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The Phytochemical and Antimicrobial Effect of Citrus sinensis (Orange) Peel Powder Extracts on Some Animal Pathogens as Eco-Friendly

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Abstract

A noticeable potency of many species of plants present against bacterial and fungal pathogens. Bacterial resistance against antibiotics is consider one of the common problems in the medical world, so one of the most important steps in microbiological researches is to find a new antimicrobial compound with minimal side effects. So the aim of this study is to investigate the antimicrobial activity of *Citrus sinensis* (orange) peel aqueous and organic solvent extracts on some medically important animal pathogens and to determine some phytochemical compounds to be recycled to added in animals ration. Hot and cold aqueous in addition to ethanol extracts of *Citrus sinensis* (orange) peel were evaluated for their antimicrobial activity against some medically important pathogens isolated from animals and poultry farms (*Staphylococcus aureus, Escherichia coli, Pseudomonas aerogenes, Bacillus cereus* and *Candida albicans*) by agar well diffusion method. Both hot, cold aqueous and ethanol peel extracts showed high antibacterial and antifungal effect against the all examined pathogenic samples. Also, phytochemical compound of aqueous and ethanol peel extracts were determined, results of the chemical tests explain the extracts of *Citrus sinensis* (orange) peel contain alkaloids, flavonoids, tannins and saponin compounds while glycosides not found. So it could be concluded that the *Citrus sinensis* (orange) peel extracts possess remarkable antibacterial activity against gram positive and gram negative bacteria in addition to its antifungal activity against *Candida albicans* and to be introduced as an alternative to chemical antimicrobial drugs, is required wider investigation also ecologically as recycling of fruits peel can be used as food additive in animal ration.

Keywords: *Orange; Antimicrobial; Extract; Citrus sinensis; Fruits Peel*

Introduction

The main food source for some essential nutrients is fruits and vegetables and also includes a series of bioactive components, which might have multiple effects in the fields of health [1,2]. Fresh, in juices, and cider fruits are different forms of fruit consumption including orange.

Orange as one of the family Rutaceae it is called botanically *Citrus sinensis* where it is known as tasty, juicy fruit. It has total global production about 120 million tons so it considered one of the most important and widely grown fruit crop and due to its tasty juice and medicinal value it has been cultivated widely in tropical and subtropical climates [3,4].

Orange peel extract has a lot of medicinal properties which have been reported just like against cancer, diuretic, stomachic, immuno – enhancing, colic, cormunative, upset stomach, tonic to digestive system, immune system and skin. It is also used to treat and prevent vitamin deficiencies, colds, flu, and scurvy and helping to fight viral and bacterial infections [3,5]. In addition to its antibacterial effect which also have been reported in the literature. As its potent antibacterial effect against Enteric pathogens that were reported by Mehmood., *et al*. (2015) [3,6]. Moreover what have been mentioned by Akdemir (2015) about its potent effectiveness against *Klebsiella pneumonia* [3,7].

Via literature search did not imply any studies investigating the effect of orange peel extract on animal disease pathogens.

More over traditional herbal therapy can be a satisfying option where some of the pathological condition where the scientific drugs become crippled but demands an ample amount of research [8].

So the aim of this study is to investigate the antimicrobial activity of *Citrus sinensis* (orange) peel aqueous and organic solvent extracts on some medically important animal pathogens and to determine some phytochemical compounds to be recycled to added in animals ration

Materials and Methods

Collection of plant materials: *Citrus sinensis* (orange)used in this study were obtained from local market in Alquieiya, KSA, 2017.

Aqueous extraction: The dried plant peels were added to the boiled distilled and left to cool. Then were mixed by the blender and filtered to get clear aqueous extracts then, were kept at 4°C until to be used. But the hot aqueous extract has been prepared directly after boiling and filtration according to [9].

Solvent extract: 5g of dried plant peel was extracted with 10 ml of each solvent (Ethanol) kept for 24h. After that it was filtered using Whatman No.1 filter paper. The solvent was evaporated to make the final volume as 1/2 of the original according to [10].

Preparation of inoculums: From large animals and poultry farms on the outskirts of Cairo the inoculums (bacterial strains and fungi) were isolated. The strains of bacteria (*Bacillus cereus, Escherichia coli, Pseudomonas aerogenes, Staphylococcus aureus*) and fungi (*C. albicans*) were inoculated Sabouraud dextrose agar (SAB). (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) and nutrient broth (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) for overnight at 37°C for bacteria and 25°C for fungi according to [9].

Antimicrobial screening: The agar well diffusion method was used for the determination of antibacterial activity of *Citrus sinensis* (orange) peel aqueous extracts in addition to ethanol extract by using bacterial isolates taken from animals and poultry (*Staphylococcus aureus, Escherichia coli, Pseudomonas aerogenes, Bacillus cereus*) and fungi (*C. albicans*) to evaluate its effects on the isolated bacteria. Bacterial isolate loopful growth was inoculated into nutrient broth incubated at 37°C for 18 hours. The dilution of bacterial suspensions with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to get a uniform suspension containing 1.5×10^8 CFU/ml. Muller- Hinton agar was inoculated with 0.1 ml of bacterial inoculum. Using cork borer, wells were made on the cultured media. The aqueous and solvent extracts were considered as the 50% concentration. Then, 0.1 ml of extracts were added to wells, then the plates left for 30 minutes in refrigerator at 4°C, thereafter, they were incubated at 37°C for 24 hrs. The activity of extracts was determined by measuring the diameter of inhibition zone in millimeter. All experiments were duplicated. Ciprofloxacin (10 µg) and penicillin (10 µg) used as positive control while distilled water (100 µg) used as negative control for antibacterial screening aqueous extract. Nystatin (10 µg) was used as positive control while distilled water (100 µg) used as negative control for antifungal screening. All chemicals used (Purchased from Witan – Biolife Company produced by Jalil Medicals Company) [9,11].

Phytochemical Tests

1. **Tannins Test:** A modified methods stated in [9,11] was used to be presented of tannins on the extracts, A few drops of Ferric chloride reagent were added for 3 ml of extract. A blue black color refereed to the present of tannins.
2. **Alkaloids Test:** A few drops of Marqus reagent (prepared from mixing 0.5 ml of Formaldehyde with 5 ml of concentration H_2SO_4), added to the 5 ml of extract. Turbidity refereed to the present of alkaloids [9,12].

3. **Saponins Test:** 3 ml of extract was added to the 2 ml of Ferric chloride, a white residue to be formed as evidence to the present of Saponins [9,13].
4. **Flavonoids Test:** Flavonoids test were implement in conformity with [9,13]. 2 ml of extract mix with Alcoholic KOH (0.5 mol.), a yellow color as proofed to the present of Flavonoids.
5. **Glycosides Test:** 0.5g of grinded *Citrus sinensis* (orange) dried peel was dissolved in 2 ml of glacial acetic acid containing one drop of Ferric chloride solution, and then underlaid with 1 ml of concentration H_2SO_4. A brown ring indicated the present of Glycosides [9,14]. All chemicals used (Purchased from Witan – Biolife Company produced by Jalil Medicals Company).

Results

The results given in table 1 indicate that the hot and cold aqueous in addition to ethanol extracts of *Citrus sinensis* (orange) dried peel were evaluated for their antimicrobial activity against some medically important pathogens isolated from animals and poultry farms bacteria (*Staphylococcus aureus, Escherichia coli*, *Pseudomonas aerogenes, Bacillus cereus*) and fungi (*C. albicans*) by agar well diffusion method. Both hot, cold aqueous and ethanol peel extracts showed high antibacterial and antifungal effect against the all examined pathogenic samples. Also, phytochemical compound of aqueous and ethanol peel extracts were determined, results of the chemical tests explain the extracts of *Citrus sinensis* (orange) peel contain alkaloids, flavonoids, tannins and saponin compounds while glycosides not found as shown in table 2.

Examined fruits and vegetables peels powder	Types of extracts	Types of examined microbes				
		S. aureus	Bacillus	E. coli	Ps. aerogenes	C. albicans
Citrus sinensis	Hot aqueous extract	24	14	23	25	17
	Cold aqueous extract	12	12	21	23	20
	ethanol	15	21	25	27	21
Control +ve	Ciprofloxacin	25	34	20	30	0
Control +ve	Bacitracin	33	36	17	22	0
Control +ve	Nystatin	0	0	0	0	16
Control –ve	Distilled water	0	0	0	0	0

Table 1: *Antimicrobial activity of Citrus sinensis (orange) peel aqueous and ethanol extracts against some animal pathogen in mm.*

Plant extracts Phytochemical tests	Citrus sinensis
Flavonoids Test	+
Alkaloids Test	+
Glygosides Test	-
Saponins Test	+
Tannins Test	+

Table 2: *The phytochemical compounds in Citrus sinensis peel aqueous extracts.*

+: Contain this phytochemical compound

Discussion

Resistant bacteria act as defy in a lot of infections treatments which require finding novel antimicrobial substances to be used against these microorganisms. The mother earth long before man can set his feet on it has been anchored by plants. As a source of Mankind existence on earth as a gift earlier to his arrival to life. According to The World Health Organization (WHO) 80% of the population use herbal medicines as a various diseases treatment because it's available, economic and have lesser side effect. The basics of medical pharmacology have been established by herbal remedies for ages and these herbal medicines popularity due to the better acceptance of patient [3,15].

Scientific studies available on medicinal plants indicate that promising phytochemicals can be developed for many health problems [16,17]. More over some of the pathological condition where the scientific drugs become crippled but traditional herbal therapy can be a satisfying option which demands an ample amount of research [8,16].

Normally peels of fruits and vegetables are discarded as waste product. But its importance for pharmaceutical purpose has been reported by different studies. These documented components activities just like antiproliferative, antioxidant, anti-inflammatory and antimicrobial etc [18].

Even through Citrus juice processing industries the peels are generally wasted while the yielded juice of citrus is lesser half of the fruit weight. Where oranges byproduct wastes, such as peels produced in very large amount every year [19,20]. Now there is increasing attention to recycling the waste products to produce useful materials and this is the tee for citrus recycling into value-added products but the suitable methods for utilization have to be researched [19,21]. That will lead to reduction in pollution environmentally. In addition to the phytochemicals and nutrients constituents of the citrus peels which increase its importance as drugs and food supplements [19,22-24].

The present study aimed to investigate the antimicrobial activity of *Citrus sinensis* dried peel powder aqueous and organic solvent extracts on some medically important animal pathogens and to determine some phytochemical compounds to be recycled to added in animals ration.

The results given in table 1 indicate that the hot and cold aqueous in addition to ethanol extracts of *Citrus sinensis* dried peel were evaluated for their antimicrobial activity against some medically important pathogens isolated from animals and poultry farms bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerogenes, Bacillus cereus)* and fungi (*C. albicans)* by agar well diffusion method. Both hot, cold aqueous and ethanol peel extracts showed high antibacterial and antifungal effect against the all examined pathogenic samples

Orange peel extract has a lot of medicinal properties which have been reported just like against cancer, diuretic, stomachic, immuno – enhancing, colic, cormunative, upset stomach, tonic to digestive system, immune system and skin. It is also used to treat and prevent vitamin deficiencies, colds, flu, and scurvy and helping to fight viral and bacterial infections [3,5]. In addition to its antibacterial effect which also have been reported in the literature. As its potent antibacterial effect against Enteric pathogens that were reported by Mehmood., *et al*. (2015) [3,6]. Moreover what have been mentioned by Akdemir (2015) about its potent effectiveness against *Klebsiella pneumonia* [3,7].

Cowan (1999) [3,25] stated that the patent of the antimicrobial property of citrus peel depending on the used solvent type of extract especially due to its constituents of aromatic and organic antibiotic compounds as plant which easily soluble in organic solvents.

In the present study, ethanol and hot extracts were found to be more effective than cold extracts. This results in agreement with the results which have been obtained by Jeyaseelan and Jashothan (2012) where the hot ethanol extract of *Ricinus communis* L leaf showed high antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli* which explained by authors due to the production biomolecules as a result of heating and this product active than the cold extract [3,26]. Fruits, as rich sources of phenolic compounds have been paid a special attention [1,27-29].

As the preliminary phytochemical investigation done by Mamta (2013) demonstrated the presence of tannins, saponins in both the citrus peel and pulp. These constituents play an important role in the antibacterial activity but it is difficult to correlate their action to a which one [19].

In regard to the previous results phytochemical compound of aqueous and ethanol peel extracts were determined, results of the chemical tests explain the extracts of *Citrus sinensis* contain alkaloids, flavonoids, tannins and saponin compounds while glycosides are not found as shown in table 2.

Tannins, saponins, phenolic compounds, essential oils and flavonoids play an important role the antimicrobial potency of plants as a secondary active metabolites of plants for example the role of tannin which extracted from citrus peel it can produce irreversible complexes with proline which inhibit the cell protein syntheses [3,30].

Moreover the role of alkaloid as a toxic compound for the foreigner organisms to cell [3,31]. Just., *et al*. (1998) [3,32] reported that the saponins which have been extracted from Citrus peel have inhibitory effect on inflamed cells. In addition to the role of flavonoids, just like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties [3,33].

Conclusion

One of the most novel way to produce new and safe compounds with low cost especially in human, animal and plant nutrition as well as in the pharmaceutical industry is the recycling of fruit waste and *Citrus sinensis* is an interesting example of a plant used in traditional medicine for many year especially its peel which contain active phytochemical constituents just like tannins, saponins etc. which can act as antimicrobial agents.

So the *Citrus sinensis* peels can be recycled as an available for no cost, have no side effects effective and economical antimicrobial agent for both man and animal not treated only as wastes but it considered eco-friendly.

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