International Conference of Science, Engineering & Environmental Technology (ICONSEET), 3(2): 13-17, 2018
ISSN 0794-9650

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Comparative analysis of the antimicrobial activities of the extract of peels of Citrus species (Orange, Lemon and Lime) against selected pathogens

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Abstract- ***The antimicrobial activity of the peel extract of three citrus species was investigated. The citrus species were Citrus sinensis (Sweet orange), Citrus limon (Lemon) and Citrus limetta (Lime). The extraction was carried out using ethanol and sterile distilled water. 2 g each of the pulverized peel was added to 20 ml of each solvent and left for 48 hours after which it was evaporated using the rotary evaporator. The antimicrobial assay was carried out using the agar well diffusion method. The ethanolic extraction revealed a zone of inhibition of a range of 8-10.5 mm while the aqueous extraction was between 7 and 7.25mm. The study revealed the antimicrobial activities of the citrus peels which confirm the usefulness of the so called waste that would have been discarded naturally.***

Keywords: Antimicrobial activity, antimicrobial assay, citrus peels, extraction and zone of inhibition

1.0 Introduction

The Citrus fruit belongs to the family Rutaceae with several species ranging from *Citrus sinensis*, *Citrus limon* and *Citrus limetta* to mention but a few. They are rich source of minerals, vitamins (specifically vitamin C), flavonoids, fiber and folic acid (Adeneye *et al*., 2006). The origin of Citrus fruits cannot be precisely identified but researchers believed they emerged in south East Asia from where they slowly spread to Northern Africa mainly through migration and trade (Holland *et al*., 2009). Citrus species were introduced to Nigeria by the Federal Department of Agriculture (FDA) and missionaries in 1930s (Adigun, 1993). Subsequently, it spreads throughout the country and is currently rated as the most widely planted fruit in Nigeria (Holland *et al*., 2009).

For quite a long period of time, there have been naturally occurring materials which tend not to be important but having biologically active substances with high biological activity and with great potential for producing new drugs. These natural materials include peels of fruits and vegetables (Djeussi *et al*., 2013). It has been discovered that orange and lemon peels could be used in eliminating bacteria causing acne (Tumane *et al*., 2014) and also to control sebum production (Suryawanshu and Saonere, 2011). Dried peels could also be turned into an exfoliating powder while banana and avocado peels could be used as moisturizer on the skin (Sato *et al*., 2007).

The emergence of antibiotic resistant microorganisms as a result of misuse of antibiotics in combating several diseases and infections and with several side-effects has prompted the use of less important materials such as fruit peels in treating some of these infections (Adeneye *et al.*, 2006). This will help in

dealing with less chemicals and also guide against the use of expensive drugs or products which could drain our purse, hence this study.

2.0 Methodology

2.1 Collection and preparation of plant material

The three species of Citrus namely sweet orange (*Citrus sinensis*), lemon (*Citrus limon*) and lime (*Citrus limetta*) were purchased from Oje market in Ede, Osun State, Nigeria. They were gently washed under running water and neatly peeled. The peels were placed on a clean tray and were air dried at room temperature (25^0C) for one month. The air dried peels from each of the Citrus specie was separately pulverized to a fine powder using a sterile blender. The powdered products were then stored separately in air tight bottles.

2.2 Test organisms

Pure clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were collected from the Microbiology unit of the Science Laboratory Department, Federal Polytechnic, Ede, Osun State, Nigeria.

2.3 Extraction process

2 g of each of the powdered samples were weighed into different conical flasks after which 20 ml each of ethanol and sterile distilled water were added respectively into the separate flasks. The mixtures were allowed to stand for 48 hours at room temperature. After this, filtration was carried out with the use of filter paper; the filtrate was taken as the extract. The extracts were then evaporated using rotary evaporator.

2.4 Antimicrobial activity of plant extract on test microorganisms

The antimicrobial activity of the plant was assayed using agar diffusion technique as described by Cheesbrough (2004). Mueller Hinton Agar was used for culturing the bacterial species while Potato Dextrose agar was used for the fungal isolate. 0.1ml of each of the test isolate was spread on the agar plate with the aid of a glass spread. The cork borer was used to make eight holes (8 mm in diameter) on the medium that has been seeded with microorganism in the plate. 0.1ml of each extract was dropped into each of the holes. The procedure was repeated for all the six extracts on different plate with the different organism. The plates were incubated and the zone of inhibition was measured after 24hours.

3.0 Results and discussion

The results of this study revealed the inhibitory effect of the extract from the peels of the Citrus species against the selected pathogens. The ethanolic extract gave an average zone of inhibition of 8.67mm against *Escherichia coli* while aqueous extract gave an average zone of inhibition of 7.25mm (Table 1). Similar results were observed in the other tested organisms with ethanolic extract giving a better performance (Tables 2 and 3). Figures 1 and 2 compared the ethanolic and aqueous extraction of the peels of the Citrus species against the tested organisms respectively. The fungal pathogen, *Candida albicans* gave the highest zone of inhibition while *Staphylococcus aureus*, a bacterial pathogen gave the least effect using ethanolic extraction. Similar trend was also observed for aqueous extraction. Ofloxacin and fluconazole used as positive controls for the bacterial (*E.coli*, *S.aureus*) and fungal (*C.albicans*) pathogens gave zones of inhibition of 23, 28 and 22mm respectively. Ethanol used only on the test organisms was only effective on *E.coli* while the use of distilled water only on the organisms showed no effect at all. Though ethanol used only on the test organisms was only effective on *E.coli*, the bactericidal activity of the solvent has been found to be effective on some other organisms as confirmed in other

studies (Fried and Novick, 1973; Barker and Park, 2001 and Silveira *et al*., 2004). The antimicrobial activity of fruit peels had also been confirmed by Narender *et al*., 2018.

Table 1: Antimicrobial activities of the peel extract against *Escherichia coli*

Extract/ specie	Diameter of zone of inhibition (mm)				Mean ±S.D
$E_{O.P}$	7	9	9	9	8.50 ± 1.00
$A_{O.P}$	7	7	7	7	7.00 ± 0.00
$E_{Li.P}$	9	9	9	10	9.25 ± 0.50
$A_{Li.P}$	7	7	9	8	7.75 ± 0.96
$E_{Le.P}$	7	8	8	9	8.00 ± 0.82
$A_{Le.P}$	7	7	7	7	7.00 ± 0.00

Keys: $E_{O.P}$ = Ethanolic extract of orange peels, $A_{O.P}$ = Aqueous extract of orange peels, $E_{Li.P}$ =Ethanolic extract of lime peels, $A_{Li.P}$ =Aqueous extract of lime peels, $E_{Le.P}$ = Ethanolic extract of lemon peels, $A_{Le.P}$ = Aqueous extract of lemon peels

Table 2: Antimicrobial activities of the peel extract against *Staphylococcus aureus*

Extracts	Diameter of zone of inhibition (mm)				Mean ±S.D
$E_{O.P}$	9	10	9	8	9.00 ± 0.82
$A_{O.P}$	7	8	8	7	7.50 ± 0.58
$E_{Li.P}$	8	7	7	9	7.75 ± 0.96
$A_{Li.P}$	7	7	7	7	7.00 ± 0.00
$E_{Le.P}$	7	8.5	9.8	10	8.75 ± 1.38
$A_{Le.P}$	7	7	7	7	7.00 ± 0.00

Keys: $E_{O.P}$ = Ethanolic extract of orange peels, $A_{O.P}$ = Aqueous extract of orange peels, $E_{Li.P}$ =Ethanolic extract of lime peels, $A_{Li.P}$ =Aqueous extract of lime peels, $E_{Le.P}$ = Ethanolic extract of lemon peels, $A_{Le.P}$ = Aqueous extract of lemon peels

Table 3: Antimicrobial activities of the peel extract against *Candida albicans*

Extract	Diameter of zone of inhibition (mm)				Mean ±S.D
$E_{O.P}$	10	10	9	11	10.00 ± 0.82
$A_{O.P}$	7	8	8	8	7.75 ± 0.50
$E_{Li.P}$	12	11	11	13	11.75 ± 0.96
$A_{Li.P}$	7	7	7	7	7.00 ± 0.00
$E_{Le.P}$	10	10	11	11	10.50 ± 0.50
$A_{Le.P}$	7	7	8	7	7.25 ± 0.50

Keys: $E_{O.P}$ = Ethanolic extract of orange peels, $A_{O.P}$ = Aqueous extract of orange peels, $E_{Li.P}$ =Ethanolic extract of lime peels, $A_{Li.P}$ =Aqueous extract of lime peels, $E_{Le.P}$ = Ethanolic extract of lemon peels, $A_{Le.P}$ = Aqueous extract of lemon peels

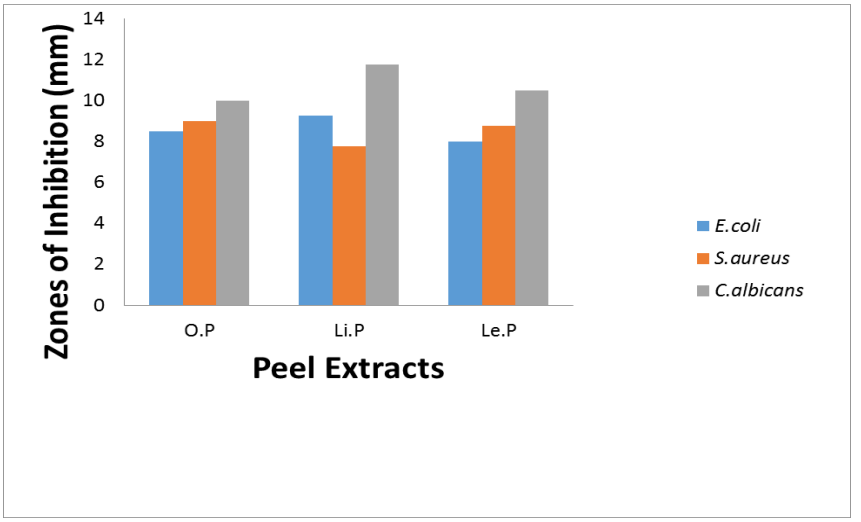

Figure 1: Comparative analysis of the ethanolic extraction of the three species of the citrus peels against the pathogens

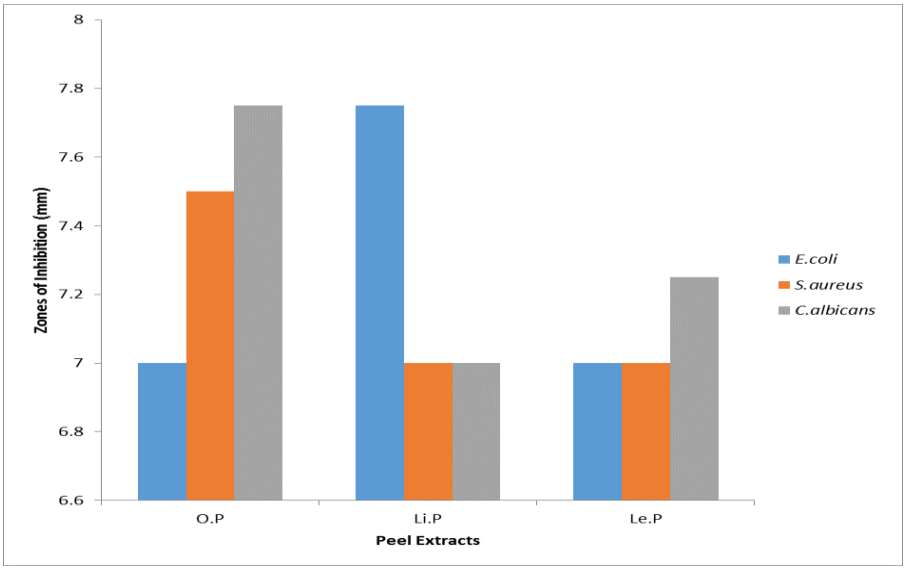

Figure 2: Comparative analysis of the aqueous extraction of the three species of the Citrus peels against the pathogens

4.0 Conclusion and recommendations

The results of this study had confirmed the antimicrobial activity of the extract of Citrus peels against the selected pathogens whether it was extracted using ethanol or water. This is confirming the fact that some of these waste materials could still be of benefits to mankind especially in solving some health challenges. In as much as this has been confirmed, further studies should still be carried out on the extract especially on different concentrations of the solvent to be used, in vivo application of the extract etc. it is hereby suggested that clinician should be consulted before use especially the dosage to be used in topical application.

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