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PHYTOCHEMICAL COMPOSITION AND IN VITRO ANTIOXIDANT ACTIVITIES OF THE GENUS CITRUS PEEL EXTRACTS: A SYSTEMATIC REVIEW

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ABSTRACT

India is the leading producer of fruits worldwide. The major problem after citrus fruits consumption is their peel that are hazardous to our environment and mainly regarded as a solid waste however, they are rich sources of fibres, large amount of Vitamin C, phenolics and flavonoids which are best agents of antioxidant. In this paper, we have discussed about the orange peel waste which has many beneficial roles in our daily life. Citrus peel has the highest number of Phytochemicals such as flavonoids, terpenoids, quinolines and having highest antioxidant activity as compared to the pulp and seed as well as identified from the extraction. The different Phytochemicals test analysis have been discussed in this paper (steroids testing, saponins testing) and different antioxidant test used to check the antioxidant activity of the citrus fruit peel extract are Hydrogen peroxide scavenging test, Nitric oxide scavenging test, DPPH test etc. The main objective of this paper is that the higher antioxidant activity of different mixture of Citrus fruit peel can help to reduce the generation of free radicals and can act as an anti-proliferative agent. The search for novel drugs and alternative medicine especially against cancer has led to increased research in medicinal plants. This review also indicated that Citrus peels contains antioxidant compounds which could be exploited as value added products in the food and cosmetic industry.

Keywords: Phytochemical Composition, Genus Citrus peel, Antioxidant activities, Extraction, Applications.

I. INTRODUCTION

Orange have been the most frequently employed fruit in the world because of its gratifying taste and nourishing values. Citrus sinensis or sweet orange originated from south East Asia what is consumed all over the world as a powerful natural anti-oxidant that enhance the activity of our immune system. Today it is grown all over the world as a source of food for human consumption because of its high nutritional value vitamin rich sources [1]. Orange are most commonly utilized fruit worldwide due to its taste. Because of large consumption of the fruit a huge amount of waste is produced. Orange peels are mainly included in this waste. The orange peel contains number of compounds having various properties. Therefore, throwing of these peels considering waste can be used to produce those compounds [2]. For discovering newer therapeutically active moieties natural sources has provided us with an excellent hunting ground. Many diseases such as inflammation, atherosclerosis and cancer are caused by lipid peroxidation and free radical generation inside human body. Citrus fruits including grape fruit, lemon, oranges are principle source of nutrient which is responsible for prevention of degenerative [3]. The accurate grouping of the citrus fruit is very essential in kingdom Plantae. On the basis of species, genus, order and family, the classification of citrus fruit is done. The classification tells about the characteristic features and its relation with other fruit taxonomically. The citrus fruit belongs to division Magnoliophyta. Magnoliophyta includes angiosperms i.e. all flowering plants. This division is further divided in Magnoliopsida and liliopsida. Oranges are placed in genus citrus. All fruits belongs to particular species are economically same. [4]. Some of species cultivated are in Table.1.

A wide section is mainly directed for the citrus juice extraction to produce huge number of remnants at industrial level which includes segment membranes and Peel. Orange peel has many active phytochemicals due to which it shows antioxidant property and includes vitamins, flavonoids, Lignin's,

Carotenoids, Saponin, Plant Sterols, Terpenoids. The main compounds in citrus peel such as ascorbic acid, flavonoids are beneficial for the human Health. The flavonoids have mainly three types occur in Orange peel species are Eriocitrin, Narirutin, Hesperidin and Naringin. Citrus peel also contains lemon Oil, D-limonene, Vitamin-C and pectin's. Orange peel contains 1.5% phytoconstituents essential oil [3]. The citrus fruit contains plenty of alkaloids, flavonoids, limonoids groups and coumarins most dominant are synephrine, flavanones, limonin and, auraptene are the most respectively [5]. Some Phytochemicals of some Citrus species in Table 2 [3].

In citrus peel various anti-oxidants are found which shows various anti-oxidant effects that includes metal chelation and free radical scavenging activities. Reactive oxygen species plays vital role in number of diseases which includes cardiovascular dysfunction, process of ageing, neurodegenerative disease and cancer, so it is encouraging to study the phytochemicals present in *C. sinensis*. [6]. *C. sinensis* contain rich amount of GOFA, that previously showed dietary feeding colon cancer chemo preventive and neuroprotective effects in rats, revealed in recent studies of citrus peel extract during the identification of GOFA(4- Gernyloxyferulic). [7,8]. Due to presence of wide range of properties in poly methyl flavones(PMFs) and alkaloids in citrus peels have given great attention to citrus peels.

For exploitation and analysis of bioactive compounds of plants, extraction is the major step. A quantitative, time effective and non-destructive method is the ideal type of extraction method. The traditional method for the usage of medicinal plants was by consuming the extracts in food or by boiling in water due to ease of access of water and less toxicity, but the effectiveness was a doubt of consumption of medicinal plants by these methods. [9]. Due to simplicity, Conventional Solvent Extraction (CSE) is used for recovering biologically active compounds, but it also have some disadvantages like large consumption of solvents, hazardous liquid organic solvents, long extraction time and exposure to flammable. The anti-oxidant activities and total phenolic content of extract extracted by CSE were comparable to the extracts extracted by non-conventional methods shown in study of ref. [10]. The properties of extracting solvents greatly affects the anti-oxidant and total phenolic content. Commonly used extracting solvents are acetone, ethyl acetate, methanol, ethanol and propanol. [11]. The solvents with high polarity dissolves the phenolic compounds easily like in methanol. Ethanol is also used as food-grade solvent because some solvents identified are toxic like methanol, is preferred as extracting solvent for phenolic compounds from citrus fruit peel. [12].

Due to their high availability and low cost in world *C. sinensis* peels and the phytochemicals present in them serves as a nutritional dietary supplement and cheap or a therapeutic agent. The reliable and systematic study proves the health benefits of *C. sinensis* peels. The conventional extraction with different solvents like acetone, methanol and ethanol, the useful biologically active compounds with high anti-oxidant activities was hypothesized. [13].

This study aims to examine various capabilities of *C. sinensis* peel extracts with the correlation to their phytochemical content.

SEARCHING METHODS

This survey incorporates investigations of phytochemical and anti-oxidant analysis extract from Citrus peel, distributed in the English language. The EMBASE databases were looked through PubMed and Google Scholar. In the hunt, the keywords comprising of "Phytochemical" and "anti-oxidant", "Citrus peel", or "Extraction method" were utilized. Different keywords "Anti- Cancer", "Flavonoids" and "Applications" were likewise utilized as search refinement. These keywords would cover however much data about phytochemical and anti-oxidant analysis extract from Citrus peel as could be expected without ignoring related inquiries about.

II. EXTRACTION METHODS

Distillation by Steam method: Prepared 155 grams of the citrus peels were added into the fractioning flask which was then attached to the rounded bottom flask having water. With the tubing the flask was attached to the condenser, with a heating mantle. Extraction of essential oils with the help of distillation unit using steam that was percolating inside the peels. Settling of the water and oil was withdrawn from

the distillation unit. Final product of the steam distillation contains oil and water mixture that was isolated by using separating funnel. Separation of the water at the bottom layer and on the top layer contains essential oils. Separation was continued until oil was left with negligible amount of water. [14]

Water distillation method: By using digital weighing balance, 150 gm of the orange peel powder was weighed. These orange peel powders were transferred into the round bottom flask and to cover the peels huge quantity of water was added [15]. The flask was Attached to the column which was further attached to the condensing unit. The essential oil was extracted when the water being heated with the help of steam generation. While passing through the condenser the water was condensed as a part of steam. Distillate contain oil and water mixture which was collected and added into the separating funnel. By using separating funnel, two layers of mixture was separated (upper layer: Essential oil, Lower layer: aqueous solution). This two-layer separation will further help to separate the water and the extracted oil which was accumulated in a bottle. At different temperature interval, the experimental run up was carried out and at regular interval of time volume of oil was taken at constant level. [16]

Solvent extraction method: For this procedure, using a standard particle size 0.6mm sieve, the grounded peels were sieved. The two factors: Time and Temperature, were considered in experimental design. The extraction was carried out by utilizing extraction solvent (Hexane). For every experiment, 10g of the sample was taken. Using Soxhlet apparatus, having heating mantle the round bottomed flask with solvent was heated to evaporate the sample and condensed to extract the oil. So, oil and solvent mixture was separated later. The extraction was carried out by using extraction solvent (Hexane). For every experiment, 10g of the sample was taken. Using Soxhlet apparatus, having heating mantle the round bottomed flask with solvent was heated to evaporate the sample and condensed to extract the oil. So, oil and solvent mixture was separated later. The experimental design parameters for solvent extraction were given in the table shown below Table. 3 [16].

Extraction of phenols by Ethanol and Aqueous methods: 2gm of frozen citrus powder peel was placed in 50ml centrifuge tube. Added 16ml of solvent or aqueous. The experiment was left for 3 hours at various temperature in the range from 20-80°C. By using a Mistral 1000 centrifuge the given mixture were centrifuged for 10min. at room temperature. With the help of Whatman filter paper no. 42, the supernatant was filtered. After filtration a filtrate of 10ml aliquots was concentrated by evaporation of the solvent using a rotatory evaporator partial vacuum at less than 1ml of fixed filtrate at 40°C. In 10ml of milli-Q water the extract was re dissolved and stored at 4°C prior to purification step. Triplicates were prepared of all the extracts. [16]

Extraction by using Trichloroethane: Use Trichloroethane (b.-74°C) as an extraction solvent and dilute sodium sulphate as a desiccant. Separation of mixture with oil and water is carried out by using separatory funnel [17].

Extraction by Soxhlet Method: Orange fruit was washed and peeled off. Dry the crust for 48hours at 41°C in a Ventilated oven. Crush the crust into fine powder and pass through a 24-Mesh filter. Add 850ml of ethanol, methanol at room temperature for 6hours by Soxhlet method. Whatman filter paper no.2 was skipped for the removal of peel particles. To ensure complete extraction the residues was re-extracted 2 or 3 times under same conditions. The mixture was filtrated and the extract was evaporated to dryness using rotary evaporator at a low pressure of 60°C. The extract was stored in a dark bottle of the refrigerator at 4°C. [18]

III. PHYTOCHEMICAL ANALYSIS

Alkaloid testing: 1% HCL was mixed with 2ml of filtrate and add 5-6 drops of Mayers Chemical agent. If the Alkaloids are present then there was a creamish or pale-yellow colored precipitation occurs. [19]

Testing by dragendroff's reagent: To 2-3ml of filtrate add few drops of dragendroff's reagent. Orange brown precipitates confirms the presence of alkaloids. [18]

Amino acids testing: Add 2-3 drops of ninhydrin in orange peel extract. Violet color appears shows the amino acids are present in the orange peel. [18]

Testing by xanthoproteic reagent: To the extract add 3-4 drops of concentrated nitric acid. Precipitate were formed of yellow color shows the amino acids presence. [18]

Testing for saponin: Boil the extract and sieved the mixture. In a test tube add

distilled water of volume 20 ml and then mix 2.5 ml of the filtrate. The mixture was stirred for few minutes and then allowed to stand for some time. The formation of honeycomb along with frothing shows the existence of saponins [19].

Tannins Testing: Take 6ml of deionized water in a small beaker and 3g of extract is filtered. Add 10% ferric chloride solution dropwise with the help of a dropper. Appearance of bluish color shows the existence of tannins.[3]

Testing by Gelatin: Take sodium chloride in a test tube and add 1% of gelatin. Add 2ml of extracted sample. White clumps were formed confirms the presence of tannins. [20]

Steroids testing: Take 3ml of chloroform along with 0.8ml of extract in a beaker and filter it. Mixed concentration Sulfuric acid to the filtrate along the sides of test tube which form bottom layer. A brown reddish color circle indicates the existence of steroids. It is also known as Salkowski test. [20]

Glycoside Testing: Take test tubes having 5ml of extract then add 3ml of Anhydrous acetic acid. Add 1ml of Iron (III) chloride solution. The mixture was treated with strenuous Sulfuric acid formation of brown circle at the interfacing region shows the presence of glycosides. [3]

Testing for Anthraquinones: Extract solution was hydrolyzed accompanied by benzene extracted with dilute concentrated H_2SO_4 . The mixture was then treated with 1-2 ml of dilute ammonia. The positive response for anthraquinones was detected by rose pink coloration. [3]

Flavonoids Testing: In extract 2-3 drops of ferric chloride is added. Blackish red color was produced which shows the existence of flavonoids. [18]

Test for reducing sugars: Add some amount of Fehling solution in to the extract. Boil the mixture for about 3-4 minutes. Existence of reducing's sugars was detected by brick red color appearance. [20]

IV. ANTIOXIDANT ANALYSIS

Scavenging activity by using DPPH method: Due to the delocalization of the spare electrons it is mainly characterized as stable free radical. So, the molecules of 1,1-diphenyl-2-picrylhydrazyl does not get dimerized which is mainly occurred in other free radicals. The formation of the deep violet color denotes the delocalization of the electrons. At 517nm, it is mainly characterized by the formation of an absorption band in ethanol solution. Mixing of the solution of DPPH with respect to the substrate (responsible for donation of hydrogen atom) fades the violet color. Evaluation of the antioxidant property has been done through the generation of the free radicals which mainly scavenge by the test samples. [21]

Using FRAP Method (Ferric reducing-antioxidant power): It mainly helps to detect the antioxidant property by reducing ferric iron. Ferric ions are converted into ferrous ions with the reduction of complex TPTZ at low pH. At 593nm, change in absorption maxima can be measured by the conversion of iron (II) into iron (III) (by using diode spectrophotometer). [22]

Scavenging activity by using Hydroxyl Radical: The most potent reactive oxygen species is Hydroxyl ions which mainly react with cell membrane phospholipids made up of polyunsaturated fatty acid molecules and responsible to cell damage.[22]

By using method of TEAC (Trolox equivalent antioxidant capacity) / Method of ABTS radical decolorization: When an oxidant is mixed to the blue green chromophore the loss of the color is studied by using diode spectrophotometer. Decolorization of the ABTS mainly by the presence of the antioxidant compounds. [22]

By using Hydrogen peroxide scavenging assay: Hydrogen peroxide can initiate lipid deoxidation by its decomposition into oxygen and water which produces hydroxyl ions and damages the DNA. The hydrogen peroxide concentration is measured by using spectrophotometer at 230nm. [22]

By using nitric monoxide scavenging activity: With the help of specific nitric monoxide synthases, the arginine is metabolized into citrulline by the formation of nitric oxide by 5 electron oxidative reaction. Nitric oxide reacts with oxygen under aerobic condition to form steady products (NO_3^- or NO_2^-). The absorbance is measured at 546nm in spectrophotometer by adding Griess reagent. [22]

By using Phosphomolybdate assay: It is the quantitative method and the antioxidant capacity is represented by equivalents of ascorbic acid. The increasing order of capacity of antioxidant in various solvents is: n-hexane (TLH)< residual aqueous fraction (TLA)< *Torilis leptophylla* (TLM)< ethyl acetate (TLE)< n-butanol (TLC)< n-butanol (TLB).[1]

By using superoxide anion scavenging assay: Riboflavin –light-NBT system supports the assay for superoxide's anion scavenging activity. The absorbance is measured at 560nm by using spectrophotometer taking ascorbic acid as standard. [22]

By using reducing power: In the presence of solvent fractions the reducing power depends on the transformation of Fe^{3+} into Fe^{2+} . This conversion is detected by spectrophotometer at 700 nm with the formation of Perl's. [1]

By using oxygen radical absorbance capacity (ORAC) method: The oxygen radical absorbance capacity is new test tube analysis to check the antioxidant power. In this method beta-phycoerythrin or fluorescein is used as a target molecule. The fluorescence is shown at excitation wavelength of 485nm and at emission wavelength of 520nm taking torolox as a standard. [22]

By using DMPD method (N,N-Dimethyl-p-phenylene Diamine dihydrochloride): The N,N-Dimethyl-p-phenylene Diamine dihydrochloride is a decolorization cation radical method to measure antioxidant activity. In this method, there is the elevation of buffer solution in acetate buffer and Iron (II)chloride. The absorbance maximum is measured at 500 nm in the presence of scavengers. The % reduction of DMPD expressed the activity of antioxidant. [22]

By using conjugated Diene assay/beta-carotene Linoleic acid method: Linoleic acid is a polyunsaturated omega-6 fatty acid which is produced through oxygenated water by getting oxidized with the help of reactive oxygen species. The products formed in the reaction Initiate beta-carotene oxidation and Decolorize it. The antioxidant activity is measured at 434 nm. [22]

By using chelating activity of metal: Ferrozine form chelates with iron (II)complex of reddish color. In the existence of other sequestering agents, the reaction is restricted which results in the depletion of reddish color of the compound formed. The reduction of the color is measured at absorbance of 562nm. The positive control used in this method is EDTA or citric acid. [21]

By using cupric ion reducing antioxidant capacity (CUPRAC) method: The chromogenic oxidizing reagent that is bis(neocuproine) Cu^{2+} chloride reaction with polyphenols liberate protons in the buffer solution of concentrated ammonium acetate. The reactive groups of polyphenols are oxidized into quinoline and Cu^{2+} -Neocuproine then reduced into highly colored chelate of Cu^{+} -Neocuproine. The maximum absorption is shown at 450nm. [22]

By using Thiobarbituric acid (TBA) method: In this method the concentration of sample used is 0.025% w/v . 21% trichloroacetic acid and 0.7% of Thiobarbituric acid was added in 2ml of solution. In the boiling water bath, the mixture was placed for 15min. After cooling centrifuge, the mixture at 3500rpm for 15mintues. The maximum absorbance was measured at 552nm. [22]

V. APPLICATIONS

Oranges shows more antioxidant property, are rich in Phenolic compounds, Vitamin-C, Flavonoids, and pectin. Hesperidin, Narutotum, naringin and erythroprin are the important Flavonoids. One orange can fulfil the 114% Requirement of the vitamin-C in our daily routine. Vitamin-C is known to be an antioxidant which is water soluble and prevents the formation of free ions in the Human body. Citrus fruit shows anti-obesity effects by elevating the beta-oxidation in adipose tissue [23].

Its also shows a anticarcinogenic property i.e. the constituents of orange, limonene reduces various cancers risk of mouth, lungs, skin, Colon, Stomach and breast. Hesperidin and its analogue also have some Anti cancerous activity which is shown in various in-vivo studies [24-25]. Anticarcinogenic property depends on the ability of molecules to modulate the detoxifying hepatic enzyme activity and antioxidant property of those molecules. The polyethoxylated flavones shown antiproliferative action against cancer cell. In orange, Beta-cryptoxanthin is present in higher amount. It also lowers the risk of developing lung cancer [3].

Citrus fruit offers protection against cardiovascular diseases [4]. There are various carotenoids, Flavonoids and Vitamin-C which acts as cardioprotective. Limonene present in orange peel have cholesterol lowering effect. Poly methoxylated Flavones (PMF) present in citrus fruit peel lowers the cholesterol more efficient, without showing any allergic reactions. The most PMFs present in citrus fruit peel are tangeretin and nobiletin. These PMFs inhibits the synthesis of cholesterol and triglycerides in the

liver so act as statin drugs. The orange peel is used on the acne. It also used during Pyrexia fever. Skin diseases can be cured by preparing poultice of orange peel. It reduces the allergic reactions by suppressing the pathway signals [26]. It is also good anti-microbial properties by inhibiting the pathogenic and spoiling microorganisms [27].

Bioflavonoids present in orange fruit is have antidiabetic property. These orange peels place an antidiabetic role by the regulation of regulatory enzymes of glucose in mice. The bioflavonoids present elevates the activity of phosphoenolpyruvate and phosphatase in glycolysis. The chronic treatment of diabetic rats with naringenin prevent the functional changes in vascular reactivity in diabetic rats in vivo [28].

Antiperoxidation can be mediated the potential of controlling glucose level in orange peel by the inhibition of alpha-amylase enzyme activity which helps mainly in the conversion of complex carbohydrates into glucose. Orange peel possess larvicidal property due to presence of saponins .. The citrus peel is mainly used to extract orange oil which is further used as tranquilizer by aromatherapists. The sweet orange oil is also used as a anxiolytic agent [29].

Citrus flavonoids play a significant role in inhibiting the progress of hyperglycemia, somewhat by binding to starch, enhancing hepatic glycolysis and glycogen concentration, and diminishing hepatic gluconeogenesis [30]. Hesperidin and naringin both significantly reduced the blood glucose level [31]. Intravenous inoculation of diosmin lessened hyperglycemia caused by alloxan in rats [32].

VI. HELPFUL HINTS

Figures and Tables

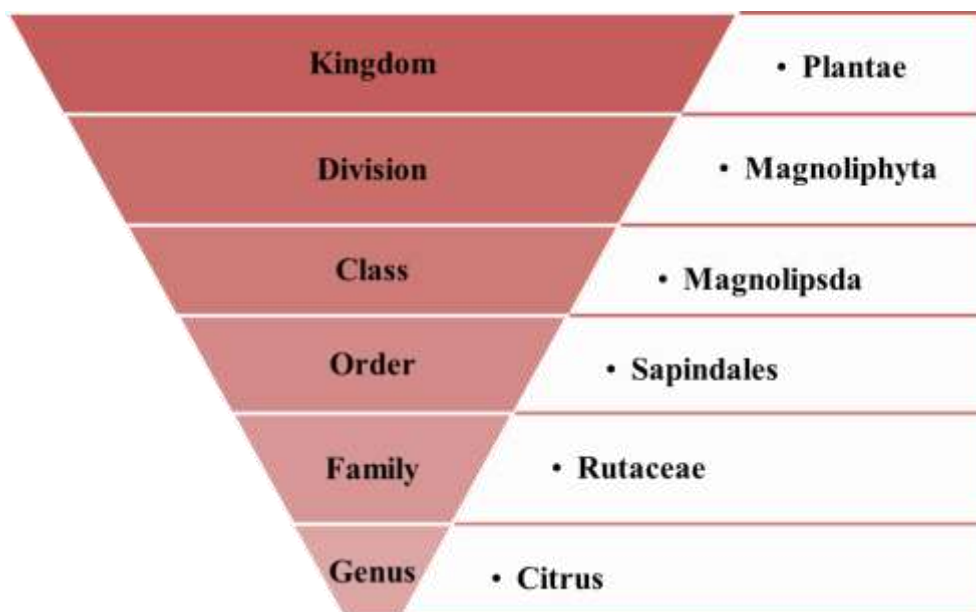


Fig-1: Scientific classification of Orange Fruits.

Table-1: Some species of Citrus with common name [3]

Scientific Name	Common Name
<i>Citrus limetta</i>	Mosambi
<i>Citrus reticulata</i>	Mandarin orange
<i>Citrus aurantium</i>	Bitter orange
<i>Citrus sinensis</i>	Malta
<i>Citrus bergamia Risso</i>	Bergamot orange
<i>Citrus trifoliata</i>	Trifoliolate orange
<i>Citrus sinensis osbeck</i>	Sathgudi

Table-2: Phytochemicals of some Citrus species [3].

Compounds	Phytoconstituents
<i>Flavone Glycosides</i>	Neo Hesperindine, Naringin, Hesperidine, Narirutin
<i>Pigment</i>	Anthocyanin, Beta-cryptoxanthin, Cryptoxanthin, Zeaxanthin
<i>PMFs</i>	Nobiletin and Tangeretin
<i>Flavonoids</i>	Noradrenaline, Citracridone and Citbrasine.

Table-3: The experimental design parameters for solvent extraction [7].

Factor	1	2
Name	Period of Time	Temperature coefficient
Units	Minutes	°C
Type	Numerical	Numerical
Low actual	64.5	39.6
High actual	177.9	69.5
Low coded	-2	-1
High coded	1	-1
Mean value	121.1	55.5
Std. Dev.	43.6	15.77

VII. CONCLUSION

Citrus plants are understood to have biological actions beneficial to human health. In expanding, ethnopharmacological purposes of plants are a good accessory for their bioactivity and the detection of active composites. Citrus plants are readily accessible and show their effect in the treatment of different diseases, so more research attention is needed on the safety and potency of this genus which will enhance its use in therapeutic purpose to some areas. Entire, the present review has concluded the current status that the Orange peel which mostly regarded as a waste material used in multitargeted-pharmacological strategy which suggest their role in the prevention of various diseases like loss of appetite, dyspeptic complaints. The phytochemical analysis indicates the presence of various kinds of phytochemicals present in the orange peel which is used in fruit juice processing industry to minimize the waste produced by orange peel. The bioactive phytochemicals present in the peel produces some physiological action on the human body. The alkaloids and glycosides present in fruit peel also possess some anti cancerous activity and can be used as drug supplement. The recycling of fruit peel utilizes it into yielding of new products and meet the requirements of essential products in human, animals and Pharmaceutical industry. The antioxidant property of the orange peel can be proved as a better substitute as compared to synthetic antioxidant to increase the shelf life of food preventing the peroxide formation. The dried orange peels have been used for treatment of psychosis and nervous system disease. Orange peel along with other natural products in a capsule form used to sooth the liver, nourish the stomach, remove stasis, stop pain and cure various gastric diseases. Oral liquid containing orange peel among other things used for the treatment of neurasthenia, chronic bronchitis, asthma, coronary heart disease, hepatitis and immune dysfunction. All the studies evaluating the antioxidant and anti-cancerous properties of orange peel which prevent to cure various type of cancer like colon cancer, breast cancer.

VIII. CONFLICT OF INTEREST

The authors report no conflict of interest.

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