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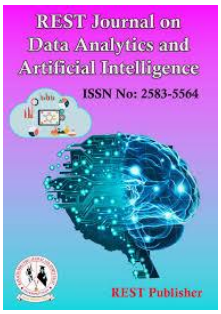

Susceptibilities of *Salmonella typhi* and Other Bacterial Pathogens to Antibiotics and Hot Aqueous Extract of *Hibiscus sabdariffa*

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Abstract: *This study investigated the susceptibility of Salmonella typhi and other pathogens to antibiotics and the hot aqueous extract of Hibiscus sabdariffa using agar diffusion and agar well diffusion methods. Salmonella typhi showed sensitivity to ampicillin, ceftriaxone, ciprofloxacin, gentamicin, ofloxacin, and perfloxacin, while being resistant to nitrofurantoin, ampicillin, clarithromycin, and augmentin. Additionally, Escherichia coli, Klebsiella spp., and Staphylococcus aureus were sensitive to 50%, 70%, and 60% of the antibiotics, respectively, while Pseudomonas aeruginosa was resistant to all tested antibiotics. The Hibiscus sabdariffa extract (0.6g in 6ml sterile distilled water) exhibited antimicrobial activity against S. typhi at concentrations of 100mg/ml, 50mg/ml, and 25mg/ml, with inhibition zone diameters (IZDs) of 23mm, 20mm, and 16mm, respectively. Staphylococcus aureus was susceptible to extract concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml, with IZDs of 29mm, 18mm, 17mm, and 14mm. Klebsiella spp. responded to 25mg/ml and 12.5mg/ml of the extract with IZDs of 15mm and 10mm. However, Escherichia coli and Pseudomonas aeruginosa were resistant to all concentrations of the extract. These findings suggest that medicinal plants like Hibiscus sabdariffa should be considered for therapeutic applications by both government and industry.*

Keywords*: Salmonella typhi, Hibiscus sabdariffa, antibiotic resistance, antimicrobial activity, medicinal plants.*

1. INTRODUCTION

Long before the discovery of microbes, the healing potential of plants, particularly their antimicrobial properties, was widely recognized (Doughari, Mahmood, & Tyoyina, 2011). Although early humans may not have understood the mechanisms of plant-based cures, they were able to identify and utilize plants for their therapeutic benefits long before detailed botanical classifications existed (Sofowora, 2008). The use of plants and their extracts to treat infectious diseases is an ancient tradition in African medicine (Onyeagba, Ugbogu, Okeke, & Iroakasi, 2004), with traditional medical practices varying across countries (Sofowora, 1984). The World Health Organization (WHO) defined traditional medicine in 1978 as the collective knowledge and practices used in the diagnosis, prevention, and treatment of diseases, based on experience passed down through generations, whether verbally or in writing.

Throughout history, humans have relied on plants to treat common infectious diseases, and many traditional remedies are still used today (Doughari et al., 2011). In Nigeria, numerous plants are utilized as phytomedicine by traditional practitioners. Plant extracts are often administered singly or as mixtures in various forms, such as powders, liquids, or liniments, for treating diverse ailments (Apata, 1979). Over 70% of Nigerians depend on herbal concoctions for treating certain diseases (Kimbi-Beyioku, 1996). Several studies have demonstrated the antimicrobial activity of constituents in higher plants (Akobundu & Agykara, 1987; Rocio & Rion, 1982; Almagboul et al., 1988; Misra et al., 1992; Hablemariam et al., 1993), with numerous plant-derived compounds showing antimicrobial properties (Corthout, Piefers, & Cleays, 1992). One such plant is Hibiscus sabdariffa.

Hibiscus sabdariffa, commonly known as Roselle (or Zobo in northern Nigeria), belongs to the mallow family (Malvaceae) and is native to West Africa. It thrives in loamy, well-drained soil in tropical climates with consistent rainfall. The plant is renowned for its medicinal properties, including its anti-hypertensive effects. In traditional medicine, Hibiscus sabdariffa has been used as a diuretic, mild laxative, and for treating cardiac and nerve diseases, as well as certain cancers. The plant is rich in anthocyanins and flavonoids, including gossypetin, hibiscetin, sabdaretine, and daphniphylline, which contribute to its therapeutic qualities, making it a potential chemotherapeutic agent against pathogens (Mohammed et al., 2007).

Typhoid Fever and *Salmonella typhi*

Salmonella typhi is an enteric bacterium responsible for typhoid fever, a disease that has plagued humanity since population densities became sufficient to contaminate water supplies. Typhoid fever is primarily contracted through the consumption of food or water contaminated with the bacterium (Jerry, 2007). Infections can arise from various sources, including contaminated food, poor kitchen hygiene, excretions from infected individuals (both symptomatic and asymptomatic), and polluted water sources.

The symptoms of typhoid fever occur in four phases. In the first week, patients experience a slow rise in temperature, headaches, coughing, malaise, and abdominal pain. During the second week, high fevers (around 40°C or 104°F) occur, accompanied by bradycardia and delirium. In the third week, patients may develop complications such as intestinal hemorrhages, encephalitis, neuropsychiatric symptoms, metastatic abscesses, and endocarditis. By the fourth week, patients enter the "typhoid state," the final stage of the disease.

The incidence of *Salmonella* infection is higher in developing countries compared to developed nations, largely due to differences in sanitation and hygiene practices. Typhoid fever can be diagnosed through bone marrow or stool cultures, and the Widal test, which detects antibodies against *Salmonella* O and H antigens. Treatment typically involves antibiotics such as ampicillin, chloramphenicol, trimethoprim, sulfamethoxazole, and ciprofloxacin. Preventive measures include improved sanitation and the use of vaccines, such as the live oral Ty21a vaccine (Vivotif Berna).

Given the growing concern over antibiotic-resistant bacteria, there is an increasing interest in exploring the antimicrobial properties of medicinal plants. This study aims to evaluate the in vitro effects of *Hibiscus sabdariffa* extract on a clinical isolate of *Salmonella typhi* and other pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.

2. STUDY AND OBJECTIVES

1. To investigate the in vitro effects of *Hibiscus sabdariffa* extract on *Salmonella typhi* and other clinical pathogens.
2. To determine the antibiotic susceptibility profiles of *Salmonella typhi* and other pathogens.
3. To compare the antimicrobial activity of *Hibiscus sabdariffa* extract with standard antibiotics.
4. To assess the potential of *Hibiscus sabdariffa* as a complementary or alternative treatment for antibiotic-resistant pathogens.

Objectives

- Collect and identify *Hibiscus sabdariffa* flowers.
- Conduct antibiotic sensitivity tests on the target organisms.
- Screen the antimicrobial properties of *H. sabdariffa* extract against the test organisms.
- Investigate the susceptibility of *Salmonella typhi* and other pathogens to hot aqueous extracts of *H. sabdariffa* flowers.

3. LITERATURE REVIEW

Plant Extracts: From the earliest times, humanity has recognized the healing properties of plants, though ancient people lacked the extensive botanical knowledge available today (Sofowora, 1984). It is estimated that the earth is home to 250,000 to 500,000 plant species (Borris, 1996), with only a small percentage (1-10%) serving as food for humans and animals. Many more plants are used for medicinal purposes (Moerman, 1996). Hippocrates, in the late fifth century B.C., referred to 300-400 medicinal plants (Schultes, 1978). The use of higher plants and their extracts

to treat infections has been a traditional practice in many parts of the world, especially in Africa (Sofowora, 1984). However, these medicinal practices vary across different countries (Onyeagba et al., 2004).
Natural substances have been a source of medicinal agents for thousands of years. Several modern drugs have their origins in traditional plant-based medicine. This system continues to play a crucial role in health care (Owolabi, Omogari & Obasuyi, 2007). The World Health Organization (WHO) acknowledges that medicinal plants are an important source of various drugs and advocates further research into these plants to better understand their properties, safety, and effectiveness (Nascimento et al., 2000).

The use of herbal remedies is one of the most ancient healing practices known to humankind. These remedies come in various forms, including powders, liquids, and liniments, as described by Apata. Numerous researchers have demonstrated the antimicrobial properties of the compounds found in higher plants (Akobundu & Agykara, 1987). A variety of chemical compounds derived from plants have been shown to exhibit antimicrobial activity (Corthout, Piefers & Cleays, 1992).

A Brief History of Medicinal Plants:
The Bible mentions approximately 30 healing plants. Historical records indicate that frankincense and myrrh were highly valued for their medicinal properties, such as antiseptic qualities that made them useful as mouthwashes (Majorie, 1999). The fall of ancient civilizations led to a decline in Western knowledge of medicinal plants, and many of the records were lost or destroyed (Stockwell, 1988). During the Dark Ages, the Arab world continued to build on earlier knowledge of medicinal plants, while Asian cultures developed their own pharmacopeia.

India is the largest producer of medicinal herbs and is often referred to as the "botanical garden of the world" (Uma & Sudersaman, 2011). With its diverse climate and biodiversity, India has long used medicinal plants to treat ailments. Ayurveda, Unani, Siddha, and folk medicine are the primary traditional medical systems practiced in India.

In North America, indigenous cultures (Native Americans) have used medicinal plants for food and medicine since prehistoric times (Weiner, 1980). Native American knowledge of plant medicine has been extensively reviewed, with over 2,500 plant species documented as having medicinal uses (Klink, 1997).

According to Okigbo and Mmeka (2006), traditional medicine is a major part of Africa's socio-cultural heritage. It has been practiced for hundreds of years, with hunters historically acting as custodians of effective herbal remedies. The World Health Organization defines traditional medicine as the sum of knowledge, skills, and practices used to diagnose, prevent, and treat physical, mental, or social diseases. This knowledge is passed down through generations, either verbally or in writing.

In Nigeria, the integration of traditional medicine with conventional health care is now a priority. It is believed that orthodox medicine alone cannot achieve effective health care unless complemented by traditional medicine (Elujoba, Odeleye & Ogunyemi, 2005). Over 70% of the population in Nigeria relies on herbal remedies for disease treatment (Kimbi & Fagbenro-Beyioku, 1996).

African Medicinal Plants:

Throughout history, medicinal plants have played a critical role in African healing traditions. Below are examples of some African medicinal plants and their uses:

TABLE 1.

Plant Name	**Disease Cured**	**Action**	**Usage**
Xylopia aethiopica	Intestinal spasms, cough, postpartum tonic, headache	Soothing, antispasmodic, sedative	Poultice of the plant
Garcinia kola	Bronchitis, throat infections, liver disorders	Antibiotic, expectorant, choleretic	Eating the seed of the plant
Crytolepis sanguinoleta	Malaria, urinary tract infections, venereal diseases	Antiplasmodial, antiviral, anti-inflammatory	Hot poultice of dried root

The knowledge and use of these plants have been passed down through generations and remain integral to health care in many parts of the African continent. Even today, people continue to rely on these traditional remedies due to their effectiveness and accessibility.

Phytochemical Components with Antimicrobial Activity:

i. Alkaloids: The term "alkaloids" was coined in 1819 by German chemist Carl F.W. Meissner. Alkaloids are a diverse group of over 12,000 nitrogen-containing cyclic compounds present in more than 20% of plant species. These compounds typically feature a nitrogen-containing ring and are known for their profound effects on the central nervous system. For example, caffeine, an alkaloid, has a mild stimulating effect, while alkaloids from Datura can cause severe intoxication or even death. Alkaloids are widely used in the development of various drugs (Okwu, 2005).

ii. Terpenes: Terpenes represent a large class of organic compounds found primarily in plants, especially conifers. An example of a terpene is Vitamin A. When terpenes undergo chemical modifications, such as oxidation or rearrangement of their carbon skeleton, they become terpenoids (Uma and Sudarsanam, 2011). Terpenes consist of units of isoprene, and their classification includes monoterpenes, sesquiterpenes, diterpenes, and triterpenes, depending on the number of isoprene units.

iii. Phenolics: Phenolics, sometimes referred to as phenols, are a class of chemical compounds characterized by a hydroxyl group (-OH) directly attached to an aromatic hydrocarbon group. The simplest member of this class is phenol, also known as carbolic acid (C6H5OH). Phenolics exhibit higher acidity due to the interaction between the aromatic ring and the oxygen-hydrogen bond. They are widely recognized for their antioxidant properties and roles in plant defense.

iv. Flavones, Flavonoids, and Flavonols: Flavones are phenolic structures with a single carbonyl group, unlike quinones, which contain two carbonyl groups. When a 3-hydroxyl group is added to a flavone, it yields a flavonol (Fessenden, 1982). Flavonoids are hydroxylated phenolic compounds composed of a C6-C3 unit connected to an aromatic ring. Plants synthesize flavonoids in response to microbial infections (Dixon & Dey, 1983). These compounds demonstrate antimicrobial properties by interacting with extracellular proteins and bacterial cell walls, making them effective against various microorganisms.

Characteristics of Phytomedicine

Phytomedicine, derived from plants, holds unique characteristics that distinguish it from synthetic drugs (Calixto, 2000):

- The active principle or compound is often unknown.
- Availability and quality control are frequently challenging.
- Standardization, stability, and quality control are feasible but complex.
- Phytomedicine is widely used in chronic treatments due to its broad therapeutic applications.
- Well-controlled clinical and toxicological studies to verify the safety and efficacy of phytomedicines are rare compared to synthetic drugs.
- They are often more affordable than synthetic pharmaceuticals.

Why the Demand for Phytomedicine?

The growing interest in phytomedicine can be attributed to several factors (Blumenthal, 1999 & Grunwald, 1995):

- Consumers prefer natural therapies.
- There is a rising interest in alternative medicine.
- Phytomedicine is believed to have fewer side effects, as it has been used by millions over thousands of years.
- It is often considered effective in treating certain diseases where conventional medicine may fail.
- Improvements in the quality, safety, and efficacy of phytomedicine are being observed.
- The high cost of synthetic drugs makes phytomedicine a more affordable option.

Challenges in the Use and Development of Phytomedicine

Several challenges hinder the development and broader acceptance of phytomedicine, especially in Africa:

- Developing drugs from natural sources is more complex than synthesizing them chemically. Creating phytomedicine in its crude-drug form requires specialized expertise (Elujoba et al., 2005).
- Lack of standardization and quality control during clinical trials is a significant issue (Calixto & Makhubu, 2006).
- Risks of side effects due to toxicity, over-dosage, or interactions with conventional drugs exist, as well as problems in manufacturing, such as plant misidentification, contamination, poor packaging, and environmental conditions (Elujoba et al., 2005).
- Imprecise diagnosis and dosing for phytomedicine remain problematic (Calixto, 2000).
- There is a lack of collaboration between traditional medical practitioners (TMPs), orthodox medical practitioners, and scientists (Elujoba et al., 2005 & Makhubu, 2006).
- Inadequate patient randomization in studies, insufficient sample sizes for trials, and insufficient statistical significance in most research (Calixto, 2000).
- The lack of attention, resource mobilization, and political will also obstruct progress (Elujoba et al., 2006).
- Communication barriers between TMPs and scientists pose further obstacles (Makhubu, 2006).
- There is variation in treatment durations using herbal medicine (Calixto, 2000).
- Public mistrust of phytomedicine after long-term use of orthodox medicine is common (Makhubu, 2006).
- Many plant species are at risk of extinction due to indiscriminate harvesting and inadequate cultivation practices, while traditional healers are aging and their knowledge is fading (Makhubu & McGee, 1998; Elujoba, 2003).
- Outdated and restrictive legislation, such as the Witchcraft Act of 1901, further limits the advancement of phytomedicine (Makhubu, 2006).

Possible Solutions:

To enhance the development and use of phytomedicine, the following measures are recommended:

- Ensuring the use of fresh plants and controlling physical factors such as temperature, light, and water availability improve the quality and stability of phytomedicine.
- Cultivating plants instead of harvesting them from the wild can reduce variations in their active constituents.
- Standardization of phytomedicine can be achieved using chromatography and spectroscopic techniques like infrared (IR) and ultraviolet (UV) spectrometry (Calixto, 2000).
- Pharmacognosists, pharmacologists, and physicians must document and use traditional knowledge to prevent the extinction of valuable medicinal plants and practices (Elujoba, 2003).
- Workshops between TMPs and scientists can help overcome communication barriers and foster collaborative research (Makhubu, 2006).
- Collaborative efforts could involve staff exchanges, training, and joint research projects funded by governments, private sectors, and NGOs.
- Public awareness campaigns should be organized to promote the benefits of medicinal plants and remove misconceptions about scientists' intentions (Makhubu, 2006).
- Outdated laws like the Witchcraft Act should be replaced with modern legislation that protects traditional knowledge and resources.

A Review of Hibiscus sabdariffa:

Hibiscus sabdariffa, commonly known as rosella, Jamaican sorrel, or java jute, belongs to the Malvaceae family. Its fiber is part of a large group of fibers with significant commercial value. The plant is believed to be native to West Africa, where two main varieties exist: H. sabdariffa variety altissima, grown primarily for its fiber, and H. sabdariffa variety sabdariffa, cultivated for its edible calyx. The plant was already known in the West Indies by the 16th century and spread to Asia by the 17th century. In the 1920s, cultivation was extended in Indonesia for fiber production, especially for making sugar sacks.

Scientific Classification

- Kingdom: Plantae
- Unranked: Angiosperms
- Unranked: Eudicots
- Unranked: Rosids
- Order: Malvales
- Family: Malvaceae
- Genus: Hibiscus
- Species: Hibiscus sabdariffa
- Binomial name: Hibiscus *sabdariffa*

Description

H. sabdariffa is typically grown as an annual plant, though it is a perennial by nature. Propagated by seed, it grows best in loamy, well-drained soil, typically in tropical climates with an average of 10 inches (25 cm) of rainfall per month during the growing season. The plant ranges from 3 to 5 meters in height with little branching when grown closely together for fiber production, whereas for fruit crops, it is more widely spaced and shorter with many branches. Its flowers are creamy white or pale yellow, and its stalks and leaves vary from dark green to reddish hues.

The retting process is used to extract fiber from the plant. Roselle fiber is lustrous, creamy to silvery-white in color, and moderately strong. It is often combined with jute and used for bagging fabrics and twines. Major producers of H. sabdariffa fiber include India, Java, and the Philippines.

Origin and Distribution

Native to the regions from India to Malaysia, H. sabdariffa was likely brought to Africa early on. It has since been distributed throughout the tropics and subtropics and has become naturalized in many parts of the West Indies and Central America. The plant's introduction to the New World is said to have been via African slaves. H. sabdariffa was grown in Brazil in the 17th century and Jamaica by 1707. By the 19th century, the plant was used for food in Guatemala and Mexico.

FIGURE 1.

Several attempts were made to cultivate H. sabdariffa commercially, such as in Queensland, Australia, where factories produced jam for export to Europe, though these enterprises were short-lived. It was also introduced to California and Florida, though cultivation in the latter was impacted by frost.

Today, H. sabdariffa is gaining interest in the food, beverage, and pharmaceutical industries as a potential natural product and alternative to synthetic dyes.

Various Names of Hibiscus sabdariffa

The plant is known by various names across different regions and languages, including:

- Australia: Rosella
- India: Meshta
- Nigeria: Zobo (western Nigeria), Isapa (white variety)
- Caribbean: Sorrel
- Mexico: Flor de Jamaica
- France: Bissap
- Thailand: KraJiabDaeng
- Sudan and Egypt: Karkade
- Namibia: Omutete
- Burma: Chin baung

In Zambia, it is called lumanda in ciBemba and katolo in kiKaonde, while in China, it is known as Luo Shen Hua.

Climate

Hibiscus sabdariffa thrives in tropical and subtropical regions up to 3,000 ft (900 m) in elevation. It requires a growing season with at least 72 inches (182 cm) of rainfall. The plant is sensitive to frost and can be grown as a summer crop in temperate regions, though fruit may not ripen in such climates.

Cultivation and Harvesting

While it prefers deep, fertile sandy loam, H. sabdariffa can also grow in less ideal soils, as observed in the oölitic limestone of Dade County. The plant tends to reseed itself and can become invasive. It is usually propagated from seed, though cuttings are also used, especially in India, where the plant is intercropped with trees.

Seedlings can be transplanted, but most often, seeds are sown directly in the field, with spacing depending on the intended use (fiber or fruit). For herbage, the plants can be cut multiple times during the growing season. Fruits are harvested when full-grown but still tender, and seeds are collected from the mature fruits later in the season.

Pests and Diseases

The root-knot nematode (Heterodera rudicicola) is the primary pest affecting H. sabdariffa. Other pests include mealybugs, beetles (Nisotra breweri, Lagris cyanea, and Rhyparida discopunctulata), and the cocoa beetle (Steirastoma breve), which infests roselle in some regions. Additional minor pests include scales, aphids, and the cotton stainer (Dysdercus suturellus).

In Florida, mildew (Oidium) may be an issue, and Phoma sabdariffae has caused minor damage in the Philippines.

General Uses of Hibiscus sabdariffa Hibiscus sabdariffa is known for its antihypertensive properties and is cultivated in some regions for the fiber derived from its stem, which can be used as a jute substitute in burlap production. In folk medicine, Roselle (a variety of Hibiscus) has been used as a diuretic, mild laxative, and treatment for cardiac, nervous system disorders, and cancer. Its red calyces are exported to America and Europe, especially Germany, for use in food coloring. In countries like France, particularly in areas with Senegalese immigrants, the flowers and syrup are also available in markets. Additionally, the green leaves of the plant are used in Senegal as a spicy flavoring for the traditional fish and rice dish, thiéboudieune.

In East Africa, the plant’s calyx is brewed into an infusion known as "Sudan tea," which is consumed to alleviate coughs. A juice made from Roselle, seasoned with salt, pepper, asafetida, and molasses, is used to treat biliousness. The plant's leaves are also used in traditional treatments, where heated leaves are applied to foot cracks, boils, and ulcers to speed recovery. The seeds have diuretic properties, and the oil extracted from them is claimed to heal sores in camels. In India, a decoction of the seeds is used to treat urinary problems and mild digestive disorders, while Brazilians believe the plant’s roots help with stomach issues.

Culinarily, Hibiscus sabdariffa is versatile. The fruits are prepared by removing the tough base of the calyx with the seed capsule attached. The calyces are eaten raw in fruit salads, cooked as a side dish, or used as a filling for tarts and pies. When cooked with sugar, the calyces resemble cranberry sauce in both taste and appearance. The plant is also used in jams, marmalades, syrups, and other condiments. The calyces contain 3.19% pectin, making them suitable for jam production, and in Pakistan, the plant is recommended as a source of pectin for the fruit-preserving industry.

The plant's leaves and tender stems are also consumed, either raw in salads or cooked with vegetables, meat, or fish. In West Africa, the leaves are sold in large quantities. The juice from boiled leaves and stems is used for similar purposes as that of the calyces. Roselle seeds, although bitter, have been ground into flour in Africa and roasted as a coffee substitute. After extracting oil from the seeds, the residue is consumed in soups or blended with bean meal for patties. The seeds are high in protein.

Hibiscus sabdariffa has been used in traditional medicine across various countries, from Mexico to Africa and India to Thailand, where it is said to treat diseases like hypertension and urinary tract infections. However, scientific evidence on its effectiveness in reducing blood pressure or cholesterol levels is still inconsistent. It has shown antimicrobial properties against E. coli in vitro, and some studies suggest that certain extracts of H. sabdariffa have activities against atherosclerosis, liver disease, cancer, diabetes, and metabolic syndromes.

Phytomedicine of Hibiscus sabdariffa H. sabdariffa is rich in anthocyanins and protocatechuic acid. The dried calyces contain flavonoids such as gossypetin, hibiscetine, and sabdaretine. The major pigment is daphniphylline, along with small amounts of myrtillin, chrysanthenin, and delphinidin. Roselle seeds are also a significant source of lipid-soluble antioxidants, particularly gamma-tocopherol.

Various Research on Hibiscus sabdariffa Researchers have explored the plant's potential benefits. Dried flower extracts of Hibiscus sabdariffa have demonstrated antioxidant activity, particularly in protecting rat liver cells from oxidative stress and free radical toxicity. The calyces of the plant have been found to possess anti-spasmodic properties, reducing the tone of certain isolated muscles in animal studies. Additionally, the plant has shown a hypocholesterolemic effect by lowering lipid fractions even in the presence of continued cholesterol intake.

Description of Salmonella typhi Salmonella typhi, the bacterium responsible for typhoid fever, is an ancient pathogen that has affected human populations for centuries, particularly through contaminated water. Typhoid fever remains a significant health issue in developing countries where access to clean water and sanitation is limited. The disease is typically transmitted through poor hygiene or direct contact with an infected person. Typhoid fever is characterized by a systemic infection that can progress to septicemia.

Scientific Classification of Salmonella typhi

- Superkingdom: Bacteria
- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: Salmonella
- Species: S. typhi

History of Typhoid Fever In ancient times, typhoid fever was not well understood due to its non-specific and systemic symptoms. It is also referred to as enteric fever. One of the earliest known outbreaks of what is believed to be typhoid occurred in Athens between 430-426 B.C., killing a third of the population. The disease's cause was only properly understood in the mid-19th century, and it was then distinguished from other illnesses like typhus and malaria.

Typhoid fever is transmitted primarily through contaminated water and poor hygiene. One of the most notorious cases of the disease was that of Mary Mallon, known as "Typhoid Mary." She was an asymptomatic carrier of the disease who caused several typhoid outbreaks in New York City in the early 20th century. Despite being warned to stop

working as a cook, she continued, eventually causing more outbreaks before being quarantined for the remainder of her life.

Causative Agent:

Typhoid fever is primarily caused by Salmonella typhi, a bacterium that was once considered human-specific, along with certain non-typhoidal salmonella (NTS), particularly strains A, B, and C. Paratyphoid fever, a milder form of the disease, is caused by S. paratyphi A, B, and C, also referred to as Horsellafeldil. Other serotypes like S. typhimurium can cause gastroenteritis in humans, commonly known as salmonellosis.

Salmonella belongs to the Enterobacteriaceae family. These bacteria are Gram-negative, flagellated, non-sporulating, facultative anaerobic bacilli that ferment glucose, reduce nitrate to nitrite, and produce peritrichous flagella when motile. They typically range from 0.7 to 1.7 micrometers in diameter and 2 to 5 micrometers in length. As chemoorganotrophs, they derive energy from organic sources through oxidation and reduction reactions, and they can thrive in both aerobic and anaerobic environments. Most Salmonella species produce sulfide, detectable by culturing on media containing ferrous sulfate such as Triple Sugar Iron (TSI) agar. Isolates typically exhibit two phases: a motile phase and a non-motile phase, with motility tested using the Cragie tube method. Salmonella, closely related to the Escherichia genus, is found worldwide in cold and warm-blooded animals (including humans) and in the environment, causing diseases like typhoid fever and paratyphoid fever, as well as other foodborne illnesses.

Mode of Transmission

Typhoid fever is contracted through the ingestion of bacteria found in contaminated food or water. Common sources of infection include:

- Infected food, often originating from production sites and entering the commercial food supply.
- Poor kitchen hygiene, especially in institutional kitchens and restaurants, which can lead to significant outbreaks.
- Excretions from infected individuals or asymptomatic carriers, particularly those handling food.
- Polluted surface water and standing water (e.g., in neglected water dispensers).
- Contaminated poultry and the meltwater from these sources.
- An association with reptiles, especially aquatic turtles, which can carry the bacteria.

Salmonella can survive for weeks in dry conditions and months in water, making it prevalent in polluted environments. Birds and reptiles, along with livestock like poultry, cattle, and sheep, are significant vectors for transmission, often contaminating food items such as milk and meat.

Signs and Symptoms

Typhoid fever typically presents with a sustained fever as high as 40°C (104°F), profuse sweating, gastroenteritis, and non-bloody diarrhea. Symptoms are classically divided into four stages, each lasting about one week:

- First Week: Initial symptoms include a gradual temperature rise, severe headache, cough, malaise, and abdominal pain. Bloody noses (epistaxis) may occur in about 25% of cases. Blood tests may show leucopenia and relative lymphocytosis, with cultures positive for S. typhi.
- Second Week: Patients often experience high fevers plateauing at around 40°C (104°F), bradycardia, and delirium, which can be calm or agitated. This stage is often referred to as “nervous fever.” Rose spots may appear on the abdomen, and abdominal pain is common, particularly in the right lower quadrant. Patients may experience diarrhea or constipation, with six to eight stools per day that are green and have a distinctive odor. Hepatosplenomegaly is noted, and liver enzymes may elevate.
- Third Week: Complications can arise, including constipation, intestinal hemorrhage, intestinal perforation, encephalitis, neuropsychiatric symptoms, and metastatic abscesses. Fever remains high with minimal fluctuation, and the patient may experience dehydration.
- Fourth Week: The fever typically begins to decrease (defervescence), continuing into the final stage of the illness.

Epidemiology of Typhoid Fever:

Estimates of Salmonella typhi infections may be unreliable, as most patients are treated as outpatients, leading to underreporting. In the United States, there are about 1.4 million cases and over 500 deaths annually. The incidence is significantly higher in developing countries, with WHO estimates suggesting 33 million cases of typhoidal salmonellosis occur in regions like Africa and Southeast Asia. Worldwide, approximately 20 million cases and 700,000 deaths occur each year due to typhoid, highlighting it as a major health concern in developing nations.

Pathogenesis of Typhoid Fever

The pathophysiology of typhoid fever is complex and occurs in several stages. The incubation period lasts 7 to 14 days, with severity inversely related to the size of the infecting dose. During this time, bacteria invade macrophages and spread through the reticuloendothelial system. Initial symptomatic disease is characterized by a progressive rise in temperature and subsequent bacteremia. The second week sees the emergence of rose spots, abdominal pain, and splenomegaly, while the third week may involve serious complications like perforation and hemorrhage.

Infection begins when the bacteria survive the acidic stomach environment, penetrate the intestinal epithelium, and interact with phagocytic cells. However, macrophages fail to eliminate the bacteria. Salmonella uses a type 3 secretion system to inject effector proteins into innate immune cells, stimulating both pro-inflammatory and anti-inflammatory responses. After the incubation period, the bacteria proliferate, spreading to the liver, spleen, bone marrow, and gallbladder, with inflammation leading to tissue necrosis, a key feature of typhoid fever.

Prevention and Control:

Preventive measures for typhoid fever focus on sanitation and hygiene. The disease spreads primarily in environments where human feces or urine contaminate food or drinking water. Therefore, proper food preparation and handwashing are vital. Effective water treatment methods, including filtration and chlorination, are essential since food is often prepared with contaminated water. Vaccination is also a crucial preventive strategy. Two vaccines recommended by the World Health Organization include:

- Live oral Ty2la vaccine (Vivotif Berna)
- Ingestible Vi capsular polysaccharide vaccine (Typhim VI)

Both vaccines offer 50-80% protection and are advised for travelers to areas where typhoid is prevalent.

Diagnosis

Diagnosis is typically achieved through blood, bone marrow, or stool cultures, as well as the Widal test, which detects antibodies against Salmonella O-somatic and H-flagella. Despite the availability of several serological assays for detecting S. typhi antigens or antibodies, no non-culture tests have proven consistently sensitive and specific. The Widal test is especially unreliable in endemic areas due to single titers. Confirmatory diagnosis requires the identification of S. typhi in clinical specimens, which can be isolated from over 90% of patients if blood, stool, rose spots, and bone marrow samples are cultured. Other serological tests, like Enzyme-linked immunosorbent assays (ELISA) and dipstick assays, have been developed for rapid diagnosis.

Risk Factors: The age group at highest risk for typhoid fever is typically between 11-30 years. However, this finding has been challenged by a WHO study indicating that the disease affects individuals of all ages and genders.

Treatment: While most cases of typhoid fever are not fatal, antibiotics such as ampicillin, chloramphenicol, trimethoprim, sulfamethoxazole, and ciprofloxacin are effective treatments. Prompt antibiotic intervention can reduce the case fatality rate to about 1%.

Other Pathogens: Other pathogens that can cause similar illnesses include Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae.

Escherichia coli, named after Theodor Escherich, is a Gram-negative, rod-shaped bacterium commonly found in the intestines of warm-blooded organisms. While most E. coli strains are harmless, some, like serotype O157 can cause

severe food poisoning in humans. Harmless strains are part of the gut's normal flora, providing benefits like Vitamin K2 production and preventing pathogenic bacteria colonization.

4. RESULTS

Scientific Classification of *E. coli*:

- Kingdom: Bacteria
- Phylum: Proteobacteria
- Class: Gammaproteobacteria
- Order: Enterobacterales
- Family: Enterobacteriaceae
- Genus: Escherichia
- Species: *E. coli*

E. coli can survive briefly outside the human body, making it an ideal indicator organism for testing environmental samples for fecal contamination. It is easily cultured and genetically manipulatable, making it a key organism in biotechnology and microbiology.

E. coli is a Gram-negative, facultative anaerobic, non-sporulating bacterium, typically rod-shaped, measuring about 2 micrometers long and 0.5 micrometers in diameter, with a cell volume of 0.6-0.7 cubic mic.

Collection and Confirmatory Tests of Test Organisms

A pure culture of *Salmonella typhi* was obtained from the Department of Pharmaceutics at the Faculty of Pharmacy, University of Nigeria, Nsukka. *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella spp.*, and *Pseudomonas aeruginosa* were sourced from the Microbiological Laboratory Unit of the University of Nigeria Teaching Hospital in Ituku Ozalla, Enugu State. The microorganisms were subcultured, and pure isolates were confirmed through gram staining and several biochemical methods.

Gram Staining*:* This method differentiates bacteria into Gram-positive and Gram-negative cells based on their color reactions.

Procedure:

- A flamed wire loop was used to collect a colony from a plate, and a thin film of the organism was prepared on a clean, grease-free glass slide.
- The film was heat-fixed by waving it over a Bunsen burner flame.
- The smear was stained with crystal violet for one minute, then rinsed with water.
- Lugol's iodine was applied for about one minute, followed by rinsing with water.
- Acetone, as a decolorizing agent, was applied briefly for 10 seconds and rinsed quickly to prevent over-decolorization.
- Safranin was used as a counterstain to provide a contrasting color.

Results:

- Gram-positive cells appeared purple.
- Gram-negative cells appeared red.

Biochemical Tests:

The following tests were conducted:

1. **Indole Test:** This test identifies *E. coli*, which can convert the amino acid tryptophan into indole. Method:
    - Tubes of peptone water were inoculated with a young culture of the isolates.
    - After 24 hours of incubation at 37°C, four drops of Kovac's reagent were added to 1 ml of each culture.

Results:

- A red surface layer indicates a positive indole test.
- No red layer indicates a negative indole test.

2. **Oxidase Test:** This test determines if the microorganisms produce the enzyme oxidase, which is characteristic of *Neisseria spp.* and can also indicate the presence of *Pseudomonas spp.* The oxidase reagent (blue) is used to impregnate filter paper, and the test organisms are smeared onto this paper. A color change from blue to purple within 30 seconds to 1 minute indicates a positive result, while no color change indicates a negative result.

3. **Catalase Test:** This test demonstrates which organisms produce the enzyme catalase that decomposes hydrogen peroxide.
Method:
    - A loopful of pure culture was placed on a clean glass slide and mixed with a drop of 3% hydrogen peroxide.

Results:
    - Gas bubbles indicate a positive catalase test (e.g., *Staphylococcus spp.*).
    - Absence of bubbles indicates a negative test.

4. **Coagulase Test**: This test identifies organisms that produce coagulase, which causes plasma to clot.
Method:
    - A drop of distilled water was placed on two clean glass slides, and a colony of the test organism was emulsified in each.
    - A loopful of plasma was added to one suspension and mixed gently.
    - Clumping observed within 10 seconds indicates the presence of coagulase.

Results:
    - Clumping indicates *Staphylococcus aureus*.
    - No clumping indicates *Staphylococcus spp.*

5. **Sugar Fermentation Test:** This test detects acid production from sugar fermentation.
Method:
    - Peptone water was prepared according to the manufacturer's instructions, with drops of methyl red indicator added.
    - Various sugar fermentation tests were conducted using sterile peptone broth.
    - Acid production was indicated by yellow color changes, while gas production was indicated by bubbles in the inverted Durham tube.

Collection and Identification of Plant Material: Dried calyces of *Hibiscus sabdariffa* (zobo) flowers were purchased from Ogbete Market in Enugu. The material was identified by Emeritus Prof. J.C. Okafor of the Department of Botany at the University of Nigeria, Nsukka.
Extraction of Plant Material: Using the maceration method:

- 25g of *H. sabdariffa* was weighed and placed in a conical flask.
- 125ml of hot distilled water (100°C) was added to the flask and stored for 24 hours.
- The mixture was filtered through Whatman No. 1 filter paper.
- The filtrate was placed in glass Petri dishes and dried in a laboratory oven at 45°C to 70°C to concentrate the extract.
- The dried extract was scraped into a sterilized amber bottle and stored in the refrigerator.

Preparation of McFarland's Standard:
This involved mixing 1% Tetraoxosulphate (VI) acid (sulfuric acid, H_2SO_4) and Barium Chloride (1.17%). To create the 0.5 McFarland's standard, 9.95ml of H_2SO_4 was mixed with 0.05ml of $BaCl_2$ to form a precipitate suspension. This standard served as a turbidity reference for the test organisms.

Preparation of Cell Suspension (Inoculum): The test organisms were subcultured on nutrient agar plates and incubated at 37°C for 15-24 hours. Growth from each plate was transferred to a test tube with 5ml of 0.9% sterile saline, adjusting the volume to match the turbidity of the 0.5 McFarland's standard, which corresponds to approximately 1.5×10^8 colony-forming units per ml (cfu/ml).

Serial Dilutions of the Extract: An electronic balance was used to weigh 0.6g of *H. sabdariffa* extract, which was then dissolved in 6ml of distilled water, resulting in a concentration of 100mg/ml. Serial dilutions were performed by transferring 3ml of this solution to another container with 3ml of sterile distilled water, creating a 50mg/ml solution. This process continued to prepare solutions of 25mg/ml, 12.5mg/ml, and 6.25mg/ml.

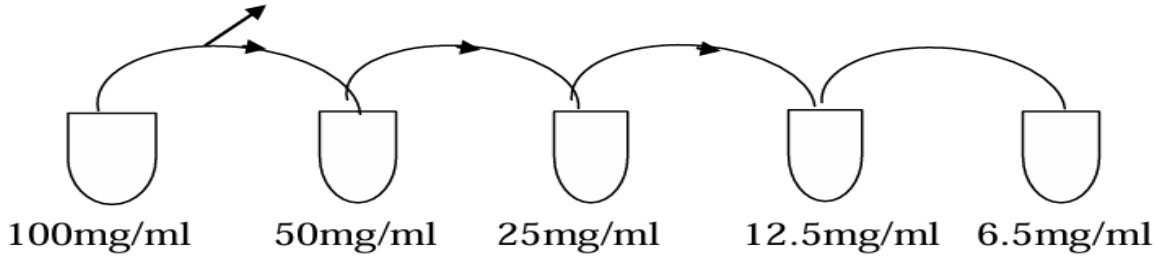

FIGURE 2.

Susceptibility Testing of Test Organisms with Antibiotics Using the Disc Diffusion Method:
The susceptibility of the test organisms to various antibiotics was assessed using nutrient agar plates. After inoculating the plates with the organisms in an anticlockwise manner, multidisc antibiotics were placed on the media.
Gram-negative organisms were tested against ciprofloxacin (10µg), ofloxacin (10µg), perfloxacin (30µg), nitrofurantoin (100µg), ceftriaxone (30µg), gentamicin (10µg), Augmentin (30µg), ampicillin (30µg), chloramphenicol (10µg), and clarithromycin (30µg). Gram-positive organisms were screened against erythromycin (10µg), ceftriaxone (30µg), ampicillin, cloxacillin (35µg), levofloxacin (5µg), norfloxacin (10µg), ciprofloxacin (5µg), gentamicin (10µg), ofloxacin (5µg), and clindamycin (10µg).
Using the Kirby-Bauer disc diffusion method, the prepared cell suspension was used to swab the surface of nutrient agar plates, rotating the swab in an anticlockwise manner. The antibiotic discs were allowed to diffuse for one hour before the plates were incubated at 37°C for 24 hours. After incubation, the inhibition zone diameters (IZDs) around each antibiotic were measured in millimeters and recorded, calculating the mean of IZDs from duplicate plates. The results were interpreted according to CLSI standards.

Susceptibility Testing of Test Organisms Using Hibiscus Sabdariffa Extracts with the Agar Well Diffusion Method:
Nutrient agar plates were inoculated with a suspension of test organisms using the swab method, as described in section 3.8. The susceptibility of the test organisms to Hibiscus sabdariffa extract was evaluated using the agar well diffusion method. Wells were created using a sterile cork borer (8mm) near a Bunsen burner. Following this, 0.1ml of solutions with varying concentrations of H. sabdariffa extract (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml) were dispensed into the labeled wells. The plates were allowed to stand for 1 hour to ensure proper diffusion before being incubated for 24 hours at 37°C. After incubation, the plates were examined, and the inhibition zone diameters (IZDs) were measured using a ruler calibrated in mm to assess the susceptibility of the test organisms. The IZDs (mm) were recorded by calculating the mean of the inhibition zone diameters for each set of duplicate plates.

5. RESULTS

TABLE 2. Results of Identification Tests

Organism	Gram	INDO	CAT	COA	OXI	CIT	GLU
S. typhi	-	-	+	-	-	-	AG
E. coli	-	+	+	-	-	-	-
P. aeruginosa	-	+	+	-	+	-	-
S. aureus	+	-	+	+	-	+	AG
Klebsiella spp	-	-	+	-	-	+	-

Key:

- Positive Result = +
- Negative = -
- AG = Acid Gas
- A+ = Acid positive
- G = Gas

- A = Acid
- OXI = oxidase test
- CAT = catalase test
- INDO = indole test
- COA = coagulase test
- CIT = citrate test
- GLU = glucose test
- Gram = Gram reaction test.

TABLE 3. Inhibition Zone Diameters (IZDs) of Antibiotic Agents on Test Organisms (mm)

Test Organism	C	AM	N	CT	CIP	GN	OF	AU	PF	CM	%R	%S
S. typhi	0	12	0	15	26	16	20	0	≥28	14	50	50
E. coli	0	0	20	28	20	14	0	0	20	20	50	50
Klebsiella spp	0	13	11	22	22	16	24	16	≥28	18	30	70
P. aeruginosa	0	0	0	0	0	0	0	0	0	0	100	0
S. aureus	18	12	0	10	30	14	28	18	≥28	28	40	70

Keys for Gram Negatives:

- C - chloramphenicol
- Am - ampicillin
- N - nitrofurantoin
- Ct - cetriaxone
- Cip - ciprofloxacin
- Gn - gentamycin
- Of - ofloxacin
- Au - augmentin
- Pr - perfloxacin

Keys for Gram Positive Organisms:

- E - Erythromycin
- Ct - cetriaxone
- Ap - ampicillin
- Ce - cifixmine
- Lv - levofloxacin
- Nb - norfloxacin

Cip - ciprofloxacin
Gn - gentamycin
Of - ofloxacin

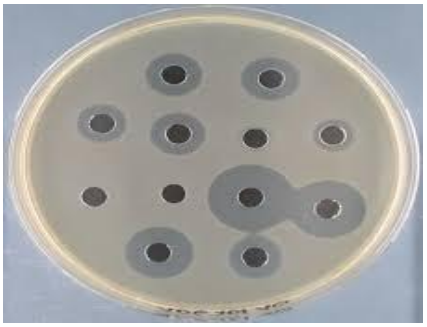

FIGURE 3. Agar plate showing the effect of antibiotics on S. typhi.

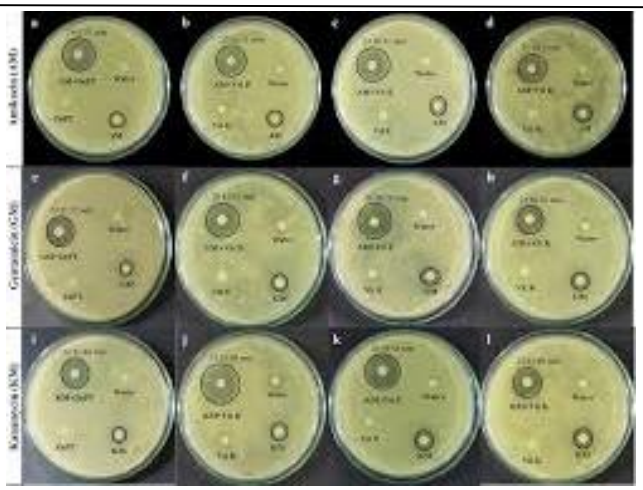

FIGURE 4. Agar plates showing the effect of antibiotics on other test organisms.

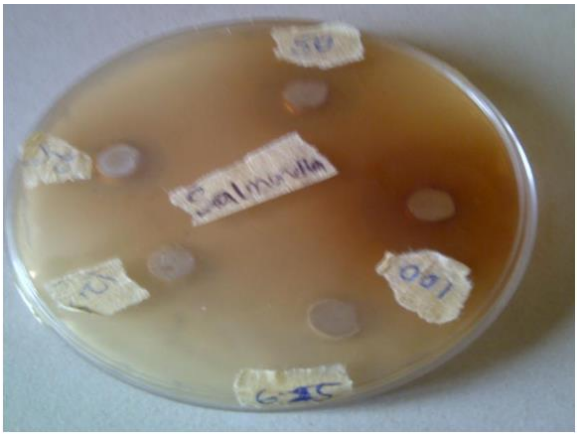

FIGURE 5. Agar plate showing the effect of H. sabdariffa extract on S. typhi.

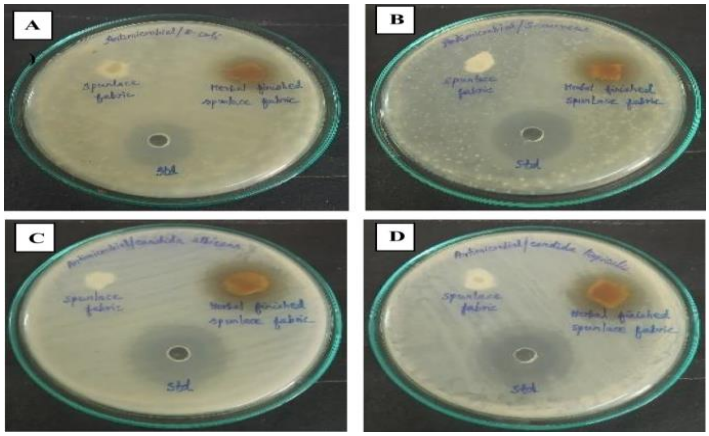

FIGURE 6. Agar plate showing the effect of H. sabdariffa on other test organisms.

6. DISCUSSION OF RESULTS

The results from this study indicate that the aqueous extract of Hibiscus sabdariffa calyces exhibits effective antimicrobial activities. The microorganisms tested included Salmonella typhi, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Staphylococcus aureus. Antibiotics are synthetically produced substances capable of inhibiting or killing microorganisms, primarily targeting bacteria and fungi. Their mechanisms of action include inhibiting cell wall synthesis (e.g., penicillin), protein synthesis, nucleic acid synthesis, and disrupting nuclear membrane synthesis. However, some microorganisms have shown resistance to certain antibiotics, underscoring the need for further investigation and the development of new substances to combat these pathogens to achieve the health standards outlined by the World Health Organization.

The antibiotic sensitivity tests revealed varying susceptibility among the organisms. Salmonella typhi was sensitive to ceftriaxone, ciprofloxacin, gentamicin, ofloxacin, and perfloxacin, but resistant to nitrofurantoin, ampicillin, chloramphenicol, and augmentin. Escherichia coli demonstrated sensitivity to nitrofurantoin, ceftriaxone, ciprofloxacin, perfloxacin, and clarithromycin, but showed resistance to chloramphenicol, ampicillin, ofloxacin, gentamicin, and augmentin. Klebsiella spp. was sensitive to six antibiotics but resistant to four. Pseudomonas aeruginosa displayed resistance to all antibiotics, while Staphylococcus aureus was sensitive to six of the tested antibiotics and resistant to four.

It is crucial to recognize that conventional medicine cannot solely address microbial resistance; thus, the exploration of medicinal plants is essential for enhancing antibiotic efficacy in treating various diseases caused by these microorganisms. H. sabdariffa, a plant native to West Africa, is not only consumed as a beverage but also possesses antimicrobial properties due to its constituents, which include moisture, crude protein, fat, fiber, and various acids.

The screening results indicated that H. sabdariffa extract effectively inhibited some tested organisms. For Salmonella typhi, the inhibition zone diameters (IZDs) for concentrations of 100mg/ml, 50mg/ml, and 25mg/ml were 23mm, 20mm, and 16mm, respectively, with a minimum inhibitory concentration (MIC) of 25mg/ml. Escherichia coli showed resistance at all concentrations. In Klebsiella spp., IZDs of 15mm and 10mm were observed at 12.5mg/ml and 6.25mg/ml, respectively, resulting in an MIC of 12.5mg/ml. Pseudomonas aeruginosa demonstrated resistance to all concentrations of H. sabdariffa extract, while Staphylococcus aureus was the most sensitive, with IZDs of 29mm, 18mm, 17mm, and 14mm at concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml, respectively.

The findings indicated that Staphylococcus aureus was most sensitive to H. sabdariffa aqueous extract, followed by Salmonella typhi and Klebsiella spp., consistent with Marbel's 1996 study. However, Pseudomonas aeruginosa and Escherichia coli showed resistance to all concentrations of the extract. Notably, some antibiotic-resistant organisms were susceptible to various concentrations of H. sabdariffa extract, highlighting the potential of medicinal plants in the development of new antibiotics.

7. CONCLUSION

Based on this study's findings, the aqueous extract of H. sabdariffa exhibits antimicrobial activity. The extract showed the most significant effect on S. aureus, followed by S. typhi and Klebsiella spp., while E. coli and P. aeruginosa displayed resistance. The results indicate that H. sabdariffa can both inhibit and promote the growth of certain microorganisms due to its rich variety of constituents, including moisture, crude protein, fat, fiber, carbon, and phosphorus. Given its nutritional and therapeutic properties, H. sabdariffa should be produced on an industrial scale. It is essential for manufacturers to adhere to standard operational procedures (SOP) and to pasteurize the final product.

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