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PHYTOCHEMICAL ANALYSIS OF *CITRUS SINENSIS* PEEL

PANKAJA S. CHEDE

Department of Botany, Narsamma's Arts, Commerce and Science College, Kiran Nagar, Amravati. M.S.,India.

ABSTRACT

Citrus Sinensis peel was screened for its phytochemical composition. The aqueous as well as the ethanoic extracts of the peel revealed the presence of carbohydrates, alkaloids, tannins, fixed oils and lipids, sugars, proteins, terpenoids, steroids, and amino acids whereas the saponins are present only in the ethanolic extract.

KEYWORDS: ***Citrus Sinensis,*** **Phytochemicals, Aqueous extract, Ethanolic extract.**

PANKAJA S. CHEDE
Department of Botany, Narsamma's Arts, Commerce and Science College, Kiran Nagar, Amravati. M.S.,India.

Corresponding author

INTRODUCTION

Phytochemicals can be defined as any compound found in plants (the ancient Greek word *phyton* means plant). Phytochemicals are certain non-nutritive plant chemicals which have some disease preventive properties[1]. However, 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. Numerous epidemiological studies have indicated that a diet rich in fruit and vegetables offers considerable health benefits to humans. Among these benefits are:

1. Reduction of the risk of developing many forms of cancer (lung, prostate, pancreas, bladder and breast).
2. Reduction of the risk of cardiovascular diseases.

The majority of these beneficial effects are at least in part due to the presence of phytochemicals in vegetables and fruits. In this context phytochemicals may be defined as "non-nutrient" chemicals found in plants that have biological activity against chronic diseases[2].

Orange (*Citrus sinensis*.), a hesperidium belonging to the Rutaceae family, is the most widely grown and commercialized citrus specie. Orange is composed by an external layer (peel) formed by flavedo (epicarp or exocarp) and albedo (mesocarp), and an inner material called endocarp (pulp) that contains vesicles with juice. Orange peel contains citral, an aldehyde that antagonizes the action of vitamin A. Therefore, anyone eating quantities of orange peel should make certain that their dietary intake of vitamin A is sufficient[3]. Sweet orange oil is a by-product of the juice industry produced by pressing the peel. It is used as a flavoring of food and drink and for its fragrance in perfumes and aromatherapy. Sweet orange oil consists of about 90% d-limonene, a solvent used in various household chemicals.[4,5]. Phytochemicals are already a part of our diet through vegetables and fruits. Citrus fruits are found to be rich in phytoconstituents[5]. *Citrus sinensis* (sweet orange) is one of them. Limonene now is known as a significant chemopreventive agent (Crowell, 1999) with potential value as a dietary anticancer tool in humans[6,7]. Orange peel is medically used against fungi[8].

MATERIALS AND METHODS

Collection of sample: Fresh

Sweet orange were collected from Amravati (Central region of India) in the month of April 2012. The handpicked Sweet orange were washed well using tap water and twice using distilled water. Then the peel and pulp of Sweet orange were separated by cutting them into small pieces and it was dried in shade for a period of 20-25 days, at an ambient temperature of 30˚C. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form.

Preparation of extracts

Aqueous extract

25 gm of both sample was suspended in 200 ml of distilled water. Extraction was done at 70˚C for 30 minutes, followed by filtering of the extracts using Whatman filter paper No.1. Extracts were then evaporated at 45˚C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use[9].

Ethanolic extract

95% ethanol was added to 25 gm of sample. Extraction was allowed to stand for 72 hours at 27˚C, after which they were filtered using Whatman filter paper No.1. Extracts were then evaporated at 45˚C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use[10,11].

Phytochemical analysis (Qualitative analysis)

Test for carbohydrates

Molisch's reagent was added to 2 ml of both extract. A little amount of concentrated sulphuric acid was added to it and allowed to form a layer. The mixture was shaken well, and allowed to stand for few more minutes,

which was then diluted by adding 5 ml of distilled water. Purple precipitate ring showed the presence of carbohydrates[11],[12,13].

Test for reducing sugars

A little amount of Fehling's reagent was added to the both extract, and the mixture was boiled for 2 minutes. A brick red colour indicated the presence of glycosides[11,12,13].

Test for proteins

0.5 ml of each extract was treated with equal volume of 1% sodium hydroxide, to which a few drops of copper sulphate solution was gently added. The solution turning to purple colour, indicated the presence of proteins[12,13,14].

Test for tannins

Gelatin test:-

3 gm of both extract was added to 6 ml of distilled solution was added to it. A bluish green colour indicated the presence of tannins[13,14].

Test for steroids

0.5 ml of the each extract was dissolved in 3 ml of chloroform and was filtered. To the filtrate, concentrated sulphuric acid was added by the sides of the test tube, which formed a lower layer. A reddish brown colour ring with a slight greenish fluorescence was taken as the indication for the presence of steroids[15].

Test for proteins and amino acids

Ninhydrin Test:-

To the sample extract, few drops of Ninhydrin reagent were added. After mixing it well, the solution was boiled in water for 2-3 minutes. A bluish-blackish colour indicated the presence of proteins[16].

Test for terpenoids (Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids[12,13].

Test for anthraquinones (Borntrager's test)

1 ml of the extract solution was hydrolyzed with diluted Conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones[11,12].

Test for alkaloids

0.5 gm of each extract was stirred with 4 ml of 1% dilute hydrochloric acid. It was boiled and filtered.

Dragendorff's test

1 ml of the filtrate was treated with few drops of Dragendorff's reagent. Orange brown precipitate indicated the presence of alkaloids[17,18].

Test for Chalcones

2 ml of Ammonium hydroxide was added to 0.5 g each extract of each sample. Appearance of reddish color showed the presence of chalcones[12,13].

Test for saponins

Froth test for saponins was used. 1g of the each sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins[11,12].

Test for fixed oils and lipids

Small quantity of each extracts were separately pressed between two filter papers, and allowed to dry. Appearance of an oil stain or a grease spot on the filter paper when observed under direct sunlight, indicated the presence of fixed oils[12,13].

RESULTS AND DISCUSSION

The Sweet Orange peel extracts were rich in phytochemical activity, as shown in Table 1.

Table 1
Phytochemical analysis of Citrus Sinensis peel

S.N	Phytochemicals	Tests performed	Aqueous Extract	Ethanolic Extract
1.	**Carbohydrates**	Molisch's Test	+	+
2.	**Sugar**	Dragendorff's Test	+	+
3.	**Protein**	Ninhydrin Test	+	+
4.	**Tannins**	Gelatin Test	+	+
5.	**Steroids**	Ring test	+	+
6.	**Amino acids**	Ninhydrin Test	+	+
7.	**Terpenoids**	Salkowski test	+	+
8.	**Anthraquinones**	Borntrager's test	_	_
9.	**Alkaloids**	Dragendorff's test	+	+
10.	**Chalcones**	Spot test	_	_
11.	**Saponins**	Froth test	_	+
12.	**Fixed oils and lipids**	Spot test	+	+

CONCLUSION

The aqueous as well as the ethanoic extracts of the peel revealed the presence of carbohydrates, alkaloids, tannins, fixed oils and lipids, sugars, proteins, terpenoids, steroids, and amino acids whereas the saponins are present only in the ethanolic extracts.

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