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Assessment of Antimicrobial Competence of Epiphytes and Endophytes from Osmium basilicum and Trigonella foenum graecum

ASSESSMENT OF ANTIMICROBIAL COMPETENCE OF EPIPHYTES AND ENDOPHYTES FROM *OSMIUM BASILICUM* AND *TRIGONELLA FOENUM GRAECUM*

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ABSTRACT

Plant-associated bacteria are an unexplored group of microorganisms that has enormous potential. These bacteria are the source of finding new antimicrobial substances. The present study aimed to isolate and characterize the epiphytes and endophytes from *Osmium basilicum* and *Trigonella foenum graecum* plants and to determine their antimicrobial potential against pathogenic bacteria from Nishtar Hospital Multan, Pakistan. The sum of 18 stems and roots along leaf specimens were assembled as of the plant's *Osmium basilicum* plus *Trigonella foenum graecum* as of the distinct locales of the Multan city. Overall 73 bacterial strains were isolated and their colony morphology, gram staining, spore staining, and characterization were done. Further 10 epiphytic strains and 14 endophytic strains were selected based on their antimicrobial potential intended for a thorough examination. The activity of the selected microbial isolates was determined against pathogenic bacteria *K.pneumoniae, MRSA, E.coli, S .aureus, S.typhimurium* as of Nishtar hospital. Out of all strains, only one epiphytic and two endophytic bacterial isolates (methanolic extracts) (obr, obl3, and tfen2 were isolated from root, leaf, and root of *Osmium basilicum* and *Trigonella foenum graecum* correspondingly inhibited altogether gram-positive besides gram-negative pathogenic microbes involved in this investigation. The hunt to find novel compounds from plant bacteria will open new horizons in the industrial and agricultural sectors.

Keywords: Antimicrobial activity, endophytes, epiphytes medicinal plants, microbial secondary metabolites.

INTRODUCTION

Medicinal plants have been utilized for thousands of years for a variety of purposes, such as food preservation, pharmaceuticals, herbal treatments, and alternative medicine. It's common knowledge that substances created organically as opposed to artificially break down more quickly and are therefore more environmentally friendly. These days, consumers are using and valuing natural antibacterial, antioxidant, cytotoxic, and nutritional agents more and more because of their popularity and favorable perception. The extensive use of commercial antimicrobial drugs to treat infectious diseases has led to the emergence of diverse drug resistance to pathogenic bacteria in both plants and people in recent years. Herbs and natural products are major sources of medications because it is believed that 60 % of the world's population relies on them for treatment (Sahu et al., 2022).

Most of the herbs and shrubs of the Lamiaceae family's Ocimum genus are aromatic, including *Ocimum basilicum* (sweet basil), *Osmium tenuiflorum* (tulsi/holy basil), *Osmium gratissimum* (African basil), *Osmium campechianum*. There are numerous recognized

pharmacological, biological, therapeutic, and positive health applications of *Osmium basilicum*. It is also regarded as a crucial element in the Ayurvedic and Unani medical systems for the prevention, diagnosis, and treatment of a variety of illnesses (Dhama et al., 2021). Linalool, eugenol, methyl chavicol, methyl cinnamate, ferulate, methyl eugenol, triterpenoids, and steroidal glycoside are all present in *Occimum basilicum* and are renowned for their antibacterial and bactericidal properties (Mishra et al., 2022). Basil has powerful antibacterial, antifungal, antiviral, and antiparasitic properties that are indicative of its antimicrobial activity. These properties are due to the presence of many bioactive chemicals (Brandão et al., 2022).

The annual, herbaceous, and fragrant plant known as fenugreek, or *Trigonella foenum graecum*, is a member of the leguminosae family (Zeena G. Faisal, 2022). Fenugreek is well known for its medicinal benefits around the world (Ansari and Patel, 2022). The inclusion of numerous bioactive compounds, including fibers, fatty acids, alkaloids, flavonoids, saponins, and flavonoids, increases the medicinal potential of fenugreek. In terms of its therapeutic effectiveness, fenugreek has antibiotic, antioxidant, and anticarcinogen properties. It also decreases hyperglycemia in individuals with diabetes (Kumar et al., 2022). Additionally, it has been utilized as a folk remedy for tuberculosis, cellulitis, and boils. As a detoxifying agent for eliminating toxic wastes, dead cells, and trapped protein through the lymphatic system, fenugreek is also believed to help clear congestion (Visuvanathan et al., 2022).

MATERIALS AND METHODS

Specimen Collection

The present study was carried out from September 2016 to April 2017. During this period microbial flora of two medicinal plants *Osmium basilicum* (Niazbo) and *Trigonella foenum graecum* (Methi) were chosen for the investigation. The plant specimens were assembled from the various locales of Multan City via the technique of (Bibi et al., 2017). Healthy stems, roots along leaves were carted and stored in sterilized containers then transported to the laboratory and stored below 4 °C.

Table 1: Plants description.

Common name	The botanical name, family, and description	Medicinal Reputation
Niazbo (Basil)	*Osmium basilicum* (Lamiaceae) Descriptions: Common herb, grow to the size of 1-2 feet in height	As a folk remedy, it is used in a substantial number of illnesses, including tediousness, cancer, deafness, diarrhea, epilepsy, gout, convulsion insanity, queasiness, sore throat, toothaches, and whooping cough.
Methi (Fenugreek)	*Trigonella foenum graecum* (Fabaceae) Descriptions: Common herbs and vegetables, famous cuisine of the Indian subcontinent	It is being used to promote digestion, lowers blood sugar levels in diabetics, induce labor, and liver tonic.

Isolation of Epiphytes and Endophytes

Ten grams of the root, stem along leaves were assembled meant for the isolation of epiphytes. Sections of the plant matter were submerged in distilled water (9 ml) for 60 minutes. Of that 9 ml, 100 µl was plated onto nutrient agar medium. Intended for the isolation of endophytes, roots leave and stems were rinsed with water. Next, the plant sections were surface disinfected by ethyl alcohol (70 %) for one minute in addition to sodium hypochlorite for three minutes then rinsed three to four intervals with distilled water. The plant material was crushed, and 9 ml sterile distilled water was added to make a solution of roots, stems, and leaves. The mixture was plated onto lauria-bertani agar medium. Inoculated plates were incubated overnight at 37 °C. Following incubation, bacterial colonies were observed, tallied, and purified for more categorization (Ali et al., 2016).

Assessment of Antibacterial Potential

Clinical pathogens, gram positive (*Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus), as well as gram negative bacteria (Salmonella typhimurium, Klebsiella pneumoniae,* and *Escherichia coli,),* were exploited to assess the antimicrobial action of epiphytic and endophytic bacteria. All pathogenic microbial strains (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhimurium, Methicillin-resistant Staphylococcus aureus)* were acquired from Nishtar Hospital, Multan, Pakistan. Selection of epiphytic along with endophytic bacteria was accomplished in two stages i.e. primary selection coupled with secondary selection.

Initial Screening via Lawn Spot Technique

Initially, the multidrug-resistant bacteriological species (*Salmonella typhimurium, E.coli, Klebsiella pneumoniae, Methicillin-resistant Staphylococcus aureus, Staphylococcus aureus)* as of Nishtar Hospital were cultivated in nutrient broth overnight at 37 °C afterward were swabbed onto nutrient agar plates. Subsequently, the isolated microbial strains were spotted on the agar plates and then incubated overnight at 37 °C. Following incubation, bacterial species were examined via the inhibition area (Bottone and Peluso, 2003).

Secondary Selection via Agar Well Diffusion

Intended for secondary selection, agar diffusion approach was employed for the isolated bacterial specimen to scrutinize the antimicrobial action utilizing diverse solvents (chloroform, methanol, hexane). Twenty-four hours old cultures of multidrug-resistant microbial species (*Salmonella typhimurium, Methicillin-resistant Staphylococcus aureus, Klebsiella pneumoniae, E. coli, Staphylococcus aureus)* were exploited for the lawn preparation. Onto nutrient agar plates, the lawn was made with the aid of cotton swabs. For well-making, cork borer was utilized. With the assistance of a micropipette, a supernatant of microbial isolates (80 µl) was added to wells. Plates were incubated overnight at 37 °C, inhibition zone of every well was evaluated (Magaldi et al., 2004).

Antibiotic Sensitivity test for Pathogenic bacteria

The antibiotic susceptibility of pathogenic microbes was evaluated via the Kirby-Bauer disk diffusion method used against various antibiotics (Amoxicillin 30 µg, Streptomycin 10 µg, Vancomycin 30 µg, and Ciprofloxacin 5 µg).

Table 2: Physical parameters of isolated strains.

Bacterial isolates	Plant source	Part	Type	Temperature	Humidity	Dilution used
Ob1	*Osmium basilicum*	Leaf	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Ob2	*Osmium basilicum*	Leaf	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Ob3	*Osmium basilicum*	Leaf	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Ob4	*Osmium basilicum*	Leaf	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Ob5	*Osmium basilicum*	Leaf	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Obs1	*Osmium basilicum*	Shoot	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Obs2	*Osmium basilicum*	Shoot	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Obr3	*Osmium basilicum*	Root	Epiphytic	37 °C	++	10^{-2}, 10^{-4}
Tf1	*Trigonella foenum graecum*	Leaf	Epiphytic	37 °C	+++	10^{-2}, 10^{-4}
Tf2	*Trigonella foenum graecum*	Leaf	Epiphytic	37 °C	+++	10^{-2}, 10^{-4}
Obl1	*Osmium basilicum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Obl2	*Osmium basilicum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Obl3	*Osmium basilicum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfl1	*Trigonella foenum graecum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfl2	*Trigonella foenum graecum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfl4	*Trigonella foenum graecum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfl6	*Trigonella foenum graecum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfl7	*Trigonella foenum graecum*	Leaf	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfe2	*Trigonella foenum graecum*	Shoot	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfe3	*Trigonella foenum graecum*	Shoot	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfe4	*Trigonella foenum graecum*	Shoot	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfe5	*Trigonella foenum graecum*	Shoot	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfen3	*Trigonella foenum graecum*	Root	Endophytic	37 °C	+++	10^{-2}, 10^{-4}
Tfen5	*Trigonella foenum graecum*	Root	Endophytic	37 °C	+++	10^{-2}, 10^{-4}

The organisms to be tested were evenly swabbed on the surface of the Muller Hinton agar medium. After swabbing, with the assistance of sterilized forceps, the discs were positioned on the MH agar and incubated overnight at 37 °C. The plates were observed for their areas of growth inhibition (EL-Mehalawy et al., 2005).

Morphologic and Biochemical Categorization

Benchmark morphologic, as well as biochemical analyses, were done for the detection of epiphytic and endophytic bacterial cultures. Bacterial cultures were categorized by gram staining, endospore development, along with biochemical assessments (catalase, starch hydrolysis, salt tolerance, Voges Proskauer test, Sugar

fermentation test, and citrate utilization) (Cappuccino and Sherman, 2002).

RESULTS

Isolation of Epiphytic and Endophytic Bacteria

A sum of 18 leaves, stems, and root samples of two medicinal plants Basil (*osmium basilicum*) and Fenugreek (*Trigonella foenum graecum*) were assembled from distinctive areas of Multan, from September 2016 to April 2017. 10 epiphytic bacteria were found as distinct portions of two medicinal plants viz *Osmium basilicum* along with *Trigonella foenum graecum.* Out of 10, 8 epiphytic bacteria were isolated as distinct sections of *Osmium basilicum* (leaf 5, shoot 2, and root 1). Out of 10, 2 epiphytic bacteria were isolated from *Trigonella foenum graecum.* Both strains were isolated from leaves. 14 endophytic bacteria were found as of various segments of two medicinal plants viz *Osmium basilicum* as well as *Trigonella foenum graecum.* Out of 14, 3 endophytic bacteria were isolated as distinct divisions of *Osmium basilicum* (3 as of leaf). 11 endophytic bacteria were isolated from different parts of *Trigonella foenum graecum* (leaf 5, shoot 4, and root 2) as shown in table 2.

Table 3: Antimicrobial activity of isolated strains.

Bacterial isolate	*Klebsiella pneumoniae*	*Escherichia coli*	*MRSA*	*Salmonella typhimurium*	*Staphylococcus aureus*
Ob1	+	-	-	-	-
Ob2	+	-	+	+	+
Ob3	+	-	-	+	+
Ob4	+	+	-	+	+
Ob5	+	-	-	+	+
Obs1	-	-	+	-	+
Obs2	-	-	+	+	+
Obr	+	+	+	+	+
Tf1	+	+	+	+	+
Tf2	-	+	+	-	+
Obl1	-	+	+	-	-
Obl2	-	+	+	+	-
Obl3	+	+	+	+	+
Tfl1	+	+	+	+	-
Tfl2	+	+	-	-	-
Tfl3	-	+	-	+	+
Tfl4	+	+	+	-	+
Tfl5	+	+	+	-	+
Tfe1	+	+	-	-	-
Tfe2	-	+	-	+	-
Tfe3	-	+	-	+	+
Tfe4	-	+	+	-	-
Tfen1	+	+	-	+	-
Tfen2	+	+	+	+	+

+, Inhibition; -, No inhibition

Assessment of Antimicrobial Effect

Antimicrobial action of epiphytes and endophytes were studied in contrast to gram positive (*Methicillin-resistant Staphylococcus aureus, Staphylococcus aureus*) and gram negative (*Klebsiella pneumoniae, E. coli, Salmonella*

typhimurium). Out of all strains, only one epiphytic and two endophytic bacterial isolates (methanolic extracts) (obr, obl3, and tfen2 were isolated from the root, leaf, and root of *Osmium basilicum* and *T. foenum graecum* correspondingly inhibited both gram positive as well as gram negative pathogenic microbes employed in this investigation. The rest of the isolated epiphytes and endophytes revealed diverse findings as indicated in table 3. George et al., (2011) achieved the testing of fruit extracts of *F. inermis* versus multidrug-resistant bacteria and then stated that acetonic extract of the fruit has significant antimicrobial action (Shibumon and Benny, 2010).

Antibiotic Sensitivity of Pathogenic Bacteria

To inspect the efficacy of antimicrobial action of components formed by the most effective strains obr, obl3, and tfen2, a comparative study with antibiotics was done in contradiction of pathogenic bacterial species (*Methicillin-resistant Staphylococcus aureus, Staphylococcus aureus*, *Klebsiella pneumoniae*, *E. coli*, *Salmonella typhimurium*). Only *Methicillin-resistant Staphylococcus aureus and Salmonella typhimurium* were found to be sensitive to vancomycin. *Staphylococcus aureus* was found to be susceptible against ciprofloxacin and *Klebsiella pneumoniae* was only found to be sensitive against amoxicillin shown in table 4. However, obr, obl3, and tfen2 were found to inhibit all the pathogenic strains and can lead to the development of effective compounds having antimicrobial properties.

Morphological and Biochemical Characterization

Numerous biochemical procedures were accomplished following Bergey's scheme. Whereas most of the bacterial cultures were examined for their biochemical classification based on their enzyme production. Various biochemical tests catalase, citrate utilization, glucose fermentation, hydrogen sulfide production, Voges Proskauer, and starch hydrolysis (Table 5, 6) were performed. Corresponding to the morphologic as well as biochemical categorization of all the 24 strains were observed to be gram positive. Out of 24 strains, 15 (Ob2, Ob4, Ob5, Obl3, Obs1, Obs2, Obr, Tfl2, Tfl3, Tfl4, Tfe2, Tef3, Tfe4, Tfen1, Tfen2) showed encouraging findings of endospore formation assessment along with exhibited antimicrobial activity against pathogens test. Out of twenty-four strains, fifteen belonged to *Bacillus* sp, six from *Lactobacillus* sp, and three belonged to *Corynebacterium* sp.

Table 4: Antibiotic susceptibility test of pathogenic bacteria through disc diffusion method

Abx	*MRSA*	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*	*Salmonella typhimurium*
Amx	R	R	S	R	R
St	R	R	R	R	R
Van	S	R	R	R	S
Cip	R	S	R	R	R

R, resistant; S, sensitive; Amx, Amoxicillin; St, Streptomycin; Van, Vancomycin; Cip, Ciprofloxacin

Table 5: Biochemical characterization of epiphytes and endophytes

Bacterial isolate	Catalase	6.5% NaCl	Voges Proskauer	Starch hydrolysis
Ob1	-	+	-	+
Ob2	+	+	-	+
Ob3	+	+	-	+
Ob4	+	+	-	+
Ob5	-	+	-	+
Obs1	-	+	-	+
Obs2	+	+	-	+
Obr	+	+	-	+
Tf1	-	+	-	+
Tf2	-	+	-	+
Obl1	+	+	-	+
Obl2	+	+	-	+
Obl3	+	+	-	+
Tfl1	-	+	-	+
Tfl2	+	+	-	+
Tfl3	+	+	-	+
Tfl4	-	+	-	+
Tfl5	-	+	-	+
Tfe1	-	+	-	+
Tfe2	-	+	-	+
Tfe3	-	+	-	+
Tfe4	+	+	-	+
Tfen1	-	+	-	+
Tfen2	+	+	-	+

+, Positive; -, Negative

Table 6: Carbohydrate fermentation test of selected epiphytes and endophytes

Bacterial isolate	Mannitol	Lactose	Glucose	Fructose	Sucrose
Ob1	+	+	+	+	-
Ob2	+	+	+	+	-
Ob3	+	+	+	+	+

Ob4	+	+	+	+	+
Ob5	-	+	+	-	+
Obs1	+	-	+	-	+
Obs2	-	-	+	+	+
Obr	-	-	+	+	+
Tf1	-	+	+	+	+
Tf2	-	+	+	+	+
Obl1	+	-	+	-	+
Obl2	+	-	-	-	-
Obl3	+	+	+	-	-
Tfl1	-	+	+	-	-
Tfl2	-	+	+	+	+
Tfl3	-	-	+	+	+
Tfl4	+	-	+	+	-
Tfl5	+	+	+	-	+
Tfe1	-	+	+	-	-
Tfe2	-	+	+	+	-
Tfe3	+	-	+	+	+
Tfe4	+	-	+	-	+
Tfen1	+	-	+	-	+
Tfen2	+	-	+	+	-

+, Positive; -, Negative

DISCUSSION

Human beings have made astonishing progress in science besides technology, even now illnesses especially contagious ones pose a risk to human beings and happen to be the leading trigger of fatality in the advanced along with underdeveloped nations. Plants are believed as the ecological environment for countless unique varieties of microbes. Plants have additional three kinds of settings, rhizospheric endospheric phyllosphere (Hirano and Upper, 2000). Worldwide plus notably in underdeveloped nations, where maximum number of individuals expire owing to bacteriological infectivity of gram positive and gram negative bacteria like *MRSA* and *Escherichia coli*. Such microbes can persist in critical ecological circumstances. At the same time, synthetic antibiotics come up with shortcomings of expense, multidrug resistance to bacteria, along with non-availability to the public in undeveloped nations. Antibiotics have a certain extent of undesirable impacts consequently as of such because biological

compounds are getting the limelight nowadays. Microbial secondary metabolites of plants are the latest foundation of medications. For this aim, numerous approaches of isolation coupled with a biological selection have been established (Bibi et al, 2011). For the current investigation, methods involving certain stages of isolation, antimicrobial assay via lawn spot and agar well diffusion, and biochemical categorization of isolates was employed. The choice of the microbial strains was performed based on their antimicrobial capacity. The initial selection was done via pathogenic species (*Salmonella typhimurium, Klebsiella pneumoniae, MRSA, E. coli, Staphylococcus aureus).* Antimicrobial action of the epiphytes along with endophytes was verified by agar well diffusion of 3 strains (obr, obl3, tfen2) and was observed to be effective against all the gram-positive and gram-negative pathogens. A massive variety of bacteria inhabits the inner sections of medicinal plants, and this might indicate that such microbes could signify the foundation of biologically effective antimicrobial components. Even now, there are numerous queries linked to the ecological task of bacteria in the endophytic settings involving their distribution, isolation, and evolution of enormously bioactive metabolites (Qin et al, 2009).

CONCLUSION

The findings achieved from this investigation can be deliberated as enough meant for advanced analysis as well as experiments for the formation of novel antimicrobial substances as it is very necessary because of the spread of resistant pathogenic strains. Comprehensive analysis is yet essential concerning the sustainability, probable side effects, and antimicrobial capacity of these components rather than their addition to an inventory of efficient medicinal vehicles.

AUTHORS CONTRIBUTION

Atia Iqbal designed the study, Iram Asim performed the experiments and writing of manuscript, Muhammad Ikrama Tanveer edited the manuscript.

ONFLICT OF INTERST

The author declares no conflict of interest.

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