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SHORT COMMUNICATION

Antimicrobial Activity of the Ethanolic and Petroleum Ether Extracts of Tangerine Seed on Selected Bacteria

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ABSTRACT

The antimicrobial activity of the ethanolic and petroleum ether extracts of tangerine seeds were carried out on three test bacteria namely *Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae*. The tangerine seed extracts were obtained by first drying the seeds followed by soxhlet extraction using petroleum ether and ethanol. The extracts were used for antimicrobial studies by the agar-well diffusion method. The various zones of inhibition displayed on agar plates containing the bacteria were taken as a measure of the susceptibility of the test organisms to the extract. From the results, the zone of inhibition of the ethanolic extract on *Staphylococcus aureus* was 20 mm, *Escherichia coli* 15 mm and *Klebsiella pneumonia* 12 mm; while the zones of inhibition shown by the petroleum extract were 15 mm for *Staphylococcus aureus,* 9 mm for *Escherichia coli* and 7 mm for *Klebsiella pneumonia*. Ciprofloxacin was used as the control and the zones of inhibition it displayed were *Staphylococcus aureus* 40 mm, *Escherichia coli* 35 mm and *Klebsiella pneumoniae* 29 mm. Even though the zones of inhibition displayed on the agar plates were relatively small, the potential to use the tangerine seed extracts in the treatment of diseases produced by these bacteria especially skin diseases was established.

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INTRODUCTION

The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control (Lino and Deogracious, 2006; El-Mahmood *et al.,* 2008). This is based on the belief that natural products are intrinsically less dangerous and can be obtained at a lower cost (Ayoola *et al.,* 2008). The antimicrobial activities of medicinal plant extracts have been linked to the presence of bioactive compounds which sometimes serve to protect the plants themselves against bacteria, fungi and viral infections as well as exhibiting their antimicrobial properties on these organisms (El-Mahmood and Amey, 2007). Identification of the active principle can lead to the synthesis of more potent analogues that can be readily formulated into more useful dosage forms. Volatile oils, also known as essential oils are lipophilic compounds containing volatile aroma compounds. The antimicrobial agents when isolated by distillation methods, solvent extraction or cold expression are contained in the phytochemical constituents (flavonoids, citric acid and tannins) of the plants (Edeoga *et al*., 2005; Ayoola *et al.,* 2008). Citrus oils are very versatile and lime is an essential ingredient in the preparation of most herbal concoctions which are sometimes used to suppress stomach ache and possess antimicrobial activities in conjunction with other extracts (Onyeagba *et al.,* 2004).

Tangerine oil has been traditionally used as an antiseptic, antispasmodic, stomachic, sedative, diuretic agent (Odugbemi, 2006). Tangerines are good sources of vitamin C, folate, β-carotene, potassium, magnesium and vitamins B_1, B_2 and B_3.

Active principles are found in various parts of tangerine fruits such as the seeds, peels and pulp, which include citric acid, coumarins, flavonoids and flavonones which have antibacterial, anti-inflammatory and insecticidal properties (Habib *et al*., 1986).

The present situation in the Nigeria necessitates the need to intensify researches on the use of alternative strategies for the effective control of diseases using products obtained from plant extracts.

It is intended in this work to ascertain the antimicrobial activity of ethanolic and petroleum ether extracts of tangerine seed by testing them against three clinically important bacteria namely: *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae.*

MATERIALS AND METHODS

Sample Collection and Processing

The tangerine fruits were obtained locally from Nnokwa in Idemili South Local Government Area of Anambra State in South-eastern Nigeria. They were prepared for use by harvesting the seeds and drying them at room temperature. 200g of dried tangerine seeds were weighed and ground into particulate sizes using Qlink electronic blender (Model No: QBL-20L330). The ground seeds were packed in a thimble and inserted into a Soxhlet Extractor and 40-60^{o}C petroleum ether (BDH Analar Grade) and 80% ethanol were used as the extracting solvents. After the extraction process, the solvent was removed from the extract by steam distillation leaving behind the neat extracts. The extracts were then stored in a well sealed dark-brown coloured glass bottle.

Test Organisms

Escherichia coli, Klebsiella pneumonia and *Staphylococcus aureus* were used as the test organisms. The bacteria were obtained from the Culture Collection Unit of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State.

Antimicrobial Test

Pure cultures of the 24 hours test organisms were each seeded onto three different freshly prepared Mueller-Hinton agar plates, by means of a sterile swab stick. With a sterile cork borer, wells of 10 mm in diameter were punched in the agar plates. An aliquot of the extract (100µl) was dropped into each well. Ciprofloxacin antibiotic suspension (0.005%) was equally dropped into another well to a volume of 100µl which served as the control. Incubation was done at 37^{o}C for 24 hours and the zones of inhibition recorded in millimetres (Ayoola *et al*., 2008).

RESULTS

The results of the study are presented in Table 1.

Table 1: Zones of Inhibition (mm) of Test Organisms to Various Extractions of Tangerine Seed

Organisms	Zones of Inhibition of Various Extractions in (mm)		
	TSEE	TSPE	Cip
Staphylococcus aureus	20	15	40
Escherichia coli	15	9	35
Klebsiella pneumonia	12	7	29

KEY: TSEE = Tangerine Seed Ethanolic Extraction; TSPE = Tangerine Seed Petroleum Ether Extraction; Cip = Ciprofloxacin

DISCUSSION

The antimicrobial activity of tangerine seed oil was tested on *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The extracts were found to possess some degree of antimicrobial activities as shown in the various zones of inhibition observed on agar plates. The ethanolic solutions of the extract displayed broad spectrum activity on the test organisms. The zones of inhibition obtained were 20 mm for *Staphylococcus aureus,* 15 mm for *Escherichia coli* and 12 mm for *Klebsiella pneumoniae*. Nonetheless, the petroleum ether extract had an appreciable activity on *Escherichia coli* and *Klebsiella pneumoniae*, but showed a potent activity on *Staphylococcus aureus* (15 mm). Ethanol may be a better solvent which fostered the diffusion of the oil into the bacteria cell. The complexity of the cell wall of the Gram-negative bacteria may have been the reason for the reduced effect of the extracts on them. The fact that ciprofloxacin displayed more potency than the various solvent extracts, is in concordance with the results of Ayoola *et al*. (2008), who showed that ciprofloxacin was active against *Proteus mirabilis* than tangerine peel oil extracts.

The results obtained for the antibacterial testing were compared with the findings of Ayoola *et al*. (2008), who demonstrated the antimicrobial activity of tangerine peel oil obtained by steam distillation on six Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella paratyphi, Proteus mirabilis and Citrobacter spp*), two Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and a fungus *Candida albicans* and showed that the methanolic oil of *Citrus reticulata* peel was potent against all the organisms tested save *P. mirabilis,* with a minimum zone diameter of 9 mm for *S. aureus* ATCC 25923 and a maximum of >30 mm for *E. faecalis* and *C. albicans.*

The potential of the petroleum ether extract to have less inhibitory activity on the growth of the test bacteria *in vitro* can be equated with the findings of Martinez *et al.* 2003, who showed that the antibacterial activity of tangerine oil (*Citrus reticulata* Blanco) variety Dancy at a concentration of >1% was bioactive against *Bacillus subtilis, Staphylococcus aureus* and *Listeria monocytogenes,* but had no activity against *Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Escherichia coli.*

Cvetni and Vladimir-Kne'evi (2004), performed antibacterial and antifungal testing of the ethanolic extracts of grape seed against ten Gram-positive and ten Gram-negative bacteria strains, as well as ten yeast strains. The antibacterial tests were based on the standard serial dilution assay and the agar diffusion method using Mueller Hinton agar for bacteria and Saboraud dextrose agar for yeasts. The diameters of the clear growth inhibition zones obtained ranged from 8-22 mm. When compared with the results obtained for tangerine seed oil, the range were similar.

Conclusion

The study has established the potential for the industrial extraction and commercialisation of the seed extracts from tangerine fruits and its subsequent use in the manufacture of useful products such as pharmaceuticals, manufacture of foods, beverages, skincare products and antioxidants.

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