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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL EFFECTS OF LEAF EXTRACTS OF *Pterocapus erinaceus* (POIR) ON *Salmonella typhi* AND *Escherichia coli*

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Abstract

The incidence of drug resistant strains of pathogenic microbes remained a rising global concern to the pharmaceutical world. This was consequent upon adaptation due to continuous use of conventional drugs with numerous associated setbacks. The phytochemical screening and antibacterial effects of ethanolic leaf extracts of Pterocapus erinaceus on Salmonella typhi and Escherichia coli was investigated, using standard procedures. Saponins, phenols, steroids, reducing sugar and tannins were bioactive compounds obtained at resonance frequencies of 0.022, 2.812, 0.001, 0.232 and 0.720 respectively. The sensitivity response of the test organisms to control drug compared significantly ($p<0.05$) with the leaf extract across the concentrations. Average zones of inhibition were 5.00, 10.00, 12.00 and 17.00mm for Escherichia coli, 12.00, 15.00, 20.00 and 21.00mm for Salmonella typhi at 25, 50,100 and 200mg/ml respectively. The antibacterial potential ranged from moderate to strong and strong to very strong for Escherichia coli and Salmonella typhi respectively. The ethanolic leaf extract gave ≤25mg/ml and ≥200mg/ml as minimum inhibitory and minimum bactericidal concentrations against Salmonella typhi and Escherichia coli respectively, with bacteriostatic antibiotic power effects on the microbes. These findings clearly revealed potential antibiotic alternative to conventional options, while raising afforestation need to guarantee sustainable management of the plant.

Key Words: *Phytochemical, Ethanolic, Extract, Pterocapus erinaceus, bactericidal, Bacteriostatic*

Introduction

Herbal medicine also known as herbalism or botanical medicine, is a medical system based on the use of plant extract (stem bark, seed, leaves etc.), that are eaten or applied to the skin for medicinal purposes (Habtom and Gebrehiwot, 2019).

Escherichia coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals and human. Not all *E. coli* strains are pathogenic. Most pathogenic strains are transmitted by infected food. The signs and symptoms of *E. coli* exposure includes diarrhoea, nausea, ulcerative colitis, abdominal pain and, in some cases, kidney disorders or death, especially among children. Pathogenic strains are responsible for urinary tract infections, neonatal meningitis, and intestinal diseases. The intestinal pathogen could be enterotoxigenic(causing diarrhea in infants and travelers), enteroinvasive (causing dysentery-like diarrhea with fever), enteropathogenic (responsible for watery, sometimes bloody, diarrhea especially in children), and enterohemorrhagic (causing hemorrhagic diarrhea and/ or food poisoning which may develop into hemolytic uremic syndrome (HUS) and includes the invasive 0157:H7 strain making up 80% of the EHEC serotypes producing the verotoxin or Shiga toxin) (Croxen *et al*., 2013; Amin, 2019).

Salmonella species and strains are pathogenic to humans and animal when acquired by oral route. They are transmitted from animal and animal products and human where they cause anteritides systemic infections and enteric fever (Geo *et al*., 2007). The evolution and spread of antibiotic resistance, as well as the evolution of new strains of disease-causing agents, is of great concern to the global health community (Frank and Meyers, 2010).

Medicinal plants constitute an effective resource for traditional and modern medicines, and herbal medicine has been shown to have genuine utility. In Nigeria, many plants are used in traditional medicine as antimicrobial agents but only few are documented. Plants based system of traditional medicine has continued to play an essential role in health care in many cultures. The increased use of plant derived products as alternatives to orthodox or synthetic drugs and increasing awareness of beneficial effect of natural product has resulted in increased interest in alternative therapies. Anti-bacterial compounds with herbal sources have a wide range of therapeutic use. These compounds are not only efficient for the treatment of infectious diseases, but also currently diminish existing side effects via their anti-bacterial compounds (Nas *et al*., 2017).

Pterocarpus erinaceus is deciduous endangered species of tree that is native to the Sahelian region of West Africa, with a high, open, few-branched crown; usually growing 12 - 15 metres tall with some specimens reaching 25 metres. It bears dark, scaly bark and yellow flowers. The bole is straight and cylindrical under good conditions, but is often twisted, fluted and low-branched under poorer conditions. It is slightly buttressed when old and up to 75cm in diameter (Hutchinson *et al.,* 1985). It is used for fuel wood, for medicinal purposes, as a woodworking material, and is useful as a nitrogen-fixing plant to improve nutrient-depleted farming land. The resin obtained from the tree used to be exported to Europe for medicinal usage but has now been replaced by newer drugs (Noufou *et al.*, 2016).

The tree has been seriously over-exploited in the past, attempts have been made at reforestation of the savannah and the establishment of stands on farms

(Kossi *et al*., 2019). The tree has considerable potential as an ornamental, completely covered with copious racemes of bright golden yellow flowers during the dry season. The plant has been reported to show anti-inflammatory, analgesic, anti-plasmodial activities of *P. Erinaceus* (Noufou *et al*., 2016), antidiarrheal activity antiulcerogenic properties, Antimalarial activity, and antifungal, antimycotic activity, its antimicrobial activities, based on inherent phytochemical constituents (Olaleye *et al*., 2013; Patrick, 2018; Tittikpina *et al*., 2019; Alqethami and Aldhebiani, 2021). Paucity of literatures on efforts relating to the use of the leaf extract as antimicrobial against the selected microorganisms, coupled with the rising challenges of multiple drug resistance form the major impetus for the study.

This study, therefore, investigated the phytochemical constituents of leaf extracts of *Pterocapus erinaceus* and tested the effects of the plant extract on *Salmonalla typhi* and *Escherichia coli*. *Pterocapus erinaceus* has been reported to show positive effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Tittikpina *et al*., 2017; Okoli et.al. 2023) even at low concentrations (4µg/ml) and may be an important herbal solution to many bacterial infections.

Materials and Methods

Collection of Plants Materials and Extraction

Leaf materials of *Pterocarpus erinaceus* were collected at Shere Hills located about 10 km to the East of Jos metropolis, of Plateau State, Nigeria (latitude of 9°571N 9°031E and longitude 9.950°N 9.050°E and have an elevation of 1829 m (6001 ft) above sea level) mount Dimlang (Vogel peak) on the Shebshi mountains reaching a height of about 2042 m or 6699 feet above sea level (Olowolafe, 2002). The samples were identified at Federal College of Forestry Herbarium, Jos (with voucher specimen number FHJ062022), thereafter, washed with distil water and air-dried for 4 weeks under shade to maintain it compositional integrity (Ncube *et al*., 2008). The samples were pulverized with mortar and pestle packed in airtight glass jar until required for analysis.

Twenty grams (20g) of pulverized samples were mixed in 100ml of ethanol, allowed to stand for 24hours, before filtering. The filtrate was concentrated using a rotary evaporator and dried in a hot air oven at 50°C and kept at 4°C (refrigeration) until used.

Percentage Yield of Extracts

Extract yield was evaluated (in %) according to Ardzard *et al.* (2009) as described below:

$$\%\ yield = \frac{x_2 - x_1}{WSE}\ X\ 100\%$$

Where :

X_1 = Weight of empty beaker; X_2 = Weight of beaker + final dried extract

WSE = Weight of sample before extraction.

Phytochemical Screening

The presence of some basic secondary metabolites in pulverized a plant material will be determined by using standard method (Sofowora, 1993; Trease and Evans, 2009).

Test for Saponin

Five millilitres of each plant extract was placed into a test tube and diluted with 5ml of distilled water, the mixture was shaken was shaken vigorously for two minutes. Persistent appearance of form listing for at least fifteen minutes or the

forming of an emulsion when olive oil was added confirmed the presence of saponins.

Test for Tannins

Two (2) drops of 50% felling solution was added to 1.0ml of extract in separate test tube. The appearance of a dirty-green precipitates will be considered as indication for the presence of tannins.

Test for Phenols

The extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black coloration indicated the presence of phenols.

Test for Flavonoids

1.0g magnesium powder and 1-5 drops of concentrated Hel will be added to 3.0ml of each extract in separate test tube. The presence of red colour in the methanol and water extracts indicated the presence of flavonoids.

Test of Alkaloids

To 3.0ml of each extract mixture in a test tube, 1.0ml of Hel was be added to 2.0ml of extracts mixture 2 drops of Mayer' swarner's and dragend roof reagent was added separately. A creamy white (Mayer), a reddish brown (Wagner) and an organ brown (Dragendroff) precipitates in the ethanol and water extracts was taken as evidence of the presence of alkaloids (Endimiani *et al.*, 2009).

Standardization of Test Organisms

Separate cultures *Salmonella typhi* and *Escherichia coli* were obtained from National Veterinary Research Institute (NVRI), Vom, Jos South LGA, Plateau State, Nigeria. Their identities were confirmed using cultural morphological and biochemical tests (Akimnibosun *et al*., 2009). The bacteria isolates were maintained on nutrient agar at 4°C.

The methods of McFarland modified by Olajubu *et al*. (2012) was use for the preparation of the broth culture of the inoculi at an adjusted suspension density equal to 0.5 McFarland turbidity standards (Andrews, 2001), with cell density equivalent to 1.5×10^6 colony forming units (cfu)/ml. 10ml of the pure cultures of each test organism was separately inoculated in 10 ml of nutrient broth in sterile McCartney bottles and incubated at 37°C overnight.

Determination of Antimicrobial Activity Sensitivity Tests

The antimicrobial effects of and ethanol extract on *Pterocapus erinaceus* was determined individually by using the agar diffusion method according to Ardzard *et al*. (2009). Nutrient agar was swabbed using sterile wire loop with the broth culture of respective bacterial isolate. Into each of the four equi-distant wells (6.0mm in diameter) made using a sterile cork borer on the nutrient agar, were introduced 0.1ml of each of the extract concentrations (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml), while the control was introduced into the centre well. It was allowed to diffuse at room temperature for 2hours before incubation at 37^0C for 24 hours (Pradeepa *et al*-., 2014; Chomini *et al*., 2021a). Diameter of the zones of inhibition was measured (mm) as an indicator of sensitivity of the test microbe to the extracts.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The four extract concentrations (25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml) were diluted two folds with nutrient broth in a series of nine test tubes. An aliquot of 1ml of each bacterial suspension (bacteriological peptone - 1.5 × 106 cfu/ml) was inoculated into each tube. All tubes were incubated at 37°C for

18 - 20 h. The lowest concentration that did not permit any visible growth when compared with the control was considered as the MIC (Cheesbrough, 2000; Aboaba *et al*., 2006).

The MBC was determined by sub-culturing all the tubes in each set showing no visible turbidity during the test for MIC. A loop full of the contents of the tubes showing no microbial growth were sub-cultured by streaking over the surface of already set nutrient agar plates without extracts. The plates were incubated at 37°C for 24hours. The MBC was recorded as the lowest concentration with no growth observed after sub-culturing. All plates showing no growth on the nutrient agar indicated bactericidal effect of the extracts concentration (Chesbrough, 2000, Chomini *et al.,* 2021a).

The MBC was determined by sub-culturing all the test tube in each set showing no visible turbidity during the test for MIC. A loop full of content for the tube showing, no microbial growth will be sub-cultured by streaking over the surface of already set nutrient agar will be an indication of bactericidal effects of the extract (Cheesbrough, 2000).

Statistical Analysis

Data obtained were subjected to analysis of variance, using SPSS version 16, to determine the level of significance, while significant means were separated using least significant difference.

Results

Percentage Yield of Plant Extracts

The percentage yield of the ethanolic leaf extracts of *Pterocarpus erinaceus* (ELEPE) was 41.56% (Table 1). The phytochemical screening of ELEPE showed the presence of saponins (R_f 0.022), phenols (R_f 2.812), steroids (R_f 0.001), reducing sugar (R_f 0.232) and tannins (R_f 0.720), based on their respective UV absorbance (Table 2). Flavonoids, alkaloids, glycosides and terpenoids were absent.

Table 1: Percentage Yield of leaf extracts of *Pterocarpus erinaceus*

Plant part	Solvent	Percentage yield (%)
Leaf	Ethanol	41.56

Table 2: Phytochemical Constituents of Ethanolic leaf Extracts of *Pterocarpus erinaceus*

Phytochemical	Observation	R_f value
Saponins	+	0.022
Phenols	+	2.812
Steroids	+	0.001
Reducing sugar	+	0.232
Tannins	+	0.824
Terpenoids	-	-
Alkaloids	-	-
Flavonoids	-	-
Glycosides	-	-

Key: + = present, − = Absence, R_f = Resonance frequency

Sensitivity Tests

The ethanolic leaf extracts of *Pterocarpus erinaceus* (ELEPE) has engendered different sensitivity responses by the test microorganisms, based on the test concentrations investigated. The average inhibition zone (AIZ) increases with extract concentration. 5.0 and 12.0, 10.0 and 15.0, 12.0 and 20.0 and 17.0 and 21.0mm were the AIZs of *Escherichia coli* and *Salmonella typhi*, recorded at 25mg/ml, 50mg/ml, 100mg/ml and 200mg/ml concentrations respectively. The control drug (Ciprofloxacin) gave 25.0 and 27.0mm as AIZ values for *E. coli* and *S. typhi* respectively. The effects of the test drug and the seed extracts were significantly different ($p<0.05$) on the sensitivity of the test organisms (Table 3).

Table 3: Average Zone of Inhibition (ZOI) (cm) of the Leaf extract of *Pterocarpus erinaceus* on the test organisms

Extract Concentration (mg/ml)	Test Organism	
	Escherichia coli	*Salmonella typhi*
25	5.00±0.82[a]	12.00±1.71[a]
50	10.00±1.14[b]	15.00±0.41[b]
100	12.00±0.98[c]	20.00±1.06[c]
200	17.00±1.22[d]	21.00±1.63[c]
Control (ciprofloxacin)	25.00±1.06[e]	27.00±0.98[d]
LSD	1.91	2.10

Value were means of triplicate observation (n = 3, ± SD), means followed by different superscripts are significantly different (P =0.05) based on LSD

Antibacterial Activities and Indices

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The antibacterial activities assessed, based on minimum inhibitory concentration (MIC) of ethanolic leaf extracts of *Pterocarpus erinaceus* (ELEPE) on *Salmonella typhi* and *Escherichia coli* were ≤ 25mg/ml for both test organisms. However, the minimum bactericidal concentrations (MBC) were ≥200mg/ml for *S. typhi and E. coli* respectively.

The ratio of MBC: MIC evaluated, also known as antibiotic power, (AP), was 8.0 for *S. typhi* and *E. coli* respectively, which indicated a bacteriostatic effect of the ELEPE on the test microorganisms (Table 4).

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of leaf extracts of *Pterocarpus erinaceus* against test organisms

	Incubation	Leaf extract concentration (mg/ml)				Antimicrobial Indices	
Test organism	Time(hour)	200	100	50	25	**MIC**	**Remark**
Escherichia coli	24	-	-	-	-	<25	
Salmonella typhi	24	-	-	-	-	<25	
						MBC	
Escherichia coli	24	+	+	+	+	≥200	
Salmonella typhi	24	+	+	+	+	≥200	
						MBC/MIC	
Escherichia coli						>8.0	Bacteriostatic
Salmonella typhi						>8.0	bacteriostatic

MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration, MBC/MIC = Ratio of Minimum Bactericidal Concentration to Minimum Inhibitory Concentration (antibiotic power (AP))
+ = Growth, - = No Growth

Discussion

The yields of plant extracts have been found to vary with plant species, plant parts, as well as the types of solvents used. The methanolic flower and leaf extracts of *Securidaca longipedunculata* were found to be19.88% and 24.43% (Shemishere *et al*., 2020). Senthilkumar *et al.* (2020), obtained 0.71 and 0.54 as yields from stem bark extracts of *P. indicus* using methanol and acetone as organic polar solvents.

The results of bioactive compounds assayed from botanicals have been found to be dependent on species, plant parts, provenance, solvent used among other factors (Felhi *et al*., 2017; Essou *et al.*, 2017). Patrick *et al.* (2016), obtained tannins, saponins and phenols from the stembark of *E. erinaceus*, using methanol as solvent, while Glycosides and terpenoids were absent, which were similar to the results of the present study.

Senthilkumar *et al.* (2020), reported the presence of tannins, phenols, steroids and carbohydrate among other phytochemicals from the methanol and acetone stembark of *Pterocarpus indicus*. These phytochemicals are known to exhibit medicinal as well as physiological activities (Shrestha *et al*., 2015). The quantitative phytochemistry of assayed ELEPE revealed the bioactive components, which according to Ogundana *et al*. (2008) were responsible for the antibacterial activities. Tannins has been reported to show lethal tendency against bacteria Shaimaa (2014), this is due to their ability to form protein complex which result to microbial cytoplasmic leakage. According to Arabski *et al*. (2012), saponins have the capacity to alter the permeability, structure and function of cell membranes, resulting in microbial cell destruction.

Phenols are described to exhibit antioxidant and enzyme inhibitory tendencies against pathogenic microbes (Maria *et al*., 2013). This, as explained by Roger *et al*. (2015), was due to their ability to exhibit distinct reactivity property with proteins-related polyamides polymers.

Sensitivity Test

The microbial sensitivity response test is one of the easiest means of assessment of antimicrobial effects of plant extracts on the test microorganisms (Chomini *et al*., 2021a). This was achieved by evaluating the average inhibition zones (AIZs) due to extract effects on the growth of the microbes. According to Sarjono *et al.* (2019), the zones of inhibition were categorized based on AIZ. An AIZ values of < 5.0 mm, 5.0 – 10.0 mm, 10.0 – 20.0 mm and ≥ 20.0 mm are described as weak antibacterial potential (WAP), moderate antibacterial potential (MAP), strong antibacterial potential (SAP) and very strong antibacterial potential (VSAP) respectively. Consequent upon this study, the ELEPE showed MAP (at 25 and 50mg/ml) and SAP (at 100 and 200mg/ml) against *E. coli;* and SAP (25, 50 and 100mg/ml) and VSAP (200mg/ml) *S. typhi*. These observations corroborated the findings of Chomini *et al.* (2021d), who described the effects of methanolic seed extracts of *Garcinia kola* as indicative of SAP on *S. typhi* and *Klebsiella pneumoniae.* However, the control drug gave VSAP for both E. coli and *S. typhi*, which agreed with the findings of Nas *et al* (2017) and Chomini *et al.* (2021d), who reported VSAP (28.2 and 29.2 mm) for *S. typhi* and *K. pneumoniae* subjected to ciprofloxacin (control drug). Ogodo *et al*. (2017), posited that low concentration of inhibition indicated better antibacterial activity, suggesting stronger effects against microorganisms.

Antibacterial Activities and Indices

The MIC values recorded for *E coli* and *S. typhi* were lower than 100mg/ml reported for each of the organisms by Habtom and Gebrehiwot (2019), using ethanolic leaf of *Vernonia amygdalina*. In a related study, MICs of 97.22 ± 27.78 and 32.40 ± 9.26mg/ml for *E coli* and *S. typhi* subjected to methanolic extract of aerial parts of *Sida rhombifolia* (Debalke *et al*., 2018). These variations could be attributed to different plant types (Habtom and Gebrehiwot., 2019), age and geographical location of collected plant (Debalke *et al*., 2018). According to Habtom and Gebrehiwot (2019), the relatively low MIC values are considered very effective antimicrobial agents as higher values are indications of increased resistance to some of the bioactive constituents of the extracts. The MBC showed higher values than the MIC. This was corroborated by the findings of Chomini *et al.* (2021b), who obtained higher MBC values for *E. coli* and *S.typhimurum* subjected to ethanolic leaf extracts of *Anogeissus leiocarpa*. In a related study, similar pattern was observed with methanolic extract of *A. hispidum* D. C. on four strains of *Salmonella* spp (Chomini *et al.,* 2021a). Findings of other workers also agreed with current results (Shameem *et al*., 2015; Abubakar and Usman, 2016; Venkateswarulu *et al*., 2019).

The ratio of MBC to MIC, which describes the antibiotic power (AP), showed that antibacterial activities of ethanolic leaf extracts of *Pterocarus erinaceus* (ELEPE) on *E. coli* and S. typhi were both bacteriostatic, as the AP values were both >8 for the test organisms. This

corroborated the findings of Noumedem *et al.* (2013), describing the outcome as bactericidal and bacteriostatic when the ratio MBC/MIC is ≤4 and >4 respectively. Chomini *et al.* (2021a) obtained a bactericidal effect on *S. typhi S. typhimorum*, *S. gallinarum* and *S. paratyphi,* based on effects of methanolic leaf extracts of *A. hispidum* D.C. on the bacterial strains. Furthermore, a bacteriostatic effect was reported for combined methanolic seed extracts of *Aframomum melegueta* and *Garcinia kola* on *S. typhi* (Chomini *et al*., 2021c).

Conclusion

The study has revealed that ethanolic leaf extracts of *Pterocarpus erinaceus* (ELEPE) exhibit antibacterial activities against *Salmonella typhi and Escherichia coli*. The bioactive compounds screened include saponins, phenols, steroids, reducing sugar and tannins, at different UV absorbance. The control drug had significant sensitivity effects on the test organisms, relative to different concentrations ELEPE. The effects of the extract elucidated a moderate to strong antibacterial potential on *E. coli*, and a strong to very strong antibacterial potentials on *S. typhi* based on the extract concentrations. This gives a pointer to its antimicrobial potential for pharmaceutical industries, while raising afforestation need for this valuable botanical.

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