A comparativestudy onPhytochemical evaluation of peels of *Citrus auriantum, Citrus maxima*and *Citrus sinensis*using different solvents – Profiting Fruit Waste

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ABSTRACT: *Citrus fruits have been appreciated for their refreshing juice and health benefits. They alsoknown to have phytochemicals which are not of nutritional use but can work towards the deterrence of diseases. As they can be absorbed by human body, they show numerous therapeutic proprieties like minimizing free radical damage to the cells due to oxidative stress, antiviral, anticancer, anti-inflammatory activities, inhibition of platelet aggregation, capillary fragility and minimizing age related muscular degradation. This study provides a clear perception about Citrus maxima, Citrus auriantum and Citrus sinensis peels, as known to have a greater number of phytochemicals. The present phytochemical screening of citrus peels extracts using different solvents revealed the presence of tannins, flavonoids, carotenoids, saponins and carbohydrates. Flavonoids were highly detected in all extracts of Citrus maxima. Tannins, triterpenoids and saponins were also found in C. maxima peel extracts. Citrus auriantum peels showed the presence of tannins, flavonoids, saponins, and triterpenoids in comparatively less amount from different solvents. Whereas Citrus sinensis revealed the presence of alkaloids, triterpenoids, tannins and flavonoids. This work shows the availability of phytochemicals from the peels ofCitrus maxima, Citrus auriantum and Citrus sinensis for various industrial application.*

Key Words: *Citrus maxima, Citrus auriantum,Citrus sinensis, phytochemical, Soxhlet extracts. aqueous extract.*

I. INTRODUCTION

Citrus fruits belonging to the family, Rutaceae,are one of the main fruit crops in the world. Even though Sweet orange (*Citrus sinensis*) is the major fruit (about 70%), the family also includes fruits like grape fruit (*Citrus maxima*), bitter orange (*Citrus auriantum*), lemon (*Citrus sinensis*) and tangerine (*Citrus reticulata*) (Okwu, 2006 and Marzouk, 2013). Citrus fruits are consumed as fresh fruits, also used to produce juices and byproducts (Ferguson,1990 and Kamal et al. 2011). They are rich source of vitamin C and found to be rich in phytochemicals. Phytochemicals are some non-nutritive plant chemicals, that are not essentially required for sustenance of life but offer benefits against pathogens (Roghini, 2018 and Kokate et al. 2006). Phytochemicals work in different ways. They protect the cells against free radical damage by acting as an antioxidant, eg:- Polyphenols, carotenoids etc. They reduce the risk of breast cancer by stimulating certain enzymes, e.g.;- terpenes (Baghurst, 2003, Guimaraes et al. 2010 andAtolani et al. 2012). They act as antibacterial and hormonal stimulant. They even act as binders which prevent the adhesion of pathogen to human cell wall (Roghini, 2018).

Citrus peels are abundant source of flavones and polymethoxylated flavones that are rare in other plants (Rekha, 2013).

Phytochemical screening avail us to know the bioactive compounds in the plant material and the possible range of bioactivities the plant product may possess. It is the primary step in research protocol aiming isolation, purification and use of inhererent compounds in the plant material for medical, pharmaceutical and agro industrial use.

Citrus peels constitute 50-65% of total weight of the fruits. If it is not processed, it becomes a waste causing serious environmental pollution (Mandalari et al. 2006 and Hegazy, 2012). This study aims to the possible waste-to-wealth utilization of citrus peels with the advantage of providing sources of phytochemicals, pharmaceuticals and thereby reducing environmental issues.

II. MATERIALS AND METHODS

2.1 Collection of samples

Healthy disease free citrus fruits(grape fruit- *Citrus maxima*, bitter orange- *Citrus auriantum*, orange- *Citrus sinensis*) were collected from forest areas of Shimoga and Chikkamagalur districts of Karnataka. The fruits

were washed well using tap water and rinsed thrice using distilled water. Then peel and pulp of the fruits were separated. The peels were cut into small pieces and dried in sunlight for
3-4 days. The dried samples were grinded using mortar and pestle and further reduced to powder using electric blender. The powder was stored in air tight containers.

2.2 Preparation of extracts

Aqueous exctract

15g of the dried powder was mixed with 200ml distilled water. Boiled for 10 min. on water bath. Filtered through Whatman filter paper No.1. The extract was cooled and kept in refrigerator at 4°C. This was used for further tests (Lin et al. 1999).

Soxhlet extracts

15g of the dried powder was taken for solvent extraction using 80% methanol, 100% ethyl acetate and 80% hexane separately in a standard soxhlet apparatus set-up for 2-3 hours at a temperature not exceeding the boiling point of the solvent(Lin et al. 1999). Each extract was transferred to sterile glass vials and kept at 4°C before use.

2.3 Phytochemical screening

Test for tannins

1ml of extract was treated with 2 to 3 drops of 10% ferric chloride and observed for dark blue or brownish green coloration (Ajayi et al. 2011).

Test for flavonoids

Sodium hydroxide test

To 1 ml of extract, 5 to 10 drops of 20% NaOH was added. Formation of yellow or orange yellow color indicated the presence of flavonoids (Ayoola et al. 2008).

Lead acetae test

1 ml of extract was taken. To this few drops of 10% lead acetate was added. Appearance of yellow precipitate showed the presence of flavonoids.

Test for triterpenoids

0.5 ml of extract was treated with 2 ml of chloroform followed by few drops of concentrated H_2SO_4. Red brown color at interface showed the presence of triterpenoids (Ayoola et al. 2008).

Test for coumarins

To 1ml of extract few drops of 10% ferric chloride solution is added. Green color solution is formed. To this few drops of concentrated nitric acid is added. Formation of yellow color further indicated the presence of coumerins (Lin et al. 1999).

Test for alkaloids

Hager's test

1 ml of extract was treated with few drops of saturated picric acid solution and observed for the formation of yellow precipitate.

Test for carotenoids

To 1ml of extract 10ml of chloroform was added and was shaken vigorously. To this, 2-3 drops of 85% H_2SO_4 was added. Appearance of brown color at interface indicated the presence of carotenoids.

Test for carbohydrates

Benedict's test

1ml of Benedict's solution was added to 4 ml of extract. Then it is heated to boil. Formation of green, yellow, orange, red or brown color in the order of increasing concentration of simple sugar in the extract, due to the formation of cuprous oxide indicated the presence of carbohydrates (Ayoola et al. 2008).

Test for proteins and amino acids

Ninhydrin test

Few drops of 0.2% ninhydrin was mixed with 2 ml of extract and heated for 5 minutes. Formation of blue or violet color indicated the presence of proteins (Ayoola et al. 2008).

Biuret test

To 1ml of sample 5-8 drops of NaOH solution was added, followed by 2 drops of $CuSO_4$ solution and observed for the formation of violet red color (Ayoola et al. 2008).

Lead sulphide test

1ml of extract was treated with 1ml of 20% NaOH solution. To this few drops of lead acetate was added and boiled on water bath for few minutes. Formation of black precipitate indicated the presence of sulphur containing amino acid.

Test for saponins

10 ml of distilled water was added to 1ml of boiled and filtered extract. It was shaken well for few minutes and was allowed to stand for some time. Formation of honeycomb frothing indicated the presence of saponins (Roghini, 2018).

III. RESULTS AND DISCUSSION

Phytochemical screening of different extracts of the peels of *Citrus maxima*, *Citrus auriantum* and *Citrus sinensis* using different solvents yielded different results in each of the experiment conducted in this study. There were differences in inherent phytochemicals in the extracts obtained in various solvents. The yield is considered based on the appearance of dark or light colour in the tests.

Table 1, shows the phytochemical constituents of *Citrus maxima* extracts obtained from different solvents. It showed the highest yield of phytochemical flavonoids, in methanol extract and aqueous extract followed by fewer amounts in hexane. Tannin was found more in aqueous, methanol and ethyl acetate and moderately in hexane. It also showed the presence of saponins in aqueous, methanol and ethyl acetate extracts. The work of Khan (2018), revealed the absence of flavonoids, and tannins in hexane extract of *Citrus maxima*. But this study showed their presence in the same extract. The absence of saponins, alkaloids and terpenoids in hexane extract shows that this work is in line with the work of Khan (2018), who reported the same result.

Phytoconstituents of *Citrus sinensis* is shown in Table 2. Kumar et al (2011) and Ghongade (2013) reported the presence of various phytochemicals in *Citrus sinensis*. Comparing their results with our study, it revealed the highest yield of carbohydrates followed by tannins and then by flavonoids in each solvent extract. However, the difference is,Kumar et al (2011) reported the absence of tannin in ethylacetate, but this study shows the presence of tannin in the same solvent. Similarly, the report of Ghongade(2013), showed the absence of saponins in aqueous extract, but this study revealed its presence in the same extract. Phytochemicals in *Citrus auriantum* is shown in table 3.By observing the results, it is found that *Citrus auriantum* is rich in sugars and tannins followed by flavonoids in different solvents. It showed the least presence of coumerins and triterpenoids and absence of alkaloids in different solvents. All the three samples showed the presence of carbohydrates in almost all the extracts.

Table 1: Phytochemical screening of peel of *Citrus maxima* using different solvents.

Sl. No.	Phytochemicals	Tests performed	Solvents			
			Aqueous	Methanol	Ethyl acetate	Hexane
1	Tannins	Ferric chloride Test	++	++	++	+
2	Flavonoids	Sodium hydroxide Test	++	++	+	++
		Lead acetate Test	++	++	+	+
3	Triterpenoids	Chloroform Test	++	++	-	-
4	Coumarins	Ferric chloride Test	+	-	+	+
5	Alkaloids	Hager's Test	+	-	-	-
6	Carotenoids	Chloroform Test	-	+	++	-
7	Proteins and amino acids	Ninhydrin Test	+	-	-	-
		Lead sulphide Test	-	-	-	-
		Biuret Test	-	-	-	+
8	Carbohydrates	Benedict's Test	++	++	++	+
9	Saponins	Foam Test	+	+	++	-

Table 2: Phytochemical screening of peel of *Citrus sinensis* using different solvents.

Sl. No.	Phytochemicals	Tests performed	Solvents			
			Aqueous	Methanol	Ethyl acetate	Hexane
1	Tannins	Ferric chloride Test	++	+	+	+
2	Flavonoids	Sodium hydroxide Test	++	++	+	++
		Lead acetate Test	++	-	-	+
3	Triterpenoids	Chloroform Test	+	+	-	-
4	Coumarins	Ferric chloride Test	-	-	-	+
5	Alkaloids	Hager's Test	-	-	-	-
6	Carotenoids	Chloroform Test	++	+	-	-
7	Proteins and amino acids	Ninhydrin Test	+	+	-	-
		Lead sulphide Test	-	+	-	-
		Biuret Test	-	-	-	+
8	Carbohydrates	Benedict's Test	++	++	+	+
9	Saponins	Foam Test	+	+	+	-

Table 3: Phytochemical screening of peel of *Citrus auriantum*using different solvents.

Sl. No.	Phytochemicals	Tests performed	Solvents			
			Aqueous	Methanol	Ethyl acetate	Hexane
1	Tannins	Ferric chloride Test	++	++	+	+
2	Flavonoids	Sodium hydroxide Test	++	+	+	+
		Lead acetate Test	++	+	-	+
3	Triterpenoids	Chloroform Test	+	+	++	-
4	Coumarins	Ferric chloride Test	-	-	-	+
5	Alkaloids	Hager's Test	-	-	-	-
6	Carotenoids	Chloroform Test	-	++	+	-
7	Proteins and amino acids	Ninhydrin Test	+	-	-	-
		Lead sulphide Test	-	-	-	-
		Biuret Test	-	+	-	-
8	Carbohydrates	Benedict's Test	++	++	++	+
9	Saponins	Foam Test	+	+	+	-

Key:++ =Highly detected: based on dark color,**+ =**Less detected: based on light color,**-** = Not detected
All tests were carried out in triplicates.

This variation in phytoconstituents explains the solubility of different plant compounds in different solvents. The medicinal value of these citrus fruit peels lies in the bioactive components, that produce definite physiological action on human body (Akinmoladum et al. 2007). This comparative analysis revealed the presence of various constituents of citrus peels. The presence of flavonoids showed that peels of *Citrus maxima*, *Citrus auriantum* and *Citrus sinensis*could act as antioxidant, anti-inflammatory and immune enhancers. The presence of saponin which has cholesterol binding and hemolytic activities supports the use of these citrus peels in medicinal field (Kumar et al. 2011). As this study is in agreement with the studies of Kumar et al. (2011), Ghonade(2013) and Khan (2018), which says that citrus peels are highly nutritive, they can be used as drugs and even as food supplements. This study also suggests the dual benefit of citrus wastes (peels), one enhancing industrial economics and other reducing environmental pollution (Mandalari et al. 2006 and Hegazy, 2012).

CONCLUSION

This study shows that, the citrus fruit wastes contain potentially useful biological products. They are invaluable sources of raw materials for traditional medicine, drug production and production of food supplements. This preliminary analysis of various citrus revealed the presence of phytochemicals in different extracts. Most of the phytochemicals were detected in all the three citrus samplesshowing strong presence of flavonoids in all extracts. Since the nature and number of bioactive compounds in each extract are not clear, further evaluation with pure compound is required for the definite conclusion.

REFERENCES

1. Ajayi, I.A., Ajibade, O. and Oderinde, R.A., 2011. Preliminary phytochemical analysis of some plant seeds. Res. J. Chem. Sci, 1(3), pp.58-62.
2. Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O., 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimumgratissimum. Scientific Research and Essays, 2(5), pp.163-166.
3. Atolani, O., Omere, J., Otuechere, C.A. and Adewuyi, A., 2012. Antioxidant and cytotoxicity effects of seed oils from edible fruits. Journal of Acute Disease, 1(2), pp.130-134.
4. Ayoola, G.A., Coker, H.A., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C. and Atangbayila, T.O., 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research, 7(3), pp.1019-1024.
5. Baghurst, K., 2003. The health benefits of citrus fruits. Horticulture Australia.
6. Ferguson U., 1990 "Citrus fruits processing." Horticultural Science, Florida pp.117-118.
7. Ghongade, R., 2013. Phytochemical analysis of citrus karna fruit. International Journal of Pharma and Bio Sciences, 4(2), pp.1162-1167.
8. Guimarães, R., Barros, L., Barreira, J.C., Sousa, M.J., Carvalho, A.M. and Ferreira, I.C., 2010. Targeting excessive free radicals with peels and juices of citrus fruits: grapefruit, lemon, lime and orange. Food and Chemical Toxicology, 48(1), pp.99-106.
9. Hegazy, A. E., and. Ibrahium M. I. 2012 "Antioxidant activities of orange peel extracts." World Applied Sciences Journal 18.5 pp.684-688.
10. Kamal, G.M., Anwar, F., Hussain, A.I., Sarri, N. and Ashraf, M.Y., 2011. Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. International Food Research Journal, 18(4), p.1275.

11. Kawaii, S., Tomono, Y., Katase, E., Ogawa, K. and Yano, M., 1999. Quantitation of flavonoid constituents in citrus fruits. Journal of Agricultural and Food Chemistry, 47(9), pp.3565-3571.
12. Khan NH, Qian CJ, Perveen N. 2018. Phytochemical screening, antimicrobial and antioxidant activity determination of citrus maxima peel. Pharm Pharmacol Int J. 2018;6(4):279–285
13. Kokate, C.K., Purohit, A.P. and Gokhale, S.B., 2006. Chapter V-Experimental pharmacognostic evaluation in. The Text Book of Pharmacognosy", Page, (67).
14. Kumar, K.A., Narayani, M., Subanthini, A. and Jayakumar, M., 2011. Antimicrobial activity and phytochemical analysis of citrus fruit peels-utilization of fruit waste. International Journal of Engineering Science and Technology, 3(6), pp.5414-5421.
15. Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jäger, A.K. and Van Staden, J., 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. Journal of Ethnopharmacology, 68(1-3), pp.267-274.
16. Mandalari, G., Bennett, R.N., Bisignano, G., Saija, A., Dugo, G., Lo Curto, R.B., Faulds, C.B. and Waldron, K.W., 2006. Characterization of flavonoids and pectins from bergamot (Citrus bergamiaRisso) peel, a major byproduct of essential oil extraction. Journal of agricultural and food chemistry, 54(1), pp.197-203.
17. Marzouk, B., 2013. Characterization of bioactive compounds in Tunisian bitter orange (Citrus aurantium L.) peel and juice and determination of their antioxidant activities. BioMed research international, 2013.
18. Okwu, D.E. and Emenike, I.N., 2006. Evaluation of the phytonutrients and vitamin contents of Citrus fruits. Int. J. Mol. Med. Adv. Sci, 2(1), pp.1-6.
19. Rekha, S.S. and Bhaskar, M., 2013. In vitro screening and identification of antioxidant activities of orange (Citrus sinesis) peel extract in different solvents. Int. J. pharm. Bio. Sci, 4(4), pp.405-412.
20. Roghini, R. and Vijayalakshmi, K., 2018. Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of citrus paradisi. International journal of pharmaceutical sciences and research, 9(11), pp.4859-4864.