Antidiarrheal Activity of Aqueous and 80% Methanol Extracts of The Leaves of *Leucas abyssinica* (Lamiaceae) in Mice.

Tadele Abera (B.pharm)

A Thesis Submitted to The Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences in Partial Fulfillment of The Requirements for The Degree of Master of Science in Pharmacology.

Addis Ababa University

Addis Ababa, Ethiopia

November, 2020

Antidiarrheal Activity of Aqueous and 80% Methanol Extracts of the Leaves of *Leucas abyssinica* (Lamiaceae) in Mice

Tadele Abera (B.pharm)

A Thesis Submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences in Partial Fulfillment of The Requirements for The Degree of Master of Science in Pharmacology

Addis Ababa University

Addis Ababa, Ethiopia

November, 2020

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Tadele Abera, entitled "Antidiarrheal activity of aqueous and 80% methanol extracts of the leaves of *Leucas abyssinica* (Lamiaceae) in mice" and submitted in partial fulfillment for the requirements of the Degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Name	**Signature**	**Date**
External Examiner: Dr. Tadesse Eguale	_____	_____
Internal Examiner: Dr. Shemsu Oumer	_____	_____
Advisor: Professor Teferra Abula	_____	_____

_____

Chair of Department or Graduate Program Coordinator

Abstract

Antidiarrheal Activity of Aqueous and 80% Methanol Extracts of the Leaves of *Leucas abyssinica* (Lamiaceae) in Mice

Tadele Abera

Addis Ababa University, 2020

*Leucas abyssinica (*Lamiaceae family*)* is one of the medicinal plants used for the management of diarrhea in Ethiopia but it lacks scientific evidence. Thus, this study was aimed to evaluate the antidiarrheal activities of aqueous and 80% methanol extracts of the leaves of *Leucas abyssinica* using experimental models in mice. castor oil induced diarrhea, misoprostol induced enteropooling, normal and castor oil induced gastrointestinal transit in mice models were used to evaluate antidiarrheal activity of the plant. Five groups of mice (six animals per group) were used for each model. These groups were negative control group (received distilled water 10 ml/kg), positive control group (administered loperamide 3 mg/kg) and three test groups (treated with graded dose of 100, 200 and 400 mg/kg aqueous and methanol 80% extracts respectively). Pretreatment of mice with stated doses of both extracts of the plant significantly reduced the frequency of wet and total feces, the weight of wet and total feces as well as increased diarrheal free periods compared to negative controls. Both extracts produced significant reduction in both the weight and volume of intestinal contents induced by misoprostol in a dose dependent manner. The percentage of inhibition of gastrointestinal transit induced by castor oil for both extracts were significant ($p < 0.001$) at all the tested doses in a dose dependent fashion. But none of the tested doses of both extracts significantly reduced normal gastrointestinal transit in mice. Finally, the results from this study suggests that both extracts of the plant have significant antidiarrheal effect on animal models and this finding supports the antidiarrheal traditional use of the plant for management of diarrhea.

Keywords: Antidiarrheal activity, castor oil induced diarrhea, gastrointestinal transit, mice, misoprostol induced enteropooling, *Leucas abyssinica* and 80% methanol

Acknowledgments

First and foremost, I would like to thank the almighty God along with the Saint Virgin Marry for protecting and providing me strength, patience and health.

Next, I would like to extend my sincere and heartfelt thanks to my advisor Professor Teferra Abula for his valuable comments, insightful suggestions, guidance, and supervision throughout my thesis work.

I wish also to express my appreciation to the Department of Pharmacology and Clinical Pharmacy for giving of the necessary laboratory facilities and the Department of Pharmacognosy for providing some laboratory materials.

I would also like to thank Mr. Molla Wale, Mrs. Fantu Assefa, Mrs. Etetu Mamo, and Mr. Eyasu Dukemo for their valuable technical assistance during my laboratory work.

Besides, I would like extend my heartfelt gratitude to my families for their priceless moral and financial support.

I would also thank all colleagues and friends for their enthusiastic assistance and encouragements.

Finally, I would like to thank Addis Ababa University for funding and Mizan-Tepi University for sponsoring me for the sponsorship.

Table of Contents

Abbreviations and Acronyms

ANOVA	Analysis of Variance
AQLA	Aqueous Extract of *L. abyssinica*
BAD	Bile Acid Induced Diarrhea
FDA	Food and Drug Administration
GIT	Gastrointestinal Tract
IBS	Inflammatory Bowel Syndrome
IVT	Intravenous Therapy
LCM	Length of Charcoal Meal
LSI	Length of Small Intestine
MELA	80% Methanol Extract of *L. abyssinica*
NSAIDs	Nonsteroidal Anti-inflammatory Drugs
OECD	Organization for Economic Cooperation Development
ORS	Oral Rehydration Salt
PG	Prostaglandins
PGE_2	Prostaglandins Type E two
SEM	Standard Error of The Mean
SIBO	Small Intestine Bacterial Overgrowth
UNICEF	United Nations Children's Fund
WGO	World Gastroenterology Organization
WHO	World Health Organization

List of Tables

List of Figure

1. Introduction

1.1 Overview and Classification of Diarrhea

Diarrhea is defined as having transit of watery feces at least three times in a twenty-four-hour period or more repeatedly than is the usual for that person (WHO and UNICEF). Fecal frequency, consistency or weight could be considered to explain the term diarrhea. Stool consistency is a common concept referred by most patients for diarrhea. Certainly, this fecal consistency is estimated by the water-retaining capacity of the stool. However, the consistency of stool is problematic to estimate with simple tests and the extent of looseness feces are fairly subjective (Arasaradnam *et al.,* 2018). Increased fecal frequency usually occurs in liquid stools and this property is regularly used in the definition of diarrhea. The frequency of stool relies greatly on diet and it differs significantly from individual to individual (Schiller, 2012). The daily stool weight more than 200 gram may indicate diarrhea (Navaneethan and Giannela, 2010). Diarrhea is also distinguished by increased gastrointestinal motility and secretion, and reduced electrolytes and fluid absorption frequently accompanied with increased propulsion of intestine (Mekonnen *et al.,* 2018).

Depending on the time course, diarrhea can be classified into three and these are acute, persistent, and chronic. Acute diarrhea is defined as the sudden onset of the passage of three or more loose stools in twenty-four hours of period and lasts not more than fourteen days (Riddle, 2018). There are two forms of acute diarrhea, acute watery diarrhea (which results in considerable loss of fluid and causes fast dehydration), and bloody diarrhea or dysentery (which is characterized by the presence of blood in stool and related with the injury of the intestine and waste of nutrients) (WHO and UNICEF). The time course of persistent diarrhea is between two and four weeks (Schiller *et al.,* 2014). while chronic diarrhea is defined as the passage of increased frequency of liquid stool that lasts for more than four weeks of duration and frequently linked with noninfectious cause (Fernández-Bañares *et al.,* 2016).

1.2 Epidemiology of Diarrhea

Diarrheal disease is the second top causes of childhood mortality and resulted an approximately 688 million morbidity and 499,000 mortality of children under-five years of age globally. And the Sub-Saharan Africa and South Asia share 90% of all death in 2015 (Kotloff *et al.,* 2017) Another

study revealed that globally, approximately 1.339 billion children under five years old and 79 thousand newborns aged 0–27 days were died as result of diarrhea (Kamath *et al.,* 2018).

In developing countries, diarrhea is a principal reason for morbidity and mortality of young children (Asrie *et al.,* 2016). In Africa, 19% of deaths of children younger than five years old was caused by diarrhea and it accounts for a quarter of pediatric death of children below five years old in Ghana (Akuffo *et al.,* 2017). Approximately, 75% of the total death among the children below five years old due to diarrhea occurred annually in fifteen countries (WHO and UNICEF). In this report, Ethiopia was fifth ranked in which 73,700 children of under-five aged death was due to diarrhea (Kamath *et al.,* 2018).

In East Africa the prevalence of childhood diarrhea was 13–32% (Asfaha *et al.,* 2018). And in Ethiopia the problem is higher than any other world, and the death rate of Ethiopian children by the age of the fifth birthday was thirty times more than death of children in Western Europe due to diarrhea (Anteneh *et al.,* 2017). The 2011 Ethiopian Demographic and Health Survey data showed that the magnitude of childhood diarrhea was 13% and it was a major public health problem that accounts for morbidity and mortality in children (Demographic E. Health survey, 2011).

1.3 Etiology, Transmission Routes and Prevention of Diarrhea

Diarrhea is often a consequence of gastrointestinal infection and a range of bacteria, virus, and parasitic organisms can cause it (Bahekar and Kale, 2015). *Escherichia coli, Shigella flexneri, Staphylococcus aureus and Salmonella typhi* are the main pathogens of diarrhea, and *Candida albicans* also causes diarrhea in humans (Umer *et al.,* 2013). Enteric microorganisms such as viruses, bacteria and parasites largely result acute diarrhea while inflammatory bowel disease and malabsorption disorder commonly cause in chronic diarrhea. The responsible causative agents for persistent diarrhea are *Giardia lamblia*, *Cryptosporidium* and enteropathogenic bacteria intestine (Shrivastava *et al.,* 2017). In developing nations, rotaviruses and *Escherichia coli* are the two recognized causative agents that result in moderate to severe diarrhea (Kareem *et al.,* 2016).

The major transmission route for such organisms is feces-contaminated with drinking-water and food, and the infection is higher in the area where there is less access to clean drinking-water, cooking and cleaning, and poor hygiene and sanitation (Anteneh *et al.,* 2017). The crucial

interventions to prevent diarrhea are proper hand-washing with soap, providing clean drinking-water, using improved sanitation, proper individual and food cleanliness, education of parents how infections are transmitted, and exclusive breastfeeding for at least six months (Cairncross *et al.,* 2010; Hart and Umar, 2000). Using probiotics such as lactobacillus GG and Saccharomyces boulardii decreases the course and reduces stool frequency in acute diarrheal infections (Guarino *et al*., 2015).

The antimicrobial prophylaxis for travelers should be not be used routinely in travelers but it should be considered for travelers at high risk of health-associated complications of travelers' diarrhea. Once antibiotic prophylaxis is appropriate, rifaximin is recommended but the use of fluoroquinolones is not suggested. Bismuth subsalicylate may be suggested for any traveler to prevent travelers diarrhea (Riddle *et al.,* 2017).

1.4 Normal Physiology of Intestine

The ion transport through the plasma membrane is essential for the normal function of the cell. The absorption and secretion of electrolytes across diverse arrangements of the ion transporters are regulated by epithelial cells of the intestine, which function in concert to sustain the equilibrium of fluid in the body. During the diarrheal disease this fluid equilibrium is compromised and the function of transporters are altered by infections and inflammatory disorders, or might be triggered by mutations of gene (Das *et al.,* 2018).

The main purpose of the small intestine is to absorb the nutrients and there is much known variance among the proximal and distal intestine in the absorption of nutrients. The large intestine also has a part in maintaining the fluid and electrolytes and there are segments differences in the transporting electrolytes. The sodium absorption in the cecum is via cation channels, in proximal colon its absorption coupled with sodium chloride and the distal bowel and rectum possess an amiloride-sensitive sodium absorption. These differences might have subtle consequences and in diarrhea, the sodium absorption is reduced and active chloride secretion is enhanced (Sellin, 2001).

1.4.1 Physiology of Intestinal Absorption and Secretion

The absorptive and secretory function of the intestine is differentiated by intestinal tracts with the absorption mainly from superficial cells and the active secretion mostly from the crypt cells. The numerous proteins situated on the brush border membranes of the small and large intestine are

accountable mechanisms for solute transport across gastrointestinal tract (GIT) (Kiela and Ghishan, 2016). In the usual state, the daily input of the small intestine is nearly about 9 liters of which 1.8 liters is from the oral ingestion. Most of this is reabsorbed in the small intestine and 1.5 to 2 liters usually reaches the large intestine, and in healthy individuals majority of this is reabsorbed (Kelly *et al.,* 2018).

The jejunum is the most permeable portion of the small intestine and fast changes occur in luminal osmolality when food is digested and nutrients are absorbed. The sodium-coupled cotransport of organic substrates such as glucose, galactose, amino acids, and tripeptides are the important absorptive mechanism in the jejunum. The permeability of the ileum to water is minor, although the absorption of sodium and organic substrates are similar as in the jejunum. But ion absorptive mechanisms like the sodium chloride transport is further important in ileum (Whyte and Jenkins, 2012).

1.5 Pathophysiology of Diarrhea

Diarrhea results when the delicate balance between the absorptive and secretory functions inside the intestine is impaired (Kelly *et al.,* 2018). The major mechanisms that are accountable for pathophysiology of diarrhea are raised luminal osmolality, enhanced electrolyte secretion and reduced electrolyte absorption and accelerated intestinal motility (Bahekar and Kale, 2015).

For better understanding of the pathophysiology of diarrhea, diarrheal disorders are categorized as secretory, osmotic or malabsorption induced, inflammatory and functional diarrhea. The etiologies of diarrhea will have a complex pathophysiology including one or more of the following mechanisms.

1.5.1 Secretory Diarrhea

Secretory diarrhea results when the intestinal mucosa excessively secretes fluid because of the irritation of the enterocytes by a toxin (such as cholera toxin), inherent abnormalities in the enterocytes or when other inflammatory processes take place in the intestine (Kelly *et al.,* 2018). This diarrhea shows increased volume of stools which continue despite the lack of oral ingestion. Usually small intestine is the primary site for over secretion of fluid and electrolyte, and the function of colon for water and ions absorption is surmounted by the process (Deepak and Ehrenpreis, 2011).

Diarrhea ensuing from overstimulation of intestine's secretory capacity may develop to pure form (e.g. cholera) or as a component of a more complex ailment process (e.g. Celiac and Crohn disease). Large volumes of stool (which is more than one liter per hour in typical hydrated individuals), disappearance of red or white blood cells, persistence of diarrhea despite fasting and lack of large gap in stool electrolytes are the signs of secretory diarrhea. Bacterial enterotoxins, hormones produced by endocrine tumor, dihydroxy bile acids, hydroxylated fatty acids, and inflammatory mediators are some of the stimuli for secretory diarrhea (Field, 2003).

1.5.2 Osmotic Diarrhea

Osmotic diarrhea results when a poorly absorbed substance with osmotic activity is consumed. Examples of such substances include magnesium, phosphate or sulfate-containing compounds, poorly absorbed carbohydrates and polymers, such as polyethylene glycol, which can exert abnormal osmotic activity (Schiller, 2012).

When large numbers of osmotic substance are existing in the lumen, more liquid passively passes into the bowel lumen down the osmotic gradient. The consequence is the solute load beyond the absorptive ability of the intestine and hence diarrhea happens. Therefore, osmotic diarrhea stops when the patient is fasting (Kelly *et al.,* 2018).

Sugar alcohols such as mannitol or sorbitol are nonabsorbable solutes whereas magnesium, sulfates and phosphates are poorly absorbable solutes. The osmotic activity of unabsorbed solutes in the intestine causes in driving water and electrolytes in to the bowel lumen hence results in diarrhea. Osmotic diarrhea may occur in patients with malabsorption and these mal-absorbed substances acting as poorly absorbed solutes (Navaneethan and Giannela, 2010).

Antidiabetic drugs like acarbose and miglitol which prevent carbohydrates metabolism by inhibiting alpha-glucosidases, causes 30% of diarrhea in patients. Psyllium and bran are bulk laxatives, synthetic polymers like polycarbophil, and methylcellulose stimulate fluid retention and might enhance intraluminal volume by rapid stool transit time via the bowel. When soluble fibers are fermented by bacteria in the colon, short chain fatty acid are produced. Which in turn enhance osmolarity in the lumen of the intestine and may result laxative outcome (Deepak and Ehrenpreis, 2011).

Most case of antibiotic-associated diarrhea are because of impaired metabolism of carbohydrate or bile acid. Broad spectrum antibiotics, poorly absorbed antibiotics and parenteral antibiotics with enterohepatic circulation are more likely causes of diarrhea. Augmentin, cefixime, ampicillin, cephalosporin and clindamycin have higher chance of causing diarrhea (Bartlett, 2002). Carbohydrate related diarrhea is component of antibiotic-associated diarrhea due to reduced normal gut flora. These altered flora causes impaired metabolism poorly absorbed carbohydrate; hence osmotic diarrhea will result (Deepak and Ehrenpreis, 2011).

1.5.3 Motility Related Diarrhea

Motility related diarrhea results from motility disorder triggered by increasing intestinal transit in post-vagotomy diarrhea or by delaying transit, which makes small intestine prone to small intestine bacterial overgrowth. This diarrhea could be either secretory or osmotic (Camilleri *et al.,* 2017). Irritable bowel syndrome with diarrhea is the most predominant forms of motility associated diarrhea. Accelerated colonic transit or increased number of high amplitude propagated contractions are the features of irritable bowel syndrome with diarrhea (Field, 2003).

In disorders like coeliac disease, inflammatory bowel syndrome and carcinoid syndrome, excess serotonin might contribute for diarrhea. Intestinal secretions and peristalsis induced by serotonin that act on serotonin subtype receptor three and four have significance in inflammatory bowel syndrome and carcinoid syndrome. This is further supported by serotonin subtype receptor three antagonists efficacy in reducing intestinal transit and symptoms of diarrhea (Spiller, 2007).

1.5.4 Inflammatory Diarrhea

The digestive tract of epithelium is defended by several mechanisms constituting barrier of GIT. The disruption GIT epithelium is commonly related with extensive destruction of absorptive epithelium that results in the discharge of serum and blood to the lumen of intestine. In this situation, absorption of water becomes impaired and hence diarrhea occurs (Philip *et al.,* 2017).

Epithelial inflammatory signaling pathway induction such as nuclear factor of kappa B leads in calcium/cyclic nucleotide signaling and activation of chloride secretion or blocking sodium absorption. The discharged inflammatory mediators like tumor necrosis factor and interleukin six from stimulated T cells and neutrophils, leads to mucosa mast cells degranulation and discharges prostaglandins and histamine, which further activate chloride secretion. Inflammation possibly

influences absorption of sodium by downregulating sodium hydrogen exchanger (NHE1) and (NHE3) in the irritable bowel syndrome with diarrhea. Inflammatory diarrhea mostly observed in bacterial pathogens like Clostridium difficile, which also stimulate epithelial inflammatory signaling pathways and recruit immune cells (Thiagarajah *et al.,* 2015).

Moreover, parasympathetic nervous system stimulation, mechanical distension, and antigen presentation consequences the discharge of inflammatory mediators. The released inflammatory mediators like histamine, serotonin, prostaglandins, platelet-activating factor, adenosine, reactive oxygen metabolites and endothelin result the enhanced secretion of water and electrolytes (Sellin, 2001).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to cause drug induced diarrhea by irritating epithelial mucosa, immunosuppressive and chemotherapeutic agents cause disrupt and compromise epithelial integrity, and ergotamine and cocaine causes vascular compromise (Philip *et al.,* 2017). The presence of blood or pus in stool with systemic symptoms (recurring fever, weight loss) or additional manifestations of intestinal inflammation with increased acute phase reactants (which involve C-reactive protein, erythrocyte sedimentation rate and platelet count) or stool calproctin more than 150 mg/kg of two samples obtained not the at the same time in patients who are not receiving NSAIDs are some of signs of inflammatory diarrhea (Fernández-Bañares *et al.,* 2016).

1.5.5 Functional Diarrhea

The Rome criteria sets a standard for the diagnosis and classification of functional gastrointestinal disorders (Drossman and Dumitrascu, 2006). According to Rome III criteria, functional diarrhea is defined as more than 75% bowel movement (recurrent mushy stools) occurred prior to three months in absence of pain and the onset of symptoms is more than six months. Microscopic colitis, carbohydrate impaired absorption or colorectal diarrhea may trigger functional diarrhea (Fernández-Bañares *et al.,* 2016; Tack 2012).

1.6 Management of Diarrhea

The treatment of diarrhea includes both nonpharmacological and pharmacological interventions (Bahekar and Kale, 2015).

1.6.1 Nonpharmacologic Management of Diarrhea

1.6.1.1 Dietary Modifications

The progression of certain gastrointestinal state can be modified by adjusting diet. For example, in patients with lactose intolerance or celiac disease, avoiding lactose or gluten-rich diet respectively has advantage. Also, in extensive small bowel injury, preventing lactose is recommended to reduce transport of bulky lactose to the large intestine and successive retention of water (Li and Vaziri, 2012).

Food items such as fructose, sorbitol, lactose, caffeine, and another diarrhea aggravating diets should be avoided. Besides, alcohol intake should be reduced, which might precipitate diarrhea in vulnerable individuals, and restrictions of fiber containing diet are advised (Tack, 2012).

1.6.2 Supportive Treatments for Diarrhea

1.6.2.1 Fluid and Electrolyte Substitution

Fluid and electrolyte substitution are mainstay therapy in diarrhea and its substitution by oral route are enough if the patient is not vomiting or the losses are not very severe. Early administration of oral rehydration solution (ORS) is suggested for infants and children because they are prone to dehydration. Intravenous fluid therapy is required only when the infants and young children are severely dehydrated. Fluid replacement can correct acidosis that can happen due to severe dehydration and specific bicarbonate replacement are not needed (Farthing and Casburn-Jones, 2004).

In the case of severe dehydration, shock/altered mental state and not responding to ORS, isotonic intravenous fluids like lactated Ringer's and normal saline solution should be given. In ketonemic patients, intravenous hydration might be required due to the tolerance to oral rehydration. Intravenous rehydration should be maintained until the patient has no aspiration risk, no indication of ileus and the patient's pulse, perfusion and mental condition is corrected (Shane *et al.,* 2017).

1.6.2.2 Oral Rehydration Therapy

Oral hydration therapy is giving proper solutions orally to avoid dehydration due to diarrhea. Its major benefit is cost-effective and minimize hospitalization in treating gastroenteritis in both developing and developed countries (Farthing *et al.,* 2012). ORS is suggested regardless of age

and type of diarrhea involving cholera. It contains rehydration water and electrolytes given to substitute the losses. Currently World Health Organization (WHO) and United Nations Children's Fund (UNICEF) recommends hypo-osmolar (245 milliosmoles per liter) to promote water absorption (Thaigarajah *et al.,* 2015).

Oral hydration therapy includes glucose containing fluids because glucose enhances the intestinal electrolytes absorption. Sodium glucose linked transporter one cotransport sodium and glucose, followed by water. The standard ORS does not decrease stool weight and periods of disease; conversely, it increases fecal fluid loss. However, the use of hypo-osmolar ORS decreases the fecal output by nearly 40% in children with persistent diarrhea. In adolescents and adults, the incorporation of unabsorbable starch in ORS decrease stool fluid loss and disease period (Kent and Banks, 2010).

The use of ORS for early treatment of severe dehydration, children with paralytic ileus, recurrent vomiting, and painful oral circumstances like moderate to severe thrushes (oral candidiasis) are contraindicated (Farthing *et al.,* 2012).

1.6.2.3 Zinc Supplement

The shortage of zinc is prevalent in the children of low socioeconomic countries. Zinc supplementation decreases diarrheal incidence and nonaccidental deaths to 50 %. In treatment of persistent diarrhea and undernourished children, zinc supplementation is vital. In developing countries, as the combination therapy of zinc and ORS has modest decrease in severity but it highly decreases diarrheal episodes. The mechanism for this effect is not clear but it blocks chloride secretion by inhibiting potassium channels and activating sodium hydrogen exchanger three in vitro. The recommended dose of zinc for children with diarrhea is 20 mg and 10 mg for infants and children less than 2 months age (Farthing *et al.,* 2012; Thiagarajah., 2015).

1.6.3 Pharmacological Agents Used in the Treatment of Diarrhea

Antibiotics

Febrile patients with moderate to severe diarrhea, travelers' diarrhea, persistent diarrhea, suspected small intestine bacterial overgrowth and cases related to parasites or more serious bacterial infections frequently requires antibiotic treatment. Also, in chemotherapy induced diarrhea, the antibiotic therapy is recommended only when the diarrhea lasts for longer than one day (Lee,

2015). The safety and efficacy of antimicrobials are limited due to the increased incidence of antibiotic-resistant infections, treatment adverse effects, disruption of normal gut bacterial flora, and the association with complications (Kent and Bank, 2010).

The specific antibiotic treatment suggested by World Gastroenterology Organization (WGO) are, for cholera (doxycycline, azithromycin and ciprofloxacin), for shigellosis (ciprofloxacin, pivmecillinam and ceftriaxone), for Campylobacter (azithromycin and fluoroquinolones like ciprofloxacin), for amoebiasis of invasive intestine (Metronidazole) and for giardiasis (metronidazole, tinidazole, ornidazole and secnidazole) (Farthing *et al.,* 2012).

Antimotility Agents

Loperamide and diphenoxylate are the main antimotility drugs utilized in the management of acute diarrhea and among these, loperamide is the most used drug with minor central opiate effects. Loperamide act as antidiarrheal by producing segmental contractions in the intestine (slows gut transit), which reduces the movement of luminal fluid and results in greater absorption. Another effect of loperamide is by blocking calmodulin thereby decreasing mucosal secretion. Study also revealed that morphine and loperamide inhibit chloride secretion in rabbit ileal mucosa and studies in humans showed that loperamide reduced prostaglandin E type two (PGE_2) induced intestinal secretion (Kent and Banks, 2010; Riddle, 2018).

Antimotility agents are contraindicated in bacterial diarrhea primarily owing to the risk of paralytic ileus, and diphenoxylate also has considerable central opioid effects. Moreover, these drugs have low therapeutic index and linked with intestinal problems like toxic dilatation of the large intestine or extended ailment when used in bacterial inflammatory pathogens. The common adverse effect of loperamide is constipation (Riddle *et al.,* 2016; Thiagarajah *et al.,* 2015).

Antisecretory Agents

The endogenous opioid, enkephalins have both proabsorptive and antisecretory activity in the small intestine (Farthing, 2006). The production of Cyclic Adenosine Monophosphate (cAMP) is directly blocked by endogenous peptide, enkephalins by attaching to opioid receptors on the enterocyte. Because of antisecretory and proabsorptive potential of enkephalins, they are indicated in treatment of diarrhea. Racecadotril is the blocker of enkephalinase, which is used in the

management of acute diarrhea (Kent and Banks, 2010). Its efficacy is equal with that of loperamide in the management of diarrhea but prevalence of constipation is significantly reduced with using racecadotril and used in management of diarrhea in children (Farthing *et al.,* 2012; Kent and Bank, 2010).

Anti-inflammatory Agents

In inflammatory bowel disorder, diarrhea is common symptom. An anti-inflammatory drug, mesalazine or 5-aminosalicylic acid is used to manage inflammatory bowel syndrome with diarrhea such as Crohn's disease and ulcerative colitis. It is also commonly employed in the treatment of patients with microscopic colitis but the mesalazine efficacy in the management of microscopic colitis is weak. Budesonide is corticosteroid with less systemic action because its first pass effect is extensive. Budesonide use is approved by Food and Drug Administration (FDA) for the management of mild to moderate Crohn's disease of ileum and ascending large intestine. The evidence for the use of budesonide in the treatment of microscopic colitis is stronger compared to mesalazine (Lee, 2015; Li and Vaziri, 2012).

Bile Acid Sequestrants (Binding Agents)

For the treatment of bile acid induced diarrhea (BAD), cholestyramine and colestipol are considered as the choice of drug but the unpleasant teste results in reduced patient adherence (Camilleri, 2015). Cholestyramine and related bile acid sequestrants decreased fecal frequency and weight, and improved the urgency and fecal constituency. Cholestyramine is mainly used in treatment of watery diarrhea induced by bile acid malabsorption regardless of the cause and severity (Borghede *et al.,* 2011; Tack, 2012).

Anticholinergics (Antispasmodics) Drugs

Anticholinergic and antispasmodic are the choice of drugs in the management of mild to moderate diarrhea. Mechanism of action of anticholinergic drugs is by blocking acetylcholine receptors, thereby reducing both bowel movement and mucosal secretion. Dry mouth, dizziness and retentions of urine are some of the prominent adverse effects (Dellon and Ringel, 2006).

Antispasmodic drugs such as alverine citrate, otilonium bromide, peppermint oil, pinaverium bromide, and mebeverine have influence on calcium channels and directly affect smooth muscles

of gut. Alverine citrate, antispasmodic agent which regulate activities of smooth muscle by blocking the uptake of calcium. Decreased flow of calcium might lead to reduced chemical sensitivity, which results in relaxation of smooth muscle of intestine. Thus, it decreases both the frequency and amplitude of smooth muscle of GIT contractions (Lee, 2015).

1.6.4 Traditional Medicinal Plants Used for the Treatment of Diarrhea

Medicinal plants have been used as the traditional remedies for several human illnesses for many years in numerous countries. In developing countries, about 80% of people practice traditional medicines for health care. The natural products obtained from medicinal plants have confirmed a plentiful biological constituent, which made them the source for the development new novel compounds (Palombo, 2006).

Countries with poor economic status encounter many economic constraints, thus it is important to emphasize on special interventions that are cheap and decrease the problem of disease accountable to particular risk factor (Gutiérrez *et al.,* 2007). To overcome such difficulties, WHO introduced Diarrhea Disease Control Program, which involves all aspects of traditional medicine practices, along with evaluation of health education and preventive approaches (Syder *et al*., 1982).

Medicinal plants have been utilized as a source of medicine from ancient time to manage various diseases and traditional medicine is one of essential cultural component in Ethiopia. About 80% of Ethiopian population relies on traditional medicine and among this 95% of the traditional medicine sources are plants. The extensive use of this traditional medicine in societies of Ethiopia may be due to the cultural acceptability, accessibility, economic affordability and efficacy of traditional medicine. And diarrhea is one of known disease treated by traditional medicine (Woldeab *et al.,* 2018).

The main components of medicinal plants which are chiefly accountable for antidiarrheal activity are tannins, alkaloids, flavonoids and terpenoids, and there are variety of medicinal plants that have been broadly practiced for the treatment of diarrhea and gastrointestinal associated disorders by traditional practitioners in Ethiopia (Sisay *et al.,* 2017).

Conyza pyrrhoppapa, Ficusthonningii, Tamarindus Indica (Khan *et al.,* 2018), *Ziziphus spina-Christi* (Fenetahun *et al.,* 2017), *Cordia africana Lam*. (Abrha *et al.,* 2018), *Brucea antidysenterica Swiss Chard., Clutia abyssinica Jaub. and Spach, Capsicum annuum L., icus sur*

Forssk., Leonotisocymifolia, Lepidium sativum, Ocimumgratissimum L., (Amsalu *et al.,* 2018), *Amaranthus caudatus L., Croton macrostachyus Hochst. ex Delile, Ensete ventricosum (Welw.), Cheesman, Grewiavillosa and Nicotiana tabacum L.* (Mesfin *et al.,* 2014) are some of reported medicinal plants used to treat diarrheal disease in Ethiopia.

1.7 The Experimental Plant

Lamiaceae or Labiatae family is referred as minty family, a family of angiosperms. This family has widespread distribution around the world and it comprises of around 236 genera and 6900 to 7200 species (Raja, 2012). This family is the most wide-ranging and diverse plant families used in traditional medicine (Venkateshappa and Sreenath, 2013).

The genus Leucas belongs to the family Lamiaceae, one of the greatest genera in subfamily of Lamioideae. It has around 100 species growing on dry in tropical to Southern Africa, and tropical and subtropical parts of Asia and Australasia. Northern part of East Africa shares the largest species the genus Leucas (Scheen and Albert, 2009).

Leucas abyssinica shown in Figure 1 belongs to the family of Lamiaceae and it is widely dispersed to the semi-arid areas of Eritrea and eastern Ethiopia. And it occurs in these countries at the altitude of 200–300 meters and grows in semi-arid to dry montane bushland and in deforested areas of *Juniperus procera* Hochst. ex Endl. forest. It is also extensively dispersed to Somalia where it occurs in this area on the altitude of 200 to 700 meter, and grows in deciduous to evergreen bushland (Paton *et al.,* 2018).

Leucas abyssinica has different vernacular names for instance, ‘Tseadakurneaa’ in Eretria, ‘PeloTsala’ in Maale and Ari ethnic communities, ‘Aychedamo’ in Fiche, ‘Siwakarni’ in Tigray and ‘Kirikkissaa’ in Dawuro Zone. The traditional uses of *L. abyssinica* are the leaves are used to treat endoparasites and cold (Yemane *et al.,* 2017), stomachache, amoebiasis, stomach bloating, headache, food poisoning, rheumatism and vomiting (Kidane *et al.,* 2014). Leaves are used to treat eye infection and the root is used to treat urine retention of cattle (Teklay *et al.,* 2013). In Dawuro Zone, Southwestern Ethiopia traditional ly the leaves of *L. abyssinica* is crushed and mixed with water, then a cup and half of cup drunk by adults and children respectively for the treatment of diarrhea (Andarge *et al.,* 2015).

Figure 1: The image of *Leucas abyssinica* (photo captured by principal investigator during plant collection)

1.8 Rationale of the Study

In the treatments of gastrointestinal disorders such as diarrhea and constipation, medicinal plants are frequently preferred due to their numerous components with synergetic effect or adverse effect counteracting capacity, and thus chosen comparatively safe for extended treatment (Birru *et al.,* 2016).

Similar to other developing countries, Ethiopian people are also highly dependent on traditional medicine for their health care (Asrie *et al.,* 2016; Birru *et al.,* 2016). There are several medicinal plants used for management of diarrhea, which have been practiced in native societies of Ethiopia. But most of these medicinal plants with antidiarrheal effects has not been scientifically proved (Woldeab *et al.,* 2018).

The associations of conventional drugs and homegrown medicines have been promoted by WHO to invent and detect active biologically compounds that are safe and effective (Collise and Goduka, 2012). Also, WHO encouraged studies of traditional medical practices together with health education and preventive approach for the management and prevention of diarrheal diseases (WHO*.,* 1987).

The use antisecretory agents like racecadotril is not recommended in adults with cholera infection, and the use of loperamide in bloody or inflammatory diarrhea is contraindicated and not recommended for children (Farthing *et al.,* 2012). Moreover, there is an emerging antibiotic resistance, adverse effects, contraindications and superinfection by anti-infectives limiting the use of existing conventional antidiarrheal agents (Kent and Banks, 2010).

L. abyssinica is one the several medicinal plants traditionally used for treatment of diarrhea. Thus, this study endeavored to validate the traditional use of the claimed plants as medicines for diarrhea. The results from this study might contribute in searching for novel antidiarrheal agents which might work out the problems related to modern antidiarrheal drugs. It might help the traditional users of the community by giving direction on alternative ways of preparation of the plant. Furthermore, the finding from this study might show the demand for scientific community to conduct further advance in investigation on molecular mechanisms and specific agents accountable for antidiarrheal effect of the plant.

2. Objectives of the Study

2.1 General Objective

- To evaluate the antidiarrheal activity of 80% methanol and aqueous extract of the leaves of *Leucas abyssinica* (Lamiaceae) in mice.

2.2 Specific Objectives

- To test the acute oral toxicity of 80% methanol and aqueous extract of the leaves of *L. abyssinica*;
- To evaluate the effect of 80% methanol and aqueous extract of the leaves of *L. abyssinica* on castor oil induced diarrhea in mice;
- To assess antienteropooling effect of 80% methanol and aqueous extract of the leaves of *L. abyssinica* on misoprostol induced enteropooling in mice;
- To investigate the effect of 80% methanol and aqueous extract of the leaves of *L. abyssinica* on castor oil induced gastrointestinal motility using charcoal meal test;
- To evaluate the effect of 80% methanol and aqueous extract of the leaves of *L. abyssinica* on normal gastrointestinal motility in mice;
- To test preliminary phytochemical screening of the 80% methanol and aqueous extracts of *L. abyssinica*;

3. Materials and Methods

3.1 Chemicals, Drugs and Reagents

The following chemicals, drugs and reagents were used in the study: Loperamide HCl (Medochemie-Cyprus), misoprostol (Mylan Laboratories, India) castor oil (Amman Pharmaceutical Industries Co, Jordan), activated charcoal (Lab. Reagent, India), distilled water (Social Pharmacy and Pharmaceutics Laboratory, Addis Ababa University), chloroform, ethyl acetate and mercuric chloride (Bulex Laboratory, India), sulphuric acid (Park scientific Ltd, UK), methanol (Carlo Erba Reagents S.A.S, France), Tween 80 (UNI-CHEM, India), diethyl ether (BDH Laboratory Supplies, England) and acetic anhydride (Lot A13/45/67/A), ammonia and ferric chloride anhydrous (Sisco Research Laboratories, India) and Potassium iodide (Calibre Engineering, India). All the chemicals and reagents used were laboratory grade.

3.2 Apparatus and Instruments

The following apparatus and instruments were used during the study: Rotary evaporator (Heidolph, Germany), lyophilizer (OPERON, OPR-FDU-5012, Korea) and electronic balance (KERN-ALJ 220-4, Germany), metabolic cage, ruler, scissors and forceps.

3.3 Collection of Plant Material

The fresh leaves of *L. abyssinica* were collected from around Tercha Town, Dawuro Zone, Southwestern Ethiopia which is located 497 km away from Addis Ababa, capital city of Ethiopia in January 2018. Meanwhile, the fresh aerial part of the plant was sent to and authenticated by a taxonomist at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University and voucher specimen was deposited with voucher number of (001TA) for future reference.

3.4 Experimental Animals

Healthy adult swiss albino mice of either sex, weighing (20-30 gram) were obtained from animal house unit of the Department of the Pharmacology and Clinical Pharmacy, School of Pharmacy, Addis Ababa University. The animals were housed under standard environmental conditions and with free access to pellet food and water *ad libitum*. Maintaining the laboratory under standard conditions, the mice were acclimatized for seven days before the actual experiment. All the

experiments were carried out according to the guideline for the care and use of laboratory animals (Herling, A. W. (2016)).

3.5 Preparation and Extraction of the Plant Material

3.5.1 Preparation of 80% Methanol Extract

After collections of the leaves of *L. abyssinica*, the collected leaves were washed with tap water and air-dried under shade at room temperature. Next the dried leaves were coarsely pulverized by using mortar and pestle. One hundred gram of powdered leaves were weighed using an electronic balance (KERN-ALJ 220-4, Germany) and subjected to cold maceration with 500 ml of 80% methanol for the three days with regular manual shaking and stirring. After seventy-two hours, the supernatant was filtered by Whatman filter paper number one. Then, the residue/marc was remacerated with the same volume of solvent (500 ml of 80% methanol) and duration (72 hrs.) two times for exhaustive extraction. The filtrates from the three batches were combined and the methanol was removed from the filtrate by evaporation under reduced pressure by using a rotary evaporator (Heidolph, Germany) at 40°C. After removing methanol, the filtrate was frozen by deep freezer. The remaining water from the filtrate was removed by using a lyophilizer (OPERON, OPR-FDU-5012, Korea). 12.4% yield (12.4 gm) of the 80% methanol extract was obtained and this dried extract was transferred into small glass vials and placed in a refrigerator till used for the experiment.

3.5.2 Preparation of Aqueous Extract

One hundred gram of coarse powder of the dried leaves of plant was soaked with one liter of distilled water and placed for the three consecutive days at room temperature with occasional manual shaking and stirring. Then, the mixture was filtered first by using muslin cloth and next by Whatman number one filter paper. The marc/residue of plant was remacerated with same amount of solvent (one liter of distilled water) and duration (three days) for two time to extract exhaustively. The filtrates from the three batches were combined and stored in deep freezer. The water from the filtrate was removed by lyophilizer (OPERON, OPR-FDU-5012, Korea). Finally, 11.6% yield (11.6 gm) of dried aqueous extract was obtained and transferred in to small glass vials and placed in a refrigerator until used for experiment.

3.6 Acute Toxicity Study

Acute oral toxicity test was conducted in accordance with the Organization for Economic Cooperation and Development (OECD) guideline 425 (Ocde, 2008) . Firstly, two female mice were administered at the limit test dose for each extract and survived for one day follow up. The other eight mice were sequentially administered 2000 mg/kg of each extract, so that a total of ten animals were tested for aqueous and methanol extracts respectively. Then, the animals were carefully observed individually for signs of toxicity at continuously in the first half hour, periodically during the first day, and daily for an additional thirteen days after dosing, for a total of fourteen days.

3.7 Grouping and Dosing of Animals

Total of forty-eight mice were used for each antidiarrheal model and the mice were randomly allocated into eight groups (controls remain the same) comprising of six mice of either sex. Four antidiarrheal models were castor oil induced diarrhea, misoprostol induced enteropooling, normal transit and castor oil induced transit in mice. And the five groups of animals were (group I, negative controls), (group II, positive control), test groups (group III, IV and V). The negative control group, group I was treated with the distilled water 10 ml/kg and the positive controls, group II was administered with the standard drug, loperamide 3 mg/kg and the test groups received (group III, 100 mg/kg), (group IV, 200 mg/kg) and (group V, 400 mg/kg) each extract orally.

3.8 Determination of Antidiarrheal Activity

3.8.1 Castor Oil Induced Diarrhea

Castor oil induced diarrhea model was conducted according to the method described previously with slight modifications (Oghenesuvwe et.al., 2018). Accordingly, the mice which were found diarrheic when they received 0.5 ml castor oil in the early screening test were involved in this experiment. Forty-eight mice were deprived of food and water for twelve hours and divided into five groups randomly with six animals per group. Firstly, the negative control group treated with distilled water 10 ml/kg and the positive control group received the standard drug, loperamide (3 mg/kg) by orally. The three test groups received grade doses of the test substance as per grouping. After one hour, all the mice received 0.5 milliliter (ml) per mouse of castor oil orally. Then the mice were kept individually in transparent metabolic cages, the bottom of which was lined with white sheet of clean paper to observe for the frequency and consistency of stools. Throughout observation period of four hours, the onset of diarrhea, the number and weight of both dry and wet

stools excreted by the animals were noted and compared to negative control for investigating the antidiarrheal effect. The onset diarrhea (diarrheal free periods) was determined as the time interval in minutes between the administration of castor oil and the appearance of the first diarrheal feces. The total number of diarrheal feces of the negative control group was considered as 100%. Diarrheal consistency was as graded as numerical score as follows: normal stool = 1, semi-solid stool = 2, and watery diarrhea = 3 (Tadesse *et al.,* 2014). And the percentage of inhibition was calculated as follow,

$$\% \text{ of Inhibition} = \frac{\text{Average weight of wet stools in the (control - treated) group}}{\text{Average weight of wet stools in the control group}} * 100$$

3.8.2 Misoprostol Induced Enteropooling

Enteropooling test was determined using the method used previously with some adjustments (Oghenesuvwe et.al., 2018). Forty-eight mice were fasted food for twelve hours while water was *ad libitum*. The mice were grouped and treated in the similar way as described for castor oil induced diarrhea. 20 µg/kg of misoprostol was administered to all mice in all groups orally (Birru *et al.,* 2016). One hour after misoprostol administration, all of mice were sacrificed by cervical dislocation, and the small intestine each mouse was ligated both at the pylorus and cecum, and was isolated and weighed. Then, the intestinal contents of all individual animal was collected by emptying into a measuring cylinder and the volume of intestinal contents were measured. Just after removal of the intestinal content, the intestine of each mouse was reweighed. Then, percent reductions in the weight and volume of intestinal content, relative to the negative control group, was calculated using the following formulas (Mekonnen *et al.,* 2018).

Percentage of reduction in weight of intestinal contents =

$$\frac{\text{Weight of intestinal contents (gm) of (negative controls - treated group)}}{\text{Weight of intestinal contents (gm) of negative controls}} * 100$$

Percentage of reduction in volume of intestinal contents =

$$\frac{\text{Volume of intestinal contents (ml) of (negative controls - treated group)}}{\text{Volume of intestinal contents (ml) of negative controls}} * 100$$

3.8.3 Normal Gastrointestinal Transit in Mice

Normal gastrointestinal transit in mice was carried out by adapting the method explained by (Yacob *et al.,* 2016) with little modifications. The mice were fasted for twelve hours but water was *ad libitum*. One hour after giving the vehicle (distilled water 10 ml/kg) to group I, extract, or reference drug (loperamide 3mg/kg), each animal was treated with one ml standard charcoal meal (10% activated charcoal suspension in 2% tween 80). The mice were, then sacrificed sixty minutes after administration of charcoal meal, the abdomen of each mouse was opened and the small intestine was immediately removed. The length of small intestine from pylorus to the caecum and the length transversed by the charcoal marker was measured. The peristaltic index for each mouse was calculated, expressed as percentage of the distance transversed by the charcoal meal relative to the total length of the small intestine. The percentage inhibition relative to the control was also calculated as the following;

$$\text{Peristaltic index} = \frac{\text{Length of charcoal meal}}{\text{Length of small intestine}}$$

$$\%\ \text{of inhibition} = \frac{\text{Average distance traveled by charcoal marker (controls - treated) group}}{\text{Average distance traveled by charcoal marker of negative controls}} * 100$$

3.8.4 Castor Oil Induced Gastrointestinal Transit in Mice

This experiment was conducted according to the method described by (Mekonnen *et al.,* 2018) with little modification. The mice were randomly divided into five groups per six mice and the mice were deprived of food for twelve hours while water was provided *ad libitum*. Each group (negative, positive and three test groups) were then administered as per grouping. After one hour of treatment, 0.5 ml of castor oil was given orally per mouse for all groups. Again, after an hour, one ml of standard charcoal meal (10 % activated charcoal suspension in 2 % tween 80) was given to each mouse orally. Then, all mice were sacrificed after an hour and the small intestine of each mouse was removed. The intestinal length moved by the charcoal meal from the pylorus toward the caecum and the length of small intestine were measured, and the percent of intestinal length moved by the charcoal meal and percent inhibition of intestinal transit was determined as follows;

$$\%\ \text{of inhibition} = \frac{\%\ \text{of intestinal transit by charcoal meal (control – treated) group}}{\%\ \text{of intestinal transit by charcoal meal in the control group}} * 100$$

3.8.1 Antidiarrheal Index

The *in vivo* antidiarrheal index was computed according to the formula described by Sisay *et al.,* (2019*)*.

Antidiarrheal index *in vivo* =

$$\sqrt[3]{\text{Delay in diarrheal onset} * \text{Gut meal by travel reduction} * \text{Reduction in wet the frequency of wet stools}}$$

Delay in defection time =

$$\frac{\text{Onset of diarrhea in minute of the (treated – control) group}}{\text{Onset of diarrhea in minute of control group}} * 100$$

Gut meal travel reduction =

$$\frac{\text{Distance transversed by charcoal marker in the (negative control -treated) group}}{\text{Distance transversed by charcoal marker in the negative control}} * 100$$

Reduction in the frequency of stools =

$$\frac{\text{Average numbers of wet stools in (negative control- treated) group}}{\text{Average numbers of wet stools in negative control}} * 100$$

3.9 Preliminary Phytochemical Analysis

The aqueous and 80% methanol extract of the *L. abyssinica* was tested for the presence of preliminary phytochemicals such as alkaloids, saponins, flavonoids, glycosides, steroids, terpenoids and tannins using the following standard methods (Fentahun *et al.,* 2017; Sheel *et al.,* 2014).

Test for Saponins (Frothing Test)

Five hundred milligram (mg) of powdered each of test substances or extracts in a measuring cylinder, 5 ml of distilled water was added and the mixture was vigorously shaken. And the formation of a froth persisting for thirty minutes prove the presence of saponin.

Test for Glycosides (Concentrate H_2SO_4 Test)

In 5 ml of each of the extract, 2 ml of glacial acetic acid, a drop of FeCl3 and 1 ml of Conc. H_2SO_4 were followed. Appearance of brown ring at interface confirm the existence of glycosides

Detection of Alkaloids

Five hundred mg of powdered extracts boiled in 10 ml of prepared acid alcohol and filtered, about 5 ml of the filtrate was taken and 2 ml of dilute ammonia added. Then 5 ml of chloroform was added and shaken gently. 10 ml of acetic acid was used to extract the layers of chloroform. The presence of a cream (with Mayer's reagent) or reddish-brown precipitate (with Dragendorff's reagent) confirms the presence of alkaloids.

Detection of Flavonoids

In 200 mg of each extract about 10 ml of ethyl acetate was added and heated on a water bath for three minutes. The mixture was cooled and filtered. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia solution. The layers could separate and the appearance of yellow coloration that disappeared on standing indicates the existence of flavonoids.

Test for Sterols (Liebermann-Burchard Test)

About 2 ml of acetic anhydride was added to 250 mg each of crude extract and mixed with 2 ml chloroform. Then, 1 ml of concentrated sulphuric acid was followed. The change color from violet to blue or green indicates the presence of steroids.

Test for Tannins

About 500 mg of each powdered extract was added in 10 ml distilled water in beaker and then mixture was heated and filtered. Few drops of 0.1% ferric chloride (FeCl3) were added to the filtrate. A brownish green or a blue-black coloration confirms the existence of tannins.

Test for Terpenoids (Salkowski Test)

In 5ml of each extract of 2ml of chloroform was added and in this mixture 3 ml of concentrated H_2SO_4 was judicially added to form a layer. The formation a reddish-brown color of interface confirms the presence of terpenoids.

3.10 Ethical Considerations

The handling of the animals and all experimental procedures were conducted in accordance with internationally accepted standard guidelines for the use of laboratory animals (Herling, A. W., 2016) and the proposal was approved by Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Science, Addis Ababa University.

3.11 Data Analysis

The experimental data was analyzed using Software Statistical Package for Social Sciences, version 25 for window. Results were expressed as means ± standard error of the mean (SEM). Comparisons between groups were made by using one-way analysis of variance (ANOVA) followed by post hoc Tukey's multiple comparison test. Linear regression was used to show dose dependency of the extracts. At 95% confidence interval ($p < 0.05$), the difference between the compared groups were considered as statistically significant. The analyzed data were then presented using tables.

4. Results

4.1 Acute Oral Toxicity Test

In acute oral toxicity test, administration of the limit test dose (2000 mg/kg) of both aqueous and 80% of methanol extracts of the leaves of *L. abyssinica* resulted in neither death nor notable signs of toxicity in the first twenty-four hour and also in following thirteen days. Thus, the result showed that the LD_{50} of both extracts were higher than 2000 mg/kg.

4.2 The Effects of 80% Methanol and Aqueous Extract of the leaves of *L. abyssinica* on a Castor Oil Induced Diarrhea in Mice

Both 80% methanol and aqueous extract of *L. abyssinica* significantly extended diarrheal free periods at the dose of 400 mg/kg ($p < 0.001$) compared to negative control group. Also, the 200 mg/kg of methanol extract significantly delayed the onset of defection ($p < 0.01$). But in delaying onset of diarrhea, the lower doses of aqueous and methanol extracts, and the middle dose, 200 mg/kg of aqueous extract had lower effects ($p < 0.05$) compared to the higher doses of 400 mg/kg of aqueous and methanol extract, and the middle dose, 200 mg/kg of methanol extracts.

All the tested doses of methanol, and middle and higher doses of aqueous extracts significantly reduced the weight of wet and total weight of defection ($p < 0.001$) compared with vehicle treated group. In reducing the weight of wet stool, the lowest dose, 100 mg/kg aqueous extract had lower activity compared with positive control, loperamide 3mg/kg ($p < 0.001$) and highest dose of 80 % methanol extract ($p < 0.01$).

Moreover, all the tested doses of both extracts significantly reduced both the frequency of wet and total frequency of stools ($p < 0.001$). And both extracts produced dose dependent ($R^2 = 0.97$ for methanol and 0.77 for aqueous extract) and significant inhibition of diarrheal defection but the percentage inhibition of methanol extract for the three doses were relatively higher than that of respective doses of aqueous extract as depicted in Table 1.

Table 1: The effects of 80% methanol and aqueous extract of the leaves of *L. abyssinica* in castor oil induced diarrhea in mice

Groups	Onset of diarrhea (min)	Average weight of wet stools (gm)	Total weight of stools (gm)	Average No. of wet stools	Total no. of stools	% of inhibition diarrhea
NC	60.50 ± 7.08	1.34 ± 0.15	1.46 ± 0.10	7.83 ± 1.30	10.67 ± 0.61	-
PC	158.33 ± 9.49 ^{a3}	0.23 ± 0.47 ^{a3}	0.48 ± 0.16 ^{a3}	2.17 ± 0.40 ^{a3}	5.17 ± 0.48 ^{a3}	82.84
MELA 100	118.17 ± 8.33 ^{a1}	0.56 ± 0.10 ^{a3}	0.63 ± 0.10 ^{a3}	3.33 ± 0.49 ^{a3}	6.67 ± 0.49 ^{a3}	58.21
MELA 200	124.17 ± 10.16 ^{a2}	0.46 ± 0.57 ^{a3}	0.59 ± 0.13 ^{a3}	3.17 ± 0.40 ^{a3}	6.50 ± 0.43 ^{a3}	65.67
MELA 400	148.50 ± 10.16 ^{a3}	0.33 ± 0.48 ^{a3f2}	0.57 ± 0.09 ^{a3}	3.00 ± 0.26 ^{a3}	5.83 ± 0.31 ^{a3}	75.37
AQLA 100	113.00 ± 14.89 ^{a1}	0.87 ± 0.11 ^{a1b3e2}	0.83 ± 0.12 ^{a1}	3.50 ± 0.34 ^{a3}	7.00 ± 0.77 ^{a3}	35.07
AQLA 200	115.67 ± 13.80 ^{a1}	0.59 ± 0.11 ^{a3}	0.61 ± 0.17 ^{a3}	3.33 ± 0.33 ^{a3}	6.67 ± 0.56 ^{a3}	55.97
AQLA 400	138.50 ± 14.59 ^{a3}	0.51 ± 0.74 ^{a3}	0.60 ± 0.09 ^{a3}	3.17 ± 0.31 ^{a3}	6.17 ± 0.60 ^{a3}	61.94

Data were expressed as mean ± SEM. (n = 6 mice), NC = Negative control group (10ml/kg distilled water), PC. = Positive control group (3 mg/kg Loperamide), MELA = 80% of methanol extract of the leaves of *L. abyssinica*, AQLA= aqueous extract of the leaves *L. abyssinica* a= against negative control group b= against positive control group, e= against 400 mg/kg MELA, f = against 100 mg/kg of AQLA, ^{1}= $p < 0.05$, ^{2} = $p < 0.01$, ^{3} = $p < 0.001$ numbers refers to dose in mg/kg

4.3 The Effects of 80% Methanol and Aqueous Extracts of the Leaves of *L. abyssinica* on a Misoprostol Induced Enteropooling in Mice

All tested doses of the extract significantly reduced the weight of intestinal contents ($p < 0.001$) in dose dependent manner (R^2 0.97 and 0.96 for methanol aqueous extract respectively) compared with negative controls. In reducing the volume of secretions, the lowest dose, 100 mg/kg of both extracts significantly showed an effect but had lower activity ($p < 0.01$) compared to the middle and highest doses of both extracts. The percentage of reduction of both intestinal weight and volume of fluid secretions of methanol extract was relatively higher than that of aqueous extracts. The reference drug, loperamide 3mg/kg also revealed a noticeable reduction in intestinal weight and volume of fluid secretion by 71.11% and 64.71%, respectively, compared to the negative control group, which was the so far higher than that of both extracts. Beside this, both extracts significantly reduced the fluid contents ($p < 0.001$) in dose dependent manner ($R^2 = 0.99$ and 0.73

for methanol and aqueous extracts respectively) in castor oil induced diarrhea, which was computed from the difference of wet and dry feces after 48 hours as unveiled in Table 2.

Table 2: The effect of 80% methanol and aqueous extracts of leaves of *L. abyssinica* on misoprostol induced enteropooling assay in mice

Groups	Net weight of intestinal contents (gm)	The volume of intestinal contents (ml)	Water/ fluid contents in (gm)	% of reduction in weight (gm)	% of reduction in volume
NC	0.90 ± 0.11	0.85 ± 0.09	1.01 ± 0.14	-	-
PC	0.26 ± 0.03 ^{a3}	0.30 ± 0.03 ^{a3}	0.19 ± 0.05 ^{a3}	71.11	64.71
MELA 100	0.35 ± 0.04 ^{a3}	0.50 ± 0.06 ^{a2}	0.37 ± 0.06 ^{a3}	61.11	41.18
MELA 200	0.34 ± 0.05 ^{a3}	0.43 ± 0.04 ^{a3}	0.30 ± 0.06 ^{a3}	62.22	49.41
MELA 400	0.29 ± 0.04 ^{a3}	0.33 ± 0.50 ^{a3}	0.13 ± 0.05 ^{a3}	67.78	61.18
AQLA 100	0.42 ± 0.04 ^{a3}	0.52 ± 0.07 ^{a2}	0.45 ± 0.06 ^{a3}	53.33	38.82
AQLA 200	0.36 ± 0.07 ^{a3}	0.45 ± 0.05 ^{a3}	0.31 ± 0.09 ^{a3}	60.00	47.06
AQLA 400	0.30 ± 0.08 ^{a3}	0.41 ± 0.05 ^{a3}	0.28 ± 0.08 ^{a3}	66.67	51.76

Data were expressed as mean ± SEM. (n = 6 mice), NC = Negative control group (10ml/kg distilled water), PC. = Positive control group (3 mg/kg loperamide), MELA = 80% of methanol extract of the leaves of *L. abyssinica*, AQLA= aqueous extract of the leaves *L. abyssinica,* a= against negative control group ^{1}= $p < 0.05$, ^{2} = $p < 0.01$, ^{3} = $p < 0.001$ and numbers refers to dose in mg/kg.

4.4 The Effect of 80% Methanol and Aqueous Extracts of the Leaves of *L. abyssinica* on Normal Gastrointestinal Transit in Mice

None of the tested doses of both methanol and aqueous extracts did produce significant ($p > 0.05$) reduction of small intestine transit compared with vehicle treated groups as revealed in Table 3. The reference drug, loperamide 3mg/kg significantly reduced the transit of small intestine with the peristaltic index of 30.66 ($p < 0.001$) compared to negative control group. Except for highest dose of the methanol extract, all the tested doses showed significant difference in reducing the gastrointestinal transit compared to the positive control. The maximum nonsignificant percentage inhibition was obtained at higher dose of methanol extract (31.46 %) compared to negative control group.

Table 3: The effects 80 % methanol and aqueous extracts of the leaves of *L. abyssinica* on normal gastrointestinal transit in mice.

Groups	Mean length of small intestine (cm)	Mean distance of charcoal transited (cm)	Peristaltic index	Percentage of inhibition
NC	56.33 ± 1.99	41.83 ± 4.08	74.47	-
PC	56.00 ± 2.31	17.17 ± 1.08 ^{a3}	30.66	58.95
MELA 100	56.17 ± 2.70	37.00 ± 3.53 ^{b2}	65.68	11.55
MELA 200	49.50 ± 1.86	32.00 ± 3.21^{b1}	64.65	23.50
MELA 400	50.50 ± 0.85	28.67 ± 2.53	56.77	31.46
AQLA 100	47.33 ± 2.43	34.67 ± 1.98 ^{b2}	73.25	17.12
AQLA 200	51.17 ± 1.19	37.33 ± 2.76 ^{b3}	72.95	10.76
AQLA 400	50.00 ± 2.03	34.17 ± 4.07 ^{b2}	68.34	18.31

Data were expressed as mean ± SEM. (n = 6 mice), NC = Negative control group (10 ml/kg distilled water), PC. = Positive control group (3 mg/kg loperamide), MELA = 80% of methanol extract of the leaves of *L. abyssinica*, AQLA= aqueous extract of the leaves *L. abyssinica,* cm = centimeter, a= against negative control group b= against positive control group, ^{1}= $p < 0.05$, ^{2} = $p < 0.01$, ^{3} = $p < 0.001$ and numbers refers to dose in mg/kg.

4.5 The Effect of Methanol and Aqueous Extracts of the leaves of *Leucas abyssinica* on Castor Oil Induced Gastrointestinal Transit in Mice

The methanol extract of *L. abyssinica* significantly reduced the gastrointestinal transit induced by castor oil at 42.80 %, 46.70 %, and 54.47 % at doses of 100, 200, and 400 mg/kg ($p < 0.001$), respectively while the aqueous extract inhibited the charcoal meal transit by 41.23%, 44.36 % and 51.37% at the identical graded doses ($p < 0.001$) compared with vehicle treated group. Peristaltic index (30.66) resulted by loperamide was the least of all, rather the percentage inhibition was highest (61.7 %) and followed by higher dose of methanol extract (54.47 %). Both extracts inhibited the charcoal meal transit in dose dependent fashion (R^2= 1 for methanol and 0.999 for aqueous extract respectively) as depicted in Table 4.

Table 4: The effect of 80% methanol and aqueous extracts of L. abyssinica on castor oil induced transit in mice

Groups	Mean length of small intestine (cm)	Mean distance of charcoal transited (cm)	Peristaltic index	Percentage of inhibition
NC	55.33 ± 2.86	42.83 ± 3.61	77.41	-
PC	60.83 ± 1.11	16.5 ± 0.76 [a3]	27.12	61.47
MELA 100	55.67 ± 2.17	24.50 ± 3.14 [a3]	44.01	42.80
MELA 200	60.00 ± 1.48	22.83 ± 2.50 [a3]	38.05	46.70
MELA 400	55.50 ± 1.36	19.50 ± 2.70 [a3]	35.14	54.47
AQLA 100	55.75 ± 1.71	25.17 ± 3.05 [a3]	45.15	41.23
AQLA 200	53.67 ± 1.33	23.83 ± 2.52 [a3]	44.40	44.36
AQLA 400	55.50 ± 2.06	20.83 ± 2.10 [a3]	37.53	51.37

Data were expressed as mean ± SEM. (n = 6 mice), NC = Negative control group (10 ml/kg distilled water), PC. = Positive control group (3 mg/kg loperamide), MELA = 80% of methanol extract of the leaves of *L. abyssinica*, AQLA= aqueous extract of the leaves *L. abyssinica,* cm = centimeter, a= against negative control group, 1= $p < 0.05$, 2 = $p < 0.01$, 3 = $p < 0.001$ and numbers refers to dose in mg/kg.

4.6 *In Vivo* Antidiarrheal Index

Effects of the *in vivo* antidiarrheal indices for methanol extract were 61.66, 66.38, and 78.77 at the doses of 100, 200 and 400 mg/kg while indices for the aqueous extract were 58.72, 60.40 and 73.31 at the similar graded doses. The highest dose of methanol extract resulted a maximum index of 78.77 but less than the *in vivo* antidiarrheal index of loperamide (Table 5). Both extracts resulted in antidiarrheal activity *in vivo* antidiarrheal index in a dose dependent fashion (R2=0.996 and 0.949 respectively for methanol and aqueous extracts).

Table 5: The *in vivo* anti diarrheal index of *L. abyssinica*

Groups	Average onset of diarrhea in minute (%)	Gut meal travel reduction (%)	Reduction in the frequency of stools (%)	Antidiarrheal index
PC	161.70	61.47	72.29	89.44
MELA 100	95.32	42.80	57.47	61.66
MELA 200	105.24	46.70	59.51	66.38
MELA 400	145.45	54.47	61.68	78.77
AQLA 100	88.78	41.23	55.30	58.72
AQLA 200	91.19	44.36	54.47	60.40
AQLA 400	128.93	51.37	59.51	73.31

AQLA = aqueous extract of *L. abyssinica,* MELA = 80% methanol extract of *L. abyssinica*

4.7 Preliminary Phytochemical Analysis

The preliminary phytochemical analysis of both aqueous and methanol extract showed that the existence of terpenoids, tannins, alkaloids, flavonoids, and steroids but saponins and glycosides were not identified as depicted Table 6.

Table 6: Preliminary phytochemical analysis of the aqueous and 80% methanol extracts of *L. abyssinica*

Secondary metabolites	Extracts of the leaves of *L. abyssinica*	
	MELA	AQLA
Saponins	-	-
Terpenoids	+	+
Tannins	+	+
Flavonoids	+	+
Glycosides	-	-
Steroids	+	+
Alkaloids	+	+

MELA= 80% methanol extract of *L. abyssinica*, AQLA= aqueous extract of *L. abyssinica*, - = absence, + = presence

5. Discussion

Several human ailments have been treated by using medicinal plants as components of traditional treatment for many years and in various parts of the world (Mohamed *et al.,* 2017). In rural regions, traditional remedies are chief sources of treatment and in these areas these traditional medicines are mainly used in the management of diarrhea. Among traditional medicines, the use of medicinal plants for the management of diarrheal diseases have been reported by several studies (Rawat *et al.,* 2017; Semenya and Maroyi, 2012). In Ethiopia, there are diverse plants with antidiarrheal value that have been consumed by native communities, but the efficacy of these several antidiarrheal medicinal plants have not been scientifically proved (Woldeab *et al.,* 2018).

The objective of this study was to experimentally evaluate the traditional acclaimed use of leaves of *L. abyssinica*, which were considered to confer its traditional value for the diarrhea protection. And numerous studies validated the use antidiarrheal medicinal plants by examining the biological activity of the extracts such as plants that have antispasmodic effects, delay the gut transit, suppress intestinal motility, enhance water absorption, or reduce intraluminal fluid accumulation (Pérez-Gutiérrez *et al.,* 2013). Therefore, the present study employed different experimental antidiarrheal models in mice to prove the efficacy of *L. abyssinica*. And the current study revealed that both extracts of *L. abyssinica* have antidiarrheal activity on different antidiarrheal models.

In this study, both extracts of the *L. abyssinica* leaves significantly increased the diarrheal free periods (delayed onset of diarrheal defection) in concentration dependent way compared with the vehicle treated groups. The 400 mg/kg of both methanol and aqueous extracts significantly produced maximum effect in extending the diarrheal free periods ($p < 0.001$) compared with negative controls. All the tested doses of 80% methanol extract and the middle and higher dose of aqueous extract significantly reduced the weight of wet and total weight diarrheal stools ($p < 0.001$) compared with the vehicle treated groups.

In reducing the weight of wet and total diarrheal feces, there was significant difference between lowest dose of aqueous extract and standard drug, loperamide 3mg/kg ($p < 0.001$). Again, the percentage inhibition of diarrhea produced by loperamide (3mg/kg) was highest of all doses of both extracts. This might be because loperamide produces antidiarrheal effects by different mechanisms such as regulating gastrointestinal function, decreasing colonic flow rates and intestinal transit (Tadesse *et al.,* 2014).

Moreover, all the tested doses of both extracts significantly reduced both frequency of wet and total diarrheal feces ($p < 0.001$) compared to negative control group. The percentage reduction of weight of wet diarrheal stools was increased with corresponding increase of dose both methanol and aqueous extracts of the plant. And the percentage inhibition of diarrhea by the methanol extract was comparable with the percentage inhibition produced by the standard drug, loperamide (3mg/kg). In general, in delaying the diarrheal free periods, in reducing the weight of wet and total of stools, in decreasing frequency of wet and total stools, 80% methanol extract produced relatively higher effect than that of the aqueous extracts. This may be due to methanol efficiently penetrates the plasma membrane, allowing the extraction of large amounts of intracellular phytochemicals in contrast to solvents with lower and higher polarity (i.e. it has medium polarity). As result, polar components along with medium and low polar constituents of the extract are mainly dissolved by methanol (Panda *et al.,* 2012). Meanwhile the prostaglandin biosynthesis inhibitors delay the castor oil induced diarrhea, it is possible mention that the plant extract may have capacity to attenuate the effects of ricinoleic acid in the gut mucosa. Thus, the antidiarrheal effect of both extracts of *L. abyssinica* on castor oil induced diarrhea may result from the inhibition of prostaglandin biosynthesis or by substituting the antisecretory mechanism (Wansi *et al.,* 2017).

The finding obtained from this model agrees with reports elsewhere, where the methanol extracts of *Calpurnia aurea* leaves (Umer *et al.,* 2013), methanol extract of the leaves of *Justicia schimperiana* (Mekonnen *et al.,* 2018) and aqueous extracts of leaves of *Pyrena canthastaudtiim* (Awe *et al.,* 2011) showed significant extension of diarrheal free periods, reducing the frequency of diarrheal and total defecation, and decreased the wet stools weight. Phytochemical constituents like flavonoids and alkaloids are recognized for inhibiting the release of autocoids and prostaglandins, thus blocking secretion induced by castor oil induced diarrhea (Qnais *et al.,* 2007).

The antisecretory activity of diarrhea was investigated using misoprostol induced enterpooling model. Regarding misoprostol induced enterpooling, both methanol and aqueous extracts of the leaves of *L. abyssinica* reduced diarrhea significantly by decreasing both the weight of intestinal contents and volume intraluminal fluid accumulation at all doses respectively in dose dependent fashion. Beside this, both extracts showed significant reduced fluid contents in castor oil induced diarrhea, which was computed from weight difference of wet diarrheal stool and dried feces after two days (48 hours). Moreover, the percentage reduction of weight and volume of intestinal

contents of the higher of doses of 80% methanol extract were comparable with the reference drug loperamide 3mg/kg.

Prostaglandins might be partly contributing to the pathophysiological state of the digestive tract and particularly PGE_2 causes diarrhea in both investigational animals and humans. And it has both effects on both motility of intestine and transport of electrolytes and water (Araújo *et al.,* 2015). The effects of reducing both the weight and volume of intestinal contents by both methanol and aqueous extracts possibly is due to the ability of the extracts inhibiting intestinal secretion and/or enhancing the absorption of water and electrolytes through attenuating the biosynthesis and release of prostaglandins. The secretory diarrhea is related with chloride channel stimulations, causing the out flow of chloride from cell. The efflux of chloride causes the huge secretion of water and electrolytes into the lumen of intestine hence results watery diarrhea (Yacob *et al.,* 2016). From this observation, it is plausible to suggest that the extracts probably inhibit the secretion of water into the lumen of intestine by reverting this pathophysiological mechanism.

The secondary metabolites such as flavonoids (Hämäläinen *et al.,* 2011), terpenoids (Prakash, 2017) and steroids (Awad *et al.,* 2004) have been revealed to show antienteropooling effects by inhibiting the biosynthesis of PGE_2, which has key role in the secretions of intestine by enhancing electrolytes and water secretions (Araújo *et al.,* 2015). Flavonoids also has antioxidant properties which are accountable for the inhibitory effects that exerted over numerous enzymes involving the metabolism of arachidonic acid, as result decreasing prostaglandin induced secretion of fluid (Yacob *et al.,* 2016). Moreover, tannins denature proteins in the gut mucosa by producing protein tannates complexes that make gut mucosa more resistant to the alteration by chemicals and may decrease PGE_2 induced secretion (Pandey *et al.,* 2012). Thus, these phytochemical constituents could be responsible for antienterpooling activity and detected for this plant during preliminary phytochemical analysis. Moreover, ricinoleic acid, hydrolytic metabolite of castor oil induces diarrhea by liberating nitric oxide nitric oxide synthesis, which in turn stimulates PGs biosynthesis, increasing peristalsis (Kauer *et al.,* 2014) and mediate gut secretion (Degu *et al.,* 2016). Study revealed that bioactive molecules like terpenoids, flavonoids (Sisay *et al.,* 2019) and alkaloids (Rho *et al.,* 2007) are implicated in attenuation of NO synthesis. Thus, reduction of both the weight and volume of gastrointestinal contents by the extracts might be attributed to attenuation of nitric oxide.

The autonomic nervous system, both the sympathetic and parasympathetic fibers extrinsically innervates the gastrointestinal tract (Shahed-Al-Mahmud *et al.,* 2018). The stimulation of intestine by the alpha two adrenergic agents such as enkephalins and somatostatins results in reduction peristaltic activity and gastrointestinal absorption (Degu *et al.,* 2016; Mbagwu and Aydeyemi, 2008) while the innervation of intestine by acetylcholine and vasoactive intestinal peptides stimulates the secretion of sodium chloride and fluid, and gastrointestinal motility (Cooke, 2000; Rahman *et al*., 2013). Degu *et al* (2016) also stated the phytochemical constituents like flavonoid from the plant source may activate alpha two adrenergic receptors in absorptive cells of intestine. From this observation, it is possible to suggest that plant extracts reduced intestinal contents likely by either enhancing the absorption water and electrolytes, and via stimulation of sympathetic nerve fibers or by inhibiting gut secretion through inhibiting parasympathetic nerves.

All the tested doses of both 80% methanol and aqueous extracts of *L. abyssinica* significantly inhibited ($p < 0.001$) the castor oil induced gastrointestinal transit in dose dependent fashion but did not produce any significant outcome on the normal gastrointestinal transit in mice compared to negative controls. From this observation, it is possible to suggest that the plant extracts have markedly affect the gut propulsion in a diseased condition but do not affect at health state. One of the mechanisms of action for antidiarrheal drugs is the attenuation of gastrointestinal propulsion (Schiller *et al.,* 1984). Study revealed that the activated charcoal absorbs drugs and chemical on the surface of charcoal particles hence inhibiting absorption (Shahed-Al-Mahmud *et al.,* 2018). Gastrointestinal propulsion test by activated charcoal, usually referred as charcoal meal test is used to assess the effects of extracts on normal and castor oil induced gastrointestinal transit of mice.

Since decreasing gastrointestinal propulsion is one of principal aims in therapy of diarrhea, antimotility and antispasmodic agents like opiates (e.g. loperamide) and anticholinergic blockers (atropine and hyoscine) are used in the managements of diarrhea (Salako *et al.,* 2015). The reduction of gut propulsion increases the time of stay of the nutrients which delay the gastrointestinal contents in the intestine and this might enhance gastrointestinal absorption of water and electrolytes (Mekonnen *et al.,* 2018). The delaying of gastric propulsion causes absorption of water from feces and contribute by decreasing watery textures of diarrheal stool. Thus, decrease in the castor oil induced intestinal transit might be due to the antimotility and antispasmodic properties of the extracts, which in turn responsible for antidiarrheal effects of the plant.

The secondary metabolites such as phenolics, tannins, terpenoid, flavonoids and alkaloids are accountable in inhibiting the gastrointestinal propulsion (Sagar *et al.,* 2005; Yacob *et al.,* 2016). Among these phytochemical constituents, flavonoids result antidiarrheal activities by inhibiting gut propulsion, and water and electrolytes secretions. In *ex vivo* and animal studies, flavonoids also have revealed that it enables to inhibit gut secretions caused by PEG_2 and contractions caused by electricity and certain agonists like acetylcholine, serotonin, and histamine (Sarin *et al.,* 2013). Tannins and tannic acid are widely available in many plants and they denature the proteins by forming protein tannate complex, which coats and results more resistant gut mucosa while decreasing the secretions and intestinal propulsion (Devi *et al.,* 2002; Hassan *et al.,* 2011). Also, tannin reduces intestinal secretion and propulsion by either decreasing intracellular calcium influx or by stimulating the system of calcium pumping which in turn results relaxation of smooth muscle (Umer *et al.,* 2013). The reduced castor oil induced gastrointestinal transit maybe due to the presence of these secondary metabolites such as flavonoids, tannins and terpenoids which were identified during preliminary phytochemical analysis of extracts.

Both extracts of *L. abyssinica* resulted in the concentration dependent *in vivo* antidiarrheal index which reveals that both plant extracts produced dose dependent antidiarrheal effect. Generally, the *in vivo* anti diarrheal index value reveals that the given extracts potential to treat diarrhea (Sisay *et al.,* 2019). It measures the integrated results of different parameters of diarrhea like reducing the number of wet stools, diarrheal free periods, and the frequency of gut propulsion (Tadesse *et al.,* 2017). As revealed in literature, it also adds up the antidiarrheal effects and the higher *in vivo* antidiarrheal index value of extract, the better effects in management of diarrhea (Akindele *et al.,* 2014). The higher antidiarrheal effect was produced by 80% methanol extracts of the plant. This might be due to its expanded polarity range which efficiently extracts medium to polar phytochemical components (Panda *et al.,* 2012).

Moreover, during acute oral toxicity studies for the two weeks on both extracts at the limit test dose showed no signs of toxicity and death of animals were not recorded after of administration of the test extracts. This indicates both extracts of the plant have wide margins of safety (i.e. LD_{50} > 2000 mg/kg) and this also confirms the safety of traditional use of the plant as antidiarrheal remedy.

6. Conclusion

The results obtained from the current study suggest that both aqueous and 80% methanol extract of the leaves of *L. abyssinica* showed antidiarrheal activity probably by its antisecretory and antimotility effect. The present findings provide a scientific evidence for the traditional use of the plant as an antidiarrheal remedy with alternative using of alcoholic solvents for the preparation diarrheal remedy from the plant.

7. Recommendations

Depending on the findings of the current study, the following recommendations are forwarded for further studies:

- Further studies should be carried out to isolate, purify, and identify bioactive compounds(s) which are responsible for the antidiarrheal activities of both extracts of the plant;
- Further studies should be conducted to confirm the precise mechanism of action of the antidiarrheal activity of the extracts of plant;
- Further toxicological studies should be conducted to confirm the absence of sub-chronic and chronic toxicity although both extracts of the plant revealed wide margin of safety in acute toxicity study;

References

Abrha, G., Hintsa, S. and Gebremedhin, G. (2018). Indigenous knowledge-based identification of medicinal plants in Central Zone of Tigray, North Ethiopia. *International Journal of Biodiversity and Conservation,* Vol.10 (6), 265-275.

Akindele, A., Salako, O., and Ohonbamu, U. (2014). Evaluation of the antidiarrhoeal activity of the hydroethanolic leaf extract of *Pupalia lappacea* Linn. Juss.(Amaranthaceae). *Journal of Ethnopharmacology, 151*(2), 984-989.

Akuffo, R., Armah1, G., Clemens, G., Kronmann, K., Jones, A., Agbenohevi, P., Sagoe, K., Puplampu, N., Nzussouo, N.T. Ampofol, W., Koram, K., Duplessis, C., and Dueger, E.(2017). Prevalence of enteric infections among hospitalized patients in two referral hospitals in Ghana. *BioMed Central Research Notes, 10*(1), 292.

Amsalu, N., Bezie, Y., Fentahun, M., Alemayehu, A., and Amsalu, G. (2018). Use and conservation of medicinal plants by indigenous people of Gozamin Wereda, East Gojjam Zone of Amhara region, Ethiopia: an ethnobotanical approach. *Evidence-Based Complementary and Alternative Medicine*.

Andarge, E., Shonga, A., Agize, M., and Tora, A. (2015). Utilization and conservation of medicinal plants and their associated indigenous knowledge (IK) in Dawuro Zone: An ethnobotanical approach. *International Journal Medicinal Plant Res, 4*, 330-337.

Anteneh, Z. A., Andargie, K., and Tarekegn, M. (2017). Prevalence and determinants of acute diarrhea among children younger than five years old in Jabithennan District, Northwest Ethiopia, 2014. *BioMed Central Public Health, 17*(1), 99.

Arasaradnam, R.P., Brown, S., Forbes, A., Fox, M.R., Hungin, P., Kelman, L., Major, O'Connor, M., Sanders, D.S., Sinha, R., Smith, S.C., Thomas, P. and Walters1, J.R. (2018). Guidelines for the investigation of chronic diarrhoea in adults: British Society of Gastroenterology. *Gut, 67*(8), 1380-1399.

Araújo, T. S., Costa, D. S., Sousa, N. A., Souza, L. K., de Araújo, S., Oliveira, A. P., Sousa, F.B., Silva, D.F., Barbosa, A.L., J R. Medeiros Leite, J. R. S. (2015). Antidiarrheal activity of cashew

GUM, a complex heteropolysaccharide extracted from exudate of *Anacardium occidentale* L. in rodents. *Journal of Ethnopharmacology, 174*, 299-307.

Asfaha, K. F., Tesfamichael, F. A., Fisseha, G. K., Misgina, K. H., Weldu, M. G., Welehaweria, N. B., and Gebregiorgis, Y. S. (2018). Determinants of childhood diarrhea in Medebay Zana District, Northwest Tigray, Ethiopia: A community based unmatched case–control study. *BioMed Central pediatrics, 18*(1), 120.

Asrie, A. B., Abdelwuhab, M., Shewamene, Z., Gelayee, D. A., Adinew, G. M., and Birru, E. M. (2016). Antidiarrheal activity of methanolic extract of the root bark of *Cordia africana*. *Journal of Experimental Pharmacology, 8*, 53.

Awad, A. B., Toczek, J., and Fink, C. S. (2004). Phytosterols decrease prostaglandin release in cultured P388D1/MAB macrophages. *Prostaglandins, leukotrienes and essential fatty acids, 70*(6), 511-520.

Awe, E. O., Kolawole, S. O., Wakeel, K. O., and Abiodun, O. O. (2011). Antidiarrheal activity of *Pyrenacantha staudtii* Engl. (Iccacinaceae) aqueous leaf extract in rodents. *Journal of Ethnopharmacology, 137*(1), 148-153.

Bahekar, S. E., and Kale, R. S. (2015). Antidiarrheal activity of ethanolic extract of *Manihot esculenta* Crantz leaves in Wistar rats. *Journal of Ayurveda and integrative medicine, 6*(1), 35.

Bartlett, J. G. (2002). Antibiotic-associated diarrhea. *New England journal of medicine, 346*(5), 334-339.

Birru, E. M., Asrie, A. B., Adinew, G. M., and Tsegaw, A. (2016). Antidiarrheal activity of crude methanolic root extract of Idigofera spicata Forssk.(Fabaceae). *Complementary and Alternative Medicine, 16* (1), 272.

Borghede, M. K., Schlütter, J. M., Agnholt, J. S., Christensen, L. A., Gormsen, L. C., and Dahlerup, J. F. (2011). Bile acid malabsorption investigated by selenium-75-homocholic acid taurine (75SeHCAT) scans: causes and treatment responses to cholestyramine in 298 patients with chronic watery diarrhoea. *European journal of internal medicine, 22*(6), 137-140.

Cairncross, S., Hunt, C., Boisson, S., Bostoen, K., Curtis, V., Fung, I. C., and Schmidt, W.-P. (2010). Water, sanitation and hygiene for the prevention of diarrhoea. *International journal of epidemiology, 39* (suppl_1), 193-205.

Camilleri, M. (2015). Bile acid diarrhea: prevalence, pathogenesis, and therapy. *Gut and liver, 9*(3), 332.

Camilleri, M., Sellin, J. H., and Barrett, K. E. (2017). Pathophysiology, evaluation, and management of chronic watery diarrhea. *Gastroenterology, 152*(3), 515-532. e512.

Collise, N., Goduka, N.I., (2012). Treatment of diarrhea in rural African Communities: an overview of measures to maximise the medicinal potentials of indigenous plants. International *Journal of Environmental Research and Public Health,* 9, 3911–3933.

Cooke, H. J. (2000). Neurotransmitters in neuronal reflexes regulating intestinal secretion. *Annals of the New York Academy of Sciences, 915*(1), 77-80.

Das, S., Jayaratne, R., and Barrett, K. E. (2018). The role of ion transporters in the pathophysiology of infectious diarrhea. *Cellular and molecular gastroenterology and hepatology, 6* (1), 33-45.

Deepak, P., and Ehrenpreis, E. D. (2011). Diarrhea. *Disease-a-month: DM, 57*(9), 490.

Degu, A., Engidawork, E., and Shibeshi, W. (2016). Evaluation of the anti-diarrheal activity of the leaf extract of *Croton macrostachyus* Hocsht. ex Del. (Euphorbiaceae) in mice model. *BioMed Central Complementary and Alternative medicine, 16* (1), 379.

Dellon, E. S., and Ringel, Y. (2006). Treatment of functional diarrhea. *Current treatment options in gastroenterology, 9*(4), 331-342.

Devi, B. P., Boominathan, R., and Mandal, S. (2002). Evaluation of anti-diarrheal activity of *Cleome viscosa* L. extract in rats. *Phytomedicine, 9*(8), 739-742.

Demographic E. Health survey (2011). Addis Ababa. Ethiopia and Calverton, Maryland, USA: Central Statistical Agency and ICF International; 2012.

Drossman, D.A. and Dumitrascu, D.L. (2006). Rome III: New Standard for Functional Gastrointestinal Disorders. J*ournal Gastrointestin Liver Disease*.Vol.15 No.3, 237-241.

Farthing, M., M. Salam, M., Dite, P., Salazar-Lindo, E., Ramakrishna, B.S., Thomson, A., Khan, A.G., Krabshuis, J. and LeMair, A. (2012). Acute diarrhea in adults and children: a global perspective. *Journal of clinical gastroenterology, 47*(1), 12-20.

Farthing, M. and Casburn-Jones, A., (2004). Management of infectious diarrhoea. *Gut, 53*(2), 296-305.

Farthing, M. J. (2006). Antisecretory drugs for diarrheal disease. *Digestive diseases, 24*(1-2), 47-58.

Fenetahun, Y., Eshetu, G., Worku, A., and Abdella, T. (2017). A survey on medicinal plants used by traditional healers in Harari regional State, East Ethiopia. *Journal of Medicinal Plants Studies, 5*, 85-90.

Fentahun, M., Ayele, Y., Amsalu, N., Alemayehu, A., and Amsalu, G. (2017). Antibacterial evaluation and phytochemical analysis of selected medicinal plants against some pathogenic enteric bacteria in Gozamin District, Ethiopia. *Journal of Pharmacovigilance, 5*(5).

Fernández-Bañares, F., Accarino, A., Balboa, A., Domènech, E., Esteve, M., Garcia-Planella, E., Ruiz-Cerulla, A., Jordi Guardiolaf, J., Molerob, X., Rodríguez-Lunag, A., Ruiz-Cerullaf ,A., Santosb, J., Vaquero, E., (2016). Chronic diarrhoea: Definition, classification and diagnosis. *Gastroenterología y Hepatología (English Edition), 39*(8), 535-559.

Field, M. (2003). Intestinal ion transport and the pathophysiology of diarrhea. *The Journal of clinical investigation, 111*(7), 931-943.

Guarino, A., Guandalini, S., and Vecchio, A. L. (2015). Probiotics for prevention and treatment of diarrhea. *Journal of clinical gastroenterology, 49*, S37-S45.

Gutiérrez, S. P., Sánchez, M. A. Z., González, C. P., and García, L. A. (2007). Antidiarrhoeal activity of different plants used in traditional medicine. *African journal of Biotechnology, 6*(25).

Hämäläinen, M., Nieminen, R., Asmawi, M. Z., Vuorela, P., Vapaatalo, H., and Moilanen, E. (2011). Effects of flavonoids on prostaglandin E2 production and on COX-2 and mPGES-1 expressions in activated macrophages. *Planta medica, 77*(13), 1504-1511.

Hart, C., and Umar, L. (2000). Diarrhoeal disease. *Tropical doctor, 30*(3), 170-172.

Hassan, K. A., Brenda, A. T., Patrick, V., and Patrick, O. E. (2011). *In vivo* antidiarrheal activity of the ethanolic leaf extract of *Catharanthus roseus* Linn.(Apocyanaceae) in Wistar rats. *African Journal of Pharmacy and Pharmacology, 5*(15), 1797-1800.

Herling, A. W. (2016). Guidelines for the Care and Use of Laboratory Animals. In Hock F.J. (eds). Drug Discovery and Evaluation: Pharmacological Assays; 4th edition: 4259-4280.

Kamath, A., Shetty, K., Unnikrishnan, B., Kaushik, S., and Rai, S. N. (2018). Prevalence, patterns, and predictors of diarrhea: a spatial-temporal comprehensive evaluation in India. *BMC public health, 18*(1), 1288.

Kareem R, Irshad S, Asad R, Alam S. Cultural and traditional practices for the management of diarrhea in children under 5 years of age in selected suburbs of Khyber Pakhtunkhwa (2016). *Journal Rehman Medical Institute*; 2(3):12-23.

Kaur, M., Singh, A., and Kumar, B. (2014). Comparative antidiarrheal and antiulcer effect of the aqueous and ethanolic stem bark extracts of Tinospora cordifolia in rats. *Journal of Advanced Pharmaceutical Technology and Research, 5*(3), 122.

Kelly, L., Jenkins, H., and Whyte, L. (2018). Pathophysiology of diarrhoea. *Paediatrics and Child Health, 28*(11), 520-526.

Kent, A. J., and Banks, M. R. (2010). Pharmacological management of diarrhea. *Gastroenterology Clinics, 39*(3), 495-507.

Khan, M. A., Agize, M., Shonga, A., and Tora, A. (2018). The Utilization and Conservation of Plants of Medicinal Value by Local Traditional Medicinal Practitioners and the Associated Indigenous Knowledge in Dawuro Zone of Ethiopia: Northeast Africa—An Ethnobotanical Approach. *In Plant and Human Health, Volume 1* (267-321).

Kidane, B., van Andel, T., van der Maesen, L. J. G., and Asfaw, Z. (2014). Use and management of traditional medicinal plants by Maale and Ari ethnic communities in southern Ethiopia. *Journal of ethnobiology and ethnomedicine, 10* (1), 46.

Kiela, P. R., and Ghishan, F. K. (2016). Physiology of intestinal absorption and secretion. *Best Practice and Research Clinical Gastroenterology, 30*(2), 145-159.

Kotloff, K. L., Platts-Mills, J. A., Nasrin, D., Roose, A., Blackwelder, W. C., and Levine, M. M. (2017). Global burden of diarrheal diseases among children in developing countries: Incidence, etiology, and insights from new molecular diagnostic techniques. *Vaccine, 35*(49), 6783-6789.

Lee, K. J. (2015). Pharmacologic agents for chronic diarrhea. *Intestinal research, 13*(4), 306.

Li, Z., and Vaziri, H. (2012). Treatment of chronic diarrhoea. *Best Practice and Research Clinical Gastroenterology, 26*(5), 677-687.

Mbagwu, H., and Adeyemi, O. (2008). Anti-diarrhoeal activity of the aqueous extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae). *Journal of Ethnopharmacology, 116* (1), 16-20.

Mekonnen, B., Asrie, A. B., and Wubneh, Z. B. (2018). Antidiarrheal activity of 80% methanolic leaf extract of *Justicia schimperiana*. *Evidence-Based Complementary and Alternative Medicine, 2018*.

Mesfin, F., Seta, T., and Assefa, A. (2014). An ethnobotanical study of medicinal plants in Amaro Woreda, Ethiopia. *Ethnobotany Research and Applications, 12*, 341-354.

Mohamed, A., Aldaw, M., Ismail, E., Abu-algasim, A., and Karar, E. (2017). Evaluation of antimicrobial activity of different solvent extracts of *Saussurea lappa*, *World Journal of Pharmacy and Pharmaceutical Sciences, 6*(9), 12-18.

Navaneethan, U., and Giannella, R. A. (2010). Definition, Epidemiology, Pathophysiology, Clinical Classification, and Differential Diagnosis of Diarrhea. *In Diarrhea* (1-31): Springer.

Ocde, O. (2008). Acute oral Toxicity: up and down procedure. *OECD. Guideline for the Testing of Chemicals, 425*, 1-2.

Oghenesuvwe, E. E., Tedwins, E. J. O., Obiora, I. S., Lotanna, A. D., Treasure, U. N., Ugochukwu, O. M., and Emmanuel, I. E. (2018). Preclinical screening techniques for anti-diarrheal drugs: a comprehensive review. *American Journal of Physiology, 7*(2), 61-74.

Palombo, E. A. (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 20*(9), 717-724.

Panda, S. K., Patra, N., Sahoo, G., Bastia, A. K., and Dutta, S. K. (2012). Anti-diarrheal activities of medicinal plants of Similipal Biosphere Reserve, Odisha, India. *International Journal of Medicinal and Aromatic Plants, 2*(1), 123-134.

Pandey, P., Mehta, A., and Hajra, S. (2012). Antidiarrhoeal activity of ethanolic extracts of Ruta graveolens leaves and stem. *Asian Journal of Pharmaceutical and Clinical Research, 5*(4), 65-68.

Paton, A. J., Friis, I., and Demissew, S. (2018). A new species of Leucas, *L. gypsicola* (Lamiaceae), from gypsum outcrops in eastern Ethiopia. *Kew bulletin, 73*(4), 59.

Pérez-Gutiérrez, S., Zavala-Mendoza, D., Hernández-Munive, A., Mendoza-Martínez, Á., Pérez-González, C., and Sánchez-Mendoza, E. (2013). Antidiarrheal activity of 19-deoxyicetexone isolated from *Salvia ballotiflora Benth* in mice and rats. *Molecules, 18*(8), 8895-8905.

Philip, N. A., Ahmed, N., and Pitchumoni, C. S. (2017). Spectrum of drug-induced chronic diarrhea. *Journal of clinical gastroenterology, 51*(2), 111-117.

Prakash, V. (2017). Terpenoids as source of anti-inflammatory compounds. *Asian Journal of Pharmaceutical and Clinical Research, 10*(3), 68-76.

Qnais, E., Elokda, A., Abu Ghalyun, Y., and Abdulla, F. (2007). Antidiarrheal Activity of the Aqueous extract of *Punica granatum*. (Pomegranate) peels. *Pharmaceutical Biology, 45*(9), 715-720.

Rahman, M. A., Sultana, R., Akter, R., and Islam, M. S. (2013). Antidiarrheal and antidiabetic effect of ethanol extract of whole *Ageratum conyzoides* L. in albino rat model. *African Journal of Pharmacy and Pharmacology, 7*(23), 1537-1545.

Raja, R. R. (2012). Medicinally potential plants of Labiatae (Lamiaceae) family: an overview. *Research journal of medicinal plant, 6*(3), 203-213.

Rawat, P., Singh, P. K., and Kumar, V. (2017). Evidence based traditional anti-diarrheal medicinal plants and their phytocompounds. *Biomedicine and Pharmacotherapy, 96*, 1453-1464.

Rho, J.-R., Jun, C.-S., Ha, Y., Yoo, M.-J., Cui, M.-X., Baek, H.-S., . . . Chai, K.-Y. (2007). Isolation and characterization of a new alkaloid from the seed of Prunus persica L. and its anti-inflammatory activity. *Bulletin of the Korean Chemical Society, 28* (8), 1289-1293.

Riddle, M. S., DuPont, H. L., and Connor, B. A. (2016). ACG clinical guideline: diagnosis, treatment, and prevention of acute diarrheal infections in adults. *American Journal of Gastroenterology, 111*(5), 602-622.

Riddle, M.S., Connor, B.A., Beeching, N.J., DuPont, H.L., Hamer, D.H., Kozarsky,P., Libman, L., Steffen, R., Taylor, D., Tribble, D.R., Vila, J., Zanger, P. and Ericsson, C.D. (2017). Guidelines for the prevention and treatment of travelers' diarrhea: a graded expert panel report. *Journal of Travel Medicine*, Vol. 24, Suppl 1, S63–S80.

Riddle, M. S. (2018). Current management of acute diarrheal infections in adults. *Polish archives of internal medicine, 128* (11), 685-692.

Sagar, L., Sehgal, R., and Ojha, S. (2005). Evaluation of antimotility effect of Lantana camara L. var. acuelata constituents on neostigmine induced gastrointestinal transit in mice. *BioMed Central Complementary and Alternative Medicine, 5* (1), 18.

Salako, O. A., Akindele, A. J., Shitta, O. M., Elegunde, O. O., and Adeyemi, O. O. (2015). Antidiarrhoeal activity of aqueous leaf extract of *Caladium bicolor* (Araceae) and its possible mechanisms of action. *Journal of Ethnopharmacology,* 176, 225-231.

Sarin, R. V., Narwal, S., and Bafna, P. A. (2013). Anti-diarrhoeal activity of aqueous extract of *Ocimum kilimandscharicum*. *Journal of Ethnopharmacology, 148* (1), 223-228.

Scheen, A.-C., and Albert, V. A. (2009). Molecular phylogenetics of the Leucas group (Lamioideae; Lamiaceae). *Systematic Botany, 34*(1), 173-181.

Schiller LR, Ana CAS, Morawski SG, Fordtran JS (1984). Mechanism of the antidiarrheal effect of loperamide. Gastroenterology.; 86:1475-1480

Schiller, L. R. (2012). Definitions, pathophysiology, and evaluation of chronic diarrhoea. *Best Practice and Research Clinical Gastroenterology, 26*(5), 551-562.

Schiller, L. R., Pardi, D. S., Spiller, R., Semrad, C. E., Surawicz, C. M., Giannella, R. A., Kreis, J.G., Farthing, J.G. and Sellin, J. H. (2014). Gastro 2013 APDW/WCOG Shanghai working party report: chronic diarrhea: definition, classification, diagnosis. *Journal of gastroenterology and hepatology, 29*(1), 6-25.

Sellin, J. H. (2001). The pathophysiology of diarrhea. *Clinical transplantation, 15*, 2-10.

Semenya, S., and Maroyi, A. (2012). Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa. *Journal of ethnopharmacology, 144*(2), 395-401.

Shahed-Al-Mahmud, M., Jahan, T., and Islam, M. T. (2018). Antidiarrheal activities of hydroalcoholic extract of *Sida cordifolia* roots in Wister albino rats. *Oriental Pharmacy and Experimental Medicine, 18*(1), 51-58.

Shane, A. L., Mody, R. K., Crump, J. A., Tarr, P. I., Steiner, T. S., Kotloff, K., M. Langley, J., Wanke, C., Warren, A., K. Pickering, L. Cheng, A. C. (2017). 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. *Clinical Infectious Diseases, 65*(12), e45-e80.

Sheel, R., Nisha, K., and Kumar, J. (2014). Preliminary phytochemical screening of methanolic extract of Clerodendron infortunatum. *Journal of Applied Chemistry, 7*(1), 10-13.

Shrivastava, A.K., S.Kumar, N.K. Mohakud, M. Suar, and P.S. Sahu, "Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India," *BioMed Central Gut Pathogens*,vol.9.

Sisay, M., Bussa, N., and Gashaw, T. (2019). Evaluation of the Antispasmodic and Antisecretory Activities of the 80% Methanol Extracts of *Verbena officinalis* L: Evidence From *In Vivo* Antidiarrheal Study. *Journal of evidence-based integrative medicine, Journal of Evidence-Based Integrative Medicine Volume 24: 1-9*.

Sisay, M., Engidawork, E., and Shibeshi, W. (2017). Evaluation of the antidiarrheal activity of the leaf extracts of *Myrtus communis* Linn (Myrtaceae) in mice model. *BioMed Central complementary and alternative medicine, 17*(1), 103.

Spiller, R. (2007). Chronic diarrhoea. *Gut, 56*(12), 1756.

Syder JD, Merson MH (1982). The magnitude of the global problems of acute diarrheal disease: a review of active surveillance data. Bulletin of the WHO, 60:605–613.

Tack, J. (2012). Functional diarrhea. *Gastroenterology Clinics, 41*(3), 629-637.

Tadesse, E., Engidawork, E., Nedi, T., and Mengistu, G. (2017). Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara Linn* (Verbenaceae) in mice. *BioMed Central complementary and alternative medicine, 17*(1), 190.

Tadesse, W. T., Hailu, A. E., Gurmu, A. E., and Mechesso, A. F. (2014). Experimental assessment of antidiarrheal and antisecretory activity of 80% methanolic leaf extract of *Zehneria scabra* in mice. *BioMed Central Complementary and Alternative medicine, 14*(1), 460.

Teklay, A., Abera, B., and Giday, M. (2013). An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. *Journal of ethnobiology and ethnomedicine, 9* (1), 65.

Thiagarajah, J. R., Donowitz, M., and Verkman, A. S. (2015). Secretory diarrhoea: mechanisms and emerging therapies. *Nature Reviews Gastroenterology and Hepatology,* 12 (8), 446.

Umer, S., Tekewe, A., and Kebede, N. (2013). Antidiarrhoeal and antimicrobial activity of C*alpurnia aurea* leaf extract. *BMC complementary and alternative medicine,* 13 (1), 21.

UNICEF and WHO, D. (2009). Why children are still dying and what can be done. New York: The United Nations Children's Fund (UNICEF). *World Health Organization (WHO)*.

Venkateshappa, S., and Sreenath, K. (2013). Potential medicinal plants of Lamiaceae. *American international journal of research in formal, applied and natural sciences,* 1 (3), 82-87.

Wansi, S. L., Deumeni, C. R. M., Laure, S., Kamani, P., Sama, L. F., and Tchoumba, L. M. (2017). Antidiarrhoeal activity of aqueous and methanolic *Alchornea laxiflora* (Euphorbiaceae) leaves extracts in rats. *Journal of Medicinal Plants, 5* (1), 205-211.

Whyte, L., and Jenkins, H. (2012). Pathophysiology of diarrhoea. *Paediatrics and child health, 22*(10), 443-447.

Woldeab, B., Regassa, R., Alemu, T., and Megersa, M. (2018). Medicinal plants used for treatment of diarrhoeal related diseases in Ethiopia. *Evidence-Based Complementary and Alternative Medicine, 2018*.

Yacob, T., Shibeshi, W., and Nedi, T. (2016). Antidiarrheal activity of 80% methanol extract of the aerial part of *Ajuga remota Benth* (Lamiaceae) in mice. *BioMed Central Complementary and Alternative medicine, 16*(1), 303.

Yemane, B., Medhanie, G., and Surender Reddy, K. (2017). Survey of some common medicinal plants used in Eritrean folk medicine. *American Journal of Ethnomedicine,* 4 (2), 14.

W.H.O. (1987). Report of the Second Meeting of Directors of WHO Collaborating Centres for Traditional Medicine. *WHO, Geneva, Swiszerland*. (Document WHO/ TRM/88.1)