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Comparative phytochemical profiling and antimicrobial activity of different plant parts of *Ocimum basilicum* L.

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Abstract

Ocimum basilicum L. (Basil) is one of the most common plants used traditionally all over the world. Present research consists of phytochemicals analysis and antimicrobial activities of leaves and stem of *Ocimum basiliscum.* It belongs to the *Lamiaceae* family. An attempt has been made to screen the phytochemicals and antimicrobials potential *Ocimum Basilicum* using ethanol extract. The powder obtained from dry part of leaves and stem of plant were exerted with ethanol solvent. The extract was analyzed for phytochemicals and antimicrobials (antibacterial & antifungal). Phytochemicals characterization of the different extracts revealed the presence of the phytochemicals–phenol, tannins, flavonoids, steroid, terpenoid, alkaloids, proteins, carbohydrates, and glycosides. Alkaloids, proteins and terpenoids were present in both stem and leaves of ethanol extracts of *o. basilicum.* Saponins compound were absent in both stem and leaves of ethanol extracts. While steroids, tannins and phenols were only present in ethanol extracts of *o. basilicum* leaves. Likewise, glycosides and carbohydrates were present in stem of ethanol extracts of *O. basilicum* and absent in leaves. Total phenolic and flavonoid content observed higher in *Ocimum Basilicum* leaves and lower in stem. TPC in *Ocimum Basilicum* leaves (35.7 ±0.36) shows higher value then *Ocimum Basilicum* stem (16.8±0.2). TFC in *Ocimum Basilicum* leaves (28.59±0.37) shows higher value then *Ocimum Basilicum* stem (26.53±0.37). Antibacterial activity**,** *Ocimum Basilicum* leaves (11.16±0.76) shows large zone of inhibition against *B. subtilis* and *E. coli* (8.26±1.12) as compared to *Ocimum Basilicum* stem against *B. subtilis* (4.13±0.41) and *E. coli* (6.16±0.56).Antifungal activities of ethanolic extract of leaves of *O. basilicum* shows minimum value against *Alternaria* after 48 hours (1.43±0.022) and shows maximum value after 72 hours (3.12±0.02),while *Colletotrichum* shows (1.5±0.5) after 48 hours and (3.19±0.03) after 72 hours & stem shows minimum value against *Alternaria* (1.61±0.017) after 48 hours and shows maximum value (3.28±0.026) after 72 hours while *Colletotrichum* shows maximum in both after 48 hours (2.69±0.005) and (3.86±0.028) after 72 hours.

1 Introduction

Plants include a wide range of medicines, nutraceuticals, nutritional supplements, pharmaceutical intermediates, and chemical compounds that may be used to create new drugs. There is a long history of the use of plants and plant compounds as medicinal substances (Issazadeh *et al*., 2012). There is no doubt in anyone's mind about the value of plants. Plants have a wealth of potential medicines, and recent years have seen a rising appreciation for medicinal plants. Natural remedies are readily accessible, less expensive, and associated with less risks and negative effects than conventional pharmaceuticals. One of the most apparent choices when looking at the search for therapeutically effective new pharmaceuticals like anticancer treatments and antibacterial drugs is to look at plants that have been used for medicinal purposes for thousands of years (Yadav and Agarwala, 2012). To cure a variety of ailments and to improve an array of cosmetic products, several plants include naturally occurring antioxidants such phenolic and polyphenolic chemicals that may be found in abundance in the botanical world (Mousavi *et al*., 2020). Medicines for a broad variety of human ailments may be

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found in abundance in the plant kingdom. In impoverished countries, 80% of people use herbal medicine to cure their health issues, according to a WHO survey (Ahmed and Aujla, 2012). Over 85 percent of the 175 medicinal substances are natural or derived straight from nature.

New pharmacologically active chemicals may still be isolated from plants in the early years of the twenty-first century (Rubab *et al*., 2021). *O. basilicum* is pubescent from the bottom to the top. Foliage is shiny and glabrous, oblong and toothed, with vein patterns. Its petiolate edge is angulate and narrow. Verticillaster constitutes the inflorescence. Labiate flowers come in six distinct varieties. The pedicel is a tethered object. 5-lobed bilabiate calyx with the uppermost lobe spreading over the others. The white bilabiate corolla has four lobes on each of its four petals. It is 1 centimeter long and may have petals that are independent or united. Its bottom lip has four stamens, and the stamens are supported aloft by the corolla (Rubab *et al*., 2021). Fragrance, culinary spice, and medicine have all relied on it for a long time. When used as a carminative, stomach galactogogue, antispasmodic, antioxidant and anti-inflammatory in traditional medicine its leaves and blooming tips are highly prized (Lal *et al*., 2017; Moghaddam *et al*., 2011). *Ocimum basilicum* has traditionally been used to cure a wide range of ailments, including coughs, warts, diarrhea, stomach aches, and even hepatic diseases, sunstroke, inflammation, snake bites, rheumatism, constipation, and worms (Bahadur *et al*., 2020). As a flavoring component, it has a distinct scent. It is employed in the cosmetics and pharmaceutical industries because to its unique aroma (Gohari *et al*., 2020). The agricultural, veterinary, and pharmaceutical sectors all use phytochemicals (Ahmed *et al*., 2019). There are several antimicrobial and antidiarrheal properties in flavonoids and polyphenols as well as tannins and coumarins, which are antiviral. Terpenoids and essential oils, which are antibacterial, antidiarrheal, and antiviral, are also included (Adeyanju *et al*., 2011; Tiwari *et al*., 2011).

2. Materials and Methods

2.1 Description of Study Area

The study was place over the course of a year in Pakistan's southern Punjab province's Cholistan Desert, an area of 2600 square kilometers with a hot and dry environment in the district of Bahawalpur. The Cholistan desert is between latitudes 75240 East and 27420 North. The monsoon season brings the bulk of the region's annual precipitation, which ranges from 100 to 250 millimeters. The average summer temperature is 34 to 38 degrees Celsius, while the average winter temperature is 14 to 16 degrees Celsius. During the summer (May–July), the temperature reaches 52 degrees Celsius, while in the winter it drops below zero degrees Celsius (Metrology Department, Government of Punjab, Pakistan). Its sandy silt and sandy clay soil make up the Cholistan desert's landscape (Riaz *et al*., 2021).

2.2 Identification and Collection of Plant Leaves and Stem

We chose *Ocimum basilicum* (Lamiaceae (mints) family) as the subject of our pharmacological research. An experienced taxonomist from the Forestry Laboratory identified the plants (Forestry, Range, and Wild Life Management). The research took place at the Islamia University of Bahawalpur's Phytochemical Laboratory, which is part of the Department of Forestry, Range, and Wildlife Management. Fresh leaves and stems of a variety of plants were taken from the Islamia University of Bahawalpur's Nursery. The leaves were rinsed in distilled water and dried for 3 to 5 days in the shade, while the stems were dried for 5 to 10 days. After that, the leaves were dried in an oven at temperatures ranging from 40 to 45 degrees Celsius for 4-5 days. The dried leaves and stems were then ground into powder using an electric grinder. The powdered leaves and stem were kept in sealed containers to be analyzed chemically later.

2.3 Preparation of Ethanol Extract of Leaves & Stem of Plant

The maceration extraction method was used to make the ethanol extract. For this, 130gm of leaf and stem powder was steeped for one week in an apparatus containing 650ml of ethanol, or until the solvent changed colour. Preparations were filtered using Whatman No. 1 sterilized filter paper. The extract was then reduced in volume by evaporation to a third of its original volume at room temperature. Additionally, aqueous extracts of leaves and stems were produced and kept in the refrigerator at 4°C for future use.

2.4 Qualitative Phytochemicals Analysis

Crude extract was combined with 2ml of Millon's reagent, protein was found in the white precipitate that formed. When the precipitate was heated gradually, it developed a reddish-orange color. 2ml Benedict's solution was boiled with the crude extract, the precipitate that formed indicated that carbohydrates were present in the sample. It was mixed with 2 percent $FeCl_3$ solution to make 2 mL of crude extract. To identify the presence of phenols and tannins, the material was dyed a blue-green or black color. HCl was added to the combination of crude extract and magnesium ribbon by the smallest amount possible. Flavonoids formed a rosy scarlet color after a short time, suggesting their existence. Crude extract was dissolved in 5ml of distilled water and shaken rapidly in a test tube before being stored in the refrigerator. Saponins were credited with the development of long-lasting foam. Chloroform was added to the crude extract to dilute it. After that, it was safe to add 2ml of concentrated H_2SO_4 and give it a good shake. Color of the glycoside indicated the existence of a steroidal ring, which is the glycone part of the glycoside. Chloroform was added to the crude extract and evaporated until it was entirely dry, then the mixture was centrifuged. For around two minutes, the mixture was heated with 2ml of concentrated H2SO4. There was a tinge of grey to suggest the presence of terpenoids. Using a sideways motion, concentrated H_2SO_4 was poured into a mixture of 2 mL chloroform and crude extract. The bottom chloroform layer was stained red as a result of the presence of steroids. Two milliliters of one percent hydrochloric acid were added to the crude extract and warmed to room temperature. Mayer and Wagner chemicals were then added to the mixture. Alkaloids may be detected by looking at the precipitate's turbidity (Yadav and Agarwala, 2012).

2.5 Quantitative Phytochemical Analysis

2.5.1 Determination of total flavonoid content

The aluminum chloride colorimetric technique was used to measure the total flavonoid content. 1 mL of plant extracts at varied concentrations was mixed for 30 minutes at room temperature in n-hexane, aluminum chloride and potassium acetate, which were then diluted with 5.6 mL of distilled water. A spectrophotometer set at 415 nm was used to measure the reaction mixture's absorbance. In order to guarantee the accuracy of the findings, methanol was used as a negative control. Applying the formula below, we determined the flavonoid content of plant ethanol extracts in terms of quercetin equivalents:

$$C = (C \times V)/m$$

the concentration of quercetin measured using the calibration curve is "c" and the amount of flavonoid content overall in mg/g plant extract is "m," the volume and weight of crude plant extract are "V" and "M," respectively.

2.5.2 Determination of total phenolic content

Using the Ciocalteu method, Folin-colorimetric Total phenol content (TPC) was measured. We added 1.5 ml of Folin-reagent Ciocalteu's (10%) and 1.2 ml of sodium carbonate (7.5 percent w/v) to the extracted solution (0.3 ml in triplicate). After 30 minutes in the dark, the absorbance of the mixture was measured at 765 nm. The quantity was calculated using a conventional acid curve. The data were represented by gallic acid equivalents (GAE) (mg of gallic acid per 100 ml). There was a total of three retests [14].

2.6 Antimicrobial Activity

2.6.1 Antibacterial Activity

Bacterial strains

Islamia University's University College of Veterinary and Animal Sciences in Bahawalpur have cultured bacteria from the bacteriology section of Bahawal Victoria Hospital. *Escherichia coli* and *Bacillus subtilis* are among the pathogens under consideration. With the use of Eppendorf tubes, we created 150-liter stocks of glycerol and 850-liter cultures of bacteria.

Media preparation

The following media were used for the antibacterial studies:

Nutrient broth:

To make nutritional broth, 100 mL of distilled water was mixed with 1.3 grams of nutrient broth. 20 minutes at 121°C in an autoclave for sterilization.

Nutrient agar:

Dissolving 2.8 grams of nutrition agar in distilled water resulted in the creation of nutrient agar. Disinfect the item for 20 minutes at 121°C using an autoclave.

Extraction of plant materials

Following extracts were made to take antibacterial assay:

Ethanol extract:

In order to prevent ethanol evaporation, a conical flask was filled with 10 grams of powdered plant and 100 mL of 100 percent ethanol. The flask was then coated with aluminum foil. A normal Whatman filter paper was used to purify the extract after 42 hours of extraction.

Cutting of discs from disc diffusion method

Normal Whatman filter paper was used to produce the discs. Plant extracts were utilized to test the discs' ability to inhibit the growth of microbes in culture. When conducting negative controls, discs were impregnated with ethyl alcohol. Pre-soaked discs of ciprofloxacin (a commercially available antibiotic) were used as a benchmark.

Antibacterial Assay

Extracted strains of bacteria from glycerol stocks were fed to sterilized broth in a loop, and the resulting broth was sterilized. For 24 hours, the cultures were kept at 37 degrees Celsius and shaken constantly. Look for turbidity in the samples in the tubes. Adjust the OD600 readings of the cultures to 0.4. It takes 10 minutes to spin 1 mL of culture at 12000 revolutions per minute (RPM). Leave around 100 liters of supernatant on the particle. Place the pellet on agar and dissolve it with water.

Determination of zone of inhibition

Testing for antibacterial activity was carried out using the agar disc diffusion technique. Nutrient agar melted and was placed into sterilized petri plates. Using a glass spreader, plates were infected with 100 L of the appropriate organism and incubated at 37oC for one hour. Agar media was seeded with different microorganisms and then pre-soaked discs (6 mm in diameter) in various extracts. While Ciprofloxacin discs were utilized as positive controls, discs coated with ethanol were employed for negative control. In order for the bacteria to thrive, the plates were incubated at 37°C for 24 hours. Test samples' antibacterial activity was quantified by measuring the width of the inhibition zone in millimeters. Antibacterial activity was found in the clear zones, whereas bacterial resistance was found in the clear zones that included micro-colonies (Nawwal *et al*., 2021).

2.6.2 Antifungal Activity

Preparation of Potato Dextrose Agar (PDA)

Researchers in the Plant Pathology laboratory created Potato Dextrose Agar medium for the cultivation of fungus (Agriculture Department of Islamia University of Bahawalpur). Petri dishes of the appropriate size were utilized throughout the experiment. PDA was made by mixing 39 grams of PDA powder with 1000 cc of distilled water and stirring until the liquid was homogenized. After that,

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an autoclave at 121°C for 25 minutes under 15 psi pressures sterilized the PDA combo. It was then placed in petri dishes at a 20-ml/dish ratio and left on the table to cool down to room temperature so that agar could harden.

Poisoned Food Technique Assay

A little adjustment was made to the antifungal test employing poisoned food.1mL of each plant concentration was added to sterilized melted PDA and gently swirled in circular motion to get homogenized on sterilized petri plates. The similar technology was used to create controls for a PDA-filled plate. Let them cool down and solidify before using them. After that, a 7-day-old culture's growing hyphae were used to make 5 mm diameter mycelial discs using a sterile 5 mm diameter cork borer. All of the petri plates containing treatments had mycelial discs inserted in the middle of them. Petri plates containing inoculum were taped shut and left to incubate at room temperature. Each therapy was carried out three times in total. Vernier caliper was used to measure the development of mycelium.

Tools for Data Analysis

The formula was used to determine the percentage of mycelial inhibition:

$$\%\ inhibition = \frac{C - T}{C} x100$$

It is important to note that C represents mycelium growth, and T is mycelium growth minus inoculum disc diameter for both sets of mycelium growth (Hussain *et al*., 2016).

***2*.7 Statistical analysis**

For Statistical analysis, the plant samples were investigated using analysis of variance (ANOVA) and the Tukey's HSD test was used to separate the means.

3. Results

3.1 Quantitative Phytochemical Analysis Experiment

Total Phenolic Content (TPC) of *Ocimum basilicum*

Significant results were found for total phenolic contents. The Fig.1 indicates highly significant differences for ethanol extract (leaves and stem). The TPC of stem and leaves of *O. basilicum* was observed to be statistically highly significant using one-way ANOVA ($P < 0.01$)

Total Flavonoid Content (TFC) of *Ocimum basilicum*

Significant results were found for total Flavonoid contents. The Fig.1 indicates highly significant differences for ethanol extract (leaves and stem). The TPC of stem and leaves of *O. basilicum* was observed to be statistically highly significant using one-way ANOVA ($P < 0.01$)

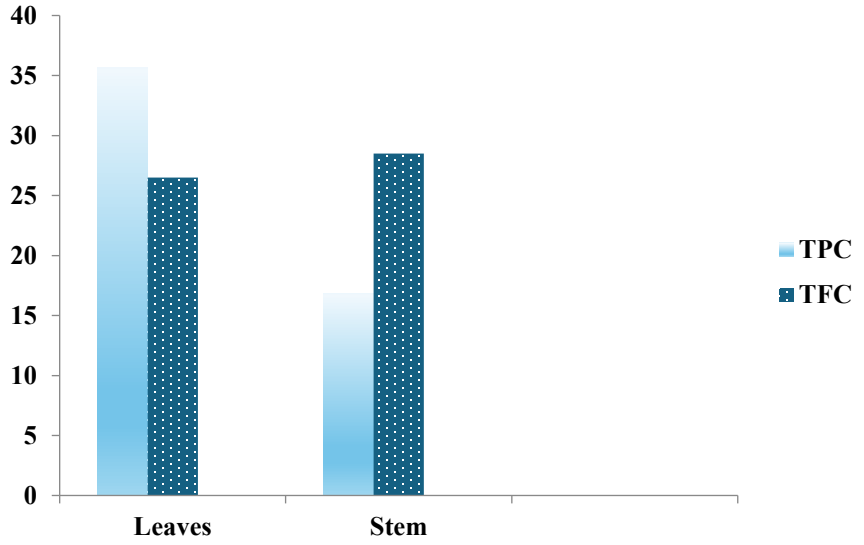


Fig 3.1 Total Phenolic Content (TPC) and (TFC) of ethanolic extract of leaves and stem (*Ocimum basilicum)*

3.2 Qualitative phytochemical analysis

Alkaloids, proteins and terpenoids were present in both stem and leaves of ethanol extracts of *o. basilicum.* (Table: 1). Saponins compound were absent in both stem and leaves of ethanol extracts. While steroids, tannins and phenols were only present in ethanol

extracts of *O. basilicum* leaves. Likewise glycosides and carbohydrates were present in stem of ethanol extracts of *O. basilicum* and absent in leaves (Table: 1).

Table-1: Phytochemical analysis of the leaves and stem of ethanol extracts of *O. basilicum*

Sr No:	Phytochemicals	Leaves	stem
1	Carbohydrate	_	+
2	Phenols	+	_
3	Tannins	+	_
4	Flavonoids	+	+
5	Saponins	_	_
6	Glycosides	_	+
7	Steroids	+	_
8	Terpenoids	+	+
9	Alkaloids	+	+
10	Proteins	+	+

+: Present, - : Absent

3.3 Antibacterial Activity

Antibacterial assay of ethanolic extract of *O. basilicum* against *B. subtilis*

Ethanol leaves generated the largest inhibition zone (11 mm) against *B. subtilis*, whereas the stem created a 4 mm zone of inhibition. *B. subtilis* was killed by an ethanol extract of *basilicum* leaves (11 mm mean value) and modest activity was found in the stems. *O. basilicum* ethanol extract was observed to be statistically highly significant using Completely Randomized ANOVA ($P < 0.01$). Ciprofloxacin's zone of inhibition on *S. subtilis* was considerable (29.5 mm) but that on *E. coli* was less so (23 mm) as shown in table 2.

Antibacterial assay of ethanolic extract of *O. basilicum* against *E.coli*

The findings revealed that the largest inhibition zone (8 mm) against *E. coli* was created by the ethanol leaves, whereas the stem formed a 6 mm inhibition zone. Ethanol extract of *basilicum* stem revealed moderate activity against *E. coli* (i.e. 8 mm mean value) and minimal activity was found in the stems. *O. basilicum* was observed to be statistically significant using Completely Randomized ANOVA ($P < 0.04$)

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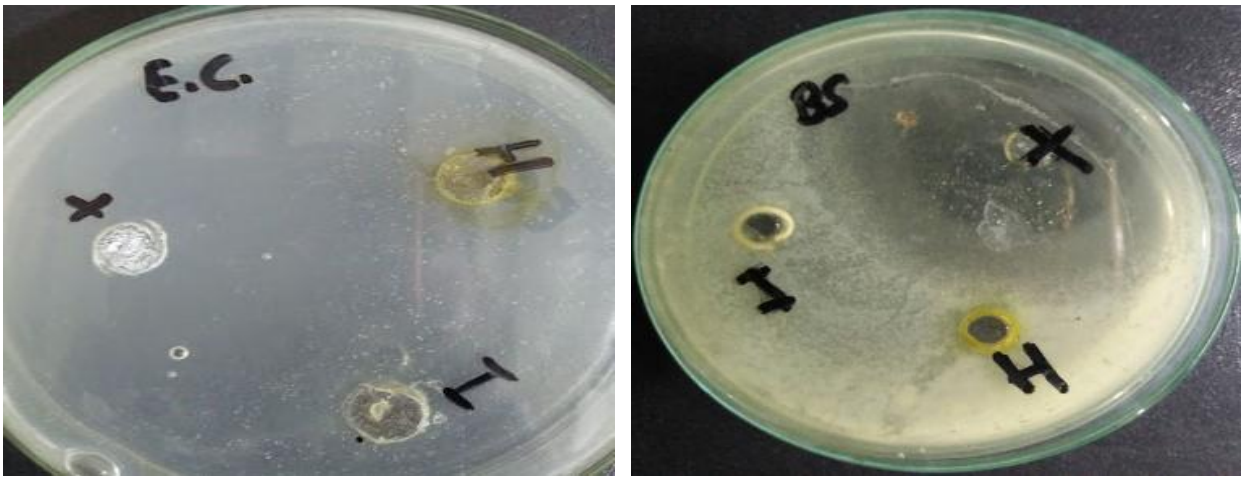


Fig 3.2 +: control, H: leaves, I: stem S (zone of inhibition activity against *E. coli* and *B. subtilus*)

Table-2: Value of Zone of inhibition *B. Subtilis*, *E. Coli* and Control

Treatment	*B.Subtilis*	*E.Coli*
+ control	29.5mm	23mm
Leaves	11mm	8mm
Stem	4mm	6mm

Bacterial strain used: *Esherichia.Coli*: *E.Coli*, B*acillus subtilis*: *B.Subtilis.*
Standard Antibiotic: Ciprofloxacin

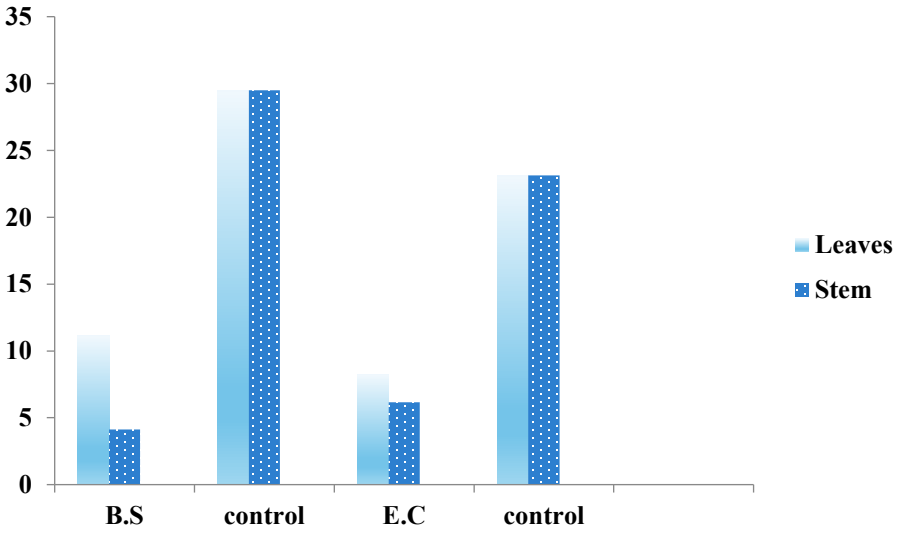
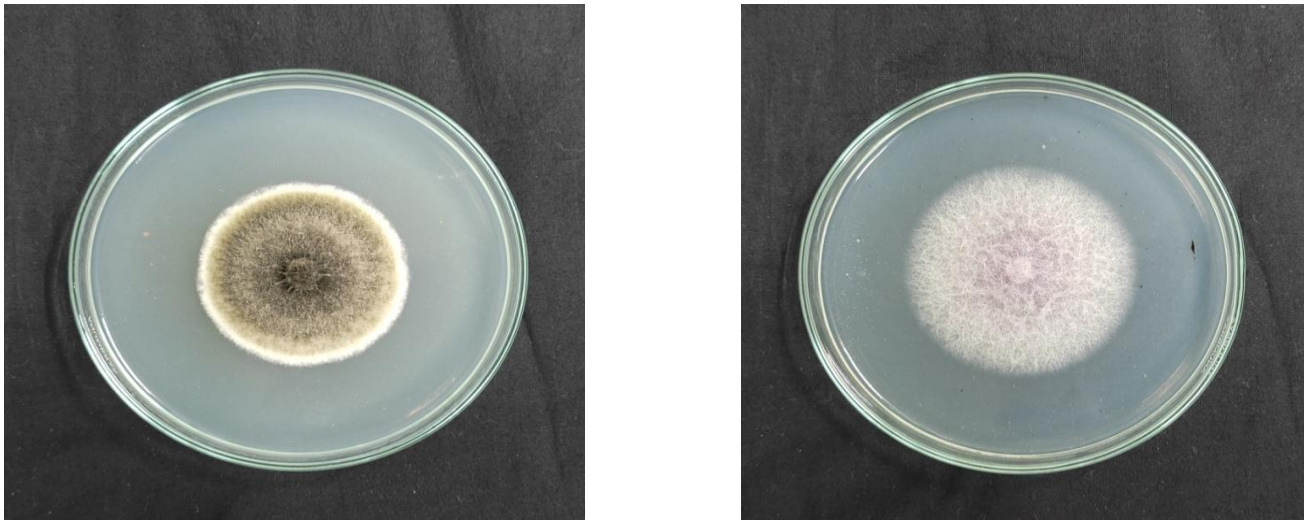


Fig 3.3 Antibacterial activities of ethanolic extracts of leaves and stem against (*E.coli* and *B.subtilis*)

3.4 Antifungal Activity

Antifungal activity of ethanolic extract of leaves of *O. basilicum*

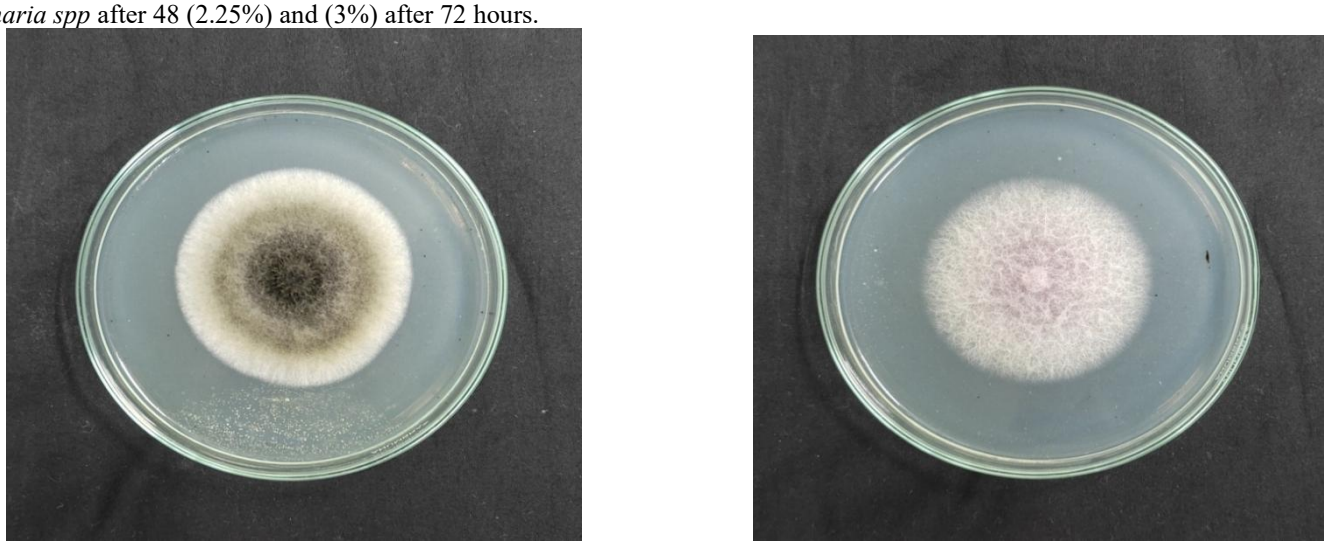
For *Colletotrichum* species, the ethanol leaves exhibited maximum inhibition at 48 hours1.50% and 3.20 % at 72 hours, whereas for *Alternaria spp*, the minimum inhibition was at 1.45% and the maximum inhibitory concentrations were at 3.15% at 72 hours. The ethanol extract of *O. basilicum* leaves was shown to be the most effective against *Colletotrichum spp.* Leaves of *O. basilicum* was observed to be statistically highly significant using Completely Randomized ANOVA ($P < 0.001$).



(a) *Alternaria* (b) *colletotrichum*

Fig 3.5 Inhibition activity of ethanolic extract *O. basilicum* leaves (a) *Alternaria*, (b) *colletotrichum*

A control showed significant inhibition against *Colletotrichum spp* after 48 (3%) and (6.15%) after 72 as compared to *Alternaria spp* after 48 (2.25%) and (3%) after 72 hours.



(a) *Alternaria* (b) *Colletotrichum*

Fig 3.6 Inhibition activity of control (a) *Alternaria*, (b) *Colletotrichum* spp)

Table-3: Value of inhibition of *Alternaria* and *Colletotrichum* spp.

Extracts	**Fungal**	**After 48 hours**		**After 72 hours**	
		Treatment	control	treatment	Control
Leaves	*Alternaria spp*	1.45	2.25	3.13	4.46
	Colletotrichum spp	1.50	3.0	3.20	6.15

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Stem	*Alternaria spp*	1.60	2.25	3.29	4.46
	Colletotrichum spp	2.69	3.0	3.85	6.15

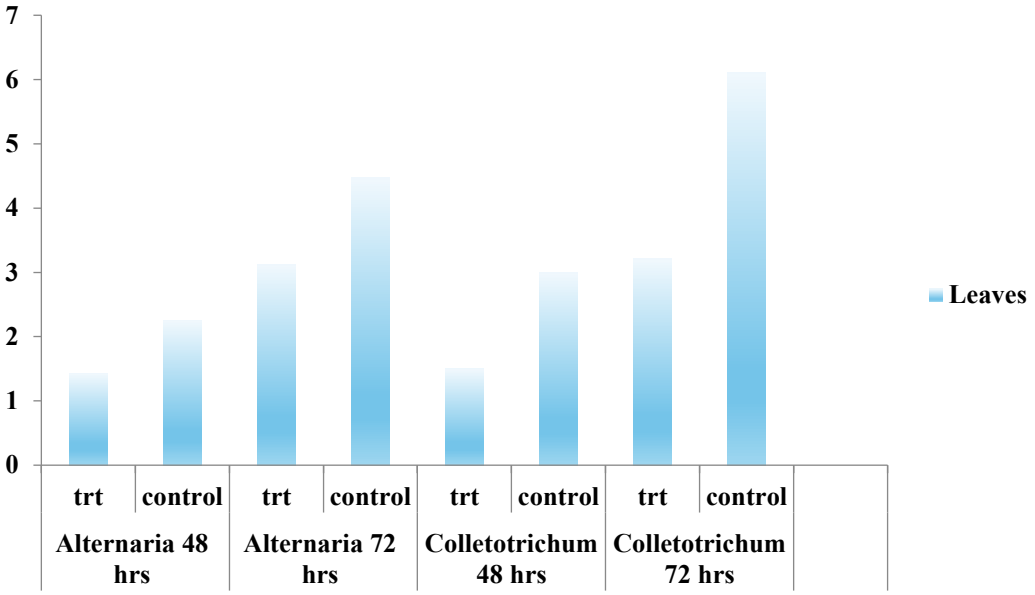
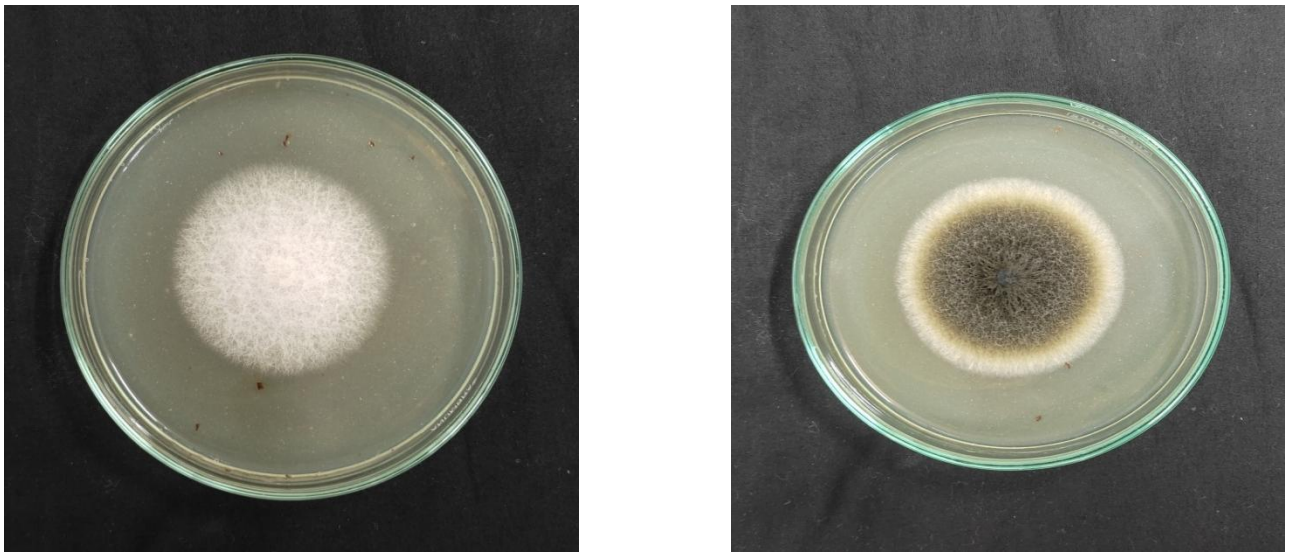


Fig 3.7 Antifungal activities of leaves of *O. basilicum*

Antifungal activity of ethanolic extract of stem of *O. basilicum*

There was a 2.69 percent inhibition of *Colletotrichum spp* after 48 hours and a 3.85 percent after 72 hours with the stem of ethanol, whereas *Alternaria spp* was generated at 1.60 percent and 3.29 percent after 48 hours and 72 hours. Compared to *Alternaria spp*, the Ethanol extract of *O. basilicum* stem was more effective against *Colletotrichum* species. Stem of *O. basilicum* was observed to be statistically highly significant using Completely Randomized ANOVA ($P < 0.001$).



(a) *Colletotrichum* (b) *Alternaria*

Fig 3.8 Inhibition activity of ethanolic extract *O. basilicum* stem (a) *Colletotrichum*, (b) *Alternaria*

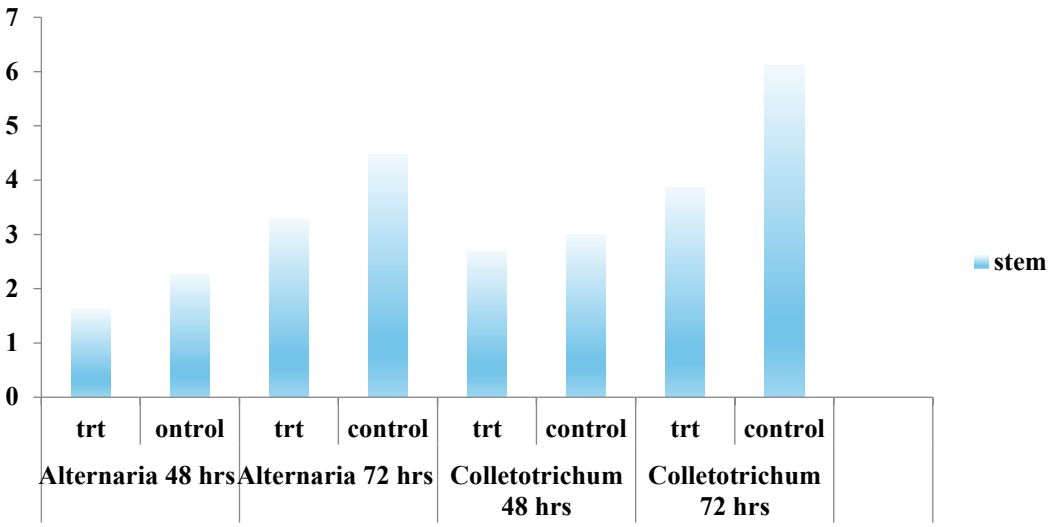


Fig 3.9 Antifungal activity of stem of *O. basilicum*

4. Discussions

As a demonstrated by our research *O. basilicum* leaves has a considerable quantity of phenol, tannins, flavinoids and other phytochemicals as well as steroids, terpinoids, alkaloids and proteins. Carbohydrates, favinoids, glycosides, terpinoids, alkaloids, and proteins are all included in the stem's ethanolic extract. According to previous work, tannins, terpernoids, glycosides, cardiac glycosides. Extracts of *O. basilicum* leaves are rich in a variety of phytochemicals, and these compounds are increasingly being used in pharmaceuticals and food items alike (Gebrehiwot *et al*., 2016). Alkaloids, glycosides, saponins, phenols, proteins, carbohydrates, steroids, flavonoids, and tannins are only some of the secondary metabolites found in *Ocimum basilicum's* ethanol and aqueous extracts. Alkaloids, saponins, flavonoids, and tannins have been shown to help treat infections (Juliet *et al.,* 2019).

Evaluation of antibacterial activity of *O. basilicum* extracts against microorganisms examined in this study. In *Ocimum basilicum* (leaves and stem), the extracts were shown by using the presence or absence of inhibition zones, as well as MIC values. The ethanolic extract of leaves show more resistance against *B. subtilis* and show less resistance against *E. coli*. The ethanolic extract of stem show resistance against *E. coli* and show very less resistance against *B. subtilis*. These findings can be compared with those of (Juliet *et al.,* 2019). Ethanol extract of *O. basilicum* showed antimicrobial action against nine bacteria from the families *Acinetobacter, Bacillus*, *Escherichia*, and *Staphylococci*. When compared to water extracts, ethanol extracts of *Ocimum basilicum* were shown to be more effective in killing bacteria. Agar well diffusion experiments with different concentrations of *Ocimum basilicum* ethanol extract (4200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml) showed discrete zones of inhibition against *Escherichia coli*, *Salmonella typhi*, and *Shigella flexneri*. Based on the findings of this analysis, the extract was shown to be sensitive to *Escherichia coli* and *Shigella flexneri*, with an inhibition zone at 200 mg/ml of 11, while *Salmonella typhi* had an inhibition zone at 200 mg/ml of 9. At concentrations of 25 mg/ml and 12.5 mg/ml, the ethanol extract had no impact on the test organisms. Gram-positive Leaf extracts had a substantial impact on *Staphylococcus aureus* and *Bacillus subtilis*, as well as Gram-negative *Shigella dysentriae*. *Salmonella typhi* was not killed by the leaf extracts. The methanolic extract showed a 30-millimeter inhibitory zone against *Staphylococcus aureus*, with a MIC and MBC of 1.56mg/ml and 3.125mg/ml, respectively. An abundance of flavonoids and a little amount of cardiac glycoside may be responsible for the tea's antibacterial qualities. Flavonoid-rich extracts may suppress certain bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. Flavonoids (94.00 percent and 51.29 percent, respectively) were found in both aqueous and methanolic extracts of the plant's leaf (Isa *et al*., 2019).

Ocimum basilicum ethanolic extracts of (stem and leaves) were shown to be ineffective against *Alternaria alternate*. These findings can be compared with Ahmad et al. (2016) the modest antifungal action of *Ocimum basilicum* is effective against *Aspergillus fumigates, Alternaria alternata*, and *Cladosporium*. *Ocimum basilicum* methanolic extracts were shown to be ineffective against *Aspergillus flavus, Penicillium*, and *Alternaria.* (Sharma et al., 2019). The *Alternaria alternata* and *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Alternaria solani* and *R. solani*, were shown to be much more resistant to the boiling extract. This research supports the use of a boiled *Ocimum sanctum* leaf extract to suppress fungus. The crude form of *Ocimum sanctum* leaf extract reduced the development of *Phoma sorghina*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum f.sp. ciceri, and Fusarium oxysporium f.sp. pallidoroseum* and *ciceri*, *pallidoroseum* more successfully than the other four, whereas the powdered form better controlled the growth of Sclerotin. These findings by showing *Ocimum basilicum* ethanolic extracts of (stem and leaves) were shown to be effective against *Colletotricum gloeosporioides.*

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5. Conclusions

Phytochemicals are mostly obtained from medicinal plant. Medicinal plants are used to screen and find secondary metabolites, which are extremely valuable in the development of new medicines. Pharmaceutical businesses and research institutions are also interested in the phytochemical study of medicinal plants for the creation of new medications for a wide range of illnesses. *O. basilicum* active in tests against both gram-negative and gram-positive microorganisms, respectively. *O. basilicum* considered a medicinal plant in future bioassay. As a whole, the findings of this study lend credence to traditional medicine's use of plant extracts to treat a variety of fungal infections. As a result of our research, we discovered that this plant may be a new source of natural antibacterial agents. *O. basilicum* can be utilised as an antimicrobial alternative. Natural bioactive phytochemicals may be responsible for *O. basilicum* extract's therapeutic efficacy.

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