https://doi.org/10.53656/nat2021-5.02

POTENTIAL APPLICATIONS OF ANTIBACTERIAL COMPOUNDS IN EDIBLE COATING AS FISH PRESERVATIVE

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Abstract. Fish is a product that breaks down quickly due to biochemical reactions that cause a decrease in the quality of its nutritional and sensory values. Natural preservatives make fish safer for consumption than fish preserved with formalin. Edible coating is a preservative that can be eaten, can prevent biological, chemical and physical changes, is able to prevent mass transfer, acts as a moisture barrier, be contained antimicrobial/antibacterial and antioxidants, increases shelf life, as well as protects food from microbial contamination. Antimicrobial/antibacterial compounds added to edible coatings are able to inhibit food degradation and/or remodel toxic compounds such as free radicals. Antimicrobials can be obtained from plant extracts such as alkaloids, flavonoids, saponins, tannins, phenolic acids, and eugenol. These compounds can slow the growth of bacteria in fish namely *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus* sp., *Staphylococcus aureus,* Psychrotrophic and Psychrophilic bacteria counts, *Enterobacteriaceae*, and lactic acid bacteria. This article reviews the application of various antibacterial compounds from various plants that are added to edible coatings as preservatives in fish.

Keywords: edible coating; antibacterial; preservative

Introduction

Fish is a perishable product and its freshness quickly declines after death compared to other high protein foods. This is due to biochemical reactions, namely changing lipid and protein content, formation of hypoxanthine, biogenic amines as well as microbiological spoilage which causes a decrease in the quality of nutritional and sensory values in fish. Nutrients in fish can be maintained by preserving fish (Dehghani et al. 2018). Preservation of fish can be done using formalin to extend shelf life. This technique can have the advantage of long shelf life over ice preservation. However, excessive exposure to formalin in food can be potentially lethal (Sanyal et al. 2017). Generally, consumers will choose foods that contain natural preservatives over synthetics, because they are safer to consume. Natural preservatives are produced from animals, microorganisms, and plants (May et al. 2019). One example of a natural preservative is edible coating.

Edible coating is a natural preservative in the form of a thin layer, biodegradable and edible. This layer is able to prevent mass, vapor and gas transfer, can block moisture (Sucharitha et al. 2018), be contained antimicrobial/antibacterial and antioxidants, (Dehghani et al. 2018), increase shelf life, and protect food from microbial contamination. Edible coating and edible film can be eaten, but both have differences, namely edible coatings that are used on the surface of food directly, while edible films are applied as food wrappers (Suhag et al. 2020). Edible coatings have been studied because they are important by bringing several functional compounds such as antimicrobials, plasticizers, and antioxidant agents that can minimize the risk of pathogenic microflora contamination and quality degradation. Plant sources are widely applied to edible coating formulations because they contain polyphenolic compounds that behave as antioxidant and antimicrobial/antibacterial agents in edible coatings (Bhagath & Manjula 2019).

Many bio-based polymers have been studied to produce sustainable edible coating structures. Hydrocolloids, either proteins or polysaccharides, are biopolymers commonly used to make edible coatings sourced from animals, plants, or microorganisms. Materials for making edible coatings can be taken from cellulose, starch, alginate, pectin, chitosan, and carrageenan derivatives which include polysaccharides (Galus et al. 2020).

There are various review articles that discuss edible coatings, such as (to only mention recent examples) the review of Dehghani et al. (2018) which discusses the application of natural biopolymers namely polysaccharides, proteins and lipids in edible coatings and films for seafood preservation. Bhagath & Manjula (2019) also discussed about applications of various lipids, polysaccharides, proteins, herbs, microbes, and nanomaterials as well as their multi-component coating system as a preservative for cuts of meat. The last, Hauzoukim et al. (2020) reviews the progress and characteristics of protein-based edible coatings as food packaging. However, until now there has been no specific review that discusses the application of antibacterial on edible coatings for fish preservation. Thus, it is necessary to study the application of various compounds that act as antibacterials added to edible coatings for fish preservation.

Basic Materials for Edible Coating Productions

Edible coatings are biopolymers that are commonly used as food packaging. The use of a thin layer as a food protector can increase the shelf life because it reduces gas exchange and the rate of oxidative reactions (Pinzon et al. 2020). Edible coating has advantages such as being edible, biodegradable, preventing moisture loss and fading food colour, reducing oxidation reactions, and preventing the appearance of unpleasant odours in food. The basic material for making edible coatings comes from biopolymers produced by food waste or other materials containing polysaccharides, proteins, or lipids (Umaraw et al. 2020).

Polysaccharides as a matrix in edible coatings act to prevent oxygen because it has a neatly arranged hydrogen bond network structure. However, polysaccharide-based edible coating cannot be used as a moisture barrier. The resulting edible coating generally has no colour, is free from oil content, and has fairly low calorie content. Cellulose and pectin derivatives are the two main groups of edible coatings of polysaccharides (Panahirad et al. 2020). In addition, polysaccharide-based edible coatings can be made from gum (Moreira et al. 2020), carrageenan (Dwivany et al. 2020), pullulan (Ganduri 2020), starch and alginate (Sondari et al. 2018), and chitosan (Riaz et al. 2021).

Protein is a potential biopolymer as a basic material for making edible coatings. The characteristic of protein-based edible coating is its ability in protecting from oxygen and carbon dioxide. However, it is not able to block water vapour and has low mechanical properties (Galus et al. 2021). Several types of protein used as edible coatings are soy protein and whey protein (Obiri et al. 2018). Fish collagen (Liu et al. 2020), gelatine (Gallego et al. 2016), casein (Bonnaillie et al. 2014), and egg protein can be used as edible coating biopolymers (Pilli 2020).

Lipids in edible coatings are useful for increasing water vapour or moisture holding properties because they have hydrophobic properties (Miri et al. 2021). Lipids act as a barrier to moisture loss because they have low polarity. The type of lipid that is often used as an edible coating matrix is wax. Wax is a soft solid of lipophilic organic compounds. This material is hydrophobic due to the presence of esters of long-chain aliphatic with long-chain aliphatic alcohol. Beeswax based edible coating is a good protection against moisture because of its viscosity and elasticity properties also its long chain fatty acid composition. In addition, beeswax reduces the interaction between hydrophilic groups and water vapour in the polymer (Rodriguez et al. 2020). The combination of polysaccharides and beeswax increases the flexibility and characteristics of edible coatings as protection from moisture (Bahrami et al. 2019).

Edible Coating Application Methods

The technique of application of edible coatings on food products can be in the form of liquid solutions, suspensions, emulsions, and powders. Adjustment of edible coating is done by drying, coagulation, cooling, and heating processes. Previous research reports the use of precipitation methods from the application of edible coatings to food products to improve their quality and increase their shelf life (Suhag et al. 2020). Various edible coating application methods are depicted in Figure 1.

Figure 1. Edible Coating Application Methods

1. Dipping Method

The dipping method was popular in the early 12th century in China for wax coating on lemon and orange (Dhall 2016). The product to be preserved is immersed in the coating material and the excess solution on the surface of the food product is removed by drying method as illustrated on Figure 2 (Sipahi et al. 2012).

Figure 2. Dipping Techniques of Edible Coating for Fish

2. Spray Method

Spray is a common method used for coating food. This method increases the coated area of the coating material in the form of droplets. Then, it is distributed on the surface of the food through nozzles. The advantages of this application technique are that the coated area is uniform, can manage thickness, the chance of multilayer application, does not pollute the coating solution, allows temperature management of the coating material solution, and can facilitate prolonged production automation (Andrade et al. 2012).

3. Fluidized-bed Method

This method is carried out by "fluidizing" the particles upward in a furnace. When the set fluidization temperature has been reached, the edible coating in the form of dry pellets is slowly thrown into the fluidization zone and the holding time is adjusted (Kim 2014).

4. Panning Method

This method is done by placing the product into a rotating container and then adding edible coating in the form of liquid or powder then the container is closed and rotated. Automatically the product will be coated or coated with edible coating, after which the layer is dried with a certain pressure (Guimarães et al. 2020).

Active Compounds in Edible Coating

Edible coating functions as a carrier for additives and active ingredients, as a barrier to gas and moisture transfer. Plant sources are widely applied to edible coating formulations because they contain polyphenolic compounds that act as antimicrobial and antioxidant agents in edible coatings (Bhagath & Manjula 2019). The addition of antibacterial ingredients in edible coatings increases its functionality as a preservative. This is due to the antimicrobial release process that reduces sensory changes, but increases its interaction with the surface components of the material so as to maintain its activity (Ortega et al. 2016). Antimicrobial compounds are able to inhibit food degradation and/or break down unwanted compounds such as free radicals (Dehghani et al. 2018). Antimicrobials can be obtained from plant extracts or essential oils. Antimicrobial compounds can be flavonoids (Karak 2019), saponins (Irum et al. 2021), alkaloids (Yan et al. 2021), tannins (Kaczmarek 2020), phenolic acids (Liu et al. 2020) and eugenol (Ulanowska & Olas 2021). Various studies of edible coating researches on fish bacteria are presented in Table 1.

Table 1. Edible Coating Researches on Fish Bacteria

Basic Material	Active Compound Source	Antibacterial Agent	Fish Bacteria	Reference
Bio-nanocomposite (carboxy methyl cellulose, Arabic gum, and gelatin)	Garlic Extract (Allium sativum L.)	Saponin, tannin, and flavonoid	Staphylococcus sp.	(Youssef et al.,2021)
Carboxy methyl cellulose	Propolis	Flavonoid	Psychrotrophic bacteria counts, Enterobacteriaceae, Pseudomonas sp., Lactic acid bacteria	(Gilani et al. 2021)
Carboxy methyl cellulose	Etlingera elatior Flower	Flavonoid, saponin, and alkaloid	E. coli, Staphylococcus aureus, Bacillus subtilis	(Naufalin et al. 2019)
Farsi Gum	Clove	Eugenol	Psychrotrophic bacteria, Pseudomonas sp, Lactic acid bacteria (LAC)	(Dehghani et al. 2017)

Pectin	Clove Extract	Eugenol	Psychrophilic bacteria counts, Enterobacteria-ceae, Pseudomonas sp., Lactic acid bacteria	(Nisar et al. 2019)
Pectin	Pomegranate peel extract	Flavonoid and tannin	E. coli and S.aureus	(Ghorbani et al. 2021)
Chitosan	Pomegranate peel extract	Alkaloid, tannin, and flavonoid	Enterobacteriaceae, coliform, Salmonella, E. coli, and S.aureus	(Alsaggaf et al. 2017)
Chitosan	Lemon verbena extract	Pheno-lic acid, saponin, flavonoid, tannin, and alkaloid	Psychrotrophic bacteria and Enterobacteriaceae	(Rezaeifar et al. 2020)
Fish gelatin and chitosan	Clove extract	Eugenol	Salmonella typhimuri-um and E. coli	(Xiong et al. 2021)
Whey protein	Common Nettle extract	Tannin, flavonoid, and phenolic acid	Psychrotrophic bacteria and Enterobacteriaceae	(Erbay et al. 2017)

Garlic extract contains saponins, tannins, and flavonoids as antibacterial agents. Tilapia coated with 4% bio-nanocomposite and 8% garlic extract successfully delayed bacteriological development and had a one week longer storage time than samples without coating (Youssef et al. 2021).

Carboxymethyl cellulose has the characteristics of a film that is transparent, flexible, and resistant to oils. Research by Gilani et al. (2021), propolis increases the functionality of CMC-based edible coatings. Propolis contains flavonoids that act as antibacterials that inhibit the growth of psychrotrophic bacteria, Lactic acid bacteria (LAC)*, Pseudomonas* sp., and *Enterobacteriaceae*. According to research by Naufalin et al. (2019), the content of flavonoids, saponins, and alkaloids of *Etlingera elatior* flower extract in CMC-based edible coatings inhibited the growth of bacteria in gourami fillets.

One type of gum that can be used as an edible coating is farsi gum. Farsi gum-based edible coating with clove extract inhibits bacterial growth in trout. During 12 days of storage, trout fish coated with edible coating had a TVC value of more than 7 log CFU/g. Eugenol in cloves causes leakage of lipids and proteins after damage to the cytoplasmic membrane in bacteria (Dehghani et al. 2017).

Pomegranate peels have bioactive phytochemical compounds: alkaloids, tannins, and flavonoids which are safe to be used by humans as food antimicrobials. Research by Ghorbani et al (2019), pomegranate skin effectively acts as antimicrobials for *E.coli* and *S.aureus* bacteria that cause fish spoilage. Pectin-based edible coating with pomegranate peel extends the storability of foods. Tilapia soaked in chitosan edible coating with the addition of 2% pomegranate peel extract has an average sensory characteristic of 4 which is in accordance with the acceptability limit of fish for human consumption after 30 days (Alsaggaf et al. 2017).

In the research of Rezaeifar et al. (2020) it is known that Lemon verbena extract contains saponins, phenolic acids, alkaloids, tannins, and flavonoids. The use of chitosan is also capable of acting as an antibacterial due to positive molecules that can be reacted with negative molecules on the bacterial cell membrane. The result of this research is that the maximum amount of TVC (Total Viable Count) that can be accepted is 7 log CFU/g. The control sample had a TVC value more than 7 log CFU/g in 6 days, while the sample treated with lemon verbena had a TVC value less than 7 log CFU/g in 16 days.

Cloves contain the bioactive component eugenol which inhibits bacterial growth. In the research of Nisar et al. (2019), Bream (*Megalobrama amblycephala*) fish coated with edible coating with 1.5% clove extract showed a slowdown in bacterial growth. TVC values in Psychrophilic bacteria counts were 5.7 log CFU/g, while TVC value in *Enterobacteriaceae* between control and sample treated with edible coating have a difference of 2.3 log CFU/g, in *Pseudomonas sp.* have a difference of 4.6 log CFU/g, and a difference of 1.5 log CFU/g on lactic acid bacteria. While in the research of Xiong et al. (2021), salmon fillet coated with fish gelatine and chitosan-based edible coating with the addition of clove extract was shown to inhibit the increase in pH of fish fillets. In addition, the TVC value of fish fillets with edible coating was less than 7 log CFU/g after 15 days of storage.

The addition of common nettle extract in edible coatings based on whey protein has the potential as a natural fish preservative. Research by Erbay et al. (2017), showed that rainbow trout was stored in temperature 4^0C, decomposed in 6 days of storage, the TVC value was 6.27 log CFU/g. The bioactive components in nettle extract have strong antimicrobial properties, which could extend the shelf life and freshness of fish up to 15 days of storage.

1. Flavonoids as Antibacterial Agents

Flavonoids are phenolic substances found in all vascular plants with a C6-C3-C6 chemical structure. Flavonoids have anti-inflammatory, antioxidant, antiviral, antidiabetic, antimicrobial, anti-cancer, and cytotoxic properties. Apigenin, flavone and flavonol glycosides, flavanones, isoflavones, galangin, and chalcones have been shown to have strong antimicrobial activity. Antimicrobial activity works by its potency to inactivate microbial adhesins, cell envelope transport proteins, and enzymes (Karak 2019). The antioxidant effect of flavonoids is due to the scavenging of free radicals through the donor of a hydrogen atom from the hydroxyl group of flavonoid. Phenol compounds are able to play a role as antioxidants by acting as reducing agents that donate H+ ions from their hydroxyl groups (Naufalin et al. 2019). Flavonoids can be found in garlic extract (Youssef et al. 2021), pomegranate peel (Alsaggaf et al. 2017), lemon verbena (Rezaeifar et al. 2020), common nettle (Erbay et al. 2017) and others. The flavonoid structure is depicted in Figure 3 (Rajarathinam et al. 2018).

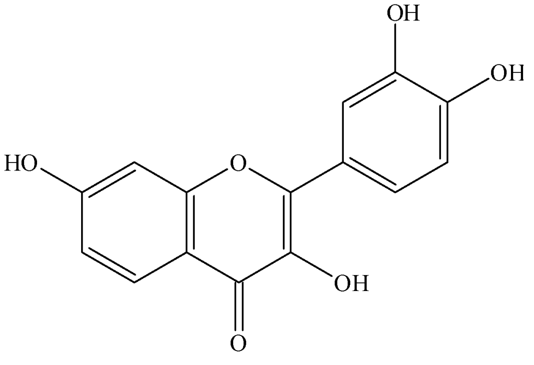

Figure 3. Flavonoid Structure

2. Saponins as Antibacterial Agents

Saponins are glycosides with high molecular weights that can reduce the surface tension of the cell wall thereby increasing membrane permeability or cell leakage, resulting in the release of intracellular compounds (Muniyan et al. 2017). Saponins are natural surfactants from plant extracts. The molecular structure of saponins consists of hydrophilic sugars attached to aglycones (steroids and triterpenoids) (Paul et al. 2020). In nature, saponins are known to have antimicrobial, anti-tumor, antifungal, antioxidant, and anti-diabetic properties (Irum et al. 2021). Saponins are widely contained in garlic extract (Youssef et al. 2021), lemon verbena (Rezaeifar et al. 2020) and others. The structure of saponins is as shown in (Figure 4) (Bassir & Shadizadeh 2020).

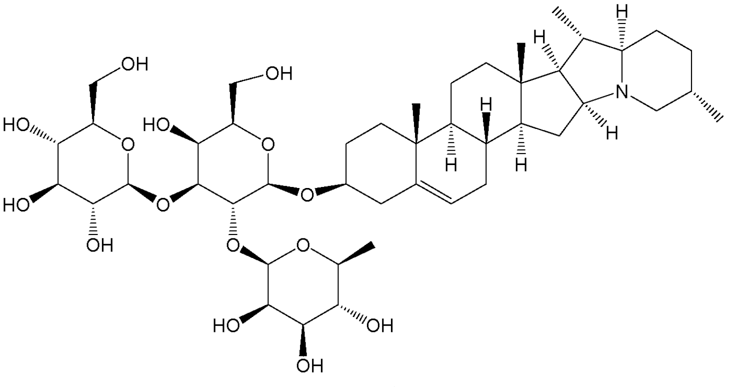

Figure 4. Saponin Structure

3. Alkaloids as Antibacterial Agents

According to Raji et al. (2019), alkaloids have antibacterial activity that can inhibit the process of transcription and toxin production. Alkaloids play a role in controlling development in living systems. Natural antibacterial alkaloids show that they can disrupt bacterial cell membranes, affect DNA function, and inhibit protein synthesis. Thus, natural alkaloids are potentially active against various bacteria, including *Staphylococcus aureus* (Yan et al. 2021). The mechanism of alkaloids is to prevent the essential components of peptidoglycan in bacterial cells, which thus the cell wall layer is not fully formed and causes cell death (Rahayu et al. 2019).

4. Tannins as Antibacterial Agents

Tannins are vital phenolic compounds that are present in the plant body, naturally as secondary metabolites and have a very essential function in suppressing antimicrobial resistance. Tannins have a certain ability that can precipitate proteins, inhibit the accessibility of substrates for bacterial cells, insufficiency of extracellular microbial enzymes, direct action on mechanisms of microbial, and the unavailability of iron make them useful for controlling the proliferation of resistant bacteria (Javed et al. 2020). Tannins in bacterial cells cause disruption of cell metabolism which ultimately leads to the destruction of bacteria. Tannins inhibit the attachment of bacteria which reduces the adhesion of bacteria to surfaces. Lack of adhesion causes bacterial cells to die (Kaczmarek 2020). Tannins can be found in garlic extract (Youssef et al. 2021), pomegranate peel extract (Alsaggaf et al. 2017), lemon verbena (Rezaeifar et al. 2020), common nettle (Erbay et al. 2017) and others.

5. Phenolic Acids as Antibacterial Agents

Phenolic acids are secondary metabolites derived from shikimic acid and benzoic acid. Phenolic acids can be found in plants, especially in the hydroxy benzoic and hydroxy cinnamic derivatives, which lead to their organoleptic properties, i.e. sour and bitter tastes (Spiegel et al. 2020). Phenolic acids are secondary metabolites in plants that can destroy microorganisms and/or inhibit bacterial growth. Inhibition mechanisms of bacterial growth by phenolic acids include destabilization of the bacterial cytoplasmic membrane, changing the permeability of the bacterial plasma membrane, inhibiting extracellular microbial enzymes that straight alter metabolism of microbial, and removing microbes from substrates needed for growth. Phenolic acids can change the surface physicochemical properties of bacteria. For instance, ferulic acid has been revealed to reduce the hydrophobicity of *Pseudomonas aeruginosa*. Phenolic acids can similarly change the polarity of bacteria by changing the electron acceptor of the bacterial surface in Gram-positive (increased acceptor component) and Gram-negative strains (decrease acceptor component) (Liu et al. 2020). Phenolic acids can be found in extracts of lemon verbena (Rezaeifar et al. 2020) and nettle (Erbay et al. 2017).

6. Eugenol as Antibacterial Agent

Research on eugenol (4-allyl-2-methoxyphenol) has shown its ability to fight bacteria. Eugenol is nonpolar which can change the nature of proteins and react with phospholipids in the membranes and envelopes of bacterial cells. The mechanism of action of eugenol is carried out by influencing the process of transporting ions and ATP and the structure of fatty acids from bacteria (Pathirana et al. 2019). The research report of Nisar et al. (2019) shows that the application of clove extract containing eugenol in edible coating was able to inhibit the growth of Psychrophilic bacteria counts, *Enterobacteriaceae, Pseudomonas* sp., and Lactic acid bacteria.

7. Antibacterial Potential as a Preservative in Fish

Of the several antibacterial compounds above, more flavonoids are contained in a plant (Donadio et al. 2021). In its role as an antimicrobial, flavonoids are very effective against various microorganisms (Karak 2019). The mechanism of flavonoid compounds is to inhibit the role of cell membranes by creating complexes with bacterial extracellular proteins through hydrogen bonds, causing the cell wall structure and bacterial cell membranes to become unstable and result in the release of intracellular compounds. Flavonoid compounds can also inhibit energy metabolism by reducing the use of oxygen by bacteria, inhibition of C reductase occurs so that the formation rate of metabolism is decreased. The mechanism of flavonoids as antibacterial is to inhibit energy metabolism, while the mechanism of phenol is to denature proteins, and the mechanism of tannins is to form bonds with proteins so that protoplasmic coagulation occurs in bacteria (Apridamayanti et al. 2021).

Future Trends and Advancement

Edible coating is a safe preservative for consumption which is efficient in preserving the quality of food and environmentally friendly. The challenges in the application of edible coatings are the complex reactions of edible coatings that can cause changes in quality and safety as well as its potential to be treated with increasingly sophisticated technologies such as ultrasonic processing, gamma radiation. Research in the field of nanotechnology in edible coating systems can be an innovation in the future. Therefore, further research is required to optimize the formulation of edible coatings. Future researchers should focus on the potency of the edible coating matrix to release biologically active compounds and nanomaterials gradually, the chemical changes that occur at the molecular level with the use of edible coating complexes, as well as enzyme reactions in food with the edible coating matrix and other secondary metabolites.

Conclusion

Based on literature study, edible coatings added with plant extracts can be potential as natural preservatives for fish because they contain secondary metabolite compounds that have high antibacterial properties i.e. flavonoids, alkaloids, saponins, tannins, eugenol, and phenolic acids. These compounds can inhibit the growth of bacteria in fish such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus* sp, Psychrotrophic, Psychrophilic bacteria counts, *Enterobacteriaceae*, and lactic acid bacteria. Some basic materials containing protein, pectin, lipids, or polysaccharides can be potential as materials for making edible coatings because they have the unique properties of each of these materials. The application of edible coatings on fish is expected to increase the shelf life of fish because it can reduce gas exchange and the rate of oxidative reactions. The presence of antibacterial in plant extracts is also expected to replace synthetic preservatives such as formalin so as to decrease the negative impact of long-term use of synthetic preservatives.

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