Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 11[10] September2022 : 143-150
©2022 Academy for Environment and Life Sciences, India
Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
REVIEW ARTICLE **OPEN ACCESS**

Conversion of Orange Peel into Value Added Product for Sustainable Utilization of Waste – A Review

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ABSTRACT
Municipal wastes generated from urban centres either go uncollected or dumped in landfills. Around 4 metric tonnes of citrus wastes are produced from food industries and households each year. If these wastes are to be utilized for the production of substances that are useful to mankind, the amount of waste dumped each year will drastically decreased leading to reduction of pollution and diseases. The orange peel which is a primary waste in the juice manufacturing industry can be employed as animal feed or compost and can also be utilized to produce various value-added compounds like biofuels, enzymes, bioactive substances, organic acid. The major application of orange peel is to produce disinfectant solution which acts as an alternate for chemical disinfectants. The antimicrobial activity of the disinfectant obtained can be tested by adopting the agar well diffusion technique. Minimum bactericidal concentration and minimum inhibitory concentration of the disinfectant can also be determined. The goal of this review is to exploit the characteristics orange peel to yield disinfectant solutions and options available for converting into other value-added compounds.
Keywords: *Orange peel, Citrus sinensis, Disinfectant solution, Anti-microbial activity, Vitamin C*

Received 11.08.2022 Revised 20.08.2022 Accepted 25.09.2022

INTRODUCTION

With the technological growth, the usage of chemical compounds in everyday life has significantly increased. Excessive utilization of chemical compounds in daily life, like chemical pesticides and detergents, may contribute to increased pollution and potentially dangerous health conditions [31]. There has been an increasing tendency in recent years towards effective usage and quality enhancement of agro-industrial wastes [3]. Preservatives and antimicrobial drugs have been traditionally obtained from plant extracts since they are regarded viable sources of bioactive substances [45]. The structure of secondary metabolites found in plants can affect their ability to suppress the development of pathogenic microorganisms [38].

Citrus fruits have become a staple of the human diet for centuries due to their nutritional and therapeutic properties, and they are the most prominent fruit crops that are cultivated commercially on all seven continents [3]. Globally, approximately 124.73Metric Million Tonnes of citrus are produced [40]. They belong to the kingdom Plantae, family the Rutaceae, which consists of 1600 species of climbers, trees, and shrubs belonging to 150 genera [23]. It belongs to the genera Citrus. Grapefruit (*Citrus vitis*), tangerine (*Citrus reticulata*), lemon (*Citrus limonum*), lime (*Citrus aurantifulia*), and sweet orange (*Citrus sinensis*) are examples of citrus fruits [7]. Sweet orange accounts for more than 61% of global citrus fruit produced [41]. For its excellent juice and medicinal properties, it is commonly cultivated in most places having appropriate climates: subtropical, tropical, and borderline temperate/subtropical [10]. Sweet orange fruits are high in fibre, vitamin C, potassium, folic acid, pectin, and several bioactive components like carotenoids and phenolic compounds [37]. Industries processing juice mostly employ citrus fruits and since by-product waste is more than half of the fruit weight, peels, segment membranes, pith residues, and seeds accounts for 50-70% of by-product waste every year [25]. Citrus waste has a greater concentration of polyphenols when compared to consumable part of the fruit making it a major source of phenolic compounds [40]. Peels constitute the majority of this waste, accounting for 40-55% [25].

Epicarp (flavedo) and mesocarp (albedo) are the two components of orange peel. The flavedo, which is coloured is the peel's outermost layer, and the albedo, which is soft and white, is the peel's interior layer [20]. Orange peels are abundant in sugars like fructose, sucrose, and glucose, and insoluble polysaccharides like pectin and cellulose [29]. Vitamin C, vitamin B complex, minerals, flavonoids, carotenoids, limonoids, acridone alkaloids, essential oils are among the other compounds present in them [20]. They are used to yield "homemade enzyme" solutions. Additionally, it can be utilized to partially replace household cleaning agents to clean floors and dishes and to do laundry [31]. The application of citrus peel waste in bioprocesses provides other new substrates and it helps to prevents the solution of the pollution issues [3].

MANAGEMENT OF WASTE MATERIALS

One of the most important concerns facing every municipality is management of waste. The quantity of municipal solid waste produced individually every day has grown significantly. It has nearly doubled in the last ten years, to 1.2 kg from 0.64 kg, and by the year 2025, it is anticipated to reach 1.42 kg [18]. Food waste is taking up more and more area in waste treatment plants and landfills [8]. The utilization of natural resources can reduce the weight of the final dump (landfill), where 70% of waste on landfills is dominated by organic garbage [27].

Vitamin C waste management

Fruit and vegetable waste are the most common wastes produced in the kitchen [31]. The agro-food and food processing industries create a significant quantity of by-products or waste (pulps, peels, and seeds), accounting for 50% of the raw processed fruit [10]. By weight, citrus waste accounts for 5-70% of the fruit, of which 60-65% is peel, 30-35 % interior tissues, and nearly 10% being seeds [21]. The large quantity of waste produced during the manufacture of citrus juice has resulted in major environmental and economic problems. By transforming citrus juice manufacturing waste into value-added compounds, these issues may be substantially reduced [25]. Reflux distillation, stirring, shaking, ultrasonic extraction, microwave, and various other processes are used to extract industrially significant compounds from peels of citrus. Rapid and modern techniques that use less energy and solvent include ultrasound extraction, supercritical fluid extraction, subcritical water extraction and controlled pressure drop procedure [21]. The most significant value-added product recovered from processing waste of citrus juice is citrus essential oil [25]. Numerous studies have found that the essential oil from peels of orange has biological activity effects, like antifungal, anticancer, antidiabetic, antioxidant, anti-microbial, and anti-inflammatory activities and are made up of many components like alcohols, hydrocarbons, aldehydes, and esters [21, 50].

CONTENT OF ORANGE PEEL

Oranges have high levels of vitamin b1, b2, b3, b6, and b9, besides vitamin C. It also contains substantial mineral resources like iron, magnesium, calcium, phosphorous, selenium, potassium, and zinc [27]. It contains a lot of polymethoxylated flavones and flavanones which are not common in other plants [16]. Flavonoids contain various chemicals, one of which, hesperidin, is said to protect against viruses and bacteria [27]. Composition of various components of orange peel is represented in (Figure 1).

Orange peel has plenteous bioactive compounds, like flavonoids and phenolic compounds. Citrus fruits have immense flavonoid contents in the seeds and peel. Flavonoids are proven to have cardioprotective, antiproliferative, antioxidant and anti-inflammatory properties [17]. These biological activities of the orange peel can be exploited to produce various substances which in turn reduce the solid waste that is produced in the juice manufacturing industries.

USES OF ORANGE PEEL

The presence of many important components in the peel of the orange makes it an ideal component to be used in the production of many economically important substances and various other processes. Orange peel can be used directly and indirectly.

Direct uses

The easiest approach to treat the raw material is to use it in processes that utilize the entire orange peel without separating individual constituents. It is the simplest method for implementation as it requires no investment or infrastructure and it potentially raises the price of the waste material significantly [19]. Orange peel maybe directly used as feed for animals, organic fertilizer, and so on. Citrus peel, a major waste, contains limonene, pectin, and molasses and is generally dried, combined along with dried pulps, and is used as cattle feed [37]. Orange peel when fed to cattle, has been shown to significantly lower the presence of harmful bacteria like *Salmonella* and *E. coli* in the cattle's intestine which ultimately reduces the number of internal bacteria in cows which results in reduced levels of hazardous microorganisms in the meat. Orange peel can be composted and can be utilized as an organic fertilizer [31].It is also used to prevent and cure flu, colds, scurvy, and vitamin deficiencies, as well as aiding in the battle against bacterial and viral infections [44].

Indirect uses

With greater knowledge about orange peel, a biorefinery strategy may now be used to maximize the value of this substantial agricultural waste. A biorefinery is a tool that combines methods of biomass conversion and equipment to create fuels, electricity, and chemicals, primarily from discarded biomass [19].

Bioactive compounds

Orange peels are highly nutritious and can be utilized as food supplements and drugs [2]. Method of extraction and uses of various bioactive compounds recovered from orange peel is shown in (Table 1).

Enzymes

Peel of orange, pomegranate and pineapple were used to produce invertase enzyme using the fungi *Aspergillus flavus* [48]. Funguses namely *Trametes hirsute* and *Pleurotus sp.* (oyster mushroom)are used in the production of laccases enzyme using orange peel. *Aspergillus niger* is used to produce pectinase and xylanases enzyme from orange peel [40]. Pectinase finds its application in the extraction of vegetable oil, wastewater treatment, food and beverage industry [22]. Citrus peel is also used to produce α-amylases [40]. Solid-state fermentation technique is utilized in the manufacturing of cellulase enzymes from orange peel [24].

Organic acids

Orange peels may be used to make various organic acids, such as lactic acid, employing *Lactobacillus delbrueckii* bacteria. Lactic acid has cosmetic, food, chemical, and pharmaceutical applications [28]. *Aspergillus niger* is used to produce citrus acid using orange peel [42]. The extraction and fermentation technique is used to produce succinic acid from orange peel [35].

Biofuel

The rapid depletion of global petroleum reserves, rising fossil fuel prices, and the issue of global warming have prompted scientific community to seek out renewable and sustainable resources as a means of meeting energy demand without risking food security [1]. Fuel that is produced using biomass is known as biofuel. Citrus waste is a lignocellulosic biomass that is non-food biomass and belongs to feedstock of second generation of biofuels. Orange peel has more carbohydrates than other citrus fruits, which means it can produce more biofuels [14]. Microbial flora of methane production (MFMP) along with armed *Clostridium cellulovorans* degraded orange peel without any pretreatment to produce biomethane [47]. Bioethanol and biobutanol were produced using orange peel by steam explosion process using *Saccharomyces cerevisiae* and *Clostridium acetobutylicum*[14]. *Enterobacter aerogenes* and *Enterobacter cloacae* isolated from wastewater can be used to produce biohydrogen from orange peel [1]. The processes involved in production of various biofuels using orange peel are shown in (Figure 2).

Single-cell protein (SCP)

Intensive protein and food production from animal sources necessitates enormous quantities of nitrogenous fertilizers, which can result in a range of health and environmental issues. As a result, single-cell protein (SCP) technology is emerging as a potential alternative to current protein production methods [11]. SCP means total proteins or dead, dried microbial cells recovered from pure microbial cell culture and are made from a variety of microorganisms like fungi, algae and bacteria. They contain fats, nucleic acids, carbohydrates, vitamins, and minerals along with high protein content (60-82% of dry cell weight). SCPs were produced from *Saccharomyces cerevisiae* by submerged fermentation using orange peel as substrate [26]. *Rhizopus oryzae* and *Aspergillus niger* can also be used to produce SCP by utilizing orange peel as a substrate [11]. The processes involved in production of Single-cell protein (SCP) using orange peel are shown in (Figure 3).

Disinfectant solution

Lastly, the peel of orange can be used as a disinfectant solution too. With the increased impacts of chemical disinfectants on the environment and the mutation of microorganisms paving the way for the discovery of new disinfectants, the production of disinfectants from natural sources is gaining more importance. The antimicrobial and antibacterial properties of the orange peel make it an ideal to produce disinfectants.

DISINFECTION

The significance of sanitation and the usage of disinfectants has escalated in the everyday lives of people [9]. The main aim of disinfection is to prevent the infectious diseases from spreading. Antimicrobial agents applied on non-living items are known as disinfectants. It inhibits or prevents the growth of microorganisms [5]. An effective disinfectant should be non-irritating, non-corrosive, low in toxicity, affordable and have a broad antibacterial range [9]. Antimicrobials are chemicals that inhibit the proliferation of microorganisms or kill microorganisms including bacteria, protozoans, and fungus [2]. Microorganisms are becoming extremely resistant to present disinfectants as a consequence of mutations in their genetic structure and environmental changes, prompting the search for new disinfectants [5]. Lately, many scientists have been looking at microbial and plant extracts, secondary metabolites, essential oils, and newly synthesized compounds as possible antibacterial agents [4]. Orange peel extract possessed antimicrobial activity against *S. aureus*, *S. pyogens*, *A. niger*, *C. albicans, Salmonella*, and *Streptococcus*[50].

PROTOCOL

Production of disinfectant using fermentation method

This disinfectant is composed of water, orange peel, and sugar [27]. It is the fermentation process in which brown sugar is converted into alcohol by bacteria, which is then reduced to vinegar or acetic acid [31]. A pint of water, orange peel and java sugar were added to the plastic bottle containing mineral water during the manufacturing process. After that, seal the used bottled water and store it in areas with direct sunlight and adequate air circulation for three months and open it daily to remove gas so that it does not explode on the seventh, thirtieth, and ninetieth days [27].

Production of disinfectant using a solvent

Extraction

To get rid of the dust particles on the fruits, they were washed many times with clean water [7]. Peels were removed and sliced into tiny pieces [20]. The peels were dried at room temperature (32-35^{0}C) in the shade after collection for 5 days to achieve a consistent weight. 15g of each plant part was finely ground with a mortar and pestle before being powdered with an electric blender. The powder was stored in sealed containers [16].

Production

25g of peel powder was poured into different conical flasks with 100ml of methanol and is kept for 48 hours with periodic stirring. The flask's contents were evaporated to dry after filtering with sterile Whatman No.1 filter paper. Antimicrobial activity was determined using condensed extracts of peel [20].

TESTING FOR ANTIMICROBIAL ACTIVITY

To achieve complete pathogen disinfection, the antimicrobial efficiency of disinfectants must be evaluated [9]. Different microorganisms give diverse response to disinfectants produced using different solvents. The antimicrobial activity of these disinfectants can be found out using various techniques like the agar well diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) techniques.

Agar well diffusion

The antibacterial activity of orange peel extracts was detected using an agar well diffusion assay [7]. Test microorganisms that were cultured for 24 hours in nutrient broth were swabbed aseptically on sterile nutrient agar plates. Then, using a sterile cork borer, 6-8mm diameter wells were punched on nutrient agar. Peel extracts of 20-100μL were loaded into designated wells [4]. In aerobic conditions, the plates were incubated for 24 hours at 37^{0}C in a vertical position [7, 44]. Generally, the suppression of test microorganisms' growth and germination occurs as the diffusion of antimicrobial agents occurs into the agar [4]. The zone of inhibition created surrounding the wells were quantified and utilized to determine positive bioactivity [30]. The lack of zone of inhibition suggests disinfectant was unsuccessful towards the test organisms [9]. The zone of inhibition (in cm) of *Citrus sinensis* peel extracted using various solvents for different bacterial strains (Table 2).

Minimum Inhibitory Concentration (MIC) determination

The minimum inhibitory concentration (MIC) of an extract/compound/drug is described as the minimal concentration that effectively suppresses microorganism growth in 24 hours[44]. Antimicrobial susceptibility testing requires bacteria that have been recovered in pure culture and classified to the genus and species level [49]. It is crucial to standardize the bacterial cell number that is used in susceptibility testing for generating reliable and repeatable findings, and in agar dilution tests, the optimal cell number is established as 10^4cfu per spot**.** All quantitative approaches employ Mueller-Hinton (MH) medium in the form of broth (MHB) or agar (MHA) to calculate MIC values. In agar dilution technique, each concentration of antimicrobial agent is added at a temperature of 45-50^{0}C to 19mL of still liquid MHA medium and is poured on petri plates having a diameter of 9cm [15]. Inoculate the plate with bacteria and incubate for 16-20h at 37^{0}C and check for bacterial growth at various antimicrobial concentrations [49].

Minimum Bactericidal Concentration (MBC) determination
It is the minimal antimicrobial agent concentration needed to kill microbes [32]. From the plate well that showed no apparent signs of growth of microorganisms during determination of MIC, a loopful of broth was collected and streaked onto sterile nutrient agar. The test organisms which acts as a control were streaked on nutrient agar [16]. After that, the plates were incubated at 37^{0}C for 24 hours [32]. The concentration which showed no evident growth after incubation was recorded as the minimum bactericidal concentration [16].

Table 1. Method of extraction and uses of various bioactive compounds recovered from orange peel

Sl. No.	Bioactive compound	Extraction method	Uses	Source
1.	Hesperidin flavonoid	Subcritical water extraction	Has an antioxidant and antimicrobial capacity Associated with low risks of certain cancer types	[17]
2.	Limonene	Solvent extraction using cyclopentyl methyl ether (CPME), 2-methyl-tetrahydrofuran (2-MeTHF)	Used in cosmetics, pharmaceutical, and food industry Has antioxidant properties and fragrance character	[34]
3.	Methane	Fermentation/ anaerobic digestion	Used as fertilizer and to generate electricity Used as fuels in vehicles	[36]
4.	Narirutin flavonoid	Subcritical water extraction	Has antioxidant properties	[17]
5.	Pectin	Fermentation/ anaerobic digestion	Used in pharmaceutical and food industry	[13]
6.	Polyphenolic compounds (natural antioxidants)	Solid-liquid extraction	Used in production of cosmetics and paints Used in food and pharmaceutical industry	[33]

Table 2. Zone of inhibition (in cm) of *Citrus sinensis* peel extracted using various solvents for different bacterial strains

Bacteria	Methanol	Acetone	Petroleum ether	Ethanol	Ethyl acetate	Source
B. cereus	1.2	-	-	2.2	-	[20, 43]
B. subtilis	1.0	1.4	-	0.7	1.0	[6, 46]
E. coli	1.1	1.6	1.0	1.8	0.9	[2, 16, 43]
K. pneumoniae	1.4	1.4	0.9	0.8	1.1	[16, 20]
S. aureus	1.0	1.3	-	1.8	1.1	[2, 16, 43]
S. flexneri	1.3	-	-	-	-	[20]
S. typhi	-	1.5	0.7	0.8	0.9	[16]

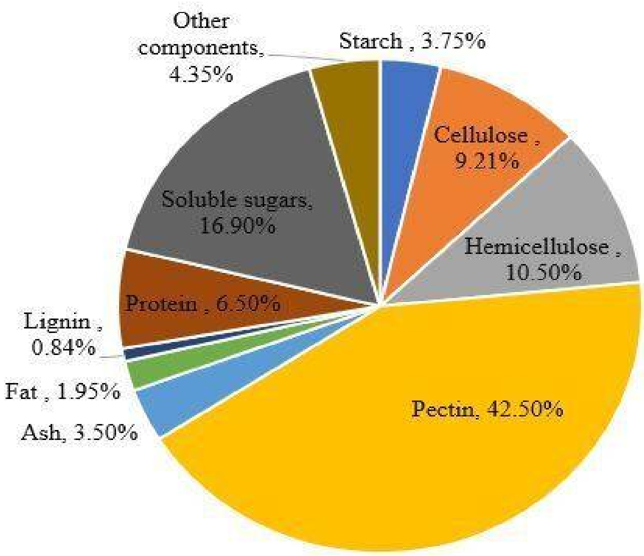

Figure 1. Composition of various components of orange peel; Source: [39]

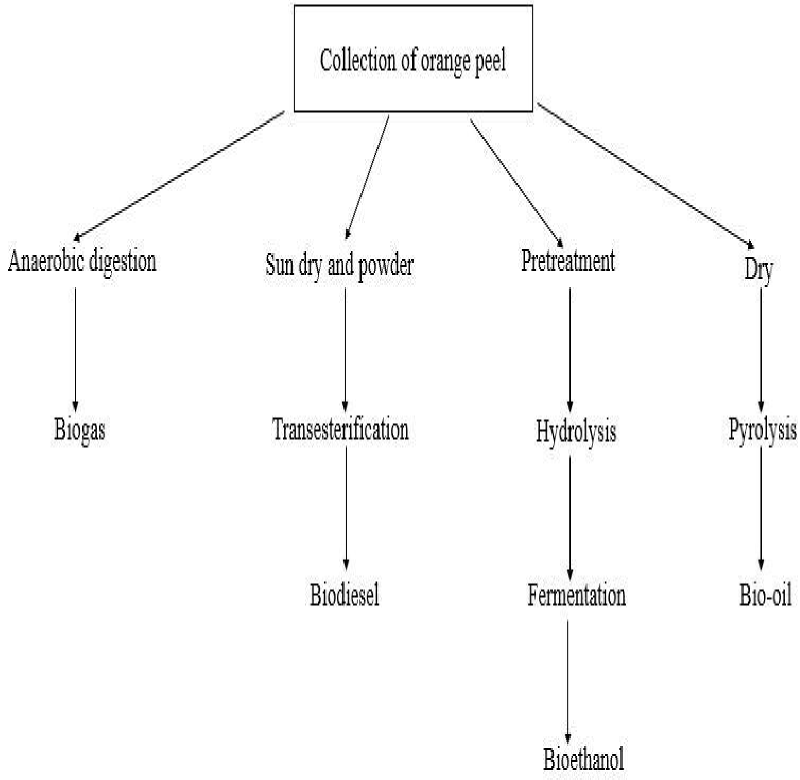

Figure 2. Flowchart showing processes involved in production of various biofuels using orange peel

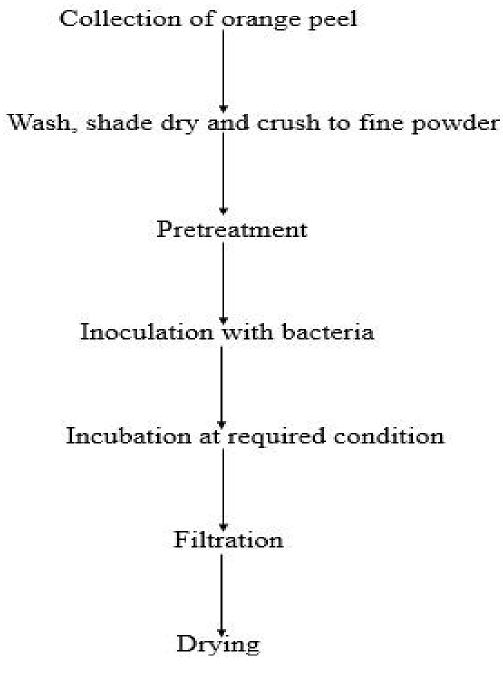

Figure 3. Flowchart showing processes involved in production of Single-cell protein (SCP) using orange peel

CONCLUSION

Considering the composition of chemical disinfectants and the increased risk of allergens, it is imperative to look for a natural alternative. In this regard, citrus peel, with its high phenolic content and antimicrobial and antiviral activity are found to be the ideal candidate. The extraction and production of disinfectant from citrus peel follows a considerably simple protocol and does not need any sophisticated instruments. Thus, with the implementation of some simple cleanliness protocols, this could be easily established into a small-scale industry. This process also enables efficient waste management in the citrus juice industry. This would enable the utilization of resources to the maximum and increase revenue.

FUTURE SCOPE OF WORK

The citrus peel acts as a major substrate for the synthesis of disinfectant solution as it contains many bioactive compounds. An ideal protocol for the extraction of disinfectant using orange peel has not yet been developed. A protocol for large-scale extraction is the need of the hour towards this. The establishment of proper concentrations of compounds to enable the broad-spectrum action of the disinfectant is also needed.

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CITATION OF THIS ARTICLE

Hinduja K, Lingaraju H G, Rakesh Sharma MS and S Kalikeri.Conversion of Orange Peel into Value Added Product for Sustainable Utilization of Waste – A Review. Bull. Env. Pharmacol. Life Sci., Vol 11 [10] September 2022: 143-150