INVITRO ANTIOXIDANT AND ANTIDIARRHEAL PROPERTIES OF POLYHERBAL FORMULATION (*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum*)

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PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR DEGREE (BSc.) IN SCIENCE LABORATORY TECHNOLOGY

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

(PHYSIOLOGY AND PHARMACOLOGY TECHNIQUES)

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

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CERTIFICATION

This is to certify that this project work, titled "**INVITRO ANTIOXIDANT AND ANTIDIARRHEAL PROPERTIES OF POLYHERBAL FORMULATION (*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum*)**" was carried out by Chinonso Augustine EZIKE with Matriculation Number LSC1807220, of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, under the supervision of Dr. D. O. Uwaya.

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DEDICATION

This Project work is dedicated to almighty God for his love and to my family for their support during the course of my research work.

ACKNOWLEDGEMENTS

Firstly, I will love to show my utmost appreciation to Almighty God who has made it a huge success for me to complete my project work with ease.

I also wish to thank my very intellectual and vibrant supervisor, Dr. D. O. Uwaya, who supported me all through the research. Thank you so much for filling the gap between a mentor, a friend, and a tutor. I am also grateful to the Head of Department, Prof. E. O. Oshomoh, who has been there for the department to grow. and to my special and amiable lecturers, Dr. Peter Obaro, Dr. (Mrs.). Obaro, Mr. James Oseyomon, Mr. Osazuwa Ekhator, Mrs. Ema, and Dr. G. O. Benjamin.

My profound gratitude goes to my ever supportive father, Chief Mr. Titus Ezike for all the love, support, encouragement and understanding all through my stay in the university. And also to my amazing mother Lolo Mrs Lilian Ezike , who was always supportive throughout my academic struggles both financially, prayerfully and through emotional support and words of encouragement and also to my entire family including my brothers and sister and my cousin who is more like an elder brother to me, the person of Mr Okonkwo Ambrose Ikenna who always came through for me whenever i was going through a problem and had no one else to help me, I say thank you. I would also like to thank my fellow project mates under the supervision of Dr. D. O. Uwaya, who later became family. I love you all, thank you and God bless you.

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ABSTRACT

Traditional herbal medicine refers to the use of plants or plant material, either in crude or processed form, to treat illnesses or injuries. Presently, there is ongoing investigation into the therapeutic potential of medicinal plants with ethnomedicinal properties.The aim of this study is to evaluate the in-vitro anti-oxidants and anti-diarrheal properties of poly herbal formulated tea.*(Citrus Lemon, Curcuma Longa, Zingiber Officcinale, Allium Sativum, Moringa Deifera, Syzygium aromaticum*). The formulation, consisting of multiple herbal extracts, was subjected to antioxidant assays using ascorbic acid as the standard. Additionally, its effect on GIT motility was assessed using loperamide as the standard in an anti-diarrheal model induced by castor oil, with water serving as the control. Results from the antioxidant assay revealed significant antioxidant activity of the poly-herbal formulation, comparable to that of ascorbic acid. However, in the anti-diarrheal model, the formulation did not exhibit significant antidiarrheal properties when compared to the control (water) and the standard (loperamide). Interestingly, the poly-herbal formulation demonstrated a significant effect on GIT motility, indicating its potential to modulate intestinal transit time. These findings suggest that while the poly-herbal formulation lacks antidiarrheal properties, it possesses notable antioxidant activity and influences gastrointestinal motility. Further research is warranted to elucidate the mechanisms underlying its effects on GIT motility and explore its potential therapeutic applications in gastrointestinal disorders.

CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND OF STUDY

From ancient times up to the nineteenth century, herbal remedies were the predominant form of medical treatment until synthetic drugs emerged. During this period, their use gradually diminished in developed nations (Dahanukar *et al*., 2000). However, there has been a recent revival of interest in herbal medicine as their effectiveness is being reassessed and validated. Plant-derived formulas and substances for treating a range of diseases are becoming increasingly popular. Additionally, between 75 and 80 percent of people worldwide still depend on herbal remedies as their primary medical recourse in developing nations. Regrettably, thorough assessments of herbal medications are frequently absent, posing a critical need for ensuring user safety. Traditional herbal medicine involves using plants or plant-based substances, whether in their natural form or after processing, to treat illnesses or injuries. Presently, there is ongoing exploration into the healing potential of medicinal plants with ethnomedicinal properties (Kunwar *et al*., 2010). The ethnopharmacological approach to understanding and evaluating traditional and herbal remedies integrates both social and natural sciences. This method typically starts with conducting ethnographic field studies to document the traditional use of naturally occurring medicines (Leonti and Casu, 2013). Throughout history, herbal products have been widely utilized in treating various health conditions, mainly due to the diverse range of biologically active secondary metabolites produced by microbial and plant species. This emphasizes the importance of natural products and their associated structures as valuable sources of innovative medications.

For centuries, Chinese medicine has utilized blends of herbs, known as poly-herbal treatments, yet scientific evidence supporting their medicinal efficacy remains scarce (Che *et al.,* 2013). Conversely, the combination of medications often shows greater promise in treating diseases than single drugs. In Western medicine, the practice of combining drugs is firmly entrenched, with notable success achieved through pharmacological combination therapy for infectious diseases and cancer in recent years (Risberg *et al.,* 2011). Research has illustrated that naturally occurring herbs and herbal compounds, when formulated into specific blends, can produce various interaction effects, such as mutual amplification, support, inhibition, or even hostility (Ramaiah *et al.,* 2013).

In the Ayurvedic medical tradition, combinations of multiple herbs, known as poly-herbal compounds, are predominantly employed for treating various infections. For instance, the Bharangyadi poly-herbal blend contains *Clerodendrum serratum*, *Hedychium spicatum*, and *Inula racemosa* (Kajaria, 2011). Another example is *Indukantha Ghritha* (IG), a poly-herbal preparation composed of 17 plant constituents, commonly prescribed by Ayurvedic practitioners for diverse ailments (George *et al*., 2008). The Unani system of medicine is gaining increasing global recognition due to the notable clinical efficacy of its formulations. However, despite their extensive historical use, there is minimal documented evidence regarding the safety and effectiveness of Unani medicines. This lack of evaluation has impeded the establishment of regulations and legislation in the field (Ajazuddin *et al.,* 2010). An example of such a formulation is Majoon Suranjan (MS), a poly-herbal mixture containing herbs like *Lawsonia inermis*, *Foeniculum vulgare*, *Capparis spinosa*, utilized in the Unani system for treating rheumatoid arthritis (RA) (Singh *et al*., 2011;

Krishnan, 2005). Efforts have been made to investigate extracts from plants like *Cissus rotundifolia*, *Cassia abbreviate*, and *Zanthoxylum chalybeum* in laboratory studies as part of poly-herbal formulations (Krishna, 2005). Despite the widespread global use of poly-herbal formulations, scientific evidence supporting their effectiveness remains limited. Many herbal therapies, including poly-herbal blends, are still undergoing in vivo evaluation and have yet to undergo clinical trials. Moreover, safety assessments such as toxicological studies are often absent. It is crucial to employ scientific methodologies such as clinical trials to assess poly-herbal formulations, identify potential bioactive compounds, and elucidate their mechanisms of action for the advancement of herbal medicine.

1.2. AIM OF THE STUDY

The aim of study is to evaluate the in-vitro anti-oxidants and anti-diarrheal properties of poly herbal formulated tea.*(Citrus Lemon, Curcuma Longa, Zingiber Officcinale, Allium Sativum, Moringa Deifera, Syzygium aromaticum*).

1.3. SPECIFIC OBJECTIVES OF THE STUDY

I. To assess the antioxidant and anti-diarrheal effects of a polyherbal tea blend (comprising *Citrus Lemon*, *Curcuma Longa*, *Zingiber Officinale*, *Allium Sativum*, *Moringa Deifera*, and *Syzygium aromaticum*) in mice with induced diarrhea caused by castor oil in an in vitro setting.

II. To evaluate antioxidants such as super-oxide scavenging activity , DPPH , Hydroxyl free radicals and Total oxide capacity.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Citrus limon*(Lemon)

2.1.1 Description of *Citrus limon* (Lemon)

Lemon is scientifically known as *Citrus limon*, is a small evergreen tree belonging to the Rutaceae flowering plant family. It is native to Asia, particularly Northeast India (Assam), Northern Myanmar, and China (Morton, 1987). Widely recognized for its oval-shaped yellow fruit, the lemon is valued globally for its versatile applications in both culinary and non-culinary domains. Its juice is particularly prized for its acidic properties, commonly utilized in cooking and cleaning tasks (Morton, 1987). The pulp and peel are also commonly used in various culinary preparations. Lemon juice typically contains approximately 5-6% citric acid, giving it a sour taste with a pH of around 2.2 (Spencer *et al*., 2010). This tangy flavor, attributed to its high citric acid content, renders lemon juice a key ingredient in numerous beverages and dishes, such as lemonade and lemon meringue pie (Elsevier, 2010). Lemons are rich in various phytochemicals, including polyphenols, terpenes, and tannins (Rauf *et al.,* 2014). In comparison to lime juice, lemon juice contains slightly higher levels of citric acid (around 47 g/L), nearly double that of grapefruit juice, and approximately five times more citric acid than orange juice (Penniston *et al.,* 2008).

Plate 2.1 : Fresh lemon fruits Source (Rauf *et al*., 2014)

2.1.2. Distribution of *Citrus limon* (Lemon)

Lemons are thought to have reached Europe around the second century AD, possibly introduced through southern Italy during the Ancient Roman period (Morton, 1987). They later spread to Persia, Iraq, and Egypt by approximately 700 AD (Morton, 1987). References to lemons in literature can be traced back to the 10th century, as noted in an Arabic agricultural text, which also highlighted their decorative role in early Islamic gardens (Morton, 1987). Between 1000 and 1150, lemons became widely distributed throughout the Arab world and the Mediterranean region (Morton, 1987). Ibn al-'Awwam's 12th-century agricultural manuscript, "Book on Agriculture," contains a section on lemon and lime tree cultivation in Andalusia, Spain (Ibn al-'Awwam, 1864).

Significant lemon cultivation in Europe began in Genoa during the mid-15th century. They were later brought to the Americas in 1493 by Christopher Columbus, who transported lemon seeds to Hispaniola during his voyages. The dissemination of lemon seeds was facilitated by Spanish colonization across the New World, primarily for decorative and medicinal purposes. In the 19th century, there was a notable increase in lemon cultivation in Florida and California (Morton, 1987).

2.1.3. ETHNOMEDICINAL PROPERTIES OF *CITRUS LIMON*(LEMON)

Lemons (*Citrus limon*) have been utilized in various ethnomedicinal practices globally due to their numerous health benefits. These practices often incorporate different parts of the lemon, including the fruit, juice, peel, and essential oil.

Historically, lemon juice has been used to aid digestion and relieve constipation (Rajkumar *et al.,* 2011). Lemons are renowned as a rich source of vitamin C, essential for enhancing the immune system, maintaining skin health, and promoting iron absorption (Penniston *et al.,* 2008). The essential oil derived from lemon peel is employed in traditional medicine for its antimicrobial properties, commonly applied in treating skin infections (Takahashi *et al.,* 2010).

A popular traditional remedy involves mixing lemon juice with honey to soothe sore throats and coughs (Morton, 1987). Lemon water is traditionally consumed for detoxification and weight loss, although scientific evidence supporting these claims is limited (Bousquet *et al.,* 2017). The flavonoids present in lemons possess antioxidant properties, believed to combat free radicals and reduce inflammation (González-Molina *et al.,* 2010).

Citrus fruits contain no cholesterol, sodium, or fat. They have a low energy content, which may be beneficial for individuals concerned about obesity. Citrus fruits are rich in vitamin C and contain moderate amounts of carotenoids, folate, and fiber, some of which can convert to vitamin A.

2.2. *Curcuma Longa* (Turmeric)

2.2.1. Description of *Curcuma Longa* (Turmeric)

Turmeric, extracted from the *Curcuma Longa* plant, is a prevalent component in Asian cooking and holds a significant role in traditional medicine. This review aims to evaluate the scientific literature concerning turmeric, with a particular focus on its active ingredient, curcumin, and its potential health advantages. Curcumin, the primary active component in turmeric, is renowned for its robust anti-inflammatory and antioxidant properties (Hewlings and Kalman, 2017). It is primarily responsible for the myriad health benefits associated with turmeric, thanks to its chemical composition, which enables it to scavenge free radicals and mitigate oxidative stress (Menon and Sudheer, 2007). Turmeric is distinguished by its thick, underground rhizomes, which are the source of the spice. These rhizomes are encased in tough brown skin and contain deep orange flesh (Sasikumar, 2005). The plant also features large, elongated leaves, measuring up to 1 meter in length, which are bright green and lance-shaped, arranged in two rows (Ravindran *et al.,* 2007). Turmeric plants produce pale yellow flowers in spike-like clusters, often concealed by leaf sheaths. Thriving in warm, humid climates with temperatures ranging from 20°C to 30°C, turmeric necessitates substantial rainfall or irrigation and prefers well-drained, fertile soils. Typically propagated through rhizome cuttings, turmeric requires approximately 7 to 10 months from planting to harvesting (Salvi *et al.,* 2002).

Plate 2.2 : Turmeric plant rhizome.

Source:(Sasikumar,2005)

2.2.2. Distribution of *Curcuma Longa* (Turmeric)

Turmeric (*Curcuma Longa*) is believed to have its origins in South Asia, particularly in regions of India and Pakistan. India stands as the leading producer, consumer, and exporter of turmeric worldwide (Sasikumar, 2005). Historically, turmeric has been an integral part of Indian culture for over 2500 years, primarily utilized as a culinary spice and in traditional Ayurvedic medicine. Turmeric cultivation has extended to various regions globally, including Southeast Asia, East Africa, West Africa, and the Caribbean. Countries such as Indonesia and Malaysia in Southeast Asia have emerged as significant producers, while African nations like Tanzania and Nigeria also cultivate turmeric. Jamaica, within the Caribbean region, is particularly known for its turmeric production. India dominates the global turmeric market, accounting for approximately 80% of the world's supply (Prasad *et al.,* 2014). Key turmeric-producing states in India include Andhra Pradesh, Tamil Nadu, and Karnataka, with Bangladesh, Pakistan, Sri Lanka, Thailand, and China also being noteworthy producers. The global trade of turmeric is primarily propelled by its widespread use as a spice in culinary practices and its incorporation into health and cosmetic products. India, as the largest exporter, fulfills demand in regions such as the Middle East, the United Kingdom, the United States, and others. Among the largest importers, the United States primarily imports turmeric for inclusion in food items and dietary supplements.

2.2.3. Ethnomedicinal Uses of *Curcuma Longa* Turmeric

Numerous research endeavors have delved into the health advantages of turmeric, particularly focusing on its anti-inflammatory and antioxidant attributes. The effectiveness of curcumin as an anti-inflammatory agent has been likened to that of conventional over-the-counter anti-inflammatory medications (Chainani-Wu, 2003). Additionally, there is evidence linking curcumin to improved brain function, primarily by elevating levels of brain-derived neurotrophic factor (BDNF), which could potentially impact brain-related ailments and age-related cognitive decline (Kulkarni *et al.*, 2009). Turmeric exhibits promise in enhancing endothelial function, which is critical for cardiovascular well-being (Akazawa *et al.,* 2012). Moreover, considerable attention has been directed towards exploring curcumin's potential in cancer therapy, with studies indicating its capability to modulate the growth and progression of cancer cells at the molecular level (Gupta *et al.*, 2013). Given its anti-inflammatory characteristics, turmeric has gained popularity as an alternative remedy for arthritis (Chainani-Wu, 2003). Furthermore, its potential role in mental health, particularly in addressing depression, is under scrutiny, with evidence suggesting that curcumin may influence brain neurotransmitters associated with mood regulation (Sanmukhani *et al.,* 2014).

2.3. *Zingiber Officinale* (Ginger)

2.3.1. Description of *Zingiber Officinale* (Ginger)

The ginger plant, scientifically known as *Zingiber Officinale*, is a perennial herb extensively cultivated for its aromatic, culinary, and medicinal rhizome, commonly referred to as ginger root. Growing from a rhizome, ginger can attain heights of up to one meter. It produces annual pseudo stems, composed of rolled leaf bases originating from the rhizome. Its leaves, characterized by their long, slender, green appearance and distinctive lanceolate shape, emerge directly from the rhizome on individual shoots. While ginger flowers are infrequent in cultivation, they manifest as small, yellow blooms densely clustered in cone-like inflorescences. Flourishing in warm, humid conditions, the ginger plant prefers partial shade or filtered sunlight (Mohammad and Hamed, 2012).

As part of a botanical family that encompasses turmeric, cardamom, and galangal, ginger boasts a rich history of cultivation and utilization. Historical records underscore its significance as one of Asia's earliest exported spices, traversing the spice trade routes to Europe and finding extensive usage among ancient civilizations such as the Greeks and Romans (Mohammad and Hamed, 2012). The rhizome stands out as the most prized part of the ginger plant, utilized in both fresh and dried forms. Renowned for its robust, spicy flavor, ginger holds a revered status in various cuisines worldwide. Medicinally, ginger has been employed for millennia, particularly in Ayurvedic, Chinese, and Arabic traditional medicine, valued for its diverse array of benefits including anti-inflammatory, anti-emetic, and digestive properties (Ali *et al*., 2015; Ernst and Pittler, 2000).

Cultivated in diverse tropical and subtropical regions, ginger necessitates fertile, well-drained soil, consistent moisture, and shelter from strong winds. Its propagation primarily involves rhizome division, requiring meticulous cultivation practices due to susceptibility to various pests and diseases (Ali *et al*., 2015).

Plate 2.3: *Zingiber Officinale* (Ginger) rhizome Source (Ali *et al*., 2015)

2.3.2. Distribution of *Zingiber Officinale* (Ginger)

Ginger is believed to have originated in Southeast Asia, with India being acknowledged as a significant hub of its diversity. However, due to its extensive cultivation and adaptation in various tropical regions, the exact origins of the plant remain somewhat uncertain (Mohammad and Hamed, 2012). Originating in Asia, ginger has been dispersed globally, particularly in tropical and subtropical regions. It is extensively cultivated in countries such as India, China, Indonesia, Nigeria, and Thailand, among others, all of which are prominent global producers of ginger, highlighting its widespread cultivation and importance (Kizhakkayil and Sasikumar, 2011).

While ginger thrives in warm, humid climates, it has adapted to diverse tropical and subtropical environments. Typically grown at lower elevations, it requires well-drained soil, consistent moisture, and partial shade. Its cultivation extends to regions characterized by alternating wet and dry seasons, with planting commonly occurring at the onset of the rainy season (Mohammad and Hamed, 2012). In addition to its primary cultivation areas in Asia and Africa, ginger is also grown in the Caribbean, South America, and other tropical regions. Its expansion has been facilitated by its popularity as both a spice and medicinal herb, leading to its introduction to numerous tropical colonial territories by European traders and settlers (Kizhakkayil and Sasikumar, 2011).

2.3.3. Ethnomedicinal uses of *Zingiber Officinale* (ginger)

The ginger plant, scientifically referred to as *Zingiber Officinale*, holds a significant position in various traditional and ethnomedicinal practices worldwide. Its rhizome, the underground stem, is particularly valued for its aromatic, culinary, and therapeutic properties. Ginger has garnered widespread acclaim for its roles in aiding digestion, relieving nausea, and alleviating symptoms of the flu and common cold. It is commonly utilized in herbal teas, soups, and as a culinary spice to promote digestion (Mashhadi *et al.,* 2013). Ginger contains gingerol, a compound well-known for its potent anti-inflammatory and antioxidant properties. Across diverse traditional medicinal systems, ginger has been utilized to reduce inflammation, manage pain, and in some cases, address conditions such as rheumatoid arthritis and osteoarthritis (Grzanna *et al.,* 2005). Acknowledged for its effectiveness in alleviating nausea and vomiting, ginger has historically been used in traditional medicine to alleviate morning sickness during pregnancy and post-operative nausea (Marx *et al.,* 2017). Additionally, ginger has been embraced in traditional medical practices for its antimicrobial properties, which can combat a range of bacteria and fungi (Gull *et al.,* 2012). Traditional medicinal practices have also explored ginger's potential benefits in enhancing cardiovascular health, including lowering cholesterol levels, regulating blood pressure, and preventing blood clot formation (Nicoll and Henein, 2009).

2.4. *Syzygium aromaticum* (Clove)

2.4.1. Description of *Syzygium aromaticum* (Clove)

The *Syzygium aromaticum*, commonly referred to as the clove tree, is an evergreen plant belonging to the Myrtaceae family. It is indigenous to the Maluku Islands, also known as the Spice Islands (Kamatou *et al.,* 2012). Standing at heights ranging from 8 to 12 meters (26–39 feet), it features large, glossy, leathery leaves. The clove buds, typically measuring 1 to 2 centimeters long, appear as reddish-brown spikes with a bulbous top surrounded by four spreading sepals. Emitting a fragrant aroma, the leaves contribute to the tree's distinctive scent, while its branches are noted for their quadrangular shape. Initially pale in color, the clove buds transition from green to a vibrant red when they are ready for harvest.

Plate 2.4: ***Dried Syzygium aromaticum (Clove)*** ***Source*** (Kamatou *et al., 2023)*

Harvesting involves hand-picking the buds before blooming, followed by either sun-drying or mechanical drying, which results in the cloves turning brown. Clove trees typically start flowering between 5 to 8 years of growth and can remain productive for over 50 years, rendering them a valuable, enduring crop for tropical farmers (Abdulaziz *et al*., 2023). Eugenol, a compound found in cloves, is responsible for their distinctive aroma and taste and has been subject to research due to its potential health-promoting properties. With their robust, aromatic, and slightly sweet flavor, cloves are widely used in baking, cooking, and the formulation of fragrant oils (Kamatou *et al*., 2012). Beyond eugenol, cloves are rich in various nutrients and compounds, including vitamins, omega-3 fatty acids, fiber, as well as minerals like magnesium and calcium, offering diverse health benefits (Kusuma *et al*., 2023).

2.4.2. Distribution of *Syzygium aromaticum* (Clove)

The clove plant, scientifically known as *Syzygium aromaticum,* originated from the Maluku Islands, also known as the Spice Islands, situated in Indonesia, and has since spread across tropical regions worldwide (Hussain, *et al.,* 2017). Today, cloves are cultivated in numerous tropical countries, including Indonesia, Madagascar, Zanzibar (part of Tanzania), India, Sri Lanka, and Brazil, where warm and humid climates are conducive to their growth. Indonesia, particularly the Maluku Islands, remains the primary producer due to its favorable environmental conditions (Kusuma *et al.,* 2023). The expansion of clove cultivation beyond Indonesia was facilitated by the colonial and trade activities of European powers, such as the Portuguese, Dutch, and British, during the 16th century. They introduced clove plants to other regions, including their colonies, aiming to diversify spice sources and monopolize the profitable spice trade (Zubair *et al.,* 2017). Despite the global dissemination of clove cultivation, the Maluku Islands continue to be renowned for their production of premium-quality cloves, preserving their historical significance as the epicenter of clove farming. The widespread cultivation of cloves worldwide has cemented their status as a fundamental ingredient in cuisines and traditional medicines across various cultures, highlighting their enduring importance and widespread popularity (Hussain *et al.,* 2017).

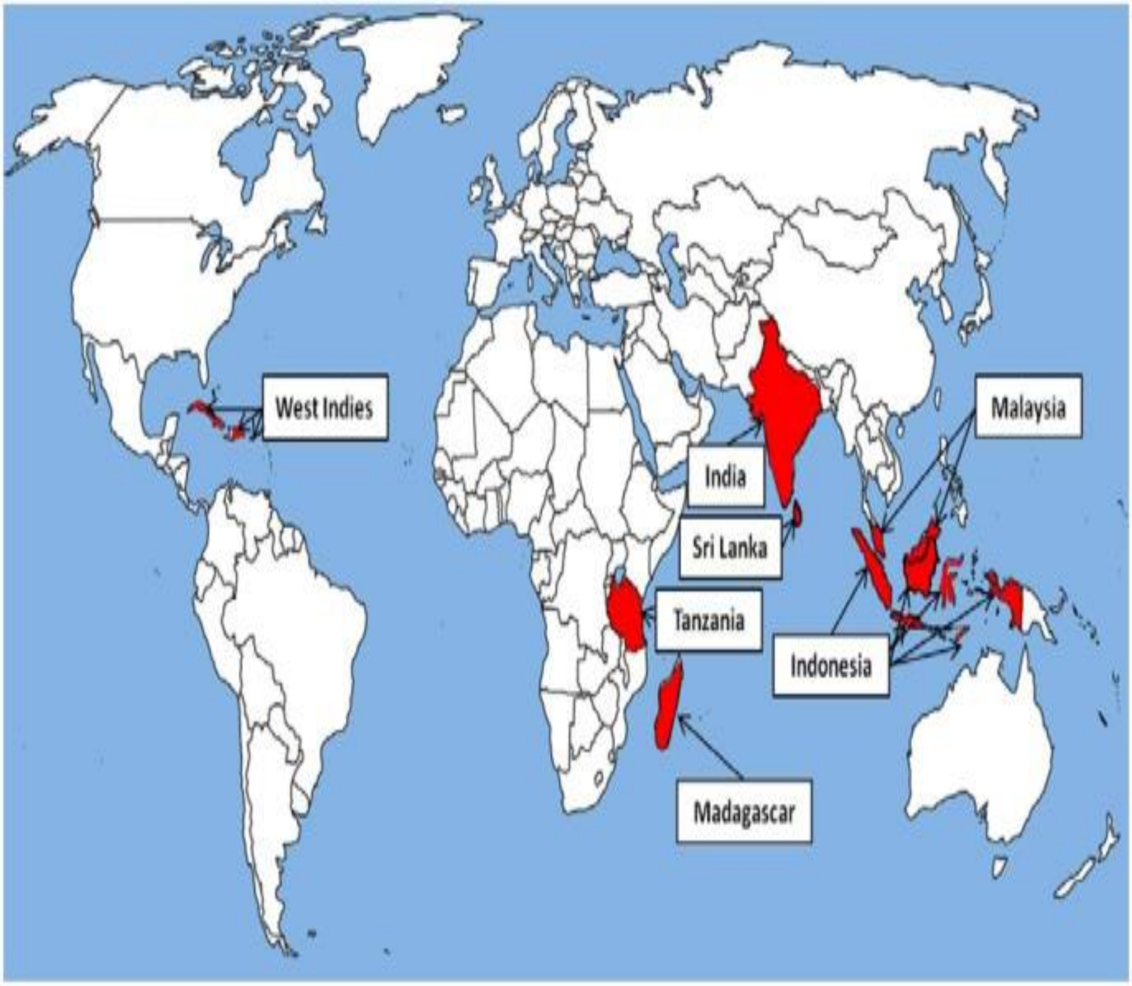

Figure 2.1*:* A map showing clove producers (Kamatou *et al.,* 2012)

2.4.3. **Ethnomedincinal of *Syzygium aromaticum* (Clove)**

Clove (*Syzygium aromaticum*) holds a significant place in ethnomedicine, having been utilized across diverse cultures for its therapeutic attributes. Among its well-documented traditional applications, clove has been prominently featured in dental care. The clove bud yields clove oil, known for its antiseptic and analgesic properties, employed to alleviate toothache, dental discomfort, and oral infections. Eugenol, the primary active component in clove, persists in modern dentistry, often utilized in temporary fillings (Hussain *et al*., 2017).Various traditional medicine systems, such as Ayurveda and Chinese medicine, incorporate cloves to aid digestion, soothe gastric irritation, and mitigate symptoms like flatulence and nausea. Cloves are thought to enhance the secretion of digestive enzymes, facilitating the digestive process (Zubair *et al*., 2017).Clove's antimicrobial prowess, credited to eugenol and other compounds, positions it as a popular remedy against microbial infections. Traditional practices harness clove's potential in combating infections caused by bacteria, fungi, and viruses (Kusuma *et al*., 2023; Cortés-Rojas *et al*., 2014). In traditional medicine, cloves have been utilized as a means to alleviate inflammation and provide natural pain relief. Its anti-inflammatory properties hold particular relevance in managing conditions like arthritis and mitigating inflammatory pain (Kamatou *et al*., 2012).

Abundant in antioxidants, cloves play a pivotal role in shielding the body against oxidation stress and lowering the risk of chronic ailments. With one of the highest antioxidant capacities among spices, clove's traditional medicinal use extends to potentially bolstering the immune system and enhancing overall well-being (Zubair *et al*., 2017).

Within traditional medicine, cloves have been administered to address various respiratory issues. Their expectorant properties make them beneficial in clearing respiratory passages and easing symptoms associated with coughs, colds, and asthma (Hussain *et al*., 2017).

Derived from the plant's buds, leaves, and stems, clove oil finds application in aromatherapy and serves as a natural remedy for pain relief and diverse health concerns (Zubair *et al.,* 2017; Kamatou *et al.,* 2012).

2.5 *Moringa oleifera* (Moringa)

2.5.1. Description of the Moringa (*Moringa oleifera*)

Moringa oleifera, commonly known as Moringa, Drumstick tree, or Horseradish tree, is a small to medium-sized evergreen or deciduous tree that can grow up to heights of 10-12 meters. It typically has a spreading open crown with an umbrella-shaped canopy and deep roots. The trunk is often crooked, usually singular but sometimes bifurcated from the base (Pareek *et al.,* 2023). Its bark has a rough texture and appears whitish-grey in color. The leaves are tripinnate, oval-shaped, and typically range from 20 to 40 cm in length (Srivastava *et al.,* 2023). Each year, the Moringa tree produces fragrant, hermaphroditic flowers. These small, white flowers form drooping panicles that are about 10-25 cm long (Prajapati *et al.,* 2022). The tree's fruit starts off green and changes to brown as it matures. It is a three-sided brown capsule, measuring between 20 to 45 cm in length, and contains numerous round, dark brown seeds (Kumar and Sharma, 2023).

Plate 2.5 : Fresh Moringa leaves. Source: (Srivastava *et al*., 2023)

2.5.2. Distribution of the *Moringa oleifera* (Moringa)

Moringa oleifera originated in the southern foothills of the Himalayas in northwestern India (Khan and Ali, 2023). Since its initial introduction, the species has spread to various regions worldwide, particularly across tropical and subtropical areas. Currently, it is extensively grown in Africa, South and Central America, the Caribbean, Southeast Asia, and select parts of the Middle East (Tshabalala *et al.,* 2019). Moringa demonstrates remarkable adaptability to different climatic conditions, thriving in warm, arid environments while also showing tolerance to frost and cooler temperatures (Devkota and Bhusal, 2020). It particularly flourishes in arid and semi-arid settings, often found in regions with impoverished, sandy soils where other crops struggle to grow (Abdoun *et al.,* 2022).

In Africa, Moringa has been introduced to countries such as Nigeria, Ethiopia, Kenya, and Tanzania, where it serves both nutritional and medicinal purposes (Leone *et al.,* 2015). In Southeast Asia, especially in the Philippines and Indonesia, Moringa holds a significant role in traditional cuisine and medicine (Khan and Ali, 2023). The introduction of Moringa into diverse ecosystems has generally yielded positive outcomes, with minimal reports of invasiveness or adverse effects on local biodiversity. Its contribution to soil enhancement and assistance in reforestation initiatives targeting degraded lands has also been acknowledged (Shami *et al.,* 2022).

2.5.3. Ethnomedicinal Uses of *Moringa oleifera* (Moringa)

The utilization of the Moringa plant in traditional medicine systems across different cultures has a long-standing history spanning centuries. In Ayurvedic medicine, originating from India, Moringa has been highly regarded for its therapeutic capabilities, purportedly addressing over 300 conditions (Patil *et al.,* 2022). Across Africa, various parts of the plant are utilized to treat ailments such as diabetes, hypertension, and infections (Pareek *et al.,* 2023). Similarly, within Southeast Asian traditional medicine, Moringa is prized for its anti-inflammatory and antiseptic properties (Tanaka and Kashiwada, 2021). Various components of Moringa, including its leaves, seeds, bark, and roots, contain compounds with significant therapeutic potential.

Moringa leaves, rich in antioxidants, vitamins, and minerals, are commonly used to enhance immunity and improve overall health (González-Burgos *et al.,* 2021). Regarding Moringa seeds, their discovery of possessing anti-inflammatory and analgesic properties makes them beneficial in alleviating conditions such as arthritis and other painful ailments (Paikra, 2017). Notably, Moringa has reported hypoglycemic effects, which are beneficial in managing diabetes. Regular consumption of Moringa leaf powder has shown significant reductions in blood glucose levels among diabetic patients. Additionally, its high potassium content contributes to its anti-hypertensive properties, assisting in controlling elevated blood pressure (Aekthammarat *et al.,* 2019).

Moringa demonstrates antimicrobial activity, exhibiting efficacy against various bacterial and fungal strains, particularly evident in its leaf extracts (Anzano *et al.,* 2022). Traditionally, the roots and bark are highly valued for their detoxifying effects and have been used to treat digestive disorders and liver ailments (Paikra, 2017).

2.6 *Allium Sativum* (Garlic)

2.6.1 Description of *Allium Sativum* (garlic).

The garlic plant (*Allium Sativum*), a perennial bulbous plant from the Alliaceae family, is closely related to onions, shallots, leeks, and chives. Renowned for its robust flavor and aroma, garlic finds extensive use worldwide for both culinary and medicinal purposes. Typically reaching a height of about 60 cm (24 inches), the plant produces hermaphroditic flowers, although in many varieties, these flowers are sterile and incapable of producing seeds. Instead, garlic primarily reproduces through its cloves (Nasir *et al*., 2020). The garlic bulb, comprised of numerous cloves enveloped in a papery skin, is the most utilized part of the plant. Thriving in well-drained, fertile soils and preferring full sun exposure, garlic has been revered for its therapeutic properties for millennia. It contains allicin, a compound believed to contribute to its various health benefits (Singh *et al*., 2014; Patel, 2019).

Plate 2.6: Fresh garlic bulbs (Nasir *et al*., 2020)

2.6.2 Distribution of *Allium Sativum* (garlic)

The garlic plant (*Allium Sativum*) has gained global popularity, cultivated in almost every corner of the world due to its widespread use as both a cooking ingredient and a medicinal remedy. Originating from Central Asia, particularly Iran and neighboring areas, garlic has been adapted and grown in various climates and regions worldwide (Rivlin, 2001). Its cultivation spans temperate, subtropical, and tropical zones. In temperate regions, garlic is typically planted in autumn, goes dormant during winter, and is harvested in late spring or early summer. Conversely, in warmer areas, planting times vary depending on local climate conditions and the specific garlic variety. China stands out as the world's largest garlic producer, significantly contributing to global garlic production. Other major garlic-producing countries include India, South Korea, and Egypt. The United States also has significant garlic cultivation, especially in California, known for the Gilroy Garlic Festival, which celebrates the crop's importance to the regional economy. Garlic's adaptability to diverse soil types and climates underscores its widespread cultivation (Ding *et al.,* 2023). However, garlic thrives best in well-drained, fertile soil and full sunlight. Its resilience and simple cultivation process make it a staple crop in both backyard gardens and large-scale agricultural operations. Despite its global presence, the variety of garlic types exhibits regional differences influenced by factors such as local culinary traditions, climate conditions, and historical trade routes (Vuković *et al.,* 2023).

2.6.3 Ethnomedicinal uses of *Allium Sativum* (garlic)

Garlic (*Allium Sativum*) has been esteemed in numerous cultures worldwide for its ethnomedicinal attributes, with its usage dating back millennia. Traditional remedies harness garlic's bioactive components, such as allicin, to address and prevent a broad spectrum of ailments. Its potent antimicrobial properties are widely acknowledged, enabling it to combat bacteria, viruses, fungi, and parasites. Garlic has served as a natural remedy for various infections, including respiratory, digestive, and dermatological ailments (Nakamoto *et al.,* 2020). Moreover, traditional medicine has recognized garlic's potential cardiovascular benefits, employing it to reduce blood pressure, lower cholesterol levels, and enhance overall heart health. Its ability to inhibit platelet aggregation has made it a sought-after remedy for averting heart disease and strokes across diverse cultures (Patel, 2019; Ried, 2016). Ethnomedicine also values garlic for its immune-boosting capabilities, often utilized to fortify the body against colds, flu, and other illnesses, particularly during seasonal transitions when respiratory infections are prevalent (Bayan *et al.,* 2014). Additionally, garlic finds its place in promoting digestive well-being within many traditional medicinal systems. It has been employed to combat intestinal parasites, aid digestion, and alleviate gastrointestinal disorders like dyspepsia and bloating (Ansary *et al.,* 2020). Some cultures regard garlic as a detoxifying agent, attributing its efficacy to sulfur-containing compounds believed to support liver health and facilitate detoxification processes (Palani *et al.,* 2014). Externally, garlic has been topically applied in various traditional practices to address fungal infections, wounds, and even earaches. Its antimicrobial prowess renders it effective in treating skin conditions and infections when used externally (Ashfaq *et al.,* 2021).

2.7. OVERVIEW OF DIARRHEA

Diarrhea, a prevalent condition, can vary in its severity and underlying causes. Evaluation of diarrhea hinges on factors such as its duration, severity, and accompanying symptoms. Treatment approaches also vary, but hydration therapy constitutes a crucial component in managing individuals with diarrhea. The normal stool water content is approximately 10 ml/kg/day in infants and young children, or around 200 g/day in teenagers and adults. Diarrhea occurs when there is an increase in stool water content due to disruptions in the physiological processes responsible for absorbing ions, substrates, and subsequently, water, within the small and large intestines.

Acute diarrhea is characterized by the sudden onset of three or more loose or watery stools per day, lasting for up to 14 days. Conversely, chronic or persistent diarrhea persists beyond the 14-day mark. Infections frequently underlie acute diarrhea, while noninfectious causes become more prevalent with chronic diarrhea. This differentiation is crucial as it informs treatment strategies based on duration and specific causative factors. Hydration therapy remains integral in managing individuals with diarrhea (Chen *et al.,* 2018). Preventive measures against infectious diarrhea include proper hand hygiene to mitigate the transmission of pathogens (Null *et al.,* 2018).

The term "acute gastroenteritis" is often interchangeably used with "acute diarrhea"; however, the former is somewhat misleading. Gastroenteritis implies involvement of both the stomach and small intestine, yet gastric involvement is rarely observed in cases of acute diarrhea, even if it is of infectious origin. Furthermore, enteritis is not always present. For instance, cholera and shigellosis represent examples of infectious diarrhea devoid of enteritis. Consequently, it is more clinically accurate to employ the term acute diarrhea rather than acute gastroenteritis.

2.7.1. TYPES OF DIAHOREA

Diarrhea is the result of reduced water absorption by the bowel or increased water secretion. A majority of acute diarrheal cases are due to infectious etiology. Chronic diarrhea is commonly categorized into three groups; watery, fatty (malabsorption), or infectious.

1. **Watery diarrhea**

Lactose intolerance presents as a form of watery diarrhea characterized by heightened water secretion into the intestinal lumen (Szilagyi and Ishayek, 2018). Patients commonly experience symptoms such as bloating, flatulence, and watery diarrhea. In the intestines, lactose is normally broken down by the enzyme lactase, with its byproducts being readily absorbed by epithelial cells. However, when lactase levels are reduced or absent, lactose absorption is impaired, resulting in its accumulation within the gut lumen. Lactose, being osmotically-active, retains and attracts water, thereby causing the onset of watery diarrhea.

2. **Fatty diarrhea**

Common causes of fatty diarrhea include celiac disease and chronic pancreatitis. The pancreas releases enzymes that are necessary for the breakdown of food. Enzymes are released from the pancreas and aid in the digestion of fats, carbohydrates, and proteins. Once broken down, the products are available for uptake in the gut. Patients with chronic pancreatitis have insufficient enzyme release leading to malabsorption. Symptoms often include upper abdominal pain, flatulence, and foul-smelling, bulky pale stools due to malabsorption of fats.(Nikfarjam , *et al* 2017).

3. **Infectious diarrhea**.

Bacterial and viral infections commonly underlie the secretory type of diarrhea. In this scenario, the presence of watery stool stems from damage inflicted upon the gut epithelium. The intestinal tract is lined with epithelial cells that play a crucial role in the absorption of water, electrolytes, and various solutes. When infectious agents attack, they harm these epithelial cells, resulting in heightened intestinal permeability. Consequently, the impaired epithelial cells fail to effectively absorb water from the intestinal lumen, culminating in loose stool.

2.7.2. Treatment / Management

An essential component of managing diarrhea involves replenishing lost fluids and electrolytes (Gauchan and Malla, 2015). Patients are advised to consume diluted fruit juice, Pedialyte, or Gatorade. In severe cases, intravenous fluid rehydration may be necessary (Santos, 1986). Consuming foods low in fiber can help firm up stools. A diet consisting of bananas, toast, oatmeal, white rice, applesauce, and soup or broth is generally well-tolerated and may alleviate symptoms (Dekate *et al.,* 2017). Anti-diarrheal medications, such as anti-secretory or anti-motility agents, may be initiated to reduce stool frequency. However, they should be avoided in adults experiencing bloody diarrhea or high fever, as they can exacerbate severe intestinal infections. Empirical antibiotic treatment with an oral fluoroquinolone may be considered for patients with more severe symptoms. Probiotic supplementation has been demonstrated to lessen symptom severity and duration and should be recommended for those with acute diarrhea. Proper diagnosis and management of diarrhea require identifying the causative agent, emphasizing its importance in treatment decisions :

i. Stool characteristics differ among various causes, including differences in consistency, color, volume, and frequency.
ii. Presence or absence of associated intestinal symptoms, such as nausea/vomiting, fever, and abdominal pain.
iii. Exposure to child daycare where commonly encountered pathogens are rota-virus, astrovirus, calicivirus; Shigella, Campylobacter, Giardia, and Cryptosporidium species.
iv. History of the ingestion of infected food, such as raw or contaminated foods.
v. History of water exposure from swimming pools, camping, or marine environment.
vi. The history of travel is essential as specific regions are prone to common pathogens; enterotoxigenic Escherichia coli being the prevalent pathogen in many cases (Jiang and DuPont, 2017).
vii. Animal contact has traditionally been associated with diarrhea, for instance,

contact with young dogs or cats has been linked to Campylobacter infection, while contact with turtles has been associated with Salmonella infection (Hoelzer *et al.,* 2011).

viii. Underlying factors like hospitalization, antibiotic usage, and immunosuppression (Ghosh *et al.,* 2017).

Typically, patients experiencing acute diarrhea usually follow a self-limiting course and typically do not necessitate laboratory tests or imaging. However, if a patient presents with bloody diarrhea or severe illness, a stool culture is recommended to exclude bacterial causes. Further investigations for Shiga toxin and lactoferrin are necessary in cases of bloody stools.

Treatment for chronic diarrhea is tailored to the underlying cause (Schiller, 2017). The initial step involves categorizing the diarrhea into watery, fatty, or inflammatory types. Once categorized, an algorithm can be employed to determine the subsequent steps in management. Many cases require additional fecal studies, laboratory tests, or imaging. In some instances, more invasive procedures such as colonoscopy or upper endoscopy may be necessary.

Indications for referral and further medical evaluation of children include the following:

i. Under 3 months old

ii. Weighs less than 8 kg (17.6 lbs)

iii. History of premature birth, chronic illnesses, or concurrent medical conditions

iv. Fever of 38ºC (100.4 F) or higher in children less than 3 months old or 39ºC (102.2 F) or higher in children between 3 and 36 months of age

v. Grossly bloody stool

vi. High-output diarrhea

vii. Persistent vomiting

viii. Signs of dehydration, such as sunken eyes, decreased tear film, dry mucous membranes, and oliguria/anuria.

ix. Mental status alterations

x. Inadequate response to oral re-hydration or the caregiver is unable to administer oral re-hydration

xi. For children that are less than in 10 kg body-weight - give 60-120 ml of oral re-hydration solution for each episode of loose stool or vomiting.

xii. For more than 10 kg body-weight - give 120-140 ml of oral re-hydration solution for each episode of loose stool and vomiting.

2.7.3. Prevention and Patient Education

Education plays a pivotal role in both the prevention and treatment of diarrhea. Ensuring proper oral rehydration therapy is crucial to prevent dehydration. Early initiation of re-feeding aids in faster healing of the intestinal mucosa. Caregivers should be educated on the importance of hygiene and adhering to proper food preparation practices to prevent future infections and their transmission.

Effective hand-washing practices are essential in preventing the spread of infectious diarrhea. Individuals with infectious diarrhea should refrain from returning to work, school, or daycare until their symptoms have completely resolved. Professionals should emphasize the significance of vaccinating children against rotavirus, a common cause of viral diarrhea. Probiotic therapy may be considered for patients taking antibiotics to prevent C. difficile colitis (Lau and Chamberlain, 2016).

To reduce the risk of traveler's diarrhea, patients should be advised to consume bottled water, avoid consuming raw fruits and vegetables, and opt for hot, thoroughly cooked foods when traveling to developing countries. It is advisable to use bottled water even for brushing teeth. Prophylactic antibiotics for traveler's diarrhea are generally not recommended. However, antibiotics may be considered for individuals with underlying medical conditions who may be more severely affected by diarrhea (Bolia, 2017).

2.8 ANTIOXIDANTS

Free radicals and antioxidants have become common used terms in contemporary discussions surrounding disease mechanisms (Lobo *et al.,* 2010). In today's rapidly advancing world marked by civilization, industrialization, and overpopulation, understanding antioxidants is crucial as many diseases are mediated through Reactive Oxygen Species (ROS). Antioxidants have garnered significant attention due to their involvement in various biological processes such as tissue protection, immunity, maintaining homeostasis, aging, growth, and development. Antioxidants are broadly defined as substances that delay, prevent, or remove oxidation damage to a target molecule (Khlebnikov *et al.,* 2007). They can directly scavenge ROS or indirectly act to enhance antioxidant defenses or inhibit ROS production.

Put simply, antioxidants are molecules that inhibit the oxidation of other molecules. Oxidation, a chemical reaction involving the loss of electrons and an increase in the oxidation state, leads to the formation of unstable atoms and molecules known as free radicals, which are deficient in electrons. In the late 19th and early 20th centuries, extensive research focused on the use of antioxidants in significant industrial processes, including preventing metal corrosion, rubber vulcanization, and fuel polymerization to prevent fouling in internal combustion engines (Matill, 1947). Early studies on antioxidants in biology primarily explored their role in preventing the oxidation of unsaturated fats, which causes rancidity (German, 1999). However, the discovery of vitamins A, C, and E as antioxidants revolutionized the field and underscored their importance in the biochemistry of living organisms (Jacob, 1996; Knight, 1998). This led to the recognition of antioxidants as reducing agents that prevent oxidation reactions, often by scavenging reactive oxygen species before they can harm cells (Wolf, 2005).

2.8.1. Classification of Antioxidants

Antioxidants can be categorized as either enzymatic or non-enzymatic antioxidants (Mehta and Gowder, 2015).

2.8.2.Enzymatic (Endogenous)

The antioxidant enzymatic system directly/indirectly contributes to defense against the ROS. Catalase, superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, etc., are enzymatic antioxidants.

2.8.3. Non-Enzymatic

A variety of antioxidants exist, including vitamins (such as A, C, E, and K), enzyme cofactors (like Q10), minerals (such as Zn and Se), organosulfur compounds (like those found in allium plants and allium sulfur), nitrogen compounds (such as uric acid), peptides (like glutathione), and polyphenols (including flavonoids and phenolic acid).

2.8.4. The DPPH (4-2,2-Diphenyl-1-Picrylhydrazyl) assay

A quick, straightforward, and cost-effective technique for gauging the antioxidant potential of food entails utilizing the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is extensively employed to assess compounds' capacity to function as scavengers of free radicals or donors of hydrogen, thereby enabling the assessment of antioxidant activity in foods. Additionally, it has been utilized in recent years to quantify antioxidants in intricate biological systems. The DPPH assay stands as a widely utilized method for appraising the antioxidant activity of compounds. DPPH itself is a stable free radical distinguished by its characteristic purple hue. The principle underlying the assay is that antioxidants can furnish hydrogen atoms or electrons to DPPH radicals, causing a visual transition from purple to yellow as the radicals are neutralized (Mahomoodally and Mootoosamy, 2021). This assay holds significance in antioxidant investigations, facilitating the screening and comparison of antioxidant activities among compounds or blends. It furnishes a swift and dependable means of assessing substances' capability to scavenge free radicals and guard against damage associated with oxidative stress (Shalaby and Shanab, 2013).

2.8.5. Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) denotes the collective capability of a substance or blend to counteract free radicals and oxidative stress within a biological milieu. It serves as an indicator of the overall antioxidant efficacy encompassing all constituents within a given sample. TAC evaluations are commonplace in antioxidant investigations, offering insights into the comprehensive antioxidant potential of various items like foods, beverages, supplements, and other compounds (Benzie and Strain, 1996). Numerous methodologies exist for assessing TAC, including the oxygen radical absorbance capacity (ORAC), ferric reducing ability of plasma (FRAP), Trolox equivalent antioxidant capacity (TEAC), and total radical-trapping antioxidant parameter (TRAP), among others. These techniques typically involve gauging antioxidants' ability to impede oxidation or scavenge free radicals within specific experimental conditions.

2.8.6. Hydroxyl free radicals (OH)

Hydroxyl free radicals (OH) represent highly reactive entities characterized by an unpaired electron on the oxygen atom, rendering them among the most potent and detrimental free radicals within biological systems. These radicals are produced during various physiological processes, including oxidative stress, and possess the capability to induce oxidative damage to biomolecules such as lipids, proteins, and DNA (Halliwell and Gutteridge, 1984).

In antioxidant investigations, hydroxyl radicals are frequently employed as a prototype oxidant to evaluate the capacity of antioxidants to scavenge or counteract these radicals. This assay typically entails quantifying the ability of antioxidants to provide electrons or hydrogen atoms to hydroxyl radicals, thereby impeding their interaction with biomolecules and averting oxidation damage. Hydroxyl radical scavenging assays serve as standard methodologies in antioxidant research, facilitating the assessment of substances' potential to shield against oxidative damage and shedding light on their prospective therapeutic uses.

2.8.7. Super-oxide scavenging activity(SSA)

The super-oxide scavenging activity assay evaluates the ability of a substance to neutralize or scavenge super-oxide radicals ($O2-$), which are generated during oxidation stress and are implicated in various diseases and aging processes. Super-oxide radicals are highly reactive and can cause damage to bio-molecules such as lipids, proteins, and DNA.

In the assay, the super-oxide radicals are typically generated by enzymatic or non-enzymatic methods, and the scavenging activity of the tested substance is measured by its ability to inhibit the production of super-oxide radicals or to directly neutralize existing radicals.(Kaur and Kapoor ,2020). Super-oxide scavenging assays play a crucial role in antioxidant research by providing insights into the antioxidant potential of substances and their potential therapeutic applications in combating oxidation stress-related diseases.

2.9. Mechanism of Action

Antioxidants neutralize free radicals by donating one of their electrons, which ends the electron stealing reaction. Antioxidants have been reported to work through single or combined mechanisms, namely, free radical scavenging, reducing activity, complexing of pro-oxidant, scavenging lipid peroxyl radicals, and quenching of singlet oxygen (Mehta and Gowder , 2015).

2.9.1 Chain-breaking mechanism

Chain-breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems, or it simply decays into a harmless product. These antioxidants target free radicals and disrupt the chain reaction in the oxidation propagation phase. These make up most antioxidants in the industry (Lobo , 2010).

2.9.2. Preventive antioxidant mechanism

These antioxidants block the formation of free radicals. This group includes metal chelators, which add to the efficacy of secondary or chain-terminating antioxidants. It prevents oxidation by reducing the rate of chain initiation. They can also prevent oxidation by stabilizing transition metal radicals such as copper and iron (Singh , *et al*., 2013).

2.9.3 Beneficial Effects of Antioxidants

1. Protect Against Heart Disease

The American Heart Association advises consuming a diet rich in fruits, vegetables, and other antioxidant-containing foods to combat cardiovascular disease. However, they do not endorse antioxidant supplements due to the lack of scientific evidence supporting their efficacy in preventing heart disease (Rice-Evans and Diplock, 1993).

2. Protect Against Cancer

Lycopene is found in high concentrations in tomato-based products like soups, sauces, and paste, with smaller amounts present in fresh tomatoes, watermelon, and pink grapefruit. Lycopene has been linked to a reduced risk of cancers affecting various parts of the body, including the mouth, pharynx, esophagus, stomach, colon, and rectum.

Additionally, lutein, another compound found in these foods, may contribute to a decreased risk of macular degeneration.

3. Boost Immunity

Vitamin C's ability to reduce the severity of the common cold is indicative of its effect on the immune system, according to experts at the Cleveland Clinic. Most fruits and vegetables provide some Vitamin C. Citrus fruits, kiwi, tomatoes and sweet peppers are particularly good sources.

4. Fight Aging

While there's no definitive evidence to suggest that antioxidants directly extend lifespan, they do offer protection against the degenerative effects of age-related diseases, which can contribute to premature mortality. Research conducted by the U.S. Agricultural Research Service on laboratory animals indicates that a diet rich in antioxidants, particularly those present in blueberries, strawberries, and spinach, may mitigate age-related cognitive decline. Incorporating a diverse range of fresh, vibrant fruits and vegetables into one's diet—such as broccoli, spinach, tomatoes, sweet peppers, carrots, mangoes, kiwi, berries, and cantaloupe—along with other plant-based foods like grains, legumes (beans, lentils, and split peas), and nuts, is considered the safest and most effective strategy to enhance antioxidant intake and harness the potential health benefits associated with these compounds.

2.9.4 SOURCES OF ANTIOXIDANTS

1. Carotenoids (a form of vitamin A)

Apricots, peaches, broccoli, pumpkin, cantaloupes, carrots, spinach and sweet potatoes

2. Beta-Carotene

Fruits, grains, oils and vegetables (carrot, green plants, spinach)

3. Lycopene: Tomatoes

4: Alpha Tocopherol(Vitamin E)

Nuts and seeds, whole grains, green leafy vegetables, vegetable oil and liver oil, eggs, poultry meat.

5. Ascorbic acid (Vitamin C)

Citrus fruits like oranges and lime etc, green peppers, broccoli, green leafy vegetables, strawberries and tomatoes.

6. Selenium: Fish and shellfish, red meat, liver, yeast, grains, eggs, chicken and garlic

7. Flavonoids: Green tea, grapes, apple, cocoa, berries, onion, broccoli.

8. Resveratrol: Grapes, red wine, purple grape juice, peanuts, and some berries.

Most Commonly Known Antioxidants and Their Food Sources. (Singh , *et al*., 2013)

2.9.5 ANTI DIARRHEA

Anti-diarrheal medications are a category of drugs designed to alleviate or halt diarrhea symptoms. They do not address the underlying cause of diarrhea but provide relief from associated symptoms like increased stool frequency and urgency. Ceasing the use of anti-diarrheal medication typically results in the return of diarrhea unless the underlying cause resolves naturally. Some anti-diarrheal drugs function by slowing intestinal contractions, prolonging the transit time of bowel contents. This facilitates increased water absorption from the bowel back into the body, thereby reducing stool water content. Others operate by adding bulk to the stool, enhancing its volume through fiber-like substances. In essence, an anti-diarrheal drug refers to any medication offering symptomatic relief for diarrhea (Ernst and Monika, 2001). Oral rehydration agents, although sometimes classified as antidiarrheal, do not impede or decelerate diarrhea; instead, they replenish lost fluids during episodes of diarrhea. Additional agents employed to alleviate diarrhea symptoms encompass anti-motility and antispasmodic agents. While antibacterial agents may occasionally be used to treat diarrhea resulting from specific infections like campylobacter or giardia, their routine use or necessity is not recommended.

2.9.6 Types of anti diarrhea agents

1. **Electrolyte solutions agents**

   Electrolyte solutions are not considered genuine antidiarrheal medications; however, they are administered to replenish lost fluids and salts during acute cases.

2. **Bulking agents**

   Methylcellulose, guar gum, and plant fibers such as bran, sterculia, and isabgol serve as bulking agents utilized in managing diarrhea associated with functional bowel diseases and regulating output in ileostomies.

3. **Absorbent agents**

   Absorbents absorb toxic substances that cause infective diarrhoea, methylcellulose is an absorbent.

4. Anti-inflammatory agents

Anti-inflammatory compounds such as bismuth subsalicylate.

5. Anticholinergics agents

Anticholinergics reduce intestinal movement and are effective against both diarrhoea and accompanying cramping.

6. **Opioids**

Opioids, traditionally recognized for their analgesic properties, are also employed as anti-diarrheal agents. They exert agonistic effects on intestinal opioid receptors, which, upon activation, lead to constipation. Substances like morphine or codeine can effectively alleviate diarrhea through this mechanism. Loperamide stands out as a notable opioid for diarrhea relief, as it selectively acts as an agonist on μ opioid receptors in the large intestine. Unlike other opioids, loperamide does not produce central nervous system effects because it does not readily penetrate the blood-brain barrier in significant quantities. This unique feature allows loperamide to offer the same therapeutic benefits as other opioid medications for diarrhea without the associated central nervous system side effects or potential for abuse.

7. **Octreotid**

Octreotide, a synthetic analogue of somatostatin, could be administered to hospitalized individuals for managing diarrhea caused by excessive secretion.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Apparatus and Equipment Used

The apparatus and equipment used are as follow:

Measuring cylinder (100 ml), stirring, masking tape, centrifuge, conical flask (500 ml), industrial blender(KENWOOD Model; KCB239K), plain bottle, transparent cylindrical (45 X 40 X 30), dissecting set, water bath, mortar and pestle, beaker (50 ml and 250 ml), cotton wool, universal bottles (10 ml), hand gloves, analytical weighing balance (Ohaus Corp Pine Brook. NJ USA. China), chopping board, Spectrophotometer (Model: T80 + UV/VIS), mice cage, syringes and needles (1 ml, 2 ml, and 5 ml), storage container, knife, dehydrator (Model: SF-4006 China).

3.2. Chemicals and Solvents

The following chemicals and reagents used are:

KH_2PO_4–KOH, deoxyribose, ferric chloride ($FeCl_3$ ascorbic acid, H_2O_2, castor oil, loperamide, ascorbic acid, activated charcoal, hydroxyl, 2, 2- diphenyl -1- picrylhydrazyl hydrate, super-oxide, total antioxidant.. All chemicals and reagents used were of analytical grade and purchased from a reputable chemical store in Benin city.

3.3. Plant Collection

Allium satirum (Garlic),*Curcuma Longa* (tumeric), *Syzygium aromaticum*(Clove) and *Zingiber Officinale* (ginger) were purchased from Kurmi Market within Kano Municipal Local Government Area, Kano State. *Moringa oleifera*(Moringa leaves) was gotten from Ikpoba-Okha Local Government Area in Edo State. *Citrus* *limon*(lemon) was gotten from New Benin market Oredo local Government Area. The plants were identified and authenticated by Dr. H . Akinnibosun in the Department of plant biology and biotechnology in the faculty of life science.

3.4. Preparation Of Plant Materials/Polyherbal Tea Formulation

The Cirus limon (lemon),Curcuma Longa (tumeric), *Allium satirum* (Garlic) and *Zingiber Officinale* (ginger) were washed with running water to remove dirt and debris and then they were chopped into smaller bits. The *Moringa oleifera*(Moringa leaves), was washed and air dried. The *Citrus limon* (lemon), the chopped *Allium satirum* (Garlic), *Curcuma Longa* (tumeric) and the *Zingiber Officinale* (ginger) were dehydrated using a dehydrator. After dehydration, the dehydrated *Citrus limon*(lemon), *Zingiber Officinale* (ginger), *Allium satirum* (Garlic),*Curcuma Longa* (tumeric) , *Syzygium aromaticum*(Clove) and *Moringa oleifera*(Moringa leaves) were ground to powder separately using an industrial blender. The powders of *Citrus limon* (lemon), *Allium satirum* (Garlic), *Zingiber Officinale* (ginger),*Moringa oleifera*(Moringa leaves), *Syzygium aromaticum*(Clove) and *Curcuma Longa* (tumeric) were weighed and mixed in an equal proportion (1:1:1:1:1:1) to formulate the herbal tea. The herbal tea was formulated in such a way that a tea bag contained 200 mg of each powdered plant material. The poly-herbal formulated tea was formulated using Uwaya and Effiong, 2024 with slight modifications.

3.5. POLYHERBAL TEA EXTRACTION

Formulated poly-herbal tea (1,485g) was weighed into an extracting jar and 8.15 liters of distilled water was added. The mixture was stirred using a stirrer and allowed to macerate for 72hours. the mixture was filtered using a strainer into a storage container with a lid. The filtrate was concentrated using a water bath. The extract was concentrated using a water bath to yield 473.9g. the extract was stored in an amber bottle in the refrigerator at 4°c prior to use.

3.6. EXPERIMENTAL ANIMALS

Healthy adult albino mice of either sex weighing 15.31–24.16 g were purchased from commercial farm in Ibadan, Oyo State. The mice were housed within the animal facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, of the University of Benin and given two weeks of acclimatization under normal laboratory conditions with a 12-hour light/dark cycle. They were fed normal animal pellets spontaneously. The animals were handled in accordance with normal protocols for laboratory animals (National Institute of Health, USA, Public Health Service Policy on Humane Care and Use of Laboratory Animals, 1986).

3.7. ANTI DIARRHEA EXPERIMENTAL DESIGN

Healthy adult albino male rats of either sex weighing 120g–250 g was purchased from a commercial farm in Ibadan, Oyo State. The rats were housed within the animal facility of the Department of Animal and Environmental Biology, Faculty of Life Sciences, of the University of Benin and given two weeks of acclimatization under normal laboratory conditions with a 12-hour light/dark cycle. They were fed normal animal pellets spontaneously. The animals were handled in accordance with normal protocols for laboratory animals (National Institute of Health, USA, Public Health Service Policy on Humane Care and Use of Laboratory Animals, 1986).

3.7.1 Intestinal transit time in albino mice

Sixteen (16) Healthy adult albino mice of either sex were allotted into 4 groups of 4 mice in each group as follows:

Group 1- Received 10 ml/kg of distilled water.

Group 2- Received 5 mg/kg extract of poly-herbal-formulated tea.

Group 3- Received 10 mg/kg extract of poly-herbal-formulated tea.

Group 4- Received 2 mg/kg of loperamide (standard drug).

After one hour, all animals were given 10ml/kg of charcoal meal (10% charcoal suspension in 5% normal saline) orally. All animals were euthanized (sacrificed) 20 minutes after being given the charcoal meal via cervical dislocation. The over all length and distance traveled by the amount of charcoal meal in the gut from the pyrolus to the caecum was measured and expressed as a percentage of the small intestine's total length.

3.7.2. Assessment of anti diarrhea activity Castor oil induced diarrhea

Mice of either sex were fasted overnight. They were separated into four groups (n = 4).

Group 1- Received 10 ml/kg of distilled water.

Group 2- Received 5 mg/kg extract of poly-herbal-formulated tea.

Group 3- Received 10 mg/kg extract of poly-herbal-formulated tea.

Group 4- Received 2 mg/kg of loperamide (standard).

After 60 minutes of treatment, the animals in each group were given 0.3ml of castor oil orally, and the watery stool material and number of stool were recorded for 4 hours in clear metabolic cages with filter paper at the base. The time of administration of castor oil and the time of first watery stool were noted. The number of watery stool and the weight of watery stool were recorded.The weight of paper before and after defecation was recorded.

3.7.3 DETERMINATION OF 2,2-DIPHENYL-1-PICRYLHYDRAZYL HYDRATE SCAVENGING ACTIVITY

The procedure to investigate the ability to resist oxidation of the poly-herbal formulated tea was carried out as described by (Tran *et al* , 2022) with modification. The reaction mixture consisted of 100 µL of DPPH (6x10-4 M) and 100 µL of extract (concentration from 0 to 10 mg/mL). The reaction mixture was incubated for 60 min in the dark and at 30ºC. The reaction solution was then measured for the absorbance of DPPH at 517 nm. The positive control in the treatment was Ascorbic acid.

The formula to calculate the DPPH free radical scavenging effect is as follows: E (%) = (ODc – ODm)/ODc x100.

Where: E: Antioxidant efficacy (DPPH free radical neutralization ability) (%)

ODc : The O.D. value of the negative control sample

ODm: O.D. value of test sample.

3.7.4. DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY

The antioxidant activity of poly-herbal formulated tea was investigated using the phosphomolybdenum method (Prieto *et al*., 1999). The procedure was started with the preparation of a mixture including a test sample (100 µL) at different concentrations, reagent solution (0.6 M sulfuric acid, 2 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was covered tightly and incubated at 95ºC for 90 min. The reaction solution was kept cool at room temperature, and its absorbance was measured at 765 nm. Ascorbic acid was used to compare the result in the treatment.

3.7.5. DETERMINATION OF SUPEROXIDE SCAVENGING ACTIVITY

The poly-herbal formulated tea were mixed with 0.2 ml of 0.1 m EDTA, 0.1 ml of 1.5 mM NBT in 1.5 mg of NaCN, 0.05 ml of riboflavin, and 2.64 ml of phosphate buffer. The control tube contained distilled water instead of plant extracts. Optical density was determined in a spectrophotometer at 560 nm.
The super-oxide anion scavenging activity was estimated as follows: Scavenging activity (%) = ABc −**MN**t **MN**c x 100,

where ABc represents absorbance of control and ABt represents absorbance of control. (uwaya *et al*., 2021)

3.7.6. DETERMINATION OF HYDROXYL RADICAL SCAVENGING ACTIVITY

Hydroxyl radical scavenging assay the hydroxyl radical scavenging activity of poly-herbal formulated tea was measured according to the method described by Ozyurek Bektasoglu, Guclu and Apak. The reacting mixture for the deoxyribose assay contained in a final volume of 1mL the following reagents: 0.1ml KH_2PO_4–KOH, 0.1mL deoxyribose, 0.1mL $FeCl_3$, 0.1mL EDTA, 0.1mL ascorbic acid, 0.1mL H_2O_2 and 0.1L sample. Reaction mixtures were incubated at 37 ◦C for 1h. At the end of the incubation period, 1mL of 1% (w/v) TBA was added to each mixture. The solutions were heated on a water bath at 95˚C for 20 min to develop the pink colour. The absorbance of the resulting solution was measured at 532 nm against a blank containing. Ascorbic acid used as reference standard.

The percentage inhibition and IC50 were calculated. The percentage inhibition of herbal extract was calculated using the following formula Percentage inhibition= A0 – A x 100 A0 Where A0 is the absorbance of control and A is the absorbance of test /standard. The IC50 value of the sample, which is the concentration of sample required to inhibit 50% of the hydroxyl free radical was calculated using linear regression analysis and used to indicate antioxidant capacity(Merlin and Jyoti, 2018)

3.8. STATISTICAL ANALYSIS

The data were presented as mean ± standard error of the mean (SEM), with ‘n indicating the number of mice in each experimental group. One-way analysis of variance (ANOVA) was conducted, followed by the Newman Keuls post hoc test. GraphPad Prism software version 6 from the UK was used for all data analysis. A significance indicated notable differences between the compared data.

CHAPTER FOUR

RESULTS

4.0 ANTIOXIDANT ASSAY

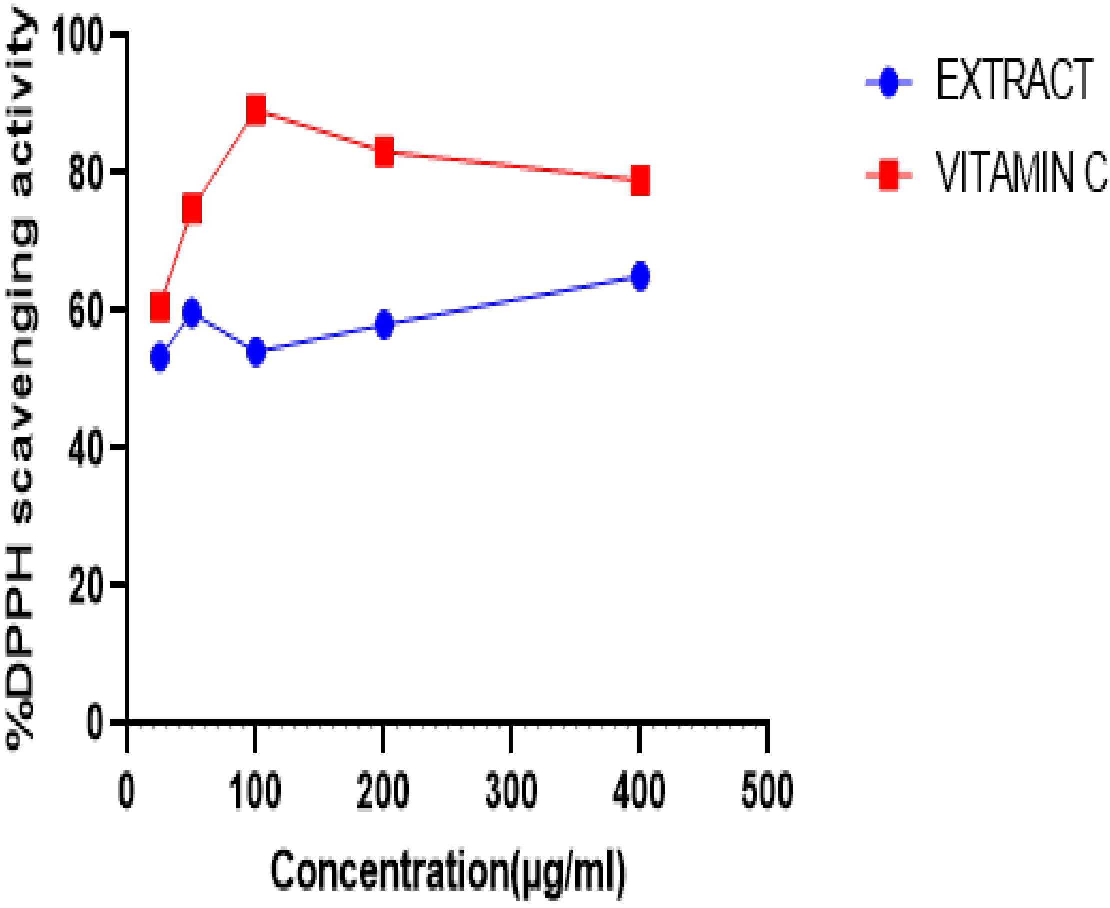

FIGURE 4.1: The 2,2-diphenyl-2-picrylhydrazyl hydrate scavenging activity of ascorbic acid and polyherbal formulated tea (*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum*).The scavenging activity of polyherbal formulated tea and ascorbic acid is comparable at 25µg/ml and 400µg/ml.

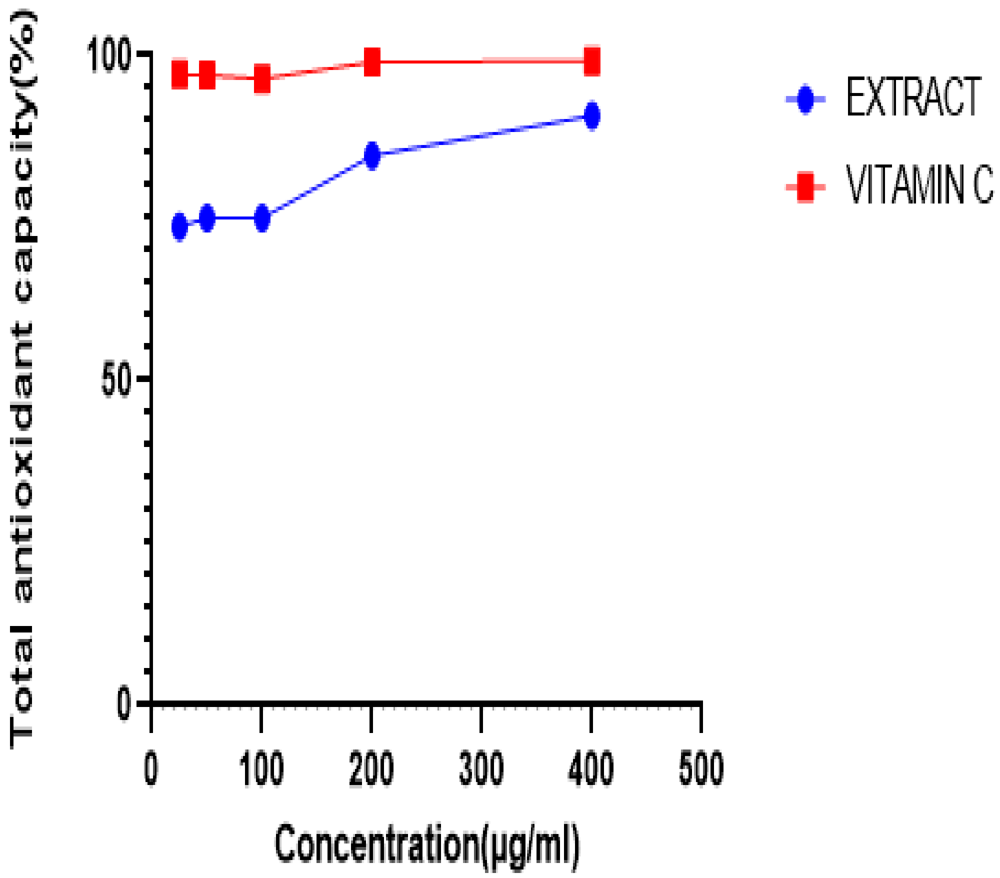

Figure 4.2 : The total antioxidant capacity of ascorbic acid and polyherbal formulated tea(*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum).*The total antioxidant capacity of polyherbal formulated tea and ascorbic acid is comparable at 400µg/ml.

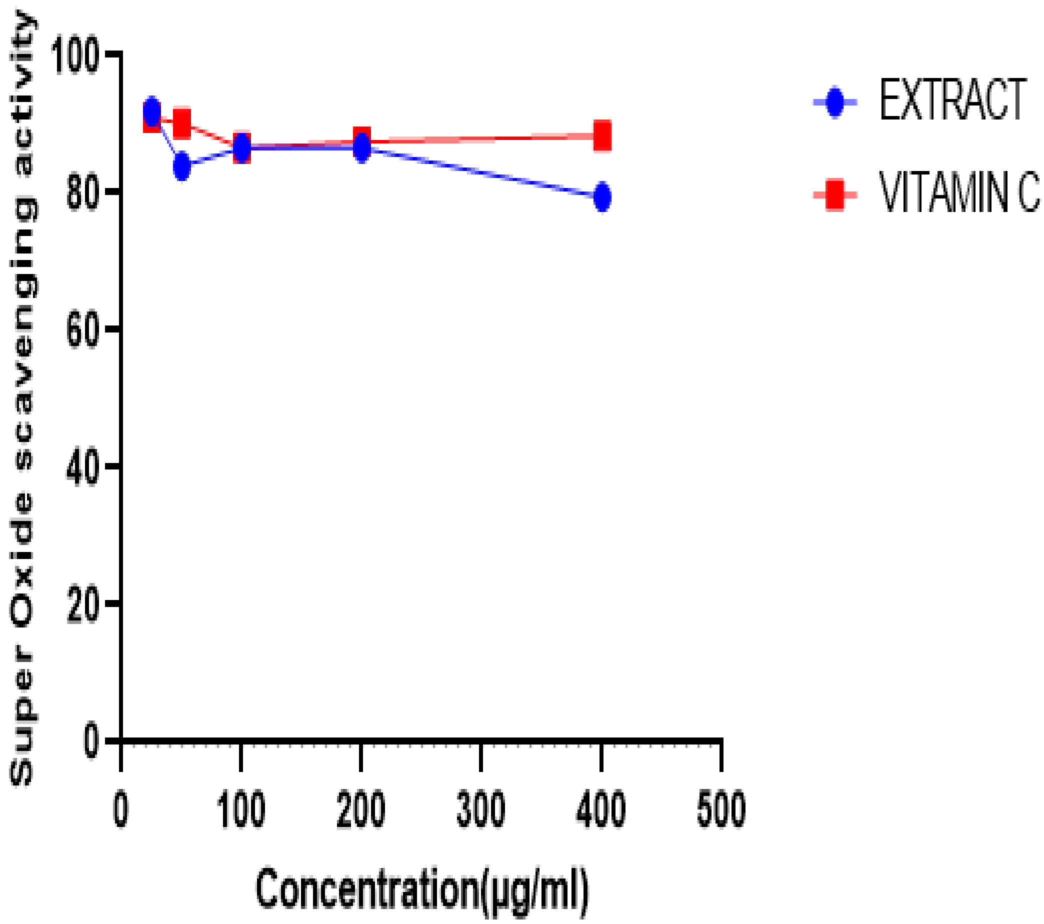

Figure 4.3 : The superoxide scavenging activity of ascorbic acid and polyherbal formulated tea (*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum). The scavenging activity of polyherbal formulated tea and ascorbic acid is comparable at 25*µg/ml, 100µg/ml and 200µg/ml.

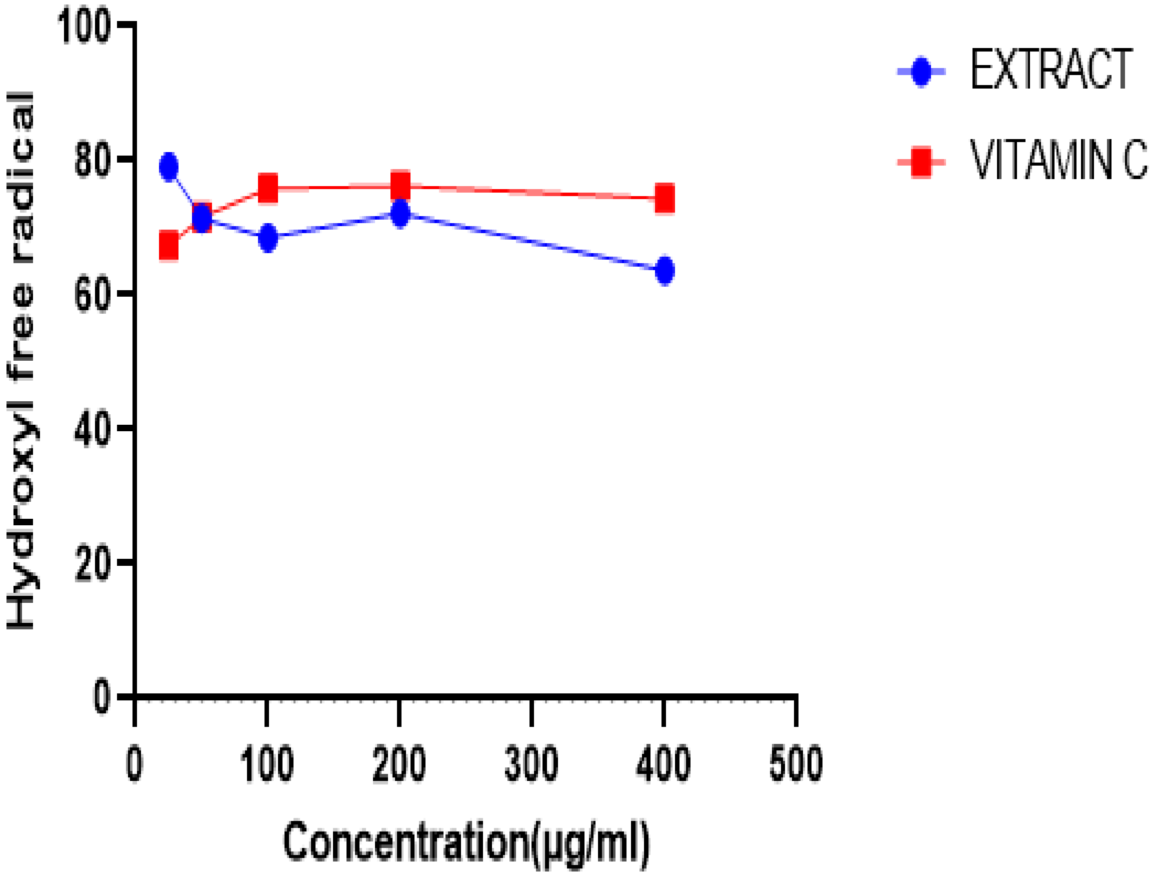

Figure 4.4 : The hydroxyl scavenging activity of ascorbic acid and polyherbal formulated tea (*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum). The scavenging activity of polyherbal formulated tea and ascorbic acid is comparable at 50*µg/ml, 100µg/ml and 200µg/ml.

ANTIDIRRHEAL

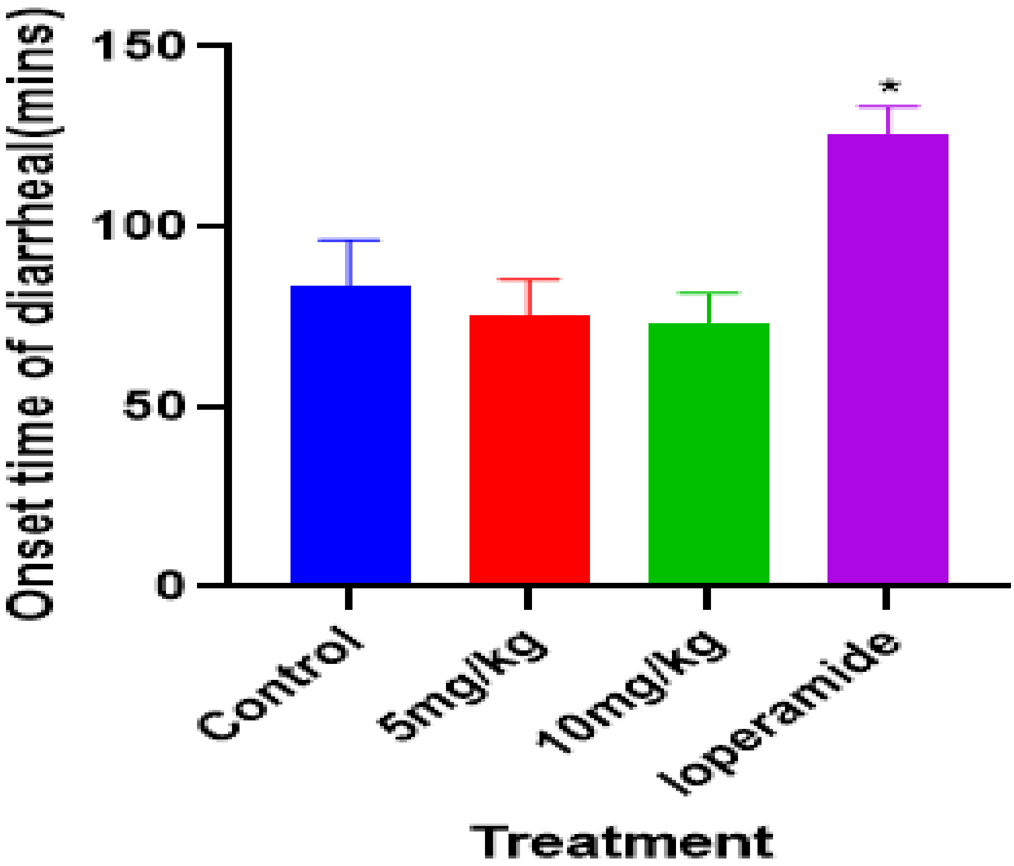

Figure 4.5: The effect of poly herbal formulated tea(*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum)* and loperamide on the onset time of diarrhea in albino mice with castor oil induced diarrhea. The polyherbal formulated tea has no effect on the onset time of diarrhea compared to control. However,loperamide shows a significant increase on the onset time of diarrhea compared to control(***p<0.05**) . Values are represented as; mean ± SEM n=4

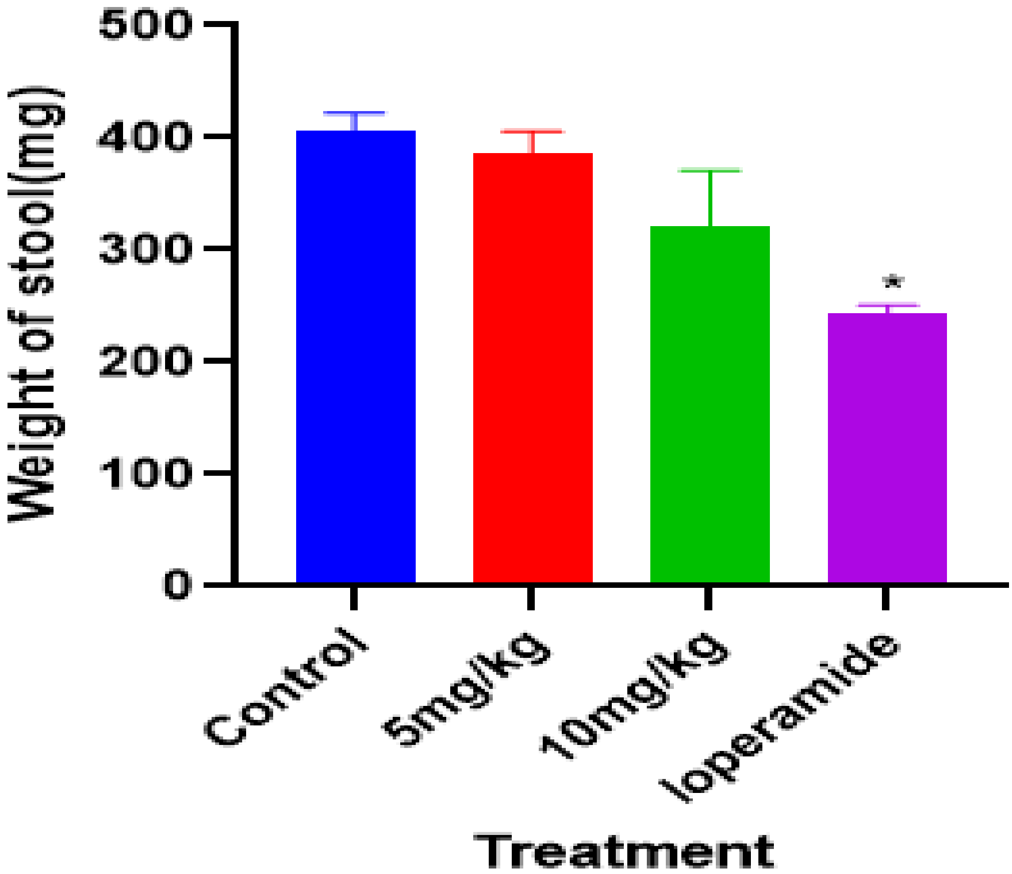

Figure 4.6 : The effect of poly herbal formulated tea(*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum)* and loperamide on the total weight of stool in albino mice in castor oil induced diarrhea.The polyherbal formulated tea has no effect on the total weight of stool compared to control. However,loperamide shows a significant decrease on the total weight of stool compared to control(*$p<0.05$) . Values are represented as; mean ± SEM n=4

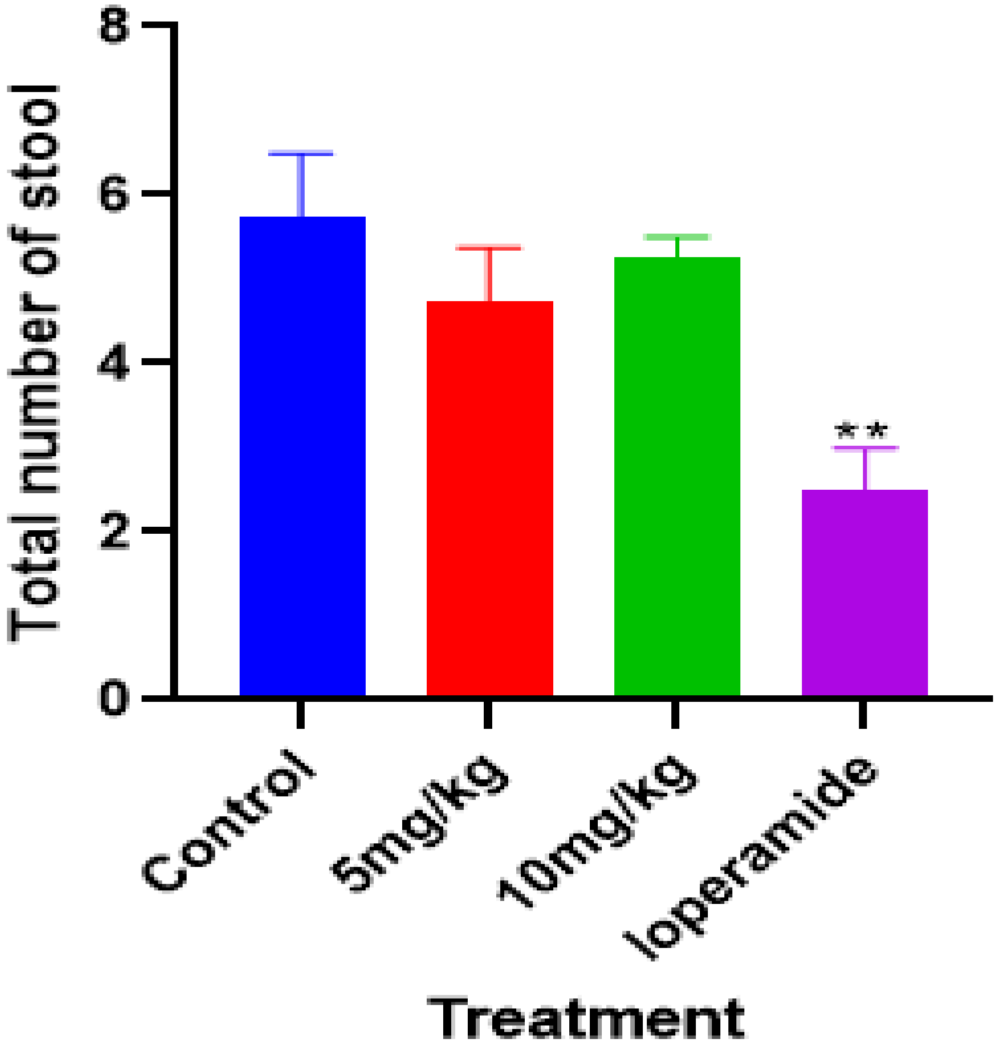

Figure 4.7: The effect of poly herbal formulated tea(*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum)* and loperamide on the total number of stool of albino mice in castor oil induced diarrhea. The poly-herbal formulated tea has no effect on the total number of stool compared to control. However,loperamide shows a significant decrease on the total number of stool compared to control(**$p<0.01$) . Values are represented as; mean ± SEM n=4.

Table 4.1: The effect of the poly herbal formulated tea(*Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera and Syzygium aromaticum)* and loperamide on intestinal transit time in albino mice.

Group	Treatment	Total Length of Intestine (Cm)	Distance Travelled by Charcoal	% Distance of Charcoal Travelled	% Inhibition Of Git Motility
Control	Castor Oil + Distilled Water (2ml/Kg P.O)	48.90 ± 0.8708	39.20 ±2.884	80.03 ±5.00	-
Standard	Loperamide (5mg/Kg P.O)	48.63 ± 2.322	23.95 ±1.144**	53.18 ±2.238**	33.54
5mg/Kg	Castor Oil + Plant Extract	53.13 ± 1.532	33.60 ±0.8124	60.90 ±1.489*	23.9
10mg/Kg	Castor Oil + Plant Extract	45.78 ± 2.011	36.73 ±3.221	79.20 ±4.666	1.03

The poly-herbal formulated tea showed significant reduction on the percentage distance travelled by the charcoal when compared to the control (*p<0.05) and also showed no statistical difference on the distance travelled by the charcoal when compared to the control, however in loperamide 5mg/kg, there was significant reduction in the distance travelled by charcoal(**p<0.01) and the percentage distance of charcoal travelled. therefore, when compared to control, (p**<0.01). Values are represented as mean ± SEM

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. DISCUSSION

Poly-herbal teas blend diverse plant materials, each selected for their unique medicinal properties, to craft a comprehensive beverage that fosters overall health. Furthermore, research has associated poly-herbal teas with various health advantages beyond antioxidant characteristics, including enhanced digestion, immune support, stress reduction, and improved cognitive function (Kumar *et al.,* 2018).

The findings of this study reveal the antioxidant scavenging activity of a poly-herbal formulated tea (comprising *Citrus limon*, *Curcuma Longa*, *Zingiber Officinale*, *Allium Sativum*, *Moringa oleifera*, and *Syzygium aromaticum*) compared to ascorbic acid (Vitamin C). The poly-herbal tea formulation effectively scavenges the 2, 2-diphenyl-2-picrylhydrazyl hydrate radical activity (Figure 2.1). Notably, the scavenging ability of the tea aligns with that of ascorbic acid. These findings are consistent with prior studies by (Bayan *et al.,* 2014) and (Shubhra *et al.*, 2014), which reported that plant-based antioxidants increase the concentration of DPPH free radical scavenging activity. The assay is based on measuring the scavenging activity of antioxidants towards the odd electron of the nitrogen atom in DPPH, resulting in the formation of the corresponding hydrazine (Contreras-Guzman and Strong, 1982).

Figure 4.1 illustrates that the poly-herbal tea, composed of *Citrus limon*, *Curcuma Longa*, *Zingiber Officinale*, *Allium Sativum*, *Moringa oleifera*, and *Syzygium aromaticum*, exhibits a robust total antioxidant capacity. This finding corroborates a previous study by (Uwaya *et al.,* 2021). The total antioxidant capacity increases with the concentration of the tea. An essential parameter for assessing the overall antioxidant potential of plant extracts is the total antioxidant capacity (TAC). TAC measures the substance's ability to neutralize free radicals and prevent oxidative damage to cells and tissues, serving as a crucial indicator of the overall antioxidant status of biological samples (Prior *et al.,* 2003). The rise in the total antioxidant capacity of the tea suggests its potential to scavenge free radicals and counteract oxidative stress. Various chronic diseases and aging processes involve oxidative damage, underscoring the significance of a higher TAC in offering potential protective effects against oxidative stress (Jones and Lee, 2021).A higher TAC indicates as a potential protective effect against oxidative stress (Jones and Lee, 2021).

Figure 4.2 illustrates the effectiveness of ascorbic acid and poly-herbal teas, comprising *Citrus limon*, *Curcuma Longa*, ginger, *Allium Sativum*, *Moringa oleifera*, and *Syzygium aromaticum*, in scavenging superoxide radicals. Both the poly-herbal-formulated tea and ascorbic acid demonstrate similar scavenging activity against superoxide radicals. This finding aligns with previous research studies (Jose and Kuttan, 1995).

Ascorbic acid and poly-herbal teas (*Citrus limon*, *Curcuma Longa*, *Zingiber Officinale*, *Allium Sativum*, *Moringa oleifera*, and *Syzygium aromaticum*) are effective in scavenging hydroxyl radicals. The scavenging activity of the poly-herbal-formulated tea and ascorbic acid is comparable at concentrations of 50µg/ml, 100µg/ml, and 200µg/ml. This indicates similar efficacy in scavenging hydroxyl free radicals. The hydroxyl scavenging assay evaluates a substance's capacity to neutralize or scavenge hydroxyl radicals, highly reactive and damaging free radicals produced during various biological processes, including oxidative stress. These radicals can inflict harm on cellular components such as lipids, proteins, and DNA, contributing to oxidative stress-related diseases and aging. The hydroxyl scavenging assay is essential for assessing the antioxidant potential of compounds, as it gauges their ability to neutralize highly reactive hydroxyl radicals, thereby mitigating oxidative stress (Halliwell, 1991).

In this anti diarrhea studies, the poly-herbal tea, does not have any effect on the onset time of diarrhea, weight of stool and number of stools in castor oil induced diarrhea (Figure 5 - 7). However, loperamide delayed the onset of diarrhea, reduced weight of stool and number of stool (Figure 4.4 – 4.7). The formulated tea, have no effect on percentage distance travel by charcoal and decreased percentage charcoal travel (Table 1). Loperamide at 5mg/kg decreased percentage distance travel by charcoal and percentage charcoal travel (Table 1). Poly-herbal-formulated tea at 5 mg/kg and loperamide 5mg/kg suppresses GIT motility by 23.9% and 33.54 respectively.

5.2 MECHANISM OF ACTION OF CASTOR OIL ON INDUCING DIARRHEA

Castor oil works as a laxative due to its high concentration of ricinoleic acid, which activates EP3 prostanoid receptors in the intestines, leading to increased smooth muscle contractions and fluid secretion into the intestines, ultimately stimulating bowel movements.(Soriano *et al*., 2013)

5.3 MECHANISM OF ACTION OF LOPERAMIDE ON TREATING DIARRHEA

Loperamide, an opioid receptor agonist, acts on the mu-opioid receptors in the gut, inhibiting peristalsis and decreasing intestinal motility. This results in increased transit time through the intestines, allowing for greater absorption of water and electrolytes, ultimately reducing diarrhea symptoms.(García-Martín *et al* ., 2022)

5.4 ROLE OF LOPERAMIDE RELATING TO INTESTINAL TIME

Loperamide functions as a mu-opioid receptor agonist within the gastrointestinal tract, extending the duration of intestinal transit. This action results in the deceleration of content movement through the intestines, facilitating enhanced absorption of water and electrolytes. Consequently, this process leads to the formation of more solid stools and a decrease in symptoms associated with diarrhea (Bueno *et al.,* 1992).

5.5 CONCLUSION

The findings of the research demonstrate that poly-herbal teas, comprising extracts from *Citrus limon, Curcuma Longa, Zingiber Officinale, Allium Sativum, Moringa oleifera, and Syzygium aromaticum,* exhibit antioxidant properties. While the formulated tea does not exhibit anti-diarrheal effects, it does show the capability to decrease gastrointestinal motility.

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