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SCREENING OF TOTAL PHENOL, CELLULOSE AND TANNIN CONTENT IN ORANGE PEEL BY USING DIFFERENT PARAMETERS IN ETHANOL EXTRACT

P. Karthiga[1], M. Bimonisha[2], A. Chandra Mohan*[3] and R. Gajalakshmi[4]

M. Sc Student[1&2], Guide[3], Assistant Professor[4], PG Research, Dept of Biochemistry and Chemistry, Jaya College of Arts and Science, Thiruninrayur, Chennai, Tamilnadu.

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***Corresponding Author**
Dr. A. Chandra Mohan
Guide, Dept of Biochemistry and Chemistry, Jaya College of Arts and Science, Thiruninrayur, Chennai, Tamilnadu.

ABSTRACT

Orange belongs to *Citrus* species. Its botanical name is *Citrus sinensis* belonging to family *Rutaceae* Citrus fruits are sweet in nature and *Citrus aurantium* is considered to be bitter than other varieties. Present day oranges are hybrids between pomelo and mandarin. The citrus peels are rich in nutrients and contain many phytochemicals they also can be efficiently used as drugs or as food supplements. There is an increase in the number of antibiotic resistance pathogens, there is always a search of an alternative drug that is regarded as safe. Orange juices are a rich source of vitamin C which is considered as a most important water soluble antioxidant. The purpose of the present study was to evaluate the effect of using different parameters with ethanol extract and estimate the efficiency of effective compounds, such as polyphenolic, cellulose and tannin compounds from the orange extracts.

KEYWORDS: Citrus peels, polyphenolic, cellulose and tannin.

INTRODUCTION

Since 1987, Orange is the most cultivated citrus varieties of world. They mainly grow in subtropical climate for their sweet fruit. The fruit can be eaten fresh, processed for its juice and peel. Citrus is the general term for plants belonging to the family Rutaceae. Plants have large shrubs or small trees that can reach a height of 5-15 m with branches covered with thorns and evergreen leaves (Ladaniya, 2008). They are important source of many bioactive compounds, such as phenolic acids and flavanone glycosides. Naringin and hesperidin, so

called citrus flavonoids, are the two main glycosidic flavanones presented in citruses (Abeysinghe *et al*., 2007). Caffeic, chlorogenic, ferulic, sinapic and p-coumaric acid are the most abundant phenolic acids present in citruses (Tokusoglu & Hall, 2011).

Citrus, is one of the most consumed type of fruit over the world, due to low cost and bulk productivity as well as for their wholesome nutritional properties consisting of vitamin C, A and B, minerals (calcium, phosphorus, potassium), dietary fiber and many phytochemicals such as flavonoids, amino acids, triterpenes, phenolic acids and carotenoids (Melèndez *et al*., 2008 and Roussos PA, 2011). However, the consumption of citrus fruits have led to the generating of residue (peel, pulp, seeds) which accounts approximately a 50% of the fruit weight and its moisture content (Garcia-Castello EM *et al*.,2011, Fernandez-Lopez J *et al*., 2004 and Pe rez-Alvarez JA *et al*., 2001).

This huge amount of waste can be considered as an agricultural waste, by the fact that it was discarded and contributes to the environmental pollution. Cellulose can also be used as starting materials for nanocellulose production via strong acid hydrolysis. The citrus peels are rich in nutrients and contain many phytochemicals they also can be efficiently used as drugs or as food supplements. There is an increase in the number of antibiotic resistance pathogens, there is always a search of an alternative drug that is regarded as safe. (Wilson CL & Droby GG, 2000 and Friedman *et al*., 2002).

A Pulp and their seed contribute to bulk of the fruit weight comprising about 46% and 44% while peel constitutes about 10%. The orange fruit is highly nutritious and rich in minerals, proteins, carbohydrates, and fat (Prasad K.N *et al*., 2010). Orange juices are a rich source of Vit. C which is considered as a most important water - soluble antioxidant. The major role of Vit C is the prevention of scurvy; this causes the disease which leads to the formation of spots on the skin, spongy gums and bleeding from the mucous membranes. Vit C is unstable compounds which are degraded by both aerobic and anaerobic pathways. The loss of Vit C might be a critical factor for the shelf life of some products as citrus juice concentrates (Micucci *et al*., 2011).

MATERIALS AND METHODS

Collection of orange peel

Collection, separation and drying of orange peel. The orange fruits were purchased. The peels were manually separated from the fruit. The peels were shade dried. The dried peels were

collected and ground well to form a powder. The powdered orange peel was stored in an airtight container and used for various tests.

Preparation of the peel extract

Preparation of the extracts one gram of dried bitter orange peel powder was extracted with 20 ml of aqueous, ethanol was soaked overnight at room temperature. The sample was then filtered through Whatman.No.1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota -vapor at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extract was approximately 100%. The extracts were used for further tests.

Qualitative phytochemical analysis The phytochemical tests were carried out using standard methods of analysis of tannins, saponins, quinones, flavanoids, glycosides, cardiac-glycosides, terpenoids, phenols, coumarins, steroids, alkaloids, anthocyanin and betacyanin.

MATERIALS REQUIRED

- Acetic/Nitric Reagent: Mix 150ml of 80% acetic acid and 15mL of concentrated nitric acid.
- Anthrone: Dissolve 200mg anthrone in 100mL of ice-cold 95% sulphuric acid. Prepare fresh and chill for 2h before use.
- 67% sulphuric acid.
- Folin-Denis Method: This is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group.
- Sodium carbonate, Tannic acid and Folin-ciocalteau reagent.
- Distilled water.

METHODS

SONICATION

Sonication is the process of converting an electrical signal into a physical vibration that can be directed toward a substance. Sonicators are vital lab equipment and are used for a number of purposes. Sonication is usually performed to break apart compounds or cells for further examination. The vibration has a very powerful effect on solutions, causing their molecules to break apart and cells to rupture. A prime example is in DNA testing, where the cells that may contain DNA information are subjected to sonication to break them apart and release the DNA proteins so they can be tested.

The primary part of a sonication device is the ultrasonic electric generator. This device creates a signal (usually around 20 KHz) that powers a transducer. This transducer converts the electric signal by using piezoelectric crystals, or crystals that respond directly to the electricity by creating a mechanical vibration. This vibration, molecular in origin, is carefully preserved and amplified by the sonicator, until it is passed through to the probe. The sonication probe transmits the vibration to the solution being sonicated. This probe is a carefully constructed tip that moves in time with the vibration, transmitting it into the solution. The probe moves up and down at a very high rate of speed, although the amplitude can be controlled by the operator and is chosen based on the qualities of the solution being sonicated.

MAGNETIC STIRRER

A magnetic stirrer or magnetic mixer is a laboratory device that employs a rotating magnetic field to cause a stir bar (also called "flea") immersed in a liquid to spin very quickly, thus stirring it. The rotating field may be created either by a rotating magnet or a set of stationary electromagnets, placed beneath the vessel with the liquid. Magnetic stirrers are often used in chemistry and biology, where they can be used inside hermetically closed vessels or systems, without the need for complicated rotary seals. They are preferred over gear-driven motorized stirrers because they are quieter, more efficient, and have no moving external parts to break or wear out other than the simple bar magnet itself.

Magnetic stir bars work well in glass vessels commonly used for chemical reactions, as glass does not appreciably affect a magnetic field. The limited size of the bar means that magnetic stirrers can only be used for relatively small experiments, of 4 liters or less. Stir bars also have difficulty in dealing with viscous liquids or thick suspensions. For larger volumes or more viscous liquids, some sort of mechanical stirring is typically needed. Because of its small size, a stirring bar is more easily cleaned and sterilized than other stirring devices. They do not require lubricants which could contaminate the reaction vessel and the product. Magnetic stirrers may also include a hot plate or some other means for heating the liquid.

Quantitative phytochemical analysis

Total phenol content

Determination of total phenol content The Folin-Ciocalteu’s reagent method has been used for estimation of total phenolic content, according to Lister and Wilson with slight modification. 100µl of crude extract of the bitter orange peel was mixed with 0.5 ml of

Folin-Ciocalteu's reagent (1/10) dilution and 1.5 ml Na2Co3 (2% w/v). The blend was incubated in a dark place at room temperature for 15 minutes. The absorbance of blue coloured solution of all samples was measured at 765 nm using a UV spectrophotometer. The results were expressed in mg of gallic acid equivalent (GAE) per gram dry weight of plant.

Total cellulose content

Add 3mL acetic/nitric reagent to a known amount (0.5g or 1g) of the sample in a test tube and mix in a vortex mixture. Place the tube in a water bath at 100°C for 30min. Cool and then centrifuge the contents for 15-20min. Discard the supernatant and Wash the residue with distilled water. Add 10mL of 67% sulphuric acid and allow it to stand for 1h. Dilute 1mL of the above solution to 100mL. To 1mL of this diluted solution, add 10mL of anthrone reagent and mix well. Heat the tubes in boiling water bath for 10min. Cool and measure the color at 630nm. Set a blank with anthrone reagent and distilled water. Take 100mg cellulose in a test tube and proceed from step No. 6 for standard. Instead of just taking 1mL of the diluted solution (Step 7) take a series of volumes (say 0.4 to 2mL corresponding to 40-200mg of cellulose) and develop the color.

Total tannin content

Determination of total tannin content Tannin content of bitter orange peel extract was estimated by the method described by Fagbeme *et al*., 2005. The peel extract (1 ml) was mixed with Folin-Ciocalteu's reagent (0.5 ml) followed by the addition of saturated Na2Co3 solution (1ml) and distilled water (8 ml). The reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant was obtained by centrifugation and absorbance was recorded at 725nm using UV – visible spectrophotometer. Tannin content was calculated as mg tannic acid equivalent obtained from a calibration curve. Abs 725 nm = 7.061 x (TA) mg Where, (TA) mg is the concentration of tannic acid taken as standard.

Determination of flavanoid content The Aluminium Chloride calorimetric method was modified from the procedure reported by Woisky and Salantino. Quercetin was used to make the calibration curve, 10 mg of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95 % ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance

of the reaction mixture was measured at 415 nm with a spectrophotometer. The amount of 10% aluminium chloride was substituted by the same amount of distilled water in blank.

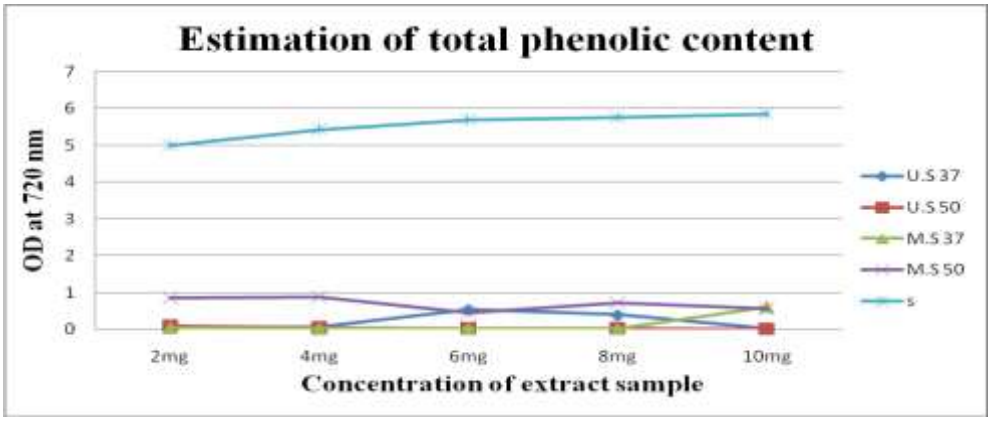
RESULT AND DISCUSSION

Phytochemical screening and analysis carryout with standard procedures of ethanol solvent in orange peel extracted and with different parameter a) ultrasonicator b) magnetic stirrer at various temperatures (37ºc and 50ºc) evaluated the presence of phytochemicals such as Phenol, celluloseand tannins, Harborne (1973).

Estimation of Total phenolic content (TPC)

The concentrations of total phenolic content in the extracts were expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract the total phenolic content of the orange of peel extract sampleswere determined using the Folin-Ciocalteu reagent method. The reduction of FCR by phenols to a mixture of blue oxides which have a maximal absorption in the region of 765 nm was measured spectro-photometrically.

The results revealed the presence of highest total phenol content in the ethanol extract of orange peel in different parameter at various temperature in 37˚C and 50˚C is shown in (Fig. 4). The increasing concentration of ethanolic extract 2mg, 4mg, 6mg, 8mg and 10mg showed increasing OD value respectively. The highest OD valuefor ultra sonicator at 37˚C in 6mg is (0.5254), where as in magnetic stirrer the highest OD value is seen at 50˚C in 4 mg is (0.8810). The highest total phenolic content were obtained in magnetic stirrer at 50˚C in 4mg. Phenolic compounds possess different biological activities, but most important are antioxidant activities. Phenols are able to scavenge reactive oxygen species due to their electron donating properties.

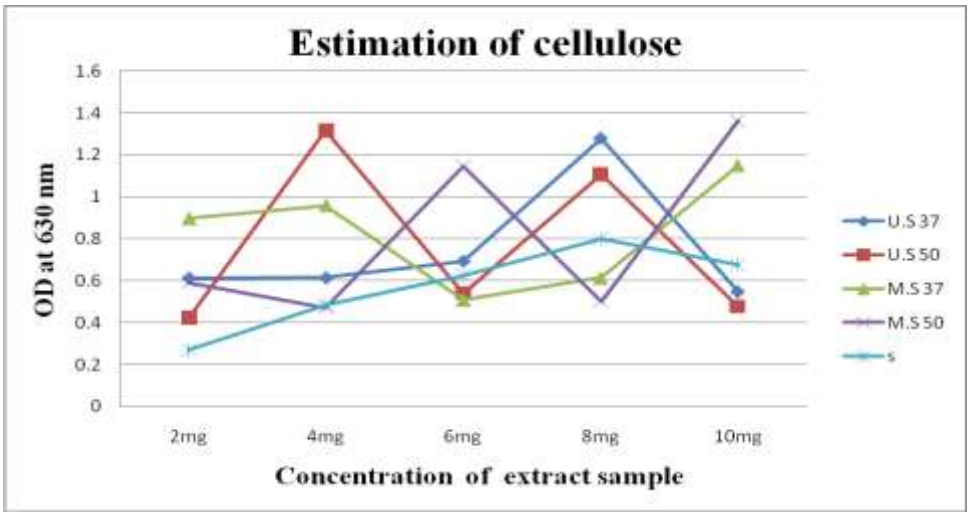


"Fig. 4" Estimation of total phenolic content from ethanol extracts.

Estimation of Total cellulose content

The total cellulose content of the orange peel extract samples were determined using the anthrone method. The reduction of AR by cellulose to a mixture of blue oxides which have maximal absorption at 630nm was measured spectrophotometrically. The result revealed the presence of highest total cellulose content in the ethanol extract of orange peel in different parameters at various temperature in 37˚c and 50˚c is shown in the (fig. 5).

The increasing concentration of ethanolic extract 2mg, 4mg, 6mg, 8mg & 10mg showed increasing increasing OD value respectively. The highest OD value of ultrasonicator at 50˚c in 4mg is (1.3187), where as in magnetic stirrer the highest OD value seen at 37˚c in 10mg (1.1499). The highest total cellulose content were obtained magnetic stirrer at 37˚c in 10mg.



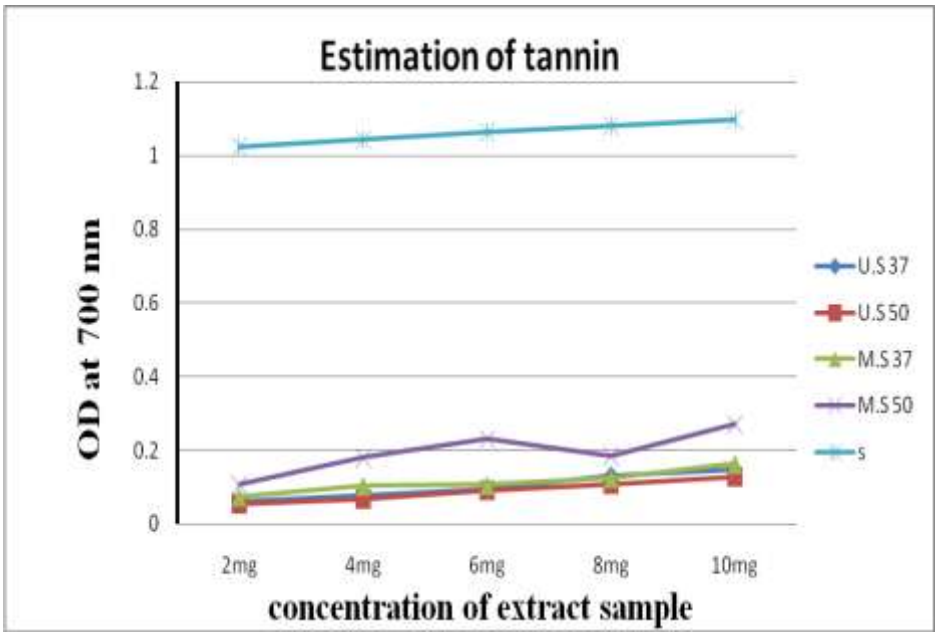
"Fig. 5" Estimation of total cellulose content from ethanol extracts.

Estimation of total Tannin

The concentration of total tannin content was expressed as mg of Tannic acid equivalent/g of dry weight (mg E/g) of extracts. The total Tannin content of the orange peel extract samples were determined using by Folin–Denis method. The reduction of FDR by tannin to a mixtue of blue oxides which have a maximal absorption in the region at 700nm was measured spectrophotometrically.

The result revealed the presence of highest total-Tannin content in the ethanol extract of orange peel in different parameters at various temperature in 37˚c and 50˚c in shown in (fig. 6). The increasing concentration of ethanolic extract, 2mg, 4mg, 6mg, 8mg and 10mg showed increasing OD value respectively. The highest OD value of ultrasonicator at 37˚c in 6mg is

(0.0959), where as in magnetic stirrer the highest OD value is seen at 50˚c in 10mg (0.2703). The highest total tannin content were obtained in magnetic stirrer at 50˚c in 10mg.



"Fig. 6" Estimation of tannin from ethanol extract.

CONCLUSION

Recycling of fruit waste is one of the most important means of utilizing it in a number of innovative ways yielding new products and meeting the requirements of essential products required in human, animal and plant nutrition as well as in the pharmaceutical industry. . Orange fruit and its peel exhibit a high antioxidant potential. They have gain edawide acceptance for their pharmacological activities against serious maladies such asprostrate, colon and liver cancers, stomach ulcers, cardiovascular diseases and digestive disorders. The cytoprotective and inhibitory effects of this fruit and its peel demonstrate the potential to prevent some human carcinomas. As ethnopharmacological utilization of the fruit and peel extract is prevalent in a variety of cultures to cure common disorders without any consideration to its phytochemical profile and toxicological limit, safety verification and clinical trials are needed prior to its pharmacological exploitation by modern medicine.

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