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## POTENTIAL UTILIZATION OF HOLY BASIL LEAVES (OCIMUM SANCTUM) IN FISHERY ACTIVITIES

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#### **KeyWords**

antibacterial, antioxidant, fish transportation, holy basil, preservative

#### ABSTRACT

Holy basil leaves can be used for more than just human nutrition; they can also be employed in aquaculture. Several studies have found that the biochemical composition of holy basil can be employed as an antibacterial, a preservative for fresh and processed fish, and an anesthetic in the transport of fish. Alcohol compounds, essential oils, and phenols are just a few of the chemical substances found in holy basil leaves that can stop germs from growing. Bacteriostatic and bactericidal properties are possible with these substances. Bacteriostatic traits allow bacteria to grow again after the antibacterial material has been removed, but bactericidal capabilities prevent germs from reproducing even after the antibacterial material has been removed. Ascorbic acid, b-carotene, b-sitosterol, eugenol, palmitic acid, and tannins are the primary chemical components in holy basil that have antioxidant characteristics.

#### INTRODUCTION

Indonesia is a tropical country that has a very high diversity of herbal plants. The use of herbal plants by the Indonesian people is very diverse, ranging from their application as medicine, spices, and to increase immunity. One of the plants that are widely grown and used by the people of Indonesia is the ruku-ruku (*Ocimum sanctum*) plant. Plants of the genus Ocimum are shrubs that grow upright and have a large number of branched. The group of plants from the genus Ocimum has a characteristic smell of fragrant leaves. Several species of members of this genus have been cultivated for a long time, but there are still many species that grow wild. Two types of plants from the genus Ocimum often cultivated are hoary basil (*Ocimum canum*) and holy basil (*Ocimum sanctum*) or *ruku-ruku* in local Indonesian language. These two types of plants are generally planted in gardens, yards or roadsides [1].

The use of holy basil leaves is not limited to providing food for humans, but can also be used in aquaculture activities. Several studies have shown that the biochemical content in the holy basil plant can be used as an anti-bacterial, a preservative for fresh and processed fish, as well as an anesthetic in the process of transporting fish. As also stated in [2], the benefits and efficacy of the holy basil plant lie not only in the leaves but in all parts including stems, flowers, and roots. Holy basil plant has activity as an antioxidant, antimicrobial, anti-inflammatory, antimetastatic, and many more, hence that there are many uses of the ruku-ruku plant in the broader community, it is necessary to provide information about the safety of the holy basil plant from the results of general and specific toxicity.

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#### PLANT MORPHOLOGY AND DISTRIBUTION OF HOLY BASIL

Holy basil plants grow in the tropics and subtropics. This plant grows upright with a height between 30-75 cm, has many branches and has fine hairs. The stems of young plants are green and will turn brown after the plants age [3], [4]. The leaves of this plant are single, opposite each other, and arranged from bottom to top. The length of the petiole is about 0.25-3 cm with each leaf blade ovate to elliptical, elongated, and the tip is tapered or blunt. The base of the leaves of this plant is peg-shaped to rounded and has fine hairs on both surfaces. Leaf edges are weakly serrated, wavy, or flat [5]. The leaf blade is oval in shape, the tip is tapered, looks wavy and there are 3-6 branch bones with slightly jagged leaf edges. The leaves are about 2.5-7.5 cm long and 1-2.5 cm wide [6]. The shape of the holy basil plant can be seen in Figure 1.



Figure 1. Holy basil (Ocimum sanctum)

Holy basil plants according to [7] are classified as follows:

Kingdom	: Plantae
Phylum	: Spermatophyta
Class	: Magnoliopsida
Order	: Lamiales
Family	: Lamiaceae
Genus	: Ocimum
Species	: Ocimum sanctum

This plant considered as a shrub, has a taproot, a woody, rectangular, hairy, branched, light green stem and has a height of 60 cm. The flowers of this plant are compound in the form of bunches, hairy, have protective leaves that are elliptical in shape, with purplish white flowers [8]. The fruit part is box-shaped, dark brown, upright, with the tip forming a circular hook with a petal length of 6-9 mm. The fruit seeds are small, hard type, dark brown, and when taken can immediately expand. Each fruit consists of four seeds [5], [9].

In general, there are several species of holy basil plants. The morphological differences of various Ocimum species can be seen in Table 1.

Туре	Local Name	Height	Number of Branch	Stem diameter	Leaf Width	Leaf Length
Ocimum gratissimum	Ruku-ruku	140,58	21	2,3	9,56	12,78
	hutan					
Ocimum minimum	Selasih	81,47	19,4	1,23	2,41	6,38
	ngombol					
Ocimum basilicum (green	Basil	90,03	16,18	1,17	3,54	5,57
leaves)						
Ocimum basilicum	Basil	97,89	29	1,47	3,36	6,70
(purplish leaves)						
Ocimum canum	Kemangi	97,54	11,6	1,44	2,50	4,31
Ocimum sanctum	Ruku-	80,58	12,44	1,33	2,16	3,82
	ruku					

#### Table 1. Morphology of various species of Ocimum

Source: [10]

Holy basil plants are widely found in Java and Madura, easy to grow in dry places at an altitude of 1 - 1,100 meters above sea level, grow on roadsides, edges of gardens or fields, dry rice fields, parks, open forests, and meadows. Holy basil can grow wild or be cultivated [5], [11], [12]. Ruku-ruku leaves from the ruku-ruku plant (*Ocimum sanctum*), the Labiate family, are known by different names in several areas, including on the island of Sumatra: balakama, kemangi utan, ruku-ruku (West Sumatra), Java: klampes, lampes (West Java), kemangen (Central Java), Madura: kemangi, ko-roko, Nusa Tenggara/Bali: uku-uku, Sulawesi (Celebes): balakama (Manado), Maluku: lufe-lufe (Ternate) [13]. Holy basil leaves have a clove-like aroma and taste spicy in the mouth [14].

Holy basil plant comes from India and other South Asian countries. Holy basil plants are found growing naturally in moist soils almost all over the world [15]. Medicinal plants have been widely used by pharmacists. Holy basil are known as Tulsi "queen of plants" because they have great potential to prevent and cure disease [16].

#### THE CONTENT OF THE RUKU-RUKU PLANT AND ITS BENEFITS

Traditionally this plant can be used as a medicine for bronchitis, malaria, diarrhea, dysentery, skin diseases, arthritis, eye diseases, insect bites, and so on [17]. Basil and ruku-ruku have uses as spices in cooking. The parts of the holy basil plant that can be used as medicine are leaves, stems, flowers, roots, and seeds.

The benefits as a medicinal ingredient or as a spice from these two types of plants are more closely related to their aroma. The aroma that comes from the leaves of these two types of plants can reduce the fishy smell of wet fish. Based on studies on the genus Ocimum, generally plants of this genus contain compounds of alkaloids, flavonoids, tannins, saponins, triterpenoids, and essential oils [18].

The holy basil plant has aromatic, gastric, carminative (relieves flatulence), diuretic (urine formation) and expectorant (cough with phlegm) functions [19]. The content that plays a very important role is the essential oil. Holy basil essential oil has antifungal activity against *Aspergillus niger*, *Rhizopus stolinifera* and *Penicillium digitatum*. The water extract of the holy basil plant showed inhibition of the growth of *Klebsiella* sp., *Eschericia coli*, *Proteus* sp. and *Staphylococcus aureus*, even the alcohol extract of holy basil can inhibit the growth of *Vibrio cholera* [20]. Holy basil plants contain a lot of nutrients and their contents can be seen in Table 2.





Several groups of chemical compounds contained in holy basil leaves can inhibit the growth of bacteria such as alcohol compounds, essential oils and phenols. These compounds can be bacteriostatic and bactericidal [22]. Bacteriostatic properties have the characteristic that when the antibacterial material is removed, bacteria can grow again, while in bactericidal properties, bacteria that have died cannot reproduce even though the antibacterial material has been removed [23]. The main chemical compounds of holy basil which have antioxidant properties are ascorbic acid, b-carotene, b-sitosterol, eugenol, palmitic acid, and tannins [24].

#### HOLY BASIL LEAVES AS AN ANTIMICROBIAL AGENT

Antimicrobial compounds are biological or chemical compounds that can inhibit the growth and activity of microbes. Antimicrobial compounds as food additives are commonly used for the purpose of preventing spoilage or poisoning caused by microorganisms in food [25]. Based on their activity, antimicrobial/antibacterial substances are divided into two types, namely those with bacteriostatic activity (inhibiting bacterial growth) and those with bactericidal activity (killing bacteria) [26].

Antimicrobial substances as food preservatives must meet the criteria, namely having broad antibacterial activity, non-toxic, economical, antibacterial activity does not decrease with the presence of food components, does not cause changes in the taste of food and not only inhibits bacterial growth but can also kill bacteria. [27]. The use of antimicrobials in fish will inhibit microbial activity, so the number of bacteria will be lower. Antimicrobials are substances that can interfere with the growth and metabolism of bacteria, so that these compounds can inhibit or even kill bacteria.

The addition of antimicrobial treatment can affect the growth of the number of bacteria because the growth of bacteria can be inhibited. The active substances contained in various types of plant extracts are known to inhibit several pathogenic microbes and food spoilers. These active substances can come from plant parts such as seeds, fruit, rhizomes, stems, tubers and leaves [25]. Like the leaves of the holy basil plant, which contain chemicals such as alkaloids, flavonoids, tannins, triterpenoids, steroids, glycosides, saponins, and essential oils [28]. Alkaloids are plant chemical compounds resulting from secondary metabolites that are formed based on the principle of mixture formation [29]. Alkaloids are amino acid derivatives that are basic because they contain one or more hydrogen atoms [30].

The use of alkaloids for plants is as a protector from pest attacks, plant boosters, and hormone regulators [29]. Alkaloids have the ability as an antibacterial. Its mechanism as an antibacterial is related to its ability to interact with bacterial DNA by placing itself between DNA strands. This position causes DNA damage so that the bacteria undergoes lysis [31]. Flavonoids are glucoside compounds consisting of sugars and flavones which are toxic. Flavonoids are the largest class of natural phenolic compounds, apart from simple phenols (phenylpropanoids and phenol quinones) [32] and are slightly acidic [33]. Flavonoids contain a conjugated aromatic system and are often found in glycosides so that they are easily extracted in polar solvents [34]. Flavonoids are widely found in plants and have many functions, flavonoids are the most common group of polyphenolic compounds. Polyphenol compounds have the ability to form complexes with proteins through hydrogen bonding so that they can inhibit enzyme activity [35].

Flavonoids have antibacterial activity because flavonoids have the ability to interact with bacterial DNA and inhibit the function of the bacterial cytoplasmic membrane by reducing the fluidity of the inner and outer membranes of bacterial cells [36]. This results in damage to the permeability of the bacterial cell wall membrane and the membrane does not function properly, including for attaching to the substrate [37]. The result of this interaction causes damage to the permeability of bacterial cell walls, microsomes and lysosomes [38].

Tannins are bitter plant polyphenols that are good and fast for binding or shrinking proteins. Tannins are built from elements C, H, and O and often form large molecules with molecular weights greater than 2000 [39]. Tannins are easily soluble in polar solvents, such as water, dioxane, acetone, alcohol and benzene. Tannins are usually found in vacuoles and the waxy layer of plant leaf surfaces [40]. Tannins can shrink cell walls or cell membranes, thereby disrupting the permeability of bacterial cells. Due to the disruption of permeability, cells cannot carry out living activities so that their growth is inhibited or even dies [41].

Triterpenoids and steroids are compounds whose carbon skeletons are derived from six isoprene units and are derived biosynthetically from acyclic  $C_{30}$  hydrocarbons. The triterpenoids are colorless crystals, have high melting points and are optically active. These compounds have a cyclic structure and are mostly alcohols, aldehydes, or carboxylic acids. Steroids are triterpenoid compounds that are commonly found in animals and plants. In higher plant tissues, steroids are mostly found as phytosterol compounds. This compound is widely used as an additive in food, medicine and cosmetics. The antibacterial mechanism of steroids/triterpenoids is not clearly known, but there is speculation that these compounds are lipophilic which can damage bacterial membranes [31].

Saponins are glycosides triterpenoids and sterols. Saponins work as antibacterial by disrupting the stability of the bacterial cell membrane, causing bacterial cell lysis which results in cell membrane damage and causes the release of important components from the bacterial cell, namely proteins, nucleic acids and nucleatides [42]. In addition, saponins are able to interact with the lipid and lipopolysaccharide layers on the outer membrane of bacteria, thereby damaging the integrity of the bacterial cell wall [43].

Essential oil is one type of plant oil that has many benefits. Its physical characteristics are viscous liquid that can be stored at room temperature. One of the main characteristics of essential oils is that they are volatile and have a distinctive aroma [44]. Essential oils in some plants have biological activity as antibacterial and antifungal. This oil is so important that it can be used as a food preservative and natural antimicrobial [36]. The chemical content of holy basil that has antimicrobial properties is thymol, which is a component of thyme oil apart from carvacrol, (ro)-cymene, and (gamma)-terpiene. Thymol compounds are known to have an effect as an antimicrobial compound against bacterial growth. Bacteria that can be inhibited include *Salmonella* sp., *Staphylococcus aureus, Eschericia coli, Listeria monocytogenes, Campylobacter jejuni* and *Bacillus cereus* [45]. Many factors affect the inhibition of microorganisms by antimicrobials. These factors are the concentration of antimicrobial substances, the number of microorganisms, temperature, species of microorganisms and acid-base properties [46].

#### HOLY BASIL LEAVES AS AN ANESTHETIC FOR FISH TRANSPORTATION

The transportation of live fish from the hatchery to the grow-out pond or point of sale is one of the important things to consider. Fish are kept alive until their destination by lowering their metabolism. One of the methods used in fish transportation is to stun the fish during the trip. So far, fish have been anesthetized in the transportation process using synthetic anesthetics such as Trichaine sufanosulphate (MS-222) and Quinaldine (2-4 Methychinolin). Some of the obstacles to the use of synthetic anesthetics for fish transportation are: 1) the wrong dose can cause fish poisoning, 2) the use of chemicals that accumulate in the fish's body and are dangerous for humans who consume them, 3) the price of synthetic anesthetics is expensive.

Based on this, the use of natural anesthetics is something that needs to be considered in fish transportation. Some natural ingredients such as clove oil, nutmeg seed oil, rubber seed oil and holy basil leaves have been researched and proven to be natural anesthetics. These plants have active ingredients that are anesthetizing the central nervous system, so that the organism is unconscious or experiences fainting. The use of natural ingredients is safer because it has no residue and is not toxic to fish and humans as consumers.

The use of holy basil leaves as an anesthetic has been carried out in the transportation of tilapia [47]. The results showed that 20% solution of holy basil leaf extract was able to stun fish for 1 hour (100%), 3 hours (90%), 5 hours (75%) and 9 hours (20%). The ability of holy basil is anesthetic due to the presence of natural compounds such as essential oils, saponins, galavoind and tannins in it. In addition, holy basil contains 64.5% methyl eugenol, 4% cineol, 2.3% linalol and 1% terpinol [48].

#### HOLY BASIL LEAVES AS FISH PRESERVATIVES

Fish is a food that is easily spoiled. Efforts that can be made to maintain the freshness of fish in a long period of time is by handling low temperatures (5-10°C). The use of low temperatures in fishery products can inhibit enzyme activity and bacterial growth, so that the deterioration of fish quality will run much more slowly and remain fresh for a long time [49]. The use of low temperatures also prevents chemical reactions and the loss of water content from foodstuffs [50].

Another effort that can be made to maintain the quality of fresh mackerel is to combine cold treatment and the addition of preservatives. Preservatives are compounds that are able to inhibit and stop the process of fermentation, acidification, or other forms of food spoilage, or compound that can provide food protection from spoilage [51].

Holy basil leaf is one of the natural ingredients that can be used as a preservative. According to [52] natural ingredients have the potential to preserve fish because they have microbial inhibiting activity caused by certain components in them. Some of the chemicals contained in all parts of the holy basil plant include 1,8-cineol, anthol, apigenin, stigmaasterol, tryptophan, tannin, sterol, and boron [53], while the leaves contain flavonoids, glycosides, gallic acid and its esters, caffeic acid, and essential oils containing eugenol (70.5%) as the main component [54].

Eugenol in holy basil leaf essential oil can act as an antibacterial pathogen [55]. This eugenol also gives a distinctive aroma to the holy basil plant [48]. Holy basil acts as an anti-bacterial especially on *Eschericia coli, Streptococcus mutans* and *Staphylococcus aureus*, in addition, chewed holy basil leaves can prevent thrush and provide protection against bacteria and germs. Holy basil also has anti-tubercular activity and inhibits the in-vitro growth of *Mycobacterium tuberculosis*, even holy basil leaf extract shows strong anti-fungal activity against *Aspergillus* sp. [56]. Based on the content of flavonoids, tannins, essential oils, and others as well as their antimicrobial and antibacterial properties, holy basil leaves can be used as an alternative natural preservative for fish.

Research that proves the effect of storing fish with and without antimicrobials has been carried out by [57], who used holy basil leaf extract to preserve mackerel. The results of the research by [57] are presented in Table 3.

No	Observed Parameters	Holy Basil Solution Concentration (%)					
NO.	Observed Farameters	0	10	30	50		
1.	Shelf life (day)	7	9	13	13		
2.	Number of bacteria at acceptance limit (cfu/g)	1,61 × 10 <sup>7</sup>	1,70 × 10 <sup>7</sup>	6,90 × 10 <sup>7</sup>	5,10 × 10 <sup>7</sup>		
3.	Degree of acidity at acceptance limit	6,40	6,60	6,95	7,40		
4.	Weight loss at acceptance limit (%)	6,59	8,25	9,52	7,37		
5.	Moisture content at acceptance limit (%)	67,06	71,65	65,32	68,51		

Table 3	The Effect	of Holy	hasil on	the shelf	life of	mackerel
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Source: [57]

#### Conclusion

Based on the results of the review that has been done, it can be concluded that holy basil leaves have great potential to be used in fishery activities, especially as a preservative for fresh fish, antimicrobial and anesthetic in the fish transportation process.

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