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Isolation and Identification of Endophytic Bacteria from Stem Bark of the Medicinal Plant *Dialium Guineense* (*WILD*)

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Abstract: *Fresh healthy stem bark samples of Dialium guineense were collected from a partially bushy area along Warrake road, Auchi. The plant material were transferred into well labeled clean plastic bags and immediately transported to the laboratory for analysis. The plant material was thoroughly washed under slow running tap water for about 30 minutes to remove dust, soil particles and debris before carrying out surface sterilization immediately followed by culturing on nutrient agar for 24 hours at 37 °C. Macroscopic observation was carried out by directly observing the colony characteristics of bacterial isolate including color, shape, edge and elevation of the colony. Microscopic observations were done by gram staining method. From the results, five (5) endophytic bacteria were isolated from the stem bark of Dialium guineense after macroscopic and microscopic characterization which fell under three genera namely Pseudomonas, Bacillus and Streptococcus in the ratio 3:1:1. Only two of the endophytic bacteria isolates of the genera Bacillus and Streptococcus were gram positive (BS 4 and BS 5); while the other isolates belonged to the genera Pseudomonas. It was evident that Dialium guineense contains different types of gram positive and negative endophytic bacteria isolates.*

Keywords*: Endophytic bacteria; Dialium guineense (Wild); Isolation; Surface sterilization.*

I. INTRODUCTION

Microorganisms known as endophytes live in plant tissues such as leaves, stems, and roots without causing harm to their host plant (Anteneh, 2020). Numerous types of plants contain endophytic organisms which spend all or a portion of their developmental cycles inside the tissues of living plants and may be no visible symptoms of sickness in the host plant as a result of endophyte infections (Chijioke, Nwamaka, Dina and Udoma, 2022). According to Santoyo, Moreno-Hagelsieb, Del Carmen Orozco-Mosqueda and Glick (2016), endophytic bacteria are those that are gotten after surface-sterilization of plant tissues. These bacteria have been discovered to persist throughout the entire plant, including the seeds and above-ground and underground portions thus having a good impact on plant growth (Chebotar *et al*., 2015). *Dialium guineense* belongs to the Leguminosae family and grows in dense forests in Africa along the southern edge of the Sahel and other African countries (Akinpelu, 2011). The bark, leaves and fruits of the plant have medicinal properties and are used to treat diseases such as stomatitis, toothache, fever, diarrhea, palpitations, and microbial infections. As a result of their importance in diet and medical care, humans are largely dependent on the plant (Uusiku, Oelofse, Duodu, Bester and Faber, 2010). In Africa, for example, more than eighty percent of the population relies exclusively on plants for healing (WHO, 2013). Whilst it is true that this practice has existed for thousands of years on the whole

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surface of the globe, it persists more in traditional areas mainly because of the satisfaction attained by the populations resorting to medicinal plants. Therefore, there is a real culture of traditional medicine in most part of the world. *Dialium guineense* grows in dense forests in Africa along the southern edge of the Sahel and it can be found in West African countries such as Ghana where it is known as “Yoyi”, Sierra Leone, Senegal, Guinea-Bissau and Nigeria where it is known as “Awin” or "Igbaru" in Yoruba, “Icheku” in Igbo, “Tsamiyarkurm”in Hausa and “Amughen” in Edo (Abu, Adeogun and Ebhohon, 2019). In most developing countries of the world, these antibiotics are not readily affordable, which makes compliance difficult. This calls for research into alternative sources of antimicrobials.

II. MATERIALS AND METHODS

2.1 Materials

Fresh healthy samples of stem bark of *Dialium guineense* were collected from a partially bushy area along Warrake road, Auchi. The plant materials were transferred into well labeled clean plastic bags and immediately transported to the laboratory for analysis. The plant samples were authenticated at the Plant Biology and Biotechnology Unit (Herbarium Curation Sub-Division, Department of Biological Science, Edo State University, Uzairue, Edo State. Nigeria.

2.2 Methods

2.2.1 Isolation of endophytic bacteria

The plant material was thoroughly washed under slow running tap water for about 30 minutes to remove dust, soil particles and debris. This was followed by surface sterilization to remove epiphytes according to Renugadevi, Ayyappadas, Subhapriya, Floryshobana and Vivekanandhan (2021). Using a sterile scalpel, the plant material were cut into pieces of 3.5 cm separately placed with sterile forceps on already prepared solidified nutrient agar plates and then incubated at 37 ^{o}C for 24 hours. Growth cultures were observed for morphologically different bacteria colonies which were selected and streaked on fresh nutrient agar plates to obtain a pure culture of distinct colonies.

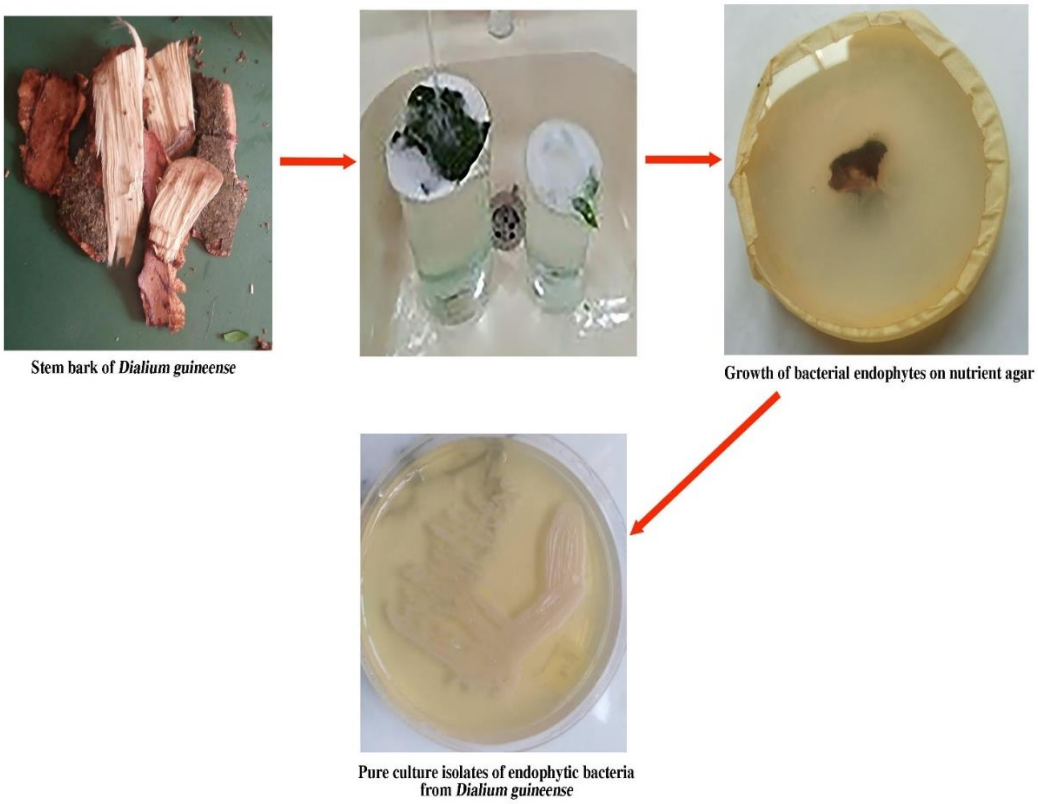

Plate 1: Isolation of bacterial endophytes from the stem bark of *Dialum guineense.*

2.2.2 Identification of bacteria isolates

Identification of bacterial isolates was done by observing macroscopic and microscopic characteristics of bacteria. Macroscopic observation was carried out by directly observing the colony characteristics of bacterial isolate including color, shape, edge and elevation of the colony. Microscopic observations were carried out through gram staining and some biochemical characterization such as catalase, oxidase, citrate utilization, motility test, hydrogen sulfide production, glucose and lactose tests.

III. RESULTS AND DISCUSSION

About five endophytic bacteria (gram-positive and gram-negative) were isolated from the stem bark of the medicinal plant *Dialium guineense*. After morphological and biochemical characterization, the endophytes were placed under the genera Pseudomonas, Bacillus and Streptococcus. From the three genera, Pseudomonas was more prominent which is not surprising as many species of the genera are known opportunistic organisms. A diverse range of endophytic bacteria have been documented to reside on various plant types. Endophytic bacteria come in a wide variety, from gram-positive to gram-negative (Sun, Wang and Li, 2016). According to Chaturvedi, Singh and Gupta (2016), Bacillus and Pseudomonas are the most common types of bacteria, followed by Burkholderia, Micrococcus, Pantoea, and Stenotrophomonas. In addition, large growth of bacterial endophytes on culture media after standard surface sterilization is an indication of minimal damage to the endophytic population as a result of the sterilization technique (Eevers *et al.*, 2015). *Pseudomonas* and *Bacillus* species showed heavy growth on agar plates after carrying out a pure culture of all the stated organisms from their mixed culture. Table 1 below depicts the morphological characterization of endophytic bacteria isolates to ascertain their identity.

Table 1: Morphological characterization of endophytic bacteria isolates

Characteristic	SB1	SB2	SB3	SB4	SB4
Shape	Rod	Rod	Rod	Cocci	Rod
Edge	Wavy	Entire	Entire	Entire	Wavy
Elevation	Flat	Slightly raised	Raised	Flat	Flat
Colony	Large	Large	Small	Small	Medium
Surface	Fruity	Round	Round	Round	Round
Consistency	Slimy	Slimy	Dry	Dry	Dry
Transparency	Opaque	Opaque	Opaque	Opaque	Opaque

The result in table 2 showed the microscopic and biochemical characterization of endophytic bacteria isolates, where SB1, SB2, and SB3 were negative to gram staining while SB4 and SB5 were positive to gram staining. When bacteria penetrate and establish a close relationship with plants, endophytic development takes place (Perotti, 1926). In order to survive in their natural surroundings, plants may establish beneficial relationships with inhabitants of their ecological system, including microorganisms (Santoyo *et al.*, 2016). It is well recognized that certain plant-beneficial bacteria provide their host plants a variety of advantages, assisting them in overcoming biotic and abiotic difficulties (Miliute, Buzaite, Baniulis and Stanys, 2015). The plant endosphere offers a stable and constant habitat for endophytic bacteria, which are protected from the variable factors affecting rhizospheric and epiphytic bacteria in the environment (Senthilkumar, Anandham, Madhaiyan, Venkateswaran and Sa, 2011).

Table 2: Microscopic and biochemical characterization of endophytic bacteria isolates

Test	SB1	SB2	SB3	SB4	SB5
Gram reaction	-	-	-	+	+
Catalase	+	+	+	-	+
H_2S production	-	-	-	-	-
Oxidase	+	+	+	-	+

Citrate	+	+	+	-	+
Urease	-	-	+	-	-
Glucose	-	-	-	+	+
Motility	+	+	-	-	+
Lactose	-	-	-	+	+
Suspected identity	*Pseudomonas* sp	*Pseudomonas* sp	*Pseudomonas* sp	*Streptococcus* sp	*Bacillus* sp.

IV. CONCLUSION

A total of 5 genera of endophytic bacteria were isolated from stem bark of *Dialium guineense* (SB1 – SB5) based on macroscopic and microscopic characteristics. As much as three out five of the bacteria were classified as gram negative while the other two were gram negative. All the genera of endophytic bacteria isolated are common bacteria endophytes associated with most medicinal plants.

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