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Research Article

ANTIMICROBIAL ACTIVITY OF GARLIC (*Allium sativum*) AND ITS POTENTIAL USE IN FISH PRESERVATION AND DISEASE PREVENTION

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Abstract- The high-water content in fish makes prone to microbial-spoilage by growth and proliferation of food-spoilage bacteria, thus crosses the limit of acceptability for safe human consumption. Consumers, now-a-days, demand natural but effective preservation of food free of potential health risk. So, to satisfy the consumers' preference of minimally-processed, fresh-alike, tasteful, nutritious and economic food free from antibiotic and/or harmful chemical preservative with longer shelf-life and necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by the use of biological, natural and organic antimicrobial compounds derived from plants and other natural table spices, such as garlic, either alone or in combination with mild physicochemical treatments and low concentrations of traditional chemical preservatives. The use of such antimicrobial compounds inhibits the growth of spoilage and pathogenic bacteria. This improves the shelf life of the product and ensures food safety. The main component responsible for the antimicrobial activity of garlic is allicin which also provides garlic its distinct flavour and aroma. Garlic inhibits the pathogenic disease-causing organisms, increases growth performance and disease resistance in fish. Garlic helps to improve the flesh quality and food calling effect when applied as a food additive; and extend the shelf-life of fish fillet. Thus, garlic extract, especially raw garlic juice, can be used as a natural antimicrobial agent: a potential natural fish preservative suitable for commercial application to extend the shelf-life of fish and fishery products as well as in modern aquaculture practices.

Keywords- *Antimicrobial activity, Garlic, Allium sativum, Allicin, Fish, Microbiological spoilage in fish, Fish preservation with garlic, Fish disease and prevention*

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Introduction

Fish is perishable in nature due to its high-water content (about 60-80% of water) which makes fish prone to microbial spoilage associated with a combination of factors. Upon storage of fish, due to production of volatile amines, pH starts to rise and finally crosses the limit of acceptability of safe human consumption of fish leading to spoilage. Higher pH associated with high water activity of fish muscle leads to spoilage by growth and proliferation of food spoilage bacteria. The extent of microbial spoilage depends on the microbial load, available metabolites in tissue, storage conditions and species concern [1]. Started from fish harvesting to processing via handling pathogens cause deterioration of the fish tissues [2] which are expressed by strong odour, texture changes with slime production and gross discoloration, thus fish being rejected by consumers [3].

As temperature accelerates the bacterial spoilage in fish, one of the major concerns in food industries in tropical countries like India is the contamination by food spoilage and pathogenic bacteria [4] causing serious food borne illness. The bacterial pathogens that account for these cases include *Salmonella, Campylobacter jejuni, Escherichia coli* 0157:H7, *Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis* and *Clostridium botulinum* [5]. Moreover, higher temperature accelerates oxidation and hydrolysis of fats [6], protein denaturation and autolysis which are associated with microbial spoilage [4]. Among the biochemicals changes associated with fish deterioration, the low molecular weight nitrogenous compound Biogenic amine (BA) such as histamine and tyramine, are produced due to the decarboxylation of the amino acids by decarboxylase enzyme of spoilage bacteria present in fish. Higher level of BA leads to several symptoms like nausea, vomiting, burning sensation in mouth, respiratory disease, itching, headache, allergy, heart palpitation, hypertension etc. [7]. Likewise, the common fish pathogens causing fish diseases are *Aeromonas* sp., *Flavobacterium* sp., *Streptococcus* sp., *Vibrio* sp., and *Photobacterium* during aquaculture practices as reported by Benhamed *et al*. [8]. Bacterioses is a limiting factor when it comes to fish farming which eventually results in decreasing productivity, delayed fish growth, eventually causing a considerable mortality during aquaculture practices [9]. Chemotherapeutic agents and antibiotics are generally used to control infection caused by bacteria and other parasites during aquaculture [10]. Although, during storage and preservation of fish with antibiotics effectively reduce the chances of microbial spoilage, but development of antibiotic resistant bacterial strains is of great concern and hard to control. Moreover, use of these antibiotics and other chemotherapeutics exerts drug residue in treated fish and causes significant environmental pollution [11]. The random use of antibiotics and synthetic chemicals can cause severe health hazard like allergic reactions and immune suppression. Many countries including Singapore and the European Union has banned the import of aquaculture produces susceptible to have antibiotic residues [12].

Present day food preservation is viewed as a 'convenience' product as well as key to ensure the availability of food as a vital benefit. Consumers, now-a-days, demand natural but effective preservation of food free of potential health risk. With the industrial revolution and the subsequent development of food industries, food processing moved from kitchen or cottage industries to large scale technological operations with increased need for food preservation. This stimulated the use of food additives, especially those preserve food and enhance food quality.

In recent years, the addition of chemical preservatives has fallen into disfavour with consumers who seek for foods that are of high quality, less severely processed (less intensive heating and minimal freezing damage), less heavily preserved, more natural (free of artificial additives) and safe [13]. Thus, presently, a major concern of food industry is use of synthetic preservatives [14]. This has resulted in the emergence of a new generation of chill stored, minimally processed foods. So, to satisfy the consumers' preference of minimally processed, fresh-alike, tasteful, nutritious and economic food free from antibiotic and/or harmful chemical preservative with longer shelf life and necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in food are being replaced by the use of biological, natural and organic antimicrobial compounds derived from plants and other natural table spices [12], such as garlic, either alone or in combination with mild physicochemical treatments and low concentrations of traditional chemical preservatives. Addition of natural antimicrobial compounds such as garlic is expected to improve the shelf life of the food through inhibition of spoilage microorganisms. These compounds also inhibits the growth of pathogens in food, thus ensuring food safety without adversely effecting the nutritional quality of the food products [15].

Garlic (Allium sativum) and its constituents have been known to have antimicrobial properties since long back. The main component responsible for the antimicrobial activity of garlic is allicin which also provides garlic its distinct flavour and aroma [16]. This review focuses on the forms, variations and potential uses of garlic while being used as a natural antimicrobial agent in fish preservation as well as in modern aquaculture practices.

Garlic as A Potential Antimicrobial Agent

Garlic (*Allium sativum*), a member of family Liliaceae, is considered as one of the oldest medicinal plants or table spices that has been used by different countries to treat a number of common infectious diseases since long back. In ancient days, garlic was used to treat heart diseases, and even tumour which was documented in the Egyptian Codex Ebers during 1550 BC [17]. Garlic is thought to be of Central Asian origin and features are widely spread in the legends and folklore of both India and China. History reveals that garlic was a staple diet of the Egyptian pyramid builders [18]. The antimicrobial activity of garlic was observed by Louis Pasteur in 1858. Garlic was used as an antiseptic to prevent gas gangrene during the World War II. It has also been known to show reasonable action against Cryptosporidium infection in AIDS patients [19]. The nomenclature of garlic reveals that 'gar' refers to the spear like foliage and the 'lic' refers to leek.

Food borne illness is a serious issue in present day food industry and garlic has excellent potential in inhibiting food borne pathogens and spoilage microorganisms especially in low acid foods like meat, fish and fishery products. The microbial spoilage in fish and fishery products results the production of volatile bases like ammonia as a result of proteins denaturation, enzymatic degradation as well as autolysis, associated with putrefactive smell that leads to lowering the shelf life [20]. Garlic is known to be one of the edible spices as well as medicinal plants with antimicrobial activity against a broad spectrum of microorganisms including bacteria, fungi, protozoa and viruses. In 1944, Cavallito and Bailey, [21] specified that allicin (diallyl thiosulfinate) is the potential compound responsible for the antimicrobial activity of garlic [22]. Parallelly, Rios and Recio [23] reported that the essential oil present in garlic is responsible for the antimicrobial activity. The antimicrobial activity of the essential oil varies among the food pathogens depending on their chemical composition [24]. Garlic is having antihypertensive, antihyperlipidemic, hypoglycaemic, anticancer antidote activity during heavy metal poisoning, anticarcinogenic, hepatoprotective and immunomodulation effects [25, 26, 27]. The higher concentration of sulphur compounds is responsible for antibiosis effect of garlic. Moreover, reduction of blood lipids and anti-cancer property of garlic was also reported by Mauti *et al*. [28]. Fresh garlic and its essential oil are applied as natural antimicrobial agent, flavouring agent and antioxidant in chicken and processed meat products as reported by El-Sayed *et al*. [29].

There are apparently two varieties of garlic available: the white skin garlic, principally grown in Egypt for centuries and is exported throughout the world; and the purple skin garlic was introduced only around twenty years ago in the market by the Egyptians. These two varieties seem to possess differences both in their chemical as well as antimicrobial activities. Studies suggest the white skinned garlic oil is stronger as compared to that of purple skinned variety in terms of antimicrobial activity against a wide range of tested microorganisms due to higher content of trisulfide compounds [29]. The concentration of diallyl sulphide, derivative of which plays an important role in governing the antimicrobial activity of garlic, is influenced by garlic cultivar and its geographical origin [29]. Fresh garlic filtrates are used as alternatives to antibiotics in poultry and cattle industries as they inhibit growth of Salmonella typhimurium and mastitis bacteria [30].

Garlic has strong inhibition against both Gram-positive and Gram-negative bacteria. Research on garlic showed that aqueous extract of garlic can inhibit 17 types of human pathogenic bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteous* sp. that easily acquires resistance to several types of antibiotics [30]. Erguig *et al*. [31] reported that crushed garlic showed broad spectrum antimicrobial properties against both Gram-positive and Gram-negative bacteria which include Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, and Clostridium. Even acid-fast bacteria Mycobacterium tuberculosis is sensitive to garlic too [31]. Garlic extract, in combination with antimicrobial substance like nicin, synergistically inhibit foodborne pathogen Listeria monocytogenes. Moreover, edible films containing garlic essential oil confer antimicrobial property [29].

Chemistry of Garlic

Although garlic has been known to mankind for a long time but the quest to explore its chemistry, structure and action has only began in the early 19th century. The German chemist Wertheim was the first person to carry out chemical analysis on garlic in the year 1844. During steam distillation, an oil with pungent smell was obtained from the garlic; the hydrocarbon chain present in the oil was named as 'allyl' by him. The extracted oil had both antibacterial and antifungal properties [32]. Later on, in 1892, another steam distillation was carried out by Semmler. In 1944, Cavallito and Bailey, [21] first isolated diallyl disulfide, the major component of garlic oil allicin [18]. Allicin is chemically unstable colourless liquid. Although, the characteristic odour of garlic is due to allicin, garlic bulbs are odourless until its cutting or crushing which may be due to the development of allicin takes place when an enzyme acts on the odourless precursor molecule (+) -S-allyl -L- cysteine sulfoxide [32]. Out of the 600 known allium species, garlic is most rich in allicin. The precursor molecule on which alliinase acts is a non-protein amino acid alliin. Allicin is known to be very unstable specially in presence of other garlic derived compounds that camouflage the activity of allicin, releasing in lesser amount comparatively; thus, researchers face major constrain while trying to specify the antimicrobial activity of alliin, alliinase and other sulphur rich compounds in garlic extract. This phenomenon justifies the very low or no activity of garlic extract powder, tablets, and capsules due to low release of allicin [33].

Challenges in Modern Aquaculture

Fish is an excellent source of high nutritional value protein and contains omega-3 PUFA (poly unsaturated fatty acids), especially, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [34]. The omega-3 fatty acids are essential for human growth and is reported to prevent inflammatory and autoimmune disorders, hypertension, heart diseases and cancer [35]. Fish is also a good source of vitamins like A, D, B6, and B12. Minerals such as iron, zinc, iodine, selenium, potassium, sodium, etc. are also present in fish; thus, fish can meet up the daily requirement of omega-3 PUFA as well as other vital nutrients [36]. India ranks the second position in aquaculture contributing 6.3% of global fish production [37] with a fish diversity of 10% worldwide [38].

Thus, in the recent years we have witnessed a dramatic rise in the aquaculture industry primarily due to this increasing demand for incorporation of fish in human nutrition. As a result, a high stress environment has been generated as the previously semi-intensive fish farming has turned into a more intensive culture practices that makes fishes more vulnerable and susceptible to infectious diseases [39]. A number of pathogens of different origins, such as freshwater, marine, feral, cultured, sport and ornamental fish culture, causing disease to fish [40].

Common pathogenic species affecting fish

The pathogenic microbes associated with diseases during aquaculture practices are listed below. The aerobic disease-causing strains includes *Acinetobacter johnsonii, Enterobacter aerogenes, Flavobacterium psychrophilum, Flexibacter* sp., *Micrococcus luteus, Moraxella* sp., *Pseudomonas fluorescens, Psychrobacters, Acinetobacter calcoaceticus, and Alcaligenes faecalisto*. The facultative aerobic and facultative anaerobic disease-causing strains includes *Aeromonas hydrophila, Aeromonas bestiarum, Aeromonas caviae, Aeromonas jandaei, Aeromonas schubertii, Vibrio fluvialis, Vibrio harveyi, Vibrio albensis, Vibrio ordalii, Vibrio fischeri, Photobacterium logei, Photobacterium angustum, Photobacterium damselae, Photobacterium damselae* subsp., and *Photobacterium piscicida*. Other disease-causing microbes in fish includes *Alcaligenes piechaudii, Escherichia coli, Bacillus cereus, Bacillus firmus, Caulobacter, Coryneforms, Cytophaga / Flexibacter, Hyphomicrobium vulgare, Prosthecomicrobium, Pseudomonas marina, Alteromonas, Plesiomonas shigelloides,* and *Moraxella* [8]. The disease causing Gram-positive and Gram-negative microbes with their primary host are listed out here [41].

Table-1 *Gram-positive pathogens causing disease in fish*

Pathogen	Disease	Primary Host
Enterococcus seriolicida	Lactococcosis	Yellowtail, eel
Streptococcus iniae	Streptococcosis	Yellowtail, seabass
Streptococcus parauberis	Streptococcosis	Turbot
Streptococcus phocae	Streptococcosis	Atlantic salmon
Renibacterium salmoninarum	BKD	Salmonids
Mycobacterium marium		Atlantic salmon, Turbot
Piscirickettsia salmonis	Piscirickettsiosis	Salmonids

Table-2 *Gram-Negative pathogens causing disease in fish*

Pathogen	Disease	Primary Host
Vibrio anguillarium	Vibriosis	Salmonids, seabass, turbot
Vibrio ordalii	Vibriosis	Salmonids
Vibrio vulnificus	Vibriosis	Atlantic salmon, cod
Vibrio salmonicida	Vibriosis	Eels
Moritella viscosa	Winter ulcer	Atlantic salmon
Photobacterium piscicida	Photobacteriosis	Seabass, shoal
Pasteurella skyensis	Pasteurellosis	Atlantic salmon
Flexibacter maritimus	Flexibacteriosis	Turbot, salmonids, shoal
Aeromonas salmonicida	Furunculosis	Salmonids, Turbot
Pseudomonas anguilliseptica	Winter disease	Seabream, turbot, eel

The clinical manifestations of infection with *Flexibacter* and *A. salmonicida* include rotting of gills, fin and surface ulceration which are similar to that of viral diseases where increased mortality may be the first sign of systemic disease. Some of the common symptoms include darkened pigmentation, exophthalmia, ascites with abdominal swelling or distention, lack of feeding, lethargy, and swimming difficulties. Severity results subcutaneous haemorrhage of the skin and gills. Some cases may show gastrointestinal involvement that leads to inflamed and oedematous vent and faecal casts, enteritis, and intestine is devoid of food but filled with yellow mucus and sloughed epithelial cells. Gram-negative bacteria generally cause severe organ and tissue necrosis [40].

As a treatment of bacterial diseases in fish, the intensive fish farming practices ensure the sound use of antibiotics which results the development of resistant pathogens, harmful residues in fish flesh with a serious impact on the aquatic environment. Moreover, the transmission of antibiotic resistance from the aquaculture environment to human and animal is a matter of severe concern [16]. Thus, the understanding, prevention and control measures to reduce risks of diseases as well as production losses in the commercial, semi-commercial and small-scale aquaculture system has thus garnered some serious attention in the recent times [42]. Treatment is done by applying antimicrobial drugs to the diseased fish shows immediate benefit. The significant use of antimicrobial drugs has prophylactic and growth enhancing properties in aquaculture practices [43].

Fish Preservation

The major challenge to the food processing industry is to maintain food safety standard and reduce or eliminate the occurrence of food related illness and food spoilage. Seafood is often consumed fresh and precooked. Moreover, seafoods are mostly imported to developed nations from developing countries. In either context, the seafood may suffer temperature abuse resulting in outbreak of food borne illness [44]. Hence, in Europe, morbidity from food borne illness is second only to respiratory diseases, with estimates of 50,000 to 300,000 cases of acute gastroenteritis per million populations every year [45]. Several bacterial pathogens including *Salmonella, Campylobacter jejuni, Escherichia coli* 0157:H7, *Listeria monocytogenes, Staphylococcus aureus* and *Clostridium botulinum* are found associated with such outbreaks [15]. Food spoilage occurs mostly due to biochemical, microbial and enzymatic actions [46]. Spoilage of fish initiates immediately after harvesting and accelerates in higher ambient temperature. The microbial spoilage in fish and fishery products results production of volatile bases like ammonia as a result of proteins denaturation, enzymatic degradation as well as autolysis, associated with putrefactive smell that leads to lowering of shelf life [20]. Fish spoilage is associated with formation of new compounds causing discoloration of fish muscle which is an indicator of deteriorating the freshness of fish, thus consumer's acceptability decreases [47]. Therefore, preservation of food in sound and safe condition continues to be an ongoing challenge for human.

Gradual Development of Food Preservation

The development of optimum preservation techniques requires a thorough understanding of the mechanism by which the degradation occurs [46]. Drying, salting and fermentation are the age-old traditional methods of preservation. Canning and freezing are relatively recent developments, dating back to Napoleonic times and the 1920s, respectively. Food fermentations developed by default rather than by design. Food, that is spoiled during storage and the product is acceptable for consumption, is the most probable basis for the development of fermented foods. Lactic acid bacteria (LAB) play an important role in food fermentations, causing the characteristic flavour changes and exercising a preservative effect on the fermented product. It is estimated that 25% of the European diet and 60% of the diet in many developing countries consists of fermented foods [13].

Preservation by modified atmospheric packaging (MAP) by restricting the growth of aerobic bacteria, irradiation, freeze-drying and high-pressure packaging are modern fish preservation techniques. In recent years the addition of chemical preservatives has fallen into disfavour with consumers who seek for foods with high quality, less intensively heating and minimal freezing damage, less heavily preserved, more natural (free of artificial harsh chemical additives) and safe [13]. Thus, the demand of minimally processed food led to the obvious choice of natural alternatives: the use of biological, natural and organic antimicrobial compounds which includes medicinal plants like garlic and use lactic acid bacteria (LAB), other beneficial microbes and their metabolites such as bacteriocin, which is otherwise defined as biopreservation [48, 49]. So, to satisfy the consumers' preference of minimally processed, fresh-alike, tasteful, nutritious and economic food free from antibiotic and/or harmful chemical preservative with longer shelf life and necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by the use of biological, natural and organic antimicrobial compounds derived from plants and other natural table spices such as garlic to retain its nutritional value, original odour and aroma, texture and flavour, safety and quality. Therefore, adequate preservation techniques must be applied and addition of natural antimicrobial preservatives need to be ensured.

Garlic in Fish Disease Control

Garlic (*Allium sativum*), containing the active compound allicin, has been receiving the focus for a long time due to is activity not only as a table spice but also as a remedial for a variety of diseases. The role of garlic is not only limited to inhibit pathogenic disease-causing organisms in fish, but its effects on growth performance and disease resistance has been evaluated. Garlic can efficiently eliminate the primary pathogenic bacteria such as *Pseudomonas fluorescens, Myxococcus piscicola, Vibrio anguillarum, Aeromonas punctate,* and *F. intestinalis* that are found in freshwater fish [31]. The aqueous extract garlic bulbs prepared and sterilized with mercuric chloride, showed inhibitory effects against two important fish pathogens, *Aeromonas* sp. and *Pseudomonas* sp. with the former being more susceptible to garlic action than the later.

Garlic also has role in improvement of disease resistance particularly against *Pseudomonas fluorescens* infection in fish. However, the bacteriological parameters of normal and garlic fed fish showed no difference because the pH of gastric cavity of fish is 3 or lower, which can irreversibly neutralize alliinase responsible for the formation of allicin from alliin; which implies that garlic administration through feed does not provide significant effects on gut microbiota of fish [50].

Garlic in Fish Preservation

Garlic helps to improve the flesh quality and food calling effect when applied as a food additive [31]. The antimicrobial activity of raw garlic extract in tuna (*Thunnus tonggol*) fillets in extending shelf life proves its efficacy as a potential fish preservative suitable for commercial application [51]. Literature review reveals that raw garlic juice shows excellent antioxidant property due the high phenolic content compared to the boiled forms of garlic juice. Although at 100oC the antioxidant capacity of garlic reduces, at 65°C the sulphur containing volatile compounds are present resulting optimum antioxidant activity [24, 52, 53]. While studying the optimum concentration and form of garlic juice effective most in inhibiting 13 fish pathogenic and spoilage bacteria, raw garlic juice exhibited the highest antibacterial activity against both Gram- positive and Gram-negative bacteria. Both water bath boiled form and microwave oven boiled form of garlic juice showed varied levels of activity against Gram-positive bacteria [51]. The fish spoilage and pathogenic bacteria are inhibited by garlic extract are *Pseudomonas aeruginosa, Aeromonas hydrophila, Escherichia coli, Salmonella typhi, Yersinia enterocolitica, Vibrio cholera, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Micrococcus* sp.*, Bacillus pumilus, and Bacillus licheniformis* [51]. Addition of raw garlic juice was able to significantly delay the rate of microbial spoilage and extended the shelf life of fish fillets by 6 days under specific storage conditions compared to control [51]. Thus, it can be inferred that garlic extract, especially raw garlic juice, can be used as a potential natural fish preservative to extend the shelf life of fish and fishery products.

Conclusion

Recently there is a high demand among the consumers for fish with ensured quality and safety, presence of minimal amount of harmful preservatives, low cost and the absence of pollutants, antibiotics and carcinogens. In order to achieve improved food safety and to harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods as well as use of harmful chemical preservatives are being replaced by combinations of innovative technologies that include natural antimicrobial substance, free of potential health hazards; such as garlic. Garlic (*Allium sativum*) and its constituents have been known to have antimicrobial properties since long back but plenty of literatures are not available regarding the extent of inhibition of spoilage bacteria in food system, especially during preservation of fish. Moreover, scope is there to quantify the ability to increase the shelf life of stored fish and fishery products due to application of garlic by correlating the texture profile parameters with microbiological, biochemical and sensory evaluation of fish fillet upon storage and to standardize the dosage to be applied for preservation of fish. Thus, the role of garlic to be a potential natural fish preservative and its global commercial application must necessarily be explored vividly in near future.

Application of research: Garlic (*Allium sativum*) and its constituents have antimicrobial properties and there is scope to use it to increase the shelf life of stored fish and fishery products without adversely affecting the nutritional quality.

Research Category: Antimicrobial activity

Abbreviations: AIDS -Acquired Immunodeficiency Syndrome;
MAP - Modified Atmospheric Packaging; LAB -Lactic Acid Bacteria

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