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PHARMACOGNOSTIC STANDARDIZATION AND ANTIMICROBIAL STUDIES OF THE LEAVES AND SEEDS OF *DELONIX REGIA* (BOOJER .HOOK.) RAF. AND *PELTROPHORUM PTEROCARPUM* (DC) BACKER EX K HEYNE

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ABSTRACT

Standardization is a key process to evaluating the quality, purity and efficacy of natural drugs. The constant rise in antibiotic resistance of certain causative organisms to popular antibiotics has increased the search for alternatives. This study aims to establish pharmacognostic standards for the leaves and seeds of *Delonix regia* and *Peltrophorum pterocarpum* and also to evaluate the antimicrobial potential of their methanolic extracts. The samples were found to contain saponins, alkaloids, flavonoids, and tannins. The microscopic evaluation revealed various epidermal features including the presence of trichomes, venation patterns and stomata cells on the abaxial and adaxial surfaces. The physico-chemical and heavy metal results obtained were within the standard limits. The extracts exhibited both antibacterial and antifungal activities against tested microorganisms showing varying zones of inhibition from 13.7mm - 27.0mm. The Minimum Inhibition Concentration (MIC) of the plant drugs was consistently low ranging from 0.6125 - 10% w/v and this can indicate that the test drugs have high resistance mechanisms to these microorganisms. The Minimum Bactericidal Concentration (MBC) showcase varying antimicrobial strengths against selected microbial strains with *D. regia* leaf extract showing effectiveness at low MBC value of 1.25% W/V. The results underscore the antimicrobial potential of both species, supporting their use as natural alternatives to conventional antibiotics, especially in the treatment of resistant microbial strains. Their phytochemical profiles are key contributors to their efficacy, providing a strong basis for their pharmacognostic and medicinal evaluation.

INTRODUCTION

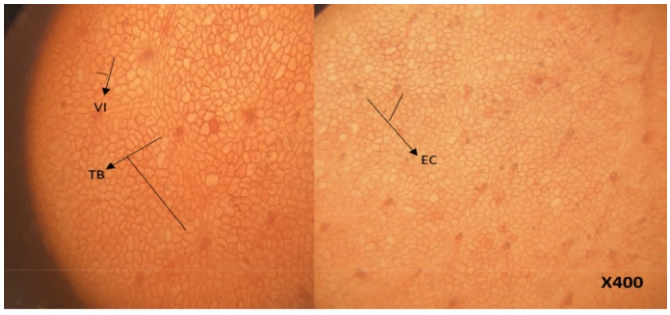
Natural products are integral to pharmaceutical drug development. Approximately 80% of populations in Africa, Asia, Europe, and the Americas depend on traditional medicines for treating ailments [1, 2]. Plant-based traditional medicines are widely used for primary healthcare globally, influenced by sociocultural factors and often linked to disease-related complications. Over 50% of clinical drugs are of natural origin, with many derived from vegetables, fruits, herbs, and spices. Also, WHO estimates 25% of modern pharmaceuticals in the United States is plant-based and over 120 active compounds from higher plants are used in modern medicine, with 80% supporting therapeutic applications of their source plants [3]. However, issues such as adulteration, poor quality controls, inadequate labeling, and untested adverse effects pose risks to consumers as reported in previous research [4, 5, 6].

Commercialization has made standardization vital for ensuring the safety, efficacy, and quality of medicinal plants. Variability in raw materials arises from factors such as plant identity, seasonal changes, and storage conditions [7]. Standardization provides guidelines for adjusting herbal drugs to known therapeutic content, enhancing consumer safety and legitimizing herbal healthcare [8]. This is the reason why this research is aimed at establishing pharmacognostic standards for the leaves and seeds of *Delonix. regia* and *Peltophorum pterocarpum,* aiding their identification and validating their antimicrobial potential.

Delonix regia or "flame of the forest," is a semi-deciduous tree valued for uses such as livestock forage, firewood, and remedies for piles, asthma, and helminthiasis [9, 10]. Its ethnomedicinal applications include fever treatments and bronchitis therapy, and studies indicate its antirheumatic potential [9, 10] *Peltophorum pterocarpum* is a deciduous tree with fragrant yellow flowers, known for treating insomnia, dysentery, skin issues, and pain. Its flowers are sleep inducers, while its bark is used for conditions such as sores and stomatitis [11].

RESULTS

Microscopic Evaluation



(A) (B)

Figure 1: Upper Epidermal Layer (Adaxial layer) of P. pterocarpum x400 showing Vein Islets (VI), Trichome Base (TB) and Epidermal cells (EC)

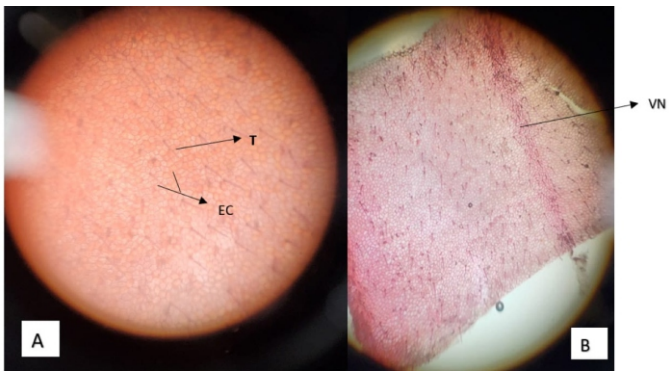


Figure 2: Upper Epidermal Layer (Adaxial layer) of *D. regia* at x100 (A) and x40 (B) showing key features such as Unicellular Trichomes (T), Simple Epidermal Cells (EC) and Venation (VN)

The epidermal features of *D.regia* and *P. pterocarpum* consist of stomata and trichomes. The adaxial surface of *D. regia* shows a large number of epidermal cells (polygonal shaped) with many unicellular trichomes while in *P. pterocarpum* also shows a large amount of epidermal cells (polygonal shaped) and has a lot of trichome bases but no trichome was seen. Many vein islets were also observed on the adaxial surface of *P. pterocarpum.* The adaxial surface of *D. regia* and *P. pterocarpum* has no stomata on them.

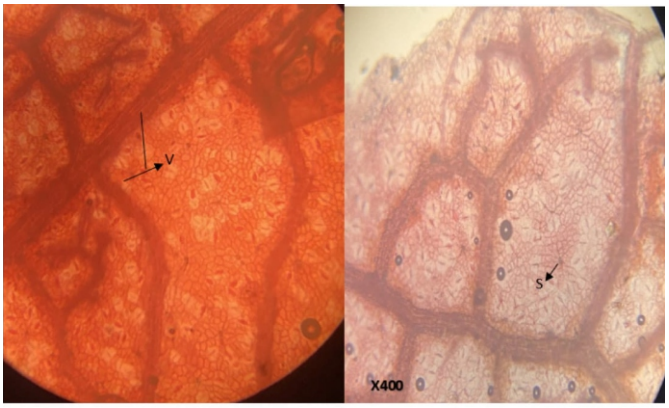


Figure 3: Lower Epidermal Layer (Abaxial layer) of *P. pterocarpum* at x400 showing Anomocytic Stomata cells (S) and Veination pattern (V)

The stomatal (Anomosytic) were found to be present on the abaxial surface of the epidermis of *P. pterocarpum*. Hence, the plant is hypostomatic. The venation pattern of the leaf was also very prominent in the abaxial surface. The abaxial surface of *D. regia* also showed the presence of few deposits of anomocytic stomata between the epidermal cells. There were also unicellular trichomes present in the abaxial surface

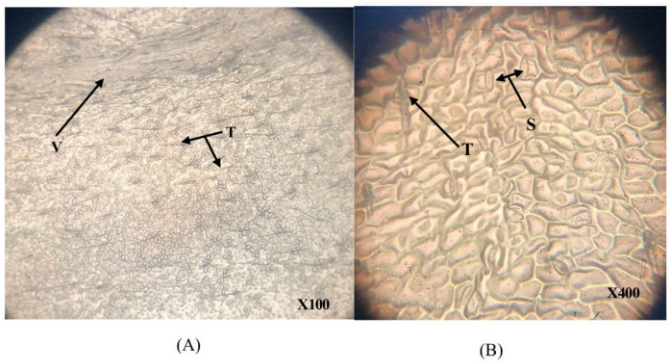


Figure 4: Lower Epidermal Layer (Abaxial layer) of *D. regia* at x100 and x400 respectively showing Venation pattern (V), Anomocytic stomata (S), and Trichomes (T)

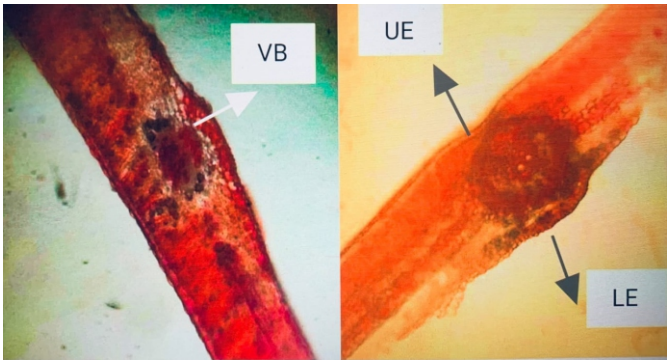


Figure 5: Transverse sections of *D. regia* (A) and *P. pterocarpum* (B) leaves at x100 showing key features such as lower epidermis (LE), upper epidermis (UE), and vascular bundles (VB).

A transverse section in the leaflets shows upper and lower epidermises on *D. regia* and *P. pterocarpum* enclosed within the mid rib. The transverse section of the mid rib of both plants revealed the presence of palisade tissues, cortex and epidermal cells. *D. regia* and *P. pterocarpum,* mesophyll is heterogenous, differentiated into palisade and sponge tissue. The palisade is formed of one row of columnar, closely packed cells having straight walls.

Table 1: Physical constant for Leaves and Seeds of *D. regia* and *P. pterocarpum*

	DRL (%)	DRS (%)	PPL (%)	PPS (%)
Total Ash	7.35	5.14	5.80	5.20
Water Soluble Ash	1.43	1.21	1.05	1.18
Acid Insoluble Ash	2.50	2.12	2.80	2.10
Alcohol Extractive Values	3.15	2.06	2.15	2.05
Water Extractive Values	13.20	12.60	11.75	12.20

Table 2: Chemo-Microscopy analysis of the leaves of *D. regia* and *P. pterocarpum*

TEST	OBSERVATION	*D. regia*		*P. pterocarpum*	
LIGNIN TEST **(Phloroglucinol + Conc. HCl)**	Red Colour Observed	Present (+)	Present (+)	Present (+)	Present (+)
STARCH TEST **(+N50 Iodine)**	Blue-black coloration	Absent (-)	Absent (-)	Absent (-)	Absent (-)
MUCILAGE TEST **(+Ruthenium Red)**	Red or dark pink coloration	Present (+)	Present (+)	Present (+)	Present (+)
CELLULOSE TEST	Blue coloration	Absent (-)	Absent (-)	Absent (-)	Absent (-)
FATTY OIL **(+ Sudan IV reagent)**	Pink color	Present (+)	Present (+)	Present (+)	Present (+)
PROTEIN **(+1% Picric acid + Millions Reagent)**	Red color	Present (+)	Present (+)	Present (+)	Present (+)
CALCIUM OXALATE **(+hydrochloric acid)**	Bright crystals observed	Present (+)	Present (+)	Present (+)	Present (+)

Table 3: Proximate analysis of powdered Leaves and Seeds of *D. regia* and *P. pteroca rpum*

	DRL	DRS	PPL	PPS
Moisture content (%)	7.15	10.05	7.25	7.78
Crude Protein (%)	22.77	20.14	16.20	15.32
Crude Fibre (%)	28.02	27.90	18.72	20.29
Crude Fat (%)	2.47	2.52	0.86	2.60

DRL (*D. regia* leaf**), DRS (***D. regia* seed**), PPL (***P. pterocarpum* leaf**), PPS** (*P. pterocarpum* Seed)

Table 4: Phytochemical screening result for Leaves and Seeds of *D. regia* and *P. pterocarpum*

TEST	DRL	DRS	PPL	PPS
SAPONINS				
Frothing test	+	+	+	++
ALKALOIDS				
Meyers Test	+	+	+	+
Wagners Test	+	+	+	+
Dragendoffs Test	+	+	+	+
TANNINS	+	++	+	+
FLAVANOID	+++	+	+	+
GLYCOSIDES				
Keller-Kehlani	-	+	+	+
Keddes Test	-	+	-	+
ANTHRAQUINONE	-	-	-	-

DRL (*D. regia* leaf**), DRS (***D. regia* seed**), PPL (***P. pterocarpum* leaf**), PPS** (*P. pterocarpum* Seed). *Note:* (+) indicates the presence while (-) indicates the absence

The phytochemical screening of *Delonix regia* and *Peltophorum pterocarpum* highlights consistent presence of saponins, alkaloids, flavonoids, and tannins across samples, selective absence of anthraquinones, and notable variations in glycosides.

Table 5: Heavy metal analysis of *D. regia* and *P. pterocarpum* Leaf and Seeds

Heavy Metal	DRL (mg/kg)	DRS (mg/kg)	PPL (mg/kg)	PPS (mg/kg)	WHO Limit (mg/kg)
Lead (Pb)	7.95	6.70	5.25	5.80	10.0
Cadmium (Cd)	--	--	--	--	--
Nickel (Ni)	4.15	1.90	6.70	9.55	N/A
Cobalt (Co)	--	--	--	--	3.4
Copper (Cu)	9.45	8.40	4.70	12.45	20.00

DRL (*D. regia* leaf**), DRS (***D. regia* seed**), PPL (***P. pterocarpum* leaf**), PPS** (*P. pterocarpum* Seed). *Note:* (-) indicates the absence

ANTIMICROBIALASSAY

The extracts were tested against four Gram-negative bacteria (*Eschericia coli, Klebsiella pneumonia, Acinetobacter baumannii and Pseudomonas aeruginosa*), one Gram positive bacteria (*Methicillin Resistant Staphylococcus aeurus MRSA*) and a fungus (*Candida albicans*). The extracts were administered at 20% w/v.

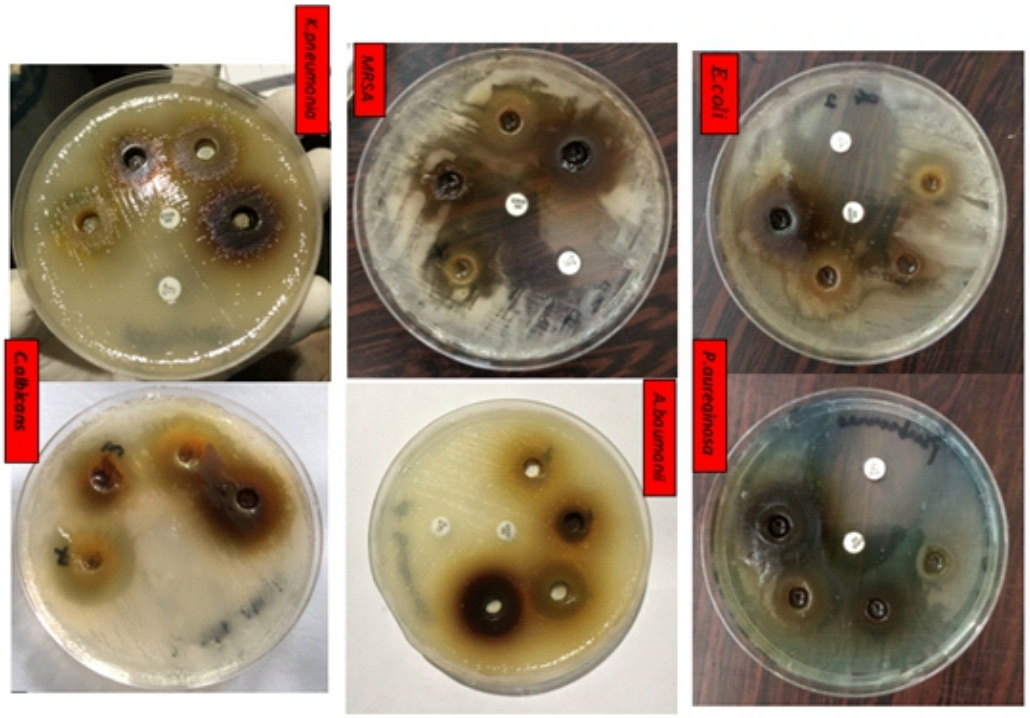


Figure 6: showing zones of inhibition of the leaves and seeds of *D. regia* and *P. pterocarpum* against microorganisms

Table 6: MIC of Leaves and Seeds of *D. regia* on selected strains of Microorganisms (mm)

Test Organisms	DRL	DRS	CIP	CRO
Escherichia coli (2)	25.0 ± 0.83	22.5 ± 1.41	30.0±0.00	24.0±0.00
Pseudomonas aureginosa	24.3 ± 0.52	21.3 ± 0.86	20.0±0.00	23.0±0.00
Meticillin Resistant Staphlococcus aureus (MRSA)	25.0 ± 0.53	19.3 ± 0.91	25.0±0.00	20.0±0.00
Klebsiella pneumonia (3)	19.2 ± 0.81	23.0 ± 0.78	R	R
Acinetobacter baumannii	23.0 ± 0.00	20.5 ± 1.7	R	R
Candida albicans	20.0 ± 0.81	26.8 ± 0.47	--	--

DRL (*D. regia* leaf**), DRS (***D. regia* seed**), CIP (**Ciprofloxacin), **CRO** (Cotrimoxazole). Values represent mean ± Standard Deviation (SD) from three (3) independent replicates. (n = 3)

Table 7: MIC of leaves and seeds of *P. pterocarpum* on selected strains of Microorganisms (mm)

Test Organisms	PPL	PPS	CIP	CRO
Escherichia coli (2)	13.7 ± 0.51	18.7 ± 0.50	30.0±0.00	24.0±0.00
Pseudomonas aureginosa	16.0 ± 0.00	14.7 ± 0.52	20.0±0.00	23.0±0.00
Meticillin Resistant Staphlococcus aureus (MRSA)	24.0 ± 0.49	20.0 ± 0.81	25.0±0.00	20.0±0.00
Klebsiella pneumonia (3)	18.3 ± 0.53	17.0 ± 0.00	R	R
Acinetobacter baumannii	18.7 ± 0.78	17.3 ± 0.53	R	R
Candida albicans	23.0 ± 0.00	27.3 ± 0.94	--	--

PPL (*P. pterocarpum* leaf**), PPS (***P. pterocarpum* seed**), CIP (**Ciprofloxacin), **CRO** (Cotromoxazole), Values represent mean ± Standard Deviation (SD) from three (3) independent replicates. (n = 3)

Table 8: Minimum Inhibition Concentration of Leaves and seeds of *D. regia* and *P. pterocarpum*

Test Organisms	DRL (% W/V)	DRS (% W/V)	PPL (% W/V)	PPS (% W/V)
Escherichia coli	2.50	0.6125	5.0	2.5
Pseudomonas aureginosa	1.25	2.50	0.6125	1.25
Meticillin Resistant Staphlococcus aureus (MRSA)	2.50	1.25	5.0	2.50
Klebsiella pneumonia	5.0	2.50	1.25	0.6125
Acinetobacter baumannii	1.25	0.6125	2.5	0.6125
Candida albicans	10.0	2.50	10.0	5.0

DRL (*D. regia* leaf**), DRS (***D. regia* seed**), PPL (***P. pterocarpum* leaf**), PPS** (*P. pterocarpum* Seed)

Table 9: Minimum Bacterial Concentration of Leaves and Seeds of *D. regia* and *P. pterocarpum*

Test Organisms	DRL (% W/V)	DRS (% W/V)	PPL (% W/V)	PPS (% W/V)
Escherichia coli	5.0	1.25	10.0	5.0
Pseudomonas aureginosa	2.5	5.0	1.25	2.50
Meticillin Resistant Staphlococcus aureus (MRSA)	5.0	2.50	10.0	5.0
Klebsiella pneumonia	10.0	5.0	2.50	1.25
Acinetobacter baumannii	2.50	1.25	5.0	2.50
Candida albicans	20	5.0	20.0	5.0

Delonix regia leaves extract shows the highest zone of inhibition of 25.0mm, 24.3mm, and 25.0mm against *Escherichia coli, Pseudomonas aeruginosa,* and MRSA, respectively, when compared to the other extracts. All the extracts had activity against *Klebsiella pneumoniae* and *A. baumanii,* while the standards were resistant to these organisms. The highest zone of inhibition for *Klebsiella pneumonia* was observed in the seeds of *Delonix regia* at 23.0mm, while for *A. baumanii, D. regia* seeds had the highest zone of inhibition at 23.0mm. For the antifungal activity, the highest zone of inhibition against *Candida albicans* was observed in the seeds of *P. pterocarpum* at 27.3mm, while the antibiotic used (Fluconazole) had a zone of 20mm.

DISCUSSION

Herbal drugs, derived from plants or their parts, undergo simple processes from harvest to production to transform them into phytopharmaceuticals. Standardization is vital in evaluating such crude drugs, encompassing aspects like raw material selection, handling, safety, efficacy, quality, and stability assessments of the final products. According to Mukherjee et al. [12] and as corroborated by much of the literature, medicines, whether synthetic or plant-based, must adhere to basic principles of safety, efficacy, and quality to ensure therapeutic effectiveness. The growing challenge of antibiotic resistance continues to impact healthcare systems worldwide. This problem is especially pronounced in the context of multidrug-resistant pathogens, which compromise the efficacy of current antibacterial therapies. Consequently, the demand for alternative sources of antimicrobial agents has surged, with plants serving as promising candidates due to their production of bioactive compounds with established therapeutic properties.

Micromorphological variations observed in the study provide a taxonomic basis for distinguishing these plants, aligning with prior reports by Albert and Sharma [13] on the significance of leaf epidermal and anatomical features in plant taxonomy. These features include stomata, trichomes, and vein islets, which hold substantial taxonomic importance. Specifically, the adaxial epidermis of *Delonix regia* exhibits polygonal epidermal cells with numerous unicellular trichomes, but no stomata were observed on this surface. *Peltophorum pterocarpum*, while also featuring polygonal epidermal cells, displays numerous trichome bases but lacks visible trichomes. Multiple vein islets were identified on the adaxial surface. These observations correlate with findings from Sonibare et al. [14], who documented similar characteristics in the microscopic analysis of *Erythrophleum suaveolens* and *Peltophorum pterocarpum.* Such micromorphological markers enhance plant identification and support their taxonomic classification.

Chemomicroscopic examination of the powdered samples revealed the presence of lignin, mucilage, calcium oxalate crystals, mucilage, fatty oil, and protein. All samples tested negative for the presence of cellulose and starch. The presence of these features (lignin, mucilage, calcium oxalate crystals, mucilage, fatty oil, and protein) can also be used as a standard for the identification and detection of adulterants in the plant drug. Bruce, Onyegbule, and Ezegwu [15] also revealed the presence of lignin, starch, mucilage, calcium oxalate crystals, cellulose, fatty oil, and protein in the leaves of *Fadogia cienkowski*.

The samples of leaves and seeds of *Delonix regia* and *Peltrophorum pterocarpum* were screened for saponins, alkaloids, flavonoids, tannins, anthraquinones, and glycosides. The result is summarized in Table 4. All the samples of the selected plant parts were found to contain saponins, alkaloids, flavonoids, and tannins. *Delonix regia* leaf tested negative for glycosides. All the samples tested negative for anthraquinones. The presence of alkaloids in the plants can indicate its use as a potential antimicrobial agent as they work by inhibition of DNA topoisomerase [16]. The high content of saponins in the seed of *Peltrophorum pterocarpum* and all the other samples has suggested the industrial values of these bioactive ingredients present in the leaf [17]. Karem, German-Shashoua, and Yarden [18] earlier revealed that saponins have shown anticarcinogenic, neuroprotective, hypoglycemic, immunomodulatory, hypocholesterolemic, hepatoprotective, antimutagenic, anticoagulant, anti-inflammatory, and antioxidant activities in *Cicer arietinum* following a microwave-assisted bioactive saponin extraction. According to the review done by Pikhtirova, Pecka-Kielb, and Zigo [19], the Saponin level and antimicrobial effect may vary from one plant to the other.

The heavy metal analysis of *Delonix regia* (DR) and *Peltophorum pterocarpum* (PP) (Table 5) reveals that Lead (Pb) concentrations in all samples (DRL: 7.95 mg/kg, DRS: 6.70 mg/kg, PPL: 5.25 mg/kg, PPS: 5.80 mg/kg) are below the WHO permissible limit of 10.0 mg/kg, indicating safety for medicinal use in terms of lead content. Nickel (Ni) was detected in varying amounts (DRL: 4.15 mg/kg, DRS: 1.90 mg/kg, PPL: 6.70 mg/kg, PPS: 9.55 mg/kg), though no specific WHO limit is provided. Literature suggests that excessive nickel can inhibit plant growth and secondary metabolite production, potentially affecting antimicrobial efficacy [20]. Copper (Cu) was found in safe concentrations (DRL: 9.45 mg/kg, DRS: 8.40 mg/kg, PPL: 4.70 mg/kg, PPS: 12.45 mg/kg), well below the WHO limit of 20.0 mg/kg. Copper is essential for enzymatic activities and secondary metabolite synthesis, which may enhance antimicrobial properties. Conversely, nickel stress has been shown to disrupt metabolic pathways, potentially reducing the synthesis of bioactive compounds [20]. Environmental pollution from nickel may be due to industry, the use of liquid and solid fuels, as well as municipal and industrial waste. Nickel contact can cause a variety of side effects on human health, such as allergy, cardiovascular and kidney diseases, lung fibrosis, lung and nasal cancer [21]. The findings align with research emphasizing the need for controlled environmental conditions to optimize the medicinal quality of plants [22].

The absence of Cadmium (Cd) and Cobalt (Co) in all samples indicates no toxicological concerns from these metals as far as the study is concerned. The absence of cadmium and cobalt, along with permissible levels of lead and copper, ensures the safety of these plant species for medicinal use. The presence of nickel, while not exceeding critical thresholds, warrants attention as it may influence secondary metabolite production. Environmental factors such as soil composition and pollution levels could have contributed to the observed heavy metal concentrations, potentially impacting the quality of the antimicrobial assay results [22].

The low level of moisture content in the leaves of *Delonix regia* (7.15%) and *Peltophorum pterocarpum* (7.25%), as observed in Table 3 shows their likelihood to reduce microbial growth and spoilage, thereby enhancing extract stability and shelf life. The high protein content and substantial crude fibre of *D. regia* leaves at 22.77% suggest a potential source of bioactive peptides or enzymes that could disrupt microbial cell walls as well as serve as a physical barrier against microbial invasion. Alagbe, Adeoye, and Oluwatobi [23] had earlier explored the nutritional and phytochemical composition of *D. regia,* highlighting its therapeutic applications.

The pharmacognostic physical constant analysis of *Delonix regia* and *Peltophorum pterocarpum,* as observed in Table 1, revealed key differences in their leaves and seeds. *D. regia* leaves consistently showed higher mineral content, water-soluble compounds, and alcohol extractives, making them richer in phytochemicals. Conversely, *P. pterocarpum* leaves were notable for their higher acid-insoluble ash, indicating a greater presence of siliceous material. The research of Aulifa et al. [24] highlighted those siliceous materials in plant extracts, primarily composed of silica, play a significant role in the

therapeutic applications of medicinal plants. Silica is known for its beneficial effects on human health, particularly in promoting skin, hair, and nail health, as well as supporting bone strength and connective tissue integrity.

Antimicrobial assay results for all the extracts showed activity against the organisms which confirms the works of [14, 25] where the antimicrobial activity of both leaves (*Peltophorum pterocarpum* and *Delonix regia*) was tested and confirmed active. The MIC results for *D. regia* and *P. pterocarpum* demonstrate their antimicrobial activity against selected microbial strains, with varying degrees of efficacy compared to the standards Cotrimoxazole (CRO) and Ciprofloxacin (CIP). In Comparison with Standards, *D. regia* leaf extract (DRL) showed comparable inhibition against *Escherichia coli* and MRSA to CIP and CRO, indicating its potential as an alternative antimicrobial agent. In like manner, *P. pterocarpum* seed extract (PPS) exhibited strong activity against *Candida albicans*, surpassing the inhibition levels of CIP and CRO. On one hand, the presence of flavonoids and tannins in *D. regia* aligns with its strong inhibition against *E. coli* and MRSA, as these compounds are known to disrupt microbial cell walls and inhibit enzymatic activity, as reported by [26, 27]. On the other hand, Saponins and alkaloids in *P. pterocarpum* contribute to its efficacy against *Candida albicans* and other strains, enhancing membrane permeability and leading to microbial cell death [28].

The Minimum Bactericidal Concentration (MBC) results for *Delonix regia* (DR) and *Peltophorum pterocarpum* (PP) in Table 9 showcase their varying antimicrobial strengths against selected microbial strains, with specific concentrations required to eliminate microbes. MBC values showed that *D. regia* seed extract (DRS) exhibited strong bactericidal activity against *Escherichia coli* and *Acinetobacter baumannii*, requiring lower concentrations (1.25% W/V). However, *P. pterocarpum* seed extract (PPS) showed the most effective activity against *Klebsiella pneumonia* and *Candida albicans,* with MBC values of 1.25% W/V and 5.0% W/V, respectively. Studies have shown that MBC levels could differ in their effects. For instance, one study examined the ethanolic and aqueous extracts of *Alchornea cordifolia* and *Sida acuta* leaves, and found that the MBC values for these extracts ranged from 1.563mg/mL to 12.5mg/mL depending on the type of extracts and bacterial strain tested [29]. The 1.25% W/V MBC level observed in this study implies higher effectiveness since it requires a smaller concentration to eliminate the bacteria. The effectiveness of the selected samples can be attributed to their phytochemicals. From this study, *D. regia* seeds showed high levels of tannins and flavonoids, contributing to its effectiveness against bacterial strains like *E. coli.* The saponins and alkaloids in *P. pterocarpum* seeds are likely responsible for their activity against *Candida albicans*, disrupting fungal cells and increasing their permeability. The review of Hency and Vijay [30] has highlighted that saponins, flavonoids, and alkaloids, among other phytochemicals, have medicinal implications.

The results underscore the antimicrobial potential of both species, supporting their use as natural alternatives to conventional antibiotics, especially in the treatment of resistant microbial strains. Their phytochemical profiles are key contributors to their efficacy, providing a solid foundation for their pharmacognostic and medicinal evaluation.

MATERIALS AND METHODS

Plant collection

The leaves and seeds of *Delonix regia* and *Peltrophorum pterocarpum* was collected at the premises of the University of Ibadan, Oyo State, Nigeria, in the month on July, 2021. The plants were identified at the Forest Herbarium, Ibadan (FHI), and the authentication was carried out at the Forest Research Institute of Nigeria (FRIN), Ibadan, with voucher number: FHI. 113593 and FHI. 113597.

Preparation of Plant Extracts

The leaves of the two plants were air dried separately for about two weeks, after which they were oven dried at 40°c The powdered sample of the leaves and seeds of both plants was exhaustively extracted at room temperature (280-320 °C) by maceration, using distilled methanol. The solvent was left for 72 hours while stirring using a glass rod and then filtered using a Buchner funnel. This extraction procedure was repeated until the solvents obtained were clear in each case. Filtrate was concentrated using a rotary evaporator at 50 °C. The dried extracts were weighed and used for the calculation of the extraction or percentage yield and then stored at 4°C in a refrigerator until used for other analyses.

Pharmacognostic Studies

Fresh leaves were cut and placed on a microscope slide and held with the left hand. To get the adaxial layer, the abaxial surface (lower surface) of the leaves was scraped until a translucent layer was obtained, and vice versa for the abaxial layer (upper layer). A clean and sharp razor blade was used for this purpose. The epidermal surface was afterwards cleared by placing them in 2% sodium hypochlorite solution in a petri dish. The epidermal layers were rinsed several times to remove the sodium hypochlorite and thereafter cleared with a soft camel hair brush to dissociate tissue remains from the surfaces. The epidermal surfaces were stained with Safranin O for about 2 min and then transferred into 3 different concentrations of ethanol (50, 70, and 100%). The epidermal layers were placed on a microscope slide, glycerol was added (served as the mountant), and then covered with a microscope slips. The slide was viewed under a light microscope. A photomicrograph of the microscopic features was taken at different magnifications with a clear camera.

Transverse section of a leaf

The transverse section (T.S.) of the samples was obtained through the midrib with a small portion of lamina and a thin section, which was bleached with 2% sodium hypochlorite and stained with Safranin. The stained sections were further observed under a microscope, and photomicrographs were taken with the aid of a clear camera. The powdered sample was also mounted in different reagents, and cellular diagnostic and diagnostic cell inclusions were observed [31].

Organoleptic study

The organoleptic investigations were performed on the odor, color, taste, surface characteristics, and texture. The tongue was used for tasting, the nose for smelling, the eyes for color detection, while the hands were used for the texture of the leaves.

Physical Constant
Physicochemical analysis was carried out on the leaf and seed powdered samples following standard procedures [32, 33]. Total ash value, water soluble ash value, acid insoluble ash value, alcohol, and water-soluble extractive values were evaluated.

Heavy Metals Analysis
Heavy Metal contents of the powdered samples of the leaves and seeds of both plants were determined using wet digestion standard procedures [34]. Acceptable limits were used as the reference standard.

Phytochemical Screening
The powdered leaves of the two plants were screened phytochemically for the presence of saponins, alkaloids, flavonoids, tannins, anthraquinones, and glycosides using standard procedures [34].

Antimicrobial Screening
The bacterial and fungal strains used for the study were pure cultures obtained from the Department of Medical Microbiology, University College Hospital (UCH), Ibadan. The organisms were selected due to their medical relevance. The microbial species include four Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa*), one Gram-positive bacterium (*Methicillin-Resistant Staphylococcus aureus, MRSA*), and a fungus (*Candida albicans*). Bacterial strains were subcultured onto fresh nutrient agar (Oxoid (CMO405), England) plates and stored in a refrigerator at 4 ^{0}C for use. Well-isolated colonies of the same morphological type of each organism were enumerated in 5 mL Mueller-Hinton Broth (Difco, 275730, France) and incubated at 37^{0}C °C for 16h before antibacterial testing to attain the turbidity of 0.5 McFarland standard (approximately 1-2 ×106 CFU mL^{-1} for *E. coli*) for the antimicrobial assay. Similarly, the fungus was cultured overnight at 37^{0}C in Sabouraud Dextrose Agar.

Dilution of Extracts: The plant samples were tested against microorganisms at 20% w/v. The antimicrobial assay was tested using the agar well diffusion method, with zones of inhibition measured for Minimum Bacterial Concentration (MBC) and Minimum Inhibitory Concentration (MIC).

Culture Media: 38g of Mueller-Hinton Agar (MHA) and 65g of Sabouraud Dextrose Agar (SDA) were dispensed each into 1000 mL of distilled water, which was allowed to soak for 10 min according to the manufacturer's specification and autoclaved for 15 min at 121^{0}C, allowed to cool at 47 ^{0}C. Pouring of the agar was poured gently after cooling the nutrient agar and allowed to solidify. Standard and sterile core borers of 6mm diameter were used to bore holes into the plate. The wells were at equidistant positions to each other.

Determination of Antibacterial and Antifungal Activity
The antibacterial activity of both plant species *Delonix regia* (leaf and seed) and *Peltrophorum pterocarpum* (leaf and seed), was determined using the Agar Well diffusion method. The microorganisms were introduced using a sterile swab stick test solution (0.2 ml). 100 µl of each extract (20% w/v) was added to the respective wells. Antibiotic sensitivity discs (Ciprofloxacin and Ceftriaxone) were used as positive control, while the negative control was DiMethylSulfoxide DMSO (DMSO 10%, v/v). The experiment was carried out in triplicate for each bacterium.
The plates were left on the bench for 30 min to ensure adequate diffusion of the extracts and thereafter were incubated at 37 °C for 24 h, after which the diameter of all the resulting zones of inhibition was measured to the nearest millimeter along two axes using a millimeter ruler and the result tabulated. The results were expressed in means ± SD of the means.
The antifungal activity of both plant species *Delonix regia* (leaf and seed) and *Peltrophorum pterocarpum* (leaf and seed), was determined using the Agar well diffusion method. The organism was introduced over the SDA plate.
The plates were left on the bench for 30 min to ensure adequate diffusion of the extracts and thereafter were incubated at 37 °C for 24 h after which the diameter of all the resulting zones of inhibition were measured to the nearest millimeter along two axes using a millimeter ruler and the result tabulated against standard antibiotics (Fluconazole) which was used as positive control while the negative control was DiMethylSulfoxide DMSO (DMSO 10%, v/v). The experiment was carried out in triplicate for each bacterium. The results were expressed in means ± SD of the means.
The Minimum Inhibitory Concentrations (MIC) of the extracts were determined by the agar well diffusion method. The MIC was prepared by two-fold serial dilution of the extracts with decreasing concentrations at 20, 10, 5, 2.5, and 1.25% of each extract was transferred to the respective wells. Then, five wells were made on each plate, and 100 µl of each extract was transferred to the respective wells. Plates were kept in the refrigerator for 30 min and then incubated at 37^{0}C for 18h. The MIC was considered the lowest concentration that inhibited the growth of the respective microorganisms. All assays were performed in triplicate. DMSO served as a control for the extracts.
Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration of antimicrobial agent that will prevent the growth of an organism after subculture onto antibiotic-free media. 100 µl from the tube showing MIC was placed on an MHA plate, antibiotic-free, and was spread over the plate. After incubation at 37°C for 24h, the plates were examined for the growth of bacteria to determine the concentration of extract at which 99.9% killing of bacterial isolates was achieved.

Statistical Analysis
The data represent the mean of three replicates ± standard deviation (SD). Results were subjected to multivariate analysis of variance, and the mean comparisons were performed using the Statistical Software Package (SPSS).

Author contributions
JAS conducted the experiments and drafted the manuscript. TOA supervised the experimental procedures and oversaw the overall research process. ASO was responsible for the preparation and finalization of the manuscript. All authors have read and approved the final version of the manuscript.

Declaration of competing interest
The authors declare no competing interests.

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List of non-standard abbreviations

- **FHI**: Forest Herbarium Ibadan
- **SEM**: Standard Errors of Means
- **SPSS**: Statistical Package for the Social Sciences
- **DRL:** *D. regia* leaf
- **DRS:** *D. regia* seed**,**
- **CIP:** Ciprofloxacin
- **CRO:** Cotrimoxazole
- **PPL:** *P. pterocarpum* leaf
- **PPS:** *P. pterocarpum* Seed

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