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Antioxidant and antimicrobial activity of essential oils extracted from aromatic plants

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ABSTRACT

The antimicrobial and antioxidant activities of extracted essential oils in some aromatic plants were determined. Phytochemical screening was conducted using standard qualitative techniques, while the essential oils was extracted from the *Citrus limon* linn leaf, *Vitex trifolia* seed and *Cananga odorata* by modified steam distillation apparatus. Furthermor, the plant materials were tested using Antioxidant activity and Antimicrobial activity test. *Citrus limon* linn produced large volume essential oil (1.4 ml), *Vitex trifola* (0.7 ml) and *Cananga odorata* (0.5 ml) after 3hours of steam distillation. Antioxidant activities test using DPPH method reveals that all the essential oil determined showed positive effect, but the oil extracted from *Citrus limon* linn leaf could be a better antioxidant than that of *Vitex tifolia* and *Cananga odorat* when compared to commercial Ascorbic acid. The antioxidant activities of the essential oils of different plants under investigation showed a variation which may be as a result of the difference in their chemical compositions. Antimicrobial activities showed that all the essential oils were inhibited on the entire five microorganisms being used. However, *Citrus limonlinn* showed highest inhibition on the organisms (*E. coli, S. typhi C. Kleb, P. avengunosa* and *S. aureus*) compared to *Cananga odorata* and *Vitex trifolia*.

***Keywords*:** Antioxidant, Antimicrobial, Essential oils, Phytochemical screening, bioactive agents and Alkaloids, *Citrus limon, Vitex trifolia, Cananga odorata*,

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1. INTRODUCTION

Essential oils are such volatile substances from plants containing a complex mixture of terpenenes and oxygenated hydrocarbons derivatives such as aldehydes, ketones, esters and alcohols [1]. Over 1,200 compounds such as terpenes plus their corresponding aldehydes, phenylpropanoids, alkanones, alcohols, oxides, sulfur, esters e.t.c have been identified in essential oils. In general, the essential oils constitutes terpenes (monoterpenes and sesqutterpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids) [1, 2]. They contain volatile compounds of plant origin with unique properties that have been prized worldwide for long time. Due to the higher amount of plant required in the production of natural essential oils, products of essential oils in the market are often adulterated with low grade oils or synthetic compounds. These reduce the costs in order to increase profit margin. Issues concerning essential oils dilution and adulteration of the pure essential oils with those with lesser value were also determined [3].

Extraction of essential oils from plant materials is done through different methods including steam distillation, vacuum distillation, solvent extraction, cold-pressing and hot pressing [4]. Current investigation showed that steam distillation is being used to extract essential oils from plant material in small scale. Essential oils are used in cosmetics, deodorants, pharmaceuticals, food flavors and embalming antiseptics [5]. Oils are used in medicine, purification and embalming process also. Investigations confirmed practical use of essential oil posses compounds with a long range of biochemical effects. Professional practitioners have been using over 300 essential oils [6]. Essential oils can be used to protect our bodies and homes against pathogenic organisms. Essential oils can also support our immune system [7].

Essential oils can be use to serve as natural additives in foods and food products because it possesses antioxidant and antimicrobial activities. Modern technology is being developed to continuously overcome the limitation of conventional methods, and to enhance the extraction efficacy. More attention is being paid to essential oil from several plants as a source of natural additives. Therefore, they can serve as processing aid and/or alternative to additives for sustainable development [8].

2. MATERIALS AND METHODS

2. 1. MATERIALS

2. 1. 1. *PLANTS MATERIALS*

The *Vitex trifolia seeds* and *Citrus limon* leaves were collected from Girei Local government of Adamawa State, while the flower of *Cananga odorata* was collected from Kaduna State in October 2016.

2. 2. METHODS

2. 2. 1. *COLLECTION AND PREPARATION OF PLANT MATERIALS*

Fresh *Vitex trifolia* seeds, *Citrus limon Linn* leaves, and *Cananga odorata* flower were collected from Moddibo Adama university of technology, Yola, located in the North-Eastern part of the country and Unguwan Rimi, Doka district in Kaduna state respectively. The

specimen were shade-dried at 30 °C to obtain a constant weight and then crushed them into powder using an electric blender so as to enhance effective contact of solvent with sites of the plant materials.

2. 2. 2. *PREPARATION OF PLANT EXTRACTS*

A portion (100g) of each powdered plant material that was shade-dried was soaked in 300 cm^3 of methanol for at least 24 hours. Each extract was filtered using Whatman filter under vacuum. The filtrates were further concentrated at 40 °C using a rotary evaporator and later stored at 4 °C for further use [9].

2. 2. 3. *SCREENING THE EXTRACT FOR BIOACTIVE AGENTS*

The phytochemical screening was carried out for some major constituent of the plant extracts using standard qualitative techniques as described by Odebiyi *et al*., [10], Trease *et al*., [11], Kubmarawa *et al*., [12]; Harbone [13]; and Runde *et al*., [9] as follows;

2. 2. 3. 1. *FLAVONOIDS TEST*

Into 1 ml of 10% lead acetate solution, 1 ml of aqueous extract was added. Formation of a yellow precipitate indicated the presence of flavonoids [13].

2. 2. 3. 2. *TERPENOIDS TEST*

2 ml of chloroform was used to dissolved the 2 ml of the organic extract and allowed to dry. Another 2 ml of conc. sulfuric acid was added and heated for about 2 min. A greenish coloration developed and that showed the presence of terpenoids [13].

2. 2. 3. 3. *TEST FOR TANNINS*

2 ml of the aqueous extract combined with 2 ml of de-ionized water and two drops of $FeCl_3$ were added. The formation of a green precipitate indicated the presence of tannins [14].

2. 2. 3. 4. *SAPONINS TEST*

5 ml of aqueous extract plus 5 ml of distilled were put into a test tube. It is then thoroughly shook and warmed for some time. The formation of stable foam was considered as indication for the presence of saponins [10].

2. 2. 3. 5. *GLYCOSIDES TEST*

2 ml of chloroform was added to the 2 ml of the extract until it dissolved. Then another 2 ml of sulfuric acid was then added gently and shaken for some time. Appearance of a reddish-brown colour showed that a steroidal ring is present [9].

2. 2. 3. 6. *ALKALOIDS TEST*

3 ml of 1% HCl was added in to the 3 ml of aqueous extract, placed on a steam bath and stirred for some time. Mayer's and Wagner's reagents were also added. Turbidity of the resulting precipitate indicated the presence of alkaloids [11].

2. 2. 3. 7. *PHENOLS TEST*

3 ml of ferric chloride was added to 3 ml of extract. A deep bluish colour indicated the presence of phenols [13].

2. 2. 3. 8. *ESSENTIAL OILS TEST*

Two drops of $FeCl_3$ were added to 90 % alcohol containing small quantity of the extracts and greenish coloration appeared which also indicated the presence of essential oils [9].

2. 2. 3. 9. *RESINS TEST*

2 ml of extract plus 2 ml of acetic acid were put into a test tube, and 3 drops of sulfuric acid were added. The presence of resins was authenticated by the observed violet color [10].

2. 3. EXTRACTION OF ESSENTIAL OILS

Steam distillation was used to carry out the extraction of the oil as described by Runde *et al*., [9]. The percentage yield of oil was calculated by using:

$$\text{Yield (\%)} = (\text{Oil (mL)}) / (\text{Plant (g)}) \times 100$$

2. 4. ANTIMICROBIAL INVESTIGATION FOR ESSENTIAL OILS

2. 4. 1. *ANTIMICROBIAL ACTIVITY TEST*

Antimicrobial activity test was investigated by adopting disc-diffusion method [15]. 10 ml of Mueller Hinton agar medium in a petri plate were seeded with one day old culture of a selected bacteria strain. Sterile filter paper disc (9 mm in diameter) containing 1000 ppm of the oil was dissolved in dimethyl sulpuroxide (DMSO) place on the medium, where the Dimethyl sulphuroxide and water were served as a negative controls. A standard disc containing chloramphenicol antibiotic drug (30 µg / dics) was used as a + control. It was allowed to incubate at 34 °C for 24 hours, and the antimicrobial activity, as diameter of incubation zone around the disc, was measured using a simple ruler. (Diameter of incubation zone minus that of the dics). Average zone of incubation were calculated for three replicates and an incubation zone of 8 mm or greater was considered a better antimicrobial activity [16]. Essential oils that showed positive activity in the initial screening was diluted serially (two fold) and loaded on the filter paper disc. The serially diluted concentrations of oil, assayed in triplicate to determine the minimum inhibitory concentration (MIC) i.e. the minimum concentration per dics to inhibit the growth of the microorganism [17].

2. 5. DETERMINATION OF ANTI-OXIDANT ACTIVITY OF ESSENTIAL OILS

2. 5. 1. *DPPH FREE RADICAL SCAVENGING ASSAY*

The essential oil was dissolved in methanol at various concentrations (2, 6, 12, 24, and 50 µl / ml). The assay mixture contain in a total volume of 1 ml, 50 µl of the oil, 125 µl prepared DPPH (1 mM in methanol), and 375 µl solvent (methanol). After 30 min incubation at 25 °C, a sharp decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was deduced from the equation below:

% RSA = Abs control – Abs Sample / Abs control × 100

The method was described by Brand-Williams, [18] and Wei *et al.,* [19] and adopted by Runde *et al*., [9] going by the volume of the oil and the value of reagent described above.

3. RESULTS AND DISCUSSIONS

3. 1. PHYTOCHEMICAL SCREENING TEST

From Table 1, the result of phytochemical screening test of methanolic extracted seeds, leaves and flowers of *Vitex trifolia, Citrus limon* and *Cananga odorata* revealed that they have essential oils respectively. All the plants contain Tannins except *Citrus limon*. Phenol, essential oils, alkaloid, flavonoid, terpenoids and glycoside were present in *Citrus limon, Vitex trifolia,* and *Cananga odorata* plants. Saponin was present in *Citrus limon* and *Cananga odorata* while absent in *Vitex trifolia* seeds. *Cananga odorata* contain all the basic phytochemicals as listed in this experiment.

Related work on ethanolic peel extract of *Citrus limonum* revealed that they contain carbonhydrates, saponins, tannins, cardiac glycosides, fixed oils, steroids, phytosteroids, proteins, amino acids and so on [20]. Whereas the ethanolic peel extract of *Citrus sinensis* evaluated the presence of alkaloids, carbohydrate, saponins, tanins, fixed oils, steroids, phenols were present [21].

Table 1. Summary of the phytochemical analysis of methanol extracts.

Plant extract	Alk	EO	Ph	Gly	Sap	Flav	Tan	Terp
C. limon	+	+	+	+	+	-	-	+
V. trifolia	+	+	+	+	-	+	+	+
C. odorata	+	+	+	+	+	+	+	+

Key: Alkaloids = Alk, Essential oil = EO, Phenol = Ph, Gly = Glycosides, Sap = Saponnins, Flav = Flavonoids, Tan = Tannins, Terpenoids = Terp

Similar research was done on phytochemical screening of *Vitex trifolia* with various qualitative chemicals tests on it leaf extract which indicated that ncarbonhydrate, flavonoids, proteins, tannins, phytosterols and saponinsphytoconstituent were present [22]. It was concluded that the phytochemical activities of leaves extract of *Vitex trifolia* revealed that it may act as an antibacterial agent [23].

Another study of solvent extracted *Vitex trifolia* leaves discovered the presence of phytochemicals. Saponi and steroid were said to be present when it was extracted using

petroleum ether as a solvent. Extraction in benzene medium showed that alkaloids (0.25 mg), Flavonoids (0.367 mg), phenol (0.30 mg), steroids, tannins (0.231 mg), terpenoids (0.461 mg) were present. So also Acetone extract showed that flavonoids (0.432 mg), steroids, tannins (0.521 mg) and Terpenoids (0.321 mg) were present. Furthermore, Ethanol extract showed that alkaloids, flavonoids (0.232 mg), phenols (0.163 mg), saponins (0.123 mg), tannins (0.461 mg) and terpenoids (0.361 mg) were present. However, water extract showed that only flavonoids (0.423 mg) and tannins (0.62 mg) were present [23].

Cananga odorata leaves extract shows antimicrobial activity, antibiofilm activity, anti-inflammatory activity, antivector acitivity, antidiabetic activity, antifertility activity and antimelanogenesis activity [24]. As saponin are reported to have anti-imflamatory,

Hypocholesterolemic and immune-stimulating properties [24].

Going by the above facts as observed by various authors on the medicinal profile of phytochemicals, it is therefore possible to attribute the therapeutic properties of these tested plants to the presence of either or both saponin, tannins, flavonoids, alkaloids, essential oils, glycosides and phenols

3. 2. PERCENTAGE YIELD OF THE ESSENTIAL OILS

Percentage yields of 0.175, 0.088 and 0.075 % (V/W) of the oils of *Citrus limom* leaves, *Vitex trifolia* seeds and *Cananga odorata* flowers were obtained by the steam distillation method of the oil extraction of 800 g of the plant materials respectively. The findings showed that *Citrus limon* leaves has the highest % yield of 0.175 %, followed by *Vitex trifolia* seeds of 0.088 % and *Cananga odorata* flowers with percentage yield of 0.075 %.

Similar work was reported on the percentage yield of the oils which varies with factors like site of collection, time of collection, part and form of plant used and the type of extraction method adapted among others [25]. Different percentage yield have been reported for leaves of *Ocimum americanus*, *Vosia cuspidata*, *Eucalyptus camaldulensis* and the stem bark of *Bosweiliadalzielii* to have 0.16 %, 0.05 %, 0.12 % and 0.12 % respectively [9]. Nevertheless, the percentage yield of the oils from leaves, stem bark and flowers of *Eucalyptus camaldulensis* obtained from Malaysia were 1.4, 0.5 and 0.46 % respectively [26].

Similar report shows that hydrodistillation of *Boweilliadalzielii* was reported to have yielded 1.25 % essential oils [12]. Similar study also identified the yields of essential oils from Citrus were significantly ($p<0.05$) affected by drying treatments. The highest volume of the oil was found in the oven-dried sample of *C. Sinensis* peel (1.07 %) where as lowest volume was found in fresh sample of *C. paradisii* peel (0.20 %). The effect of drying on essential oil percentage was observed in *C. sinensis* (0.24-1.07 %) followed by *C. reticulate* (0.30-0.50 %) and *C. paradisii* (0.20-0.40 %) [27].

It was reported that after 180 minutes of heating more 20 ml of essential oil was extracted from 370g of the orange peels over those from equal mass of lemon and lime peel at the rate and time of heating, while lime had the least quantity of extracted essential oils (5 ml) [28].

These findings concurred with the findings of [27], and the variation of the oil yield could be as a result of the environment condition during the extraction, because it was observed that the lesser the temperature of the environment during extraction the more quantity of essential oils would be obtained.

Table 2. Percentage Yield of Essential oils

Plants	Plant parts and form	Volume (ml)	Appearance	% yield (V/W)
C. limonlinn	Fresh leaves	1.4	Greenish yellow	0.175
V. trifolia	Fresh seeds	0.7	Yellow viscous	0.875
C. odorata	Fresh flowers	0.6	Colourless	0.075

3. 3. ANTI-OXIDANT ACTIVITIES OF ESSENTIAL OILS FROM VARIOUS PLANTS

The antioxidant activities tests of the essential oils of, *Citrus limon* leaves *Vitex trifolia* seeds and *Cananga odorata* flowers were obtained using 2, 2 – Diphenyl-1-picrylhydrazyl. The percentage Scavenging ability for each essential oil and that of the standard (Ascorbic acid) were measured at varying concentrations (2, 6, 12, 24 and 50 µL). The result from Table 3 and Figure 1 showed that all the samples have exhibited antioxidant activity. However, the minimum scavenging ability for each of the sample is observed in the corresponding minimum concentrations. Ascorbic acid a water-soluble has the maximum scavenging ability followed by *Citrus limon* linn, *Vitex trifolia* and *Cananga odorata* at 50 µL / ml concentration. A research work showed that the antioxidant activity of *Ficus sycomorus* plant extract via DPPH radical scavenging posed stronger antioxidants properties at concentrations from 20 - 100 mg / ml compared to L-Ascorbic [29].

Similar research revealed that *Vosia cuspidata* showed some degree of antioxidant activities with values above 80 %. Conversely, the minimum scavenging property for each of the essential oils was observed in the corresponding minimum concentrations determined from vitamins E (80.32 %), the least scavenging property was found in water insoluble antioxidant, while the highest scavenging activity was found in vitamins C (98.67%) and *Vosia cuspidata* (97.42 %) at of 50 µL/ml [9].

Similar research discovered that *Vitex trifolia* extract were determined and compared with the known antioxidant ascorbic acid. Chloroform extract showed better hydroxyl radicals, lipid peroxidation, DPPH radical scavenging ability compared to methanol extract (Sreedhar *et al*., 2010). DPPH assay was used to evaluate the antioxidant property of *C. odorata* extracts to determine the free radical scavenging properties of the extracts. The ethyl acetate extract of the stem bark of *C. odorata* reveled to exhibit the maximum % of DPPH inhibition (79 %) when compared to other investigations [30]. Meanwhile, the antioxidant property of that methanol extract of *C. odorata* leaves was studied using ferric ion reducing power assay. The result indicated overall of 290.0 ± 13.1 % ferric reducing power around 0.5 μg /mL [31].

Similar research also identified the level of % inhibition from linoleic acid oxidation as exhibited by the citrus peel essential oils. High absorption of iron based peroxides indicated that a higher formation of peroxides during the reaction, and low antioxidant activity. The tested citrus oils inhibited the oxidation of linoleic acid by 54.98 %-67.80 %. *Citrus sinensis*

showed the highest antioxidant activity (67.80 %) in linoleic acid system then *Citrus paradisii* (57.12 %) and *Citrus reticulate* (54.98 %), respectively [32]. From the reported literature it can be deduced that *Citrus limon* would have the highest antioxidant activity then followed by *Vitex trifolia* and finally *Cananga odorata* as we obtained from table below.

Table 3. Result of Antioxidant activities using DPPH method.
Percentage scavenging property per microliter.

Plant essential oil	2 ul	6 µL	12 µL	24 µL	50 µL
Vitextrifolia	60.88	62.02	67.62	77.13	97.32
Citrus limon	59.91	63.39	80.01	89.19	98.89
Canangaodorata	55.01	60.11	66.60	77.48	86.56
Ascorbic acid	80.23	83.19	85.01	92.44	98.74

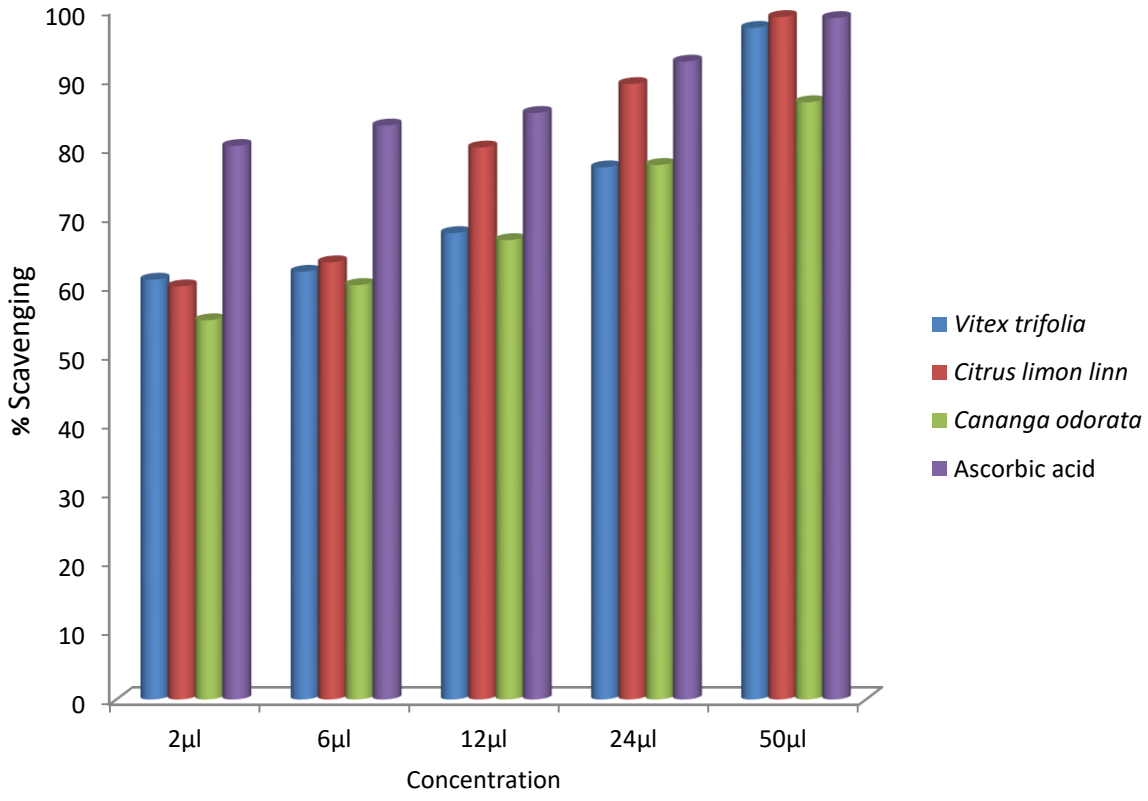

Figure 1. DPPH Scavenging properties of *Vitex trifolia, Citrus limon*, and *Cananga odorata* essential oils

3. 4. ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS

The antimicrobial property of oils was investigated adapting disc-diffusion method against five selected microorganisms, namely Escherichia coli, *Staphylococcus aureus*, *S. typhy*, *Klebsiella peumoniae* and *Pseudomonas aeruginosa.* However, all the examined oils indicated varying degrees of antimicrobial activities against the organisms. Table 4 below shows the results of the antimicrobial activity.

Similar work revealed that the antimicrobial a property of *V. trifolia (*extracted from acetone) against Escherichia coli had its zone of inhibition at exactly 0.15 cm, those extracted using ethanol and water had their zone of inhibition at 0.25 cm and 0.35 cm respectively [23].

Table 4. Antimicrobial properties of the isolated essential oils.
Microorganisms/Area of inhibition (mm)

Plant essential oil	*E. coli*	*S. aureus*	*S. typhi*	*P. aeruginosa*	*Kleb*
Citrus limonlinn	+++	++	+++	+++	+++
Cananga odorata	++	++	+++	+	+++
Vitex trifolia	+++	+++	+++	++	+++
DMSO	-	-	-	-	-

Key: 1 - 5 = +, 5 - 10 = ++, 10 – Above = +++

Table 5. Minimum Inhibitory Concentration (MIC) of the essential oil from *Citrus limonlinn* leave.

Organism	50% Essentialoil	30% Essential oil	20% Essential oil	10% Essential oil
E. coli	9	7	6	6
S. aureus	8	7	6	6
S. typhi	7	6	6	6
P. aeruginosa	10	11	10	9
K. peumoniae	10	8	6	6

Recent work from Indonesia showed that the stem bark extracts of *C. odorata* exhibited a great antimicrobial property using the agar well disc diffusion assay. The research pointed that *C. odorata* stem bark (n-hexane, ethyl acetate, and ethanolic extracts) possessed a great activity against *Propionibacterium acnes* and *Candida albicans*. *C. odorata* (ethanolic

extract) at 400 μg / well exhibited an inhibition zone of 19 ± 1.58 mm when tested against *P. acnes*. In fact, the activity index stands at 0.63 when being relative to the control which is known as chloramphenicol [30].

It is observed from reviewed work cited above, the result of antimicrobial activity of the plant materials used were almost similar to the result obtained in our work.

Table 6. Minimum Inhibitory Concentration (MIC) of the essential oil from *Vitex trifolia* seed.

Organism	50% Essential oil	30% Essential oil	20% Essential oil	10% Essential oil
E. coli	10	8	6	6
S. aureus	8	7	6	6
S. typhi	8	6	6	-
P. aeruginosa	-	-	-	-
K. peumoniae	7	6	6	6

Table 7. Minimum Inhibitory Concentration (MIC) of the essential oil from *Cananga odorata* flower.

Organism	50% Essential oil	30% Essential oil	20% Essential oil	10% Essential oil
E. coli	9	7	6	6
S. aureus	11	8	6	6
S. typhi	10	8	7	6
P. aeruginosa	-	-	-	-
K. peumoniae	-	-	-	-

4. CONCLUSION

The findings of antioxidant activities using DPPH method confirmed that essential oil isolated from Citrus limonleaves is a better antioxidant than that of *Vitex tifolia* and *Cananga odorat* when compared to commercial Ascorbic acid. Therefore, it may be said that the variation in antioxidant properties of the oils of different plants under investigation could be as a result of difference in their chemical constituents which solely depends on their unique

genetic makeup. However, the findings of antimicrobial properties in this study confirmed all the essential oils were inhibited on the entire five microorganism being used. Also, *Citrus limon* showed highest inhibition on the organisms (*E. coli, S. typhi C. Kleb, P. avengunosa* and *S. aureus*) compared to *Vitex trifolia* and *Cananga odorata* which has the least zone inhibition.

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