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Comparative Studies of Various Phytonutrients in Citrus Fruits

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ABSTRACT
The current piece of research was undertaken to investigate the phytochemicals constituents such as carbohydrates, proteins, alkaloids, anthraquinone, glycosides, Saponin, glycosides, steroid, cyanogenetic glycosides, tannins, phlobotannins, saponins, terpenoids, cardiac glycosides, etc in the species of citrus genus (Citrus sinensis, Citrus reticulate Blanco, Citrus aurantium L). All these plants were collected locally. Qualitative analysis of these phytochemicals was performed. All three types of citrus are rich with phytochemicals. Quantitative screenings is in progress in our lab.

Keywords: Citrus fruits, phytochemicals, qualitative screenings**.**

1. INTRODUCTION
Any compound found in plants is known as phytochemicals (the chemicals found in plants according to Greek word Phyton). Phytochemicals are plant chemicals which are non-nutritive in nature but have good preventive action against certain diseases[1]. Natural and medicinal plants are gifted to living things in this world. Medicinal plants becoming more important with time due to the presence of potential drug compounds. The potential of medicines obtained from these plants is due to biologically active compounds known as phytochemicals[2]. Phytochemicals has remarkable effect on the human body. Phytochemicals in the human body combines with the nutrients and fibers present and provide strength against certain diseases and stress conditions[3].

On the basis of function in plants metabolism, phytochemicals obtained from plants are classified into two categories, e.g. primary phytochemicals and secondary phytochemicals. Primary phytochemicals comprises of carbohydrates, proteins, amino acids, proteins and chlorophylls. Whereas second category consists of alkaloids, terpenoids, steroids and flavonoids, etc[4].

The core values of large number of plants have been published but still many of them remained undiscovered[5]. Nature is filled with a treasure of medicines to overcome every type of ailments[6]. Phytonutrients are one of the most important and widely distributed groups of components in plant[7].

The word phytochemical is used to a wide range of biologically active substances found in plants. Due to phytochemicals plants a particular colour, flavour and special protection against harmful agents. Nutritional studies in plants reveal that a food rich in fruit and vegetables gives numerous health benefits to human beings, e.g., reducing the risk of causing various kinds of cancer (pancreas, lungs, prostate, and breast as well as reducing the risk against cardiovascular diseases[8].

Phytochemicals have certain functions in metabolism of plants. The medicinal effects in different parts of plants are due to the secondary group phytochemicals present in them. The phytochemicals in this group are defined as "non-nutrient substances" in plants that show special biological activity against some lethal diseases[8].
Fruits and vegetables are the major source of phytochemicals in our diet. Citrus fruits also contain a large number of phytoconstituents. *Citrus sinensus* which is the sweet orange is one of them[9].

2. CITRUS FRUITS
Citrus trees are evergreen trees full of fragrance and have juicy and flavoured fruits. Fruits of citrus have a skin which protects the fruit from damages. The pulpy part of the citrus fruits is rich source of soluble sugars, ascorbic acid, pectin, fibres as well as various organic acids and potassium salts[10-11]. The origin of citrus fruits is not known well, although South East Asia is given as its origin place by most of researchers[11].

The most valuable product of the citrus fruit is its juice[7, 10, 12]. Jams can also be prepared from citrus fruits. Some essential oils can also be obtained from citrus fruits as a side product. They are used to flavour the drinks and foods. They also have pharmaceutical and industrial uses[10].

Citrus fruits are rich in phytochemicals. Phytochemicals are good for health and give protection against diseases. The phytonutrients are defined as the chemicals obtained from plants having good response to human health. These phytochemicals have been utilised as food additives, flavors, colours, pesticticides, medicines, and other chemicals[12].

3. CITRUS FRUITS & PHYTOCHEMICALS
Phytochemicals are the chemicals of plants. Phytochemical is diverse group having a large numbers of members. Plant's secondary metabolites have no major roles in growth of plant but have major roles in defence of plant from

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insects and microbes. Along with alkaloids the members of this group are cyanogenic glycosides, flavonoids, terpenoids and phenolic compounds[10, 12].

The flavonoid of the citrus fruits provides human body strong action against allergies, viruses, and carcinogens[10]. Some flavonoids present in citrus fruits include Quercetin, myricitin, rutin, tangeritin, narigin, and hesperidin[10]. The flavonoids also have antioxidant activity[14]. The citrus flavonoids enhance the ascorbic acid in them. They also protect the vascular system of humans[10]. The citrus fruits are classified as weight reducing diet because they have low fat content[10, 15]. Citrus fruits provide dietary fibers which help in softening the stools[10]. Pulp of mature citrus fruits contains a high content of water and other different constituents, including acids and sugars[16].

4. MATERIALS AND METHODS

Collection of Samples: Fresh oranges of species (*Citrus sinensis, Citrus reticulata Blanco,* and Citrus *aurantium L*) were collected from local market of district Peshawar, Pakistan. These fresh fruits were washed properly with fresh water and later twice with distilled water. The epicarps of all three different species were peeled and disposed. The mesocarps and endocarps parts were cut in to small pieces with the help of clean cutter which helps and enhances drying. These were dried further in shade for ten days. These dried oranges were put to mortar and pestle and grinded to get powdered form.

Preparation of extract: Aqueous extract 20gm of powdered samples of all three citrus fruits was weighed and suspended in 200 millilitre of water for half hour and followed by filtering through Whattman filter paper. These extracts were evaporated to get paste which was transferred and stored in sterile bottles under refrigerator for further use.

Ethanolic extracts: 25gm of powdered samples were added to 200 milliliter of ethanol and extraction was done for 72 hours, followed by filtering through Whattman filter paper. The extracts were later evaporated to get paste of samples which were stored and refrigerated for further tests.

5. PHYTOCHEMICAL TESTS (QUALITATIVE TESTS)

5.1 *Carbohydrate test*

Two drops of alpha naphthol in alcohol was added to twenty milliliter of both citrus extract samples. The sample was added with small quantity of concentrated H_2SO_4 and allowed for short time to make layers. If violet ring is present in between two layers shows the presence of carbohydrates.

5.2 *Reducing sugars Test (Benedict's test)*

A few milliliters of both juice extract and Benedict's test solution were heated in boiling water few minutes. If yellow, red or green colour precipitate appears shows the presence of reducing sugars in the test solution.

5.3 *Fehling tests*

One millilitre of Fehling solution was taken in a small test tube and boiled for a short time and an equal amount of test sample juice was added into it. Mixture was allowed to warm in boiling water for 5-10 mins. Yellow colour and then a brick red precipitate found shows the presence of reducing sugars.

5.4 *Test for Monosaccharide: Barfoed's Test*

Same volume of sample and Barfoed's reagent were properly mixed. Solution was heated for one to two minute in boiling water bath and cooled. After three minutes the reddish precipitate was formed which confirm the presence of monosaccharide.

5.5 *Test for Hexose Sugar*

Three millilitre of test sample juice was mixed with 2 millilitre solution of cobalt chloride. Then it was boiled and cooled and adding few drops of NaOH solution, purplish (fructose) or greenish blue (glucose) or lower layer purplish while upper layer is greenish blue indicates the mixture of fructose and glucose.

5.6 *Selwinoff's Test*

Six millilitre of Selwinoff's reagent solution and 2 millilitre of test sample juice was gently heated in boiling water bath for 1-2 minutes which gives the results of red colour which shows the presence of hexose sugars.

5.7 *Non-Reducing Polysaccharides: Starch test*

Three millilitre of test sample's juice and few drops of iodine dilute solution are slowly mixed. Blue coloration appearance indicates the presence of starch. It disappears if boiled and reappears when cooled.

5.8 *Protein Test: Biuret Test (General Test)*

Three millilitre test samples were added with 4% NaOH solution and then slowly adding 1%$CuSO_4$ solution. The solution appears to violet or pink colour which shows presence of protein.

5.9 ***Million's Test***
Three milliliter of sample was mixed with five milliliter of Million's reagent, if white ppt appears which turns into dark red or the precipitate get dissolves shows the presence of protein.

5.10 ***Tests for amino acids***
Three millilitre of samples juice and 3 drops of five percent ninhydrin solution was warmed in boiling water for ten minutes. Purplish or blue colour appears shows the presence of amino acids.

5.11 ***Steroid Test. Salkowski Reaction***
Two millilitre acetic anhydride was added to five milliliter extract of each sample with two milliliter of H_2SO_4. If the colour changes from violet to blue or green shows the presence of steroids.

5.12 ***Cardiac glycosides***
Five milliliter of each sample juice was treated with two milliliter of glacial acetic acid containing one drop of $FeCl_3$ solution, this was under layered with 1 milliliter of conc. H_2SO_4. A brown ring shows the deoxysugar characteristics of caredenolides. Sometime a violet ring may form indicating cardiac glycoside.

5.13 ***Test for Anthraquinone Glycosides: Borntrager's Test***
To three milliliters of samples was added small amount of dilute sulphuric acid and then boiled and filtered. To cool filtrate equal volume of benzene and $CHCl_3$ was added. It was shaken well in the separating funnel and organic layer was separated and ammonia was added. Ammoniacal layers turns pink or red which represent the presence of anthraquinone glycoside.

5.14 ***Modified Borntrager's Test***
To a five milliliter sample solution five milliliters of five percent $FeCl_3$ and five millilitre of dilute HCl was added. After that it was heated for 1 hour in boiling water bath. It was then cooled and benzene was added and on shaking organic layer was separated. The dilute ammonia was added in equal amount and ammoniacal layer has pinkish red colour.

5.15 ***Saponin Glycosides: Foam Test***
Sample's juice and water was shaken vigorously. Persistent foam observed shows saponin.

5.16 ***Test for Cyanogenetic Glycosides: Sodium Picrate Test***
Filter paper was first soaked in 10% picric acid and in 10% sodium carbonate solution and dried it. After that in a conical flask citrus sample was taken and corked it. After that filter paper was soaked in to the flask. The filter paper turns brick red or maroon which represents cyanogenetic glycoside.

5.17 ***Test for Alkaloid: Hager's test***
When three milliliter of saturated aqueous solution of picric acid (Hager's reagent) is added to one milliliter sample, precipitate of yellow colour indicates the presence of alkaloids.

5.18 ***Wagner's Test***
To three milliliter filtrate adding few drops of Wagner's reagent if reddish brown precipitate appears shows the presence of alkaloids.

5.19 ***Harborne Method***
If we add to a five milliliter sample, 10% CH_3COOH and C_2H_5OH and kept for four hours and then filtered and extract concentrated on water bath to one fourth of its original volume. On adding concentrated NH_4OH, appearance of precipitate shows the presence of alkaloids.

5.20 ***Test for Tannin***
About five millilitre of citrus sample was taken and boiled in 20 milliliter of $CHCl_3$. To the filtrate on addition of 0.1 % $FeCl_3$ appearance of brownish colour shows the presence of tannin.

5.21 ***Test for Saponin***
About five millilitre of citrus sample was taken and boiled in 20 milliliter of $CHCl_3$ and filtered. To the 10 milliliter filtrate added five milliliter of double distilled water with 3 drops of olive oil, emulsion formation shows the presence of saponins.

5.22 ***Test for Phlobotannins***
For phlobotannins test citrus sample was boiled with 1% HCl and was then observed for red precipitate.

5.23 *Test for Flavonoids*

To a portion of filtrate of $CHCl_3$ five milliliter of ammonia solution was added followed by addition of concentrated H_2SO_4, if yellow color appears which disappear on standing shows the presence of flavonoids.

5.24 *Test for Terpenoids*

Five milliliter of the sample was added to 2 milliliter of $CHCl_3$ and three millilitre of concentrated H_2SO_4. If a reddish brown colour formed shows the presence of terpenoids.

6. Results and discussions

The pulp of sweet orange extract was rich with phytonutrients as shown in Table 1.

Table-1: Experimental Results of Phytochemicals Screening of Citrus Fruits.

S. No.	TESTS		Citrus *sinensis* JUICE	Citrus *reticulate* *Blanco* JUICE	Citrus *aurantium L* JUICE
1	**Carbohydrates**	Molish Test	+	+	+
2	**Test For Reducing Sugars**	Benedict Test	+	+	+
		Fehling's Test	+	+	+
3	**Test for Monosaccharide**	Barfoed's Test	-	+	-
4	**Test for Hexose Sugar**	Cobalt Chloride Test	+	+	-
		Selwinoff's Test	+	+	+
5	**Test for Non-reducing Sugars**		-	-	-
6	**Non-Reducing Polysaccharides (STARCH) Test**	Iodine Test	-	+	+
7	**Protein Test**	Biuret Test	-	+	-
		Million's Test	+	+	-
8	**Tests For amino acids**	Tyrosine Test	+	+	+
		Ninhydrin Test	+	+	+
9	**Steroids Test**	Salkowski Reaction	-	-	-
		Liebermann-Buchard Reaction	-	-	-
10	**Cardiac Glycosides Test**		+	+	+
11	**Anthraquinone Glycosides Test**	Borntrager's Test	-	-	-
		Modified Borntrager's Test	-	-	-
12	**Saponins Glycosides**	Foam Test	-	-	-
13	**Cyanogenetic Glycosides Test**	Sodium Picrate Test	-	-	-
14	**Alkaloids Test**	Hager's Test	-	-	-
		Wagner's Test	-	-	-
		Harborne Method	-	-	-
15	**Tannins Test**		+	+	-
16	**Saponins Test**		+	-	+
17	**Phlobotannins Test**		-	-	+
18	**Flavonoids Test**		-	+	-
19	**Terpenoids Test**		+	+	+

From the above work it was concluded that citrus fruits obtained from local market of district Peshawar as well obtained from garden of Islamia College, University Peshawar are rich with phytochemicals.

Some tests were positive while others were negative for selected species of citrus fruits as shown in Table 1. Qualitative phytochemicals screening tests like for tannins, phlobotannins, saponins, steroids, cardiac glycosides, terpenoids and flavonoids were performed for the species Citrus sinensis, Citrus reticulata Blanco and Citrus aurantium Qualitative as well as some quantitave phytochemicals screening test for some other fruits and vegetables are in progress in our lab.

7. REFERENCES

1. Chede, P. S., Phytochemical Analysis of Citrus sinensis Pulp. **(2012-13)**, Vol 4(4); pp. 221-223 ISSN: 0975-4873.
2. Krishnaiah, D., Devi, T., Bono, A., and Sarbatly, R., Studies on Phytochemical Constituents of Six Malaysian Medicinal Plants. *Journal of Medicinal Plants Research* **(2009)**, Vol.3 (2) pp. 067 – 77.
3. Afolabi, F., and Afolabi, O. J., Phytochemical Constituents of Some Medicinal Plants in South West, Nigeria. IOSR-JAC **(2013)**, Volume 4, Issue 1 (Mar. – Apr. 2013), pp. 76-78.
4. Dhawale, P. G., Photochemical Analysis of some Medicinal Plants from Yavatmal District (Ms) India. *The International Journal of Engineering and Science*. (**2013)**, 2 (1) pp. 65 – 66.
5. Mushtaq, A. Q., Rahmatullah, A., Mirand, M. A. K., and Muhammad, Z., Traditional Herbal Remedies used for the Treatment of Diabetes from District Attock (Pakistan) *Pak. J. Bot*., **(2009)**, 41 (6):pp. 2777 – 2782.
6. Kokate, C. K., Purohit, A. P., Gokhale, S. B., Pharmacognosy, 31st ed.Pune (India): Nirali Prakashan; **(2005)**, pp. 593-597. 12.
7. Okwu, D. E., Awurum, A. N., & Okoronkwo, J. I., Phytochemical composition and In Vitro Antifungal Activity Screening of extracts from citrus plants against Fusarium Oxysporum of Okra Plant (Hibiscus esculentus). African *Crop Science Conference* Proceeding. **(2007)**, Vol.8.pp.1755-1758.
8. Kushad, M., Masiuymas, J., Smith, M., Kalt, W., and Eastman, K., Health Promoting Phytochemicals in Vegetables. *Horticultural reviews* **(2003)**, 28:pp.125-185.
9. Crowell, P. L., Prevention and therapy of cancer by dietary monoterpenes. *The J. of Nutr***. (1999)**, 129(3): pp775-778.
10. Okwu, D. E., Citrus Fruits: A Rich Source of Phytochemicals. *Int. J. Chem. Sci*.: **(2008)**, 6(2), pp451-471.
11. Roger, G. D. P., *Encyclopedia of Medicinal Plants* (Vol. 1). Education and Health Library Editorial Saf eliz S. L. Spsin, **(2002)**, 265, pp153-154.
12. Zhao, J., Nutriceuticals, Nutritional Therapy, Phytonutrients and Phytotherapy for Improvements of Human Health, A Perspective on Plant Biotechnology Applications. *Recent Patients on Biotechnology*, **(2007)**, 1, pp.75-97, http://dx.doi.org/10.2174/187220807779813893.
13. Chose, D. C., and McArthur, C., Rethinking the Role of Many Plants Phenolics Protection from Photodamage, Oikos, **(2002)**, 999, pp.161-172.
14. Krishnaiah, D., Sarbarly, R., and Bono, A., Phytochemical and Antioxidant for Health and Medicine. A Move Towards Nature. *Biotechnology and Molecular Biology Review* **(2007)**.
15. Okwu, D. E., and Emenike, I. W., Nutritive Value and Mineral Content of Different Varieties of Citrus Fruits, *J. Food Technology*, **(2007)**, 5(2), pp. 105-108.