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Antimicrobial and phytochemical properties of grape fruit mesocarp and juice against Some Pathogenic Microorganisms

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Abstract
This study investigated the antimicrobial and phytochemical properties of grape fruit mesocarp and juice against some pathogenic microorganisms. The ripe fruits of *Citrus paradisi* (grapefruit) were collected after identification by a Botanist. The juice was squeezed out while the fruit mesocarp was extracted using methanol. Phytochemical sereening revealed the presence of saponins, athranoids, tannins, alkaloids and phenols were detected with grape juice while anthranoids, phlobatannins, cardiac glycosides, alkaloids and phenols were detected with grape mesocarp extract. Agar well diffusion technique was adopted in the determination of the antimicrobial activities of the extracts against *Salmonella, Escherichia, Pseudomonas, Enterococcus* and *Candida species*. Zones of inhibition recorded ranged from 16mm to 24mm with grape fruit mesocarp extract and 12mm to 22mm with grape fruit juice. Inhibitory effect recorded ranged from 125mg/ml to 250mg/ml with grape fruit mesocarp extract and 250mg/ml to 500mg/ ml with grape fruit juice. Bactericidal activity was recorded with grape fruit mesocarp extract at 500mg/ml against *Salmonella* and *Escherichia species*. Grape fruit juice showed bactericidal effect at 500mg/ml against *Enterococcus species*. The extracts showed bacteriostatic and fungiostatic effect against the other test organisms. The result of the phytochemical screening of grape fruit mesocarp and juice suggests the possible pharmacological importance of this plant part. The antimicrobial activity recorded against the test organisms showed that the plant extract is effective against *Escherichia species* and *Salmonella species*. The use of this plant part in traditional and pharmaceutical industries could be of health improvement through infection prevention and management.

Keywords: Mesocarp, phytochemical, antifeedant

Introduction

Background to the study

There have been increased interests in the use of plants as sources of medicines due to their fewer, non-existent or less severe side effects, and their relative abundance and therapeutic potency over orthodox medicines. Also, the increasing problems of antibiotic drug resistance by pathogenic organisms in the past few decades and recently have led to the continuous exploration of natural plant products for new antibiotic agents (Dahpour, Rahdari & Sobati, 2012 [5]; Belmekki, Bendimerad, Bekhechi & Fernandez, 2013) [3]. Many of these products are produced in plants as secondary metabolites and often used in plants for defense against microbial attack. The antimicrobial activities of medicinal plants can be attributed to the secondary metabolites such as flavonoids, terpenoids, tannins, alkaloids that are present in these plants (Sher, 2009) [14]. Some plants have been investigated scientifically for antimicrobial activity and a large numbers of plant products have been shown to inhibit the growth of pathogenic microorganism. Citrus is considered an important fruit in world production because its great value for human diet. Citrus is member of Rutaceae family from sub-tropical origin and is known for its semi-sweet taste. Citrus is the largest genus belong to the family Rutaceae and is the most traded horticultural Citrus fruits, are one of the main fruit tree crops grown throughout the world. Although sweet orange (Citrus) 1s the major fruit in this group accounting for about 70% of citrus output. The group also encompasses small citrus fruits such as tangerine tree (*Citrus reticulata*), grapefruit tree (*Citrus paradisi*), lime tree (*Citrus arantifulia*) and lemon tree (*Citrus limonum*). Citrus paradisi (Grapefruit) is the one of the most important member of the genus citrus (Rutaceae). It is native to the island of Barbados (Dahpour *er al.,* 2012) [5] The fruit has not only been enjoyed for its palatable qualities, but its medicinal values were known to ancient Greeks. The flesh of this fruit is used as a cure in poisoning and also used to refresh the break. The seed extracts of *Citrus paradisi* have been used for the treatment of ulcers, cataracts, urinary and alimentary tract infections. The oil from the peel of grapefruit has been used as insecticide and antifeedant. The extracts of different parts of grapefruits have been reported to demonstrate antimicrobial properties and as such shows penetrating application in food, drug formulation and cosmetics industries (Kanaze *er al.,* 2008) [7]. Different parts of grape fruit is utilized in herbal medicine. In Sudan Citrus paradisi internal fruit peel is used to treat for malaria, gastro protective and antiulcer (Gupta *er al.,* 2011 [6]: Somesh. Swati, Rupali, Jose & Manish, 2015) [15]. Antibacterial potency and synergistic effect of crude aqueous and methanolic extracts of parts of grapefruit extracts showed that the plant materials possessed antimicrobial activity with greater efficacy when used synergistically on the test organism (Anthonia & Olumide, 2010) [1]. Citrus is considered an important fruit in world production because

its great value for human diet. Many citrus plants parts such as; peels and seeds are discarded as wastes. Little information exists about the utilization of mesocarp of grape as antimicrobial agent. This study will therefore evaluate antimicrobial and phytochemical properties of grape fruit mesocarp and juice against some pathogenic microorganisms.

Materials and methods

Collection of plant materials

The ripe fruits of *Citrus paradisi* (grapefruit) were collected from Relief market, Owerri, Imo State and transported to the laboratory. The plant materials were identified by a Botanist and were processed for extraction and further studies.

Extraction of grapefruit mesocarp and juice

The method described by Somesh *er al.* (2015) [15] was adopted in the extraction of the active ingredients in the grapefruits mesocarp and juice. The grapefruits were washed and the rind was peeled off with the aid of the knife and the seeds removed. The mesocarp were dried under room temperature and ground using sterile grinder and kept for extraction. or the fruit juice extract, the cut fruit was squeezed into a sterile beaker after which the filtrate was filtered using a Whatmann No. 1 filter paper and the filtrate was transferred into sterile Bijou bottle. 20 grams of the dried inner peel powder was soaked in 180 ml of methanol for 72 hours with constant stirring at intervals of 24 hours. Finally, the extracts were filtered through Whatmann No. and concentrated by boiling. filter paper and the filtrate was collected into a sterile Bijou bottle

Preparation of test organisms

The microorganisms used for the antimicrobial activities were: *Salmonella, Enterococcus, Escherichia* and *Pseudomonas aeruginosa* and *Candida species*. These microorganisms were isolated from clinical samples such as; wound infection and urines samples.

Preparation of McFarland's Standard

1ml of concentrated sulfuric acid was added to 99ml of distilled water to make 1% solution sulfuric acid. Similarly, 0.5g of dehydrated barium chloride (BaCl, $2H_20$) was dissolved in 50ml of distilled water to make 1% w/v solution of barium chloride. Then 0.5ml barium chloride was added to 99.5ml sulfuric acid solution and mixed properly (CLSI, 2010).

Antimicrobial susceptibility testing

The agar well diffusion technique as described by Somesh *er al.* (2015) [15] was adopted for this study to evaluate the antimicrobial activity of the mesocarp and juice extracts. A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland's turbidity standards on the surface of already prepared and dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. Two wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. The extracts were then poured into the wells and the plates were allowed to stand for about 30 minutes for proper diffusion of the solutions before being incubated at 37°C for 24 hours (CILSL. 2010), After 24 hours, antibacterial activity was evaluated by measuring the diameter of the zones of 1inhibit produced by the extracts against the test organisms in millimeters.

Tests for minimum inhibitory concentrations (MIC)

For the MIC tests, about two milliliters (2ml) of the mesocarp and juice extracts were added in four milliliters (4ml) of peptone water; this gives 500mg'ml. Thereafter, two fold serial dilutions were carried out from the 500 mg/ml concentration by transferring 2 ml of the 500 mg/ml concentration to 4 ml of peptone water contained in a test tube and homogenized properly. This procedure of transferring 2 ml of the tube to 2 ml of peptone water contained in the subsequent tube was continued until the eighth tube. The following concentrations were thereafter obtained: 500mg/ml, 250 mg/ml, 125 mg/ml, 62.50 mg/ml, 31.25 mg/ml, 15.62 mg/ml, 7.81 mg/ml and 3.90 mg/ml. Having obtained the different Concentrations and dilutions, three drops of overnight broth cultures of the test organisms were inoculated into the dilutions in each case of the test organisms (Chegini *er al.,* 2018). The tubes were then incubated at 37 ^oC for 24 hours. The lowest concentration of each of the extracts in each case that inhibited the growth of the test organisms were recorded as the MIC.

Test for minimum bactericidal/fungicidal concentrations (MBC)

Tubes showing no visible growth from the MIC test were sub cultured onto sterile nutrient agar plates and incubated at 37 °C for 24 hours. The lowest concentration of the extracts yielding no growth was recorded as the minimum bactericidal Concentration as the case may be.

Qualitative phytochemical screening of the mesocarp and juice extracts

The methods described by Prasad (2014) were adopted in the determination of the phytochemicals present in the mesocarp and juice extracts.

Test for saponins

Ten milliliters (10ml) of distilled water was added to about two milliliters (2ml) of each of the extracts in a test tube and shaken vigorously (Prasad, 2014). Persistent frothing even after heating is an indication of the presence of saponins.

Test for anthropoids

Two milliliters (2ml) of each of the extracts, five milliliters (5ml) of 0.5M potassium hydroxide was added and mixed properly. Then 6 drops of acetic acid was added followed by 2ml of toluene. To the upper layer formed, 2ml of 0.5M potassium hydroxide was added. A change in colour of the mixture was an indication of a positive test while no colour change was an indication of a negative test.

Test for anthraquinone

To about 2ml of each of the extracts, 5ml of 10% ammonia was added and shaken Vigorously. 2ml of benzene was thereafter be added. A colour change was indication of a positive test while none was an indication of a negative test.

Test for phenol
5ml of each of the extracts was mixed with 8ml of distilled water in a test tube and 6ml of Ferric chloride was added to the mixture. A colour change to light brown is an indication of a positive test while none indicates a negative test.

Test for alkaloids
To about 2ml of each of the extracts, Sml of 1% aqueous hydrochloric acid was added and placed in a water bath for 3 minutes and thereafter 3 drops of Mayer's reagent was added. A white precipitate was an indication of a positive test while none was an indication of a negative test.

Test for tannins
To about 1ml of each of the extracts, 2ml of 1% ferric chloride was added. A colour change was an indication of a positive test while none was an indication of a negative test.

Test for phlobatannins
To about 2ml of each of the extracts, 2ml of 1% aqueous hydrochloric acid was added and boiled. The presence of white precipitate was an indication of a positive test while none was an indication of a negative test.

Test for cardiac glycoside
The Salkowski test was employed in this test. To 1ml of the extracts, 2ml of chloroform was added and then 2ml of concentrated tetraoxosulphate (VI) acid was added to form a lower layer. A reddish brown colour at the interphase was an indication of a positive test while none was an indication of a negative test.

Results
The results of this study on antimicrobial and phytochemical properties oI grape fruit mesocarp and juice against some pathogenic microorganisms are shown in
Table 1 to Table 4 below. Table 1 showed the phytochemical constituents of the grape fruit mesocarp and juice. The presence of saponins, athranoids, tannins, alkaloids and phenols were detected with grape juice while anthranoids, phlobatannins, cardiac glycosides, alkaloids and phenols were detected with grape mesocarp extract.
Table 2 showed the antimicrobial susceptibility pattern of the grape fruit mesocarp and Juice against the test organisms. Zones of inhibition recorded ranged from l6mm to 24mm with grape fruit mesocarp extract and 12mm to 22mm with grape fruit juice.
Table 3 showed the minimum inhibitory concentration of the grape fruit mesocarp and juice against the test organisms. Inhibitory effect recorded ranged from 125mg/ml to 250mg/ml with grape fruit mesocarp extract and 250mg/ml to 500mg/ml with grape fruit juice.
Table 4 showed the minimum bactericidal/fungicidal concentration of the grape fruit mesocarp and juice against the test organısms. Bactericidal activity recorded with grape fruit mesocarp extract at 500mg/ml against *Salmonella* and was *Escherichia species*. Grape fruit juice showed bactericidal effect at 500mg/ml against *Enterococcus* species. The extracts showed bacteriostatic and fungiostatic effect against the other test organisms.

Table 1: Qualitative phytochemical screening of the grape fruit mesocarp and juice

	Extracts/Result	
	Juice	**Mesocarp**
Phytochemical Constiturents		
Saponins	+	-
Anthranoids	+	+
Anthraquinones	-	-
Tannins	+	+
Phylpbsatannins	-	+
Cardiac Glycosides	-	+
Alkaloids	+	+
Phenols	+	+

Key: + = Presence of phytochemical
- =Absence of phytochemical

Table 2: Antimicrobial Suceptibility testing with the extract

	Extract/ Zone of inhibition (mm)	
Testing Organism	**Mesocarp**	**Juice**
Salmonella species	24	18
Enterococcus species	16	22
Escherichia species	22	16
Pseudomonas species	26	12
Candida species	22	18

Key: mm = Millimeter CLSI = Guidelines for antimicrobial agents
R=Resistant (0-12mm) S= Susceptible (16mm and above)

Table 3: Minimum Inhibitory Concentration of the extract

	Extract/Concentrations (mg/ml)	
Test Organism	**Mesocarp**	**Juice**
Salmonella species	125	500
Enterococcus species	250	250
Escherichia species	125	500
Pseudomonas species	125	ND
Candida species	125	500

Key: Mg/ml= Milligram per milliliter ND = Not Detected

Table 4: Minimum Bactericidal Concentration of the extract

	Extract/Concentrations (mg/ml)	
Test Organism	**Mesocarp**	**Juice**
Salmonella species	500	ND
Enterococcus species	ND	500
Escherichia species	500	ND
Pseudomonas species	ND	ND
Candida species	ND	ND

Key: Mg/ml= Milligram per milliliter ND = Not Detected

Discussion

Plant parts have been used as herbal medicine for their healing properties since ancient times. This study investigated the antimicrobial and phytochemical propertiesof grape fruit mesocarp peels and juice against some pathogenic microorganisms.

Table 1 showed the phytochemical constituents of the grape fruit mesocarp and juice. The presence of saponins, athranoids, tannins, alkaloids and phenols were detected with grape juice while anthranoids, phlobatannins, cardiac glycosides, alkaloids and phenols were detected with grape mesocarp extract. Azhari (2017) [2] has previously reported the presence of phytochemicals such as terpenes, flavonoids, alkaloids and tannins from grape fruit extracts.

Table 2 showed the antimicrobial susceptibility pattern of the grape fruit mesocarp and juice against the test organisms. Zones of inhibition recorded ranged from 16mm to 24mm with grape fruit mesocarp extract and 12mm to 22mm with grape fruit juice.

Azhari (2017) [2] reported antibacterial activities of peel and juice extracts of *Citrus aurantifolia* against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*. The zones of inhibition recorded in their study ranged from 13mm to 18mm with the juice extract showing better antimicrobial activity. Mehra *er al.,* (2015) reported zones of inhibition with grape fruit inner peel to range from 7mm to 16mm and 8mm to 17mm with pulp extracts.

Table 3 showed the minimum inhibitory concentration of the grape fruit mesocarp and juice against the test organisms. Inhibitory effect recorded ranged from 125mg/ml to 250mg/ml with grape fruit mesocarp extract and 250mg/ml to 500mgml with grape fruit juice.

Table 4 showed the minimum bactericidal/fungicidal concentration of the grape fruit mesocarp and juice against the test organisms. Bactericidal activity was recorded with grape fruit mesocarp extract at 500mg/ml against *Salmonella* and *Escherichia species*. Grape fruit juice showed bactericidal effect at 500mg/ml against *Enterococcus species*. The extracts showed bacteriostatic and fungiostatic effect against the other test organisms.

The antimicrobial activity recorded with this extracts is not surprising. This could be traced to the phytoconstituents of the plant. A variety of rich secondary metabolites such as tannins, alkaloids, phenols, cardiac glycosides etc (Table 1) could have resulted to its efficacy. The presence of phytochemicals in plants is an indication that they have functions of biological activity (Epidi *er al.,* 2016).

Saponins are natural glycosides that act as hypo-glycemic, antifungal and serum cholesterol lowering agents in animals. They are essential elements in ensuring hormonal balance and synthesis of sex hormones (Rahman *er al.,* 2011) [13]. Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. They have physiological role by acting as antioxidants through free radical scavenging activity, chelation of transition metals, inhibition of prooxidative enzymes and lipid peroxidation, hence modulating oxidative stress and preventing degenerative diseases. They also inhibit tumor growth by inducing apoptosis and inhibiting mutagenecity of carcinogens (Ngozi *er al.,* 2011) [10]. They exhibit anti-microbial activity by complexing nucleophilic proteins by hydrogen bonding, covalent bonding, and nonspecific interactions.

Tannins form complexes with proline-rich proteins that inhibit cell protein Synthesis. Synergistic action of tannins, flavonoids, alkaloids and saponins are known to inhibit the growth of pathogens (Nwankwo *er al.,* 2014) [11]. Tannins exert antimicrobial activities by iron deprivation, hydrogen bounding or specific interactions with vital proteins such as enzymes in microbial cells. Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery. Alkaloids can alter DNA, selectively deform cells, and cause locoism. Some alkaloid molecules, both natural and synthetic, can act as narcotics (Kittakoop *er al.,* 2014) [8]. An alkaloid is a plant-derived compound that is toxic or physiologically active. Some alkaloids such as isopteropodine, pteropopine have anti-microbial activity whereby they act by promoting white blood cells to dispose harmful micro-organisnms and cell debris (Ogunwenmo *er al.,* 2017) [12]. Phenols protect against pathogens, prevent heart ailments and acts as anti-inflammatory agents.

Conclusion

The result of the phytochemical screening of grape fruit mesocarp and juice suggests the possible pharmacological importance of this plant part. The antimicrobial activity recorded against the test organisms showed that the plant extract is effective against *Escherichia species* and *Salmonella species.* The use of this plant part in traditional and pharmaceutical industries could be of health Improvement through infection prevention and management.

Recommendations

1. The use of this plant part in traditional medicine should be encouraged other than its normal use as a spice.
2. There should be more research with the extract on other pathogenic bacteria and fungi so as to determine its efficacy against those organisms.
3. 3.Research on the toxicity of the seeds should be evaluated so as to justify its antimicrobial properties so as to encourage its safe use.

Conflict of interest

The authors declared that there is no conflict of interest regarding the publication of this manuscript.

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