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Anti-Bacterial Activity On Vibrio Spp. By Plant Extracts In Thaijo Database

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Abstract

Thailand is currently regarded as one of the top 10 countries in the world for both seafood consumption and exports. Consumption of raw seafood also carries a high risk of foodborne bacterial outbreaks, which can significantly affect the quality of life for consumers such as Vibrio spp. contamination. Bacterial infections in the gastrointestinal system can contribute to the development of antimicrobial resistance, a significant public health concern in Thailand and many countries in the world. Resistance to antimicrobial agents has led to insufficient effectiveness in controlling outbreaks, particularly those caused by Vibrio spp. bacteria. Resistance to antimicrobial agents is therefore an important part of the interest in searching for new antimicrobial agents to treat bacterial infections. The plant has medicinal properties, and its antibacterial properties have been widely studied. Thus, it is hypothesized that humans may utilize these substances to inhibit Vibrio spp. and potentially develop to another resistance bacteria. This article therefore aims to explore the antibacterial activity of Vibrio spp., especially in Thailand, to disseminate the data to researchers and those interested in seeing progress in research on plant extracts, that have been tested against Vibrio spp., also information on bioactive compounds, from the Thai Journals Online (ThaiJO) database via "vibrio and extract" keywords. This review may be a starting point for exploring the use of plant extracts, that have been tested for their ability to inhibit Vibrio spp. in Thailand and can be the information to other researchers to screening or finding the new plant for gastrointestinal and other bacterial infection.

Keywords: Vibrio spp., Bioactive compounds, Antibacterial activity

Introduction

Thailand is currently ranked among the top ten countries globally for the consumption and export of seafood products. The issue of bacterial contamination in seafood and fresh food remains common, with a high risk of cross-contamination to other foods, increasing the likelihood of foodborne bacterial outbreaks, such as contamination by *Vibrio* spp. in fresh food (1). This issue represents a significant public health concern affecting consumer quality of life and leading to the emergence of antimicrobial-resistant bacteria, a major public health problem in Thailand and many countries worldwide.

The emergence of antimicrobial resistance can result from several factors, including the use of antimicrobial agents, especially broad-spectrum antibiotics, improper, incomplete, or unnecessary consumption of these medications, and the excessive use of antimicrobials in agriculture. These practices have contributed to the increased development of drug-resistant bacteria, rendering antimicrobial agents ineffective in controlling the spread of diseases (2). This is particularly concerning for bacteria in the *Vibrio* spp. group, prompting interest in discovering new antimicrobial agents to treat bacterial infections, such as combining other molecules with existing antimicrobial agents (3).

Plants are another source with medicinal properties, and there has been extensive research on their antibacterial properties (4). For example, essential oils contain bioactive compounds that can destroy bacterial cell walls due to their ability to dissolve in oils. Furthermore, phytochemicals or bioactive compounds, such as flavonoids, alcohols, aldehydes, aromatic compounds, phenolics, steroids, and terpenoids, have all been reported to possess antibacterial properties. This suggests the potential use of these substances to combat *Vibrio* spp. and other bacteria (5).

Given the prevalence of *Vibrio* spp. contamination in food, the authors have become interested in the incidence of antimicrobial resistance in these bacteria and in exploring the antibacterial effects against *Vibrio* spp. through a review of the literature available in the Thai Journals Online (ThaiJO) database, which serves as the central electronic journal database of Thailand. The aim is to highlight the progress of research on plant extracts tested against *Vibrio* spp. that have been published in the ThaiJO database by searching for the keywords "vibrio and extract."

Additionally, the article provides general knowledge about bacterial antimicrobial resistance, specifically in *Vibrio* spp., and information on bioactive compounds in plant extracts that have been reported in ThaiJO concerning *Vibrio* spp. This article could serve as a starting point for exploring the use of plant extracts tested for their effectiveness in inhibiting *Vibrio* spp. in Thailand. It also offers preliminary information on the types and essential substances that other researchers have tested, allowing for further research or exploration of new plants to develop treatments for pathogenic bacteria in various systems in the future.

1. Antimicrobial Resistance in Bacteria

Currently, bacterial infections resistant to antimicrobial agents are a significant and ongoing threat to human health, being a major cause of death worldwide. Antimicrobial resistance reduces or nullifies the effectiveness of existing medications (2). As a result, various studies are being conducted to find methods to control bacterial resistance to antimicrobials, focusing on combining other molecules with existing antimicrobial agents. These may include substances without intrinsic antibacterial properties, such as Clavulanic acid, which is used to inhibit bacteria capable of producing β-Lactamases (3).

The common mechanisms of bacterial inhibition by antimicrobial agents include:

Bacterial Protein Biosynthesis: This occurs by inhibiting ribosomal subunits of bacteria, which includes antimicrobial agents in the Macrolides, Tetracyclines, Aminoglycosides, and Oxazolidinones groups (2).

Cell-Wall Biosynthesis: This is achieved by inhibiting Transglycosylases and Transpeptidases, which are target enzymes of the Penicillins and Cephalosporins antimicrobial groups. Additionally, Glycopeptide antimicrobials, such as Vancomycin, inhibit Peptidoglycan synthesis (6).

Nucleic Acid Synthesis: This is done by inhibiting the enzyme DNA gyrase, preventing bacteria from unwinding, replicating, and repairing their DNA strands. Antimicrobials in this group include Nalidixic acid and Fluoroquinolones, such as Ciprofloxacin (7).

Destruction of Bacterial Membrane: Agents like Polymyxins bind to the Lipid A of Lipopolysaccharides, affecting the transport of substances and ions into and out of bacterial cells. The destruction of the cell membrane leads to leakage and aggregation of substances in the cytoplasm (2).

Bacterial resistance to antimicrobial agents can be divided into two main mechanisms: resistance through the synthesis of various substances or other molecules by the cell and natural evasion mechanisms commonly

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found in bacteria. The general mechanisms of bacterial resistance to antimicrobials include:

Efflux Pump: A primary mechanism that allows bacteria to resist multiple types of antimicrobials by rapidly transporting substances out of the cell. Both Lipophilic and Amphipathic molecules can be expelled from the cell.

Porin Modification: This mechanism limits the entry of antimicrobials into the cell by controlling the transport through porin channels, which are the main pathways for water and nutrient transport into and out of the cell, particularly in Gram-negative bacteria (8).

Destroying the Antibacterial Agents: This occurs through the production of hydrolytic enzymes, such as β-lactamase, which break down the β-lactam ring found in drugs like Penicillins, Cephalosporins, and Carbapenems (2).

Modification of Antibiotics: This involves altering the functional groups of antimicrobial agents, such as Aminoglycosides, preventing the drug from binding to RNA and thereby failing to inhibit bacterial protein synthesis (9).

Altered Target: For example, resistance to Penicillin may occur due to changes in Penicillin-binding proteins (PBPs), reducing the specificity between the target molecule and the antimicrobial agent (2).

Due to bacterial resistance to antimicrobials, there is a growing interest in developing phytochemicals derived from plants to combat this resistance. These substances can act alone or in combination with antibiotics to enhance their effectiveness against multiple bacterial species. However, the relationship between the structure and mechanism of action of most natural compounds remains poorly understood (2).

2. Vibrio spp.: Bacterial Ecology and Public Health Significance

Vibrio spp. is Gram-negative, curved rod-shaped bacteria that move using polar flagella. They belong to the Proteobacteria phylum, Gamma-proteobacteria class, Vibrionales order, and Vibrionaceae family. These bacteria inhabit environments containing water and can tolerate varying levels of salinity, including freshwater, brackish water, and seawater. Most Vibrio spp. is classified as chemoorganotrophic bacteria, using organic compounds as their carbon source. They can break down nutrients through both aerobic respiration and fermentation, producing acids such as formic, lactic, acetic, and succinic acids, with end products like ethanol and pyruvate. This metabolic diversity distinguishes them from the Enterobacteriaceae family, which can break down fewer types of sugars.

The Vibrio genus is highly diverse, comprising approximately 147 species. The species pathogenic to humans include V. cholerae, V. parahaemolyticus, and V. vulnificus. Other species are often pathogenic to animals, such as fish, shrimp, coral, and crustaceans, while most species in the genus are non-pathogenic, with some being symbionts (10). Vibrio spp. can produce enzymes like oxidase, indole, and citrate, and are capable of reducing nitrate to nitrite and breaking down urea compounds. Additionally, they can degrade substances such as gelatin, starch, chitin, and alginate using extracellular enzymes (11).

The public health significance of Vibrio spp. is primarily due to species such as V. cholerae and V. parahaemolyticus, which are enteropathogenic bacteria that cause gastrointestinal diseases in humans. These infections range from mild to moderate severity in healthy individuals but can be severe in people with underlying health conditions such as diabetes, liver disease, cancer, HIV, thalassemia, and immunodeficiencies, as well as in infants. Furthermore, V. parahaemolyticus outbreaks commonly occur in coastal areas of temperate and tropical regions. This species does not produce enterotoxins but can cause wound infections in humans similar to V. vulnificus, which often results from contamination in seafood, water, consumption of contaminated or undercooked seafood (especially shellfish), or contact of wounds with contaminated water.

Most outbreaks occur during warm weather when the optimal growth temperature for Vibrio spp. ranges from 30 to 37 degrees Celsius (10). Virulence factors that increase the pathogenicity of Vibrio spp. include the ability to produce various enzymes, such as hemolysins, which are toxins significant in the disease process. For example, Thermostable Direct Hemolysin (TDH) in V. parahaemolyticus and Hemolysin A (HlyA) in V. cholerae are important in disease development (12). Additionally, extracellular proteases, such as collagenases and gelatinase, play roles in virulence (13).

3. Antimicrobial Resistance and the Antibacterial Activity of Plant Extracts Against Vibrio spp. in Thailand

Currently, the antimicrobial agents used to treat Vibrio spp. infections (vibriosis) include Tetracycline, Oxytetracycline, Quinolones, Trimethoprim, Potentiated sulfonamides, Oxolinic acid, and Sarafloxacin (14). The incidence of antimicrobial resistance remains a growing concern, which may be due to the use of first-line drugs, especially broad-spectrum antibiotics, which may be taken incorrectly, incompletely, or unnecessarily, leading to an increase in drug-resistant bacteria. Additionally, the excessive use of antimicrobials in agriculture is another factor contributing to the reduced effectiveness of these agents in controlling the spread of diseases caused by Vibrio spp. (15).

Furthermore, according to Sapcharoen and Duangrak (2024), Thailand has implemented a national strategic plan to manage antimicrobial resistance for the years 2017-2021. This plan aims to reduce the incidence of antimicrobial-resistant infections by 50%, decrease the use of antimicrobials in humans by 20%, reduce the use of antimicrobials in animals by 30%, increase public awareness of antimicrobial resistance and proper antimicrobial use by 20%, and achieve a national antimicrobial resistance management system that meets international standards, at least at level 4. This highlights that antimicrobial resistance remains a significant public health concern in Thailand.

Therefore, the challenge for scientists is to find new drugs to combat bacterial resistance. Medicinal plants have emerged as a promising source and have been increasingly studied (4). For example, essential oils contain bioactive compounds capable of inhibiting cell walls due to their good solubility in oils. Bioactive compounds such as flavonoids, alcohols, aldehydes, aromatic compounds, phenolics, steroids, and terpenoids have shown inhibitory effects against bacterial growth and may be used to counteract Vibrio spp. and other bacteria (5).

In Thailand, various plants and herbs have been studied for their antibacterial effects against Vibrio spp. For instance, the "Samut Noi" herbal formula, which includes black cumin (Nigella sativa L.), long pepper (Piper retrofractum Vahl), pomegranate (Punica granatum L.), and galls of oak (Quercus infectoria G. Olivier) (16). A review of the literature on the inhibition of Vibrio spp. using plant extracts published in Thai Journals Online (ThaiJO), the central electronic journal database of Thailand, found 19 articles (Table 1). The data indicate that most studies on plants and herbs published in the ThaiJO database in Thailand still focus on using crude extracts to inhibit the growth of Vibrio spp. Most studies lack the identification and classification of key bioactive compounds derived from plants.

Table 1: Studies on the Antibacterial Activity Against Vibrio spp. Of Plant Extracts Published in the Thai Journals Online (ThaiJO) Database

Scientific names	Common names	Bioactive compounds	References
Curcuma longa Linn.	Turmaric	Curcuminoids	(17–20)
Suaeda maritima (L.) Dumort	Annual seablit	Total phenolic content (TPC), Total Flavonoid content (TFC)	(21)
Terminalia catappa L.	Bengal Almond	-	(22)
Psidium guajava Linn.	Guava	-	
Andrographis paniculata (Burm.f.) Nees	Kariyat	-	

Clidemia hirta (L.) D. Don	Khlong khleng yuan	Tannins, Flavonoids, Saponins, Terpenoids	(23)
Lantana camara Linn.	Lantana	-	
Melastoma malabathricum L.	Malabar melastome	-	
Caulerpa racemosa var. corynephora	Caulerpa sertulaeioides	-	(24)
Artocarpus lakoocha Roxb.	Lok Hat	-	(25)
Chlorella minutissima (Chm1)	Chlorella	TPC	(26)
Ulva intestinalis	Ulvaintestinalis Linnaeus	-	(27)
Gracilaria fisheri	Gracilaria seaweed	-	
Bruguiera cylindrica (L.) Blume	White Kidneys Beans	Anthraquinone, Terpenoid, Flavonoid, Saponin, Phenolic, Alkaloid	(28)
Cannabis sativa L. subsp. sativa	Hemp	-	(29)
Phyllanthus urinaria L.	Chamber bitter	-	(30)
Allium tuberosum Rottler ex Spreng.	Garlic chives	-	(31)
Caesalpinia sappan Linn.	Sappan Tree	-	(32)
Stevia rebaudiana Bertoni.	Stevia	-	(33)
Sargassum sp.	Sargassum	-	(34)
Capparis zeylanica Linn.,	Chinese Tallow Tree	-	(35)
Caryota maxima,	Jaggery palm	-	
Markhamia stipulata,	Artemisia	-	
Amphineurion marginatum	Aganosma acuminata (Roxb.)	3′-Acetyllycopsamine, Lupeol	
Tristaniopsis burmanica	Tristaniopsis rufescens (Hance)	-	
Allium sativum L.	Garlic	-	(36)
Allium chinense G.Don.	Garlic	-	
Elephantopus scaber L.	Prickly-leaved elephant's foot	Phenolic, Flavonoid, Terpenoid, Steroid	(37)

Elephantopus mollis Kunth.	false elephant's foot		
Nigella sativa L.	Turmeric	-	(38)
Eurycoma longifolia Jack	Eurycoma longifolia Jack	TPC	(39)
Jasminum officinale L. f. *var. grandiflorum* (L.) Kob.	Spanish jasmine	TPC	(40)
Musa sp. *(Kluai Thepanom, Kluai Som, Kluai Hak Muk, M. acuminata* Colla.)	Banana (Kluai Thepphanom, Didymoplexiella siamensis (Rolfe), Silver Bluggoe, The Wild Banana)	-	(41)
Punica granatum L.,	Pomegranate	-	
*Sandoricum koetjape (*Burm.f.*)* Merr.	Sentul		
Dimocarpus longan Lour.	Longan	-	
Coffea arabica L.,	coffee	-	
Hevea brasiliensis Müll.Arg.	Para rubber	-	
Terminalia catappa L.	Tropical Almond	-	
Psidium guajava L.	Guava.	-	

4. Bioactive Compounds with Significant Roles in Inhibiting Vibrio spp.

The key bioactive compounds can be classified into Polyphenols, Alkaloids, Terpenoids, Organosulfur compounds, and Nitrogen-containing compounds (42). Based on a review of articles on the antibacterial activity against Vibrio spp. published in the ThaiJO database, the following bioactive compounds from plants were identified:

Curcumin or Diferuloylmethane (Curcuminoids): A polyphenolic flavonoid extracted from the roots of turmeric (C. longa L.) with bioactive properties against V. vulnificus, including inhibiting growth, motility, and preventing adhesion between the bacteria and host. Curcumin also interferes with the activity of RTX toxin, a toxin produced by bacteria to destroy host cells. Therefore, Curcumin is of interest as an alternative treatment for V. vulnificus and other bacterial infections (43).

Alkaloids: Heterocyclic nitrogen compounds that are colorless and slightly soluble in water. They can inhibit enzymes such as dihydrofolate reductase or topoisomerases, which are essential for DNA synthesis and cell division through the FtsZ protein. They also inhibit Sortase A enzyme, causing bacterial homeostasis loss, inhibit the outer membrane and cytoplasmic membrane, efflux pumps, and bacterial virulence factors (4).

Phenolic Compounds: Bioactive compounds that inhibit the growth of both Gram-positive and Gram-negative bacteria. These compounds are commonly found in plants and include Quercetin, Catechin, Ferulic acid, Vanillic acid, and Protocatechuic acid. They have been reported to reduce bacterial biofilm formation, inhibit motility, exopolysaccharide (EPS) production, and interfere with bacterial cell communication. Studies on V. parahaemolyticus have shown that Quercetin and morin can reduce bacterial motility and inhibit the enzyme Thermolabile hemolysin (TLH), which bacteria use to lyse human red blood cells (44).

Flavonoids: The largest group of bioactive compounds found in plants that can inhibit bacterial cell wall function. They act on the bacterial cell wall by binding to hydrogen atoms of the lipid molecules in the hydrophobic interior and hydrophilic interface. The binding of flavonoids with phospholipids can induce loss of bacterial cell wall function (45).

Tannins: A group of polyphenols comprising two subgroups: hydrolysable tannins (HT) and condensed tannins (CT). Tannins have iron-chelating properties, inhibit cell wall formation, and disrupt cell wall function. They also inhibit bacterial virulence factors, such as biofilm formation, bacterial cell communication, and enzymes like protease, phospholipase, urease, neuraminidase, and collagenase (46).

Saponins: Composed of a lipophilic sapogenin moiety and a hydrophilic sugar moiety, they exhibit foaming characteristics (47). There is no definitive study confirming the properties and mechanisms of saponins in inhibiting Vibrio spp.; however, plant extracts with high concentrations of saponins have shown antibacterial activity and the ability to inhibit bacterial virulence factors (48).

Alkaloids: A group of secondary metabolites in plants, with nitrogen atoms as the main structural components of their molecules, exhibiting significant biological activity, including antibacterial properties (47).

Terpenoids (Terpenes): Composed of hydrocarbon groups derived from a 5-carbon isoprene (C_5H_8) structure, terpenoids generally inhibit microbial growth through two mechanisms: reduced oxygen uptake, which disrupts growth, and interference with oxidative phosphorylation at the cytoplasmic membrane (49).

Anthraquinones: Polycyclic compounds with a quinone core structure, including cyclohexadiene diketone or cyclohexadiene dimethyl. They exhibit antibacterial activity by inhibiting biofilm formation, toxin production, and the synthesis of lipopolysaccharides and peptidoglycans in bacteria (50).

Acetyl Lycopsamine: Exhibits antibacterial activity. Additionally, Lupeol has anti-inflammatory and anticancer properties and is isolated from certain medicinal plants (37).

Steroids: Bioactive compounds characterized by a molecular structure containing at least one aromatic ring, which provides diverse biological properties, such as in medicine and pharmacology. Steroids are also found in other organisms like fungi and animals (51). However, steroids from plants and animals may not possess general antibacterial properties (52).

Conclusion

Currently, bacterial infections resistant to antimicrobial agents pose a significant threat to human health and are a leading cause of death worldwide. Antimicrobial resistance reduces or eliminates the effectiveness of existing drugs, prompting research into various methods to control bacterial resistance. One approach involves developing phytochemicals derived from plants to combat bacterial resistance, particularly against Vibrio spp.

Vibrio spp. are bacteria that live in aquatic environments and tolerate various levels of salinity, including freshwater, brackish water, and seawater. There are approximately 147 species of Vibrio, with species of significant public health concern, such as V. cholerae and V. parahaemolyticus, which are enteropathogenic bacteria causing gastrointestinal diseases in humans. Other species typically cause diseases in animals such as fish, shrimp, coral, and crustaceans.

In Thailand, various plants and herbs have been studied for their antibacterial effects against Vibrio spp. A review of the literature published in the Thai Journals Online (ThaiJO) database on the inhibition of Vibrio spp. by plant extracts revealed several studies using crude extracts. Most of these studies focused on compounds such as TPC (Total Phenolic Content), TFC (Total Flavonoid Content), tannins, flavonoids, saponins, terpenoids, anthraquinones, terpenes, phenolics, alkaloids, and steroids. However, there remains a lack of identification and classification of key bioactive compounds, resulting in an unclear understanding of the relationship between the

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structure and mechanisms of action of plant-derived bioactive compounds against Vibrio spp.

This study serves as a starting point for exploring the use of plant extracts to inhibit Vibrio spp. in Thailand. The review of literature published in the ThaiJO database indicates a need for further research to identify key bioactive compounds that effectively inhibit Vibrio spp. This research could also lead to the development of applications for other pathogenic bacteria in various systems in the future.

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