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RESEARCH ARTICLE

Antibacterial, Antioxidant, and Phytochemical Screening of Essential Oil

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ABSTRACT

Essential oils (EOs) are liquid mixtures of volatile compounds obtained from aromatic plants. Many EOs have antioxidant properties, and the use of EO as natural antioxidants is a field of growing interest. In the present study was also undertaken with the four different EO, namely, Peppermint oil, Patchouli oil, Frankincense oil, and Ylang Ylang oil were collected from local market. To procure the five pathogens, *namely, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis,* and *Escherichia coli* from the Department of Medical Microbiology, RMMCH, Annamalai University. To study the antibacterial activity of four EO against certain pathogenic bacteria by agar well diffusion method. Among the four tested EO ylang ylang EOs exhibited maximum Zone of inhibition against all the tested human pathogens. Based on the maximum antibacterial activity of ylang ylang oil was further subjected to the phytochemical screening and Antioxidant activity.

Keywords: Antibacterial, clinical pathogens, essential oil, phytochemical and DPPH

INTRODUCTION

Essential oils (EOs) are concentrated natural extracts derived from plants, which were proved to be good sources of bioactive compounds with antioxidative and antimicrobial properties (Man *et al*., 2019)[1]. EOs produced by plants have been traditionally used for respiratory tract infections and are used nowadays as ethical medicines for colds. In the medicinal field, inhalation therapy of EOs has been used to treat acute and chronic bronchitis and acute sinusitis. Inhalation of vapor of EOs augmented the output of respiratory tract fluid, 3 maintained the ventilation and drainage of the sinuses, 4 had an anti-inflammatory effect on thetrachea5 and reduced asthma (Inouye *et al*., 2001)[2].

An important role of EOs in nature is protection of plants by acting as antifungal, antibacterial, antiviral, and insecticidal agents and also protection against herbivores by reducing appetite of herbivores for plants with such properties. Health and Human Services Public Health Services have recognized EOs as safe substances and some EOs contain compounds that can be used as antibacterial additives (Stefanakis *et al*., 2013)[3].

Mentha piperita L., a medicinally important plant belongs to the Family *Lamiaceae* (African pharmacopoeia, 1985; The Wealth of India, 1962), it was cultivated by the ancient Egyptians and documented in the Icelandic pharmacopoeia of the 13th century. It is widely grown in temperate areas of the world, particularly in Europe, North America, and North Africa but nowadays cultivated throughout all regions of the world (Singh *et al*., 2015)[4] Kalp peppermint EO is steam distilled from Peppermint plants. Peppermint EO can be used for health and beauty purpose. Peppermint EO has antiviral antibacterial anti-inflammatory, antispasmodic and carminative properties.

Patchouli EO can be attributed as an anti-depressant, antiphlogistic, antiseptic, astringent, deodorant, antifungal, and tonic substance. Patchouli is an important herb which possesses many therapeutic properties and is widely used in

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the fragrance industries (Yang *et al*., 2013)[5]. In traditional medicinal practices, it is used to treat colds, headaches, fever, nausea, vomiting, diarrhea, abdominal pain, insect, and snake bites.

Frankincense oil mostly used in aroma therapy. This oil also used to make many perfumes. The *Yersinia pestis* outbreak known as "The Great Plague" became widespread and was responsible for killing one-third of the European population (Perry *et al*., 1997)[6]. It was said that people exposed to EOs were "immune" to deleterious effects of this bacteria (Chun *et al*., 2016)[7]. This traditional medicine of the east is believed to have anti-inflammatory, expectorant, antiseptic, and even anxiolytic and anti-neurotic effects.

Ylang Ylang is highly popular among the Common wealth of the Northern Marianas Islands (CNMI) and is frequently used in local Medicine. This can be attributed to its properties as an antiseptic, antidepressant, anti seborrheic, hypotensive, sedative, and nervine substance. It is also used in aromatherapy treatments, perfume, and cosmetic products all over the world. The key chemical constituents of the Ylang Ylang are linalool, germacrene, geranyl acetate, methyl benzoate, and p-cresyl methyl ether, which all contribute to its medicinal effects. With the presence of antibacterial properties, it may be used as an alternative to conventional medicine, but it has yet to be tested (Dizon *et al*., 2016)[8].

The DPPH analysis is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers.

Phytochemical analysis refers to the extraction, screening. and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants, and phenolic compounds. Phytochemicals, that is, secondary metabolites of plants are an essential component of our diet. Phytochemicals are responsible for the beneficial properties which plants possess such as anti-microbial, anti-inflammatory, cholesterol lowering, blood sugar lowering, insecticidal, and fungicidal properties. Even the anti-oxidant properties of fruits, vegetables and spices are a result of the biochemical process which these phytochemicals undergo. Whereas flavonoids and tannins impart anti-oxidant properties, saponins are anti-cancerous and lower cholesterol levels. Alkaloids are used as anti-malarial compounds and as analgesics. Terpenoids are used for cardiac related disorders for being a source of Vitamin A.

In the present study, the phytochemistry, antibacterial, antioxidant activities of four EO, ylang ylang *were* investigated. The current study also aim to investigate the antibacterial activities of four EOs viz; Peppermint oil (*M. piperita L*), Patchouli oil (*Pogostemon cablin*) Frankincense oil (*Boswellia carterii*) and Ylang Ylang oil (*Cananga odorota*) against five pathogenic bacteria (*Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Proteus mirabilis, and Escherichia coli*).

MATERIALS AND METHODS

General Methods

Cleaning of glasswares

All the glasswares were soaked in cleaning solution (100 g potassium dichromate was added to 100 ml of distilled water followed by addition of 500 ml of concentrated sulfuric acid) for about 12 h and washed in tapped water. They were thoroughly rinsed in tapped water and dried. They were sterilized at 108°C for 3 h in a hot air oven.

Sterilization

All the media were sterilized in an autoclave under 15 lbs pressure for 20 min. The glasswares were sterilized in autoclave under 15 lbs for 20 min.

Chemicals

All the chemicals used in this experiment were of analytical reagent (AR) grade and distilled water was used throughout the period of study.

pH Adjustment

All media used in this experiment has been checked for their pH by using Universal pH indicator paper

or pH meter and we are adjusted either using 0.1 N NaOH or 0.1 N HCl.

Sample Collection

Four EOs (Peppermint oil, Patchouli oil, Frankincense oil and Ylang Ylang oil) marketed by Exim Enterprise 196, Bhagyoday Ind. Magob, Surat, Gujarat, were purchased for the study. The quality of the oil was determined as 100% pure.

Inoculum Preparation

Stock cultures of bacterial strains were maintained at 4°C on slopes of nutrient agar, active cultures for experiments were prepared by transferring a loopful of cells the stock cultures to test tubes of Mueller Hinton broth for bacterial strains that were incubated without agitation for 24 h at 37°C. After 24 h of incubation, the turbidity of the test bacterial suspension is compared with that of 0.5 MacFarland Standard against a white background with contrasting black lines under adequate light.

Determination of Antibacterial Activity

The antibacterial test of EOs was investigated by Agar Well Diffusion method Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extract. Mueller Hinton Agar (MHA) was prepared for Agar Well Diffusion method. The pH of each preparation of MHA was checked which lies in between 7.2 and 7.4. The media was also checked for its ability to support the growth of most relevant control strains. MHA was prepared with distilled water. The media are sterilized by autoclaving at 121°C for 15 min. After sterilization the agar medium is allowed to cool to 40–50°C. The media are then poured into a sterile Petri plate and allowed it to solidify. Agar Well Diffusion test of EO was performed using a 24 h cultured bacteria at 37°C in Nutrient broth or Mueller Hinton broth. Similarly to the procedure used in disk diffusion method, the agar plate surface is inoculated by spreading a volume of microbial inoculum over the entire agar surface. Then a hole with a diameter of 6–8 mm is punched aseptically with a sterile cork borer and a volume of the EOs at a desired concentration (25 µl, 50 µl, 75 µl, and 100 µl) is introduced into the well. Chloramphenicol is used as positive control and DMSO is used as negative control. The plates were then incubated at 37°C for 24 h. After 24 h incubation of the plates, zones were examined. The diameters of the zone of inhibition were measured through a ruler graduated to 0.5 mm. Each zone was read twice (at right angles) and the average result was recorded to the nearest mm (averages of 0.5 mm was rounded up).

Phytochemical Analysis of Ylang Ylang EO

Qualitative analysis of phytochemicals

Detection of carboxylic acid

To 1ml plant extract, 2 ml of sodium bicarbonate solution was added. Color changes occur indicates the presence of carboxylic acid.

Detection of tannins

To 2 ml of plant extract, 2–3 ml of 10% HCL was added and boiled for 5–6 min. Formation of red color indicates the presence of tannins.

Detection of steroids

To 0.5 ml extract, 5 ml of chloroform was added and equal amount of conc. H2so4 was added. In the upper layer formation of red color and in the lower layer, yellow with green color is formation indicates the presence of steroids.

Detection of flavonoids

To 0.5 ml extract, 4 ml of 1% ammonia was added and to this 1ml of conc. H2so4 was added. The formation of yellow color indicates the presence of flavonoids.

Detection of glycosides: Borntrager's test

Taken 2 ml of hydrolysate, 3 ml of chloroform was added, shaken vigorously, and then the chloroform layer gets separated. Then 10% ammonia solution was added. The formation of pink color indicates the presence of glycosides.

Detection of proteins (Bradford method)
To 500 µl of plant extract, 5 ml of the Bradford reagent was added, incubated at dark for 10–15 min. Taken the OD at 575 nm.

Detection of phenol (ferric chloride test)
To 50 mg of extract, 5 ml of distilled water was added and a few drops of 5% ferric chloride solution were added. The formation of dark green color indicates the presence of phenol.

Saponin test
To 50 mg of plant extract, 20 ml of distilled water was added and shaken vigorously for 15 min, formation of 2 cm layer of foam indicates the presence of saponins.

Test for Alkaloids-Mayer's test
To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of the test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

Saponification test
To 1 or 2 ml of 10 N sodium hydroxide, 2 ml of extract is added and boiled for 2 min formation of soap or fat indicates the positive test for saponification.

Gum test
The 100 mg of plant extract was dissolved in 2 ml of distilled water. 2 ml of absolute alcohol with constant stirring. White color cloudy precipitate indicates gums and mucilage's.

Detection of flavanoglycoside
The 50 mg of plant extract was dissolved in 5ml ethanol. Added few drops of magnesium sulfate and few drops of conc. HCL. The formation of pink color indicates the presence of flavanoglycoside.

Detection of carbohydrates
To 0.5 ml of extract, 0.5 ml of Benedict reagent was added ad boiled for 2 min. Color changes and precipitate are formed. It indicates the presence of carbohydrate.

Detection of resins
To 0.5 ml of plant extract, 3 ml of CuSO4 solution is added. Shaken for about 1–2 min, formation of green color precipitate indicates the presence of resins.

Biuret test
To 2 ml of extract, 1 drop of 2% CuSO4 solution. Add 1 ml of 95 % ethanol add 2 to 3 sodium hydroxide pellets. Formation of pink color indicates the test positive.

DPPH Radical Scavenging Activity of Ylang Ylang EO

1 mM of DPPH solution was briefly prepared in methanol and add 100 µl of this solution to 300 µl of the solution of Ylang Ylang EO (*C. odorota*) at different concentration (500, 250, 100, 50, and 10 µg/mL). The mixtures was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference). Lower absorbance values of reaction mixture indicate higher free-radical scavenging activity. The capability of scavenging the DPPH radical can be calculated using the following formula. DPPH scavenging effect (% inhibition) = ([absorbance of control-absorbance of reaction mixture]/absorbance of control) ×100.

RESULTS

Antibacterial Activities of Four Different EOS Extracted from Plants

The results of the antibacterial activities of EOs (Peppermint oil, Patchouli oil, Frankincense oil, Ylang Ylang oil) including the inhibition diameter are summarized in Tables 1-4 and Figures 1-5 respectively.

Antibacterial activity of Ylang-Ylang EO
In results shows that the EO of Ylang Ylang oil was found to have high inhibitory effect at 100 µl

Table 1: Antibacterial activity of Ylang Ylang essential oil

S. No.	Sample µl	Zone of Inhibition (mm)				
		Gram positive bacteria		Gram negative bacteria		
		Staphylococcus aureus	*Enterococcus faecalis*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Escherichia coli*
1	25	15±1.52	16±1.15	10±1.00	10±1.00	13±1.52
2	50	18±1.15	19±1.15	15±1.52	12±1.73	16±1.52
3	75	20±0.57	21±0.57	19±1.15	16±1.15	18±0.57
4	100	24±1.52	24±1.52	21±1.52	20±1.15	19±1.15
5	Pc	25±1.52	25±1.52	25±1.52	24±1.15	25±0.57
6	Nc	-	-	-	-	-

Positive control: Chloramphenicol, Negative control: DMSO, Na: Not affected

Table 2: The table qualitative phytochemical screening methods

S. No.	Name of the Sample	Phytochemical compound	Result
1.	Ylang ylang essential oil	Resins	-
2.		Carboxylic acid	-
3.		Tanins	-
4.		Steroids	-
5.		Flavonoid	-
6.		Carbohydrates	+
7.		Glycosides	-
8.		Saponification	-
9.		Protein	0.59
10.		Phenol	-
11.		Biuret	-
12.		Soponin	-
13.		Gum	+
14.		Flavanoglycosides	-
15.		Alkaloids	-

Table 3: OD Value at 517 nm Control Mean OD value: 0.817

S. No	Tested sample concentration (µg/ml)	OD Value at 517 nm (in triplicates)		
1.	Control	0.724	0.756	0.971
2.	500 µg/ml	0.134	0.137	0.138
3.	250 µg/ml	0.138	0.152	0.160
4.	100 µg/ml	0.163	0.173	0.165
5.	50 µg/ml	0.180	0.185	0.189
6.	10 µg/ml	0.199	0.213	0.228
7.	Ascorbic acid	0.08	0.11	0.12

against *S. aureus* (23 ± 1.52 mm) and *E. faecalis* (24 ± 1.52 mm) which are gram positive bacteria. Ylang Ylang oil also shows inhibition zone (100 µl) against Gram-negative bacteria, namely, *K. pneumoniae* (21 ± 1.52 mm), *P. mirabilis* (0.57 mm), and *E. coli* (19 ± 1.15 mm) which indicates that both Gram-positive bacteria and Gram-negative bacteria are susceptible to ylang ylang *(Cananga odorata*) EO. However, different concentrations of Ylang Ylang oil were used.

Table 4: Percentage of inhibition

S. No	Tested sample concentration (µg/ml)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	Ascorbic acid	90.20	86.53	85.31	87.35
2.	500 µg/ml	83.59	83.23	83.10	83.31
3.	250 µg/ml	83.10	81.39	80.41	81.64
4.	100 µg/ml	80.04	78.82	79.80	79.55
5.	50 µg/ml	77.96	77.35	76.86	77.39
6.	10 µg/ml	75.64	73.92	72.09	73.88

Phytochemical Analysis

The phytochemical screening was done with the EO Ylang Ylang oil. Table 5 showed the results of phytochemical analysis.

DISCUSSION

Natural products have been studied aiming to understand their biological properties. The antimicrobial activity of plants oils and extract has been recognized for many years. EO has been traditionally used as treatment of infections and to disease all over the world for centuries (Rios *et al*., 2005)[9]. Today, the used of EOs is a growing market and there are a considerable range of applications. However, few investigations have been compared large number of oils and extract using methods

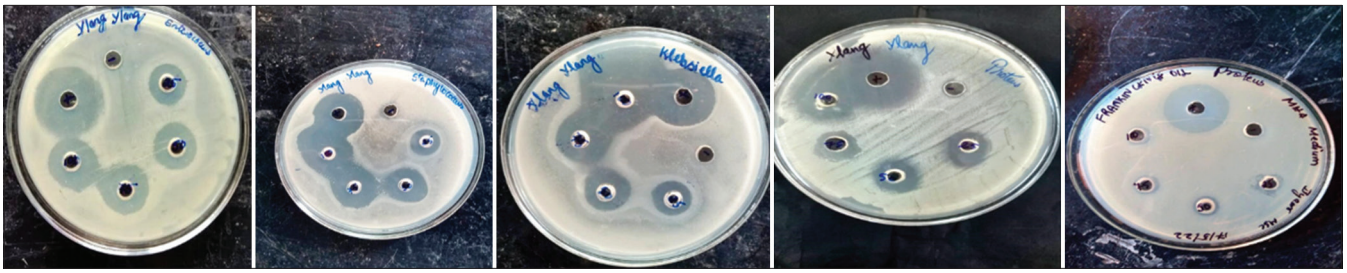

Figure 1: Antibacterial activity of Ylang Ylang Essential Oil

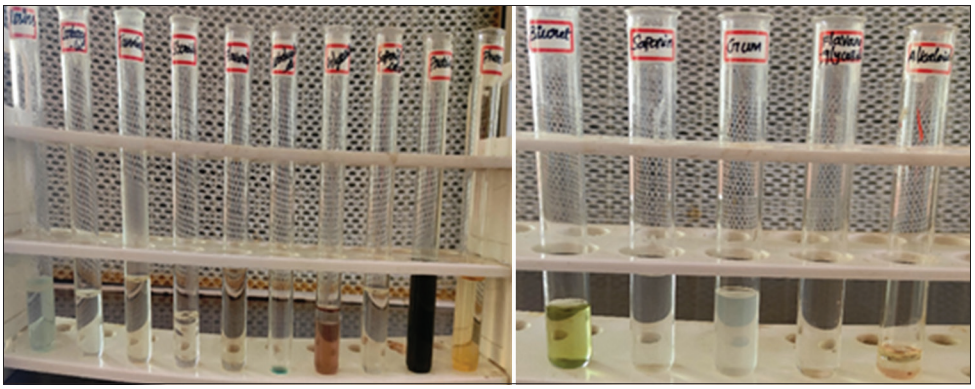

Figure 2: Images of qualitative phytochemical activity

Table 5: IC_{50} Value of tested sample: 75.22 µg/ml

log (inhibitor) versus normalized response-Variable slope		
Best-fit values		
$LogIC_{50}$		1.876
HillSlope		-1.535
IC_{50}		75.22
Std. Error		
$LogIC_{50}$		0.05021
HillSlope		0.2854
95% Confidence Intervals		
$LogIC_{50}$		1.768 to 1.985
HillSlope		-2.152 to -0.9186
IC_{50}		58.59 to 96.56
Goodness of Fit		
Degrees of Freedom		13
R square		0.9233
Absolute Sum of Squares		1510
Sy.x		10.78
Number of points		
Analyzed	3	15

that are directly comparable. In the present study four EOs that is, Ylang Ylang oil, Peppermint oil, Frankincense oil, and Patchouli oil were investigated for antibacterial activity against five pathogens, namely, two Gram Positive bacteria (*S. aureus* and *E. faecalis*) and three Gram negative bacteria (*Klebsiella* sp, *P. mirabilis* and *E. coli*) using agar well diffusion method. Chloramphenicol is used as positive control and kl DMSO is used as negative control. The results show that ylang ylang oil presented the higher activity against *S. aureus* (24 ± 1.52 mm) and *E. faecalis* (23 ± 1.52 mm) which are Gram positive bacteria. Ylang Ylang oil also found to have a good zone against Gram negative, namely, *Klebsiella* sp (21 ± 1.15 mm), *P. mirabilis* (20 ± 1.15 mm), and *E. coli* (19 ± 1.15 mm) at 100 µl. This indicates that Ylang Ylang EO (*C. odorota*) are susceptible against the tested bacterial strains (both Gram-positive and Gram-negative bacteria). It can therefore be indicated that the observed antibacterial activity of the extracts against these bacteria strains could be due to the presence of bioactive compounds such as flavonoids, tannins, alkaloids, and polyphenol compounds which were reported to possess antibacterial properties (Fouche *et al*., 2015)[10].

The phytochemistry of Ylang Ylang oil is well documented. Ylang Ylang oil is well known for its EO. EOs are referred as the natural, complex, and volatile compounds which exhibit distinctive scent that are produced by aromatic plants as secondary metabolites (Umaru *et al*., 2019)[11].

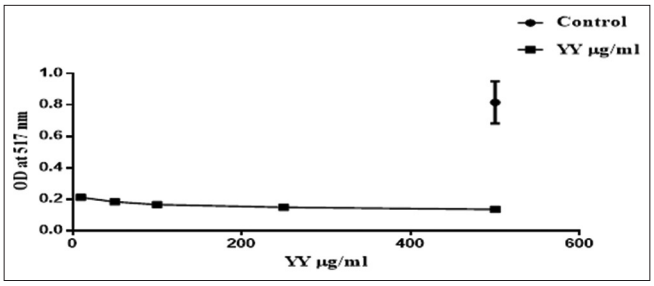

Figure 3: The percentage of inhibition

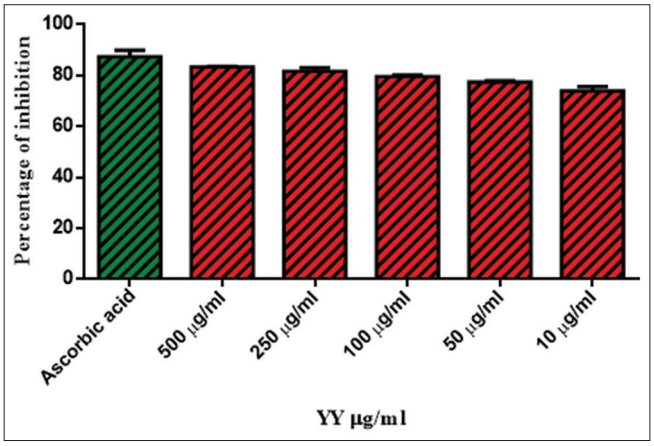

Figure 4: DPPH Radical scavenging screening

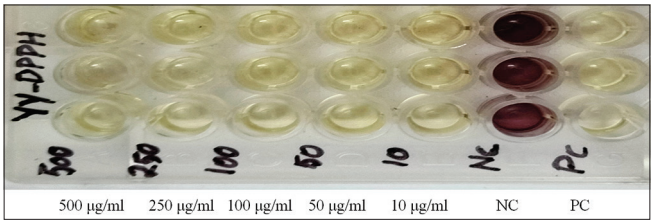

Figure 5: DPPH

The generation of free-radical intermediates through oxidative stress has been known to cause disturbances in metabolic processes. They are known to be responsible for cellular injuries and disease formation due to the destruction of unsaturated lipids, proteins, and DNA. The implications of oxidative damage have been linked to many human diseases such as cancer, cardiovascular diseases, inflammatory processes, cataracts, and even the normal ageing process (Miguel 2010)[12]. Recently, natural occurring antioxidants have been of great interest because of people's concerns over the use of synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, and tert-butylhydroquinone which may have adverse effects on human health (Shahidi *et al*., 2000)[13]. The antioxidant activity of Ylang Ylang oil extracts was evaluated using DPPH assay to determine the free radical scavenging abilities of the extracts. The result of the study revealed that the ethyl acetate extract of the Ylang Ylang oil exhibited the highest percentage of DPPH inhibition (79%) as compared to other tested plant extracts (Kusuma *et al*., 2014)[14]. Besides the DPPH assay, the antioxidant activity of was also determined by ferric ion reducing power assay. The extract showed a total of 290.0 ± 13.1% of ferric reducing power at 0.5 µg/Ml. Normally, a series of antioxidant assays will be utilized to examine different aspects of antioxidant property of plant extract. In a particular study, antioxidant activity of the Ylang Ylang oil EOs was assessed using free radical-scavenging, β-carotene bleaching, and the luminol-photochemiluminescence assays.

CONCLUSION

Among the four EOs, Ylang Ylang EO exhibited the maximum zone of inhibition against bacteria, followed by Frankincense (*Boswellia Cartiri*), patchouli (*P. cablin*), and peppermint oil (*M. piperita L*). However, peppermint oil did not affect the tested bacterial strains that are gram negative bacteria (*Klebsiellasp*, *P. mirabilis*, *and E. coli*) at lower concentration.

The result from the present study concluded that four EO purchased from local market and it was tested against five human clinical pathogens. Among all the tested four EO Ylang ylang oil exhibited maximum activity toward all the tested pathogens. Based on the antibacterial activity Ylang ylang constitutes of chemical components that reflect the maximum antibacterial potential. These components have the most important application against the human pathogens. The results of phytochemical and antioxidant properties of EO have some significant inhibitory action against pathogens.

The chemical structure of EOs components is also a crucial characteristic since it affects the precise mode of action. There are two main chemical groups responsible for antimicrobial activity of EOs, terpenes and terpenoids and aromatic and

aliphatic constituents.It should be noticed that the biosynthesis route of terpenes is independent of that of aromatic compounds, even though, these two types of substances might coexistence.

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