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PHYTOCHEMICAL EVALUATION OF PEEL OF *CITTRUS RETICULATA* BLANCO USING VARIOUS SOLVENT EXTRACTS

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ABSTRACT

Objectives: The aim of the present study is phytochemical evaluation of peel of *Citrus reticulata* using various solvent extract.

Methods: In the present investigation both qualitative and quantitative phytochemical analysis were carried out as per standard procedures.

Results: Phytochemical screening reveals the presence of tannins, phenolics, flavonoids, steroids, glycosides and terpenoids in ethanolic and methanolic extracts, where as glycosides is absent in acetone extract and all the present compounds are found in very minute level. Of the three extract tried for the phytochemical screening methanolic extract showed the maximum number of bioactive compounds followed by ethanol and acetone.

Conclusion: Result approves the presence of phytochemical compounds and amount of primary and secondary metabolites in three extracts of *Citrus reticulata* peel. Among the three extracts methanolic extract showed high level of bioactive compounds compared to other two extracts. This is the first report using peel extracts of *Citrus reticulata.* From the results it is clear that determination of biological active compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure.

Key words: Phytochemical, Bioactive compounds and *Citrus reticulata*

INTRODUCTION

Medicinal plants, fruits and herbal remedies are re-emerging medical aids whose contribution and significance in the maintenance of good health and well- being is widely accepted (Edgar *et al*., 2002). Plants are regarded as the pharmaceutical factories of natural origin for most of the drugs used by human beings. India is the largest producer and consumers of the medicinal drugs and is rightly called as botanical garden of the world (Dubey *et al*., 2000).

Citrus plants belong to the family Rutaceae are one of the fruit crop grown throughout world. India produces 8.70 million tonnes of citrus fruits annually and rank third in the production. The most important commercial citrus cultivation in India is the Mandarin- *Citrus reticulata* Blanco followed by *Citrus sinesis* Osbeck and *Citrus auroantifolia* Swingle.

Currently, there is much biomedical interest in citrus fruits because consumption of them appears to be associated with low risk of cancer, cardiovascular diseases, obesity and other diseases (Levi *et al*., 1999 and Mc Cullough *et al*., 2001). Naturally occurring phytochemical is concentrated in the peel of mandarian and other tangerine oranges. Mandarian peel contains higher level of vitamin C, flavonoids and antioxidants than its juice. *Citrus reticulata* mature and dried peel has been recorded in the Chinese pharmacopoeia as appropriate for medical use.

The citrus peel are usually processed as by-product or wasted, resulting in environment pollution. Studies conducted on several fruit peel have shown that peels are the major sources of natural antioxidants and other phytochemical compounds. Peels and fruits by-product can be used for medicinal application, food products and cosmetics etc.

Interaction between plants and their environment lead to production of different biologically active substances. Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites which are naturally synthesized in all parts of the plant body: barks, leaves, stem, root, flowers, fruits, seeds etc i.e. any part of the plant body may contain active components (Tiwari *et al*., 2001). The quantity and quality of phytochemical present in plant parts may differ from one part to another. The presence of a phytochemical of interest may lead to its future isolation, purification and characterization. Then it can be used as the basis for new pharmaceutical products. Successful determination of biological active compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure (Prashant *et al*., 2011)

Citus reticulata tree is growing abundantly in Kodaikanal, it is also known as kodai orange or kamala orange. They resembles as common orange in shape but smaller in size. They grow in higher altitude and in cool climate. Since the present study was focussed on

Citrus reticulate peel. In the present investigation *Citrus reticulate* peel has been taken and used for qualitative and quantitative analysis to find out the important bioactive compounds.

MATERIALS AND METHODS

Collection and preparation of sample

Citrus reticulata (Kodai orange) was collected from local farmers in Kodaikanal. The peel was washed thoroughly to remove dust particles and dried in hot air oven at 30° – 35° C. The dried peel was powered using mixer grinder. The dried finely ground powder was subjected to soxhlet extraction with acetone, ethanol and methanol. The residual extracts were evaporated to dryness and stored in refrigerator for further analysis.

Phytochemical screening

The extracts was analysed for the presence of tannins, saponins, phenolics, starch, flavonoids, terpenoids, steroids, glycosides, anthraquinone and phlobatannins.

(a)Test for tannins (Ferric chloride test)

One or two drops of ferric chloride solution were added to 1 ml of each extract. Brownish green or blue black colour solution indicates the presence of tannins.

(b)Test for saponins (Foam test)

About 0.2 ml of extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy mess of small bubbles) shows the presence of saponins.

(c)Test for phenolics (Ferric chloride test)

One ml of 1% ferric chloride solution was added to the extract. Blue or green colour indicates the presence of phenolics.

(d)Test for starch (Iodine test)

Few drops of iodine solution were added to 1 ml of each extract. Appearance of blue colour indicates the presence of starch in the extract.

(e)Test for flavonoids (Shimoda test)

One ml of 1% ammonia solution was added to 2 ml of each extract. Appearance of yellow colour indicates the presence of flavonoids.

(f)Test for terpenoids (Terpenoids test)

Five ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was then added to form a layer. A reddish brown precipitate colouration at the interface formed indicates the presence of terpenoids.

(g)Test for steroid (Salkowski test)

Two ml of acetic acid anhydride was added to 0.5 ml of each extract with 2 ml of H_2SO_4. Bluish green colour indicates the presence of steroids.

(h)Test for glycosides (Glycosides test)

Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. Brown ring at the interface indicate the presence of glycosides.

(i)Test for anthraquinone (Borntragers test)

About 0.5 g of each extract was boiled with 10% HCl for few minutes in water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of rose – pink colour indicates of n-hexane, chloroform, ethyl acetate and methanol of the presence of the anthroquinone.

(j)Test for phlobatannins (Phlobatannins test)

Deposition of a red precipitation when an aqueous extract of each extract was boiled with 1% aqueous HCl was taken as evidence for the phlobatannins.

Estimation of bioactive compounds

Bioactive compounds include primary and secondary metabolites such as carbohydrates, phenolics, flavonoids, vitamin C, tannins, protein and lipid, etc.

(a)Estimation of phenols

One ml of the extract was taken in triplicates and the volume was made up to 1 ml with methanol. One ml of water served as blank. To this, 5 ml of Folin's phenol reagent was added followed by 4 ml of 7.5% sodium carbonate and kept at room temperature for 1.5 hours. The intensity was read at 765 nm. A standard graph of gallic acid was plotted, from which the phenol content of the extract was determined (Lachman *et al*., 2000)

(b)Estimation of flavonoids

To 1 ml of extract, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was added and incubated for 30 min at room temperature. The absorbance was measured at 415 nm. For this Catechin is used as standard (Zhisen *et al*., 1999)

(c)Estimation of vitamin C

One ml of extract was taken and made up to 3 ml with methanol: water (1:1). To this 0.2 ml DTC reagent was added, mixed well and incubated at 37°C for 3 hours. 1.5 ml of 85% H_2SO_4 was added and kept at room temperature for 30 min. Colour intensity was read at 520 nm. A standard graph of ascorbic acid was plotted, from which the vitamin C content of the extract was determined (Yen and Chen, 1995)

(d)Estimation of tannins

One ml of the extract was taken in triplicates and the volume was made up to 1 ml with methanol. One ml of water served as blank. To this, 5 ml of Folin's phenol reagent was added followed by 5 ml of 3.5% sodium carbonate and kept at room temperature for 5 min. The intensity was read at 640 nm. A standard graph of tannin was plotted, from which the tannin content of the extract was determined (Sun *et al*., 1998)

(e)Estimation of carbohydrates

One ml of each extract was pipetted out into 25 ml standard flask, 2 ml of freshly prepared anthrone reagent was added to each extract and finally the volume was made upto 25 ml with distilled water. Blank was maintained without adding extract. A standard calibration curve was plotted using Glucose as standard. Absorbance recorded at 750 nm against reagent blank. From the standard curve, concentrations of carbohydrates were calculated (Lowry *et al*., 1951)

(f)Estimation of proteins

Five ml of alkaline copper sulphate reagent was added to 1 ml of each extract and mixed thoroughly. After 10 min of incubation 0.5 ml of Folin's reagent was added. After incubating for 30 min the absorbance was recorded at 660 nm against the blank. Bovine serum albumin was used as standard (Kafeel *et al*., 2008)

RESULTS AND DISCUSSION

Phytochemical Screening

The invigorating properties of remedial plants are perhaps due to the presence of discrete secondary metabolites such as tannins, saponins, phenolics, starch, flavonoids,

terpenoids, steroids, Glycosides, anthraquinone, phlobatannins etc. The three different extract of *Citrus reticulata* fruit peel has revealed the presence of tannins, phenolics, flavonoids, terpenoids and steroids. Glycosides is present in methanolic and ethanolic extract, where it is absent in extract of acetone. Saponins, starch, anthraquinone and phlobatannins were found to be absent in all the three extracts. From this scrutiny, methanolic extract was found to have more fractions compared to acetone and ethanolic extracts. Table.1 shows the qualitative analysis of bioactive compounds in the different extract.

Table 1: Shows Phytochemical screening of *Citrus reticulata* peel

CHEMICAL TESTS	ACETONE	ETHANOL	METHANOL
Tannins	+	++	++
Saponins	_	_	_
Phenolics	+	++	++
Starch	_	_	_
Favonoids	+	++	++
Terpenoids	+	+	++
Steriods	++	+	++
Glycosides	_	++	++
Anthraquinone	_	_	_
Phlobatannins	_	_	_

Flavonoids, tannins, phenolics are dominant group of bioactive compounds that deed as primary antioxidants or free radical scavengers. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Mc Donald, 2001).The preliminary phytochemical screening is accessible in finding chemical constituents of orange fruit peel that will lead to the quantitative estimation in spotting pharmaceutically effective compounds.

Estimation of Bioactive Compounds

Bioactive compounds are extra nutritional constituents that typically occur in small quantities in foods. They are being intensively studied to evaluate their effects on health (Kris *et al*., 2002).It is very important to quantify the bioactive compounds to ensure the medicinal properties of the extract. The quantitative analysis of bioactive compounds in the extracts is given in Table 2. From the table it was evident that bioactive compounds such as phenols, flavonoids, vitamin C, tannins, proteins and carbohydrates were found in maximum quantity in methanolic extract of *Citrus reticulate* peel. This was followed by ethanolic extract when compared to both the extract acetone extract showed a little quantity of this compound except vitamin C.

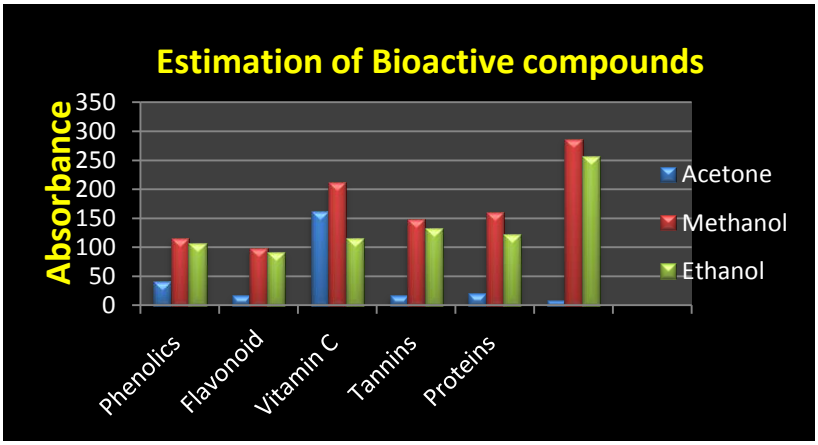
Table 2: It shows estimation of bioactive compounds of *Citrus reticulata* peel

Bio active compounds	Acetone	Methanol	Ethanol
Total phenolics (mg GAE/gm extract)	41.25	115.5	107.30
Flavonoids (mg catechin/gm extract)	16.5	99	92.4
Vitamin C (mg AAE/gm extract)	161.7	211.2	115.5
Tannins (mg TAE/gm extract)	16.5	148.5	132
Proteins (mg/gm extract)	20	160	122
Carbohydrates (mg glucose /gm extract)	8.25	287.1	257.4

The total phenolic content in the investigated extracts was expressed in terms of Gallic acid equivalent. The phenol content in extracts ranged from 41.25 - 115.5 mg GAE/g. Plant foods are rich source of phenolics, which are molecules that can act as antioxidant, to prevent heart diseases, lower the incidence of cancers (Sawadogo *et al*., 2012, pieme *et al*., 2010, Ramos, 2008 and Silvova *et al*., 2005). The protection afforded by the consumption of plant products such as fruits, vegetables and legumes is mostly associated with the presence of phenolic compounds. Flavonoids are expressed as Catechin equivalent, high flavoinoid content was found in methanolic extract (99mg CT/gm) followed by ethanolic extract (92.4mg CT/gm) and least amount of flavonoids are found in acetone extract (16.5mg CT/gm). A growing number of epidemiological studies suggest that high flavoind intake may be correlated with a decreased risk of cancer (Le Marchand, 2002).Vitamin C one of the predominant compound in all citrus fruits, is also predominant in analysed extracts compared to other active compounds. Methanolic extract found to have high level of Vitamin C (211.2mg AAE/gm), acetone extract (161.7mg AAE/gm) and ethanolic extract (115.5mg AAE/gm). Vitamin C is the major water-soluble antioxidant (Sies *et al*., 1995). Numerous analysis have shown that an adequate intake of vitamin C is effective in lowering the risk of developing cancers of the breast, cervix, colon, rectum, lung, prostate and stomach (Block., 1991, Frei , 1994 and Leine *et al*., 1996). The reducing activity of vitamin C has led to its diverse roles in human health (Buettner *et al*., 1993). Tannins are expressed as Tannic acid equivalent, high tannin content was found in methanolic extract (148.5mg TAE/gm), ethanolic extract (132mg TAE/gm) and negligible amount of tannin was found in acetone extract (16.5mg TAE/gm) Tannin and tannin like substances are poly- phenolic compounds which are divided into two group; water soluble hydrolysable tannins and condensed tannins (Khairusy *et al*., 2012). Different chemical constituents of poly-phenols (flavonoids, phenolics, hydrolysable and condensed tannins) are major bioactive components responsible

for the prevention of chronic diseases and health care (Vasundara *et al*., 2013). All of our body organs are built from proteins therefore protein is essential part of our diet, vital to development and correct functioning of the body. The presence of higher protein level in the plant points towards their possible increase food value (Thomsen *et al*., 1991). Total level of protein were found to maximum in methanolic extract (16omg/gm), followed by ethanolic extract (122mg/gm) and acetone extract (20mg/gm). Carbohydrates are macromolecules used as an ideal source of energy. Carbohydrates can be readily converted into glucose which can be easily transported in the body. Methanolic extract showed high level of carbohydrates (287.1 mg glucose/gm) followed by ethanolic extract (257.4mg glucose/ gm) and low level of carbohydrates was found in acetone extract (8.25mg glucose/gm) (Fig. 1).

Fig. 1: Shows quantification of plant metabolites of *Citrus reticulata* peel



CONCLUSION

Present analysis confesses that *Citrus reticulata* peel will be a perfect effective source of various bioactive compounds. Since the peel extracts shows the presence of important phytochemicals such as flavonoids, phenols, tannins, terpenoids, glycosides and steroids. Extracts of such an important bioactive compounds from the peel itself instead of fruit is notable worthy from the economic point of view as well as eco-friendly manner. This attempt is a step to open the door of exploring fruit wastes as strong clinically biotherapeutic agents. It also reveals fruit peel could be used as alternative useful agents in food industries.

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