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Exploration of bio-active components in *Citrus reticulata* peel powder

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Abstract
Nagpur mandarin is one of the best mandarins in the world. In India Citrus is the third largest fruit industry after Banana and Mango. Citrus fruits are commonly used for juice production, where tons of pulp peel and rags are thrown solid waste during the extraction process. The fruit waste generation not only results in financial losses but also adds to the expense of waste management and disposal. Orange peel, which is the primary waste fraction in the production of orange juice, contains are fiber, vitamin C, folate, vitamin B6, calcium and other essential nutrients. The skin of the oranges contains a good amount of polyphenols that protect against several diseases. Due to their low cost and high availability in the world, *C. reticulata* peels and their phytochemical compounds could serve as a cheap and yet nutritional dietary supplement or even as a catalyst in the synthesis of green nanoparticles. This study aims to assess the photo chemical compounds of *C. reticulate* peel powder extract. TPC was found to be higher in ethanolic extract (190 mg/g) than in aqueous extract (132 mg/g) according to the phytochemical screening results. DPPH radical scavenging activity was highest in ethanol extract (73.64%) and aqueous extract (52.18%). In comparison to aqueous extract, ethanol extract exhibited the greatest concentration of phenolic compounds in the HPLC profile. However, there is a need exploit further studies on plant origin of *C. reticulata* peel powder is still needed.

Keywords: *Citrus reticulata*, phytochemicals, extract

1. Introduction
Nagpur orange is a variety of mandarin orange (*Citrus reticulata*) grown in Nagpur, Maharashtra, India. The fruit has a pockmarked exterior and sweet and juicy pulp. Nagpur oranges blossom during the monsoon season and are ready to be harvested. The orange crop grows twice a year. The fruit available from September to December is Ambiya which has a slightly sour taste. It is followed by the sweeter Mrig crop in January. Normally, farmers go for either of the two varieties. In India, in terms of area under cultivation, Citrus is the third largest fruit industry after Banana and Mango. Nagpur mandarin is one of the best mandarins in the world. Production of this fruit crop in central and western part of India is increasing every year. Maharashtra is the country's largest producer and exporter of oranges. The area under orange cultivation in the state is about 1.21 lakh hectares and the total production exceeds 7 lakh tons annually, through two seasons Ambia and Mruga (decitrus.com).

Citrus fruits are commonly used for juice production, where 2 million tons of pulp peel and rags are thrown solid waste during the extraction process. Wastes of processing fruits are of two types; solid waste (peel, skin, seeds, stones etc.) and liquid waste (juice and washed water). In horticulture, some fruit have high discarded portion like mango 30-50%, pineapple 40-50%, orange 30-50% and banana 20%. Large amounts of fruit waste from the fruit processing sector are typically disposed of in landfills or rivers, posing environmental risks. As a result, recycling and producing livestock feed resources, as well as extracting or developing value-added products are required (Wadhwa and Bakshi, 2013) [17]

The fruit waste generation not only results in financial losses but also adds to the expense of waste management and disposal. In fact, it is commonly believed that the use of efficient technology and cautious handling during various operations can result in a loss reduction of more than 50 per cent. In general, food processing wastes are either not used at all or have been largely used as animal feed, fertilizer, and in the preparation of byproducts to a limited extent. Many waste products from the fruit processing industry, such as cuttings and shreds, can be used as animal feed. Fruit wastes can also be utilized to extract starch, pectin, natural colorants, fat, and essential oils, among other things (Helkar *et al.*, 2016) [4].

Orange peel, which is the primary waste fraction in the production of orange juice, contains are fiber, vitamin C, folate, vitamin B6, calcium and other essential nutrients. The skin of the oranges contains a good amount of polyphenols that protect against several diseases (www.sciencedirect.com). Orange peel is beneficial for healthy skin, as they possess anti-microbial, anti-inflammatory and anti-fungal and anti-oxidant properties with active phytochemicals such as flavonoids, vitamins, Coumarin, and terpenoids, carotenoids, Saponin, Carotenoids, Saponin, lignin and plant sterols. The products developed from sweet orange have an important and physiological role because of its commercial value in pharmaceutical, food industries and folk medicine in the entire world. Due to their low cost and high availability in the world, C. reticulate peels and their phytochemical compounds could serve as a cheap and yet nutritional dietary supplement or even as a catalyst in the synthesis of green nanoparticles. This study aims to examine antioxidant capabilities of *C. reticulata* peel extracts and the correlation to their phytochemical content.

2. Materials and Methods

2.1 Selection and preparation process of *Citrus reticulata* peel powder

For the study Orange fruits of the Nagpur variety were collected from the local market of Dharna. Collected fruits were cleaned thoroughly using distilled water the peel and their edible portions were separated. The peels were oven-dried at 35°C for 48 h, and ground to a fine powder with a laboratory grinder, sieved with ASTM standard mesh of 600 microns of 8 number size and stored in an air container for further experimentation (Mahmoud *et al*, 2016) [5]. The details of the extraction yield were represented in Table 1.

Table 1: Yield of the *Citrus reticulata* peel extract

Weight of fruits	Wet weight (gm)	Dry weight (gm)	Yield of powder after sieving (gm)
1 kg	135.4	81.4	42.4

2.2 Analysis of bio-active components of *C. reticulata* peel powder

Phytochemical is mainly concerned with enormous varieties of secondary plant metabolites which are biosynthesized by plants. The plant kingdom respected a treasure trove of structurally diverse bioactive molecules. The beneficial physiological and therapeutic effects of plant materials typically result from the combinations of these secondary products present in the plant (Shilpa *et al.*, 2009) [13].

2.2.1 Extraction procedure for testing bioactive components

Extraction of phenolic compounds was done according to the method described by Vastrad *et al*., (2015) [16] with little modification. In brief, 0.5 g of sample was extracted with ethanol/distilled water (2 x 25 mL) with agitation for 3h each time, the supernatants were obtained by centrifugation at 12,000 rpm for 10 min at 4 ˚C and the sample extract was used for further analysis.

2.2.1.1 Total phenolic content (TPC)

TPC in the *C. reticulata* peel extracts were determined using the Folin-Ciocalteu assay method (Singleton and Rossi 1965) [11]. The sample extracts were diluted to appropriate volumes and were mixed with 2 ml of 10% sodium bicarbonate solution. Incubated at room temperature for 3 min, 100 µl of Folin-Ciocalteu reagent was added to the mixture. The resulting solution was incubated for 90 min at room temperature under dark; the absorbance was measured at 765 nm using the UV-Vis Spectrophotometer. The TPC was expressed as Gallic acid equivalent (GAE) in milligrams per gram.

2.2.1.2 DPPH assay

It was determined by using the procedure (Manne *et al.* 2021) [7]. A freshly prepared DPPH solution in 0.5 ml ethanol were added to 3 ml of diluted each orange peel powder extract to start the antioxidant reaction. The decrease in absorbance was measured at 517 nm. The absorbance is correlated with the scavenging action of the test compound. The radical scavenging activities were expressed as percentage of inhibition and calculated according to the following formula equation:

DPPH radical scavenging activity (%) = [Abs control − Abs sample/Abs control} x100

Where, Abs control is the absorbance of sample at t = 0 min
Abs sample is the absorbance of sample at t = 30 min

2.2.1.3 HPLC analysis

Extraction of phenolic compounds was done according to the method of (Goudar and Sathisha, 2016) [3] with little modification. In brief, 0.5 g of sample was extract with ethanol & distilled water (2 x 25 mL) with agitation for 3h each time, the supernatants were obtained by centrifugation at 12,000 rpm for 10 min at 4˚C and the volume of extract was reduced using a rotary evaporator. The concentrated supernatants were hydrolyzed with 4 M NaOH (20 mL) for 2 h under nitrogen; pH was set to 2.00 using HCl. The separation was carried out using ethyl acetate (50 mL x 2) and then the fractions were pooled by treating with sodium Sulphates and dried. The residue obtained is reconstituted with methanol (5mL) and filtered through 0.45 µm, used for HPLC analysis.

3. Results and Discussion

3.1 Quantitative screening of Phenolics

Phenols, sometimes called Phenolics, are a class of chemical compounds consisting of one or more hydroxyl groups (-OH) bonded directly to an aromatic hydrocarbon group. The simplest is phenol, C_6H_5OH. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Phenolic compounds are referred to as phytochemicals found in a large number of foods and beverages. The relative high diversity of these molecules produced by plants must be taken into account when methods of preparation are employed to obtain industrial or homemade products. Their antioxidant capacities are related to these hydroxyl groups and phenolic rings. Despite the antioxidant activity, they have many other beneficial effects on human health (Minatel *et al.*, 2017) [8]. Different procedures are used to detect the presence of total phenolics, spectrophotometric and chromatographic techniques are utilized to identify and quantify individual phenolic.

3.1.1 Total Phenolic Content (TPC) of *C. reticulata* peel powder

Phenolic compounds are widely distributed in the plant kingdom. These compounds serve as important antioxidants because of their ability to donate a hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules (Cavalier *et al*. 1992). The total phenolic content (TPC) values were summarized in Table 2 based on the linear equation obtained from the Gallic acid standard calibration curve. Thus, TPC values were expressed as Gallic acid equivalents (mg GAE/100 g samples). It is observed that ethanol extract is characterized by a higher total phenolic content (190 mg/g) compared to aqueous extract (132 mg/g).

Table 2: TPC and DPPH radical scavenging activity of C. *reticulata* peel powder

S. No	Extraction Solvents	TPC (GAE *mg/g)	DPPH (%)
1	Ethanol	190±2.94	73.64±1.69
2	Aqueous	132±2.85	52.18±3.04

Data are mean ± standard deviation of duplicate determinations
GAE * Gallic acid equivalent

The solubility of phenolic compounds varies greatly on (hydrophilic, lipophillic, amphiphillic). As a result, their extractability varies depending on the type of extracting solvent used. Polyphenols are better solubilized and extracted in high polar solvents in most circumstances. The enzyme polyphenol oxidase degrades polyphenols in water extracts but is inactive in ethanol, is a more appropriate elucidation for the decrease in activity of aqueous extract Tiwari *et al*. (2011) [15].

3.1.2 DPPH radical scavenging activity of *C. reticulate* peel powder

The radical scavenging property of phenolic compounds is referred to their hydrogen donation properties, and their resonance stable structure, which oxygen can donate with it's per electron. Table 2 shows that ethanol extract has the highest free radical scavenging potential of 73.64 per cent than aqueous extract 52.18 per cent. The increase in radical scavenging activity may be attributed to the antioxidant capacities of plant extracts which are strongly dependent on the extract composition, conditions and mechanism of the tests used by (Liew *et al.*, 2018) [10]. The antioxidant activity of phenolic compounds is ascribed to the capacity of scavenging free radicals, donating hydrogen atoms, electrons, or chelate metal cations. Molecular structures, particularly the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings, confer to phenolic compounds. The extraction yield and antioxidant activity not only depend on the extraction method but also on the solvent used which greatly affected the properties. Ethanol has been known as a good solvent for polyphenol extraction which showed raise in DPPH radical scavenging activity compared to aqueous extraction (Spigno *et al.*, 2007) [12].

3.1.3 High performance liquid chromatography of *C. reticulate* peel powder

A high performance liquid chromatography (HPLC) method is used to identify and quantify phenolic acids in the orange peel powder extract. A total of seven phenolic compounds, were identified and quantified as shown in Table 3. Gallic acid (10.95) had the greatest concentration of all the phenolic components in the ethanol extract, followed by Catechin (3.74), 3, 4- Dihydroxybenzoic (2.47), quercetin (0.85), naringenin (0.52), rutin (0.36) and syringe acid (0.2),

Similarly in aqueous extract also Gallic acid has showed highest concentration with (5.52), followed by 3, 4-Dihydroxybenzoic (1.70), Catechin (1.36), quercetin (0.43), naringenin (0.36), rutin (0.22) and Syringe acid (0.037). It is observed that higher values are obtained in ethanol extract than aqueous. However, rich phytochemical components including Phenolics and flavonoids were found in both extracts, contributing to the antioxidant activity of *Citrus reticulata* peel extracts (Liew *et al.*, 2018) [10].

Table 3: HPLC profile of *C. reticulate* peel powder

S. No	Components	HPLC (mg/100g)	
		Ethanol extract	Aqueous extract
1	Gallic acid	10.95	5.52
2	Syringic acid	0.2	0.037
3	3,4-Dihydroxybenzoic	2.47	1.70
4	Catechin	3.74	1.36
5	Rutin	0.36	0.22
6	Naringenin	0.52	0.36
7	Quercetin	0.85	0.43

Among the phenolic compounds, gallic acid showed highest concentration in both solvents with 10.95 per cent in ethanol and 5.52 per cent in aqueous extract. Gallic acid and 3-4 dihydroxybenzoic acid are well-known natural antioxidant that is essentially a secondary polyphenol metabolite, identified in both extracts, according to the findings. Presence of syringic acid demonstrates a wide range of therapeutic uses in the prevention of chronic diseases, including anti-oxidant, anti-microbial, anti-inflammatory, anti-endotoxic, neuro-protective, and hepatoprotective properties (Srinivasulu *et al.*, 2018) [14].

Catechin, rutin, naringenin and quercetin are flavonoids contained in both extracts that have substantial antioxidant, anti-inflammatory, anti-carcinogenic and anti-cancer Properties. Phenolic chemicals, essential oils and flavonoids Are biologically substances that can from complexes with cellular proteins, inhibiting bacterial growth and disrupting bacterial cell membranes (Doan Thi *et al.*, 2020) [2].

4. Conclusion

Ethanol/water solvents present significant effect on the phenolic composition and antioxidant properties of C. *reticulate* peel extract. The solubility of different antioxidants in different extraction solvents differs. However, the determination by profiling the antioxidant type/composition is important. In addition, it is worth noting that both the extraction methods have determined with rich phytochemicals. Moreover, it is observed that increased antioxidant potential of *C. reticulata* peel extract directly correlates with an increased amount of TPC. By considering the antioxidant activity and total phenol content of extracts and HPLC profile, our study reveals that extracting *C. reticulata* peel powder with ethanol and water can increase the yield of total phenol content and hence antioxidant potential. However, there is a need exploit further studies on plant origin of *C. reticulata* peel powder is still needed.

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