



# COMPARATIVE STUDY OF PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF *OCIMUM SANCTUM* AND *OCIMUM BASILICUM* LEAVES

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## Abstract:

Leaves of the plants *Ocimum sanctum L.* and *Ocimum basilicum L.* belongs to family Lamiaceae. *Ocimum sp.* have shown various medicinal properties to treat ailments such as fever, poor digestion, nausea, abdominal cramps, gastro-enteritis, migraine, insomnia, depression, gonorrhea, dysentery and chronic stomach diseases etc. External application treats acne, insect stings, snake bites and skin infections.

Qualitative phytochemicals analysis and antibacterial analysis of two common species of *ocimum* was carried out. Phytochemical analysis revealed presence of alkaloid, tannins, phenols, steroids. Shade dried leaves of the respective plants were used for the preparation of hydro-alcoholic extract.

Antibacterial activity of hydroalcoholic leaf extracts of both the plants were studied against two species of microbial pathogens *Escherichia coli* and *staphylococcus aureus*.

## INTRODUCTION

India is known as an “Emporium of medicinal plants”. It possesses about 8% of the estimated biodiversity of the world with around 12,600 species and is one of the 12 mega biodiversity centers with 2 hot spots of biodiversity in the Western ghats and North eastern region. It is also rich ethnic diversity. There are about 67.37 million tribal people belonging to 537 tribal groups living in different geographical location with various subsistence pattern.

Plants are raw resource material for traditional medicines, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediate and chemical entities for synthetic drugs. Traditional medical system has great value as around 80% of the world’s population depends on traditional medicinal system for their health. (WHO,2022) *Ocimum* genus is native to tropical and warm temperate region of all 6 continents with the greatest number of species in Africa (Alhasan, *et al* 2020). Members of *Ocimum* are aromatic annual and perennial herbs and shrubs which belong to family Lamiaceae. There are about 150 species of *Ocimum*. The genus *Ocimum* has a number of species that are used for the treatment of different types of ailments from ancient times (Yaldiz, G., & Camlica, M. *et al* 2021). *Ocimum sp.* are called as ‘Basil’ which refers to royal or king in Greek because of its number of medicinal uses. It has numerous potent activities due to the active principles present in it.

Pungent smell of both sp. of *ocimum* represents its essential oil content. The extracts of essential oils of *ocimum* are used as flavors for the food products (Alhasan, *et al* 2020). It has also been used as commercial fragrances, flavors and to improve the food products shelf life. (Purushothaman, B., PrasannaSrinivasan, R., Suganthi, P., Ranganathan, B., Gimbut, J., & Shanmugam, K. *et al* 2018)

### ***Ocimum sanctum L.***

According to Indian mythology *Ocimum sanctum* is also called as holy basil as it has significant health applications. *Ocimum* is an erect, many branched subshrub, 30-60cm tall with hairy stems. It is recommended for treatment of respiratory, digestive and skin diseases. It is a highly promising plant which works as an immunomodulator anti-cancer agent. The *O. sanctum* is even known to purify or de-pollute the atmosphere and also works as a repellent to mosquitoes, flies and other harmful insects. (Sundaramurthy, *et al* 2012). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. *Ocimum* genus is well known for its antibacterial activity against various bacteria (Salleh, *et al* 2021; Suppakul, *et al* 2003).

It is believed that in India *Ocimum sanctum L.* can be used for the treatment of diseases like bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, arthritis, painful eye diseases, insect bite etc most importantly it has anticancer, antifungal, antihyperglycaemic, antibacterial, in treatment of nausea and vomiting, protection against liver and heart, analgesic, adaptogenic and diaphoretic (*Ocimum. Sanctum*) (Bhooshitha A N\*, *et al* 2020; Sharma, *et al* 2016; Rathnayaka *et al* 2013; Almatroodi *et al* 2020).

### ***Ocimum basilicum L.***

*Ocimum basilicum L.* also known as sweet basil, sabja is native to Asia and is widely grown as an ornamental or field crops from seed in the Mediterranean countries, including Turkey. Leaves and flowering parts are traditionally used as antispasmodic, aromatic, carminative, digestive, stomachic, and tonic agents (Adigüzel, *et al* 2005). They are also used as folk remedy to treat various ailments such as feverish illness, poor digestion, nausea, abdominal cramps, migraine, insomnia, depression, gonorrhoea, dysentery (Adigüzel, *et al* 2005). They have been applied for the treatment of acne, loss of smell, insect stings, snake bites, and skin infections (Adigüzel, *et al* 2005).

*O. basilicum* leaves or seeds acts as vermifuge also treats dysentery, nausea and flatulence. The oil of the plant is beneficial for the alleviation of spasm, rhinitis mental fatigue, cold, and as first aid treatment for wasp stings and snakebites. It has been used as a folk remedy for boredom and convulsion. It cures headache, improves digestion and is also good for toothache, earache and for the curing epistaxis when used with camphor. Infusion of plant is effective in cephalalgia, gouty joints, fever, cough, gout and given internally to treat cystitis, nephritis and in internal piles. Infusion of *Ocimum basilicum L.* seed is used to treat gonorrhoea, chronic diarrhoea and dysentery. Plant is also used to keep away insects and snakes.

### **CHEMICAL CONSTITUENTS :**

Leaves of *Ocimum sanctum L.* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacol, sesquiterpine, caryophyllene. Ursolic acid has been isolated from the leaves. Apart from ursolic acid, isolated apigenin, luteolin, apigenin-7-O-glucuronide, mollustidin. Isolation of two flavonoids, orientin and vicenin from the aqueous leaf extract.

Leaves of *O. basilicum* characterized by two or three major components (20-70%) of volatile oil and other components present as minor components. It is composed of major constituents such as linalool, eugenol, methyl eugenol, carvacol and methyl chavicol. However, these major constituents have been reported to occur in different percentages according to geographical location.

However both the species contains common constituents like methyl eugenol, carvacol and eugenol.

**MATERIAL AND METHODS:**

The sample leaves were collected from local flower vendors. 500 gms of leaves of both the sp. were air and shade dried for two to three days and then measured with estimated weight of 150gms. Then this dried leaves were separately converted into fine powders to form liquid extracts.

Preparation of extract:- Hydroalcoholic extract was prepared by cold extraction method. 25ml of ethanol with 25ml water was mixed with 2gm of powdered leaf sample separately and then extract was concentrated. Filtered extracts were stored in glass bottle in the refrigerator for further experimental use.

**METHODS:****a. PHYTOCHEMICAL ANALYSIS**

Phytochemicals are secondary metabolites produced by plants, which are further classified into major categories such as alkaloids, flavonoids, tannins, saponins, phenols, etc. Phytochemical analysis was carried out using standard phytochemical test

**STANDARD TEST FOR PHYTOCHEMICAL ANALYSIS**

sr.no	Secondary metabolite	Test
1	Alkaloid	<b>Wagner's test-</b> 5mg extract of <i>Ocimum sanctum L.</i> and <i>Ocimum basilicum L.</i> was taken in the test tube than 0.5 of Wagner reagent was added to the solution shaken well. Appearance of reddish brown colour showing the alkaloids present. Reddish brown colour because of iodine forms a complex in insoluble and has the colour brown reddish
2	Flavonoid	<b>Ferric chloride test-</b> ferric chloride test was performed for checking the presence of flavonoids in the aqueous extract <i>Ocimum sanctum L.</i> and <i>Ocimum basilicum L.</i> 5mg extract was mixed with 1ml of distilled water than 0.5ml of dilute ammonia solution was added to it. After addition of dilute ammonia few drops of conc sulfuric acid was mixed. Yellow colour formation
3	Glycoside	<b>Keller-kilani test-</b> for the confirmation of the glycoside in the extract 5mg extract was taken in the test tubes than the 1ml of glacial acetic acid was added. Few drop of 2% sol of ferric chloride were mixed into it. Then 1ml of conc sulfuric acid were into the mixture. A brown ring at the

		edge will be formed in presence of glycosides.
4	<b>Tannin</b>	<b>Ferric chloride test-</b> 5mg aqueous extract of both <i>Ocimum</i> sp. was mixed with 0.5ml of ferric chloride solution. Formation of black ppt in the presence of tannins.
5	<b>Saponin</b>	<b>Foam test-</b> was performed for the identification of saponin in the aqueous extract in which 1ml extract was dissolved into 5ml distilled water it was shaken properly until foam was formed. It should produce emulsions with saponins.
6	<b>Steroids</b>	<b>Salkowski's test-</b> 3 drops of conc sulfuric acid was added into 0.5ml extract. The formation of red colour indicates the presence of steroids.
7	<b>Phenols</b>	<b>Ferric chloride test-</b> 0.5ml of extract, few drops of neutral 5% ferric chloride solution was added black colour indicate presence of phenols.
8	<b>Anthraquinone</b>	0.5ml of extract, few drops of 2% HCl was added. Red colour precipitate indicated presence anthraquinone.

#### b. ANTIBACTERIAL ACTIVITY

Antibacterial activity of a molecule is completely associated with the compounds that kill bacteria or slow down their rate of growth, without being extensively toxic to nearby tissues. Antibacterial agents are most important in fighting infectious diseases. as a result of extensive use and abuse, bacteria have developed resistance against antibacterial agents which become a major problem for today's pharmaceutical industry.

##### Bacteria used:

In this study, two bacterial strains were used to study the antibacterial activity. One gram negative and one-gram positive bacteria viz. *Escherichia coli* and *Staphylococcus aureus* have used. The pure culture was obtained from the laboratory and was sub-cultured on the nutrient agar slants to maintain viability.

##### Preparation of bacterial inoculum:

For preparing the bacterial suspension, a single well isolated colony was picked from each of the sub cultured slants and was dissolved in nutrient agar broth holding the test tube under sterile condition for all strains. These tubes were then incubated for 37°C for 24 hrs. these suspension was used as inoculum for further test.

##### Screening of antibacterial activity:

For determining the antibacterial activity of *Ocimum sanctum L.* and *Ocimum basilicum L.* using well diffusion method(Rathnayaka, *et al* 2013). In this method, molten MH agar was inoculated with 0.3ml of bacterial inoculum and was poured into a sterile petri dish of 100mm diameter. the agar was allowed to solidify. After solidification 4 wells each of 8mm diameter was punched in each plate using a sterile cork borer. 100 µl of samples of hydro-alcoholic plant extract was added in each well respectively. All the procedure was carried out in laminar air flow. All the plates were prepared in triplicates. The plates were then incubated at 37°C for 24 hours. After the incubation, zone of inhibition was measured in mm. Positive standard used was (add name of the antibiotic with its concentration)



## RESULT

### PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of *O. sanctum* Showed that it is rich in alkaloids, tannins, phenols and anthraquinone.

Phytochemical analysis of *O. basilicum* showed that it is rich in alkaloids, flavonoids, tannins, phenols.

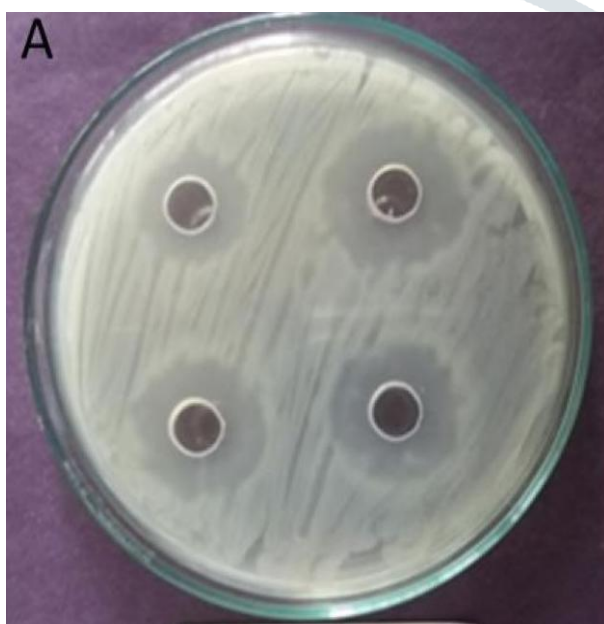
Both the species show common phytochemical constituents such as presence alkaloids, tannins and phenols.

Sr no	Test	<i>O. sanctum</i>	<i>O. basilicum</i>
1	Alkaloid Wagner's test	+	+
2	Flavonoids Ferric chloride test	-	+
3	Glycoside Keller Kilani test	-	-
4	Tannin Ferric chloride test	+	+
5	Saponin Foam test	-	-
6	Steroids Salkowski's test	-	-
7	Phenols Ferric chloride test	+	+
8	Anthraquinone	+	-

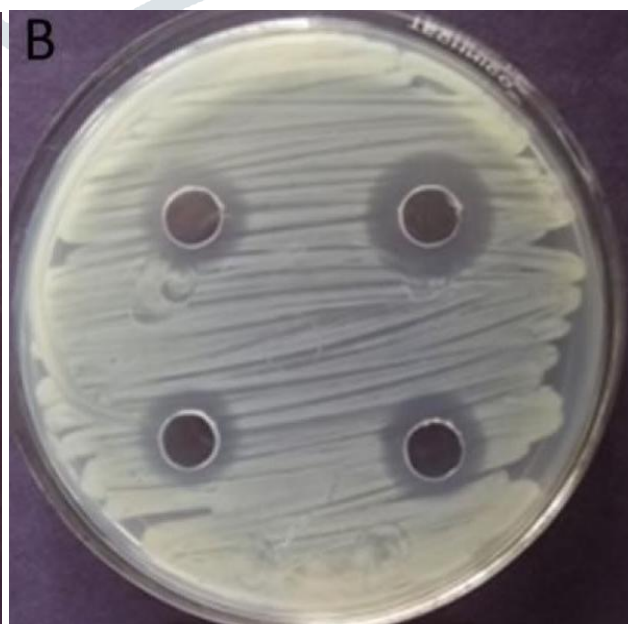
(‘+’ indicates positive and ‘-’ indicates negative)

### Antibacterial activity

The zone of inhibition was found to be 3mm for *Escherichia coli* and 2mm for *Staphylococcus aureus* for *Ocimum sanctum* L.



E.coli



S. aureus

The zone of inhibition was found to be 2.5mm for *Escherichia coli* and 3mm for *Staphylococcus aureus* for *Ocimum basilicum* L.

Species	Bacterial culture	Zone of inhibition
A. <i>Ocimum sanctum</i> L	<i>Escherichia coli</i>	1mm
		2mm
	<i>Staphylococcus aureus</i>	2.5mm
		2.5mm
B. <i>Ocimum basilicum</i> L	<i>Escherichia coli</i>	3.5mm
		2.5mm
	<i>Staphylococcus aureus</i>	2mm
		1mm

## DISCUSSION

Phytochemical studies Qualitative phytochemical investigation exhibited presence of alkaloids compounds, flavonoids, tannins in the extracts of plant. The presence of these phytochemical components may be responsible for the observed antibacterial activity of the plant leaf extract. antibacterial activity was studied against two species of bacteria *Escherichia coli* and *Staphylococcus aureus* using the well diffusion method and it showed effective results against *Staphylococcus aureus* in *Ocimum sanctum* L and against *Escherichia coli* in *Ocimum basilicum* L.

## CONCLUSION

In recent years, traditional system of medicines have emerged as potential source to cope with the growing rate of chronic, degenerative, environmental, lifestyle and stress related diseases. Hydroalcoholic extract is used as they are least toxic in nature and cause less toxic effects on human body. Both the species of *ocimum* have been used as whole herb to treat a good number of diseases. The wide range of secondary metabolites can result in synergistic effect which cannot be fully duplicated with the isolated extracts or constituents.

## FUTURE PROSPECTS

Further experiments can be done to check the cumulative effect of both the extracts on the same microorganism as well as on other bacteria and fungi . Antimicrobial activity of different extracts using different solvents can be tested which can help us to develop more potent antibacterial medicine. Also other pharmacological activities of the the different extracts of *ocimum* sp. can also be compared.

## BIBLIOGRAPHY

- Shasany, A. K. (2016). The Holy basil (*Ocimum sanctum* L. L.) and its genome. *Indian J. Hist.Sci*, 51(2), 343-350.
- Pandey, G., & Madhuri, S. (2010). Pharmacological activities of
- 61-66.
- Rathnayaka, R. M. U. S. K. (2013). Antibacterial activity of *Ocimum sanctum* L. extracts against four

- food-borne microbial pathogens. *Scholars Journal of Applied Medical Sciences*, 1(6), 774- 777.
- Adigüzel, A., Güllüce, M., Şengül, M., Ögütçü, H., Şahin, F., & Karaman, İ. (2005). Antimicrobial effects of *Ocimum basilicum* L. (Labiatae) extract. *Turkish Journal of Biology*, 29(3), 155-160.
  - Harichandan, S. P., Sahu, A. K., Gautam, S., & Nemani, R. (2019). Phytochemical screening and antioxidant activity of methanolic extract of *Ocimum sanctum* L. Linn. Leaves. *GSC Biological and Pharmaceutical Sciences*, 8(2), 022-033.
  - Ch, M. A., Naz, S. B., Sharif, A., Akram, M., & Saeed, M. A. (2015). Biological and pharmacological properties of the sweet basil (*Ocimum basilicum* L.). *Br. J. Pharm. Res*, 7, 330-339.
  - Marwat, S. K., Khan, M. S., Ghulam, S., Anwar, N., Mustafa, G., & Usman, K. (2011). Phytochemical constituents and pharmacological activities of sweet Basil-*Ocimum basilicum* L. (Lamiaceae). *Asian Journal of Chemistry*, 23(9), 3773.
  - Ch, M. A., Naz, S. B., Sharif, A., Akram, M., & Saeed, M. A. (2015). Biological and pharmacological properties of the sweet basil (*Ocimum basilicum* L.). *Br. J. Pharm. Res*, 7, 330-339.
  - Purushothaman, B., Srinivasan, R. P., Suganthi, P., Ranganathan, B., Gimbun, J., & Shanmugam, K. (2018). A comprehensive review on *Ocimum basilicum* L.. *Journal of Natural Remedies*, 18(3), 71-85.
  - Panchal, P., & Parvez, N. (2019). Phytochemical analysis of medicinal herb (*Ocimum sanctum* L.). *International Journal of Nanomaterials, Nanotechnology and Nanomedicine*, 5(2), 008-011.
  - Sotiriou, G. A., & Pratsinis, S. E. (2010). Antibacterial activity of nanosilver ions and particles. *Environmental science & technology*, 44(14), 5649-5654.
  - Daniel, V. N., Daniang, I. E., & Nimyel, N. D. (2011). Phytochemical analysis and mineral elements composition of *Ocimum basilicum* L. obtained in jos metropolis, plateau state, Nigeria. *IJET-IJENS*, 11(6), 161-165.
  - Bhooshitha, A. N., Ghosh, A. R., Chandan, H. M., Nandhini, H. S., Pramod, B. R., & Krishna, K. L. (2020). Review On Nutritional, Medicinal and CNS Activities of Tulsi (*Ocimum. Sanctum*). *Journal of Pharmaceutical Sciences and Research*, 12(3), 420-426.
  - Jamshidi, N., & Cohen, M. M. (2017). The clinical efficacy and safety of Tulsi in humans: a systematic review of the literature. *Evidence-Based Complementary and Alternative Medicine*, 2017
  - Panchal, P., & Parvez, N. (2019). Phytochemical analysis of medicinal herb (*Ocimum sanctum* L.). *International Journal of Nanomaterials, Nanotechnology and Nanomedicine*, 5(2), 008-011.
  - Borah, R., & Biswas, S. P. (2018). Tulsi (*Ocimum sanctum* L.), excellent source of phytochemicals. *International Journal of Environment, Agriculture and Biotechnology*, 3(5), 265258.
  - Utican, K., Nimham, N., Yingyongnarongkul, B. E., & Koontongkaew, S. (2020). Ethanolic extract of *Ocimum sanctum* L. leaves reduced invasion and matrix metalloproteinase activity of head and neck cancer cell lines. *Asian Pacific Journal of Cancer Prevention: APJCP*, 21(2), 363.
  - Almatroodi, S. A., Alsaqli, M. A., Almatroudi, A., & Rahmani, A. H. (2020). *Ocimum sanctum* L.: role in diseases management through modulating various biological activity. *Pharmacognosy Journal*, 12(5).
  - Shah, G. M., Ahmad, F., Panwar, S., Khadka, M. S., Ali, A., & Bisht, S. (2019). *Ocimum sanctum* L. [Tulsi]—An Alternative Additional Livelihood Option for the Poor and Smallholder Farmers. *Sustainability*, 11(1), 227.
  - Reddy, M. S., & Venkataramanaiah, P. (2021). The Effect of Infertility From Some Biochemical Parameters in Eugenol and *Ocimum sanctum* L. linn. Leaf Extract on Female Albino Rats. *Biology and Medicine*, 13(5).
  - Salleh, N. N., Yahya, H., Ariffin, N., & Yahya, H. N. (2021). Antibacterial Properties of *Ocimum* Spp.(*Ocimum Basilicum* L. and *Ocimum Basilicum* Var. *Purpurascens*) Against Selected Bacteria. *East African Scholars Journal of Agriculture and Life Sciences*.
  - Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of agricultural and food chemistry*, 51(11), 3197-3207.
  - Sundaramurthi, P., Dhandapani, S., Ponnusamy, S., & Subbaiyan, M. (2012). Effect of Tulsi (*Ocimum sanctum*) as a disinfectant for water treatment. *Hitek Journal of Bio Sciences and Bioengineering*, 1(1), 1-7.