International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-7, Issue-7, July 2021
ISSN: 2395-3470
www.ijseas.com

Contrastive Study of the Antimicrobial And Insecticidal Activities Of Essential Oils From Peels Of Citrus Species On Some Bacterial Isolates And A Household Pest

[1]Okafor-Elenwo, E.J. and [1]Enaigbe, A.A.

[1] Department of Biological Sciences, College of Natural and Applied Sciences, Igbinedion University, Okada, Nigeria

Corresponding author: *Enaigbe, A.A; Phone+2348131230105

Email: andrewenaigbe89@gmail.com ; https://orcid.org/0000-0002-3146-1999

ABSTRACT

The antimicrobial activities of five ethyl acetate Citrus peel oil extracts were tested against pathogenic bacterial isolates of *Bacillus. Subtilis, Streptococcus faecalis* and its insecticidal effects on *Musca domestica* (Housefly). This was performed by inoculating the isolates into the pure essential oil extract, contained in test tubes, flooded with nutrient agar media and observed for growth at stipulated standards. The sensitivity test was done by the disk diffusion method and minimum inhibitory concentration (MIC) was conducted to determine the lowest drug concentrations that inhibited the bacterial growths, while the insecticidal action on test pest was done by contact action. The sensitivity screening showed that, the peel oil extracts of *Citrus aurantifolia (lime), Citrus Medin*a (lemon) and *C. paradise (grape)* indicated no visible growths (-) of the test organisms *B. subtilis* and *S. faecalis*; while high growths (++) of the test bacteria were observed in the extract of *C. reticulate (tangerin).* The sensitivity test recorded the lowest inhibition zone of *C. medina* (16.3 mm) and *C. aurantifolia* (32.0 mm) against *B. subtilis;* C. paradise (10 mm) and *C. sinensis* (sweet orange) (35.3 mm) against *S. faecalis*. Consequently, the lowest minimum inhibitory concentration (MIC) value was recorded in the extract of *C. autantifolia* against the test bacterial isolates (1: 10000). More so, the mean lethal dose (LD_{50}) of the extract at 12, 18 and 24 hr exposure time on the pest ranged from 16.7µl to 39.6 µl, with the lowest scores (16.7 µl) and highest (39.6 µl) recorded in the extracts of *C. aurantifolia* and *C. paradise* respectively.

Keywords: Antimicrobial, insecticidal, bacterial isolates, peel oil extracts, sensitivity screening and minimum inhibitory concentration .

INTRODUCTION

Absolutely, the efficacious and discovery rates of synthetic therapeutic drugs and insecticides are on a decline and have resulted in deleterious clinical health challenges in recent times. So, natural chemical constituents of herbs may provide alternative

sources of antimicrobial and insecticidal properties with innate characteristic modes of activities (Edogbanya *et al.*, 2019).

The natural plants' parts; the leaves, roots, fruits, bark and seeds consist of essential oils with main chemical components (terpenoids) such as diterpenes, monoterpenes, sesquiterpenes. They are volatile secondary metabolit+es in plants that impart the usual aroma, taste and inhibitory effects on Citrus against antibiotic resistant bacteria and insect pests. However, the basic fulcrum and active molecular structures for drug synthetic fields are supplied by the rich indigenous plant sources (Preeti *et al.*, 2010).

Herbs have been utilized for the treatment of numerous infectious and non infectious diseases due to their easy accessibility, reliability, low toxicity and general acceptance by all religions and traditions globally. Meanwhile the World Health Organization (WHO) stated that, 80 % of the world population depends on herbal remedies for some aspect of primary health-care service delivery (Nayan and Shukla., 2011). In addition, a larger number of persons use plant derived concoction as the initial primary attempt of action to curb human ailments because of its reduced or insignificant side effects (Jia *et al.*, 2016). The other needs that promote herbal remedies include; resistance of certain organisms and insect pests to these synthetic drugs and insecticides. Subsequently, herbal preparations retaining life-giving nutrients such as vitamins and minerals contained in the original plant's composition and potential hazards associated with excessive intake and external application chemically synthesized therapeutics..

The oil peel of Citrus fruit is very rich in alkaloids, flavonoids, glycerols, phenols and volatile oils with huge pharmacological potentials against increasing trends of fungal infections, cancer, ulcer, migraine, inflammation and cell body radicals. Specifically, the Citrus fruit peels usually considered as agricultural and allied industrial by-products, serves as valuable precursor of essential oils and secondary metabolites with inhibitory plants 'active principles (Lawal *et al.*, 2016; Preeti *et al.*, 2017).

The ubiquitous nature of Citrus endowed it with numerous species (more than a thousand species) and entrusted as one of the world's prominent fruit crops that are produced in several countries with tropical or non tropical environmental conditions.

Environmentally safe approaches for insect control has been developed from plants that possess rich source of novel natural compounds. The effects of plants' extracts on household pests; insects, fleas, flies, lice, ticks cockroaches and bugs can be established in several aspects that include toxicity, growth inhibitor, suppression of reproductive process and decreased fecundity and fertility rates. The housefly (*Musca domestica*) is the commonest of all domestic flies, accounting for about 90 % in human habitations, considered a vector of serious diseases such as cholera (Muhammad *et al*., 2013). However, it has been reported that, there is a connectivity between plants used for pest control and medicinal or antimicrobial plants (Zettler and Cuperus, 2010).

More so,, a good number of studies also revealed insecticidal potential of citrus oils extracted from different Citrus species and their constituents at separate periods, a few of which are produced to be applied by the consumers against insect pests. Essential oils extracted from Citrus peels contained insecticidal substances, but the most active and predominant is the limonene, that ranges between 95 to 98 % of the Citrus peel oil by weight (Preeti *et al.*, 2017). Bernhoft (2010) indicated that, it is a naturally occurring compound with low toxicity for warm blooded animals and high toxic affinity for household pests.

Nevertheless, some of the key compounds for controlling microbes and insect pests definitely lie within the secondary metabolites that mainly occur in the diverse aromatic oil part of the plant. The aim of this study is to validate the claims by herbal medicine practitioners in their folklore, as to the efficacy of plants' materials in the treatment of various kinds of diseases.

MATERIALS AND METHODS

Collection of plant materials

The Citrus fruits employed in this analysis were purchased from Okada main market, Mission Road, Ovia North East Local Government, Edo State, Nigeria. five different species of ripe Citrus fruits; *Citrus aurantifola* (lime) *Citrus medina,* (lemon) *Citrus paradise* (grape), *Citrus reticulate* (tangerin) C. sinensis (sweet orange) were collected in clean state and immediately transported to the Microbiology laboratory, Igbinedion University, Nigeria.

Collection of test microorganisms

Sterile cultures of pathogenic bacterial isolates; *Bacillus subtilis* and *Streptococcus faecalis* were obtained from the Department of Clinical Pathology, University of Benin Teaching Hospital, Nigeria for the study. These test isolates were sub-cultured in nutrient agar and stored in nutrient agar slants in a refrigerator at 4 ℃ until required for use.

Collection and rearing of Household pests

The Houseflies (*Musca domestica*) were collected at the New Girls Hostel, Igbinedion University, Okada, Nigeria. The test pest samples were put onto three different sterilized glass jars containing 200 g wheat flour culture medium. The mouth tops of the jars were covered with muslin cloth, tied with rubber bands to prevent the escape of the pest. The houseflies were allowed to remain in the culture medium for 14 days for maturity, egg laying and population to be of approximate uniform age for the investigation (Muhammad *et al*., 2013).

Insect pest toxicity assay (mortality)

Insecticidal properties of the different varieties of Citrus essential oils used in this work were tested against *Musca domestica* (houseflies) by contact action. Whatman No1 filter papers were cut prior to size and shape of petri plates inoculated with different concentrations of essential oils; 5, 10, 15, 20, 25 ug in 100 ug methanol using micro-pipette. The sterile filter papers were dried in an oven to completely remove the solvent by evaporation, inserted at the bottom of the petri plate and the culture flour uniformly spread on the whole surface part of the petri plate. This experiment was done in duplicates, while the petri plates were stored in the dark and the mortality degree was recorded after 12, 24 and 36 hr respectively. (Muhammad *et al.*, 2013).

Extraction Of Essential Oils From Citrus Fruits

The collected Citrus peels were cut into pieces and dried in the oven for 24 hr at 60 ℃ and the dried peels were grind using an automated electric blender. The ground peel powder was placed in Soxhlet apparatus for the extraction of oil,using the wet-steam distillation technique as described by (Barrow and Feltham, 2003; Cassini *et al.,* 2016).

In vitro antimicrobial screening of test extracts

The antimicrobial tests were conducted using the test tube technique: Ten milliliter of each Citrus oil peel extract was measured into 2 test tubes, 1 ml each of the two test organisms was used to inoculate each of the test tubes and left for 24 hr. Sterile nutrient agar was poured onto pre -sterilized petri plates and allowed to set. These agar plates were then seeded with 0.5 ml of the test organism which was inoculated into the pure extract in the test tubes and spread evenly with a flamed, but cool glass spreader to derive effective growth of a smooth bacterial lawn. Finally, the plates were then incubated at 37 ℃ for 24 to 48 hr. However, control plates were prepared using distilled water and 2.5 % phenol as negative and positive controls respectively (Lawal *et al.*, 2016; Hans *et al.*, 2017).

Sensitivity test

The extracts with high spectra of activities against the test organisms obtained from the test tube approach of plants' screening were further confirmed for their extent of inhibiting the growth of prevailing organisms by employing the Disc diffusion method. This was conducted by sterilizing the filter paper soaked in an appropriate extract before each was inserted at middle of the plates previously flooded with test organisms. The plates were incubated at 37 ℃ for 24 to 48 hr and the cleared zones of inhibition produced around the epicenter of the plates were observed and measured in millimeter (Cassini *et al.* 2016; Hans, *et al*., 2017).

Minimum inhibitory concentration (MIC)

Five milliliters (5 ml) of varying dilutions of the extract was prepared using peptone water as diluents; the serial dilution made ranged from 10^{-1} to 10^{-5} (1: 10 to 1: 100000). In preparing the dilution, 8 test tubes containing antibiotic concentration (Streptomycin 100 mg) was prepared and inoculated with standard quantity of each extract. These dilutions were aseptically inoculated with the test organisms and incubated at 37 ^{o}C for 24 to 48 hr. Thereafter, 1 ml of each dilution was inoculated into the 8 nutrient media plates, incubated at 37 ℃ for 24 to 48 hr (Nayan and Shukla, 2011).

Statistical analysis

The POLO programme was used to determine the LD_{50} (Cassini *et al.*, 2017). Correlation and linear regression analysis were performed to ascertain the dose responses relationship.

RESULTS

The Citrus peel essential oil extracts of *C. aurantifolia, C. medina, C. paradise* and *C. reticulate and C. sinensis completely* inhibited the *Streptococcus faecalis* growth (-) meaning that, no colonies were observed, while the extract of *Citrus aurantifolia* inhibited only the growth of *Bacillus subtilis.* High bacterial growth was reported in the extract of *C. reticulate* against the test isolates (++) (Table 1).

Table 1: Effects of essential oil extracts on growth rate of bacterial isolates.

Extracts	*Bacillus subtilis*	*Streptococcus faecalis*
C. *aurantifolia*	–	–
C.medina	–	+
C.paradise	–	+
C. reticulate	++	++
C.sinensis	+	+
Sterile water	++	++
2.5% phenol	–	–

Key: No bacterial growth (-) Slight growth (+) High growth (++)

The peel oil extracts that indicated high potential of antimicrobial properties: *C. aurantifolia, C. medina, C. paradise and C.sinensis* were further screened to determine the level of clearing zones of test bacterial isolates using antibiotic drug (Streptomycin 100 mg) and measured in millimeters (mm).

Table 2 Inhibition zones (mm) of the potential essential oil extracts on test bacterial isolates

Extracts	*B. subtilis*	*S. faecalis*
C. aurantifolia	32.0	23.2
C. medina	16.3	29.1
C. paradise	25.4	10.0
C. sinensis	26 4	35.3
Distilled water	9.00	15.0
2.5 % Phenol	40.3	33.0

The minimum inhibitory concentration (MIC) of an extract or chemotherapeutic agent is the lowest concentration of the chemical substance present in plant, that prevents or stops visible growth of an organism. At the lowest extract dilution factor, the MIC was reported as followed: *C. aurantifolia*: 1:10000; 1:10000, *C. medina*: 1:1000; 1:1000, C. paradise: 1: 1000; 1: 100 and *C. sinensis*: 1:1000; 1:100 respectively (Tables 3 - 5).

Table 3: Minimum inhibitory concentration (MIC) of oil extract (*C. aurantifolia*) for test isolates.

Extracts dilutions	***B. subtilis***	***S. faecalis***
1: 10	–	–
1: 100	–	–
1: 1000	–	–
1: 10000	–	–
1: 100000	++	+
MIC	1: 0000	1: 0000

Key: No growth (–) moderate growth (+) extreme growth (++)

Table 4: Minimum inhibitory concentration (MIC) of essential oil extract (*C. medina*) for test isolates

Extract dilutions	***C. subtilis***	***S. faecalis***
1: 10	–	–
1: 100	–	–
1: 000	–	–
1: 10000	+	+
1: 100000	+	++
MIC	1: 1000	1: 1000

Key: No growth (–) moderate growth (+) extreme growth (++)

Table 5: Minimum inhibitory concentration (MIC) of essential oil extract (*C. paradise*) for test isolates

Extract dilutions	***B. subtilis***	***S. faecalis***
1: 10	–	–
1: 00	–	–
1: 1000	–	+
1:10000	++	+
1: 00000	++	++
MIC	1: 1000	1: 100

Key: No growth (–) moderate growth (+) extreme growth (++)

On the responses of the insect pest to the activities of the oil extracts, the essential oils extracted were able to kill the pest by contact action. The mean lethal dose (LD_{50}) of extract in response to exposure time of 6 hr interval (12, 18 and 24 hr) was recorded thus: *C. autantifolia*, 16.7 µl; *C. medina*, 16.7 µl; *C. paradise*, 39.6µl; *C. reticulate*, 35.8 µl and *C. sinensis*, 32.3 µl. The mean $LD_{50,}$ ranged between 16.7 to 39.6 µl indicating that, at lowest concentration of 16.7 µl, the extracts of *C. aurantifolia* and *C.*medina exhibited more inhibitory properties than the extract of C. paradise (39.6 µl).Table 6.

Table 6: The toxicity assay of extracts against the household pest (*Musca domestica*) in response to exposure time

Oil extract	Exposure time	LD_{50} (µl)	LCL - HCL	P- Value
Citrus aurantifolia	12	24.2	17.1 - 247.4	0.543
	18	15.3	14.44 - 55.3	0.178
	24	10.1	8.41 - 58.4	0.512
		M = 16.7		
Citrus medina	12	30.4	20.4 - 115.2	0.015
	18	20.1	19.0 - 121.0	0.004
	24	25.0	16.3 - 302.1	0.103
		M = 16.7		
Citrus paradise	12	40.0	19.4 - 118.3	0.126
	18	33.4	19.0-220.0	0.102
	24	45.3	25.2 - 197.3	0.065
		M = 39.6		
Citrus reticulate	12	35.0	15.3 - 200. 0	0.277
	18	22.1	65.0 - 175.0	0.002
	24	50.4	28.0 - 221.3	0.030
		M = 35.8		
Citrus sinensis	12	30.2	28.3 - 345.2	0.240
	18	26.2	35.3 - 154.2	0.070
	24	41.0	15.0 - 231.4	0.214
		M = 32.5		

Key: LD_{50} = lethal dose, LCL = Lower confidence limit, UCL = Upper confidence limit, M = Mean. $P > 0.05$, no significant difference; $P < 0.05$, significantly different; $P < 0.01$, highly significantly different; $P < 0.001$, very highly significantly different

DISCUSSION

Inappropriate application of therapeutics for the treatment of infectious diseases has emanated to the proliferation of several multi drug-resistant bacteria, which has enhanced the increasing health challenges that, fostered or culminated the desire to search or screen for plant materials in order to identify new compounds and

antimicrobial substances naturally endowed in plants (Badawy and Abdelgaleil, 2014).

The notion that certain plants possessed curing prowess had long been known even before the advent of microbes by mankind; that they indeed contained what has presently been classified as antimicrobial and insecticidal active principles (Jia *et al.*, 2016; Hans *et al.*, 2017). Comparatively, in determining the insecticidal effects of Citrus varieties on tested pest (*Musca* domestica), the mean $LD_{50,}$ ranged between 16.7 to 39.6 µl indicating that, at lowest concentration of 16.7 µl, the extracts of *C. aurantifolia* and *C.medina* exhibited more inhibitory properties than the extract of *C. paradise* (39.6 µl).

the essential oil of *C. aurantifolia* showed more toxicity (15.3 and 10.1 µl) at exposure time of 18 and 24 hr respectively against the tested pest, than that of *C. paradise* (33.4 and 45.3 µl) which indicated less toxicity.

Antimicrobial activities of several Citrus peel essential oil extracts have been investigated and revealed potent antibacterial effects against; *Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus cereus and Escherichia coli* (Preeti *et al.*, 2010) analyzed the antimicrobial activity of *Citrus aurantifolia* (lime) and *Citrus sinensis* (sweet orange) methanolic and ethyl acetate fruit peel extracts. It was however found that, the ethyl acetate extract indicated more effect against several pathogenic bacterial isolates such as *Escherichia coli, Salmonella typhi* and *Streptococcus faecalis.*

In contrast of the antimicrobial actions of the five varieties of Citrus essential oils, the *C. aurantifolia* exhibited the most potent extract against the tested organisms; *Bacillus subtilis* and *Streptococcus faecalis,* because, there were no growths. This was followed by *C. medina* and *C. paradise*, while the *C. reticulate* displayed the least extract activity due to the very high growth recorded.

The results of this study differ from the work of same research as stated by Lawal *et al.*, (2016) that *C. sinensis* oil showed the most killing impact than *C. aurantifolia, C. medina* on the tested organisms of the five essential oils used, but in diadem with the report revealed by Badawy and Abdelgaliel (2014) which enunciated that *C. aurantifolia* and *C. paradise* oil are more effective as antimicrobial agents than the *C.*

reticulate and *C sinensis* extracts. The discrepancy in result could be due to difference in the environmental conditions of the different location sources, as clearly reported by Edogbanya *et al.*, (2019) that, location causes variation in the chemical composition of essential oils.

The growth inhibition zone measured ranged from 16.3 to 32.0 mm for the *B. subtilis* with the lowest cleared are recorded in the extract of *C. medina* (16.3 mm) and the highest was in the extract of *C. aurantifolia* (32.0 mm). Inhibition cleared zone of the tested extracts against the *Streptococcus faecalis* was between 10.0 and 35.3 mm; the lowest cleared area was recorded in the extract of *C. paradise*, while the highest killed zone was observed in the extract of *C. sinensis* (Table 2). These very high cleared zones reported in *C. aurantifolia* and *C. sinensis* showed the rich potent chemical principles contained in extracts of these essential oils, as the most active agents in preventing the growth and transmission of the tested isolates. However, the low and high inhibition levels obtained from the controls: sterile water (negative control) and 2.5 % Phenol (positive control) relatively confirmed the presence of the active compounds in plants to have been responsible for antimicrobial activities.

The minimum inhibitory concentration (MIC) described as the lowest concentration of chemical (drug) that prevents visible growth of an organism is considered to indicate; lower MIC values meant that, less drug is required to inhibit microbial growth. Therefore, drugs with lower MIC scores are more effective in the control of infectious agents (Shyla *et al.*, 2017).

The findings revealed that, *C. aurantifolia* and *C. medina.* considered extracts, with high potential antimicrobial properties against the tested isolates (1:10000; 1:1000) extract dilutions respectively) (Tables 3 and 4). Consequently, the MIC for *C. paradise.* against tested isolates were: 1:1000; 1:00 (Table 5). This showed that, this extract concentration is more potent on *B. subtilis* (lower MIC value) than the *S. faecalis.* Nevertheless, the sensitivity difference between the Gram negative (*B. subtilis*) and the Gram positive (*S. faecalis*) could be as a result of differences in their cell wall compositions, as the latter contained rigid peptidoglycan layer in its cell wall, which functions as a permeability barrier; whereas, the former possessed an outer less rigid cell membrane (Edogbanya *et al.*, 2019).

In general,the inhibitory activity of the Citrus essential oils may be a combined effect of D- Limonene, as main chemical constituent with other compounds such as flavonoids, phenolics and terpenoids, that have been revealed to inhibit the growth of numerous pathogenic bacterial isolates (Shyla *et al*., 2017).

CONCLUSION

The use of Citrus peel oil extracts from *C. aurantifolia*, *C. medica* should be considered as therapeutics for infections and associated diseases caused by the test organisms. Succinctly, the extract of *Citrus paradise* and *C. sinensis* being cost effective, availability, eco-friendly with less toxicity may be recommended as options to the use of synthetic insecticides. Hence, this study has validated the claimed viability and efficacy of plants' materials in the herbal system of health care delivery services to humanity.

REFERENCES

Barrow, G. I. and Feltham, R. K. A. (2003). *Cowan and Steel's Manual of Medical Bacteria. (*3rd ed.) Cambridge University Press, UK.. 352pp. Doi: https://doi.org/10.1017/CBO9780511527104

Cassini, A., Hathaway, S., Havelaar, A., Koopmans, M., Koutsoumanis, K., Messens, W. and Scheutz, F. (2016). Microbiological risk assessment. *European Food and Safety Association* **4**: 1 - 10. Doi: https://doi:10.2903/j.efsa.2016.s0507

Edogbanya, P,R. Suleiman, M.O., Olorunmola, B. and Ojiagbe, I.J. (2019). Comparative Study of the Antimicrobial effects of essential oils from peels of three citrus fruits. Mediterranean Journal of Biology and Medicine **4**(2): 49 - 53.

Hans, S., Yang, X., Pan, Y. and Feng, H. (2017). L – Securinine inhibits the proliferation of 549 lung cancer cells and promotes DKKI promoter methylation. *Oncology Letters* **14**(4): 4243 – 4248. doi: http://doi.org/10.3892/ol.2017.6693.

Jia, M., Chem, L., Xin, H.L., Zheng, C.J. and Han, T. (2016). A friendly relationship between endophytic fungi and medicinal plants. *Front Microbiology* **7**: 906. Doi: http://doi.org/103389/fmicb.2016.00906.

Lawal, D., Bala, J.A., Aliyu, S.Y. and Huguma, M.A. (2016). Phytochemical Screening and In Vitro Antibacterial Studies of the Ethanoic Extract of *Citrus sinensis* peel against some Clinical Bacterial Isolates. *International Journal of Innovative and Applied Studies* 2(2): 138 -145.

Muhammed, S., Dilbar, H. Rahat, H.R., Hafiz, S. and Ghulum, G. (2013). Insecticidal activities of two citrus oils against *Tribilium castaneum* (Herbst). *American Journal of Research Communication* **1**(6): 67 - 73.

Nayan, R. and Shukla, V.J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula*: An ethnomedicinal plant. *Journal of Advanced Pharmaceutical Technological Research* **2**(2): 104 – 109. http://doi.org/doi104103/2231-4040.82956.

Preeti, R. Vimal, l. Devanathan, M. (2017). Antimicrobial and Antioxidant efficacy of some medicinal plants against food borne pathogens. *Advanced Biological Research* **2**: 122 - 125.

Shyla, A. Faysun, N.F. Mushikkia, M. J. and Khokon, M.A. (2017). Antimicrobial activity of different citrus fruits. *Specialty Journal of Medical Research and Health Sciences* **2**(1): 25 -32.

Zettler, J.L. and Cuperus, G.W. (2018). Pesticide resistance in *Tribolium castaneum* and *Rhizopertha dominica* in wheat. *Journal of Economic Ethnomology* **122**: 567 - 574.