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Antibacterial Activities of Aqueous, Acetone, and Ethanolic Extracts of Lime (*Citrus aurantifolia*) Leaves against some Bacteria of Public Health importance

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ABSTRACT
Citrus aurantifolia is a common plant that is used for the production of fruit juices and preparation of decoctions used for the treatment of diseases in Nigeria and many other nations. The antibacterial activities of aqueous, acetone, and ethanolic extracts of *Citrus aurantifolia* leaves were investigated in this study. The leaves of the plant used for this study were obtained from Okoloba town in Kolokuma/Opokuma local government area of Bayelsa State. The plants were shade dried and grounded into powder using blender. The extraction was done using water, acetone, and ethanol. The study made use of agar well diffusion method for the sensitivity testing. Results showed that the zone of inhibition shown by *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus*, and *Klebsiella* species, was 10.00mm, 9.33 mm, 9.67 mm, and 9.33mm for aqueous extracts, 10.67mm, 10.00mm, 12.67mm, and 9.00mm for acetone extracts, and 13.00, 11.00mm, 13.33mm, and 10.00mm for ethanolic extracts respectively. Statistically, there was no significant deviations ($p>0.05$) in the efficacy of each of the extracts across the organisms. The efficacy of the extracts was in the statistical order: aqueous = acetone = ethanol <Ampiclox for each of the test organisms except for *Staphylococcus aureus* that showed order of aqueous < acetone = ethanol <Ampiclox. This suggests that the antibacterial potentials of the extracts are affected differently by different solvents, and are influenced by the biochemical characteristics of the isolates. Overall, the zone of inhibition demonstrated that *Citrus aurantifolia* might be exploited to build broad-spectrum antibiotics. As a result, the bioactive chemicals responsible for *Citrus aurantifolia* leaves' antibacterial activity need to be extracted.
Keywords: Antibiotics, *Citrus aurantifolia*, Medicinal plant, Microorganisms

1. INTRODUCTION

Antibiotic resistance is becoming more common, posing a huge concern in both the clinical and pharmaceutical industry (George-Okafor et al., 2019). As a result, a broad examination of plants' antimicrobial ability has been conducted to counteract this trend. Plants have proven to be a viable alternative for medicine development. Medicinal plants are plants that have the ability to heal people of one or more disease conditions (Epidi et al., 2016a,b; Izah et al., 2019a-c; 2018a-e; Kigigha et al., 2015, 2016, 2018a,b). The benefits of medicinal plants have received increased attention in recent years because of their importance in remedy, safety, efficiency, and economy, as well as their usefulness as food. As such they have long been regarded as an important source of natural elements for human health.
Authors have described medicinal plants as a gift from nature, which constitute the foundation of primary health care for the majority of people, particularly in underdeveloped nations (Momoh et al., 2014; Masih

et al., 2014). Plants have been utilized to heal several human diseases since ancient times, particularly in rural areas of many African societies. Traditional medicine is preferred due to the inaccessibility of modern drugs and economic concerns (Epidi et al., 2016a,b; Amole and IIori, 2010). This is common among rural dwellers.

The existence of many bioactive and phytochemical compounds found in plants is linked to their use as therapeutics (Hassan, 2013; Epidi et al., 2016a,b; Kigigha 2016). Several bioactive and phytochemical compounds have been isolated from different plants. Some of the metabolites and phytochemicals found in plants include isoflavoes, antocyanins, steroids, terpense, alkaloids, glycosides, tannins, phenolic compounds, flavonoids, sesquiterpenes, diterpenes, triterpene, saponins, coumarins, quinine and monoterpenes etc (Pascaline *et al.,* 2011; Kigigha et al., 2015; Doherty *et al.,* 2010; Osuntokun and Oluwafoise, 2015; Eleazu *et al.,* 2015).

Antimicrobial properties have been discovered in a number of plants. Medicinal plants are plants whose one or more parts (such as roots, stem bark, leaves, fruits etc) are used as therapeutics (Izah et al., 2019a-c; Epidi et al., 2016a,b). Several plant species have been reported to possess antimicrobial potentials. The usage of plants for the treatment of microbial infections is dependent on their availability in a given place, the users' level of education, and their understanding of their efficacy.

Citrus (in the Rutaceae family) is one of the most frequently consumed and distributed fruits in the world. *Citrus* has several species. *Citrus* fruits are one of the most popular fruits and have a significant economic impact. Because of their nutritious richness and delightful flavour, these fruits are most typically consumed as fresh whole fruits or juices (Kelebek and Selli, 2011). Some of these *Citrus* fruits and juices have also been used as functional foods and beverages with the potential to treat diet-related disorders in persons with a variety of health problems.

Citrus aurantifolia is primarily utilized in everyday consumption, as well as in the production of fruit juice (Narang and Jiraungkoorskul, 2016). It's commonly used for its antibacterial, anticancer, antidiabetic, antifungal, antihypertensive, anti-inflammation, anti-lipidemia, and antioxidant characteristics, as well as its ability to protect the heart, liver, and bones, as well as to avoid urinary illnesses (Narang and Jiraungkoorskul, 2016). Alkaloids, carotenoids, coumarins, essential oils, flavonoids, phenolic acids, and triterpenoids are its secondary metabolites. Apigenin, hesperetin, kaempferol, limonoids, quercetin, naringenin, nobiletin, and rutin are other key elements that contribute to its restorative qualities (Narang and Jiraungkoorskul, 2016).

Several studies have been carried out on the antimicrobial potentials of some parts of the *Citrus aurantifolia*. Some of the parts that is commonly studied include fresh fruit (George-Okafor et al., 2019; Aibinu et al., 2007), leaves (Al-Aamri et al., 2018; Akinnibosun and Edionwe, 2015; Ogbeide and McSionel, 2019; Abubakar et al., 2018). However, several solvents have been used to extract the active compounds but comparative study on acetone, aqueous and ethanol extract of the leaves of *Citrus aurantifolia* appears scanty in literature. Therefore, the focus of this paper is to evaluate the antibacterial efficacy of acetone, aqueous and ethanol extract of *Citrus aurantifolia* leaves against some bacteria of public health importance.

MATERIALS AND METHODS

2.1 Sample Procurement

Leaves of *Citrus aurantifolia* used in this study were obtained from Okoloba in Kolokuma/Opokuma Local Government Area of Bayelsa State, Nigeria.

2.2 Sample Preparation

Leaf samples of *Citrus aurantifolia* were dried at room temperature. Thereafter, they were macerated using sterile pestle and mortar. The samples were further blended to obtain fine powder.

2.3 Extraction Method

With minor adjustments, the extraction was performed using the soaking process described by Doherty et al., (2010), Chiejina & Ukeh (2012), Kigigha et al., (2015), Kigigha (2018a,b), Izah et al., (2019a-c). The extraction was done with both hot and cold distilled water. 5g of the combined samples were extracted

individually with 10ml of ethanol, aqueous, and acetone. After 3 days of soaking, the samples were filtered through muslin fabric and the extracts were collected in a conical flask. Whiteman filter paper was used to further filter the extracts. The filtrates that resulted were concentrated.

2.4 Source and Preparation of Organisms

The microorganisms employed in this investigation were from a stock culture at the Federal Medical Centre's Microbiology and Parasitology Unit in Yenagoa, Bayelsa State, Nigeria. Subculturing was used to ensure that the bacteria were pure. Placing *Staphylococcus aureus* on Mannitol Salt Agar, which revealed yellow colouring, was used to identify it. It was also grown on Nutrient Agar, with the results being subjected to a congulase test using the Cheesbrough guide (2004). MacConkey agar and Levine's eosin Methylene Blue (EMB) agar were also streaked with the *E.coli* employed in the investigation. The development of a greenish sheen with small nucleated colonies on plates containing EMB (Eosin Methylene Blue) agar after 24 hours of aerobic incubation at 37°C showed the presence of *E. coli*. The presence of *E.coli* was confirmed by growth on MacConkey agar with pinkish red growth that had a metallic sheen or reflection (Benson, 2002). Indole, Methly red, Catalase test utilizing the scheme provided by Benson (2002) and Cheesbrough (2004). The *Klebsiella* species employed in this study were also confirmed by performing biochemical tests on the organisms, following the guide provided by Benson (2002) and Cheesbrough (2004).

2.5 Antimicrobial Screening of the Extract

The sensitive assessment of the aqueous, acetone and ethanolic extract of *Citrus aurantifolia* leaves was carried out using agar well diffusion method previously described and used by Lino and Deogracious (2006), Kigigha et al. (2015, 2016, 2018a,b), Izah and Aseibai (2018), Izah et al. (2019a-c, 2018a-d). Approximately 20ml of sterile prepared nutrient agar was poured into sterile petri dish and allowed to harden. The test organisms were inoculated unto sterile peptone water, and it was incubated for 24 hours. Thereafter, 2ml was measured and spread over the solidified agar plates. A sterile cork borer of 6mm in diameter was used to make holes in the agar. Three wells per plate were made. Each being for the different solvents used in the study. The plates were inverted and labeled on the bottom with the various concentrations that were to be placed in each of the wells. 0.2 ml extract was poured unto the agar wells. Under aerobic conditions, all of the plates were incubated for 24 hours at 37°C. The inhibitory zones that resulted were measured using meter rule in milliliter. 1% Ampiclox was used as the control.

2.6 Statistical Analysis

The statistical analysis was carried out using SPSS software version 20. The information was presented as Mean + Standard Deviation. One-way analysis of variance was used to show significance variations at a threshold of 0.05. Multiple comparisons between the means of each of the species, as well as the solvents, were made using the Tukey Honesty Significant Difference test statistics.

3. RESULTS AND DISCUSSION

Table 1 shows the zone of inhibition (mm) of aqueous, acetone and ethanolic extracts of *Citrus aurantifolia*. For *Escherichia coli, Pseudomonas* species, *Staphylococcus aureus*, and *Klebsiella*, the zones of inhibition were 10.00mm, 9.33mm, 9.67mm, and 9.33mm, respectively (aqueous), 10.67mm, 10.00mm, 12.67mm, and 9.00mm, respectively (acetone extracts), and 13.00m, 11.00mm, 13.33m, and 10.67mm, respectively (acetone extracts) (for ethanol extracts). For *Citrus aurantifolia* aqueous and ethanol leaf extract, there was no significant variation in the zone of inhibition across the isolates ($P>0.05$). However, there was a significant difference ($P<0.05$) in the inhibitory zone of *Citrus aurantifolia* acetone leaf extract. The Turkey test revealed that the mean result for staphylococcus aureus and Klebsiella species is responsible for the considerable variation.

Table 2 shows the zone of inhibition exhibited by *Escherichia coli, Staphylococcus aureus, Pseudomonas*, and *Klebsiella* species when exposed to aqueous, acetone, ethanol extracts of *Citrus aurantifolia* and ampiclox. The zone of inhibition of *Escherichia coli* for aqueous, acetone, ethanol extracts and ampiclox were 10.00mm, 10.67mm, 13.00mm and 24.00mm, respectively. Statistically, there was no significant difference ($P>0.05$) across the various solvent extracts except for ampiclox that differs

significantly from other solvents. The zone of inhibition for *Pseudomonas* for aqueous, acetone, ethanol extracts and ampiclox were 9.33mm, 10.00mm, 11.00mm and 22.67mm, respectively. Statistically there was no significant difference (P>0.05) across the various solvent extracts except for ampiclox that differs from other solvents. The zone of inhibition of *Klebsiella* for aqueous, acetone, ethanol extracts and ampiclox were 9.33mm, 9.00mm, 10.67mm and 20.00mm respectively. Statistically, there was no significant difference (P>0.05) across the various solvent extracts for Ampiclox that differs from other solvents.

When *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas aeruginosa,* and *Klebsiella* species were exposed to aqueous, acetone, ethanol extracts of *Citrus aurantifolia*, and ampiclox, they showed a zone of inhibition. The zones of inhibition for *Escherichia coli* were 10.00mm, 10.67mm, 13.00mm, and 24.00mm, respectively, for aqueous, acetone, ethanol extracts, and ampiclox. Except for ampiclox, which differs greatly from the other solvents, there was no statistically significant difference (P>0.05) between the various solvent extracts. *Pseudomonas* inhibition zones were 9.33mm, 10.00mm, 11.00mm, and 22.67mm for aqueous, acetone, ethanol extracts, and ampiclox, respectively. Except for ampiclox, which differs from the other solvents, there was no statistically significant difference (P>0.05) between the various solvent extracts. *Klebsiella* inhibition zones were 9.33mm, 9.00mm, 10.67mm, and 20.00mm for aqueous, acetone, ethanol extracts, and ampiclox, respectively. There was no statistically significant difference (P>0.05) between the various solvent extracts for Ampiclox when compared to other solvents.

Table 1: Zones of inhibition (mm) of aqueous, acetone and ethanol water extracts of *Citrus aurantifolia*.

Isolates	**Aqueous**	**Acetone**	**Ethanol**
Escherichia coli	10.00±1.00a	10.67±2.08ab	13.00±1.00a
Pseudomonas species	9.33±1.53a	10.00±1.00ab	11.00±0.00a
Staphylococcus aureus	9.67±0.58a	12.67±0.58b	13.33±1.15a
Klebsiella species	9.33±1.53a	9.00±1.00a	10.67±1.53a

Data is expressed as mean ± standard deviation. The same letters represent no significant difference (P>0.05) according to Turkey Honesty Significant Difference Statistics.

Table 2: Zones of inhibition (mm) exhibited by *E. coli, Staphylococcus aureus, Pseudomonas* species and *Klebsiella* species exposed to aqueous, acetone and ethanolic extracts of *Citrus aurantifolia*.

Extracts	*Escherichia coli*	*Pseudomonas* species	*Staphylococcus aureus*	*Klebsiella* species
Aqueous	10.00±1.00a	9.33±1.53a	9.67±0.58a	9.33±1.53a
Acetone	10.67±2.08a	10.00±1.00a	12.67±0.58b	9.00±1.00a
Ethanol	13.00±1.00a	11.00±0.00a	13.33±1.15b	10.67±1.53a
Ampiclox	24.00±1.00b	22.67±1.53b	21.67±1.53c	20.00±1.00b

Data is expressed as mean ± standard deviation. The same letters represent no significant difference (P>0.05) according to Turkey Honestly significant Differences Statistics.

A close examination of Table 1 shows an inhibition (mm) of aqueous, acetone and ethanolic extracts of *C. aurantifolia* which identifies the potency of *C. aurantifolia* on *E.coli*, *Pseudomonas* species, *Staphylococcus aureus* and *Klebsiella*. The activities of the *Citrus* aurantifolia extracts on the test organisms suggest that they can inhibit the growth of microorganism (Aibinu et al., 2007; Al-Aamri et al., 2018; Akinnibosun and Edionwe, 2015; Ogbeide and McSionel, 2019; Abubakar et al., 2018). The ability of the *Citrus aurantifolia* extract to inhibit the growth of bacteria used in the study could be due to the presence of phytochemical and bioactive compounds they possess (Epidi et al., 2016a,b). Some of the active phytochemicals found in *Citrus aurantifolia* include saponins, tannins, flavonoids, steroids, alkaloids, Phenols, Steroids, Terpenoid and cardiac glycosides (Akinnibosun and Edionwe, 2015; Abubakar et al., 2018). Some of these phytochemicals such as alkaloids helps to wade of pests including

microbes (Kigigha et al., 2015). Some phenolic compounds found in plants also possess antimicrobial potentials.

In table 2, it was noticed that the inhibition exhibited by *E.coli, Staphylococcus aureus, Pseudomonas* and *Klebsiella* species when exposed to aqueous, acetone, ethanol extracts of *C. aurantifolia* and ampiclox. Base on the result, there was significant differences in the zone of inhibition between the known antibiotics (1% ampiclox) and the *Citrus aurantifolia* extracts. The ampiclox showed a superior activity, followed by ethanolic extract. The aqueous extract showed the least inhibition against all the test organisms. Authors have variously reported that ethanolic extracts of plant is more potent to microbes as against the aqueous extract (Abubakar et al., 2018; Kigigha et al., 2015, 2016; Epidi et al., 2016a,b). The differences could be due to polarity of the solvents used for extract. The statistical variations observed when the test organisms were compared suggest differences in the biochemical composition of the organisms.

The findings of this study is in consonance with previous studies. For instance, Abubakar et al. (2018) reported zone of inhibition of 14.90mm, 14.49mm, 13.77mm and 12.01mm for *Shigella, Klebsiella, Escherichia coli* and *Salmonella typhi*, respectively. Ogbeide and McSionel (2015) reported that leave extract of *Citrus aurantifolia* is potent against *Pseudomonas aeruginosa, Escherichiia coli, Staphylococcus aureus* and *Klebsiella* species. Aibinu et al. (2007) reported that the fruit of *Citrus aurantifolia* is potent against *Staphylococcus aureus, Salmonella paratyphi, Shigella flexnerii, Streptococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Citrobacter* and *Serratia* species,. The findings is also comparable with report from different plants including *Capsicum frutescens* (Izah et al., 2019a,b), *Costus afer* (Izah et al., 2019c), *Anacardium occidentale* (Izah et al., 2018a), *Carica papaya* (Izah et al., 2018b), *Vernonia amygdalina* and *Ocimum gratissimum* (Izah et al., 2018c), *Myristica fragrans* (Izah et al., 2018d), *Cymbopogon citratus* (Izah and Aseibai, 2018; Kigigha et al., 2018a), *Musanga cecropioides* (Kigigha et al., 2016), *Aframomum melegueta* (Kigigha et al., 2015), *Garcinia kola* and *Buchholzia coriacea* (Kigigha et al., 2018b).

4. CONCLUSION

The study assessed the antibacterial activities of leave extract of *Citrus aurantifolia.* Water, acetone and ethanolic were used as the extraction solvents. The results showed that there was significant variation in the zone of inhibitions exhibited by the bacteria isolates. The efficacy of the plant extracts were in the order: 1% ampiclox > ethanol = acetone > aqueous for *Klebsiella*, and 1% ampiclox > ethanol = acetone + aqueous for *E.coli, Staphylococcus aureus* and *Pseudomonas* species. The activities of *Citrus aurantifolia* against all the test organisms, suggest that it can be used for the development of broad spectrum antibiotics. Therefore, research should be carried out to purify the compounds that make the plant confers therapeutic potentials.

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