

CORRELATION BETWEEN ANTIBACTERIAL ACTIVITY AND YEAST EXTRACT OF ORTHOSIPHON STAMINEUS EXTRACT

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ABSTRACT

This paper investigates the boosting antibacterial effect of *O. stamineus* extracts supplemented with 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml concentration of yeast extracts. Leaves and stems of *O. stamineus* were extracted with methanol to assess their different antibacterial potential against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* through disc diffusion assay. Post-hoc comparisons using Tukey HSD test indicated the mean zone of inhibition for leaves extract (M = 8.67, SD = 0.58) was different compared to stems extract (M = 10.33, SD = 0.58) towards *S. aureus*. Zone of inhibition produced by the leaves extract (M = 6.67, SD = 0.58) was different compared to the stems extract (M = 7.00, SD = 0.00) towards *E. coli*. *O. stamineus* have no antibacterial potential against *P. aeruginosa*. This study showed the addition of yeast extract have no enhancing or reducing effect towards antibacterial potential. The Pearson correlation coefficient ranging from $r(6) = -0.003$ to -0.594 , $p < 0.212$ to 0.996 .

Keywords: correlation; antibacterial activity; yeast extract; *Orthosiphonstamineus*.

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1. INTRODUCTION

Increase in the number of multiple antibiotic-resistant bacteria and the continuing emphasis on health-care costs, many scientists have researched methods to develop new effective



antibacterial agents that are naturally safe and cost-effective [1]. Antibacterial are extremely valuable tools for the prevention and treatment of infectious diseases and screenings of new metabolites for novel antibiotics [2]. Problems and needs trigger numerous of studies to determine the anti-bacterial properties of plant as there are a lot of plants known to have antimicrobials, antibacterial, anti-inflammatory, antiviral, antioxidant and anticancer properties which linked to the presence of bioactive compounds such as alkaloids, flavonoids, phenolics and steroids [3]. The use of plants for bacterial diseases treatments are widely applied in agriculture, livestock production and human health [4].

Herbs such as *Orthosiphonstamineus*(*O. stamineus*) can be one of the solution to curb these problems. *O. stamineus* is a tropical, medicinal herb belonging to the family Lamiaceae, which is native to South East Asia [5]. It is commonly known as cat's whiskers, kidney tea plant or Java tea was traditionally use as medication for various human disease for centuries which is highly potential to be commercialised as a new natural health products [6]. The differences between molecular structures of chemical or microbes-based pharmaceutical products and the medicinal plants that exhibit antimicrobial and antioxidant activities, suggesting that plant-based may have potential to act as the alternatives [7-8]. In addition, studies also have shown that the *O. stamineus* leaves exhibit wide range of pharmacological properties such as anti-inflammatory, antioxidant and anti-bacterial properties [9]. They also stated that the herb shown to be exceptionally safe with no toxicity in vitro and in vivo, which emphasizes the pharmacological properties investigation of *O. stamineus* that could be the potential novelty source of new natural product curative medicine.

Currently, endophytic fungi are a promising source of bioactive and chemically novel compounds with potential application in medical, agricultural and industrial areas [10]. The natural products from endophytes are reported to have highly diverse chemically and the biological activities exhibited include antibiotics, anticancer, immunosuppressant, antioxidant, anti-diabetic and anti-insecticidal [11]. Some endophytes may contribute to the production of bioactive secondary metabolites by the host plant [12, 21]. Globally, there are at least one million species of endophytic fungi in all plants which including the yeast, that has potential to provide a wide variety of structurally unique, bioactive natural products such as alkaloids, benzopyranones, chinones, flavonoids, phenols, steroids, terpenoids, tetralones, xanthenes and others [13].

Yeast extract consists of the water-soluble portion of autolyzed yeast, which contains an undefined mixture of amino acids, peptides, vitamins and carbohydrates [14]. Yeasts able to produce toxic proteins or glycoproteins that has potential to inhibit several sensitive bacteria

and some of the yeast species. Some of the yeast genus that isolated from different sources have the phenotype that produce antimicrobial compound. The effective of antimicrobial substances produced by yeast are very rapid and can be used in various treatment [15].

2. MATERIALS AND METHODS

2.1. Plant Materials

The healthy and young which naturally planted of *Orthosiphonstamineus* were collected.

2.2. Extraction of *O. Stamineus*

Fresh stems and leaves of the plant were dried in a dry oven for 24 hours at 45°C. The dried sample was weighed in 0.5 g and transferred into 50 ml conical flask to extract the biomass of the sample with soaking in 5 ml of 95% methanol and added with yeast extract powder at concentration of 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml. The flasks then were transferred into incubator shaker at 180 rpm at 25°C for 24 hours. The liquid crude extracts were filtered through membrane pore (0.2 µm) directly into universal bottle after 24 hours of extraction.

2.3. Yeast Extract (YE) Preparation

The yeast extracts were prepared at different concentration; 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml. Yeast powder was weighted and added together with the *O. stamineus* powder into the solvent, methanol according to the concentration needed.

2.4. Test Organisms

The test organisms were used in this study included one gram positive, *Staphylococcus aureus* and two grams negative, *Pseudomonas aeruginosa* and *Escherichia coli*. The bacteria were provided by lab stock of the UniversitiTeknologi MARA, Perlis. The stock bacteria which in broth solution were maintained at 4°C.

2.5. Bacteria Culture Media Preparation

Mueller Hinton agar was prepared by dissolving 36 g of agar powder in 1 L distilled water in a hot plate. The solution then was autoclaved at 121°C for 15 minutes. The suspension was allowed to cool and immediately pour into sterile petri dish and was let for it to solidify in laminar flow chamber at room temperature. The plates with medium were placed upside down in an incubator at 37°C for 24 hours to detect any contamination. Then, the plates were kept at 4°C until further used.

Nutrient agar was prepared by dissolving 23 g of agar powder in 1 L distilled water in a hot plate. The solution then was autoclaved at 121°C for 15 minutes. The suspension was allowed to cool and immediately pour into sterile petri dish and was let for it to solidify. The plates

with medium were placed upside down in an incubator at 37°C for 24 hours to detect any contamination. The plates were kept at 4°C until further used.

Mueller Hinton broth was prepared by dissolving 18 g of agar powder in 1 L distilled water in a hot plate. The solution then was autoclaved at 121°C for 15 minutes. The cap of the bottles was slightly loosening and the bottles were placed in an incubator at 37°C for 24 hours to detect any contamination. Then, the bottles were kept at 4 °C until further uses.

2.6. Bacteria Culture Preparation

A loop full bacteria culture was taken from the stock and streaked the bacteria onto nutrient agar plates to obtain isolate colonies were incubated at 37°C for 24 hours. After 24 hours of incubation, a single colony was picked up by using wire loop and transferred into sterilized culture bottles containing MH broth. The bacteria then were incubated for 16 hours before proceeded to the next steps.

2.7. Disc Diffusion Assay

About 100 µL of bacteria culture was transferred and spread into the MH agar plate After 15 minutes of inoculation, sterile Whatmann no. 1 blank discs impregnated with 200 µL of each treatment plant extracts were placed on the surface of the inoculated medium. The solvent (methanol) was applied as the negative control, whereas 10 µg ampicillins were used as positive control. The plates were incubated in the incubator at 37°C for 24 hours.

2.8. Statistical Analysis

The data collections such as the zone of inhibition of leaves and stems extract of *O. stamineus* without yeast extract and the data of zone of inhibition of leaves and stems extract of *O. stamineus* supplemented with different concentration of yeast extract were recorded. Then, the raw data were analysed by using statistic version 20 software SPSS to express the value in mean ± standard error. Apart from that, One Way Analysis of Variance (ANOVA) and Pearson's correlation coefficient test also were conducted using SPSS to determine the significant of the results obtained.

3. RESULTS AND DISCUSSION

3.1. Antibacterial Activities of Leaves and Stems Extract of *OrthosiphonStamineus*Supplemented with Yeast Extract

The leaves and stems extracts of *O. stamineus*supplemented with different concentration of yeast extract were tested against three different bacteria to determine the effect of each concentrations on the antibacterial activities. Two types of control were used to act as the

positive and negative control. Methanol was used as the negative control and ampicillin was used as the positive control.

Based on Table 1, the negative control did not show any inhibition zone while ampicillin shown inhibition zone ranging from 16.67 ± 0.68 mm to 18.67 ± 0.58 mm towards *S. aureus* and *E. coli*, but no inhibition zone towards *P. aeruginosa*. The leaves and stems extract by addition of yeast extract also exhibit zone of inhibition towards both *S. aureus* and *E. coli*, but none towards *P. aeruginosa*.

Table 1 and Fig. 1 showed the zone of inhibition produced by leaves extract without yeast extract and 4.0 mg/ml concentration of yeast extract towards *S. aureus* are the highest which is 8.67 ± 0.58 mm. Next, leaves extract at concentration of 1.0 mg/ml and 5.0 mg/ml produced inhibition zone of 7.67 ± 0.58 mm and 7.50 ± 0.50 mm respectively, followed by concentration of 3.0 mg/ml by produced 6.50 ± 0.50 mm. The lowest inhibition zone of 6.33 ± 0.58 mm produced by concentration of 2.0 mg/ml.

The results for stems extract, the highest inhibition zone produced was 10.00 ± 1.00 mm without supplemented with yeast extract. Next, stems extract at concentration of 1.0 mg/ml produced 8.33 ± 0.58 mm, followed by concentration of 5.0 mg/ml at 8.00 ± 0.00 mm. At concentration of 4.0 mg/ml, the stems extract produced 7.00 ± 0.00 mm inhibition zone. Lastly, both concentration of 2.0 mg/ml and 3.0 mg/ml produced 6.67 ± 1.15 mm zone of inhibition.

According to [16], *P. aeruginosa* isolated from 270 diabetic foot ulcers were tested with several antibacterial drugs, the disc diffusion results showed that the bacteria is 100% resistance to ampicillin, cefoperazone, erythromycin and norfloxacin. The resistance toward ampicillin generally caused by ineffectiveness of β -lactams in the ampicillin to inhibit the peptidoglycan-assembling transpeptidases located on the outer face of the cytoplasmic membrane to weaken the cell wall of the bacteria [17].

Table 1 and Fig. 2 showed the zone of inhibition produced by leaves extract at concentration of 2.0 mg/ml towards *E. coli* was the highest at 8.33 ± 0.58 mm, followed by both concentration of 1.0 mg/ml and 3.0 mg/ml at 7.67 ± 1.15 mm. Next, leaves extract at concentration of 4.0 mg/ml and 5.0 mg/ml exhibit 7.33 ± 0.58 mm and 7.00 ± 0.50 mm inhibition zone respectively. The lowest inhibition zone produced by leaves extract without yeast extract at 6.67 ± 0.58 mm.

Table 1. The antibacterial activity of leaves and stems extract of *o. stamineus* supplemented with yeast extract

Bacteria	Disc	Zone of Inhibition (mm)						R-Value
		Concentration of Yeast Extract (mg/ml)						
		0.0	1.0	2.0	3.0	4.0	5.0	
S. aureus	LE	8.67±0.58	7.67±0.58	6.33±0.58	6.50±0.50	8.67±	7.50±0.50	-0.142 (0.789)
						0.00		
	SE	10.00±0.00	8.33±0.58	6.67±0.50	6.67±0.50	7.00±0.00	8.00±0.00	-0.594 (0.214)
E. coli	A	16.67±0.58	17.33±0.00	18.00±0.00	16.33±0.00	18.67±0.58	18.33±0.58	
	M	-	-	-	-	-	-	
	LE	6.67±0.58	7.67±0.50	8.33±0.58	7.67±0.50	7.33±0.58	7.00±0.50	-0.003 (0.996)
	SE	7.00±0.00	8.67±0.50	7.00±0.00	6.67±0.58	7.17±0.50	6.00±0.00	-0.596 (0.212)
P. aeruginosa	A	17.67±0.58	17.67±0.50	16.67±0.58	17.67±0.58	17.33±0.58	17.33±0.58	
	M	-	-	-	-	-	-	
	LE	-	-	-	-	-	-	
	SE	-	-	-	-	-	-	
	A	-	-	-	-	-	-	
	M	-	-	-	-	-	-	

Note: Data are expressed as $M \pm SD$; $n = 3$, -no inhibition zone, $r = 0.1$ to 0.3 ; weak, $r = 0.3$ to 0.5 ; intermediate, $r = 0.5$ to 1.0 ; strong correlation, () = significant value, LE-leaves extract, SE-stems extract, A-ampicillin, M-methanol.

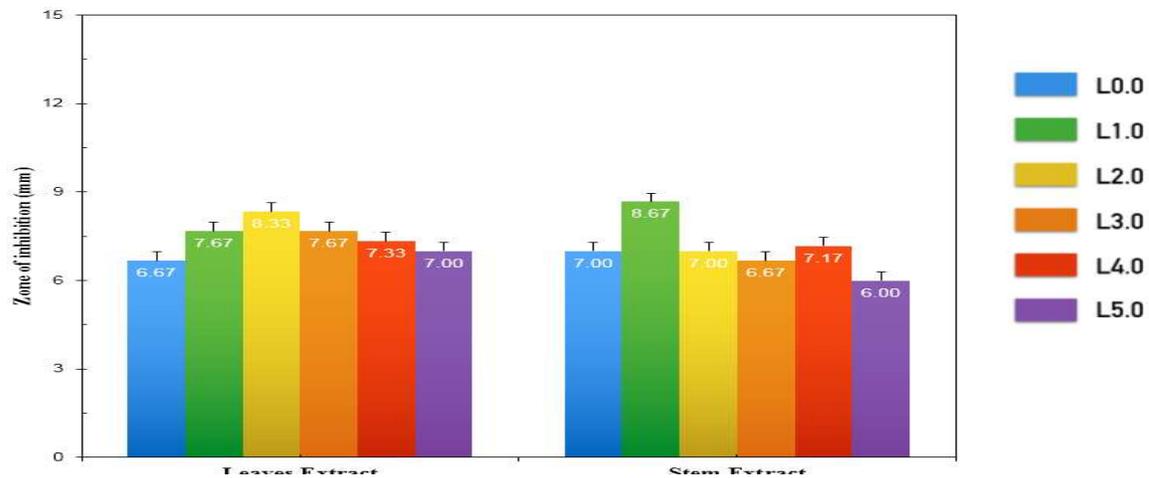


Fig.1. Zone of inhibition produced by leaves (LE) and stems extract (SE) of *O. stamineus* supplemented with different concentration of yeast extract towards *S. aureus* by using disc diffusion assay. L0.0 represent LE and SE supplemented with no yeast extract added, L1.0 represent LE and SE supplemented with 1.0 mg/ml of yeast extract, L2.0 represent LE and SE supplemented with 2.0 mg/ml of yeast extract, L3.0 represent LE and SE supplemented with 3.0 mg/ml of yeast extract, L4.0 represent LE and SE supplemented with 4.0 mg/ml of yeast extract, L5.0 represent LE and SE supplemented with 5.0 mg/ml of yeast extract

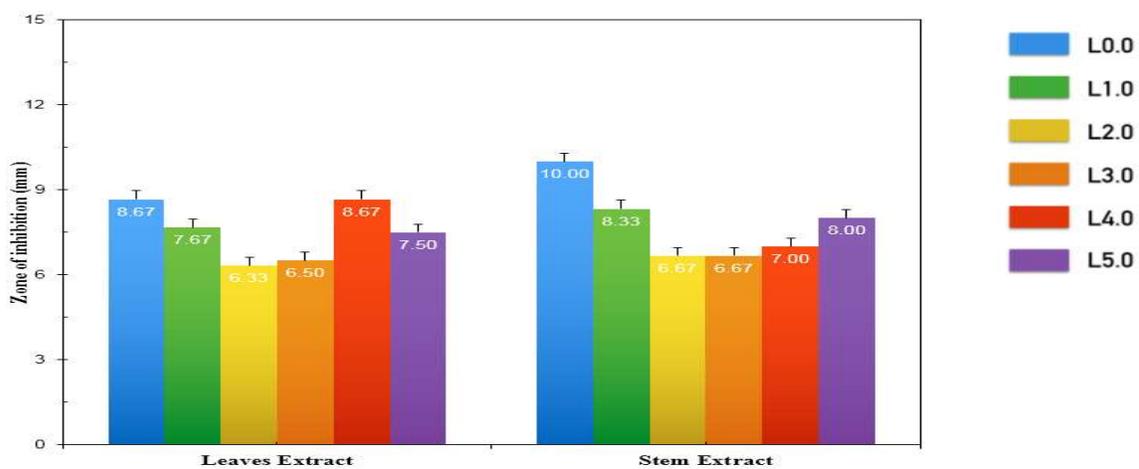


Fig.2. Zone of inhibition produced by leaves (LE) and stems extract (SE) of *O. stamineus* supplemented with different concentration of yeast extract towards *E. coli* by using disc diffusion assay. L0.0 represent LE and SE supplemented with no yeast extract added, L1.0 represent LE and SE supplemented with 1.0 mg/ml of yeast extract, L2.0 represent LE and SE supplemented with 2.0 mg/ml of yeast extract, L3.0 represent LE and SE supplemented with 3.0 mg/ml of yeast extract, L4.0 represent LE and SE supplemented with

4.0 mg/ml of yeast extract, L5.0 represent LE and SE supplemented with 5.0 mg/ml of yeast extract

Based on the results, the highest inhibition zone for stems extract was 8.67 ± 1.53 mm at 1.0 mg/ml concentration, followed by concentration of 4.0 mg/ml which exhibited 7.17 ± 1.04 mm zone of inhibition. At concentration of 2.0 mg/ml and stems extract without yeast extract, both produced 7.00 ± 0.00 mm zone of inhibition. Stems extract at concentration of 3.0 mg/ml resulted 6.67 ± 0.58 mm inhibition zone. Lastly, concentration of 5.0 mg/ml produced the lowest zone of inhibition at 6.00 ± 0.00 mm.

The study conducted by [18], methanolic extract of *O. stamineus* leaves showed no effect towards *P. aeruginosa* using agar well diffusion method. According to [19], they reported that 64.3% of the natural product used yielded larger zones of inhibition growth when well diffusion assay was used compared to disc diffusion assay which concluded in that study that well diffusion assay proved to be more sensitive than the natural products loaded disc diffusion.

Based on the hypothesis made in this study, increasing concentration of yeast extract help the plant extract to boost its antibacterial activity. However, the result obtained, the zone of inhibition produced by the increasing concentration of yeast extract were otherwise.

3.2. Correlation Antibacterial Activity and Yeast Extract

In order to determine whether the increasing concentration of yeast extract truly affect the plant extract the antibacterial activity, Pearson's correlation coefficient test was conducted. The results of the test were demonstrated on Table 1 and the graphs of correlation between zone of inhibition produced against the leaves and stems extract for bacteria *S. aureus* and *E. coli* were plotted in Fig. 3 and Fig. 4 respectively.

Based on the Table 1 and Fig. 3, the Pearson's correlation coefficient for the zone of inhibition produced by the increasing concentration of yeast extract supplemented into leaves extract of *O. stamineus* ranging from 0.0 mg/ml to 5.0 mg/ml for bacteria *S. aureus* is an insignificant weak negative correlation: $r(6) = -0.142$, $p < 0.789$.

For the zone of inhibition produced by the stems extract supplemented with increasing concentration of yeast extract for bacteria *S. aureus*, the Pearson's correlation coefficient resulted an insignificant slightly strong negative correlation: $r(6) = -0.594$, $p < 0.214$.

Based on the Table 1 and Fig. 4, the Pearson's correlation coefficient for the zone of inhibition produced by the increasing concentration of yeast extract supplemented into leaf extract of *O. stamineus* ranging from 0.0 mg/ml to 5.0 mg/ml for bacteria *E. coli* is an insignificant weak negative correlation: $r(6) = -0.003$, $p < 0.996$.

Next, for the zone of inhibition produced by the stems extract supplemented with increasing concentration of yeast extract for bacteria *E. coli*, the Pearson's correlation coefficient resulted an insignificant slightly strong negative correlation: $r(6) = -0.596$, $p < 0.212$.

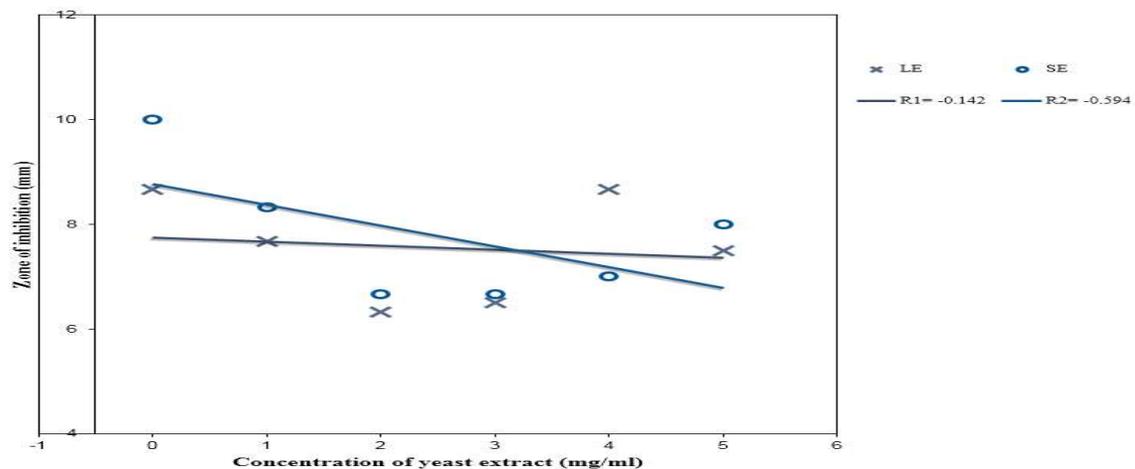


Fig.3. Zone of inhibition produced by leaves and stems extract of *O. stamineus* supplemented with increasing concentration of yeast extract towards *S. aureus* by using disc diffusion assay.

LE represents leaves extract, R1 represent r-value for leave extract, SE represents stems extract, R2 represent r-value for stems extract

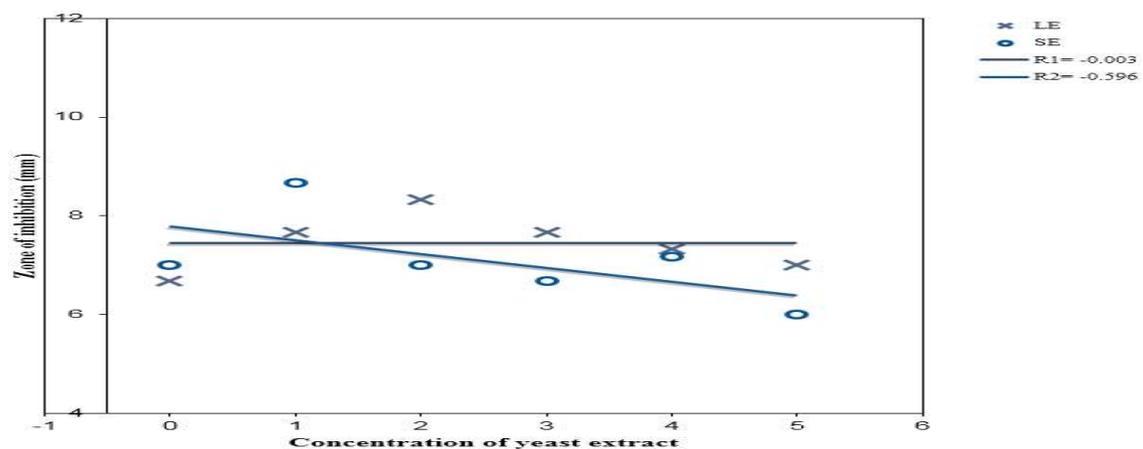


Fig.4. Zone of inhibition produced by leaves and stems extract of *O. stamineus* supplemented with increasing concentration of yeast extract towards *E. coli* by using disc diffusion assay. LE

represents leaves extract, R1 represent r-value for leave extract, SE represents stems extract,
R2 represent r-value for stems extract

Based on Pearson's correlation coefficient test conducted, both leaves and yeast extract shown negative correlation to both bacteria. This indicates that the addition of yeast extract to the plant extract, give the antagonistic effect. However, the $p > 0.05$ showed the correlations are insignificant. Although the antagonistic effect is not relevant, nevertheless several reasons can be deduced from this experiment.

According to [17], specific strain of *Saccharomyces cerevisiae* able to inhibit the growth of bacteria and molds as it acts as the bacteriocins as it secretes polypeptide toxin protein that can target sensitive cells and kills it without direct cell to cell contact. Based on the study by [18], fruit yoghurt isolates yeast shown the best antimicrobial activity with 35mm inhibition zone against *Pseudomonas aeruginosa*, 8mm inhibition zone against *Staphylococcus aureus* and 10mm inhibition zone against *Escherichia coli*.

In this study, Bacto yeast extract (Manufacturer; R&M chemicals) was used. Bacto yeast extract was manufactured from selected strains of *Saccharomyces cerevisiae*. However, the Bacto yeast extract purpose for mass cultivation of various microorganism as it contained the nutritive values, amino acids, vitamins, B group and growth factors. Bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 cultural response towards the Bacto yeast extract are luxuriant, which means the Bacto yeast extract able to promotes the growth of the organism. Therefore, this fact can be related with the antagonistic effect of the yeast extract towards *Orthosiphonstamineus* extract as it reduces the plant antimicrobial potential by slightly promote the growth of the test bacteria in this study.

In addition, the negative correlation of the plant antibacterial potential might cause from the defect of extraction method. Extraction method used in this experiment is soaking method or maceration by using solvent methanol. It is a solvent extortion using solid-liquid extraction. In solid-liquid extraction, the plant material is placed in contact with a solvent. The whole process is dynamic. At first contact between the solvent and the solid which is the plant powder containing the cells, the solvent has to diffuse into cells, in the following step it has to solubilize the metabolites and finally it has to diffuse out of the cells enriched in the extracted metabolites [20]. Bacto yeast extract is free soluble in distilled water or purified water.

However, since the solvent used in this experiment is methanol which is alcohol, Bacto yeast extract is insoluble in alcohol. Hence, there is no extraction occur between the solvent methanol and the solid yeast extract powder. Besides, the presence of yeast powder reduce the the contact surface of the *O. stamineus* powder towards the solvent methanol.

4. CONCLUSION

In conclusion, methanolic extracts of both part leaves and stems of *O. stamineus* share the same antibacterial potential towards *S. aureus* and *E. coli* as the Post-hoc comparisons using Turkey test indicated that mean of zone of inhibition produced was insignificant at $p < 0.05$ level. The leaves and stems extract of *O. stamineus* have promising potential to be develop as an alternative organic antimicrobial drug to inhibit the growth of *S. aureus* and *E. coli* as the results from the Tukey test significant differences $p < 0.01$ between antibacterial potential compared to the ampicillin.

Furthermore, there is no significant correlation on increasing the concentration of yeast extract added into the leaves and stems extract of *O. stamineus* towards antibacterial effect to inhibit the growth of *S. aureus* and *E. coli*. Hence, the addition of yeast extract show no significant effect on increasing antibacterial potential of *O. stamineus* extract.

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