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Photochemical Studies on Ficus religiosa and Ocimum tenuiflorum L. and their Antimicrobial Effect on Bacillus Subtilis and Escherichia Coli

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ABSTRACT

Phytochemical studies of Ocimum tenuiflorum and Ficus religiosa leaves and their antimicrobial effect on Escherichia coli and Bacillus subtilis" is an effort to put light on the efficient antimicrobial activity of Ocimum tenuiflorum and Ficus religiosa over Bacillus subtilis and E.coli which is the underlying organism of diverse diseases as mentioned below. We executed a number of trials such as, antimicrobial assay, sensitivity test; along with phytochemical screening which let us know the secondary metabolite answerable for antimicrobial activity. We also utilized the TLC modus operandi in attempt to bring out the antibacterial compounds present in the leaf extract for the inhibition of microbial growth. The end results obtained in the form of tables and graphs are accompanied by individual photographs to prove its legitimacy of the trials performed. The studies in real sense have not concluded yet, in fact it had divulged a number of panoramas to be carried out in our near future- The antimicrobial compounds in Ficus religiosa can be more distinctively identified and split through improved skill such as UPLC/HPLC. The complexes of the leaf extract can be altered in its chemical chattels in enhancing its antimicrobial commotion. The compounds responsible for antibacterial effect of Ocimum tenuiflorum can be taken apart through TLC to a elevated level. Plant fractions further than leaf such as root, stems can also be regarded as an aim of study for a variety of antimicrobial extracts.

Keywords: Ocimum tenuiflorum, Ficus religiosa, Bacillus subtilis, UPLC/HPLC and Antimicrobial activity.

1. INTRODUCTION

Plants apart from being the primary producers of the ecosystem are playing the most vital role in human life and origin, since the time immemorial. It has contributed not only as supplementary diets but also protected the immune system through its antimicrobial activity. This miraculous activity (hormonal, enzymatic or chemical) of plants has proved to be a boon to human civilization. This antimicrobial property has also been shown by various synthetic compounds in antibiotics produced now days. Some of those antibiotics are metronidazole, trimidazole, triazole allylamine etc. Though they show fast antimicrobial activity but these actions, in most cases are accompanied by various undesirable consequences of health hazards such as vomiting, nausea, shortage of breath, emegence of resistant bacteria, depression, hormonal changes, and allergic reactions etc by the active use of these antibiotics over a long period of time. In context to this phenomenon, the organic antimicrobial compounds do not show undesirable results. Medicinal plants are known to possess a number of secondary metabolites such as amines and alkaloids, cyclilots, fatty acids and seed oils, tannins, flavonoids, terpenoids, resins etc. These secondary metabolites are mainly responsible for the antimicrobial property of the plants. It has been reported that Ocimum tenuiflorum possess antimicrobial compounds namely ursolic acid, apigenin and leleolin. The purpose of the present study is to investigate the antimicrobial activity of leaf extracts of Ocimum tenuiflorum and Ficus religiosa against gram positive Bacillus subtilis and gram negative Escherichia coli. The extracts with the highest antibacterial effectiveness were chosen for subsequent use in pharmaceutical formulations. The selected microbes are known to be the causal organism of various diseases such as Bacillus anthracis, dental problems caused by Bacillus whereas diseases like cholecystitis, bacterimia, colengitis, Urinary infection (UTI), travellers diarrhoea, neonal meningitis, Pneumonia etc. are caused by E coli.

Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the anti microbial activity of medicinal plants. Few papers relating to the effect of these plants are being forwarded by eminent scholar researchers- Srinivasan et al, (2001), Kumaraswamy et al, (2002), Ali et al, (2001), Masika and Afolayan, (2002), Hamill et al.(2003) etc.The use of plants and plant products should be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in "Rig Veda", which is said to have been retained between 4500-1600 BC and is supposed to be the oldest evidence of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002). Keeping this point in view, solvent extraction procedure has been formulated. In consideration to the above facts it becomes important to study the biochemical components of different plant species, isolate the active components and assess their antimicrobial properties and explore the possibility of utilizing in curing diseases of plants and animals.

2. OBJECTIVE

1. To study the effect of different solvent system in isolation of the active substances.
2. To study the efficacy of O. tenuiflorum and F. religiosa leaf extract in commonly controlling diseases caused by B. subtilis and E. coli.
3. To study the active phytochemicals of the experimented plants which are responsible for controlling the diseases caused by B. subtilis and E. coli

2.1 Plant as source of antimicrobials

The use of plants and plant products as medicines could be traced as far back as beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rig-Veda", which is said to be written between 4500-1600 BC and is supposed to be the oldest repository of human knowledge. It is ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing. (Rastogi and Mehrotra, 2002).A major part of a total population in developing countries still uses traditional folk medicine obtained from plant resources (Farnsworth 1994) with an estimation of WHO that as many as 80% of world's population living in rural areas rely on herbal traditional medicines as their primary health care, the study on properties and uses of medicinal plants are getting growing interests. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Herbal medicine is still the mainstay of about 75-80 % of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituent (Akerele, 1993). The plant extracts were more active against gram positive bacteria than against gram negative bacteria.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases. In addition to this problem, antibiotics are sometimes

associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances .Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Agarwal et al., 1996) Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combination of secondary metabolite such as alkaloid, anthraquinones, flavonoid, Phlobatanins, saponins, steroid, tannin, terpenoids which are capable of producing definite physiological action on body.

2.2 Characterization of bacterial Isolates

2.2.1 Gram staining

This method was introduced by Hans Christian Gram; it is one of the most important staining technique in microbiology. It generally differentiates two large species of bacteria based on their different cell wall constituents i.e. Gram-positive and Gram-negative. The crystal violet is attracted to both Gram-positive and Gram-negative microorganism. The second step (Gram's iodine, a mordant) stabilizes the crystal violet into the peptidoglycan layer of the cell wall which is much thicker in Gram-positive bacteria than in Gram-negative bacteria. Hence, crystal violet is more extensively entrapped in the peptidoglycan layer. The third step (alcohol decolourization) dissolves the lipids in the outer membrane of the Gram-negative bacteria and removes the crystal violet from the peptidoglycan layer, which cannot be removed in Gram-positive organism. After the third step, only the colourless Gram-negative microorganism can accept the safranin (counterstain).

2.3 Antibiotic Susceptibility Test

Bioassays are typically conducted to measure the effects of a substance on a living organism. Bioassays may be qualitative or quantitative. Qualitative bioassays are used for assessing the physical effect of a substance that may not be quantified such as abnormal development or deformity. Quantitative bioassays involve estimation of the concentration of a substance by measurement of the biological response that it produces. Several methods are employed to assay bioactive property of bacterial isolates such as Agar diffusion method, Disc plate method, MIC.

2.4 Solvent Extraction Method

Solvent extraction method is a procedure for the preparation of the plant extracts using various organic solvents. Many scholars and researchers have studied the effects of various solvent extracts of plants upon different microorganisms and their activity. K.Sudharameshwari and J.Radhika studied the antimicrobial activity by using petroleum ether, chloroform, ethanol and water where petroleum ether exhibited the maximum antibacterial activity (www.Antibacterial screening of Aegle Marmelos, Lawsonia Inermis and Albizzia Libbeck.html). Peter Synder studied the antimicrobial effects of spices and herbs upon different strains of bacteria, using three different organic solvents and found out maximum antibacterial activity on ethanol (www.Antimicrobial activity of spices

and herbs.html). Salma Ahmed studied the effects of Garlic and Mint on bacteria thus concluding that maximum activity was shown in the water extracts. Keeping in view the above references, the effect of Ocimum tenuiflorum and Ficus religiosa was studied using eight different solvents.

2.5 Phytochemical Screening of Plants

Clinical microbiologists have great interest in screening of wide variety of plants for antimicrobial activities and phytochemicals as potential new therapeutics. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in variety of different compounds such as aldehydes and phenolic compounds. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, flavonoids, resins, fatty acids gums which are capable of producing definite physiological action on the body.(Middle East Journal Of Scientific Research, 2010).

2.6 Thin Layer Chromatography

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantitation. This method is referred to as HPTLC or "high performance TLC"(www.Thin layer chromatography the free encyclopedia.html).

3. MATERIALS AND METHODS

3.1 Materials

Fresh leaves of Ocimum tenuiflorum and Ficus religiosa were collected at the early hours of the day from Guwahati during the months of September and December. Fresh leaves of both the plants were washed under running tap water, extra water removed with help of paper, oven dried and then crushed to fine powder using mortar pestle and stored in air tight bottles. The ethno botanical information of the plants screened is given below.

3.1.1 Ocimum tenuiflorum: (Basil, Tulsi)

The genus Ocimum is comprised of more than 50 species of herbs and shrubs. The shape of the leaves in Ocimum tenuiflorum and its close relatives varies in size of leaves, vein and petioles. The colours of the leaves vary from bright green to dark green and sometimes almost black. Due to the difficulties in identifying the species, Massimo et al (2004) has concluded that identification can be optimized by combined analysis of morphological traits, essential oil composition and molecular markers. The plant parts that can be used are the leaves, stem and the root. Use of Ocimum tenuiflorum:

Tulsi in several formulations help to enhance immunity and metabolic functions as well as in the management of respiratory problems.

1. It has pharmacological effects.
2. It has antimicrobial, antimalarial, anti-allergic and anti-stress effect.

3.1.2 Ficus religiosa

Ficus is a genus of about 850 species of woody trees, shrubs, vines, epiphytes and hemiepiphyte in the family Moraceae collectively known as fig trees or figs. Ficus is a pan tropical genus of trees, shrubs, and vines occupying a wide variety of ecological niches; most are evergreen, but some deciduous species are endemic to areas outside of the tropics and to higher elevations. Fig species are characterized by their unique inflorescence and distinctive pollination syndrome, which utilizes wasp species belonging to the Agaonidae family for pollination.

Use of Ficus religiosa:

Phenolic compounds have been extracted from the roots of Ficus religiosa which are antibacterial in nature. Further studies are going on the species to bring out their pharmacological effects.

3.2 Identification of the Microbial Strain

Area of collection of the microbial strain

The microbial strain was collected from the microbiology department of Hyderabad Medical College and Hospital, Hyderabad, Telengana. The strain studied was Bacillus subtilis and Escherichia coli.

AN OVERVIEW OF TEST ORGANISM

Bacillus subtilis :In 1835, the bacterium was originally named vibrio subtilis by Christian Gottfried Ehrenberg, and renamed Bacillus subtilis by Ferdinand Cohn in 1872. Bacillus subtilis, known also as the hay bacillus or grass bacillus, is a gram-positive, catalase bacterium commonly found in soil. A member of the genus bacillus, B. Subtilis is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. B. subtilis can divide symmetrically to make two daughter cells or asymmetrically, producing a single endospore .The endospore is formed at times of nutritional stress, allowing the organism to persist in the environment until conditions become favorable. Prior to the process to produce the spores the bacterium might become motile, though the production of flagella and also take up DNA from the environment. Escherichia coli: E.coli was discovered by German Paediatrician and bacteriologist Theodor Escherich in 1885. E.coli is a gram negative rod shaped bacterium that is commonly found in the lower intestine of warm blooded organisms. Most E. coli strains are harmless, but some can cause serious food poisoning in humans and are occasionally responsible for product recalls. E.coli is grams negative, facultative anaerobic and non sporulating cells is typically rod-shaped and are about 2 micrometers long and 0.5 micrometer in diameter. Optimal growth of E.coli occurs at 37º C but some laboratory strains can multiply at temperatures of up to 49 ºC. Virulent strains of E.coli can cause gastroenteritis, urinary tract infections and neonatal meningitis.

3.3 Inoculum Preparation-

Pure culture of Bacillus subtilis and Escherichia coli were taken and were sub-cultured in the nutrient broth at the interval of every 1 week.

3.3.1 Composition of the nutrient broth-

For 1000ml of water, the composition is as follows-

Beef extract- 3gm, Sodium chloride- 5gm, Peptone- 5gm, Water- 1000ml

3.3.2 Composition of the agar medium-

For 1000ml of water, the composition is as follows-

Beef extract- 3gram,Sodium chloride- 5gram,Peptone- 5gram,Agar- 15 gram,Water- 1000ml

3.4 Solvent Extraction-

Two grams of the dried materials of each plant species (Ficus and Ocimum) were mixed with 20ml of the 8 solvents separately. The solvents included are-

1. Acetone, 2. Dichloromethane, 3. Chloroform , 4. Water ,5. Ethyl acetate 6. Hexane 7. Methanol 8. Ethanol The mixtures were kept in the rotatory shaker for about 72 hours. Then it was filtered with the help of muslin cloth and the filtrate was allowed to air-dry until it became quite dry. Then the left out substances were scraped out and were collected in the tubes. Its volume was made upto 1ml with the original solvent used. It was then stored for further analysis.

ABOUT THE SOLVENTS USED IN THE EXPERIMENT

1. METHANOL(CH3OH): It is the first member of the homologous series of saturated monohydric alcohol. It occurs in the nature in the form of esters.

2 . ETHANOL(C2H5OH): It is an organic compound, whose molecule contains on hydroxyl (-OH) group attached to a saturated carbon atom.

3. ETHYL ACETATE(C4H8O2): Ethyl acetate or ethyl ethanoate is the ethyl ester of acetic acid and is a typical member of the class.

4. HEXANE(C6H14): Hexane is the sixth member of the homologous series, it is a aliphatic saturated hydrocarbon which is relatively uncreative under ordinary laboratory condition.

5. ACETONE(CH3COCH3): Acetone, Dimethyl ketone or propanone is the first and the best known member of the homologous series of ketones.

6. CHLOROFORM(CHCl3): The name chloroform was given to trichloromethane by Dumas who established that its molecule contained chlorine in union with ‘formyl’,an old name for CH group .

7. WATER(H2O): Water contains two hydrogen atom bonded to the oxygen. It is a chemical compound and polar molecule which is liquid at standard temperature and pressure. Water is found almost everywhere on the earth and is required by all life form known.

8. DICHLOROMETHANE(CH2Cl2): Dichloromethane (DCM or methylene chloride) is an organic compound with the formula CH2Cl2. This colorless, volatile liquid with a moderately sweet aroma is widely used as a solvent.

3.5 Antibacterial assay-

The antibacterial activity of the different solvent extract was evaluated by the disc diffusion method. The discs were soaked with the respective solvents. The experiment was performed several times under aseptic condition. The microbial sensitivity was determined by measuring the diameter of the zone of inhibition.

3.6 Disk diffusion method-

Disc diffusion method is also known as Kirby Bauer method which contain an antimicrobial agent placed on the surface of an agar plate containing a medium that has been inoculated with the disease agent being tested. The antimicrobial agent diffuses into the medium killing some of the disease agent around where the antimicrobial agent was inoculated, depending on how susceptible the disease agent is to the antimicrobial agent. The size of the area cleared of the disease agent shows how effective the antimicrobial agent is.15-20 ml of the nutrient agar medium was poured in petriplates.The petriplates were then swabbed with bacterial suspension. After, drying in a sterile hood, 0.5cm diameter discs soaked in the different solvent extracts of O.sanctum and F.hirta were placed on the agar. Discs containing the eight different solvents were used as controls. The petriplates were incubated at 37ºC for 24hrs.

3.7 Minimum Inhibitory Concentration (MIC)

Minimum Inhibitor Concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. An MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. MIC can be determined by agar or broth dilution methods. Serial dilution of the plant extracts were made and were added to the agar media containing the organisms with the help of disc diffusion method and incubated at 37º C for 24 hours and results are noted down.

3.8 Thin Layer Chromatography

Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures. Thin layer chromatography is performed on a sheet of glass, plastic, or aluminum foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide, or cellulose (blotter paper). This layer of adsorbent is known as the stationary phase. Thin layer chromatography can be used to1. Monitor the progress of a reaction2. Identify compounds present in a given substance and determine its purity.

3.8.1 Plate preparation

TLC plates of size 18.5× 5cm was taken over which the mixture is spread as a thick slurry. The silica gel mixture is used as the adsorbent materials prepared by mixing water and silica gel powder in the ratio 2:1(10gm of silica gel

powder in 20ml of water). The resultant plate is dried and activated by heating in an oven for 30 minutes at 110ºC. The thickness of the adsorbent layer is typically around 0.5-2.0mm for preparative TLC.

Solvent Preparation

The organic solvent used for TLC was prepared by mixing chloroform and methanol in the ratio 9:1 Procedure: To run a TLC, the following procedure is carried out:A small spot of extract containing the sample is loaded to a plate, about 1.5 centimeters from the bottom edge. The solvent is allowed to completely evaporate off, otherwise a very poor or no separation will be achieved. A small amount of an prepared solvent is poured in to the separation chamber to a depth of less than 1 cm. The chamber is closed with a cover glass or any other lid and is left for a few minutes to let the solvent vapors saturate the air in the chamber. The TLC plate is then placed in the chamber so that the spot(s) of the sample do not touch the surface of the elutant in the chamber, and the lid is closed. The solvent moves up the plate by capillary action, meets the sample mixture and carries it up the plate. When the solvent front reaches a height leving a space of 1cm at the top, the plate is removed) and dried. The separated compounds on the TLC plate were observed with naked eye while to detect the invisible fluroscent phenolic compounds, it was observed under UV at the range of 258nm.

4. RESULTS AND DISCUSSIONS

After carefully performing the experiments the results obtained from the study are presented in this chapter with the help of tables, figures and graphs.

4.1 Antimicrobial activity of the plant extracts upon the organisms

4.1.1 Studying of the antimicrobial activity of Ocimum tenuiflorum upon Bacillus subtilis and Escherichia coli

4.1.2 Studying of the antimicrobial activity of Ficus religiosa upon Bacillus subtilis and Escherichia coli

4.2 Studying the effect of the solvent extracts upon Bacillus subtilis and Escherichia coli

4.3 Studying of the MIC for the solvent extracts upon Bacillus subtilis and Escherichia coli

4.3.1 MIC of Ocimum tenuiflorum upon Bacillus subtilis and E.coli

4.3.2 MIC of Ficus religiosa upon Bacillus subtilis and E.coli

Phytochemical screening of the selected plant.

4.1. Antimicrobial activity of solvent extracts:

4.1.1Ocimum tenuiflorum –The antimicrobial properties of leaf extract of O.sanctum have been studied over Bacillus subtilis and E.coli by using the using 8 different solvents by disc diffusion method. The results are summarized in the table (4.1.1).

Table- 4.1.1-Antimicrobial activity of solvent extracts of O.tenuiflorum on B. subtilis and E.coli

Serial no.	Solvents	Mean for *B. subtilis* (cm)	Mean for *E.coli* (cm)
1.	Acetone	1.03	1.05
2.	Ethyl acetate	2.46	1.89
3.	Ethanol	1.80	1.40

4.	Methanol	0	0
5.	Chloroform	0	0
6.	Hexane	0	0
7.	Dichloromethane	0	0
8.	Water	0	0

The results showed an effective control of Bacillus subtilis when treated with ethyl acetate extract ethanol and acetone, while no positive results were observed with rest of the solvents. Similar results were observed for E.coli, The standard diameter of the disc was considered to be 0.5 cm.

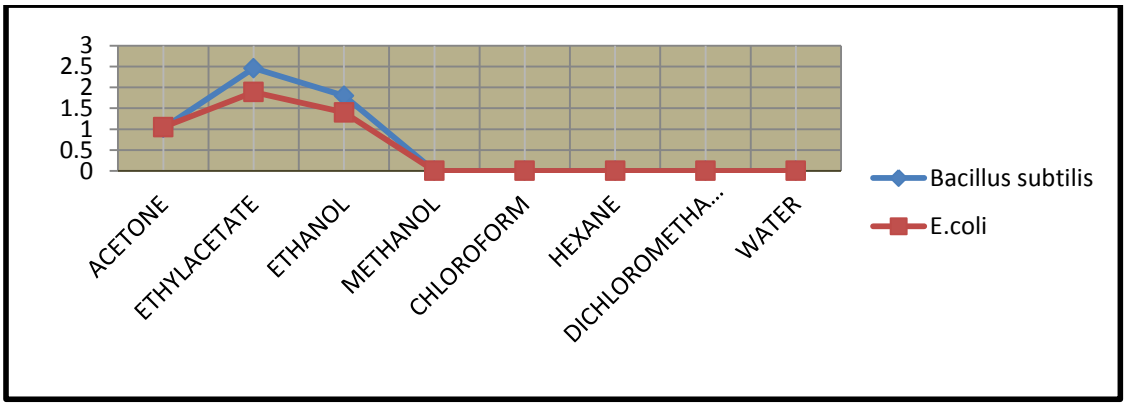


Figure: 4.1.1 Antimicrobial activity of Ocimum tenuiflorum over Bacillus subtilis and E.coli

Considering the above figure thus it can be concluded that the activity of Ethyl acetate is maximum for the respective organism followed by ethanol and acetone. While no significant effect were shown by rest of the solvents.

4.1.2. Ficus religiosa

The antimicrobial properties have been studied over Bacillus subtilis and E.coli by using the leaf extract of Ficus religiosa using 8 different solvents by disc diffusion method. The results are summarized in the table(4.1.2).

Table- 4.1.2- Antimicrobial activity of solvent extracts of Ficus religiosa on Bacillus subtilis and E.coli.

Serial no.	Solvents	Mean for Bacillus subtilis (cm)	Mean for *E.coli* (cm)
1.	Acetone	0.875	0.75
2.	Ethyl acetate	1.375	1.30
3.	Ethanol	0.25	1.00
4.	Methanol	0	0
5.	Chloroform	0	0
6.	Hexane	0	0
7.	Dichloromethane	0	0
8.	Water	0	0

The results showed an effective control of Bacillus subtilis when treated with ethyl acetate extract ethanol and acetone, while no positive results were observed with rest of the solvents. Similar results were observed for E.coli, The standard diameter of the disc was considered to be 0.5 cm

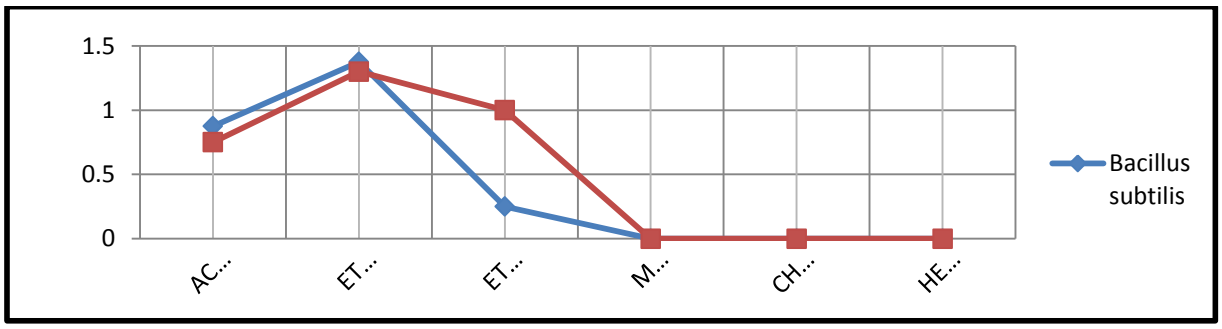


Figure:4.1.2 Antimicrobial activity of Ficus religiosa over Bacillus subtilis and E.coli

Considering the above figure thus it can be concluded that the activity of Ethyl acetate is maximum for the respective organism followed by ethanol and acetone. While no significant effect were shown by rest of the solvents.

4.2 Sensitivity test

Based on the results obtained the experiments were repeated for 3 times to test the validity of the obtained results. The results of the performed experiment were tabulated in terms of its positive (+) and negative (-) response towards the mentioned solvents in table

SL.Sl .No	SOLVENTS	*Ocimum tenuiflorum*						*Ficus religiosa*					
		Bacillus subtilis			E.coli			Bacillus subtilis			E.coli		
1.	Acetone	+	+	-	+	+	+	+	+	+	+	+	-
2.	Ethyl Acetate	+	+	+	+	+	+	+	+	+	+	+	+
3.	Ethanol	+	+	+	+	+	+	+	+	+	+	+	+
4.	Methanol	-	+	-	-	-	-	-	-	-	-	-	-
5.	Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
6.	.Hexane	+	-	-	-	-	-	-	-	-	-	-	-
7.	Dichloromethane	-	-	-	-	-	-	-	-	-	-	-	-
8.	Water	-	+	-	-	-	-	-	-	-	-	-	-

Key: (+) – present, (-) - absent

4.3 Phytochemical screening of the plants-

Phytochemicals such as alkaloids, steroids, tannins, flavonoids, etc from the leaf of Ocimum tenuiflorum and Ficus religiosa were tested and observations were presented in table (4.3).

1.Test for flavonoids- Sodium hydroxide test: Few quantity of leaf powder dissolved in water, filtered, 2ml of 10 % aqeous sodium hydroxide was added to produce yellow colouration. A change in colouration from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

2. Test for alkaloids- Pinch of leaf powder stirred with 5% aqeous hydrochloric acid on water bath and filtered. To the filtrate Mayers reagent added. Appearance of buff coloured precipitate will be an indication for presence of alkaloids.

3. Test for steroids- Liebermann-burchard Test: 2ml of acetic acid added to 0.2 gram of leaf powder, cooled well in ice, conc. sulphuric acid added by the sides.colour development from violet to blue or bluish green indicative of a positive test for steroidal ring.

4. Test for saponins- 1 gram leaf powder boiled with 5ml distilled water and was filtered. To the filtrate 3ml of distilled water was further added and shaken vigorously for 5 minutes. Frothing which is persistent on warming was taken as an evidence for the presence of saponins.

5. Test for soluble starch- Pinch of leaf powder boiled with 1ml of 5% potassium hydroxide solution, cooled, acidified with sulphuric acid. Yellow colouration indicative of positive test.

6.Test for anthraquinones:Barntragers test-0.2 gram leaf powder taken and shaken with 10ml benzene and filtered.5ml of 10% ammonia solution added to filtrate and shaken. Appearance of pink, red, or violet colour in the ammoniacal (lower) phase was taken as the presence of free anthraquinones.

7. Test for terpenoids:Salkowski test-0.2 gram leaf powder dissolved in 2ml of chloroform and mixed. To it conc. sulphuric acid of about 3ml was added along the sides to form a layer. Reddish brown colouration at the interface was formed to indicate a positive test.

8.Test for tannins- 0.5 gram of leaf powder taken, dissolved in distilled water, boiled and filtered. To the filtrate 1% ferric chloride solution was added in drops. Occurrence of blue-black green or blue green precipitate indicative of positive test

Table- 4.3- Phytochemical screening of selected plant.

Sl.No	Experiment	Observations	Inference	
			Ocimum tenuiflorum	*Ficus religiosa*
1.	Flavonoids	Yellow to colourless	-	+
2.	Alkaloids	Buff coloured precipitate	-	+
3.	Steroids	Violet-blue or bluish-green steroidal ring	-	+
4.	Saponins	Frothing persistent on warming	+	-
5.	Starch	Yellow colouration	+	+
6.	Anthraquinones	Pink, red, violet colouration in lower phase	-	-
7.	Terpenoids	Reddish brown colouration at interface	-	-
8.	Tannins	Blue-black green or blue green precipitate	-	+
9.	Carbohydrates	Red or violet colour at interface of two layer	+	+
10.	Monosaccharides	Reddish precipitate of cuprous oxide	+	+
11.	Phlobatanins	Red coloured precipitate	-	-

Key: + present,- absent

9. Test for carbohydrates:Molisch's test- Leaf powder were taken, dissolved in 10ml of water, filtered, filtrate used wherein few drops of Molisch's reagent was added followed by addition of conc. sulphuric acid. Red or dull violet colouration at interference of two layers considered as a positive result.

10.Test for monosaccharides:Barfoed's test- 0.5 gram of leaf powder taken, dissolved in distilled water, boiled and filtered.1ml of filtrate mixed with 1ml of Barfoed's reagent. Heated on water bath. Formation of reddish precipitate cuprous oxide considered as positive test.

11.Test for Phlobatanins- 0.2 gram of leaf powder boiled with 1% aqeous hydrochloric acid. Formation of red coloured precipitate indicative of a positive test.

4.4. Minimum inhibitory concentration

The Minimum inhibitory concentration has been studied over B. subtilis and E.coli by using the leaf extract of O.sanctum.

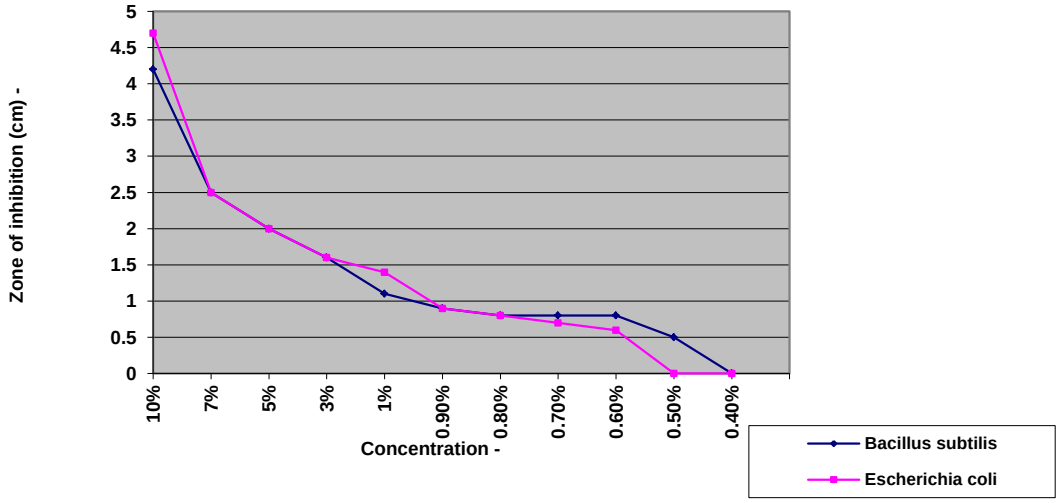
From the table given below, it was found that with increase in dilution the diameter of the ring or the zone of inhibition shows a significantly decrease, thus, it can be concluded that after attaining a particular dilution, the antimicrobial activity of the plant extract did not show any activity on the growth of Bacillus subtilis and E.coli.

The results are presented in tables-

Table 4.4.1-MIC of Ocimum tenuiflorum on Bacillus subtilis and Escherichia coli.

Dilution	Mean for *Bacillus subtilis*(cm)	Mean for *Escherichia coli*(cm)
10%	4.2	4.7
7%	2.5	2.5
5%	2.0	2.0
3%	1.6	1.6
1%	1.1	1.4
0.9%	0.9	0.9
0.8%	0.8	0.8
0.7%	0.8	0.7
0.6%	0.8	0.6
0.5%	0.5	0
0.4%	0	0

From table 4.4.1 it can be clearly seen the effect of controlling growth of the test organisms is significantly higher with 10% and minimum at 0.6% for Escherichia coli and 0.5% for Bacillus subtilis.



For statistical analysis the inhibitory concentration from 0.5% -10% dilution for Bacillus subtilis and 0.6%-10% dilution for Escherichia coli were considered since the concentration below 0.4% dilution for Bacillus subtilis and 0.4%-0.5% dilution for Escherichia coli did not show any effect.

4.4 Minimum inhibitory concentration

The Minimum inhibitory concentration has been studied over Bacillus subtilis and E.coli by using the leaf extract of F.hirta and it was found that with increase in dilution the diameter of the ring or the zone of inhibition shows a significantly decrease, thus, it can be concluded that after attaining a particular dilution, the antimicrobial activity of the plant extract did not show any activity on the the growth of Bacillus subtilis and E.coli.The results are presented in table(4.4.2)

Table 4.4.2- Minimum Inhibitory Concentration of Ficus religiosa on Bacillus subtilis and Escherichia coli.

Dilution	Mean for *Bacillus subtilis*(cm)	Mean for *Escherichia coli*(cm)
10%	1.13	1.23
7%	0.93	1.03
5%	0.9	0.96
3%	0.76	0.83
1%	0.63	0.66
0.9%	0.56	0.63
0.8%	0	0
0.7%	0	0
0.6%	0	0
0.5%	0	0
0.4%	0	0

From table(4.4.2) It can be seen clearly the effect of controlling the test organisms is significantly higher with 10% dilution and minimum at 0.8%, 0.7%, 0.6%, 0.5%, 0.4% dilutions successively. It is shown more clearly in

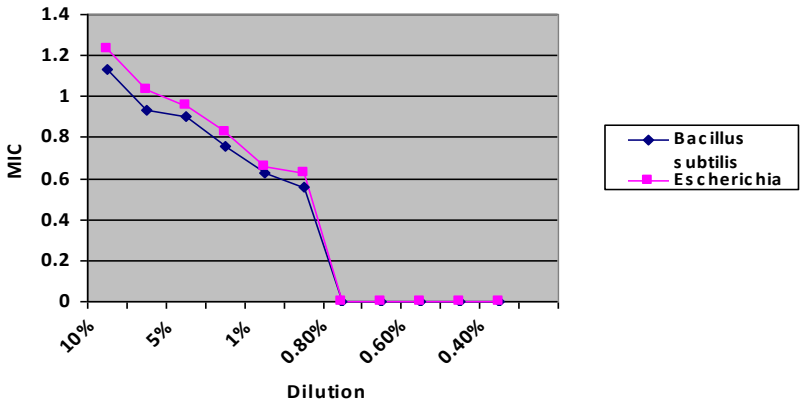


Fig:4.4.2 - MIC of Ficus religiosa on Escherichia coli and Bacillus subtilis

For statistical analysis the dilutions from 0.9% -10% (Escherichia coli and Bacillus subtilis) were considered where inhibition effect was recorded but since dilutions 0.4%,0.5%,0.6%,0.7%,0.8% did not show any effect, they were not considered.

5. CONCLUSION

Studies of Ocimum tenuiflorum and Ficus religiosa leaves and their antimicrobial effect on Escherichia coli and Bacillus subtilis" is an effort to put light on the efficient antimicrobial activity of Ocimum tenuiflorum and Ficus religiosa over Bacillus subtilis and E.coli which is the underlying organism of diverse diseases as mentioned below. Several research offers evidence that Ocimum tenuiflorum is useful against stress; it enhances stamina and increases efficient use of oxygen by body; strengthens immune system; reduces inflammation; protects from radiation; reduces aging; supports the lungs, liver and heart; it exhibits antibiotic, antiviral and antifungal, antioxidant properties.We executed a number of trials such as, antimicrobial assay, sensitivity test; along with phytochemical screening which let us know the secondary metabolite answerable for antimicrobial activity. We also utilized the TLC modus operandi in attempt to bring out the antibacterial compounds present in the leaf extract for the inhibition of microbial growth. The end results obtained in the form of tables and graphs are accompanied by individual photographs to prove its legitimacy of the trials performed. The studies in real sense have not concluded yet, in fact it had divulged a number of panoramas to be carried out in our near future. A review of the literature suggested that the main components responsible for the antimicrobial activity of Ocimum tenuiflorum were likely to be camphor, eucalyptol, and eugenol. β-caryophyllene may also have contributed to the antimicrobial activity of the Ocimum tenuiflorum but was present in smaller amounts. Since Bacillus subtilis, and E. coli are major pathogens causing SSTIs, Ocimum tenuiflorum could be a valuable topical antimicrobial agent for management of skin infections caused by these organisms or as a wound dressing to prevent infection. Early treatment or preventative measures may halt progression to more serious infection requiring systematic antibiotic therapy, and

reduce the risk of development of resistance to valuable antibiotics.The antimicrobial compounds in Ficus religiosa can be more distinctively identified and split through improved skill such as UPLC/HPLC. The complexes of the leaf extract can be altered in its chemical chattels in enhancing its antimicrobial commotion. The compounds responsible for antibacterial effect of Ocimum tenuiflorum can be taken apart through TLC to a elevated level. Plant fractions further than leaf such as root, stems can also be regarded as an aim of study for a variety of antimicrobial extracts.

6. ACKNOWLEDGEMENTS

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7. CONFLICT OF INTEREST

We declare that we have no conflict of interest

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