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Antibacterial activity of sweet orange (*Citrus sinensis*) juice extract on selected bacteria

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Plants have potentials to be developed into many new drugs yet to be discovered because of the countless chemical compositions in them. The investigation is targeted at the antibacterial activity of sweet orange juice extract on some bacteria using ethanol and ethyl ethanoate solvent to extract juice. Ditch method was used for the sensitivity testing against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Neisseria gonorrheae* with a dilution factor of 10^{-10} for inoculation from pure culture of each selected bacteria. Disc method was used to test streptomycin, ciprofloxacin, gentamycin and penicillin G against test organisms as positive controls. There was no significant difference in the effect of different concentrations of the same extract on test organisms. However, there was a significant difference in the ethyl ethanoate and alcohol extracts. The ethyl ethanoate extract showed minimum inhibitory concentration at 300 mg/ml on *E. coli* (31.5 ± 0.5 mm); *N. gonorrheae* (21 ± 0.0 mm) at 200 mg/ml; *S. aureus* (22 ± 0.0 mm) and *K. pneumoniae* (37 ± 3.0 mm) at 100 mg/ml; while ethanol extract at 100 mg/ml on *E. coli* (23.5 ± 1.5 mm) and *K. pneumoniae* (25 ± 5.0 mm); *N. gonorrheae* (13.5 ± 1.0 mm) and *S. aureus* (12.5 ± 2.5 mm) at 300 mg/ml and 200 mg/ml respectively. The zones of inhibition exhibited by streptomycin ranges from *N. gonorrheae* (14-24 mm) *E. coli*; ciprofloxacin varies from 15- 21 mm on *K. pneumonia*e and *S. aureus* respectively. Gentamycin ranges from 14-20 mm on *N. gonorrheae* and *S. aureus* respectively; and penicillin G on *N. gonorrheae* (14 mm) and *S. aureus* (28 mm). It can be concluded that sweet orange juice of ethyl ethanoate extract was more effective than the ethanol extract and the positive control.

Key words: Antibacterial activities, ethanolic extract, ethanolic extract, sweet orange and microorganisms.

INTRODUCTION

Medicinal plants can be developed into many new drugs yet to be discovered because of the extraordinarily large chemical constituents found in them. The use of herbal medicine in Africa and Asia had been traced back to the time immemorial. The part of plants used as drug vary from the roots, barks, stems, leaves and seed as extracts and concoctions (Hassan et al., 2013). Many plants were used as antimicrobial agents because of various

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chemical constituents found in them. However, recently attention had been drawn towards extracts and biologically active compound from popular plant species. Plants have ability to synthesize aromatic substances such as phenolic, (for example phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites (Alo et al., 2012). These substances serve as plant defense mechanisms against predators like microorganism, insects and herbivores (Badar et al., 2008).

Orange is one of the most important commercial fruit cultivated on all continents of the world. The importance of the orange is attributed to its diversified use and cultivation worldwide and which probably stands first among the cultivated fruits. *Citrus sinensis* (sweet orange) is widely cultivated in Nigeria and many other tropical and subtropical regions (Piccinelli et al., 2008). Sweet orange commonly called orange is a member of the family *Rutaceae* and a main source of vitamins, especially vitamin C; but also has sufficient amount of folic acid, calcium, potassium, thiamine, niacin and magnesium (Angew, 2007). Sweet orange is the major source of vital phytochemical nutrients and for a long time have been valued for their wholesome nutrition and antioxidant properties. It has been scientifically established beyond reasonable doubt that oranges are very rich in vitamins and minerals that are beneficiary to humans as nutrient and immune booster. According to Doughari and Manzara (2008), sweet orange juice can be used in the development of safe antibiotics for the treatment of bacterial infections. It was recently appreciated that other biologically active and non-nutrient compounds present in sweet orange juice such as antioxidants, as well as soluble and insoluble dietary fibers are reported to reduce the risk of cancers; while many chronic diseases such as arthritis, obesity and coronary heart diseases have been treated with sweet orange juice (Crowell, 1999).

Rehman et al. (2007) reported that essential oil of the citrus juice exhibits antifungal, antibacterial, antiviral and anti-parasitic properties. Recently, many microorganisms have developed resistance against many conventional antibiotics; because of acquisition and expression of resistant genes in them (Bakhru, 2001). Furthermore; conventional antibiotics had been associated with adverse health effects such as hypersensitivity, allergic reactions and immune suppressions (Ahmed and Beg, 2001). Hence, time had come to develop new antibiotics that are safe for the treatment of infectious disease. According to Bhardwaj and Laura (2009), fruits and plants possess secondary metabolites that can inhibit and kill most pathogens. The difference in the antibacterial activity of the various extracts showed that different extracts have varying antibacterial agents with different modes of action and bacteria susceptibility or that not all phytochemicals responsible for antibacterial activity are soluble in a single solvent (Kumar et al., 2011; Badar et al., 2008). Fruits are considered to have great potential therapeutic treatment for various microbial diseases and it is therefore necessary to carry out a study to validate the antibacterial activity of sweet orange on selected bacteria.

MATERIALS AND METHODS

Biological sample

Sample collection

Ten fresh sweet oranges (*C. sinensis*) free from insect infestation and other kinds of damage were plucked at early morning (7 am) from the Lagos State Polytechnic campus at Ikorodu area of Lagos State, Nigeria (Latitude 6.5945°N Longitude 3.3370°E).

Microorganisms

Pure cultures (clinical isolate) of test organisms were obtained from Nigeria Medical Research, Yaba (NIMER). The test organisms were purified by sub culturing and preserved on nutrient agar at 4°C before used. They included their reference numbers: *Staphylococcus aureus* (ATCC 25923) (gram positive bacteria), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883) and *Neisseria gonorrheae* (ATCC 49226) (gram negative bacteria).

Phytochemical screening of C. sinensis juice

The phytochemical analysis was carried out using the method described by Odebiyi and Sofowora (1978). The orange juices were screened for the presence of tannins, saponins, flavonoids, steroids, amino acid, terpenoids, carbohydrate, alkaloids as well as oil and fat.

Extraction of sweet orange juice

The sweet oranges were washed several times using clean water, peeled and sliced into halves and the juice were squeezed or squashed into the beaker. Orange juice (500 ml) was measured into two flasks each and 1000 ml of solvents (100% Ethyl ethanoate and 70% Ethanol) were added to the orange juice to make two different mixtures. The mixtures were left for two days to enable the solvents extract the active ingredients in the orange juice. The mixtures were filtered through Whatman filter paper (number 4) so as to obtain clean and clear filtrate from the residues of the extracts. The filtrates were concentrated in a vacuum using a rotary evaporator model (Buchi rotavapor R – 114) which ensures evaporation of the bulky solutions (filtrates) to the smaller volume concentrates (semi-solid) at temperature between 40°60°C. The resultant concentrates (extracts) were filter - sterilized using millipore filter 0.45 µm and they were ready for the antibacterial activities.

Preparation of extract concentration and the isolate

Six labeled beakers were separated into two groups. The first three breakers were used for the preparation of extract from ethyl ethanoate and the other three were used for the preparation of

Table 1. Phytochemical Constituents of *C. sinensis* juice.

Active ingrédients	Quantitative analysis	Inférence
Tannins	++	Moderate amount
Saponins	+++	High amounts
Flavonoids	++	Moderate amount
Steroids	+++	High amounts
Amino acid	+	Slightly detected
Carbohydrate	+	Slightly detected
Terpenoids	+++	High amounts
Alkaloids	+++	High amounts
Oil and fats	+	Slightly detected

extract from ethanol. In the first three beakers, 100, 200 and 300 mg/ml of extract from ethyl ethanoate was added to each beaker respectively. The same procedures were followed for preparing extract concentration from ethanol; however, the two tubes containing only the ethyl ethanoate and ethanol served as negative controls. A dilution factor of 10^{-10} of each selected organism (pure culture) was prepared and 1mL each out of these was taken for inoculation and labelled accordingly.

Antibacterial activity of the sweet orange juice extract

The extract concentrates of the sweet orange juice were screened for antibacterial activity by using a ditch (well or cup) method. Mueller-Hinton Agar was prepared based on the manufacturer prescription. Aliquot of 1 ml each of the test organism suspensions was inoculated with micro pipette onto the agar surface of each plate of the test organisms and with the aid of the hockey stick (spreader), the bacterial suspension was aseptically spread on the agar surface. The plates were allowed to absorb the organism suspensions at room temperature. A sterile cork-borer of 5mm diameter was used to punch on each agar surface in the plates to make three wells (ditches); subsequently each well was filled with 1mL of the orange juice extract of 100, 200 and 300 mg/ml respectively; and control wells containing the same volume (1 ml) of ethyl ethanoate and ethanol were made for each plate as negative control. However, positive control contained four plates of prepared Mueller-Hinton Agar based on the manufacturer prescription and the same procedure for the inoculation of test organisms was followed as early described in the ditch method and onto which discs impregnated with the following antibiotics; streptomycin, penicillin G, ciprofloxacin, and gentamycin were placed aseptically. The plates were incubated at 35°C for 24 h. Thereafter; the antibacterial activity was evaluated by measuring the diameter of the inhibition zone around the well and disc as case may be. The zone of inhibition around each well and disc was measured using a transparent metric ruler in millimeters (mm). All the tests (ditch method) were performed in duplicate and the average diameter of the two tests was calculated to give mean value and standard deviation of zone of inhibition on each organism and concentration.

Statistical analysis

$$\frac{f_1 + f_2}{2} = \text{mean value (mm)}$$

f_1 = first plate (diameter of zone of inhibition) in mm
f_2 = second plate (diameter of zone of inhibition) in mm

$$\sigma = \sqrt{\frac{\sum(x_i - \mu)^2}{N}}$$

σ = plates standard deviation
∑ = summation
N = the size of the diameter of zone of inhibition
x_i = each value of diameter of zone of inhibition
μ = mean value of the plates (diameter of zone of inhibition) in mm

The result from the above formulae (data) were then expressed as mean ± SEM (standard error mean) of duplicates and subjected to one-way analysis of variance (ANOVA), using the Statistical Analysis System (SAS 9.4 Version).

RESULTS AND DISCUSSION

Table 1 illustrates phytochemical constituents of sweet orange juice extract and the followings were present: tannins, saponin, flavonoid, terpenoid, steroid, amino acid, carbohydrate, alkaloid and oil and fat. These results were similar with the finding of Baba et al. (2018), except that amino acid was absent; while steroid and oil and fat were not analysed at all. Pytochemical analysis of the juice extracts showed that plant constituents such as alkaloids, saponins, terpenoid, tannins and flavonoid were present and that saponin in sweet orange juice was responsible for the antibacterial properties of the juice. According to Kumar et al. (2011) *Citrus sinensis* juice has large amount of saponin with haemolytic activity and cholesterol binding properties.

Table 2 shows that at 95% confidence level, there was a significant difference in the antibacterial activities (zones of inhibition) of extracts (ethyl ethanoate and ethanol respectively) on *E. coli, K. pneumoniae, N. gonorrheae* and *S. aureus*. Furthermore, according to the Duncan Post Hoc analysis of the ANOVA; there was no significant difference between the means of the zones of inhibition of the extracts (ethyl ethanoate and ethanol respectively) on *N. gonorrheae* and *S. aureus* and also there was no significant difference between the means of the zones of inhibition of the extracts (ethyl ethanoate and ethanol respectively) on *E. coli* and *K. pneumoniae*.

Table 2. Comparison of zone of inhibition of ethyl ethanoate and ethanol extract.

Organisms / Extract type	Concentration of extracts in (mg/ml)/ Means of zone of inhibition (mm)			Minimum inhibitory concentration (MIC)(mg/ml)
	100	200	300	
Escherichia coli				
Ethyl ethanoate extract	29.5 ± 0.5	29 ± 1.0	31.5 ± 0.5	300
Ethanol extract	23.5 ± 1.5	22.5 ± 0.5	22 ± 1.0	100
Klebsiella pneumoniae				
Ethyl ethanoate extract	37 ± 3.0	36 ± 1.0	36 ± 2.0	100
Ethanol extract	25 ± 5.0	21.5 ± 3.5	19.5 ± 0.5	100
Neisseria gonorrheae				
Ethyl ethanoate extract	17.5 ± 0.5	21 ± 0.0	21 ± 1.0	200 and 300
Ethanol extract	0.0 ± 0.0	6 ± 6.0	13.5 ± 1.0	300
Staphylococcus aureus				
Ethyl ethanoate extract	19 ± 2.0	21.5 ± 0.5	22 ± 0.0	300
Ethanol extract	10 ± 0.0	12.5 ± 2.5	5.5 ± 5.5	200

ANOVA of antibacterial activities of orange juice on different bacteria at different concentrations

Source	Type III sum of squares	Df	Mean square	F	P value
Model	12487.698[a]	7	1783.96	162.133	0
Effect on organism	1148.95	3	382.983	34.807	0
Conc on organism	6.812	2	3.406	0.31	0.738
Type of the extract	810.844	1	810.844	73.693	0
Error	187.052	17	11.003		
Total	12674.8	24			

Neisseria gonorrhea[a] and *Staphylococcus aureus*[a]; *Escherichia coli*[b] and *Klebsiella pneumoniae*[b].

This means that the zones of inhibition of extracts on *E. coli* and *K. pneumoniae* were higher compared to the zones of inhibition of extracts on *N. gonorrheae* and *S. aureus*.

There was no significant difference in the effect of different concentrations of the same extract on *E. coli, K. pneumoniae, N. gonorrheae* and *S. aureus*. This means that the change in the concentration of the same extract does not affect or improve the potency of the antibacterial activities of the extract but there was a significant difference in the type of extract (ethyl ethanoate and ethanol extracts) that is to say ethyl ethanoate extract was more effective than the ethanol extract. Thus ethyl ethanoate extract showed a remarkable inhibition against *K. pneumoniae* (37±3.0 mm) and *E. coli* (29.5±0.5 mm) compared to ethanol extract on the same test organisms; which showed lower zones of inhibition. Gram negative bacteria have been reported to be more resistant to antibacterial agents due to the possession of an outer-membrane permeability barrier that prevents the antimicrobial agents to reach inner part of the bacterial cell. The antibacterial activity against *E. coli* (gram negative) and *S. aureus* (gram positive) bacteria used in this study is an indication of its broad spectrum activity. This observation is in agreement with the report of Doughari and Manzara (2008) and Kumar et al. (2011). Ethyl ethanoate at various concentrations (mg/ml) demonstrated the highest antibacterial activity against *K. pneumoniae* (37 ± 3.0 mm), *E. coli* (29.5 ± 0.5 mm), *S. aureus* (22 ± 0.0 mm) and *N. gonorrheae* with the minimum zone of inhibition (21 ± 0.0 mm) at 100 mg/ml, 100 mg/ml, 300 mg/ml and 200 mg/ml respectively. This result concurs with the Kumar et al. (2011) findings.

Kumar et al. (2011) reported a maximum zone of inhibition (16 mm) against *E. coli* with ethyl ethanoate extract of the sweet orange juice. The variation in the antibacterial activity of the various extracts showed that different extracts have varying antibacterial agents with different modes of action and bacteria susceptibility or that not all phytochemicals responsible for antibacterial activity are soluble in a single solvent (Kumar et al., 2011 and Badar et al., 2008). Ethyl ethanoate extract was found to be a good solvent for the extraction of antibacterial agent in this study as it had shown the

Table 3. Zone of inhibition of conventional antibiotics using disc method.

Antibiotics	Disc code	Organisms per Zone of Inhibition (mm) on each Antibiotics				Interpretation
		Neisseria gonorrheae	***Staphylococcus aureus***	***Klebsiella pneumoniae***	***Escherichia coli***	**Resistant / Susceptible**
Streptomycin	S – 10	14	23	20	24	Susceptible
Ciprofloxacin	CIP – 5	18	21	15	17	Susceptible
Gentamycin	GM	14	20	17	15	Susceptible
Penicillin G	P	18	28	15	14	Susceptible

highest yield in the antibacterial activity of the sweet orange juice. However, none of the conventional antibiotics (positive control) as illustrated in Table 3 could match the inhibition zone of extract from ethyl ethanoate on the test organisms. According to Hassan et al. (2013), the ethanol extracts of *Ocimum gratissimum (E. coli* 17 mm; *S. aureus* 19 mm*)* and *Vernonia amygdalina* (*E. coli* 12mm; *S. aureus* 5mm) were the most effective on majority of test organisms among the water extracts of *Ocimum gratissimum* (*E. coli* nil, *S. aureus* 13 mm) and *Vernonia amygdalina* (*E. coli* nil, *S. aureus* nil) and the drugs; teteracycine (*E. coli* 16 mm, *S. aureus* 17mm) and flagy (*E. coli* nil, *S. aureus* 11 mm) used in their study. It can be concluded that *K*. *pneumoniae* and *E. coli* were more susceptible to the extracts (ethyl ethanoate and ethanol) compared to the zones of inhibition shown by *N*. *gonorrheae* and S*. aureus*. This finding concurs with Kumar et al. (2011) who asserted the highest zone of inhibition (16 mm) against *E. coli* with ethyl ethanoate extract of sweet orange juice.

Conclusion

The results obtained from this research proved that ethyl ethanoate and ethanol extracts of sweet orange juice have varying degree of antibacterial activity against the test organisms. This suggested that extracts of sweet orange juice can be useful in developing a new drug, which can be used in treating bacterial infections caused by the test organisms in this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Ahmed A, Beg AZ (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. Journal of Ethnobiology 74:113-123.

Alo MN, Anyim C, Igwe JC, Elom M, Uchenna DS (2012). Antibacterial activity of water, ethanol and methanol extracts of *Ocimum gratissimum, Vernonia amygdalina* and *Aframomum melegueta*. Advances in Applied Science Research 3(2):844-848.

Angew ON (2007). Functional funds. Trends in Food Science and Technology 30:19-21.

Baba J, Mohammed SB, Ya'aba Y (2018). Antibacterial activity of sweet *Citrus sinensis* (orange) on some clinical bacteria species isolated from wound. Journal of Family Medicine and Community Health 5(4):1154.

Badar N, Arshed M, Farooq U (2008). Characteristics of *Anethum graveolens* (Umbeliferae) seed oil: Extraction, composition and antimicrobial activity. International Journal of Agriculture and Biology 10:329-332.

Bakhru HK (2001). Herbs that heal: Natural remedies for good health. Orient Paper backs Rakesh Press, New Delhi -110028.

Bhardwaj SK, Laura JS (2009). Antimicrobial activities of some plant extracts against plant pathogenic bacteria *Xanthomonas campestris*. Indian Journal of Agriculture Research 43(1):26-31.

Crowell PL (1999). Prevention and therapy of cancer by dietary monoterpenes. The Journal of Nutrition 129(3):775-778.

Doughari JH, Manzara S (2008). In vitro antibacterial activity of crude extracts of *Mongifera indica*. African Journal of Microbiological Research 2:67-72.

Hassan IA, Ashiru WA, Ajetunmobi OA (2013). Sensitivity of *Vernonia amygdalina* and *Ocimum gratissimum* on some selected bacteria. International Journal of Scientific Research and Reviews 2(4):188-196.

Kumar KA, Narayani M, Subanthini A (2011). Antimicrobial activity and phytochemical analysis of Citrus fruit peels-Utilization of fruit waste. International Journal of Engineering Science and Technology 3(6):5414-5421.

Odebiyi A, Sofowora AE (1978). Phytochemical screening of Nigeria medicinal plant. Lylodia 4(13):234-246.

Piccinelli AL, Mesa MG, Armenteros DM (2008). HPLC-PDA-MS and NMR characterization of c-glycosy / flavones in a hydroalcholic extract of citrus aurantifolia leaves with antiplatelet activity. Journal of Agriculture 25:30-35.

Rehman S, Hussain S, Nawaz H (2007). Inhibitory effect of citrus peel essential oils on the microbial growth of bread. Pakistan Journal of Nutrition 6:558-561.