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Screening and Quantification of Bioactive Compounds and Antimicrobial Activities of Fresh Juice, Methanolic Peel and Pulp Extract of *Citrus sinensis* L. (Sweet Orange).

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ABSTRACT

In this work, phytochemical examination of fresh juice and methanolic peel, pulp extract revealed the presence of diverse bioactive organic components. The antioxidant activity of *Citrus sinensis* L. (Sweet Orange) juice extract (JE) and crude methanolic extract of pulp and peel (MPPE) were tested. Two fungal strains and six bacterial strains (3 gramme positive and 3 gramme negative) were tested. *Citrus sinensis* plant extracts show antibacterial action against both bacterial strains, according to the research, but there is no efficacy against fungal strains. MPPE has higher antibacterial activity than J.E, this may be because phenolic and flavonoid compounds are found.

INTRODUCTION

The Rutaceae family includes roughly 160 genera and 1700 species. Citrus was one of the Rutaceae family's most important economic genera, producing high levels of polyphenolic and vitamin C. (Mulero *et al.*, 2012). Citrus fruits have a cell-protective effect because antioxidants such as vitamin C and flavonoids are present (Mokbel and Hashinaga 2006). The botanical name for the frequently cultivated sweet orange is *Citrus sinensis*. It belongs to the Citrus genus, which accounts for a considerable percentage of flowering plants (Jansz *et al.*, 1994).

The orange is the most well-known fruit in the world; it can be eaten raw or juiced (Topuz *et al.*, 2005). The peel, pulp, seeds, and juice make up the fruit. In some places, in contrast to other fruits, *C. sinensis* can be used not just as a fruit but more as a medicinal plant (Blauer, 2003). Approximately thirty-four percent of the fruit can be used to make juice, resulting in approximately as a by-product, forty -four percent of the peels (Li *et al.*, 2006). Citrus sinensis has a great number of secondary bioactive compounds, which play an important part in the plant's pharmacological activity. Many different sections of the plant, contain fatty acids, steroids, alkanes, and hydroxyamides (Rani *et al.*, 2009), flavonoids, and other phytochemicals (Gattuso *et al.*, 2007), peptides **(**Matsubara *et al.*, 1991**),** carotenoids (Aschoff *et al.*, 2015**),** and alimentary elements as sodium, potassium, calcium, and magnesium (Niu *et al.*, 2009).

The peel and seeds are sensitive to microbial deterioration; they produced a high amount of by-products that could pollute the environment. Citrus by-products, which are high in dietary fibre and bioactive chemicals, can be utilized in nutrition as useful elements in the development of efficient meals (Marin *et al.,* 2002). Peeling is used in a variety of industrial sectors since it is a valuable resource of phenolic bioactive compounds that can be employed in the composition of foods or isolated as natural antioxidants to keep certain foods from oxidising (Albishi *et al.,*2013). phenolic compounds present in a high amount in Citrus peels and seeds, such as phenolic acids and flavonoids; while flavonoids are found in greater quantities in peels than in seeds (Sawalha *et al.,* 2009)**,** Citrus fruits are regarded to be the essential sources of natural antioxidant, containing high amounts of bioactive compounds (Al-Juhaimi & Ghafoor, 2013). In addition, volatile essential oils found in *C. sinensis* peels are efficient at inhibiting bacterial growth and disinfecting wounds. Rios and Recio (Rios and Recio, 2005).

MATERIALS AND METHODS

Preparation of Extract:

Fresh fruits of *Citrus sinensis* were purchased from a local market for this study, at Benha City, Egypt. Whole Citrus fruits (300 g) were washed in the laboratory then surface sanitized with 70% alcohol, washed with sterile distilled water, peeled the fruit and juice extracted using the juice extractor into a separately sterilized container and after that, it was filtered into a new sterile container to eliminate any remaining tissues and weight. (70 ml). It was peeled and cut into smaller pieces then grinded with pulp. The fresh peels with pulp (230 g) were soaked in 300 ml methanol at room temp and shaking for 24 hours and filtrated. The filtrate was concentrated to dryness below vacuum pressure using a rotary evaporator at 35 Celsius degrees. The plant residue that resulted was considered as a crude extract. It weighed (22.8 gm), was collected in glass stoppered tubes, and stored at -10 °C in a deep freezer till use. The whole two studied extracts included (1) juice extract (JE) and (2) crude methanolic extract of pulp and peel (MPPE).

Phytochemical Screening:

Alkaloids:

A-Wagner Test: The formation of reddish-brown precipitate appears when(1ml) of Crude extracts are treated with Wagner's reagent.

B-**Mayor's test**: When we mixed 2 ml filtrate with 1 percent HCl and approximately 6 drops of Mayor's reagents, we got a creamish or pale-yellow precipitate.

Terpenoid and Sterol Test (salkowski test):

20 mL of ethanol were added to Crude extracts and then boiled in a water bath. Subsequently, the filtration and vaporization were done then 10 mL of diethyl ether was added into the residue and filtered once more. At room temperature, the filtrate was dried. After that few drops of acetic acid were added and then a few drops of conc.H_2so_4 were added. The formation of blue or greenish-blue color indicated the existence of steroids and the presence of triterpenoids was revealed by the production of reddish-brown color.

Saponins Test (Foam Test): The crude extracts were mixed and shaked with distilled water then heated to boiling point. The formation of bubbles indicates that the saponins are present.

Flavonoids Test (ammonia test): In a test tube, 1-2 drops of 1% NH_3 (ammonia) solution are added to the aqueous extract of each sample. If flavonoids are present, they produce a yellow color.

Phenolic Content Test: We added 5 mL distilled water to the crude extracts then added few drops of 10% iron (III) chloride solution to the mixture When the color of the solution changed to blue or green, it meant the phenolic content was positive.

Tannins Test (ferric chloride test): Added 2 mL of distilled water to the Crude extracts and heated on a water bath, followed by adding of 1-2 drops of diluted $FeCl_3$. The presence of

tannins was shown by the formation of a dark green tint

Test for amino acids (Ninhydrin test): 1-3 drops of Ninhydrin reagent were added to 1 ml of the extract. The presence of amino acids is indicated by the occurrence of purple color.

Quantification of Total Phenolic Compounds: -

To evaluate the concentration of total phenolic compounds in dry extracts, the Folin-Ciocalteu technique (Singleton and Rossi, 1965; Kähkönen *et al.,* 1999**)** was employed.

Quantification of total flavonoids compounds:

Aluminum chloride colorimetric assay (Zhishen *et al.,* 1999; Zou *et al.,* 2004) was used to determine the total flavonoid content.

Antioxidant Activities Assays:

According to (Pérez–Jiménez *et al.,* 2008), a DPPH radical scavenging assay was used. The DPPH radical scavenging activity (percent RSA) of substances was estimated using the equation from the absorbance at the start (0) and after a certain reaction time (T) (1).

(% RSA) = (ABS-ATS) / ABS x 100 (1)

Where; The absorbance of the blank sample (DPPH) solution without the substance to be tested is **ABS,** and the absorbance of the tested sample is **ATS**.

Antimicrobial Activity Test:

Antimicrobial activity tests were conducted at Benha University's Faculty of Sciences' Department of Botany and Microbiology. The tested samples were determined using a modified Kirby-Bauer disc diffusion method (Bauer *et al.,* 1966). 100 l of the test bacteria/fungus were cultivated in 10 ml of fresh media until they reached roughly 108 cells/ml for bacteria and 105 cells/ml for fungi (Pfaller, *et al.,* 1988). On plates, 100 µl of microbial suspension was spread corresponding to the broth in which they were preserved. Inoculated fungi Plates *as Aspergillus flavus* at 25 C for 48 hours; Inoculated bacterial plates; Gram (+) bacteria as *Staphylococcus aureus, Bacillus subtilis*; Gram (-) bacteria as *Escherichia coli, Pseudomonas aeuroginosa* they were incubated at 35-37 °C for 24-48 hours and yeast as *Candida albicans* incubated at 30 °C for 24-48 hours and, then the diameters of the inhibition zones were measured in millimeters (NCCLS, 1993)**.**

The Type Strain of Microorganisms:

Six bacterial strains (3 grams positive and 3 grams negative (Table1), and two fungal strains were tested (Table 2).

Table 1: Bacterial species

Name	Gram reaction	ATCC
Bacillus cereus	G +	14579
Staphylococcus aureus	G +	6538
Streptococcus faecalis	G +	25175
Escherichia coli	G -	8739
Pseudomonas aeruginosa	G -	9027
Salmonella typhimrium	G -	14028

Table 2: Fungal species

Name	ATCC
Candida parapsilosis	22019
Aspergillus flavus	9643

RESULTS AND DISCUSSION

Phytochemical screening

Extraction is a necessary step in the analysis and utilization of the plant's bioactive compounds. **Table (3)** summarizes the phytochemical test findings for J.E. and MPPE, which indicate positive results for Terpenoids, Steroids, Alkaloids, Tannins, Phenolic, Protein Compounds, and Flavonoids, but no results for Saponin. Citrus JE and MPPE are high in phytochemicals, according to these findings. *Citrus sinensis* fruits contain carbohydrates, coumarin glycosides, flavonoids, volatile oils, organic acids, lipids fixed oils, and glycosides in varying proportions, according to Oikeh *et al.,* 2013. Phytochemicals have a wide range of health advantages, including anti-inflammatory, antibacterial, and anti-diabetic properties (Ayoola *et al.,* 2008; Oikeh *et al.,* 2013).

Table 3: Phytochemical analysis of JE and MPPE of Citrus sinensis

Plant Constituent	Juice	Peel &pulp extract	Name of test
Alkaloids	+	+	Wagner's
	+	+	Mayer
Flavonoids	+	+	Ammonia
Phenolics	+	+	Ferric Chloride
Tannins	+	+	Ferric Chloride
Sterols	+	+	Salkowski
Protein & amino acids	+	+	Ninhydrin
Saponin	-	-	Foam
Terpenoid	+	+	Salkowski

(+): Present (-):Absence

Quantification of Total Flavonoid and Phenolic Content:

Anti-inflammatory action, antibacterial activity, enzyme inhibition (Harborne and Baxter 1999), anti-allergic effect, and antioxidant activity are only a few of the therapeutic qualities of flavonoids (Middleton and Chithan 1993). Total flavonoid content was reported to be 0. 07 g/100g in JE and 0. 06 g/100g in MPPE, respectively. Follin Ciocalteu reagent method using to calculate the content of total phenol, which was represented as g/100 g. Figure **(1)** revealed the total phenolic content of JE and MPPE was 8.43 mg/g and 11.24 mg/g, respectively. The peel of *C. sinensis* is high in vitamin C, fibre, and many nutrients, including phenolics and flavonoids. (Favela-Hernández *et al.,* 2016).

Antioxidant Activity: Because of their chemical structures and redox properties, plants high in flavonoids, phenolics, and carotenoids have antioxidant action (Shoib and Malik 2018). Because of its stability and simplicity, the DPPH Free Radical Scavenging Activity technique is commonly utilized (Brand Williams *et al.,* 1995). The results showed that when the concentration increased, the free radical scavenging activity of both JE and MPPE increased (Fig. 1). This research backs up the findings of (Cillard and Cillard 1998), who found that the ethanolic extract of *C. sinensis* Linn had anti-inflammatory and antioxidant properties. The presence of key phytochemical components such as phenolic acid, a flavonoid, was linked to the antioxidant efficiency of *C. sinensis* peel extract in the current study. The antioxidant activity which belongs to the phenolics and flavonoids content in plants is a critical aspect in the treatment and prevention of disease. As a result, the

therapeutic potential of a plant source may be determined by its bioactive phytochemical components (Dai & Mumper, 2010).

Antimicrobial Activities of Juice and Methanolic Extracts of *Citrus sinensis*.

Many medications have been discovered and designed using natural bioactive compounds derived from plants (Hafidh *et al.,* 2011). *Citrus sinensis* JE and MPPE were examined for antimicrobial properties, and the results are displayed in Figures (2&3) and Table (1,2&4). Herbal medicine is regarded as a major source for the discovery of novel antibiotic therapy for the medication of bacterial infection-related disorders. The antibacterial and antifungal properties of *C. sinensis* JE and MPPE were investigated in this study. Citrus sinensis JE and MPPE were tested against six bacterial strains (three Gram (+ve) and three Gram (-ve)) and two fungal strains. The findings of this investigation revealed that the *C. sinensis* extracted sample examined displayed antibacterial properties versus Gram +ve and Gram-ve bacterial strains, but no possible antifungal activity. Table 4 and Figures 2&3 demonstrate the maximum inhibitory concentration (MIC) and lowest inhibitory concentration (MBC) values. The occurrence of significant natural bioactive compounds such as tannins, flavonoids, polyphenols, and alkaloids is linked to the antibacterial activities of JE and MPPE in *C. sinensis*. In many of the plants investigated, the main antioxidants are phenolic substances. Components that support high antioxidant action (Cai *et al.,* 2004; Zheng and Wang, 2001)

Rahman *et al.* (2011) supported our findings, stating that the phytochemicals responsible for plant antibacterial action are connected to the presence of tannins, flavonoids, saponins, phenolic compounds, and essential oils. In contrast to JE, MPPE performed better in all bacterial strains, and this is an explanation that MPPE has a greater phenolic content than JE (11.24 mg/g and 8.43 mg/g) respectively. The antibacterial activity of plant phenolic aromatic compounds is diverse. Plants that deal with microbial infestation produce these chemicals. Their capability to form complexes with extracellular and soluble proteins, as well as bacterial cell walls, has been postulated as a possible explanation for their action (Dhiman *et al.,* 2012). These results are consistent with those of Ehigbai *et al.* (2020), who discovered a significant content of tannin, flavonoids, and phenols in fresh *C. sinensis* peel extract.

Citrus sinensis peel extracts were also shown to have antibacterial properties by El-Desouky *et al.,* 2018; Baba *et al.,* 2018). Many biological activities of flavonoids have been discovered, including antibacterial, antioxidant, and anti-inflammatory properties (Gorniak *et al.,* 2019). Tannins create complexes with proline-rich proteins that stop cells from making proteins. The synergistic activity of alkaloids, flavonoids, tannins, and saponins is linked to pathogen growth inhibition (Nwankwo *et al.,* 2014).

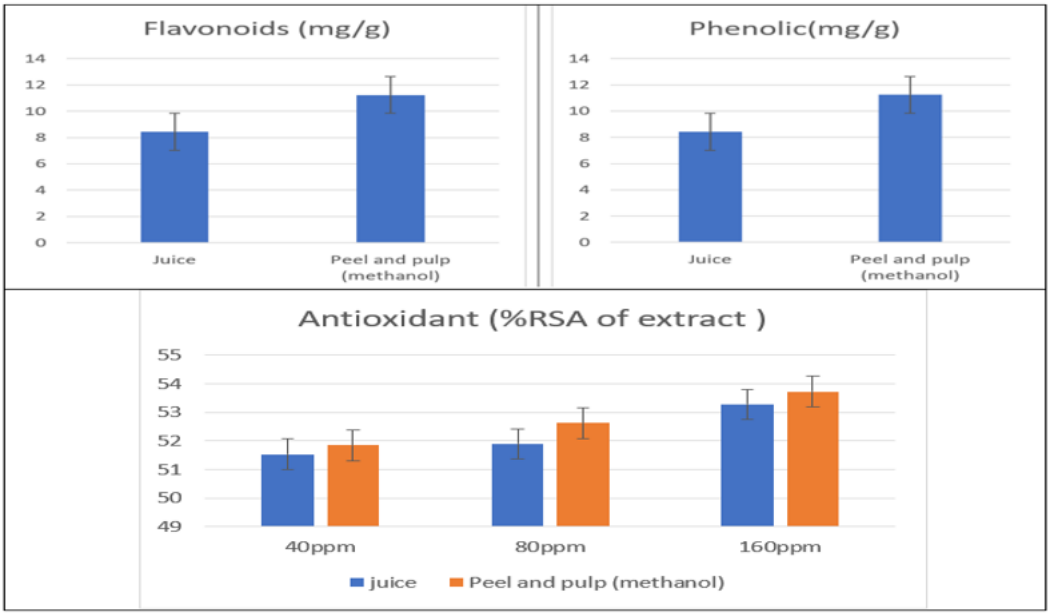

Fig. 1: Total Flavonoids, Total Phenolic and Total antioxidant content of JE and MPPE of *Citrus sinensis*

Table 4: Antimicrobial activities of *Citrus sinensis* JE and MPPE against some bacterial and fungal strains tested by disc diffusion

	Bacillus Cereus (G+)	Strepto-coccus faecalis (G+)	Staphylo-coccus Aureus (G+)	Escherichia Coli (G-)	Pseudo-monas Aeruginosa (G-)	Salmonell typhimrium (G-)	Aspergillus flavus	Candida parapsilosis
Ampicillin antibacterial agent	26	20	21	25	26	20	-	-
Amphotericin antifungal agent	-	-	-	-	-	-	17	23
juice	0	5	10	5	9	0	0	0
Peel and pulp (methanol)	10	12	14	13	16	18	0	0

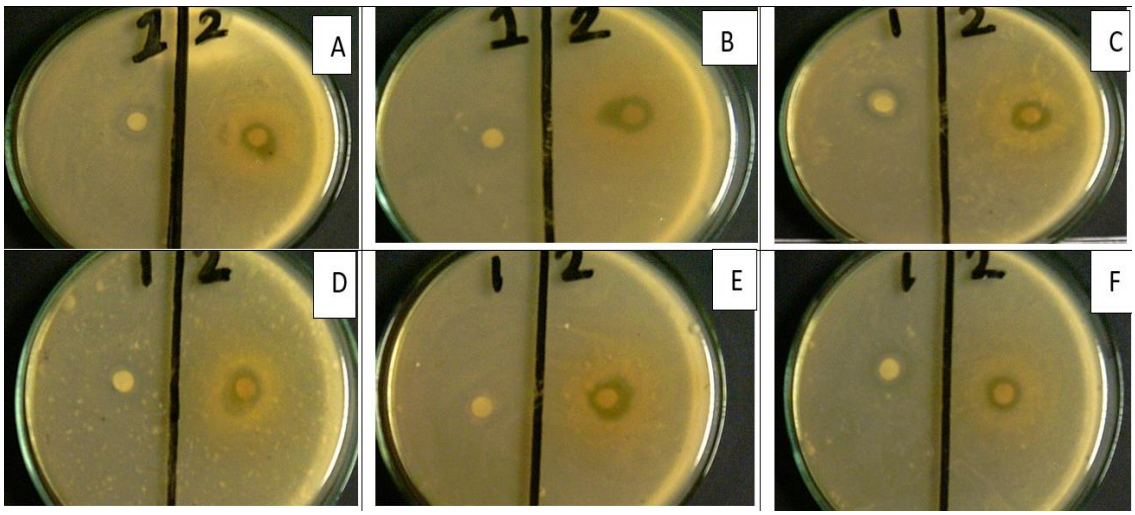

Fig. 2: The agar diffusion method for the antimicrobial activity to Juice (1) and peel and pulp extract (2) against pathogenic bacteria (a) *Streptococcus mutans* (b) *Pseudomona aeruginosa* (c) *Staphylococcus aureus*, (d) *Bacillus cereus* (e) *Salmonellatyphimrium* (f) *Escherichi coli*.

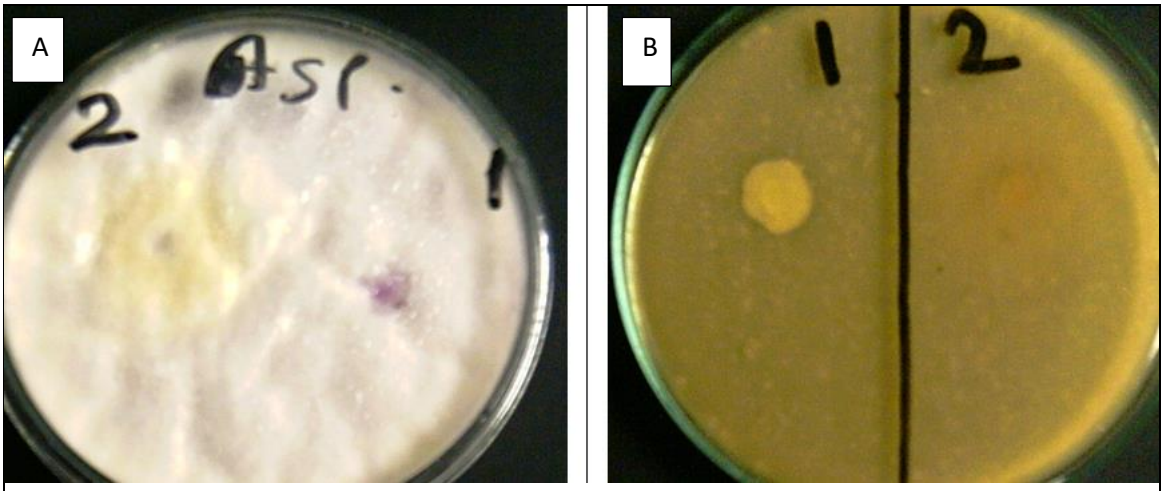

Fig. 3: The agar diffusion method for the antimicrobial activity to Juice (1) and peel and pulp extract (2) against pathogenic (a&b) *Aspergillus flavus* (b) *Candida parapsilosis*

Conclusion:

This research investigated that the antimicrobial efficiency of MPPE and JE of the *C. sinensis* related to the synergistic action of alkaloids, flavonoids, tannins, and phenol. According to the findings of this investigation, MPPE is an effective antibacterial agent.

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