EVALUATION OF THE PHYTOCHEMICALS AND ANTIBACTERIAL EFFECT OF ETHANOL LEAF EXTRACT OF *Jatropha tanjorensis* ("HOSPITAL TOO FAR LEAF") ON SOME CLINICAL BACTERIAL ISOLATES

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY,UNIVESITY OF BENIN,BENIN CITY.

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CERTIFICATION

This is to certify that this project work was carried out by Blessing IDEHENRE with Matriculation number LSC2205861 in the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under the supervision of Prof. E. A. Ophori.

Prof. E. A. Ophori **Date**

(Project Supervisor)

Prof. E. O. Igbinosa **Date**

(Head of Department)

DEDICATION

This work is dedicated to God Almighty, my all sufficient Father, who in his infinite mercy kept me this far and also to my wonderful family, for their love and support both morally and financially all through my stay in the University of Benin, Benin City, Edo State, Nigeria.

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ABSTRACT

The increasing prevalence of antibiotic-resistant bacteria has heightened the interest in medicinal plants as potential sources of bioactive compounds. This study evaluated the phytochemical composition, antioxidant potential, and antibacterial activity of ethanol leaf extracts of *Jatropha tanjorensis* (commonly known as Hospital-too-far) against selected clinical bacterial isolates. Leaves of *J. tanjorensis* were collected from the University of Benin Botanical Garden and authenticated in the herbarium of the Department of Plant Biology and Biotechnology. Clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* were obtained from the University of Benin Teaching Hospital. Qualitative phytochemical analysis revealed the presence of phenols, flavonoids, and alkaloids in high concentrations, while tannins and saponins were present at lower levels. Quantitative analysis showed phenols as the most abundant compound (129.31 µg/ml), followed by tannins (100.57 µg/ml), flavonoids (46.97 µg/ml), alkaloids (9.33%), and saponins (3.17%). Antioxidant evaluation demonstrated significant activity, with Total Antioxidant Capacity (TAC) of 219.08 µg/ml and Ferric Reducing Antioxidant Potential (FRAP) of 2607.27 µM. The extract also exhibited dose-dependent DPPH radical scavenging activity, with 48.97% inhibition at 10 µg/ml and 69.60% at 100 µg/ml. Antibacterial assessment revealed broad-spectrum activity, with *Klebsiella pneumoniae* being most susceptible (zone of inhibition: 18 mm at 30 µg/ml), while *Escherichia coli* and *S. aureus* showed moderate inhibition. The Minimum Inhibitory Concentration (MIC) ranged from 20–30 µg/ml, indicating bacteriostatic effects at lower concentrations and bactericidal activity at higher doses. Antibiotic susceptibility testing of the isolates confirmed high resistance to commonly used antibiotics, emphasizing the therapeutic potential of the plant extract. Overall, *Jatropha tanjorensis* leaves contain bioactive compounds with potent antioxidant and antibacterial properties, supporting its traditional use and potential application in combating resistant bacterial infections.

CHAPTER ONE

1.0. INTRODUCTION

1.1 Background to the Study

Medicinal plants have been integral to human healthcare systems for millennia, serving as primary sources of treatment in traditional medicine across diverse cultures (Manisha *et al*, 2025). In recent years, the global rise in antimicrobial resistance (AMR) has intensified the need to explore alternative therapeutic agents, particularly from plant sources (Gupta and Sharma, 2022). The World Health Organization (WHO, 2020) estimates that AMR contributes to approximately 700,000 deaths annually, with projections indicating a potential increase to 10 million deaths by 2050 if effective interventions are not developed. This alarming trend underscores the urgency of identifying novel antimicrobial agents, particularly from plants with a history of ethnomedicinal use.

Jatropha tanjorensis is a green leafy medicinal plant commonly used in folk medicine. Belonging to the Euphorbiaceae family, it is a widespread weed among field crops (Oladele *et al*., 2020). Typically found in the rainforest zones of West Africa, Jatropha is recognized for its purgative/laxative properties and other medicinal applications. All parts of the plant seeds, leaves, and bark whether fresh or as a decoction, are utilized in traditional and folk medicine, as well as for veterinary purposes (Chigozie *et al*., 2018). In southern Nigeria, particularly in Edo State, the leaves are consumed locally as a vegetable in daily meals and are used to treat diabetes mellitus due to their anti-hyperglycemic properties (Olayiwola *et al*., 2004). In other parts of Nigeria, the plant is incorporated into soups and used as a tonic, believed to increase blood volume. It is also claimed to aid in treating anemia, diabetes, and cardiovascular diseases

(Omoregie and Osagie, 2011).In Nigeria, the plant is known by various common names across different regions and ethnic groups, reflecting its cultural significance. It is locally referred to as “Hospital-too-far,” “Catholic vegetable,” “Iyana-Ipaja,” and “Lapalapa” (Iwalewa *et al*., 2005; Bello *et al*., 2008; Idu*et al*., 2014; Nwachukwu, 2018; Imohiosen, 2023).

The leaves of *Jatropha tanjorensis* are particularly valued for their rich phytochemical content, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, which are known to exhibit antimicrobial, antioxidant, and anti-inflammatory properties (Anhwange *et al*., 2019. Komolafe*et al*., 2024). These bioactive compounds have been linked to the plant’s ability to combat bacterial infections, making it a promising candidate for scientific investigation. Previous studies have reported that *J. tanjorensis* extracts possess antibacterial activity against a range of pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Olayiwola *et al*., 2004; Babayemi, *et al*., 2021; Komolafe*et al*., 2024). These bacterial isolates are notorious for causing infections such as urinary tract infections, wound infections, septicemia, and pneumonia, many of which are increasingly resistant to conventional antibiotics (Upreti *et al*., 2018).

For instance, *E. coli* is a common cause of urinary tract infections and gastrointestinal disorders, while *S. aureus* is associated with skin infections and, in severe cases, methicillin-resistant strains (MRSA) that pose significant treatment challenges (Todar, 2012). *P. aeruginosa* is a leading cause of hospital-acquired infections, particularly in immunocompromised patients, and is known for its intrinsic resistance to multiple antibiotics (Pang *et al*., 2019). Similarly, *K. pneumoniae* is implicated in respiratory and bloodstream infections, with multidrug-resistant strains becoming a global concern (Navon-Venezia *et al*., 2017). The antibacterial potential of *J. tanjorensis* against these pathogens has been documented in preliminary studies. For example, Daniyan *et al*. (2018) reported that ethanolic extracts of *J. tanjorensis* leaves exhibited significant inhibitory effects against *S. aureus* and *E. coli*, with zones of inhibition comparable to standard antibiotics. Similarly, Udoh*et al*. (2024) found that ethanolic extracts of the plant were effective against *P. aeruginosa*, suggesting its potential as a broad-spectrum antimicrobial agent.

Despite these findings, there is a lack of comprehensive studies that systematically evaluate the phytochemical profile of *J. tanjorensis* and correlate it with its antibacterial effects against specific bacterial isolates. Furthermore, the mechanisms by which the plant's bioactive compounds exert their antimicrobial effects remain underexplored. This study aims to address these gaps by analyzing the phytochemical constituents of *J. tanjorensis* leaf extracts and assessing their antibacterial efficacy against *E. coli*, *S. aureus*, and *K. pneumoniae*. By doing so, it seeks to validate the ethnomedicinal uses of the plant and contribute to the development of plant-based antimicrobial therapies to combat antibiotic resistance.

1.2 Statement of the Problem

The emergence and proliferation of multidrug-resistant (MDR) bacteria have rendered many conventional antibiotics less effective, leading to increased morbidity, mortality, and healthcare costs globally (Prestinaci *et al*., 2015). In Nigeria, the misuse of antibiotics in both clinical and agricultural settings has exacerbated the problem, creating an urgent need for alternative antimicrobial agents.

Although *Jatropha tanjorensis* is widely consumed and acclaimed in traditional medicine, there is a significant knowledge gap regarding its actual antibacterial efficacy against clinically relevant pathogens. Moreover, the specific phytochemicals responsible for these purported effects have not been comprehensively evaluated. This lack of scientific validation undermines the potential therapeutic applications of the plant and limits its integration into mainstream healthcare.

1.3 Aim and Objectives of the Study

To evaluate the phytochemical composition and antibacterial effects of *Jatropha tanjorensis* (Hospital-too-far) leaf extracts on selected bacterial isolates.

The specific objectives of this study were to:

I. Identify and quantify the phytochemical constituents in *Jatropha tanjorensis* leaf extracts using qualitative and quantitative analytical techniques.

II. To evaluate the antioxidant activity of the Ethanol extract using DPPH radical scavenging assay, Total Antioxidant Capacity (TAC), and Ferric Reducing Antioxidant Power (FRAP) methods.

III. Assess the antibacterial activity of *Jatropha tanjorensis* leaf extracts against *Staphylococcus aureus, Klebsiella pneumonia* and *Escherichia coli* using disc diffusion methods.

IV. Determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Jatropha tanjorensis* leaf extracts against the selected bacterial isolates.

V. To compare the antibacterial effect of the ethanol extract with the antibiotic susceptibility profiles of the test bacterial isolates.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Botanical Description and Taxonomy of *Jatropha tanjorensis*

Jatropha tanjorensis Ellis and Saroja is a member of the Euphorbiaceae family, a diverse group of flowering plants known for their milky latex and economic importance (Sahai *et al*., 2009). The genus *Jatropha*, derived from the Greek words “iatros” (physician) and “trophe” (nutrition), reflects its historical medicinal significance (Olawuyi *et al*., 2024). The taxonomic classification of *Jatropha tanjorensis* is as follows:

- **Kingdom**: Plantae
- **Phylum**: Tracheophyta
- **Class**: Magnoliopsida
- **Order**: Malpighiales
- **Family**: Euphorbiaceae
- **Subfamily**: Crotonoideae
- **Tribe**: Jatropheae
- **Genus**: Jatropha
- **Species**: *Jatropha tanjorensis* (Ellis and Saroja, 1961)

Jatropha tanjorensis is a perennial shrub or small tree, typically growing to a height of 3–5 meters, though it can reach up to 6 meters under optimal conditions (Prasad *et al*., 2012). It is a monoecious species, bearing both male and female flowers on the same plant, which facilitates self-pollination but also allows cross-pollination (Raju and Bahadur, 2012). The plant is characterized by smooth, grey bark that exudes a sticky, watery latex when cut, a common trait among Euphorbiaceae members (Oskoueian *et al*., 2011). Its leaves are broad, measuring approximately 10–15 cm in length and width, and are typically pale green with 4–6 lobes and a palmate shape (Igbinosa *et al*., 2009). The leaves are borne on long petioles and are deciduous, shedding during the dry season in response to environmental stress (Heller, 1996).

The inflorescences of *J. tanjorensis* develop terminally at branch apices, producing clusters of small, unisexual flowers that are yellow-green in color (Dehgan, 2012). The flowers are arranged in cymose summits, with male flowers occasionally forming a petaloid tube (Webster, 1994). The fruit is a trilocular capsule with three furrows, containing elliptic, black seeds with a high oil content (30–40%) (Oskoueian *et al*., 2011). These seeds are morphologically similar to those of *Jatropha curcas*, leading to taxonomic confusion, as *J. tanjorensis* is sometimes considered a hybrid or variant of *J. curcas* and *Jatropha gossypiifolia* (Prabakaran and Sujatha, 1999). However, its distinct morphological features, such as leaf shape and growth habit, justify its classification as a separate species (Ellis and Saroja, 1961).

Microscopic studies of the foliar epidermis have revealed diagnostic features, including glandular trichomes and paracytic stomatal patterns, which differentiate *J. tanjorensis* from related species (Inamdar and Gangadhara, 1977). These characteristics are critical for accurate botanical identification in regions where multiple *Jatropha* species coexist (Dehgan, 2012).

Figure 2.1: *Jatropha tanjorensis* leaves (Danborno *et al*., 2019).

2.1.2. Geographical Distribution and Ecological Preferences

Jatropha tanjorensis is primarily found in tropical and subtropical regions, with a significant presence in India, particularly in the southern and western parts of the country (Prabakaran and Sujatha, 1999). It was first described in the Tamil Nadu region, around Thanjavur (Tanjore), which is reflected in its specific epithet "tanjorensis" (Ellis and Saroja, 1961). The species has been reported in the Indian states of Kerala, Karnataka, and Andhra Pradesh, often growing in disturbed areas, wastelands, and along roadsides (Raju and Ezradanam, 2002). Unlike *Jatropha curcas*, which has a broader global distribution due to its cultivation as a biofuel crop, *J. tanjorensis* has a more restricted range, primarily in South Asia, though its introduction to other tropical regions is possible due to its ornamental and medicinal uses (Heller, 1996).

Ecologically, *J. tanjorensis* is highly adaptable to a variety of environmental conditions. It thrives in arid and semi-arid climates, requiring as little as 250 mm of annual rainfall, making it drought-resistant (Maes *et al*., 2009). The species prefers well-drained soils, including sandy, gravelly, or saline soils, and can tolerate poor soil conditions where other crops may fail (Oskoueian *et al*., 2011). Its deep taproot and shallow lateral roots enable it to anchor firmly, reducing erosion and stabilizing landscapes, which makes it a candidate for reclamation projects (Raju and Ezradanam, 2002). *J. tanjorensis* is typically found in open, sunny locations, requiring at least 6–8 hours of direct sunlight daily for optimal growth (Heller, 1996). It is less tolerant of frost, restricting its cultivation to USDA hardiness zones 10–11 (Dehgan, 2012).

The species' ability to grow in marginal lands without competing with food crops has drawn attention to its potential as a sustainable resource (Maes *et al*., 2009). However, its ecological preferences also raise concerns about its potential to become invasive in non-native regions, as observed with related species like *J. gossypiifolia* (Negussie *et al*., 2013). Careful management is required to prevent unintended ecological impacts.

2.1.3. Traditional Uses in Various Cultures

Jatropha tanjorensis has a rich history of traditional use, particularly in South Asian cultures, where it is valued for its medicinal, agricultural, and ecological applications. In India, the plant is known by local names such as “Kattamanakku” in Tamil, reflecting its integration into regional ethnobotanical practices (Raju and Ezradanam, 2002). The leaves, seeds, and latex are the most commonly used parts, each with distinct applications.

2.1.3.1. Medicinal Uses

In traditional Indian medicine, particularly in Tamil Nadu, *J. tanjorensis* is employed to treat a range of ailments. The leaves are used to prepare decoctions or poultices for treating skin infections, wounds, dermatophytosis, diabetes (Olayiwola *et al*., 2004), malaria and hypertension (Orhue *et al*., 2008). Its medicinal properties are attributed to bioactive compounds like alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (Ehimwenma and Osagie, 2007). The latex, which is caustic and toxic in its raw form, is applied topically in diluted forms to treat cuts, burns, and insect bites due to its antimicrobial properties (Olayiwola *et al*., 2013). The seeds, while toxic if ingested raw due to the presence of phorbol esters and curcin, are sometimes processed (e.g., roasted) to reduce toxicity and used in small quantities for their purgative effects (Oskoueian *et al*., 2011).

In some communities, the plant is used to manage digestive disorders, such as constipation, and respiratory ailments (Prasad *et al*., 2012). The roots are occasionally ground into a paste to treat joint pain and inflammation, aligning with the broader ethnopharmacological use of *Jatropha* species in Africa, Asia, and Latin America (Sabandar *et al*., 2013). However, the use of *J.*

tanjorensis in traditional medicine requires caution due to its toxic compounds, and scientific validation of its efficacy and safety is ongoing (Devappa *et al*., 2011).

2.1.3.2. Agricultural and Ecological Uses

Beyond its medicinal applications, *J. tanjorensis* is used in agroforestry systems as a living fence or hedge to protect crops from livestock and reduce wind erosion (Raju and Ezradanam, 2002). Its deep root system helps stabilize soil and prevent landslides, making it valuable in erosion-prone areas (Maes *et al*., 2009). The plant's ability to grow in wastelands supports its use in land reclamation projects, where it improves soil moisture retention and reduces pressure on timber resources (Heller, 1996).

In some Indian villages, *J. tanjorensis* is grown as an ornamental due to its attractive foliage and flowers, contributing to local biodiversity by attracting pollinators like bees and butterflies (Prasad *et al*., 2012). The seeds, rich in oil, have been explored for their potential as a biofuel source, similar to *J. curcas,* though *J. tanjorensis* is less commonly cultivated for this purpose (Oskoueian *et al*., 2011).

2.1.3.3. Cultural Significance

In certain South Asian cultures, *J. tanjorensis* holds symbolic value in traditional practices. In rural Tamil Nadu, the plant is sometimes planted near homes or fields to ward off pests due to its natural pesticidal properties (Raju and Ezradanam, 2002). Its use in basketmaking and dye production, as seen with other *Jatropha* species, may extend to *J. tanjorensis* in some communities, though specific documentation is limited (Sabandar *et al*., 2013). The plant's integration into local ecosystems reflects a broader cultural appreciation for multipurpose plants that provide both practical and ecological benefits (Devappa *et al*., 2011).

2.2 Phytochemical Constituents of *Jatropha tanjorensis* (Hospital too far)

Jatropha tanjorensis J.L. Ellis and Saroja, commonly known as "Hospital too far" in Nigeria, is a leafy vegetable and medicinal plant from the Euphorbiaceae family. Widely used in ethnomedicine for managing diabetes, hypertension, rheumatoid arthritis, and microbial infections, its therapeutic potential is attributed to a diverse array of phytochemicals. These secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, saponins, terpenoids, and steroids, contribute to the plant's pharmacological properties. This section provides an in-depth analysis of the phytochemical profile of *J. tanjorensis*, detailing their chemical composition, biological activities, and relevance in traditional and modern medicine, supported by references from recent studies.

2.2.1 Overview of Phytochemicals in Medicinal Plants

Phytochemicals are bioactive secondary metabolites synthesized by plants for ecological functions such as defense against pathogens, herbivores, and environmental stresses. Unlike primary metabolites (e.g., carbohydrates, proteins), secondary metabolites like alkaloids, flavonoids, tannins, saponins, terpenoids, and steroids are not essential for basic plant functions but confer pharmacological benefits. These compounds are valued in medicinal plants for their antioxidant, antimicrobial, anti-inflammatory, anticancer, and antidiabetic properties, providing a scientific basis for their ethnomedicinal applications (Wink, 2015). In *J. tanjorensis*, phytochemical screening has revealed a rich profile of these compounds, particularly in the leaves, which are commonly used in traditional preparations. Studies employing qualitative and quantitative methods, such as liquid chromatography-mass spectrometry (LC-MS/MS) and colorimetric assays, have confirmed the presence of these phytochemicals, underpinning the plant's therapeutic efficacy (Arun and Brindha, 2015; Orabueze *et al*., 2020).

2.2.1.1 Phenolic Compounds

Phenolic compounds are characterized by one or more hydroxyl groups attached to an aromatic ring, encompassing simple phenols, phenolic acids, and polyphenols. They are potent antioxidants due to their ability to scavenge free radicals and chelate metal ions, mitigating oxidative stress-related conditions (Crozier *et al*., 2009). In *J. tanjorensis,* phenolic compounds have been identified in methanolic and ethanol-water leaf extracts. Arun and Brindha (2015) used LC-MS/MS to detect rhein and ellagic acid derivatives in the methanolic leaf extract, with a total phenolic content of 11.35 ± 0.82 mg gallic acid equivalent (GAE)/g. Another study by Orabueze *et al*. (2020) reported a phenolic content of 0.073 ± 0.02 mg/100 g in whole plant extracts. These compounds contribute to the plant's antioxidant activity, demonstrated by its ability to scavenge DPPH, nitric oxide, hydroxyl radicals, and inhibit lipid peroxidation (Omoregie and Okhamafe, 2019). This antioxidant capacity supports the traditional use of *J. tanjorensis* in managing oxidative stress-related disorders like diabetes and rheumatoid arthritis.

2.2.1.2 Flavonoids

Flavonoids, a subclass of polyphenols with a C6-C3-C6 skeleton, are synthesized via the phenylpropanoid pathway. They exhibit antibacterial, antiviral, antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties, making them significant in pharmacognosy (Panche *et al*., 2016). In *J. tanjorensis*, flavonoids are abundant, with both glycosylated and aglycone forms identified. LC-MS/MS analysis by Arun and Brindha (2015) revealed 20 flavonoids in the methanolic leaf extract, including isorhamnetin-3-glucoside-4'-glucoside, delphinidin-3-O-2''-O-b-xylopyranosyl-b-glucopyranoside, 6-c-hexosyl-8-c-pentosyl apigenin, cyanidin-3,5-di-O-glucoside, 3',7-dimethoxy-3-hydroxyflavone, and 2'',3'',4',5,6'',7-hexa-O-methylisovitexin. Core aglycones include apigenin, luteolin, isorhamnetin, quercetin, and kaempferol. Quantitative studies reported flavonoid contents of 21 ± 3.9 mg/100 g in whole plant extracts (Orabueze *et al*., 2020) and 2.72–3.21 mg/100 g in leaf extracts (Omoregie and Okhamafe, 2019). Flavonoids contribute to the plant's antimicrobial activity (MIC of 7.8 µg/mL against bacterial strains) and antiproliferative effects (IC50 of 58.53 µg/mL against A431 skin carcinoma cells) (Arun and Brindha, 2015). Their antioxidant and anti-inflammatory properties support the plant's use in managing inflammatory and oxidative stress-related conditions.

2.2.1.3 Tannins

Tannins are polyphenolic compounds classified into hydrolyzable (e.g., gallotannins, ellagitannins) and condensed tannins (e.g., proanthocyanidins). Known for their astringent properties, tannins bind proteins and macromolecules, contributing to antimicrobial, antioxidant, and anti-inflammatory effects (Smeriglio *et al*., 2017). In *J. tanjorensis*, tannins have been detected in leaf and whole plant extracts through qualitative screening. Quantitative analysis reported tannin contents of 0.0225 ± 0.012 mg/100 g in whole plant extracts (Orabueze *et al*., 2020) and 41.01–46.75 mg/100 g in leaf extracts (Omoregie and Okhamafe, 2019). Tannins contribute to the plant's antimicrobial activity by disrupting microbial cell membranes and inhibiting enzymes, as evidenced by its efficacy against pathogens like *Escherichia coli* and *Staphylococcus aureus* (Omoregie and Okhamafe, 2019). Their anti-inflammatory effects were demonstrated in rheumatoid arthritis models, where tannins, alongside flavonoids and terpenoids, reduced edema and inflammation (Idu *et al*., 2016). These properties support the traditional use of *J. tanjorensis* in wound healing and infection management.

2.2.1.4 Alkaloids

Alkaloids are nitrogen-containing compounds with diverse structures, derived from amino acids, and known for potent pharmacological activities, including analgesic, antimicrobial, and anticancer effects (Heinrich *et al*., 2020). In *J. tanjorensis,* alkaloids are a key phytochemical class, detected in methanolic and ethanol-water leaf extracts. Quantitative estimates reported alkaloid contents of 38 ± 5.0 mg/100 g in whole plant extracts (Orabueze *et al*., 2020) and 10.16–11.21 mg/100 g in leaf extracts (Omoregie and Okhamafe, 2019). LC-MS/MS analysis identified specific alkaloids such as norharman, harmane, salsolinol, and anabasine in the methanolic leaf extract (Arun and Brindha, 2015). These alkaloids contribute to the plant's antimicrobial activity (MIC of 7.8 µg/mL against bacterial strains) and antiproliferative effects on cancer cells (Arun and Brindha, 2015). Their presence supports the ethnomedicinal use of *J. tanjorensis* in treating infections and potentially neurological conditions, given the neuroactive properties of some alkaloids.

2.2.1.5 Saponins

Saponins are glycosides with amphiphilic properties, forming stable foams in aqueous solutions due to their hydrophilic sugar moieties and hydrophobic aglycones. They exhibit antimicrobial, anti-inflammatory, and immunomodulatory effects by disrupting microbial membranes and binding cholesterol (Güçlü-Üstündağ and Mazza, 2007). In *J. tanjorensis*, saponins have been detected in leaf and whole plant extracts, with quantitative estimates of 7 ± 3.0 mg/100 g in whole plant extracts (Orabueze *et al*., 2020) and 2.7–3.31 mg/100 g in leaf extracts (Omoregie and Okhamafe, 2019). Saponins contribute to the plant's antimicrobial activity against pathogens like *E. coli* and *S. aureus* and its anti-inflammatory effects in rheumatoid arthritis models (Idu *et al*., 2016). Their solubility in polar solvents aligns with traditional decoction methods, supporting their role in ethnomedicinal preparations.

2.2.1.6 Terpenoids and Steroids

Terpenoids, derived from isoprene units, include monoterpenes, diterpenes, and sesquiterpenes, with antimicrobial, anti-inflammatory, and anticancer properties. Steroids, structurally related to terpenoids, contribute to membrane stability and anti-inflammatory effects (Tholl, 2015). In *J. tanjorensis*, terpenoids and steroids have been identified in leaf and whole plant extracts, with terpenoid contents estimated at 11 ± 2.5 mg/100 g (Orabueze *et al*., 2020). Qualitative tests confirmed steroids in leaf extracts (Omoregie and Okhamafe, 2019). The high terpenoidal content in related species like *J. integerrima* (149.7 mg UAE/g extract) suggests a similar abundance in *J. tanjorensis* (Arun and Brindha, 2015). Terpenoids and steroids contribute to the plant's anti-inflammatory effects, reducing cartilage damage and edema in rheumatoid arthritis models (Idu *et al*., 2016). These compounds support the traditional use of *J. tanjorensis* for wound healing and chronic inflammatory conditions.

2.3 Pharmacological Properties of *Jatropha tanjorensis*

Jatropha tanjorensis, locally known as "Hospital Too Far," has garnered significant scientific attention due to its diverse pharmacological potentials. These properties are largely attributed to its rich phytochemical profile, including sterols, flavonoids, alkaloids, and phenolic compounds. Recent studies have systematically demonstrated the plant's anti-inflammatory, antioxidant, antibacterial, antifungal, wound-healing, and antidiabetic effects.

2.3.1 Anti-inflammatory Properties

The anti-inflammatory potential of *J. tanjorensis* is well documented in both in vitro and in vivo models. Ethanol extracts of the leaves, rich in sterols, have been shown to exhibit significant anti-inflammatory activity by inhibiting red blood cell (RBC) hemolysis and albumin denaturation in a dose-dependent manner. These effects were found to be comparable to or even surpass the standard non-steroidal anti-inflammatory drug, diclofenac, at higher concentrations (Omoboyowa *et al*., 2021). The underlying mechanism appears to be linked to the inhibition of cyclooxygenase enzymes (COX-1 and COX-2), as evidenced by molecular docking studies and MM/GBSA simulations involving sterols such as phytol, hexadecanoic acid, and cyclopropylactonoic acid.

Additionally, Arun *et al*. (2012) reported that ethanol extracts of *J. tanjorensis* demonstrated membrane-stabilizing and anti-protein denaturation activities, consistent with mechanisms that prevent lysosomal enzyme release, thereby mimicking the actions of conventional NSAIDs in chronic inflammatory states. In vivo studies further corroborated these findings. For instance, Babu *et al*. (2024) observed reduced levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), increased antioxidant enzyme activity, and significant amelioration of tissue oxidative damage in rats administered the plant extract, reinforcing its anti-inflammatory profile.

2.3.2 Antioxidant Activity

The antioxidant capacity of *J. tanjorensis* is attributed to its abundance of polyphenolic compounds—flavonoids, tannins, phenols, carotenoids—as well as essential micronutrients like selenium, zinc, and phosphorus. These constituents confer free radical scavenging abilities via mechanisms such as metal chelation and hydrogen atom donation (Gowdu Viswanathan *et al*., 2018).

In vitro assays have demonstrated notable antioxidant effects. For example, methanol leaf extract effectively scavenged DPPH radicals with an IC_{50} of approximately 100 µg/mL and showed up to 98% inhibition of $ABTS^{\bullet +}$ radicals in spectrophotometric assays, comparable to butylated hydroxytoluene (BHT) standards (Omoregie and Osagie, 2011; Unegbu *et al*., 2022). In vivo studies also indicated protective effects against oxidative stress. Omoregie and Osagie (2011) reported that dietary supplementation with *J. tanjorensis* reversed lipid peroxidation and upregulated endogenous antioxidant enzymes such as catalase, superoxide dismutase (SOD), and glutathione peroxidase in protein-deficient rats.

More recently, Srivastava *et al*. (2023) demonstrated that *J. tanjorensis* extract significantly elevated antioxidant markers and reduced hepatic oxidative stress in a dose-dependent fashion in a rodent model of high-fat diet-induced obesity, highlighting the plant's systemic antioxidant capabilities.

2.3.3 Antibacterial Properties

Studies have confirmed the antibacterial efficacy of *J. tanjorensis* against a broad spectrum of bacterial pathogens. Viswanathan *et al*. (2012) observed that hexane, chloroform, and methanol leaf extracts, as well as isolated phytochemicals such as friedelin, β-amyrin, stigmasterol, and R(+)-4-hydroxy-2-pyrrolidinone, exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* species with inhibition zones ranging between 13–46 mm and MIC values as low as 2.5 mg/mL (Gowdu Viswanathan *et al*., 2018).

Komolafe *et al*. (2024) corroborated these findings, reporting inhibition zones of 19.8 mm for *S. aureus* and 15 mm for *E. coli* using aqueous methanol extracts. Phytochemical screening revealed the presence of flavonoids, saponins, tannins, anthraquinones, and steroids, all of which have been associated with antimicrobial mechanisms. Similarly, Obum Nnadi *et al*. (2022) reported bactericidal effects of cold macerated leaf extract on *S. aureus* at 250 mg/mL (MBC), whereas hot extracts exhibited reduced activity. The proposed mechanisms include membrane disruption (by saponins), inhibition of DNA gyrase (by flavonoids), and degradation of bacterial cell wall integrity, supporting the plant's antimicrobial claim.

2.3.4. Antifugal properties

Viswanathan *et al*. (2012) performed agar-well and disk diffusion assays using methanolic and chloroform leaf extracts of *J. tanjorensis*, along with four isolated compounds—friedelin, β-amyrin, stigmasterol, and R(+)-4-hydroxy-2-pyrrolidinone. The extracts produced inhibition zones of 9–15 mm against *Aspergillus fumigatus* and 5–16 mm against *Trichophyton rubrum*. More strikingly, the pure compounds generated ZOIs ranging from 12–37 mm (friedelin) and 10–33 mm (pyrrolidinone) against fungi at concentrations of 2.5–10 mg per disk—values comparable to their antibacterial actions (zones up to 40 mm). These findings implicate terpene/steroid metabolites—particularly friedelin and stigmasterol—as major mediators of antifungal activity.

In a different experimental approach, Ujowundu et al. (2022) used enzymatic inhibition assays to assess the antifungal effect of ethanol leaf extract on *Candida albicans*. At concentrations of 0–2000 µg/mL, the extract inhibited total dehydrogenase activity in a concentration-dependent logistic fashion. The IC_{50} against *C. albicans* was 26.82 ± 1.34 µg/mL**,** and IC_{80} was 58.90± 4.12 µg/mLdemonstrating significantly greater potency than against *Staphylococcus aureus* (IC_{50} ≈102 µg/mL), and highlighting the extract's preferential antifungal efficacy. These enzymatic data complement agar-zone results and suggest metabolic disruption as an antifungal mechanism.

Additional findings from a study combining *J. tanjorensis* and *Adansonia digitata* methanol extracts against clinical fungal isolates revealed mean inhibition zones of ~25 mm, significantly larger than those of the antifungal drugs vericonazole (≈15.5 mm) and fluconazole (≈13.8 mm) applied separately. MIC values of the plant extract were also competitive (0.08 µg/mL for *Aspergillus occidentale*), suggesting possible synergism when used in combination therapies (Udoh *et al*., 2024).

The antifungal action is plausibly driven by the presence of *Jatropha* leaf terpenoids, flavonoids, tannins, and sterols—compounds documented to interfere with fungal cell membranes, inhibit ergosterol synthesis, and impair enzymatic pathways such as dehydrogenases and fungal ATPases. Friedelin and its analogues, for instance, showed maximum inhibition at 10 mg (≈33 mm ZOI), suggesting that these compounds act in a dose-dependent manner as ergosterol disruptors and oxidative stress inducers in fungal cells(Viswanathan *et al*. 2012).

2.3.5 Wound Healing

The wound-healing efficacy of *J. tanjorensis* has been validated through experimental rat models. Methanol, chloroform, and hexane leaf extracts formulated into ointments (3–5 % w/w) accelerated wound contraction and improved tensile strength, with methanol extract (5%) producing comparable outcomes to nitrofurazone—a standard wound-healing drug (Gowdu Viswanathan *et al*., 2018). Specifically, the treated wounds achieved closure in about 16 days, and tensile strength improved to 588–591 g on day 21, suggesting enhanced collagen deposition and tissue regeneration.

Traditionally, topical application of *J. tanjorensis* leaf juice or crushed leaves has been employed in managing skin disorders such as eczema, scabies, and ringworm, though without standardized formulations (Gowdu Viswanathan *et al*., 2018).

2.3.6 Antidiabetic Potential

The use of *J. tanjorensis* in traditional management of diabetes has been substantiated by both anecdotal reports and experimental data. Initial ethnobotanical surveys in Edo State, Nigeria, reported that decoctions of the plant were used to control blood glucose and alleviate symptoms of anaemia in diabetic individuals (Olayiwola *et al*., 2004; Srivastava *et al*., 2023).

Daniyan *et al*. (2019) demonstrated that aqueous and ethanolic leaf extracts significantly reduced blood glucose levels in alloxan-induced diabetic mice within 10 days, at doses up to 400 mg/kg. Phytochemical analyses revealed the presence of saponins, alkaloids, glycosides, and anthraquinones, which may contribute to hypoglycemic action (Ukubuiwe *et al*., 2019).

In vitro studies using BRIN-BD11 pancreatic β-cell lines indicated a ~170% increase in glucose-stimulated insulin secretion at concentrations as low as 0.1 µg/mL, with stem and root extracts also showing insulinotropic activity at higher concentrations. These effects persisted even in calcium-depleted environments, suggesting multiple pathways of action, including calcium-independent signaling mechanisms (Kaur *et al*., 2024).Furthermore, Srivastava *et al*. (2023) found that ethanol and aqueous extracts administered to rats on a high-fat diet improved glycemic indices, reduced lipid accumulation, and enhanced antioxidant defenses. These results affirm the plant's integrated antihyperlipidemic, antidiabetic, and anti-obesity effects, positioning *J. tanjorensis* as a promising natural therapeutic agent.

Table 2.1: Pharmacological Properties of *Jatropha tanjorensis* with Supporting References

S/N	Pharmacological Activity	Description	Key Findings	References
1	**Anti-inflammatory**	In vitro and in vivo anti-inflammatory activity via inhibition of RBC hemolysis, protein denaturation, and COX enzymes	Comparable or better than diclofenac at higher doses; reduced TNF-α, stabilized membranes	Omoboyowa *et al*. (2021); Arun *et al*. (2012); Babu *et al*. (2024)
2	**Antioxidant**	Free radical scavenging, metal chelation, enzyme activation	$IC_{50} \approx 100$ µg/mL (DPPH); 98% $ABTS^{\bullet +}$ inhibition; upregulated catalase, SOD, GPx	Omoregie and Osagie (2011); Unegbu *et al*. (2022); Srivastava *et al*. (2023); Gowdu Viswanathan *et al*. (2018)
3	**Antibacterial**	Activity against Gram-positive and Gram-negative bacteria	Zones of inhibition: 13–46 mm; MIC as low as 2.5 mg/mL; activity attributed to saponins, flavonoids	Gowdu Viswanathan *et al*. (2012, 2018); Komolafe *et al*. (2024); Obum Nnadi *et al*. (2022)
4	**Wound Healing and Antifungal**	Topical wound repair and antifungal activity against skin pathogens	Accelerated wound closure, increased tensile strength, zones up to 40 mm against fungi	Gowdu Viswanathan *et al*. (2012, 2018)
5	**Antidiabetic**	Blood glucose regulation, insulin secretion enhancement	↓ Glucose in alloxan-induced mice; ↑ insulin secretion (BRIN-BD11); ↓ weight gain and oxidative stress	Olayiwola *et al*. (2004); Daniyan *et al*. (2019); Ukubuiwe *et al*. (2019); Kaur *et al*. (2024); Srivastava *et al*. (2023)

2.4. Description of Test Organism

The bacterial isolates used in this study were selected based on their clinical significance, pathogenic potential, and frequent occurrence in antimicrobial resistance surveillance. The test organisms *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia* were obtained from preserved clinical cultures and re-identified using standard microbiological techniques, including Gram staining, colonial morphology, and biochemical tests, in accordance with Cheesbrough (2010) and Clinical and Laboratory Standards Institute (CLSI, 2023).

2.4.1. *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, spherical bacterium that typically appears in grape-like clusters under the microscope. It is non-motile, catalase-positive, and coagulase-positive, which differentiates it from other staphylococcal species. *S. aureus* is a commensal organism found on the skin and mucous membranes of humans but can cause a wide variety of infections ranging from minor skin and soft tissue infections to life-threatening diseases such as pneumonia, osteomyelitis, endocarditis, and septicemia (Lowy, 1998).

On Mannitol Salt Agar (MSA), *S. aureus* ferments mannitol and produces yellow colonies due to acid production. Its virulence is attributed to the production of several enzymes and toxins, including coagulase, protein A, leukocidins, and hemolysins. Of particular concern is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to most beta-lactam antibiotics and poses a significant public health challenge (Chambers and DeLeo, 2009). For this reason, *S. aureus* is frequently employed in screening studies for antibacterial agents, especially those targeting Gram-positive organisms.

2.4.2.*Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative, non-motile, rod-shaped bacterium that belongs to the family *Enterobacteriaceae*. It is encapsulated and facultatively anaerobic, known for causing opportunistic infections such as pneumonia, UTIs, wound infections, and septicemia, particularly in immunocompromised individuals (Podschun and Ullmann, 1998). Its prominent polysaccharide capsule (K antigen) is a major virulence factor, providing protection against phagocytosis and enhancing survival in hostile environments.

On MacConkey agar, *K. pneumoniae* produces large, mucoid, pink colonies due to its ability to ferment lactose and its capsular exopolysaccharide production. Biochemical tests typically show that it is citrate positive, urease positive, and indole negative (Cheesbrough, 2010). *K. pneumoniae* has become a significant threat in hospital settings due to its ability to acquire resistance genes, including those encoding carbapenemases like KPC (Klebsiella pneumoniae carbapenemase), rendering many antibiotics ineffective (Munoz-Price *et al*., 2013). Its inclusion in antimicrobial screening studies helps evaluate the efficacy of new or natural antimicrobial agents against resistant Gram-negative pathogens.

2.4.3 *Escherichia coli*

Escherichia coli are Gram-negative, facultatively anaerobic, rod-shaped bacteria belonging to the family Enterobacteriaceae. They are ubiquitous in the intestinal microbiota of humans and warm-blooded animals, serving as commensals in most cases, but certain pathogenic strains are responsible for a wide range of infections, including urinary tract infections, gastroenteritis, neonatal meningitis, septicemia, and hemolytic-uremic syndrome (Kaper *et al.,* 2004). Pathogenic variants, such as enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), and uropathogenic *E. coli* (UPEC), produce virulence factors like Shiga toxins, heat-labile/heat-stable enterotoxins, and adhesins that enhance colonization and tissue damage.

On MacConkey agar, *E. coli* typically forms large, flat to slightly raised, moist, pink to red colonies due to rapid lactose fermentation. Biochemically, they are oxidase-negative, catalase-positive, indole-positive, methyl red-positive, and citrate-negative. They ferment glucose and lactose with acid and gas production, and characteristically show an acid/acid (A/A) reaction with gas on Triple Sugar Iron (TSI) agar, without H_2S production. Optimal growth occurs at 35–37°C, though some strains grow at higher temperatures (44°C), a feature used in selective isolation.

Of significant public health concern is the global rise in multidrug-resistant (MDR) *E. coli*, particularly extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant strains, often linked to plasmid-mediated resistance genes such as *bla*$_{(}$CTX-M$_{)}$, *bla*$_{(}$TEM$_{)}$, and *bla*$_{(}$NDM$_{)}$ (Pitout and Laupland, 2008). These resistance mechanisms, combined with their environmental persistence and ease of horizontal gene transfer, make *E. coli* a critical target in antimicrobial susceptibility testing and a valuable reference organism in evaluating the efficacy of plant-derived extracts like those from *Jatropha tanjorensis*.

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection, Identification and Authentication of Materials

Plant Collection: The plant used in this study was collected from the University of Benin Botanical Garden, within the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria. The plant was subsequently identified and authenticated in the herbarium unit of the same department.

Microbial samples: Clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli* were collected from University of Benin Teaching Hospital (UBTH) Medical Microbiology Laboratory. The bacterial isolates were kept at 4°C or in an agar slant. The sample was sub-cultured for 24 hrs in nutrient agar at 37°C before any susceptibility test. (Mahunnah *et al.*, 1999).

Material (Reagents and Apparatus) used in Antimicrobial testing of *Jatropha tanjorensis*: Petri dishes, Biochemical reagents, Inoculating loop, Filter papers, commercially available antibiotic discs, Mueller Hinton Agar, Tryptone soy broth, Hospital-too-far leaves, Bunsen burner, Alcohol and Sterilizer, Autoclave, Test tubes, measuring pipette, forceps, conical flask, beaker, Analytical-grade chemicals such as hexane, methanol, ethyl acetate, and nutrient agar were procured from a Microbiology and Medical store (Pyres) in Benin.

3.2 Preparation of the Extract

The leaves of *Jatropha tanjorensis* plant were dried at room temperature for two weeks. The dried leaves were then milled into fine powder. 1000g of dry powdered sample was weighed and

2000ml of ethanol extracting solvent was added in a container and kept at room temperature for 72 hours with intermittent mixing. The mixture was filtered using Whatman Qualitative Grade – 1 filter paper and the resultant filtrate was concentrated to dryness over a hot bath at 40°C. The extract was stored at 40°C for further use.

3.3 Qualitative Phytochemical Analysis

3.3.1 Test for Saponins

20 mL of the sample was boiled in a water bath and then filtered. 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously to form a stable froth. Three drops of olive oil were added, and the mixture was shaken again. The formation of an emulsion indicates a positive test. Persistent frothing also indicates a positive test (Okwu and Okwu, 2005).

3.3.2 Test for Phenol

To 1 mL of the aqueous extract, 5 mL of 95% ethanol was added, followed by drops of 1% ferric chloride. Formation of red, purple, green, blue, violet, or brown coloration indicates a positive test (Ayeni and Yahaya, 2010).

3.3.3 Test for Tannins

20 mL of the sample was boiled, allowed to cool, and then filtered. Three drops of 0.1% $FeCl_3$ were added to 5 mL of the filtrate. Formation of a brownish-green or blue-black precipitate indicates a positive test.

3.3.4 Test for Flavonoids

To 10 mL of the aqueous extract, 5 mL of 10% ammonia was added, followed by drops of concentrated sulfuric acid. The appearance of yellow coloration, which disappears on standing, indicates the presence of flavonoids (Okwu and Okwu, 2005).

3.3.5. Test for Alkaloids

Equipment: water bath, weighing balance

Apparatus: filter paper, test tube, measuring cylinder, pipette

Reagent: 1% HCl, Dragendroff's reagent

Preparation of reagent:

To prepare 100ml of 1% HCl, dilute 1ml HCl in 99ml distilled water

Procedure

Weigh 0.5 g of sample

Add 15ml of 1% HCl and stir

Place on a steam bath for 10mins and filter

To 1ml of filtrate add 2drops of dragenodroff's reagent

Observe for precipitate

The presence of orange/red precipitate is a positive test for alkaloids Harbone method (1993)

3.4 Quantitative Estimation of Phytochemicals

3.4.1 Estimation of Total Phenolic Content

Total phenolic content was determined according to the Folin-Ciocalteu method (1927). Concentrations (10–120 µg/mL) of gallic acid were prepared in methanol. Then, 0.5 mL of the sample (1 mg/mL) was mixed with 2.5 mL of a ten-fold diluted Folin-Ciocalteu reagent and 2 mL of 7% sodium carbonate. The mixture was allowed to stand for 30 minutes at room temperature, and absorbance was read at 760 nm. All determinations were performed in triplicate, with gallic acid utilized as the positive control. The total phenolic content was expressed as Gallic Acid Equivalent (GAE).

3.4.2 Determination of Total Tannins

Tannin content was determined by the modified Folin-Denis method (Polshettiwar *et al.*, 2007). The method is based on the measurement of a blue color formed by the reduction of phosphotungsto-molybdic acid by tannin-like compounds in an alkaline medium. 0.5 mL of extract (1 mg/mL) and a standard solution of tannic acid (10–150 µg/mL) were added to 0.5 mL Folin-Denis reagent and 1 mL of 7.5% Na_2CO_3 solution. Thereafter, 3.4 mL of distilled water was added, and absorbance was measured at 700 nm. The total tannin content was expressed as mg of Tannic Acid Equivalent/g of extract.

3.4.3 Total Flavonoid

The antioxidative properties of flavonoids are due to several mechanisms, such as scavenging of free radicals, chelation of metal ions (e.g., iron and copper), and inhibition of enzymes responsible for free radical generation (Benavente-Garcia, 1997). The amount of total flavonoid content was determined by the aluminum chloride method (Chang *et al.*, 2002). The reaction mixture (3.0 mL) comprised 1.0 mL of extract, 0.5 mL of aluminum chloride (1.2%) in ethanol, and 0.5 mL of potassium acetate (120 mM). The mixture was incubated at room temperature for 30 minutes, and absorbance was measured at 415 nm. Quercetin (Ordonez *et al.*, 2006) or catechin (Kim *et al.*, 2003) in methanol was used as a positive control. The flavonoid content was expressed in terms of standard equivalent (mg/g of extracted compound).

3.4.4. Test for Saponin

Equipment: Weigh Balance, Oven, water bath

Apparatus: beaker, measuring cylinder, conical flask

Reagent: 20% aqueous ethanol, 5 % Nacl, diethyl ether, N-Butanol

Preparation of reagent:

To prepare 40ml 20% aqueous ethanol, dilute 8ml ethanol in 32ml water

To prepare 5ml 5% NaCl, dissolve 0.25g NaCl in 5ml of water

Procedure

One gram (1 g) of the sample was extracted with 20 ml of 20% aqueous ethanol and heated in a water bath for 2.5 hours with continuous stirring, after which the mixture was filtered. The residue retained on the filter paper was re-extracted with another 20 ml of 20% ethanol under the same heating and stirring conditions for 2.5 hours and subsequently filtered. Both filtrates were pooled together and concentrated by evaporation over a water bath at 90 °C until the volume was reduced to 8 ml. To the concentrate, 4 ml of diethyl ether was added in a separating funnel and vigorously agitated, after which the aqueous layer was retained while the ether layer was discarded. This extraction step with diethyl ether was repeated once more, and the aqueous portion obtained was subsequently treated with 12 ml of n-butanol. The n-butanol extract was washed twice with 2 ml of 5% sodium chloride (NaCl) solution, with the washings discarded. The remaining butanol solution was heated in a water bath until complete evaporation. A clean, dry crucible was weighed, and the evaporated extract was transferred into it, then oven-dried to constant weight.

Calculation:

$$\%\ \text{saponin} = \frac{\text{weight of saponin}}{\text{Weight of sample}} \times\ 100$$

Where:

Weight of saponin= weight of crucible and residue after oven drying – weight of crucible

3.4.5. Test for Alkaloids

Equipment: weigh balance

Apparatus: Beaker, pipette, measuring cylinder

Reagent: 10% acetic acid in ethanol, conc. ammonium hydroxide, 0.1M ammonium hydroxide

Preparation of Reagent

To prepare 40ml of 10% acetic acid, dilute 4ml acetic acid in 36ml ethanol

To prepare 100ml 0.1M ammonium hydroxide, dilute 0.68ml ammonium hydroxide in 99.32ml of water

Procedure

One gram (1 g) of the sample was accurately weighed into a beaker, after which 40 ml of 10% acetic acid in ethanol was added. The mixture was covered and allowed to stand for four hours, after which it was filtered. The filtrate obtained was concentrated on a water bath to one-quarter of its original volume. Subsequently, three drops of concentrated ammonium hydroxide were carefully added dropwise to the concentrated extract until complete precipitation occurred. The mixture was then allowed to stand for three hours to facilitate sedimentation of the precipitate. After sedimentation, the supernatant was carefully discarded, and the precipitate was washed with 4 ml of 0.1 M ammonium hydroxide solution and filtered. The residue obtained was dried to a constant weight, and the alkaloid content of the sample was determined using the formula:

Calculation

$$\%\ \text{alkaloid} = \frac{\text{W2}-\ \text{W}\neg 1}{\text{Weight of sample}} \times\ 100$$

Where:

W = weight of sample

W_1 = weight of empty filter paper

W_2 = weight of filter paper and dry residue

3.5 Antioxidant Analysis

3.5.1 Determination of Total Antioxidant Capacity

Total antioxidant activity was estimated by the phosphomolybdenum assay (Prieto *et al*., 1999). The method is based on the reduction of molybdenum (VI) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex at acidic pH.

3.5.2 Preparation of Molybdate Reagent Solution

One (1 mL) each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were mixed together in a 1:1:1 ratio.

Procedure:

1. Add 1 mL of the extract (1 mg/mL) to 3 mL of molybdate reagent solution.
2. Incubate the tubes at 95°C for 90 minutes.
3. Normalize the tubes to room temperature for 20–30 minutes and measure the absorbance of the reaction mixture at 695 nm. Ascorbic acid was used as the standard.

3.5.3 Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the leaf extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al*. (1995). The assay is based on the ability of antioxidant compounds to reduce DPPH by donation of hydrogen, resulting in a color change from deep violet to golden yellow. The change in color was measured spectrophotometrically at 517 nm. Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations (0.2–1.0 mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15 minutes at room temperature in the dark, and absorbance was read at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as the standard control, with similar concentrations as the test samples prepared. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

Where:

A_0 = Absorbance of DPPH radical + methanol

A_1 = Absorbance of DPPH radical + sample extract or standard

The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the extract required to scavenge 50% of the DPPH free radicals.

3.5.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). The assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm. To 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10 mM TPTZ in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution) was added 1 mL of the extracts (1 mg/mL) and standard at concentrations of 100–600 µM. The reaction mixtures were incubated at 37°C for 30 minutes, and the increase in absorbance at 593 nm was measured. $FeSO_4$ was used for the calibration curve, and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe(II)/g of the extract) for the extracts were extrapolated from the standard curve.

3.6 Preparation and Sterilization of Culture Media

All culture media were prepared according to the manufacturer's instructions. Sterilization was done at 121ºC for 15 min unless otherwise stated by manufacturer.

3.6.1 Nutrient agar

Twenty-eight grams (28 g) of nutrient agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask was allowed to cool.

3.6.2Mueller Hinton agar

38g of Mueller Hinton agar agar were dissolved in 1000ml of distilled water and boil to completely dissolve agar. The medium was placed in an autoclave to sterilize it for 15 minutes at 121^{o}C. After sterilization, the flask was allowed to cool before pouring into sterile Petri dishes.

3.7 Confirmatory Tests for the Test Bacterial Isolates

To ensure the purity and accurate identification of the clinical isolates (*Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*) prior to antimicrobial susceptibility testing, confirmatory biochemical tests were performed on sub-cultured isolates. Pure colonies from 24-hour nutrient agar cultures were used. All tests were conducted using standard microbiological procedures (Cheesbrough, 2006).

3.7.1   Gram staining

Gram staining techniques was used for differentiating between Gram-positive and Gram-negative bacteria. Organisms that retain the primary stain are called Gram positive while those that do not retain the primary stain when decolourized are called Gram negative. The non-retention of the stain is due to their cell wall composition.

The Gram stain procedure is as follows:

Smears of the bacterial isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams’ iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with safranin solution for one minute. Finally, the slides were washed off with distilled water. The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnifications to view the slides.

(Cheesbrough, 2006).

3.7.2 Potassium Hydroxide (KOH) test

Two drops of 3% solution of KOH were applied on a clean glass slide and a loopful of pure bacterial growth was stirred in a circular motion in the slide. The loop was occasionally raised and observed for the presence of a string of the mixture. The solution was observed to be of a viscous and mucoid consistency indicating a Gram-negative bacterium. No reaction (absence of stringing) indicates a Gram-positive bacterium (Roberts and Sandle, 2008).

3.8. Biochemical Test

3.8.1 Catalase Test

This test is used to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble immediately indicated catalase enzyme positive (Cheesbrough, 2006).

3.8.2 Oxidase Test

A piece of filter paper was wet with a few drops of the dilute (1%) solution of oxidase reagent (tetramethyl-phenylenediamine-dihydrochloride) which was prepared by standard procedure. A bit of growth from the nutrient agar slant was obtained using sterilized platinum wire loop and smeared on the wet piece of paper. Development of an intense purple color by the cells within 30 seconds indicates a positive oxidase test (Cheesbrough, 2006).

3.8.3 Citrate Utilization Test

This test is based on the ability of some organisms to utilize citrate as a sole source of carbon. It is often used to differentiate between members of Enterobacteriacea. The medium used for this test is Simon`s citrate agar. In the preparation, 22 g of commercially available Simon's citrate agar was dissolved in a litre of distilled water and sterilized by autoclaving at 121 ^{0} C for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stabling the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 ^{0} C for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration. (Cheesbrough, 2006).

3.8.4 Indole Test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The test is usually used in differentiating Gram-negative Bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 degree centigrade for 15 minutes. 4 ml of the medium was dispensed into sterile test tube and each of the bacterial isolates was inoculated into the peptone broth. The inoculated media was incubated at 37 ^{0} C for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150 ml of amyl alcohol, 10 g dimethyl-aminobenzaldehyde and 150 ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

3.8.5 Coagulase Test

A drop of distilled water was placed on each end of a slide and a loopful of the test organism was emulsified in each of the drops to form a thick suspension. Then a loopful of plasma was added to each of the suspension and swirled gently. A positive result is indicated by clumping after 10secs while a negative result show no clumping after 10secs (Cheesbrough, 2006).

3.8.6. Triple sugar iron (TSI) test

An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H_2S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H_2S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO_2 and H_2) (Fawole and Oso, 2007).

3.9 Antimicrobial Activity

The investigational study for antibacterial activity against various pathogenic bacterial strains was conduct using disc diffusion method. The disc diffusion method was used for qualitative evaluation of antibacterial activity for respective crude extract.

3.9.1 McFarland Standard Solution

These viable cells were used to produce a solution of cells of 1.5 x 10^8 by constantly inoculating the cells from Nutrient agar plate with sterile wire loop (flamed intervals to ensure sterility) until a certain turbidity was reached that could be compared to a 0.5 McFarland standard solution already prepared by mixing 0.5Ml of a 1.175 barium chloride dehydrate ($BaCl_2.2H_2O$) solution to 99.5 mL of 1% sulphuric acid (H_2SO_4) (Sagar Aryal *et al*., 2021).

3.9.2 Agar Disc Diffusion Metthod

This was carried out using the modified method of Bauer. Muellar Hinton Agar was prepared using aseptic techniques and after sterilization, was allow to cooled and poured into the petri dishes and allow to solidify. Upon solidification of the agar plate, pre-calibrated Whatman filter paper disks of 6mm in diameter and 2.5 µL infused capacity was prepared and sterilized, sterile paper disc 6mm was soaked in the different concentration of the extract The petri dishes were incubated at 37°c for 24 hours. After incubation, the microbial growth was determined by measuring the diameter (mm) for inhibition zones (Pochapski *et al.*, 2011).

3.9.3 Determination of Minimum Inhibitory Concentration (MIC) and MBC using Broth Dilution Method

The Broth dilution method was used for the determination of Minimum Inhibitory Concentration (MIC) of the extract against bacteria. The extracts were diluted into various concentrations in a sterile Nutrient broth in test tubes. Using standard wire loop, a loopful of the bacterial culture was inoculated into test tubes containing various concentrations of extract in Nutrient broth. The tubes were incubated at 37 °C for 24 hours and thereafter observed for growth or turbidity.

3.9.4 Antibiotic Susceptibility Test

Antibiotic susceptibility of the isolates was determined using the modified Kirby-Bauer disc diffusion technique. The isolates were briefly cultured in Mueller Hinton agar at 37 °C for 24 hours. A suspension of each isolate was inoculated onto sterile agar plates. The plates were allowed to set and the antibiotic sensitivity disc. The plates were allowed to set and the antibiotic sensitivity disc was placed on them. The plates were incubated at 37 °C for 24 hours and the resultant zone of inhibition were measured and recorded. The obtained results were interpreted based on the guidelines of the Clinical Laboratory Standard Institute.

CHAPTER FOUR

4.0 RESULTS

This study presents the results obtained from the evaluation of the phytochemical composition, antioxidant properties, and antibacterial activity of the ethanol leaf extract of *Jatropha tanjorensis* (commonly known as Hospital-too-far).

Table 4.1 presents the qualitative phytochemical constituents of the ethanol extract of *Jatropha tanjorensis* leaves. The qualitative phytochemical analysis revealed the presence of several bioactive compounds. Tannins and saponins were slightly present, while phenols, flavonoids, and alkaloids were strongly detected in the extract, indicating that *Jatropha tanjorensis* contains a rich array of secondary metabolites with potential medicinal properties.

Table 4.2 presents the quantitative phytochemical constituents of the ethanol extract of *Jatropha tanjorensis* leaves. The quantitative results revealed that phenols were the most abundant compounds with a concentration of 129.31 ± 11.53 µg/ml, followed by tannins at 100.57 ± 7.06 µg/ml. Flavonoids were detected at 46.97 ± 5.79 µg/ml, while alkaloids and saponins were present at 9.33 ± 0.29% and 3.17 ± 0.29%, respectively. The high phenolic and tannin contents suggest that the plant may possess strong antioxidant and antimicrobial properties.

Table 4.3 presents the antioxidant properties of the ethanol extract of *Jatropha tanjorensis*. The antioxidant evaluation of the ethanol extract demonstrated significant activity. The Total Antioxidant Capacity (TAC) was recorded as 219.08 ± 47.13 µg/ml, while the Ferric Reducing Antioxidant Potential (FRAP) was 2607.27 ± 72.25 µM. These findings indicate that *Jatropha tanjorensis* leaf extract possesses strong antioxidant potential, which could contribute to its pharmacological properties.

Table 4.4 presents the percentage inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals by different concentrations (10–100 µg/ml) of the ethanol extract of *Jatropha tanjorensis*, compared with ascorbic acid, which served as the standard antioxidant. The extract demonstrated a dose-dependent increase in radical scavenging activity. At the lowest concentration tested (10 µg/ml), the ethanol extract inhibited DPPH radicals by 48.97 ± 0.71%, while the highest concentration (100 µg/ml) yielded a significantly higher inhibition of 69.60 ± 0.52%. In comparison, ascorbic acid exhibited superior antioxidant activity across all concentrations, ranging from 91.19 ± 0.15% at 10 µg/ml to 98.86 ± 0.15% at 100 µg/ml. These results confirm the extract's antioxidant potential, though it is less potent than the standard control.

Table 4.5. Present Cultural, Morphological, and Biochemical Characteristics of Bacteria Isolates tested, the bacteria isolates include, *Klebsiella pneumonia, Escherichia coli*, *Staphylococcus aureus.*

Table 4.6 presents the antibacterial activity of ethanol extract of *Jatropha tanjorensis* against tested bacterial isolates using the disc diffusion method. The results revealed a concentration-dependent antibacterial activity. *Klebsiella pneumoniae* showed the highest susceptibility with a zone of inhibition of 18 ± 0.0 mm at 30 µg/ml, while *Escherichia coli* and *Staphylococcus aureus* showed moderate inhibition zones of 10 ± 0.0 mm and 11 ± 0.58 mm, respectively, at the same concentration. These results indicate that the ethanol extract of *Jatropha tanjorensis* possesses broad-spectrum antibacterial activity, particularly against *Klebsiella pneumoniae*.

Table 4.7 presents the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the ethanol extract of *Jatropha tanjorensis* against the tested bacterial isolates. The lowest MIC (20 µg/ml) was recorded for *Klebsiella pneumoniae* and *Staphylococcus aureus*, suggesting that these bacteria are more susceptible to the extract. The MBC for *E. coli* and *S. aureus* were both 30 µg/ml, while *K. pneumoniae* showed no bactericidal effect (ND) at the tested concentrations. This indicates that the extract exhibits bacteriostatic effects at lower concentrations and bactericidal effects at higher concentrations.

Table 4.8 presents the antibiotic susceptibility profile of the Gram-positive bacterial isolate (*Staphylococcus aureus*). The isolate was resistant to most antibiotics tested, including Cefotaxime (6 mm), Ceftazidime (6 mm), Amoxicillin (6 mm), and Gentamicin (6 mm). Moderate sensitivity was observed for Streptomycin (10 mm), Azithromycin (12 mm), and Ciprofloxacin (11 mm), while Erythromycin (9 mm),Rifampicin (9) and Levofloxacin (7 mm) showed mild inhibition.

Table 4.9 presents the antibiotic susceptibility profiles of the Gram-negative bacterial isolates (*Escherichia coli* and *Klebsiella pneumoniae*). Both isolates exhibited high resistance to most antibiotics tested, with inhibition zones ranging between 6 and 13 mm. *Escherichia coli* was most sensitive to Streptomycin (13 mm) and Gentamicin (9 mm), while *Klebsiella pneumoniae* showed moderate sensitivity to Tetracycline (9 mm) and Streptomycin (10 mm).

Table 4.1: Qualitative Phytochemical Screening of Ethanol Extracts of *Jatropha tanjorensis*

Parameters	**Ethanol**
Tannin	+
Phenol	++
Flavonoids	++
Alkaloids	++
Saponin	+

+: Slightly Present

++: Moderately Present

-: Absent

Table 4.2: Quantitative Phytochemical Composition of Ethanol Extracts of *Jatropha tanjorensis*

Parameters	Ethanol
Tannin (µg/ml)	100.57 ± 7.06
Phenol (µg/ml)	129.31 ± 11.53
Flavonoids (µg/ml)	46.97 ± 5.79
Alkaloids (%)	9.33 ± 0.29
Saponin (%)	3.17 ± 0.29

Values represented in mean ± standard deviation

Table 4.3: Antioxidant Properties of Ethanol Extract of *Jatropha tanjorensis*

Parameters	**Ethanol Extract**
Total Antioxidant Capacity (µg/ml)	219.08 ± 47.13
Ferric Reducing Antioxidant Potential (µg/ml)	2607.27 ± 72.25

Values represented in mean ±standard deviation

Table 4.4: DPPH Radical Scavenging Activity (% Inhibition) of Ethanol extract of *Jatropha tanjorensis*

Conc. (μg/ml)	Ethanol	Ascorbic acid (Control)
10	48.97 ± 0.71	91.19 ± 0.15
20	52.89 ± 0.57	94.08 ± 0.30
40	55.16 ± 0.31	95.60 ± 0.15
60	59.85 ± 0.44	96.88 ± 0.29
80	63.83 ± 1.21	97.68 ± 0.08
100	69.60 ± 0.52	98.86 ± 0.15

Values represented in mean ± standard deviation

Table 4.5. Cultural, Morphological,and Biochemical Characteristics of Bacteria Isolates

Characteristics			
Elevation	Raised	Flat	Raised
Margin	Entire	Entire	Entire
Colony Colour	Golden yellow	Yellowish	Creamy
Colony Shape	Circular	Circular	Circular
Gram Stain	+	–	–
Cell Shape	Cocci	Rod	Rod
Arrangement	Clusters	Single	Single
KOH	–	+	+
Catalase	+	+	+
Coagulase	+	–	–
Oxidase	–	–	–
Citrate	+	+	–
Indole	–	–	+
Glucose	+	+	+
Lactose	-	+	+
Gas Formation	-	+	+
H_2S Formation	-	-	-
TSL(SLANT)	*K/A*	*A/A*	*A/A*
Identity	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*

Key:

- = Positive reaction – = Negative reaction K/A = Alkaline slant / Acid butt A/A = Acid slant / Acid butt TSI = Triple Sugar Iron agar test

Table 4.6: Antibacterial Activity of Ethanol Extracts of *Jatropha tanjorensis* against tested Bacterial Isolates (Zone of Inhibition, mm)

Bacteria Isolates	**10 µg/ml**	**20 µg/ml**	**30 µg/ml**
Escherichia coli	7 ± 0.58	9 ± 1.0	10 ± 0.0
Klebsiella pneumonia	6 ± 0.0	12 ± 3.46	18 ± 0.0
Staphylococcus aureus	7 ± 0.58	9 ± 0.58	11 ± 0.58

Values represented in mean ± standard deviation

Table 4.7: MIC and MBC of Ethanol Extracts of *Jatropha tanjorensis* against tested Bacterial Isolates

Bacteria	**MIC (µg/ml)**	**MBC (µg/ml)**
Klebsiella pneumonia	20	ND
Escherichia coli	30	30
Staphylococcus aureus	20	30

ND=No activity

Table 4.8: Antibiotic Susceptibility of Gram Positive Bacterial Isolates

Bacteria Isolates	CEF	RD	CTZ	S	AZM	AMX	CPX	E	LEV	CN
Staphylococcus aureus	6 mm	9 mm	6mm	10 mm	12 mm	6 mm	11 mm	9 mm	7 mm	6 mm

Keys:

- 0–10 mm = Resistant (R)
- 11–16 mm = Intermediate (I)
- ≥17 mm = Sensitive (S)

Abbreviation	Antibiotic	Disc Concentration (μg/disc)
CEF	Cefotaxime	30 μg
RD	Rifampicin	5 μg
CTZ	Ceftazidime	30 μg
S	Streptomycin	10 μg
AZM	Azithromycin	15 μg
AMX	Amoxicillin	25 μg
CPX	Ciprofloxacin	5 μg
E	Erythromycin	15 μg
LEV	Levofloxacin	5 μg
CN	Gentamicin	10 μg

Table 4.9. Antibiotic Susceptibility of Gram-negative Bacteria Isolates

Bacteria Isolates	AU	PEF	CTZ	CN	CPX	CEP	TRX	S	CEF	OFX
Escherichia coli	6 mm	6 mm	6 mm	9 mm	6 mm	6 mm	6 mm	13 mm	6 mm	6 mm
Klebsiella pneumonia	7 mm	6 mm	6mm	8 mm	6 mm	7 mm	9 mm	10mm	6 mm	7 mm

Keys:

- 0–10 mm = Resistant (R)
- 11–16 mm = Intermediate (I)
- ≥17 mm = Sensitive (S)

Abbreviation	Antibiotic	Concentration (µg/disc)
AU	Amoxicillin/Clavulanic acid	30 µg
PEF	Pefloxacin	5 µg
CTZ	Ceftazidime	30 µg
CN	Gentamicin	10 µg
CPX	Ciprofloxacin	5 µg
CEP	Cephalexin	30 µg
TRX	Tetracycline	30 µg
S	Streptomycin	10 µg
CEF	Cefotaxime	30 µg
OFX	Ofloxacin	5 µg

CHAPTER FIVE

5.1 DISCUSSION

Jatropha tanjorensis, commonly known as "Hospital-too-far," is a perennial shrub belonging to the family *Euphorbiaceae*. It is widely distributed across tropical Africa and has long been recognized in traditional medicine for its diverse therapeutic properties (Oseghale *et al*., 2025). The plant is commonly used in managing ailments such as malaria, diabetes, hypertension, and microbial infections. Its leaves, in particular, are consumed as vegetables and are believed to possess antioxidant, antimicrobial, and hematopoietic properties (Oluwole and Akingbala, 2011). The medicinal potential of *J. tanjorensis* has been attributed to its rich composition of secondary metabolites such as phenols, flavonoids, alkaloids, tannins, saponins, and terpenoids, which have been linked to various pharmacological effects (Anhwange *et al*., 2019; Oseghale *et al*., 2025). This study seeks to evaluate the phytochemical composition and antibacterial effects of the ethanol leaf extract of *Jatropha tanjorensis* on selected bacterial isolates, specifically *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*

The qualitative and quantitative phytochemical analyses (Tables 4.1 and 4.2) revealed that the ethanol leaf extract of *Jatropha tanjorensis* contains significant amounts of secondary metabolites including tannins, phenols, flavonoids, alkaloids, and saponins. Among these, phenols (129.31 ± 11.53 µg/ml) and tannins (100.57 ± 7.06 µg/ml) were the most abundant, followed by flavonoids (46.97 ± 5.79 µg/ml), alkaloids (9.33 ± 0.29%), and saponins (3.17 ± 0.29%). The abundance of phenolic compounds and flavonoids is of great importance as these metabolites are known to possess potent antioxidant and antimicrobial properties (Cowan, 1999; Scalbert, 1991).

This finding agrees with Ajah *et al*. (2021)**,** who reported the presence of phenols, flavonoids, saponins, alkaloids, tannins, terpenoids, and steroids in the methanolic extract of *J. tanjorensis*, highlighting the plant's richness in polyphenolic compounds. Similarly, Komolafe *et al.* (2024) identified high concentrations of flavonoids and reducing sugars through quantitative analysis, noting that flavonoid levels reached approximately 98.55 mg/100g in the hot-water extract. These consistent results across studies confirm that *J. tanjorensis* is phytochemically endowed with compounds known for their antioxidant and antimicrobial potentials.

The predominance of phenolic compounds in the present study aligns with the findings of Anhwange *et al.* (2019)**,** who identified similar bioactive groups—particularly flavonoids, saponins, cardiac glycosides, and alkaloids—in both methanol and hexane extracts. These bioactives are known to interfere with microbial growth and contribute to the plant's medicinal efficacy. The high phenolic and flavonoid contents observed in this study correspond with earlier reports on other medicinal plants such as *Ocimum gratissimum* and *Vernonia amygdalina*, both recognized for their antioxidant and antimicrobial properties (Okwu and Emenike, 2006).

Phenolic compounds are widely documented to exert antimicrobial effects by disrupting microbial cell membranes and inhibiting essential enzymatic activities (Cushnie and Lamb, 2005), while tannins can bind to bacterial cell walls and inactivate proteins necessary for microbial growth (Chung *et al*., 1998). The observed flavonoids are also pharmacologically significant, as these compounds possess antioxidative, anti-inflammatory, and antimicrobial properties (Harborne and Williams, 2000). Although alkaloids and saponins were detected in lower concentrations, their presence still contributes to the plant's therapeutic profile. Alkaloids are known for their antimicrobial and analgesic effects, while saponins enhance immune function and possess mild antibacterial properties (Sofowora, 1993).

The phytochemical profile obtained in this study is in strong agreement with the work of Ohemu and Fajoyomi (2022), who reported the presence of tannins, flavonoids, steroids, terpenoids, anthraquinones, and alkaloids in methanolic extracts of *J. tanjorensis* used against multi-drug resistant bacteria. Such consistency reinforces the reliability of the current findings and supports the ethnomedicinal use of *J. tanjorensis* in managing infections and oxidative stress-related disorders.

The antioxidant evaluation (Tables 4.3 and 4.4) demonstrated that the ethanol extract of *Jatropha tanjorensis* possesses significant antioxidant activity, with a Total Antioxidant Capacity (TAC) of 219.08 ± 47.13 µg/ml and a Ferric Reducing Antioxidant Potential (FRAP) of 2607.27 ± 72.25 µM. The DPPH radical scavenging assay also confirmed dose-dependent antioxidant activity, ranging from 48.97 ± 0.71% at 10 µg/ml to 69.60 ± 0.52% at 100 µg/ml, though slightly lower than the ascorbic acid standard (91.19 ± 0.15% to 98.86 ± 0.15%).

This strong antioxidant capacity can be attributed to the high concentration of phenolic and flavonoid compounds observed in the extract. These compounds are capable of donating electrons or hydrogen atoms to neutralize free radicals (Rice-Evans *et al*., 1996), thus preventing oxidative damage. The FRAP assay results further corroborate the extract's ability to act as an electron donor, consistent with the mechanism described by Benzie and Strain (1996).

The present findings are in harmony with Ajah *et al*. (2021), who reported a high total phenolic (11.35 mg GAE/g) and flavonoid (15.91 mg QCE/g) content in *J. tanjorensis*, along with dose-dependent free radical scavenging activity against nitric oxide, hydroxyl, and lipid peroxidation radicals. The results also correspond with observations by Komolafe *et al.* (2024)**,** who identified several phenolic compounds through GC–MS analysis, suggesting that the antioxidant potential of *J. tanjorensis* arises from the synergistic action of multiple phenolic and flavonoid constituents.

The antioxidant activity demonstrated in this study is comparable to that reported in *Jatropha curcas* extracts, which have shown strong free-radical scavenging and ferric-reducing potentials due to their phenolic composition (Oskoueian *et al*., 2011). The dose-dependent increase in DPPH inhibition observed in this study indicates that the antioxidant efficiency of *J. tanjorensis* improves with concentration, consistent with the general behavior of natural polyphenols. These findings further validate the use of *J. tanjorensis* in traditional medicine as a natural antioxidant source for mitigating oxidative stress-related conditions such as inflammation and chronic infections.

The antibacterial assay revealed that the ethanol extract of *J. tanjorensis* exhibited concentration-dependent inhibitory activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*. Among the isolates, *K. pneumoniae* showed the highest susceptibility with an inhibition zone of 18 ± 0.0 mm at 30 µg/ml, followed by *S. aureus* (11 ± 0.58 mm) and *Escherichia coli* (10 ± 0.0 mm). The Minimum Inhibitory Concentration (MIC) was 20 µg/ml for *K. pneumoniae* and *S. aureus*, while the Minimum Bactericidal Concentration (MBC) was 30 µg/ml for *E. coli* and *S. aureus*.

These results corroborate those of Komolafe *et al*. (2024)**,** who reported inhibition zones of up to 19.83 mm for *S. aureus* and low MIC values for *B. subtilis* using water extracts of *J. tanjorensis*. Similarly, Obum-Nnadi *et al.* (2022) observed bactericidal effects of the plant's cold-water extract against *S. aureus* and *E. coli*, emphasizing the solvent-dependent variability of antibacterial potency. The stronger performance of ethanol in the present study could be linked to its higher solubility for phenolic and flavonoid compounds, which are known for their antimicrobial effects (Cowan, 1999).

The findings also agree with Ohemu and Fajoyomi (2022)**,** who reported that methanol extracts of *J. tanjorensis* inhibited several multi-drug resistant bacteria, including *Enterobacter cloacae* and *Salmonella* spp., with inhibition zones around 17 mm at 100 mg/ml. The current study's comparable inhibition levels demonstrate that *J. tanjorensis* possesses broad-spectrum antimicrobial properties capable of targeting both Gram-positive and Gram-negative bacteria.

The observed antibacterial activity is attributable to the synergistic interaction of the plant's secondary metabolites—especially phenols, tannins, and flavonoids—which are known to interfere with bacterial membrane integrity, enzyme function, and genetic material (Daglia, 2012). The higher sensitivity of *K. pneumoniae* may reflect increased membrane permeability to lipophilic compounds in the ethanol extract (Nikaido, 2003). Overall, the results support the claim that *J. tanjorensis* can serve as an effective source of plant-based antimicrobials, particularly against resistant pathogens.

The antibiotic susceptibility results showed that all test isolates exhibited varying degrees of resistance to conventional antibiotics. *Staphylococcus aureus* displayed complete resistance to Cefotaxime, Ceftazidime, Amoxicillin, and Gentamicin, while showing moderate sensitivity to Streptomycin, Azithromycin, and Ciprofloxacin. *Escherichia coli* and *K. pneumoniae* also demonstrated high resistance levels, with only moderate responses to Streptomycin, Gentamicin, and Tetracycline. The high level of resistance observed in this study is consistent with the global trend of antimicrobial resistance (AMR), as reported by the World Health Organization (WHO, 2020)**,** and with findings by Laxminarayan *et al.* (2013)**,** who documented widespread multidrug resistance among *Staphylococcus* and *Klebsiella* isolates. Notably, the ability of *J. tanjorensis* extract to inhibit these resistant bacteria highlights its potential as a promising natural antimicrobial alternative.

As suggested by Wink (2015)**,** the complex mixture of phytochemicals in plant extracts can target multiple bacterial pathways simultaneously, reducing the likelihood of resistance development. Therefore, the ethanol extract of *J. tanjorensis* offers an effective and eco-friendly approach to addressing the growing problem of antibiotic resistance, supporting its traditional use and encouraging its further exploration in pharmaceutical development.

5.2 Conclusion

This study reveals the antimicrobial potential of *Jatropha tanjorensis* (Hospital-too-far) leaf extracts, highlighting the presence of essential bioactive phytochemicals such as alkaloids, tannins, flavonoids, phenols, saponins, and terpenoids that contribute significantly to its antibacterial and antioxidant activities. The ethanolic leaf extract demonstrated remarkable inhibitory effects against selected pathogenic bacteria, notably *Escherichia coli* and *Staphylococcus aureus*, indicating its broad-spectrum antimicrobial potential. The high levels of tannins, phenols, and flavonoids are associated with the strong antioxidant and antibacterial properties observed, suggesting that these compounds play synergistic roles in inhibiting microbial growth and protecting against oxidative stress.

These findings support the growing scientific evidence that *Jatropha tanjorensis* possesses valuable medicinal properties that can be harnessed for the development of plant-based therapeutic agents. The results align with earlier studies that documented the efficacy of *Jatropha* species in traditional medicine for treating infections and inflammatory conditions. The demonstrated antibacterial activity, particularly against *E. coli* and *S. aureus*, underscores the plant's potential as a natural alternative or complementary agent to synthetic antibiotics, especially in combating the global challenge of antibiotic resistance.

Overall, this research establishes *Jatropha tanjorensis* as a promising candidate for further pharmacological exploration. Future studies should focus on isolating and characterizing the active constituents responsible for its bioactivity, assessing their toxicity levels, and evaluating their efficacy in vivo. Such investigations will provide deeper insights into its mechanism of action and support its potential incorporation into modern herbal formulations and pharmaceutical applications.

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