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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, ANTIOXIDANT, ANTIBACTERIAL ACTIVITIES OF *CITRUS SINENSIS* PEEL EXTRACTS

[1,*]Mr. Karthikeyan, G., [2]Mr. Velusamy K. and [3]Ms. Manju, S.

[1]Assistant Professor, Department of Biochemistry, Kongu Arts and Science College (Autonomous), Erode
[2&3]I-M.Sc Biochemistry, Kongu Arts and Science College (Autonomous), Erode

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ABSTRACT

In the present-day scenario perishable fruit peels are considered as a new era of pharmaceutical products as they are rich in phytochemicals and act as antioxidant agents. In this work, the phytochemicals are analysedusing the various extracts of *Citrus sinensis* peel (aqueous, acetone, ethanol and hexane). The identified phytochemicals are alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides, steroids and phenolic compounds. These phytochemicals are widely used for cure many diseases (liver, cardiac and brain related diseases) as well as used in preparation of medicines. The antioxidants are very important for our body because they reduce the free radicals. The present study also evaluates the antioxidants found in *Citrus sinensis* peel extracts using DPPH assay and total phenolic assays. The hexane and water extracts have more antioxidant activity in DPPH assay and total phenolic assays respectively. The extracts of orange peel *C.sinensis* exhibited potent antibacterial activity against *E.coli, Klebsiella pneumoniae* and *Bacillus subtilis*. From this, the zone of inhibition of is higher in acetone extractthan others. Findings from this study support the useless orange peels are very supportive for medicinal purposes.

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INTRODUCTION

A medicinal plant has similar properties as conventional pharmaceutical drugs. Humans have used them throughout history to either cure or lessen symptoms from an illness. A pharmaceutical drugis produced in a laboratory to cure an illness (Kumar, 2011). Humans commonly used spices because of their high essential oil content that helped to keep food from becoming diseased by bacteria or other microbes (Muhammad Ali, 2018).Sweet orange (*Citrus sinensis*) is a small evergreen tree 7.5 m high and sometimes up to 15 m. Its origin is China and it is grown commercially worldwide in tropics, semitropical and some warm temperate regions and has become the most widely planted tree fruit in the world (Ali Sadeghian, 2011). Some herbs and spices like orange peel, capsicum, pumpkin skin, cardamom, and cloves have some special compounds helps to prevent infections as well as diseases from microorganism (Nessma Ahmed El Zawawy, 2015). The orange peel contains antioxidant and anti-inflammatory activity is also present in the plant materials due to the presence of many active phytochemicals such as flavonoids, phenolicsvitamins, coumarins, terpenoids, carotenoids, saponins, lignin and plant sterols and so on (Nessma Ahmed El Zawawy, 2015). Thus they offer protection against pathogens (Suja, 2017) and these peels and pomace are a source of sugars, minerals and organic acids, dietary fibers and phenolic which have a wide range of actions which includes antioxidants, antimutagenic, cardio preventive, antibacterial and antiviral activities (Kumar, 2011). The essential oils comprise phenolic compounds such as terpenes. (Hajoori, 2014). *Citrus* fruit products act as antimicrobial agents against the bacteria and fungus. The sweet orange product has an important and physiological role because of its commercial value in pharmaceutical and food industries of the entire world (8). Use of waste as a source of polyphenols and antioxidants may have considerable economic benefit to food processors, while the vegetable processing in India generates substantial quantities of waste, income and employment (Basharat Mehmood, 2015). In past, many research have been done on antimicrobial potential of orange peel extract in different solvents, (Mutahar Shiban, 2012) cold water, Ethyl acetate, Acetone and Ethanolic extract of peel shows significant result against S. typhimurium, P. aeruginosa, E. coli, S.aureus, S. typhi, B. subtilis, K. pneumonia. The present investigation is aimed to investigate and characterize the fruit

Corresponding author: **Mr. Karthikeyan, G.,**
Assistant Professor, Department of Biochemistry, Kongu Arts and Science College (Autonomous), Erode.

peel wastes of lemon and oranges using phytochemical analysis, antioxidant property and antibacterial activity (5).

MATERIALS AND METHODS

Collection and processing of peel of *citrus sinensis:* The Orange were collected from local market and remove the peel of it, thenthe peel was subjected to shade drying for about two to five days. The dried peel of Orange was further crushed to powder and the powder was stored in air tight container.

Preparation of extracts: The dried and powdered plant materials (15 g) were extracted successively with 200 ml of each solvent separately by using soxhlet extractor for 5h. The solvents used for the study was Water, Ethanol, Acetone and Hexane. The extracts were filtered and then concentrated to dryness using a steam bath at 37ºC. Each extract were transferred to glass vials and kept at 4º C before use. Yield of the extract obtained was calculated as

Yield % = Weight of extract recovered / Weight of dried Powder ×100

Preliminary phytochemical screening

Test for carbohydrates: To a few drops of extract, 2 ml of Molish's reagent is added. The mixture is shaken well and 2.0 ml of Conc. H2SO4 is added slowly along the sides of the test tube and allowed to stand. A reddish ring formed at the junction of two solutions indicates the presence of carbohydrates.

Test for reducing sugars: To a few drops of extract, 2 ml of Fehling's reagent is added. The mixture is shaken well and boils for 5 minutes. Brick red precipitate indicates the presence of sugar.

Test for tannins: To a few ml of extract, few drops of 1% Lead acetate is added. The mixture is shaken well. A yellowish precipitate indicates the presence of tannins.

Test for flavonoids: To a few ml of extract, few drop of Dilute H2SO4 is added. Orange colour develops which indicates the presence of flavonoids.

Test for terpenoids: To 2 ml of extract, 2 ml of acetic anhydride and Conc. H2SO4 is added. Formation of blue, green rings indicate the presence of terpenoids.

Test for protein: To a few ml of extract, few drop of Millon's reagent is added. White precipitate indicates the presence of protein.

Test for glycosides: To 2 ml of extract, 2ml of chloroform and 2 ml of acetic anhydride is added. Formation of violet to blue to green reddish brown ring indicates the presence of glycosides.

Test for cardiac glycosides: In a test tube added 5 ml of extract and 2 ml of glacial acetic acid and 1 drop of ferric chloride and 1.0 ml of Conc. H2SO4 is added slowly along the sides of the test tube and allowed to stand. Formation of brown, violet, greenish rings indicate the presence of cardiac glycosides.

Test for coumarin: To 2 ml of extract, 10% of 3 ml NaOH is added. Formation of yellow indicates the presence of coumarin.

Test for totalphenols: To 2 ml of extract, 3% of FeCl2 is added. Formation of deep blue colour indicates the presence of total phenol.

Test for Phenols: To 2 ml of extract, 3 ml of ethanol and a pinch of ferric chloride are added. A greenish yellow colour appears which indicates the presence of Phenols.

Determination of antioxidant activity

Dpph Assay: Different volumes (2 - 20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. Experiment was done in triplicate.

$$\% \text{ RSA } = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Total phenolic test: Phenolic contents (mg/100ml of extracts) were determined using the Folin-Ciocalteu reagent method. The reaction mixture was made with extract (100µl), Folin-Ciocalteu reagent (100µl) and 20% sodium carbonate (3 ml). Reaction mixture was incubated at room temperature for 1h and the absorbance of deep blue complex was measured at 765 nm. Gallic acid was used as a standard with varied concentration from 200ppm to 1000ppm. The total phenolic content was expressed as mg gallic acid equivalents per gram extract weight (mg/100gm).Experiment was done in triplicate.

Antibacterial assay of orange peel: The agar well diffusion method is used to determine the antibacterial activity of various extracts of C. sinensis peel. Nutrient agar and Nutrient Broth Media were used for bacterial culture (*E.coli, Klebsiella pneumoniae* and *Bacillus subtilis*). Thirty five millilitres of seeded nutrient agar media was transferred into each Petri plate and solidify. The organisms were streaked in different petri plates. Four wells were made in each plate. Test solution of 50µL was poured into each respective well. These plates were incubated at 37^{o}C. After 24 hours of incubation, the diameter of the clear zones that showed inhibition of bacterial growth was measured in millimetre (mm). Experiment was done in triplicate.

RESULTS AND DISCUSSION

In the present study, evaluated the phytochemical analysis, antioxidant activity and antibacterial activity of different extracts of orange (*Citrus sinensis)* peel. The results are given below.

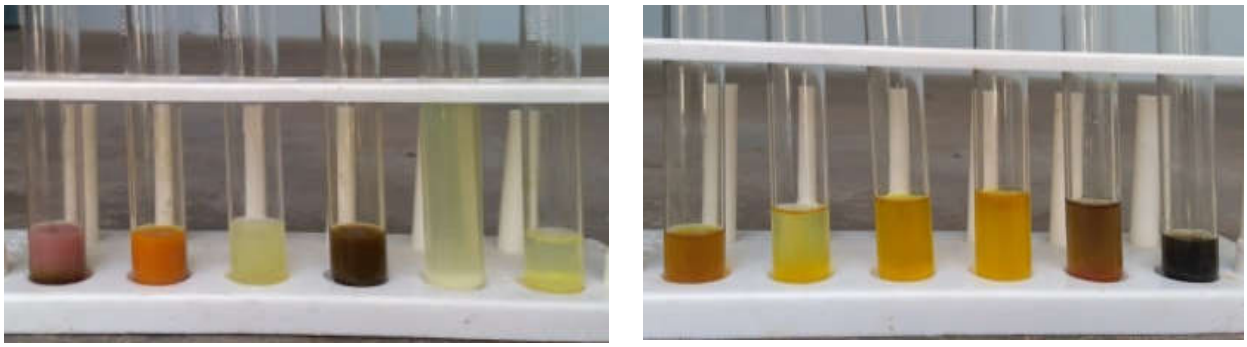
Yield of Extract: The percentage of extracts obtained from different solvents are shown as follows

Table 3.1.Yield of Extracts

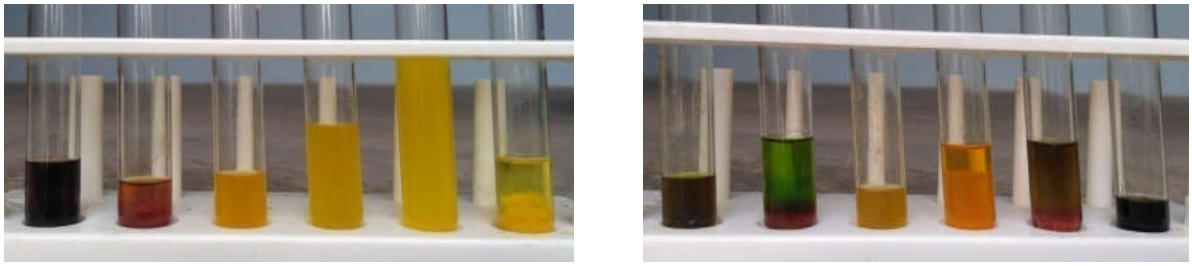
SOLVENTS USED	% OF EXTRACTS OBTAINED
Water	75.0
Acetone	60.0
Ethanol	50.0
Hexane	32.0

Table 3.2. Phytochemical screening of various extracts of *Citrus sinensis*

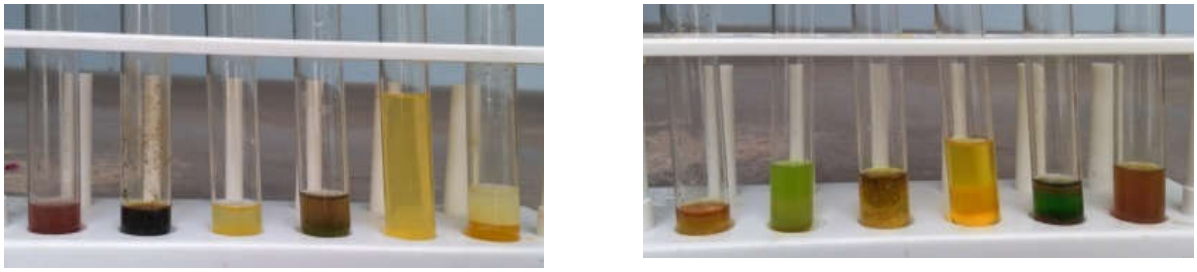
S. No	Test for Phytochemicals	Extracts			
		Water	Acetone	Ethanol	Hexane
1	Carbohydrates	+	-	-	+
2	Reducing sugars	-	-	+	+
3	Tannins	+	-	+	-
4	Flavonoids	+	-	-	-
5	Terpenoids	+	+	+	+
6	Test for Protein	+	-	-	+
7	Glycosides	+	+	-	-
8	CardiacGlycosides	-	+	+	+
9	Coumarin	+	+	+	-
10	Cycloglycosides	-	+	+	-
11	Totalphenols	-	-	+	-
12	Phenols	-	+	+	+



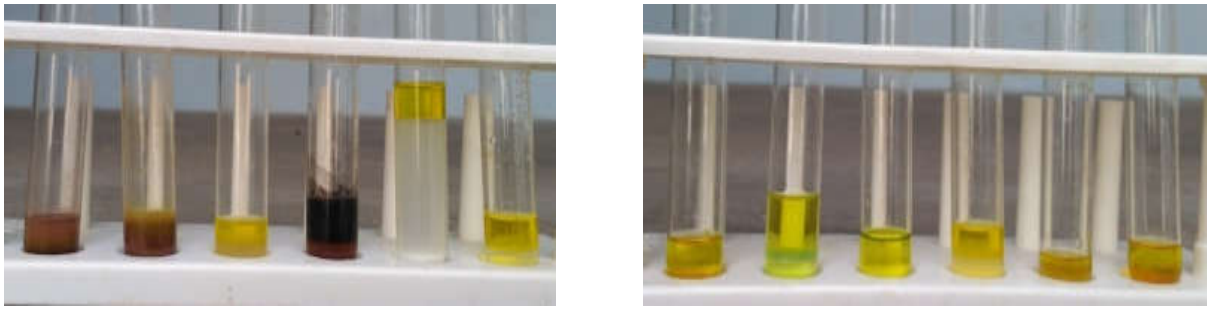
Figures 3.1. Phytochemical screening of Aqueousextract



Figures 3.2. Phytochemical screening of Acetone extract



Figures 3.3. Phytochemical screening of Ethanol extract



Figures 3.4. Phytochemical screening of Hexaneextract

Table 3.5. Values of DPPH Assay

EXTRACTS	DPPH (%)
Water	4.25± 0.01
Acetone	36.17± 0.22
Ethanol	27.65± 0.15
Hexane	40.42± 0.31

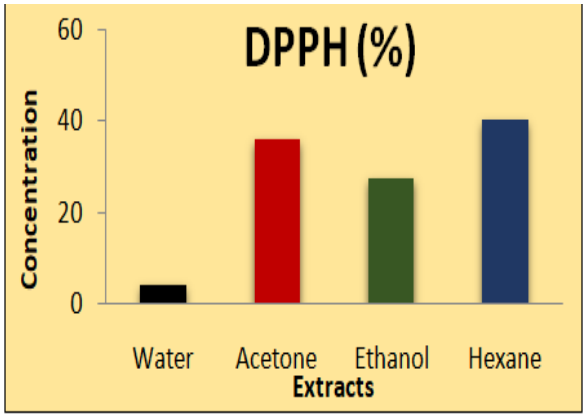


Figure 3.7 & 3.8. Showing the results of Total phenol activity

Table 3.7. Zone of inhibition of *Citrus sinensis* peel extract against microorganism

S.No	EXTRACTS	ZONE OF INHIBITION in mm		
		Bacillus subtilis	*Klebsiella pneumoniae*	*Escherichia coli*
1	Aqueous	7.0 ± 1.0	3.0 ± 0.2	10.0 ± 1.0
2	Acetone	30.0 ± 2.0	10.0 ± 1.0	18.0 ± 2.1
3	Ethanol	5.0 ± 0.3	5.0 ± 1.2	13.0 ± 0.8
4	Hexane	-	-	6.0 ± 0.1

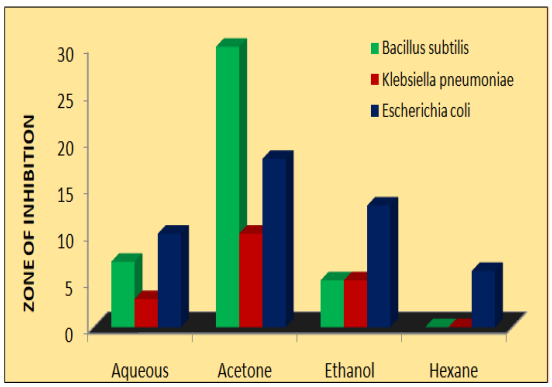


Figure 3.7 & 3.8. Showing the results of antibacterialactivity

Phytochemical screening: The Phytochemical screening of various extracts shows the presence of certain important components such as Terpenoids, Coumarin, Cycloglycosides, Cardiac Glycosides and Phenols.
The above phytochemical constituents were highly present in the acetone and hexane extracts.

Antioxidant assay of orange peel: The antioxidants of Orange peel weredone using DPPH test and Total phenol in different extracts such as water, acetone, ethanol and Hexane.

DPPH Assay: From the result it is clear that Hexaneextract of *Citrus sinensis* peel shows maximum DPPH activity when compared to other extracts.

Total phenol activity: From the result it is clear that water and acetone extracts of *Citrus sinensis* peel shows maximum Total phenol activity when compared to other extracts.

Antibacterial assay of orange peel (*citrus sinensis*): The peel extract of the *Citrus sinensis* had been tested for their antibacterial activities and an interesting antibacterial profile has been observed against *Bacillus subtilis*,*Klebsiella pneumoniae* and *Escherichia coli*.The peel extracts showed enormous activity against all three bacteria tested. The activities of extracts are mentioned in the terms of zones of inhibitions (mm). The zone of inhibition against *Bacillus subtilis* was 7.0 ± 1.0mm, 30.0 ± 2.0mm,5.0 ± 0.3mm and no activity for Aqueous, Acetone, Ethanol and Hexaneextracts of *Citrus sinensis* peel respectively. The zone of inhibition against *Klebsiella Pneumoniae* was 3.0 ± 0.2 mm, 10.0 ± 1.0mm, 5.0 ± 1.2 mm and no activity for Aqueous, Acetone, Ethanol and Hexaneextracts of *Citrus sinensis* peel respectively. The zone of inhibition against *Escherichia coli* were 10.0 ± 1.0mm, 18.0 ± 2.1mm, 13.0 ± 0.8mm and6.0 ± 0.1mm for Aqueous, Acetone, Ethanol and Hexaneextracts of *Citrus sinensis* peel respectively. From the result, we observed that the zone of inhibition of *Bacillus subtilis* and *Escherichia coli* is higher in acetone extract whereasthe zone of inhibition of *Klebsiella pneumoniae* is higher in ethanol extract.

Summary

In this research we inference the presence of certain phytochemicals in *Citrus sp*. (orange) peel powder like Alkaloids, saponins, Tannins, Flavonoids, Terpenoids, Glycosides, Steroids and Phenolic compounds. Theantibacterial activity of orange peel extracts exhibits the enormous results against *Bacillus subtilis, Escherichia coli*, and *Klebsiella pneumonia* by agar well diffusion methodand concluded that the fruit peel having antibacterial substance.This study exhibited almost antioxidant activity which could be due to the presence of polyphenols compounds. Thus, these wastes of the orange could be utilised as a source of supplement or further exploited for value addition as they are rich in phytochemicals and antioxidant components. Hence citrus peel is one of the most underutilised and geographically diverse bio-waste residues on the planet.

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