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**Akinro EB**  
Department of Science  
Laboratory and Technology,  
Osun State Polytechnic, Iree

**Oladipupo OA**  
Department of Science  
Laboratory and Technology,  
Osun State Polytechnic, Iree

**Abejaye OA**  
Department of Science  
Laboratory and Technology,  
Osun State Polytechnic, Iree

**Busari T**  
Department of Science  
Laboratory and Technology,  
Osun State Polytechnic, Iree

**Aboderin EA**  
Department of Applied  
Sciences, Osun State  
Polytechnic, Iree

**Olarewaju SO**  
Department of Science  
Laboratory and Technology,  
Osun State Polytechnic, Iree

**Adetuberu IA**  
Department of Applied  
Sciences, Osun State  
Polytechnic, Iree

**Corresponding Author:**  
**Akinro EB**  
Department of Science  
Laboratory and Technology,  
Osun State Polytechnic, Iree

## Comparative antibacterial activity of orange seed oil and antibiotics of choice against some food borne pathogens

**Akinro EB, Oladipupo OA, Abejaye OA, Busari T, Aboderin EA, Olarewaju SO and Adetuberu IA**

### Abstract

In this present study, crude antibacterial potential of citrus seed oil was investigated against some selected food borne pathogens (*Bacillus cereus*, *Klebsiella* sp., *E.coli*, *Staphylococcus aureus* and *Pseudomonas* sp.) using standard procedures. The antibacterial activity (zones of inhibition (mm) ranged from  $6.3 \pm 0.03$ - $11.00 \pm 0.0$  with *E.coli* and *Pseudomonas* sp. been the most resistant and *Staphylococcus* sp. been the most susceptible. The MIC assay showed that the oil at 6.25 and 3.12mg/ml only had effect on *Bacillus* sp. *Staphylococcus* sp. and *Klebsiella* sp. At 12.5mg/ml, all the test organisms were susceptible. The orange seed oil compared slightly with the antibacterial activity of antibiotic of choice. Antibacterial efficacy shown by the seed oil provides a scientific basis and thus validates their use as medicinal remedies. Isolation and purification of different phytochemicals may further yield significant antibacterial agents.

**Keywords:** Comparative antibacterial, pathogens, phytochemicals, antibacterial agents

### Introductions

For a long period in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs. Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Abeyasinghe, 2010) [1]. Finding new naturally active components from plants or plant-based agricultural products has been of interest to many researchers. Hence, a great deal of attraction has been paid to the antibacterial activity of citrus as a potential and promising source of pharmaceutical agents (Jo *et al.*, 2004; Ortuño *et al.*, 2006) [14, 25]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones 1996) [13]. These purposes vary from the use of rosewood and cedarwood in perfumery, to flavouring drinks with lime, fennel or juniper berry oil (Lawless 1995) [17], and the application of lemongrass oil for the preservation of stored food crops (Mishra and Dubey 1994) [22]. In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans 1997) [18].

While some of the oils used on the basis of their reputed antimicrobial properties have well documented *in vitro* activity, there are few published data for many others (Hili *et al.* 1997) [10]. Some studies have concentrated exclusive Lyon one oil or one micro-organism. While these data are useful, the reports are not directly comparable due to methodological differences such as choice of plant extract(s), test microorganism(s) and antimicrobial test method (Janssen *et al.*, 1987) [11].

Lemon is an important medicinal plant of the family Rutaceae. It is used mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (leaves, stem, root, juice, peel and flower) of Lemon against clinically significant bacterial strains has been reported (Kawaii *et al.*, 2000) [16].

Citrus flavonoids have a broad spectrum of biological activity including antibacterial, antifungal, anti-diabetic, anticancer and antiviral activities (Ortuño *et al.*, 2006) [25]. Antimicrobial activity of the peel extract is directly concerned with the components that they contain. The studies showed that essential oils, *protopine* and *corydaline alkaloids*, *lactons*, *polyacetylene*, *acyclic sesquiterpenes*, *hypericin* and *pseudo hypericin* compounds are effective toward various bacteria (Maruti *et al.*, 2011) [21]. Furthermore, citrus fruit had been used in traditional Asian medicines for centuries to treat indigestion and to improve bronchial and asthmatic conditions. Johann *et al.*, (2007) [12] and Ghasemi *et al.*, (2009) [6] have shown that citrus varieties are considered and containing a rich source of secondary metabolites with the ability to produce a broad spectrum of biological activities.

Giuseppe *et al.*, (2007) [7] have reported the presence of limonoids in Citrus species, which can be considered responsible for activity against many clinically, isolated bacterial strains.

Limonoids obtained from *C. limon*, showed good antibacterial and antifungal activity. Extracts of citrus fruit (e.g. lemon, orange and grape fruit) are among the most studied natural antimicrobials for food applications), and it has shown to be effectively decrease the growth of bacteria. There are several *Citrus (C.)* species, of these *C. limon (lemon)*, *C. Aurantium (bitter orange)*, *C. Limetta (sweet lemon)*, *C. Jambhiri (Rough lemon)* and *C. paradise (grape fruit)*.

Due to rapid increase of antibiotic resistance in our country, plants that have been used as medicines over hundreds of years, constitute an obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. The aim of this study is to compare antibacterial activity of orange seed oil and antibiotics of choice against some food borne pathogens.

## Materials and methods

**Collection of fruit seeds:** Orange fruits were purchased from Oja Oba in Iree, Osun state, Nigeria. The orange fruits were rinsed to remove dirt and soaked in potassium permanganate to remove germs and cold pressed to obtain orange juice containing orange seeds. The orange juice containing orange seeds were separated by decanting the orange juice and leaving the orange seeds behind in the bowl.

**Extraction of seed oil:** The orange seed was subjected to oven drying for 3 hours, in which the dried orange seeds were dried mulled in a cleaned attrition mill and subjected to solvent extraction process of extracting the oil content of the mulled orange seed.

**Source of Microorganisms:** The following bacteria species were used: *Bacillus cereus*, *Klebsiella sp.*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas sp.* They were maintained on nutrient agar slant and stored at 4°C. Bacteria were sub-cultured onto fresh media at regular intervals until used.

**Preparation of Inoculum:** The method of Oliveora *et al* (2005) were followed with some modifications to prepare the microbial inoculum

**Antimicrobial Activities:** The screening of antimicrobial activities of orange seed oil used in this investigation was determined on nutrient agar media (all tested organism grow on agar media), by the using agar well diffusion method. (CLSI, 2002) [5]. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

**Determination of minimum inhibition concentration:** The method of was used for the determination of the MIC. The MIC was considered as the lowest concentration that could not produce a single bacterial colony.

## Results and Discussion

Table 1 shows the antibacterial activity of crude orange seed oil using paper disc diffusion method against some selected food poisoning organisms. The zones of inhibition (mm) ranged from  $06.03 \pm 0.03$  -  $12.6 \pm 0.14$ . The most susceptible organisms are *Staphylococcus sp.* and *Klebsiella sp.* while the least susceptible organisms are *Bacillus sp.*, *Pseudomonas sp.* and *E.coli*. Table 2 shows the minimum inhibitory concentration (mg/ml) of crude orange seed oil on selected food poisoning organisms. The concentration range used were from  $-3.12$  -  $50.00$  mg/ml. The results of this assay showed that the higher the concentration, the better the antibacterial activity in terms of zones of inhabitation. Only *Staphylococcus sp.* was susceptible to all the different concentration while 3.12 and 6.25 mg/ml has no effect on other bacteria pathogen tested with only 50.00 and 25.00 having potential activity on *Pseudomonas sp.*

**Table 1:** Antibacterial activity of crude orange seed oil on selected food borne organisms

| Test organisms               | Antimicrobial activity/ zones of inhibition (WN) |
|------------------------------|--|
| <i>Bacillus cereus</i>       | 08.5±0.7   |
| <i>Klebsiella sp.</i>        | 11.00±0.0  |
| <i>E. coli</i>               | 06.03±0.03                                       |
| <i>Staphylococcus aureus</i> | 12.6±0.14  |
| <i>Pseudomonas sp.</i>       | 06.3±0.13  |

Values are mean ± S.D. of three replicate results.

**Table 2:** Minimum inhibitory concentration activity of crude orange seed oil on selected food borne organisms (mg/ml)

| Test organisms/Conc.(mg/ml)  | Minimum Inhibitory Concentrate/zones of inhibition |         |         |          |          |
|------------------------------|--|---------|---------|----------|----------|
|                              | 50   | 25.0    | 12.5    | 6.25     | 3.12     |
| <i>Bacillus cereus</i>       | 1.9±0.2  | 1.4±0.0 | 1.2±0.1 | 0.5±0.2  | 0.20±0.1 |
| <i>Klebsiella sp.</i>        | 1.4±0.0  | 1.2±0.2 | 0.8±0.1 | 0.31±0.1 | 0.0±0.0  |
| <i>E. coli</i>               | 0.9±0.0  | 0.6±0.2 | 0.3±0.2 | 0.0±0.0  | 0.0±0.0  |
| <i>Staphylococcus cereus</i> | 1.8±0.2  | 1.2±0.3 | 0.6±0.3 | 0.3±0.2  | 0.0±0.0  |
| <i>Pseudomonas sp.</i>       | 0.6±0.1  | 0.4±0.3 | 0.0±0.0 | 0.0±0.0  | 0.0±0.0  |

Values are mean ± S.D. of three replicate results.

**Table 3:** Antibacterial activity of antibiotics of choice against the selected food borne organisms

| Organisms                 | Antibiotics/zones of inhibition |          |          |          |          |          |          |
|---------------------------|---------------------------------|----------|----------|----------|----------|----------|----------|
|                           | AMX                             | TET      | CHL      | ERY      | AUG      | OFL      | NAL      |
| <i>Bacillus sp.</i>       | 2.1±0.03                        | 1.2±0.0  | 1.3±0.02 | 2.2±0.0  | 1.8±0.5  | 1.6±0.03 | 0.6±0.0  |
| <i>Klebsiella sp.</i>     | 1.6±0.02                        | 0.6±0.0  | 1.6±0.02 | 1.8±0.03 | 0.9±0.02 | 1.8±0.2  | 1.3±0.0  |
| <i>E. coli</i>            | 0.8±0.0                         | 0.3±0.0  | 0.9±0.0  | 0.4±0.3  | 0.6±0.01 | 12±0.2   | 0.9±0.1  |
| <i>Staphylococcus sp.</i> | 0.6±0.02                        | 0.3±0.22 | 0.4±0.1  | 0.6±0.00 | 0.2±0.0  | 0.0±0.0  | 0.2±0.0  |
| <i>Pseudomonas sp.</i>    | 2.1±0.02                        | 1.8±0.03 | 2.2±0.3  | 1.6±0.00 | 1.3±0.4  | 0.9±0.00 | 0.3±0.00 |

AMX = Amoxycillin (25ug), TET = Tetracyclin (30ug), CHL chloramphenicol (30ug), ERY = Erythromycin (10ug), AUG = Augmentin (30ug), OFL = ofloxacin (30ug), NAL = Nalidixic acid (3ug).

Table 3 shows the antibacterial activity of antibiotics of choice against the selected food poisoning organisms. *Bacillus sp.*, *Klebsiella sp.* and *Pseudomonas* were the least susceptible test organism in terms of their high zones of inhibition (mm). *E. coli* and *Staphylococcus sp.* to be most susceptible.

Anecdotal evidence and the traditional use of plants as medicines provide the basis for indicating which oil and extract of plant may be useful for specific medical conditions. Historical, oils and extracts of plants have been used as tropical antiseptics or has been reported to have antimicrobial properties (Lawless, 1995) [17]. It is important to investigate scientifically those plants, which have been used in traditional medicine as potential source of novel antimicrobial compounds which citrus plant is among.

When comparing data in different studies most publications, provide generalization about whether or not a plant oil or extract possess activity against gram positive and gram negative bacteria. Comparing the data obtained in this study previously published results in problematic. First, the composition of plant oils and extracts is known to vary according to locality and environmental conditions. Secondly the method used to assess antimicrobial activity, and the choice of test organism(s) varies between publications (Janssen *et al.*, 1987) [11]. A method frequently used to screen plant extracts for antimicrobial activity is the agar well diffusion technique (Smith-Palmer, *et al.*, 1998) [29]. The usefulness of this method is limited to the generation of preliminary, qualitative data only as the hydrophobic nature of most essential oils and plant extracts prevents the uniform diffusion of these substances through the agar medium (Rios *et al.*, 1988) [30].

Agar and broth dilution methods are also commonly used. The results obtained by each of these methods may differ as many factors vary between assays (Janssen *et al.*, 1987; Hili *et al.*, 1997) [11, 10]. These and other elements may account for the large differences in MICs obtained by the agar and broth dilution methods in this study. *In vivo* studies may be required to confirm the validity of some of the results obtained.

Several authors (Mann and Markham, 1998) [20] have stressed the need for a standard, reproducible method for assessing oils. In view of this, many methods have been developed specifically for determining the antimicrobial activity of essential oils (Carson *et al.*, 1995) [4]. The benefits of basing new methods on pre-existing, conventional assays such as the NCCLS methods are that these assays tend to be more readily accepted by regulatory bodies (Carson *et al.*, 1995) [4]. Also, these methods have been designed specifically for assessing the activity of

antimicrobial compounds, and factors affecting reproducibility have been sufficiently investigated.

The prevalence of antibiotic resistance is a continual problem due to the evolution of a potent defense mechanism against antibiotics. Therefore, it is necessary to exploit and develop a novel inhibitory agent against those bacteria (Caballo, 2006) [3]. Plants and plant products have been used extensively throughout history to treat medical problems. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity. The results indicated that the extracts of all the sorts studied showed antibacterial activities towards the Gram-positive, negative bacteria and yeast, but with variability related to the bacterial genus and species. Some significant components are abundantly available in citrus peel, including ascorbic acid, phenolic acids, polyphenols, and dietary fiber (Gorinstein *et al.*, 2001) [8]. Constituents with antioxidant, antiviral, antibacterial, antifungal, and anticancer activities have also been reported in citrus (Mahmud *et al.*, 2009) [19]. Numerous studies have described the inhibitory activities of citrus against human pathogens, fungi, and yeasts and food pathogens. The reason for the different sensitivity of the Gram-negative bacteria compared to that of Gram-positive bacteria could be due to differences in their cell wall composition. Gram-positive bacteria contain an outer peptidoglycan layer, which is an effective permeability barrier, whereas Gram-negative bacteria have an outer phospholipidic membrane (Samarakoon *et al.*, 2012) [26]. Hayes and Markovic (2002) [9] investigated the antimicrobial properties of lemon and found that lemon possesses significant antimicrobial activity against *S. aureus*, *Klebsiella*, *Escherichia coli*, *P. aeruginosa* and *C. albicans*. Nevertheless, these results unmatched with our results as these organisms showed resistance to the oil except that of *S. aureus* and *Klebsiella* as it matched with these results with inhibition zone (2-15mm). Moreover, showed good bacterial inhibition by *C. limon* especially against *S. aureus*, *P. aeruginosa* and *P. vulgaris*.

### Conclusion

In summary, this study confirms that crude oil extracted from the seeds of citrus plant possess in-vitro antibacterial activity at very low concentrations, which has potential use as novel of systems food preservation. Antibacterial efficacy shown by the seed oil provides a scientific basis and thus validates their use as medicinal remedies. Isolation and purification of different phytochemicals may further yield significant antibacterial agents.

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