



INVITRO AND INVIVO ANTIOXIDANT AND ANTIDIARRHEA PROPERTIES OF POLYHERBAL TEA IN RODENT

Dickson O. Uwaya^{1*}; Toluwani AJAYI¹

*Email: dickson.uwaya@uniben.edu

ISSN: 3121-9837

www.ujbas.uniosun.edu.ng/ujbas

ujbas@uniosun.edu.ng

Authors Affiliation:

¹Department of Science
Laboratory Technology,
Faculty of Life Sciences,
University of Benin, Benin
City, Nigeria

History:

Volume 1, Number 5
Published: 10/04/2026

Keywords:

Polyherbal-tea, in-vitro, in-vivo, antioxidant, antidiarrheal

ABSTRACT

Various traditional medicinal practices have historically valued polyherbal formulations for their therapeutic potential. This study aims to evaluate the antioxidant and antidiarrheal properties of a polyherbal-formulated teas containing *Zingiber officinale*, *Ageratum conyzoides*, *Anthocleista djalonensis*, *Allium sativum*, and *Thespesia garckeana*. The in vitro antioxidant potential was assessed using the DPPH radical, superoxide radical activity, hydroxyl radical activity, and total antioxidant capacity methods. Antidiarrheal activity was evaluated using the castor oil-induced diarrhea and intestinal transit time model in mice. The aqueous extract of polyherbal tea scavenges DPPH radicals, superoxide radical activity, hydroxyl radical activity, and overall antioxidant capacity. The tea's scavenging ability was comparable to that of ascorbic acid. Compared to the control, the polyherbal-formulated tea showed no effect on the onset time of diarrhea, weight of feces, or number of feces ($p > 0.05$). Loperamide substantially extended the onset time of diarrhea and decreased the weight and number of stools compared to the control ($p < 0.05$) in castor oil-induced diarrhea. In transit time, aqueous extract polyherbal-formulated tea (10 and 20 mg/kg) has no influence on the distance traveled by charcoal or the proportion of charcoal trip compared to the control ($p > 0.05$). Loperamide at 5 mg/kg exhibits a statistical difference in distance traveled by charcoal and percentage of charcoal travel compared to control (** $p < 0.01$, * $p < 0.05$). The polyherbal-formulated tea displayed impressive in vitro antioxidant activity and had no antidiarrheal properties.

1. INTRODUCTION

In recent years, herbal remedies and traditional medicines have gained popularity due to their perceived effectiveness and minimal side effects compared to synthetic drugs (Karimi *et al.*, 2015; Kazemipoor *et al.*, 2012; Philomena, 2011). Polyherbal formulations, which blend multiple herbs, have become particularly popular in various traditional medicinal systems around the world. The synergistic effects of their bioactive ingredients lead to the health benefits of polyherbal teas, ((Adhami, and Mukhtar, 2006; Panahi *et al.*, 2015; Haniadka *et al.*, 2013; Jurgens *et al.*, 2021).

Zingiber officinale, or ginger, is a perennial herbaceous

plant from the Zingiberaceae family. The primary part used is the pale yellow, knobby rhizomes (Singh *et al.*, 2008). Originally from Southeast Asia, particularly India, China, and Indonesia, tropical and subtropical regions worldwide, including Africa, the Caribbean, and South America, now cultivate ginger. It thrives in warm, moist climates with well-drained soil (Ali *et al.*, 2008). Ginger has long been used to treat digestive issues like nausea and indigestion (Grzanna *et al.*, 2005). Its compounds, gingerol and shogaol, possess strong anti-inflammatory properties, helping with conditions like arthritis (Marx *et al.*, 2017). It is a



natural remedy for cold and flu symptoms, as well as menstrual pain, and may benefit cardiovascular health by lowering cholesterol and improving blood circulation (Allen *et al.*, 2010; Al-Amin *et al.*, 2006; Daily *et al.*, 2015).

Ageratum conyzoides, also known as whitehead beggarticks or tropical fleabane, is a perennial herb in the Asteraceae family (Ojewole 2006). Native to tropical climates, it is widespread globally, found in countries like Brazil, Nigeria, Malaysia, and India. It thrives in diverse environments, including waste areas, roadsides, open fields, and moist disturbed regions, aided by its environmental adaptability (Ojewole 2006). Extracts from *Ageratum conyzoides* demonstrate antimicrobial activity against bacteria, fungi, and viruses, making them a traditional treatment for wounds and infections. Various cultures use it to treat diarrhea and gastrointestinal diseases because of its astringent qualities that help regulate bowel motions. The plant acts as a febrifuge, with its leaves used in infusions or decoctions to reduce fever from infectious disorders. It serves as an anthelmintic agent to expel intestinal worms, often administered as teas or poultices.

Anthocleista djalonensis, known as "Djalono bitters," is a tall flowering plant from the family Gentianaceae, native to the forests of West Africa. For its therapeutic qualities, West African indigenous groups use the bark, leaves, and roots of *A. djalonensis*. West African indigenous groups use decoctions and infusions from the plant to treat fever, gastrointestinal problems, and malaria symptoms.

Extracts from its leaves and roots are valued for their analgesic, antibacterial, and anti-inflammatory properties. *Anthocleista djalonensis* is notable for its bioactive properties, containing phenolic chemicals, alkaloids, flavonoids, and terpenoids (Kone *et al.*, 2004). The plant's bark has yielded anthocleistone A, an alkaloid with antimalarial properties (Kone *et al.*, 2004). However, its habitat faces threats from degradation and deforestation, necessitating conservation efforts (Myers *et al.*, 2000).

Allium sativum, or garlic, is a perennial plant from the Amaryllidaceae family. It grows up to 0.6 meters, has flat, linear leaves, and produces spherical flowers. Its distinct odor and taste come from sulfur compounds like allicin, which are released when the cloves are crushed (Rabinowitch *et al.*, 1990). China is the largest producer of garlic, followed by South Korea, Egypt, India, and Russia (FAO, 2020). It thrives in well-drained, fertile soils and can grow wild in temperate regions. Traditional medicine across cultures has used garlic for centuries, citing its various health benefits. Due to its cardioprotective qualities, people use garlic to treat illnesses like high blood pressure, poor circulation, and high cholesterol (Ried *et al.*, 2013). Garlic's allicin provides broad-spectrum antibacterial properties (Ankri *et al.*, 1999). It may also boost immune function by enhancing cytokine production and immune cell activity (Nantsch *et al.*, 2012). Additionally, garlic's antioxidant compounds help reduce oxidative stress (Bayan *et al.*, 2014). Traditional uses of garlic include alleviating digestive issues and promoting the growth of beneficial gut bacteria (Gebhardt, 1997).



Thespesia garckeana, known as the African tulip tree or bastard rosewood, is a medium-sized deciduous tree from the Malvaceae family (Louppe et al., 2008). East Africa (Kenya, Tanzania, and Uganda) and West Africa (Nigeria to Cameroon) are home to *Thespesia garckeana*, a native of tropical and subtropical Africa. It also grows in the Indian Ocean Islands and Madagascar. The tree thrives in riverine forests, coastal forests, and woodland savannas, often near water sources (Beentje et al., 2005). Traditional medicine, across its range, uses various parts of *Thespesia garckeana*. East Africa uses the leaves and bark to treat fever, gastrointestinal issues, and malaria, and applies the bark to wounds and skin diseases due to its antibacterial properties (Kokwaro et al., 1976). In West Africa, especially Nigeria, leaf teas treat asthma, bronchitis, and cough, while bark decoctions address dysentery and diarrhea. Seed extracts are used as a laxative or purgative (Oladunmoye et al., 2011). This study aims to evaluate the antioxidant and antidiarrheal properties of polyherbal-formulated teas containing *Zingiber officinale*, *Ageratum conyzoides*, *Anthocleista djalensis*, *Allium sativum*, and *Thespesia garckeana*.

MATERIALS AND METHODS

Plant Collection

Allium sativum (garlic) and *Zingiber officinale* (ginger) were purchased from Kurmi Market within Kano Municipal Local Government Area, Kano State. *Thespesia garckeana* (Goron Tula) was

purchased at Tula village in the Kaltungo Local Government Area, Gombe State. *Anthocleista djalensis* and *Ageratum conyzoides* (goatweed) were gotten from Ikpoba-Okha Local Government Area in Edo State.

Preparation of Plant Materials/Polyherbal Tea Formulation

Allium sativum (garlic), *Zingiber officinale* (ginger), *Anthocleista djalensis*, and *Ageratum conyzoides* (goatweed) were washed with running water to remove dirt. *Allium sativum* (garlic) and *Zingiber officinale* (ginger) were chopped into smaller bits, and *Anthocleista djalensis* and *Ageratum conyzoides* (goatweed) were air dried. The chopped *Allium sativum* (garlic), *Zingiber officinale* (ginger), and *Thespesia garckeana* (Goron Tula) seeds removed from the fruit were dehydrated at 50°C. After dehydration, the dehydrated *Zingiber officinale* (ginger), *Allium sativum* (garlic), and *Thespesia garckeana* (Goron Tula) seed and the air-dried *Ageratum conyzoides* (goatweed) and *Anthocleista djalensis* were ground to powder separately using an industrial blender (KENWOOD Model: KCB239K).

Polyherbal Tea Formulation

The powders of *Allium sativum* (garlic), *Zingiber officinale* (ginger), *Thespesia garckeana* (Goron Tula), *Ageratum conyzoides* (goatweed), and *Anthocleista djalensis* were weighed and mixed in an equal proportion (1:1:0.3:1:1) 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 plants were identified and authenticated by Dr. H. Akinnibosun in the Department of Plant Biology and Biotechnology, Faculty of Life Science, Uniben Benin City, Edo State, Nigeria. The polyherbal-formulated tea was formulated using Uwaya and Efiog (2024) with slight modifications.

Polyherbal tea extraction

Formulated polyherbal tea (1,161 g) was weighed into an extracting jar, and 12.25 litres of distilled water were added. The mixture was stirred using a stirrer and allowed to stand for 72 hours. The solution was filtered using a strainer into a storage container with a lid. The filtrate was concentrated using a water bath to yield 40.82% of the extract. The extract was stored in an amber bottle and in a refrigerator at 4⁰ °C prior to use.

Experimental Animals

Healthy adult albino mice of either sex weighing 20–35 g were purchased from a 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, University of Benin, Benin City, Edo State. The animals were given two weeks of acclimatization under normal laboratory conditions with a 12-hour light/dark cycle. They were fed normal animal pellets and clean water, and the cages were cleaned regularly. 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).

Determination of Total Antioxidant Capacity, Superoxide Scavenging Activity, Hydroxyl Scavenging Activity, and 2, 2-Diphenyl-2-Picrylhydrazyl Hydrate Scavenging Activity.

Determination of 2-Diphenyl-2-Picrylhydrazyl Hydrate Scavenging Activity

With some changes, the method outlined by Tran *et al.* (2022) was used to examine the polyherbal-formulated tea's resistance to oxidation. 100 µL of DPPH (6x10⁻⁴ M) and 100 µL of extract (concentrations ranging from 0 to 10 mg/mL) made up the reaction mixture. The reaction mixture was left to incubate at 30 °C in the dark for 60 minutes. The absorbance of DPPH in the reaction solution was then measured at 517 nm. Ascorbic acid served as the treatment's positive control (Tran *et al.*, 2022).

Determination of Total Antioxidant Capacity

The polyherbal tea's antioxidant content was examined through the application of the phosphomolybdenum technique. The process began with the creation of a combination including a reagent solution (0.6 M sulfuric acid, 2 mM sodium phosphate, and 4 mM ammonium molybdate) and a test sample (100 µL) at various concentrations. After covering the mixture tightly, it was incubated for 90 minutes at 95 °C. The absorbance of the reaction solution was measured at 765 nm while it was kept cold at room temperature. Ascorbic acid was used to compare the treatment's outcomes. (Tran *et al.*, 2022).

Determination of Superoxide Scavenging Activity

The tea that was made using polyherbal infusions was combined with 2.64 ml of phosphate buffer, 0.05 ml of riboflavin, 0.1 ml of 1.5 mM NBT in 1.5 mg of NaCN, and 0.2 ml of 0.1 M EDTA. Instead of plant extracts, distilled water was used in the control tube. In a spectrophotometer, optical density was measured at 560 nm (Shinde *et al.*, 2006; Uwaya *et al.*, 2021).

Determination of Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging assay: Elizabeth and Rao (1990) method, modified by Shinde *et al.* (2006) and Uwaya *et al.* (2021) was used to study hydroxyl radical scavenging activity of tea made with polyherbals. A final volume of 1 mL was filled with the following reagents in the reaction mixture for the deoxyribose assay: 0.1 mL $\text{KH}_2\text{PO}_4\text{-KOH}$, 0.1 mL deoxyribose, 0.1 mL FeCl_3 , 0.1 mL EDTA, 0.1 mL ascorbic acid, 0.1 mL H_2O_2 , and a 0.1 mL sample. For one hour, reaction mixtures were incubated at 37 °C. Each combination received 1 mL of 1% (w/v) TBA at the conclusion of the incubation time. To get the pink colour, the solutions were heated for 20 minutes at 95 °C in a water bath. The resultant solution's absorbance was measured at 532 nm (Merlin and Jyoti, 2018).

Anti-Diarrhoea Experimental Design

Castor oil causes diarrhoea.

Sixteen (16) albino mice were allotted into 4 groups of 4 mice in each group and fasted overnight.

Group 1 received 0.5 ml/kg of distilled water.

Group 2 received a 10 mg/kg extract of polyherbal-formulated tea.

Group 3 received a 20 mg/kg extract of polyherbal-formulated tea.

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

Following an oral medicine regimen for 60 minutes, each group of animals received 0.3 millilitres of castor oil. For a maximum of four hours, the amount of watery faeces and the quantity of defecations were recorded in transparent metabolic cages, including filter paper at the bottom. The first stool's time and the amount of paper deposited both before and after were noted (Sharma *et al.*, 2010).

Intestinal Transit in Mice

Sixteen (16) albino mice were allotted into 4 groups of 4 mice in each group and fasted overnight.

Group 1 received 10 ml/kg of distilled water (the control group) orally.

Group 2 received a 10 mg/kg extract of polyherbal-formulated tea orally.

Group 3 received a 20 mg/kg extract of polyherbal-formulated tea orally.

Group 4 received 2 mg/kg of loperamide (standard) orally.

Following an hour, 10 ml/kg of charcoal meal (10% charcoal solution in regular saline) was administered orally to each animal. The animals were sacrificed via cervical dislocation twenty minutes later. As a percentage of the small intestine's overall length, the length and distance covered by the amount of charcoal meal in the gut from the pylorus to the caecum were measured (Uwaya *et al.*, 2021).

Statistical Analysis

The mean \pm standard error of the mean (SEM) was used to show the data, and the number of mice in each experimental group was indicated by the letter "n." A one-way analysis of variance (ANOVA) and the Newman-Keuls post hoc test were then performed. All data analysis was performed using the UK-sourced GraphPad Prism software version 6. Notable differences between the compared data were indicated by a significance level.

3. RESULT

Invitro Antioxidant Activity of Polyherbal Formulated Tea

Figure 1 illustrates the 2,2-diphenyl-2-picrylhydrazyl hydrate scavenging activity of ascorbic acid and polyherbal-formulated tea. The scavenging activity

of polyherbal-formulated tea and ascorbic acid is comparable at 400 $\mu\text{g/mL}$. Figure 2 demonstrates the overall antioxidant capacity of ascorbic acid and polyherbal-prepared tea. The total antioxidant capacity of polyherbal-formulated tea and ascorbic acid is comparable at 400 $\mu\text{g/mL}$. Figure 3 exhibits the superoxide scavenging activity of ascorbic acid and polyherbal-formulated tea. The scavenging activity of polyherbal-formulated tea and ascorbic acid is comparable at 100 $\mu\text{g/mL}$. Figure 4 exhibits the hydroxyl-scavenging action of ascorbic acid and polyherbal-formulated tea. The scavenging action of polyherbal-formulated tea and ascorbic acid is equivalent at 25 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 200 $\mu\text{g/ml}$, and 400 $\mu\text{g/ml}$.

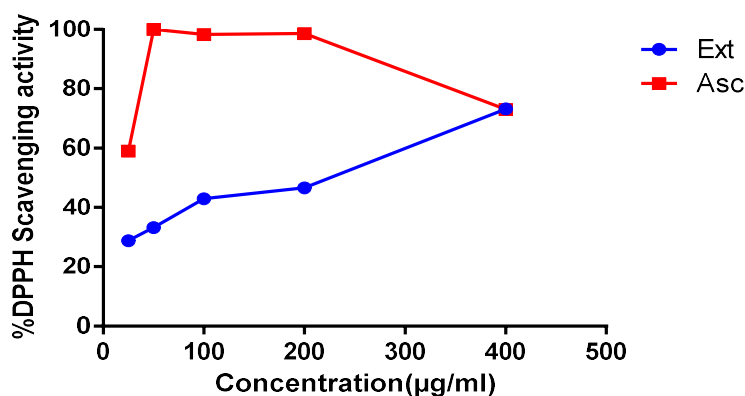


Figure 1: The 2, 2- diphenyl -2-picrylhydrazyl hydrate scavenging activity of ascorbic acid and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalensis*, *Allium sativum*, *Thespesia garckeana*). The scavenging activity of polyherbal formulated tea and ascorbic acid is comparable at 400 $\mu\text{g/ml}$.

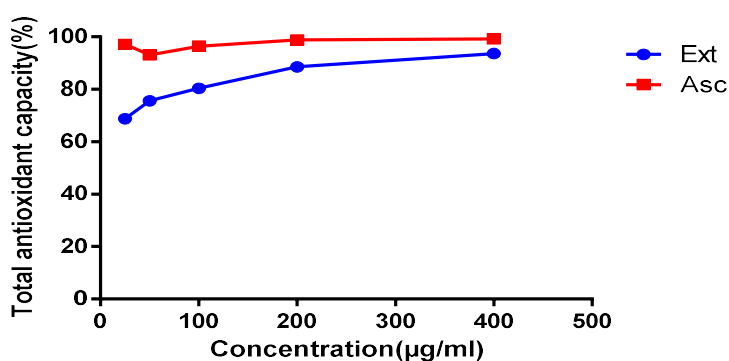


Figure 2: The total antioxidant capacity of ascorbic acid and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalensis*, *Allium sativum*, *Thespesia garckeana*).

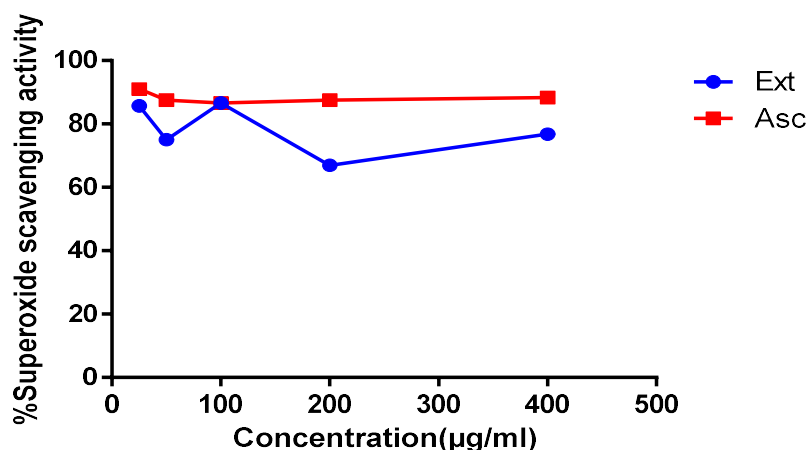


Figure 3: The superoxide scavenging activity of ascorbic acid and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalonensis*, *Allium sativum*, *Thespesia garckeana*).

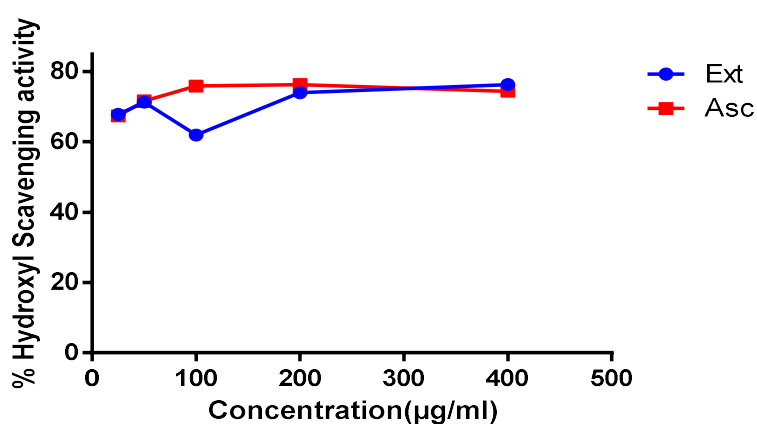


Figure 4: The hydroxyl scavenging activity of ascorbic acid and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalonensis*, *Allium sativum*, *Thespesia garckeana*).

Antidiarrhoea Activity of Polyherbal Formulated Tea

Castor Oil Induced Diarrhoea Activity of Polyherbal Formulated Tea

Figure 5 illustrates the effect of loperamide and polyherbal-formulated tea on the onset time of diarrhea in mice with castor oil-induced diarrhoea. Loperamide significantly increased the onset time of diarrhoea compared to the control ($p < 0.05$), while the polyherbal-formulated tea had no effect on the onset time of diarrhoea compared to the control ($p > 0.05$). Figure 6 shows how loperamide and polyherbal tea changed the amount of waste that mice with diarrhoea caused by castor oil threw up. Loperamide significantly decreased the weight of faeces compared to the control ($p < 0.05$), but the polyherbal-formulated tea had no effect on the weight of faeces compared to the control ($p > 0.05$). Figure 7 demonstrates the effect of loperamide and polyherbal-formulated tea on the total number of faeces in mice with castor oil-induced diarrhoea. Loperamide significantly lowered the quantity of faeces compared to the control ($p < 0.05$), but the polyherbal-formulated tea had no effect on the amount of faeces compared to the control ($p > 0.05$).

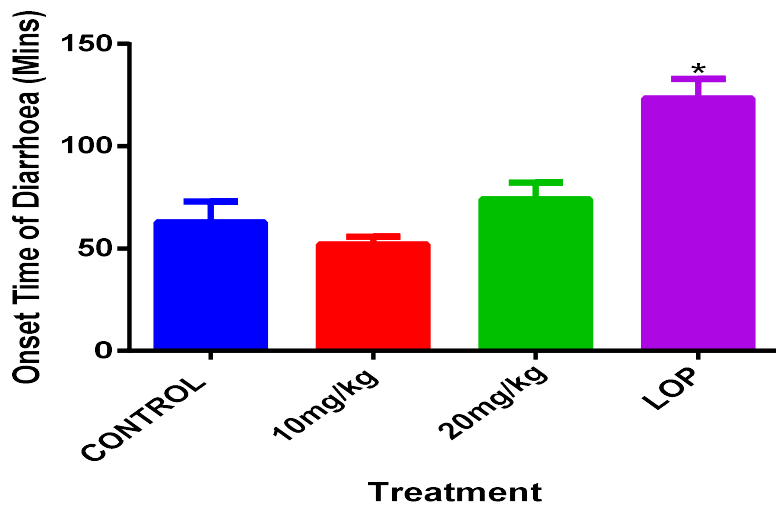


Figure 5: The effect of loperamide and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalonenensis*, *Allium sativum*, *Thespesia garckeana*) on the onset time of diarrhoea in mice in castor oil induced diarrhoea. Values are represented as mean \pm S.E.M, n=4.

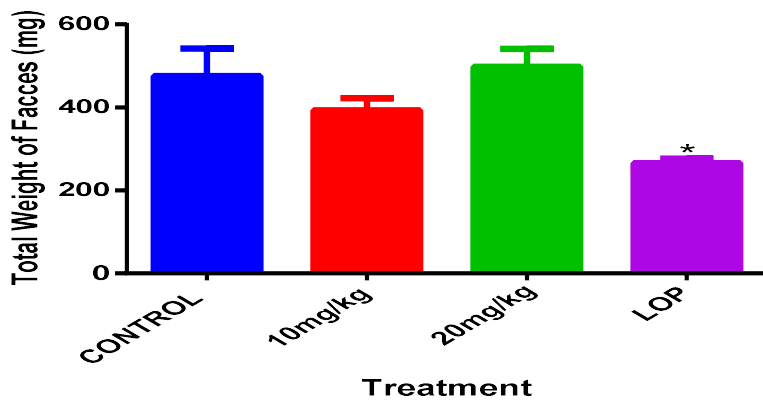


Figure 6: The effect of loperamide and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalonenensis*, *Allium sativum*, *Thespesia garckeana*) on the total weight of faeces in mice in castor oil induced diarrhoea. Values are represented as mean \pm S.E.M, n=4.

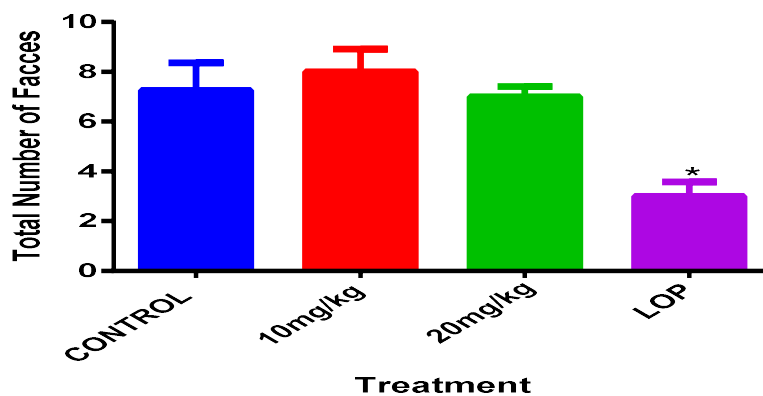


Figure 7: The effect of loperamide and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalonenensis*, *Allium sativum*, *Thespesia garckeana*) on the total number of faeces in mice in castor oil induced diarrhoea. Values are represented as mean \pm S.E.M, n=4.

Castor Oil Induced Intestinal Transit Time In Mice of Polyherbal Formulated Tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalonenensis*, *Allium sativum*, *Thespesia garckeana*)

Table 1 shows the effect of loperamide and polyherbal-formulated tea on castor oil-induced intestinal transit time in mice. Loperamide at 5 mg/kg shows statistical difference at distance traveled by charcoal and % of charcoal travel compared to control (**p<0.01, *p<0.05),

while the polyherbal-formulated tea has no statistical difference compared to control ($p > 0.05$).

Table 1: The effect of loperamide and polyherbal formulated tea (*Zingiber officinal*, *Ageratum conyzoides*, *Anthocleista djalonsensis*, *Allium sativum*, *Thespesia garckeana*) on castor oil induced intestinal transmit time in mice.

GROUPS	TREATMENT	Total Length of Intestine (cm)	Distance Traveled by Charcoal (cm)	% of Charcoal Travel	% Suppression on Git Motility
CONTROL	CASTOR OIL + DISTILLED WATER (2ml/kg p.o.)	46.70±2.82	38.23 ± 3.57	81.58 ± 4.34	-
STANDARD	LOPERAMIDE (5mg/kg p.o.)	46.56 ±1.73	26.97±2.52*	58.60±3.34**	28.17
10mg/kg	Castor oil + plant extract	51.53± 1.32	37.50 ± 2.40	72.79 ± 4.18	10.77
20mg/kg	Castor oil + plant extract	51.80± 2.17	38.87 ± 1.96	74.98 ± 1.47	8.09

Loperamide at 5mg/kg shows statistical difference at distant traveled by charcoal and % of charcoal travel (** $p < 0.01$, * $p < 0.05$), while the polyherbal formulated tea has no statistical difference compared to control ($p > 0.05$). Values are represented as mean \pm S.E.M., $n=4$.

4. DISCUSSION

Herbs used in combination or individually, comprised of various medicinal plants, offer diverse health benefits, including antioxidant activity, anti-inflammatory effects, digestive support, and metabolic enhancement (Khan *et al.*, 2019, Panahi *et al.*, 2015; Haniadkaet *al.*, 2013; Jurgens *et al.*, 2021). A report has shown the health benefits of medicinal plants rely on their phytochemical constituents, non-phytochemical constituents, and antioxidant activity (Chang, 2014). The *in vitro* antioxidant activity result of this investigation shows that the polyherbal tea suppresses the activity of 2,2-diphenyl-2-picrylhydrazyl hydrate radicals

(Figure 1). This outcome is consistent with earlier research (Baliyan *et al.*, 2022). At 400 μ g/mL, the tea prepared with many herbs is equivalent to ascorbic acid. The free radical DPPH is a quick, easy, and affordable way to test antioxidant capacities. It is frequently used to assess a compound's capacity to function as a hydrogen source and free-radical scavenger. The DPPH test depends on the stabilised free radical DPPH being eliminated. In fact, DPPH is a crystalline substance with a black colour that is made up of stable free radical particles. It's a well-known antioxidant test for radicals. The DPPH radical is dark purple in solution when it is reduced and changes into DPPHH, but it becomes colourless

or light yellow when it is reduced and changes into DPPH-H (Baliyan *et al.*, 2022).

Figure 2 reveals the polyherbal-formulated tea has a good total antioxidant capacity, which is comparable to ascorbic acid. This result agrees with a previous study (Uwaya *et al.*, 2021). A crucial parameter for evaluating the overall antioxidant potential of plant extracts is total anti-oxidative capacity (TAC), which represents the cumulative antioxidant activity of all compounds present in the extract. The high TAC of certain plant extracts has been associated with numerous health benefits. For instance, Rana *et al.* (2022) have linked polyphenol-rich extracts from fruits like berries and pomegranates to a reduced risk of chronic diseases such as cardiovascular disease and cancer. Additionally, TAC-rich herbal extracts have shown promising effects in alleviating symptoms of age-related cognitive decline and improving overall brain health (Uddin *et al.*, 2013). This assay measures the ability of polyherbal-formulated tea to scavenge free radicals and inhibit oxidative damage.

The polyherbal-formulated tea scavenges superoxide-free radicals and is comparable with ascorbic acid (Figure 3). This outcome agrees with previous studies (Hazra *et al.*, 2008). The ability of this polyherbal-formulated tea to scavenge superoxide radicals has important implications for human health. By mitigating oxidative stress and inflammation, superoxide scavenging may contribute to the prevention and management of various chronic

diseases, including cardiovascular disorders, neurodegenerative diseases, and cancer. Additionally, antioxidant activity is associated with the preservation of cellular integrity and function, thereby promoting overall well-being and longevity. (Valko *et al.*, 2007). The role of free radicals and antioxidants in normal physiological functions and human disease is well documented.

The tea scavenges hydroxyl free radicals and is comparable with that of ascorbic acid at 25µg/ml, 50µg/ml, 200µg/ml, and 400µg/ml (Figure 4). This comes in agreement with previous studies (Hazra *et al.*, 2008). Hydroxyl free radicals ($\bullet\text{OH}$) are highly reactive oxygen species implicated in oxidative damage to biomolecules and the onset of numerous diseases. Various cellular processes form them, which can initiate lipid peroxidation, DNA damage, and protein oxidation. The ability of this polyherbal-formulated tea to scavenge hydroxyl radicals holds significant implications for human health, like preventing and managing various diseases, including cardiovascular disorders, neurodegenerative diseases, and cancer (Sies, 2017).

On its antidiarrhoea effect, the polyherbal-formulated tea shows no effect on castor oil-induced diarrhea. Figure 5-7 reveals that the polyherbal-formulated tea has no effect on the onset time of diarrhoea, total weight of faeces, or total number of faeces, while the standard drug (loperamide) delayed the onset time of diarrhoea total, reduced weight of faeces, and number of. Castor oil consumption by rats sets off a chain

reaction that results in diarrhea. In this process, chykinins such as substance P (SP) and neurokinin A (NKA) play a crucial role. These neuropeptides control a number of biological processes, including secretion and gastrointestinal motility. The metabolization of ricinoleic acid found in castor oil causes the gastrointestinal system to release tachykinins, including NKA and SP. These tachykinins cause diarrhoea by binding to receptors such as NK2 and NK1. They increase secretion and motility within the gut, which decreases the absorption of water and electrolytes and causes watery stools. Furthermore, they may aggravate diarrhoea by inducing inflammation and harm to the intestines (Raul and Saul, 2002).

Likewise, on its effect on the induced intestinal transit time in mice, the polyherbal-formulated tea shows no statistical difference compared to the control (Table 1). For loperamide at 5 mg/kg, there was a greater reduction in the percentage travelled by charcoal and the distance travelled by charcoal. Loperamide has been seen to suppress GIT motility with 28.17%, and polyherbal-formulated tea at 10 mg/kg with 10.77% and at 20 mg/kg with 8.09%.

5. CONCLUSION

This study shows that the polyherbal-formulated tea demonstrated impressive in vitro antioxidant activity and no antidiarrhoea properties.

Acknowledgement

We deeply appreciate Mr. A. Barnabas, a member of the laboratory staff of the Department of Science Laboratory Technology, Faculty of Life Sciences, for his invaluable cooperation with this study.

Conflict of Interests

There are no conflicting interests between the authors and how the study is carried out.

Authors' Contributions

Dickson Uwaya participated in the manuscript's drafting, as well as the study's design and supervision. Both the experimentation and the manuscript's drafting were actively undertaken by Ajayi Toluwai. Before submitting the work, all authors reviewed it and gave their approval.

References

- Adhami, V.M & Mukhtar, H.(2006) Polyphenols from green tea and pomegranate for prevention of prostate cancer. *Free radical research*,40(10): 1095-1104.
- Al-Amin, Z. M, Thomson, M, Al-Qattan, K. K, Peltonen-Shalaby, R & Ali M. (2006) Anti-diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin-induced diabetic rats. *British Journal of Nutrition*,96(4): 660-666.
- Ali, B. H, Blunden, G, Tanira, M. O & Nemmar, A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research. *Food and Chemical Toxicology*. **46**(2): 409-420.
- Allen, S.J, Martinez, E.G, Gregorio, G.V & Dans, L. F.(2010). Probiotics for treating acute infectious diarrhoea. *Cochrane Database of Systematic Reviews*,**11**.
- Ankri, S & Mirelman, D.(1999) Antimicrobial properties of allicin from garlic. *Microbes and Infection*,1(2): 125-129.
- Baliyan, S, Mukherjee, R, Priyadarshini, A, Vibhuti, A, Gupta, A, Pandey, R.P & Chang, C.M.(2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules*, **27**: 1326.
- Barr, W & Smith, A.(2014). Acute diarrhoea. *Am Fam Physician*, 89(3): 180-9.



- Bayan, L, Koulivand, P.H & Gorji, A. (2014). Garlic: a review of potential therapeutic effects. *Avicenna Journal of Phytomedicine*, 4(1): 1-14.
- Beentje, H.J, Jeffrey, C & Hind, D.J.N. (2005). Flora of Tropical East Africa. Compositae, (Part 3).
- Canani, R.B, Cirillo, P, Terrin, G, Cesarano, L, Spagnuolo, M.I, De Vincenzo, A, Albano, F, Passariello, A, De Marco, G, Manguso, F & Guarino, A.(2007) Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *Biomedical journal*,335(7615): 340.
- Chang Sam, K. C.(2014) Antioxidant and Antiproliferative Properties of Extract and Fractions from Small Red Bean (*Phaseolus vulgaris* L.).*Journal of Food Nutrition*, 1: 1-11
- Daily, J.W, Zhang, X, Kim, D.S& Park S.(2015) Efficacy of ginger for alleviating the symptoms of primary dysmenorrhea: A systematic review and meta-analysis of randomized clinical trials. *Pain Medicine*,16(12): 2243-2255.
- Dauchet, L, Amouyel, P, Dallongeville, J & Ruidavets, J.B. (2006). Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *The Journal of nutrition*, 136(10): 2588-2593.
- Di Stefano, M, Miceli, E, Gotti, S, Missanelli, A, Mazzocchi, S & Corazza, G.R.(2007). The effect of oral alpha-galactosidase on intestinal gas production and gas-related symptoms. *Digestive diseases and sciences*,52(1):78-83.
- Elizabeth, K & Rao, M.N.A .(1990). Oxygen radical scavenging activity of curcumin. *International Journal of Pharmaceutics*. 58:237-240.
- Fletcher, S.M, Stark, D, Harkness, J & Ellis, J. (2012). Enteric protozoa in the developed world: a public health perspective. *Clinical microbiology reviews*, 25(3):420-449.
- Ford, A.C, Moayyedi, P, Chey, W.D, Harris, L.A, Lacy, B.E, Saito, Y.A & Quigley, E.M. (2018). American College of Gastroenterology monograph on management of irritable bowel syndrome. *Official journal of the American College of Gastroenterology| ACG*, 113:1-18.
- Ford, A.C, Talley, N.J, Spiegel, B.M, Foxx-Orenstein, A.E, Schiller, L, Quigley, E.M & Moayyedi, P.(2008). Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. *Biomedical journal*, 337.
- Food and Agriculture Organization of the United Nations. (2020). FAOSTAT. Retrieved 20 August 2019
- Gebhardt, R. (1997). Antioxidative and protective properties of extracts from leaves of the artichoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicology and applied pharmacology*,144(2): 279-286.
- Grzanna, R, Lindmark, L & Frondoza, C.G. (2005). Ginger—an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*, 8(2): 125-132.
- Guarino, A, Ashkenazi, S, Gendrel, D, Vecchio, A.L, Shamir, R & Szajewska, H. (2014). European Society for Pediatric Gastroenterology, Hepatology, and Nutrition/European Society for Pediatric Infectious Diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe: update (2014). *Journal of pediatric gastroenterology and nutrition*,59(1): 132-152.
- Guerrant, R.L, Hughes, J.M, Lima, N.L & Crane, J.(1990). Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. *Reviews of infectious diseases*,12(Supplement_1): S41-S50.
- Guerrant, R.L, Van Gilder, T, Steiner, T.S, Thielman, N.M, Slutsker, L, Tauxe, R.V, Hennessy, T, Griffin, P.M, DuPont, H, Bradley, S.R & Tarr, P.(2001). Practice guidelines for the management of infectious diarrhea. *Clinical infectious diseases*, 32(3): 331-351.
- Halliwell, B & Gutteridge, J. M.(2015). Free radicals in biology and medicine. Oxford university press, USA.
- Haniadka, R, Saldanha, E, Sunita, V, Palatty, P.L, Fayad, R & Baliga, M.S. (2013). A review of the gastroprotective effects of ginger (*Zingiber officinale* Roscoe). *Food and function*, 4(6): 845-855.

- Hazra, B, Biswas, S & Mandal, N. (2008). Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC complementary and Alternative Medicine*, 8: 1-10.
- Hill, D.R & Ryan, E.T.(2008). Management of travellers' diarrhoea. *Biomedical Journal*, 2008; 337.
- Kamboj A, & Saluja A.K, *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. *International Journal of Green Pharmacy (IJGP)*, 2(2).
- Kaur, G.J & Arora, D.S. (2009). Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*. *BMC complementary and alternative medicine*, 9: 1-10.
- Kazemipoor, M., Radzi, C. W., Cordell, G. & Yaze, I.(2012) Safety, efficacy and metabolism of traditional medicinal plants in the management of obesity: a review. *International Journal of Chemical Engineering and Applications* 3(4):288–92.
- Karimi, A., Majlesi, M. & Rafieian-Kopaei, M.(2015). Herbal versus synthetic drugs; beliefs and facts. *Journal of Nephro pharmacology* 4(1):27-30.
- Khan, N, Mukhtar, H & Afaq, F. (2019). Polyphenols from tea and pomegranate for prevention of prostate cancer. In F. Afaq (Ed.), *Polyphenols in Human Health and Disease*. Academic Press. pp. 143–162
- Kokwaro, J.O.(1976). *Medicinal Plants of East Africa*. East African Literature Bureau, Nairobi, Pp 292-294
- Kone, M. W, Atindehou, K. K, Terreaux, C, Hostettmann, K, Traore, D, Dosso, M & Kone, T. (2004). Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*. 93(1): 43-49
- Kuete, V, Dongfack, M.D, Mbaveng, A.T, Lallemand, MC, Van-Dufat, H.T, Wansi, J.D & Wandji, J.(2010). Antimicrobial activity of the methanolic extract and compounds from the stem bark of *Drypetestessmanniana*. *Chinese journal of integrative medicine*, 16: 337-343.
- Loftus, Jr. E.V.(2004). Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology*, 126(6): 1504-1517.
- Louppe, D, Oteng-Amoako, A. A & Brink, M. (2008). *Plant Resources of Tropical Africa (7)1 – Timbers 1* Dominique Louppe, Andrew Oteng-Amoako, Martin Brink, Eds. PROTA Foundation, Backhuys Publishers, Wageningen. *Afrika Focus*, 22(2):105-106
- Marx, W, Ried, K, McCarthy, A.L, Vitetta, L, Sali, A, McKavanagh, D, Isenring, L.(2017). Ginger—Mechanism of action in chemotherapy-induced nausea and vomiting: A review. *Critical Reviews in Food Science and Nutrition*, 57(1): 141-146.
- McKay, D.L & Blumberg, J.B.(2006). A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 20(8): 619-633.
- Merlin, M & Harindran, J.(2018). Antioxidant and free radical scavenging activity of *Peperomia pellucida* (L.) Kunth: an in vitro study. *World Journal of Pharmaceutical Research*, 7(17): 1218-1227.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*. 403(6772): 853-858.
- Ojewole, J.A.(2006). Antiinflammatory and analgesic effects of *Psidium guajava* Linn.(Myrtaceae) leaf aqueous extract in rats and mice. *Methods and findings in experimental and clinical pharmacology*, 28(7): 441-446.
- Oladunmoye, M.K. & Kehinde, F.Y.(2011). Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South Western Nigeria. *African Journal of Microbiology Research*, 5(19): 2991-3004.
- Panahi, Y., Hosseini, M.S., Khalili, N., Naimi, E., Majeed, M. & Sahebkar, A.(2015). Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: a randomized controlled trial and an updated meta-analysis. *Clinical nutrition*, 34(6): 1101-1108



- Patel, MM., Widdowson, M.A., Glass, R.I., Akazawa., K., Vinje, J. & Parashar, U.D.(2008). Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerging infectious diseases*,**14**(8): 1224.
- Pérez-Jiménez, J., Neveu, V., Vos, F& Scalbert, A.(2010). Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *European journal of clinical nutrition*, **64**(3): S112-S120.
- Philomena, G. (2011). Concerns regarding the safety and toxicity of medicinal plants - An overview. Journal of Applied Pharmaceutical Science, 1*(6):40–44.
- Rabinowitch, H. D & Brewster, J. L. (1990). Onions and Allied Crops: Volume 2: Botany, Physiology, and Genetics. CRC Press Inc. Boca Raton, FL, USA, 273 pp.
- Rana, A, Samtiya, M, Dhewa, T, Mistra, V & Aluko, R. (2022) Health Benefits of Polyphenols: A Concise Review. *Journal of Food Biochemistry*, 46, e14264.
- Raul, A. & Wapnir, S .T.(2002). Regulation mechanisms of intestinal secretion: implications in nutrient absorption. *The Journal of Nutritional Biochemistry*,13(4): 190-199
- Ried, K., Toben, C. & Fakler, P. (2013). Effect of garlic on serum lipids: an updated meta-analysis. *Nutrition reviews*, **71**(5): 282-299.
- Savino, F., Cresi, F., Castagno, E., Silvestro, L. & Oggero R. A.(2005). Randomized double-blind placebo-controlled trial of a standardized extract of *Matricariae recutita*, *Foeniculum vulgare* and *Melissa officinalis* (ColiMil®) in the treatment of breastfed colicky infants. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **19**(4): 335-340.
- Scalbert, A., Johnson, I.T. & Saltmarsh, M.(2005). Polyphenols: antioxidants and beyond. *The American journal of clinical nutrition*, 2005; **81**(1): 215S-217S.
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L. & Griffin, P.M.(2011). Foodborne illness acquired in the United States—major pathogens. *Emerging infectious diseases*,**17**(1): 7.
- Shahidi, F. & Zhong, Y.(2015). Measurement of antioxidant activity. *Journal of functional foods*,**18**: 757-781.
- Sharma, P., Vidyasagar, G., Singh, S., Ghule, S. &Kumar, B.(2010). Antidiarrhoeal activity of leaf extract of *Celosia Argentea* in experimentally induced diarrhoea in rats. *Journal of advanced pharmaceutical technology and research*,**1**(1): 41-48.
- Shinde, V, Kamlesh, D, Paradkar, A. R, Mahadik, K. R & Kadam, S.S. (2006). Evaluation of in-vitroantioxidant activity of human placental extract. *Pharmacologyonline*. 3: 172-179
- Sies, H. (1997) Oxidative stress: oxidants and antioxidants. *Experimental Physiology: Translation and Integration*,**82**(2): 291-295.
- Sies, H.(2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox biology*,**11**: 613-619.
- Singh, G., Kapoor, I.P.S., Singh, P., de Heluani, C.S., de Lampasona, M.P.&Catalan, C.A.(2008). Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *Food and chemical toxicology*, **46**(10): 3295-3302.
- Spencer, J.P.(2010). Beyond antioxidants: the cellular and molecular interactions of flavonoids and how these underpin their actions on the brain. *Proceedings of the Nutrition Society*,**69**(2): 244-260.
- Srivastava, J.K., Shankar, E. & Gupta, S.(2010). Chamomile: A herbal medicine of the past with a bright future. *Molecular medicine reports*,**3**(6): 895-901.
- Subcommittee, O.A.G.(1996). Practice parameter: the management of acute gastroenteritis in young children. *Pediatrics*,**97**(3): 424.
- Szajewska, H., Horvath, A. & Kołodziej, M. (2015). Systematic review with meta-analysis: *Saccharomyces boulardii* supplementation and eradication of *Helicobacter pylori* infection. *Alimentary pharmacology and therapeutics*, **41**(12): 1237-1245.
- Tran Men, T., Phien, H.H., Tu, L.T.K., Anh, N.T.K., Thu, N.T.A., Quy, T.N. & Nhu, N.T.B.(2022).



- Antioxidant and in vitro antidiabetic activities of *Peperomia pellucida* (L.) Kunth extract. *Veterinary Integrative Sciences*, **20**(3): 4
- Trinh, P.C., Thao, L.T.T., Ha, H.T.V. & Nguyen, T.(2020). DPPH-scavenging and antimicrobial activities of Asteraceae medicinal plants on uropathogenic bacteria. *Evidence-Based Complementary and Alternative Medicine*, 2020 May 14:2020:7807026. doi: 10.1155/2020/7807026
- Uddin, R., Kim, H.H., Lee, J H. & Park, S.U.(2013). Neuroprotective effects of medicinal plants. *Excli Journal*, 2:541-545
- Uwaya, O.D., Omozuwa, P.O. & Inegbedion, R.E.(2021). Evaluation of in-Vitro antioxidant and antidiarrheal activities of *Peperomia Pellucida* methanol extracts on Albino mice. *Journal of Applied Sciences and Environmental Management*, **25**(9): 1681-1688.
- Uwaya, D.O & Effiong, O. N (2024). Quantification of phytochemical constituent and non-enzymatic antioxidant of polyherbal-formulated tea on antitussive, expectorant, and analgesic activity in rodent. *Research in Biotechnology and Environmental Science*, **3**(1): 9-17.
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M. & Telser, J.(2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of biochemistry and cell biology*, **39**(1): 44-84.
- Van Tilburg, M.A., Palsson, O.S., Ringel, Y. & Whitehead, W.E.(2014). Is ginger effective for the treatment of irritable bowel syndrome? A double blind randomized controlled pilot trial. *Complementary therapies in medicine*, **22**(1): 17-20.
- Vitorino, M.D.R.(2021). Taxonomic implications of the genus *Ageratum* L. (Asteraceae: Eupatorieae) in Brazil based on nuclear ribosomal DNA and morphological data. *Plant Systematics and Evolution*, **307**(7): 1-16.
- World Health Organization. The treatment of diarrhoea: a manual for physicians and other senior health workers (No. WHO/FCH/CAH/05.1). World Health Organization, (2005).
- World Health Organization. Oral rehydration salts: Production of the new ORS (No. WHO/FCH/CAH/06.1). World Health Organization, 2006.

