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Enzymatic and antimicrobial activities of endophytes in *Khaya senegalensis*

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Abstract

Khaya senegalensis is a plant widely used in Benin's traditional medicine for treating various diseases. Due to overexploitation, it faces anthropogenic pressure, threatening its extinction. This study aimed to evaluate the enzymatic and antimicrobial activities of endophytes in *Khaya senegalensis*. Fresh leaves, stems, and roots collected from Abomey-Calavi were sterilized and cultured on specific media to isolate bacterial and fungal endophytes. The isolation rate of these endophytes was determined. Their enzymatic activities (amylase, lecithinase, lipase, cellulase) were explored, and the antimicrobial activity of these endophytes against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* was evaluated by agar diffusion. The results revealed that the leaves of *Khaya senegalensis* had the highest endophyte isolation rate, with *Bacillus* spp. (46.67%) dominating among bacteria and *Alternaria* spp. (35.71%) predominating among fungi. All isolated bacterial endophytes showed varied enzymatic activities (catalase, amylase, lecithinase, hemolysin) but no antimicrobial activity against the tested strains. However, the fungal endophytes inhibited bacterial strains to varying degrees but had no effect on *Candida albicans*. Fungal isolates F1c, F3a, and T2c exhibited antibacterial activity against all tested bacterial strains, with F1c showing the greatest potential. This study highlights the diversity of bacterial and fungal endophytes in *Khaya senegalensis*, which exhibit different enzymatic and antimicrobial profiles.

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Introduction

Medicinal plants hold a central place in the traditional pharmacopoeia of many cultures around the world (Salmerón-Manzano *et al.*, 2020; Süntar, 2020). They are used to treat a variety of conditions, ranging from infectious diseases to inflammatory and metabolic disorders (Nyakudya *et al.*, 2020; Ugboko *et al.*, 2020). Traditional medicine relies on the empirical use of plants, and many active substances of plant origin have led to the discovery of modern drugs (Khumalo *et al.*, 2022; Mbuni *et al.*, 2020).

In Africa, medicinal plants play a crucial role in primary healthcare. Approximately 80% of the African population relies on medicinal plants for their health needs due to limited access to modern healthcare and the high cost of pharmaceutical drugs (WHO, 2022). The richness of African flora, with around 45,000 species of vascular plants, offers a vast reservoir of biodiversity for traditional medicines (Ghazal *et al.*, 2021). More than 5,000 species of African plants are used for medicinal purposes, highlighting the importance of this resource for local populations (Saliu *et al.*, 2023). However, increasing pressure on natural resources and deforestation endanger these valuable sources of traditional remedies (Nuwagira *et al.*, 2022; Umar *et al.*, 2020). Moreover, the global phenomenon of antimicrobial resistance exacerbates the need to discover new sources of bioactive compounds (Salam *et al.*, 2023). Consequently, research has gradually highlighted the crucial role of endophytes, these microorganisms (bacteria and fungi) that live symbiotically inside plants without causing them harm (Agrawal and Bhatt, 2023). Endophytes contribute to the health and resilience of their host plants by helping them defend against pathogens, enhancing their growth, and protecting them against environmental stresses (Adeleke *et al.*, 2021; Khare *et al.*, 2018). They are capable of producing bioactive metabolites, similar to or even identical to those of their host plants, with antimicrobial, antioxidant, anticancer, and anti-inflammatory properties (Sharma *et al.*, 2020; Strobel, 2018).

The study of endophytes from medicinal plants represents a new frontier in pharmaceutical and biotechnological research (Upadhyay *et al.*, 2016). These microorganisms can synthesize potential bioactive molecules that offer promising alternatives to conventional treatments, especially in the context of increasing antibiotic resistance.

Additionally, the identification of new endophyte metabolites can lead to the discovery of innovative pharmaceutical compounds (Fontana *et al.*, 2021). Medicinal plants sought for their endophytes often have a long history of traditional use and produce a wide variety of secondary metabolites with antimicrobial and antioxidant properties (Fadiji and Babalola, 2020). Adapted to diverse environments, they provide a broad range of microenvironments conducive to the discovery of microorganisms producing potentially therapeutic bioactive compounds (Kuźniar *et al.*, 2019).

Khaya senegalensis, also known as African mahogany, is a well-known medicinal plant in West and Central Africa (Langa *et al.*, 2024). Traditionally used to treat malaria, bacterial and fungal infections, as well as various digestive and inflammatory disorders (Adamu *et al.*, 2022; Gouissi *et al.*, 2021), the tree is also valued for its wood, making it an ecologically and economically important resource (Langa *et al.*, 2024). Recent studies on *Khaya senegalensis* have focused on evaluating the biological properties of its various extracts, derived from leaves, bark, and seeds. The results have revealed significant therapeutic potential, suggesting that *Khaya senegalensis* could provide effective natural alternatives for treating infections and preventing oxidative stress (Elbana *et al.*, 2024; Faoziyat *et al.*, 2020; Kandeda *et al.*, 2022). Thus, *Khaya senegalensis* is an ideal candidate for the study of its endophytes.

However, its endophytes represent an underexplored but potentially rich avenue for the discovery of new therapeutic agents. This study was initiated within this context and aims to explore the diversity and

biological potential of endophytes isolated from *Khaya senegalensis* in Benin. Specific objectives include exploring the diversity of bacterial and fungal endophytes of *Khaya senegalensis*, determining the total polyphenol and flavonoid content of isolated endophytes, and evaluating the antibacterial and antifungal properties of endophytes isolated from *Khaya senegalensis*.

Materials and methods

Biological strains and reagent

Staphylococcus aureus ATCC 6528*, Escherichia coli ATCC 25922, Pseudomonas* aeruginosa ATCC 9027, *Candida albicans* ATCC 90028 were either provided by the Research Unit in Applied Microbiology and Pharmacology of Natural Substances at the University of Abomey-Calavi, Benin.

The leaves, stems, and roots of *Khaya senegalensis* were collected at the University of Abomey-Calavi, Benin, following the methodology of Zerroug (2021). They were placed in sterile bags to be used within 24 hours.

Antibiotic discs of chloramphenicol at 30 µg were purchased from Sigma-Aldrich and used as controls for the antimicrobial test.

Surface Sterilization of Khaya senegalensis organs

The fresh organs of *Khaya senegalensis* collected underwent surface sterilization to eliminate epiphytes on the surface of the organs, allowing for healthy and optimal growth of endophytes (Martinez-Klimova, Rodríguez-Peña, et Sánchez 2017). To achieve this, the carefully collected organs were thoroughly washed with tap water for 15 minutes (Marchut *et al.*, 2023).

After washing, they were placed in a first bath of 70% ethanol, then in a second bath of 6% sodium hypochlorite (NaOCl), and finally in a third bath of 70% ethanol, for six and four minutes, respectively. The samples were then rinsed three times with sterile distilled water for 1 minute each and dried on sterile filter paper (Khan *et al.*, 2017; Ratnaweera *et al.*, 2015).

The effectiveness of the organ sterilization was checked by inoculating aliquots from the third rinsing bath onto a nutrient agar plate to ensure that the isolates obtained indeed come from the internal tissues. The absence of colonies on the media indicates that the surface sterilization was successful and that all epiphytic microorganisms have been eliminated (Marsola *et al.*, 2022).

Culturing Khaya senegalensis organs

In total, three leaves, three stems, and three roots were used. Fragments, specifically three of 1 to 1.5 cm from each organ, were cut and then aseptically placed on Petri dishes containing Potato Dextrose Agar (PDA) (Zakaria *et al.*, 2016) supplemented with chloramphenicol (100 µg/mL) to prevent bacterial growth for fungal endophytes, and on Petri dishes containing Nutrient Agar (NA) for bacterial endophytes. The Petri dishes were incubated at 37°C for 5 to 7 days and checked daily until the appearance of mycelium or colonies for fungal endophytes, and for 24 to 48 hours for bacterial endophytes.

Isolation and purification of fungal and bacterial endophytes

All fungal and bacterial endophytes that formed colonies were then isolated three times and placed into new Petri dishes containing PDA and NA, respectively, until pure colonies were obtained (Jinxiu *et al.*, 2018).

The percentage of colonization (PC) and isolation of fungal endophytes was calculated using the following formula:

PC (%) = Number of colonized fragments / Total number of fragments × 100 (Pimentel *et al.*, 2006; Jayatilake *et al.*, 2020)

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Morphological and biochemical characterization of fungal and bacterial endophytes

Macroscopy

Fungal and bacterial endophytes were identified from pure colonies. For macroscopic observation, the general appearance of the colony, its color, and growth diameter were the criteria considered (Bouyaiche and Guedjal, 2018).

Microscopy

For fungal endophytes microscopy, a simple staining with methylene blue was performed using the Scotch-tape method following Duan *et al.* (2019). This method involves placing a drop of methylene blue on a clean, dry microscope slide. Young structures of the fungal colonies were then picked up using the Scotch tape and placed on the slide with a drop of methylene blue so that the fungal structures were stained and visible under observation. Excess methylene blue around the slide was gently removed using sterile tissue paper. The slide was examined under the microscope at 400x and 1000x magnification to observe the characteristic fungal structures. Structures such as hyphae, septa, and spores were looked for to identify different strains.

For bacterial endophytes microscopy, a fresh preparation was made. This method involves placing a portion of a young colony on a clean, dry slide with a drop of physiological saline, then covering it with a cover slip. Observation was conducted at 40X magnification.

Additionally, a Gram staining was performed, and biochemical characteristics were studied using catalase and oxidase tests, as well as the Api 20 E gallery (Tassadaq *et al.*, 2013; Celiwe *et al.*, 2020).

Analysis of antibacterial activity of fungal and bacterial endophytes of Khaya senegalensis

To evaluate the antibacterial activity of fungal endophytes, the agar cylinder method as described by Pelo *et al.* (2020) was used. The different strains to be tested were revived in nutrient broth and incubated at 37°C for 24 hours. Once these broths showed turbidity, they were streaked onto nutrient agar plates using a three-way streak technique, which were then incubated at 37°C for 24 hours. After incubation, morphologically identical colonies from each plate were scraped using a sterile platinum loop and transferred to 5 ml of sterile physiological saline. These suspensions were well homogenized, and the turbidity was adjusted to 0.5 McFarland (Jayatilake *et al.*, 2020). The bacterial suspensions were inoculated using a sterile swab by the Kirby-Bauer method onto nutrient agar plates.

The swab was dipped into the bacterial suspension and then rotated against the inner wall of the tube to remove excess suspension. The nutrient agar surface was streaked densely from top to bottom, with a 60° rotation of the Petri dish each time (Jayatilake *et al.*, 2020). Once the reference strains were inoculated, 6 mm diameter cylinders of young fungal cultures (7 days old) were carefully placed aseptically in the center of the previously inoculated plates (An *et al.*, 2020). The inoculated plates were kept at room temperature for 2 hours to allow pre-diffusion of the active substances secreted by the fungal endophytes before being incubated at 37°C for 24 hours. After incubation, the diameters of the inhibition zones around the cylinders were measured (Munasinghe *et al.*, 2020).

Analysis of antifungal activity of fungal and bacterial endophytes of Khaya senegalensis

The antifungal activity was assessed using the dual culture technique described by Orole and Adejumo (2009). This method involves placing a 5 mm diameter disk of the endophyte fungus from a 6-day culture on a PDA plate, and then placing another disk of the same diameter of the phytopathogenic fungus (6-day-old culture) at the opposite end of the medium, with a 50 mm distance between the two disks. The plates inoculated with the endophyte and the control plates, which did not contain the endophyte, were incubated at 28°C. After eight days of incubation, the radial growth of each pathogenic fungus was measured in relation to the endophyte fungus as well as in the control plates. The percentage of inhibition was calculated using the following formula:

$$\text{Percentage of Inhibition (PI)} = (R1 - R2) / R1 \times 100$$

Where:

R1: Radial growth of the pathogen in the control;

R2: Radial growth of the pathogen in the dual culture.

Enzymatic activity of isolated endophytes

Catalase activity

The isolated endophytes were tested for hydrogen peroxide (H_2O_2) hydrolysis using the slide method. Fungal strains grown on PDA agar and incubated at 30°C for 7 days were used for the test. A drop of hydrogen peroxide was placed on the test slides, and then a 1 mm² fragment of a young fungal endophyte culture was picked with a sterile platinum loop and placed into the hydrogen peroxide drop. The production of peroxidases was visually detected by observing immediate bubbling (Attia *et al.*, 2020).

Cellulase activity

To test the production of cellulase by fungal strains, PDA medium supplemented with 0.5% carboxymethylcellulose was used. After 3 to 5 days of fungal growth, the plates were flooded with a 0.2% aqueous Congo Red solution and decolorized with 1M NaCl for 15 minutes. The appearance of yellow zones around the fungal colonies indicates cellulase production by the strains (Marsola *et al.* 2022).

Protease activity

A PDA medium containing 0.4% casein (pH=6) was used. After an incubation period of 3 to 5 days, the degradation of casein appears as a clear zone around the colonies, indicating protease production by the fungal strain (Usman *et al.*, 2023).

Lipase activity

To test this activity, Peptone Agar medium (peptone 10 g, NaCl 5 g, $CaCl_2 \cdot 2H_2O$ 0.1 g, agar 16 g, distilled water 1L; pH 6.0) supplemented with 1% Tween 20 was used. After an incubation period of 3 to 5 days, the appearance of a visible precipitate around the colonies indicates positive lipase activity, facilitated by the formation of calcium salts of lauric acid released by the enzyme (Sunitha *et al.*, 2013).

Amylase activity

A PDA medium containing 0.2% starch (pH=6) was used. After an incubation period of 3 to 5 days, starch degradation appears as a clear zone around the colonies after staining with 2% Lugol's solution, indicating amylase production by the fungal strain (Taneja *et al.*, 2023).

Determination of total polyphenol and flavonoid content in fungal and bacterial endophytes of Khaya senegalensis

Total polyphenol assay of Khaya senegalensis endophytes

The total polyphenol content was measured using spectrophotometry, following the colorimetric method with the Folin-Ciocalteu reagent as described by Singleton *et al.* (1999). Briefly, 50 µL of the diluted (1/100 in distilled water) extract solution at 25 mg/mL was added to 250 µL of 10% Folin-Ciocalteu reagent (diluted 1:10 in distilled water) and 750 µL of a 75 g/L sodium carbonate (Na_2CO_3) aqueous solution. After an 8-minute incubation, 950 µL of distilled water were added and mixed by vortexing, then incubated in the dark at room temperature for 2 hours. After incubation, the optical densities (OD) were read at 760 nm using a CECIL CE 2041 spectrophotometer. The reading was performed against a blank consisting of a mixture of 250 µL of Folin-Ciocalteu reagent and 750 µL of Na_2CO_3 and 1 mL of distilled water.

Total flavonoid assay of Khaya senegalensis endophytes

The quantification of flavonoids was carried out using a method adapted from Zhishen *et al.* (1999) and Kim *et al.* (2003), using aluminum chloride ($AlCl_3$) as the reagent. Thus, 500 µL of a 2% $AlCl_3$ solution were added to 500 µL of the sample. To this mixture, 3 mL of ethanol was added. The blank consisted of 500 µL of $AlCl_3$ and 3.5 mL of ethanol. Absorbance readings were taken at 415 nm using a spectrophotometer after a 10-minute incubation. The samples were prepared in triplicate for each analysis. The total flavonoid content was determined in mg of rutin equivalent per gram of extract (µg RuE/g) using the formula employed by Ahmed *et al.* (2019).

Results

Isolation of endophytes from Khaya senegalensis

The results of the isolation of endophytes from *Khaya senegalensis* are presented in Table 1.

Table 1. Frequency of isolation of endophytic bacteria from *Khaya senegalensis* based on organs

Endophytes	Leaves	Stems	Roots
Bacterial	46.67%	33 ;33%	20.00%
Fungal	50.00%	28.57%	21.43%

The results show that the leaves have the highest frequency of endophytic bacteria (46.67%), followed by the stems (33.33%) and the roots (20%). Similarly, fungal endophytes are more frequently isolated from the leaves (50%), with lower frequencies observed in the stems (28.57%) and roots (21.43%).

Pre-identification of endophytes from Khaya senegalensis

The analysis of Fig. 1 and Fig. 2 reveals the distribution of endophytic bacterial species and their occurrence in different plant organs. Fig. 1 indicates that the isolated species are either *Bacillus* spp. (46.67%) or *Listeria* spp./*Corynebacterium* spp. (53.33%). In Fig. 2, it is shown that *Bacillus* spp. is more frequently isolated from the leaves (57.14%), whereas *Listeria* spp./*Corynebacterium* spp. is more commonly found in the stem (50%).

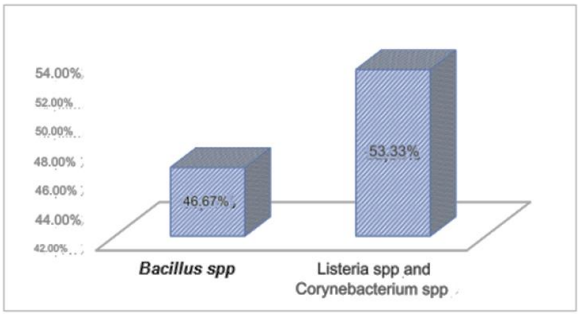


Fig. 1. Distribution of endophytic bacterial species

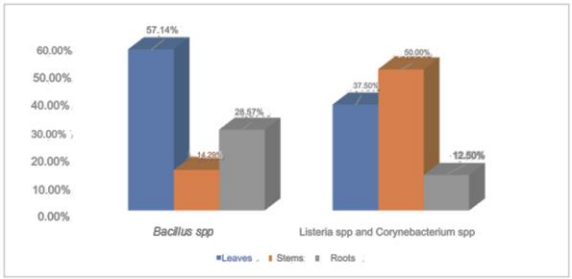


Fig. 2. Distribution of endophytic bacterial species according to the plant organs

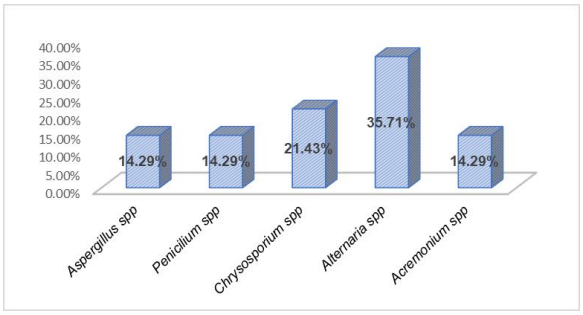


Fig. 3. Distribution of endophytic fungal species

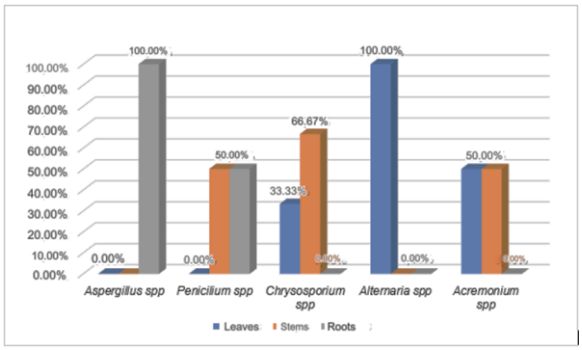


Fig. 4. Distribution of endophytic fungal species according to plant organs

Similarly, Fig. 3 and Fig. 4 illustrate the distribution of endophytic fungal species and their presence in various plant organs. Fig. 3 identifies five different fungal genera: *Alternaria* spp. (35.71%), *Chrysosporium* spp. (21.43%), *Acremonium* spp. (14.29%), *Aspergillus* spp. (14.29%), and *Penicillium* spp. (14.29%). Fig. 4 demonstrates that all fungi of the genus *Aspergillus* spp. have been isolated from the roots (100%), while all fungi of the genus *Alternaria* spp. have been isolated from the leaves (100%). Fungi of the genus *Penicillium* spp. have been found in both the stems and the roots, whereas fungi of the genus *Acremonium* have been found in the leaves and stems. *Chrysosporium* spp. has been predominantly found in the stems (66.67%).

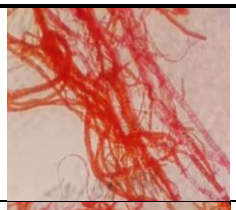
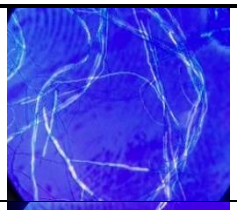
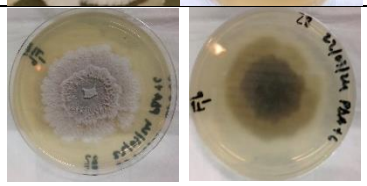
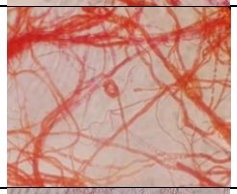
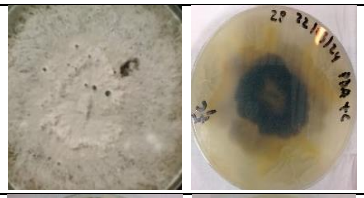
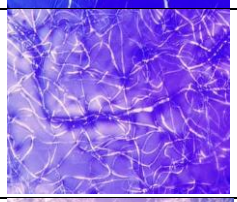
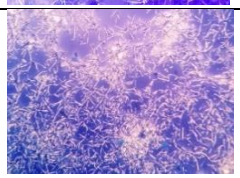
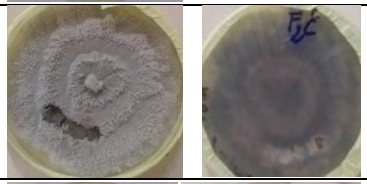
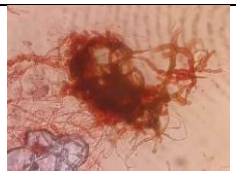
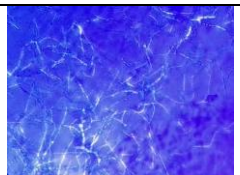
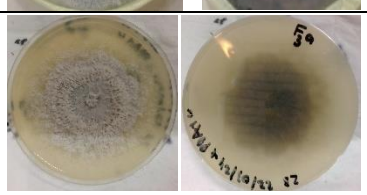
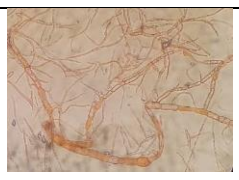
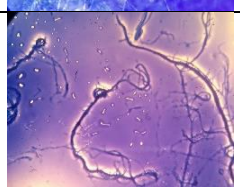
The macroscopic and microscopic characteristics of the various isolated endophytes (bacterial and fungal) are presented in Table 2.

Antimicrobial activities of Khaya senegalensis endophytes

The evaluation of the antimicrobial properties of the different isolated endophytes showed that none

of the bacterial endophytes exhibited antimicrobial activity against the tested strains. However, the fungal endophytes demonstrated variable antimicrobial activity depending on the targeted microorganism. For *E. coli*, endophytes F1a, F1b, F1c, and R1a showed strong activity with inhibition diameters greater than 20 mm, while F2c, F3a, F3b, T1c, T2b, and T2c exhibited moderate activity (10 to 16 mm), and F2a, F2b, and T2a showed low activity (6 to 7 mm). Regarding *S. aureus*, F3a and F3b displayed strong activity (10 mm), while F1c, F2a, F2c, and T2b showed moderate activity (5 to 9 mm). The endophyte T2a showed low activity (4 mm), and several endophytes (F1a, F1b, F2b, T1c, R1a, and R1b) showed no activity (Table 3). For *Pseudomonas aeruginosa*, the endophyte R1a exhibited activity with an inhibition diameter of 6.67 mm. Endophytes F1a, F1c, F3b, T1c, and T2c demonstrated moderate activity (5 to 6.67 mm), while F2b, F3a, and T2a showed low activity (1.67 to 3.67 mm). Several endophytes (F2a, F2c, T2b, and R1b) showed no inhibition against Pseudomonas. None of the tested endophytes exhibited activity against *C. candida*.

Table 2. Macroscopic (on PDA medium) and microscopic (stained with Congo Red and Methylene Blue) characteristics of endophytes from *Khaya senegalensis*

Code	PDA medium	Microscopy (Congo Red)	Microscopy (Methylene Blue)
F1a *Alternaria* spp. 1			
F1b *Alternaria* spp. 2			
F1c *Alternaria* spp. 3			
F2a *Acremonium* spp.			
F2c *Alternaria* spp. 4			
F3a *Alternaria* spp. 5			

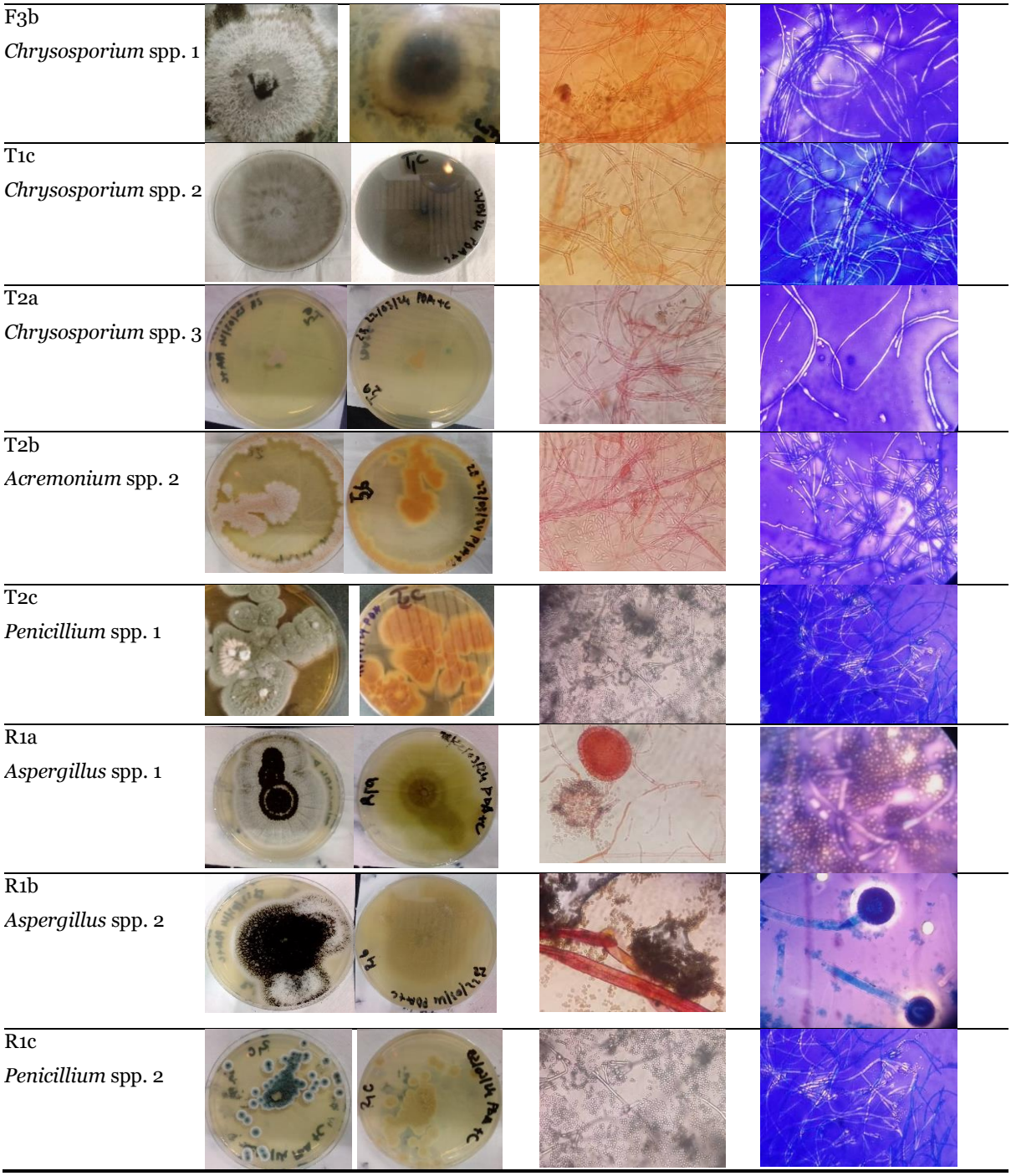


Table 3. Inhibition Diameter of microbial strains by *Khaya senegalensis* fungal endophytes

Fungal Endophytes	Inhibition diameter (mm) *E. coli*	Inhibition diameter (mm) *S. aureus*	Inhibition diameter (mm) *Pseudomonas aeruginosa*	Inhibition diameter (mm) *C. Candida*
F1a	20.33	0	6.66	0
F1b	22.33	0	5	0
F1c	20.33	7.33	6.66	0
F2a	7	5	0	0
F2b	6.33	0	5	0
F2c	12.66	8,33	0	0
F3a	12.33	10	3.66	0
F3b	11.33	10	5	0
T1c	14	0	5	0
T2a	7	4	1.66	0
T2b	13.33	9	0	0

T2c	15.66	3.66	5.33	0
R1a	23	0	6.66	0
R1b	10.66	0	0	0
R1c	13.66	5	0	0

Table 4. Enzymatic production based on bacterial endophyte species

Enzyme	*Bacillus* spp	*Listeria* spp/ *Corynebacterium* spp	Total
Catalase	100% (7)	100% (8)	100% (15)
Amylase	85.71% (6)	87.5% (7)	86.66% (13)
Protease	57.14% (4)	62.5 (5)	60% (9)
Lipase	0% (0)	0% (0)	0% (0)
Lecithinase	85.71% (6)	87.5% (7)	86.66% (13)
Hemolysin	100% (7)	37.5% (3)	66.66% (10)
Cellulase	0% (0)	0% (0)	0% (0)

Table 5. Enzymatic activity of fungal endophytes

Enzyme	*Aspergillus* spp	*Penicillum* spp	*Chrysosporum* spp	*Alternaria* spp	*Acremonium* spp	Total
Amylase	0% (0)	50% (1)	66.66% (2)	100% (4)	50% (1)	61.53% (8)
Protease	0% (0)	50% (1)	33.33% (1)	0% (0)	0% (0)	15.38% (2)
Esterase	100% (2)	50% (1)	0% (0)	0% (0)	0% (0)	23.07% (3)
Lecithinase	0%(0)	0% (0)	0% (0)	0% (0)	0% (0)	0% (0)
Hemolysin	100% (2)	50% (1)	33.33% (1)	75% (3)	100% (2)	69.23% (9)
Cellulase	0% (0)	0% (0)	33.33% (1)	100% (4)	0% (0)	38.46% (5)

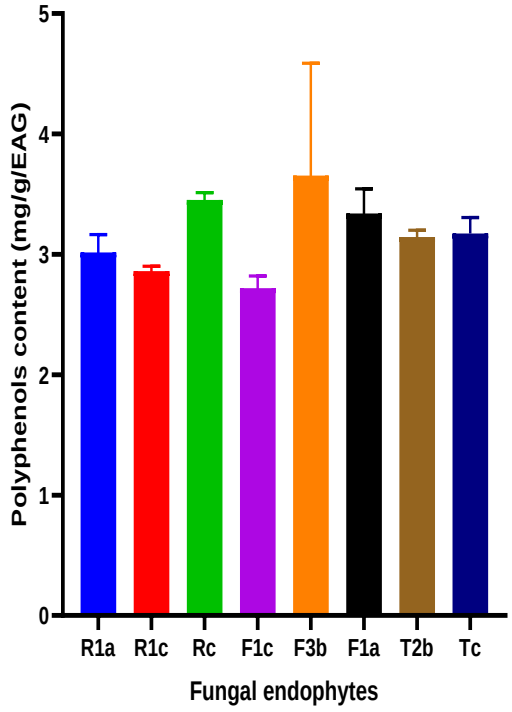


Fig. 5. Polyphenol content of fungal isolates

Enzymatic activities of isolated endophytes

The enzymatic production of bacterial and fungal endophytes exhibits distinct characteristics. Catalase production was observed in all isolated bacterial endophytes.

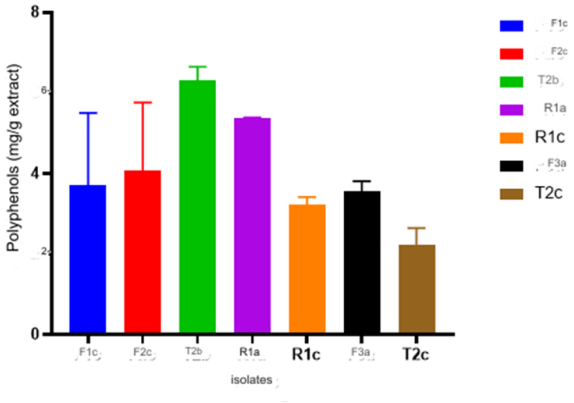


Fig. 6. Polyphenol content of bacterial isolates

All bacterial endophytes (100%) produced catalase, and the majority also produced amylase and lecithinase (86.66%). None of the isolated bacterial endophytes produced lipase or cellulase (0%) (Table 4). More than 60% of the fungal endophytes produced hemolysin (69.23%) and amylase (61.53%). All fungal endophytes isolated from the genus *Aspergillus* produced esterase and hemolysin. All fungal endophytes isolated from the genus *Alternaria* produced amylase and cellulase. Fungal endophytes isolated from the genus *Acremonium* produced hemolysin. No isolated fungal endophyte produced lecithinase (0%) (Table 5).

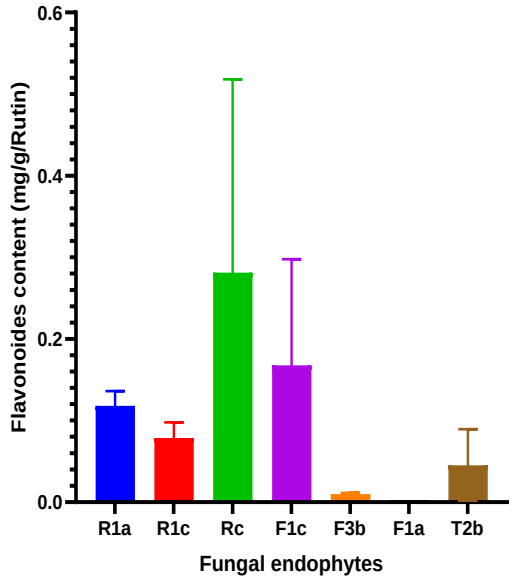


Fig. 7. Flavonoids content of bacterial isolates

Determination of total polyphenol content in fungal endophytes

Fig. 5 illustrates the polyphenol content of fungal isolates. The isolates F1a, Rc, and F3b exhibited the highest levels of total polyphenols. In contrast, the isolates with lower polyphenol content were R1c and F1c. Fig. 6 displays the polyphenol content of bacterial isolates. Analysis of this figure shows that there is no significant difference in the total polyphenol content among the various bacterial isolates.

Determination of total flavonoid content in endophytes

Fig. 7 shows the flavonoid content of different endophyte strains. The endophytes exhibit very low levels of flavonoids, comparable to trace amounts.

Discussion

Endophytes present in medicinal plants represent a new frontier in pharmaceutical and biotechnological research (Upadhyay *et al.*, 2016). These microorganisms have the capacity to produce bioactive molecules, offering innovative and promising solutions to replace conventional treatments, particularly in the face of growing antibiotic resistance. The present study aims to explore the diversity and biological potential of endophytes isolated from *Khaya senegalensis* in Benin.

Medicinal plants studied for their endophytes generally have a long history of traditional use and are known to produce a wide variety of secondary metabolites with antimicrobial and antioxidant properties (Fadiji and Babalola, 2020). Adapted to various environments, they provide a broad range of microenvironments conducive to the discovery of microorganisms capable of synthesizing potentially therapeutic bioactive compounds (Kuźniar *et al.*, 2019). The results of isolating endophytes from *Khaya senegalensis* reveal colonization of different parts of the plant by both bacterial and fungal endophytes. Results show that leaves have the highest frequency of endophytic bacteria (46.67%), followed by stems (33.33%) and roots (20.00%). Similarly, fungal endophytes are more frequently isolated from leaves (50.00%), with lower frequencies observed in stems (28.57%) and roots (21.43%). These results indicate a strong presence of endophytes in the aerial parts of the plant, suggesting a specific adaptation of these microorganisms to the ecological niches provided by *Khaya senegalensis* (Lin *et al.*, 2022). Similar results have also been obtained in several plants from the African pharmacopoeia (Ntemafack *et al.*, 2021).

The diversity of microbial species among endophytes sometimes reveals interesting trends in distribution based on the organs (Duan *et al.*, 2019). The isolated species primarily include *Bacillus* spp. (46.67%) and *Listeria* spp./*Corynebacterium* spp. (53.33%). Analysis shows that *Bacillus* spp. is more frequently isolated from leaves (57.14%), while *Listeria* spp./*Corynebacterium* spp. is more commonly found in stems (50%).

Regarding the fungal endophytes isolated in this study, the analysis of fungal species diversity highlights the presence of five different genera: *Alternaria* spp. (35.71%), *Chrysosporium* spp. (21.43%), *Acremonium* spp. (14.29%), *Aspergillus* spp. (14.29%), and *Penicillium* spp. (14.29%). All fungi of the genus Alternaria spp. were isolated from leaves (100%), while those of the genus *Penicillium* spp. were found both in stems and roots.

Fungi of the genus *Acremonium* were detected in leaves and stems, and *Chrysosporium* spp. was predominantly found in stems (66.67%). These results suggest an ecological preference of these microorganisms for specific plant organs, possibly reflecting distinct microenvironmental niches (Jain *et al.*, 2021; Mamangkey *et al.*, 2022).

Bacterial endophytes are known for their ability to produce a range of enzymes such as cellulase, lecithinase, and proteases (Marsola *et al.*, 2022). These enzymes play essential roles in key biological processes, such as cellulose degradation, lipid modification, and protein degradation (Tidke *et al.*, 2017). Their detection and in-depth study in research are crucial not only for understanding their functioning in the plant ecosystem but also for exploring their potential in various biotechnological applications, including agriculture and medicine (Agrawal and Bhatt, 2023). The analysis of enzymatic activities and biochemical properties of bacterial and fungal endophytes from *Khaya senegalensis* reveals distinct characteristics. All isolated bacterial endophytes demonstrated catalase production (100%) and a majority also produced amylase and lecithinase (86.66%), while none showed activity for lipase and cellulase (0%). Regarding fungal endophytes, more than 60% produced hemolysin (69.23%) and amylase (61.53%). All isolates of the genus *Aspergillus* spp produced esterase and hemolysin, while those of the genus *Alternaria* spp. produced amylase and cellulase. Endophytes of the genus *Acremonium* spp. showed hemolysin production.

None of the isolated fungal endophytes produced lecithinase (0%). These results highlight the functional diversity of bacterial and fungal endophytes from *Khaya senegalensis*, underscoring their potential in the production of various enzymes and toxins, which could have significant implications in biotechnology and medicine.

Conclusion

This study highlights a differentiated distribution of bacterial and fungal endophytes according to plant organs, with a significant predominance in the leaves for both groups. *Bacillus* spp. dominates among the isolated bacteria, primarily in leaves, while *Listeria* spp./*Corynebacterium* spp. is more frequently found in stems. Among fungi, *Alternaria* spp. is exclusively isolated from leaves. Catalase is produced by all bacteria, while fungi show variability in the production of enzymes such as amylase and hemolysin. These results suggest promising pathways to explore the interactions between endophytes and plants and their potential in agricultural and environmental biotechnology.

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