








The beneficial effects of citrus peel waste and its extract on fish performance and health status: A review

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Abstract

Food production and processing in developing countries produce a huge amount of fruit waste by-products, which is costly and pose detrimental effects on the environment. Proteins, lipids, starch, micronutrients, bioactive compounds and dietary fibres are found in many of these fruit wastes. Among fruit wastes, citrus fruits play an important role in generating a wide range of health benefits. Citrus L. of the Rutaceae family are common fruits cultivated and consumed globally both as fresh fruits and as a juice. Citrus peel wastes (CPW) are considered the main by-products, with an average of 60% of processed fruits; hence, CPW have a promising role in the food production industry. CPW contain high concentrations of polyphenol and essential oils, which have nutritional importance and pharmaceutical usage. There is a concern on the increasing prevalence and incidence of different fish infections and a growing interest in shifting from synthetic to natural antimicrobial agents, leading to the use of citrus peel wastes for identification of novel compounds for use as fish feed additives. Although the antimicrobial properties of EOs have been reviewed extensively, the antimicrobial properties of citrus peels oil have not been extensively discussed. In fish farming, feeding strategies that employ phytochemicals as modulators of immunological and physiological responses such as growth, antioxidant activity and gene expression have received attention. In the past years, several studies have reported positive results of using citrus peel extracts as a nutritional additive in aquafeeds. Recently, these dietary functional feed additives have been evaluated and reported to increase disease resistance and improve fish growth, animal welfare and feed utilization. This review elucidates the global production, bioactive compounds, natural sources, chemical structures, physical properties, practical applications of citrus peel wastes and extracts as a desirable and sustainable route in fish nutrition.

KEYWORDS

aquafeed additive, citrus peel wastes, growth performance, health status, phytochemicals

1 | INTRODUCTION

Sweet orange (*Citrus sinensis*), tangerine or mandarin (*Citrus reticulata*), grapefruit (*Citrus vitis*), lime (*Citrus aurantiifolia*) and lemon

(*Citrus limon*) are among the most widely cultivated and consumed citrus fruits in the world. Citrus fruits have a distinct flavour, taste and fragrance and are high in vitamins and minerals, such as vitamin C, folic acid, carotenoids, potassium, pectin and fibre. Citrus fruits

also contain active ingredients such as polyphenolic compounds (e.g. phenolic acid and flavonoids) (Guimarães et al., 2009; Manthey & Grohmann, 2001). Antioxidants, anti-inflammatory and antibacterial properties are all provided by these bioactive substances. These substances can also help to alleviate the symptoms of some significant chronic diseases, such as cardiovascular disease and age-related muscle degeneration. Furthermore, several of these compounds contain antigenotoxic properties, which prevent the genetic material in cells from being damaged (López-Romero et al., 2018). As a result, they play an important physiologic function in human health and vitality (Pellegrini et al., 2003; Peterson et al., 2006).

Citrus crops are grown throughout the world in tropical and subtropical regions. They were initially grown in the warm southern slopes of the Himalayas in northern India and Myanmar. China, Brazil, India, Mexico, Spain and the United States are the top citrus-producing countries, accounting for nearly two-thirds of global production. In 2019, citrus plant production reached 157 million tons, with 60% used as fresh fruit for human consumption and the other 40% for industry (World Data Atlas, 2021).

Citrus peel wastes (CPW) are the most common by-products, accounting for 60% of all processed fruits; hence, CPW have a promising role in the food industry (Chavan et al., 2018). They have a high concentration of polyphenol and essential oils, both of which have nutritional and pharmaceutical use. Traditionally, CPW have been used as low-value feedstuffs for ruminant animals or as organic soil fertilizer. An excessively high load of CPW may compromise soil fertility or cause damaged aquatic ecosystems because of the antibacterial activities of the essential oils. CPW is generally discarded by either incineration or landfilling, causing environmental pollution and economic loss. As a result, the treatment and processing of these readily available and inexpensive wastes is a promising approach to saving alternative sources of nutrients for human food and animal feed while also keeping the environment clean (Satari & Karimi, 2018; Zema et al., 2018).

Citrus by-products have been investigated as an unconventional source of vital nutrients, such as soluble sugars, fibre, pectin, polyphenol and essential oils. Previous studies have explored the use of citrus

by-products in food, pharmaceuticals and biotechnology processes. This paper presents a review of the nutritional and economic benefits of using citrus peel wastes as a viable source of vital nutrients and high-value bioactive compounds for improvement of fish health.

2 | GLOBAL CITRUS PRODUCTION

Citrus fruits were first cultivated in Southeast Asia, then introduced and disseminated over Africa, southern Europe, the Mediterranean and the Americas during the Middle Ages (Gmitter & Hu, 1990; Webber, 1967). Citrus production has grown rapidly in more than 140 countries throughout the tropical and subtropical regions of the world because of efficient growing, harvesting and processing techniques (Baldwin, 1993; Ladaniya, 2008a).

Oranges, tangerines, mandarins and grapefruit accounted for 51.9%, 32.6%, 7.2% and 8.34% of global production respectively. Approximately 68.44% of citrus is consumed, with the remaining 21.5% used for processing. Mostly, sweet oranges (*C. sinensis*) account for over 70% of total citrus production and consumption globally, with one-third utilized for juice production and the rest marketed as whole fruit (Figure 1; FAO, 2017; USDA, 2019). Citrus production in China increased to 40 million tons in 2017, more than doubling that of Brazil, the world's second-largest citrus producer. In China, the most popular citrus fruits are tangerines, oranges and grapefruits. The two biggest citrus producers in America, particularly oranges, are Brazil and the United States. After China and Brazil, India is the third largest producer of citrus fruits, with oranges accounting for 88% of total production (Figure 2; FAO, 2019; Ladaniya, 2008b).

3 | THE STRUCTURE OF CITRUS FRUITS

The citrus fruit consists of the peel or rind (the flavedo—the outer coloured part that has either yellow or orange colour, and the albedo—the interior white spongy part), the pulp or rag, and seeds, which make up to 10%, 17%, 71% and 2% of the total fruit weight respectively.

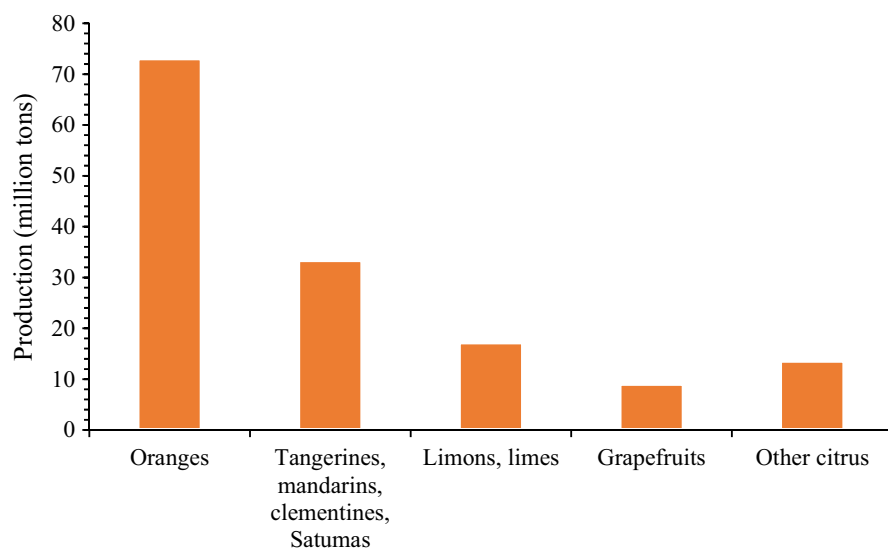


FIGURE 1 Global citrus fruit production in 2017 (FAO STAT, 2017).

Mostly, 87% of the citrus fruits is water, and the rest consists of several micronutrients (10% minerals, 5% essential oils, 3% fats, 9% proteins, 11% fibres, 16% citric acids, 21% pectin, 10% glucosides and 15% pentosans; Mahato et al., 2018; Oikeh et al., 2013). The edible components of the citrus are the pulp and juice, while the non-edible parts include the segment walls, peel, pith residues and seeds.

4 | CITRUS BY-PRODUCTS (WASTES)

Citrus by-products are the solid material that remain after juice extraction from the fruits. These wastes include the peel, pulp, rag and seeds (Figure 3). Citrus juice yield and citrus by-products chemical

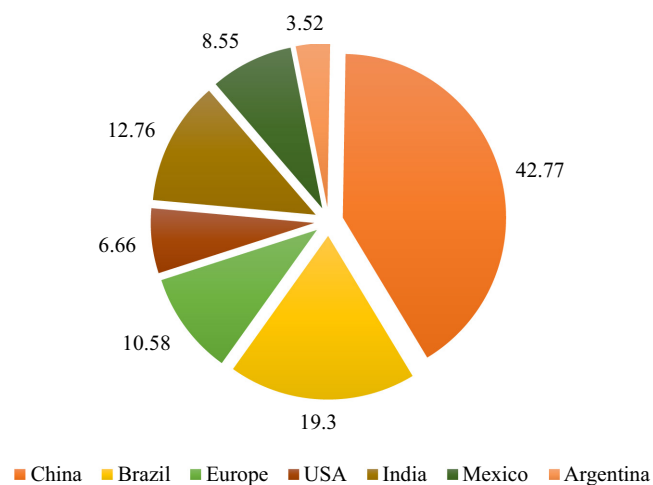


FIGURE 2 Citrus production by country in 2019 (in metric tons) (FAO, 2019).

composition depend on the juice extraction method, citrus fruit, climate, and location of cultivation, and amount of water that remained after processing (Tables 1 and 2). Citrus by-products represent 50%–70% of the total treated citrus weight. Citrus by-products contain 60% peel, 30% pith residues and 10% seeds (Bampidis & Robinson, 2006; Chavan et al., 2018; Marín et al., 2002). Also, these by-products contain high moisture (85%) and organic matter content. Moreover, they are a rich source of natural valuable compounds such as soluble sugars (glucose, fructose and sucrose), carbohydrates (cellulose, starch, pectin and dietary fibres), proteins, organic acids (citric, malic and oxalic acids), lipids (linolenic, oleic, palmitic and stearic acids), essential oils (*D*-limonene, α -terpinolene and α -pinene), carotenoids (carotene and lutein), vitamins (vitamin C and vitamin B complexes) and polyphenols (flavonoids and phenolic acids; Mahato et al., 2018).

Citrus wastes have been either directly dumped in landfills and rivers or burned, which poses an important environmental issue of soil degradation. Soil is degraded due to the destruction of soil flora by the antimicrobial properties of the essential oils from the citrus peels and oxygen depletion of water.

Citrus wastes may be used as an agricultural fertilizer or a low nutritional value animal feed for ruminants, chickens and pigs. Nevertheless, the drying process for the citrus wastes is costly and energy intensive. The low nutritional value of citrus wastes not only limits their use as an animal feed, but also are characterized with low palatability for animals, mainly attributed to the bitter flavour, foul odour and high acidity of limonene. Hence, to make the most of the use of citrus wastes, the removal of oils serves as an indispensable step in the processing. Currently, a biorefinery framework capitalizes on the value of citrus wastes, either by extracting high-value products and food items, or by recycling such renewable resources using low-cost techniques to produce valuable products (Panwar et al., 2019).

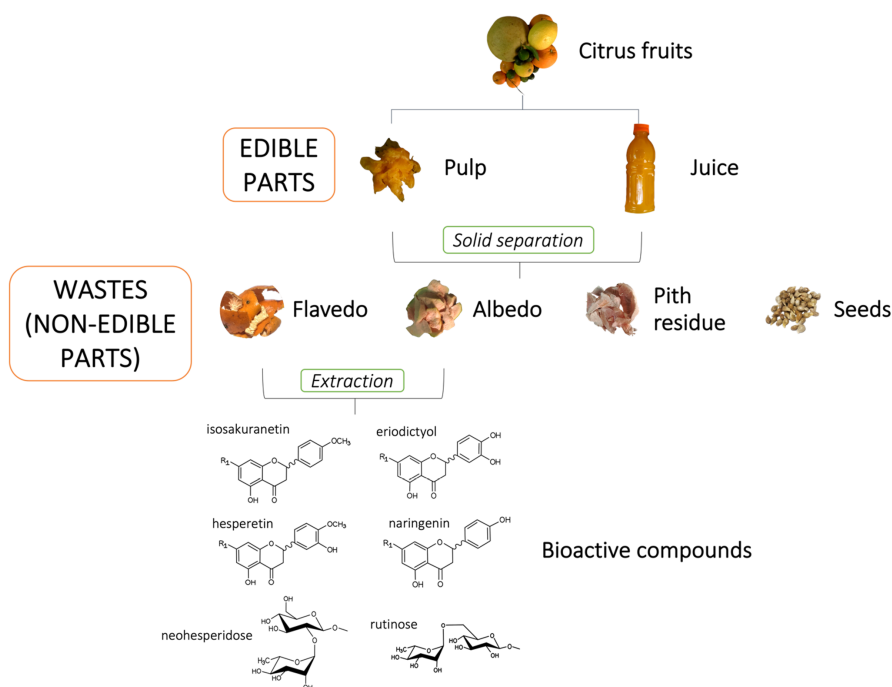


FIGURE 3 Different citrus wastes and selected extracts thereof from citrus fruits processing.

TABLE 1 Nutritive value of different citrus wastes (expressed as % dry basis)

Citrus wastes	Ash	Sugar	Fat	Protein	Pectin	Lignin	Cellulose	Hemicellulose	Sources and references
Lemon peels	2.5	6.5	1.5	7.0	13.00	7.56	23.06	8.09	Marín et al. (2007)
Lemon pulp	2.5	9.0	3.1	8.7	22.53	7.55	36.22	11.05	
Orange peels	1.7–2.6	9.6–41.4	3.9–4.0	7.9–9.1	14.4–23.02	1.0–7.52	16.2–37.08	11.04–13.08	Marín et al. (2007); Mamma et al. (2008)
Orange pulp	2.6	6.0	1.5	6.6	12.07	7.51	24.52	7.57	Marín et al. (2007).
Kinnow mandarin waste	3.2	31.6	n.d.	5.8	22.6	0.6	10.1	4.3	Oberoi et al. (2011)
Mandarin peel waste	3.0	21.6	n.d.	n.d.	14.2	8.9	20.8	17.2	Boluda-Aguilar et al. (2010)
Grapefruit Peels	3.3	n.d.	n.d.	n.d.	16.1	8.2	19.8	18.3	Boluda-Aguilar and López-Gómez (2013)

Abbreviation: n.d., not detected.

5 | BIOACTIVE COMPOUNDS OF CITRUS WASTES

Citrus wastes contain a wide variety of biologically active compounds, such as essential oils, pectins, carotenoids and limonoids that can be extracted and used in food, feed, pharmaceutical and cosmetic industries. There are certain limitations to extracting these valuable compounds such as the lack of an efficient and low-cost extraction technique, among others, that preserves the biological activity of the extracted compounds. The process of extraction has a significant impact on the biological activity of the extracted compounds (Panwar et al., 2019). Furthermore, the toxicity of the organic solvents used for the extraction of the lipophilic compounds, such as hexane, petroleum ether and diethyl ether, poses a health issue. The choice of the extraction technique depends on the nature of the biological compounds to be extracted and the type of citrus fruits treated. The yield of the extracted compound is also affected by the solvent used, and the operation duration, temperature, pH and the ratio of solute to solvent.

5.1 | Pectins

Pectin is a gelatinous polysaccharide with a high molecular weight that is found in the citrus cell wall and middle lamella. It binds the plant cells to provide the plant with strength and flexibility. It comprises of a linear chain of D-galacturonic acid units linked together by α -1,4 glycosidic linkages, which are partially esterified at the carboxylic acid using methanol or acetic acid to produce methyl esters. Citrus wastes contain a high proportion of pectin (20%–30%) in the overall dry weight of peels compared with those of apples by-products (10%–15%). In citrus wastes, there are two types of pectins: insoluble pectins and soluble pectins, which range in colour from white to light brown. Pectins are used in food manufacture as a thickening, emulsifying, texturizing or stabilizing agent and as a fat substitute. Pectins can be used in the processing of jams, jellies, bakery fillings, confectionary products and biodegradable films (Min et al., 2010; Satari et al., 2017).

5.2 | Phenolic substances

Citrus wastes have higher levels of polyphenols ranging from 0.67% to 19.62% on dry basis when compared with the edible portion of the fruit (M'hiri et al., 2017). As a result, they have a variety of health-related characteristics, including antioxidant, anti-inflammatory, anticancer, and antibacterial activities. The polyphenol composition varies depending on the type of citrus by-products. Gallic acid, ferulic acid, chlorogenic acid, hesperidin, rutin and naringenin are all found in lemon (*C. limon*) peels, whereas caffeic acid and eriocitrin are found in its seeds. The main flavanones found in bergamot, lemon and orange peels are neoeriocitrin, naringin and neohesperidin. On the contrary, the most abundant flavonoids in sweet orange are hesperidin and narirutin.

The most abundant polyphenols in the peels or flavedo are flavonoids. They include four groups, flavanones present in high

TABLE 2 Concentration of bioactive compounds in citrus by-products, peel, pulp and juice

Bioactive compounds	Peel	Pulp	Juice	Sources and references
Total phenolics ($\mu\text{g ml}^{-1}$)	147.6	57.7	673.9	Rapisarda et al. (1999); Goulas and Manganaris (2012)
Total flavonoids ($\mu\text{g ml}^{-1}$)	49.2	1.15	260.1	Rapisarda et al. (1999); Wang et al. (2008); Goulas and Manganaris (2012)
Antioxidant activity	656.18	456.7	46.35	Goulas and Manganaris (2012)
Total carotenoids ($\mu\text{g g DW}^{-1}$)	2.04	-	-	Wang et al. (2008)

quantities followed by flavones (luteolin, apigenin and diosmin), flavonols, isoflavones, flavanols and anthocyanins. Dry citrus peels contain 2%–3% flavanone and 0.91%–4.92% of total polyphenols. They have a protective role against Alzheimer's and Parkinson's diseases as they elicit a strong antioxidant impact and lipid peroxidation inhibition (Mulvihill & Huff, 2012; Wang et al., 2014).

The peels also contain phenolic acids, including hydroxybenzoic (gallic, vanillic and syringic acids) and hydroxycinnamic acids (caffeic, ferulic, p-coumaric and sinapic acids). They have a potent antioxidant effect as these substances act as free radical scavengers and heavy metals chelating agents. Peroxides such as reactive oxygen species, superoxide radical, hydrogen peroxide, hypochlorous acid and hydroxyl radical initiate oxidation and auto-oxidation of lipid in the food, which then cause fat rancidity and breakdown. This indeed negatively change the nutritional value and physical properties of the food (i.e. colour, flavour, odour and texture) especially those of high-fat content, such as in meat products. Also, peroxides can decrease the shelf half-life of food. They have toxic effects on human health, through the peroxidation of the phospholipids of the cell membranes found in different body cells and organs (resulting in cell ageing). This, in turn, will cause serious health problems, such as cancer, diabetes, nervous and cardiac diseases (Aruoma, 2003; Hool, 2006; Huang et al., 2001; Perry et al., 2000).

There are synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene and natural antioxidants. Synthetic antioxidants are used to restore the food nutrient quality from oxidation. It has been reported that BHA helps in the growth of cancer cells in the rat. Therefore, it is necessary to extract natural antioxidants from food by-products to resolve the adverse effects of synthetic antioxidants (Burlow, 1994; Moure et al., 2001). Orange (*C. sinensis* L. and *Citrus aurantium* L.) by-products and phenolic acids isolated from kinnow rind powder have been used as natural antioxidants (Benamrouchea & Madania, 2013; Devatkal et al., 2010). Also, 40-geranyloxyferulic and boropinic acids are found to be abundant in *C. sinensis* while kumquats possess antibacterial properties (Genovese et al., 2014).

5.3 | Carotenoids

Carotenoids are natural pigments synthesized by plants, algae, some bacteria and fungi. They are the most common type of isoprenoids

and responsible for colours found in fruits and vegetables. Citrus wastes contain two types of carotenoids: oxygenated xanthophylls such as lutein and violaxanthin, and hydrocarbon carotenoids such as carotene and lycopene. There are approximately 115 carotenoids in citrus fruits, which give them their appealing colours (Namitha & Negi, 2010). Carotenoids are characterized by their high antioxidant and provitamin A functions. Also, they may be used as colouring agents in food and beverage preparation. Carotenoid extraction is difficult due to the presence of rigid cell walls in citrus wastes. Hence, before the extraction procedure, citrus wastes must be cooked, dried, frozen and treated with enzymes (Zou et al., 2016).

5.4 | Limonoids

Citrus wastes contain limonoids, which include limonoid aglycones (e.g. limonin and nomilin) and limonoid glucosides (e.g. limonin glucoside and nomilic acid glucoside). The limonoid aglycones, which are present predominantly in citrus seed or peels, are insoluble in water and induce the bitter flavour of citrus fruits and juices. Limonoid glucosyltransferase transforms limonoids into tasteless limonoids during maturation, which decreases the bitterness of citrus fruits (Qin et al., 2018).

Citrus juice and pulp include limonoid glucosides, which are water soluble. Citrus liquids develop a delayed bitterness over time, lowering the quality and acceptability of the fruit juice. To increase the quality and acceptability of citrus juice, physical, chemical and microbiological approaches have been investigated (Bi et al., 2019; Breksa et al., 2009; Manners, 2007).

Limonoids have antibacterial, antioxidant, anticancer and cholesterol-lowering properties. Compared with any other by-product of citrus, citrus molasses contains the highest concentration of limonoids. Furthermore, the viscous liquid shape of molasses makes it the most promising source for limonoids recovery (Hasegawa & Miyake, 1996; Russo et al., 2016).

5.5 | Essential oils

At 0.6% and 3.8% dry basis, citrus essential oils (CEO) are aromatic liquids comprising mixtures of 20–60 different types of volatile compounds found in the oil sacs situated in the depths of citrus peels

(M'hiri et al., 2016). CEO are lipophilic compounds with low water solubility and are fluid at room temperature. CEO comprise primarily of isoprenoids and terpene carbohydrate derivatives (i.e. monoterpenes and sesquiterpenes), alkensenes, acids, alcohols, ketones, aldehydes, esters, phenols and nitrogen compounds. Limonene constitutes up to 90%–98% of CEO. These compounds are used in the dairy, medical, medicinal and cosmetic industries as flavouring agents. CEO have antibacterial, antioxidant and biocidal effects against fungi, viruses, protozoa, insects and plants (Negro et al., 2016; Schieber et al., 2001). Essential oils derived from citrus peel wastes have been examined for their antibacterial and antifungal properties (Afzalani Zein et al., 2015; Callaway et al., 2011a, 2011b; Nam et al., 2006).

5.6 | Dietary fibres

Citrus fruits are high in soluble dietary fibres (SDF) and insoluble dietary fibres (IDF) that are mostly concentrated in the peel. Gums, pectins, glucans and a variety of biological and synthetic polysaccharides are examples of SDF, whereas cellulose, hemicellulose and lignin are examples of IDF. Furthermore, citrus waste fibres have a high nutritional value, a well-balanced composition and are high in SDF and bioactive elements including flavonoids and carotenoids.

Gorinstein et al. (2001) found that lemon peels and peeled fruits have the highest antioxidant activity when compared with oranges and grapefruits. For lemon and orange peels, the fibre content is 14g/100g in dry matter (DM) and 57g/100g DM respectively (Fishman et al., 1999). The cellulose and hemicellulose contents of citrus peel can range from 50% to 60%. Pectins, lignins, celluloses and hemicellulose of lemon and orange peels range are as follows: 13.00–23.03g/100g DM, 7.52–7.56g/100g DM, 23.06–37.08g/100g DM and 8.09–11.04g/100g DM respectively (Marín et al., 2007). Citrus fibres, which contain polysaccharides and polyphenol-like components, have antioxidant qualities that inhibit lipid oxidation in meat products, maintain oxidative stability and extend the shelf life of meats. Orange juice fibre has also been utilized as a fat substitute in ice cream and the meat industry due to its ability to retain water and oil (de Moraes Crizel et al., 2013; Fernández-Ginés et al., 2003; Mehta et al., 2015; Sáyo-Ayerdi et al., 2009).

6 | EFFECTS OF SUPPLEMENTAL CITRUS WASTE PRODUCTS AND EXTRACTS ON FISH GROWTH

Several studies have recently been carried out to assess the impact of citrus waste products (CWP) on the growth performance of several fish species (Table 3). Supplementing diets with lemon peel (*Citrus aurantiifolia*) or bergamot (*C. bergamia*) peel oil enhanced the growth performance of Nile tilapia (*Oreochromis niloticus*) and European sea bass (*Dicentrarchus labrax*) respectively (Acar et al., 2019; Toutou et al., 2018). The antibacterial and antioxidant characteristics of dietary lemon peel may explain the better growth

performance and food conversion efficiency of fish given various doses of the peel (Milos et al., 2000). Additionally, lemon peel might be used to supplement fish feed with a low-cost supply of vitamin C. Furthermore, bergamot essential oils are also a good source of bioavailable substances, such as, limonene, linalool, linalyl acetate and β -pinene (Costa et al., 2010). In the same context, the addition of grapefruit (*Citrus paradise*) peel extract to Caspian white fish (*Rutilus frisii kutum*) diets at different levels (0, 6.25, 12.5 and 25mgkg⁻¹ diet) increased fish growth performance (Samavat et al., 2019). This is most likely linked to the increased nutritional digestion and absorption capabilities of the fish as a result of the healthy gut microbiota (MacLennan et al., 2002). Furthermore, it is well known that the inclusion of plant products or plant-based bioactive compounds to fish feeds improves the digestive system of the fish. In particular, supplementations improved the absorption surface in the digestive tract, leading to greater nutritional absorption and growth (Demirci et al., 2021). It has been noted that the digestive system of tilapia fed feeds containing D-limonene-rich lemon and orange essential oils was strengthened, resulting in increased growth performance (Mohamed et al., 2021). Gültepe (2020) found that adding D-limonene obtained from sweet orange (*C. sinensis*) peel to rainbow trout diets at concentrations of 0.5, 1 and 3mlkg⁻¹ improved fish growth performance. Moreover, the inclusion of essential oils extracted from orange (*C. aurantium*) peel at 0.25, 0.5, 1.0 and 2.0mlkg⁻¹ in silver catfish (*Rhamdia quelen*) diets improved the production performance and antioxidant capacity (Lopes et al., 2019). Also, there was a significant improvement in growth of Nile tilapia fed diets supplemented with essential oils extract from sweet orange (*C. sinensis*) and/or lemon (*C. limon*) peels (Mohamed et al., 2021). Moreover, increased dietary levels of lemon essential oil loaded in chitosan nanoparticles increased growth indices and feed utilization of rainbow trout, *Oncorhynchus mykiss* (Gheytsi et al., 2021). The addition of 0.25% of the essential oil (Neroli oil) derived from bitter orange (*C. aurantium*) to the diets improved the growth of common carp (*Cyprinus carpio*) (Acar et al., 2021). These may be attributed to the quick absorption of essential oils in the gastrointestinal tract of fish (Gültepe, 2018). There was also an improvement in the growth performance and feed utilization of Siberian sturgeon (*Acipenser baerii*) fed lemon verbena (LV) (*Aloysia citrodora*) extract at the rate of 10–20mgkg⁻¹ (Adel et al., 2021). Dietary LV is high in phytochemicals and aromatic tastes, which improve diet palatability and feed ingestion and utilization (Bahramsoltani et al., 2018). Also, the LV protects the intestinal epithelial layer resulting in increased mucus secretion and improved passage of the digested nutrients through villi until they reach the circulation; these lead to the improvement of the growth performance (Zhu, 2020). In addition, ingested nutrients contribute to many metabolic roles necessary for important processes, resulting in improved growth performance (Dawood et al., 2021). Furthermore, the polyphenols in LV have an antibacterial impact, allowing good bacteria to breakdown nutrients via released digestive enzymes (Furné et al., 2005). On the contrary, the study of Abdel Rahman et al. (2019) found non-significant reduction in growth of Nile tilapia (*O. niloticus*) fed 1% and 2% dried lemon peel *C. limon*

TABLE 3 Biological activities of citrus wastes and essential oils thereof when used for diets of different fish species

Citrus species and products	Fish species	Dosage level	Effects and benefits	Sources and references
Dehydrated lemon peel powder	Gilthead seabream (<i>Sparus aurata</i> L.)	1.5% and 3%	↑ Growth ↑ Expression of some immune-related genes: antioxidant gene <i>nkefa</i> , pro-inflammatory gene <i>il1b</i> , antibody <i>igth</i> and macrophage stimulator gene <i>csfr1</i>	Beltrán et al. (2017)
Dehydrated lemon peel powder	Nile tilapia (<i>Oreochromis niloticus</i>)	1% and 2%	↑ Serum glucose ↑ catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) ↑ Serum lysozyme activity (LYZ), myeloperoxidase activity (MPA) ↔ Serum cortisol (COR) ↓ Growth	Abdel Rahman et al. (2019)
Dehydrated lemon peel powder	African catfish (<i>Clarias gariepinus</i>)	1% and 2%	↑ CAT, SOD, GSH ↑ Serum LYZ, MPA ↔ Growth ↔ Serum glucose (GLU) ↔ Serum COR	Abdel Rahman et al. (2019)
Lemon (<i>C. aurantiifolia</i>) peel	Nile tilapia (<i>O. niloticus</i>)	0.5%, 1%, and 2%	↑ Growth ↓ Serum GLU	Toutou et al. (2018)
Lemon (<i>C. aurantiifolia</i>) peel	Thin-lip mullet (<i>Liza ramada</i>)	0.5%, 1%, and 2%	↑ Growth ↓ Serum GLU	Toutou et al. (2018)
Bergamot (<i>C. bergamia</i>) peel oil	Nile tilapia (<i>O. niloticus</i>)	0.5%, 1.0%, and 2.0%	↑ Growth ↑ Red blood cells (RBC), hematocrit (HCT), haemoglobin (Hb) ↑ Serum total protein (TPRO) ↓ Serum glucose ↓ Serum cholesterol (CHO), triglyceride (TRI) ↓ Glumatic oxaloacetic transaminase (GOT), glumatic pyruvic transaminase (GPT), alkaline phosphatase (ALP) and lactate dehydrogenase	Kesbiç et al. (2020)
<i>C. aurantium</i> essential oil	Silver catfish (<i>Rhamdia quelen</i>)	0.25, 0.5, 1.0, and 2.0 ml kg ⁻¹ diet	↑ Growth ↑ GLU, lactate (LAC), and protein levels in liver and muscle ↑ SOD activity in liver ↑ Growth hormone ↔ Prolactin, somatolactin ↓ Thiobarbituric acid reactive substance and lipid hydroperoxides ↓ Glutathione S-transferase activity in liver	Lopes et al. (2019)
Dehydrated lemon peel	Gilthead seabream (<i>S. aurata</i> L.)	1.5% and 3%	↑ Growth ↑ Serum GLU, urea, total antioxidant status, alanine transaminase (ALT), LAC ↑ Oxidative stress and apoptosis: Hsp 70, Hsp 25 and Phospho-c-jun in skin mucus ↑ Serum TPRO ↑ Serum SOD, CAT, GPX and LYZ	Beltrán et al. (2017)
Bergamot (<i>C. bergamia</i>) peel oil	European sea bass (<i>Dicentrarchus labrax</i>)	0.5%, 1.0%, and 2.0%	↔ RBC, HCT and Hb ↓ Serum GLU, ALP, GOT, CHO	Acar et al. (2019)

(Continues)

TABLE 3 (Continued)

Citrus species and products	Fish species	Dosage level	Effects and benefits	Sources and references
Citrus × latifolia essential oil	Tambaqui (<i>Colossoma macropomum</i>)	0.25, 0.5, 1.0, and 2.0 ml essential oil kg ⁻¹ diet	<ul style="list-style-type: none"> ↑ Lipid peroxidation in muscle ↑ Reactive oxygen species (ROS) in liver ↑ SOD in liver ↔ SOD in muscle ↔ GPX in liver and muscle ↓ ROS in muscle 	Lopes et al. (2020)
Dried lemon (<i>Citrus lemon</i>) pomace	Common carp (<i>Cyprinus carpio</i>)	1.5%, 3%, and 5%	<ul style="list-style-type: none"> ↑ Growth ↑ RBC, GSH, GPX, SOD ↑ Plasma ferric reducing antioxidant power (FRAP) ↔ Plasma ALT, aspartate aminotransferase (AST), ALP, TPRO, albumin (ALB), bilirubin, urea ↓ Plasma malondialdehyde (MDA) 	Laein et al. (2021)
Bitter lemon (<i>C. limon</i>) peels	Nile tilapia (<i>O. niloticus</i>)	0.75% and 1%	<ul style="list-style-type: none"> ↑ Growth ↑ LYZ, phagocytic activity, total leucocytes ↑ SOD and CAT ↑ Somatotrophic axis growth-mediation gene <i>igf-1</i>, antioxidant enzyme activity gene <i>sod</i>, and immune-related pro-inflammatory cytokine gene <i>tnf-α</i> expression in liver ↔ RBC, Hb, packed cell volume (PCV), TPRO, and ALB ↔ Lymphocytes, heterophils, monocytes ↓ GLU, globulin (GLO), CHO, TRI, ALT, AST 	Mohamed et al. (2020)
Grapefruit (<i>C. paradisi</i>) peel extract	Caspian white fish (<i>Rutilus frisii kutum</i>)	6.25, 12.5, and 25 mg peel extract kg ⁻¹	<ul style="list-style-type: none"> ↑ Growth ↑ RBC, white blood cells (WBC), PCV and Hb ↑ SOD ↓ GLU, CHO, AST, ALP, and ALT 	Samavat et al. (2019)
Dried lemon (<i>C. limon</i>) peel	Rohu (<i>Labeo rohita</i>)	1, 2.5, and 5 g kg ⁻¹	<ul style="list-style-type: none"> ↑ Growth ↑ WBC, TPRO, ALB, and GLO ↑ SOD, CAT, GPX, MDA and GSH ↑ Phagocytic activity, respiratory burst activity, peroxidase activity, alternative complement pathway, serum complement C3 level, LYZ, serum total immunoglobulin M (IgM) ↑ Hsp 70 and Hsp 90, SOD, glutathione peroxidase, glutathione, interleukin-1β and interleukin-8, tumour necrosis factor alpha, inducible nitric oxide synthase, transforming growth factor beta, and IgM 	Harikrishnan et al. (2020)
Lemon (<i>C. aurantifolia</i>) peel	Common carp (<i>C. carpio</i>)	1.5% and 3%	<ul style="list-style-type: none"> ↑ Growth ↑ WBC, TPRO, ALB ↑ Serum LYZ ↑ CAT, SOD, GPX ↔ RBC, HCT, Hb, lymphocyte and neutrophil ↔ CHO, TRI, GLO, AST, ALT, and ALP ↓ MDA 	Sadeghi et al. (2021)

TABLE 3 (Continued)

Citrus species and products	Fish species	Dosage level	Effects and benefits	Sources and references
Citrus limon peel	Ningu (<i>Labeo victorinus</i>) fingerlings	10, 20, 50 and 80 g kg ⁻¹	↑ Growth, ↑ ALB, RBC, WBC, HCT, Hb, TPRO, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), serum Ig, LYZ, respiratory burst activity ↓ Plasma COR, GLU, TRI, CHO, lymphocytes, neutrophils	Ngugi et al. (2017)
Limonene by citrus peel origin	Nile tilapia (<i>O. niloticus</i>) fingerlings	200, 400, and 600 ppm	↑ Growth ↑ Gene expression levels of insulin growth factor I <i>igf-I</i> , alkaline phosphatase <i>alp</i> , lipoprotein lipase <i>lpl</i> , oligo-peptide transporter 1 <i>pept1</i> , mucin-like protein <i>muc</i> , catalase <i>cat</i>	Aanyu et al. (2018)
<i>C. sinensis</i> fragment	Nile tilapia (<i>O. niloticus</i>) (31 g)	0.2%, 0.4%, 0.6%, and 0.8%	↑ Lymphocyte, neutrophil, monocyte ↔ Growth ↔ RBC, Hb, HCT, MCV, MCHC, TPRO, ALB, GLO, CAT ↓ SOD, GPX	Vicente et al. (2019)
Dehydrated <i>C. sinensis</i> peel powder	Catla (<i>Catla catla</i>)	2, 6, and 10 g kg ⁻¹	↑ Growth ↑ Fillet quality, fillet amino acid amount, digestive system protease, amylase, lipase ↔ Serum GOT, GPT, fillet fatty acid amount	Shabana et al. (2019)
Dehydrated (<i>C. sinensis</i>) peel powder	Common carp (<i>C. carpio</i>)	1, 3 and 5 g kg ⁻¹	↑ Growth	Allah Beygi et al. (2019)
Lemon (<i>C. lemon</i>) peel and pulp powder	Common carp (<i>C. carpio</i>)	1.5%, 3%, and 5%	↑ Growth ↑ MDA, FRAP ↓ Protein carbonyl contents	Safaeian Laein et al. (2018)
<i>C. sinensis</i> peel	Rainbow trout (<i>Oncorhynchus mykiss</i>)	0.5, 1, and 3 g kg ⁻¹	↑ Growth	Gültepe (2018)
Lemon (<i>C. aurantiifolia</i>) peel powder	Nile tilapia (<i>O. niloticus</i>)	0.5%, 1% and 2%	↑ Growth ↑ Fillet quality, creatinine (CRE) ↔ Lipase (LIP) ↓ CHO, TRI, amylase (AMY), GLU	Toutou et al. (2018)
Lemon (<i>C. aurantiifolia</i>) peel powder	Thin-lip mullet (<i>L. ramada</i>)	0.5%, 1% and 2%	↑ Growth ↑ Survival rate, fillet quality, CRE, CHO, TRI, LIP ↓ AMY, GLU	Toutou et al. (2018)
<i>C. sinensis</i> fragment	Nile tilapia (<i>O. niloticus</i>)	1, 2, and 4 g kg ⁻¹	↑ Growth ↑ fillet quality, digestive system surface area	Salem and Abdel-Ghany (2018)
Dehydrated lemon (<i>C. limon</i>) peel	Rainbow trout (<i>O. mykiss</i>)	0.5%, 1.5%, and 2.5%	↑ Growth After crowding stress: ↑ Serum CAT, SOD, MDA, GPX, Total Ig, IgM, LYZ, alternative complement	Chekani et al. (2021)

diets and in African catfish (*Clarias gariepinus*) fed 2% *C. limon* diet. Similarly, there was a significant decrease in growth efficiency of gilthead sea bream (*Sparus aurata*) using 3% dehydrated lemon peel (DLP) in diets for 30 days (Beltrán et al., 2017). One explanation for

the observed decrease in fish development might be connected to lemon's diuretic properties in mammals, which means it promotes urine output in the kidneys and thus aids in the elimination of excess fluid (Menichini et al., 2015; Wu et al., 2015). Another explanation

might be connected to the antilipogenesis action of lemon and the genus *Citrus* in *in vivo* studies in rats and in *in vitro* studies (Menichini et al., 2015; Miyake et al., 2006). New studies may assist to figure out why these outcomes are happening, and show if DLP has diuretic or antilipogenesis properties in fish.

7 | EFFECTS OF CWP AND EXTRACTS ON HEMATO-BIOCHEMICAL INDICES

Haematological, serum biochemical and immunological parameters, antioxidant enzyme activities and gene expressions have been well-documented to determine the effects of citrus by-products supplementation in fish feeds used for *in vitro* feeding experiments (Table 3). The addition of essential oil extracted from *C. limon* peel up to 5% in diet was enhanced the biochemical, haematological and immunological responses in cyprinid fish *Labeo victorianus* fingerlings (Ngugi et al., 2017). Moreover, Samavat et al. (2019) observed that adding grapefruit (*C. paradise*) peel extract (GFE) at a level of 25 mg kg⁻¹ markedly enhanced the hemato-biochemical indices including red blood cell (RBC) and white blood cell (WBC) counts, packed cell volume and haemoglobin (Hb) values of Caspian white fish (*R. frisii kutum*). As a result, it is possible that GPE activates the hematopoietic tissue of the fish. However, further research works are required to illustrate the mode of action.

On the contrary, supplemental diets with *C. aurantiifolia* peel and *C. sinensis* fragment did not induce any marked effects on the haematological parameters of common carp (*C. carpio*) and Nile tilapia (Mohamed et al., 2021; Sadeghi et al., 2021). Also, orange essential oil and/or lemon essential oil had no marked effects on the haematological indices including Hb and RBC of the cultured fish (Acar et al., 2019; Vicente et al., 2019). Blood parameters may differ between species, and even within fish of the same species, depending on a variety of factors, such as water quality, sampling method and feeding (Gallaughan & Farrell, 1998). Despite these variables, CWP utilization in fish feeds for various cultured species has been found to have no deleterious impacts on fish haematological parameters. Using bitter lemon (*C. lemon*) peel at 0.75% and 1% levels decreased serum glucose, globulin, cholesterol, triglycerides, alanine transaminase and aspartate aminotransferase values of Nile tilapia (Mohamed et al., 2021). Similarly, Toutou et al. (2018) reported that the serum glucose level of Nile tilapia and thin-lip mullet (*Liza ramada*) fed diets supplemented with *C. aurantiifolia* peel was dramatically reduced when fed at 0.5%, 1% and 2% levels because stress was avoided under high levels of (vitamin C) lemon peel. Also, serum glucose, cholesterol, alkaline phosphatase and aspartate aminotransferase values were decreased using bergamot (*C. bergamia*) peel oil (BPO) up to 1% in European seabass (*D. labrax*) diets due to the presence of limonene, which is a key component of BPO that has a protective effect on the liver health status of sea bass (Acar et al., 2019). While, adding lemon verbena (*A. citrodora*) extract was increased the RBC, WBC, Hb, hematocrit and total protein contents of Siberian sturgeon (*A. baerii*; Adel et al., 2021). This increase might be attributed to the

availability of vitamin C, amino acids and natural immunostimulants in the lemon verbena extract, which benefit the general health of the Siberian sturgeon (Bahramsoltani et al., 2018). In line with the previous studies, the serum glucose and cholesterol levels were lowest in Mozambique tilapia (*O. mossambicus*) and rainbow trout fed diets containing sweet orange (*C. sinensis*) essential oil and lemon essential oil loaded in chitosan nanoparticles (Acar et al., 2015; Gheytsi et al., 2021). The potential of plant oils to mitigate the impacts of stressors in fish farming might explain the decrease in glucose concentrations (Güleç et al., 2013). Indeed, polymethoxylated flavones and hesperetin (a flavanone derivative) compounds found in lemon essential oil decrease cholesterol and triglyceride biosynthesis (Baba et al., 2016; Kurowska & Manthey, 2004; Ngugi et al., 2017).

8 | EFFECTS OF CWP AND EXTRACTS ON IMMUNE RESPONSE

The study of phagocytic activities, phagocytic indices, lysozyme activities, WBC and differential WBC counts are standard tools to assess the immunological responses of fish (Hassaan et al., 2019, 2020, 2021; Mohammady et al., 2021). In fish, phagocytosis is an important part of cell-mediated immunity. It detects the pathogen, inhibits its transmission and thereby lowers disease outbreaks (Kawahara et al., 1991). Lysozyme activity is a crucial defence mechanism that causes lysis of pathogenic bacteria (Saurabh & Sahoo, 2008). The immunological response of Nile tilapia including lysozyme activity, phagocytic activity and phagocytic index was improved by adding essential oil extracted from lemon at 3% and/or sweet orange peels at 0.75% (Mohamed et al., 2021). Furthermore, the dietary administration of oregano essential oils (OES) extracted from *Origanum vulgare* at a concentration of 15 g kg⁻¹ improved these immunological parameters in common carp (*C. carpio*) (Abdel-Latif et al., 2020) due to the probable function of the primary components of OES and thymol and carvacrol in enhancing fish disease defence (Saurabh & Sahoo, 2008; Sivropoulou et al., 1996). Moreover, there was an improvement in the lysozyme activity of Mozambique tilapia (*O. mossambicus*) by adding essential oils extracted from lemon and orange peels (*C. sinensis*) (Acar et al., 2015; Baba et al., 2016). Indeed, increasing the amount of lemon essential oil loaded with chitosan nanoparticles in rainbow trout (*O. mykiss*) diets steadily increased serum lysozyme activity (Gheytsi et al., 2021).

9 | THE INFLUENCE OF CWP AND EXTRACTS ON ANTIOXIDANT ACTIVITY

Catalase (CAT) and superoxide dismutase (SOD) are essential enzymes in modulating and relieving antioxidant mechanisms and cellular immunity in fish (Al-Deriny et al., 2020). However, there was an increase in the activities of SOD and CAT in Nile tilapia fed essential oils extracted from sweet orange (*C. sinensis*) and/or lemon (*C. limon*) peels, indicating the enhancement in fish resistance against

any oxidative stress (Mohamed et al., 2021). The study on the effects of bergamot (*C. bergamia*) peel oil on European sea bass (*D. labrax*) showed a similar pattern on the increase in SOD and CAT levels (Acar et al., 2019). This increase in SOD could maintain the level of superoxide anion or it may facilitate the process of conversion to O₂ or OH⁻ through metal facilitate interaction to improve the microbial exterminate potential of phagocytes (Yilmaz, 2019a, 2019b).

In Nile tilapia and African catfish fed *C. limon* peels (1% and 2%), Abdel Rahman et al. (2019) found a considerable increase in the activities of CAT and SOD enzymes, except for SOD in Nile tilapia, which remained unaltered. Additionally, after feeding a mixture of *Bacillus licheniformis* and lemon peel powder to common carp, serum SOD, CAT and glutathione peroxidase (GPX) levels were increased (Sadeghi et al., 2021). Rohu (*Labeo rohita*) fed with 2.5 and 5 g kg⁻¹ dried lemon peel diets markedly regulated SOD, CAT and GPX while malondialdehyde did not change in any of the treatments according to Harikrishnan et al. (2020).

10 | EFFECTS OF CWP AND EXTRACTS ON GENE EXPRESSION

Several studies have revealed the influence of various forms of bioactive compounds derived from plant sources on growth performance and blood metabolites in fish, but information about the pathways in which the changes occur is still lacking. Therefore, the measurement of the level of gene expression may help in elucidating the growth and/or metabolic differences.

Limonene obtained from orange peel had effects on growth (growth hormone, growth hormone receptor I and insulin growth factor I), feed utilization (mucin-like protein, oligo peptide transporter 1, alkaline phosphatase, chymotrypsinogen A-like, phospholipase, glucose transporter 2, aminopeptidase N-like and pancreatic alpha-amylase), lipid metabolism (lipoprotein lipase, peroxisome proliferator-activated receptor alpha, sterol regulatory element binding transcription factor 1 and fatty acid synthase) and oxidative stress (GPX, CAT, and SOD2) associated with gene expression levels of Nile tilapia *O. niloticus* (Aanyu et al., 2018).

Dehydrated citrus peel is another form of citrus wastes that has been utilized as an aquafeed. Rohu (*L. rohita*) fed diets supplemented with dehydrated lemon peel at 2.5% and 5% levels have exhibited improved expression levels of immuno-antioxidant genes such as heat-shock proteins 70 and 90, SOD, GPX, interleukins 1β and 8, tumour necrosis factor alpha, inducible nitric oxide synthase, transforming growth factor beta, immunoglobulin M and hepcidin (Harikrishnan

et al., 2020). Moreover, Nile tilapia fed with essential oils extract from sweet orange (*C. sinensis*) and/or lemon (*C. limon*) peels showed increased somatotrophic axis growth-mediation gene, antioxidant enzyme activity gene (SOD) and immune-related pro-inflammatory cytokine gene expressions (Mohamed et al., 2021). In common carp (*C. carpio*), dietary supplementation of oregano essential oil (15 g kg⁻¹) elevated the relative expression of hepatic interleukin-1beta (IL-1β) and interleukin-10 (IL-10) genes (Abdel-Latif et al., 2020). In general, using immunostimulants in common carp diets can boost IL-1 and IL-10 expression (Hoseinifar et al., 2017; Watanuki et al., 2006).

11 | IN VITRO STUDIES RELATING TO FISH PATHOGENS

Studies on the use of CWP for in vivo aquaculture applications have been limited. Generally, citrus peel products, particularly essential oils, have antibacterial properties against various pathogens. Citrus products, particularly the essential oils produced from citrus peel, have been demonstrated to be prospective products that can be employed against bacterial fish pathogens in studies using citrus products against pathogens isolated from fish. D-limonene, the primary component of citrus peel essential oil and lime peel essential oil, has been evaluated as a viable alternative to antibiotics in an in vitro experiment of pathogens identified from flounder, one of the most important aquaculture species (Pathirana et al., 2018). Citrus peel essential oils have been shown in experiments to exhibit an antibacterial impact on various of fish infections. As a result, Mancuso et al. (2019) suggested that *Vibrio* sp. and *Photobacterium* sp., which cause serious losses in aquaculture systems, are sensitive to essential oils and can be utilized to fight fish infections. In vitro studies have mainly covered the use of citrus essential oils, which have shown to exhibit antibacterial activity against different fish infections (Table 4).

12 | CONCLUSION

Waste products are becoming more prevalent in today's society and can constitute a significant health and environmental concern. Therefore, this paper contains a review of the current research on the utilization of citrus wastes in different aquaculture-related studies. Although a number of studies have already been published on the use of citrus wastes (especially the peel or flavedo) in aquafeeds, while it was in fact quite a more recent undertaking. Every

TABLE 4 Antimicrobial effects of *Citrus sinensis* peel essential oils against fish pathogens

Fish pathogens	Sources and references
<i>Klebsiella pneumoniae</i> , <i>Yersinia enterocolitica</i> , <i>Klebsiella pneumoniae</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Bacillus subtilis</i> , <i>Yersinia enterocolitica</i> , <i>Staphylococcus aureus</i> , <i>Yersinia ruckeri</i> , <i>Aeromonas hydrophila</i> , <i>Lactococcus garvieae</i> , <i>Listonella anguillarum</i> , <i>Edwardsiella tarda</i> , <i>Citrobacter freundii</i>	Ozogul et al. (2015); Debbarma et al. (2013); Öntaş et al. (2016)

year, various citrus products, primarily orange juice and lemonade, are manufactured and marketed as raw materials. Citrus residues, which would otherwise be dumped as trash in the environment, should be considered as prospective nutraceutical resources capable of providing low-cost nutritious dietary supplements due to their cheap cost, accessibility and availability. These undesired industrial cast-offs, which are rich in bioactive components, might be valuably recycled into value-added functional food supplements that provide beneficial dietary fibre and polyphenols with potential perspectives for preventive therapies for some diseases. They act as non-caloric bulking agents, increase water and oil retention, improve emulsion and may protect from oxidative stress-related disorders. Fruit peel extracts have potential as bioactive chemical sources in the food industry. Furthermore, a well-established citrus peel utilization would aid in the reduction in pollution caused by improper disposal of such wastes. Thus, finding novel uses for these waste materials not only contributes to environmental protection but also for tapping in potential economic gains from converting low-value wastes into high-value and marketable products. Aquaculture is one of the ideal industries that could benefit from the use of products and extracts obtained from citrus wastes. In addition, further research (in vitro and in vivo) is needed to determine the bioavailability and practical advantages of using citrus peel extracts.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

AUTHORS CONTRIBUTIONS

The authors state that their individual contribution is equal.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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