technologies). The GC-MS was operated under a temperature program that started at 60 °C and was ramped up to 240 °C at 3 °C/min. The injection temperature was 250 °C. The injection volume was 1 μL in the split mode with a split ratio of 50:1. The quadrupole temperature was 150 °C and the transfer-line temperature 280 °C. Helium was used as a carrier gas and was maintained at a constant pressure. Identification of the volatile constituents was performed by comparing their mass spectra with those of the database using W8N08 and NIST 98 mass spectral libraries. The identification was also confirmed by comparison of their Kováts retention indices relative to $C_8$-$C_{22}$ *n*-alkanes, and comparison of the mass spectra of individual components with the reference mass spectra in the Wiley 275 and NIST05 databases.

### The Investigation of Selected Biological Activities

Two Gram-positive bacterial strains (*Micrococcus luteus* TISTR884, *Staphylococcus aureus* TISTR 1466) were obtained from the Thailand Institute of Scientific and

Technological Research, and two Gram-negative bacteria (*Pseudomonas aeruginosa* DMST 781 and *Escherichia coli* DMST 780) were also obtained from the Department of Medical Science, Ministry of Health, Bangkok, Thailand. The antimicrobial activities of the oils sample leaves had been analysed in the microbiological laboratory, Mae Fah Luang University, Thailand.

**Analysis of Antibacterial Activity**

The essential oil was tested for their antibacterial activities using the disc diffusion technique. *M. luteus*, *S. aureus*, *Ps. aeruginosa* and *E.coli* were testing of the antibacterial activity of *P. amaryllifolious* leaves essential oil. The standard discs containing tetracycline and penicillin were used as positive control while used dichloromethane solvents as negative control.

Paper disc diffusion method: Essential oil of *P. amaryllifolious* leaves was tested for their antibacterial activity by disc diffusion method. To prepare the testing bacteria, a single colony of each bacterial culture was transferred to 3 mL nutrient broth (NB) pH 6.9 (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and incubated for overnight at 37 °C and each bacterial culture was then spread on the surface of the nutrient agar medium (NA) obtaining from 8.0 g/L of NB and 15.0 g/L of agar (Union Science Co. Ltd, Chiang Mai, Thailand) using sterile cotton swab. Subsequently, filter paper discs (6 mm in diameter (Whatman No.1, Maidstone, UK)) were placed on surface of each inoculated plate. The crude extracts were prepared at two fold concentrations (31.25, 62.5, 125, 250, 500, and 1,000 µg/mL). A small amount, 20 µL, of each was then added into a disc plate using a sterile micropipette. These plates were then incubated overnight at 37 °C, but *M. luteus* was incubated at 30 °C. The diameter of the clear zone around each disc plate was measured in mm after incubation and was expressed as the mean value +/- the standard deviation (± SD). This experiment was performed 3 times on each extract and essential oil.

## Results and Discussion

The leaves of *P. amaryllifolius* afforded a yellowish colored essential oil with a percentage yield of 0.002% (w/w) (calculated on a dry weight basis). The identified constituents of the oils are listed in Table 1, demonstrating a variety of constituents. The gas chromatography–mass spectroscopy (GC-MS) analysis of *P. amaryllifolius* leaves essential oil showed the presence of 54 compounds were identified, representing 98.28 % of the total oil composition (see Table 1). It was observed in essential oils that phytol was the major component, with the highest concentration in oil (21.35%), followed by $\alpha$-thujaplicin (18.64%), dodecanol (12.55%), *n*-tetradecanol (8.93%), benzyl acetate (8.08%).This compound has been found as the main component for the aroma and quality of *P. amaryllifolius* leaves essential oils. Benzyl benzoate (3.38%), eugenol (3.22%), $\alpha$-cresol (2.84%), linalool (2.45%), indole (2.14%), *4Z*-decen-1-ol (1.72%), benzyl alcohol (1.55%), polygodial (1.50%), *n*-heptadecane (1.34%) were also found to be minor components of the essential oil of the leaves. Figure 1have been shown for the chromatogram of the essential oil of pandanus leaves. Among the identified compounds, the dominant component; phytol is an acyclic diterpene alcohol which is formed by enzymatic degradation of chlorophyll in plants. Phytol is widely used in the synthesis of vitamin E and K, in the fragrance industry, food and cosmetics industry etc. The use of phytol in human body is essential in activating enzymes that have a positive effects on the production of insulin and enzymes that decrease blood cholesterol Ghazali (2011). Recent reports suggest that phytol has antimicrobial activity, cancer preventive, diuretic, anti-inflammatory (Li & Ho, 2003). An earlier report showed the presence of phytol (42.14%) as major constituent in *P. amaryllifolius* essential oil (Chen & Ge, 2014) and ) and Ghazali (2011) also reported that 2-acetyl-pyrroline as the main

constituents. According to literature data, it was obvious that the chemical composition of plants differs according to developmental stage, extraction method, conditions of the analysis (Kim & Lee, 2004). The chemical composition could be due to several environmental conditions such as climatic, seasonal, geographical and genetic variations (Perry et al., 1999). Moreover, Karousou et al. (2005) noted that plant habitat affected on the content of volatile constituents. In general, the application of essential oils such as the biocide action are depend on the composition of these oils (Chamorro, Zambón, Morales, Sequeira, & Velasco, 2012).

The antibacterial activities of the essential oil were evaluated by the diameter of inhibition and MIC values compared with those obtained from the standard drugs, tetracycline. The antibacterial activities of sample and drugs on Gram-negative and Gram-positive bacteria are summarized in Table 2 respectively, while MIC values of the sample and drugs are shown in Table 3. The essential oil provided various efficiencies of antibacterial activity depending on the tested bacterial strains. The essential oil had the greatest antibacterial activity against all Gram-negative bacteria. The essential oil had the largest inhibition zones against *E. coli* and *M. luteus*, measured at 15.3, 10.7, respectively (see Table 2). These results are confirmed by the MIC values in Table 3. All Gram-negative bacteria were inhibited by the essential oil. It is interesting to note that the essential oil exhibited the strongest inhibitory effects against all Gram-positive bacterial strains. An increased inhibition zone of the essential oil was observed with *E. coli* measured at 15.33 mm (see table 2). The lowest MIC value (31.2 µg/mL) was observed for the drugs, while the essential oil showed higher MIC values at concentrations of 31.25 µg/mL for *E. coli* and *M. luteus*, 62.5 µg/mL for *Ps. aeruginosa* and 125 µg/mL for *S. aureus*, respectively. The essential oil showed antibacterial activity which was more selective for Gram-negative bacterial strains than Gram-positive bacterial strains.

Normally, Gram-positive bacteria are more sensitive to the essential oil than Gram-negative bacteria, which contain lipopolysaccharides in their external walls which limit access to compositions of the essential oil (Burt, 2004). This is the first report on the antibacterial activities of *P. amaryllifolius* leaves in which their antibacterial activities may be attributed to the major components, phytol, $\alpha$-thujaplicin (Aoyagi, Kimura & Murata, 1974). Another major compound, tetradecanol, dodecanol, benzyl acetate and linalool, also have antibacterial properties, as reported by Shahverdi and coworkers at 2004. The antibacterial properties of *P. amaryllifolius* leaves also resulted from various oxygenated monoterpene compounds such as citronellyl formate, eugenol. These components were identified as possessing antibacterial activity in previous reports. It is noted that the antibacterial activities of *P. amaryllifolius* leaves were related significantly to qualitative and quantitative variations in chemical compositions of leaves oil.

The presence of essential oil, i.e. mixtures consisting predominantly of mono- and sesquiterpene derivatives, account for the insect-repellent and attractant properties associated with some aromatic plants (Herout, 1970; Rice, 1983).

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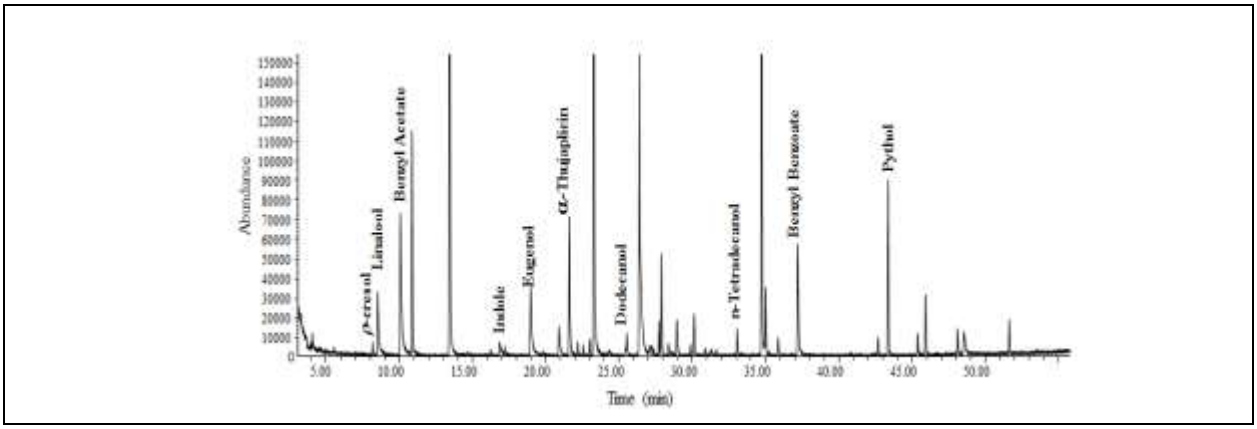



**Figure 1.** GC Chromatogram of essential oils of *P. amaryllifolius* leaves

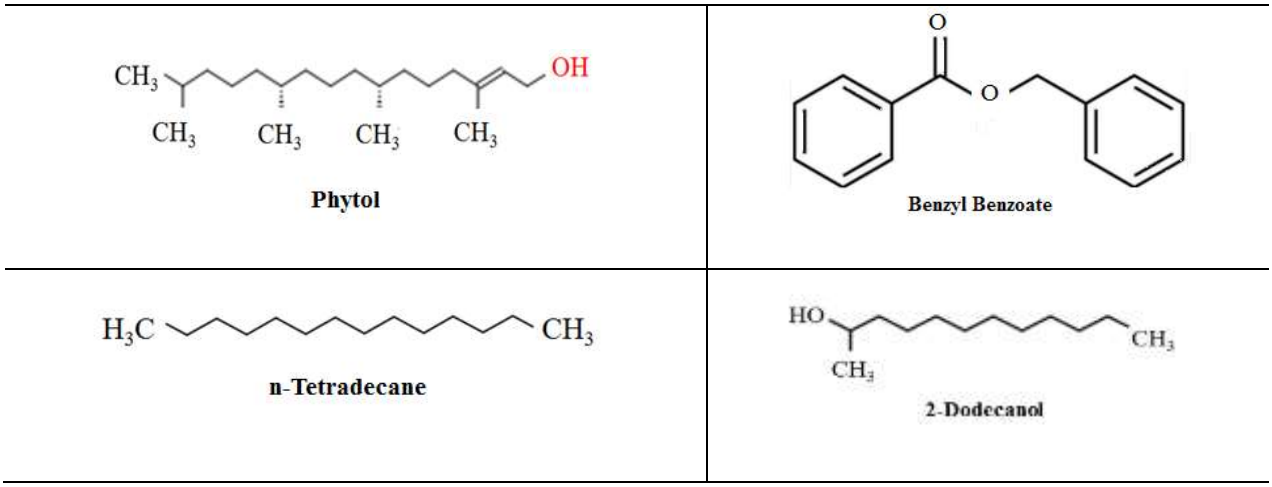
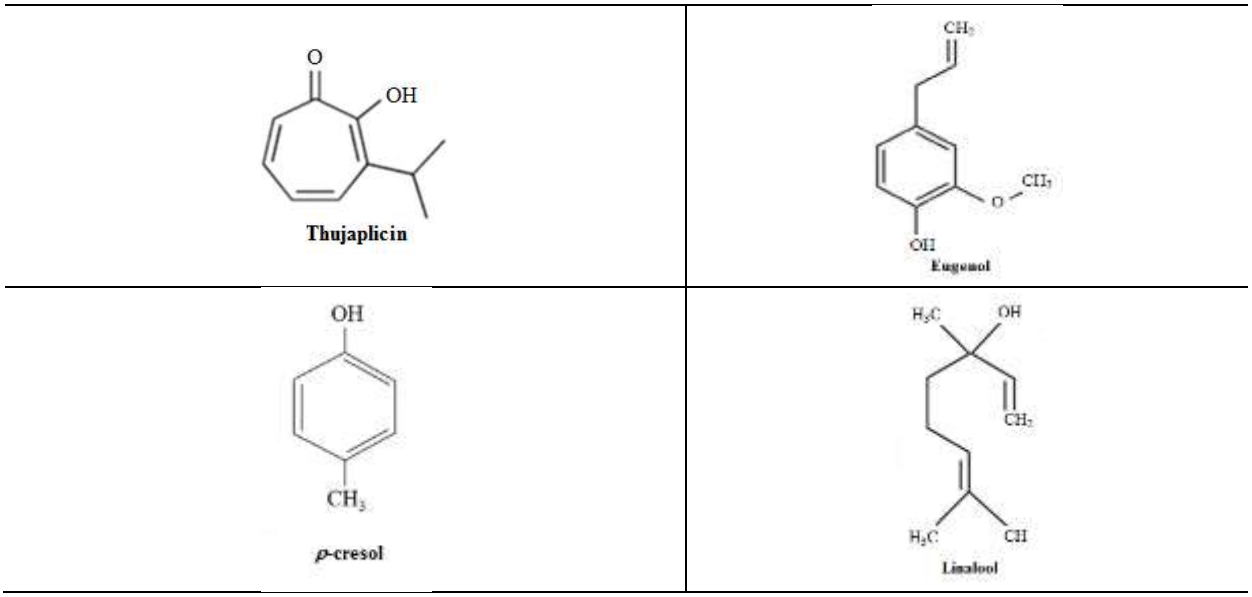
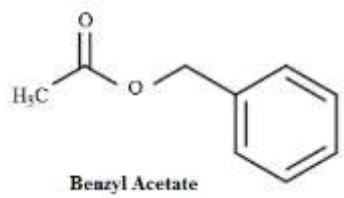



**Figure 2.** The Chemical Structure of Major Components in Essential Oil of *P. amaryllifolius* Leaves





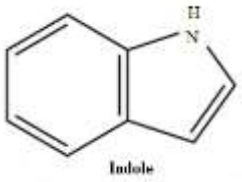




**Figure 2.** The Chemical Structure of Major Components in Essential Oil of *P. amaryllifolius* Leaves (continued)

**Table 1.** Chemical constituents, their relative peak area percentage and retention indices (KI) of essential oil of *Pandanus amaryllifolius* Leaves

| No. | Compound | MW | KI | % content |
|---|---|---|---|---|
| 1 | *n*-Hexanol | 100 | 871 | 0.17 |
| 2 | 5-hydroxy-Pentanal | 102 | 890 | t |
| 3 | 4*Z*-Heptanol-1-ol | 114 | 966 | t |
| 4 | Ethyl-3E- hexenoate | 142 | 1004 | t |
| 5 | 2*E*-Octen-1-ol | 128 | 1066 | t |
| 6 | Benzyl alcohol | 108 | 1031 | 1.55 |
| 7 | $\rho$-Cresol | 108 | 1076 | 2.84 |
| 8 | Linalool | 154 | 1096 | 2.45 |
| 9 | Benzyl acetate | 150 | 1162 | 8.08 |
| 10 | 4*Z*-Decen-1-ol | 156 | 1259 | 1.72 |
| 11 | $\gamma$-Octalactone | 142 | 1254 | t |
| 12 | Ambersage | 184 | 1266 | t |
| 13 | Indole | 117 | 1291 | 2.14 |
| 14 | Z-Isosafrole | 162 | 1339 | t |
| 15 | Dihydroisojasmone | 168 | 1342 | t |
| 16 | $\alpha$-Longipinene | 204 | 1352 | 0.08 |
| 17 | Eugenol | 164 | 1359 | 2.68 |
| 18 | dihydro-Jasmone | 166 | 1380 | t |
| 19 | *E*-Jasmone | 164 | 1391 | t |
| 20 | *E*-$\alpha$-Damascone | 192 | 1393 | t |
| 21 | Dodecanal | 184 | 1408 | t |
| 22 | *Z*-Jasmone | 164 | 1392 | t |
| 23 | $\alpha$-Thujaplicin | 204 | 1412 | 18.64 |
| 24 | *n*-Tetradecane | 204 | 1400 | t |
| 25 | *E*-Caryophyllene | 204 | 1419 | t |
| 26 | Phenyl hexan-3-one<1-> | 220 | 1423 | t |
| 27 | Linaloo butanoate | 224 | 1423 | t |
| 28 | Dodecanol | 186 | 1470 | 12.55 |
| 29 | <*trans*->Cadina-1-6,4-diene | 204 | 1472 | t |
| 30 | Lavandulyl isovalerate | 238 | 1509 | 0.01 |
| 31 | Butyllated hydroxytoluene | 220 | 1515 | t |

| | | | | |
|---|---|---|---|---|
| 32 | $\delta$-Amorphene | 204 | 1512 | t |
| 33 | 1-Phenyl heptan-3-one | 190 | 1526 | t |
| 34 | 4-diene-trans-Cadina | 204 | 1534 | 0.23 |
| 35 | Geranyl butanoate | 224 | 1564 | t |
| 36 | 3Z-Hexenyl benzoate | 204 | 1566 | 0.15 |
| 37 | 1-Hexadecane | 224 | 1589 | t |
| 38 | *Z*-methyl Jasmonate | 224 | 1649 | t |
| 39 | Khusinol | 220 | 1680 | t |
| 40 | n-Tetradecanol | 214 | 11672 | 8.93 |
| No. | Compound | MW | KI | % content |
| 41 | Massoia dodecalactone | 196 | 1686 | t |
| 42 | *n*-Heptadecane | 240 | 1707 | 1.34 |
| 43 | 1-phenyl hepta-1,3,5-triyne | 164 | 1720 | t |
| 44 | Benzyl benzoate | 212 | 1759 | 3.38 |
| 45 | $\rho$-Cresol octanoate | 234 | 1777 | t |
| 46 | Benzyl salicylate | 228 | 1865 | t |
| 47 | Methyl hexadecanoate | 270 | 1921 | 0.02 |
| 48 | Phytol | 296 | 1943 | 21.35 |
| 49 | Isohibaene | 272 | 1934 | t |
| 50 | *n*-Eicosane | 282 | 2000 | 0.03 |
| 51 | Polygodial | 234 | 2017 | 1.5 |
| 52 | Methyl linoleate | 294 | 2095 | 0.07 |
| 53 | *n*-Heneicosane | 296 | 2100 | 0.09 |
| 54 | Sclareolide | 250 | 2066 | 0.03 |
| | **% of Compounds** | | | 90.03% |

KI, Kováts retention indices on DB-5 column, t- trace amount <0.1%.

**Table 2.** Zone of Inhibition produced by essential oil of *P. amaryllifolius* leaves, Dichloromethane and Tetracycline

| **Bacteria** | **Diameter (mm ± SD)** | | |
|---|---|---|---|
| | **Tetracycline** | **Dichloromethane** | **ESS** |
| **$G^-$** | | | |
| *Ps. aeruginosa* (DMST 4739) | 14.3 ± 1.3 | 0.0 ± 0 | 8.0 ± 1.6 |
| *E. coli* (DMST 4212) | 22.3 ± 2.1 | 0.0 ± 0 | 15.3 ± 2.1 |
| **$G^+$** | | | |
| *M. luteus* (TISTR884) | 14.7 ± 1.3 | 0.0 ± 0 | 10.7 ± 0.9 |
| *S. aureus* (DMST 8840) | 27.0 ± 2.9 | 0.0 ± 0 | 8.0 ± 0.8 |

**Table 3.** Minimum Inhibitory Concentration (MIC) of Essential oil of *P. amaryllifolius* leaves on $G^-$ bacteria and $G^+$ bacteria

| | *Ps. aeruginosa* | *E. coli* | *M. luteus* | *S. aureus* |
|---|---|---|---|---|
| **Tetracycline** | 31.25 | 31.25 | 31.25 | 31.25 |
| **ESS** | 62.5 | 31.25 | 31.25 | 125 |

## Conclusions

In summary, the essential oil possesses the important antibacterial potential, especially against Gram-negative bacteria. *P. amaryllifolius* leaves oil is rich in phytol, *α*-thujaplicin, tetradecanol, dodecanol, benzyl acetate, and linalool, which may play important roles as antibacterial compounds. Other minor constituents, such as eugenol, *n*-heptadecane, benzyl benzoate, indole, and benzyl alcohol may also have important antibacterial properties. The results of this work indicate that the essential oil of *P. amaryllifolius* leaves may be considered as an alternative drug for the screening and development of natural bactericides.

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