

Antibacterial activities of Parinari Macrophylla Seed Extracts against Salmonella Typhimurium Infection (Gastro-Enteritis) Induced in Wistar Rats.

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Antibacterial activities of *Parinari Macrophylla* Seed Extracts against *Salmonella Typhimurium* Infection (Gastro-Enteritis) Induced in Wistar Rats

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ABSTRACT

Bacterial infection is the major cause of concern worldwide. Moreover, Bacterial drug resistance still remains a major challenge in the health system, thereby giving rise to an increased search for antibacterial drugs. Hence the need to explore the potentials in Plants as a valuable source of antimicrobial agents. This study evaluated the *Invitro/Invivo* antibacterial and toxicological potentials of *P. macrophylla* seed extracts against *S. typhimurium* infection (gastro-enteritis) induced in Wistar rats. Thirty (30) female Wistar rats were randomly grouped into 6 (A-F). The groups were infected with *S. typhimurium* except the negative control group (F). A-C were administered 100, 200, and 400 mg/kg body weight of hydro-ethanolic extract of seed *P. macrophylla* respectively, while group D was given 6 mg/kg body weight of ciprofloxacin while group E wistar rats served as positive control. The rats were then infected with 10^6 *S. typhimurium* and observed for nine consecutive days for symptoms of gastroenteritis, before starting the treatments. Fecal droppings from all rat groups were collected randomly, over a period of five days to evaluate the microbial load before and during the five days' treatment. There was a progressive significant decrease in fecal bacterial load in groups treated with *P. macrophylla* seed extract compared to the antibiotic and positive control group throughout the five-day treatment period. The rats were later euthanized and serum obtained from the collected blood were utilized for biochemical assay. The globulin concentration, activity of aspartate and alanine amino transferase in the animal administered the extract showed a significant increase ($p < 0.05$) compared to the group treated with ciprofloxacin. There was a significant decrease in creatinine in the groups administered hydro-ethanolic seed extract of *P. macrophylla* compared to the negative control group and antibiotics treated group. The antibacterial effect of the seed extract of *P. macrophylla* could be adduced to its bioactive compounds, with the potentials to inhibit the multiplication of the bacterial cell in the gastrointestinal tract of the wistar rats. Furthermore, the antioxidant effect of the extract could have mediated a mitigative effect against the pathogenesis associated with the infection. There was no alteration in the liver function and kidney function indices which suggest the safety of the extract in the rats. The hydro-ethanolic seed extract of *P. macrophylla* displayed a potential antibacterial activity against the test organism *S. typhimurium* compared with the standard drug (Ciprofloxacin).

Key words: Medicinal Plant, *Parinari Macrophylla*, Seed Extracts, *Salmonella Typhimurium* Infection, Induced, Wistar Rats.

1. Introduction

Africans have traditionally used medicinal plants, also known as botanical medicine, herbal medicine, or phytomedicine, to treat a variety of ailments. *Pentaclethra macrophylla*, a tropical fruit plant with root, stem, leaf, flower, fruit, and seed parts, is among the underutilized plants in Nigeria. Numbers of people are not aware of the seed's immense potential to combat infections and malnutrition. Most medicines in the world are primarily derived from plants, and they continue to provide humans with new treatments. Nigeria is expected to have the world's healthiest population due to its abundant supply of fruits, vegetables, herbs, and medicinal plants. We might have also emerged as pioneers in the field of drug discovery (Yusuf et al., 2019).

Before the advent of modern medicine, various societies, including indigenous peoples, developed traditional knowledge based on their folk beliefs over many generations. This body of knowledge, also known as traditional medicine, indigenous medicine, or folk medicine, includes the collective wisdom, skills, and practices used by different cultures to maintain health and treat illnesses. The World Health Organization (WHO) defines traditional medicine as the accumulation of knowledge, skills, and practices based on cultural theories, beliefs, and experiences, regardless of scientific explanation, used for health maintenance and the prevention, diagnosis, improvement, or treatment of physical and mental illnesses. Traditional medicine frequently contrasts with scientific or modern medicine, which is based on scientific principles, research, and clinical trials, both systems play critical roles in healthcare, with traditional medicine providing culturally significant practices and modern medicine providing scientifically validated treatments.

About 25% of all prescribed drugs are derived from plants worldwide. Among the 252 pharmaceuticals on the WHO list of essential medicines, plants make up 11% of the total. Actually, opium from poppies' seedpods was used to make morphine, the first medicinal substance, about 200 years ago. Since then, researchers have studied plants to create the pharmaceuticals we use today. People are beginning to pay more attention to natural, herbal medicine, however, after years of overmedicating, dealing with resistant bacteria in the microbiome, and treating symptoms rather than the cause of illness. Plants are now being used to treat a variety of illnesses and ailments, including cancer, gastrointestinal problems, allergies, rheumatism, migraines, exhaustion, skin infections, wounds, and burns (Susan et al., 2019). This emphasizes the long-standing truth that food and natural remedies can be effective medicines. Many people are returning to the traditional use of herbs and plants for treatment because they are frequently less expensive and safer than conventional medications, botanical medicine is still relevant in the modern Western world.

According to a recent World Health Organization estimate, around the world, 80% of people get some portion of their primary care from herbal medicines. Because of this pervasive dependence on botanical remedies, there is a \$60 billion yearly global market for herbal medicine, indicating the important role that herbal medicine still plays in contemporary health practices. Americans' interest in herbal therapy has grown as a result of the growing expense of prescription medications

and a resurgence of interest in organic and natural cures. Numerous components found in whole herbs can aid in the treatment of diseases and the reduction of their symptoms. Herbal medicine, another name for botanical medicine, refers to the therapeutic use of a plant's seeds, berries, roots, leaves, bark, or flowers. The biological characteristics of these plants provide a variety of health benefits, which are influenced not only by the plant's inherent properties but also by factors such as the growing environment, harvesting methods, and processing techniques. Plants can be purchased either unprocessed or processed, with some of their beneficial compounds extracted via maceration in water, alcohol, or other solvents, thereby increasing their therapeutic potential. Herbal medicines contain a variety of chemicals, including fatty acids, sterols, alkaloids, flavonoids, glycosides, and saponins, which contribute to their therapeutic properties (Susan et al., 2019).

This practice is especially common among low-income people in developing countries, who frequently rely on traditional herbal practices for their basic healthcare needs. This preference persists despite significant technological advances in modern medicine and efforts by organisations such as the World Health Organisation (WHO) to combat diseases like diarrhoeal infections in these areas. Traditional herbal medicine continues to play an important role in these communities, alongside modern medical practices. The preference for traditional and herbal medicine in low-income developing countries has prompted the World Health Organisation (WHO) to fund research aimed at advancing traditional medical practices. This includes treating and preventing common diseases such as diarrhoea, which is a major cause of death in these areas. Research has looked into the effectiveness of various traditional medicinal herbs in combating such diseases, emphasizing the importance of incorporating these practices into larger healthcare strategies. Encouraging this research, the WHO hopes to increase the use of effective traditional remedies, improving health outcomes and lowering mortality rates in developing countries. With the global market for medicinal plants generating billions of dollars annually and continuing to grow, Nigeria does not benefit significantly. The few drugs developed from these plants have not been included in the list of critical medications used by conventional doctors. Historically, Nigerians relied heavily on regional herbal remedies before the advent of conventional medicine. However, significant barriers to the use of African medicinal herbs include poor quality control and safety concerns. Traditional medical practices are often secretive, and adverse effects are rarely reported or documented (WHO, 2019). Despite these challenges, the majority of people worldwide continue to manage illnesses with herbs and other traditional treatments, underscoring the persistent reliance on and potential of traditional medicine in global healthcare.

The World Health Organization emphasizes that businesses in the United States and Europe face stringent regulations and entry barriers that necessitate rigorous testing before they can begin mass production. Nigeria, on the other hand, has fertile land suitable for growing medicinal plants, which could help to create jobs and boost the economy significantly. However, the lack of regulations and incentives has slowed progress in this field. The national physical fitness garden in villages provides an excellent opportunity for drug development because it has helped

communities improve their health, prevent disease, and provide treatments. Guided by a taxonomist, a traditional healer, and a local focus person, a variety of medicinal plants were discovered, each with potential active ingredients. These plants are easily accessible, inexpensive, and well-liked by the locals, indicating a significant opportunity for utilising traditional knowledge in drug development and healthcare advancements.

A team of researchers led by a distinguished academic and researcher made a significant breakthrough a few years ago that could lead to the discovery of a cancer treatment drug derived from Nigerian medicinal plants (Marte et al., 2019). This study identified eight local herbs or plants that, according to a pharmacology professor, are more effective than current cancer treatments. The professor presented these findings at the Nigerian Academy of Science's induction ceremony, emphasizing that some Nigerian herbal plants may be more effective than current cancer treatments. This development emphasizes the potential of traditional Nigerian plants in cancer treatment and the need for further research in this area. In 2014, the World Health Organization (WHO) passed a resolution advocating for the integration of traditional medicine into the global healthcare system. This resolution represents a strategic plan aimed at enhancing the safety, quality control, and policy frameworks surrounding traditional medicine. It emphasizes the importance of accumulating and organizing knowledge about traditional medicinal practices to improve their application. Additionally, the resolution calls for the creation of a national database to catalog the appropriate uses of various herbal and medicinal plants. This initiative seeks to ensure that traditional medicine is utilized effectively and safely, providing a structured approach to incorporating these practices into mainstream healthcare.

Signs and symptoms of gastroenteritis include nausea, vomiting, diarrhoea, stomach discomfort, fever, headaches, loss of appetite, weight loss, and sweating. Currently, the plant's main bioactive components have been identified as steroids and flavonoids, which include glycosides related to stigmasterol, quercetin, and catechin. The extracts of the plant have anti-venom, antimicrobial, analgesic, anti-inflammatory, anti-mycobacterial, anthelmintic, and antioxidant properties. Further research on *P. macrophylla* is required to discover additional biological functions that traditional healers attribute to it, as well as to isolate more of its bioactive compounds. Despite its widespread use, extensive safety and tolerability studies are required to ensure that *P. macrophylla* is both safe for consumption and effective in medicinal applications. This additional research will help validate traditional healers' claims and ensure that the plant's use is supported by scientific evidence, thereby increasing its potential as a therapeutic resource while protecting public health.

According to Al Jassas et al. (2018), gastroenteritis is an infectious condition characterised by inflammation of the gastrointestinal tract's mucous membrane, which includes the stomach and intestine's infection with parasites, viruses, and bacteria, which are the main causes of gastroenteritis in children. Bacterial agents include *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio*; viral causes include norovirus and rotavirus. In addition to chemical poisons, parasites such as *Giardia lamblia* and *Entamoeba histolytica* can cause the illness. Poor sanitation and personal

hygiene play a major role in the spread of these intestinal parasites, which are particularly common in poor resource settings. Numerous symptoms, including vomiting, diarrhea, nausea, stomach discomfort, fever, headaches, loss of appetite, weight loss, and sweating, are indicative of gastroenteritis (Al Jassas et al., 2018). These symptoms highlight the need of putting both preventive measures and efficient therapies into practice because they can have a major impact on a child's health and well-being. It falls into one of three categories: chronic, acute, persistent, or non-infectious. Death rates can still surpass 17,000 per year even with improved care (Al Jassas et al., 2018).

The diarrhea brought on by intestinal bacteria may be caused by various mechanisms. These mechanisms may involve the generation of toxins, adhesion, and mucosal invasion. Understanding the pathophysiology of the illness is crucial for developing management protocols for gastroenteritis. The small intestine's role in fluid absorption is crucial. Due to the effects of toxins on the intestines, small intestines in cases of gastroenteritis are unable to perform this function (Al Jassas et al., 2018).

Adherence is an important virulence factor to consider when thinking about gastroenteritis. Many bacteria need to stick to the intestinal mucosa, especially at first. They create a number of adhesive factors and proteins that aid in creating the necessary attachment to the intestinal walls in order to establish this adherence, for instance, the surface adhesions that the *Vibrio cholerae* bacteria use to adhere to the intestinal borders. Another illustration would be ingesting toxic *E. coli*, which causes watery diarrhoea by producing the adherence protein known as the colonization factor antigen. The pathology of enterotoxigenic *E. coli* depends on this step. *Shigella* and enteroinvasive *E. coli* infections can result in dysentery. This happens as a result of the small intestine's mucosa being invaded and destroyed (Al Jassas et al., 2018). Production of toxins, including enterotoxins, is the final key virulence factor that contributes significantly to the onset of a gastroenteritis attack. Due to enterotoxins' direct secretory effects on the small intestine's mucosa, they can cause watery diarrhea (Al Jassas et al., 2018).

One of the most prevalent illnesses worldwide that leads to infectious diseases is gastroenteritis. Among the warning signs and symptoms are vomiting, stomach pain, and—most importantly—diarrhea. There could be acute, recurring, or chronic gastroenteritis. In the United States alone, there are about 350 million instances of acute gastroenteritis recorded annually. Viral infections cause gastroenteritis in the great majority of cases. Antibiotic use based on symptoms is therefore generally not recommended. However, empiric antibiotic therapy is recommended in a small number of patients and has been linked to significant improvement and mortality reduction. The primary objective of gastroenteritis management is to treat dehydration (Al Jassas et al., 2018).

Antibiotics Resistance factors necessities the concept of this Research

Most current medications are no longer useful due to antimicrobial resistance, which has arisen as a result of extensive, incorrect, inconsistent, and excessive antibiotic use. The World Health

Organization stated that this new trend could be the most pressing issue confronting medical science. From medicinal plant-derived antimicrobials, there is significant therapeutic potential and additional approaches to cost-effective therapy. Furthermore, there is a growing need for novel antimicrobial agents that can curb antibiotic usage while simultaneously battling resistance to microbes. To tackle this issue, researchers have been tasked with isolating and characterizing novel bioactive compounds from plants. Medicinal plant extracts have the potential to be a helpful substitute treatment option when drug resistance and numerous side effects occur (Manandhar et al., 2019).

Novel beneficial compounds from medicinal plants must be quickly identified and isolated due to the growing threat that drug-resistant diseases pose to public health. The development of resistance mechanisms in bacteria is rendering traditional antibiotics less effective, hence requiring the development of novel treatment medicines. Due to their profusion of bioactive chemicals, medicinal plants hold great potential as a source of novel antibiotics. Standardized modern analytical procedures enable systematic and reliable extraction, identification, and characterization of these compounds. This approach improves our ability to discover new, effective strategies to combat pathogenic bacteria and address the critical issue of antimicrobial resistance (WHO, 2021b).

Epidemiological profile of Gastroenteritis

In the US, acute gastroenteritis is typically regarded as a mild condition that rarely results in serious, life-threatening complications. Acute gastroenteritis and upper respiratory tract infections are really two of the most common causes of infectious illnesses in the United States. The CDC used data from the National Centre for Health Statistics to report the total number of fatalities from all causes related to acute gastroenteritis has been rising since 1999 and has now reached about 17,000 per year. In the elderly population, we found that more than 80% of these deaths occurred, and more than 60% of them were attributed to infections with *Clostridium difficile*. The important conclusion that results from this is that, after an acute gastroenteritis attack, older ages are linked to higher rates of morbidity and mortality.

Some of the essential things that need to be established include: the recent contact with affected persons, the type and frequency of bowel movements, vomiting and fluid intake and output, travelling, and the use of antibiotics and other drugs. Children frequently experience chronic constipation, and false diarrhea might be mistaken for faecal overflow incontinence. In young children, vomiting and diarrhea are nonspecific symptoms. If a child has a high fever, persistent symptoms, or indicators of a surgical etiology (such as severe stomach pain, bilious nausea, or abdominal mass), the diagnosis of gastrointestinal disease should be reevaluated. Youngsters with inborn metabolic abnormalities and diabetes mellitus may throw up. Referrals to pediatric services should be taken into consideration for children with underlying conditions, since they may be more susceptible to problems (Koutri et al., 2019).

During outbreaks, stool samples from children have to be collected, particularly in residential homes, child care centers, schools, or hospitals where determining the pathogen's identity and tracking its origin is crucial for maintaining public health. The samples should be subjected to both viral pathogen detection and bacterial culture by using quick antigen detection techniques. A prompt diagnosis enables a child to be isolated and nosocomial infection to be prevented, which is a common and often used method of assessing the effectiveness of contact infection prevention techniques. Children who have bloody diarrhoea, have recently gone overseas, or have a high fever should also have their stool samples taken. Clinicians are required by law in many countries to report a range of bacterial and viral illnesses to public health authorities.

According to the World Health Organisation (WHO), traditional medicinal plant medicines continue to benefit 80% of the developing world's population. In comparison to the 28,187 medicinal species that humans use, there are an estimated 374,000 total plants (Christenhusz & Byng, 2016). More precisely, Amina et al. (2021) report that *P. macrophylla* seed extracts exhibit strong anti-venom, antimicrobial, analgesic, anti-inflammatory, anti-mycobacterial, anthelmintic, and antioxidant characteristics. Steroids and flavonoids, which include glycosides linked to stigmasterol, quercetin, and catechin, are currently the plant's primary active ingredients (Musa et al., 2021). There has been a lot of studies on *P. macrophylla*'s leaves, stems, and bark, but not much on the extracts from the seeds. The medicinal value and effectiveness of the seed extracts as a gastroenteritis treatment will be further elucidated by the in vivo study.

Aim of Study

The aim of this study is evaluating the antibacterial and toxicological effects of *Pentaclethra macrophylla* seeds plant extract on gastroenteritis infection induced in Wistar rats. This study is expected to add value to these seed extracts to be considered as a natural tool for new drug discovery through its potent antibacterial gastroenteritis therapy.

Salmonella typhimurium

Salmonella typhimurium is a rod-shaped, partially anaerobic, gram-negative bacterium belonging to the Enterobacteriaceae family. Their main dwelling is the intestines of humans and other animals. Certain species are present without producing symptoms of illness, while others can lead to a range of moderate to serious diseases commonly referred to as salmonellosis. Most human cases of *Salmonella* infections are brought on by eating or drinking dirty water (Zeyu et al., 2023).

Salmonellosis, a foodborne illness, is caused by the bacterium *S. typhimurium*. It can form biofilms on food contact surfaces and develop multidrug resistance (Zeyu et al., 2023). *S. typhimurium* can colonise tumour tissues and has been shown to have oncolytic and immune system-regulating properties, making it a potential cancer therapy approach (Sara et al., 2022). Infection with *S. typhimurium* can cause a decreased immune response, downregulation of pro-inflammatory

cytokine receptor genes, and inhibition of interferon I and II signalling pathways (Liping et al., 2023).

S. typhimurium infections can result from polluted environments, unhealthy food sources, or consumption of infectious prey. including the veterinary hospital. The number of pathogenic microorganisms, the cat's immune system, and the presence of coexisting conditions all influence the clinical signs that are produced. There is no difference between songbird fever and other Salmonella infections, despite the fact that infection rates in cats (and people) have been linked to seasonal bird migrations. Clinical symptoms usually start to show up three to five days after exposure. They usually start with fever (which is usually higher than 40° C [104° F]), fatigue, and anorexia and then move on to vomiting, diarrhea, and stomach discomfort. Biochemistry findings are usually vague, while haematology may show non-regenerative anemia and leukopenia with a left shift. To make the diagnosis, the organism is isolated by culturing and identified using PCR; however, since the organism may be isolated from healthy animals, as is the case with most GI infections, it is crucial to correlate the detection of pathogens with clinical indications.

Antibiotic resistance, like *E. coli*, is widespread, according to one survey conducted in the United Kingdom, the most common bacteriophage type identified was multiple drug-resistant strain DT104. Since frequent use of antibiotics in the treatment of salmonellosis increases drug resistance and lengthens the convalescent excretion period, treatment should be limited to cats presenting systemic symptoms. Since resistance is so prevalent and erratic, sensitivity results alone should be used to guide antibiotic selection. This implies that excrement needs to be cultured as well, even if the bacterium was detected by PCR. Treatment must be long enough—up to 28 days—to eradicate the organism's faecal discharge, avoid relapse, and lessen the possibility that resistance may emerge. The zoonotic potential makes these precautions more crucial. Because of the significant zoonotic potential of salmonellosis, these measures are essential.

Food items from sick pigs, cattle, and poultry as well as healthy food that has subsequently come into contact with infected food are the two primary causes of infection. While *S. Dublin* is more locally distributed, *S. typhimurium* is the most commonly seen serovar isolated from cattle in several countries worldwide. The latter serovar has very infrequently been found in cattle in Canada, although it has been found in the United States, the United Kingdom, and other European nations. For most of the 1960s, the common serovar isolated from cattle in the United Kingdom was *S. Dublin*. Following this, there was a significant drop in the number of infections, and currently, *S. typhimurium* is the most frequently isolated serovar in cattle. Australia has seen a rise in *S. Dublin* spread in dairy cattle within the past 20 years. A major cow pathogen in South Africa and South America, *S. typhimurium* was the most often isolated serovar from cattle in Canada during the 1990s; a comparatively smaller number of isolates were serotyped as *S. Anatum*, *S. Muenster*, *S. Give*, *S. Mbandaka*, *S. Heidelberg* and *S. Cerro* during the 1960s.

In the early 1990s, *S. typhimurium* was the most often isolated serovar from cattle in the United States. Outbreaks and cases in cattle have recently been documented (Schlumberger et al., 2005). Many methods can be used to further identify these strains, including strain investigation during the detection of *Salmonella* plasmid virulence (spv) gene sequences on a virulence-associated plasmid of approximately 75 Kb, plasmid profile analysis, clinical trials, and detrimental features of the infection in calves and cattle (Sara et al., 2022).

Methodology

Materials

Chemicals and Reagent

All Regents used were analytical grades purchased from Sigma-Aldrich, chemical Co. (St Louis, Mo, USA). Chemicals were newly prepared on the day of experiments according to standard protocol as directed by the procedures that were followed. Distilled water was prepared under sterile condition in the microbiology laboratory

Media

Culture media used were Mac conkey agar, nutrient agar, desoxycholate citrate agar, from the Oxoid brand was used for microbial assays. All of the media were prepared by figuring out how much was needed, according to the manufacturer's instructions, suspended in distilled water, heated to a boil while stirring to completely dissolve the powder, and then autoclaved for 15 minutes at 121°C to sterilize the media before using.

Plant Material

Fresh *P. macrophylla* fruit was obtained from Egbe, West Central, Kogi State. The fruit was sent to Omu Aran, Kwara State, Nigeria, to the Department of Food Science and Microbiology at Landmark University.

Ethical Report

Ethical permit was obtained from Landmark University's ethics committee, Omu-aran, Kwara state, Nigeria. With an approval number (LMUIREC/ACSC/019/2023). All animals were handled humanely following the Institution's values and Guidelines for the care of animals and use of Laboratory were strictly observed throughout the research work.

Methods

Collection and Identification of *P. macrophylla* Seeds

Fresh *P. macrophylla* fruit was obtained from Egbe, West Central, Kogi State. The fruit was sent to Omu Aran, Kwara State, Nigeria, to the Department of Food Science and Microbiology at Landmark University.

The seed was authenticated in the University of Ilorin, Herbarium Center, identified using a voucher specimen from the herbarium as a guide, (UILH/001/1789/2024).

Preparation of Crude Extract

The fresh seeds of *P. macrophylla* were collected, air-dried for three weeks, and pulverized using a blender. The extracting solvent was hydro-ethanol. Five hundred grams of the seed powder was soaked in the solvent using cold maceration extraction method for 72 hours and mixed intermittently. The filtrate was collected and a rotary evaporator was used to expel the solvents. The paste was freeze-dried and stored in air-tight glassware in the refrigerator at 4 °C. The concentration at which bacterial development was suppressed was recorded as the minimum inhibitory concentration (MIC). The zone of inhibition for each extract and the control was measured and recorded (Balouiri et al., 2021).

Phytochemical Screening

Standard test procedures were used to determine the presence of qualitative phytochemical in the seeds extract of *P. macrophylla*. The alkaloid, flavonoids, saponin, tannins and phenolics were determined according to the methods of Harborne (1973) and Sofowora (1992). The extract was subjected to phytochemical analysis in accordance with the established protocol for the identification of saponins, tannins, and terpenoids (Lawal et al., 2019).

Quantitative Phytochemical Analysis

Total Phenolic Content.

The method outlined by Singleton and Rossi [2020] for determining the total phenolic content of the extracts was applied to the Folin and Ciocalteu reagent. The sample and standard readings at 765 nm were measured using a wavelength spectrometer in respect to the reagent blank. A mixture of 0.6 mL of water and 0.2 mL of Folin-Ciocalteu's phenol reagent (1:1) was added to the test sample (0.2 mL). The combination was added to one milliliter of saturated sodium carbonate solution (8% w/v in water) after five minutes, and the volume was increased to three milliliters using filtered water. The reaction was centrifuged after a half-hour dark period, and the blue absorbance of the different samples was measured at 765 nm. The phenolic content was calculated as Gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of Gallic acid (5–500mg/L, $Y = 0.0012x + 0.138$, $R^2 = 0.969$). All determinations were carried out in triplicate (Penchala et al., 2012).

Total Flavonoids Content

The sample's total flavonoid concentration was ascertained using the aluminium chloride colorimetric technique. The accepted calibration curve for the measurement of total flavonoids was created using quercetin. After 5.0 mg of quercetin was dissolved in 1.0 mL of methanol to create a stock quercetin solution, methanol was serially diluted to create the standard quercetin solutions (5–200 µg/mL). Separately, 0.6 millilitres of diluted standard quercetin extracts or solutions were combined with 0.6 millilitres of 2% aluminium chloride. The mixture was then allowed to sit at ambient temperatures for 60 minutes. At 420 nm, the absorbance of the reaction mixtures was measured in relation to a blank. The concentration of total flavonoid content in the test samples was calculated from the calibration plot ($Y = 0.0081x + 0.0696$, $R^2 = 0.9997$) and

expressed as mg quercetin equivalent (QE)/g of dried plant material. All the determinations were carried out in triplicate (Talari *et al.*, 2012).

Saponins

Spectrophotometric determination of saponins was done according to the method of Brunner (1984) At this, to a 250 ml beaker, 1g of the dried and ground sample was taken and 100 ml of isobutyl alcohol was added to it. From the results one can conclude that the mixture was shaken on a UDY shaker for 5 hours in order to accomplish a perfect randomization of the liquids. After that the mixture was filtered by using Whatman No. 1 filter paper in a 100 ml beaker containing 20 ml of 40% saturated solution of $MgCO_3$. The filtrate was then obtained in order to produce a scholarly colourless solution as the end product. Laboratory test I of each sample was prepared by pipetting 1ml of the colourless filtrate into a 50ml volumetric flask, adding 2ml of 5 % $FeCl_3$ and then distilled water to the mark. After that it was allowed to stand for 30 minutes to give a blood red color as described above. Saponin standard solution was prepared from saponins stock solution with the degree of saponin concentration as 0-10ppm. As in the earlier descriptions, 2ml of 5% $FeCl_3$ solution was also added like the standard solutions. Percentage absorption of developed samples and standard saponin solutions was measured at the wavelength of 380nm using Jenway V6300 spectrophotometer. Percentage saponin was calculated using the formula:

$$\% \text{ saponin} = \text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}$$

$$\text{Weight of sample} \times 10,000$$

Tannins

The method developed by Swain (1979) was used to estimate the quantity of tannins. 0. 20g of test was put on the volumetric of 50ml measuring glass. 20ml of half methanol was added and covered with paraffin, and then placed in a water bath at 77-80 degrees Celsius for an hour. To make sure, it was violently shaken. homogeneous mixing After the extraction was finished the arrangement was sifted through a twofold layered Whatman NO 41 channel paper into a 100ml volumetric carafe. After that, 20 milliliters of water and 2 After adding 5 milliliters of Folin-Denis reagent and 10 milliliters of 17% Na_2CO_3 solution, the mixture was thoroughly stirred. Following this procedure, water was added to the mixture, it was thoroughly stirred, and it was left to stand for twenty minutes. The color at the extreme end of the range will be bluish-green. Test focus under 0-10ppm was additionally regarded equivalent to test fixation more noteworthy than 1ml. Using a spectronic 21D spectrophotometer at 760 nm, the absorbances of the Tannic acid standard solutions and samples after color development were measured. Percentage Tannin was calculated using the formula:

$$\text{Tannin} = \text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}$$

$$\text{Weight of sample} \times 10,000$$

***In-Vitro* Study**

The test microorganism *S. typhimurium* a pure culture was obtained from Federal University of Technology, (FUTH) Minna.

Antibacterial Activity of the Extract / In-Vitro Study

The antibacterial activity of the concentrates was resolved utilizing the Kirby Bauer procedure; the concentrates and divisions were resolved utilizing the stock miniature weakening technique; the examples were gathered at various fixations; the unrefined concentrates were disintegrated in 1 milliliter of Dimethyl sulfoxide (DMSO) arrangement; the culture was blended in with 5 milliliters of saline water utilizing a vortex blender to accomplish a consistently turbid appearance, which was contrasted with that of the 0.5 Mcferland standard; desoxycholate citrate agar was ready and administered in each petri-dish and permitted to cement; a q-tip was utilized to get a settlement of microscopic organisms contained in the stock, until it imagined consistency with 0.5 Mcferland standard, A cleaned paper circle was given opportunity to retain the different concentrate focuses. Subsequent to vaccinating the dried agar plate multiple times with a swab, turn the plate by around 60 degrees each chance to ensure an even conveyance of the inoculum. The plates were then left to sit at room temperature for something like three to five minutes. A short time later, 6.0 mm openings were exhausted utilizing plug drills, and concentrate at different focuses was added to each well. Following six hours of standing time, the plates were brooded at 37°C for eighteen to 24 hours. The grouping of the example at which bacterial development was smothered, to approve the movement, was the base inhibitory focus (MIC). The zone of restraint for each concentrate and the control was estimated and recorded (Balouiri *et al.*, 2021).

Preparation of McFarland Standard

McFarland standards are suspensions of latex or barium sulfate that make it possible to compare the density of bacteria visually. By combining 0.05 mL of 1.175% barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) with 9.95 mL of 1% sulfuric acid (H_2SO_4), a turbidity indicator was seen while creating a 0.5 McFarland standard. The accuracy of the turbidity standard was confirmed by measuring absorbance using a spectrophotometer equipped with a matching cuvette and a 1-cm light path. For the 0.5 McFarland standard, the absorbance at 625 nm ranged from 0.08 to 0.13 (Balouiri *et al.*, 2021).

Grouping model for the experimental Animals

The research used 30 female Wistar rats weighing 100-120 g in which were in good health, the Wistar rats were randomly grouped into 6 groups: A to E and each consisting 5 Wistar rats (Table 3.1). *Salmonella typhimurium* (10^6 cfu/ml) causing gastroenteritis was induced orally with the use of cannula, incubation period for the manifestation of infection gastro-enteritis in Wistar rat was 9 days after induction. Treatment took place after the infection started manifesting, for 5 to 7 days.

Table 3.1: Experimental rat batches: infection against types of treatment administered

Groups of rats		Infected treated with 100 mg extract	Infected treated with 200 mg extract	Infected treated with 400 mg extract	Infected treated with Ciprofloxacin	Infected withnot treated	Not infected, Not treated
No of Wistar rats		5	5	5	5	5	5
<i>S. typhimurium</i> (10^6 cfu/ml)		pos	Pos	pos	pos	pos	neg
On Ciprofloxacin treatment at 6 mg/kg b.w.		neg	Neg	neg	Pos	neg	neg
On <i>P. macrophylla</i> extracts treatment		Pos	Pos	pos	ne	neg	neg

Instrument Used in Sampling

An animal house was constructed, where the animals were kept, with good access to food and water with standard humidity levels (55–65%), light and temperature cycles (12–14 hours). A week prior to the commencement of the study, all rats were acclimatized to the experimental conditions. Guidelines and standard operational procedures in the laboratory were followed when it comes to animal care and experimental procedures throughout the course of the project work. to check the potency and efficacy of the seed extracts, weight of the rats was determined and the dosage of the extracts. The rat received 1ml of the isolates (10^6 Cfu/ml) using cannula via oral administration, after comparing the turbidity to MacFerland standard with the isolates and confirm using UV Spectrophotometer, the incubation period for symptoms of gastroenteritis will occur 7 to 14 days. Symptoms of gastroenteritis start manifesting after 7 days of inducing the rats with *S. typhimurium* , I confirmed gastroenteritis infection using the following four parameters;

End use of Parameters

The following parameters was established to confirm the infection of gastroenteritis in induced Wistar rat prior to treatment;

- Culturing of the stool sample using a selective media (desoxycholate citrate) that will enhance the growth of *Salmonella typhymurium*.
- Swab sticking the colon using a sterile swab stick sand grown on selective media (desoxycholate citrate) incubating it for 24hours for visible growth of *Salmonella typhymurium*.
- Physical parameters; observing the infected rats for dizziness/sleepiness
- A differential test was carried out on the blood of the infected rat that revealed the level of infections.

Treatment of infected rats began using different concentrations of the seed extract of *P. macrophylla* (100 mg, 200 mg, 400 mg), respectively. with antibiotic (ciprofloxacin) which was a standard drug, the treatment was done five days before the rats were sacrificed and the serum was prepared for biochemical assay.

Antioxidant Assessment using DPPH Radical Scavenging Assay

A widely used spectrophotometric technique for assessing the antioxidant potential, pure materials, and herbal extracts is the DPPH radical scavenging. The methodology explained by (Siddhartha *et al.*, 2022).

The antioxidant assessment test for DPPH radical scavenging activities of *P. macrophylla* seed extract had a higher antioxidant property, ascorbic acid was used as a standard.

Calculation

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$$0.743 - 0.321 = 0.422$$

$$\text{Abs of control} = 1.10$$

$$0.422 - 1.10 = 0.678 \times 100 = 67.8$$

$$67.8 / 1.10 = 61.64\%$$

Purification and Identification of Isolates

In line with the procedure described by (Duarte *et al.*, 2019). Isolating and verifying the bacteria from samples is a step in the colony identification process for *S. typhimurium*.

Standard microbiological procedures, such as the International Organization for Standardization (ISO) 6579:2002 method, were used to isolate *S. typhimurium*. Using this technique, animal excrement was placed in buffered peptone water and left to incubate for eighteen hours at 37°C.

After that, the enhanced culture was put into Rappaport Vassiliadis Soya (RSV) broth and left to incubate for a whole day at 42°C. An aliquot of the enriched culture was streaked onto XLD (Xylose Lysine Deoxycholate) agar and incubated at 37°C for 24 hours.

The colonies of *S. typhimurium* on XLD agar appeared as pink to red with a black center due to the production of hydrogen sulfide (H₂S) from the fermentation of xylose and lysine. This distinctive appearance helps in the differentiation of *S. typhimurium* from other microorganisms present on the agar plate.

Biochemical Identification

The biochemical identification of *S. typhimurium* involves a series of tests to determine the presence of specific enzymes and metabolic characteristics associated with the bacterium. One of the tests used was the Triple Sugar Iron (TSI) agar test. In this test, a TSI agar medium was inoculated with the suspected *S. typhimurium* isolate. The medium contains sugars (glucose, lactose, and sucrose) and ferrous sulfate. The generation of hydrogen sulphide (H₂S) gas, which combines with ferrous sulphate and produces a black precipitate, confirmed the occurrence of *S. typhimurium*. This was then followed by another test which was the Lysine Decarboxylase (LDC) test. In this test, a LDC broth is inoculated with the isolate, and the presence of *S. typhimurium* was indicated by the production of lysine decarboxylase enzyme, which led to the formation of a purple color. Standard protocols were followed for the motility test and Biochemical identification assay.

Toxicology Assay

A wide range of assessments are utilized in the evaluation of patients with hepatic dysfunction; the liver is essential in the metabolism of carbohydrates, proteins, and fats. The blood sample was kept at -20°C for the analyses of Total Protein, Albumin, Globulin, Glucose, Creatinine, Bilirubin, Urea, Sodium, and Calcium, Potassium Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST), and Alanine Amino Transferase (ALT).

The analysis was performed using Sigma assay kits (Sigma Co. St. Louis, Missouri, USA).

Data Analysis

IBM Statistics SPSS version 23 was used for both the statistical analysis and graph. The data was analyzed via one-way ANOVA, preceded by the Tukey post hoc mean comparison test. The findings were reported as the mean of three replicates $\pm$ SD. $P < 0.05$ was considered statistically significant.

4. Results

4.1.1 Results for qualitative and quantitative phytochemicals

The qualitative analysis and quantitative analysis showed the presence of saponin, terpenoids, flavonoids, tannins, and alkaloids as shown in table 4.1 and 4.2 respectively.

Table 4.1: Result of qualitative analysis for phytochemicals in *P. macrophylla* seed extract

Phytochemicals	Result
Saponin	Strongly present
Terpenoids	Strongly present
Flavonoid	Strongly present
Alkaloids	Present
Phenols	Present
Tannins	Slightly present

Table 4.2: Result for quantitative analysis for phytochemicals in *P. macrophylla* seed extract

Phytochemicals	(mg)
Phenol	5.53±0.01
Flavonoids	40.808±0.01
Alkaloids	8.18±0.01
Tannin	10±0.01
Terpenoids	30±0.01
Saponin	45±0.01

DPPH Radical Scavenging Activity

P. macrophylla hydroethanolic extract showed a better DPPH scavenging activity compared to ascorbic acid (Figure 4.1)

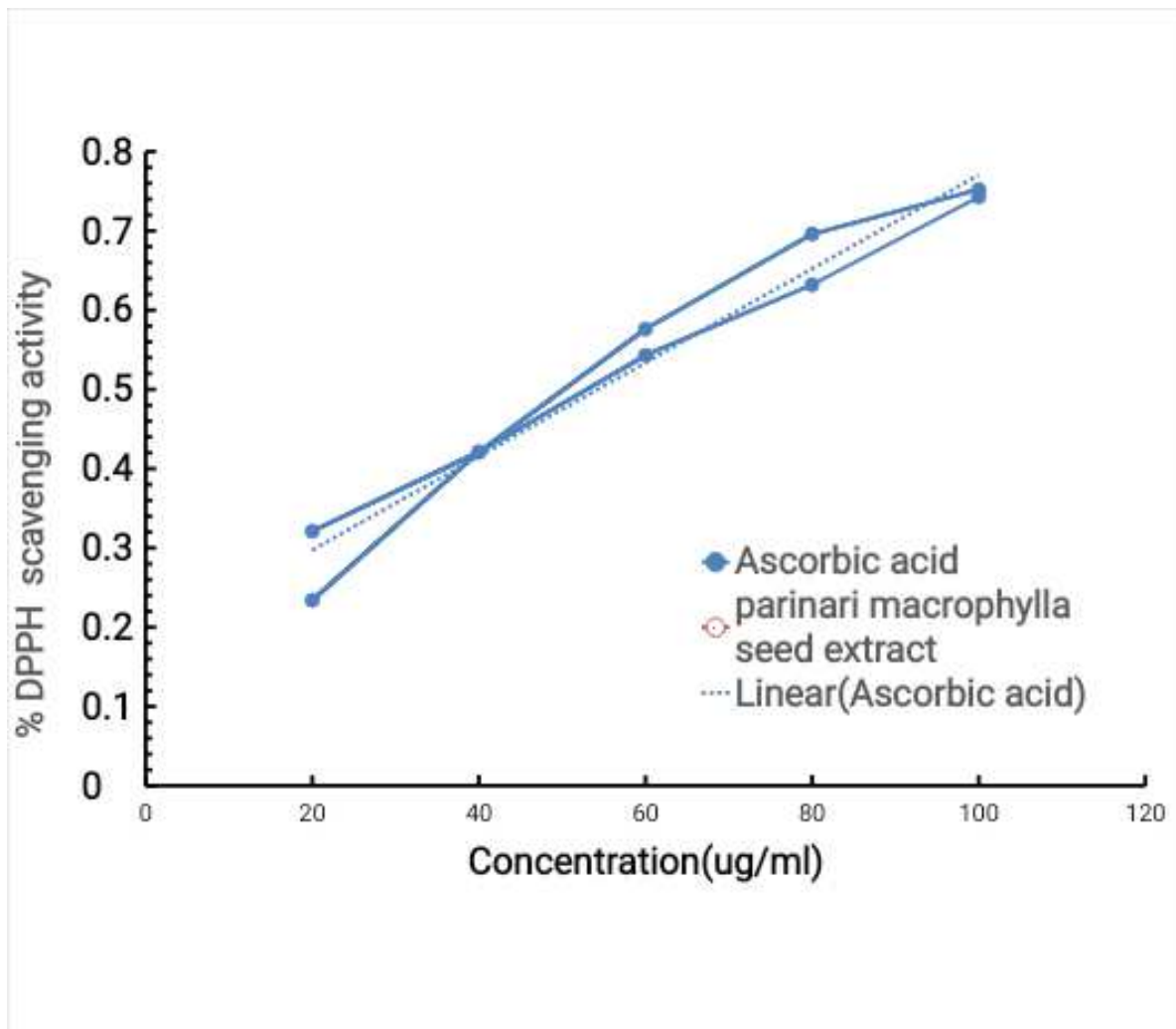


Figure 4.1: Antioxidant assessment of the seed extract of *P. macrophylla* using DPPH radical scavenger.

***In vitro* Antibacterial activity of *P. macrophylla* seed extract**

The extract of *P. macrophylla* against *S. typhimurium* showed a significant decrease in diameter of zone of inhibition compared to the antibiotic's standard. On the other hand, the extract's zone of inhibition width increased in a dose-dependent manner (400 mg > 200 mg > 100 mg) (Figure 4.2).

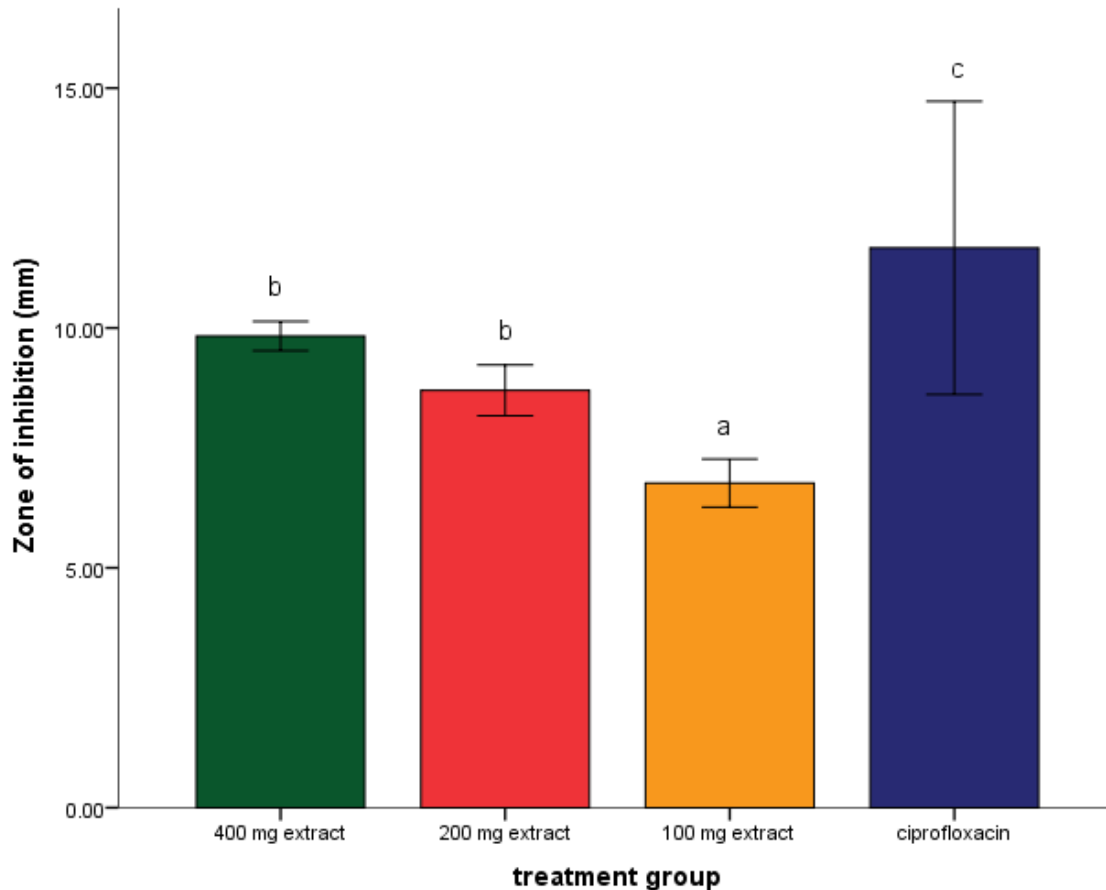


Figure 4.2: Invitro anti-bacterial activity of *P. macrophylla* seed extract on *S. typhimurium*. Data were displayed as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

***In vivo* Antibacterial Activity of *P. macrophylla* Seed Extract on *Salmonella typhimurium* Infected Wistar rat**

Day 1-3, there was a significant decrease (Figure 4.3) in fecal bacteria load in the groups administered *P. macrophylla* hydroethanolic seed extracts and antibiotics compared to the negative control. At day 4 & 5, the extract 400 milligrams per kilogram body weight showed progressively decrease in the fecal bacterial load compared to standard drug (ciprofloxacin).

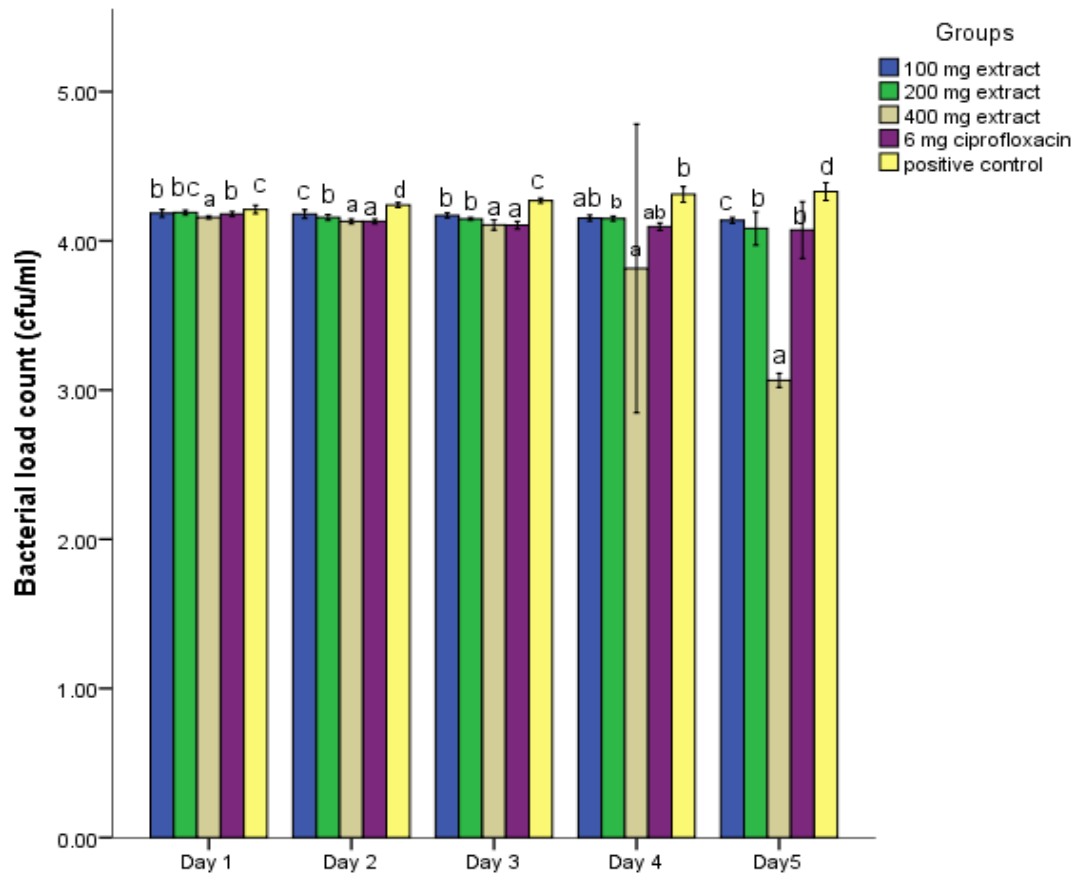


Figure 4.3: Effect of *P. macrophylla* seed extract on the bacterial load of fecal sample in *S. typhimurium* infected Wistar rat. Data were displayed as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

Liver Function Indices

The Concentration of Globulin in Wistar Rat

There was a significant decrease ($p < 0.05$) in globulin concentration in the animal administered antibiotic compared to the group treated with the extracts (Figure 4.4).

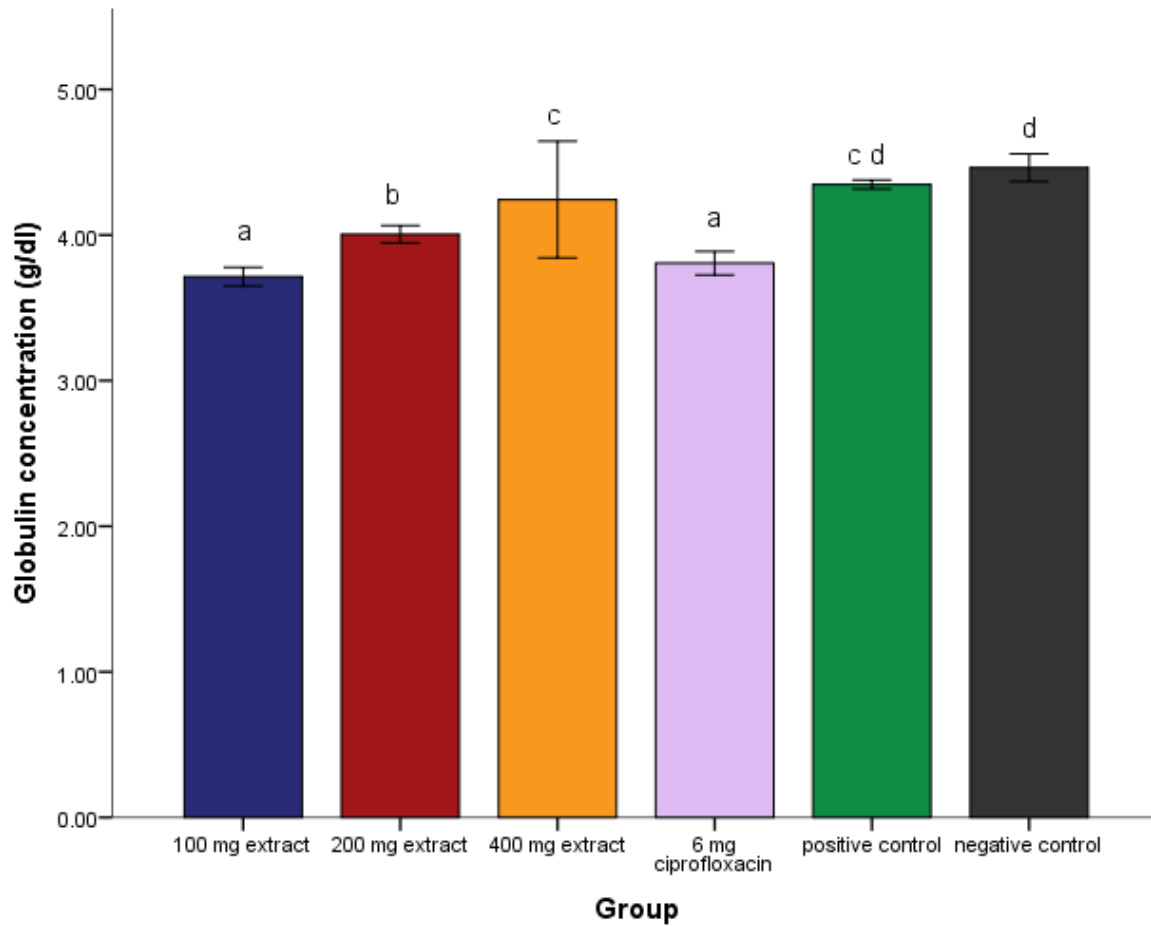


Figure 4.4: Effect of *P. macrophylla* hydro-ethanolic extract on serum globulin concentration of *S. typhimurium* induced gastro- enteritis in rats. Data were displayed as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

Concentration of Aspartate-Amino-Transferase Concentration in Wistar Rat

The aspartate amino transferase activity significantly decreased in Wistar rats administered with ciprofloxacin when compared to the groups given extract (Figure 4.5).

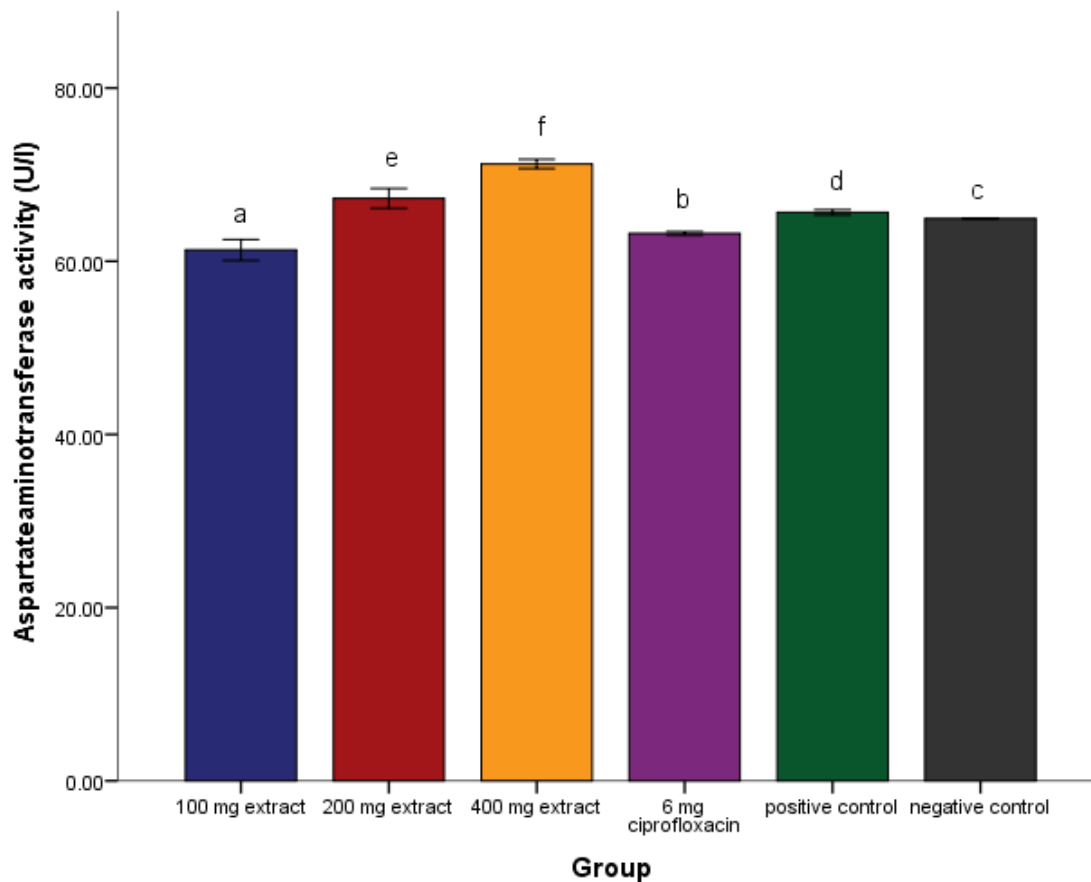


Figure 4.5: Effect of *P. macrophylla* hydro-ethanolic extract on serum aspartate amino transferase concentration of *S. typhimurium* induced gastro- enteritis rat. The data were represented as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

Level of Alanine-amino-Transferase Activity in Wistar Rat

Compared to the groups administered with extract, the ciprofloxacin-administered groups showed a significant decrease in the activity of alanine amino transferase (Figure 4.6).

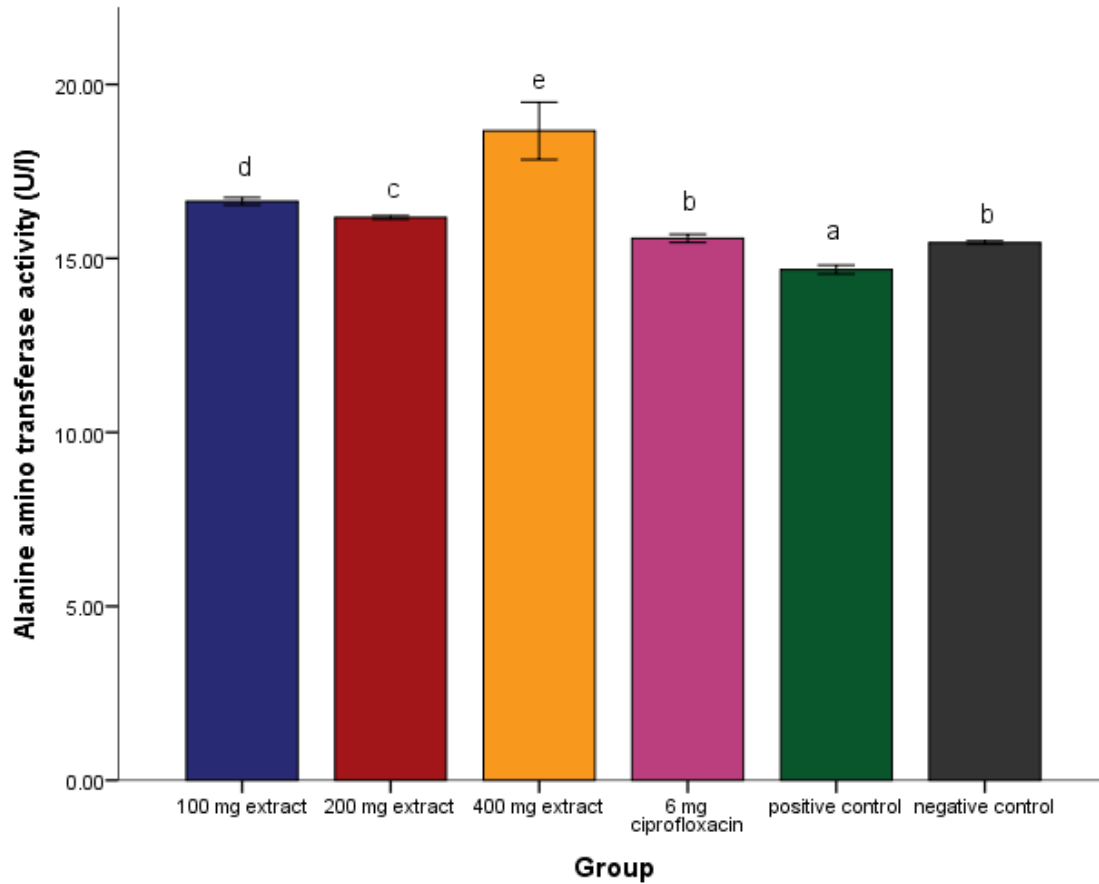


Figure 4.6: Effect of *P. macrophylla* hydro-ethanolic extract on alanine amino transferase activity concentration of *S. typhimurium* induced gastro-enteritis rat. The data were represented as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

The Concentration of Albumin in Wistar Rat

Comparing the group given the standard drug to the extract and negative control, there was a significant decrease (Figure 4.7).

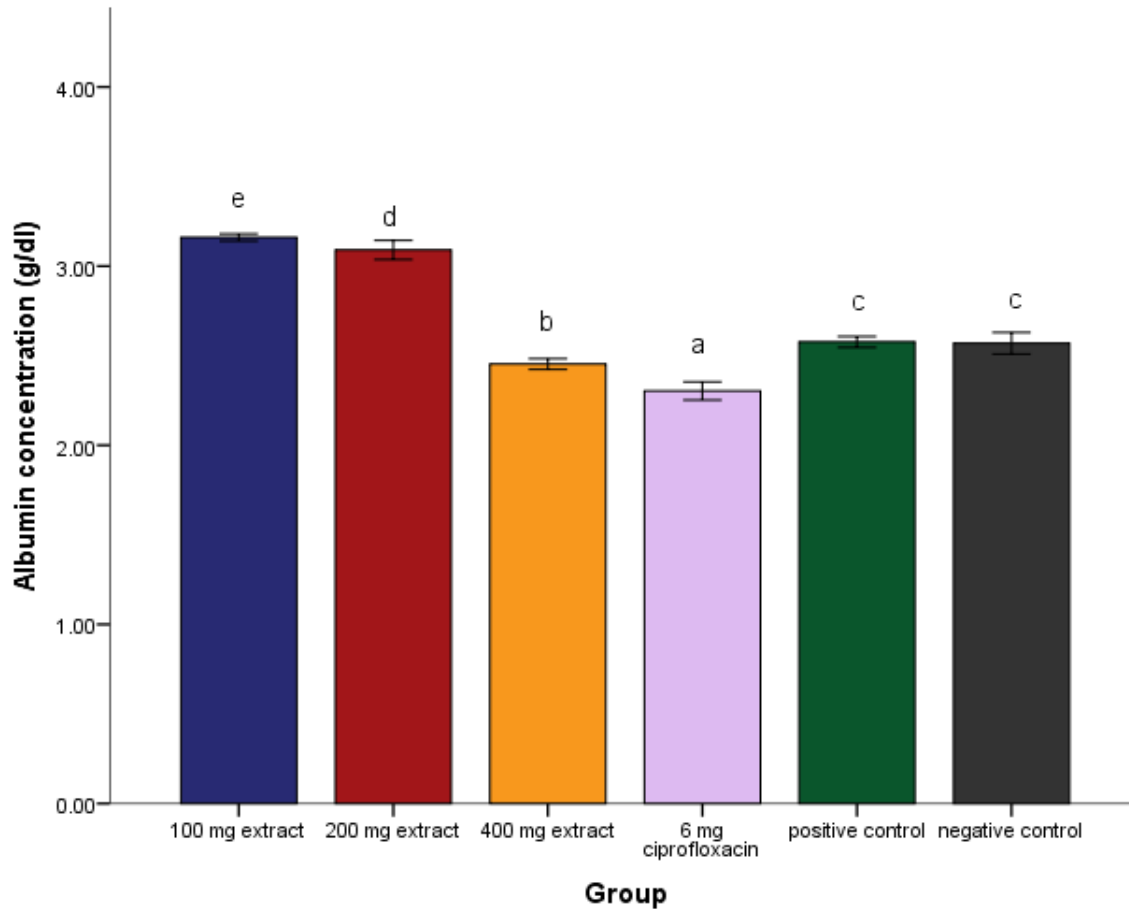


Figure 4.7: Effect of *P. macrophylla* hydro-ethanolic extract on albumin concentration of *S. typhimurium* induced gastro- enteritis rat. The data were represented as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

4.6 Kidney Function Indices

Level of Creatinine in Wistar Rat

There was a significant decrease in activity, in the groups administered the extract compared to the groups given standard drug and negative control. (Figure 4.8).

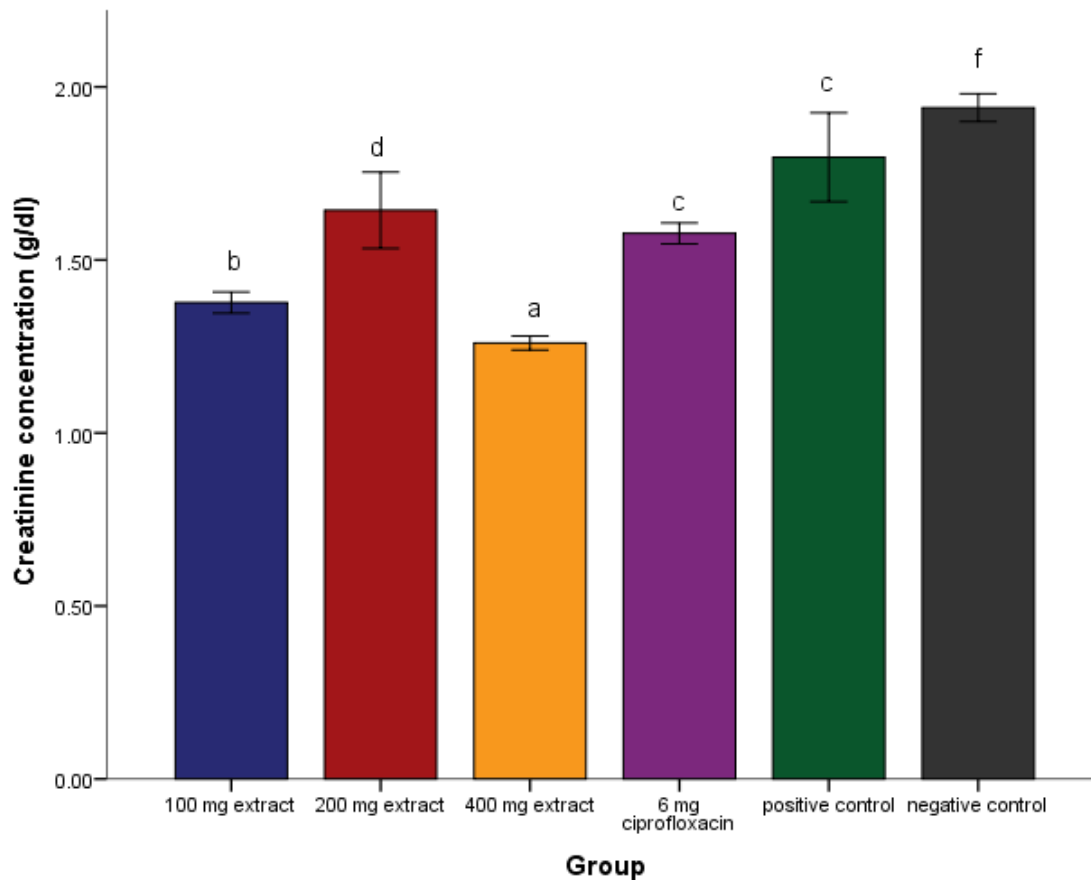


Figure 4.8: Effect of *P. macrophylla* hydro-ethanolic of creatinine concentration of *S. typhimurium* induced gastro-enteritis rat. The data were displayed as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

Concentration of Urea in Wistar Rat

When comparing the groups treated with the extract to those treated with the standard drug and the negative control, there was a significant increase in activity (Figure 4.9).

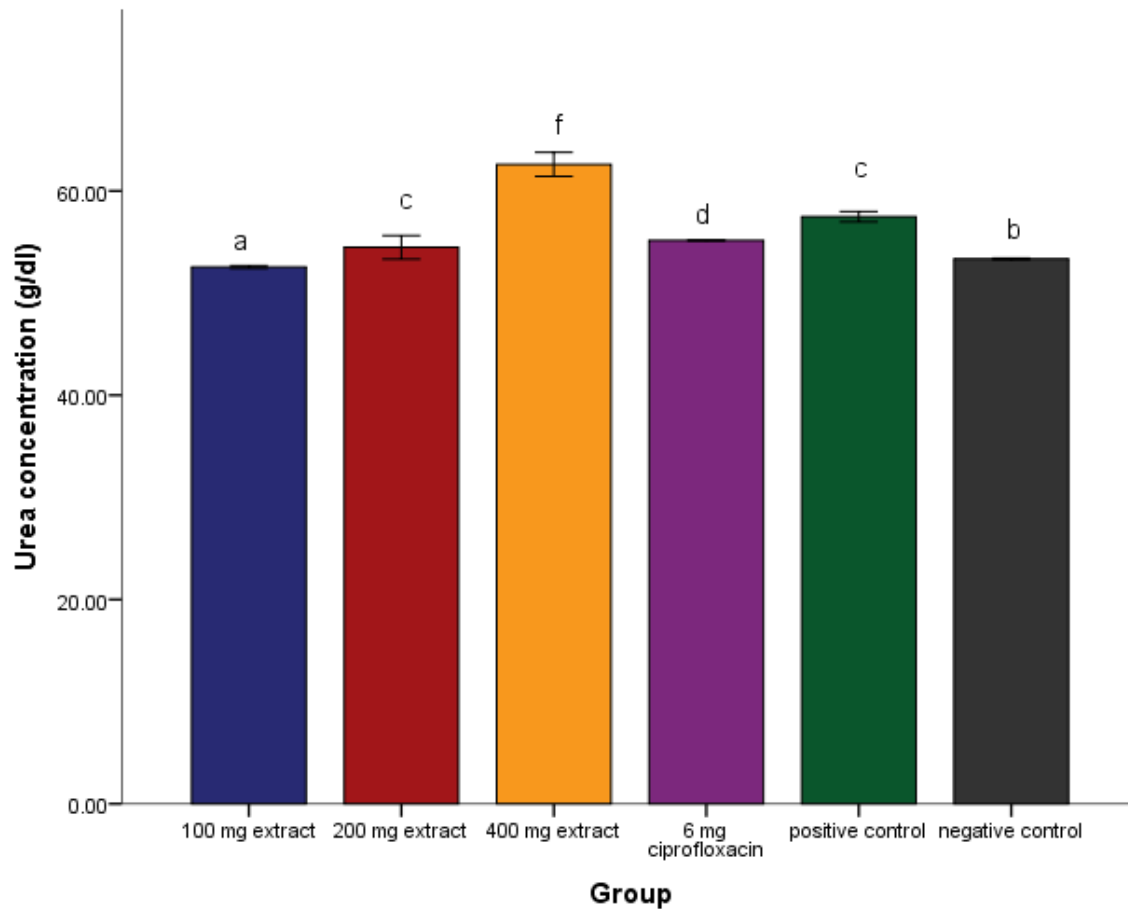


Figure 4.9: Effect of *p. macrophylla* hydro-ethanolic extract on serum urea concentration of *S. typhimurium* induced gastro-enteritis rat. The data were represented as mean $\pm$ SD, and bars with distinct superscripts indicate significant differences at $p < 0.05$.

Discussion

Some plants in herbal medicine have therapeutic and pharmaceutical applications, including the treatment of antibiotic-resistant infections. They contain phytochemicals with antioxidant activity (Owa *et al.*, 2014). These plants are rich in carbs and minerals. *P. macrophylla* seed extract contains compounds like flavonoids, alkaloids, saponin, phenols, tannins, and terpenoids that might give rise to its antioxidant properties. Certain phytochemicals are bioactive compounds that give herbal plants therapeutic properties. Mallikharjunah *et al.* (2007) reported the effectiveness of preliminary qualitative tests in detecting bioactive principles, which may lead to drug discovery and development. Phytochemicals are non-essential nutrients derived from plants; the majority of them are physiologically active. Numerous phytochemicals have been shown to have therapeutic effects such as antibacterial, antioxidant, antifungal, antispasmodic, anticancer, and liver-protective properties. Chemical substances with antimicrobial activity derived from plants have enormous therapeutic potential and have proved successful in the treatment of infectious diseases while attracting many of the side effects that are frequently caused by synthetic antimicrobial agents.

Flavonoids are abundant and have a variety of bioactivities, including anti-inflammatory and antioxidant effects, as well as changes in redox-sensitive signal transduction pathways and gene expression (Meskin *et al.*, 2008). Terpenoids were abundant in *P. macrophylla* seed extracts, indicating that a decoction of dried kernel can be used to treat diabetes mellitus, as stated for cashew nut oil (Mbatchou and Kosoono, 2012). Tannins have various functions, including serving as chelating agents for metal ions, antioxidants in the body, and protein precipitating agents.

Saponins in *P. macrophylla* seed extracts are used industrially as a cleansing agent and in mining due to their surfactant nature, as well as their high antibacterial properties (Rigano *et al.*, 2009). Alkaloids, a very prominent class of defence compounds, were also present in the seed extract of *P. macrophylla*, indicating their functionality in repellent, deterrence, toxicity, and growth inhibition by microbes, as well as as a metabolite that is used for UV-protection and nitrogen storage. (Fattorusso *et al.*, 2008).

The reduction in fecal bacterial load reported in the current indicates that hydroethanolic extract of *P. macrophylla* had an antibacterial effect in the gastro intestine of the rats. The decrease in the bacteria could be due to an inhibitory effect on bacterial cell division by the bioactive compounds of the extract. Supporting the antibacterial efficiency of the extract is the *In vitro* bacterial zone of inhibition carrying less efficiency as the *in vivo* antibacterial effects in this study, According to (Obinna *et al.*., 2022), *Terminalia glaucescens* root and leaf extracts exhibited significant inhibitory activity against *E.coli* and *S.typhi*. Ethanol exteriors of medicinal plants show the lowest antimicrobial activity against five clinical bacterial isolates including two negative stained Gram-positive Bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, and three negative stained Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia*. Bark

extracts of *Azadirachta indica* (A. Juss.) at 25-400 mg/ml exhibited substantial antibacterial property in interaction with 14 MDRT *Salmonella typhi* strains within the zone of 8-31mg/ml. The improved antibacterial property of the In vivo in the current study may be related to the drug biotransformation mechanism. The process of drug metabolism is also called biotransformation.

According to (Hassan *et al.*, 2022) drug metabolism simply means the biotransformation of therapeutic agents or chemotherapeutic agents in the body in order to facilitate their elimination. The majority of drug related metabolic activities occur in the liver as the enzymes that help facilitate the reactions are found therein (Noor *et al.* 2023). This is also in agreement with other work done with plants (Wangchuk *et al.*, 2011). Leaf extracts from *Verbena officinalis* and *Myrtus communis* were discovered to have strong antibacterial properties against *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*. Significant action against *Pseudomonas aeruginosa* has also been demonstrated by *M. communis*.

The ability of the extract to scavenge DPPH, which was reported in this study, have shown its antioxidant property that may offset the pathogenesis brought about by bacterial infection. Some phytochemicals with antibacterial interest have molecular characteristics which are allegedly consistent with antioxidant effects 'in vivo and are believed to influence cellular signaling responses related to RONS by scavenging or preventing the formation of active oxygen or through the inhibition of relevant enzymes or chelation of trace metals with capacity to generate free radicals. Baliyan *et al.*, 2022)

The high globulin concentration as much as it has been established in the current study in the rats administered the extract, gives out the fact that there was a humoral immunity in the rat which helped in protecting the rat against the infection. Globulin are a group of proteins found in the blood, they have significant function in liver disease and blood coagulability also in immunological reaction to bacterial (Pati *et al.*, 2023). By using Total Protein percentage amount of total protein such as albumin or globulin fraction depending its availability in the serum. Either high or low levels of total protein can be indicative of immunodeficiency-hence acute infection or organ disease (Kumar, and Gill., 2018).

Aspartate aminotransferase (AST) is an enzyme found in many tissues and erythrocytes, namely the heart, liver, muscles, and kidneys. Diseases affecting these tissues, such viral hepatitis, muscular dystrophy, and myocardial infarction, are associated with elevated serum levels (Vishwanath *et al.*, 2023). The multifunctional, non-glycosylated, negatively charged plasma protein known as human serum albumin (HSA) is widely distributed and has been linked to ligand-binding, transport, antioxidant, and enzymatic activities. It is primarily generated in the liver and is believed to be an acute-phase negative protein. Apart from its physiological role in preserving colloid osmotic pressure, albumin may also have an impact on the elements of the inflammatory cascade, such as neutrophil adherence and cell signaling moieties' function, as well as microvascular integrity. Although data from meta analyses and the currently published SAFE trial

indicates albumin may not give a survival benefit over crystalloid solutions when supplied to the critically sick, albumin has been used clinically as a plasma expander in various patient categories. Nonetheless, there have been some positive findings from research on the use of albumin as a volume expander as well as albumin dialysis treatment in liver disease patients.

Albumin, Globulin level and the activities of Alanine amino transferase and Aspartate amino transferase was not altered, which may imply that the extract might not be toxic at the dose reported in the study. This might be a better alternative to the conventional antibiotic which has been reported to have side effects. The kidney function indices were within the normal range supporting the none toxicity of the extract (Bai., *et al* 2021). The body typically produces creatinine at a fairly consistent pace; it is a breakdown product of creatinine phosphate in muscle (Harita *et al.*, 2009). The kidneys filter creatinine; if the kidneys' filtering capacity is compromised, blood creatinine levels will rise. Since ammonia is poisonous, it is converted to urea via the urea cycle and excreted from the body as urea. Urea is a byproduct of amino acid deamination in the liver (Bai *et al.*, 2021).

Conclusion

Medicinal plant antibacterial activity offers a novel method of reducing the grave concerns associated with the growing body knowledge about antibiotic resistance. Consequently, it is imperative to discover and separate novel bioactive substances from plants that haven't received enough research attention in the past. The wide range of applications for these compounds as antimicrobials and antimicrobial resistance modulators has been shown.

It is really challenging to fully utilize the potentials of novel compounds that are bioactive. To ensure the discovery of safe and efficient antibacterial plant-derived compounds, a significant amount of in vitro and in vivo testing needs to be done. Utilizing the chemicals' possible antagonistic or synergistic effects within and across medicinal plant extracts is another important concern. The results have shown that, in comparison to the standard medication (ciprofloxacin), the hydro-ethanolic seed extract of *P. macrophylla* is rich in phytochemicals and antioxidants that support the demonstration of possible antibacterial action against the test organism *S. typhimurium*.

This study adds value to these seed extracts to be considered as a natural tool for new drug discovery through its potent (antibacterial), gastroenteritis therapy, and the management of infective diseases triggered by resistant micro-organisms. Therefore, further study needs to be done to unravel more bioactive compounds, antibacterial activities in the seed of *P. macrophylla* which can be utilized industrially and therapeutically specially to combat resistant micro-organism. Based on the conclusion, further research is recommended to explore the antibacterial and therapeutic potential of *P. macrophylla* seed extract as a treatment for gastroenteritis. Clinical trials and investigations should be conducted to evaluate its efficacy, safety, and optimal dosage, paving the way for its potential integration for gastro enteritis therapy

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Declarations:

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Contributions: Methodology: S.O.O., J.A.N. and A.O.O.; Experiment, record data: B.I.A., S.K.O, S.O.O; Software: G.U.J and S.I.A.; Writing—original draft preparation: J.A.N. and B.I.A.; Writing—review and editing: B.I.A., C.J.N, J.A.N and S.O.O. All authors have read and agreed to the published version of the manuscript.

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REFERENCES

- Al Jassas, B., Khayat, M., Alzahrani, H., Asali, A., Alsohaimi, S., Al Harbi, H., Al Qadi, M., Al Qassim, M., Mutahar, A., & Mahbub, M. (2018). Gastroenteritis in adults. *International Journal of Community Medicine and Public Health*, 5(11), 4959–4964.
- Amina, B., & Shazia, B. (2021). Phytochemical evaluation and therapeutic potential of underutilized tropical seeds. *Journal of Medicinal Plants Research*, 15(4), 180–189.
- Bai, Y., Wang, X., & Zhang, L. (2021). Assessment of plant extract safety margins: Liver and kidney physiological indices in animal models. *Journal of Toxicological Sciences*, 46(3), 112–121.
- Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM.(2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*. 16;27(4):1326. doi: 10.3390/molecules27041326.
- Brown, J. E., Khodr, H., Hider, R. C., & Rice-Evans, C. A. (1998). Structural dependence of flavonoid interactions with ions: implications for their antioxidant and pro-oxidant activities. *Biochemical Journal*, 330(3), 1173–1178.
- Christenhusz, M. J., & Byng, J. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa*, 261(3), 201–217.
- Fattorusso, E., Taglialatela-Scafati, O., & Lanzotti, V. (2008). Plant defense metabolites: Structural diversity and biological functionality. *Phytochemistry Reviews*, 7(2), 245–259.
- Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry*, 177(2), 751–766.
- Harita, N., Hayashi, T., Sato, K. K., Nakamura, Y., Yoneda, T., Endo, G., & Okamoto, M. (2009). Lower serum creatinine is a risk factor for type 2 diabetes in kidney-healthy populations. *Diabetes Care*, 32(3), 424–426.
- Hassan, M., Mir, H., & Lone, I. A. (2022). Biotransformation mechanisms and drug metabolism updates of modern and therapeutic agents. *Current Drug Metabolism*, 23(4), 289–301.
- Kumar, V., Gill, K.D. (2018). To Estimate the Amount of Total Protein and Albumin in Serum and to Find A/G Ratio. In: Basic Concepts in Clinical Biochemistry: A Practical Guide. Springer, Singapore. https://doi.org/10.1007/978-981-10-8186-6_10

Mallikharjunah, P. B., Rajanna, L. N., Seetharam, Y. N., & Sharanabasappa, G. K. (2007). Phytochemical studies and antimicrobial activity of *Azadirachta indica*, *Pentaclethra macrophylla*, and related medicinal species. *Journal of Plant Sciences*, 2(3), 321–327.

Marte, R., Arsalan, T., & Nnamdi, C. (2019). Evaluation of cytotoxic profiles of selected indigenous Nigerian medicinal plants against cancer cell lines. *Journal of Pharmacognosy and Phytochemistry*, 8(2), 114–122.

Mbatchou, V. C., & Kosoono, I. (2012). Aphrodisiac activity of oils from *Anacardium occidentale* L. seeds and kernels of *Pentaclethra macrophylla* Benth. *Alexandria Journal of Medicine*, 48(4), 341–345.

Meskin, M. S., Bidlack, W. R., & Davies, A. J. (2008). *Phytochemicals: Mechanisms of Action and Bioactivities*. CRC Press.

Musa, S., Bello, U., & Abdullahi, Z. (2021). Isolation of bioactive steroidal glycosides from Nigerian market spices and medicinal seeds. *African Journal of Pure and Applied Chemistry*, 15(3), 45–52.

Noor, A., Ahmad, S., & Khan, M. A. (2023). Hepatic enzyme systems and their primary role in xenobiotic biotransformation. *Journal of Biochemical and Molecular Toxicology*, 37(2), e23241.

Obinna, E. C., Timothy, C. O., & Chinedu, P. A. (2022). *In vitro* evaluation of *Terminalia glaucescens* extracts against enteric pathogens. *Nigerian Journal of Clinical and Biomedical Research*, 14(1), 45–53.

Owa, S. O., Onuminya, T. O., & Olubire, F. A. (2014). Phytochemical constituents and qualitative antioxidant capacities of local Nigerian botanical species. *Journal of Scientific Research in Medical and Biological Sciences*, 5(2), 88–95.

Pati I, Cruciani M, Candura F, Massari MS, Piccinini V, Masiello F, Profili S, De Fulvio L, Pupella S, De Angelis V.(2023). Hyperimmune Globulins for the Management of Infectious Diseases. *Viruses*. 15(7):1543. doi: 10.3390/v15071543.

Rigano, D., Sirignano, C., & Taglialatela-Scafati, O. (2009). Industrial and Biomedical applications of plant-derived surfactants and saponins. *Phytochemistry Letters*, 2(4), 181–187.

Susan, A., Kayalvizhi, R., Kaviarasi, S., & Uma, M. K. (2019). An overview of plant-based interventions to ameliorate arsenic toxicity. *Biomedicine & Pharmacotherapy*, 109, 838–852.

- Vishwanath, M., Sharma, R., & Joshi, K. (2023). Clinical enzymology: Aspartate aminotransferase and diagnostic biomarkers in tissue injury. *Journal of Laboratory Medicine*, 47(1), 12–21.
- Wangchuk, P., Keller, P. A., Pyne, S. G., Taweechotipatr, M., Tonsomboon, A., & Rattanajak, R. (2011). Evaluation of the antimicrobial and antioxidant activities of leaf extracts of *Verbena officinalis* and *Myrtus communis*. *Journal of Ethnopharmacology*, 137(1), 436–442.
- World Health Organization. (2014). *WHO Traditional Medicine Strategy: 2014-2023*. World Health Organization.
- World Health Organization. (2019). *Monitoring health for the SDGs, Sustainable Development Goals*. World Health Organization.
- World Health Organization. (2021). *Global priority pathogens list of antibiotic-resistant bacteria*. World Health Organization.
- Yang, Z., Zou, L., Yue, B., & Hu, M. (2019). *S. typhimurium* may support cancer treatment: a review. *Acta Biochimica et Biophysica Sinica*, 55(3), 331–340.
- Yusuf, R. O., Adeniran, J. A., & Sonibare, J. A. (2019). Phytomedicine wealth of Nigeria: Strategic potential for regional drug discovery. *Nigerian Journal of Natural Products and Medicine*, 23(1), 12–19.