



# Evaluating The Antimicrobial Properties Of *Citrus Sinensis* Extracts: Insights From A Comparative Review Of Five Studies

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## INTRODUCTION:

The research into phytochemical and antimicrobial screening of compounds from natural sources has always been of great interest for scientists looking for new sources of useful drugs against infections and diseases (Silva RP, 1998). Medicinal plants have been used in the management of microbial infections for thousands of years. Plants are natural sources for antimicrobial compounds to ward off microbial organisms competing with them in their environment (Thawabeth *et al.*, 2019). Bacteria that can cause wound infections are *Coliform bacilli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella spp.* The presence of the microorganisms in wounds significantly slows down the wound healing process (Perescacho and Rouseffm *et al.*, 2008). *Citrus* is a good source of vitamin C, carotenoids, flavonoids, essential oils, acridone alkaloids, minerals and Vitamin B (Madhuri *et al.*, 2014). Sweet orange also contains fiber, bio active components and phenolic compounds. Generally, antioxidant reduce oxidative stress which is a common feature in health dysfunctions in apparent support of the suggestion by that these bioactive compounds, including the antioxidants compounds, in sweet orange juice could reduce the risk for cancers and many chronic diseases (Crowell PL 2009).

In this review, we examine and synthesize findings from five key studies that have explored the antimicrobial activity of different *Citrus sinensis* extracts. These studies provide valuable insights into the potential use of these extracts in developing new therapeutic agents. The objective of this review is to assess the comparative efficacy of these extracts and to highlight the most promising ones for further research and application.

## **MATERIALS AND METHODOLOGY:**

Phytochemical analysis of the fresh and dry ethanolic peel extract of *Citrus sinensis* was done. Fresh and dry ethanolic extracts of sweet orange were extracted by the soxhlet extraction and then concentrated by rotary evaporator. The antibacterial activities of ethanolic extract of *Citrus sinensis* seed oil and non oil extracts are investigated against selected bacterial strains, MIC and MBC were determined (Ehigbai I.Oikeh, *et al.*, 2020).

The leaf extract of *Citrus sinensis* were screened for its antimicrobial and phytochemical activities. The solvents used for the leaves and root extraction were benzene, acetone, aqueous. The plant leaf extracts in different solvent were screened for the phytochemicals analysis is carried out for alkaloids, glycosides, tannins, saponins, flavonoids, steroids (Oikeh, EI *et al.*, 2020).

The phytochemical screening of epicarp of *Citrus sinensis* for the presence of phytochemical compounds in methanol and aqueous extract was done. Antibacterial activity of *Citrus sinensis* was carried out by disc diffusion method with varying concentrations (50, 100, 150, 200 mg/ml) of the methanol and aqueous extracts *Citrus sinensis* peels showed against the tested bacteria (Baba *et al.*, 2018).

The phytochemical screening of juice and peels of *Citrus sinensis* was carried out with the ethanol and aqueous extracts of the peel of sweet orange. Three bacterial isolates were subjected for antibacterial activity was *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Agar well diffusion method used for the antibacterial activity (Anthony Cemaluk C. Egbuonu, 2016).

The phytochemical screening of ethanolic extract of unripe *Citrus sinensis* peel shows the presence of alkaloid, saponin, tannin, flavonoid, cyanogenic glycosides and phenol. The antibacterial activity was done against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Agar well diffusion technique was employed to determine the antibacterial activity (Nwankwo, *et al.*, 2014).

**RESULT:**

1. The fresh peel extract (FPE) produced the widest zone of inhibition (ZOI) of 20 mm against *E. faecalis* followed by *S. aureus* and *P. aeruginosa* with 14 mm ZOI and *E. coli* with 13 mm ZOI. The FPE produced a 6 mm ZOI for *S. typhimurium*., the lowest observed for the bacterial strains studied. The dry peel extract (DPE) produced generally smaller zones of inhibition against the bacterial strains with a 12 mm zone of inhibition observed for *E. faecalis* and 10 mm for *S. typhimurium*. The DPE produced 4 mm, 6 mm and 8 mm zones of inhibition, respectively against *S. aureus*, *P. aeruginosa* and *E. coli*. The FPE was most effective against *C. albicans*, producing an 18 mm ZOI while the DPE was most effective against *P. notatum* with an observed ZOI of 10 mm and 2) mm zones of inhibition were observed for the FPE against *A. niger* and *P. notatum*. While 2 and 4 mm respectively for *C. albicans* and *A. niger* when exposed to the DPE. It has been concluded that the ethanolic extract of FPE showed better antibacterial activity than DPE. The minimum inhibitory concentrations (MIC) ranged from 12.5 to 100 µg/mL. The lowest MIC value (12.5 µg/mL) was observed for the FPE against *S. aureus*, *E. faecalis* and *P. aeruginosa*. MIC values were higher for the DPE against the same microbial strains. DPE had MIC value of 50 µg/mL against *P. aeruginosa* and *S. typhimurium*. MBC values were generally higher than the MIC values obtained ranging from 25 µg/mL for the FPE against *S. aureus*, *E. faecalis* and *P. aeruginosa* to 200 µg/mL for DPE against *S. aureus*, *E. faecalis* and *E. coli*. The DPE had lower MIC and MFC values against *A. niger* (50 µg/mL and 100 µg/mL respectively) compared to the FPE (100 µg/mL and 200 µg/mL for MIC and MFC respectively) Similar MIC and MFC (100 µg/mL and 200 µg/mL respectively) were observed for both FPE and DPE against *P. notatum* (Ehigbai I.Oikeh, *et al.*, 2020).

2. The result shows that *Escherichia coli* was however not susceptible to the *C. sinensis* seed oil as no inhibition of growth was observed. MIC was lowest in the *C. sinensis* seed oil against *Pseudomonas aeruginosa* and *Salmonella spp* (50 µg/ml) with an MBC of 100 µg/ml for these organisms. An MIC value of 100 µg/ml was observed for the seed extract against all the bacterial strains tested. Maximum antibacterial activity for the seed extract was observed against *Staphylococcus aureus* with a 12 mm zone of inhibition while the seed oil had Maximum antibacterial activity against *Salmonella spp* (8 mm zone of inhibition). Measurement of zones of inhibition against Gram-positive and Gram-negative bacteria strains show that the seed extract had larger zones of inhibition than the seed oil against both Gram-positive organisms (*Staphylococcus aureus* and *Enterococcus faecalis*) at the concentrations studied. The *Citrus sinensis* seed oil did not show any antibacterial activity against *Escherichia coli* as demonstrated by the lack of any visible zone of inhibition. This is in contrast to the seed extract with 1 and 2 mm zones of inhibition at 100 and 200 µg/ml of the extract respectively. These observed zones of inhibition are however not high enough to conclude that the seed extract may be a good source of antimicrobial agents against *E. coli*. (Oikeh, EI, 2020)

3. The methanol crude extracts of the epicarp of sweet orange (*Citrus sinensis*) showed activity against all the tested bacteria with the highest activity of 22.0mm against *Escherichia coli* at the highest concentration of 200mg/ml used but the methanol extract failed to exhibit activity against *Bacillus macerans* at lower concentration of 50 and 100mg/ml used. The aqueous extract showed activity against the organism at all the concentrations used with highest activity of 20.0 at 200mg/m against *S.aureus* of the concentration used but did not produce any activity on *Bacillus macerans*, chloramphenicol was used as positive control which also showed varying degree of activities against the bacteria. MIC and MBC study of both the methanol and aqueous extract showed that the epicarp of sweet orange (*Citrus sinensis*) can be bacteriostatic and bacteriocidal against the test bacteria except against *Bacillus macerans*. These results suggests that the epicarp of *Citrus sinensis* has great antibacterial potentials and can be used to formulate remedies that could treat disorders caused by these test bacteria (Baba J *et al*, 2018).

4. The isolates were tested against standard antibiotics. Gentamycin, streptomycin, norfloxacin and tetracycline exhibited broad spectrum activity against the three isolates, while benzylpenicillin showed activity against *S. aureus*, *E. coli* but no activity against *P. aeruginosa*. Both *P. aeruginosa* and *E. coli* were susceptible to the effects of ciprofloxacin and Erythromycin while *S. aureus* was resistance to the effect of both of them. The result of the study showed that ampicillin has activity against *E. coli* only while *S. aureus* and *P. aeruginosa* were both resistant to the effect of ampicillin. The minimum inhibition concentration and minimum bactericidal concentration was carried out on the juice and the two extracts. The MIC of the juice for *S. aureus* was 0.625%, 1.25% for *E. coli* and 2.5% against *P. aeruginosa*. The ethanol extract of the orange peel had MIC of 0.625mg/ml for *S. aureus*, 1.25mg/ml for *E. coli* and 2.5mg/ml for the *P. aeruginosa*. The aqueous extract of the peel had MIC of 5mg/ml for the three organisms. The minimum bactericidal concentration of the fresh juice of *C. sinensis* was 2.5% for *S. aureus*, 5% for *E. coli* and 2.5% for *P. aeruginosa*. The ethanolic extract of *C. sinensis* fruit peel, was 10mg/ml for *S. aureus*, 5mg/ml for *E. coli* and no antibactericidal activity showed against *P. aeruginosa*. The aqueous extract of the peel showed no bactericidal activity against the three isolates (Anthony Cemaluk C. Egbuonu, 2016).

5. The antibacterial assay of the extracts carried out on various concentrations such as 25mg/ml, 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml. No concentration of the ripe peel extract could inhibit the growth of any of the 3 test organisms. The antibacterial activity of the ethanolic extract of the unripe *Citrus sinensis* peel shows 7.00mm on 25mg/ml, 8.05mm on 50mg/ml, 9.75mm on 100mg/ml, 9.88mm on 150mg/ml and 11.000mm on 200mg/ml against *S. aureus*, no ZOI observed against *E.coli* on any concentrations and against *P.aeruginosa* no ZOI observed on 25mg/ml and 50mg/ml, 7.5mm on 100mg/ml, 9.0mm on 150mg/ml and 12mm on 200mg/ml was observed. It has been concluded that ethanolic extracts of unripe *Citrus sinensis* peels used as treatment for the infection caused by *S.aureus* and *P.aeruginosa* (Nwankwo,I.U., 2014).

## DISCUSSION:

### 1. Ehigbai I. Oikeh, 2020; Clinical phytoscience

Tannins, flavonoids, saponins, phenolic compounds, and essential oils are phytochemicals responsible for plants' antimicrobial effects. Flavonoids have antibacterial, antioxidant, and inflammatory properties, and can modulate enzymatic activities and inhibit cell proliferation. Tannins form complexes with proline-rich proteins, inhibiting cell protein synthesis. The fresh *C. sinensis* peel extract has higher total phenol, flavonoid, and tannin content than the dry extract, possibly due to drying process loss (Hafidh RR *et al.*, 2011) (Dhiman A., 2012).

### 2. Oikeh, EI, 2020; Journal of applied sciences and environmental management

The study reveals the antimicrobial properties of *Citrus sinensis* seed oil and extract against various bacterial strains, suggesting potential for broad-spectrum antimicrobial agents. The hydrophobic nature of the oil may hinder uniform diffusion, but its superior activity against *Salmonella* spp. suggests potent antibacterial properties (Hammer *et al.*, 1999).

### 3. Baba J, 2018; Journal of family medicine and community health

The extracts of *C. sinensis* fruit inhibited the growth of tested bacteria, indicating the presence of active antimicrobial properties. Secondary metabolites were responsible for these effects. The sensitivity pattern of organisms to these extracts was comparable to previous studies. The juice's activity was higher than previous studies (Ogueke *et al.* 2006) (Wiley JM 2008).

### 4. Anthony Cemaluk C. Egbuonu, 2016; European journal of medicinal plants

Sweet orange peels and seeds extracts demonstrated antibacterial activity against *E. coli* and *S. aureus*, with ethanol extract showing greater activity against *S. aureus*. The water and ethanol extracts of the peel were found to be more effective than the seed in combating these diseases, suggesting their broad spectrum activity. The activity of the ethanol extract against *S. aureus* for sweet orange peels (19.00 mm) compared with that (37mm at 250 mg/ml) for orange leaves (Doughari JH, 2008)(Kumar KA, 2011).

### 5. Nwankwo, I.U *et al.*, 2014; International journal of advances in pharmacy, biology and chemistry.

The extracts of *C. sinensis* peels showed antibacterial activity due to tannins, alkaloids, flavonoids, saponins, phenols, and cyanogenic glycosides. The unripe peel showed strong inhibition on isolates, but no effect on *E. coli*. The ripe peel had no effect on wound pathogens. Gram negative organisms are generally more resistant to antimicrobial agents due to their complex cell wall structure and antibiotic resistance plasmids (Almajano NP *et al.*, 2007).

**SUMMARY:**

According to the reviews of the five articles showed that the leaf, peel, and seed extracts of *Citrus sinensis* exhibit significant antimicrobial activity, particularly against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Ethanolic extracts, especially from unripe peels and seeds, show the highest antibacterial effectiveness, with juice and fresh peel extracts also demonstrating strong activity. The antimicrobial efficacy varies by extract type, with acetone extracts generally outperforming benzene and aqueous extracts. Fresh peel extracts are notably effective against multiple bacteria and fungi, while dry peel extracts show the least activity.

**CONCLUSION:**

Understanding the antimicrobial properties of natural products has become increasingly important in the fight against drug-resistant pathogens. Among these, the potential of *Citrus sinensis* (sweet orange) extracts as effective antimicrobial agents has garnered significant interest due to their wide availability and bioactive compounds. Various parts of the *Citrus sinensis* plant, including leaves, seeds, and peels, have been studied for their efficacy against a range of microorganisms, particularly those associated with wound infections. Based on the reviews, it is concluded that acetone leaf extracts, ethanol seed extracts, juice, ethanolic peel extracts, fresh peel extracts, and ethanolic extracts from unripe *Citrus sinensis* peels exhibit the highest antimicrobial activity against organisms isolated from wound infections.

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