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ORIGINAL ARTICLE

Phytochemicals Screening of Citrus Peel Extract in Relation to Different Extraction Parameters

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ABSTRACT
The present study was designed to calculate the qualitative, quantitative estimation of phytochemicals and determination of total phenol, flavonoid content in Citrus reticulata peel. The peel and pulp of the fruits were separated and subjected to cold extraction using 70% alcohol. For the extraction of plant peel aqueous, methanol, Chloroform and petroleum ether were used. Quantitative estimation was done according to the earlier protocol. Determination of total phenol content was carried out by using Folin - Ciocalteau method and total flavonoid content by using Aluminium chloride colorimetric method. The content of phenolic in 100 gm (dry weight) extract of citrus peel was 148.7µg/ml Tannic acid equivalent (TAE). Total Flavonoid content was calculated from the standard plot and is expressed as quercetin equivalents (QE). Total flavonoid content was 256.785 ± 4.337 mg/g quercetin equivalent in citrus peel. The study thus revealed that peel of citrus reticulata have potential sources of bioactive compounds which are reflected in antioxidant activity and supports their health-promoting claims of plethora of investigations. These results suggest that plant Citrus reticulate is medicinally and commercially important.
Keywords: *Citrus fruits, Peel, Qualitative and quantitative, Phytochemicals.*

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INTRODUCTION

Nowadays, there is a growing interest in finding phytochemicals as an alternative to synthetic substances, which are commonly used in the food, pharmaceutical and cosmetic industry. The uses of fruits peel extract can be used for various therapeutic purposes [1]. Citrus fruits are mainly used by juice processing industries while the peels are generally wasted in the industries. Since the juice yield of citrus is less half of the fruit weight. A very large amount of oranges by product wastes, such as peels which are formed every year [2]. The citrus peels are rich in nutrients and contain many phytochemicals; they also can be efficiently used as drugs or as food supplements. There is an increase in the number of antibiotic resistance pathogens, there is always a search of an alternative drug that is regarded as safe [3], [4], [5]. Synthetic molecules are suspected to cause or promote negative health effects; so the idea is supported by the consumer's concern about the safety of products containing synthetic chemicals because these Citrus is the main crop, mainly used in fruit industries for fresh juice production. Citrus fruit is the most abundant fruit that grows worldwide and contains very rich amounts of phytochemicals and bioactive compound [6]. Citrus fruit is a medicinal plant belonging to the Rutaceae family. They are a rich source of Vitamin C, A, E alkaloids and flavonoids, and other minerals. Citrus plants originated from tropical, subtropical and East Asia and it is consumed all over world as a rich source of Vitamin C and other minerals and good source of vitamin A and contains a powerful natural antioxidant antiviral, antifungal and antibacterial activity that builds the strong body immune system Peel wastes are highly perishable and seasonal which is a problem to the processing industries and pollution monitoring agencies. Therefore, there is an increased demand in bringing useful products from these waste materials. Utilization of wastes can also improve economy of the processing units and can also reduce the problem of environmental pollution. The citrus peel and seeds are rich in phenolic compounds such as phenolic

acids and flavonoids [7]. Due to these reasons, it is very important to develop methods for extraction of these compounds from plant/fruit wastes.

MATERIAL AND METHODS

Collection and processing of plant materials:

The fresh peel of *Citrus reticulata was* collected from the local area of the Jaipur. The peel was shed dried for few days and then kept in incubator at 50°C for 1 hour. The dried peel material was crushed in mechanical grinder in order to make fine powder which was stored at room temperature. [8]

Preparation of Extract: The Citrus *reticulata* fruit powder was extracted with varies solvents. (water, methanol, chloroform and petroleum ether) using Soxhlet apparatus for 5h at a specific temperature for each solvent but not exceeding the boiling point. The mixture was filtered through Whatman filter paper no 2 for the removal of peel particles. The residue was re-extracted twice to ensure complete extraction. Filtered extract evaporated to dryness by rotary evaporator. Further, the extract was preserved in refrigerator in glass bottle throughout the experiment (i.e. for both quantitative and qualitative analysis).

Yield Estimation

Yield of the extract obtained was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered X 100}}{\text{Weight of dry powder}}$$

Phytochemical screening test

The extracts of *Citrus reticulata* peel was analysed for the presence of various Phyto-constituents which were identified using standard phytochemical procedure. A small portion of the dry extract was used for the phytochemical test for compounds which includes alkaloids, flavonoids, and glycosides, saponins, reducing sugars, phenols, tannins terpenoids and anthroquinone [9].

Test for Alkaloids

1ml of each plant extract was added with few drops of Dragen droff's reagent. Formation of reddish color indicates the presence of alkaloids.

Test for Flavanoids

0.5ml of each plant extract was dissolved in 10ml of distilled water and 5ml NH3 was added and treated with 1ml H2SO4. Formation of yellow color indicates the presence of flavonoids.

Test for Glycosides

1ml of each extract was treated with 2ml of glacial acetic acid and 1 drop of ferric chloride solution .1 ml of concentrated $H2SO_4$ was then added. A brown ring at the interface indicated the presence of glycosides.

Test for Saponins

2 ml of each extract was added in 5ml of distilled water and the solution was shaken vigorously for 30s, stable persistent frothing indicated the presence of saponins.

Test for Reducing Sugar

1 ml of each plant sample was taken in a test tube and 10ml distilled water then few drops of Fehling solution was added and heated at 400°C. Brick red precipitate indicates positive result.

Test for Phenols

1ml of each extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Test for Tannins

0.5ml of each extract was dissolved and added to a tube containing 1ml of distilled water. A few drops of ferric chloride were added and allowed to blue-black coloration indicate the presence of tannins.

Test for Terpenoids

0.5ml of each extract was taken and 1ml $CHCl_3$ was added followed by addition of 1ml conc. H2SO4. Reddish brown interface was observed for presence of terpenoids.

Total Phenolic Content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method [10]. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of Tannic Acid equivalent per g dry weight.

Total Flavonoid Content

The total Flavonoid content of crude extract was deter-mined by the aluminium chloride colorimetric method [11]. In brief, 50 µL of crude extract (1 mg/mL ethanol) were made up to 1mL with methanol, mixed with 4mL of distilled water and then 0.3mL of 5% NaNO2solu-tion; 0.3mL of 10% $AlCl_3$solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10mL with double-

distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total Flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight.

ANTIOXIDANT ACTIVITY

DPPH Assay

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. 200 µL of methanolic extract (100–500 µg/mL) were mixed with 3.8mL DPPH solution and incubated in the dark at room temperature for 1 h. The absorbance of the mixture was then measured at 517 nm. Ascorbic acid was used as a positive control. The ability of the sample to scavenge DPPH radical was determined from:

$$\text{DPPH scavenging effect} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

RESULT AND DISCUSSION

Yield Estimation:

The yield of extracts (mg) is shown in Table: -1. the amount obtained from Methanol, Aqueous, Chloroform, Petroleum ether extracts are 7.2mg, 3.8mg, 1.7mg, 0.9mg respectively.

Table 1: Yield obtained from various solvent of plant extract

Solvents	Yield obtained (mg)
Methanol	7.2
Aqueous	3.8
Chloroform	1.7
Petroleum Ether	0.9

Phytochemical Screening

Phytochemical analysis of various solvent shown in Table 2. It has been observed that peel extracts of citrus reticulate confirmed the presence of alkaloids, Flavonoids, Glycosides, Saponins, Reducing Sugar, Phenols, Tannins, Terpenoids. Earlier findings of [12,13] support the presence of nutrients and numerous phytochemicals in citrus fruit.

Table 2: Qualitative photochemical analysis of citrus fruit

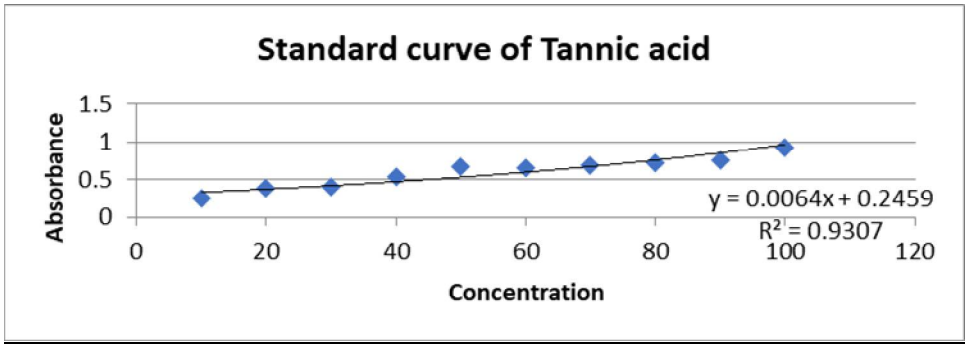
		Extract Result			
S. No.	Test	Aqueous	Methanol	Chloroform	Petroleum Ether
1.	Alkaloids	+	+	-	-
2.	Flavonoids	+	++	-	-
3.	Glycosides	++	++	+	-
4.	Saponins	++	-	++	++
5.	Reducing Sugar	+	++	+	-
6.	Phenols	+	++	-	-
7.	Tannins	+	+	-	-
8.	Terpenoids	-	-	++	-

Total Phenolic Content Estimation

The content of total phenolic in the methanol plant extract was determined using the Folin-Ciocalteu reagent. The result of total phenolic content was calculated from the standard plot. The content of phenolic in 100 gm (dry weight) extract of citrus peel was 118.7µg/ml Tannic acid equivalent (TAE). Phenolic compounds in plants play the key role as primary antioxidants or free radical scavengers. The bioactivity of phenolic may be related to their ability to chelate metals, inhibit lipoxy genase and scavenge free radical.

Table3 Absorbance of Standard Compound Tannic Acid

Concentration (µg/ml)	Absorbance (Mean) λmax =760 nm
10	0.293
20	0.354
30	0.409
40	0.523
50	0.642
60	0.56
70	0.62
80	0.702
90	0.69
100	0.812



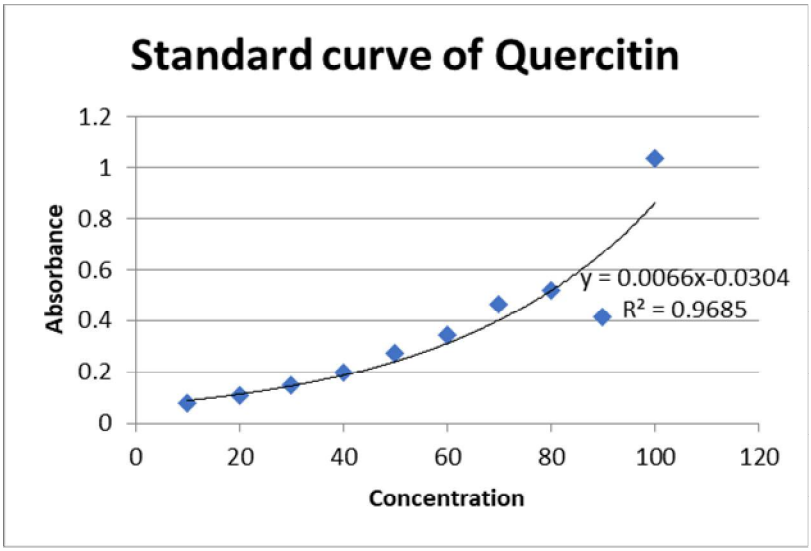
Graph 1: Calibration curve of standard Tannic Acid

Total Flavanoid Content Estimation:

Total Flavonoid content was calculated from the standard plot and is expressed as quercetin equivalents (QE). Total flavonoid content was 230.785 ± 5.439 µg/ml quercetin equivalent in citrus peel. Flavonoids are the most common and widely distributed group of plant's phenolic compounds, characterized by a benzo-γ-pyrone structure. It is ubiquitous in fruits and vegetables.

Table 4: Absorbance of Standard Compound (Quercetin)

Concentration (µg/ml)	Absorbance (Mean) λmax =760 nm
10	0.077
20	0.109
30	0.146
40	0.199
50	0.275
60	0.346
70	0.464
80	0.52
90	0.415
100	1.036



Graph 2 Calibration curve of standard quercetin for determination of Total Flavonoid Content

At present, it has been proved that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress [14]. Earlier studies support the presence of natural antioxidants, particularly in fruits, can be phenolic compounds (tannins, flavonoids, phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid [15,16]. In the present study, antioxidant activity mainly attributed to the presence of Vitamin C and phenolic. Flavonoids are the most important natural phenolic compound. These possess biological properties including radical scavenging properties [17].

DPPH Assay:

Table:5 -DPPH % scavenged

Concentration(µg/ml)	O.D.	% scavenged
100	0.724	51.47
200	0.665	56.27
300	0.609	59.11
400	0.488	65.36
500	0.415	74.64

It has been recognized that flavonoids show antioxidant activity. Effects of these on human nutrition and health are considerable. Antioxidant assay were done are DPPH assay [18] and percentage scavenged by DPPH are mentioned in table. The data therefore suggest that the extracts of Citrus are a potential source of natural antioxidants.

CONCLUSION

Present study has provided a rough estimation of antioxidant content and antioxidant capacity in the peel of citrus. Therefore, on the basis of study, it can be concluded that waste material of Citrus fruit i.e. peel could be a good source of phytochemicals and could show antioxidant properties. Hence, citrus peel can be used in further manufacturing of antioxidant drugs, and the peel extracts could be used as a drug after pharmacological evaluation and clinical trials.

Regarding future prospects, current research includes further screening of phytochemicals from different solvents and identifications and use of these constituents into drug discoveries and manufacturing.

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