Isolation and characterization of Endophytic Bacteria from *Ziziphus Spina-Christi* (Christ's Thorn Jujube) and Evaluation of their Antimicrobial Activities against common human pathogens

Candidate: Abera Bulo Diriba

A Thesis Submitted to The Department of Applied Biology School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of master's in Biology Specialization in Biotechnology

Office of Graduate Studies

Adama Science and Technology University

June, 2024

Adama, Ethiopia

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DECLARATION

I hereby declare that this Master Thesis entitled "**Isolation and characterization of Endophytic Bacteria from *Ziziphus Spina-Christi* (Christ's Thorn Jujube) and Evaluation of their Antimicrobial Activities against common human pathogens**" is my original work. That is, it has not been submitted for the award of any academic degree, diploma, or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Abera Bulo Diriba		
Name of student	Signature	Date

RECOMMENDATION

I, the advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled "**Isolation and characterization of Endophytic Bacteria from *Ziziphus Spina-Christi* (Christ's Thorn Jujube) and Evaluation of their Antimicrobial Activities against common human pathogens**" prepared under our guidance by **Abera Bulo Diriba** submitted in partial fulfillment of the requirements for the degree of Master of Science in Biotechnology. Therefore, I recommend the submission of a revised version of the thesis to the department following the applicable procedures.

Seid Mohammed (PhD)

Major Advisor	Signature	Date

APPROVAL PAGE

I, the advisors of the thesis entitled "**Isolation and characterization of Endophytic Bacteria from *Ziziphus Spina-Christi* (Christ's Thorn Jujube) and Evaluation of their Antimicrobial Activities against common human pathogens**" and developed by **Abera Bulo Diriba**, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Seid Mohammed (PhD)		
Major Advisor	Signature	Date

APPROVAL OF BROAD OF REVEIWERS

I, the advisors of the thesis entitled "**Isolation and characterization of Endophytic Bacteria from *Ziziphus Spina-Christi* (Christ's Thorn Jujube) and Evaluation of their Antimicrobial Activities against common human pathogens**" and developed by **Abera Bulo Diriba**, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Seid Mohammed (PhD)		
Major Advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by **Abera Bulo Diriba** have read and evaluated the thesis entitled "**Isolation and characterization of Endophytic Bacteria from *Ziziphus Spina-Christi* (Christ's Thorn Jujube) and Evaluation of their Antimicrobial Activities against common human pathogens**" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfilment of the requirement of the degree of Master of Science in Biotechnology.

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Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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ABBREVIATIONS AND ACRONYMS

BCG	Bromocresol green
BM	Biomass
CD	Colony diameter
CDW	Cell dry weight
CMC	Carboxy methyl cellulose
EC	*Escherichia coli*
eDT	Extended direct transfer.
EIBZ	Endopphytic isolated bacteria
FAO	Food and Agriculture Organization
IZ	Inhibition zone
LB	Luria Bertani
MALDI-TOF MS	Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry.
MR	Methyl red
NA	Nutrient agar
OD	Optical density
PA	*pseudomonas aeruginosa*
SA	*Staphylococcus aureus*
SD	Standard Deviation
SI	Solubilization index
SIM	Sulphide indole motility
SPY	*Streptococcus pyogen*
VP	Voges-proskaure
WHO	World Health Organization

ABSTRACT

Endophytes include all microorganisms that enter the internal tissues of living plants without immediately manifesting illness signs or having an adverse impact on their host. Through competition for their hosts' habitats and food supplies, endophytic bacteria help to defend their hosts from harmful other pathogens. Several endophytic microbes were isolated from the leaves of the medicinal plant Ziziphus spina and spina-Christi, which are shrubs that can grow well in drought-prone areas and is easily drought tolerant and there are microbes with which help these plants to resist drought. The recent study aimed at isolation and identification of endophytic bacterial species from Ziziphus spina-Christi. The part of Ziziphus spina-Christi samples was collected and transported into ASTU. Surface sterilization was performed. Various biochemical tests, and morphological characterizations were carried out against isolates Endophytic bacterial isolates obtained from Ziziphus spina-Christi. Phosphate and zinc solubilization were also conducted against Endophytic bacteria isolates. Further identifications of these Endophytic bacteria isolates were carried out using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry tools, a protein profiles-based technique. Growth conditions such as optimum pH values, temperature, carbon sources, nitrogen sources, and salt concentration were evaluated against these Endophytic bacteria isolates Evaluation of agro-wastes as a main carbon source against these Endophytic bacteria isolates and Antimicrobial activities that extracted from bacteria by ethyl acetate were also conducted. In this study, a total of 8 endophytic bacterial isolates were obtained from various parts of Ziziphus spina-Christi. Their colony colors were also different. Some of these are motile and others are non-motile. Some isolates were found to be fermentative. Based on protein profiles, the recent Endophytic bacteria isolate was identified as Cronobacter Sakazaki, Rhodotorula sp., *Bordetella* sp., *Rhodotorula* sp., *Enterobacter* sp., *Stenotrophomonas Maltophilia, and Enterobacter* sp., and *Pseudomonas sp*. Studies showed that the recent isolate was found to be soluble in certain phosphates. However, no single isolate can display zinc solubilization. These isolates gave various cell dry weight (g/L) and optimum density OD_{600nm} based on various growth conditions. In conclusion, various *Endophytic bacteria isolate* obtained from different parts of *Ziziphus spina-Christi* were found to produce cell dry weight (CDW (g/L)) and optimum density (OD_{600nm}), and other enzymes with a potential to promote plant growth due to mineral solubilization.

Key words: Endophytic bacteria, biochemical, morphological and Gram staining.

1. INTRODUCTION

1.1. Background

This plant has recently attracted a lot of attention due to its nutritional value and historical medicinal uses. Christ's thorn jujube is another name for *Z. spina-Christi* El Maaiden et al (2020). North and West Asia, as well as northern and tropical Africa, all support the growth of trees known as *Z. spina-christi*. As a deciduous tree, Christ's thorn jujube is indigenous to warm temperate and subtropical locations like North Africa, South Europe, the Mediterranean, Australia, and tropical America (Asgarpanah 2012). The broadest and most common definition of endophytes include all microorganisms that enter the internal tissues of living plants without immediately manifesting illness signs or having an adverse impact on their host Sharma et al (2018). Through competition for their hosts' habitats and food supplies, endophytic microbes help to defend their hosts from other pathogenic bacteria. Additionally, it acts to stop the spread of pathogenic organisms by rapidly invading their hosts and reducing the amount of nutrients that are available for their growth Ab Rahman et al (2018). Through the creation of some poisonous chemicals that make the host inedible to insects and animals, endophytic microbes can help to defend their hosts against attack by these creatures (Ahirwar et al (2020). According to several studies, endophytic microbes produce the extracellular enzymes amylase, pectinase, cellulases, laccase, and protease as a defense against infections and as a means of obtaining nutrients from the host (Hawar, 2022). Endophytic microbes can be utilized for detoxification or in the bioremediation of industrial and agricultural waste as well as other harmful substances Karaś et al (2021). Bacteria have evolved the capacity to synthesis enzymes and natural products as new metabolites Hug et al (2020). They can create industrial enzymes, antibacterial compounds, and microbial biomass Singh et al (2019). Amylases, lipases, and cellulases are examples of bacterial enzymes that can function either directly as antimicrobials or by producing intermediary compounds that have antimicrobial activity (Ramesh, 2020) Additionally, enzymes are employed in the preparation of food to extend shelf life and preserve food quality without compromising nutritional value Amit et al (2017)

One of the most recent sources of enzymes with a variety of functions and an untapped field, the commercial synthesis of enzymes from endophytic fungi. The food and beverage industry, as well as the leather, paper, textiles, and other industries, heavily rely on fungi as a source of enzymes (Sunitha, 2013). Because medicinal plants are regarded as pharmacological agents, they are crucial for the creation of new medications (Abdulrahman, 2022). Around 80% of the world's population in

underdeveloped countries uses herbal medicine and traditional medicine, according to the WHO, for primary healthcare (Alhakmani, 2014). Fractures and chest pain are treated by *Ziziphus spina* Dhanalekshmi et al (2020). Blistering, bruising, and headaches (Hussein, 2021). The Rhamnaceae family includes the genus *Ziziphus*, which includes *Z. spina-christi*, whose thorns are supposed to have been used to create the crown for Holy Christ. Due to their durability, these plants are typically prickly shrubs, although occasionally they can grow into small trees that can withstand extreme heat and drought (Alsayari, 2021). These are cultivated in patches or naturally in the tropics of Asia and Africa. *Ziziphus* is a plant genus that is well-known for its therapeutic uses as an immune system stimulant, hypertensive, antioxidant, hypoglycemic, anticancer, anti-inflammatory, and liver protector Agrawal et al (2023). Due to their oxytocic characteristics, the crushed leaves of *Ziziphus* are cooked in water and given to women who have a retained placenta or extended labour (Hawar, 2022). The leaves are rich in iron (7.2 mg 100/g dry weight), calcium (1270 mg 100/g dry weight), and magnesium (169 mg 100/g) Saied et al (2008). There are roughly 100 species of deciduous or evergreen trees and shrubs in the genus Ziziphus worldwide Abalaka et al (2010).

Z. vulgaris has historically been used in folk medicine to treat a variety of illnesses, including digestive issues, weakness, liver issues, obesity, urinary issues, diabetes, skin infections, appetite loss, fever, pharyngitis, bronchitis, anemia, diarrhea, and sleeplessness Dodangeh et al (2017). The roots are used to treat and prevent skin conditions, while the leaves are used locally to sores (Thakur, 2017). Sedative seeds are used occasionally with buttermilk to top pregnancy-related nausea, vomiting, and abdominal pain Rouhi-Boroujeni et al (2017). The leaves are used as poultices and are beneficial for fever, asthma, and liver problems. Food has become increasingly scarce recently, particularly in harsh locations where the population suffers from poor nutrition due to a lack of suitable food supplies brought on by the local environmental circumstances (Misra, 2014). Due to this, people began looking for plant sources that would provide them with the necessary nutritional value. The Food and Agriculture Organization (FAO) gathered between 15,000 and 28,000 of these plants. One of the most significant of these plants is *Ziziphus*, which is notable for its high capacity for growth under conditions of drought and high salinity, in addition to being rich in chemical compounds that make it a source for food, industry, and the environment Abdoul-Azize, (2016). As a medicinal plant in the production of many extracellular enzymes and may be considered a new source of enzymes used in industrial and pharmaceutical applications.

1.2. Significance of the study

Microbes, particularly bacteria, have an inherent capability of plant growth promotion (PGP) and bioremediation. Bacterial genera have been used as plant growth promoting agents since the 1970s with a variety of reports justifying their capability of phosphate solubilization, zinc solubilization, nitrogen fixation, biological control of plant diseases and other PGP attributes Rakshit et al (2018). The current shift in agricultural practices has thereby gradually incorporated minimal use of agrochemicals in field practices while simultaneously employing bacterial microbiota as bio-inoculant, either singly or in consortia. Taking into concern the impressive role of biopesticides and biofertilizers in the promotion of sustainable agriculture, which basically include, target-specificity, environmental safety, and biodegradability, it is believed copious research are necessary for promoting the use of these microbes. And there are in fact several research on these microbes being carried out by scientists and researchers worldwide. Although much research is being carried out worldwide, when we come to Ethiopia the use of these green inputs is significantly less. Therefore, this study significantly adds to the existing knowledge of bacterial endophytes, their taxonomy, and their potential to be used as biofertilizers and biopesticides in developing a sustainable, safe, and effective agriculture system in addition with their potential use in forestry and other fields, we believe also this research could serve as an eye opener for the potentials of the endophytic bacteria to produce different important enzymes and secondary metabolites.

1.3. Statement of the problem

Agricultural advancements, particularly the green revolution, enforced the use of chemical fertilizers and pesticides for greater production and higher yield Rakshit et al (2015). However, persistent chemo inputs into soil over the years have rendered a catastrophic effect on the soil micro-biota as well as on human health.(Singh, 2018) believes that this phenomenon is quite evident from the fact that many agricultural lands have been rendered completely unproductive, while there is a significant upsurge of certain deadly diseases such as cancer, diabetes, hypertension etc. due to a constant exposure to the chemical compounds. Degradation rates of the chemical fertilizers are quite negligible owing to their complex structures. This leads to their bioaccumulation and biomagnification thereby resulting in a loss in specific biodiversity and additionally, severe groundwater contamination. Apart from the abovementioned concerns, an exponentially increasing human population along with a persistent caveat of abiotic stress is demanding the utilization of other ecofriendly alternatives chiefly to ensure food

security Keswani et al (2017). The absence of detailed knowledge about endophytic bacteria and their potentials in our country has caused these agriculturally important microbes to be neglected and the use of potentially harmful agro-chemicals to persist.

1.4. Objectives of the study

1.4.1. General Objective

To isolate and characterize endophytic bacteria from *Z. spina-Christi* plants and evaluate their antimicrobial activities against common human pathogens at ASTU.

1.4.2. Specific Objectives

The specific objectives of this study were to:

- Determine the biochemical characterization and enzymatic activities for these newly obtained endophytic bacterial isolates.
- Identify these endophytic bacterial isolates using MALDI TOF MS techniques.
- Evaluate the optimum growth condition for these endophytic bacterial isolates.
- evaluate the antimicrobial activity of crude extracts of endophytic isolates.

2. LITERATURE REVIEW

2.1. Chemical composition of *Ziziphus spina-christi*

Geranyl acetone (14.0%), methyl hexadecanoate (10.0%), methyl octadecanoate (9.9%), farnesyl acetone C (9.9%), hexadecanol (9.7%), and ediyl octadecanoate (8.0%) are the primary components of the oil derived from *Z. spina-Christi*. Mervat et al (2018) Also discovered in the fruit oil from Z. spina-Christi was dodecanoic acid (22.4%). In the Middle East, South and East Asia, its extracts are employed in the creation of pharmaceuticals due to their pharmacological properties. Phytochemicals such as cardiac glycosides, polyphenols, saponins, and tannins were found in the extracts (Soni, 2013). Because it includes a variety of nutrients, *Z. spina-Christi* is a crucial food source. There are calories in *Z. spina Christi*. Fresh fruits have an average of 80% carbs (glucose-fructose), 3% iron, 0.9 g of fat, 140 mg of calcium, 0.04 mg of thiamin, 0.13 mg riboflavin, 3.7 mg niacin, and 30 mg of ascorbic acid, with the amount of ascorbic acid fluctuating depending on the fruit's level of maturity (Vernin, 2016). The seeds are 28.5% fat, 18.6% protein, and these proteins are notable for having high sulfur amino acid content. The leaves contain a variety of minerals, including magnesium 169 mg%, calcium 1,270 mg%, and iron 7.2 mg% Muhammad et al (2022).

Z. spina-Christi is an important food source because it contains many nutrients. *Z. spina-Christi* contains calories. Fresh fruits contain 80% carbohydrates (glucose-fructose), 3mg% iron, 0.9g fat, 140mg calcium, 0.04 mg of thiamin, 0.13 mg riboflavin, 3.7 mg niacin and 30 mg ascorbic acid, and the concentration of ascorbic acid changes according to the degree of ripeness with respect to the fruit. The seeds contain 28.5% fat, 18.6% protein, and these proteins are characterized by being rich in sulfur amino acids. The leaves contain many minerals such as calcium 1,270 mg%, iron 7.2 mg%, magnesium 169 mg% Saied et al (2008).

2.2. Multipurpose of Ziziphus spina-christi

Z. spina-Christi has various uses, and its fruits can be consumed fresh, dried, or pounded into flour for baking. The fruit pulp can also be consumed right away or saved for later use, and it can be formed into small balls for use in baked goods. Because it was utilized as food and treatment for illnesses, the ancient people were aware of its significant medical relevance (Alsayari, 2021).

They are essential genetic resources in global efforts to sustain biodiversity and are a part of local and regional food procurement and agriculture systems. *Z. spina-Christi* is a species that has evolved to thrive in North Africa's hot, arid climate. Fruits of *Z. spina-Christi* are available in most local markets in Sudan all year. Those fruits have an annual quantitative worth of about 8,890 tons, making them one of the most significant non-wood forest products. Of all the trees, this one is the most valuable for the Yemeni economy's traditional trade (Al-Fatimi, 2024). Because they are pharmacological agents and crucial for the creation of medications, interest to medicinal plants has recently expanded 80% of the world's population in underdeveloped nations, according to the World Health Organization (WHO), uses traditional and herbal medicine for their primary treatment. *Z. spina Christi* is used to treat chest pains, bruises, blisters, dandruff, and fractures. There are many forms of benefiting of *Z spina-Christi*, the fruits of which are eaten either fresh or dry and may be ground in the form of flour used in baked goods, where they are made in the form of small balls, as the fruit pulp may be eaten or stored for the future. It has great medical importance that the ancient people knew, as it was used as food, as well as a medicine for diseases Tounekti et al (2019).

2.2.1. Animal feed

Leaves are used as food for animals, especially in the dry season, where small branches are rubbed and used as feed for camels and goats (Al Shafei, 2016). The leaves provide valuable animal forage and fodder under open grazing conditions, but the nutritional value is apparently not high for most domestic livestock. The fruits are eaten by sheep and goats and the foliage by camels. From existing data of leaf biomass and nutritional values of *Z. spina-Christi* leaves, it could be therefore concluded that its leaves could replace conventional supplements, which are expensive and not easily available locally to provide protein supplements for growing ruminants like goats. This would reduce some of the major feed. Ethiopia is endowed with a huge population and many breeds of goats that serve multiple functions at household and national level. Though the purpose of keeping goats varies from area to area due to economic, cultural, and ecological factors they are mainly maintained for fulfilling multiple roles, ranging from socio-cultural purposes to providing meat, milk, and manure Ali et al (2019).

However, productivity of goats is very low in the region and because of this the country is not getting the benefits from this huge resource. Poor nutrition is one of the major obstacles which limit the performance of goats Lemecha et al (2013). In lowland areas, goats rely on browsing and grazing whereas in the highlands they depend on communal grazing, fallow lands, and crop residues. Such

types of feeds are insufficient to provide nutrients beyond maintenance requirements as a result poor grazing and low-quality feeds especially in terms of energy or protein leads to undernourishment and low productivity. Among the options, browsing plants plays a significant role in the nutrition of ruminant livestock in tropical regions. Browse species, because of their resistance to heat, drought, salinity, alkalinity, drifting sand, grazing, and repeated cutting, are the major feed resources during the dry season. *Ziziphus* species are widespread in tropical Africa as indigenous tree including Ethiopia being distributed in areas with altitude range from 400 to 1600 masl. The tree is ecologically and economically important for its tolerance to drought and salinity, and the high value of its non-wood products, particularly fruit for food and leaves and shoots for saponin and tannin substrates. In the rift valley regions of Ethiopia, ripened fruits of *Z. spina-Christi* are consumed by local shepherds, and propagation under natural conditions is affected by the problem of hard seed coat dormancy, resulting in a very low-germination percentage. The role of *Z. spina-Christi* leaves for fodder for goat has been reported (Njidda, 2012).

2.2.2. Environmental uses

It has great environmental importance as it is grown around villages and works as a safety belt and windbreaks because of its deep and extended rootstocks to raise soil fertility rates because it contains a large amount of phosphorous. Future climate change models predict that many regions of the world will experience shifts in temperature and an increase in the frequency and severity of droughts Ahmadi et al (2012) *Z. spina-Christi* has become more abundant in the east Mediterranean, presumably because of a gradual increase in winter temperatures. *Z. spina-Christi* is considered resistant to drought, soil salinity, and heat; it survives in desert areas. *Z. spina-Christi* is cross-pollinated, the cymes developing in the leaf axils of young branches, and the edible fruits, which have a fleshy mesocarp, usually ripen under warm conditions Zait et al (2020).

The tree is propagated through seeds covered with a hard woody endocarp (seed coat) that inhibits water absorption, thereby imposing physical dormancy (also known as exogenous dormancy) that limits germination (Takhti, 2012). *Z. spina-christi* seeds germinate during the winter when soil moisture is high, but temperatures are relatively low. This forces the seedling to cope with occasional chill events, which can be lethal. The relatively slow growth of the seedlings during the first year (mainly of the shoot) may be another limiting factor on the successful establishment of the saplings.

For these reasons, *Z. spina-christi* may be among those trees that might benefit from future warming. An additional limiting factor for *Z. spina-christi's* successful establishment is its incapacity to photosynthesize and respire at low temperatures, therefore limiting the production of sugars necessary for growth and other metabolic maintenance processes**.** The tree improves soil quality by increasing available phosphorus Gmedhn et al (2018).

2.2.3. Human food

There are many ways to take advantage of the plant. It may be eaten fruits, which are characterized by the sweetness of taste or may be ground, then mixed with water and made from it baked goods such as gingerbread. Recent studies have proven that honey resulting from bees that feed on dam trees is a strong bacterial antibody compared to other types of honey (Borba, 2015). *Z. spina-Christi* contains multiple nutritional components that render the plant one of the greatest food sources. The plant has been utilized in traditional medicine in the Middle East, mainly fruits, seeds, leaves, roots, and bark Dhanalekshmi et al (2022). The fruit of *Z. spina-christi* is small, orange yellow with spiked branches that are considered a rich source for important dietary components. The dry fruits are wealthy in energy compounds (80.6% carbohydrates: starch, sucrose, glucose, and fructose) Saied et al (2008) Furthermore, there are 0.9 g fat, 4.8 g protein, 140 mg calcium, 0.13 mg riboflavin, 0.04 mg thiamine, and 3.7 mg niacin per 100 gm dried fruit. The fruit also contains a high amount of vitamin C (98 mg) which is a considerably larger amount compared to those present in strawberry (59 mg), orange (50 mg), and grape 38 mg Bhardwaj et al (2014).

It was reported that the fruits have a rich number of polyphenols (24.62 mg/g) of gallic acid equivalent of dry extract). Phenolic compounds are important natural antioxidants that possess scavenging activity against free radicals. The seeds of *Z. spina-Christi* hold 28.5% fats (mostly linoleic and linoleic acid), and 18.6% protein (mostly sulphur-rich amino acids). The seeds have a sedative effect and could be used to relieve pregnancyrelated nausea, vomiting, and abdominal discomfort. The *Z. spina-Christi* stem bark has been marked to have antifungal, antibacterial, and cytotoxic actions Kassem et al (2011).

2.2.4. Nature of the plant and its content

Z. spina-Christi is a shrub that may grow as tall as 20 m and as wide as 60 cm; it has light grey, severely cracked, scaly bark; a twisted trunk; a dense crown; and white, flexible, drooping shoots with

pairs of thorns, one straight and the other curled. Fruits from *Z. spina-Christi* are often consumed fresh and are particularly nutrient-dense. Yemen and Eritrea, which includes our country Ethiopia, depend heavily on flowers as a source of honey Hegazi et al (2022). On cuts and sores, the fruits are applied. They are also used to treat diarrhea, lung conditions, fevers, and to hasten the healing of recent wounds Taghipour et al (2020). *Ziziphus* stands out for having a tree with a trunk diameter of 45–60 cm and a height of 5–20 meters. From whitish brown to pale gray, the bark's hue changes. The flowers are small greenish white flowers that are found at the top of the leaves and are distinguished by the presence of 5 sepals that measure 2 mm in length and 3 petals that measure 1.5 mm in length. The leaves are simple and curly, with a length range of 1-9 cm and a width of 1-3.5 cm. The fruit is reddish brown in color, is up to 1.5 cm in diameter, and has a firm seed surrounding it with a sweet tasting flesh. The warp is made up of 5 opposing petals encircled by a flat lobed disk. The fruit is reddish brown in color, grows to a diameter of 1.5 cm, has a solid seed encircled by a sweet tasting pulp, and bears fruit from October to April. The warp is made of five opposing petals surrounding by a flat, lobed disk Agrawal et al (2023)

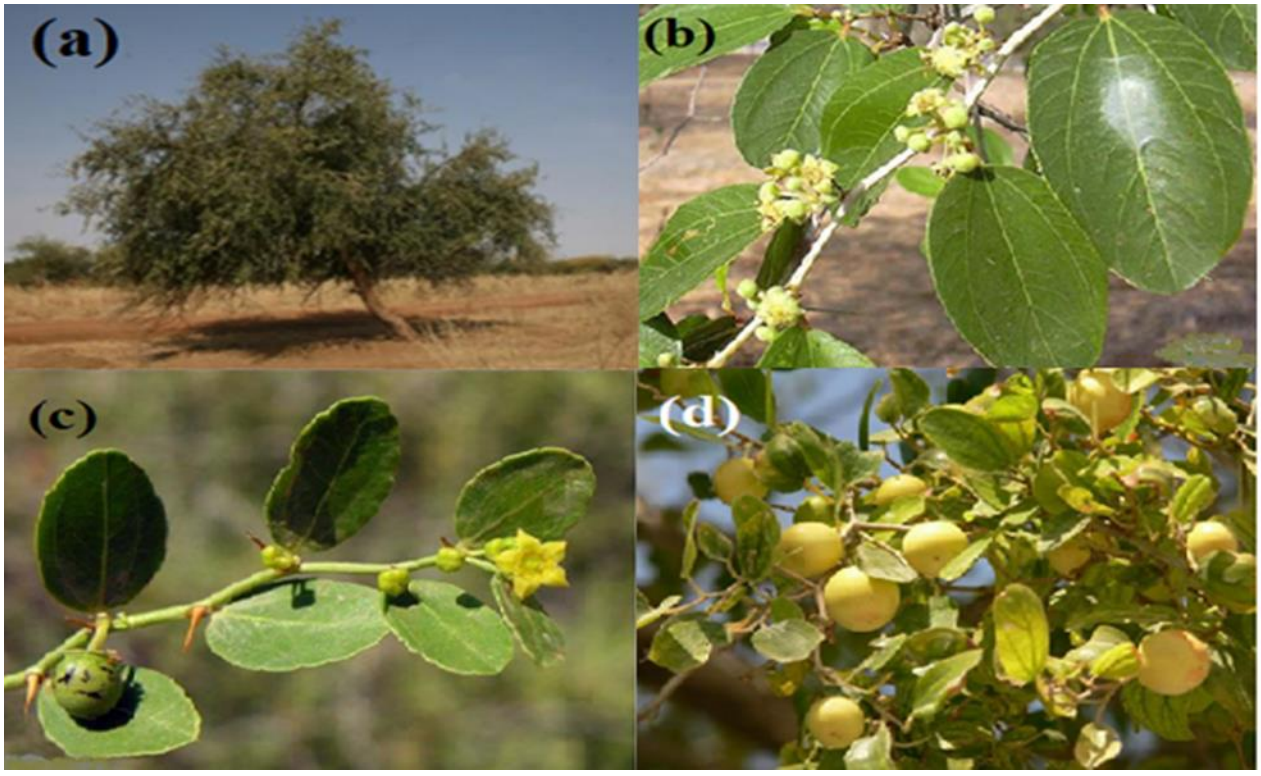


Figure 1: Figure of the ziziphus spina-Christi leaf, steam, and fruit! (a) tree of ziziphus spina-Christi (b) Its leaves are glabrous on upper surface, finely pubescent below, ovate-lanceolate, or

ellipsoid, apex acute or obtuse, margins almost entire, lateral veins conspicuous. (c) Flowers in cymes, subsessile, peduncle 1 to 3 mm. (d) Fruit about 1 cm in diameter Asgarpanah et al (2012).

2.2.5. Pharmaceutical uses

The medicinal uses of *Z. spina-christi* are versatile as hypoglycemic (Hussein, 2019). This plant has glycemic effect; research indicated that induced diabetic rats by aqueous extract of plant decrease the level of blood glucose by two mechanisms by acting on glucose homeostasis in an extra-pancreatic way or by improvement of liver action and take glucose to synthesis glycogen act as fuel of energy Jamshidi et al (2014). Saponin glycoside is known as natural product present in this plant which responsible for lowering level of glucagon which is hormone responsible for mobilization of glucose into blood stream and hence lowering level of blood glucose in indirect pathway, hypotension, anticancer, anti-inflammation is used. Because the plant contains tannins, it has an antibacterial effect Elmaidomy et al (2023). This is since tannins are associated with protein, especially proline-rich proteins. Where tannins are bound to the iron which contributes to the inhibition of metabolism inside the microbe and helps to eliminate it de Melo et al (2023).

The presence of saponins contributes to increasing the surface tension of the membranes, which contributes to increasing the permeability of cells and helps in destroying the microbe. Alkaloids are bound to DNA, which affects the process of cell division, Flavonoids bind to DNA and R N A, thereby inhibiting protein and fat formation, causing energy metabolism to be impaired, thereby affecting the growth of the microbe. The plant is used as a treatment for diarrhea and colon cramps. It is also used as a twig powder in the treatment of rheumatism, scorpion sting, strengthening the immune system and regulating its mechanism of action Abdulrahman et al (2022). The leaves are also used to treat headaches, bone pain, abscess treatment, and superficial wound dressing, and are sometimes used as an analgesic Phondani et al (2016). Small branches are used in the treatment of eye infections, its leaves are boiled in water and used as a face wash and mixed with lemon and used as a shampoo to maintain the softness of hair and the purity of the skin (Dkhil, 2018). Fruit tea is used to treat measles. Fruits are used to treat chest pain, respiratory system, and blood purification from impurities. Its leaves are used to get rid of the undesirable taste in oral medications and to keep the gums clean Saied et al (2008).

2.2.6. Potentials of Z. Spina-christi

A tropical evergreen tree of many parts of Iran, it is cultivated mainly as a dry crop for its mucilage nutritious fruits, honey production and landscaping purposes Talebi et al (2014). It serves the ecosystem by controlling erosion, acting as wind break and it improves soil quality by increasing available phosphorus Feyssa et al (2014). Traditionally, it is used in Iran as a medicinal plant; the fruits are used for the treatment of fever, pain, dandruff, wounds, and ulcers, in inflammatory conditions, asthma and to cure eye diseases, while the seeds are used as a tonic Papari et al (2020). Extracts from the plant could be useful in the treatment of nosocomial infections, opportunistic infection of the unary tract, infantile gastroenteritis, traveler's diarrhea, wound infection, meningitis, and wounds infection which are diseases caused by some of these organisms Adzu et al (2022). Additionally, *Z. spina-christi* fruit extract causes neurotransmitters release, which is probably related to presence of ascorbic acid and the leaves, may potentially be safe for use as sedative drug. A variable activity of the plant extract is against *Staphylococcus aureus* which highly infects various burns. More-over, the methanol extract of Sidir could be used not only as a safe potential natural functional food ingredient or as therapeutic drug in the treatment of diabetes, but also it is effective in reducing both hyperlipidemia and oxidative stress accompanying diabetes. It is easily domesticated and can be grown commercially for the benefit of pharmaceutical industry and vegetation purposes Jalaie-Esfandabadi et al (2022).

2.2.7. Antimicrobial properties of plant exteract

The aqueous extract of *Z. spina-christi* stem bark has shown significant antibacterial activity against *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia Proteus vulgaris, Pseudomonas aeruginosa, Salmonella para typhi* B and *S. aureus* Mervat et al (2018). Alcoholic extract of the leaves has also shown good antibacterial activity against *S. aureus* isolated from eye infections (conjunctivitis). An inhibition zone of 20 mm was recorded for 1 mg/ml of the extract (Sindi, 2020). *Z. spina Christi* (ZSC) is among the important plants in the traditional medicine of Iran Alomari et al (2016). *Z spina-Christi* is a shrub and an evergreen tree. Interims of phytochemical and antibacterial analysis, water, and ethanol extracts of *Z. spina-Christi* leaves will be tested. It has simple, broad leaves with three notices able basal veins of 2 cm to 7 cm in length (0.79 to 2.76 inches). Some species of this plant are deciduous, and others are evergreen. The phytochemical screening shows that the extract of leaves contains flavonoids, alkaloids, saponins, lipids, proteins, and free sugar as the dominant chemical composition in this plant Dhanalekshmi, et al (2022).

Plant materials significantly lead to improving human health by the prevention of diseases. Sider (*Z. spina-christi*) leaves' extracts were more significantly effective against some bacterial species, especially Pseudomonas species that most often cause infections in the lungs (pneumonia). The current research was aimed at assessing the antibacterial and anti-microbial activity of methanolic and ethanolic extracts of sider (*Z. spina-Christi*) leaves as a treatment for respiratory disorders, especially those caused by coronaviruses Taghipour et al (2020). Coronaviruses are positive stranded, large RNA, enveloped viruses infecting numerous avian and mammalian species, meanwhile capable of causing gastrointestinal and respiratory diseases. The variable effects of some plant extracts against some fungal diseases revealed that they might either stimulate or inhibit the growth of some fungi species. The variable effects of some plant extracts against some fungal diseases revealed that they might either stimulate or inhibit the growth of some fungi species. Eman et al (2020) The antifungal activity of the plant aqueous extracts of *Ziziphus sp*. Fungal growth was inversely proportional to the increment of plant extract concentrations of *Fusarium oxysporum, Helminthosporium* sp., *Alternaria* spp. and *Rhizoctonia solani,* despite there were no reported-inhibitory effects on the other species (Temerk, 2017)

2.3. The characteristics of endophytic bacteria.

The bacterial endophytes promote the growth of host plants directly via producing phytohormones, including indole-3-acetic acid (IAA), gibberellins, cytokinin's, phosphate solubilization, N_2 fixation or indirectly via the production of antibiotics, siderophores. In addition, endophytes secrete various types of secondary metabolites volatiles that significantly control and inhibit the growth of phytopathogen. Besides this, endophytes also counteract the adverse effects of abiotic stresses such as drought and salinity Kumar et al (2016) According to a previously published report, Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes are reported as predominant bacterial phyla, while *Pseudomonas, Bacillus*, *Pantoea,* Acinetobacteria are the most common bacterial genera reported in various plant species Verma et al (2021)

3. MATERIALS AND METHODS

3.1. Description of Study area

The study was conducted at Adama Science and Technology University. The leaf, steam and root of the plant were collected from Adama. Adama city is located at 8.54°N and 39.27°E, at elevation of 1712 m, about 99 km southeast of Addis Ababa. The city is located between the base of an escarpment to the west, and the Great Rift Valley to the east. It is a rapidly growing major city near Addis Ababa in central Ethiopia, with a total population of 500,000 in 2018 (Hundera et al 2020).

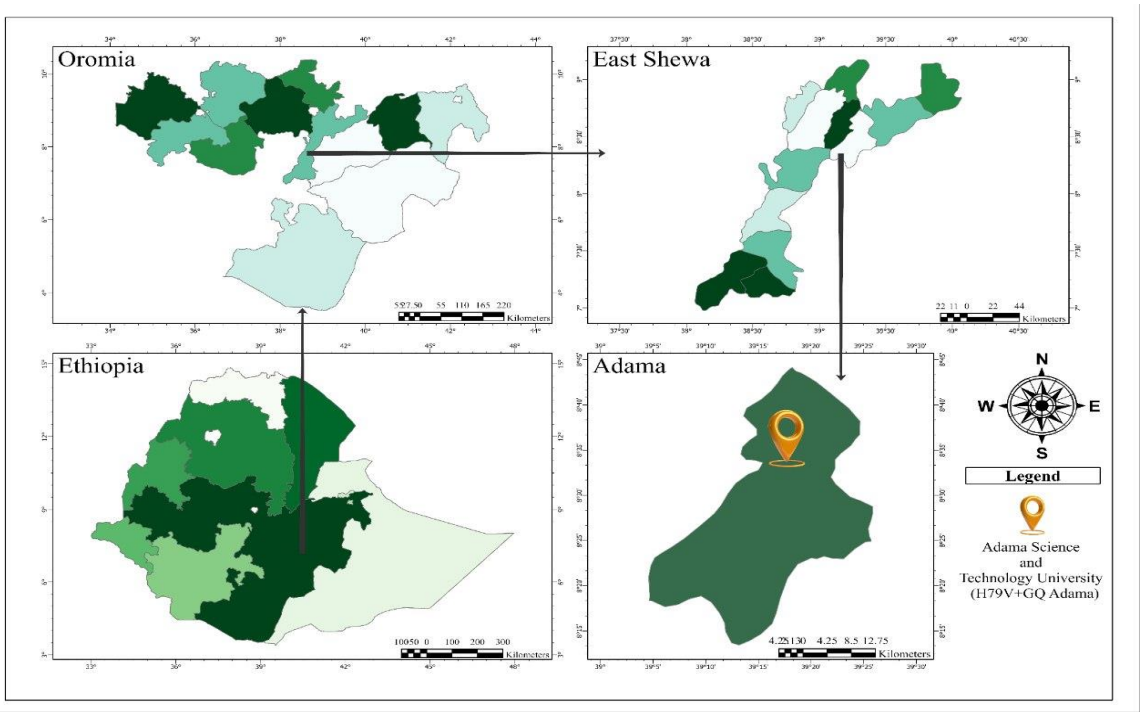


Figure 2: Map of the study area (Hulluka et al 2021)

3.2. Collection of Plant Materials

The disease free and mature plant where two leaves, two stem, and four root part of *Z. sphina-christi* were collected from Adama Science and Technology University. A fresh plant material sample was transported to the lab under aseptic conditions for further isolation of endophytic bacteria.

3.2.1. Surface sterilization of collected leaves, stems, and root samples.

The leaves of the plant separated from the twigs and washed thoroughly under plenty of running tap water to remove adhering soil particles and the microbes. Leaf, steam, and root samples were cleaned with detergent (soap), running tap water, and then distilled water have taken into laminar hood and immerged into 70 % ethanol for 30 sec and then into sodium hypochlorite (5%) solution for 3 min. Finally, leaf, steam and root samples have exposed to absolute alcohol for 10 sec and then were washed thrice with autoclaved distilled water (ADW). Surface decontaminated leaves, steam and root were cut into two pieces of about 1 cm^2, two pieces of 1 cm^2, and four pieces of 1 cm^2, root using sterile surgical blade. After cutting in to pieces the leave, steam and root part of plant material put on the nutrient agar medium on the plate and placed in the incubator at 30^oC for 24h to further growth.

3.2.2. Isolation of endophytic bacteria

After plant leaves, steam and root cut into 1cm^2 these segments were incubated on Nutrient agar (NA) for 24 h. And the bacteria growth was identified. Following the procedure of Shweta et al. (2013) these segments were incubated on Nutrient agar (NA) (Glucose 10 g/L; NaCl 5 g/L; peptone 10 g/L; yeast extract 10 g/L; Agar 18 g/L at pH 7.0-7.2) plates amended with ketoconazole (20µg/ml) for 1day at 30 ± 2^oC. The sterilized water taken from the third and final wash was also incubated using spread culture method as a positive control. A similar procedure without surface sterilization for few segments were done and used as a negative control to check for surface contamination.

3.3. Morphological characterization of isolated endophytic bacteria

Gram ‘s staining: Gram-staining was performed on the endophytic bacteria to differentiate between Gram-positive and Gram-negative species. Bacterial colonies were smeared on a drop of water on a clean glass slide then were left to air dry and heat fixed. Then the slides were flooded with crystal violate dye for 1 min followed by rinsing under tap water. Then, the slides were flooded with gram ‘s iodine for 1 min as a mordant and again rinsed under tap water. Thirdly, the slides were rinsed with 70% (v/v) ethanol for 15s and then rinsed with tap water. Lastly, the slides were flooded with safranin as a counter stain for 1 minute and rinsed with tap water. Next, the slides were allowed to dry and were viewed under a microscope x100 with oil immersion Arndt et al (2021)

3.4. Biochemical characterization of endophytic bacteria

3.4.1. Catalase test

For the catalase test, fresh 18-24 hours old pure bacterial colonies were used. A 3-4 ml of 3% (v/v) H_2O_2 was poured into sterile test tubes and then pure endophytic bacterial colonies taken with the stick side of a cotton swab were immersed fully into the test tubes. The formation of bubbles due to the conversion of H_2O_2 into H_2O and O_2 was checked for in the test tubes (Woodland, 2004). The slide method was also used for the catalase test. Briefly, a loopful of the isolated bacteria was smeared on a clean microscope slide and 2-3 drops of 3% (v/v) H_2O_2 were added directly to the smear, the formation of visible bubbles indicates a positive result (Kumar, 2012).

3.4.2. Cellulase test

The extracellular cellulase production ability of the bacterial isolates was tested on carboxymethylcellulose (CMC) agar plates. For the preparation of the CMC agar medium, NA (Glucose 10 g/L; NaCl 5 g/L; Peptone 10 g/L; Yeast extract 10 g/L; Agar 18 g/L at pH 7.0- 7.2) was supplemented with 1 g/L (w/v) of carboxymethylcellulose (CMC). The NA and CMC were autoclaved separately and mixed post sterilization. The CMC medium was poured into sterile Petri dishes and fresh bacterial colonies were inoculated with a loop at the center of the agar plates once they solidified. Then, after the plates were incubated for 48 hours at 37^{o}C the plates were flooded with 1% (w/v) Congo red solution for the visualization of clearing zones Nxumalo et al (2020).

3.4.3. Methyl red and Voges–Proskauer test

The MR-VP test was carried out to determine which fermentation pathway is used by the bacteria to utilize glucose. MR-VP broth medium (Dextrose 5 g/L; Monopotassium phosphate 5 g/L; Pancreatic digest of casein 3.5 g/L; Peptic digest of animal tissue 3.5 g/L) was prepared and inoculated with bacterial isolates. The inoculated broths were incubated for 48 hours at 37^{o}C. Then 10 ml of each of the incubated cultures was poured into clean test tubes to test to produce acetoin (VP test). To the first tube, 2-3 drops of methyl red solution were added, and the formation of red color was checked. To the second tube, 3-4 drops of the mixture of α-naphthol and KOH solution was added, and the solutions were shaken together vigorously and set aside for about 1h. After an hour the test tubes were checked for a change in color from yellow to brownish red to pink Vashist et al (2013). Biochemical tests such

as catalase test, cellulase test, protease test, indole production test, methyl red test, motility test, and VOGES-PROSKAUER test were carried out. Depending on the test results, the bacteria could be categorized into the different genera they belong to.

Motility test: Semi-solid Motility Test medium may also be used to detect motility. The agar concentration (0.3%) is sufficient to form a soft gel without hindering motility. When a non-motile organism is stabbed into motility test medium, growth occurs only along the line of inoculation. Growth along the stab line is very sharp and defined. When motile organisms are stabbed into the soft agar, they swim away from the stab line. Growth occurs throughout the tube rather than being concentrated along the line of inoculation. Growth along the stab line appears much more cloud-like as it moves away from the stab Josenhans et al (2002).

Indole test: A sterilized tube containing 4ml of tryptophan broth would be inoculated with 18-24 h old culture of endophytic bacterial isolates and incubated at 30^{o}C for 24 h. Then, 0.5 ml of kovac's reagent was added into test tube containing pure culture endophytic bacteria isolates, these efficiency for siderophores biosynthesis (Hnini, M., & Aurag, J. 2024).

3.4.4. Protease test

For the evaluation of the protease-producing ability of the isolated bacteria, Skim milk agar plates were prepared. Briefly NA (g/L) (Glucose, 10 g/L; NaCl, 5 g/L; Peptone, 10 g/L; Yeast extract, 10 g/L; Agar, 18 g/L and pH 7.0-7.2) supplemented with 1% skim milk (w/v) was prepared with the addition of 0.0015% (w/v). The medium was autoclaved with a temperature of 121oC for 15min. After autoclaving, the medium was poured into sterile petri dishes and solidified. After that, the plates were inoculated with the isolated bacteria and incubated at 37^{o}C for 48 h. A zone of proteolysis was detected on the agar plates after two days of incubation Vijayaraghavan et al (2013).

3.5. Plant growth promotion traits.

3.5.1. Phosphate solubilization test

The phosphate solubilizing ability of the isolated endophytic bacteria was tested by spot inoculating on Pikovskaya medium ($Ca_3(PO)_4$, 5 g/L; $FeSO_4.7H_2O$, 0.002 g/L; Glucose, 10 g/L; $MgSO_4.7H_2O$, 0.1 g/L; NaCl, 0.2 g/L; $(NH_4)_2SO_4$, 0.5 g/L; KCl, 0.2 g/L; Yeast extract, 0.5 g/L; $MnSO_4$, H_2O, 0.002 g/L

and Agar, 15 g/L at pH 7). The medium was dissolved by boiling and shaking gently and then sterilized by autoclaving at 121^{o}C for 15 min. The medium was poured into sterile Petri dishes and after solidifying was inoculated by fresh colonies of the isolated endophytic bacteria. The plates were incubated at 30^{o}C for 7 days and a zone of phosphate solubilization was observed and the PSI was recorded. To get the inhibition zone $\frac{Total\ diameter}{\text{Colony diameter}}$ ($PSI=\frac{TD}{CD}$) Pande et al (2017).

3.5.2. Zinc solubilization test.

The zinc solubilization ability of the endophytic bacteria was examined on minimal salts media. The minimal salt medium (Dextrose, 10 g/L; KCl, 0.2 g/L; K_2HPO_4, 0.1 g/L; $MgSO_4.7H_2O$, 0.2 g/L; $(NH_4)_2SO_4$, 1 g/L and Agar 15 g/L at pH 7-7.2) was prepared and 34 insoluble zinc 1 g/L was added in the form of zinc oxide (ZnO). The medium was boiled to dissolve and was sterilized at 121^{o}C for 15 min. The sterilized medium was poured into sterile petri dishes and after solidifying the plates were spot inoculated with fresh colonies of the isolated bacterial colonies. The plates were then incubated at 30^{o}C for 14 days and examined for halo zones after incubation. To get the inhibition zone $\frac{Total\ diameter}{\text{Colony diameter}}$ ($PSI=\frac{TD}{CD}$) Kamran et al (2017).

3.6. Identification of endophytic bacteria using MALDI-TOF MS

The isolated endophytic bacteria were investigated using the Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) which analyzes the protein or peptide constituents of a sample microorganism and enables identification of the microorganisms up to genus and species level. MALDI TOF-MS identification of the endophytic bacterial isolates was carried out at the Wudassie diagnostic center found in Addis Ababa around the stadium. The bacteria were grown overnight before analyzing. The extended direct transfer (eDT) procedure per the manufacturer's instruction was used to analyze the bacteria (Angeletti, 2017).

3.7. Optimization of growth conditions for isolated endophytic bacteria

Cultural conditions were optimized for two selected endophytic bacterial isolates (EIBZ 5, EIBZ 6) that were selected based on their high phosphate solubilizing and non-zinc solubilizing bacterial activities. Temperature, pH, Carbon source and Nitrogen source preferences of the endophytic isolates were

tested. All the results were presented as triplicates of OD600nm and Biomass (BM) as cell dry weight (CDW g/l) measurements.

3.7.1. Optimization of growth against various salt concentrations

The effect of salt on bacterial growth was determined at various concentrations (0.9, 1, 3, 5, 7). Briefly, the pure obtained endophytic bacterial isolates were inoculated into sterilized Luria Bertani (LB) broth (Glucose, 2 g/L; NaCl, 5 g/L; Tryptone, 10 g/L, and Yeast extract, 10 g/L at pH 7.00) was the medium of choice for salt optimization. The medium broth was incubated at 48 for 72h incubation temperature time. After incubation, the biomass was collected by centrifugation (10,000rpm, 25^{o}C. and 10min) and dried overnight at 40^{o}C using the oven. The CDW (g/L) was measured from dried biomass and the OD_{600nm} was measured by UV-spectrophotometer. Finally, the data was analyzed according to (Eevers et al (2015).

3.7.2. Growth conditions against various temperature

To find out the optimum growth temperature of the endophytic bacteria the isolates were all cultured on the same media, with the same volume of media, and for the same amount of time. Luria Bertani (LB) broth (Glucose, 2 g/L; NaCl, 5 g/L; Tryptone, 10 g/L and Yeast extract, 10 g/L at pH 7.00) was the medium of choice for temperature optimization. A 250 ml of volume for each isolate was prepared and 200 µl of 24 hours fresh inoculum of the isolated bacteria was incubated at 6 different temperatures of 25, 30, 32, 35, 37 and 40^{o}C for the same amount of time, 48 hours with 130 rpm. After 48 hours the optical density and biomass of the culture were taken in triplicates and recorded (Eevers et al (2015).

3.7.3. Growth conditions against various pH-value

To find out the optimum pH range suitable for the growth of the isolated endophytic bacteria, the isolates were all cultured on the same medium, with the same volume of medium for the duration. LB broth (Glucose, 2 g/L; NaCl, 5 g/L; Tryptone, 10 g/L and Yeast extract, 10 g/L at pH 7.00) was the medium of choice for pH optimization and 250 ml of volume for each isolate was prepared. A 200 µl of 24 hours old fresh inoculum of each of the isolated bacteria was inoculated into 4 different flasks with 4 pH ranges of 6, 6.5,7, 7.5, and 8 and were incubated for 48 h at 130 rpm. After 48 hours the optical density and biomass of the culture were taken in triplicates and recorded Touloupakis et al (2016).

3.7.4. Growth condition against various nitrogen source

To find out the most suitable nitrogen source for the growth of the isolated bacteria four nitrogen sources, two organic and two inorganic nitrogen sources were used. The organic nitrogen sources used were Yeast Extract and Peptone while the inorganic nitrogen sources were $(NH_4)_2SO_4$ and KNO_3. Minimal salts medium ($CaCl_2.H_2O$, 0.02 g/L; $FeSO_4.7H_2O$, 0.03 g/L; Glucose 2 g/L; K_2HPO_4, 1.73 g/L; KH_2PO_4, 0.68 g/L; $MgSO_4$, 7ml H_2O, 0.1 g/L; and NaCl, 4 g/L) was prepared and the four different nitrogen sources were added 0.6% (w/v) to the media. After autoclaving, the media were transferred into 250 ml Erlenmeyer flasks and inoculated with 200 µl of 24 hours old fresh inoculum of the isolated bacteria. Then the flasks were incubated for 48 hours with agitation of 130 rpm. After 48 h the optical density and biomass of the culture were taken in triplicates and recorded Eevers et al (2015).

3.7.5. Growth conditions against various Carbon- source

To find out the most suitable carbon source for the growth of the isolated bacteria four carbon-containing organic compounds were used. Minimal salt medium (K_2HPO_4, 1.73 g/L; KH_2PO_4, 0.68 g/L; $MgSO_4$, 7ml H2O, 0.1 g/L; NaCl, 4 g/L; $FeSO_4$, 7ml H2O, 0.03 g/L; NH_4NO_3, 1 g/L and $CaCl_2.H_2O$, 0.02 g/L) was prepared. The carbon sources Glucose, Fructose, Sucrose and Mannitol were added 2% (w/v) each to the prepared minimal salt medium. After autoclaving at 37^oC, 250 ml medium was poured into 250 ml Erlenmeyer flasks, and 200 µl of 24 h old fresh inoculum of the isolated bacteria was inoculated into the medium. Then, the flasks were incubated for 48 h under agitation of 130 rpm. After 48 hours the optical density and biomass of the cultured media were taken in triplicates and recorded Eevers et al (2015).

3.7.6. Evaluation of Agro-waste as a carbon source against EBIs

To find out the preference of carbon sources and the ability of the isolated bacteria to utilize cheap agro-wastes as non-commercial carbon sources molasses, wheat bran, and bagasse were used as substitutes for commercial carbon sources. The carbon source from the three materials was prepared by drying and grinding the materials into fine powder using a grinder. Then, the ground waste materials were sieved by 355 µm size sieves. Then, following the procedure of Patil and Salunkhe (2010) 100 g of each agro-waste material was soaked in 200 ml of hot distilled water (95^oC) for 2 hours under agitation of 200 rpm. After that, the extract was filtered with muslin cloth and centrifuged at 3000 rpm for 5 min to get rid of any suspensions. Then 20 ml l-1 of the extract was added as a carbon source onto

a carbon-free liquid media (K_2HPO_4, 0.3 g/L; KH_2PO_4, 0.3 g/L; $MgSO_4$.77ml H_2O, 0.3 g/L; NaCl, 0.05 g/L; $FeCl_3$.6ml H_2O, 0.006 g/L; $CaCl_2.H_2O$, 0.05 g/L and Yeast extract, 3 g/L at pH 7.00). After autoclaving the media were transferred into 250 ml Erlenmeyer flasks and inoculated with 200 µl of 24h old fresh inoculum of the isolated bacteria (Patil & Salunkhe, 2010). Then the inoculums were incubated for 48h with agitation of 130 rpm. After 48h the optical density and the biomass of the cultured media were taken in triplicates and recorded.

3.8. Antimicrobial activities

The crude extract of the endophytic bacteria isolate was prepared into two concentrations (1g/ml and 0.5 g/ml) for the antibacterial activity analysis. The agar well diffusion method was used to evaluate the antibacterial activity of the extracted secondary metabolites from the isolates.

The antibacterial activities of isolated endophytic bacteria were tested against several test bacteria including *(Pseudomonas aeruginosa)*, (*Streptococcus pyogene*), *(Staphylococcus aureus)* and *(Escherichia coli)* using the agar well diffusion method according to the method described by Balouiri et al (2016). Four human pathogenic bacterial strains *Pseudomonas aeruginosa*, Streptococcus pyogene, *Staphylococcus aureus* and EC *Escherichia coli* include the endophytic isolate from which the metabolite was extracted from the endophytic bacteria (*Enterobacter cloacae* and *Stenotrophomonas maltophilia)* were spread on Mueller-Hinton agar plates and 6mm wells were made using sterile corn borer. The 50 µl of the crude extracts and both the positive and negative controls were added to the wells. Ciprofloxacin 20 µg/ml was used as positive control and methanol was used as a negative control. The plates were incubated at 37^{o}C for 24h. After incubation, the inhibition zones were measured using a ruler and recorded Nxumalo et al (2020)

3.9. Data analysis

One way analysis of variance (ANOVA) was applied to ascertain significant differences between the different growth conditions on different salt concentration. And the effects of salt on bacterial growth was determined at various concentrations was measured from dried biomass and the OD_{600nm} was measured by UV-spectrophotometer. Finally, the data were analyzed by origin software. On optimum growth temperature of the endophytic bacteria the isolates optical density and biomass of the culture was taken in triplicates and recorded for generated data analysis by using the origin software. On the growth optimization of PH, the CDW g/L and OD_{600nm} generated data was analyzed using the Origin software. For the nitrogen source optical density and biomass of the culture were taken in triplicates and recorded. The generated data was analyzed using origin software. The same to the other optimization for the carbon source and agro-waste the optical density and biomass of the cultured media were taken in triplicates and recorded. The generated data was analyzed using the origin software.

4. RESULTS AND DISCUSSION

4.1. Isolation of endophytic bacteria from medicinal plant samples

In this study, a total of eight (two from leave, two from steam and four from root) endophytic bacterial isolates were obtained from the leaf, root, and stem parts of *Z. spina Christi* that were collected from Adama Science and Technology University. After *Z. spina Christi* parts were sterilized and incubated for 48 h endophytic bacteria with various colors were detected which emerged on the NA medium as indicated in Figure 3 and Figure $4_{a\text{-}c}$,. The colonies of certain of these emerged endophytic bacterial isolates were found in a different color. The colonies emerged on plates as illustrated in Figure 4 $_{a\text{-}h}$ after 48 h of their growth. In line with this study, it was reported that a total of 853 endophytic bacterial isolates were obtained from agronomic crops and prairie plants of which they belonging to both Gram-positive and negative Zinniel et al (2002). Like this isolation, locally grown *Momordica charantia* L. was collected from the B.H.U., botanical garden, in India Singh et al (2022). The sample tissues were thoroughly washed with running tap water to remove adhered soil particles and surface sterilized to remove any epiphytic microorganisms by following standard protocol; the sample tissue was firstly dipped in 70% (w/v) C_2H_5OH for 5 min followed by 0.5% NaOCl (v/v) for 3 min and again dipped in 70% C_2H_5OH (w/v) for 30 sec. Finally, tissues were washed with double distilled water Kumar et al (2016).

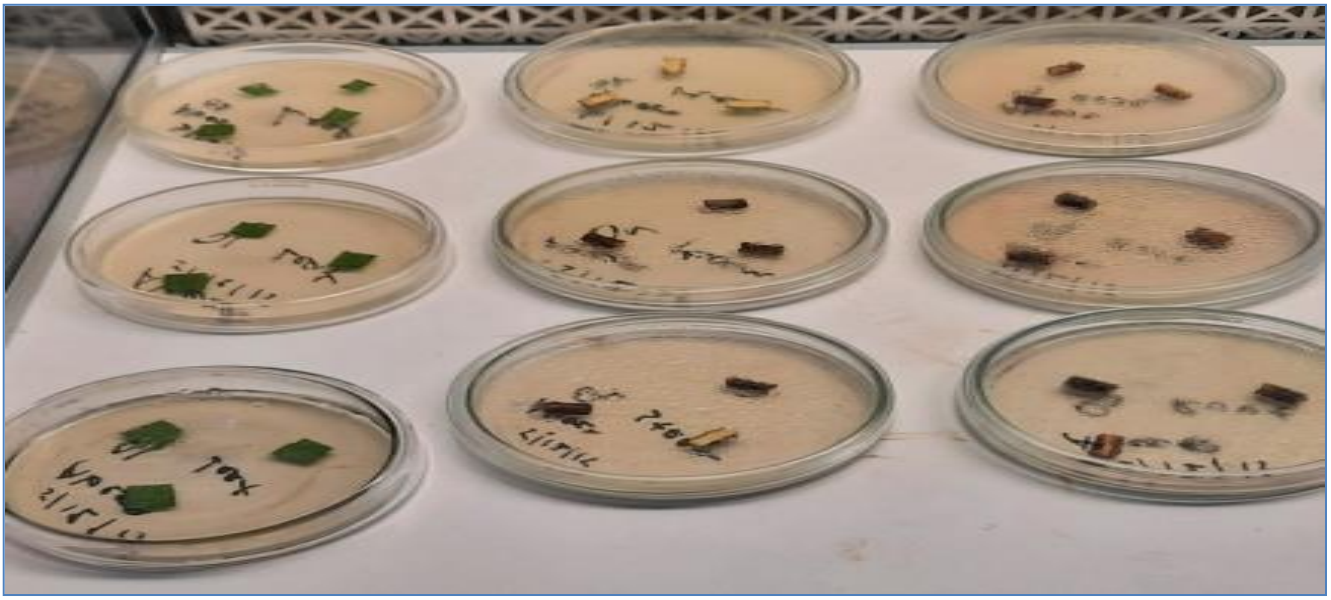


Figure 3: Surface sterilized plant material prepared for incubation.

After the plant parts were surface sterilized and incubated on NA for 48 h. The colonies of endophytic bacteria emerged on the NA plates which indicates that the grown bacteria were endophytic (Figure 3). Several types of colonies were observed on the plates with different colors, margins, or shapes (Figure 5). Various colony colors, elevations, margins, opacity of the colony, consistency, and surface of the colony were detected.

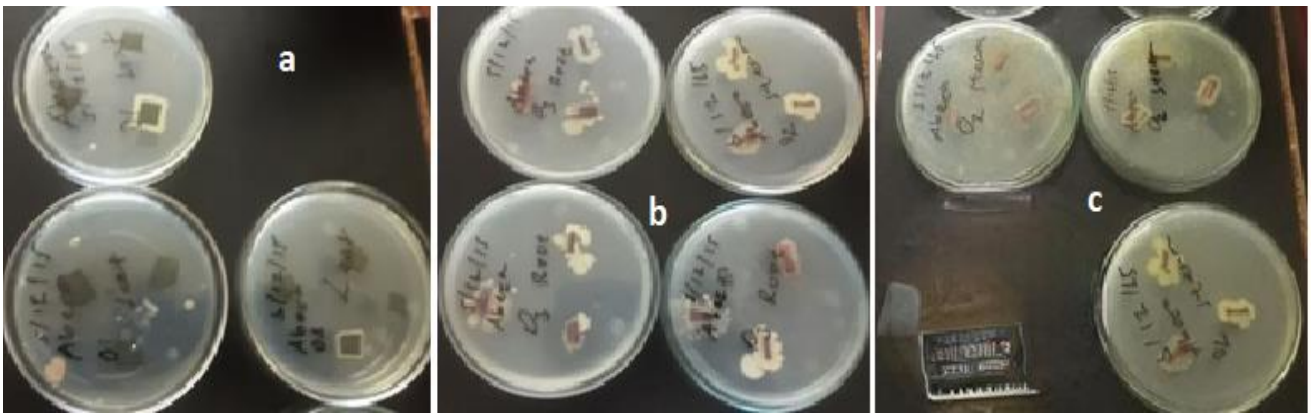


Figure 4: The endophytic bacteria isolate from leave, steam, and roots of ziziphus spina-Christi (a) Endophytic bacterial culture from leaf of the plant. b) Endophytic bacterial culture from the stem of the plant. c) Endophytic bacterial culture from the root of the plant.

4.2. Morphological characterization of Endophytic bacterial isolates

Basic morphology properties were verified for all these EBIs. Most of these EBIs were rod-shaped and Gram negative. The (EIBZ 5 &EIBZ 6) selected isolates were submitted to the biochemical tests. Their colony colors were also different as indicated in Figure 5. These all EBIs obtained from various parts of *Ziziphus spina-Christi* were found to be *Gram-positive*. Other author shows the same results of the Gram staining on isolates CEB2, CEB3, CEB4, and CEB5 were gram-positive bacilli arranged in chains. These four were also not able to produce hydrogen sulphide and were not able to ferment glucose, sucrose, and lactose Photolo et al (2020).

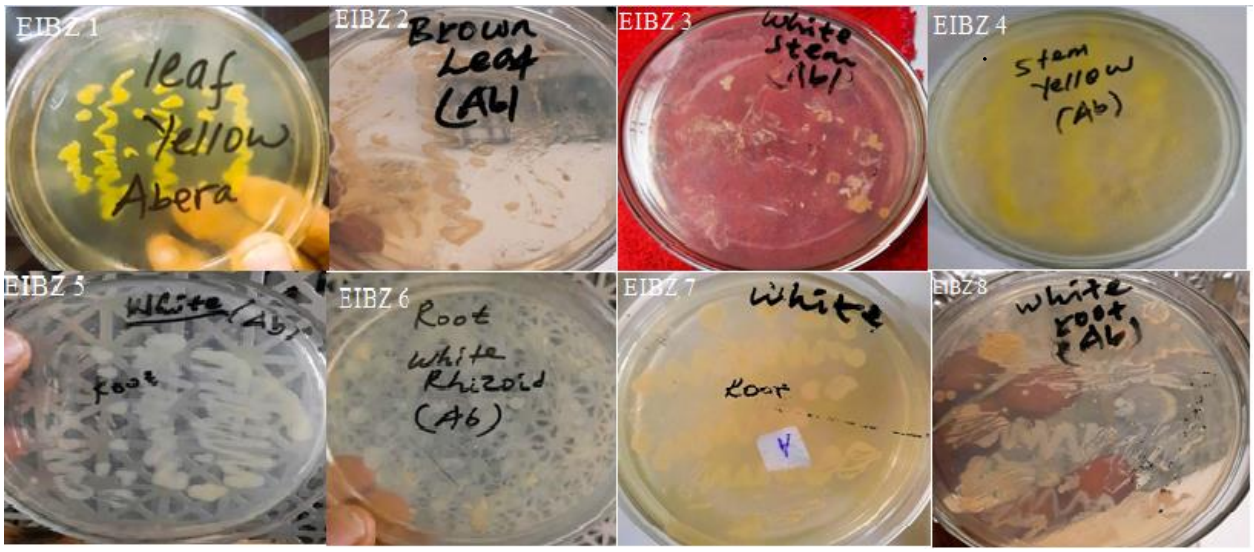


Figure 5: Different color of endophytic bacterial pure culture.

The endophytic bacteria frequently isolated were those obtained two from the leaf parts of *Z. siphina Christi*. They were identified as EIBZ-1 and EIBZ-2. Another two bacteria (EIB-3 *and* EIB-4) was obtained from the stem part of *Z. siphina Christi***,** the other four of bacteria those named EIBZ-5, EIBZ-6, EIBZ-7, and EIBZ-8 were isolated from the root part of *Z. siphina Christi.*

Gram Staining: Gram staining is one of the most crucial staining techniques in microbiology (Bartholomew, 1952). Often, the first test performed, gram staining, involves the use of crystal violet or methylene blue as the primary color. The term for organisms that retain the primary color and appear purple, and brown under a microscope is Gram-positive organisms (O'Toole, 2016). The organisms that do not take up primary stain appear red under a microscope and are Gram-negative organisms. The basic principle of gram staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. Gram-positive microorganisms have higher peptidoglycan content, whereas gram-negative organisms have higher lipid content. A Gram-positive strain appeared bluish or purple, whereas a Gram-negative strain appeared reddish. Same to these authors, on this investigation there is no Gram negative that had red color. According to the test, the bacteria were blue and purple in gram staining and were all not Gram negative (Libenson, 1955). Same to these according to Table 1: Eight bacterial isolates like (EIBZ-1), (EIBZ-5), (EIBZ-6), (EIBZ-7) Gram negative spherical shape

gram staining and (EIBZ-3), (EIBZ-8) rod-shaped, and Gram-negative bacteria were the two of (EIBZ-2), (EIBZ-4) bacteria are Gram negative.

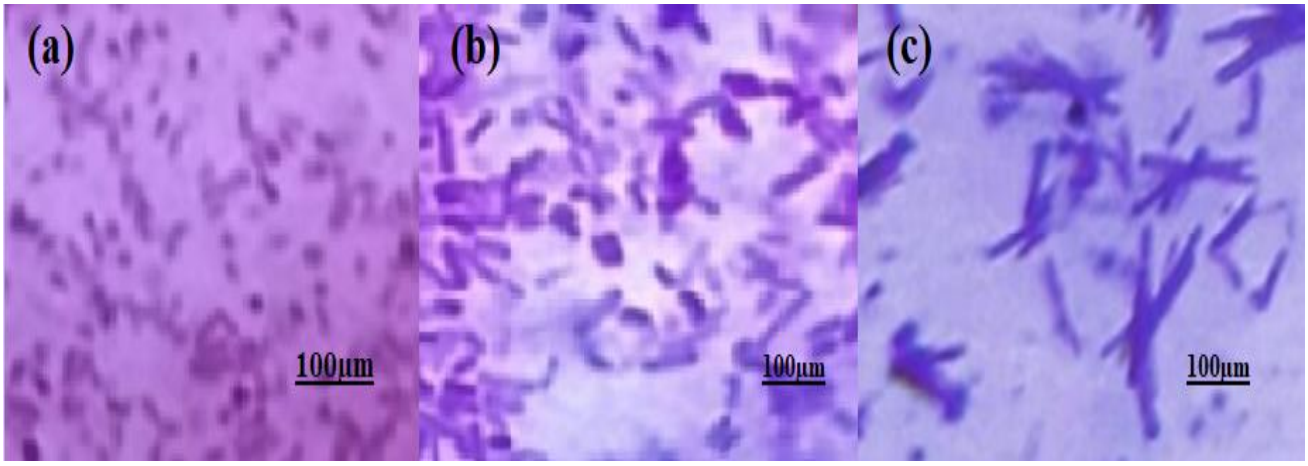


Figure 6: Few Gram staining for certain bacterial isolates which obtained from leave, steam, and roots of *Z. sphina-christi*. (a) Gram-positive short rod-shaped (EIBZ-1) (b) Gram-positive long rod-shaped (EIBZ-8) (c) Gram-positives long rod-shaped (EIBZ-9)

Motility test: Motility is a movement of cells by some form of self-propulsion. Many bacterial cells are motile as it allows them, for example, to escape from unfavorable conditions and to exploit new resources or opportunities. Combined with chemotaxis, the ability to sense a chemical gradient and direct movement accordingly, it enables bacteria to pursue nutrients and to reach specific niches. In this sense, motility is also involved in the interaction between microorganisms and their host, specifically in colonization or infectious pathogenic processes. Indeed, non-motile mutants are either impaired or completely disabled to colonize and/or cause disease (Josenhans, 2002). Recent review discusses different forms of movement observed in *Staphylococci*, including gliding, and sliding in *Staphylococcus aureus*. Motility also shows great variability among species and even strains. For example, strains from different serovars of *Salmonella enterica* showed differences up to a factor of 2.7 in swimming speed (Zawiah, 2018).

The three endophytic bacteria show positive results for the motility test. Those endophytic bacteria were included such as EIBZ-2 and EIBZ-4 which was isolated from the stem parts of *Z. spina Christi,* and EIBZ-6 which grown from the root part of *Z. spina Christi*. These three of them gave a diffuse, hazy growth that spreads across the medium which rendered as a slightly opaque one. Some of them were non-motile. These are EIBZ-1, EIBZ-3, EIBZ-5, EIBZ-7, EIBZ-8. The growth is confined to the

stab line, which has sharp defined margins and leaves the surrounding area of the medium opaque. This bacterial culture was taken from the leaf of the Z. *sphina-christi*. Other studies showed that the flagellum has traditionally been reported as the main structure responsible for bacterial motility (Martín-Moldes, 2015).

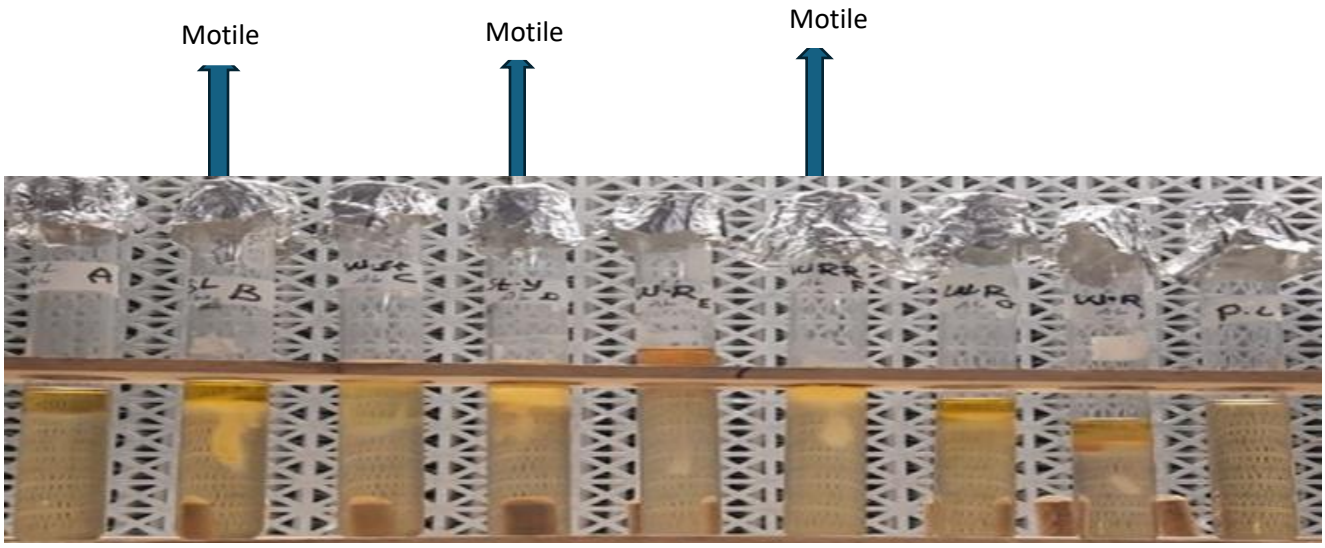


Figure 7: Motility test result

4.3. Biochemical characterization of Endophytic bacterial isolates.

Indole utilization: For this investigation, Kovacs Reagent is used to detect the presence of indole, which is one of the end products from bacterial oxidation of the amino acid, tryptophan (Figure 8). As indicates tryptophan is an amino acid that can be oxidized by some bacteria to form three major end products: indole, pyruvic acid, and ammonia. Detection of indole indicates tryptophan degradation and can be accomplished by the addition of certain aldehydes to form colored end products. The active ingredient in Kovacs Reagent, p-dimethylaminobenzaldehyde, reacts with indole to form a pinkish-red product that is highly visible**.** For this research, the results on (Picture 8a) were the most (Picture 8b) negative for indole utilization but the only one which is named *Rhodotorula mucilaginosa* (EIBZ 2) is positive this means the color of the medium was changed to red ring color.

Figure 8: Indole utilization test of endophytic isolate bacterial, (a) Negative for *indole* utilization of isolate bacteria b) Positive for *indole* utilization of isolate bacteria.

Methyl red test: Methyl red test, commonly known as MR test is used to determine the ability of an organism to produce and maintain stable acid as end products from glucose fermentation. MR test along with the VP test is performed simultaneously because they are physiologically related and are performed on MRVP broth (Barry, 1970). As indicated in Figure 9, EIBZ-2 was found to be negative for methyl red test. In line with this study, specific endophytic bacterial isolates, which screened from the Leaves, roots, and stems Parts of *Artemisia annua*, *Moringa oleifera*, and *Ocimum lamiifolium* plants were reported as methyl red positive Ebu et al (2023). In this investigation, there were 7 positive results, and 1 negative result for methyl red test (Figure 9).

Figure 9: MR tests for isolated endophytic bacteria.

Voges-proskaure test: VP is a test used to detect acetoin in a bacterial broth culture. In this study, some of them are positive for VP test as indicated in Figure 10. The test is performed by adding alpha-naphthol and potassium hydroxide to the Voges-Proskauer broth, which is a glucose-phosphate broth that has been inoculated with bacteria. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result (MacFaddin, 1980) Following this procedure all the endophytic bacteria isolate results were negative (no color change) these bacteria where a negative VP result exhibited no color change no can convert glucose to acetoin Barry et al (1967). For more information about biochemical test result summarization attached in appendices.

Figure 10 : VP test for endophytic bacterial isolates.

4.4. Identification of endophytic bacteria based on protein profiles (MALDI-TOF MS)

Various endophytic bacterial isolates were obtained from *Ziziphus spina-Christi* using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), a protein profile-based identification method. These isolates are *Cronobacter Sakazaki* EIBZ-1, *Rhodotorula* sp. EIBZ-2, *Bordetella* sp. EIBZ-3, *Rhodotorula* sp. EIBZ-4, *Enterobacter* sp. EIBZ-5, *Stenotrophomonas Maltophilia* EIBZ-6, *and Enterobacter* sp. EIBZ-7, and *Pseudomonas sp*. EIBZ-8 (Table 1). As indicated in Table 1, *Enterobacter* and *Rhodotorula* spp. were frequently isolated in this study. The previous study shown that out of 57 endophytic bacterial isolates, 49 of them were identified adequately based on protein profiles, MALDI-TOF methods Davies et al (2018). Toubal et al (2018) isolated and identified *Bacillus, Enterobacter, Enterococcus, Escherichia Paenibacillus, Pantoea,* and

Staphylococcus from internal tissues of *Urtica dioica* L. The same authors reported that most of these bacteria isolates were obtained from Tlemcen in Algeria which makes this region the richest in diversity of endophytic bacteria when compared to that harvested from Dellys. *Bacillus pumilus*-ME was also identified from the leaf part of *Urtica dioica* L. Toubal et al (2018). A recent study showed that *Rhodotorula* sp. EIBZ-2, *Bordetella* sp. EIBZ-3, *Rhodotorula* sp. EIBZ-4, *Enterobacter* sp. EIBZ-5, *and Enterobacter* sp. EIBZ-7, and *Pseudomonas sp*. EIBZ-8 (Table 1) could be the new species of endophytic bacterial isolates since their mass to charge ratio was less than 2.00 LSV. A previous study indicated that the root nodule endophytic bacterial isolates had been identified from *Arachis hypogaea* L. as the close relative and highest mass homology of *Bradyrhizobium* sp USDA 3187 *Rhizobium* sp and another *Rhizobium* sp USDA 3138 after treatment with Lysozyme and releasing of the bacterial surface signature molecules Manikandan et al (2014).

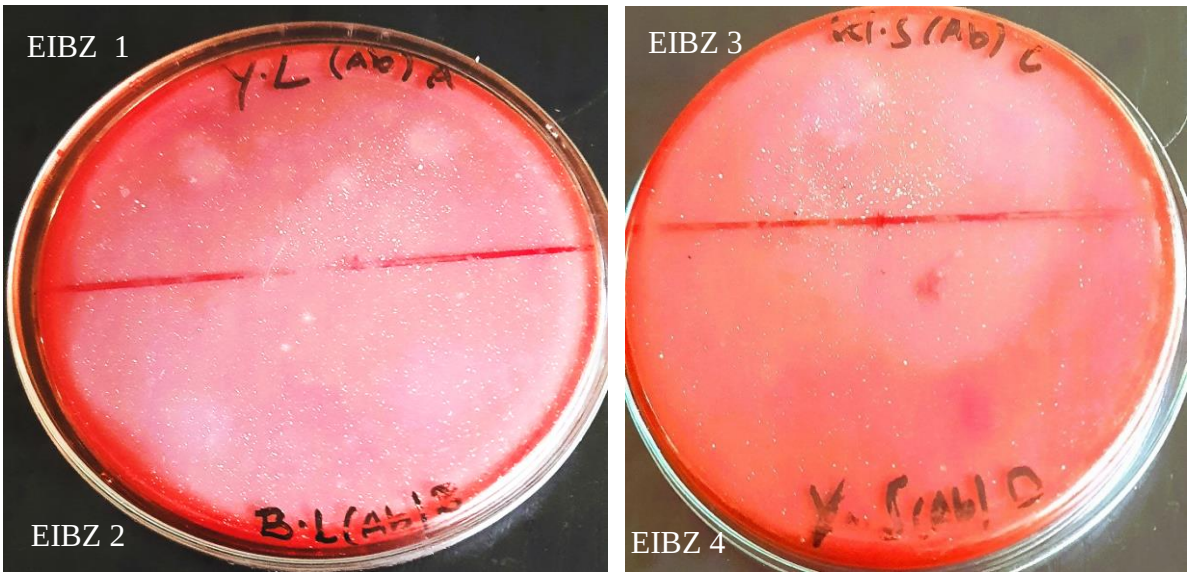
Table 1: Characterization and identification of these endophytic bacterial isolates based Various biochemical tests and MALDI TOF MS tools, respectively.

S.N.	Endophyte isolates	Various biochemical tests				Protein profile-based identification			
		Gram staining	**Indole test**	**MR test**	**VP test**	**Genus**	**Closest species**	**Score**	**Most likely isolates types**
1	EIBZ-1	-ve	-ve	+ve	-ve	*Cronobacter*	*Sakazaki*	2.04	*Cronobacter Sakazaki* EIBZ-1
2	EIBZ-2	+ve	-ve	-ve	-ve	*Rhodotorula*	*Mucilaginosa*	1.74	*Rhodotorula* sp. EIB Z-2
3	EIBZ-3	-ve	-ve	+ve	-ve	*Bordetella*	*Pertussis*	1.73	*Bordetella* sp. EIB Z-3
4	EIBZ-4	+ve	+ve	+ve	-ve	*Rhodotorula*	*Mucilaginosa*	1.74	*Rhodotorula* sp. EIBZ-4
5	EIBZ-5	-ve	-ve	+ve	-ve	*Enterobacter*	*Cloacae*	1.71	*Enterobacter* sp. EIBZ-5
6	EIBZ-6	-ve	-ve	+ve	-ve	*Stenotrophomonas*	*Maltophilia*	2.02	*Stenotrophomonas Maltophilia* EIBZ-6
7	EIBZ-7	-ve	-ve	+ve	-ve	*Enterobacter*	*Asburiae*	1.80	*Enterobacter* sp. EIBZ-7
8	EIBZ-8	-ve	-ve	+ve	-ve	*Pseudomonas*	*Stutzeri*	1.85	*Pseudomonas* sp. EIBZ-8

Key note: EBIZ- indicates Endophytic bacterial isolates from *Ziziphus spina-Chris*

4.5. Enzyme Activities for endophytic bacterial isolates

Cellulase test: As indicated in Table 1, *Bordetella* sp. EIB Z-3, *Pseudomonas sp*. EIBZ-8, and *Stenotrophomonas Maltophilia* EIBZ-6 are found to produce cellulase enzymes (Table 1, Figure 11). This indicated that these isolates could provide certain carbon sources for plant growth promotion after degrading cellulose reaches carbon wastes. The same isolates can be employed to produce cellulase enzymes which can be used for various industrial applications. A previous study showed that Georgiadou et al (2021) *Bacillus* sp. ZKA58 & ZKA60, *Cellulomonas* sp. ZKA19, *Cellulosimicrobium* sp. ZKA17 & ZKA48, *Sphingobacterium* sp. ZKA44, and *Microbacterium* sp. ZKA15, ZKA21, ZKA30 & ZKA43 showed the ability to deconstruct all cellulose and other complex substrates. The same authors stated that *Enterobacter* sp. ZKA62, *Rhodococcus* sp. ZKA 33 and *Sphingobium* sp. ZKA14 & ZKA25 can degrade cellulose containing carboxymethyl cellulose (CMC) and xylan. In a recent study, *Cronobacter Sakazaki* EIBZ-1, *Enterobacter* sp. EIBZ-5, *Enterobacter* sp. EIBZ-7, *Rhodotorula* sp. EIBZ-2, and *Rhodotorula* sp. EIB Z-4, did not deconstruct CMC and negative for cellulase tests (Table 1).



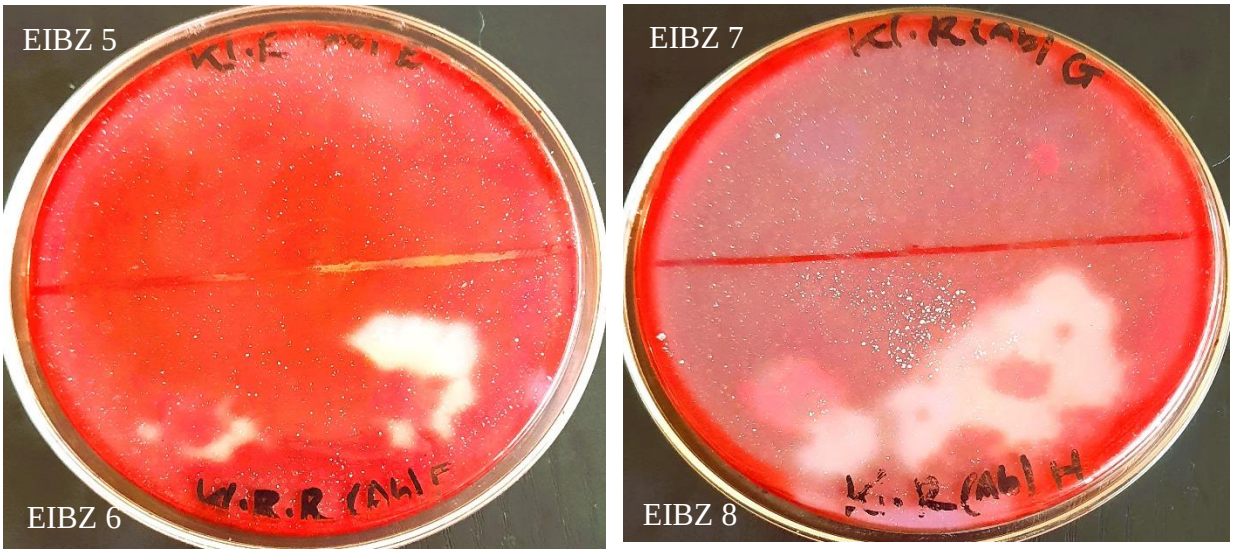


Figure 11: The four Endophytic bacterial isolate Cellulase test result.

Protease test: In this research, all endophytic isolate bacteria mean from (EIBZ-1to EIBZ-8) do not produce protease enzyme. The presence of proteolytic enzyme was determined using skimmed milk agar for protein hydrolysis. The zone of clearance around the endophytic culture was inferred to be proteins degraded into amino acids by the protease enzymes produced by the endophytes. Quantitative analysis of protease was performed by measuring from colony diameter to the inhibition zone to find the solubilizing index. This indicated that in these endophytes of an organism, there's no inhibition and no ability to solubilize protein by the endophytic organism. In the present study, C.P-20 (*B. velezensis*) exhibited very significant protease activity, producing 0.3 U/ml enzyme, and C.P-F-7 (*H. fuscoatra*) produced 0.19 U/ml. (Kumar A, 2016). On protease enzyme test all endophytic bacteria 8 isolates were negative. These eight endophytic bacteria were cronobacter sakazaki (EIBZ-1), *Rhodotorula mucilaginosa* (EIBZ-2) *Bordetella pertussis (*EIBZ-3), *Rhodotorula mucilaginosa* (EIB 4), *Entrobacter cloacea* (EIB 5), *Stenotrophomonas maltophilia* (EIBZ-6), *Entrobacter asburiae* (EIBZ-7), *Pseudomonas stutzeri* (EIBZ-8), Vijayaraghavan et al (2013).

4.6. Plant growth promotes the ability for Phosphate and Zinc solubilization.

4.6.1. Phosphate solubilization test

The endophytic bacterial isolates tend to solubilize phosphate, a nutrient that is employed for plant growth promotion (Figure 12). A similar review reported Prabhu et al (2019) that Phosphorus is recognized as the master key element among all the elements required for plant growth. *Bordetella* sp. EIBZ-3, *Enterobacter* sp. EIBZ-5, *Stenotrophomonas Maltophilia* EIBZ-6, *and Enterobacter* sp. EIBZ-7, and *Pseudomonas sp*. EIBZ-8 can solubilize phosphate at 37^{o}C which they are employed as a plant growth promoting endophytic bacterial isolates. However, *Cronobacter Sakazaki* EIBZ-1, *Rhodotorula* sp. EIBZ-2, and *Rhodotorula* sp. EIBZ-4, did not solubilize phosphate. In this study, the highest and lowest phosphate solubilization indexes were detected as indicated in Table 2. The recent isolates may produce organic acids, and acid phosphatases that used to mineralize organic phosphorous in soil which plays a significant role in plant growth promotion. Similarly, it was reported Satyaprakash et al (2017) that *Bacillus*, *Pseudomonas*, and *Rhizobium* genera are among the most powerful phosphate solubilizers since they produce organic acids, and acid phosphatases, these employed for phosphate mineralization. Forty-six *Rhizobium* isolates which were obtained from the legume root and stem nodules of *Cassia absus, Sesbania sesban, and Vigna trilobata*, were employed for phosphate-solubilizing ability when grown on Pikovskaya's agar medium Zhang et al (2013). The same authors further stated that these isolates can solubilize tricalcium phosphate. In line with this study Passari et al (2016) have reported that out of 52 isolates, of which 44 (86%) isolates were found to be positive for phosphate solubilization by forming a clear halo zone on Pikovskaya 's medium that was supplied with tri-calcium phosphate Pande et al (2017).

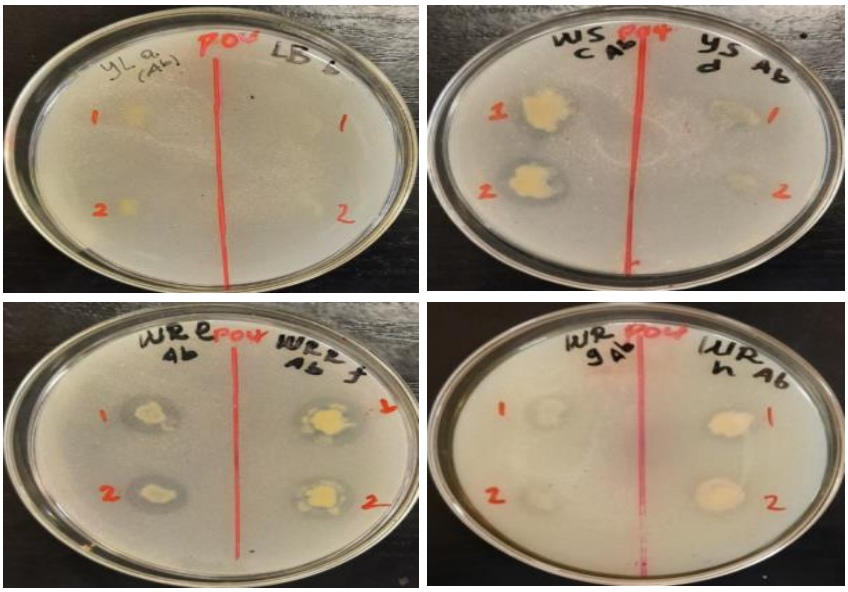


Figure 12: Phosphate soluble test for every endophytic bacterial isolated.

4.6.2. Zinc solubilization test.

From eight of the isolated bacteria, none of these endophytic bacteria can solubilize the inorganic zinc-containing compounds for these isolates which were obtained from various parts of Z. *spina Christi*. This bacterium was isolated from different Z. *spina Christi* parts were the different bacterial species. It shows some precise zone formation around the colonies (Table 2). Yasmin et al (2021) have reported that *Anabena*, *Calothrix*, *Providentia*, *Proteus*, *Serratia*, and *Trichoderma* sp can solubilize Zn and help for the growth promotion of various plants like wheat. Zinc is a micronutrient plant required to regulate the production of several proteins. It has both catalytic and structural roles in plants' metabolism in a deficient concentration. Zinc solubilizing isolates show clear zones around the colonies Yasmin et al (2021) Certain bacterial isolates can solubilize zinc. However, some of them did not solubilize the same compounds. For instance, in the present study, it was noticed that *Cronobacter sakazaki* EIB-1, *Rhodotorula mucilaginosa* EIB-2, *Rhodotorula mucilaginosa*- EIBZ 4 did not solubilize zinc. This means bacterial isolates are not Zinc solubilizer bacteria, but the other left bacterial isolates are positive or can solubilize a phosphate molecule. For Zinc solubilization all bacterial isolates are negative. (No have ability to solubilize zinc). For phosphate solubilization index there is a different clear zone formed. For more information, the following pictures show phosphate solubilization. The phosphate solubilization index is summarized as

in the table that was attached (Table: 2). On this table every point show that the individual endophytic bacterial isolated information about phosphate solubilization.

Table 2: Phosphate and Zinc solublization test result in table

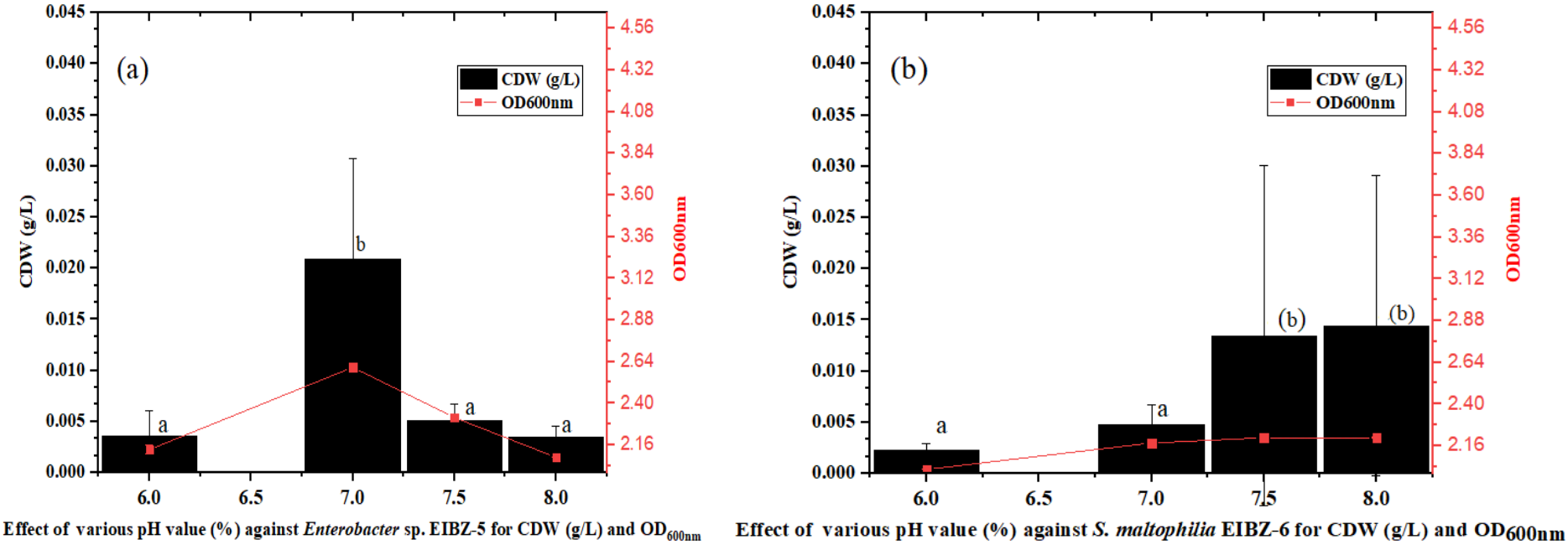
NO	Strain name	PO_4(Phosphate solubilization)	Phosphate solubilization index.
1	EIBZ-1	No	+
2	EIBZ-2	No	-
3	EIBZ-3	Yes	Reading 1, PSI = 3.75mm Reading 2, PSI=2.66mm
4	EIBZ- 4	No	-
5	EIBZ-5	Yes	Reading 1, PSI = 2.57mm Reading 2, PSI=2mm
6	EIBZ- 6	Yes	Reading 1, PSI = 3.75mm Reading 2, PSI =3.5mm
7	EIBZ-7	Yes	Reading 1, PSI = 4.3mm Reading 2, PSI = 2.5mm
8	EIBZ- 8	Yes	Reading 1, PSI= 4.3mm Reading 2, PSI= 2.1mm

4.7. Optimization of growth conditions for isolated bacterial cells

4.7.1. Effect of various pH-value against EBIs

As shown in the (Additional File: Table $A_{1\&2}$**)** and Figure 13a**,** *Enterobacter* sp. EIBZ-5, showed the highest OD_{600nm} (2.607nm**)** at pH 7. However, the maximum CDW (g/l) (0.0209±0.019799 g/l) was recorded against *Enterobacter* sp. EIBZ-5 was obtained from the root part of *Ziziphus spina-Christi* at pH 7. It was also predicted that there is no variation ($p>0.05$) among various pH values in terms of CDW (g/L) for *Enterobacter* sp. EIBZ-5 (Figure 13a, Additional File: Table $A_{1\&2}$). The same isolates (*Enterobacter* sp. EIBZ-5) gave the lowest CDW (g/l, 0.0034±0.001096) and OD_{600nm} (2.088±0.0021**)** at pH8 (Figure 13a, Additional File: Table $A_{1\&2}$), a bacterium which needs an alkaline environment to grow.

Previous research thought that the pH of the medium culture is another factor affecting the metabolic activity and growth of the fungus (Abubakar, 2013). Similarly, it was reported that (Mohammed et al 2019, 2020; Mohammed & Ray, 2022a) various pH values gave certain different CDW (g/L) and OD_{600nm} against various *Bacillus* species which can produce higher CDW when compared to the recent study.



Effect of various pH value (%) against *Enterobacter* sp. EIBZ-5 for CDW (g/L) and OD_{600nm}

Effect of various pH value (%) against *S. maltophilia* EIBZ-6 for CDW (g/L) and OD_{600nm}

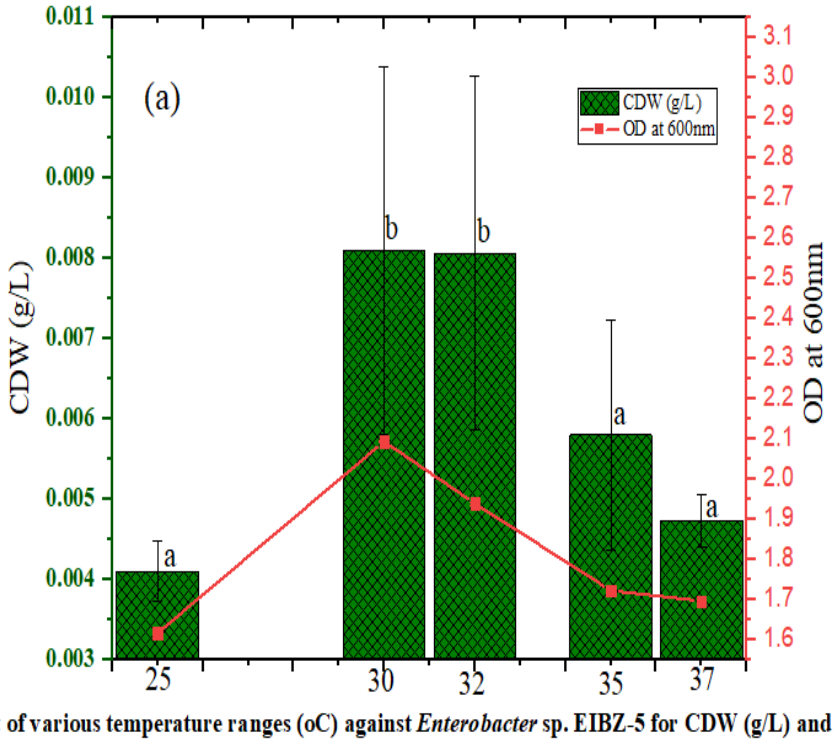
Figure 13: The effect of various pH values against EBIs with distinct CDW (g/L) and OD_{600nm}. (a) Indicates CDW (g/L) and OD_{600nm} against *Enterobacter* sp. EIBZ-5 for various pH values. (b) Indicates CDW (g/L) and OD_{600nm} against *S. maltophilia* EIBZ-6 for various pH values. Data represented mean ± SD of three replicates (n = 3) of CDW (g/ml) for various pH values. According to one-way ANOVA, there is significant variation at 95% confidence interval using LSD test (P < 0.05) with the different letters on the column bar for endophytic isolates on these pH values.

The recent study showed that the lowest CDW (g/L) (0.0022±0.00066) at pH6 and the highest CDW (g/L) (0.0144±0.01468) recorded at pH8 against *S. maltophilia* EIBZ-6 (Figure 13b, Additional File: Table $A_{3\&4}$). Similarly, the lowest and highest OD_{600nm} were recorded against S. *maltophilia* EIBZ-6 at pH6 (2.024±0.001633) and pH8 (2.207±0.001414), respectively. The results indicated that *S. maltophilia* EIBZ-6, which was obtained from Z. *spina Christi* could be alkaliphilic isolate. In line with this study, it was reported that (Ghosh et al (2024) certain alkaliphilic endophytic bacterial species were isolated from the tuber of *Dioscorea bulbifera.* Another study confirmed that alkaliphile *Bacillus, Pantoea* and *Stenotrophomonas* spp. were screened and identified from *Cistanche armena* Petrosyan et al (2022) for samples collected from saline and arid soils. As it was realized that no significant variation were detected among pH6 & pH7 (*p=0.826*), pH7 & pH7.5 (*p=0.457*), pH6 & pH8 (*p=0.305*), pH7 & pH8 *(p=0.411*), and pH7.5 & pH8 (*p=0.933*) against *S. maltophilia* EIBZ-6 for CDW (g/L) using One-way-ANOVA at 0.05 LCD and Post Hoc test with multiple Comparisons (Figure 7b, Additional File: Table $A_{3\&4}$)

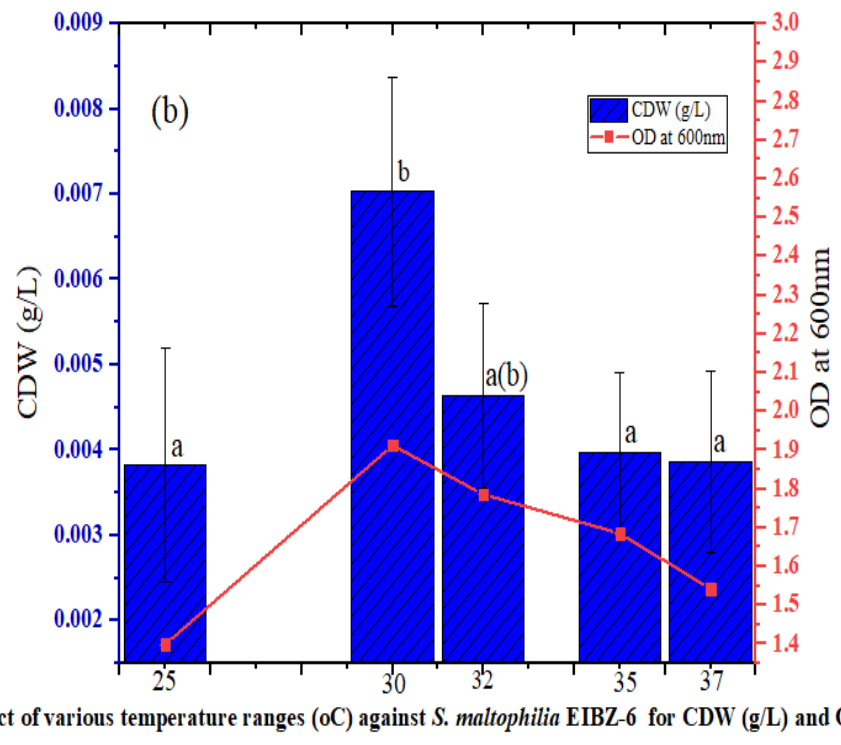
4.7.2. Growth condition against various temperature

It was found that all the selected isolate's growth and CDW (g/L) production was at its highest peak at 30^{o}C (0.0081±0.002291g/L) (Figure 14a, Additional File: Table $A_{5\&6}$). At 32^{o}C the second highest growth with CDW (g/L) (0.0081±0.00221 g/L) production and OD_{600nm} (1.9397±0.0033 nm) were observed against *Enterobacter* sp. EIBZ-5 (Figure 14a, Additional File: Table $A_{5\&6}$). The CDW (g/L) (0.0041±0.000374 g/L) production and OD_{600nm} (1.617±0.00216) of *Enterobacter* sp. EIBZ-5 was at its lowest peak at 25^{o}C (Figure 8a, Additional File: Table $A_{5\&6}$). As indicated in Figure 14a, no significant variations (*p>0.05)* were detected among 30 & 32 ^{o}C (*p=0.984*), 25 & 35 ^{o}C (*p=0.306*), 32 & 35 ^{o}C (*p=0.181*), 32 & 37 ^{o}C (*p=0*.061) and others in terms of CDW (g/L) at 0.05 LCD and Post Hoc test (Additional File: Table $A_{5\&6}$). However, variations were detected between 25 & 30 ^{o}C (*p=0.03*) and 25 & 32 ^{o}C (*p=0.031*) for the same isolate against CDW (g/L) (Figure 14a, Additional File: Table $A_{5\&6}$). Lebeaux et al. (2013) stated that the optimal growth temperature of *Citrobacter freundii* was between 30 and 37^{o}C. Uddin, et al (2008) have reported that the bacterial fish pathogen, *Citrobacter freundii* showed its peak growth at 36.1±0.4^{o}C,

meanwhile, in this experiment peak OD_{600nm} and CDW (g/l) measurement for *Enterobacter* sp. EIBZ-5 and *S. Maltophilia* EIBZ-6 were found to be higher at 30ºC and followed by 35ºC. The authors have also reported that the peak growth temperature of two strains of *Pseudomonad* sp. was detected at 24.09±0.1 and 29.2±0.5ºC.



Effect of various temperature ranges (oC) against *Enterobacter* sp. EIBZ-5 for CDW (g/L) and OD_{600nm}



Effect of various temperature ranges (oC) against *S. maltophilia* EIBZ-6 for CDW (g/L) and OD_{600nm}

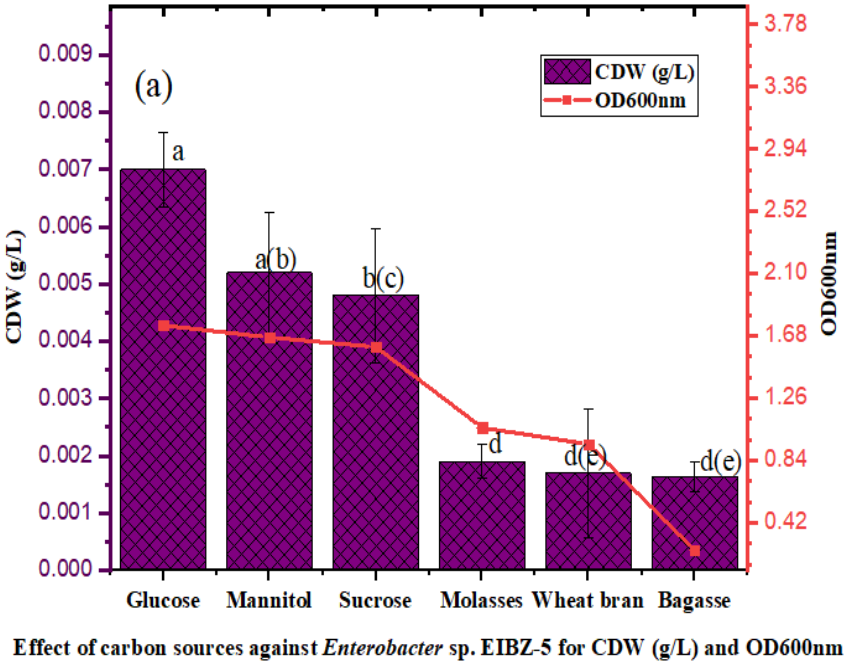
Figure 14: CDW (g/L) and OD_{600nm} against various EBIs for different temperature ranges. (a) Indicates CDW (g/L) and OD_{600nm} against *Enterobacter* sp. EIBZ-5 for various temperature ranges. (b) Indicates CDW (g/L) and OD_{600nm} against *S. maltophilia* EIBZ-6 for various temperature ranges. Data represented mean ± SD of three replicates (n = 3) of CDW (g/ml) for various temperature ranges. According to one-way ANOVA, there is significant variation at 95% confidence interval using LSD test (P < 0.05) with the different letters on the column bar for endophytic isolates on these temperature ranges.

Temperatures are among the growth parameters that affects the growth of bacterial diversities. In the present study, the highest CDW (g/L) (0.0070±0.00164) and OD_{600nm} (1.912333±0.001155) were detected against *S. maltophilia* EIBZ-6 at 30 ºC (Figure 14b, Additional File: Table $A_{7\&8}$). However, related CDW (g/L) and OD_{600nm} observed against *S. maltophilia* EIBZ-6 ($p>0.05$) (Figure 14b, Additional File: Table $A_{7\&8}$) at 25, 32, 35, and 37ºC which screened from *Ziziphus spina-Christi*. Based on the optimum temperature ranges as illustrated in Figure 14b and Additional File: Table $A_{7\&8}$, *S. maltophilia* EIBZ-6 was found to be mesophilic, an endophytic bacterial isolate that is suggested for plant growth promotion. In agreement with this study, it was found that tricalcium phosphate solubilizers, indol-3-acetic acid, and siderophores producers bacterial diversities such as *Bacillus, Pseudomonas, Rahnella, Rouxiella,* and *Serratia* were identified from *Gentianella weberbaueri* and *Valeriana pycnantha* and confirmed to grow at 24ºC Ulloa-Muñoz et al (2020). It was reported that Maalej et al. (2014) exo-polysaccharide (EPS) production and cell dry weight were reported from *Pseudomonas stutzeri* AS22. The maximum growth of *Pseudomonas stutzeri* AS22 as well as EPS production was achieved at 30ºC and elevated temperature (37ºC) with cellular growth reduction Chi et al. (2018). The same authors reported that *Enterobacter* isolate LX3 exhibited the ability to grow at 20-45ºC, but the maximum growth temperature of the *E. cloacae* was reported to be 30ºC, which is the same as the recent *Enterobacter* sp. EIBZ-5 and *S. maltophilia* EIBZ-6 isolates were obtained from Z. *Spina Christi.*

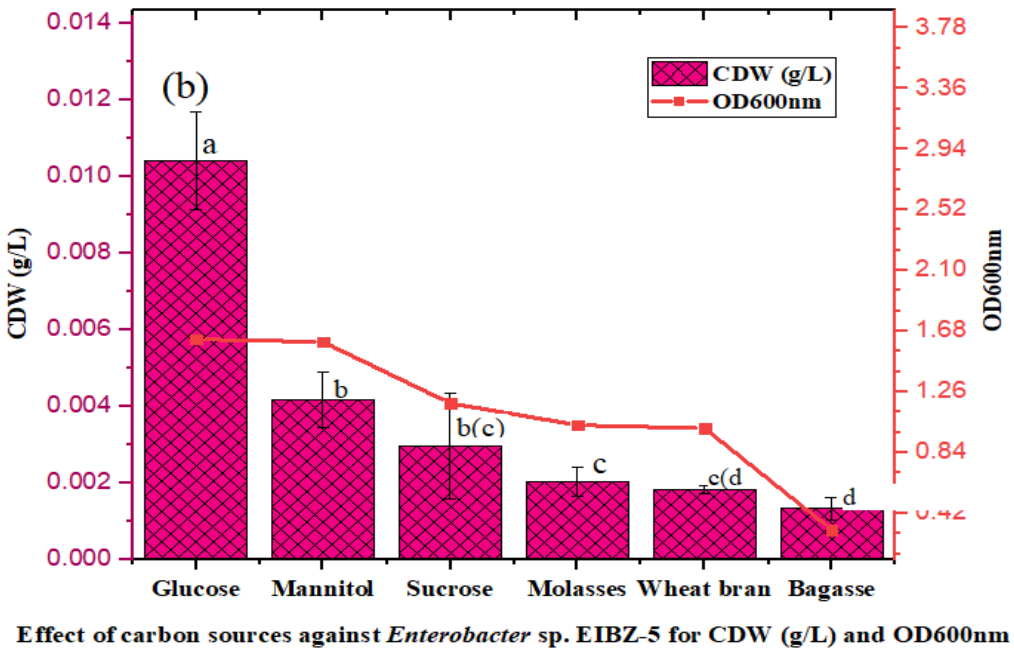
4.7.3. Effect of various carbon sources against EBIs

Carbon source is also found to be the essential nutrient for the growth of certain plant growth promoters, and endophytic bacterial optimum growth condition. As indicated in Figure 15, CDW (g/L) and OD_{600nm} were obtained using different carbon sources against various *Enterobacter* sp. EIBZ-5 (Figure 15a, Additional File: Table $A_{9\&10}$) and *S. Maltophilia* EIBZ-6 (Figure 15b, Additional File: Table $A_{11\&12}$). For instance, 0.0070 g/L CDW (g/L) was obtained from *Enterobacter* sp. EIBZ-5 (Figure 15a, Additional File: Table $A_{9\&10}$) uses glucose as the main carbon source. As indicated in Figure 11, 1.7516 which is the highest OD_{600nm} was recorded for *Enterobacter* sp. EIBZ-5 against glucose. A 0.0104 g/L (CDW) and

1.6246 OD_{600nm} were documented against *S. Maltophilia* EIBZ-6 (Figure 15b, Additional File: Table $A_{11\&12}$) for glucose that was supplied as the main carbon source. In line with this study, various carbon sources such as molasses, fructose, glucose, sucrose, and nitorgen sources such as KNO_3, $(NH_4)_32SO_4$ and peptron gave different CDW (g/L) and OD_{600nm} against *Enterobacter* sp. RPAAI-8 which are the closest results to the recent finding for *Enterobacter* sp. EIBZ-5 and *S. Maltophilia* EIBZ-6 isolates Ebu et al (2023). The same authors reported that cost-effective agro-wastes such as *Teff* straw, Corn cob, and vegetable waste were utilized by *Enterobacter* sp. RPAAI-8 and gave various CDW (g/L) and $OD_{600nm.}$ As indicated in Figure 15a, great significant varioation were observed among glucose & Molasses (*p=0.000*), glucose & Wheat bran (*p=0.000*), and glucose & Bagasse (*p=0.000*) using One-Way-Anova in terms of CDW (g/L) among carbon sources such as bagasses, glucose, molasses, mannitol, sucrose, and wheat bran. However, no significant variations were predicted among Glucose & mannitol (*p=0.056*), mannitol & sucrose (*p=0.647*), molasses & wheat bran (*p=0.818*), Bagasse & wheat bran (*p=0.939*), and molasses & bagasse (*p=0.759*) (Figure 15a, Additional File: Table $A_{9\&10}$). Figure 15a, Additional File: Table $A_{9\&10}$ showed that the lowest CDW (g/L) (0.0016±0.000249 g/L) and OD_{600nm} (0.236±0.000471) were obtained from *Enterobacter* sp. EIBZ-5. Similarly, in the previous study, it was reported that (Mohammed & Ray, 2022b) lowest CDW (g/L) was recorded when the culture medium was supplied with D-ribose as a sole carbon source against *Bacillus* sp. LPPI-18 which was obtained from soil sediment in India.



Effect of carbon sources against *Enterobacter* sp. EIBZ-5 for CDW (g/L) and OD600nm



Effect of carbon sources against *Enterobacter* sp. EIBZ-5 for CDW (g/L) and OD600nm

Figure 15: The effect of various carbon sources and other agro-waste against EBIs with distinct CDW (g/L) and OD_{600nm}. (a) Indicates CDW (g/L) and OD_{600nm} against *Enterobacter* sp. EIBZ-5 for various carbon sources. (b) Indicates CDW (g/L) and OD600nm against *S. maltophilia* EIBZ-6 for various carbon sources. Data represented mean ± SD of three replicates (n = 3) of CDW (g/ml) for commercial and cost-effective agro wastes of carbon sources. According to one-way ANOVA, there is significant variation at 95% confidence interval using LSD test (P < 0.05) with the different letters on the column bar for endophytic isolates on these carbon sources.

4.7.4. Agro-waste as carbon source against EBIs

In this study, better growth was observed for *Enterobacter* sp. EIBZ-5 (0.0019±0.000294 g/L, 1.061667±0.002357OD_{600nm}) (Figure 15a, Additional File: Table $A_{19\&10}$) and *S. maltophilia* EIBZ-6 (0.00203±0.000368 g/L, 1.03±0.000943) (Figure 15b, Additional File: Table $A_{11\&12}$). Among the cost-effective agro-wastes, molasses was found to produce maximum CDW (g/l) and OD_{600nm} against *Enterobacter* sp. EIBZ-5 (0.0019±.00036g/L, 1.061667±0.002357) (Figure 15a, Additional File: Table $A_{9\&10}$) and *S. maltophilia* EIBZ-6 (0.002033333±0.000368 g/L, 1.03133±0.000943) (Figure 15b, Additional File: Table $A_{11\&12}$). The previous study demonstrated that the CDW (g/L) obtained from *Bacillus* sp. LPPI-18 (Mohammed & Ray, 2022b) which is a PHA producer was higher than the recent CDW (g/L) that derived from *Enterobacter* sp. EIBZ-5 isolate. Similarly, it was recognized that related CDW (g/L) was obtained from *Enterobacter* sp. RPAAI-8 against mannitol supplementation for the growth of *Enterobacter* sp. EIBZ-5 and *S. maltophilia* EIBZ-6 Ebu et al (2023). Certain bacterial species can utilize wheat bran for their growth. For instance, *Bacillus cereus* sp. BNPI-92 Mohammed et al (2020), which reported to produce PHA found to utilize wheat bran for their growth, a similar study to the present finding.

4.7.5. Effect of various Nitrogen sources against EBIs

In the present study, the lowest (0.003067±0.00015) and highest peaks (0.0067±1E-04) for CDW (g/L) were observed against *Enterobacter* sp. EIBZ-5 for KNO_3 and Peptone, respectively (Figure 16a, Additional File: Table $A_{13\&14}$), these supplied as a main nitrogen source. The next higher biomass was detected for Yeast extract (YE) with 0.00657±0.00081g/L CWD (g/L) and 1.865±0.0031 nm OD_{600nm} (Figure 16a, Additional File: Table $A_{13\&14}$). The previous study showed that Peptones such as casein, gelatin, meat, soy, and yeast used to affect the generation times and yield of *Salmonella* serovar *Typhimurium* with $7{\cdot}04 \times 10^9$ CFU ml^{-1} Gray et al (2008). It was also mentioned by Seesuriyachan et al (2011) that exopolysaccharide (EPS) can be influenced by the presence of peptone, yeast extract, and beef extract against *Lactobacillus confusus* TISTR 1498. Great variations were predicted among Peptone & KNO_3, Peptone & NH_4SO_4, KNO_3 & YE, KNO_3 & NH_4SO_4,

NH_4SO_4 & YE, and others against *Enterobacter* sp. EIBZ-5 with p=0.000 (Figure 16a, Additional File: Table $A_{13\&14}$). However, insignificant differences were detected between Peptone & YE (*p=0.706*) (Figure 16a, Additional File: Table $A_{13\&14}$).

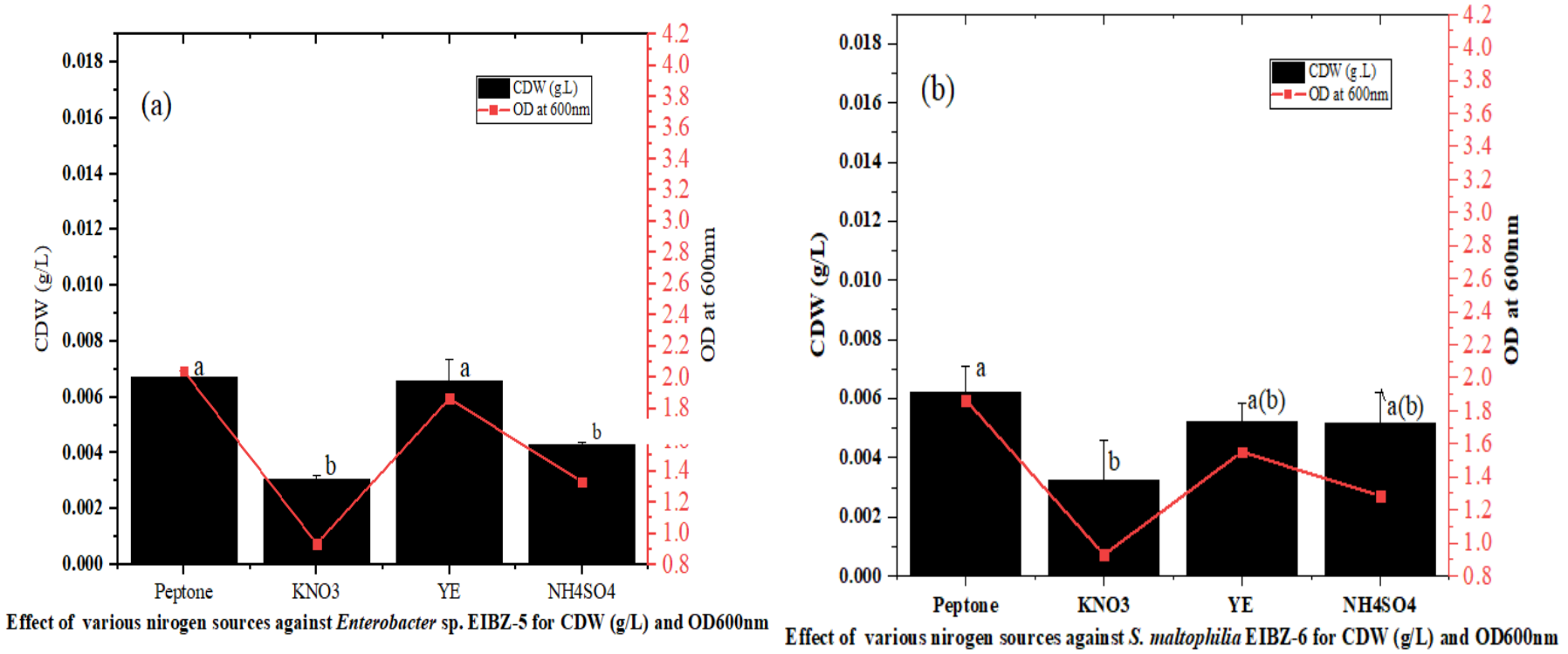


Figure 16: Effect of various nitrogen sources against different EBIs with various CDW (g/L) and OD_{600nm}. (a) Indicates CDW (g/L) and OD_{600nm} against *Enterobacter* sp. EIBZ-5 for various carbon sources. (b) Indicates CDW (g/L) and OD600nm against *S. maltophilia* EIBZ-6 for various carbon sources. Data represented mean ± SD of three replicates (n = 3) of CDW (g/ml) for commercial and cost-effective agro wastes of carbon sources. According to one-way ANOVA, there is significant variation at 95% confidence interval using LSD test (P < 0.05) with the different letters on the column bar for endophytic isolates on these carbon sources.

S. maltophilia is an emerging pathogen that can exist in the region of the rhizosphere, on animals, plants, animals, foods, and water sources (Brooke, 2012). The same bacterial species was isolated from Z. *Spina Christi* in this study. These plants can be employed for plant growth promotion like Z. *Spina Christi* with significant phosphate solubilization efficiency. In this study, the optimum CDW (g/L) and OD_{600nm} were obtained when Peptone (0.0062±0.00087g/L, 1.865±0.003 nm), YE (0.0052±0.0006 g/L, 1.550667±0.0025nm) and NH_4NO_4 (0.00513±0.00107 g/L, 1.285±0.00058nm) supplied as a main nitrogen source for the growth of *S. maltophilia EIBZ-6* (Figure 16b, Additional File: Table $A_{15\&16}$). The current study exhibited that great variation was detected between among peptones & KNO_3 (*p=0.007*) and YE & KNO_3 *(p=0.045*) (Figure 16b, Additional File: Table $A_{15\&16}$) in terms of CDW (g/L) when these nitrogen sources had been supplied as a sole source of growth. As shown in Figure 16b, Peptone, YE, and NH_4SO_4 gave related CDW (g/L) and OD_{600nm} (Additional File: Table $A_{15\&16}$)

4.7.6. Growth condition against various salt concentrations

On top of this parameter, the growth rate of bacteria is based on different amounts of salt concentration and the difference varies depending on the amount of salt. Accordingly, the amount of salt concentration which favorable for the growth *Enterobacter* sp. EIBZ-5 was found to be is 0.9, 1, 3, 5, and 7%. These isolates five have various CDW g/L (0.0060, 0.0053, 0.0051, 0.0038, & 0.0032) and OD_{600nm} (0.091, 0.077, 0.076, 0.04, & 0.0065) (Figure 17).

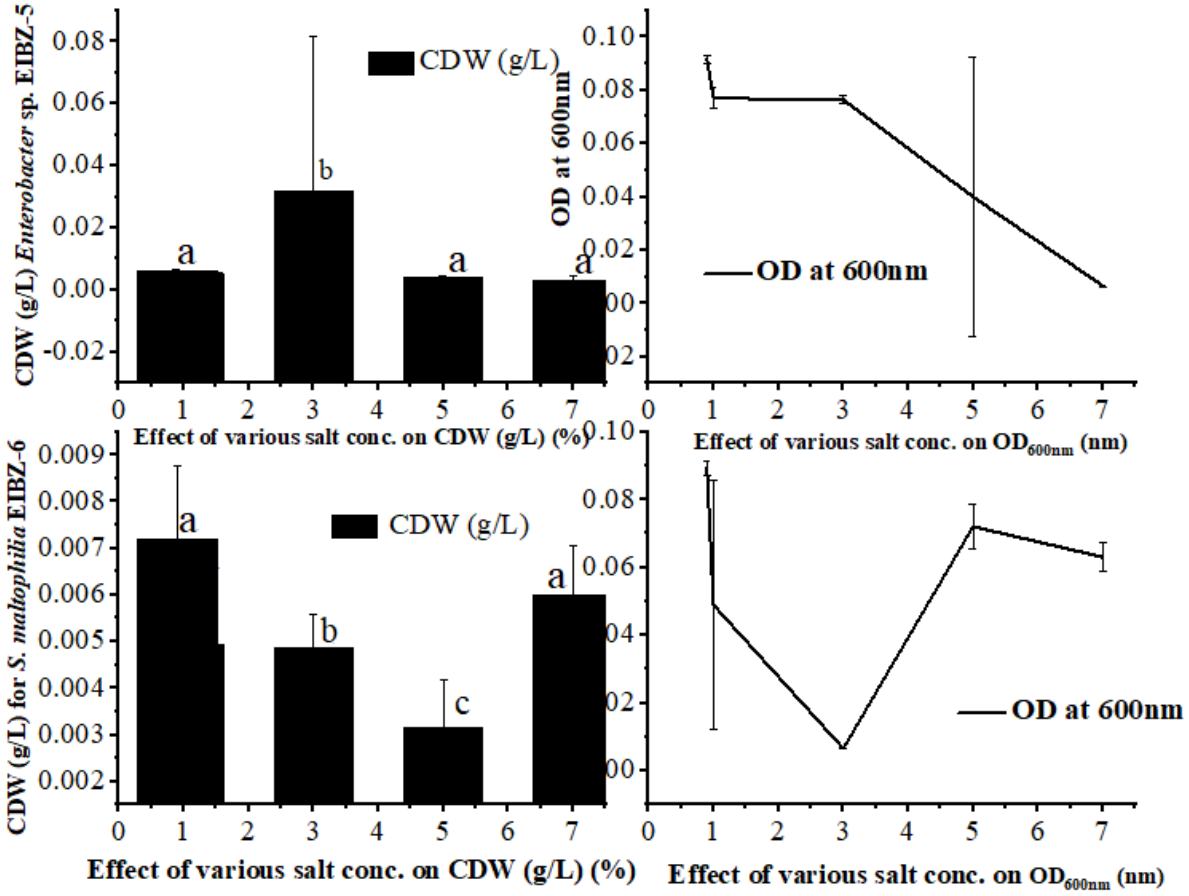


Figure 17: CDW (g/L) and OD_{600nm} against various EBIs for different salt concentrations. The Figure indicates CDW (g/L) and OD_{600nm} against *Enterobacter* sp. EIBZ-5 and *S. maltophilia* EIBZ-6 for various salt conc. Data represented mean ± SD of three replicates (n = 3) of CDW (g/ml) for various salt conc. According to one-way ANOVA, there is significant variation at 95% confidence interval using LSD test (P < 0.05) with the different letters on the column bar for endophytic isolates on these salt conc.

S. *Maltophilia* EIBZ-6 also shown considerable growth for the same salt concentration but with various CDW (g/L) (0.0056, 0.0054, 0.0048, 0.0023, 0.007) and OD_{600nm} (0.087, 0.067, 0.0066, 0.065, 0.060) (Figure 17). Salinity tolerance was obtained against the recent EBIs. at various NaCl concentrations ranging from 0.9-7% (Figure 17). There was significant inhibition in bacterial growth against *Enterobacter* sp. EIBZ-5, and *S. maltophilia* EIBZ-6 at 0.9 % NaCl concentration. *Enterobacter* sp. EIBZ-5 and *S. maltophilia* EIBZ-6 displayed to

tolerate salinity levels up to 3% NaCl concentration (Figure 17) with various CDW (g/L) and $OD_{600nm.}$.

Recently, Kumar et al (2021) screened salt-tolerant bacteria at different concentrations of NaCl and further characterized them for plant growth promotion. Also isolated rhizospheric and endophytic bacteria and reported that these bacterial isolates tolerate higher concentrations of NaCl. Salinity-affected soil is defined as soil with an electrical conductivity (EC) value greater than 4 dS m−1 (Munns and James, 2003). As the tested bacteria of the current study demonstrated salinity tolerance up to 3% NaCl concentration, the beneficial characteristics of these bacteria may remain unaffected even in a saline environment. Such bacteria are promising to be used as biofertilizers for crop production in salinity-affected soil and farmland irrigated with saline water. It is strongly suggested that biofertilizer bacteria should be tested for salt stress tolerances before application, as most irrigation water and soils are affected by high concentrations of salts Kumar et al. (2021). One-way ANOVA exhibited that great significant variation ($p<0.05$), and insignificant variation ($p>0.05$) were predicted at 0.05 LCD and Post Hoc test with multiple comparisons among variation salt concentration against *Enterobacter* sp. EIBZ-5 and *S. maltophilia* EIBZ-6 isolates which obtained from different parts of *Z. spina Christi* (Figure 17, Additional File: Table $A_{17\&18}$), medicinal plants. Similarly, it was reported that leaf extract of *Z. spina Christi* Dkhil et al (2018) can alleviate myocardial and renal dysfunction associated with sepsis in mice.

4.8. Antibacterial activity

The endophytic bacteria (Enterobacter *sp. EIBZ-5 and* S. maltophilia EIBZ-6) extract exhibited inhibitory activity against all the test organisms (*Pseudomonas aeruginosa*, *Streptococcus pyogene, Staphylococcus aureus* and *Escherichia coli)*. The highest antibacterial activity was recorded by *Enterobacter cloacae* EIBZ-5 *and the second by Stenotrophomonas* maltophilia strain against *Pseudomonas aeruginosa (ATCC-27853)* with a 17 mm and 14mm zone of inhibition respectively from the crude extract with a concentration of 1 g/ml (Figure 18).

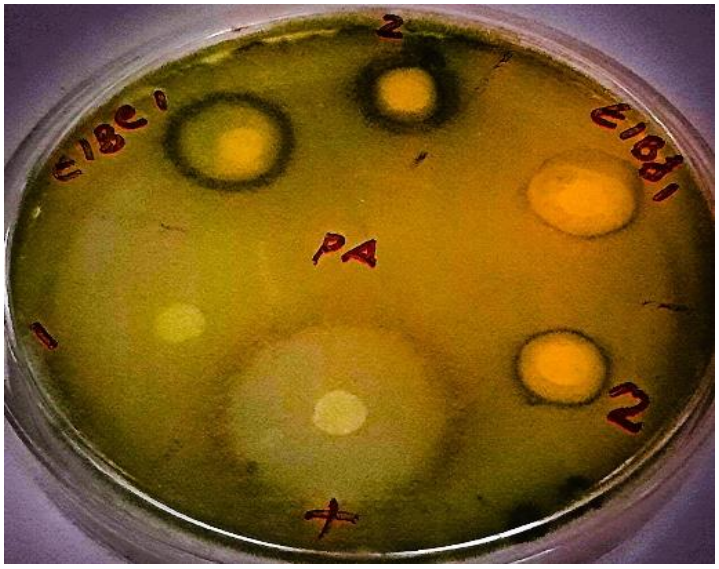


Figure 18: Antimicrobial activity result on *Pseudomonas aeruginosa* inhibition zone for EIBZ-5 and EIBZ-6.

This means that the bacteria that can apply to this human pathogen and weaken are endophytic bacteria that grew from a plant Nxumalo et al (2020) Based on this more information in detail is shown in the table on appendices. The extract exhibited inhibitory activity against all the test organisms. The highest antibacterial activity was recorded against the *Enterobacter cloacae* (EIBZ 5) strain with 17mm zone of inhibition from the crude extract with concentration of 1 g/ml. The isolate (EIBZ 5) strain from which the crude metabolite was extracted showed high levels of resistance as it covered the inhibition zone completely after being inhibited for about 10-12 h.

Table 3: Antimicrobial activity of EIBZ-5 and EIBZ- 6 result on *Pseudomonas aeruginosa* inhibition zone.

Human pathogen	**Sample code** EIBZ 5	**R_1**	**R_2**	**+ve control**	**-ve control**
PA	+	18mm	22mm	30mm	6mm
		16mm	20mm	26mm	6mm
Av		17mm	21mm	28mm	6mm
SPY	-	-	-	-	-
SA	-	-	-	-	-

Human pathogen	**Sample code** EIBZ 6	**R_1**	**R_2**	**+ve control**	**-ve control**
PA	+	15mm	11mm	30mm	6mm
		13mm	13mm	26mm	6mm
Av		14mm	12mm	28mm	6mm
SPY	-	-	-	-	-
SA	-	-	-	-	-

Shaikh et al (2015) explain that thyroiditis-producing microorganisms can protect themselves from the fatal effects of their product; this is called self-resistance. This trait of antibiotic-producing organisms is essential in understanding the similarity and the difference between drug resistance and self-resistance mechanisms, which in turn is significant in developing novel therapeutic drugs. Maliehe, et al (2023) evaluated the crude extract 's antibacterial activity against *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Proteus vulgaris,* and *Salmonella typhi,* where they found the extract showing zones of inhibition against all the test bacteria. Nxumalo et al (2020) reported that crude metabolite extracts of endophytic bacteria Isolate one strain *Pseudomonas aeruginosa* CP043328.1 demonstrated significant inhibitory ability against *E. coli*, *P. mirabilis*, *B. cereus,* and *S. aureus*. The extract was mainly effective on *S. aureus* with a 31±1.25 mm inhibition zone diameter.

5. CONCLUSIONS

- Eight Endophytic bacterial isolates were isolated from the leaf, stem, and root parts of the plant *Ziziphus sphina Christi* successfully.
- From this research, out of eight endophytic isolates two are similar species when identified based on protein profile. These isolates were recognized as *Rhodotorula* sp. EIBZ-2, and *Rhodotorula* sp. EIBZ-4.
- The emerged endophytic bacteria were purified and identified by biochemical methods as well as MALDI-TOF MS as belonging to the gener, *Cronobacter, sakazaki, Rhodotorul Mucilaginosa, Bordetella pertussis, Entrobacter cloacae, Stenotrophomonas maltophilia, Entrobacter asburiae, pseudomonas stutzeri*.
- From these isolates were EIBZ-2 and EIBZ-4 found to be Gram-positive and were another six of them are Gram negative. *Rhodotorula* sp. EIBZ-2, *Rhodotorula* sp. EIBZ-4, and *S. Maltophilia* EIBZ-6 were positive for motility test. *R. mucilaginosa (EIBZ-2)* can hydrolysis amino acid and is suggested to contain tryptophanase enzyme and negative for MR test.
- Based on enzyme tests, *Bordetella* sp, *Pseudomonas sp*, and *Stenotrophomonas sp,* were proven to be cellulase producers. A recent study confirmed that no protease enzyme for all EBIs.
- For phosphate solubilization, phosphorus is recognized as the master key element among all the elements required for plant growth. In this study *Bordetella* sp. EIBZ-3, *Enterobacter* sp. EIBZ-5, *Stenotrophomo sp.* EIBZ-6, *and Enterobacter* sp. EIBZ-7, and *Pseudomonas sp*. EIBZ-8 can solubilize phosphate at 37^{o}C which they are employed as a plant growth promoting endophytic bacterial isolates.
- In agro-waste as carbon sources a molasses, wheat bran and bagasse have the highest CDW (g/L) and OD_{600nm}, respectively.
- Glucose for carbon source optimization and peptone for nitrogen source optimization is the highest (OD600NM and CDW (g/L) respectively.
- The antibacterial activities of isolated endophytic bacteria were tested against several test bacteria, and they are effective only against *P. aeruginosa* ATCC-27853^{T}**.**

6. RECOMMENDATION

- This plant needs further research mainly because it can withstand drought for a long time and does not give up its greenness. It photosynthesizes all the time and produces more oxygen to reduce the amount of Co_2 in the atmosphere.
- Enopytic bacteria grown from this plant can produce cellulase and break down many biopolymers to accelerate plant growth and therefore extensive research is needed.
- Special research is still needed as endophytic bacteria exist inside plants without causing any harm and there is mutualism, and they prevent various diseases from plants.
- According to the antimicrobial test, the plant contains endophytic bacteria that weaken Pseudomonas aeruginosa and is useful in preventing diseases caused by Pseudomonas aeruginosa again it needs more investigation.
- This plant is often very popular with people because of its edible fruit, its leaves that stay green in winter and summer, because of its shade, but people do not understand how to prevent diseases, so it need more research.
- Studying the microorganisms that live with this plant and make it drought tolerant is necessary for other plants and requires in-depth research.
- Since this plant is not widely distributed and requires a certain place to grow or survive, all the soil properties on which this plant grows require intensive research and solutions should be found for areas without this plant.

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Additional files-1: Mean, SD and One-Way ANOVA analysis for various growth condition

Table A_1: Mean, SD, minimum and maximum value of CDW (g/L) against *Enterobacter* sp. EIBZ-5 for various pH values

CDW (g/L) against *Enterobacter* sp. EIBZ-5 for various pH values								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH6	3	.003600	.0030643	.0017692	-.004012	.011212	.0014	.0071
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH7	3	.020900	.0242489	.0140001	-.039338	.081138	.0068	.0489
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH7.5	3	.005033	.0020008	.0011552	.000063	.010004	.0030	.0070
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH8	3	.003433	.0013429	.0007753	.000097	.006769	.0019	.0044
Total	12	.008242	.0129755	.0037457	-.000003	.016486	.0014	.0489

Table A_2: Post Hoc test, significant variation at 0.05% confidence interval and Multiple Comparisons against *Enterobacter* sp. EIBZ-5 for CDW (g/L) for various pH value

Dependent Variable: CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH LSD						
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various pH values	CDW (g/L) against various pH values	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH6	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH7	-.0173000	.0100267	.123	-.040422	.005822
	CDW (g/L) against Enterobacter sp. EIBZ-5 for pH7.5	-.0014333	.0100267	.890	-.024555	.021688
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH8	.0001667	.0100267	.987	-.022955	.023288
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH7	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH6	.0173000	.0100267	.123	-.005822	.040422
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH7.5	.0158667	.0100267	.152	-.007255	.038988
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH8	.0174667	.0100267	.120	-.005655	.040588
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH7.5	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH6	.0014333	.0100267	.890	-.021688	.024555
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH7	-.0158667	.0100267	.152	-.038988	.007255
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH8	.0016000	.0100267	.877	-.021522	.024722
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for pH8	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH6	-.0001667	.0100267	.987	-.023288	.022955
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for pH7	-.0174667	.0100267	.120	-.040588	.005655
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for pH7.5	-.0016000	.0100267	.877	-.024722	.021522

Table A3: Mean, SD, minimum and maximum value of CDW (g/L) against *S. maltophilia* EIBZ-6for various pH values

CDW (g/L) against *S. maltophilia* EIBZ-6 for pH								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH6	3	.002233	.0008083	.0004667	.000225	.004241	.0013	.0027
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7	3	.004767	.0023861	.0013776	-.001161	.010694	.0021	.0067
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7.5	3	.013467	.0203814	.0117672	-.037164	.064097	.0015	.0370
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH8	3	.014433	.0179790	.0103802	-.030229	.059096	.0017	.0350
Total	12	.008725	.0128933	.0037220	.000533	.016917	.0013	.0370

Table A_4: Post Hoc test, significant variation at 0.05% confidence interval and Multiple Comparisons against *S. maltophilia* EIBZ-6 for CDW (g/L) for various pH value

Multiple Comparisons						
Dependent Variable: CDW (g/L) against *S. maltophilia* EIBZ-6 for pH LSD						
(I) CDW (g/L) against *S. maltophilia* EIBZ-6 for various pH values	(J) CDW (g/L) against *S. maltophilia* EIBZ-6 for various pH values	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH6	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7	-.0025333	.0111430	.826	-.028229	.023162
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7.5	-.0112333	.0111430	.343	-.036929	.014462
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH8	-.0122000	.0111430	.305	-.037896	.013496
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH6	.0025333	.0111430	.826	-.023162	.028229
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7.5	-.0087000	.0111430	.457	-.034396	.016996
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH8	-.0096667	.0111430	.411	-.035362	.016029
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7.5	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH6	.0112333	.0111430	.343	-.014462	.036929
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7	.0087000	.0111430	.457	-.016996	.034396
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH8	-.0009667	.0111430	.933	-.026662	.024729
CDW (g/L) against *S. maltophilia* EIBZ-6 for pH8	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH6	.0122000	.0111430	.305	-.013496	.037896
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7	.0096667	.0111430	.411	-.016029	.035362
	CDW (g/L) against *S. maltophilia* EIBZ-6 for pH7.5	.0009667	.0111430	.933	-.024729	.026662

Table A_5: Mean, SD, minimum and maximum value of CDW (g/L) against *Enterobacter* sp. EIBZ-5 for various temperature

CDW (g/L) against *Enterobacter* sp. EIBZ-5 for temperature								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against *Enterobacter* sp. EIBZ-5 at 25 ^oC	3	.004100	.0004583	.0002646	.002962	.005238	.0036	.0045
CDW (g/L) against *Enterobacter* sp. EIBZ-5 at 30 ^oC	3	.008100	.0028054	.0016197	.001131	.015069	.0054	.0110
CDW (g/L) against *Enterobacter* sp. EIBZ-5 at 32 ^oC	3	.008067	.0027062	.0015624	.001344	.014789	.0059	.0111
CDW (g/L) against *Enterobacter* sp. EIBZ-5 at 35 ^oC	3	.005800	.0017578	.0010149	.001433	.010167	.0038	.0071
CDW(g/L) against *Enterobacter* sp. EIBZ-5 at 37 ^oC	3	.004733	.0004041	.0002333	.003729	.005737	.0043	.0051
Total	15	.006160	.0023715	.0006123	.004847	.007473	.0036	.0111

Table A_6: Post Hoc test, significant variation at 0.05% confidence interval and multiple comparisons against *Enterobacter* sp. EIBZ-5 for CDW (g/L) for various temperature ranges

Multiple Comparisons						
Dependent Variable: CDW (g/L) against *Enterobacter sp.* EIBZ-5 for temperatures LSD						
(I) CDW (g/L) against *Enterobacter sp*. EIBZ-5 for various temperature	(J) CDW (g/L) against Enterobacter sp. EIBZ-5 for various temperature	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 25 oC	CDW (g/L) against Enterobacter sp. EIBZ-5 at 30^{o}C	-.0040000*	.0015772	.030	-.007514	-.000486
	CDW (g/L) against Enterobacter sp. EIBZ-5 at 32^{o}C	-.0039667*	.0015772	.031	-.007481	-.000452
	CDW (g/L) against Enterobacter sp. EIBZ-5 at 35^{o}C	-.0017000	.0015772	.306	-.005214	.001814
	CDW(g/L) against Enterobacter sp. EIBZ-5 at 37^{o}C	-.0006333	.0015772	.696	-.004148	.002881
CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 30^{o}C	CDW (g/L) against Enterobacter sp. EIBZ-5 at 25^{o}C	.0040000*	.0015772	.030	.000486	.007514
	CDW (g/L) against Enterobacter sp. EIBZ-5 at 32^{o}C	.0000333	.0015772	.984	-.003481	.003548
	CDW (g/L) against Enterobacter sp. EIBZ-5 at 35^{o}C	.0023000	.0015772	.175	-.001214	.005814
	CDW(g/L) against Enterobacter sp. EIBZ-5 at 37^{o}C	.0033667	.0015772	.059	-.000148	.006881
CDW (g/L) against *Enterobacter sp.* EIBZ-5 at 32^{o}C	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 25^{o}C	.0039667*	.0015772	.031	.000452	.007481
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 30^{o}C	-.0000333	.0015772	.984	-.003548	.003481
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 35^{o}C	.0022667	.0015772	.181	-.001248	.005781
	CDW(g/L) against *Enterobacter sp*. EIBZ-5 at 37^{o}C	.0033333	.0015772	.061	-.000181	.006848
CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 35^{o}C	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 25^{o}C	.0017000	.0015772	.306	-.001814	.005214
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 30^{o}C	-.0023000	.0015772	.175	-.005814	.001214
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 32^{o}C	-.0022667	.0015772	.181	-.005781	.001248
	CDW(g/L) against *Enterobacter sp*. EIBZ-5 at 37^{o}C	.0010667	.0015772	.514	-.002448	.004581
CDW(g/L) against	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 25^{o}C	.0006333	.0015772	.696	-.002881	.004148

Enterobacter sp. EIBZ-5 at 37°C	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 30°C	-.0033667	.0015772	.059	-.006881	.000148
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 32°C	-.0033333	.0015772	.061	-.006848	.000181
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 at 35°C	-.0010667	.0015772	.514	-.004581	.002448
*. The mean difference is significant at the 0.05 level.						

Table A7: Mean, SD, minimum and maximum value of CDW (g/L) against *S. maltophilia* EIBZ-6 for various temperatures

CDW (g/L) against *S. maltophilia* EIBZ-6 for temperatures								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against *S. maltophilia* EIBZ-6 at 25°C	3	.0038	.00168	.00097	-.0003	.0080	.00	.01
CDW (g/L) against *S. maltophilia* EIBZ-6 at 30°C	3	.0070	.00164	.00095	.0029	.0111	.01	.01
CDW (g/L) against *S. maltophilia* EIBZ-6 at 32°C	3	.0046	.00132	.00076	.0014	.0079	.00	.01
CDW (g/L) against *S. maltophilia* EIBZ-6 at 35°C	3	.0040	.00116	.00067	.0011	.0068	.00	.01
CDW(g/L) against *S. maltophilia* EIBZ-6 at 37°C	3	.0039	.00130	.00075	.0006	.0071	.00	.01
Total	15	.0047	.00175	.00045	.0037	.0056	.00	.01

Table A_8: Post Hoc test, significant variation at 0.05% confidence interval and multiple comparisons against *S. maltophilia* EIBZ-6 for CDW (g/L) for various temperature ranges

Multiple Comparisons						
Dependent Variable: CDW (g/L) against *S. maltophilia* EIBZ-6 for temperatures LSD						
(I) CDW (g/L) against *S. maltophilia* EIBZ-6 for various temperature	(J) CDW (g/L) against *S. maltophilia* EIBZ-6 for various temperature	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against *S. maltophilia* EIBZ-6 at 25 oC	CDW (g/L) against *S. maltophilia* EIBZ-6 at 30ºC	-.00320*	.00117	.021	-.0058	-.0006
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 32ºC	-.00080	.00117	.510	-.0034	.0018
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 35ºC	-.00013	.00117	.912	-.0027	.0025
	CDW(g/L) against *S. maltophilia* EIBZ-6 at 37ºC	-.00003	.00117	.978	-.0026	.0026
CDW (g/L) against *S. maltophilia* EIBZ-6 at 30 oC	CDW (g/L) against *S. maltophilia* EIBZ-6 at 25ºC	.00320*	.00117	.021	.0006	.0058
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 32ºC	.00240	.00117	.068	-.0002	.0050
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 35ºC	.00307*	.00117	.026	.0005	.0057
	CDW(g/L) against *S. maltophilia* EIBZ-6 at 37ºC	.00317*	.00117	.022	.0006	.0058
CDW (g/L) against *S. maltophilia* EIBZ-6 at 32 oC	CDW (g/L) against *S. maltophilia* EIBZ-6 at 25ºC	.00080	.00117	.510	-.0018	.0034
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 30ºC	-.00240	.00117	.068	-.0050	.0002
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 35ºC	.00067	.00117	.582	-.0019	.0033
	CDW(g/L) against *S. maltophilia* EIBZ-6 at 37ºC	.00077	.00117	.528	-.0018	.0034
CDW (g/L) against *S. maltophilia* EIBZ-6 at 35 oC	CDW (g/L) against *S. maltophilia* EIBZ-6 at 25ºC	.00013	.00117	.912	-.0025	.0027
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 30ºC	-.00307*	.00117	.026	-.0057	-.0005
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 32ºC	-.00067	.00117	.582	-.0033	.0019
	CDW(g/L) against *S. maltophilia* EIBZ-6 at 37ºC	.00010	.00117	.934	-.0025	.0027
CDW(g/L) against S.	CDW (g/L) against *S. maltophilia* EIBZ-6 at 25ºC	.00003	.00117	.978	-.0026	.0026

maltophilia EIBZ-6 at 37 oC	CDW (g/L) against *S. maltophilia* EIBZ-6 at 30^{o}C	-.00317*	.00117	.022	-.0058	-.0006
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 32^{o}C	-.00077	.00117	.528	-.0034	.0018
	CDW (g/L) against *S. maltophilia* EIBZ-6 at 35^{o}C	-.00010	.00117	.934	-.0027	.0025
*. The mean difference is significant at the 0.05 level.						

Table A_9: Mean, SD, minimum and maximum value of CDW (g/L) against *Enterobacter* sp. EIBZ-5 for various carbon sources

CDW (g/L) against *Enterobacter* sp. EIBZ-5	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against Glucose for *Enterobacter sp*. EIBZ-5	3	.007000	.0007937	.0004583	.005028	.008972	.0064	.0079
CDW (g/L) against Mannitol for *Enterobacter sp.* EIBZ-5	3	.005200	.0013000	.0007506	.001971	.008429	.0044	.0067
CDW (g/L) against Sucrose for *Enterobacter sp*. EIBZ-5	3	.004800	.0014422	.0008327	.001217	.008383	.0032	.0060
CDW (g/L) against Molasses for *Enterobacter sp.* EIBZ-5	3	.001900	.0003606	.0002082	.001004	.002796	.0015	.0022
CDW (g/L) against Wheat bran for *Enterobacter sp*. EIBZ-5	3	.001700	.0013748	.0007937	-.001715	.005115	.0002	.0029
CDW (g/L) against Bagasse for *Enterobacter sp*. EIBZ-5	3	.001633	.0003055	.0001764	.000874	.002392	.0013	.0019

Total	18	.003706	.0023087	.0005442	.002557	.004854	.0002	.0079

Table A_{10}: Post Hoc test, significant variation at 0.05% confidence interval and Multiple Comparisons against *Enterobacter* sp. EIBZ-5 for CDW (g/L)

Multiple Comparisons						
Dependent Variable: CDW (g/L) against *Enterobacter sp.* EIBZ-5 LSD						
(I) Coding	(J) Coding	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against Glucose for *Enterobacter sp.* EIBZ-5	CDW (g/L) against Mannitol for *Enterobacter sp.* EIBZ-5	.0018000	.0008507	.056	-.000054	.003654
	CDW (g/L) against Sucrose for *Enterobacter sp.* EIBZ-5	.0022000*	.0008507	.024	.000346	.004054
	CDW (g/L) against Molasses for *Enterobacter sp.* EIBZ-5	.0051000*	.0008507	.000	.003246	.006954
	CDW (g/L) against Wheat bran for *Enterobacter sp.* EIBZ-5	.0053000*	.0008507	.000	.003446	.007154
	CDW (g/L) against Bagasse for *Enterobacter sp.* EIBZ-5	.0053667*	.0008507	.000	.003513	.007220
CDW (g/L) against Mannitol for *Enterobacter sp.* EIBZ-5	CDW (g/L) against Glucose for *Enterobacter sp.* EIBZ-5	-.0018000	.0008507	.056	-.003654	.000054
	CDW (g/L) against Sucrose for *Enterobacter sp.* EIBZ-5	.0004000	.0008507	.647	-.001454	.002254
	CDW (g/L) against Molasses for *Enterobacter sp.* EIBZ-5	.0033000*	.0008507	.002	.001446	.005154
	CDW (g/L) against Wheat bran for *Enterobacter sp.* EIBZ-5	.0035000*	.0008507	.001	.001646	.005354
	CDW (g/L) against Bagasse for *Enterobacter sp.* EIBZ-5	.0035667*	.0008507	.001	.001713	.005420
CDW (g/L) against Sucrose for *Enterobacter sp.* EIBZ-5	CDW (g/L) against Glucose for *Enterobacter sp.* EIBZ-5	-.0022000*	.0008507	.024	-.004054	-.000346
	CDW (g/L) against Mannitol for *Enterobacter sp.* EIBZ-5	-.0004000	.0008507	.647	-.002254	.001454
	CDW (g/L) against Molasses for *Enterobacter sp.* EIBZ-5	.0029000*	.0008507	.005	.001046	.004754
	CDW (g/L) against Wheat bran for *Enterobacter sp.* EIBZ-5	.0031000*	.0008507	.003	.001246	.004954
	CDW (g/L) against Bagasse for *Enterobacter sp.* EIBZ-5	.0031667*	.0008507	.003	.001313	.005020
CDW (g/L) against Molasses for	CDW (g/L) against Glucose for *Enterobacter sp.* EIBZ-5	-.0051000*	.0008507	.000	-.006954	-.003246
	CDW (g/L) against Mannitol for *Enterobacter sp.* EIBZ-5	-.0033000*	.0008507	.002	-.005154	-.001446
	CDW (g/L) against Sucrose for *Enterobacter sp.* EIBZ-5	-.0029000*	.0008507	.005	-.004754	-.001046

Enterobacter sp. EIBZ-5	CDW (g/L) against Wheat bran for *Enterobacter sp.* EIBZ-5	.0002000	.0008507	.818	-.001654	.002054
	CDW (g/L) against Bagasse for *Enterobacter sp.* EIBZ-5	.0002667	.0008507	.759	-.001587	.002120
CDW (g/L) against Wheat bran for *Enterobacter sp.* EIBZ-5	CDW (g/L) against Glucose for *Enterobacter sp.* EIBZ-5	-.0053000*	.0008507	.000	-.007154	-.003446
	CDW (g/L) against Mannitol for *Enterobacter sp.* EIBZ-5	-.0035000*	.0008507	.001	-.005354	-.001646
	CDW (g/L) against Sucrose for *Enterobacter sp.* EIBZ-5	-.0031000*	.0008507	.003	-.004954	-.001246
	CDW (g/L) against Molasses for *Enterobacter sp.* EIBZ-5	-.0002000	.0008507	.818	-.002054	.001654
	CDW (g/L) against Bagasse for *Enterobacter sp.* EIBZ-5	.0000667	.0008507	.939	-.001787	.001920
CDW (g/L) against Bagasse for *Enterobacter sp.* EIBZ-5	CDW (g/L) against Glucose for *Enterobacter sp.* EIBZ-5	-.0053667*	.0008507	.000	-.007220	-.003513
	CDW (g/L) against Mannitol for *Enterobacter sp.* EIBZ-5	-.0035667*	.0008507	.001	-.005420	-.001713
	CDW (g/L) against Sucrose for *Enterobacter sp.* EIBZ-5	-.0031667*	.0008507	.003	-.005020	-.001313
	CDW (g/L) against Molasses for *Enterobacter sp.* EIBZ-5	-.0002667	.0008507	.759	-.002120	.001587
	CDW (g/L) against Wheat bran for *Enterobacter sp.* EIBZ-5	-.0000667	.0008507	.939	-.001920	.001787
*. The mean difference is significant at the 0.05 level.						

Table A_{11}: Mean, SD, minimum and maximum value of CDW (g/L) against *S. maltophilia* EIBZ-6 for various carbon sources

CDW (g/L) against *S. maltophilia* EIBZ-6							
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against Glucose for *S. Maltophilia* EIBZ-6	3	.010400	.0157667	.0091029	-.028767	.049567	.0009	.0286
CDW (g/L) against Mannitol for *S. Maltophilia* EIBZ-6	3	.004167	.0009074	.0005239	.001913	.006421	.0032	.0050
CDW (g/L) against Sucrose for *S. Maltophilia* EIBZ-6	3	.002967	.0016773	.0009684	-.001200	.007133	.0019	.0049
CDW (g/L) against Molasses for *S. Maltophilia* EIBZ-6	3	.002033	.0004509	.0002603	.000913	.003153	.0016	.0025
CDW (g/L) against Wheat bran for *S. Maltophilia* EIBZ-6	3	.001833	.0001155	.0000667	.001546	.002120	.0017	.0019
CDW (g/L) against Bagasse for *S. Maltophilia* EIBZ-6	3	.001333	.0003512	.0002028	.000461	.002206	.0010	.0017
Total	18	.003789	.0063130	.0014880	.000650	.006928	.0009	.0286

Table A_{12}: Post Hoc test, significant variation at 0.05% confidence interval and Multiple Comparisons against *S. maltophilia* EIBZ-6 for CDW (g/L)

Dependent Variable: CDW (g/L) against *S. maltophilia* EIBZ-6

LSD

(I) Coding	(J) Coding	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against Glucose for *S. Maltophilia* EIBZ-6	CDW (g/L) against Mannitol for *S. Maltophilia* EIBZ-6	.0062333	.0052974	.262	-.005309	.017775
	CDW (g/L) against Sucrose for *S. Maltophilia* EIBZ-6	.0074333	.0052974	.186	-.004109	.018975
	CDW (g/L) against Molasses for *S. Maltophilia* EIBZ-6	.0083667	.0052974	.140	-.003175	.019909
	CDW (g/L) against Wheat bran for *S. Maltophilia* EIBZ-6	.0085667	.0052974	.132	-.002975	.020109
	CDW (g/L) against Bagasse for *S. Maltophilia* EIBZ-6	.0090667	.0052974	.113	-.002475	.020609
CDW (g/L) against Mannitol for *S. Maltophilia* EIBZ-6	CDW (g/L) against Glucose for *S. Maltophilia* EIBZ-6	-.0062333	.0052974	.262	-.017775	.005309
	CDW (g/L) against Sucrose for *S. Maltophilia* EIBZ-6	.0012000	.0052974	.825	-.010342	.012742

	CDW (g/L) against Molasses for *S. Maltophilia* EIBZ-6	.0021333	.0052974	.694	-.009409	.013675
	CDW (g/L) against Wheat bran for *S. Maltophilia* EIBZ-6	.0023333	.0052974	.667	-.009209	.013875
	CDW (g/L) against Bagasse for *S. Maltophilia* EIBZ-6	.0028333	.0052974	.603	-.008709	.014375
CDW (g/L) against Sucrose for *S. Maltophilia* EIBZ-6	CDW (g/L) against Glucose for *S. Maltophilia* EIBZ-6	-.0074333	.0052974	.186	-.018975	.004109
	CDW (g/L) against Mannitol for *S. Maltophilia* EIBZ-6	-.0012000	.0052974	.825	-.012742	.010342
	CDW (g/L) against Molasses for *S. Maltophilia* EIBZ-6	.0009333	.0052974	.863	-.010609	.012475
	CDW (g/L) against Wheat bran for S. Maltophilia EIBZ-6	.0011333	.0052974	.834	-.010409	.012675
	CDW (g/L) against Bagasse for *S. Maltophilia* EIBZ-6	.0016333	.0052974	.763	-.009909	.013175
CDW (g/L) against Molasses for *S. Maltophilia* EIBZ-6	CDW (g/L) against Glucose for *S. Maltophilia* EIBZ-6	-.0083667	.0052974	.140	-.019909	.003175
	CDW (g/L) against Mannitol for *S. Maltophilia* EIBZ-6	-.0021333	.0052974	.694	-.013675	.009409
	CDW (g/L) against Sucrose for *S. Maltophilia* EIBZ-6	-.0009333	.0052974	.863	-.012475	.010609

	CDW (g/L) against Wheat bran for S. Maltophilia EIBZ-6	.0002000	.0052974	.971	-.011342	.011742
	CDW (g/L) against Bagasse for *S. Maltophilia* EIBZ-6	.0007000	.0052974	.897	-.010842	.012242
CDW (g/L) against Wheat bran for *S. Maltophilia* EIBZ-6	CDW (g/L) against Glucose for *S. Maltophilia* EIBZ-6	-.0085667	.0052974	.132	-.020109	.002975
	CDW (g/L) against Mannitol for *S. Maltophilia* EIBZ-6	-.0023333	.0052974	.667	-.013875	.009209
	CDW (g/L) against Sucrose for *S. Maltophilia* EIBZ-6	-.0011333	.0052974	.834	-.012675	.010409
	CDW (g/L) against Molasses for *S. Maltophilia* EIBZ-6	-.0002000	.0052974	.971	-.011742	.011342
	CDW (g/L) against Bagasse for S. Maltophilia EIBZ-6	.0005000	.0052974	.926	-.011042	.012042
CDW (g/L) against Bagasse for S. *Maltophilia* EIBZ-6	CDW (g/L) against Glucose for *S. Maltophilia* EIBZ-6	-.0090667	.0052974	.113	-.020609	.002475
	CDW (g/L) against Mannitol for *S. Maltophilia* EIBZ-6	-.0028333	.0052974	.603	-.014375	.008709
	CDW (g/L) against Sucrose for *S. Maltophilia* EIBZ-6	-.0016333	.0052974	.763	-.013175	.009909
	CDW (g/L) against Molasses for *S. Maltophilia* EIBZ-6	-.0007000	.0052974	.897	-.012242	.010842

	CDW (g/L) against Wheat bran for *S. Maltophilia* EIBZ-6	-.0005000	.0052974	.926	-.012042	.011042

Table A_{13}: Mean, SD, minimum and maximum value of CDW (g/L) against *Enterobacter* sp. EIBZ-5 for various carbon sources

CDW (g/L) against *Enterobacter sp*. EIBZ-5 for nitrogen sources								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against *Enterobacter* sp. EIBZ-5 for Peptone	3	.0067	.00010	.00006	.0065	.0069	.01	.01
CDW (g/L) against *Enterobacter sp*. EIBZ-5 for KNO3	3	.0031	.00015	.00009	.0027	.0034	.00	.00
CDW (g/L) against *Enterobacter sp*. EIBZ-5 for YE	3	.0066	.00081	.00047	.0046	.0086	.01	.01
CDW (g/L) against *Enterobacter sp*. EIBZ-5 for NH4NO4	3	.0043	.00010	.00006	.0041	.0045	.00	.00
Total	12	.0052	.00165	.00048	.0041	.0062	.00	.01

Table A_{14}: Post Hoc test, significant variation at 0.05% confidence interval and Multiple Comparisons against *Enterobacter* sp. EIBZ-5 for various nitrogen sources with respective CDW (g/L)

Multiple Comparisons						
Dependent Variable: CDW (g/L) against *Enterobacter* sp. EIBZ-5 for nitrogen sources LSD						
(I) CDW (g/L) against *Enterobacter sp*. EIBZ-5 for various nitrogen sources	(J) CDW (g/L) against *Enterobacter sp*. EIBZ-5 for various nitrogen sources	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against *Enterobacter sp*. EIBZ-5 for Peptone	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for KNO3	.00363*	.00034	.000	.0028	.0044
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for YE	.00013	.00034	.706	-.0007	.0009
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for NH4NO4	.00240*	.00034	.000	.0016	.0032
CDW (g/L) against *Enterobacter sp*. EIBZ-5 for KNO3	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for Peptone	-.00363*	.00034	.000	-.0044	-.0028
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for YE	-.00350*	.00034	.000	-.0043	-.0027
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for NH4NO4	-.00123*	.00034	.007	-.0020	-.0004
CDW (g/L) against *Enterobacter sp*. EIBZ-5 for YE	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for Peptone	-.00013	.00034	.706	-.0009	.0007
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for KNO_3	.00350*	.00034	.000	.0027	.0043
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for $NH4NO_4$	.00227*	.00034	.000	.0015	.0031
CDW (g/L) against *Enterobacter sp*. EIBZ-5 for NH4NO4	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for Peptone	-.00240*	.00034	.000	-.0032	-.0016
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for KNO3	.00123*	.00034	.007	.0004	.0020
	CDW (g/L) against *Enterobacter sp*. EIBZ-5 for YE	-.00227*	.00034	.000	-.0031	-.0015
*. The mean difference is significant at the 0.05 level.						

Table A_{15}: Mean, SD, minimum and maximum value of CDW (g/L) against *S. maltophilia* EIBZ-6 for various carbon sources

CDW (g/L) against *S. maltophilia* EIBZ-6 for nitrogen sources								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against *S. maltophilia* EIBZ-6 for Peptone	3	.006200	.0008718	.0005033	.004034	.008366	.0056	.0072
CDW (g/L) against *S. maltophilia* EIBZ-6 for KNO3	3	.003233	.0013429	.0007753	-.000103	.006569	.0017	.0042
CDW (g/L) against *S. maltophilia* EIBZ-6 for YE	3	.005200	.0006245	.0003606	.003649	.006751	.0045	.0057
CDW (g/L) against *S. maltophilia* EIBZ-6 for NH4NO4	3	.005133	.0010693	.0006173	.002477	.007790	.0039	.0058
Total	12	.004942	.0014145	.0004083	.004043	.005840	.0017	.0072

Table A_{16}: Post Hoc test, significant variation at 0.05% confidence interval and Multiple Comparisons against *S. maltophilia* EIBZ-6 for various nitrogen sources with respective CDW (g/L)

Multiple Comparisons						
Dependent Variable: CDW (g/L) against *S. maltophilia* EIBZ-6 for nitrogen sources LSD						
(I) CDW (g/L) against *S. maltophilia* EIBZ-6 for various nitrogen sources	(J) CDW (g/L) against *S. maltophilia* EIBZ-6 for various nitrogen sources	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound

CDW (g/L) against *S. maltophilia* EIBZ-6 for Peptone	CDW (g/L) against *S. maltophilia* EIBZ-6 for KNO3	.0029667*	.0008263	.007	.001061	.004872
	CDW (g/L) against *S. maltophilia* EIBZ-6 for YE	.0010000	.0008263	.261	-.000905	.002905
	CDW (g/L) against *S. maltophilia* EIBZ-6 for NH4NO4	.0010667	.0008263	.233	-.000839	.002972
CDW (g/L) against *S. maltophilia* EIBZ-6 for KNO3	CDW (g/L) against *S. maltophilia* EIBZ-6 for Peptone	-.0029667*	.0008263	.007	-.004872	-.001061
	CDW (g/L) against *S. maltophilia* EIBZ-6 for YE	-.0019667*	.0008263	.045	-.003872	-.000061
	CDW (g/L) against *S. maltophilia* EIBZ-6 for NH4NO4	-.0019000	.0008263	.051	-.003805	.000005
CDW (g/L) against *S. maltophilia* EIBZ-6 for YE	CDW (g/L) against *S. maltophilia* EIBZ-6 for Peptone	-.0010000	.0008263	.261	-.002905	.000905
	CDW (g/L) against *S. maltophilia* EIBZ-6 for KNO3	.0019667*	.0008263	.045	.000061	.003872
	CDW (g/L) against *S. maltophilia* EIBZ-6 for NH4NO4	.0000667	.0008263	.938	-.001839	.001972
CDW (g/L) against *S. maltophilia* EIBZ-6 for NH4NO4	CDW (g/L) against *S. maltophilia* EIBZ-6 for Peptone	-.0010667	.0008263	.233	-.002972	.000839
	CDW (g/L) against *S. maltophilia* EIBZ-6 for KNO3	.0019000	.0008263	.051	-.000005	.003805
	CDW (g/L) against *S. maltophilia* EIBZ-6 for YE	-.0000667	.0008263	.938	-.001972	.001839
*. The mean difference is significant at the 0.05 level.						

Table A_{17}: Mean, SD, minimum and maximum value of CDW (g/L) against *Enterobacter sp.* EIBZ-5 and *S. maltophilia* EIBZ-6 for various salt conc.

CDW (g/L) against *Enterobacter sp.* EIBZ-5 and *S. maltophilia* EIBZ-6 for various salt conc.								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	3	.0061	.00055	.00032	.0047	.0074	.01	.01
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	3	.0053	.00086	.00050	.0032	.0075	.00	.01
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	3	.0317	.04977	.02873	-.0919	.1554	.00	.09
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	3	.0038	.00064	.00037	.0022	.0054	.00	.00
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	3	.0032	.00118	.00068	.0003	.0062	.00	.00
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	3	.0072	.00155	.00090	.0033	.0111	.01	.01
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	3	.0049	.00045	.00026	.0038	.0061	.00	.01
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	3	.0049	.00070	.00041	.0031	.0066	.00	.01

CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	3	.0032	.00103	.00059	.0006	.0057	.00	.00
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	3	.0060	.00105	.00061	.0034	.0086	.00	.01
Total	30	.0076	.01548	.00283	.0019	.0134	.00	.09

Table A_{18}: Post Hoc test, significant variation at 0.05% confidence interval and Multiple Comparisons against *Enterobacter* sp. EIBZ-5 and *S. maltophilia* EIBZ-6 for various salt conc. with respective CDW (g/L)

Multiple Comparisons						
Dependent Variable: CDW (g/L) against *Enterobacter sp.* EIBZ-5 and *S. maltophilia* EIBZ-6 for various salt conc. LSD						
(I) CDW (g/L) against *Enterobacter sp.* EIBZ-5 and *S. maltophilia* EIBZ-6 for various salt conc.	(J) CDW (g/L) against *Enterobacter sp*. EIBZ-5 and *S. maltophilia* EIBZ-6 for various salt conc. L	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	.00073	.01287	.955	-.0261	.0276
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02567	.01287	.060	-.0525	.0012
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	.00223	.01287	.864	-.0246	.0291
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.00283	.01287	.828	-.0240	.0297
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00113	.01287	.931	-.0280	.0257
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	.00113	.01287	.931	-.0257	.0280
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	.00120	.01287	.927	-.0256	.0280
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00290	.01287	.824	-.0239	.0297
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	.00007	.01287	.996	-.0268	.0269

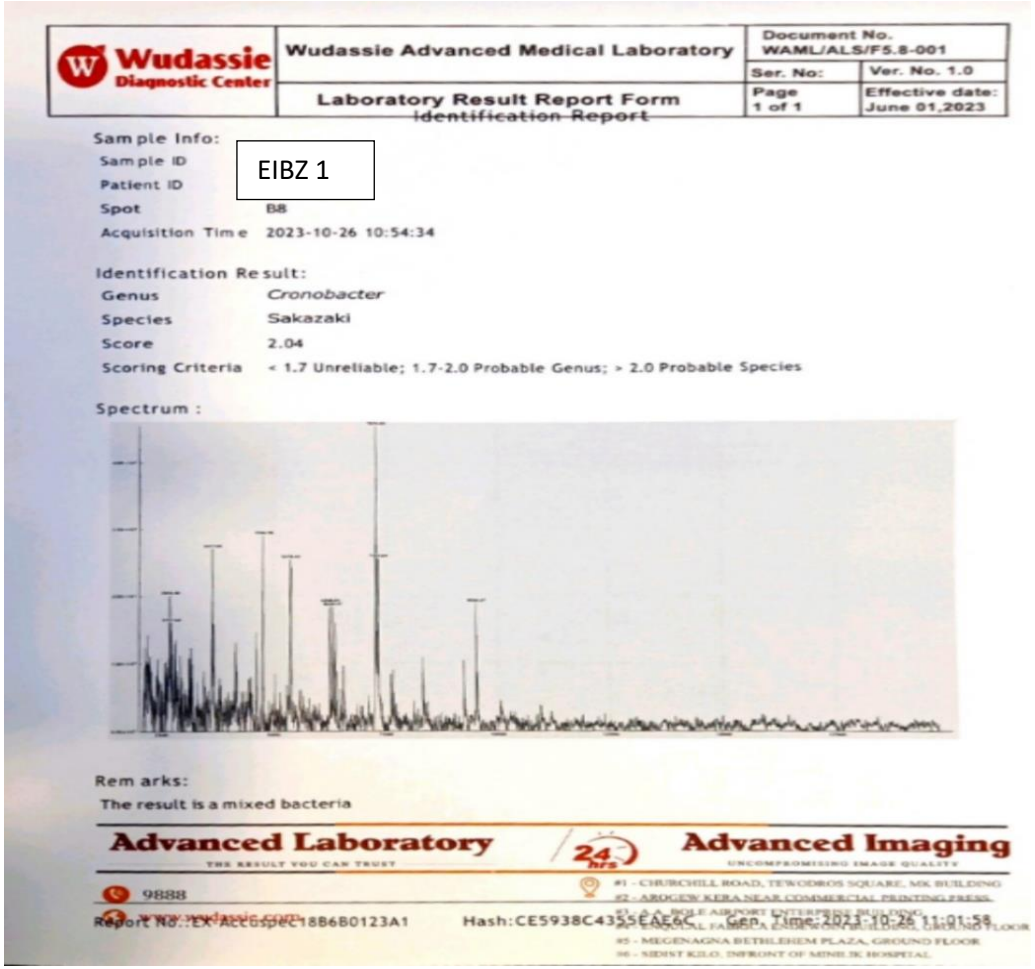
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	-.00073	.01287	.955	-.0276	.0261
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02640	.01287	.054	-.0532	.0004
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	.00150	.01287	.908	-.0253	.0283
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.00210	.01287	.872	-.0247	.0289
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00187	.01287	.886	-.0287	.0250
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	.00040	.01287	.976	-.0264	.0272
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	.00047	.01287	.971	-.0264	.0273
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00217	.01287	.868	-.0247	.0290
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	-.00067	.01287	.959	-.0275	.0262
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	.02567	.01287	.060	-.0012	.0525
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	.02640	.01287	.054	-.0004	.0532
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	.02790*	.01287	.042	.0011	.0547
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.02850*	.01287	.039	.0017	.0553
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	.02453	.01287	.071	-.0023	.0514
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	.02680	.01287	.050	.0000	.0536
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	.02687*	.01287	.050	.0000	.0537
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.02857*	.01287	.038	.0017	.0554
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	.02573	.01287	.059	-.0011	.0526
CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	-.00223	.01287	.864	-.0291	.0246
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	-.00150	.01287	.908	-.0283	.0253
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02790*	.01287	.042	-.0547	-.0011
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.00060	.01287	.963	-.0262	.0274
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00337	.01287	.796	-.0302	.0235
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	-.00110	.01287	.933	-.0279	.0257
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	-.00103	.01287	.937	-.0279	.0258
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00067	.01287	.959	-.0262	.0275
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	-.00217	.01287	.868	-.0290	.0247

CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	-.00283	.01287	.828	-.0297	.0240
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	-.00210	.01287	.872	-.0289	.0247
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02850*	.01287	.039	-.0553	-.0017
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	-.00060	.01287	.963	-.0274	.0262
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00397	.01287	.761	-.0308	.0229
	CDW (g/L) against S. maltophilia EIBZ-6 for various salt conc (1 %)	-.00170	.01287	.896	-.0285	.0251
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	-.00163	.01287	.900	-.0285	.0252
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00007	.01287	.996	-.0268	.0269
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	-.00277	.01287	.832	-.0296	.0241
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	.00113	.01287	.931	-.0257	.0280
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	.00187	.01287	.886	-.0250	.0287
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02453	.01287	.071	-.0514	.0023
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	.00337	.01287	.796	-.0235	.0302
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.00397	.01287	.761	-.0229	.0308
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	.00227	.01287	.862	-.0246	.0291
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	.00233	.01287	.858	-.0245	.0292
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00403	.01287	.757	-.0228	.0309
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	.00120	.01287	.927	-.0256	.0280
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	-.00113	.01287	.931	-.0280	.0257
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	-.00040	.01287	.976	-.0272	.0264
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02680	.01287	.050	-.0536	.0000
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	.00110	.01287	.933	-.0257	.0279
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.00170	.01287	.896	-.0251	.0285
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00227	.01287	.862	-.0291	.0246
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	.00007	.01287	.996	-.0268	.0269
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00177	.01287	.892	-.0251	.0286
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	-.00107	.01287	.935	-.0279	.0258

CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	-.00120	.01287	.927	-.0280	.0256
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	-.00047	.01287	.971	-.0273	.0264
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02687*	.01287	.050	-.0537	.0000
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	.00103	.01287	.937	-.0258	.0279
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.00163	.01287	.900	-.0252	.0285
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00233	.01287	.858	-.0292	.0245
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	-.00007	.01287	.996	-.0269	.0268
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00170	.01287	.896	-.0251	.0285
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	-.00113	.01287	.931	-.0280	.0257
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	-.00290	.01287	.824	-.0297	.0239
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	-.00217	.01287	.868	-.0290	.0247
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02857*	.01287	.038	-.0554	-.0017
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	-.00067	.01287	.959	-.0275	.0262
	CDW (g/L) against Enterobacter sp. EIBZ-5 for various salt conc (7 %)	-.00007	.01287	.996	-.0269	.0268
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00403	.01287	.757	-.0309	.0228
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	-.00177	.01287	.892	-.0286	.0251
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	-.00170	.01287	.896	-.0285	.0251
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	-.00283	.01287	.828	-.0297	.0240
CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (7 %)	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (0.9 %)	-.00007	.01287	.996	-.0269	.0268
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (1%)	.00067	.01287	.959	-.0262	.0275
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (3 %)	-.02573	.01287	.059	-.0526	.0011
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (5 %)	.00217	.01287	.868	-.0247	.0290
	CDW (g/L) against *Enterobacter sp.* EIBZ-5 for various salt conc (7 %)	.00277	.01287	.832	-.0241	.0296
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (0.9 %)	-.00120	.01287	.927	-.0280	.0256
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (1 %)	.00107	.01287	.935	-.0258	.0279
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (3 %)	.00113	.01287	.931	-.0257	.0280
	CDW (g/L) against *S. maltophilia* EIBZ-6 for various salt conc (5%)	.00283	.01287	.828	-.0240	.0297

*. The mean difference is significant at the 0.05 level.

Appendix 1: MALDTOF- MS Results for EIBZ 1



Wudassie Diagnostic Center

Wudassie Advanced Medical Laboratory

Document No. WAML/ALS/F5.8-001

Ser. No: | Ver. No. 1.0

Laboratory Result Report Form
~~Identification Report~~

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Sample Info:

Sample ID: EIBZ 1

Patient ID:

Spot: B8

Acquisition Time: 2023-10-26 10:54:34

Identification Result:

Genus: *Cronobacter*

Species: Sakazaki

Score: 2.04

Scoring Criteria: < 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum :

Remarks:

The result is a mixed bacteria

Advanced Laboratory

THE RESULT YOU CAN TRUST

24 hrs

Advanced Imaging

UNCOMPROMISING IMAGE QUALITY

9888

#1 - CHURCHILL ROAD, TEWODROS SQUARE, MK BUILDING

#2 - ABOGEW KEBA NEAR COMMERCIAL PRINTING PRESS

#3 - J. A. BOLE AIRPORT ENTERPRISE BUILDING

#4 - [illegible]

#5 - MEGENAGNA BETHLEHEM PLAZA, GROUND FLOOR

#6 - SIDIST KILO, INFRONT OF MINILIK HOSPITAL

Report No.: EX-Accuspec-18B6B0123A1 Hash:CE5938C4355EAE6C Gen. Time: 2023-10-26 11:01:56

Appendix 2: MALDTOF- MS Results for EIBZ 2

Identification Report

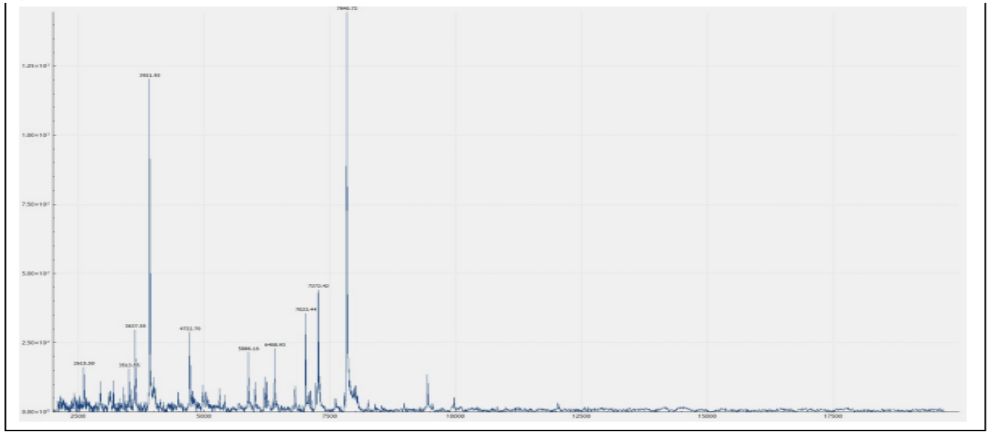
Sample Info:

Sample ID Patient ID	EIBZ 2
Spot	B10
Acquisition Time	2023-10-26 10:55:04

Identification Result:

Genus	*Rhodotorula*
Species	*mucilaginosa*
Score	1.74
Scoring Criteria	< 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum:



Remarks:

Report No.:EX-Accuspec18B6B0123ED Hash:922F482E6653AAD5 Gen. Time:2023-10-26 11:01:58

Appendix 3: MALDTOF- MS Results for EIBZ 3

Identification Report

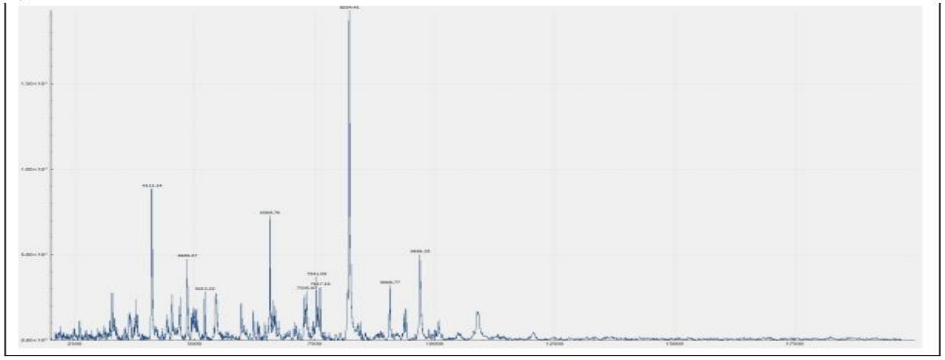
Sample Info:

Sample ID	EIBZ 3
Patient ID	
Spot	A4
Acquisition Time	2023-10-27 10:35:19

Identification Result:

Genus	*Bordetella*
Species	*pertussis*
Score	1.73
Scoring Criteria	< 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum:



Remarks:

The result is a mixed bacteria

Report No.:EX-Accuspec18B70140416 Hash:976053315FDBF571 Gen. Time:2023-10-27 10:40:41

Appendix 4: MALDTOF- MS Results for EIBZ 4

Wudassie Diagnostic Center	Wudassie Advanced Medical Laboratory	Document No. WAML/ALS/F5.8-001	
		Ser. No:	Ver. No. 1.0
	Laboratory Result Report Form Identification Report	Page 1 of 1	Effective date: June 01,2023

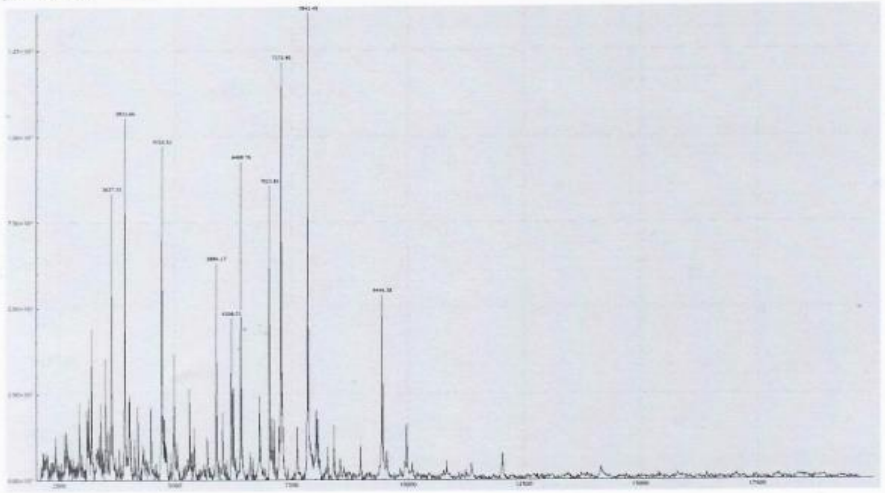
Sample Info:

Sample ID	EIBZ 4
Patient ID	
Spot	C3
Acquisition Time	2023-10-26 10:57:27

Identification Result:

Genus	*Rhodotorula*
Species	*mucilaginosa*
Score	1.74
Scoring Criteria	< 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum :



Appendix 5: MALDTOF- MS Results for EIBZ 5

Identification Report

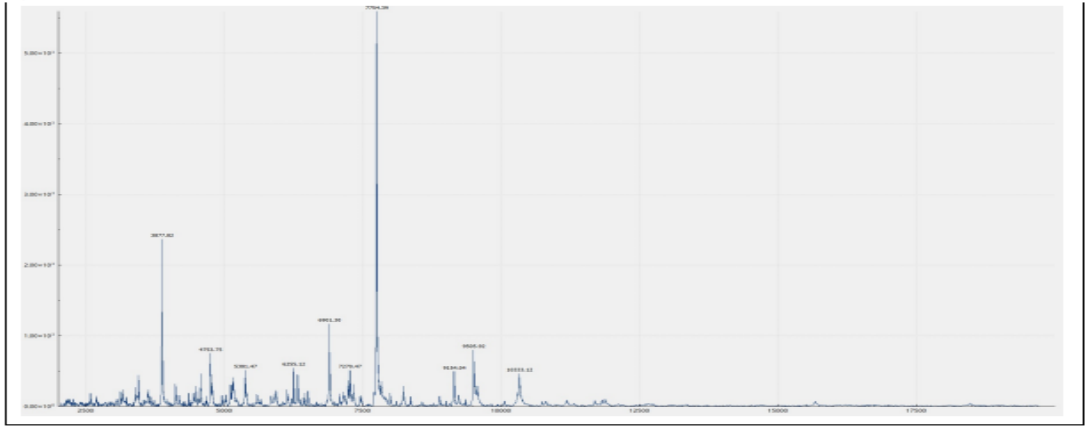
Sample Info:

Sample ID Patient ID	EIBZ 5
Spot	A5
Acquisition Time	2023-10-27 10:35:39

Identification Result:

Genus	*Enterobacter*
Species	*cloacae*
Score	1.71
Scoring Criteria	< 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum:



Remarks:

The result is a mixed bacteria

Report No.:EX-Accuspec18B701403F7 Hash:78F88B04E9A9B05E Gen. Time:2023-10-27 10:40:41

Appendix 6: MALDTOF- MS Results for EIBZ 6

Identification Report

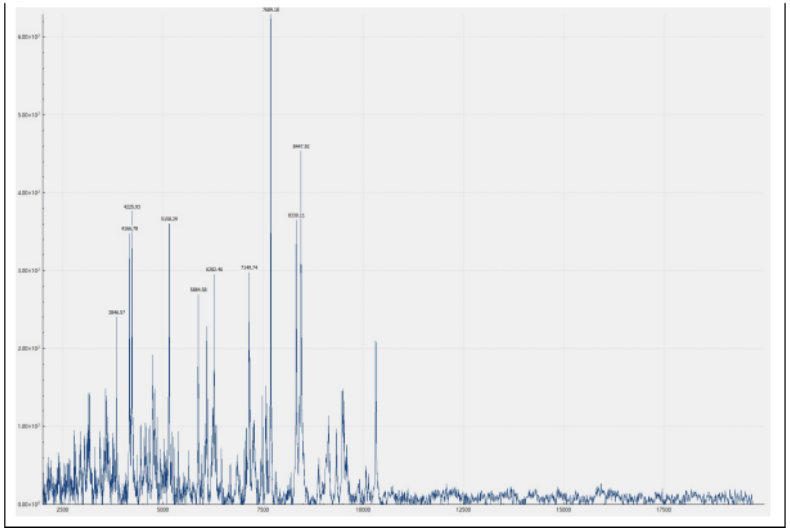
Sample Info:

Sample ID	EIBZ 6
Patient ID	
Spot	B4
Acquisition Time	2023-10-26 10:53:08

Identification Result:

Genus	*Stenotrophomonas*
Species	*maltophilia*
Score	2.02
Scoring Criteria	< 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum:



Appendix 7: MALDTOF- MS Results for EIBZ 7

Identification Report

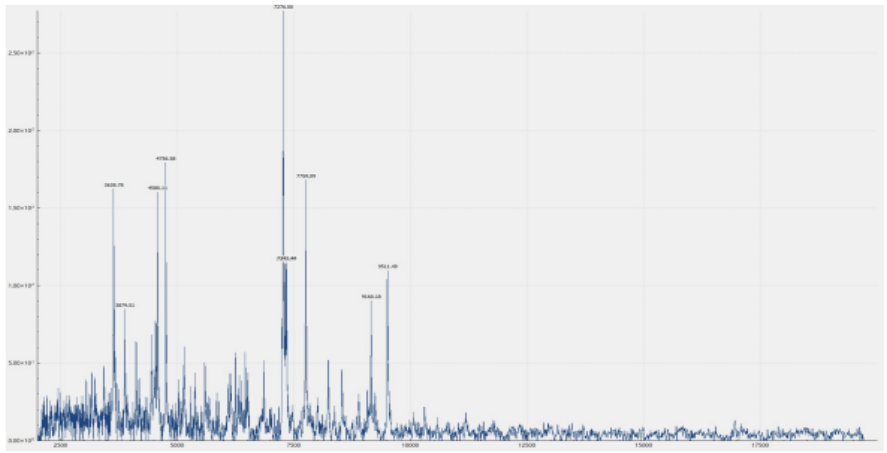
Sample Info:

Sample ID Patient ID	EIBZ 7
Spot	B1
Acquisition Time	2023-10-26 10:50:56

Identification Result:

Genus	*Enterobacter*
Species	*asburiae*
Score	1.80
Scoring Criteria	< 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum:



Remarks:

The result is a mixed bacteria

Appendix 8: MALDTOF- MS Results for EIBZ 8

Identification Report

Sample Info:

Sample ID	EIBZ 8
Patient ID	
Spot	B9
Acquisition Time	2023-10-26 10:54:58

Identification Result:

Genus	*Pseudomonas*
Species	*stutzeri*
Score	1.85
Scoring Criteria	< 1.7 Unreliable; 1.7-2.0 Probable Genus; > 2.0 Probable Species

Spectrum: