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Phytochemical Screening and Antibacterial Activity of *Citrus sinensis* Peel Extracts on Clinical isolates of *Staphylococcus aureus* and *Salmonella typhi*

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ABSTRACT

This study investigates the use of *Citrus sinensis* (Orange) peel extracts as antibacterial agents against *Salmonella typhi* and *Staphylococcus aureus*. The bacteria were isolated from typhoid fever infected patients and diagnosed using various laboratory procedures. The extracts showed antibacterial activity due to the presence of alkaloids, tannins, saponin, glycosides, flavonoids, terpenoids, and phenols. Flavonoids have anti-inflammatory, anti-hepatotoxic, and antimicrobial properties, while saponins and tannins play a role in wound healing and antimicrobial activities. The ethanol extract showed the highest antibacterial effect against the test isolate, with the highest zone of inhibition of 20 mm at 100mg/ml against S. aureus and 19 mm for *S. typhi*. The aqueous extract had the highest zone of in-inhibition of 17 mm at the same concentration for *S. typhi* and negative bacteria. The study highlights the importance of understanding the potential of *Citrus sinensis* peel extracts as antibacterial agents.

Keywords: *Staphylococcus aureus, Citrus Sinensis, S. Typhi, antibacterial activities, flavonoids, Terpenoids, and phenols*

INTRODUCTION

Natural products such as plant have been an integral part of ancient (Such as Chinese, Ayurvedic and Egyptian) traditional medicine systems [1]. Medicinal plant is any plant in which in one or more of its organs (stem, root, leaves, rhizomes, fruits, flower and seeds), contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis [2]. Such a plant (medicinal plant) will have such parts employed in the treatment or control of a diseasecondition and therefore contains biochemical components calledphytochemicals that are of medical importance. Phytochemicals are considered as bioactive substances of plant origin. Theyare regarded as secondary metabolites because they are of little need by the plant that manufactured them. The phytochemicals are naturally synthesized in all parts of plant such as bark, leavesstem, root, flower, fruits, seeds, etc. [3]. Most of the drugs enlistedas orthodox medication were originally obtained from plant.Many studies today confirmed that the herbs boost the immune system by stimulating the production of disease fighting white blood cells. Sweet orange (*Citrus sinensis* (L.) Osbeck) is a small evergreentree 7.5 m high and sometimes up to 15 m. Its origin is China and it has been cultivated over the years, but is grown commercially worldwide in tropics, semitropical and some warm temperate regions and has become the most widely planted tree fruit in the world today according to [3]. *Citrus* fruit products act as antibacterial agents against the bacteria and fungus. The sweet orange product has an important and physiological role because of its commercial value in pharmaceuticaland food industries of the entire world [4]. The antioxidant activity is also present in the plant materials due to the presence of many active phytochemicals such as flavonoids, vitamins, coumarins, terpenoids, carotenoids, saponin, lignin and plant sterols and so on [4]. The sweet orange fruits and their juices are an important source of bioactive methanol, the compound is important to human nutrition which include the antioxidant such as ascorbic acid, phenolic compound, flavonoids and pectin's [5].The present study was conducted to determine the

phytochemical constituents, antibacterial activity and activity of aqueous and ethanol extracts of sweet orange peel on clinical isolates of *Staphylococcus aureus* and *Salmonella typhi* isolated from stool samples of typhoid fever patients attending Federal Polytechnic Mubi Clinic.

MATERIALS AND METHODS

Collection of Plant Material

The plant was collected fresh from Mubi main Market, Mubi-North, Adamawa State, Nigeria. The dried orange peels were ground into fine powder using sterile pestle and mortar under laboratory condition and stored in container for further use.

Bacteria isolates

Clinical isolates of *Salmonella typhi* and *Staphylococcus aureus*,was used in this study. The bacteria were isolated from typhoid fever infected patient attending the Hospital. The isolates were diagnosed in theHospital to the species level by using different laboratory procedures including; Gram's stain, cultural characterization and Biochemical tests include (Indole, Methyl red, Vogues Proskauer, Catalase, Citrate utilization) for *Salmonella typhi* while catalase, coagulase and DNase test for *Staphylococcus aureus*. The isolates were maintained on Nutrient agar slants at 40C and transported to the Laboratory.

Extraction of Orange Peel

Aqueous and 80% ethanol solvents was used for extraction process of the phytochemical components of the orange peel. For aqueous extract, water extraction method as described by [6], was employed. During the process, 100g ofthe ground peel was weighted and mixed with 500ml of distilledwater in a sterile conical flask and kept for 4 days with intermittent shaking. The extract was filtered using Whatman filter paper and the filtrate was concentrated in water bath at 50°C. For ethanol, 100g of the powdered peel was extracted in 500ml of ethanol for 3 days. The mixture was filtered using Whatman No.1 filter paper and the extract was evaporated to dryness using rotary evaporator at 40°C. The residue obtained was diluted using10% Dimethylsulphoxide (DMSO) to produce 100 mg/ml of the extract from which various concentrations of 75, 50 and 25 mg/ml was produced.

Phytochemical Screening of the Extracts

Phytochemical screening was conducted using laboratory method as described by [7]. This was done to determine the presence of alkaloid, saponin, steroid, glycoside, tannin, terpenoid, anthraquinone, flavonoid and reducing sugar in the aqueous and ethanol extracts of the orange peel.

Antibacterial Activity of the Extracts

Agar well diffusion method was adapted to determine the antibacterial activity of the orange peel extracts against the test isolates in this study. During the process, 0.1ml of standardize organisms (0.5 MacFarland standard) was introduced onto the surface of Mueller Hinton agar in a sterile Petri dish and labelledaccordingly. A sterile corn borer 5 mm was used to produce fivewells at equal distance in the inoculated agar. The wells were filled with different concentrations of the extracts accordingly as25, 50, 75 and 100mg/l while the last well contain 50mg/ml of standard antibiotic Gentamicin (Micro lab limited) which was used as positive control in the study. The agar plates were allowedto diffuse for a period of hour and incubated at 37°c for 24 hours. After then, the diameter of the zones of inhibition around each well was measured to the nearest millimeters.

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

The MIC of the extracts was determined using broth dilution technique. Two-fold serial dilutions of the extracts was preparedby adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/ml of the extract. The process continued serially up totest tube No. 5, hence producing the following concentrations; 50, 25, 12.5, 6.25 3.125 mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 ml of 0.5 Mc-Farland equivalent standards of test organisms was introduced into the test tubes and incubated at 370 C for 24 hours. After incubation the test tubes was observed for growth by checkingfor turbidity.

Determination of Minimum Bactericidal Concentration (MBC) of the Extracts

From the result of MIC, the test tubes that did not show visible growth was used for MBC determination. About 0.1 ml was aseptically transferred onto the surface of Mueller Hinton agar plates. The plates were incubated at 37°C for 24 hours. The MBC of the extracts was recorded as the lowest concentration of the extract that had less than 99% growth on Mueller Hinton agar plates [8].

RESULTS

Phytochemical Screening

Phytochemical screening of *Citrus Sinensis* peel extracts in (Table 1) indicates the presence of alkaloid, tannin, Saponin, glycoside, flavonoid, Terpenoids, and Phenols while reducing sugar, steroid and anthraquinone were absent.

Antibacterial Activity of the Extracts

The antibacterial activity of aqueous and ethanol extract of *Citrus Sinensis* peel against Clinical isolates of *Salmonella typhi* and *Staphylococcus aureus* is presented in (Table 2). The resultshowed that the ethanol extract demonstrated higher activity of 19 mm at 100mg/ml. The zone of inhibition shown by the control(50 mg/ml of Gentamicin) is found to be 24 mm.

Table 1: Phytochemical constituents of the extracts

S/N	Phytochemical	Aqueous extract	Ethanol extract
1.	Alkaloid	+	+
2.	Flavonoid	+	+
3.	Glycosides	+	+
4.	Reducing sugar	-	-
5.	Saponin	+	+
6.	Steroids	-	-
7.	Phenols	+	+
8.	Terpenoids	+	+
9.	Anthraquinone	-	-
10.	Tannin	+	+

Key: + = presence of phytochemical, - = absent of phytochemical

Table 2: Antibacterial Activity of the *Citrus Sinensis* Peel Extract against the isolate

	Extract Conc.	Salmonella typhi	S. aureus
PAE	25	07	10
	50	08	12
	75	13	15
	100	17	16
PEE	25	10	12
	50	14	17
	75	18	20
	100	19	20
Control	50	21	22

Key: *PAE = Peel Aqueous Extract, PEE = Peel Ethanol Extract*

MIC and MBC of the Extracts

The minimum inhibitory Concentration of aqueous and ethanol extract of orange peel is represented in Table 3. The result showed dilutions of various concentrations of aqueous and ethanol extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by ethanol extract than aqueous extract with 6.25 mg/ml. MBC of the extract ranges between 12.50 - 50mg/ml.

Table 3: Minimum inhibitory concentration (MIC) and MBC of the extracts

Aqueous peel extracts			Ethanol peel extract	
Isolates	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Salmonella typhi	6.25	12.5	6.25	25
Staphylococcus aureus	12.5	50	6.25	25

Activity index of the peel extracts

The activity index of the Orange (*Citrus Sinensis*) peel extracts against standard antibiotics are presented in Table 4. The result showed that leaves ethanol extract has the highest activity index of 0.70 while the lowest activity is shown by leaves aqueousextract (0.57). The average activity index of the extracts is foundto be 0.64.

Table 4: Activity index of the extracts against standard antibioticused

Extract	Total ZOI	Average ZOI	Activity index
PAE	98	12.25	0.57
PEE	120	15.00	0.70
Total	218	13.63	0.64

Key: *PAE = Peel Aqueous Extract, PEE = Peel Ethanol Extract, ZOI = zone of inhibition*

DISCUSSION

The Phytochemical screening of the *Citrus Sinensis* (Orange) peel extracts indicated the presence of alkaloids, tannins, Saponin, glycosides, flavonoids, Terpenoids, and phenols. The above phytochemicals in the plant parts were responsible for their antibacterial activity. Flavonoids have been shown to possess anti-inflammatory, anti-hepatotoxic, and antimicrobial activities [9]. Saponins are known to possess antibacterial activities whilst tannins play an important role in wound healing and also possess some antimicrobial activities. According to this study, Alkaloid is present in the extracts. Alkaloid consists of a large group of nitrogenous compounds widely used as anticancer anesthetics and Central Nervous Stimulants. Alkaloids are known to play some metabolic roles and control development in a living system. It also interfereswith cell division, hence the presence of alkaloids in the *Citrus Sinensis* (Orange) peel could account for their use as antimicrobial agents. The antibacterial activity of the plant showed that the plant peel extracts demonstrated an antimicrobial effect against the test isolate with higher activity in ethanol extract compared to aqueous extract. The ethanol peel extract had the highest zoneof inhibition of 20 mm at 100mg/ml against *S. aureus* while 19 mm for *S. Typhi*, while the aqueous extract had the highest zone of in-inhibition of 17 mm at the same concentration for *S. Typhi* while negative bacteria. Generally, against the isolated bacteria, the higher concentration of the extract shows a greater zone of inhibition; this result is in agreement with the report of [10], which states that the higher the concentration of an antibacterial substance, the higher it shows an appreciable zone of inhibition. The antibacterial activity of aqueous extracts of peel, juice, and leaves from fresh Citrus Sinensis was evaluated against three Gram-positive (*Staphylococcus aureus, Streptococcus progenies, and Enterococcus facials*) and six Gram-negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus typhi, Proteus spp. and Moraxella catarrhal*). Citrus juices showed the highest activity against most of the studied bacteria isolates. According to the study, moderate activity was produced by *Citrus* peel, and the lowest activity was produced by *Citrus* leaf extracts. The minimum inhibitory concentration of aqueous and ethanol extract of orange peel showed dilutions of various concentrations of aqueous and ethanol of peel extracts can inhibit and/or kill the isolates. Lower MIC (3.125 mg/ml) was shown by ethanol extract than aqueous extract with 6.25 mg/ml. The MBC of the extractsranged from 12.50 – 50.00 mg/ml. The activity index of the *Citrus Sinensis* peel extracts against standard antibiotics is presented in Table 4. The result showed thatleaves ethanol extract has the highest activity index of 0.70 whilethe lowest activity is shown by leaves aqueous extract (0.57). The average activity index of the extracts is found to be 0.64 which indicated that the extract can compete with the standard antibiotic used. Statistical analysis of the result revealed that the table value (p-value at $p < 0.05$) is greater than the calculated value for analysis of variance between the extracts; therefore, there is no significant difference in the activity of the two extracts against the isolates used.

CONCLUSION

Phytochemical screening of the extracts of the seeds indicated thepresence of alkaloids, tannin, Saponin, flavonoids andphenols, Terpenoids, and glycoside. The antibacterial activity of the peel extracts against *Salmonella typhi* and *Staphylococcus aureus* showed that the peel leaves extracts demonstrated an antimicrobial effect against the isolates. The Minimum inhibitory Concentration (MIC) of aqueous and ethanol extract of orange peel showed dilutions of various concentrations of aqueous and ethanol of peel extracts can inhibit and/or kill the isolates. The average activity index of the extracts is found to be 0.64 which indicated that the extract can compete with the standard antibiotic used. Findings from this work support the use of extracts from orange peel for medicinal purposes.

RECOMMENDATION

Since the plant showed a significant presence of phenolic compounds such as tannins, complex compounds, and lots of secondary metabolites obtained from the result of phytochemical screening and antimicrobial analysis, it is necessary to expand the work to carry out antioxidant and antidiarrheal activities of the plant's extract. In addition, a toxicity assay is required to determine the toxicity level of the plant extract.

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