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PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF SEED AND COAT OF CITRUS SINENSIS

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ABSTRACT: Citrus sinensis is one of the most abundant citrus species consumed. Orange peels and seeds are a waste by-product of the fruit and may potentially contain useful phytonutrients with biological relevance. Fresh and dry peels of sweet orange were subjected to Maceration. Total alkaloid, flavonoid and tannin content were determined using standard methods. Antimicrobial activities against five bacterial strains (Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhimurium) and three fungal strains (Candida albicans, Aspergillus niger and Penicillium notatum) was carried out by observing the zone of inhibition using disc diffusion method. The total alkaloid, flavonoid and tannin content was higher in the fresh peel extract compared to the dry peel extract. Antimicrobial activities revealed that the fresh peel extract had better antibacterial activities against all bacterial strains and one fungal strain studied compared to the dry peel extract. Growth of Aspergillus niger and Penicillium notatum were however better inhibited by the dry peel extract than the fresh peel extract This study investigated the phenolic content and antimicrobial activities of fresh and dry Citrus sinensis peel extracts. The results from the study conclude that the fresh Citrus sinensis peel extract contains more phenolics and possesses better antimicrobial activities against the studied microbial strains compared to the dry peel extract. The findings in this study suggest that drying plant parts before extraction for phytonutrients may lead to loss of active phytochemicals.

KEYWORDS: Citrus sinensis, Orange, Antimicrobial, Phytochemicals, Seeds, Peels.

INTRODUCTION: Citrus sinensis (L.) Osbeck (sweet orange) is the world's most widely grown and commercialized citrus specie. The fruit of C. sinensis is mostly recognized for its vitamin C content and is also an important source of other phytochemicals such as phenolics and carotenoids which are reputed to have health benefits. The sweet orange fruit is usually eaten whole or processed into juice after the peeling of the external rind (flavedo). This peeling process leads to the generation of substantial wastes.

Sweet orange (Citrus sinensis (L.) is the world's most commonly cultivated fruit tree. It belongs to the Rutaceae family which comprises mandarins, limes, lemons, grapefruits, sour and sweet orange . Citrus fruits are of immense economic value; occupying the top position in fruit production. Orange trees are widely cultivated in tropical and subtropical climates for the sweet fruit, which is peeled or cut (to avoid the bitter rind) and eaten whole, or processed to extract orange juice. Citrus fruits are known to contain substantial quantities of vitamin C, a potent water-soluble vitamin essential for healthy living. The term

phytochemical is often used to describe a diverse range of biologically active compounds found in plants. Phytochemicals provide plants with colour, flavour and natural protection against pests. Phytochemicals are not essentially required for the sustenance of life but confer extra health benefits against pathogens. Phytochemical screening avails us with the opportunity to see at a glance the various phytochemicals present in a plant material. This may give a hint as to the possible range of bioactivities the plant product may possess. It also serves as a preliminary step in research protocol aimed at the isolation, purification and utilization of compounds inherent in the plant material for medical, pharmaceutical or agro- industrial use. The orange fruit is of unique economic importance as all portions contain potentials for diverse industrial usability. Thus far, researches into citrus wastes have been concentrated on the peels (flavedo); with little interest on the albedo and seeds. This study aims to draw attention to the possible waste-to- wealth utilization of orange fruit by-products with the added advantage of providing novel sources of nutraceuticals, phytochemicals, pharmaceuticals and reducing environmental insults.

PLANT PROFILE:

The Citrus sinensis popularly known as sweet orange seed in Igbo of Nigeria is of the Rutaceae family. The seed is best sown in a greenhouse as soon as it is ripe after thoroughly rinsing it, sow stored seed in March in a greenhouse, germination usually takes place within 2-3 weeks at 130C. seedlings are liable to damp off so they must be watered with care and kept well ventilated. Citrus sinensis. Contains a wide range of active ingredients and research is still underway in finding uses for them. They are rich in vitamin C, flavonoids, acids and volatile oils. They also contain coumarins such as bergapten which sensitizes the skin to sunlight. Bergapten is sometimes added to tanning preparations since it promotes pigmentation in the skin, though it can cause dermatitis or allergy responses in some people. Some of the plants more recent applications are as sources of anti-oxidants and chemical exfoliants in spercified cosmetics. The fruit is an appetizer and blood purifier, it is used to allay thirst in people with fever and also treat catarrh. The fruit juice is useful in treatment of bilious infections and bilious diarrhea. The fruit rind is caminative and tonic cure for acne. The dried peel is used in the treatment of anorexia, cold cough etc.

Origin And Description : The orange is unknown in the wild state; its assumed to have originated in Southern China. Northeastern India and perhaps Southeastern Asia (formally Indochina). It was carried to the mediterenian area possibly by Italian traders after 1450 of by Portuguese navigators around 1500. Up to that era citrus fruits were valued by Europeans mainly for medicinal purposes, but orange was quickly adopted as a luscidious fruit and wealthy persons grow it in private conservations, called orangeries. By 1646, it had been much publicized and was well known. The orange has become the most commonly grown fruit in the world. It is an important crop in the far east, the union of South Africa, Australia, throughout the Mediteranian area and sub tropical areas of South America and the Caribbean. The United States leads in the world production, with Florida, alone, having an annual yield of more than 200 million boxes, except when freezes occur which may reduce the crop by 20 or even 40%.

Morphology Aspect Of The Citrus Sinensis : The orange tree, reaching 25 ft (7.5m) or with great ages up to 50ft (15m) has a rounded crown of slender branches. The twigs are twisted and angled when young and may bear slender semi- flexible, bluntish spines in the leaf axils. There may be faint or conspicuous wings on the petioles of aromatic evergreen, alternate elliptic to ovate, sometimes faintly toothed “leaves” – technically solitary leaflets of compound leaves. These are 21/2 to 6m (6.5-15cm) long 1 to 33/4 in (2.5 – 9.5cm) wide. Brone singly or in clusters of 2 to 6, the sweetly fragrant white flowers, about 2 in (5cm) wide, have a saucer-shaped, 5 pointed calyx and 5 oblong, white petals, and 20 to 25 stamens with conspicuous yellow anthers. The fruit is subglobose oblate or some what oval, 21/2 to 33/4 in (6.5-9.5cm) wide. Dotted with minute glands containing an essential oil, the outer ring (epicarp) is orange or yellow when ripe, the inner ring (mesocarp) is white spongy and nonaromatic, the pulp (endocarp) yellow, orange or more less red, the sweet orange differs physically from sour orange in having a solid center.

Phytoconstituents:

1.5 % essential oil is present in the orange fruit. D-limonene (90%), citral, sinesal, n-nonanal, n-decanal, n-dodecanal, geranyl acetate, anthranil acid, citronellal, linalyl acetate, methyl ester are present.

SR.	PHYTOCONSTITUENTS	PLANT PART
1	**Flavone glycosides;** Neohesperidin, Naringin, Hesperidin, Narirutin **Triterpene;** Limonene, Citrol **Pigment;** Anthocyacin, Beta-cryptoxanthin,Cryptoxanthin, Zeaxanthin and Rutin, Eriocitrin, Homocysteine Polymethoxylted flavones; Tangeritin and Nobiletin **Flavonoids;**Citacridone, Citabrsine and Noradrenaline	Fruit peel
2	Terpanoids, Linalool, β elemene	Leaves
3	Triterpenes, Limonene	Flowers
4	**Vitamins;** B1, B2, B3, B5, B6, and Vitamin C **Minerals;**	Fruits

MATERIAL METHOD:

The peel part of citrus sinensis was collected and dried under shade. This dried material was mechanically powdered, sheaved and stored at a dry place. This powdered material was used for further phytochemical, antimicrobial and antioxidant analysis.

Collection of plant material: The fresh peels of citrus sinensis were collected from the local market in Bhopal. The peels were washed thoroughly with tab water and dried under shade for 7-10 days grounded into fine powder and sieved .

Preparation of powder: The peel part of citrus sinensis was collected and dried under shade. These dried materials were mechanically powdered, sheaved using 80 meshes and stored in an airtight container. These powder materials were used for further phytochemical analysis.

Extraction of the plant material : The peel of the plant were properly washed in tap water and rinsed in distilled water. The rinsed Peel part were hot air-dried for 7-10 days at room temperature. The dried peel part of each plant were pulverized using pestle mortar to obtain a powdered form which was stored in airtight glass containers at 4°C until used. 50 g of powdered sample was soaked in distilled water and methanol (200 mL and 100 mL) separately for 12 hrs at room temperature. The extracts were then filtered with whatsmann filter paper no 4 and concentrated to a final volume of 50 mL and subjected to phytochemical analysis.

Phytochemical Screening : The phytochemical screening involves the simple chemical test to detect the presence of secondary metabolites.. The phytochemical test include: tests for saponins, tannins, flavonoids, alkaloids, cardiac glycosides and phenol.

Test for Alkaloids :12 ml of chloroform was stirred with peel extracts separately with a few drops of dilute hydrochloric acid and filtered. The filtrates were divided into four equal portions and tested with various alkaloidal reagents namely Mayer's reagent (cream precipitate), Dragendorff's reagent (orange brown precipitate), Wagner reagent (reddish brown precipitate), and Picric acid (white precipitate).

Dragendorff's reagent: 3 drops of Dragendorff's reagent were added to first portion of the filtrate and observed for formation of the characteristic orange yellow or brown coloured precipitates served formation of the characteristic orange yellow or brown coloured precipitate.

Wagner's reagent*:* 3 drops of Wagner reagent were added to second portion of the filtrate and observed for formation of the characteristic reddish brown precipitates.

Mayer's reagent: 3 drops of Mayer's reagent were added to third portion of the filtrate and observed for formation of the characteristic cream coloured precipitates.

Test for Saponins :

Frothing test: 0.5 g of the extract was weighed and dissolved in 10 ml of sterile distilled water. The test tube was stopped and shaken vigorously for 30 seconds. It was then allowed to stand for half an hour.

Foaming test: 0.5 g of the extract was weighed and stirred in 20 ml of sterile distilled water and boiled in a water bath for 5 minutes and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Sodium bicarbonate test: 0.5 g of the extract was weighed and dissolved in 10 ml of sterile distilled water. 3 drops of sodium bicarbonate solution were added and shaken vigorously for two minutes, then observed for the formation of emulsion.

Test for Flavonoids :

Ammonia test: Filter paper strips were dipped in the ethanolic solutions of the extract and ammoniated. The filter paper changed its colour to yellow which indicates the presence of flavonoids.

Shinoda's test: To 1 ml of the each extract, a piece of metallic magnesium was added followed by addition of 2 drops of concentrated hydrochloric acid. A red colour confirmed the presence of flavonoids in the extract.

Sodium hydroxide test: Aqueous sodium hydroxide was added to 1 ml of the extract solution, dilute sulphuric acid was added slowly. A yellow colour which turned cream on addition of dilute sulphuric acid confirmed the presence of flavonoids.

Test for Tannins :

Ferric chloride test : About 0.5 g of the extract was stirred with 2 ml of distilled water and filtered. The filtrate was treated with five percent (5 %) ferric chloride reagent. A blue-green precipitation was taken as evidence for the presence of tannins.

Bromine water test: About 0.5 g of the extract solution was dissolved in distilled water in a test-tube. 5 drops of bromine water was added gently. Decolouration of the bromine water confirmed the presence of tannins.

Test for Cardiac Glycosides : Three grammes (3 g) of the extract were hydrolysed with dilute hydrochloric acid for 2 hours in water bath and allowed to cool well on ice for 2 minutes and filtered. The filtrate was subjected to different test for cardiac glycosides: Liebermann- burchard's, Keller-killani, and Salkowski tests.

Keller-Killani Test: One millilitre (1 ml) of glacial acetic acid containing traces of FeCl3 and one millilitre (1 ml) of concentrated H2SO4 was added to the extract carefully. The formation of brown ring at the interface indicated the presence of cardiac glycosides.

Salkowski test: To another portion of the solution, one millilitre (1 ml) of sulphuric acid was added carefully to form lower layer. A red brown colour at the interface indicated the presence of cardiac glycosides.

Liebermann's test: To another portion of the solution, one millilitre (1 ml) of hydrochloric acid was added carefully to form lower layer. A green colour with a ring formation on top while blue-green colour indicated the cardiac glycosides.

Test for phenols : One milliliter of the extract was added to 1ml of 10% FeCl3 and mixed together. The presence of blue precipitate confirms the presence of Phenols.

Screening for Antimicrobial Activity :

The hot and cold aqueous and ethanol extracts of the citrus sinensis were used for the antimicrobial screening using the agar well diffusion method. The media was punched with 7 mm diameter wells and were filled with various concentrations of the extracts 5 mg/ml, 10 mg/ml, 15 mg/mL, 20 mg/ mL and 25mg/mL. The plates were then incubated at 37ºC for 24 hours. After incubation, zone of growth inhibition for each extract was measured in millimeters by using a scale .

RESULT AND DISCUSSION:

Observation Teble of Phytochemical Screening

S.NO	TEST	RESULT	INFERENCE
1.	**Alkaloids Test**		
	Dragendroff Test	Present	Yellow / brown ppt
	Mayers Test	Present	Cream colour obtain
	Wagners Test	Present	Redish Brown ppt
2.	**Saponins Test**		
	Foaming Test	Present	Formation of emulsion
	Sodium Bicarbonate Test	Absent	Formation of emulsion
3.	**Flavonoids Test**		
	Ammonia Test	Absent	Yellow colour obtain
	Shinoda Test	Present	Red colour obtain
	Sodium Hydroxide Test	Present	Yellow colour obtain
4.	**Tanin Test**		
	Ferric Chloride Test	Present	Blue green colour obtain
	Bromine Water Test	Absent	Decolouration of Bromine Water
5.	**Cardiac Glycosides Test**		
	Keller Kiliani Test	Present	Brown Ring obtain
	Liebermann's Test	Present	Blue green colour obtain
	Salkowshi Test	Absent	Red brown colour obtain
6.	Phenol Test	Present	Blue colour obtain

The results show that both the FPE and DPE of C. sinensis possess varying degrees of antimicrobial activities against the test bacterial and fungal strains (Tables 1 and 2). The FPE produced the widest zone of inhibition (ZOI) of 20 mm against E. faecalis. This was followed by S. aureus and P. aeruginosa with 14 mm ZOI and E. coli with 13 mm ZOI (Table 1). The FPE produced a 6 mm ZOI for S. typhimurium., the lowest observed for the bacterial strains studied. The DPE produced generally smaller zones of inhibition against the bacterial strains with a 12 mm zone of inhibition observed for E. faecalis and 10 mm for S. typhimurium. The DPE produced 4 mm, 6 mm and 8 mm zones of inhibition, respectively against S. aureus, P. aeruginosa and E. coli.

Table 1: Antibacterial activities of Citrus sinensis peel extracts (200 µg/mL) against some bacterial strains tested by disc diffusion assay

	Zones of inhibition (mm)				
	Gram positive		**Gram negative**		
	Staphylococcus aureus	***Enterococcus faecalis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Salmonella typhimurium***
FPE	14	20	14	13	6
DPE	4	12	6	8	10

Values are mean of 3 biological replicates to the nearest mm.

Table 2: Antifungal activities of Citrus sinensis peel extracts (200 µg/mL) against some bacterial strains tested by disc diffusion assay

Zones of inhibition (mm)			
	Candida albicans	*Aspergillus niger*	*Penicillium notatum*
FPE	18	2	2
DPE	2	4	10

Values are mean of 3 biological replicates to the nearest mm

Table 2 shows that the FPE was most effective against C. albicans, producing an 18 mm ZOI while the DPE was most effective against P. notatum. with an observed ZOI of 10 mm. Two 2 mm zones of inhibition were observed for the FPE against A. niger and P. notatum. while 2
and 4 mm respectively for C. albicans and A. niger when exposed to the DPE.

SUMMARY & CONCLUSION:

This preliminary study forms the basis for further research into the identification of the antibacterial compounds present in the peels of C. sinensis. This further emphasizes the waste- to-wealth potential of sweet orange wastes. The results in this study show that fresh C. sinensis peel extract contains more phenolics and possesses better antimicrobial activities against the microorganisms studied compared to the dry peel extract. Our findings also suggest that drying of plant materials prior to extraction may not always be better as certain active pharmacological compounds may be lost during this process.

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