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COMPARISON OF ANTIBACTERIAL ACTIVITY OF *OCIMUM TENUIFLORUM L.*AND *PLECTRANTHUS AMBOINICUS (LOUR.) SPRENG* AGAINST THE CLINICAL PATHOGENS *STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA* AND *ESCHERICHIA-COLI*

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ABSTRACT

Objective: The aim of this study was to identify and compare the antibacterial activity of *Ocimum tenuiflorum* and Plectranthus *amboinicus* leaves against the human gram positive and gram negative bacteria. **Method:** The research was conducted using the ethanol extract of *Ocimum tenuiflorum* and *Plectranthus amboinicus.* The antimicrobial activity of the extract was determined by using disc diffusion methods on the gram negative and gram positive bacteria. **Results:** By the observation, *P. amboinicus* shows higher antibacterial activity towards *S. aureus, P. aeruginosa and E. coli* at the concentration of 100 mg/mL which is 14mm, 12mm and 10mm respectively. *Ocimum tenuiflorum* also shows antibacterial activity at the concentration of 100 mg/mL *S. aureus*, P. aeruginosa and E. coli which are 12mm, 10mm and 10 mm respectively. Compared to

Ocimum tenuiflorum, P. amboinicus shows higher antibacterial activity. **Conclusion:** There is a significant antibacterial activity of Ocimum tenuiflorum and Plectranthus amboinicus leaves against the selected human gram positive and gram negative bacteria.

Keywords: *Ocimum tenuiflorum, Plectranthus amboinicus*, Antibacterial and Essential oils.

INTRODUCTION

The predominant pathogen which may cause human skin and soft tissue (SSTI) disease are *S. aureus*, *P. aeruginosa*, *E. coli* and *Enterococcus spp.*[1] SSTIs range from mild infections, such as pyoderma, to serious life-threatening infections, such as necrotizing fasciitis.[2] *S. aureus* is the fourth-most-common hospital-acquired pathogen, following with *Escherichia coli, Pseudomonas aeruginosa,* and *Enterococci,* and it accounts for 9% of all nosocomial infections. Most of the *E. coli* are harmless to the human and animal however a certain specific, highly-adapted E. coli strains may lead to variety of diseases.[3] *Pseudomonas aeruginosa* is an opportunistic pathogen which may become the cause for severe systemic infections, particularly in patients with cystic fibrosis, burns, and immunosuppression.[4]

Medicinal plants are being used since ancient time without knowing about its active ingredients. The healing power of certain plant was understood and accepted before mankind discovered the existence of microorganisms. Many developed country and not forgotten our country Malaysia are still using medicinal plant for treatment and disease prevention.[5] Plants are found to be the source of many chemical compounds. The most important compound in plant is alkaloids, terpenoids, steroid phenols, glycosides and tannins. World health organization also describe that plant can be used for synthesis of useful drug and for other therapeutic uses.[6] Even though, there are many useful medicinal plant worldwide people still not aware of its benefits. By using this medicinal plant we can fulfill the demand of our medication supply at low cost.[7] Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine.[8]

According to[5], medicinal plants would be the best source to obtain a variety of natural therapies. It is proven that about 80% of individuals from developed countries are still using traditional medicine and its demand is increasing. Malaysians are still using traditional medicine for prevention and treatment of diseases.[5] Various plants which contain bioactive ingredients and Phyto-chemicals such as phenolic diterpenes, flavonoids, tannins and

phenolic acid have potential to be a source of natural antioxidant and are being used to cure disease and as a pain reliever. There were recorded that plants is placed at the top among the sources of novel drugs with antimicrobial activity.[9] Several studies conducted shows that, in many developing countries, about two thirds of the population rely heavily on traditional practitioners and medicinal plants to meet primary health care needs.[10, 11, 12] The bioactive compounds have been detected for either bacteriostatic or bactericidal property and have very minimum or no toxicity to the host. The arrival of streptomycin and many other antibiotics initiate new researches on large number of plants for antimicrobial and antibiotic which are beneficial to humans.[6]

Ocimum tenuiflorum and *Plectranthus amboiconus* belongs to the family *Lamiaceae.* They consist of many important constituents and widely used for medicinal purposes. It was proven that the essential oils of the herb belonging to *Lamiaceae* family possess biological activity against several bacteria and yeast.[13] The species of genus *Plectranthus* are always cited as antimicrobial agents that can be used to treat several infections. [14] A part from that, research on therapeutic uses of *Ocimum sanctum* has confirmed that it contains hundreds of phytochemicals which possess antioxidant, adaptogenic, immune enhancing properties and antibacterial activity against many pathogens.[9]

Figure 1 Plant of *Ocimum tenuiflorum*

Figure 2 ***Plectranthus amboiconus***

Over the time, health benefits of the available drugs are under threat as it becoming less effective not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. Thus, it became essential to investigate newer drugs with lesser resistance.[15] Plants provide a natural blueprint for the development of new drugs. They can treat many infective diseases with a less side effect and within a low budget.[16] Therefore the objective of this study is to identify and compare the antibacterial activity of *Ocimum tenuiflorum* and *Plectractus amboinicus* leaves against the human gram positive and gram negative bacteria.

METHODOLOGY

Plant collection

Plants were collected from the natural habitat at the area of Petaling Jaya. The plant was identified and authenticated by Dr. Shamsul Khamis from the Institute of Bioscience, Universiti Putra Malaysia. Upon the confirmation of plant genus, the next step of leave preparation was carried out. From the collected plant, the leaves were plucked separately without any mixture of the plants branch and flower.

Sample preparation

The leaves were then washed thoroughly and chopped into small pieces to increase its surface area. The pieces of leaves were then shade dry under a humid condition to protect them from direct sunlight. Once dried it was grounded into powder. 3 separate cleaned and dry separating funnel was taken. 30g of the ground material and 250 mL of dehydrated ethanol

was mixed thoroughly and left for 48 hours. After 48 hours the solution was filtered using standard Whats man filter paper. The filtrate was evaporated using water bath at the temperature of 70°C and the volume of the extract was reduced to 90% of its original volume. The crude was collected into a container using a clean spatula and stored at the cool room to prevent contamination with other microorganisms.[17]

Preparation of standard culture inoculum of test organism

A loopful of isolated colonies of *P. aeruginosa, S. aurues*, and *E. coli* were inoculated into 4 mL peptone water and incubated at 37°C for 4 hours. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 mL of 1.75% (w/v) barium chloride dehydrate with 99.5 mL 1% (v/v) sulphuric acid. This turbidity was equivalent to approximately 1.2×108 colony-forming units per milliliter (cfu/mL). The grown suspension was used for further testing.

Determination of Zone of Inhibition (ZOI)

The antimicrobial activity of the extracts was determined by the Kirby-Bauer agar diffusion method according to NCCLS standards. Mueller Hilton agar plate was used for the antimicrobial activity testing. Mueller Hilton agar was prepared by mixing 28g of Mueller Hilton powder with 1000mL distilled water which was then autoclaved for 4 hours. Under aseptic conditions in the biosafety chamber, 15mL of Nutrient agar medium was dispensed into pre-sterilized Petri dishes to yield a uniform depth of 4 mm and inoculated by the bacteria. The sterile discs (diameter 4mm) were impregnated with different concentration of (30 mg/mL, 50 mg/mL, 100 mg/mL). The freshly prepared inoculum was swabbed all over the surface of the agar plate using the cotton swab. The dried discs were placed on the agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface [18]. Tetracycline was used as positive control. The discs were spaced far enough to avoid reflections wave from the edges of the petri dishes and overlapping rings of inhibition. The agar plate was then incubated for 24 hours at the temperature of 37°C. The diameter of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured and recorded.

Statistical Analysis

The data obtained from different results were analyzed by two-way analysis of variance (ANOVA) procedure using the Statistical Package for the Social Science (SPSS) program (SPSS Statistics 22.0). A statistically significant difference was considered at $p < 0.05$.

RESULTS

Figure 3 shows the antibacterial activity of *Plectranthus amboinicus* toward the clinical pathogens S. aureus. It shows higher zone of inhibition toward the positive control Tetracycline which is 22 mm. Following with the zone of inhibition of 14 mm, 12 mm and 8mm at the concentration of 100 mg/mL, 50 mg/mL and 30 mg/mL of plant extract respectively.

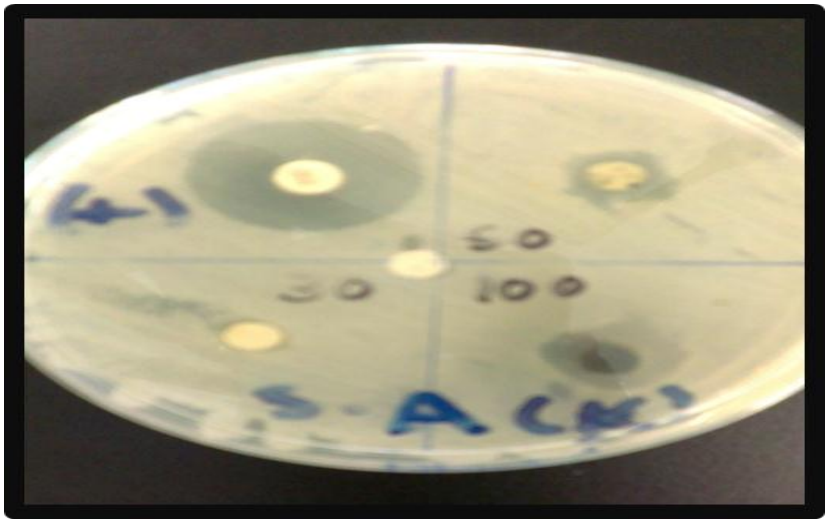

Figure 3 *S. aureus* reaction with *Plectranthus amboinicus*

Figure 4 shows the antibacterial activity of *Ocimum tenuiflorum* toward the S. aureus. It shows higher zone of inhibition toward the positive control Tetracycline which is 22 mm. Following with the zone of inhibition of 12 mm, 8 mm and 6 mm at the concentration of 100 mg/mL, 50 mg/mL and 30 mg/mL of the plant extract respectively.

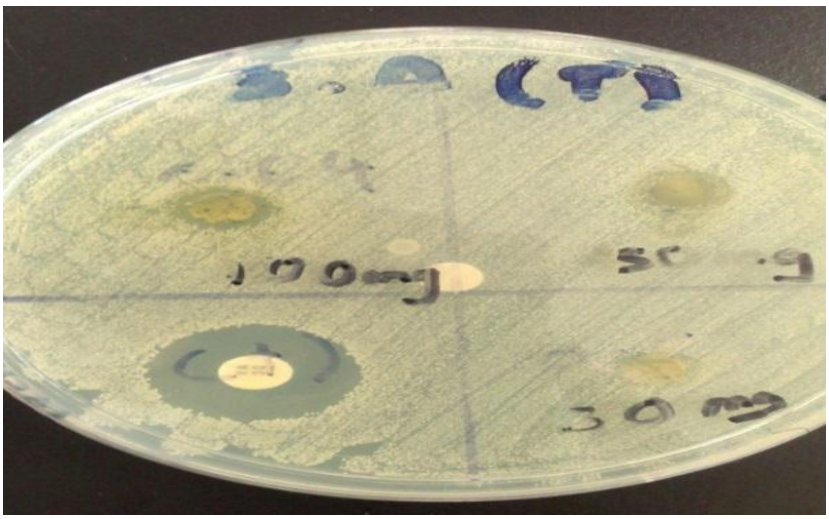

Figure 4 *S. aureus* reaction with *Ocimum tenuiflorum*

Figure 5 shows the antibacterial activity of *Plectranthus amboinicus* toward the clinical pathogen Escherichia coli. E. coli shows higher zone of inhibition toward the positive control Tetracycline which is 24 mm. Following with the zone of inhibition of 10 mm, 6 mm and 6 mm at the concentration of 100 mg/mL, 50 mg/mL and 30 mg/mL of the plant extract respectively.

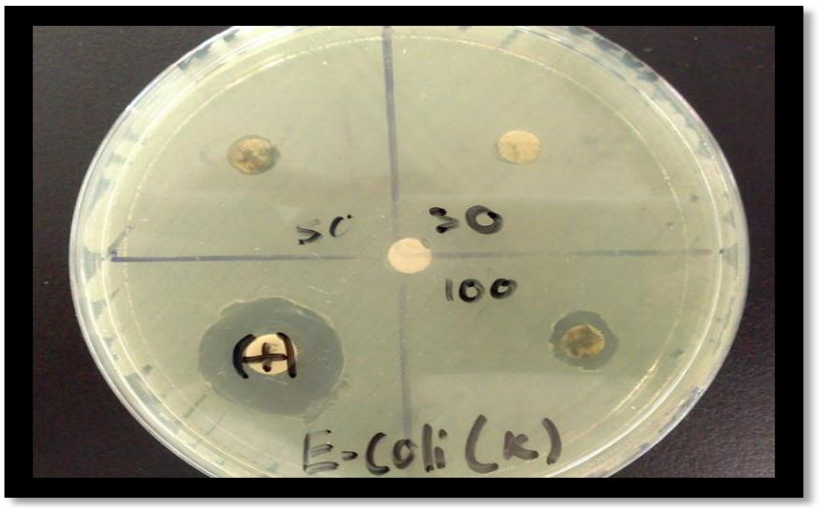

Figure 5 E-coli reaction with *Plectranthus amboinicus*

Figure 6 below shows the antibacterial activity of *Ocimum tenuiflorum* toward the clinical pathogen Escherichia coli. E.coli shows higher zone of inhibition toward the positive control Tetracycline which is 24 mm. Following with the zone of inhibition of 10 mm, 8 mm and 6 mm at the concentration of 100 mg/mL, 50 mg/mL and 30 mg/mL of the plant extract respectively.

Figure 6 *E-coli* reaction with *Ocimum tenuiflorum*

Figure 7 below shows the antibacterial activity of *Plectranthus amboinicus* toward the clinical pathogen *Pseudomonas aeruginosa*. *P. aeruginosa* shows higher zone of inhibition toward the positive control Tetracycline which is 16 mm. Following with the zone of inhibition of 12 mm, 10 mm and 8 mm at the concentration of 100 mg/mL, 50 mg/mL and 30 mg/mL of the plant extract respectively.

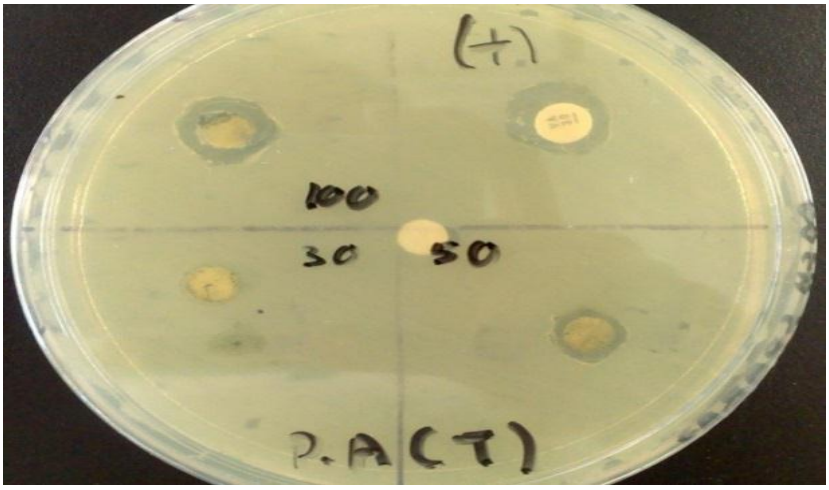

Figure 7 *P. aeroginosa* reaction with *Plectranthus amboinicus*

Figure 8 below shows the antibacterial activity of *Ocimum tenuiflorum* toward the clinical pathogen Pseudomonas aeruginosa. *P. aeruginosa* shows higher zone of inhibition toward the positive control Tetracycline which is 16 mm. Following with the zone of inhibition of 10 mm, 8 mm and 6 mm at the concentration of 100 mg/mL, 50 mg/mL and 30 mg/mL of the plant extract respectively.

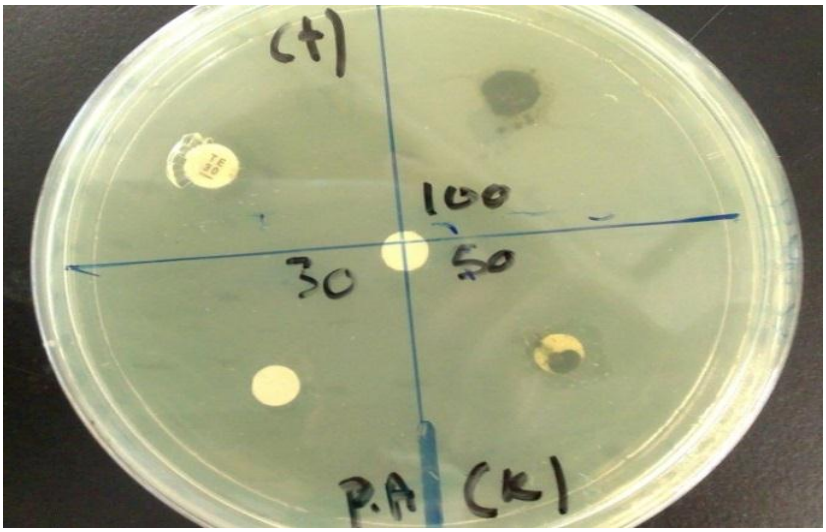

Figure 8 *P. aeroginosa* reaction with *Ocimum tenuiflorum*

Table 1 shows the ethanolic extract of *P. amboinicus* inhibitory effect on *S. aureus* with maximum (zone of inhibition = 14 mm) at concentration of 100 mg/mL. Ethanolic extract of *O. tenuiflorum* also shows inhibitory effect on the *S. aureus* with maximum (zone of inhibition = 12 mm) at the concentration of 100mg/mL. Ethanolic extract of *P. amboinicus* and *O. tenuiflorum* is resistant to *Escherichia coli* with the maximum (Zone of inhibition = 10 mm) at the highest concentration of 100 mg/mL. Ethanolic extract of *P. amboinicus* shows inhibitory effect on *Pseudomonas aeruginosa* with maximum (Zone of inhibition = 12 mm) at concentration of 100 mg/mL. *Ocimum tenuiflorum* is resistant to *Pseudomonas aeuruginosa* with maximum (zone of inhibition = 10mm) at highest concentration of 100 mg/mL.

Table 1 Ethanolic extract of *P. amboinicus* and *O. tenuiflorum*

Plant leave extract	Conc.mg/ml	Zone of Inhibition		
-	-	Pa	Sa	Ec
Plectranthus amboinicus	30 50 100 tetracycline	8 ± 0.2 mm 10 ± 0.1 mm 12 ± 0.3 mm 16 ± 0.5 mm	8 ± 0.3 mm 12 ± 0.5 mm 14 ± 0.5 mm 22 ± 0.9 mm	6 ± 0.3 mm 6 ± 0.2 mm 10 ± 0.6 mm 24 ± 1.3 mm
Ocimum tenuiflorum	30 50 100 tetracycline	6 ± 0.1 mm 8 ± 0.4 mm 10 ± 0.7 mm 16 ± 0.6 mm	6 ± 0.2 mm 8 ± 0.3 mm 12 ± 0.6 mm 22 ± 1.1 mm	6 ± 0.3 mm 8 ± 0.7 mm 10 ± 0.9 mm 24 ± 1.7 mm

Table 2 shows two-way ANOVA without replication test, the F-value is more than the F-critical (F=9.49, 5.29); (F-crit = 3.33, 4.10) respectively. Thus, the result shows that there is a significant different between the antibacterial activity of *O. tenuiflorum* and *P. amboinicus*. $P < 0.05$, thus the null hypothesis is rejected and the alternative hypothesis is accepted.

Table 2 Two-way ANOVA without replication test

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	73.77778	5	14.75556	9.485714	0.001485	3.325835
Columns	16.44444	2	8.222222	5.285714	0.027144	4.102821
Error	15.55556	10	1.555556			
Total	105.7778	17				

Figure 9 shows concentration versus zone of inhibition chart of *Plectranthus amboinicus*. At the concentration of 30 mg/mL the ethanolic extract of *P. amboinicus* show the same level of

inhibitory effect on *P. aeruginosa* and *S. aureus* = 8 mm. At the concentration of 100 mg/mL *P. amboinicus* shows higher inhibitory effect on *S. aureus* followed by *P. aeruginosa* and *E. coli*. At the concentration of 100mg/ml highest inhibition zone is shown on *S, aureus* which is 14 mm while *P. aeruginosa* and *E. coli* has same inhibitory zone of 10 mm. The positive control tetracycline has higher inhibitory effect on *E. coli* followed by *S. aureus* and *P. aeruginosa*.

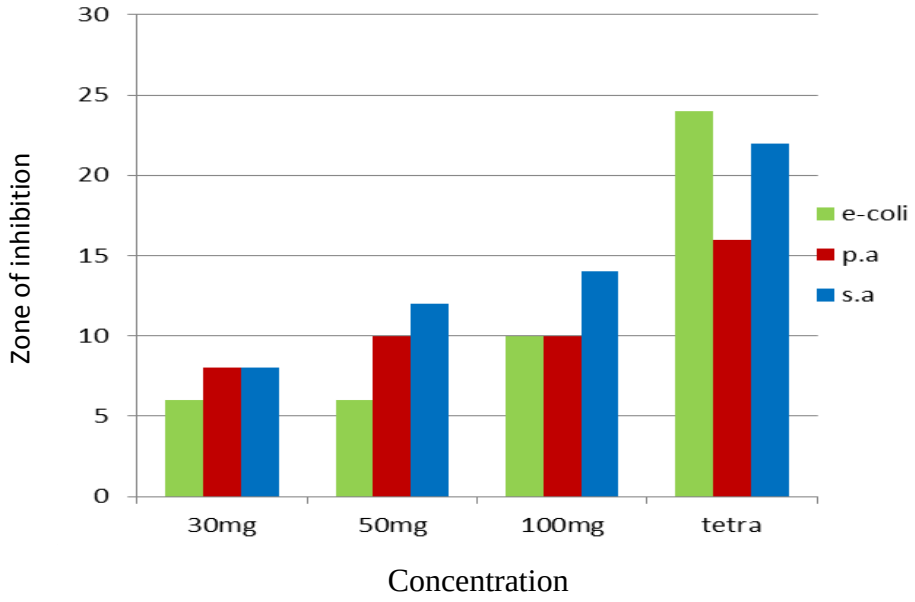

Figure 9 Concentration versus zone of inhibition of *P. amboinicus*

Figure 10 shows concentration versus zone of inhibition chart of *Ocimum tenuiflorum.* At the concentration of 30 mg/mL the ethanolic extract of *O. tenuiflorum* show the same level of inhibitory effect on *P. aeruginosa* and *S. aureus* and *E. coli* = 6 mm. At the concentration of 50 mg/mL O. tenuiflorum also shows same level of inhibitory effect on *S. aureus, P. auruginosa* and *E. coli* = 8 mm. At the concentration of 100 mg/mL highest inhibition zone is shown on *S. aureus* which is 12 mm followed by *P. aeruginosa* and *E. coli.* The positive control tetracycline has higher inhibitory effect on *E. coli* followed by *S. aureus* and *P. aeruginosa*.

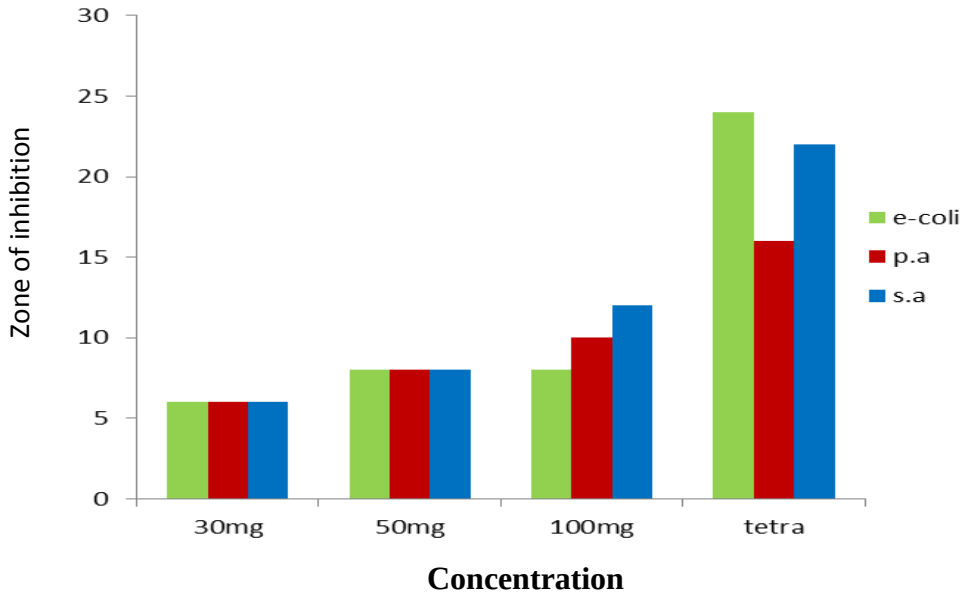

Figure 10 Concentration versus zone of inhibition of *O. tenuiflorum*

DISCUSSIONS

The *in-vitro* the antibacterial activity of the selected two plants ethanolic extracts was tested against the commonly acquired clinical pathogen of *Pseudomanas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The sensitivity test is done to determine the degree of sensitivity or resistance of the selected gram positive and gram negative pathogens toward the antimicrobial drugs.

S. aureus has shown the maximum zone of inhibition of 14 mm at the highest concentration of 100 mg/mL, which proves that *S. aureus* is susceptible toward the ethanolic extract of *Plectranthus amboinicus* which is significant to the research conducted by Ana in 2009.[21] *P. amboinicus* also has shown zone of inhibition on *P. aeruginosa* at the concentration of 100 mg/mL. Since the inhibition zone is up to 12 mm, it shows that *P. aeruginosa* is susceptible toward *Plectranthus amboinicus*. This finding is opposite to the previous research where it was stated that *P. amboinicus* essential oil was found to be active against all pathogenic bacteria except *Pseudomonas aeruginosa*.[19] This may reveal that the ethanolic extract of *P. amboinicus* has higher antimicrobial property compared to the essential oil extracts. However, *E. coli* is concluded to be resistant toward *P. amboinicus* since the maximum inhibition zone is only 10mm even at the highest concentration. In a previous study, the ethanol extract of *P. amboinicus* showed a moderate antibacterial activity toward all the selected gram positive and gram negative bacteria.[20]

Similarly, ethanolic extract of *O. tenuiflorum* also shows inhibitory effect on the *S. aureus* with maximum zone of inhibition 12mm at the highest concentration of 100mg/mL significantly; in a previous study the ethanol extract of *Ocimum sanctum* extract was moderately susceptible towards S. aureus.[21] In the presents study the ethanolic extract of O. tenuiflorum has shown a less antibacterial activity against both *E. coli* and also *P. aeruginosa*. This result is supported by the study conducted by Chirag Mosi in 2012[22], where it was stated that all of the tested gram negative bacteria including *P. aeruginosa* and *E. coli* showed zone of inhibition against ethanolic extract of *O. tenuiflorum*.[22]

The presents study revealed that the ethanolic extract of both *P. amboinicus* and *O. tenuiflorum* has antimicrobial activity against the common clinical pathogen of Pseudomonas aeruginosa, Escherichia coli and *Staphylococcus aureus*. However both plants show higher antibacterial activity in *S. aureus* compared to *P. aeruginosa* and *E. coli*. This proves that the leave extract of P. amboinicus and O. tenuiflorum has higher inhibitory effect on gram positive bacteria (*Staphylococcus aureus*) compared to gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). This finding is supported by previous research studies where it was reported that the plant extract has higher potential to inhibit gram positive bacteria compared to gram negative.[23] Gram negative bacteria are more resistant to plants extract compared to gram positive bacteria similar to the study of.[24] However, studies conducted by Rathnayaka in 2013 reported that *Ocimum tenuiflorum* has higher antibacterial activity toward gram positive bacteria compared to gram negative bacteria.[10] According to Mihaela, Gram positive bacteria have lack of additional permeability barrier compared to gram negative which makes it more susceptible toward the plant extracts.[25]

CONCLUSION

The result of current study proved that there is a significant antibacterial activity of *Ocimum tenuiflorum* and *Plectranthus amboinicus* leaves against the selected human gram positive and gram negative bacteria. However, *Plectranthus amboinicus* posseses higher antibacterial activity toward all selected common clinical pathogen when compared with *O. tenuiflorum* which shows a very less zone of inhibition toward *P. aeruginosa* and *E. coli*. Both plant extract have a higher inhibitory effect towards gram positive bacteria compared to gram negative bacteria.

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CONFLICT OF INTERESTS

Declared None

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