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# Chemical constituents, antioxidant activities and antimicrobial properties of volatile oil from different part of *Duranta repens* Linn

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**Abstract**
The volatile oil hydrodistilled from leaf, stem, fruit and root of *Duranta repens* were investigated for their chemical constituents, antioxidant and antimicrobial activity using GC-MS techniques, DPPH radical-scavenging assay and agar-diffusion method respectively. A total of 30, 12, 20 and 35 constituents were identified in the leaf, stem, fruit and root oils corresponding to 92.5%, 99.5%, 95.1% and 92.2% of the whole volatile oil respectively. Palmitic acid (55.7%, root), styrene (52.5%, fruit), *p*-vinylanisole (26.0%, fruit) and tetracosane (22.2%, stem) were the prominent components identified in the oils. The fruit and stem oils displayed good DPPH free radical-scavenging activity with $IC_{50}$ value of 29.39µg/mL and 32.02µg/mL respectively, when compared with that of synthetic antioxidants α-tocopherol (81.58µg/mL) and BHA (45.11µg/mL). The oils showed low antibacterial activity and moderate antifungal activity against the test pathogens with inhibition zone diameter (IZD) range between 2.0±0.0 and 8.5±0.5 mm, lower than the reference compounds gentamycin and ketoconazole (IZD range 9.0±1.4-21.0±1.4 mm). The chemical constituents, antifungal potential and DPPH radical scavenging activity of *Duranta repens* volatile oils suggest they can act as an adjuncts in lubricant, food, cosmetic and medicine.

**Keywords:** Volatile oils, *Duranta repens*, chemical constituents, antioxidant, Antimicrobial

## 1. Introduction
It is not contestable and not debatable, it is an everyday fact that the use of medicinal plants for the treatments of diverse ailments is becoming more acceptable to the public due to the problems associated with the synthetic drugs which include toxicity and drug resistant. According to various studies, extracts from medicinal plants possess potent antioxidant and antimicrobial properties in which many of them have long history of use in conventional medicine [1, 2]. Since oxidation deterioration and microbial infections are the common cause of diseases that have led to the death of many, researchers in the pharmaceutical field have diverted their focus on antioxidant and antimicrobial agents from plants [3]. Volatile oils are one of the most attractive, effective and superlative extracts from plants that have shown a very good antioxidant property and antimicrobial activity against a wide range of human pathogens. Besides, they are used as therapeutic agents in ethno, conventional and complementary alternative medicine as analgesic, anesthetic, antiseptic, anti-inflammatory, anthelmintic, antipruritic, antispasmodic and antiviral agents [4]. Apart from their medicinal values, they are also important materials in food and cosmetic industries as flavor and fragrance agents.

*Duranta repens* Linn. (Syn. *Duranta erecta* Linn.), commonly known as pigeon berry or golden dewdrop of the Verbenaceae is a shrub, herb or small tree widely cultivated as ornamental plant in tropical and subtropical gardens throughout the world [5]. Some of the pharmacological properties of the extracts from the plant include antioxidant, antibacterial, antifungal, antiviral, antifeedant and insecticidal [6, 7, 8]. The whole plant, fruit, leaf and bark are all used in traditional medicine. The plant is widely used as repellent against malaria vectors [9]. The stem and fruit are effective natural larvicides, which are active against *Culex quinquefasciatus* [10]. Furthermore, the fruit and the leaf are used in the treatment of intestinal worms and abscesses [11, 12], respectively. The flowers are used as stimulant [13]. Other ailments treated with *D. repens* are pneumonia, neuralgic disorder, infertility, fever, itches and as antidote to poisoning [14]. Phytochemicals from the plant crude extracts, physicochemical and

micronutrients composition of non-volatile oils from leaf and seed [11,15] have been reported. Up to now, there is dearth of information on the biological activity of the volatile oils from the plant. Thus, this study was undertaken to investigate the chemical profile, antimicrobial and antioxidant activity of the volatile oils extracted from the leaf, stem, fruit and root of *Duranta repens* in order to validate its ethnomedicinal uses.

## 2. Material and Methods

### 2.1 Plant collection and volatile oils extraction

*Duranta repens* was collected at Saundal road, University of Ibadan (7° 0.2" N/3° 28.8" E), Nigeria and was authenticated at Forestry Research Institute of Nigeria where voucher specimen was deposited (FHI 112525). The leaf, stem, fruit and the root of the plant were air dried to reduce the moisture and pulverized to increase the surface area. They were later subjected to hydrodistillation using all glass Clevenger-type apparatus for 3 h according British Pharmacopeia specification (1980) [16]. Extracted volatile oils were dried over anhydrous sodium sulfate, stored in sealed glass vials and kept under refrigeration at 4 ℃ prior to analysis and bioassay.

### 2.2 Analysis of essential oils

The volatile oils were analyzed by gas chromatography-mass spectrometry techniques using a Shimadzu GC-MS-QP2010 Ultra operated in the electron impact (EI) mode of 70 eV, scan rate-3.0 scan/s, scan range-40-400 atomic mass units and GC-MS solution software v. 4.20 (Shimadzu Scientific Instruments, Columbia, MD, USA). The GC column was a ZB-5 fused silica capillary column (Phenomenex, Torrance, CA, USA) with a (5% phenyl)-polydimethylsiloxane stationary phase and a film thickness of 0.25 µm. The initial temperature of the injector 50 ºC was increased at a rate of 2 ºC/min to 260 ºC. A 5% w/v solution of the sample in $CH_2Cl_2$ as prepared and 0.1 µL was injected with a splitting mode (30:1). The carrier gas was helium with the column head pressure of 552 kPa and flow rate of 1.37 mL/min. Identification of the volatile oil constituents was based on the comparison of their mass spectral fragmentation pattern with those reported in the literature [17] and stored in the home data base library [18].

### 2.3 Antimicrobial assay

The antimicrobial activity of the volatile oils was determined using agar diffusion method. The volatile oils were tested against one Gram-positive bacterium (*Staphylococcus aureus*, ATCC 6571), one Gram-negative bacterium (*Escherichia coli*, ATCC 25925) and three fungi (*Aspergillus niger*, *Fusarium solani* and *Candida albicans*), which were clinical isolates. Diluted overnight cultures ($10^{-2}$ CFU/mL) were inoculated into sterile agar using. Mueller-Hinton Agar for bacteria strains and Sabourand Dextrose Agar for fungi. Wells of uniform diameter were created in a seeded agar plates using 8 mm cork borer. Volatile oils of varied concentrations were allowed to diffuse into the seeded agar for 1 h before incubating for 24 h at 37 ºC and 48 h at 25-32 ºC for bacteria and fungi respectively. Zones of inhibition around the wells were observed and recorded and the activity of the oils was compared with standard drugs that is gentamycin (10 µg/mL) and ketoconazole (200 µg) for bacteria and fungi, respectively.

### 2.4 DPPH free radical-scavenging assay

The antioxidant activity of the oils was studied via DPPH free radical-scavenging assay using the method described by Saleh *et al.*, (2010) [19] with little modifications. The volume 1.5 mL of three different concentrations of the volatile oils (5 mg/mL, 25 mg/mL and 100 mg/mL) were separately mixed with 1.5 mL of 0.2 mM DPPH dissolved in methanol and incubated in the dark for 20 min at room temperature. The absorbance of the mixture at 517 nm was recorded as $A_{(sample)}$ using CE 2021, 2000 series double beam UV-Vis spectrophotometer. Blank experiment was carried out using the same procedure in the absence of volatile oils and the absorbance was recorded as $A_{(blank)}$. Each experiment was carried out in triplicates and the free radical-scavenging activities of the oils were calculated as percentage inhibition using the formula;

% inhibition= $A_{(blank)}$-$A_{(sample)}$/$A_{(blank)}$ ×100.

The free radical-scavenging activity of the oils was compared with synthetic antioxidant, butylated hydroxyanisole (BHA) and α-tocopherol. $IC_{50}$ was calculated through Microsoft EXCEL by plotting the graph of percentage inhibition against the concentration [20].

## 3. Results and Discussion

### 3.1 Chemical composition of *Duranta repens* volatile oils

The volatile oils extracted from *D. repens* leaf, stem, fruit and root were colorless with the percentage yield range of 0.14% and 0.56%. The GC-MS analysis result is presented in Table 1 in order of elution from ZB-5 fused silica capillary column. A total of 30 constituents were identified in the leaf oil amounting to 92.5% of the entire oil. The oil was dominated by non-terpenes, which were toluene (13.2%), 1-octen-3-ol (12.9%), *p*-vinylanisole (11.7%), palmitic acid (6.8%), (9*Z*)-octadecenamide (6.4%) and 3-octanol (4.0%). Terpenes identified in the oil include geranyl acetone (4.3%), phytol (3.3%), (5*E*,9*E*)-farnesyl acetone (2.7%), linalool (1.4%) and α-terpineol (0.4%). (*E*)-α and β-ionone (2.6%, 2.7%) are apocarotenoids, which were also found in appreciable quantity. The stem volatile oil was characterized largely of long-chain alkanes majorly tetracosane (22.2%), tricosane (19.3%), pentacosane (18.0%), docosane (14.2%), heneicosane (8.1%) and eicosane (4.2%). Other compounds present in noticeable amount were 1-docosanol (5.0%) and (9Z)-octadecenamide (3.8%) and the only terpene found in the oil was phytol (0.8%) a diterpene, making a total of 12 compounds equivalent to 99.5% of the total oil. The fruit oil showed the presence of 20 components equivalent to 95.1%. The oil was mainly styrene (52.5%) and vinylanisole (26.0%). The terpenes and terpenoids in the fruit oil were identified as minor components; they were α-bisabolol (0.8%), cuparene (0.7%), linalool (0.6%), α-pinene (0.4%), limonene (0.4%), γ-terpinene (0.3%), and terpinen-4-ol (0.3%). Presence of styrene could probably be the reason the fruit was toxic as reported in the literature [21]. The major economic importance of styrene is as precursor to polystyrene and several copolymers. Like the other oils, the root oil was largely non-terpenes, consisting of 35 constituents corresponding to 93.2% of the total oil. The most abundant constituents were palmitic acid (55.7%), oleic acid (6.3%), methylcyclohexane (5.0%), linoleic acid (4.3%), (9Z)-octadecenamide (3.8%) and toluene (3.6%). All the non-terpenes found in substantial quantity in *D. repens* oil are important compounds in the industry. Higher alkanes (hexadecane upward) act as anti-corrosive agents and are important component of lubricating oil [22]. Because of their nature at ambient temperature many of them are used as electrical insulation and candles. They also form most important constituents of fuel oil. Heneicosane is used as a pheromone by termites and mosquitoes [23, 24].

Eicosane is one of the components of paraffin waxes used to form candles. Palmitic acid is used in cosmetic, soaps and production of industrial mold release agents. Palmitic acid and its sodium salt find application in food industry. Palmitic acid was also reported to strongly boost metastasis in mouse models of human oral cancer cells [25]. Oleic acid is used as emulsifying agent in soap and aerosol products [26] and as excipient in pharmaceuticals. It is an insect pheromone [27], also used as an emollient [28].

The leaf and fruit oils reported by Thomas *et al*. (2019) [29] afforded 47 and 42 constituents equivalent to 95.8% and 96.3% respectively. Notable percentage of sesquiterpenes (43.1%, 43.8%) and monoterpenes (26.3%, 36.9%) were observed in the leaf and fruit oils correspondingly. The leaf oil was dominated by limonene 11.6%, β-caryophyllene 7.5%, pentadecanal 6.7%, humulene 5.0%, α-eudesmol 4.1% while the fruit oil was mainly made up of carvacrol 16.5%, 1,10-di-*epi*-cubenol 10.1%, β-caryophyllene 10.1%, *n*-hexadecane 7.0%, limonene 4.7%, selinene 4.2%. Likewise, Silva *et al*. (2014) [30] reported δ-cadinene (18.5%), (*E*)-nerolidol (16.4%), spathulenol (16.4%), caryophyllene oxide (7.8%) and cubebol (7.4%) as prominent constituents of leaf oil of *Lippa stachyoides* (Verbenaceae) which were not detected in the *D. repens* leaf oil. The observed variation was attributed to seasonal and geographical disparities, extraction procedure and plant maturity.

**Table 1:** Chemical constituents of *Duranta repens* essential oil

| Constituents | RI | Percentage composition | | | |
|---|---|---|---|---|---|
| | | Leaf | Stem | Fruit | Root |
| *Z*-1,3-Dimethylcyclopentane | 716 | - | - | - | 0.8 |
| Methylcyclohexane | 718 | 1.3 | - | - | 5.0 |
| 2-Methyl-2-pentanol | 723 | 2.4 | - | - | 0.7 |
| Ethylcyclopentane | 726 | - | - | - | 0.2 |
| 3-Methyl-3-pentanol | 745 | 1.4 | - | - | 0.4 |
| 2-Methylheptane | 754 | - | - | - | 0.2 |
| Toluene | 760 | 13.2 | - | - | 3.6 |
| 3-Methylheptane | 763 | - | - | - | 0.3 |
| *Z*-1,3-Dimethylcyclohexane | 777 | - | - | - | 0.3 |
| *E* -1,3-Dimethylcyclohexane | 780 | - | - | - | 0.2 |
| 2-Hexanone | 785 | - | - | - | 0.3 |
| 4-Methyl-2-pentanone | 786 | 0.4 | - | - | - |
| 1-Methylcyclopentanol | 792 | 1.4 | - | - | 1.0 |
| Octane | 800 | - | - | - | 0.4 |
| Hexanal | 801 | 1.9 | - | - | 0.4 |
| (2*E*)-Octene | 828 | - | - | - | 0.1 |
| Ethylcyclohexane | 832 | - | - | - | 0.2 |
| (2*E*)-Hexanal | 849 | 1.9 | - | - | - |
| Ethylbenzene | 856 | - | - | - | 0.5 |
| *p*-Xylene | 866 | - | - | - | 0.3 |
| Ethylbenzene | 891 | - | - | 1.2 | - |
| *o*-Xylene | 895 | - | - | 1.3 | - |
| Styrene | 890 | 0.6 | - | 52.5 | - |
| α-Pinene | 935 | - | - | 0.4 | - |
| Mesitylene | 957 | - | - | 0.6 | - |
| Benzaldehyde | 960 | 1.4 | - | - | - |
| 1-(1-Methyl-cyclopentyl)-ethanone | 962 | - | - | 3.5 | - |
| 1-Octen-3-ol | 977 | 12.9 | - | 0.7 | 0.5 |
| 2-Pentylfuran | 987 | 0.4 | - | - | - |
| 3-Octanol | 995 | 4.0 | - | - | - |
| Limonene | 1027 | - | - | 0.4 | 0.3 |
| γ-Terpinene | 1050 | - | - | 0.3 | - |
| Acetophenone | 1064 | 0.5 | - | - | - |
| Linalool | 1098 | 1.4 | - | 0.6 | - |
| *n*-Nonanal | 1103 | 0.4 | - | 0.4 | 1.7 |
| *p*-Vinylanisole | 1150 | 11.7 | - | 26.0 | - |
| Terpinen-4-ol | 1175 | - | - | 0.3 | - |
| Naphthalene | 1177 | - | - | 0.4 | - |
| α-Terpineol | 1193 | 0.4 | - | - | - |
| β-Cyclocitral | 1216 | 0.4 | - | - | - |
| *p*-Ethylguaiacol | 1285 | - | - | 0.9 | - |
| 4-Ethyl-1,2-dimethoxybenzene | 1335 | - | - | 1.2 | - |
| 3,4-Dimethoxystyrene | 1362 | 0.6 | - | 2.5 | - |
| (*E*)-α-Ionone | 1418 | 2.6 | - | - | - |
| Geranyl acetone | 1444 | 4.3 | - | - | - |
| (*E*)-β-Ionone | 1475 | 2.7 | - | 0.4 | - |
| Cuparene | 1500 | - | - | 0.7 | - |
| Dodecanoic acid | 1556 | - | - | - | 0.6 |
| Bulnesol | 1662 | - | - | - | 0.2 |
| Acorenone | 1686 | - | - | - | 0.3 |
| α-Bisabolol | 1682 | - | - | 0.8 | - |
| Myristic acid | 1754 | - | - | - | 0.6 |
| Phytone | 1837 | 3.7 | - | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Nonadecane | 1900 | - | 1.8 | - | - |
| (5*E*,9*E*)-Farnesyl acetone | 1904 | 2.7 | - | - | - |
| Palmitic acid | 1957 | 6.8 | - | - | 55.7 |
| Ethyl palmitate | 1989 | - | - | - | 0.7 |
| Eicosane | 2000 | - | 4.2 | - | - |
| Abietadiene | 2080 | 0.5 | - | - | - |
| Heneicosane | 2100 | - | 8.1 | - | - |
| Phytol | 2102 | 3.3 | 0.8 | - | - |
| Linoleic acid | 2123 | - | - | - | 4.3 |
| Oleic acid | 2130 | - | - | - | 6.3 |
| (9*E*)-Hexadecanamide | 2170 | 0.9 | - | - | 0.6 |
| Docosane | 2200 | - | 14.2 | - | - |
| 2-Methyltetracosane | 2259 | - | 0.9 | - | - |
| Tricosane | 2300 | - | 19.3 | - | 0.3 |
| (9*Z*)-Octadecanamide | 2350 | 6.4 | 3.8 | - | 3.8 |
| 3-Methyltricosane | 2368 | - | 1.2 | - | - |
| Tetracosane | 2400 | - | 22.2 | - | 0.5 |
| 1-Docosanol | 2484 | - | 5.0 | - | 0.4 |
| Pentacosane | 2500 | - | 18.0 | - | 0.5 |
| Monoterpene hydrocarbons | | - | - | 1.1 | 0.3 |
| Oxygenated monoterpenes | | 6.1 | - | 0.9 | - |
| Sesquiterpene hydrocarbons | | 0.5 | - | 0.7 | - |
| Oxygenated sesquiterpenes | | 2.7 | - | 0.8 | 0.5 |
| Apocarotenoids | | 5.7 | - | 0.4 | - |
| Oxygenated diterpenes | | 3.3 | 0.8 | - | - |
| Non-terpene derivatives | | 74.2 | 98.7 | 91.4 | 91.4 |
| Percentage identified | | 92.5 | 99.5 | 95.1 | 92.2 |
| Total identified | | 30 | 12 | 20 | 35 |

RI-Retention determined with respect to a series of *n*-alkanes on a ZB-5 column.
a-Order of elution from a ZB-5 fused silica capillary column

### 3.2 Antimicrobial activity of *Duranta repens* volatile oil

The result of antimicrobial assay carried out on the leaf, stem, fruit and root of *D. repens* as presented in Table 2 showed that Gram negative bacterium, *E. coli* was resistant to all the volatile oils while weak zones of inhibition of 1.8±0.4 and 2.0±0.0 mm were observed against *S aureus* for the leaf and fruit oils respectively. The fruit oil was active against all the fungi for all the observed concentrations with inhibition zones ranging from 2.0±0.0-8.5±0.1 mm, although not as active as ketoconazole which inhibited the fungi at the range between 10.3±0.4-22.0±1.4 mm at 200 µg. *F. solani* was resistant against the leaf and root oil. The root oil was inactive against all the test organisms except *C. albicans* having 2.0±0.0 mm inhibition zone at 100 µL/mL. *C. albicans* was susceptible to all the oil samples with inhibition zone range of 2.0±0.0-8.0±0.0 mm while *A. niger* was resistant to the stem and root oils. The previous work on the leaf, stem and root extracts showed low to good antimicrobial activity against both fungi and bacteria including *E. coli* which was resistant to the present volatile oils [8, 31, 32, 33]. Sharma *et al*. (2012) [8] also reported *A. niger* to be resistant to methanol extract of *D. repens* root. The major factors that could be responsible for observed difference in their activities are the disparity in their chemical compositions, location, handling, and maturity of the plants.

**Table 2:** Antimicrobial activity of *Duranta repens* Volatile oil

| Essential oils | Conc (µL/mL) | IZ diameter (mm) | | | | |
|---|---|---|---|---|---|---|
| | | ***E. coli*** | ***S. aureus*** | ***C. albicans*** | ***A. niger*** | ***F. solani*** |
| Leaf | 100 | 0.0 | 1.8±0.4 | 7.5±0.7 | 2.1±0.1 | 0.0 |
| | 10 | 0.0 | 0.0 | 5.5±0.7 | 0.0 | 0.0 |
| | 1 | 0.0 | 0.0 | 3.9±0.1 | 0.0 | 0.0 |
| Stem | 100 | 0.0 | 0.0 | 6.0±0.0 | 0.0 | 2.0±0.0 |
| | 10 | 0.0 | 0.0 | 3.8±0.4 | 0.0 | 0.0 |
| | 1 | 0.0 | 0.0 | 2.3±0.4 | 0.0 | 0.0 |
| Fruit | 100 | 0.0 | 2.0±0.0 | 8.0±0.0 | 8.5±0.1 | 8.3±0.4 |
| | 10 | 0.0 | 0.0 | 6.3±0.4 | 6.1±0.1 | 5.8±0.4 |
| | 1 | 0.0 | 0.0 | 4.1±0.1 | 3.9±0.1 | 4.0±0.0 |
| Root | 100 | 0.0 | 0.0 | 2.0±0.0 | 0.0 | 0.0 |
| | 10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| + control | | 9.0±1.4 | 11.5±0.7 | 21.0±1.4 | 10.5±0.7 | 10.3±0.4 |
| - control | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

IZ=Inhibition zone; + control= Gentamycin (10 µL/mL) and Ketoconazole (200 µg) for antibacterial and antifungal assay respectively; -control= DMSO; Inhibition zones diameter value approximately 1 decimal place mean ± SD.

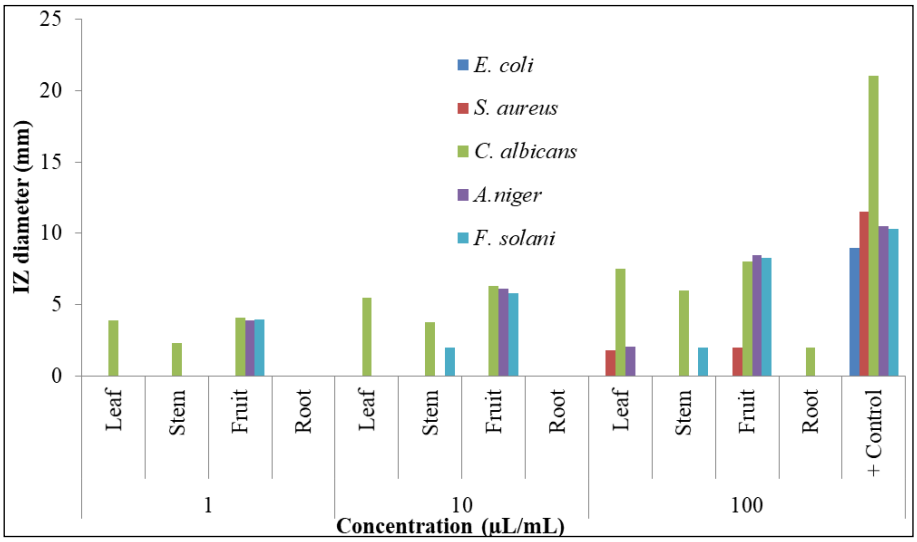


**Fig 1:** Antimicrobial activity of *Duranta repens* Essential oil

### 3.3 DPPH radical-scavenging activity of *Duranta repens* volatile oils

The DPPH free radical-scavenging test measured the ability of the extracted volatile oils to donate hydrogen atom or electron. The scavenging ability of the oils was characterized by their $IC_{50}$ value, the concentration necessary to reduce 50% of DPPH radicals. The volatile oils of *D. repens* fruit (29.39 µg/ mL) and stem (32.02 µg/mL) possess good DPPH radical-scavenging activity while the leaf oil (140.41 µg/mL) and root oil (118.72 µg/mL) activity was moderate when their $IC_{50}$ values were compared with that of the synthetic antioxidant BHA (45.11 µg/mL) and α-Tocopherol (81.58 µg/mL) that were used as positive reference.

**Table 3:** DPPH radical-scavenging activity of *Duranta repens* volatile oils

| Essential oil | Leaf | Stem | Fruit | Root | BHA | α-Tocopherol |
|---|---|---|---|---|---|---|
| $IC_{50}$ µg/ mL | 140.41 | 32.02 | 29.39 | 118.72 | 45.11 | 81.58 |

$IC_{50}$ was calculated by plotting the values of % inhibition against concentration using Microsoft EXCEL

## 4. Conclusion

The antioxidant, antibacterial and antifungal activity of volatile oils hydrodistilled from leaf, stem, fruit and root as well as the chemical compositions of the stem and root oils of *Duranta repens* are been reported for the first time. The findings from this work suggest the volatile oils could be used as adjuncts in lubricant, food, cosmetic and medicine.

## 5. References


1. Okoh SO, Asekun OT, Familoni OB, Afolayan AJ. Antioxidant and free radical-scavenging capacity of seed and shell essential oils extracted from *Abrus precatorius* (L). Antioxidants 2014;3(2):278-287.
2. Donaldson JR, Warner SL, Cates RG, Young DG. Assessment of antimicrobial activity of fourteen essential oils when using dilution and diffusion methods. Pharmaceutical Biology 2005;43(8):687-695.
3. Scur MC, Pinto FGS, Pandini JA, Costa WF, Leite CW, Temponi LG. Antimicrobial and antioxidant activity of the essential oil and different plant extracts of *Psidium cattleianum* Sabine. Brazilian Journal of Biology 2016;76(1):101-108.
4. Puri AV. *Duranta repens* Linn. (Verbenaceae): A comprehensive review of pharmacognostic, ethnomedicinal, pharmacological and phytochemical aspects. Asian Journal of Pharmaceutical and Clinical Research 2018;11(11):91-96.
5. Umaru IJ, Badruddin FA, Umaru HA. Phytochemical screening of essential oils and antibacterial activity and antioxidant properties of *Barringtonia asiatica* (L) leaf extract. Biochemistry Research International 2019;2(3):1-6.
6. Shahat AA, Nasif NM, Abousetta LM, Ibrahim NA, Cos P, Miert VS *et al*. Phytochemical investigation and antioxidant activity of *Duranta repens*. Phytotherapy Research. 2005; 19(12):1071-1073.
7. Abou-Setta LM, Nasif NM, Shahat AA. Phytochemical investigation and antiviral activity of *Duranta repens*. Journal of Applied Science Research 2007;3:1426-1433.
8. Sharma P, Khandelwal S, Singh T, Vijayvergia R. Phytochemical analysis and antifungal potential of *Duranta erecta* against some phytopathogenic fungi. International Journal of Pharmaceutical Sciences and Research 2012;3(7):2686-2689.
9. Edwin-Wosu NL, Okiwelu SN, Noutcha MAE. Traditional sources of mosquito repellents in southeast Nigeria. Journal of Biopesticide 2013;6(2):104-107.
10. Nikkon F, Saud ZA, Hossain K, Parvin S, Haque ME. Larvicidal effects of stem and fruits of *Duranta repens* against *Culex quinquefasciatus*. International Journal of Pharm Tech Research 2009;1.4:1709-1713.
11. Agomuo E, Amadi P, Ogunka-Nnoka C, Amadi B, Ifeanacho M, Njoku U. Characterization of the oils from *Duranta repens* leaf and seed. Oilseeds and fats, Crops and Lipids 2017;24(6):A601.
12. Xiao P. A pictorial encyclopedia of Chinese medical herbs. Tokyo: Chuokoron-Sha. Inc 1992.
13. Mandvi S, Showkat AG, Rajneesh KA, Rajendra S. Evaluation of *Duranta repens* for its ant fungi potential. International Journal of Medicinal Plants 2014;106:390-395.
14. Rahmatullah M, Jahan R, Azam FMS, Hosan S, Mollik MAH, Raman T. Folk medicinal uses of Verbenaceae family plants in Bangladesh. African Journal of Traditional and Complementary Alternative Medicine 2011;8(5):53-65.
15. Takeda Y, Morimoto Y, Matsumoto T, Ogimi C, Hirata E, Takushi A *et al*. Iridoid glycosides from the leaves and stems of *Duranta erecta*. Phytochemistry. 1995; 39:829-

833.
16. British Pharmacopoeia. H.M. Stationary office, London 1980;2:109.
17. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry, 5th Ed. Texensis Publishing, Gruver, Texas USA, 2017.
18. Satyal P. Development of GC-MS Database of Essential Oil Components by the Analysis of Natural Essential Oils and Synthetic Compounds and Discovery of Biologically Active Novel Chemotypes in Essential Oils. Ph.D. Dissertation, University of Alabama in Huntsville 2015.
19. Saleh AM, Savon C, Brooke W, Suziat DS. Antioxidant and free scavenging activity of essential oils. Ethnicity and Disease 2010;20:78-82.
20. Kumawat BK, Gupta M, Tarachand SY. Free radical-scavenging effect of various extracts of leaves of *Balanites aegyptiaca* (L.) Delile by DPPH method. Asian Journal of Plant Science and Research 2012;2(3):323-329.
21. Watt JM, Brandwijk MG. The medicinal and poisonous plants of Southern and Eastern Africa. 2nd Ed. E. and S Livingstone, London, United Kingdom 1962, 149.
22. Stachowiak GW, Batchelor AW. Engineering Tribology 4th Ed. Elsevier Amsterdam, Netherland, 2014, 59-119.
23. Kumar P, Lomash V, Jatav PC, Kumar A, Pant SC. Prenatal developmental toxicity study of *n*-heneicosane in Wistar rats. Toxicology and Industrial Health 2016;32(1):118-125.
24. Funaro CF, Böröczky K, Vargo EL, Schal C. identification of a queen and king recognition pheromone in the subterranean termite *Reticulitermes flavipes*. Proceedings of the National Academy of Sciences 2018;115(15):3888-3894.
25. Pascual G, Avgustinova A, Mejetta S, Martin M, Castellanos A, Attolini CS *et al*. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature 2016;541:41-45.
26. Smolinske SC. Handbook of Food, Drug and Cosmetic Excipients 1992, 247-248. ISBN 978-0-8493-3585-3.
27. Purnamadjaja AH, Russell, RA. Pheromone communication in a robot swarm: Necrophoric bee behaviour and its replication. Robotica 2005;23(6):731-742.
28. Carrasco F. Ingredientes de Cosmeticos. Dictionario de Ingredientes 4th Ed. C/Almojia, 14, 29007, Málaga, Spain, 2004, 428.
29. Thomas PS, Essien EE, Ascrizzi R, Flamini G. Composition of volatile oils from *Duranta repens* from leaves and fruits. Chemistry of Natural Compounds 2019;55(2):359-360.
30. Silva RF, Rezende CM, Santana HCD, Vieira RF, Alves RBN, Alviano DS *et al*. Composition and antimicrobial activity of the essential oils from the leaves and flowers of *Lippia stachyoides* var. Martiana (Verbenaceae). The Natural Products Journal 2014;4(4):241 247.
31. Ogbuagu AS, Okoro K, Alpuaka MU, Ogbuagu JO, Ekpunobi UE, Ohaekenyem EC. Quantitative determination of some secondary metabolites and the antibacterial effects of the leave extracts of *Duranta erecta*. American Journal of Biomedical Science and Engineering 2015;1(5):82-87.
32. Sikarwar M, Ganie SA, Agnihotri RK, Sharma R. Evaluation of *D. repens* for its antifungal potential. International Journal of Medicinal Plants 2014;106:2686-2689.
33. Jayalakshmi B, Raveesha KA, Amruthesh KN. Phytochemical investigation and antibacterial activity of some medicinal plants against pathogenic bacteria. Journal of Applied Pharmaceutical Sciences 2011;1(5):124-128.