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Research Article

PHYTOCHEMICAL ANALYSIS OF *CITRUS LIMONUM* AND *CITRUS SINENSIS* PEELS AND IDENTIFICATION OF BETA CAROTENE PIGMENT USING ETHANOLIC EXTRACT

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ABSTRACT
The *Citrus limonum* peel and *Citrus sinensis* peel is an excellent source of fiber, potassium, magnesium, calcium, and β-carotene. The ethanolic extracts of Citrus peels evaluated they contain carbohydrates, fixed oils, steroids, phenols, tannins and saponins. The phytochemical analysis of samples characterized by that is TLC and UV-Visble spectroscopy. The presence of β-carotene in sample analyzed at the absorption maxima 328 nm, whereas the R_f values for β-carotene in *Citrus limonum* peel and *Citrus sinensis* peel are found in 0.91 and 0.92 respectively.
Keywords: *Citrus limonum* Peel, *Citrus sinensis* Peel, ethanolic extracts, TLC, β–carotene, UV-Vis spectroscopy.

INTRODUCTION

Citrus limonum and *Citrus sinensis* (Rutaceae) trees most important commercial fruits crops widely cultivated in tropical and subtropical climates for their sweet fruits[1]. Citrus family had rich sources of phytochemicals. The peel of citrus fruit is having abundant source of flavanones and many polymethoxylated flavones which are very rare in other plants[2]. The peel is the byproduct of citrus juice processing with the high potential use two different tissues are found in citrus peel flavedo and albedo[3]. β-Carotene is a strongly colored red- orange pigment abundant in plants and fruits[4]. The ethanolic peel extract of *Citrus limonum* revealed that they containing carbohydrates, saponins, tannins, fixed oils, cardiac glycosides, steroids, phytosterols, phenols, flavonoids, amino acids and proteins[5]. Where as the ethanolic peel extract of *Citrus sinensis* evaluated the presence of Carbohydrates, alkaloids, saponins, tannins, fixed oils, steroids, phenols, are present[2]. Hesperidin is a falvanone glycoside found abundantly in citrus fruits its aglycone form hesperetin. Hesperetin was first isolated in 1828 by French chemist Lebreton from the white inner layer of citrus peel mesocarp and albedo[6]. An aglycone is the compound remaining after the glycosyl group on a glycoside is replaced by a hydrogen atom[7]. The phytochemical hesperidin is mainly found in *Citrus limonum* and *Citrus sinensis*. The highest concentration of hesperidin can be found in the white part of Citrus peels. Hesperetin bound to the diasaccharide rutinose. The sugar causes hesperidin to be more soluble then hesperetin[8]. Hesperetin is a bioflavonoid and to be more specific a flavonone, Hesperidin is water soluble due to the presence of sugar part in structure so on ingestion it releases its aglycone that is hesperitin[9]. However hesperidin has a low bioavailability compared to hesperetin due to the rutinoside moiety attached to the flavonoid[10].

MATERIALS AND METHODS

Collection of samples

Both samples were collected from Bhopal region (M.P.) In India. The identification of fruits was carried out by Dr. Jagrati Tripati HOD department of Botany Unique College Bhopal (M.P.), India.

Preparation of Sample

The *Citrus limonum* and *Citrus sinensis* were washed well using tap water and twice using distilled water. Then the peel of *Citrus limonum* and *Citrus sinensis* were separated by cutting them into small pieces and then the peels were dried at 100^0C for 24 h using hot air oven[3]. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered form. Phytochemical screening was carried out for extract using the standard ethanols[5].

Preparation of Extracts

Ethanolic Extract

95 % ethanol was added to 20 g of each sample. Extraction was allowed to stand for 7 days at 27˚C, after which they were filtered using Whatman filter paper No.1. Extracts were then evaporated at 45˚C using condenser to form a paste, and further transferred into sterile bottles and refrigerated until use.

Phytochemical Screening

Test for carbohydrates

Molisch's reagent was added to 2 ml of extract. A little amount of concentrated H_2SO_4 was added and allowed to stand for few minutes. Purple precipitate ring showed the presence of carbohydrates[5].

Test for alkaloids

To 0.5 ml of extract, add dilute H_2SO_4. It was boiled and filtered. Perform Hager's test[5].

Test for saponins

0.5 ml of extract was boiled and filtered. 10 ml of distilled water was added. Formation of honey comb indicated the presence of saponins[5].

Test for tannins
To 3 ml of extract was added few drops of 10 % $FeCl_3$ solution for deep blue colour[5].

Test for fixed oils
Extracts were separately pressed between two filter papers, and allowed to dry. Appearance of an oil stain on the filter paper when observed under direct sunlight indicated the presence of fixed oils[5].

Test for cardiac glycoside
0.5 ml of extract was treated with 2 ml of acetic anhydride. Then few drop of 1 % $FeCl_3$ and concentrated H_2SO_4 was added. Indicate brown ring[5].

Test for steroids
0.5 ml of the extract added 3 ml of chloroform and 2 ml concentrated H_2SO_4[5].

Test for phytosterols
1 ml extract was dissolved in 5 ml of chloroform and few drops of concentrated H_2SO_4 and few drops of dilute acetic acid and 3 ml of acetic anhydride was added. A bluish green color indicated the presence of phytosterols[5].

Test for phenols
1 ml extract was added 5 ml Folin-Ciocalteu reagent and 4 ml of Na_2CO_3. Blue color indicates the presence of phenols[5].

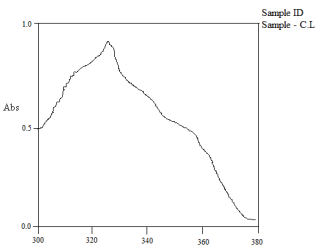
Test for flavonoids
0.5 ml extract mix with 2 ml Distilled water and also mix 0.15 ml of 5 % $NaNo_2$ solution. After 5 minutes 0.15 ml of 10 % $AlCl_3$ Solution added and allows standing for 5 minutes. Then also add 2 ml of 4 % NaoH solution to the mixture. Immediately add water to bring the final volume up to 5 ml, then mix and allows standing for 15 minutes. Appearance of pink color indicates the presence of flavonoids[5].

Test for proteins and amino acids
Millons Test - Few drops of Millon's reagent added to extract and heat, reddish-brown coloration or precipitation indicates presence of tyrosine residue, which mostly occurs in proteins[5].

Ninhydrin Test
Ninhydrin reagent added to the extract and mix, boil for few minutes. A bluish-blackish color indicates presence of proteins[5]

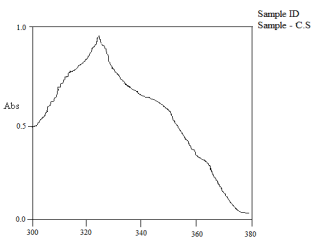
Qualitative Profile
The qualitative profile of β-carotene in the ethanolic extract is carried out by TLC[11] and UV-Vis spectroscopy[12]. TLC and UV-Vis spectroscopy are the suitable methods to show the qualitative profile of β-carotene in the ethanolic extract.

Detection of β-carotene by UV Spectroscopy
10 ml of *Citrus limonum* peel is fraction in water bath then add 25 ml of cyclohexane then shake and filtered. From this pipette out 1 ml and dilute to 25 ml. The absorbance of the resultant solution was measured at 328 nm against cyclohexane as blank. Similarly use the same process for *Citrus sinensis* Peel.



Pick Point
328 - 0.985

Figure 1: UV of *Citrus limonum* peel ethanol extract



Pick Point
328 - 0.989

Figure 2: UV of *Citrus sinensis* peel ethanol extract

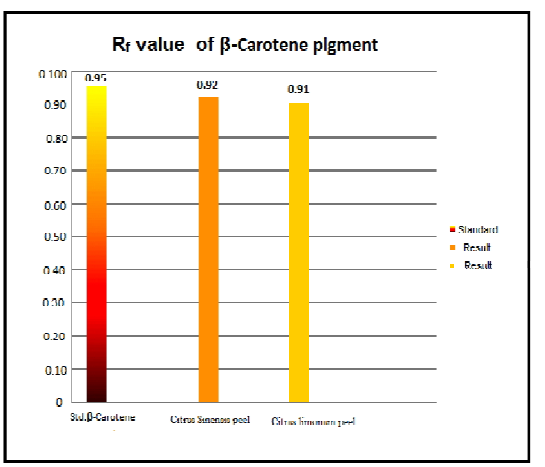


Table 1: Phytochemical Analysis of *Citrus limonum* and *Citrus sinensis Peel*

S. No.	Phytochemical	*Citrus limonum* peel	*Citrus sinensis* peel
1.	Carbohydrates	+	+
2.	Alkaloids	-	+
3.	Saponins	+	+
4.	Tannins	+	+
5.	Fixed oils	+	+
6.	Cardiac glycosides	+	-
7.	Steroids	+	+
8.	Phytosterols	+	-
9.	Phenols	+	+
10.	Flavonoids	+	-
11.	Amino acids and proteins	+	-

RESULTS AND DISCUSSION

The isolation of β-carotene was performed by TLC and UV-Visible spectroscopy. The obtained R_f values were matched with standard and reported exceptable result were obtained. The R_f values for β-carotene in *Citrus limonum* peel (1:150) and *Citrus sinensis* peel (1:150) are obtained 0.91 and 0.92 respectively. The isolated β-carotene was examined by UV absorption maxima using cyclohexane as a solvent. The peaks obtained at same intensity 328 nm. The obtained result for β-carotene was matched with Standard. The ethanolic extracts of *Citrus limonum* peel and *Citrus sinensis* peel are rich in phytochemicals, as shown in Table 1.

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