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Determination of phytochemical constituents, antibacterial and antioxidant activities of ethanolic leaf extracts of *Pterocarpus erinaceus*

EMMANUEL CHIKODIRI OKOLI♥, ISAAC JOHN UMARU, OTITOJU OLAWALE
Department of Biochemistry, Faculty of Pure and Applied Sciences, Federal University Wukari.P.M.B. 1020, Taraba 670101, Nigeria.
Tel./fax.: +2347068385043, ♥email: emmanuelokolic@gmail.com

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Abstract. *Okoli EC, Umaru IJ, Olawale O. 2023. Determination of phytochemical constituents, antibacterial and antioxidant activities of ethanolic leaf extracts of* Pterocarpus erinaceus. *Biodiversitas 24: 2272-2277*. Many people in Nigeria and West Africa have long used the leaves, stem bark, and roots of *Pterocarpus erinaceus* Poir. as a medicine to treat ailments such as malaria, ulcers, coughs, and fevers. The antioxidant, antimalarial, antiulcer, and antibacterial activities of stem bark, leaves, and roots of *P. erinaceus* have been investigated. The aim of this study was to determine the phytochemical composition, antimicrobial activity, and antioxidant activity of the *P. erinaceus* leaf. Phytochemical compounds in ethanolic leaf extracts of *P. erinaceus* were analyzed and quantified by Gas Chromatography/Flame Ionization Detector (GC-FID). The antibacterial test was performed against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and Escherichia coli* by agar diffusion. The antioxidant activity was evaluated by DPPH scavenging activity. Phytochemicals content in the extract include flavonoids (44.71%), alkaloids (19.47%), steroids (6.01%), tannins (1.73%), saponins (4.70%), glycosides (7.00%), tannins (2.60%), anti-nutrient (12.77%). Ethanol extracts of *P. erinaceus* leaves were effective against all selected bacterial strains (*E. coli, S. aureus, B. subtilis,* and *P. aeruginosa*). DPPH scavenging activity of the ethanol extract of *P.erinaceus* at a concentration of 1000 µg/mL was 49.51% and 27.20% at 40 µg/mL, categorized as moderate antioxidant activity. This study showed that crude ethanol extracts of *P. erinaceus* leaf contained pharmacologically active compounds and exhibited significant antibacterial and DPPH free radical scavenging activities in a concentration-dependent manner.

Keywords: Antioxidant, ethanolic extract, GC-FID, phytochemicals, *Pterocarpus erinaceus*

INTRODUCTION

Natural plant compounds are gaining popularity in scientific research due to their positive effects on human and animal health. Medicinal plants continue to provide new remedies in the form of active isolated molecules used as drugs (Noufou et al. 2017). The therapeutic effect of antioxidants has been studied extensively worldwide to prevent chronic diseases such as cancer, diabetes, cardiovascular problems, and liver damage (Loganayaki et al. 2013). Plant antioxidant compounds with redox properties (flavonoids, tannins, phenolic acids) act against the production of Reactive Oxygen Species (ROS). Reactive oxygen species are known for damaging membranes and proteins and causing oxidative stress (Noufou et al. 2017). This oxidative stress is linked to chronic diseases as the leading cause of death worldwide (Noufou et al. 2017).

Records show that the plant's medicinal use dates back to 4000-5000 BC, and the Chinese were the first to use natural herbal preparations as medicine. About 64% of the world's population still relies on traditional medicines and medicinal plants to meet their health needs (Okoli et al. 2022). According to a World Health Organisation (WHO) survey, about 80% of patients in India, 85% in Burma, and 90% in Bangladesh are prescribed traditional medicines (Okoli et al. 2022). Due to limited access to quality medicines, people in many developing countries use plants to treat common ailments (Tittikpina et al. 2018).

Due to emerging health challenges of pathogen resistance, searching for new drugs has become imperative worldwide (Umaru et al. 2022). Therefore, it gains the attention of researchers to explore new bioactive compounds from plants that can solve pathogen-resistant in human and animal diseases. Many studies have been conducted to reveal the active compounds in plants. The most famous example is the discovery of artemisinin from *Artemisia annua* L. However, artemisinin is not the only promising plant compound (Tittikpina et al. 2018). Many researchers from Asia and Africa have studied the bioactivity of plant extracts, pure chemicals contained in the extracts, against various microorganisms associated with bacterial and fungal diseases (Tittikpina et al. 2018).

Pterocarpus erinaceus Poir. belongs to the Fabaceae family and is a tree that usually grows between 8 and 15 m in height. This plant grows in savannas and is endemic to West and Central Africa (Noufou et al. 2012). The seeds are kidney-shaped to oblong, often oblong, and curved at the level of a small telium. The leaves and seeds are edible after cooking (Okoli et al. 2022), producing the finest wood from the country of origin. At the end of the dry season, the leaves and young bark are often cut for fodder for sheep, goats, cattle, and horses (Okoli et al. 2022). According to the ethnographic findings of Saslis-Lagoudakis et al. (2011), the leaves, bark, and roots of the plant *P. erinaceus* are used in traditional Burkinabe medicine to treat inflammatory diseases such as cough, fever, bacterial

infections, malaria, ulcers, rheumatism, and inflammation (Okoli et al. 2022). The roots of *P. erinaceus* are used to treat inflammation, ulcers, and gastric diseases (Noufou et al. 2016).

A previous study showed that the heartwood of *P. erinaceus* contains some isoflavonoids such as plum, muningin, afromosin, tectorigenin, and pseudobaptigenin. Research on the relationship between antioxidants and certain disease prevention, such as cancer, cardiovascular disease, and other inflammatory conditions, has received increasing attention in recent years. The bark, leaves, and roots of *P. erinaceus* have been studied for their anti-inflammatory, antiulcer, analgesic, antidiarrheal, antiemetic, antimalarial, antioxidant, and antifungal activities (Okoli et al. 2022). This study aimed to determine the phytochemical compounds and antibacterial and antioxidant activities of the ethanolic extract of *P. erinaceus* leaves.

MATERIALS AND METHODS

Study area

This study was carried in Federal University, Wukari, Taraba State, Nigeria, from February 2022 to July 2022. Wukari town is the headquarters of Wukari Local Government Area in Taraba State, Nigeria. It lies between latitude 7.9303°N and longitude 9.8125°E of the equator.

Materials

The leaf of *P. erinaceus* was collected from uncultivated farmland of Federal University Wukari, Wukari Local Government Area of Taraba State, Nigeria. The plant was taxonomically identified and authenticated in the Department of Plant Science of Modibbo Adama University of Technology, Yola, Nigeria.

Leaf extraction

The leaf samples were rinsed with distilled water before being air-dried for thirty days; then, it was ground into powdered using mortar and pestle. One hundred and fifty grams of powdered leaf were cold macerated in 500 mL of ethanol using an Erlenmeyer flask. Then, it was shaken at an interval of an hour and allowed to stand for 48 hours at room temperature and filtered using Whatman's filter paper No. 1. The extract was then concentrated to dryness using a rotary evaporator. It was then stored under a frozen condition until required.

GC-FID identification and quantification of phytochemical constituents

For the GC-FID (Gas Chromatograph/Flame Ionization Detector) analysis, 1 g of leaf extract of *P. erinaceus* was weighed and placed in a test tube and added with 15 mL of ethanol and 10 mL of 50% w/v potassium hydroxide. The test tube was placed in a water bath for 60 minutes at 60°C (Okoli et al. 2022). After carefully transferring the contents of the test tube into a separatory funnel, the tube was rinsed in the same funnel with 10 mL of cold water, 10 mL of hot water, 20 mL of ethanol, and 3 mL of hexane, respectively. The extract in the test tube was washed thrice with a 10 mL solution of 10% v/v ethanol. The extract solution was then dried with anhydrous sodium sulfate before removing the solvent (Okoli et al. 2022). The extract was dissolved in 100 µL of pyridine, and 20 µL was transferred into a vial on the Gas Chromatography machine for phytochemical analysis (Okoli et al. 2022).

The GC-FID phytochemical analysis was carried out using a BUCK M910 Gas Chromatograph (GC) with a flame ionization detector (BUCK Scientific, USA) (FID), with THE RESTEK 15-meter MXT^{-1} column (15m x 250µm x 0.15µm). The injector temperature was 280°C with a spitless injection of 2 µL of sample and a linear velocity of 30 cms^{-1}. Helium 5.0 Pas was the carrier gas with a flow rate of 40 $mLmin^{-1}$. The oven operated initially at 200°C, heated to 330°C at a rate of 3°Cmin^{-1}, and was kept at 320°C. The phytochemical content was calculated from the ratio of the area of the internal standard to the area of the obtained phytochemicals (Okoli et al. 2022).

Antibacterial assay

The antibacterial activity of the ethanolic leaf extract of *P. erinaceus* was determined by the agar well diffusion method by Umaru et al. (2022). 20 mL of molten nutrient agar was poured into a Petri dish and allowed to solidify. The 0.5 McFarland standardized bacterial broth was spread on the dry nutrient agar using a pre-sterilized spreader in ethanol overnight. Four wells, 6 mm in depth and about 5 cm apart, were made in the nutrient agar. Three of the wells were filled with (1) 500 µL of the *P. erinaceus* leaf extract dissolved in sterile distilled water, (2) sterile distilled water (negative control), and (3) 1% standard antibiotic, gentamicin. After 24 h incubation at 37°C, the antibacterial activities were determined by measuring the diameter of the inhibition zone. The diameter of inhibition of the extract was compared with those of the standard antibiotic, gentamicin. Experiments were performed in triplicates. The diameters of inhibition of gentamicin were compared to the diameters of the diameter of inhibition against *Escherichia coli* (Gram - ve), *Staphylococcus aureus* (Gram + ve), *Bacillus subtilis* (Gram +ve), and *Pseudomonas aeruginosa* (Gram -ve). The inhibitory zone with a 9-14 mm diameter was considered active antibacterial based on Umaru et al. (2022).

DPPH scavenging activities

Various concentrations (40-1000 µg/mL) of the leaf ethanolic extracts were put in different test tubes. The volume was adjusted to 250 µL by adding MeOH. Two milliliters of a 0.18 mM (0.005%) methanolic solution of DPPH (2,2, -diphenyl-1-picrylhydrazyl) was added to these tubes and shaken vigorously. The tubes were allowed to stand in the dark at room temperature for 30 min (Okoli et al. 2022). The negative control was prepared without any extract, and MeOH was used for the baseline correction. The absorbance of the samples was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\% \text{ Scavenging Activity} = [(A_{517} \text{ Ctrl} - A_{517} \text{ treatment}) / A_{517} \text{ Ctrl}] \times 100$$

RESULTS AND DISCUSSION

The phytochemical content in the leaf extract based on GC-FID analysis is presented in Figure 1 and Table 1. Its chemical compounds were dominated by flavonoids (44.71%), followed by alkaloids (19.47%), steroids (6.01%), tannins (2.60%), saponins (4.70%), glycosides (7.00%), resveratrol (2.96%), phytate (8.75%), and oxalate (4.02%). These compounds are usually found in vegetables, cereals, stems, and flowers. Flavonoids are organic compounds with various phenol groups. They are known for their beneficial effects on health, notably their antioxidant, anti-mutagenic, anti-inflammatory, anti-cancer, and enzyme-regulating capabilities. Flavonoids found in the extract were naringenin, anthocyanin, flavonones, flavone, flavan-3-ol, catechin, epicatechin, rutin, and kaempferol.

Flavonoids

The extract of *P. erinaceus* leaf contained flavonoids, with the highest concentration being epicatechin (38.03 µg/mL or 17.74%), whereas flavan-3-ol was the lowest concentration (3.65 µg/mL or 1.71%) (Table 1). Catechins can be found in various foods and herbs, including apples, grapes, berries, and tea. Epicatechin is mainly found in green and black tea, with cocoa having the highest quantities of epicatechin (Isemura 2019; Prakash et al. 2019). Catechin has been known to have anti-obesity, anti-cancer, hepatoprotective, anti-diabetic, and neuroprotective properties, while epicatechin has cardioprotective and anti-inflammatory properties. anti-cancer, anti-diabetic, and antioxidant (Ugoeze et al. 2020).

Anthocyanin pigments are widely distributed in terrestrial plants, acting as stress suppressors and health-promoting components (Ugoeze et al. 2020). Naringenin, present mainly in citrus fruits and tomatoes, has been used in treating cancer, cardiovascular disease, and osteoporosis (Okoli et al. 2022), significantly reducing collagen fiber production in rats with liver damage (Ugoeze et al. 2020). Naringenin also has anti-inflammatory, anti-diabetic, anti-hyperlipidemia, antioxidant, and antidepressant characteristics and can reduce oxidative stress (Ugoeze et al. 2020).

Flavones are primarily found in leaves, flowers, fruits, celery, parsley, and red peppers, whereas flavonones are present in all citrus fruits, including lemons, grapefruit, and oranges (Ugoeze et al. 2020). Flavones can bind to human serum albumin and interact with proteins to enhance plasma-mediated transport (Jiang et al. 2016). Flavonones, on the other hand, are known to have antioxidant, antihyperlipidemic, and anti-inflammatory properties (Ugoeze et al. 2020). Kaempferol is another vegetable and plant flavonoid, including grapes, green tea, potatoes, onions, and cucumbers. They, like other flavonoids, may have anti-diabetic, anti-tumor, and anti-inflammatory properties (Okoli et al. 2022). It regulates several key factors of cell signaling pathways involved in apoptosis, angiogenesis, inflammation, and metastasis; it potentially inhibits cancer cell growth and angiogenesis by inducing cancer cell apoptosis (Chen and Chen 2013).

Alkaloids

Alkaloids are one of the largest classes of natural compounds. Alkaloids are structurally unique and genetically unrelated molecules (Ugoeze et al. 2020). They have various pharmacological effects and are contained in numerous herbal medicines (De Almeida et al. 2017); among them are narcotic painkillers like morphine and codeine (Ugoeze et al. 2020). Additionally, alkaloids possess potent antibacterial, antifungal, and antiprotozoal effects (Okoli et al. 2022). The findings of the study showed that ethanol extracts of *P. erinaceus* leaf contain high content of alkaloids (19.47%), with ephedrine (Figure 2) being the highest (32.47 µg/mL or 15.15%). Lunamarin has been reported to have free radical scavenging activity (Ugoeze et al. 2020). In addition, linamarin has anti-cancer, immunomodulatory, antiestrogenic, and antiamoebic properties. Alkaloid content in *P. erinaceus* leaf extract may attribute to some pharmacological properties.

Saponins

Plants and other lower marine animals, including some microbes, are the primary sources of saponins (Ugoeze et al. 2020). Saponins are present in many legumes, including soybeans, beans, peas, and various plants and crops. Saponins have immunomodulatory, anti-inflammatory, antifungal, antiviral, antibacterial, hypercholesterolemic, and anticarcinogenic activities (Ugoeze et al. 2020). *P. erinaceus* leaf's ethanolic extract contained 4.70% saponins (Table 1). Sapogenins have several beneficial effects but also have several adverse effects. For instance, their hemolytic and cytotoxic effects have been noted (Ugoeze et al. 2020). It has been reported to significantly impair protein digestion and vitamin and mineral absorption in the small intestine, causing hypoglycemia (Ugoeze et al. 2020).

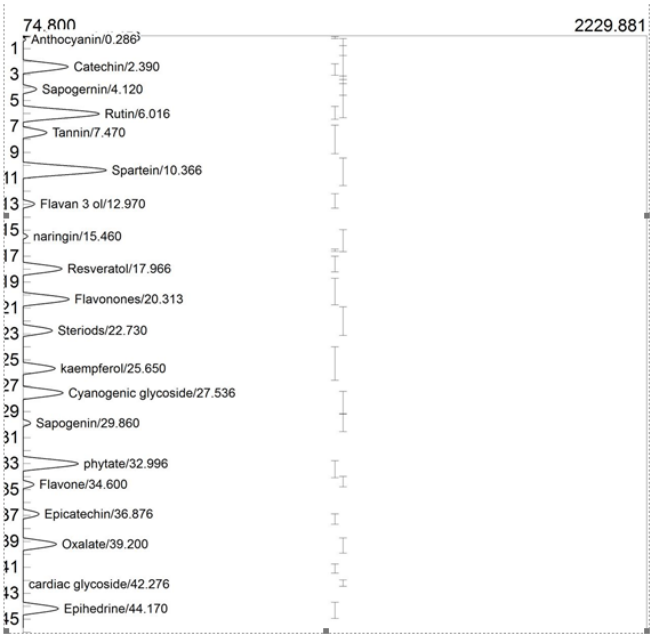

Figure 1. Chromatogram of the phytochemical constituents of ethanolic leaf extract of *Pterocarpus erinaceus*

Tannins

Tea, cocoa, vegetables, legumes, some immature fruits, and cocoa all contain tannins (Sharma et al. 2021). *P. erinaceus* leaf extract contained a low concentration of tannins (2.60%) as shown in Table 1 above. Traditional Asian medicine prioritizes tannins, and preparations from tannin-rich plants are used as astringents and diuretics (Ugoeze et al. 2020). Additionally, tannins have been used to treat tumors, stomach ulcers, and diarrhea; it exhibits antioxidant and anti-inflammatory activities (Ugoeze et al. 2020). Tannin-rich diets are usually assumed to have low nutritional value since they reduce feed intake and efficiency in experimental animals. Tannin-protein complexes can result in the inactivation of digestive enzymes and poor protein digestibility due to their interactions with protein substrates and ionized iron (Ugoeze et al. 2020).

Phytate and oxalate (anti-nutrients)

Phytate and oxalate interfere with mineral absorption and are considered anti-nutrients. Impaired nutrient absorption has been associated with headaches, rashes, nausea, bloating, and malnutrition (Popova and Mihaylova 2019). Anti-nutrients are primarily organic or synthetic, highly reactive structures, and thus poisonous. *P. erinaceus* leaf extract contained phytates and oxalates (Figure 2). Nuts, seeds, and whole grains are among the foods that contain phytic acid (myoinositol hexaphosphate). It is also present in substantial concentrations in the leaves and tubers. Phosphorylated inositol, i.e., phytic acid, is hypothesized to be important in insulin secretion (Ugoeze et al. 2020). Phytic acid may also decrease plaque growth and lower blood cholesterol and triglycerides. (Okoli et al. 2022). On the other hand, oxalates interfere with the absorption and utilization of calcium by forming calcium oxalate crystals that lead to kidney stones. They also cause irritation and swelling in the mouth and throat (Ugoeze et al. 2020).

Ethanol leaf extracts of *P. erinaceus* had different antibacterial activities against the tested strains. The bacterial strains used were clinical and laboratory isolates. All of these types of bacteria are known to be responsible for serious human infections. From a clinical perspective, *E. coli* can cause sepsis and infect the gallbladder, surgical wounds, skin lesions, and lungs (Okoli et al. 2022). *S. aureus* causes dermatitis and sialadenitis (Okoli et al. 2022). *B. subtilis* is known to cause disease in severely immunocompromised patients (Okoli et al. 2022), and *P. aeruginosa* commonly infects the pulmonary tract, urinary tract, burns, wounds, and other blood infections. Results in Table 2 above showed that the ethanolic leaf extract of *P. erinaceus* exhibited significant growth inhibition against all selected bacterial strains.

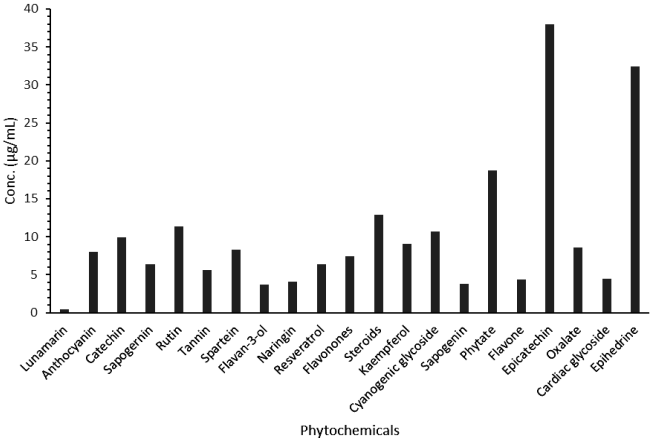

Figure 2. Concentrations of phytochemicals in ethanolic leaf extract of *Pterocarpus erinaceus* identified by GC-FID

Table 1. Identified phytochemical compounds in leaf extract of *P. erinaceus* by GC-FID

Type of phytochemical	Phytochemical constituents	RT	Area	Height	Conc. (µg/mL)	% Composition
	Catechin	2.39	12130.79	228.50	9.95	4.64
	Flavone	34.60	5899.82	111.78	4.39	2.05
	Epicatechin	36.88	6791.90	128.57	38.03	17.74
Flavonoids (44.71%)	Flavonones	20.31	12523.47	234.22	7.41	3.46
	Flavan-3-ol	12.97	6168.10	115.72	3.65	1.71
	Kaempferol	25.65	10016.25	187.04	9.02	4.21
	Naringin	15.46	4894.96	92.00	4.04	1.88
	Anthocyanin	0.29	3009.12	83.16	8.01	3.74
	Rutin	6.02	18229.86	337.71	11.31	5.28
	Lunamarin	0.08	177.35	130.84	0.41	0.47
Alkaloids (19.47%)	Spartein	10.37	19541.17	363.72	8.25	3.85
	Epihedrine	44.17	10559.76	196.23	32.47	15.15
Saponins (4.70%)	Sapogenin	29.86	5371.20	101.22	3.75	1.75
	Sapogernin	4.12	6336.68	119.93	6.33	2.95
Tanins (2.60%)	Tannin	7.47	8324.82	157.32	5.56	2.60
Steroids (6.01%)	Steroids	22.73	9463.59	177.25	12.88	6.01
Other phenols (2.96%)	Resveratrol	17.97	11156.58	209.68	6.35	2.96
Anti-nutrients (12.77%)	Oxalate	39.20	10114.97	189.47	8.61	4.02
	Phytate	33.00	14311.56	265.89	18.76	8.75
Glycosides (7.00%)	Cardiac glycoside	42.28	3498.15	65.31	4.41	2.06
	Cyanogenic glycoside	27.54	11395.41	213.41	10.72	5.00

Table 2. Diameter of growth inhibition of ethanol leaf extract of *Pterocarpus erinaceus* against several bacterial isolates

Extract (µg/mL)	*Bacillus subtilis* (Gram +ve) Inhibition zone (mm)	*Escherichia coli* (Gram -ve) Inhibition zone (mm)	*Pseudomonas aeruginosa* (Gram -ve) Inhibition zone (mm)	*Staphylococcus aureus* (Gram +ve) Inhibition zone (mm)
Gentamicin (30µg/mL)	20.40 ± 0.20^{d}	20.50 ± 0.20^{d}	30.10 ± 0.10^{d}	23.97 ± 0.15^{d}
100	7.57 ± 0.15^{a}	3.47 ± 0.15^{a}	5.13 ± 0.15^{a}	4.13 ± 0.25^{a}
150	9.20 ± 0.26^{b}	4.80 ± 0.10^{b}	6.03 ± 0.32^{b}	5.93 ± 0.35^{b}
200	13.20 ± 0.10^{c}	6.90 ± 0.10^{c}	8.07 ± 0.06^{c}	7.53 ± 0.02^{c}

Note: Result is Mean ± SD. Values with the same superscript within a column are statistically not significant, while values with different superscript within a column are statistically significant (P<0.05)

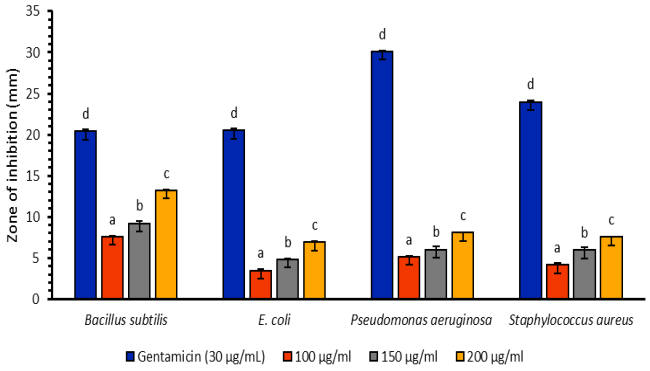

Figure 3. Diameter of inhibition of ethanolic leaves extract of *Pterocarpus erinaceus against* four isolates of bacteria

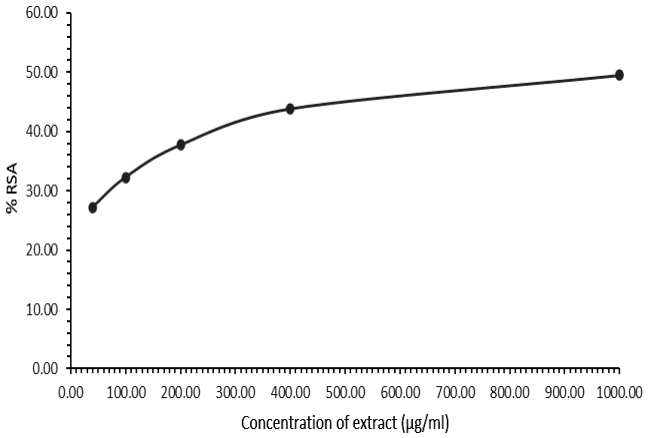

Figure 4. The percentage of Radical Scavenging Activity (RSA) of the leaf ethanolic extract of *Pterocarpus erinaceus* leaf at various concentrations

The extract showed dose-dependent increasing inhibitory activity across all concentrations (100 µg/mL, 150 µg/mL, and 200 µg/mL) against four bacterial strains used in this study. The highest inhibition was observed against *B. subtilis* with an inhibition zone of 13.20 mm at 200 µg/mL concentration. The lowest inhibition was observed against *E. coli* with an inhibition of 3.47 mm at a 100 µg/mL concentration. The inhibition zone of four bacterial isolates was significantly lower than the standard antibiotic, gentamicin. This study's result differed from that of Tittikpina et al. (2018): the MIC value of leaf ethyl acetate fractions against *S. aureus* and *E. faecalis* was 256 µL/mL. The high presence of alkaloids in the extract could be the reason for its inhibitory effects on the bacteria strains since alkaloids are reported to possess antibacterial activities.

The antioxidant activity of *P. erinaceus* leaf extract was analyzed using the DPPH method. Figure 4 revealed that the *P. erinaceus* extract has a relatively moderate free radical scavenging activity in a dose-dependent manner. It had the highest activity of 49.51% at a concentration of 1000 µg/mL (Figure 4), and the lowest was 40 µg/mL (27.20%). The presence of flavonoids and alkaloids could be the reason behind the DPPH scavenging ability of the extract as both classes of phytochemicals have been noted to exhibit antioxidant activities.

In conclusion, the ethanolic leaf extract of *P. erinaceus* was found to be rich in some classes of phytochemicals known to have many beneficial effects on human and animal health ranging from antimicrobial, antioxidant, and anti-cancer to anti-inflammatory. The extract was found to inhibit the growth of four bacterial isolates (*E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa*). The extract also exhibits a moderate radical scavenging activity in a dose-dependent manner. However, further research is needed to isolate and identify the particular bioactive molecules responsible for therapeutic effectiveness and clarify their modes of action.

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