

Comparative Study on Efficacy of the Antimicrobial Activity of *Ocimum sanctum* and *Andrographis paniculata* against Animal and Human Pathogens

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Abstract

Ocimum sanctum is an erect much branched highly aromatic, softy hairy annual, plant. Leaves of *Ocimum sanctum* contain water soluble phenolic compounds. *Andrographis paniculata* is an erect 40- 90 cm tall annual plant. *A. paniculata* was effective against bacillary dysentery. Gastro enteritis etc. Hence an attempt was made to study the antibacterial activity of *Ocimum sanctum* and *A. paniculata* against human and Animal pathogens. The study revealed that among the four organisms tested *V. cholerae* and showed maximum zone of inhibition and was followed by *Aeromonas hydrophila*.

Key words: Pathogen, Zone of inhibition, *Ocimum*, *Aeromonas sp*

Ocimum sanctum is an erect much branched highly aromatic, softy hairy annual, plant growing up to 75 cm in height found throughout India ascending up to 1900m in the Himalayas and often planted in temple and houses. It is being cultivated commercially. The plant is native to tropics of Asia and Africa being the medicinally important plant of the family *Lamiaceae*. The members of *Lamiaceae* is one of the most exploited medicinal plant family worldwide not only the source of medicinal plants but also with its valuable essential oils being used as spices and flavours for various food products. However, the traditional plants have been proved to be better source for novel antimicrobial drugs. Most of the Indian plants accounts for the richest resources of substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, all Protozoan's.

The leaves of *Ocimum sanctum* are opposite, ovate, elliptic, 3-6 cm long. Flowers are small purplish, close whorled, borne on 15-20 cm long terminal and auxillary racemes. Fruits are subglobose nutlets smooth around 1.5 mm long and 1mm broad, pale brown or reddish; with small black markings. About 85% population of the whole world partially or wholly is dependent on herbal medicines for the treatment of primary health related issues. The extract and essential oil of *Ocimum sanctum* is found to possess insecticidal and antibacterial properties.

The active constituents of *O. sanctum* include the phytochemicals such as aldehyde, and phenols also contain smaller quantity of saponins, tannins, glycosides, quinine, phlobatanin, Flavonoids, Steroids, Coumarin and Alkaloids. The aqueous extract of Tulsi possesses the primary active constituents such as eugenol, Urosolic acid, carvacrol, rosamarinic acid and B caryophyllene linalool euginal. These traditional medicinal herbs are considered as one of the most

important sources of secondary metabolites and essential oils [1]. Furthermore, studies have revealed *O. sanctum* to possess anti inflammation, analgenic, antipyretic, antidiabetic, hepato protective hypolipidemic and antistress activities. Extracted essential oil have known to contain biologically active constituents that are insecticidal, Nematicidal and fungistatic. Tulsi extract is commonly used for various medicine for therapeutic uses for treatment of many diseases such as fever headache, malaria, heart diseases and inflammation. The extract and essential oil of *O. sanctum* is found to possess insecticidal and antibacterial property.

Ocimum sanctum is a grassy annual plant which is originated from Afghanistan and India. It belongs to the order Lamiales and family Lamiaceae *O. sanctum* has antibacterial, antioxidant, antiviral properties [2]. It is also used as cardio productive renal damage recovery and wound healing [3]. Studied the antibacterial activity of *O. sanctum* and *Jasmine officinale*. Tulsi is anti-inflammatory agent as well as protects from chemicals and radiation. The chemical compounds in *Ocimum sanctum* includes flavonoids (mostly apigenin, luteolin, and their glycosides, 7-O glucuronides, luleolin 5-O glucoside apigenin 6-8-c diglycoside, luleolin 8 B D glucopyrano side triterpenoids and compounds biogenetically related eugenol.

Tulsi is highly valued in Ayurvedha for normal health maintenance and as a home remedy for minor health problems. A paste or decoction of its leaves taken internally has been recommended for the treatment of dyspepsia fever, catarrh cough, asthma, splenic disorders skin eruption toxicosis and paralysis. Tulsi leaves are also used for ophthalmic problems, parasitic infestations, hepatic affections, gastric disorders and against venomous bites. Seeds are considered as diuretic litholriptic anti-inflammatory antidiabetic, antiseptic and

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strength promoting. Plants are the primary source of medicines. Medicinal plants are considered to be very rich sources of secondary metabolites and oils which are of therapeutic importance. In India Tulsi is taken as the most sacred plant. The use of *Ocimum sanctum* as an aromatic plant has been well documented in Ayurveda [4].

In traditional system of medicine the Indian medicinal plants have been used in successful management of various disease conditions like bronchial asthma, Chorioid fever, Cold, cough, skin disease etc. [5] respiratory disorder, kidney stone, heart and vascular protection. Children teeth disorder, headaches, eye disorders, liver support, bronchial support, radiation protection, immunity tunes up, anti-inflammatory Action, antibiotic protection, nutrition high safety margin, food additive and perfume etc. *Ocimum sanctum* has specific aromatic odour because of the presence of essential or volatile oil, mainly concentrated seeds are called fixed oil and mainly composed of fatty acids. Besides oil, the plants also contain alkaloids, glycosides, saponins and tannins. The present-day information about the chemical properties is based on the various studies that have been done in different parts of the world [6]. Antibacterial activity of ethanolic extract of tulsi was tested against various gram positive and gram-negative bacterial strains. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Acinetobacter baumannii*.

In addition to physical, toxic and infective stress, modern living is associated with heightened levels of psychological stress caused by the many demands and fast of modern life. This stress compounds the toxic effects of chemical pollutants and the constant fear of pervasive toxic chemicals can itself lead to even further stress and anxiety that may be just as toxic as the chemicals causing the reality of daily chemical exposure cannot be denied. Regular consumption of Tulsi not only helps protect and detoxify the body's cells and organs, it can also help reduce toxic stress by releasing and calming the mind and offering many psychological benefits such as effects on memory and cognitive functions.

The psychotherapeutic properties of Tulsi have been explored in various animal experiments that reveal that Tulsi has anti-anxiety and antidepressant properties [7]. Perhaps one of the greatest of Tulsi's benefits in the modern world comes from its global distribution based on its cultivation in ethical fair trade, organic and ecological farming practices. There is a growing realization that in order to take issues of food security, rural poverty, higher environmental degradation and climate change a shift in agriculture is needed from a green revolution to an ecological intensification revolution. This has been highlighted in a recent United Nations document titled "Wake up Before it's Too Late" which calls for the global community to endorse and advocate for local solutions to toxicity, food insecurity and poverty, such as farming over the use of genetically modified organisms and monoculture.

Leaves of *Ocimum sanctum* contain water soluble phenolic compounds and various other constituents, such as eugenol, methyl eugenol and caryophyllene that may act as an immunostimulant. In tilapia (*Oreochromis mossambicus*) the acetone extract of *Ocimum sanctum* was found to enhance the anti-sheep red blood cell antibody response. Leaves extract of *Ocimum sanctum* affected both specific and non-specific immune responses and disease resistance against *Aeromonas hydrophila*. It stimulated both antibody response and neutrophil activity [8]. The immunostimulants enhance the level of duration of specific immune response, both cell-mediated and humoral, following vaccination. Immunostimulant support to overcome immunosuppressive effect of stress and of those infectious agents that damage or interface with the functioning

of cell of immune system. A variety of substances have been shown to have the immunostimulatory effects which are microbial derivatives, plants or animal extracts, vitamins, hormones and synthetic chemicals but herb extracts of animal origin products have a potential application as an immunostimulant in fish culture, primarily because they can be easily obtained, are not expensive and act against extracts can be given orally which is the most convenient method of immunostimulation [9].

A large proportion of the world's population depends on traditional medicine because of scarcity, high cost of orthodox medicine and unpleasant side effect. There are currently about 250 registered medical practitioners of the Ayurvedic system, as compared to about 700,000 of the modern medicine system. There are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. In addition, herbs have provided some of the very important lifesaving drugs used in the armamentarium of modern medicine. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance [10-11]. Genus *Ocimum* major molecular markers comprised β -bisabolene, (E)-bisabolene, terpineol, linalool, (2)-Cinnamic acid methyl ester, camphor exerting wide range of therapeutic effects antimicrobial, antispasmodic, bactericides, carminative, anthelmintic, hepatoprotective, antiviral, larvicidal, antinociceptive, antifungal, antipyretic, phototoxic activity, antinflatogenic, anti-diarrhetic, amylase inhibitory etc. [12].

Aquaculture is the fastest growing food production sector in the world. Aquaculture is gaining significant momentum in several parts of India. India is one of the leading countries of aquaculture practicing mainly shrimp production and its export, since flesh consists of rich proteins. Aquaculture is the organized production of crop in the aquatic medium. Aquaculture plays a vital role in the welfare and Economics of mankind. It is emerging as one of the most viable and promising area for providing national and food security for human.

Globally more than one billion live on less than one dollar a day and 840 million are classified as undernourished. In this context fish and fisheries have been playing an important role in addressing nutritional and food security of poor in developing countries. Fish are the rich source of proteins, essential fatty acids, vitamins and minerals. The fats and fatty acids in fish particularly omega 3 fatty acids, are highly beneficial and difficult to obtain from other food sources. In many developing countries, fish is the cheapest source of animal protein to the poor and fish protein is of superior quality as it contains all the essential amino acids for body building than plant proteins. India with 2.47 million tons of culture fish production stands second in world aquaculture production. It is expected that by 2025 globally one out of every two fish consumed will be from Aquaculture.

Andrographis paniculata is an erect 40-90cm tall annual, gregarious in habit and growing in wasteland throughout warmer regions of India. The leaves are lanceolate, smooth and dark green in colour, 5-7 cm and 2.5-3cm broad. Stems are quadrangular with longitudinal creases and much branched. Flowers are white and tiny, occurring in terminal racemes, flowering, fruiting and seeding takes place from August to March. Important secondary metabolites such as flurify of carbocyclic, diterpenoids, (Whole plant) flavonoids (root), some of which are glycosides. The most dominant diterpene is andrographolide, 14-deoxy andrographolide, neo andrographolide and 14-deoxy 11,12-didehydroandrographolide. Clinical trials on the efficacy of *A. paniculata* in the treatment of a variety of bacterial, viral and

other infections crude extracts of *A. paniculata* was effective against bacillary dysentery, gastroenteritis, typhoid fever and skin infections. Andrographolide as well as ethanol extract of the plant have been shown to be immune stimulant in animal models.

MATERIALS AND METHODS

Experiment plants such as *Ocimum sanctum* and *A. paniculata* were freshly collected from the Garden of Residential area of Thanjavur Tamil Nadu, India (10.46 N; 79.10 E). The pathogenic *Vibrio* species such as *V. cholerae*, *V. parahaemolyticus*, *Aeromonas hydrophila*, *Lacto bacillus sp.* were selected and isolated from diseased fish [13]. About 1gm of sterilized plant leaves were ground in a mortar and pestle with 50ml aqueous and organic solvent (Ethanol) and petroleum ether it was filtered and the supernatant was stored for antibacterial study [14].

The antibacterial activity of the leaves were tested against the selected bacterial species 30. After sterilization of TCBS agar medium and nutrient agar medium was poured into petri dishes and allowed to solidify. swab was made with test culture (10^4 cfu/ml; 18hrs) and the paper disc were loaded with test plant extract and impregnated to the discs. The plates were incubated at 37 c for 24 hrs after 24 hrs the zone of inhibition was measured and observation were recorded.

RESULTS AND DISCUSSION

The invitro antibacterial activity of *Ocimum sanctum* revealed the following observation. The study revealed that the alcoholic extract of *Vibrio cholera* showed the inhibition zone of 13.2±0.02 mm in diameter. The water extract of *Ocimum*

sanctum revealed the zone of inhibition of 12.1± 1.2mm diameter. The alcoholic extract of *V. parahaemolyticus* showed the inhibition zone at 10.4±0.01mm whereas the water Extract of *Ocimum sanctum* revealed 10.1±0.01mm diameter. The alcoholic extract of *Aeromonas hydrophila* showed the inhibition zone of 11.58±0.05mm diameter. The water extract study revealed the presence of 9.23±0.03 mm diameter. The Alcoholic extract of *Ocimum sanctum* revealed the presence of 12.6±1.2mm diameter. Whereas the water extract of *Lactobacillus sp* revealed the zone of inhibition of 12.6±1.2mm diameter and the water extract of showed the zone of 10.6±0.02 mm diameter. The present study revealed that among the four organisms tested *Vibrio cholera* showed maximum zone of inhibition, and was followed by *Aeromonas hydrophila*.

Asia is the hub of Aquaculture and has been a tradition in several parts of Asia and has been a tradition in several parts of Asia and according to FAO statistics over 80% of fish produced by aquaculture come from Asia. India is one of the leading countries of aquaculture practicing, mainly shrimp production and its export since its fish consists of rich proteins. Shrimps are cultured in water rich in natural feed. It is the world's fastest growing food production with cultured shrimp and prawn subsector growing at an annual rate of 16.8% between 1984 and 1985 [15].

Medicinal plants are one of the most commonly used natural antimicrobial agents in food and have been used traditionally for thousands of years by many cultures for controlling common health complications and antimicrobial potentials of plants are believed to be due to tannins, saponin phenolic compounds, essential oils and flavonoids plants contain numerous biologically active compounds many of which have been shown to have antimicrobial properties [16-17].

Table 1 In vitro antibacterial activity of *Ocimum sanctum*

S. No.	Organism	Alcoholic extract	Petroleum ether	Water extract
1	<i>Vibrio cholera</i>	13.2±0.02	11.3±0.01	12.1±1.2
2	<i>Vibrio parahaemolyticus</i>	10.4±0.01	10.3±0.1	10.1±0.01
3	<i>Aeromonas hydrophila</i>	11.58±0.05	10.23±0.1	9.23±0.03
4	<i>Lactobacillus sp</i>	12.6±1.2	11.2±0.2	10.6±0.02

Table 2 In vitro antibacterial activity of *Andrographis paniculata*

S. No.	Organism	Alcoholic extract	Petroleum ether	Water extract
1	<i>Vibrio cholera</i>	12.1±0.1	12.02±0.1	10.1±0.05
2	<i>Vibrio parahaemolyticus</i>	15.1±0.1	15.2±0.1	13.1±0.2
3	<i>Pseudomonas aeruginosa</i>	13.2±0.08	14.2±0.2	12.1±0.2
4	<i>Lactobacillus sp</i>	12.2±0.08	13.1±0.6	10.1±0.2

Observed maximum zone of inhibition against *Escherichia coli* and *Pseudomonas aeruginosa*. The methanolic extract effectively controlled the pathogen such as *salmonella*, *Vibrio spp*, *Yersina sp* and *Aeromonas spp* in *F. indicus*. Invitro herbal diets prepared from the five herbs such as *Adathoda vosica*, *Murraye koenigii*, *Ocimum basilicum* *Psoralea corylifolia* and *Quercus infectoria* were effectively suppressed the pathogens such as *Pseudomonas aeruginosa* *Staphylococcus aureus*, *Aeromonas hydrophila*, *Vibrio harveyi* and *Vibrio parahaemolyticus* in *P. monodon* immune system. The essential oil has been shown to exhibit high antibacterial activity against *Staphylococcus aureus*, *E. coli*, *B. subtilis* [18]. Observed that the ethanolic extract of turmeric *Curcuma longa* which contained 20.0% by dry weight of curcumin showed inhibitory effect against six species of vibrio including *V. harveyi*, *V. cholera*, *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*, *V.*

fluvialis, at concentration of 0.47, 0.47, 0.94, 0.47, 0.47 mg/disc separately. [18] reported that the *Vibrio* species isolated from *Penaues monodon*, *Sillago shigama*, in canned sea food revealed that the presence of *Vibrio cholera*, *Vibrio parahaemolyticus*, *Vibrio harveyi*. The anti-vibrio activity of selected medicinal plants revealed cumin showed maximum zone of inhibition.

CONCLUSION

In the present study on the invitro screening of the *Ocimum sanctum* against fish pathogenic microbes revealed that the *Vibrio cholera* showed maximum zone of inhibition against the alcoholic extracts of *Ocimum sanctum*. In the present study the *Andrographis paniculata* showed the maximum zone of inhibition in *Vibrio parahaemolyticus*.

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