

PHYTOCHEMICAL ANALYSIS OF *CITRUS LIMONUM* PULP AND PEEL

BLESSY B MATHEW\*, SURESH K JATAWA, ARCHANA TIWARI

School of Biotechnology, Rajiv Gandhi Technological University, Airport road, Bhopal-36, M.P., India. Email: blessing21@gmail.com

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## ABSTRACT

Plants have some specific chemicals called phytochemicals in them which may not have nutritional property as such, but they can work towards the deterrence of diseases. As they can be absorbed by the human body, they can minimize the free radical damage caused to the cells, as a result of oxidative stress. Lemon is a citrus fruit considered to be rich in phytochemicals. This study provides an explicit perception about the *Citrus limonum* portion (pulp or peel), as to which one has more number of phytochemicals. The aqueous extracts of the pulp revealed the presence of carbohydrates, alkaloids, tannins, fixed oils, reducing sugars, proteins, cardiac glycosides, steroids, phytosterols, phenols and flavonoids, whereas the ethanolic pulp extracts showed only the presence of fixed oils, reducing sugars, cardiac glycosides, steroids, phytosterols, flavonoids and amino acids. On the other hand, the aqueous peel extracts showed the presence of carbohydrates, alkaloids, tannins, fixed oils, proteins, cardiac glycosides, steroids, phenols and flavonoids and amino acids, whereas the ethanolic peel extracts revealed that they contained carbohydrates, saponins, tannins, fixed oils, cardiac glycosides, steroids, phytosterols, phenols and flavonoids.

**Keywords:** *Citrus limonum*, Phytochemicals, Aqueous extract, Ethanolic extract.

## INTRODUCTION

Phytochemicals are certain non-nutritive plant chemicals which have some disease preventive properties. They are not required by the human body for life sustenance, but they offer protection against pathogens<sup>1</sup>. There are different ways in which a phytochemical can work. It can act as an antioxidant and protect cells against free radical damage, eg. polyphenols, carotenoids etc. It can stimulate certain enzymes, thereby reduce risk for breast cancer, eg. terpenes. It may act as an anti-bacterial and hormonal-stimulant component. It may even act as binders which may prevent the adhesion of pathogens to the human cell walls<sup>2</sup>. Phytochemicals are already a part of our diet through vegetables and fruits. Citrus fruits are found to be rich in phytoconstituents<sup>3</sup>. *Citrus limonum* (lemon) is one of them. The essential oils of lemon exhibited strong antioxidant activities as well as antiproliferative activity against HeLa cell line<sup>4</sup>. The peel extracts were found to possess antibacterial and antifungal properties<sup>5</sup>, and reduced the permeability of blood vessels and in remedies for phlebitis. They even prevented development of abnormal growths on the skin and decreased the occurrence of squamous cell skin melanoma<sup>6</sup>. There have been no assured studies as to which is the component that has higher phytochemicals. This paper presents a detailed phytochemical analysis of the lemon pulp and peel.

## MATERIALS AND METHODS

## Collection of sample

Fresh lemons were collected from Bhopal (Northern region of India) in the month of May and June 2011. The handpicked lemons were washed well using tap water and twice using distilled water. Then the peel and pulp of lemons were separated by cutting them into small pieces and it was dried in shade for a period of 10-12 days, at an ambient temperature of 22°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:** 20 gm of 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 to form a paste, and further transferred into sterile bottles and refrigerated until use.

**Ethanolic extract:** 95% ethanol was added to 20 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 to form a paste, and further transferred into sterile bottles and refrigerated until use<sup>7,8</sup>.

## Phytochemical screening

## Test for carbohydrates

Molisch's reagent was added to 2 ml of aqueous 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<sup>9</sup>.

## Test for alkaloids

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

**Mayer's test** 1 ml of the filtrate was treated with few drops of Mayer's reagent. Turbidity or precipitation indicated the presence of alkaloids<sup>9</sup>.

**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<sup>10</sup>.

## Test for saponins

0.5 gm of ethanolic extract was boiled and the mixture was filtered. To 2.5 ml of the filtrate, 10 ml of distilled water was added in a test tube. It was shaken well for few minutes and was allowed to stand for sometime. Frothing alongwith the formation of honey comb indicated the presence of saponins.

## Test for tannins

**Gelatin test** 3 gm of aqueous extract was added to 6 ml of distilled water, which was filtered and few drops of 10% ferric chloride solution was added to it. A bluish green colour indicated the presence of tannins.

**Lead acetate test** Few drops of 10% lead acetate solution was added to 5 ml of aqueous extract. Formation of white precipitate indicated the presence of tannins.

## Test for fixed oils and lipids

Small quantity of 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.

**Test for reducing sugars**

A little amount of Fehling's reagent was added to the aqueous extract, and the mixture was boiled for 2 minutes. A brick red colour indicated the presence of glycosides.

**Test for proteins**

0.5 ml of aqueous 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.

**Test for cardiac glycosides**

2 ml of acetic anhydride was added to 0.5 gm of extract. Then one drop of 1% ferric chloride alongwith a little amount of concentrated sulphuric acid was added. A brown ring formation at the interphase indicated the presence of de-oxy sugars, which showed the presence of cardiac glycosides.

**Test for steroids**

0.5 ml of the 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<sup>1,11</sup>.

**Test for phytosterols**

A little quantity of the extract was dissolved in 5 ml of chloroform separately. This chloroform solution was treated with a few drops of concentrated sulphuric acid. To this a few drops of dilute acetic acid and 3ml of acetic anhydride was added. A bluish green colour indicated the presence of phytosterols<sup>12,13,14</sup>.

**Test for phenols**

An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent and 4 ml of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for colour development. An appearance of blue colour indicates the presence of phenols<sup>15</sup>.

**Test for flavonoids**

0.5 ml of sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% NaNO<sub>2</sub> solution. After 6 min, 0.15 ml of a 10% AlCl<sub>3</sub> solution was added and allowed to stand for 6 min, then 2 ml of 4% NaOH solution was added to the mixture. Water was added immediately to bring the final volume to 5 ml, then the mixture was thoroughly mixed and allowed to stand for another 15 min. An appearance of pink colour indicates the presence of flavonoids<sup>16</sup>.

**Test for proteins and amino acids**

**Millons Test** A few drops of Millon's reagents was added to the sample extract, which was then heated gently. A reddish-brown coloration or precipitate indicated the presence of tyrosine residue, which occurs in most of the proteins<sup>17</sup>.

**Ninhydrin Test** To the sample extract, few drops of Ninhydrin reagent was added. After mixing it well, the solution was boiled in water for 2-3 minutes. A bluish-blackish colour indicated the presence of proteins<sup>18</sup>.

**RESULTS AND DISCUSSION**

The lemon pulp and peel extracts were rich in phytochemical activity, as shown in Table 1. In general, the phyto-constituents of the peel extracts were found to be more than that of the pulp fractions, but only the pulp fractions consisted of reducing sugars.

**Table 1: Phytochemical analysis of *Citrus limonum* pulp and peel**

S. No.	Phytochemicals	Tests performed	Aqueous Extract		Ethanollic Extract	
			Pulp	Peel	Pulp	Peel
1.	Carbohydrates	Molisch's Test	+	+		+
2.	Alkaloids	Mayer's Test/ Dragendorff's Test	++	++		
3.	Saponins	Foam Test	-			+
4.	Tannins	Gelatin Test/Lead acetate Test	+	+		+
5.	Fixed oils	Spot Test	+	+	+	+
6.	Reducing sugars	Fehling's Test	+	-	+	-
7.	Proteins	Biuret Test	+	+		
8.	Cardiac glycosides	Ring Test	+	+	+	+
9.	Steroids	Ring Test	+	+	+	+
10.	Phytosterols	Liebermann-Burchard Test	+	-	+	+
11.	Phenols	FC Method	+	+	-	+
12.	Flavonoids	Aluminium chloride or Zhishen Method	+	+	+	+
13.	Amino acids and proteins	Millons Test/ Ninhydrin Test	+	+	+	+

+ indicates: strong presence - indicates: strong absence blank indicates: no result shown

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