

Evaluation of phytochemical screening & extraction of lycopene from *Citrullus lanatus* by using column chromatography

Lalitha Govindaraj^{*1} and Suseela Vivek²

¹Department of Biochemistry, R. V. S. College of Arts and Science, Sulur, Bharathiyar University, Coimbatore, India

²P.S.G.College of Arts and Science, Coimbatore, Bharathiyar University, Coimbatore, India

*Correspondence Info:

Lalitha Govindaraj

Department of Biochemistry,

R. V. S. College of Arts and Science, Sulur,

Bharathiyar University, Coimbatore, India

E-mail: lalithajune3@gmail.com

Abstract

The present study focused on the phytochemical constituents, isolation of lycopene of the *Citrullus lanatus* fruits which were collected from the local market in around sulur area, India. The phytochemical analysis of fruit extracts revealed the presence of bioactive compounds such as phenolics, alkaloids, saponins, tannins, steroids and flavonoids in the extract. The isolation of lycopene were quantified by using column chromatography that depicted (lycopene - 68.0285 mg/k fresh wt) respectively. Consequently, the watermelon proved as a good source of antioxidant, could be used as a raw material in drug formulation.

Keywords: *Citrullus lanatus*, phytochemistry, antioxidant activity.

1.Introduction

Plants and plant products can be used as medicines from the beginning of human civilization. Furthermore, about 80% of the world population is dependent (wholly or partially) on plant-based drugs. Fruit has been recognized as a good source of vitamins and minerals and people who eat fruit as part of an overall healthy diet generally have a reduced risk of chronic diseases. Watermelon contributes a plethora of nutritional agents as antioxidants (lycopene, beta-carotene etc.) and some specific amino acids (arginine, citrulline etc.) and Folate (folic acid) helps the body to form red blood cells[1].

Fresh watermelon consumption is considered a healthy addition to diet owing to the presence of lycopene. Additionally, it is a good source of potassium and also contains magnesium, calcium, phosphorus and iron [2].

Several ethno-medicinal plants and fruits have been documented for anti-inflammatory and antioxidant properties, these ethno-medicinal plants and fruits could serve as sources of effective medication that may be more readily accessible and inexpensive, would thus be helpful in improving the present status [3].

Considering the facts, present research project was planned to characterize locally grown watermelon (Sugar baby) with special reference to lycopene, a potent antioxidant. Hence the present study has been undertaken to evaluate the phytochemical screening and antioxidant activity of *Citrullus lanatus*.

2. Materials and methods

2.1 Collection of fruits

Fruits were collected from the wet market around Sulur area, Coimbatore, it was maintain at room temperature stored in biochemistry laboratory, department of biochemistry RVS CAS. 10 gms of the fruit pulp was taken and macerated in 100 ml of different solvents methanol, ethanol, aqueous properly and kept at room temperature for 24 hours. Thereafter the mixture was filtered through Whatmann filter paper no .1. All tests were performed in triplicates within a week and the extracts were stored at -20°C until used.

2.2 Identification tests for active compounds

The phytochemical constituents were analyzed from the *Citrullus lanatus* fruits by the standard procedures [4],[5].

Alkaloids: A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity indicates the presence of alkaloids.

Flavonoids: A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids.

Glycoside: Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Saponins: A 3 ml of test solution was added with water and shaken. Formation of foamy lather indicates the presence of Saponins.

Phenols: A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Tannins: A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

Steroids: A 3 ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4 . Purple color thus formed changes into blue or green color indicating the presence of steroids.

Amino Acids: A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

Diterpenes: A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of Diterpenoids.

Anthroquinones: A 3 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthroquinones.

2.3 Lycopene extraction using by column chromatography:

Lycopene was extracted using organic solvents i.e., hexane, acetone and petroleum ether in ratio of 2:1:1, respectively [6]. Extracted lycopene was quantified through spectrophotometer read at 503nm [7].

Weigh roughly 1.0 g of *Citrullus lanatus* fruit paste into a 15 mL centrifuge tube. Add 4 mL of a 50/50 (% volume) mixture of petroleum ether and acetone. Cap the centrifuge tube and shake until the solid becomes fluffy. Open the cap and crush the solid with a spatula. Close the tube and shake again. Repeat this crushing and shaking two more times. Centrifuge the tube to separate the extract and residue. Transfer the extract (liquid) to a clean centrifuge tube. In the original centrifuge tube, add a new 4 mL of solvent and repeat the entire extraction procedure. Add the resulting extract to the first extract (in the second centrifuge tube). Now wash the combined extracts with saturated NaCl solution (5mL), then with 10% aqueous potassium carbonate (5mL), then with saturated NaCl solution (5 mL) again. Dry the organic layer with anhydrous sodium sulfate. Decant the organic layer into a small beaker and concentrate to roughly 0.2 mL by evaporation in the hood (do not apply heat!). If the sample goes to dryness, re-dissolve in hexane (0.2 mL).

Pack your chromatography column (a 5ml pipette). Use 1.5 to 2.0 g of neutral Brockmann grade I alumina as your adsorbent. Gather your organic solution, 10 mL of hexane (the first eluent), 10mL of 10:90 (% volume) acetone: hexane (the second eluent), a small Erlenmeyer flask,(for collecting the lycopene fraction), Place the beaker

under the column. Add the first eluent, hexane, to the column until the liquid wets all of the alumina. Then add the lycopene extract via Pasteur pipette to the top of the column (use a little of the hexane to rinse the extract vial and add this to the column as well. As soon as the extract enters the alumina layer, fill the column almost all the way with hexane. Add hexane as necessary to keep the solvent level in your column relatively constant. When the first yellow band starts to drain out of the column, add your second eluent (10:90% volume of acetone: hexane) to the top of the column and keep the eluent level constant as before. When the lycopene layer (orange-red) begins to leave the column, collect the orange-red layer into the Erlenmeyer flask. When the band is almost completely off the column, remove the sample vial and replace it with the waste beaker you used earlier.

3. Result and discussion

3.1 Phytochemical analysis:

The results of preliminary phytochemical analysis of *Citrullus lanatus* fruit are showed in table 1.

Table1: Preliminary phytochemical analysis of *Citrullus lanatus*

Sr. No	Secondary metabolites	Ethanol	Methanol	Chloroform	Aqueous
1.	Alkaloids	-	-	+	-
2.	Flavonoids	-	+	+	+
3.	Glycosides	-	+	+	-
4.	Saponins	-	+	+	+
5.	Phenols	-	+	+	+
6.	Tannins	+	+	-	+
7.	Steroids	+	+	+	-
8.	Amino acid	-	-	+	+
9.	Diterpenes	-	+	-	-
10.	Anthraquinones	-	-	-	-

(+indicates presence, - indicates absence).

All the plants species subjected to phytochemical screening showed positive results for cardiac glycosides and saponins[8]. The presence of phenols in ethanol, methanol, chloroform and aqueous are found to present in all solvents. The Anthroquinone compound shows absence in all the solvents. Hence the phytochemical screening reveals that Aqueous, Methanol, chloroform extract shows high secondary metabolites. A common role of secondary metabolites in plants is defense mechanisms. They are used to fight off herbivores, pests and pathogens. Although researchers know that this trait is common in many plants it is still difficult to determine the precise role each secondary metabolite[9]. Secondary metabolites are used in anti-feeding activity, toxicity or acting as precursors to physical defense systems. A steroid is a type of organic compound that contains a characteristic arrangement of four cycloalkane rings that are joined to each other. Examples of steroids include the dietary lipid cholesterol, bile acids, the sex hormones estradiol and testosterone and the anti-inflammatory drug dexamethasone. A glucoside is a glycoside that is derived from glucose. Glucosides are common in plants, but rare in animals, these compounds give a permanent froth when shaken with water. They also cause hemolysis of red blood cells. Saponin glycosides are found in liquorice. Their medicinal value is due to their expectorant, and corticoid and anti-inflammatory effects. Alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste[10]. Flavonoids are synthesized by the phenylpropanoid metabolic pathway where the amino acid phenylalanine is used to produce 4-coumaryl-CoA, and this is then combined with malonyl-CoA to produce chalcones which are backbones of Flavonoids [11].

3.2 Extraction of lycopene, by using column chromatography:



Figure 1: column chromatography in *Citrullus lanatus*

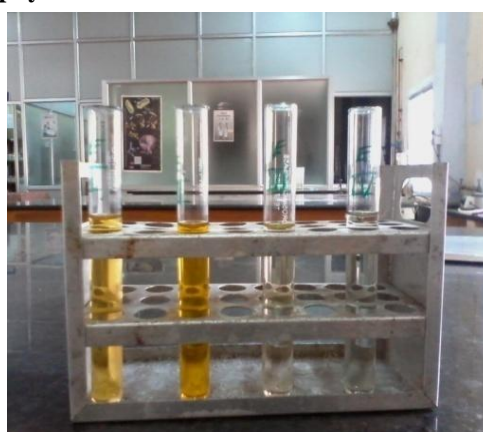


Figure 2: column chromatography in *Citrullus lanatus*

Table 2: Optical density read by colorimeter by using *Citrullus lanatus*

NM	Pigment isolated	Elution 1 (orange)	Elution 2 (white)	Elution 3 (Yellow)
503	Lycopene	0.43	0.37	0.13

3.3 Elution 1- Orange, Elution 2-White, Elution 3-Green

Lycopene was isolated from secondary antioxidants shown in (figure 1 and 2), that suppress the formation of radicals and protect against oxidative damage. This study describes a rapid and inexpensive way to estimate the lycopene content of certain foods and food products naturally rich in lycopene. The findings are in agreement with Barba *et al*[12] who computed lycopene through HPLC in various fruits and vegetables i.e., watermelon, tomato, medlar, persimmon, pepper and carrot. They expounded that watermelon and tomato had higher lycopene contents. This report revealed value for watermelon lycopene extract were 68.0285 mg/k fresh wt lycopene, respectively. The instant results for lycopene in watermelon juice are slightly lower than the findings of Oms-Oliu *et al*[13] observed 6.20 mg/100mL. They attributed watermelon juice as one of the excellent sources of lycopene. Conclusively, watermelon showed remarkable free radical scavenging and antioxidant activity owing to potent antioxidant i.e., lycopene. Consequently, the watermelon proved as a good source of antioxidant with special reference to lycopene.

4. Conclusion

This study revealed that all the phytochemical constituents were present in the *Citrullus lanatus*. It is of a great importance as it may lead to identification of a substitute for the genuine drug. Medicinal plants are natural compounds have no side effects. For further confirmation, detailed pharmacological investigations are needed. This fruit could serve as good source of nutrients when consumed.

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