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Research Article

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A comparative study of two selected *Ocimum* species with relevance to phytochemical, antimicrobial and molecular isolation

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ABSTRACT

Plants have been used for the treatment of various diseases for thousands of years. Plants and plant-products are good sources of medications and provide raw materials for modern pharmaceuticals used for various ailments. Ocimum sanctum and Ocimum gratissimumis known to be an important medicinal plants from the ancient period in India. Thisstudy was carried out to investigate the phytochemical screening, antibacterial efficacy and molecular comparison of the leaf extracts of Ocimum sanctum and Ocimum gratissimum in various solvents like hexanes, methanol, ethanol, petroleum ether and aqueous extracts. To evaluate the inhibition of the pathogens with the plant extracts, solvent extracts of the selected plant species were screened for antimicrobial activity using the agar well diffusion method. The antibacterial activity was analyzed using six different bacterial strains (Escherichia coli, Bacillus cereus, Staphylococcus aureus, Klebsiella pneumonia, Aspergillus niger and Pencillium notatum). The methanolic and ethanolic extracts of crude leaf powder were found to be most effective against the pathogenic bacterial strains selected for the study, but the hexanes and petroleum ether extracts show less activity as compared to other extracts. The qualitative phytochemical analysis was studied and compared. The results reveal that Ocimum gratissimum has more phytochemical components and antibacterial activity as compared to Ocimum sanctum. The presence of the secondary metabolites signifies the potential of Ocimum gratissimumas a source of therapeutic agents and also can be used as a source for developing antimicrobial agents. The estimation of DNA was done by using Agarose Gel Electrophoresis and it was revealed that the Ocimum sanctum showed higher molecular weight than the Ocimum gratissimum.

Keywords: *Ocimum sanctum, Ocimum gratissimum,* Phytochemistry, Antimicrobial.

INTRODUCTION

Plants are a peculiar organism which is showing unlimited benevolence and kindness to the human beings by offering the products of its life activities in many ways. Plants that are having therapeutic properties or exerting beneficial pharmacological effects on the human body are generally called as medicinal plants. There are about 260,000 plant species are present in the kingdom Plantae. Around 230,000 of them are flowering herbs, comes under the phyla *Magnoliophyta*, including most trees, shrubs, vines, flowers, fruits, vegetables and legumes [1]. The plants in this category are also called angiosperms. They differ from conifers in having their seeds inside an ovary, which is embedded in a flower or fruit. Some of the families such as Poaceae, Fabaceae, Solanaceae, Convolvulaceae, Lamiaceae (Labiatae), Oleaceaeetc. are of high medicinal importance. The current study is on the species of Lamiaceae because the family is best known for therapeutic importance as well as a source of culinary herbs [2]. Some examples include mints, thyme, tulsi, spearmint and coleus.

Ocimum sanctum

Ocimum sanctum is sacred basil. It is otherwise called Krishna tulsi. They are usually herbs, under shrubs or shrubs, strongly aromatic from oil glands. It is an aromatic herb cultivated for its medicinal value and for some religious background. It attains a height of about 4.5 feet. The herb is worshipped in many parts of India and it is carefully looked after, and when large enough to form wood, it is made into beads for rosaries [3]. It has branched tap roots, aerials, erect four angled solid, branched, hairy, woody below and herbaceous above. The leaf is ramal and cauline, estipulate, petiolate, decussate and opposite, semate, simpliovate, acute, reticulate unicostate hairy. The inflorescence is raceme of verticillaster; ebracteate, pedicellate, ebracteolate, complete, bisexual, zygomorphic, hypogynous, tetracyclic, pink, small, aromatic flowers are present [4]. Ovoid or campanulate calyx; deflexed in fruit and are usually enlarged and hardened.

The basil is cultivated all over India as culinary herbs and also as medicinal plants. These are highly aromatic. Carminative diuretic and stimulant flowers are present and the seeds are mucilaginous and are given in infusion to cure gonorrhea, dysentery and chronic diarrhea. Juice of leaves is used in cataract and bronchitis. In order to stop ear ache, it drops in the ears. Leaf infusions are useful in gastric and liver disorders [5]. Disorders of geneto-urinary system can be treated by using seeds. The decoctions of roots are given in malarial fevers. Fresh leaves, stems and roots are applied in case of mosquito bites.

Ocimum gratissimum

Ocimum gratissimum is the second species of tirtava, also called Cattu- tirtava or Rama-tulsi. The plant is five or six feet in height, which is growing in sandy soils and the roots are fibrous, black, stalks quadrangular, slightly hairy, striates with a furrow and knotted [6]. Leaves are very broad, round or oblong, serrate in the margin thin larger than those of the *Ocimum sanctum*, having very strong and pleasant smell and also sharp taste; usually arise in both twos and threes or solitarily. Flowers are the same, but white; in the incised petal from white to light green. Four white stamens are present whose apices is also having the bifurcate style (white).Calycesis large and striated apex with the nerve of the tongue shaped petal. Seeds are round, dark red, verging on brown.

It's an ornamental aromatic shrub possessing medicinal properties. It is used as a mosquito repellent and is cultivated as a culinary herb. The seeds are used for curing headache [7], [8]. The boiled roots heals fever with cough and the oil extracted from the root is expectorant which promotes the digestion of food, prevents the spoiling of the liver, promotes urine and helps in strangury (painful retention or difficulty in discharging urine), gout, pleuritis, cleanses phlegmatic and cough fluids, destroy the gas, smeared around the temple relieves headache[9], [10].

EXPERIMENTAL SECTION

Plant Collection and Identification

The plant species were collected from South Western Ghats regions of Kollam District, Kerala, India during the month of June – September in the year 2015. The plants were sent for proper identification. The plants were authenticated by the eminent botanist of the Noorul Islam Centre for Higher Education, Noorul Islam University, Tamilnadu, India. The leaf samples of *Ocimum sanctum* and *Ocimum gratissimum* were shown in Figure 1.

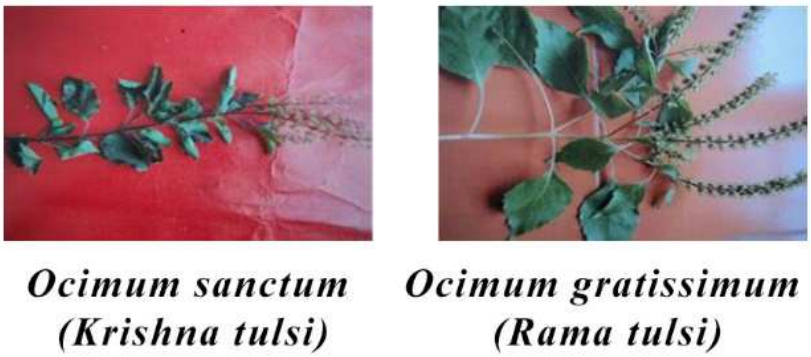

Figure 1: Leaf samples of *Ocimum sanctum* and *Ocimum gratissimum*

Preparation of leaf extracts

The leaves of *Ocimum sanctum* and *Ocimum gratissimum* were separated and cleaned well. Cleaned leaves were then dried under shade. The drying was done until all the water molecules evaporated and plants became well-dried for grinding. After drying, the plants were ground well using a mechanical blender into fine powder and transferred into airtight container with proper labelling for further use. The dried and powdered *Ocimum sanctum* and *Ocimum*

gratissimum were extracted sequentially with hexanes, methanol, ethanol, petroleum ether and aqueous using Soxhlet apparatus. Solute thus extracted were collected in a centrifuge tube and used for further studies were shown in Figure 2.

Figure 2: Leaf extracts of *Ocimum sanctum* and *Ocimum gratissimum*

Qualitative Phytochemical Screening of Secondary Metabolites

The leaf extracts of *Ocimum sanctum* and *Ocimum gratissimum* were screened for different phytochemical constituents' viz., Carbohydrates, Amino acids, Proteins, Vitamin C, Chloride, Tannins, Alkaloids, Flavonoids, Phlobatannins, Phenolic compounds, Steroids and Saponins. Phytochemical screenings of the extracts were carried out by the standard methods.

Evaluation of Antimicrobial activity of the leaf extracts

The bacterial strains obtained were inoculated in a test tube containing 5 ml of nutrient broth and the test tubes were incubated at 37ºCfor 24 hours and were referred to as seeded broth. About 20ml of the prepared Nutrient agar and Potato dextrose agar were poured into a set of well labelled sterile Petri plates under aseptic conditions and were allowed to solidify. 1 ml of seeds broth were swabbed cultured over the solidified agar surface and six wells of 6 mm were prepared on the plate and 200µl of extracts were added to the wells. Then left the plates for 1 hour and subsequently incubated at 37ºC for 24 hours. The diameter of inhibition zones was observed, measured and photographed.

Culture and Media Preparation

The five different solvent extracts of the two leaf samples were tested for antimicrobial activity using the well diffusion assay. The microbial strains used for current study *are Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Bacillus cereus, Pencillium notatum, Aspergillus niger*. The microorganisms were collected from the Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory by periodic subculture.

Antibacterial Assay

The bacterial strains obtained were inoculated in a test tube containing 5 ml of nutrient broth and the test tubes were incubated at 37ºC for 24 hours and were referred to as seed broth. About 20ml of the prepared Nutrient agar and Potato dextrose agar were poured into a set of well labelled sterile Petri plates under aseptic conditions and were allowed to solidify. 1 ml of seeds broth were swabbed cultured over the solidified agar surface and six wells of 6 mm were prepared on the plate and 200µl of extracts were added to the wells. Then left the plates for 1 hour and subsequently incubated at 37ºC for 24 hours. The diameter of inhibition zones was observed, measured and photographed.

Isolation of genomic DNA

The two fresh leaves of *Ocimum sanctum* and *Ocimum gratissimum* were collected and used for isolation of genomic DNA. Around 2 gm of the leaf tissue was crushed using mortar and pestle and is homogenized with 2 ml of the extraction buffer. The homogenate was transferred to a centrifuge tube and equal volume of phenol: chloroform: Isoamylalcohol (25:24:1) was added to the tubes and mixed gently. For 15 minutes, the tubes were centrifuged at room temperature at 15,000 rpm. The upper aqueous phase was collected in a new tube and an equal volume of chloroform: Isoamylalcohol (24:1) was added and mixed. The upper aqueous phase obtained after centrifugation, at 15000 rpm, at room temperature for 10 minutes, was transferred to a new tube. By adding 0.1 ml of 3 M Sodium acetate (pH 7.0) and 0.7 ml of Isopropanol, the DNA was precipitated from the solution. The tubes were centrifuged at 4ºC for 15 min at 15,000 rpm, after 15 min of incubation at room temperature. The DNA pellet was washed twice with 70% ethanol and then very briefly with 100% ethanol and air dried. The DNA was dissolved in TE buffer. To remove RNA, 5 µl of DNase free RNAseA (10 mg/ml) was added to the DNA.

Estimation of DNA by Agarose Gel Electrophoresis

TBE buffer is the buffer system used for the separation of nucleic acids in Agarose gel electrophoresis. Sterilize the stock solution by autoclaving. 10mg of ethidium bromide was weighed into a sterile tube and is dissolved in 1ml of distilled water. The stock solution was stored at 4ºC. 10ml of 100% glycerol or 10ml of 60% sucrose was autoclaved. 1ml of 3% bromophenol blue was prepared in a sterile tube with sterile distilled water. The sample loading dye was prepared by mixing 0.7ml of glycerol or sucrose (60%) and 0.2ml of 10X TBE and 0.1ml of 3% bromophenol blue solution. 1.5µl of the sample loading dye was mixed with 10µl of DNA sample and loaded. 0.24g of Agarose was weighed and sprinkled into 30ml of 1X TBE buffer in a 100ml Erlenmeyer flask. For about 15 minutes, the Agarose was boiled to dissolve by placing the flask in a boiling water bath. The flask was removed from the water bath when the Agarose was completely dissolved and left at room temperature to cool. The platform was washed with distilled water and then wiped dry with tissue paper. The open ends of the platform are sealed securely with cello tape. The comb was placed 1 cm away from the top end and make sure that the teeth of the comb do not touch the surface of the platform. The platform was placed on a smooth horizontal surface. Once the Agarose solution was cooled to above 50 ˚C, the solution was poured gently to cover the entire surface of the platform and let undisturbed for about 30 minutes. Once the gel was formed, the comb was removed by gently pulling up and the cello tape is also removed from both ends. Then the gel was placed along with the platform inside the gel tank and electrophoresis buffer was poured through one side of the tank to just cover the gel surface.

The DNA sample was mixed with the loading dye and using a capillary tube, the mixture was loaded into the well cared. Once the sample was loaded into the well, the cathode was connected towards the top end of the gel and towards the bottom end of the gel, the anode was connected. By switching on the D.C Power pack, the electrophoresis were started. The gel was run at approximately 5 v/cm. As the bromophenol blue (the tracking dye) has moved to~1 cm from the bottom end, the current was switched off. The power supply was then disconnected and the gel along with the platform was stained in a plastic tray containing 100ml ethidium bromide (0.5µg/ml) in distilled water (Gloves are to be used while handling ethidium bromide). After 30-45 minutes, the platform and the gel were rinsed with distilled water. By keeping the platform in a slanting position, the gel was gently pushed on to UV trans- illuminator. Finally, the Ultra Visible light, then switched on and the bands of DNA were seen and were photographed using an orange filter.

RESULTS AND DISCUSSION

The phytochemical chemical activity of *Ocimum sanctum* and *Ocimum gratissimum* were studied and the results were shown in Table 1 and Table 2. The hexanes, petroleum ether, aqueous, ethanol and methanolic extracts of *Ocimum sanctum* had showed the presence of Amino acids, Chloride, Tannins, Alkaloids, Flavanoids, Phlobatannins, Steroids and Phenolic Compounds. The hexanes, petroleum ether, aqueous, ethanol and methanolic extracts of *Ocimum gratissimum* had showed the presence of Amino acids, Chloride, Tannins, Alkaloids, Flavanoids, Phlobatannins, Steroids, Phenolic Compounds and Saponins [11].

Table 1: Phytochemical activity of *Ocimum sanctum*

S.No	Name of tests	Hexanes	Petroleum Ether	Aqueous	Ethanol	Methanol
1	Test for Carbohydrates	-	-	-	-	-
2	Test for Amino Acids	-	-	-	+	+
3	Test for Proteins	-	-	-	-	-
4	Test for Vitamin C	-	-	-	-	-
5	Test for Chloride	-	-	+	-	+
6	Test for Tannins	-	-	-	+	+
7	Test for Alkaloids	-	-	+	+	+
8	Test for Flavanoids	-	-	-	+	+
9	Test for Phlobatannins	-	-	+	-	+
10	Test for Steroids	-	-	+	-	-
11	Test for Phenolic Compounds	-	-	+	+	+
12	Test for Saponins	-	-	-	-	-

"+" represents the presence of compound and "-"represents the absence of compound.

Table 2: Phytochemical activity of *Ocimum gratissimum*

S.No	Name of tests	Hexanes	Petroleum Ether	Aqueous	Ethanol	Methanol
1	Test for Carbohydrates	-	-	-	-	-
2	Test for Amino Acids	-	-	-	+	+
3	Test for Proteins	-	-	-	-	-
4	Test for Vitamin C	-	-	-	-	-
5	Test for Chloride	-	+	+	-	+
6	Test for Tannins	-	-	+	-	+
7	Test for Alkaloids	-	-	-	+	+
8	Test for Flavanoids	-	-	+	+	+
9	Test for Phlobatannins	-	-	+	-	+
10	Test for Steroids	-	-	+	-	-
11	Test for Phenolic Compounds	-	-	+	+	+
12	Test for Saponins	-	-	-	-	+

"+" represents the presence of compound and "-"represents the absence of compound.

Table 3: Antibacterial activity of *Ocimum sanctum* against bacterial test organisms

S.No	Name of Microorganism	Hexanes	Petroleum ether	Aqueous	Ethanol	Methanol
1	*Bacillus aureus*	6mm	-	-	8mm	8.5mm
2	*Escherichia coli*	-	-	-	10mm	10mm
3	*Klebsiella pneumonia*	-	-	-	6mm	8.5mm
4	*Staphylococcus aureus*	-	9mm	-	15mm	-
5	*Aspergillus niger*	-	-	9mm	-	5mm
6	*Pencillium notatum*	6mm	9mm	14mm	12mm	11mm

Table 4:Antibacterial activity of *Ocimum gratissimum*against bacterial test organisms

S.No	Name of Microorganism	Hexanes	Petroleum ether	Aqueous	Ethanol	Methanol
1	*Bacillus aureus*	5mm	-	-	9mm	7mm
2	*Escherichia coli*	-	-	7mm	-	9mm
3	*Klebsiella pneumonia*	10mm	-	-	8mm	8mm
4	*Staphylococcus aureus*	9mm	-	-	12mm	22mm
5	*Aspergillus niger*	-	-	9mm	-	5mm
6	*Pencillium notatum*	4mm	12mm	6mm	17mm	13mm

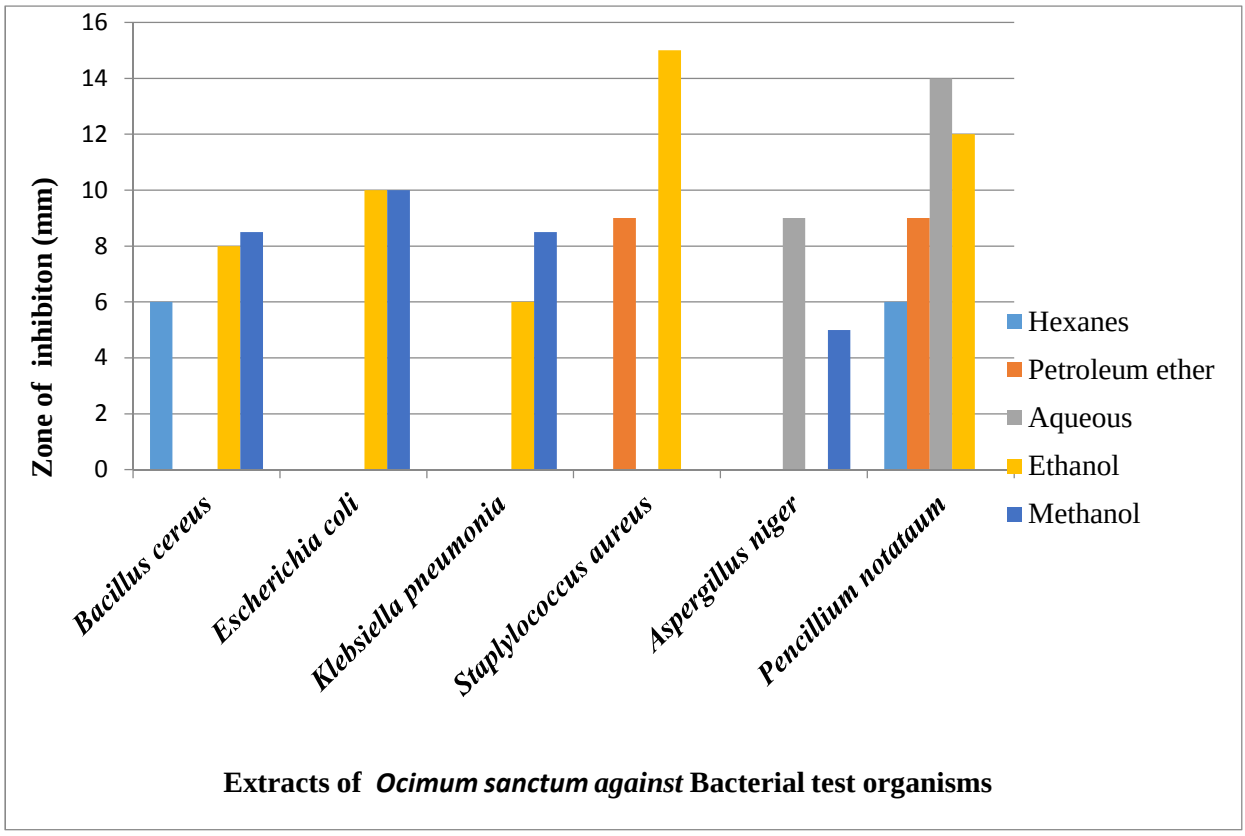

Figure 3: Antibacterial activity of leaf extracts of *Ocimum sanctum* against bacterial test organisms

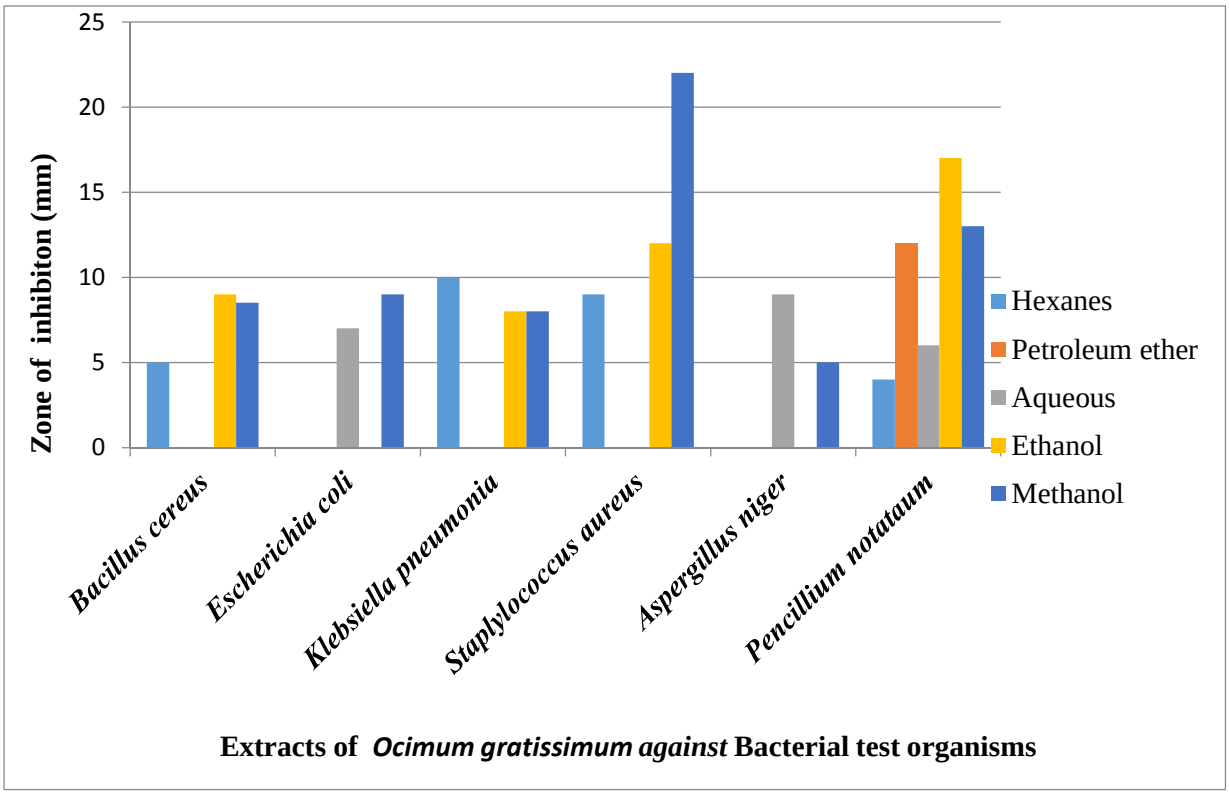

Figure4: Antibacterial activity of leaf extracts of *Ocimum gratissimum* against bacterial test organisms

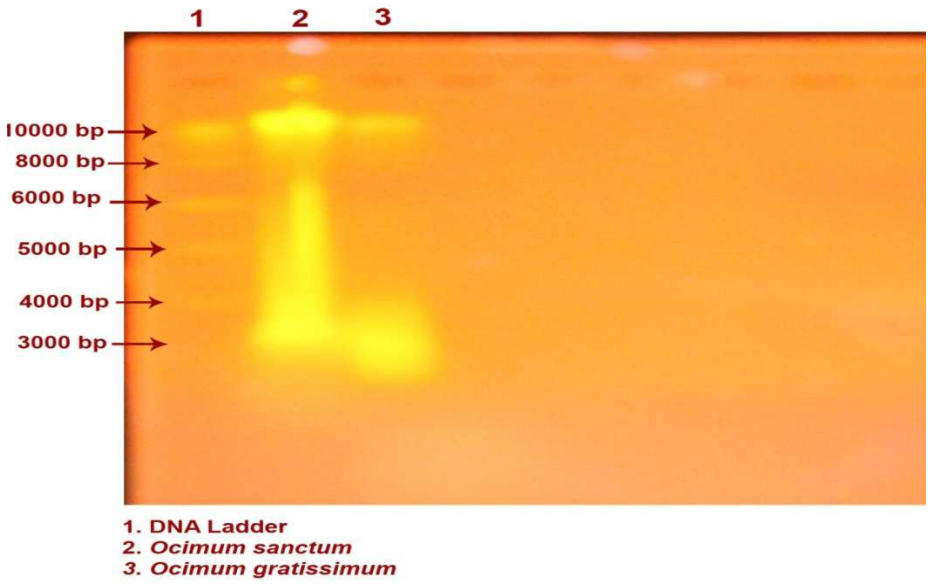

Figure 5: Agarose gel shows DNA bands at 11000 and 10000 base pairs

The extracts of *Ocimum sanctum* showed antimicrobial activity against *Bacillus aureus, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Aspergillus niger,* and *Pencillium notatum* were shown in Table 3. and Figure 3. The methanol extracts of *Ocimum gratissimum* showed antimicrobial activity against *Bacillus aureus, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Aspergillus niger*and *Pencillium notatum*; while ethanol extracts showed activity against *Bacillus aureus, Klebsiella pneumonia, Staphylococcus aureus,* and *Pencillium notatum* were shown in Table 4. and Figure 4. [12]. DNA bands of 11000 and 10000 base pairs were obtained and shown in Figure 5. Thus it was revealed that the *Ocimum sanctum* had higher molecular weight than that of *Ocimum gratissimum.*

CONCLUSION

The phytochemical analysis of *Ocimum sanctum* showed positive results for the tests for amino acids, chloride, tannins, alkaloids, flavonoids, phlobatannins, steroids and phenolic compounds, but *Ocimum gratissimum* had positive results for amino acids, chloride, tannins, alkaloids, flavonoids, phlobatannins, steroids, phenolic compounds and saponins. The methanolic extracts of *Ocimum sanctum* showed antimicrobial activity against *Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Aspergillus niger* and *Pencillium notatum*; the ethanol extracts showed activity against *Bacillus aureus, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus*, and *Pencillium notatum*; while the aqueous, hexanes and petroleum ether extracts of *Ocimum sanctum* showed less activity [13]. The methanol extracts of *Ocimum gratissimum* had showed antimicrobial activity against *Bacillus aureus, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Aspergillus niger* and *Pencillium notatum*; while ethanol extracts showed activity against *Bacillus aureus, Klebsiella pneumonia, Staphylococcus aureus*, and *Pencillium notatum*; the aqueous extract had activity against *Escherichia coli, Aspergillus niger*and *Pencillium notatum*; while the hexanes extract showed activity against *Bacillus aureus, Klebsiella pneumonia, Aspergillus niger* and *Pencillium notatum*; the hexanes, petroleum ether and aqueous extracts shoed activity against *Pencillium notatum*[14].

The DNA was isolated and estimated by Agarose Gel Electrophoresis. The molecular weight of *Ocimum sanctum* was found to be higher than that of *Ocimum gratissimum*. In the current study, it was concluded that the phytochemical activity and the antimicrobial activity was found to be high in *Ocimum gratissimum* as compared to *Ocimum sanctum.*

It could be reasoned that the comparative study among *Ocimum gratissimum* and Ocimum sanctum contains various bioactive compounds. While comparing to *Ocimum sanctum*, *Ocimum gratissimum* was found to be higher medicinal contents [15]. So it is recommended as a plant of phyto-pharmaceutical importance. Nevertheless, further studies will need to be taken on to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and organic merchandise.

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