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Qualitative and quantitative biological studies of three chemotypes of basil essential oils grown in Malaysia against *E.coli*, *S.aureus* and *P. aeruginosa*

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ABSTRACT

Different accessions of basil introduced to Malaysia as seeds and cultivated at University Malaysia Pahang (UMP) farm and their essential oils extracted by steam distillation. Three chemotypes of these essential oils with estragole, linalool and methyl cinnamate as dominant compounds were evaluated against three facultative anaerobic bacteria obtained from the National Pharmaceutical Control Bureau, Ministry of Health Malaysia: namely, *Escherichia coli, Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) by qualitative and quantitative method. Different concentration ranged from 10 µL/mL to 1000 µL/mL of basil essential oil solutions prepared and tested against bacterial strain using agar well diffusion and quantitative methods. Minimum inhibitory concentration (MIC) was performed using broth microdilution plate. Eight different concentrations of serial two-fold dilutions ranged between 250 µL/mL and 1.95 µL/mL performed using Magellan software of Tecan Infinite Series M200 Pro microplate reader. In the result of agar well diffusion test, the zone of inhibition increased as the concentration of essential oil increased. The MIC was 7.81 µL/mL for all chemotype of the oils against *E.coli* and *S.aureus* while 15.63 µL/mL for *P.aeruginosa* of linalool-rich chemotype and 31.25 µL/mL for estragole and methyl cinnamate-rich chemotypes. The results obtained in this study were considered encouraging the potential of basil essential oil for medicinal uses as antibiotics and hygienic purposes as antibacterial products.

| Essential oil| Basil (*Ocimum basilicum* L.) | minimum inhibitory concentration | *Esherichia coli* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* |

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1. INTRODUCTION

A natural product has been used since time immemorial not only as food flavors but also to treat diseases in ancient time. In recent years, there is a global attentions towards the medicinal plants and a lot of evidence has shows the promising potential of these plants which can be used in various traditional, complementary and alternate systems of treatment of human diseases [1]. Volatile oil of several plants has showed an increment in global market as antibacterial [2;3;4;5], antivirals [6], antifungals [7] and insecticides [8]. Essential oils or some of their components are used commercially in pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries [9].

An example of aromatic plant has shown an interesting properties as antimicrobial is basil. Basil (*O. basilicum L.*) is recognized as genus of *Ocimum* and includes about 50 to 150 species from different parts in the world and widely used as culinary herbs [10]. Basil oil has strong ability as insect repellent [11], antifungal [12] and nematocidal. Essential oil of basil was also known for their biological properties as antibacterial. Authors in 2009 reported on antibacterial properties of six Sudanese basil chemotypes, and all showed strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* and LD_{50} calculated was varied between 40 a nd 325 µ L [13]. Research in 2009 on antibacterial activity of essential oils obtained from different seasons showed significant results on certain types of microorganisms (*S.aureus, E.coli, B.subtilis, P.multocida, A.niger, M.mucedo, F.solani, B.theobromae and R.solani*) [14]. An antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania indicates that the oil of *O.suave (B)* has strongest antibacterial activity, followed by *O.suave (A)*, *O.kilimandscharicum* and *O.lamiifolium* were moderately active while *O.basilicum* was weakly active which tested to eight bacterial strains and three fungi [15]. Different basil essential oils chemotypes from Togo revealed that only the methyleugenol and methyleugenol/t-anethole chemotypes were active against tested fungi and bacteria where their MIC of tested bacteria varied from 200-400 µL/L and from 250-500 µL/L respectively [16]. Based on report in 2010, *E.coli* 0157:H7 was inhibited by *O.basilicum* 'Genovese' essential oil, while *Ocimum americanum* and *Ocimum x citriodorum* essential oils were the most effective against *Enterococcus faecalis*, *Enterococcus faecium*, *P. vulgaris*, *S.aureus* and *S.epidermis* [17]. This study was carried out to reveal the antibacterial activity of three chemotypes of basil growing in Malaysia against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

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2. EXPERIMENTAL

2.1 Plant Materials

The commercial seeds of *Ocimum basilicum* accessions obtained from Germany, England, United of Arab Emirates and Sudan and cultivated at Universiti Malaysia Pahang farm on 20 April 2010. Fresh leaves of all basils were collected during flowering stage. The leaves were cut into pieces and subjected to steam distillation. The oil was kept at 4 °C until analyzed or for further studies.

2.2 Preparation of Test Microorganisms

Two Gram-negative (*E.coli* and *P.aeruginosa*) and one Gram-positive (*S.aureus*) bacteria were obtained from the National Pharmaceutical Control Bureau, Ministry of Health Malaysia and cultured overnight at 37 °C on MHA agar. The bacterial isolates from the agar plate cultured before were first grown in nutrient broth for 24 hours before use and was standardized to 0.5 McFarland.

2.3 Qualitative Antibacterial Activity

The antibacterial efficacy of different major constituents of basil oil was tested against 3 strains by agar well diffusion method. Briefly, 24 hours old broth cultures of test bacteria were swabbed on sterile MHA agar plates using sterilize cotton swab. Then, 6mm wells were punched onto the agar using sterilized cork borer. Six different concentrations (10 µL/mL – 1000 µL/mL) were tested against these bacteria by carefully added to the respectively labeled well at fixed volume of 50µL per well. The plates was incubated at 37°C for 24 hours in upright position and the diameter of zone of inhibition was measured in millimeters and recorded. Tetracycline and ampicilin was used as positive control while DMSO used as negative control. The experiment was carried out triplicates and subjected to one-way ANOVA analysis.

2.4 Minimum Inhibitory Concentration (MIC)

Essential oils of linalool, estragole and methyl cinnamate sample (250 µL) were dissolved in DMSO. Later, serial of two fold dilutions was performed in a range from 1.95 µL – 250 µL in DMSO. Bacterial inoculums (200 µL) were dispensed into each well (already adjusted to approximate 0.5 McFarland) using transparent Nunc. 96 well plates. The three first well contained 200 µL bacterial inoculate which was used as positive control and another three well contained 200 µL broth as blank. The plates were covered with sterilized plate sealers. The microliter plate was incubated at 37 °C for 24 hours with 20 rpm shaking. MIC reading was taken using Biorad Infinite Series 200 Pro using Magellan software at the respective absorbance (Abs) 600 nm. Tetracycline was used as positive control. The MIC are defines as the lowest concentration of essential oil at which the microorganism does not demonstrate visible growth.

3. RESULTS & DISCUSSION

3.1 Chemotypes of Malaysian-growing Accessions of Basil (*O. basilicum L.*).

Three chemotypes of basil growing in Malaysia were classified according to the major constituents present in the essential oil is the dominant proportions and if exceeding 18% of the oil composition. Malaysian-growing basils was classified into three distinct chemotypes or groups; namely, estragole -, linalool - and methyl cinnamate –rich as shown in Table 1. Authors in 2009 reported that several types of basil was classified according to major constituents in the essential oil contents, for instance, the researchers in their classification on Sudanese basils, they based according to the major essential oil constituents present of the essential oil is the dominant proportions exceeding 50 % of the oil composition or the first two major constituents created the group, where the dominant compound named first [18].

Table 1: Top three components in all basil accessions cultivated in UMP

Accession No.	First component	Second component	Third component
1	Estragol (20.44%)	Linalool (17.12%)	Geranial (7.99%)
2*	Linalool (17.49%)	Eugenol (17.40%)	M.eugenol (13.06%)
3	Linalool (39.79%)	M.cinnamate (25.24%)	τ-cadinol (5.35%)
4	Estragol (33.30%)	Camphor (18.13%)	Eucalyptol (9.56%)
5	Linalool (27.60%)	Geraniol (22.27%)	Eucalyptol (13.45%)
6	M.cinnamate (39.52%)	Linalool (22.49%)	Eucalyptol (11.63%)
7	M.cinnamate (22.93%)	Linalool (22.25%)	Geraniol (15.86%)
8	M.cinnamate (34.09%)	Linalool (20.76%)	Eucalyptol (9.76%)
9	Linalool (27.16%)	M.cinnamate (24.20%)	Eucalyptol (9.28%)
10	Linalool (40.02%)	Eucalyptol (14.42%)	Geraniol (11.33%)

*Note: the ratio of the first component of accession No.2 (England origin) is 17.49, we consider as 18%, therefore, this accession belong to Linalool group

3.2 Qualitative and Quantitative Studies of Basil Oil

A broad variation in the antibacterial properties of investigated essential oils was observed and the results indicated in the chart represent zone of inhibition including the 6 mm well punched. The three chemotypes tested against *E.coli*, *S.aureus* and *P.aeruginosa* were shown in Figure 1A (Estragole), Figure 1B (Linalool) and Figure 1C (Methyl cinnamate), while Figure 1D and Figure 1E showed tetracycline and ampicilin respectively, that used as positive control in this study. Both chemotypes and antibiotics revealed that increasing of the concentration were also increase in their zone of inhibition significantly ($p<0.05$). Estragole-rich, linalool-rich and methyl cinnamate-rich was

found to inhibits *S.aureus* highest followed by *E.coli* and *P.aeruginosa* as lowest inhibition made by these three chemotypes while tetracycline and ampicilin showed moderately active against *E.coli* and *S.aureus*. Zone of inhibition of bacteria tested was found to be significantly low at concentration of 10 µL/mL. These three strains were resistant when test with DMSO which has been used as negative control in this study. The graph illustrated below showed the zone of inhibition in millimeters versus the concentration of the sample used in this study.

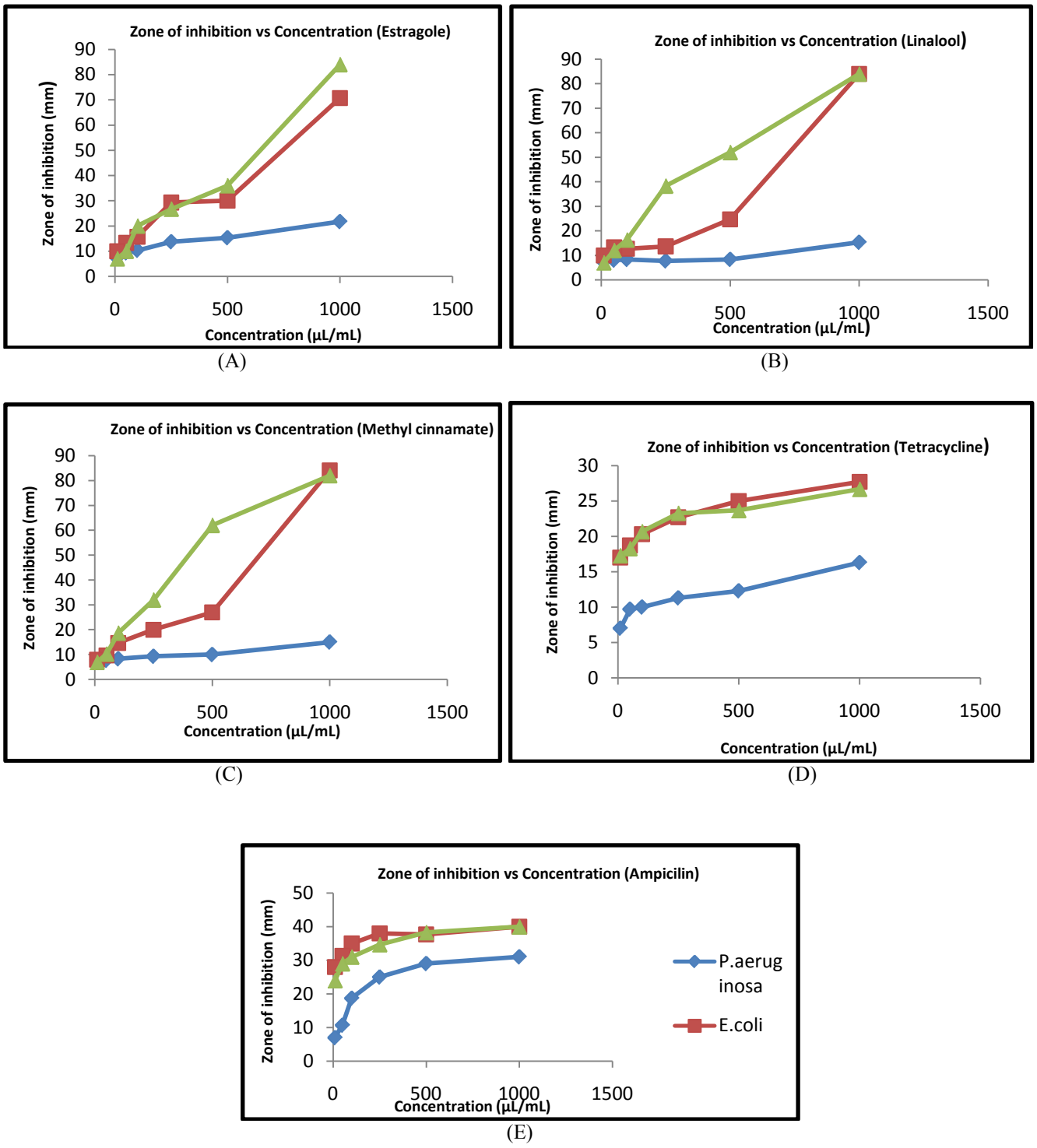


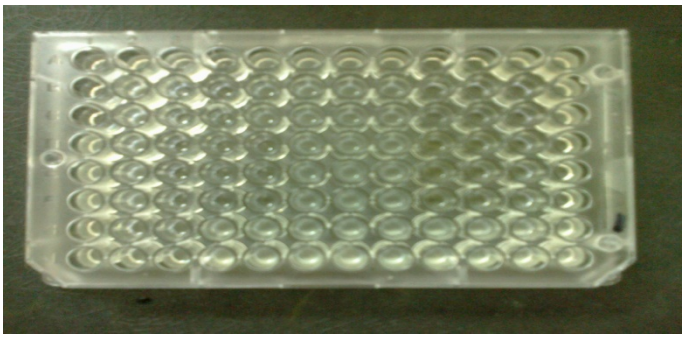
Fig. 1 (A-E) Zone of inhibition of three chemotypes and antibiotics against *P.aeruginosa*, *E.coli* and *S.aureus*

Table 2 MIC of estragole, linalool, methyl cinnamate and tetracycline measured in Abs using microplate reader at 600 nm.

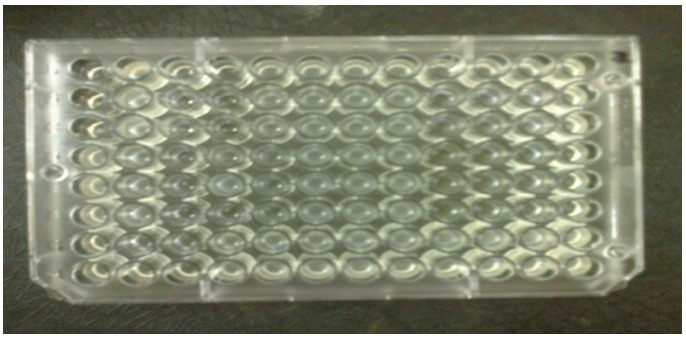
Bacteria	**Estragole (µL/mL)**								
	Blank	**250**	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**
E.coli	0.3988	0.2111	0.1273	0.1996	0.2274	0.2355	0.2793	0.4238	0.4209
S.aureus	0.5099	0.2063	0.1783	0.2520	0.2837	0.3189	0.3376	0.5171	0.5318
P.aeruginosa	0.5279	0.2854	0.3077	0.3897	0.3930	0.5260	0.5323	0.5972	0.6011
Bacteria	**Linalool (µL/mL)**								
	Blank	**250**	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**
E.coli	0.3988	0.1136	0.09453	0.1467	0.2533	0.3063	0.2834	0.4712	0.4566
S.aureus	0.5099	0.1437	0.1983	0.2681	0.3658	0.3798	0.2938	0.5084	0.5111
P.aeruginosa	0.5279	0.3531	0.2651	0.2868	0.3941	0.4391	0.5407	0.7447	0.7082
Bacteria	**Methyl cinnamate (µL/mL)**								
	Blank	**250**	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**
E.coli	0.3988	0.0916	0.1424	0.1527	0.2036	0.2599	0.3041	0.4462	0.4568
S.aureus	0.5099	0.1504	0.1831	0.1382	0.2335	0.2859	0.3279	0.5181	0.5382
P.aeruginosa	0.5279	0.2224	0.3016	0.3730	0.3685	0.5239	0.5603	0.5446	0.5629
Bacteria	**Tetracycline (µL/mL)**								
	Blank	**250**	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**
E.coli	0.3988	0.1266	0.1573	0.1819	0.2078	0.1962	0.2054	0.3718	0.4192
S.aureus	0.5099	0.1653	0.1999	0.2258	0.2373	0.2176	0.3583	0.4961	0.5583
P.aeruginosa	0.5279	0.1081	0.1647	0.2671	0.4040	0.4123	0.4758	0.6704	0.9870

As report in 2009, from five chemotypes of basil oil from Togo established, the estragole type, the linalool/estragole type and the t-anethole type was found ineffective on all fungal strain tested while methyleugenol and methyleugenol/t-anethole type was indicative their potential as possible active ingredients for use in the formulation of deodorants because their effectiveness of inhibiting feet microflora bacterial strains. Findings of Koba study, indicates that estragole and t-anethole, are much likely preserved as natural pesticides rather than used as natural fungicidal [16] but for Malaysian-growing basil oil chemotypes, an interesting antibacterial properties can be seen through the result obtained.

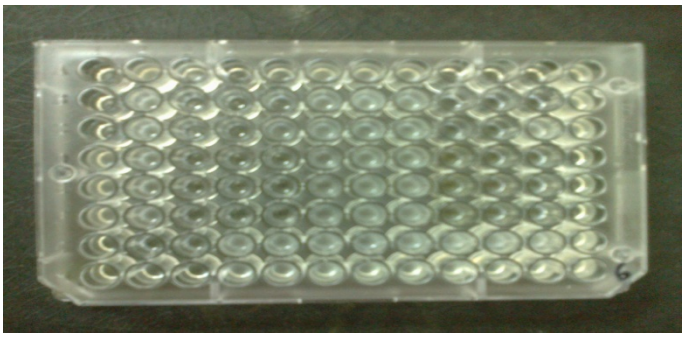
Table 2 shows serial two-fold dilutions ranged between 250 µL/mL and 1.95 µL/mL was tested against the three bacteria using 96 well microplate methods. For *Escherichia coli* and *Staphylococcus aureus*, the MIC values were found to be 7.81 µL/mL for all the three chemotypes while *Pseudomonas aeruginosa* has found to be 15.63 µL/mL as MIC value for linalool-rich and 31.25 µL/mL as minimum inhibitory concentration for estragole-rich and methyl cinnamate-rich. Figure 2 shows the visible results of MIC test of *E.coli*, *S.aureus* and *P.aeruginosa*.



(A)



(B)



(C)

Fig. 2 (A-C) MIC visible results of three chemotypes tested again: (A) *E.coli*, (B) *S.aureus* and (C) *P.aeruginosa*

4. CONCLUSION

In conclusion, the prospective investigation of the evaluation of antibacterial properties of Malaysian-growing basil oil chemotypes is quite a typical applied research and the ultimate goal of this study is to help protect plant biodiversity and validate scientific basis the potential in agricultural industries.

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