

Full Length Research

A Literature Review of Antimicrobial Activity of *Allium Sativum* (Garlic) Extract on Bacteria Isolated from Smoked Fish

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Abstract: The therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years of different microorganisms. This study was undertaken with the aim to investigate the antimicrobial activity of *Allium sativum* (garlic) extract on bacteria isolated from smoked fish. The study adopts an extensive review of literature such as conference papers, journal articles, internet sources, books to find out the effects of antimicrobial activities as reported by past authors. In this study, the authors reported that it is charming to observe how different cultures that have never come into contact with one another came to the same conclusion about the role of garlic (*Allium sativum*) in health and disease. The findings also showed that garlic has also proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *Staphylococcus aureus*. Furthermore, garlic extracts exhibited activity against both gram negative (*E.coli*, *Salmonella* species and *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Klebsiella*) and gram positive (*S. aureus*, *S. pneumonia*, Group A *Streptococcus* and *Bacillus anthracis*) all of which are causes of morbidity worldwide. Garlic is used for food preservation because of their bacteriocin based strategies. Allicin is a powerful antibiotic and antifungal in garlic. The research has provided a groundwork for other future researchers to conduct a scientific investigation into the use of *Allium sativum* in ethnomedicine and as a natural preservative agent in food.

Keywords: Antimicrobial Activity: *Allium Sativum* (garlic extract): Bacteria: Smoked Fish: Nigeria.

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1.0 Introduction of the Study

It is charming to observe how different cultures that have never come into contact with one another came to the same conclusion about the role of garlic (*Allium sativum*) in health and disease. Some of the earliest references to this medicinal and culinary plant are found on Sumerian clay tablets dating from 2600-2100 BC (Moyers, 2006; Lawson, 2008). Garlic was an important medicine to the ancient Egyptians listed in the medical text Codex Ebers (ca. 1550 BC) especially for the working class involved in heavy labor (Moyers, 2006; Lawson, 2008). There is evidence that during the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina (Lawson, 2008). Garlic has been in use since ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease (Yeh & Liu, 2001; Kris- Etherton, 2002), and regular use of garlic may help to prevent cancer, to treat malaria, and to raise immunity. Garlic has also proposed to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *Staphylococcus aureus*. Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different micro-organisms. For example, antifungal, antiviral, antibacterial, antiseptic and anti-inflammatory properties of garlic are well documented. Okhani (2018) stressed that garlic extracts exhibited activity against both gram negative (*E.coli*, *Salmonella* species and *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Klebsiella*) and gram positive (*S. aureus*, *S. pneumonia*, Group A *Streptococcus* and *Bacillus anthracis*) all of which are causes of morbidity worldwide. This study will focus on recent research on protective effects of garlic against some selected bacteria and fungi isolated from smoked fish.

Garlic qualifies as a great vegetable because not only is it an indispensable ingredient. Modern garlic are of two species, *Allium sativum* the soft neck which are excellent keeper but often mild and *Alliumophioscorodon* the hard neck which has more intense flavor but less storage capability. Taste, storage ability and suitability in growing are critical factors in selecting garlic classes of interest. According to Sofowora (2003), African medicinal plants rank highest among plants used in the investigation of antimicrobial properties and this is due to their high traditional use and also the ease of carrying out such tests. Garlic is used for food preservation because of their bacteriocin based strategies (Raman et al., 2011) and garlic is used as natural preservatives in poultry and meat products. There is extensive literature on the antibacterial effects of fresh garlic juice, aqueous and ethanolic extracts, lyophilized powders, steam distilled oil and other commercial preparations of garlic. Fenwick & Hanely (1985) undertook a thorough review of the antifungal effects of garlic and other allium vegetables up to mid 1984. More recently, the antibacterial effects of garlic have been studied by Reuter *et al.* (2006) and the *in vitro* antimicrobial properties of aqueous garlic extract against multidrug-resistant bacteria and *Candida* spp. have also been studied by Iwalokun *et al.* (2004). But this present study will test on ethanolic extract of garlic *in vitro* for its microbial activity against some bacteria isolated from smoked fish (Iwalokun *et al.*, 2004).

This study intends to explain the reason why garlic is often used to improve the shelf-life of smoked fish by inhibiting the activities of microorganisms and reducing their proliferation rate Iwalokun *et al.* (2004). The benefit in understanding the study of the phytochemical screening and antimicrobial analysis of garlic extract of *Allium sativum* is proved based on the traditional uses of plant for the preservation of foods like smoked fish, and the series of scientific investigations into various activities of the plants. However, the aim of the study is to investigate the antimicrobial activity of garlic extract on bacteria isolated from smoked fish.

2.0 Literature Review of the Study

2.1 Garlic

Allium sativum, commonly known as garlic, is a species in the onion genus, *Allium*. With a history of human use of over 7,000 years, *Allium sativum* is a bulbous plant. It grows up to 1.2m (4ft) in height (Chala et al., 2022). It produces hermaphrodite flower. Pollination occurs by bees and other insects. Garlic is native to central Asia (Enyi et al., 2022), and has long been a staple in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. It was known to Ancient Egyptians, and has been used for both culinary and medicinal purposes (Simonetti, 2011).

2.2 Scientific Classification of Garlic

Kingdom:	Plantae
Clade :	Angiosperm
Clade:	Monocot
Order:	<i>Asparagalles</i>
Family:	<i>Amaryllidaceae</i>

Sub-family: *Allioideace*
Genus: *Allium*
Specie: *Allium sativum*

Source: Kewscience Royal Botanic Garden (2005)

2.3 Properties of Garlic

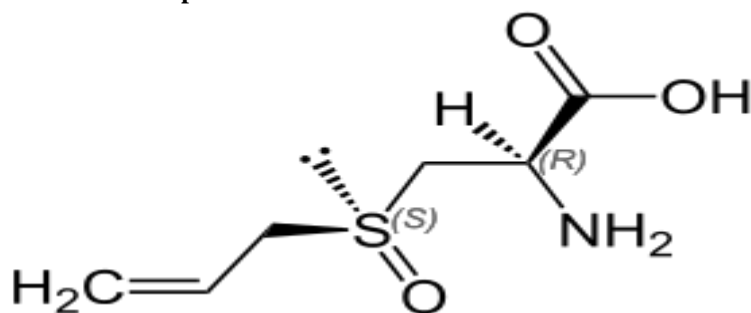


Figure 1: Alliin, a sulfur-containing compound found in garlic (Colin *et al.*, 2004 cited in Okhani, 2018).

When crushed, *Allium sativum* yields allicin, an antibiotic and antifungal compound (phytoncide) discovered by Chester J. Cavallito and colleagues in 1944 (Okhani, 2018). Fresh or crushed garlic also affords the sulfur-containing compounds alliin, ajoene, diallylpolysulfides, vinyldithiins, *S*-allylcysteine, and enzymes, B vitamins, proteins, minerals, saponins, flavonoids, and Maillard reaction products, which are not sulfur-containing compounds. Furthermore, a phytoalexin (allixin) was found, a nonsulfur compound with a γ -pyrone skeleton structure with antioxidant effects, antimicrobial effects, antitumor promoting effects, inhibition of aflatoxin B2 DNA binding, and neurotrophic effects. Allixin showed an antitumor promoting effect *in vivo*, inhibiting skin tumor formation by TPA and DMBA initiated mice. Analogs of this compound have exhibited antitumor promoting effects in *in vitro* experimental conditions. So allixin and/or its analogs may be useful compounds for cancer prevention. The composition of the bulbs is approximately 84.09% water, 13.38% organic matter, and 1.53% inorganic matter, while the leaves are 87.14% water, 11.27% organic matter, and 1.59% inorganic matter (Block, 2010). The phytochemical are responsible for the sharp flavor of garlic produced when the plant's cells are damaged (Huber, 2014). When a cell is broken by chopping, chewing, or crushing, enzymes stored in cell vacuoles trigger the breakdown of several sulfur-containing compounds stored in the cell fluids (cytosol). The resultant compounds are responsible for the sharp or hot taste and strong smell of garlic. Some of the compounds are unstable and continue to react over time. Among the members of the onion family, garlic has by far the highest concentrations of initial reaction products, making garlic much more potent than onion, shallot, or leeks. Although many humans enjoy the taste of garlic, these compounds are believed to have evolved as a defensive mechanism, deterring animals such as birds, insects, and worms from eating the plant (Macpherson *et al.*, 2009). Because of this, people throughout history have used garlic to keep away insects such as mosquitoes and slugs. A large number of sulfur compounds contribute to the smell and taste of garlic. Garlic has scientifically proven medicinal properties, it contains a substance called Allicin, which has antibacterial properties that is equivalent to weak penicillin (Okhani, 2018).

Alliin has been found to be the compound most responsible for the "hot" sensation of raw garlic. Allicin is a powerful antibiotic and antifungal in garlic, it does not occur naturally, instead garlic cloves contain the amino acid alliin (*S*-allylcysteine sulphoxide), (Huber, 2014). This chemical opens thermo-transient receptor potential channels that are responsible for the burning sense of heat in foods. The process of cooking garlic removes allicin, thus mellowing its spiciness. Allicin, along with its decomposition products diallyl disulfide and diallyltrisulfide, are major contributors to the characteristic odor of garlic, while other allicin-derived compounds, such as vinyldithiins and ajoene show beneficial *in vitro* biological activity (Block, 2010). Because of its strong odor, garlic is sometimes called the "stinking rose". When eaten in quantity, garlic may be strongly evident in the diner's sweat and garlic breath the following day. This is because garlic's strong-smelling sulfur compounds are metabolized, forming allyl methyl sulfide. Allyl methyl sulfide (AMS) cannot be digested and is passed into the blood. It is carried to the lungs and the skin, where it is excreted. Since digestion takes several hours and release of AMS several hours more, the effect of eating garlic may be present for a long time (Block, 2010).

Because of the AMS in the bloodstream, it is believed by some to act as a mosquito repellent, but no clinically reported evidence suggests it is actually effective. Abundant sulfur compounds in garlic are also responsible for turning garlic green or blue during pickling and cooking. Under these conditions (acidity, heat) the sulfur-containing compound alliinase react with common amino acids to make pyrroles, clusters of carbon-nitrogen rings (Shinsuke *et al.*, 2006). These rings can be linked together into polypyrrole

molecules. Ring structures absorb particular wavelengths of light and thus appear colored. The two-pyrrole molecule looks red, the three-pyrrole molecule looks blue and the four-pyrrole molecule looks green (like chlorophyll, a tetrapyrrole). Like chlorophyll, the pyrrole pigments are safe to eat.

2.4 Uses of Garlic

2.4.1 Culinary uses

Garlic is widely used around the world for its pungent flavor as a seasoning or condiment. The garlic plant's bulb is the most commonly used part of the plant. With the exception of the single clove types, garlic bulbs are normally divided into numerous fleshy sections called cloves. Garlic cloves are used for consumption (raw or cooked) or for medicinal purposes. They have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking. Other parts of the garlic plant are also edible. The leaves and flowers (bulbils) on the head (spathe) are sometimes eaten. They are milder in flavor than the bulbs, and are most often consumed while immature and still tender. Immature garlic is sometimes pulled, rather like a scallion, and sold as "green garlic" (Thompson, 1995). When green garlic is allowed to grow past the "scallion" stage, but not permitted to fully mature, it may produce a garlic "round", a bulb like a boiling onion, but not separated into cloves like a mature bulb (Thompson, 1995). It imparts a garlic flavor and aroma in food, minus the spiciness. Green garlic is often chopped and stir-fried or cooked in soup or hotpot in Southeast Asian (That is, Vietnamese, Thai, Lao, Cambodian, Singaporean) and Chinese cookery, and is very abundant and low-priced. Additionally, the immature flower stalks (scapes) of the hard neck and elephant types are sometimes marketed for uses similar to asparagus in stir-fries.

Borrelli *et al.* (2007) suggested that inedible or rarely eaten parts of the garlic plant include the "skin" covering each clove and root cluster. The papery, protective layers of "skin" over various parts of the plant are generally discarded during preparation for most culinary uses, though in Korea immature whole heads are sometimes prepared with the tender skins intact. The root cluster attached to the basal plate of the bulb is the only part not typically considered palatable in any form. Garlic is a fundamental component in many or most dishes of various regions, including eastern Asia, South Asia, Southeast Asia, the Middle East, northern Africa, southern Europe, and parts of South and Central America. The flavor varies in intensity and aroma with the different cooking methods. It is often paired with onion, tomato, or ginger. The parchment-like skin is much like the skin of an onion, and is typically removed before using in raw or cooked form. An alternative is to cut the top off the bulb, coat the cloves by dribbling olive oil (or other oil-based seasoning) over them, and roast them in an oven. Garlic softens and can be extracted from the cloves by squeezing the (root) end of the bulb, or individually by squeezing one end of the clove (Khoo & Aziz, 2009). In Korea, heads of garlic are fermented at high temperature; the resulting product, called black garlic, is sweet and syrupy, and is now being sold in the United States, United Kingdom and Australia. Garlic may be applied to different kinds of bread, usually in a medium of butter or oil, to create a variety of classic dishes, such as garlic bread, garlic toast, bruschetta, crostini and canapé. Oils can be flavored with garlic cloves. These infused oils are used to season all categories of vegetables, meats, breads and pasta. Garlic, along with fish sauce, chopped fresh chili, lime juice, sugar and water, is a basic essential item in dipping fish sauce, a highly used dipping sauce condiment used in Indochina. In East and Southeast Asia, chili oil with garlic is a popular dipping sauce, especially for meat and seafood. Tuongottoi Viet Nam (Vietnam Chili Garlic Sauce) is a highly popular condiment and dip across North America and Asia.

In some cuisines, the young bulbs are pickled for three to six weeks in a mixture of sugar, salt, and spices (Lissiman *et al.*, 2012). In Eastern Europe, the shoots are pickled and eaten as an appetizer. Laba garlic, prepared by soaking garlic in vinegar, is a type of pickled garlic served with dumplings in northern China to celebrate the Chinese New Year. Lightly smoked garlic is becoming increasingly popular in British and European cuisine. It is particularly prized for stuffing poultry and game, and in soups and stews. In both these cases it is important to utilize the undiscarded skin, as much of the smoke flavor is situated there, rather than in the cloves themselves. Immature scapes are tender and edible. They are also known as "garlic spears", "stems", or "tops". Scapes generally have a milder taste than the cloves (Ried *et al.*, 2006). They are often used in stir frying or braised like asparagus. Garlic leaves are a popular vegetable in many parts of Asia. The leaves are cut, cleaned, and then stir-fried with eggs, meat, or vegetables. Mixing garlic with egg yolks and olive oil produces *aioli*. Garlic, oil, and a chunky base produce *skordalia*. Blending garlic, almond, oil, and soaked bread produces *ajoblanco*. Yoghurt mixed with garlic and salt is a common sauce in Eastern Mediterranean cuisines.

2.4.2 Nutrients and Research

Some human studies found garlic supplementation to produce small reductions in blood cholesterol (Durak *et al.*, 2004), while an NCCAM-funded study found no effect. According to a meta-analysis from 2009, garlic has no beneficial effect on serum cholesterol levels either in healthy people or in people with hypercholesterolemia (Khoo & Aziz, 2009). A 2010 placebo-controlled trial, involving patients with hypertension, found a small effect of garlic extract (Ried *et al.*, 2006), but such research is considered preliminary and inconclusive (Borrelli *et al.*, 2007). As garlic may reduce platelet aggregation (Rahman, 2007), patients taking anticoagulant medication are cautioned about consuming garlic.

One news source reported garlic supplements may prevent the common cold, but there is insufficient clinical research to confirm this effect (Brinker, 2008). A 2012 report in the Cochrane Database of Systematic Reviews concluded that "there is insufficient clinical trial evidence regarding the effects of garlic in preventing or treating the common cold. A single trial suggested that garlic may prevent occurrences of the common cold but more studies are needed to validate this finding. Claims of effectiveness appear to rely largely on poor-quality evidence" (Lissiman *et al.*, 2012). Garlic was used as an antiseptic to prevent gangrene during World War I and World War II (Lissiman *et al.*, 2012).

2.4.3 Other Uses of Garlic

The sticky juice within the bulb cloves is used as an adhesive in mending glass and porcelain. An environmentally benign garlic-derived polysulfide product is approved for use in the European Union (under Annex 1 of 91/414) and the UK as a nematocide and insecticide, including for use for control of cabbage root fly and red mite in poultry (Ried *et al.*, 2006). Garlic along with cinnamon is used as a fish and meat preservative, and displays antimicrobial property at temperatures as high as 120 degree Celsius; the combination can also be used to preserve fried and deep fried foods, and in the future might be used in an inner layer of plastic (Verma, 2012; Deen *et al.*, 2013).

2.5 Smoked Fish as Source of Food

Smoked fish is fish that has been cured by smoking. Foods have been smoked by humans throughout history. Originally this was done as a preservative (Ried *et al.*, 2006). In more recent times fish is readily preserved by refrigeration, salting, fish fermentation, drying and dehydration, pickling and spicing, canning and freezing and the smoking of fish is generally done for the unique taste and flavor imparted by the smoking process. The major steps in the preparation of smoked fish are salting (bath or injection of liquid brine or dry salt mixture), cold smoking, cooling, packaging (air/vacuum or modified), and storage. Smoking, one of the oldest preservation methods, combines the effects of salting, drying, heating and smoking. Typical smoking of fish is either cold (28–32°C) or hot (70–80°C). Cold smoking does not cook the flesh, coagulate the proteins, inactivate food spoilage enzymes, or eliminate the food pathogens, and hence refrigerated storage is necessary until consumption (Alasalvar *et al.*, 2011).

Smoked fish is very important in West African coastal and lagoon fishing communities and it is an important food source in west and central Africa (Oduneye, 2010). It is usually smoke-dried for 2 to 5 days, depending on size and on the market. Smoke-drying is done over a fire. The fish is placed on sticks, bars or wire mesh trays about 1m from the floor. A fire is lit on the floor and the fish is first cooked over a high fire, then the fire is reduced to a shouldering fire which is kept going for as long as necessary smoking "ovens" can be open without walls or closed with walls either in the outside air or inside a smoke house (Okonko *et al.*, 2008). A hard-smoked fish can be kept for several months in ambient temperature. Smoke-drying of fish is essentially a drying process to preserve the product in the absence of refrigeration. It is different from fish smoking as it is known in Europe, USA, Canada etc, where it is applied to impart taste, such as smoked salmon (cold smoked) or smoked cell (hot smoked) which must be stored under refrigeration (Pekzar *et al.*, 1996).

Fish are very important resources for human worldwide because they provide a source of protein and vitamin for human, they are also used in making fish oil and various medicine. Fish helps to balance out nature because they eat plant and other fish and they serve as food to other animal. Their kingdom is Animalia and their phylum is chordate. Fishes have number of economic importance they serve as food, fish oil, fish skin and leather, medicine and disease control along with some harmful effects which include destructive, injurious and poisonous (Alasalvar *et al.*, 2011). Consumption of fish in Nigeria, demand for fish is on the increase due primarily to the health benefits of eating fish. However, fish is an extremely perishable food and this consequently resulted in colossal loss due to post harvest spoilage. A number of physical and chemical changes occur in fish after harvest (Post mortem) which causes of spoilage in fresh fish are: autolysis and bacterial decomposition (Eyo, 1993). At the death of fish certain endogenous biochemical changes occur which conditions the fish for proliferation by the spoilage organism (Adebayo, 1992), few hours after death, stiffening of the fish muscles occur whereby fish lose it flexibility through a process called rigor mortis (Wood, 1994).

According to Al-jufaili & Opara (2006) in artisanal fishery, freshly caught fish are covered with damp sacks and at times, they are mixed with wet grass or water weeds to reduce the temperature, fish treated this way is prone to contamination with microorganism such as bacteria and fungi. This indicates that spoilage of fish starts right from the aquatic Ecosystem; handling fishes are also prone to microbial attack especially of reducing temperature (Ukonu *et al.*, 2022; Ubreye Benjamin *et al.*, 2022; Ukonu *et al.*, 2022). During the drying period (smoking, sun drying, salted and drying) smoking kilns used in artisanal fishery and the over loading of the fishes on trays leads to improve processing which in turn encourages fungal attack (Eyo, 1993). During storage of dried fish products, good storage practices are not adhering by wholesales hence stores are not well ventilated and pest can easily gain access into the stores. The environment in which fish are displayed in the market is not always hygienic and this is another avenue for microbial contamination. Very often, retailers display the dried fish samples (smoked, sundried and slated and dried) in open trays beside the gutter on refuse heaps, this also encourages fungi attack and subsequent production of toxins (Thomas, 2009). This traditional method is followed for the preservation of fish especially in rural areas (Chakrabarti & Verma, 2004). Traditional drying is often rudimentary and good hygiene is rarely practiced. During the monsoon, when the humidity is high, drying cannot be achieved by

traditional methods. By this time, the fish can absorb the moisture and it serves as a habitat for microbial population such as bacteria, fungi and viruses and insect attack. In India about 17% of the total catch is being used for the production of dry fishes (JeyaShakila *et al.*, 2003). Protein available at cheaper price for the economically weaker sections of the society especially people residing in coastal areas (Prasad *et al.*, 1999).

Chichester & Graham (2011) stated that fish is harvested in relatively cleaner environments but during subsequent handling microbes of spoilage types and of public health significance type come in contact with the fish. Immediate cooling or salting of the catch is more important in tropical conditions because the ambient temperature is high and it leads to rapid spoilage (JeyaShakila *et al.*, 2003). The quality of salted and sun dried fishes are adversely affected by the occurrence of microorganisms. Determination of microbial quality of such processed fishes is very important for guarding consumer's health and hygiene (Lilabati *et al.*, 2009). Patterson & Ranjithan (2009) stated that the presence of the pathogenic loads in dried fishes is acquiring importance in views of the safety and quality of the seafood. The spoilage indicators and visible fungal attacks caused by micro-organism are known to adversely affect the quality of cured fishes. Apart from contaminated salted and dried fish other common sources of contamination are air and dust in and around fish processing place, contamination water and soil and unhygienic handling (Prabhakaran & Gupta, 2013). Curing is a simple and cheap method of processing requiring least technical expertise. But it has great significance and relevance in the socioeconomic system of small scaled fisher folk (Felix & Patterson, 2003).

3.0 Methodology of the Study

The study adopts an extensive review of literature such as conference papers, journal articles, internet sources, books to find out the effects of antimicrobial activities as reported by past authors with the help of standard literature procedures in their research work. These secondary sources of information were reviewed to understand the effect of crude ethanol extract of *Allium sativum* and isolated bacteria for susceptibility by agar. The bacteria isolated from smoked fish were *Bacillus spp.*, *Staphylococcus spp.*, *Escherichia spp.*, *Pseudomonas spp.* and *Salmonella spp.*

4.0 Conclusion of the Study

This study used an extensive review of literature to examine the determination of the antimicrobial activity determined by past authors with the help of standard literature procedures in their research work. Results of this investigation suggest that smoked fish maybe vectors in the transmission of overt or opportunistic pathogenic microorganisms as well as in the spread of multidrug resistant bacteria and fungi strains. In this literature review study, although the isolated microorganisms were susceptible to the test extract and control, authors in this study found that their presence in smoked fish requires attention. The research has provided a groundwork for other future researchers to conduct a scientific investigation into the use of *Allium sativum* in ethnomedicine and as a natural preservative agent in food. Further study could also help in discovering of new classes of substances that are of little or no toxic effects and are economically reliable and may serve as agents of infectious disease chemotherapy and control. This research has also effectively demonstrated and explicitly revealed information on the antimicrobial activity of *Allium sativum* at different concentrations.

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