ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

ANTIDIARRHEAL AND ANTISPASMODIC ACTIVITIES OF ESSENTIAL OIL OF MYRTUS COMMUNIS L

By

ADUGNA CHALA (B. pharm)

October, 2011

ANTIDIARRHEAL AND ANTISPASMODIC ACTIVITIES OF ESSENTIAL OIL OF MYRTUS COMMUNIS L.

By Adugna Chala

A Thesis Submitted to Department of Pharmacology and Therapeutics, School of Pharmacy, Addis Ababa University in partial fulfillment for the requirements of degree of Master of Science in Experimental Pharmacology

Under the supervision of Dr. Ephrem Engidawork, PH D., Associate Professor of Pharmacology, Department of Pharmacology and Therapeutics, School of Pharmacy, Addis Ababa University and Dr. Kaleab Asres, PH D., Department of Pharmaceutical chemistry and Pharmacognosy, School of pharmacy, Addis Ababa University

October, 2011

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

ANTIDIARRHEAL AND ANTISPASMODIC ACTIVITIES OF ESSENTIAL OIL OF MYRTUS COMMUNIS L

By

Adugna Chala

Department of Pharmacology and Therapeutics,

School of Pharmacy

Approved by Examining Board:

Name	Signature	Date
Dr. Ephrem Engidawork (Ph D)		
(Advisor)		
Dr. Kaleab Asres (Ph D)		31/10/11
(Advisor)		
Dr. Workineh Shibeshi (Ph D)		31/10/11
(Examiner)		

ACKNOWLEDGMENT

I would like to express my sincere thanks to my advisor, Dr. Ephrem Engidawork for his invaluable advice and guidance throughout the course of this project. I would also thank my co-advisor Dr. Kaleab Asres for his priceless advice starting from selection of plant material up to completion of the work.

Many thanks to Ato Yohanes Ayele and Kelil Haji for their help during my practical work and I greatly appreciate W/ro Fantu and Ato Haile for their kind help in technical support. I want to extend my thanks to EHNRI for their kind hospitality and permission to use premises and facilities at the Drug Research Department.

At last, special thanks to School of Graduate studies for the opportunity and financial support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

5-HT: 5-hydroxytryptamine

ADI: In vivo antidiarrheal index

cAMP: Cyclic Adenosine Monophosphate

CCK: Cholecystokinin

CF: cystic fibrosis

CFTR: Cystic fibrosis transmembrane conductance regulator

cGMP: Cyclic guanine Monophosphate

ClC-2: Chloride channel-2

Dfreq: Delay in defecation time

EHNRI: Ethiopia Health and Nutrition Research Institute

E_{max}: Maximum contraction

ENS: Enteric nervous system

EOMC: Essential oil of *Myrtus communis*

GIT: Gastrointestinal tract

GLP-2: Glucagon-like peptide-2

Gmeq: Gut motility reduction

GPI: Guinea pig ileum

ICC: interstitial cells of Cajal

LD50: Median lethal dose

NHEs: Na/H exchangers

NO: Nitric oxide

OECD: Organization for Economic Cooperation and Development

OG: Osmotic gap

ORS: Oral rehydration solution

pA_2: The negative logarithm of the concentration of antagonist that causes a concentration ratio of 2

pD_2: The negative logarithm of the concentration of agonist that produce 50% of maximum effect

Pfreq: Purging frequency

PI: Peristaltic index

PKA: Protein kinase A

VIP: Vasoactive intestinal peptide

WHO: World Health Organization

ABSTRACT

The essential oil of *Myrtus communis* (EOMC) was evaluated for its antidiarrheal and antispasmodic potential against isolated guinea ileum (GPI), *ex-vivo* antispasmodic model; normal and castor oil-induced intestinal transit in mice; castor oil-induced diarrhea in mice and prostaglandin induce enteropooling in rats. Atropine was used in GPI and normal intestinal transit test as a positive control, whereas loperamide was used in the castor oil-induced intestinal transit and castor oil-induced antidiarrheal test. EOMC inhibited normal intestinal transit siginificantly ($p<0.05$) and the effect was comparable with that of atropine. All doses (100, 200 and 400 mg/kg) of the oil employed showed significant antidiarrheal and antienteropooling activities which was comparable with that of the positive control loperamide. Different concentrations of the essential oil were used in the presence of agonist (Acetylcholine) in GPI as contraction stimulator *in ex-vivo*. The oil exhibited significant reductions in Acetylcholine-induced contractions of GPI. The agonist-induced contractions of GPI were greatly reduced by both doses of 50 µg/ml ($p<0.01$) and 100 µg/ml ($p<0.01$), suggesting a powerful spasmolytic property of the oil. The effect produced showed that the oil is much more efficacious than atropine (6.66×10^{-9} M) *in ex-vivo* model. The oil appears to be more efficacious *in ex-vivo* than *in vivo* which may be due to the difference in physiological conditions that exists between the two systems. This study suggested that the essential oil of *M. communis* possesses spasmolytic and antidiarrheal properties which are likely to be due to the α-pinene and linalool present in the oil. The spasmolytic and antidiarrheal mechanisms might be in part mediated via Ca^{+}-channel blockage. The results obtained in this study also support the traditional use of the plant for stomach pains, and diarrhea. However, further study should be conducted in order to determine the exact mechanism (s) of action of the oil and also to characterize the constituents responsible for the activity observed.

Key words: *M. communis,* essential oil, antidiarrheal, antispasmodic, antienteropooling

1 INTRODUCTION

1.1 Overview of diarrhea

Diarrhea is an increased frequency and decreased consistency of fecal discharge as compared with an individual's normal bowel pattern. Frequency and consistency are variable from person to person i.e. some individuals defecate as many as 3 times a day, while others defecate only 2 or 3 times per week (Barbara et al., 2006). Increased frequency or softer consistency of bowel movements may not lead to real diarrhea i.e. hyperdefecation, or incontinence should be distinguished from diarrhea. According to World Health Organization (WHO) (2009) diarrhea is defined as three or more watery or loose bowel movements in a 24 h period. In addition to these sometimes stool weight is used to define diarrhea and stool weight greater than 300 g per day generally indicates diarrhea (Patil et al., 2011). A combination of frequency, stool consistency, and stool weight could be taken into account for defining diarrhea.

According to World Health Organization (WHO) (1996), diarrhea is one of the main causes of high mortality rate in developing countries where over five million children under the age of five die annually from severe diarrheal diseases. WHO also documented that 3-5 billion cases occur annually, and approximately 5 million deaths are accountable to diarrhea (Heinrich et al., 2005). Acute diarrhea is the prevalent form of diarrhea and the major causes of morbidity and mortality in all age groups particularly in infants and children (Guandalini, 1997; Amole et al., 2010).

The success of the twentieth century regarding health care can be evidenced by the dramatic decline in mortality, increase in life expectancy and the eradication of smallpox. The tool for this success was scientific innovation leading to development of new drugs and medicines. Despite the emergence of a number of drugs; none has found a place in the routine management of diarrhea. Therefore, there is a need for continuous search for drugs that might inhibit the process of diarrhea development (Farthing, 2002).

1.2 Physiology of the small intestine

The alimentary tract forms the contact surface between the environment and the interior and acts as a dynamic interface allowing exchange of water, electrolytes, and nutrients but still serves as an internal barrier which prevents pathogenic microorganisms, toxins and allergenic macromolecules to enter the interior. To do these activities it is equipped with smooth nonvascular muscle cells, secretory epithelia, interstitial cells of Cajal (ICC), endocrine cells, and gut-associated lymphoid tissue (Janig, 2009; Metges, 2010).

A typical cross section of the gastrointestinal tract (GIT) wall includes the following layers starting from the lumen:

i. Mucosal layer

Mucosal layer is the largest organ in the body, covering approximately 300 to $400m^2$. It consists of epithelial cells (cubiodal or columnar cells) attached to each other and it's responsible for mucus secretion. Beneath mucosa there is submucosal layer which consists of collagen, elastin, gland and the blood vessels of GIT. The lumen of stomach, small intestine, and colon; is lined with a single layer of absorptive epithelial cells; whereas the esophagus contains a squamous epithelial cell lining (Stevens et al., 2000).

ii. Submucosal plexus and myenteric plexus

The myenteric plexus, also known as Auerbach's plexus, and the submucosal plexus, also known as Meissner's plexus, are the two ganglionated plexuses of the enteric nervous system (ENS). The myenteric plexus is located between the longitudinal and circular muscle of the GIT, whereas the submucosal plexus is situated in the submucosal region between the circular muscle and mucosa. The later one is the most prominent as a ganglionated network in the small and large intestine and does not exist as a ganglionated plexus in the esophagus and is sparse in the submucosal space of the stomach. Motor neurons innervating circular and longitudinal muscle originate from neurons that have their cell bodies in the myenteric plexus, whereas motor neurons innervating intestinal secretory glands (i.e., Brunner's glands in the duodenum and crypts of Lieberkuhn in both small and large intestines) have their cell bodies in the

submucosal plexus. The two ganglions are interconnected by neurons and form a functionally integrated nervous system. Ganglia in the ENS are similar to that of the central nervous system structurally, and functionally, but not to autonomic ganglia. In addition to the ENS, the GIT is also innervated with extrinsic nerves (Lundgren and Jodal, 1997).

iii. Circular and longitudinal muscles

Smooth muscle of the GIT is organized as two layer separated by neuronal network. The outer layer is the longitudinal muscle in which the smooth muscle cells are oriented along the length of the GIT. The inner layer is the circular muscle where the smooth muscle cells are arranged transversally to the length of GIT (Lecci et al., 2002). The longitudinal and circular muscles via their interaction with ENS and an inherent pace-maker system execute mixing and propulsive movements of the GIT; i.e. synchronized contraction of the two muscles produce peristalsis whereas independent movements (contractions) of the muscles produce mixing of chyme (Stevens et al., 2000; El-Yazbi et al., 2007).

iv. Serosa

Serosa is the outer most layer of the GIT wall which continues into the mesentery, and contains the nerve, lymphatic, and blood vessels supplying the tract. The structure of the esophagus is similar except that it has no mesentery (Guyton and Halls, 2006).

1.2.1 Normal motility of the small intestine and its function

As the small intestine is a circular tube, contraction of its muscles results in narrowing of the tube. The contraction of the muscle at different site is synchronized and as a result the content move in one direction (usually downstream, but occasionally upstream for short distances). Depolarization and redepolarization of gut smooth muscles is the base for GIT motility. These depolarization and repolarisztions occur in a cyclic pattern and are called slow waves. For action potential to develop and cause contraction the depolarization must reach a threshold which needs some kind of additional external

stimulus, like nervous or hormonal input. The muscle cells have no intrinsic characteristic of initiating the slow waves rather it is initiated in the ICCs. The ICCs are electrically coupled via gap junctions, and can function as pacemakers, setting the smooth muscle slow wave frequency. GIT motility differs in fed and fasting state. Food intake induces propagating, propulsive contractile activity. This unidirectional squeeze of GIT content is termed as peristalsis. Localized contractions and relaxation also happen after food intake and serve to mix chyme (a thick fluid mass of partially digested food and gastric secretions passed from the stomach to the small intestine) with intestinal digestive enzymes. During fasting state slow propagating contractions called migrating motor complexes predominate (Thomas, 2006; Olsson and Holmgren 2010).

Each of the small intestine functions is performed by a group of specialized cells, which can be classified as muscle, crypt, villous, Paneth and goblet cells. These functions include digestion, motility, secretion, absorption and defense. The digestion process ends in the small intestine with the help of digestive enzymes from the small intestine itself and from pancreas. Absorption of nutrients is the other function of the small intestine. The structure of small intestine is adapted for absorption. The folding, villi, and microvilli help it to have large surface area needed for absorption. A third function of the small intestine is movement of nondigested remains to the large intestine and secretion. The last function is defense and the different secretions and the physical barriers are the means by which the intestine executes this function (Mourad and Saade, 2011).

Brunner's glands which are mucous glands located in the wall of the first few centimeters of the duodenum secrete large amounts of alkaline mucus which protects the wall of duodenum from gastric content. Crypts of Lieberkühn are small pits located over the entire surface of the small intestine. They are situated in between villi and their surface is covered by epithelium made of goblet cells (secrete mucus) and enterocytes (secrete large quantities of water and electrolytes). The enterocytes of the epithelium covering the surface of villi are responsible for reabsorption of water and electrolytes along with end products of digestion (Guyton and Halls, 2006; Reed and Wickham, 2009).

The mammalian small intestine performs both absorption and secretion processes at a time. Under normal conditions actually the net process is absorption. Along with transportation of substances from the lumen into the enterocyte, there is simultaneous recirculation of water and electrolytes, exiting from the crypts into the lumen. It is

believed to involve two active secretory processes: the first one is active secretion of chloride ions into the crypts and the second is active secretion of bicarbonate ions. The secretion of both of these ions causes electrical drag as well of positively charged sodium ions through the membrane and into the secreted fluid. At last these ions all together cause osmotic movement of water (Wapnir and Teichberg, 2002; Guyton and Halls, 2006).

Many crypts of Lieberkühn are found in the mucosa of large intestine; however, unlike the small intestine, there are no villi. No digestive enzymes are secreted instead the mucous cells secrete only mucus. The large proportion of secretion in the large intestine is mucus. This mucus contains moderate amounts of bicarbonate ions secreted by a few non-mucus-secreting epithelial cells. The rate of secretion of mucus is regulated principally by direct, tactile stimulation and local nervous reflexes (Guyton and Halls, 2006; Reed and Wickham, 2009).

Around ten liters of ingested fluid and secretions enter intestine every day. Ninety percent of this fluid is absorbed in the small intestine and ninety percent of the remaining is absorbed in the large intestine. Water transport across the wall of intestine is not by active transport rather secondary to the active transport of some other solutes. When the chyme is dilute enough, water is absorbed through the intestinal mucosa into the blood of the villi almost entirely by osmosis. When it is concentrated it will be made diluted by transport of solutes out of lumen to the blood. Sodium is transported by the sodium potassium ATPase pump which ensures that the inside of enterocytes have a low sodium concentration and a negative charge. Sodium enters epithelial cells from the lumen and then taken to the paracellular space by the pumps located in the basal and side walls of these cells. Part of the sodium is absorbed along with chloride ions; in fact, the negatively charged chloride ions are mainly passively "dragged" by the positive electrical charges of the sodium ions. The electrical and osmotic gradient created by this pump drives most intestinal transport processes (Bisset, 2001; Camilleri, 2004).

The absorption of sodium from the lumen actually involves two stages. The first is the active transport that depletes sodium from the epithelial cells. This actually pave the way for the next stage to come to picture, i.e. decrease of sodium and the more negative inside the cells causes sodium from the intestinal lumen to move through the brush border of the epithelial cells to the cell interiors by a process of facilitated diffusion. That

is to mean, a sodium ion combines with a transport protein, but the transport protein will not transport sodium to the interior of the cell until the protein itself also combines with some other appropriate substance. The binding of protein with other substance is the bases for absorption of monosacchrides (glucose and galactose in particular) and amino acids. Some oligopeptides, instead of being first hydrolyzed into amino acids, are absorbed intact across the intestinal brush border by a proton-coupled mechanism. This absorptive process is indirectly coupled to Na^+ transport, since the needed protons are provided by Na/H exchange, which acidifies the unstirred layer abutting the brush border membrane. The Na^+ gradient, therefore, is the driving force for amino acid, oligopeptide, and sugar absorption (Bisset, 2001; Field, 2003; Camilleri, 2004).

Segments of jejunum and ileum involve salt absorption in the absence of nutrients. This indicates that there is a different transport mechanism in these Segments. In the jejunum, $NaHCO_3$ is absorbed via Na/H exchange (the secreted H^+ neutralizes an equivalent amount of luminal $HCO3^-$) and Cl^- movement is purely passive. In the ileum (and also in the proximal colon) NaCl is absorbed via equal rates of Na/H and Cl/HCO_3 exchanges. Three Na/H exchangers (NHEs) have since been localized to intestinal brush border membranes and cloned. NHE3 appears to be quantitatively more important, since NHE3 knockout mouse suffers from chronic diarrhea (Field, 2003).

The discovery of secretory property of intestinal epithelium was among the late twenty century achievements of human beings. Cyclic Adenosine Monophosphate (cAMP), via protein kinase A (PKA), stimulates secretion by activating or enhancing the transport activities of: apical anion channel, basolateral membrane K^+ channel and basolateral membrane NaK2Cl cotransporter (Fig 1). The inherited disease cystic fibrosis (CF) develops when dysfunctional mutations occur in the gene for the cAMP-responsive apical anion channel. Accordingly, this channel, which is present in a number of tissues, has been dubbed the cystic fibrosis transmembrane conductance regulator or CFTR for short. Studies of intestine from both humans and transgenic mice with CF show a near total absence of electrolyte secretion. This explains the secretory role of CFTR (Field, 2003).

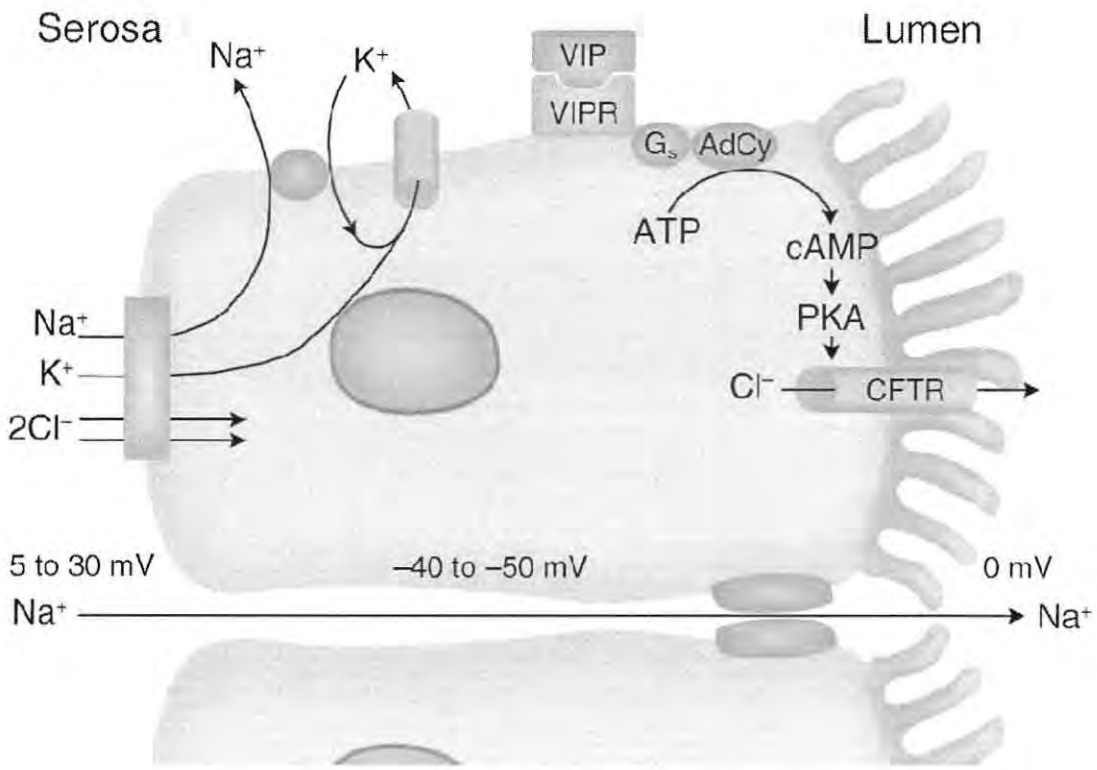


Fig 1: A model of electrolyte secretion in the intestine (The picture was taken from Field (2003)).

1.3 Regulation of small intestine motility and secretion

The ENS, the extrinsic autonomic nervous system and local hormones play an important role in regulating GIT functions. These neural and hormonal regulations are responsible for the coordinated activity of GIT. The number of neurons in this enteric system is almost exactly equal to the number in the spinal cord. This tells us that the ENS plays an important role in controlling GIT motility and secretion (Wapnir and Teichberg, 2002; Olsson and Holmgren 2010).

GI smooth muscles display cyclic patterns of electrical activity that start in ICCs and spread passively throughout the ICC network as well as to the muscle cells. Neuronal or hormonal input converts slow wave activity to muscle contractions and secretion. To understand the complex regulatory mechanisms of small intestine motility and secretion knowledge of neural reflex is crucial. Sensory cells (Enterochromaffin cells) receive and detect stimuli in the form of force or chemical or mechanical and respond by triggering release of 5-hydroxytryptamine (5-HT). 5-HT then activates the afferent neurons (both

submucosal and myenteric neurons) to initiate a neural reflex pathway to stimulate secretomotor neurons which release vasoactive intestinal peptide (VIP). VIP then acts on epithelial cells and cause Cl^- secretion and water movement into the intestinal lumen. On the other hand, stretch directly stimulates both intrinsic and extrinsic enteric neurons and causes biological response such as opening of ion channels. As a result, depolarization, firing of action potentials and initiation of the reflex which accompanied by secretion and small intestine motility will occur (Xue et al., 2007).

Although the ENS can function on its own, independently of extrinsic sympathetic and parasympathetic nervous system, stimulation by these systems can greatly enhance or inhibit gastrointestinal functions. Stimulation of parasympathetic nerves causes general increase in activity of the entire ENS and hence that of gastrointestinal functions. However, stimulation of the sympathetic nervous system inhibits activity of the gastrointestinal tract (Guyton and Halls, 2006).

Different types of neurotransmitter substances are released by the nerve endings of different types of enteric neurons. The effect of most of these transmitters is not known except the joint effect. These neurotransmitters include: acetylcholine (most often excites gastrointestinal activity), norepinephrine (almost always inhibits gastrointestinal activity), substance P, VIP, 5-HT, cholecystokinin (CCK), guanylins (mediates secretion), peptide YY, glucagon-like peptide-2 (GLP-2), enkephalins (proabsorptive) ATP, dopamine, somatostatin and others (Lundgren and Jodal, 1997; Wapnir and Teichberg, 2002; Field, 2003).

1.4 Etiology and pathogenesis of diarrhea

Diarrhea can be classified based on causative agent. The causative agents decrease absorption of solutes and water and increase secretion of electrolytes. They cause one of the four pathophysiologic mechanisms that disrupt water and electrolyte balance, leading to diarrhea. These four mechanisms are the bases for classification and include: (i) a change in active ion transport by either decreased sodium absorption or increased chloride secretion (Secretory diarrhea); (ii) a change in intestinal motility (Diarrhea due to abnormal intestinal motility); (iii) an increase in luminal osmolarity (Osmotic diarrhea); and (iv) exudation of protein and fluid from the mucosa (Exudative diarrhea). The duration of the disease can also be used to classify diarrhea as the severity parallels

the duration. The two classes of diarrhea under this classification are chronic and acute (Swamy et al., 2005, Barbara et al., 2009; Amole et al., 2010).

1.4.1 Secretory diarrhea

Secretory diarrhea can occur by over stimulation of the intestinal tract's secretory capacity ("pure" secretory diarrhea) and sometimes as component of very complex disorders. This type of diarrhea ("pure" secretory) is characterized by large stool volumes (which can exceed 1 liter per hour in well hydrated adults), absence of red or white blood cells in the stool, absence of fever or other systemic symptoms (except those due to dehydration), persistence of diarrhea with fasting (volume may diminish, however), and lack of excess osmotic gap (OG) in stool electrolytes. OG is defined as: $290 - 2\{[Na+] + [K+]\}$, where 290 is the assumed osmolarity of blood plasma, a gap greater than 50 mM is considered abnormal (Farthing, 2002; Field, 2003).

Secretory diarrhea is caused by abnormalities in both secretion and absorption of electrolytes. GIT stimulating substance like VIP, laxatives, bacterial toxin, hormones generated by endocrine neoplasms, dihydroxy bile acids, hydroxylated fatty acids, and inflammatory mediators all increase secretion or decrease absorption. The central role in these impairments is played by an increase in cAMP which has two basic effects: it inhibits neutral NaCl absorption and secondly it stimulates the secretion of chloride without impairing other transport mechanisms. The impact of the cAMP is evident e.g. in cholera. In cases of cholera, the intestinal mucosa binds the toxin which stimulates adenylate cyclase to produce cAMP. Since other transport mechanisms are intact, rehydration can be achieved by oral application of the solution of glucose with sodium (Field, 2003; Barbara et al., 2009).

Hypersecretion can be associated also with an increased intracellular calcium or cyclic guanine Monophosphate (cGMP). Impairments of the small intestine which cause atrophy of microvilli are associated with an increased secretion of electrolytes (Field, 2003).

1.4.2 Osmotic diarrhea

Osmotic diarrhea is caused by an accumulation of weakly absorbable solutes (lactulose, Mg+, SO_4^-, or PO_3^-) in the intestinal lumen, and/ or as a result of maldigestion, as seen in pancreatic insufficiency. When these molecules are present in the intestinal lumen, osmotic force develops and draws water together with ions into the lumen. On the other hand malabsorption by an individual with an absorptive defect (gluten-sensitive enteropathy, lactase deficiency) can also cause osmotic diarrhea. Osmotic diarrhea caused by the presence of weakly absorbable solutes is typical by the fact that it ceases after abstaining from their intake (Field, 2003; Barbara et al., 2009).

1.4.3 Exudative diarrhea

Inflammatory diseases of the GIT can cause exudative diarrhea by discharge of mucus, proteins, or blood into the gut (Barbara et al., 2009).

1.4.4 Diarrhea due to abnormal intestinal motility

Abnormal motility can enhance bacterial overgrowth within the intestine. Increased motility shortens the contact period of chyme with mucosa. Therefore in consequence of this change, the volume of chyme in the large intestine increases which causes faster evacuation. The consequent abbreviation of contact of chyme with mucosa results in a decrease in water absorption. Diarrhea due to abnormal intestinal motility is usually present in coincidence with irritable bowel syndrome, after gastrectomy, vagotomy, diabetic neuropathy, sclerodermia and thyrotoxicosis (Barbara et al., 2009).

1.4.5 Acute and chronic diarrhea

The four pathophysiologic mechanisms discussed can also be used as means of differentiating diarrheas. In fact duration of diarrheal disease is another parameter that should be considered to fully understand the etiology and pathogenesis of the disease.

Acute diarrhea

Acute diarrhea, (an episode of diarrhea of less than 14 days in duration) is caused by infection, drugs or develops after consuming particular substances in food (Manatsathit

et al., 2002; Barbara et al., 2006). Infectious diarrhea may affect the small intestine and/or the colon. The severity with regard to water and electrolyte loss depends on the part of intestine infected, and infections of the small intestine lead to loss of large volume of liquid stools i.e. excess loss of water and electrolyte. The pathogens most often linked to infectious diarrhea include: enterotoxigenic *Escherichia coli*, *Vibrio cholera,* Salmonella species, Shigella species, *Campylobacter jejuni*, Cryptosporidium species, enteric viruses, *Giardia intestinalis*, *Strongyloides stercoralis*, *Isospora belli*, *Sarcocystis hominis*, and *Cyclospora cayetanensis* (Farthing, 2000).

The cellular pathogenesis of these microbes is presented by taking representative examples i.e. *V. cholerae* and *E. coli*. Cholera Toxin is a protein consisting of a dimeric A subunit and five identical B subunits. The larger, A1 protein of the diameric A subunit, contains the toxic activity. Each of the B subunits functions for binding to cells in the intestinal brush border membrane. The toxin's A1 subunit covalently modifies the α subunit of Gs, the adenylyl cyclase–stimulating G protein. More specifically, the endocytosed A1 subunit catalyzes covalent bonding of adenosine diphosphoribose from NAD to the α-subunit of Gs (Gsα), inhibiting the intrinsic GTPase activity of Gsα thereby preventing self-inactivation of its adenylyl cyclase-stimulating activity. The Gsα-GTP complex separates from its membrane mooring to Gsβ and Gsγ, attaching to and activating adenylyl cyclase. This leads to continuous production of cAMP and thereby activation of PKA. The activated PKA phosphorylates and open the CFTR anion channel (Field, 2003) and anions especially chloride enters into the intestinal lumen which is followed by water being driven by osmotic force.

Toxigenic *E. coli* elaborate at least two secretion-stimulating enterotoxins, heat labile toxin and heat-stable toxin. The former one is immunologically related to cholera toxin and has the same cellular mechanism of action, whereas the latter one activates guanylate cyclase in the intestinal epithelium, thereby activating cGMP-dependent protein kinase G, which, like PKA, can open the CFTR anion channel (Field, 2003).

Chronic diarrhea

Chronic diarrhea, (diarrhea of greater than 14 days in duration) can be caused by different factors. Secretory chronic diarrhea is usually induced by drugs, hormones generated by endocrine neoplasms, bile or fatty acids; whereas Osmotic chronic diarrhea

is usually induced by laxatives, or malabsorption. Inflammatory intestinal diseases (ulcerative colitis, Crohn's disease), ischaemic colitis, parasitic invasions, motility impairments, re-infection or protracted course of intestinal infection, sclerodermia and diabetic neuropathy can also bring about chronic diarrhea (Guandalini, 1997; Barbara et al., 2009; Field, 2003).

1.5 Diarrhea management

1.5.1 Oral rehydration therapy

The main causes of concern with regard to diarrhea are the loss of body water and electrolyte imbalance. Among the population children are at greater risk than adults of life-threatening dehydration. This is so because water constitutes a greater proportion of their bodyweight. The type of dehydration (isotonic, hypotonic, or hypertonic) is independent of the causative agent. Fluid losses resulting from diarrhea and vomiting can be as high as three times the circulating blood volume. In order to keep intravascular volume constant, water is lost from the intracellular compartment to the extracellular, leading to dehydration (Sibylle and Stephanie, 2009).

Means of rehydration should therefore be the first step to treat patients especially children with secretary diarrhea. Oral rehydration solution (ORS) is used to rehydrate these victims. The use of ORS is justifiable particularly after the discovery of effect of cholera toxin. As cholera toxin inhibits nutrient-independent salt absorption, electrolyte absorption is possible when administered with glucose (WHO, 2005).

The shortcoming of such solution is that reabsorption of secreted fluid is not lessened and hence no effect on diarrhea. This may be corrected by substitution of glucose polymers for free glucose in the oral rehydration solution. These polymers release the absorbable sugar slowly as the rate of hydrolysis of starch to maltose is slow compared with the rates of hydrolysis of maltose. The end effect is that there is no enough sugar in the lumen to create hyperosmolarity which can facilitate fluid secretion (Field, 2003).

1.5.2 Antibiotics

Even though antimicrobial therapy has got many limitations (antibacterial resistance and poor tolerability), antibacterial agents are mainstays of treatment for certain forms of

diarrhea. Fluoroquinolones, azithromycin and rifaximin are commonly used for the treatment of bacterial diarrhea. *Clostridium difficile*–induced diarrhea should be treated by withdrawal of the offending antibiotic and oral administration of either metronidazole or vancomycin (Field, 2003; Herbert, 2004).

1.5.3 Antidiarrheals

i) Opioids

The antidiarrheal mechanism of opioids is by altering intestinal motility. In fact some of them may have mild proabsorptive or antisecretory activity. The intestinal motility change is mediated by peripheral μ receptors of the ENS, whereas intestinal secretion is influenced via δ receptor. They may be helpful in secretory diarrhea of mild to moderate severity by reducing the frequency and volume of stools (Manatsathit et al., 2002). Loperamide, diphenoxylate, codeine, tincture opium and other opiates are drugs used as antimotility. Among these drugs, loperamide is the most commonly recommended agent for use in uncomplicated diarrhea. It increases GIT transit time and sphincter tone. It also blocks cholera toxin induced secretion presumably via Gi-linked receptors. Diphenoxylate, difenoxin, and loperamide act peripherally and are preferred over opioids that penetrate the CNS (Manatsathit et al., 2002; Barbara et al, 2009).

Antimotility drugs are contraindicated in severe diarrhea and in diarrhea caused by invasive pathogens. This is due to the fact that pooling of large fluid volumes in paralyzed bowel loops and enhanced tissue invasion by the organisms or delay their clearance from the bowel and hence the disease condition may aggravate (Manatsathit et al., 2002; Field, 2003).

ii) Anticholinergics

Atropine, hyoscine, hyoscyamine and dicyclomine are anticholinergics which may be used to treat diarrhea. Frequency and volume of stools are not reduced by these agents, but they may have value in reducing pain from abdominal cramps. These agents have got many adverse reactions and have limited uses (Manatsathit et al., 2002).

iii) Adsorbents

These medications adsorb toxins produced by toxigenic bacteria and act by preventing their adherence to intestinal membranes. There are a variety of drugs in this group including activated charcoal, kaolin, pectin, dioctahedral smectite, attapulgite (anhydrous aluminum silicate), aluminum hydroxide and tannic acid (Manatsathit et al., 2002).

iv) Antisecretory agents

α_2 Adrenergic receptor agonists such as clonidine can interact with specific receptors on enteric neurons and enterocytes, thereby stimulating absorption and inhibiting secretion of fluid and electrolytes. These agents may have a special role in diabetics with chronic diarrhea, in whom autonomic neuropathy can lead to loss of noradrenergic innervations (Goodman and Gilman, 2006).

Serotonin receptor antagonists specifically the 5-HT_3 receptor antagonists are found to have antisecretory effect. Another newly discovered drug is racecadotril which is an enkephalinase inhibitor that produces antisecretory activity. Bismuth salicylate has been shown to be effective in the treatment of diarrhea. It reduces the number of unformed stools by approximately 50% and this is attributed to the antisecretory action of its salicylate moiety. In addition, it is also thought to have antibacterial and antiinflammatory properties (Manatsathit et al., 2002; Casburn-Jones and Farthing, 2004).

Somatostatin can be used for treatment of diarrhea induced by hormone-secreting neoplasms. Somatostatin analogues (e.g., octreotide) are used in this case. They block hormone production by the tumor cells and hence inhibit diarrhea induced by the hormones. Moreover, they also appear to have a direct antisecretory effect on the gut epithelium and have been employed for treating cancer chemotherapy–induced diarrhea (Field M, 2003).

v) Newer agents

The intestinal ion transport mechanisms which operate using intracellular signalling mechanisms are pharmacological target in the search for antisecretory agents.

Calcium and the calcium binding protein calmodulin and CFTR protein are among the targets on trial. The role of ENS in secretory diarrhea is well established. A large number of neurotransmitters in the ENS have been identified providing another potential target for antisecretory agents (Manatsathit et al., 2002; Casburn-Jones and Farthing, 2004; Sonawane et al., 2008; Alzamora et al., 2011).

1.6 Medicinal plants with antidiarrheal activity

These days the use of traditional medicines is increasing because of their safety, affordability and accessibility (Hollenberg et al., 2008). Nearly 60–80% of the world's population has been using traditional medicines for the treatment of many diseases including diarrhea (Farnsworth et al., 1985; Maikere-Faniyo et al., 1989; Almeida et al., 1995; Ziyyat et al., 1997).

In traditional Indian and Chinese medicine plants of the families Ranuculaceae and Berberidaceae are used for treatment of diarrhea. These plants contain a plant alkaloid called berberine. It has complex pharmacological actions that include antimicrobial actions, stimulation of bile flow, inhibition of ventricular tachyarrhythmias, and possible antineoplastic activity. The antidiarrheal effects of this alkaloid in part may be related to its antimicrobial activity, as well as its ability to inhibit smooth muscle contraction and delay intestinal transit by antagonizing the effects of acetylcholine (by competitive and noncompetitive mechanisms) and blocking the entry of Ca^{2+} into cells. In addition, it inhibits intestinal secretion (Goodman and Gilman, 2006). Furthermore the antidiarheal activity of: *Alhagi maurorum, Asparagus pubesons, Conyza dioscoridis, Conyza linifolia, Mentha microphylla, Moringa oleifera, Occimum grattissimum, Pergularia daemia and zygophyllum album* have been confirmed (Amole et al., 2010; Otimenyin and Uzochukwu, 2010).

These findings and others have convinced WHO to constitute a diarrheal disease control program, which focuses on traditional practices and prevention approaches (Swamy et al, 2005; Amole et al, 2010).

1.7 *Myrtus communis* L

Family Myrtaceae contains important plants used traditionally for medicinal purposes. *Psidium guajava* L. for instance, belonging to this Family and its use in traditional medicine for the treatment of various types of gastrointestinal disturbances such as diarrhea, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain was proved to have scientific bases (Mittal et al., 2010).

The genus *Myrtus* is a dominant genus of family Myrtaceae. This genus is represented by 100 species wildly distributed in the warmer parts of the world. Even though the genus is native to the Mediterranean area it is also found in almost all tropical regions. Many of the plants in this genus have showy blossoms. The family is generally considered to contain economically valuable plants. Timber, gums and resins, oils, spices and edible fruits are among the economic uses of the family (Ahmad, 2006).

Myrtle (*Myrtus communis L.*) which is an evergreen shrub grows mainly in the Mediterranean area. In Italy it grows wild in almost all the coastal areas (Flamini et al., 2004; Wannes et al., 2010; Zanetti et al., 2010). The essential oil from the leaves of myrtle has been shown to contain terpenes (α-pinene, 1, 8-cineole, linalool, α-terpineol, limonene, and myrtenyl acetate) (Monti et al., 2002; Yadegarinia et al., 2006) and several phloroglucinols which are considered responsible for the plant's antibacterial and anti-inflammatory properties (Appendino et al., 2006; Rossi et al., 2009). The plant also contains polyphenols such as flavonoids, ursolic acid, several volatile compounds and tannins (Wannes et al., 2010).

In folklore medicine myrtle is used for different illnesses including as an antiseptic, disinfectant, hypoglycaemic , anti-inflammatory, antimicrobial, antifungal, (Mahboubi et al., 2010; Mimica-Dukic et al., 2010), remedy for cough (Bruni et al., 1997; Leonti et al., 2009); and against stomach disorder, diarrhea and dysentery (Ziyyat et al., 1997; Teklehaymanot and Gidayy, 2007; Leonti et al., 2009; Mimica-Dukic et al., 2010). *In vitro* studies have also shown that the plant has antimicrobial (Zanetti et al., 2010) strong anti-inflammatory (Rossi et al., 2009) and antioxidant, antimutagenic, and anti-genotoxic activities (Hayder et al., 2004; Mimica-Dukic et al., 2010; Wannes et al., 2010). The essential oil of *M. communis* (EOMC) has been reported to possess *in vitro* antimicrobial (Zanetti et al., 2010) and strong *in vivo* anti-inflammatory activities (Rossi et al., 2009).

However, to date, there has been no report in the literature concerning the effect of the oil on diarrhea and stomachache. The present study was therefore conducted to evaluate the possible antidiarrheal and antispasmodic effects of *M. communis* essential oil using different experimental models.

2. OBJECTIVES

2.1. General objective

To investigate the antidiarrheal and antispasmodic activities of the essential oil of *M. communis* in rodents

2.2. Specific objectives

The specific objectives of this study were to

- determine the antidiarrheal activity of *M. communis* in rodents;
- assess the inhibitory effect of *M. communis* on intestinal transit time in rodents;
- find out the antispasmogenic activity of *M. communis* in *ex-vivo*; and
- measure the antienteropooling activity of *M. communis in vivo*.

3. MATERIALS AND METHOD

3.1 Materials

3.1.1 Drugs and chemicals

The following drugs and chemicals were used for the study: acetylcholine bromide, atropine, loperamide, sodium chloride, potassium chloride, magnesium chloride, sodium bicarbonate, sodium hydrogen phosphate, calcium chloride, Tween 80, Charcoal, gum aciasia (Sigma-Aldrich Inc., Germany); glucose (Eurastar Scientific Ltd., France). Castor oil was purchased from a local retail outlet in Addis Ababa and misoprostol was kindly donated by DKT, Ethiopia.

3.1.2 Plant material

The leaves of *M. communis* were collected from around Addis Ababa, Ethiopia in December 2010. The authenticity of the plant was confirmed by Ato Melaku Wondafrash, the National Herbarium, Department of Biology, College of Natural Science, Addis Ababa University where a specimen was deposited (Collection number A/01).

3.1.3 Experimental animals

Adult male and female Swiss albino mice (25-30 g), rats (150-165 g) and guinea pigs (350-500 g) were obtained from animal house of School of Pharmacy, Addis Ababa University and, Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa. The experimental animals were acclimatized for seven days. Before the start of the experiment the animals were housed at a 12 h/12 h light/dark cycle for one week with free access to food (standard pellet) and water (tap water) (Vogel, 2002).

3.2 Method

3.2.1 Essential oil extraction

The leaves of *M. communis* were subjected to hydrodistillation using Clavenger type apparatus for 4 h. A pale yellow oil (7 ml) was obtained from 1.8 kg of fresh leaves. The oil was kept in umber colored air tight container and stored in a refrigerator until used

3.2.2 Acute toxicity test

Acute toxicity study was done using the limit test dose of 2000 mg/kg as described by Organization for Economic Cooperation and Development (OECD) guideline (OECD, 2001). The procedure was carried out with six female mice (three animals per step) for the test. The Animals were fasted prior to dosing (food but not water was withheld for 3-4 h). Following the period of fasting, the animals were weighed and the test substance administered orally. After the substance had been administered, food was withheld for a further 1-2 h. Each animal was sequentially dosed at interval of 48 h and observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days (Adebiyi et *al.*, 2006; OECD, 2001).

3.2.3 Normal gastrointestinal motility test by charcoal meal

Mice were divided into control, positive control and test groups of six mice in each group and fasted for 18 h but water was freely provided. The test groups received the essential oil at 100, 200 and 400 mg/kg diluted in Tween 80/water (5% v/v) orally. Dose selection was based on the acute toxicity test and one-tenth of the limit dose used for the acute toxicity was employed as the medium dose. The lower and higher doses were then selected as half and twice of the medium dose, respectively (Deshmukh et al., 2009). The positive control group received atropine (0.1 mg/kg, i.p.), while the control group received Tween 80/water 5% v/v (5 ml/kg) orally. After 30 min each animal was given orally 1 ml of charcoal meal (3% deactivated charcoal in 5% gum acacia). Thirty minutes after charcoal meal administration, the animals were sacrificed and the movement of charcoal from pylorus to caecum was measured. Charcoal movement was expressed as peristaltic index (PI). The PI and percentage of inhibition compared with the control group were determined by using the following equation (Chatterjee, 1993; Biswas et al., 2002; Franca et al., 2008; and Amole et al., 2010).

$$Peristalitic\ Index\ (PI) = \frac{Distance\ moved\ by\ charcoal}{length\ of\ intestine} X100$$

$$Percentage\ inhibition = \frac{PI\ negative\ control\ -\ PI\ test\ drug}{PI\ negative\ control} X100$$

3.2.4 Castor oil induced gastrointestinal motility test by charcoal meal

The same procedures as described in section 3.2.3 were followed except that castor oil (0.2 ml/mouse, p.o.) was administered 30 min before administration of charcoal meal. The other difference was that the positive control group received loperamide instead of atropine at the dose of 3 mg/kg orally (Amole et al., 2010).

3.2.5 Antidiarrheal activity

Before the start of the experiment, animals were screened by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the experiment. Then overnight fasted mice were divided into control, positive control and test groups containing six mice in each group. Control group received Tween 80/water 5% v/v (5 ml/kg) orally. The positive control group received loperamide at a dose of 3 mg/kg orally; test groups received EOMC at the doses of 100, 200 or 400 mg/kg diluted in Tween 80/water (5% v/v). Each animal was placed in an individual cage, the floor of which was lined with tissue paper. The floor lining was changed every 1 h. Diarrhea was induced by oral administration of 0.5 ml castor oil to each mouse, 30 min after the above treatments. During an observation period of 4 h, diarrhea onset time, total number of fecal output and the number of diarrheic feces excreted by the animals were recorded. A numerical score based on stool consistency was assigned as follows: normal stool = 1, semi-solid stool = 2 and watery stool = 3. Calculations were made for the delay diarrheal onset, diarrheal score and percent inhibition by comparison with the control group. The *in vivo* antidiarrheal index (ADI) was then expressed according to the formula shown bellow (Shoba and Thomas, 2001; Vogel, 2002; Franca et al., 2008; Ching et al., 2008):

$$ADI = \sqrt[3]{Dfreq \times Gmeq \times Pfreq}$$

Where: Dfreq = Delay in defecation time or diarrheal onset (in % of control), Gmeq = Gut travel reduction (in % of control) and Pfreq = purging frequency as number of stool reduction (in % of control).

3.2.6 Antienteropooling activity testing

For this evaluation, rats were deprived of food and water for 18 h prior to the experiment. Five animals were randomly assigned into each group and placed in cages. The first three groups were given EOMC at the doses 100, 200 or 400 mg/kg body weight diluted in Tween 80/water (5% v/v). The fourth group (positive control) was given the standard drug loperamide (2.5 mg/kg body weight) while the fifth group (negative control) was given Tween 80/water (5% v/v) at dose of 5 ml/kg. Immediately afterwards, misoprostol was administered to all the rats (200 μg/kg). After 30 min, each rat was sacrificed and the ends of the small intestine tied at the pylorus and the caecum. This section was dissected out and its intestinal content was collected by milking into pre-weighed (M_0) graduated tubes (the cylindrical portion of a 2.5 ml syringe whose tip is sealed) and the new weight (M_1) was measured. The volume (ml) of the intestinal content was read directly from the tubes while the mass was obtained as ($M_1 - M_0$) g. % inhibition was calculated as intestinal fluid volume or mass of negative control minus that of treatment divided by the negative control and then the result was multiplied by 100 (Shoba and Thomas, 2001; Havagiray et al., 2004; Swamy et al, 2005; Thakurta et al., 2007; Franca et al., 2008).

3.2.7 Ex-vivo antispasmodic model testing

For this experiment Guinea pig ileum (GPI) was used. Guinea pigs were fasted for 24 h before the start of the experiment. The animals were killed by a gentle blow at the back and allowed to bleed. Then the abdomen was opened by midline incision and GPI 2-2.5 cm in length was removed immediately and trimmed from surrounding tissues. The contents of the intestine were washed with a physiological salt solution called Tyrode solution (composition was KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8, and glucose 5.55 mM). This procedure was repeated whenever viable tissue (GPI) was needed throughout the experiment (Venkataranganna et al., 2002; Vogel, 2002).

The isolated segments of GPI were tied with silk threads at both ends (ileum tied in opposite directions) and suspended in a thermoregulated 25 ml organ bath, maintained at 37 ^{0}C, containing the Tyrode solution. Intestinal contractions were recorded by isometric recording device (GRASS FT-03 strain gauge transducer coupled to a GRASS 7E Polygraph, U.S.A.) which is equipped with preamplifier, main amplifier, oscillograph

and time and event marker. The tissues were constantly bubbled with air and a resting tension of 1 g was applied to the individual tissue (Galvez et al., 1996; Bashir et al., 2011).

The suspended ileum was allowed to equilibrate for 30-45 min before adding agonist or antagonist or the particular plant extract. After the initial equilibration period, acetylcholine (10^{-8} to 10^{-3} M) was added to the organ bath and the concentration-response curve was constructed. Then the tissue was washed twice with Tyrode solution at the interval of 20-10 min. It was then left to resume its normal contraction. After a stabilized regular contraction, the EOMC at a dose of 50, and 100 µg/ml, were added; 5 min later for each dose of the essential oil varying concentration of acetylcholine (10^{-8} to 10^{-3} M) was added and the dose-response curve of acetylcholine in the presence of oil was constructed. Atropine (6.66 nM) was then added and the above procedure was repeated. The chart speed was set at 5 mm/min (Galvez et al., 1996, Vogel, 2002; Mohammed et al., 2009).

Different concentrations of the essential oil were prepared in physiological Tyrode salt solutions, while stock solutions of the drugs (acetylcholine and atropine) were made in distilled water and then serially diluted with the physiological salt solution. The final dilutions of the drugs were made fresh on the day of the experiment. The calculated concentrations of the essential oil and standard drugs were the final organ bath concentration. The anticholinergic activity of the EOMC was compared with that of atropine. Then pD_2 values were calculated for each dose of the essential oil and for the standard. Original computer program Graph pad prism 2.0 (GraphPad-Pism software Inc, San Diago, CA) was used for fitting non – linear curve, to draw dose response curve and for calculation of pD_2 values (Mekonnen, 1999).

3.3 Statistical analysis

All experimental data were expressed as mean ± standard error of mean (SEM) and were analyzed using one-way ANOVA followed by Tukey post test for comparing the mean differences, and were considered statistically significant when $P<0.05$. All statistical analyses were performed using standard SPSS package (version 19 for windows).

4 RESULTS

4.1 Acute toxicity test

It was observed that oral administration of the limit test dose of 2000 mg/kg of EOMC to mice neither showed mortality nor any apparent signs of toxicity like decreased food intake, changes in skin and fur, tremors, weakness, convulsions, salivation, lethargy, sleep and coma in the animals. Therefore, the acute toxicity test result revealed that the median lethal dose (LD50) value for the oil is greater than 2000 mg/kg.

4.2 Inhibition of gastrointestinal motility

The EOMC at doses of 100, 200 or 400 mg/kg produced statistically significant ($p<0.05$) inhibition of normal intestinal transit compared to the control mice. The inhibition was not dose dependent and the peak effect was produced by the 200 mg/kg dose whereas the lowest effect was produced by 400 mg/kg dose. Atropine (0.1 mg/kg) significantly reduced the intestinal transit and produced the highest effect (57%) inhibition. The inhibition produced by 200 mg/kg of the essential oil was lower than atropine, the standard drug used in the experiment (Table 1).

Table 1: Effect of the essential oil of *Myrtus communis* (EOMC) on the normal intestinal transit of charcoal meal in mice. n=6, *$p<0.05$ relative to controls.

Treatment	**Peristaltic index (%)**	**% inhibition**
Tween-80/water 5 ml/kg	75 ± 1.2	-
EOMC100 mg/kg	39.6 ± 2.1*	47
EOMC200 mg/kg	38.2 ± 2.5*	49
EOMC400 mg/kg	43.4 ± 4.3*	42
Atropine 0.1 mg/kg, i.p	32.0 ± 0.9*	57

4.3 Inhibition of castor oil-induce diarrhea

During the 4 h observation time after castor oil administration, all mice in the control group (Tween-80/water 5 ml/kg PO) produced copious diarrhea. Pre-treatment of mice with EOMC like the standard antidiarrheal agent, loperamide, caused significant ($p<0.05$) delay in the onset of diarrhea, decreased frequency of defecation (reduction in number of total stools), reduced greatly the wetness of fecal droppings (number of wet stool), and decreased the general diarrhea score when compared to the control mice. The delay in the onset of diarrhea, reduction in frequency of defecation, reduction in number of wet stools and reduction in diarrheal score caused by 400 mg/kg dose of the essential oil was comparable to that produced by the standard drug loperamide. The decrease in the wetness of stool caused by the oil was equal to that caused by the standard drug loperamide. Both the oil at a dose of 400 mg/kg and loperamide at a dose of 3 mg/kg produced the highest (92.5%) inhibition of the wetness of fecal droppings (Table 2). The highest ADI (74.5%) was obtained with the dose of 400 mg/kg of the oil which was comparable to that produced by loperamide (81%) (Table 3). Fig 2 shows the effect of the essential oil on castor oil-induced intestinal transit in mice. This model is a part of an in vivo antispasmodic model on established gut increased motility unlike the above model which measures effect on normal peristalsis. The oil caused reduction in the distance traversed by the charcoal meal and the effect was statistically significant compared to the control. The standard drug loperamide also produced statistically significant reduction in the distance traversed by the charcoal compared to the control. Whilst the effect produced by 200 and 400 mg/kg dose of the oil on castor oil induced GIT motility was comparable to that produced by loperamide, the effect produced by the lowest dose (100 mg/kg) of oil, was significantly ($p<0.05$) lower than that produced by loperamide.

Table 2: Effect of the essential oil of *Myrtus communis* (EOMC) on castor oil induced diarrheal parameters. n=6, *p<0.05 relative to controls

Treatment	**on-set time (min)**	**Total number of stool in 4h**	**% inhibition**	**number of wet stool in 4h**	**% inhibition**	**Diarrheal score**	**% Protection**
Tween-80/water 5 ml/kg	53±3.6	10.5 ± 1.1	----	6.67 ± .67	----	26.2 ± 2.89	----
EOMC 100 mg/kg	165±5.5*	5.8 ± 1.4*	44.8	1.0 ± 0.52*	85.0	9.8 ± 2.3*	62.4
EOMC 200 mg/kg	171±3.0*	4.6 ± 1.1*	55.5	1.0 ± 0.52*	85.0	9.0 ± 2.4*	65.6
EOMC 400 mg/kg	182±3.5*	3.5 ± 1.4*	66.7	0.5 ± 0.34*	92.5	5.0 ± 2.4*	80.8
Loperamide 3mg/kg	200±0.0*	3.0 ± 0.9*	71.4	0.5 ± 0.50*	92.5	4.8 ± 2.4*	81.5

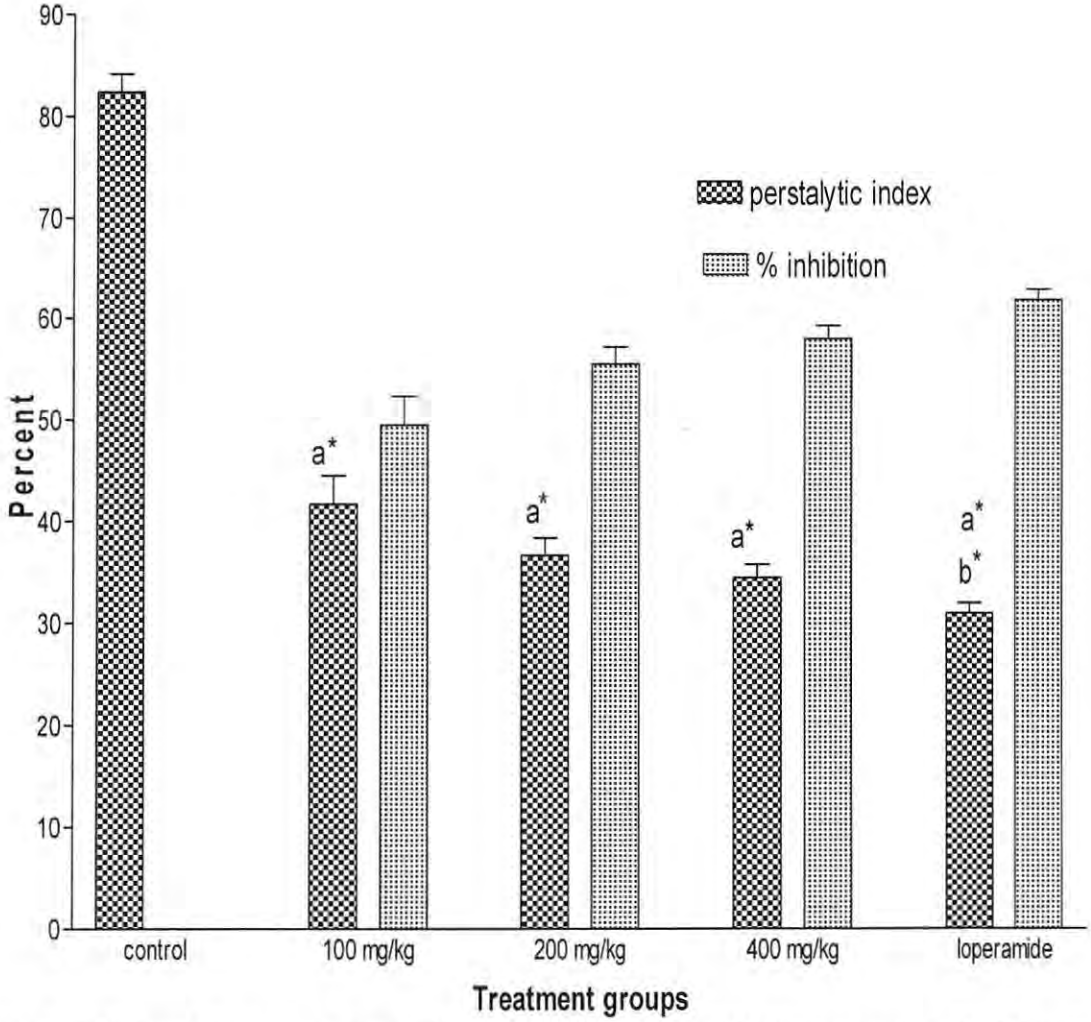


Fig 2: Effect of the essential oil of *Myrtus communis* (EOMC) on castor oil induced intestinal transit of charcoal meal in mice. n=6, a*p<0.05 relative to controls, b*p<0.05 relative to 100 mg/kg EOMC.

Table 3: In vivo antidiarrheal index of the essential oil of *Myrtus communis* (EOMC) in mice.

Treatment	Dfreq (%)	Gmeq (%)	Pfreq (%)	ADI (%)
Tween-80/water 5 ml/kg	---	----	----	----
EOMC 100 mg/kg	60.0	49.5	85.0	63.0
EOMC 200 mg/kg	66.0	55.5	85.0	67.7
EOMC 400 mg/kg	77.0	58.0	92.5	74.5
Loperamide 3 mg/kg	94.0	62.0	92.5	81.0

4.4 Anti-enteropooling activity

EOMC significantly inhibited misoprostol-induced enteropooling in rats at all doses compared to the control. The inhibition of intestinal fluid accumulation was 68% ($p<0.05$), 71% ($p<0.05$) and 73% ($p<0.05$) at doses of 100, 200, and 400 mg/kg of the oil, respectively. The standard drug, loperamide (3 mg/kg), also significantly inhibited ($p<0.05$) intestinal fluid accumulation (81%) when compared to the control. Similarly, the weight of the intestinal fluid was significantly ($p<0.05$) reduced by all doses of the oil as well as the standard when compared to the negative control. The intestinal fluid accumulation (both in terms of volume and weight) inhibited by 400 mg/kg dose of the oil was comparable to that inhibited by loperamide (Table 4).

Table 4: Anti-enteropooling effects of the essential oil of *Myrtus communis* (EOMC) in Rats. n=6, *$p<0.05$ relative to controls.

Treatment	Volume in ml	% inhibition	Weight in g	% inhibition
Tween-80/water 5 ml/kg	2.7 ± 0.2	--------	3.4 ± 0.2	---------
EOMC 100 mg/kg	0.8 ± 0.1*	68	1.4 ± 0.1*	59
EOMC 200 mg/kg	0.8 ± 0.1*	71	1.3 ± 0.1*	61
EOMC 400 mg/kg	0.7 ± 0.1*	73	1.3 ± 0.1*	62
Loperamide 3mg/kg	0.5 ± 0.1*	81	1.0 ± 0.1*	71

4.5 Ex-vivo antispasmodic activities on isolated guinea pig ileum.

EOMC caused a strong inhibition of acetylcholine induced contraction of isolated guinea pig ileum preparation. Tracing of EOMC effect on acetylcholine induced contraction is shown in Fig 3. The extent of inhibition was significant ($p<0.01$) at both 50 µg/ml and 100 µg/ml concentration of the EOMC when compared with the negative control. Spasmogenic effect of acetylcholine was significantly inhibited by the oil at all concentrations of acetylcholine. The 100 µg/ml concentration of EOMC showed 100% inhibition of contraction induced by 10^{-8}, 10^{-7} and 10^{-6} M acetylcholine. The maximum contraction induced by acetylcholine at a concentration of 10^{-3} M was decreased from 100 (for the control group) by the EOMC with concentrations of 50 and 100 µg/ml to 60±0.4 and 52±1.2, respectively. The percent spasmogenic inhibition at a concentration of 10^{-3} M acetylcholine was 40 and 48%, respectively for 50 and 100 µg/ml concentration of EOMC. Atropine (6.66 nM), on the other hand, produced much less inhibition compared to the oil which was only 2%, i.e. the E_{max} was reduced from 100 to 98 (Fig 3 and 4).

As can be seen from the dose response curve of acetylcholine (Fig 4), the oil caused depression of the E_{max} and forward shift of the graph in a non parallel manner. The pD_2 value of acetylcholine was depressed from 5.92±0.297 to 4.694±0.417 and 4.106±0.31 by 50 and 100 µg/ml concentration of EOMC, respectively. Atropine (6.5 nM) decreased the pD_2 value of acetylcholine from 5.92±0.297 to 5.053±0.232.

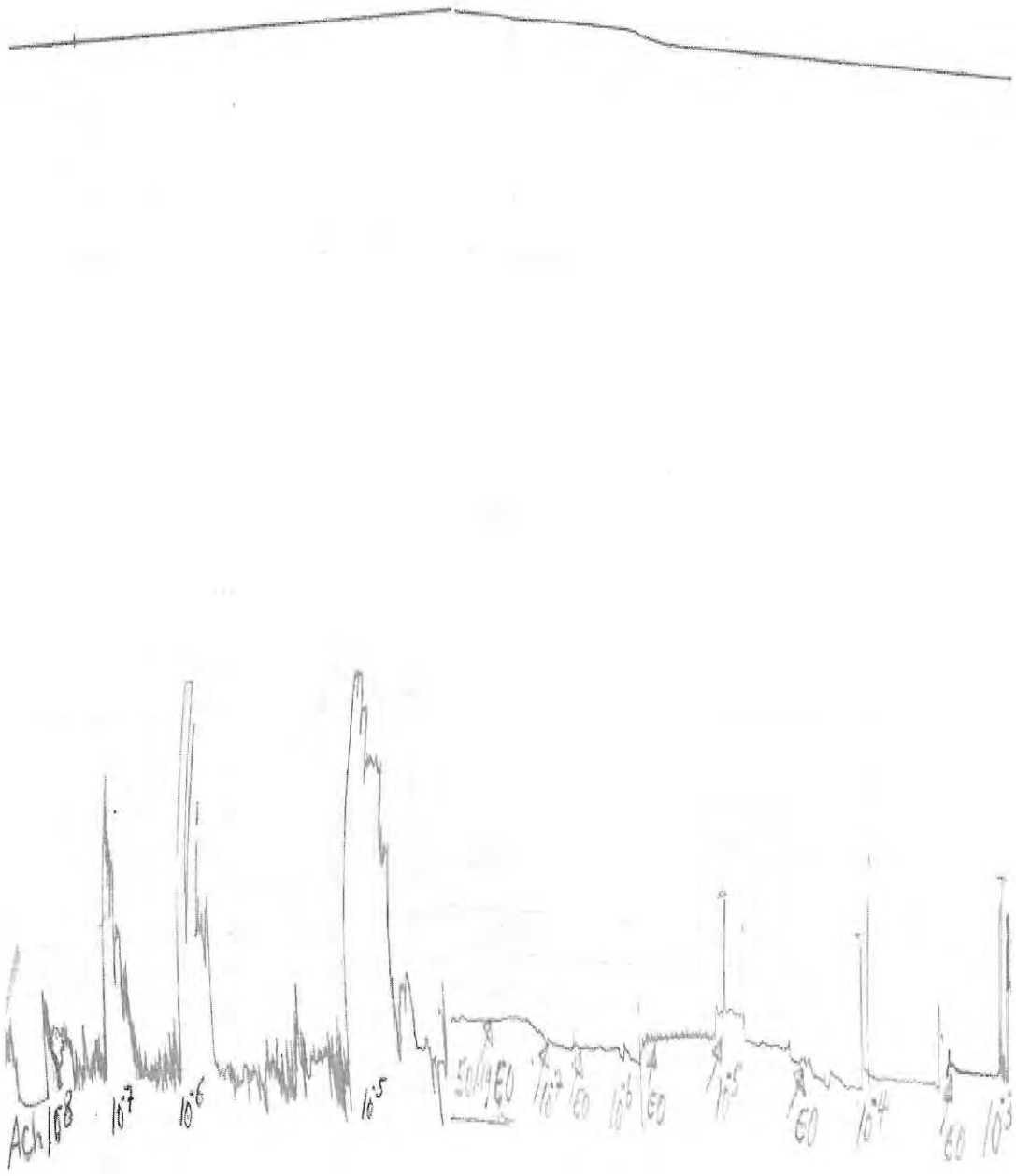


Fig 3: Effect of acetylcholine on guinea pig ileum preparation before and after treatment with 50 µg/ml of the essential oil of *Myrtus commuins* (EOMC).

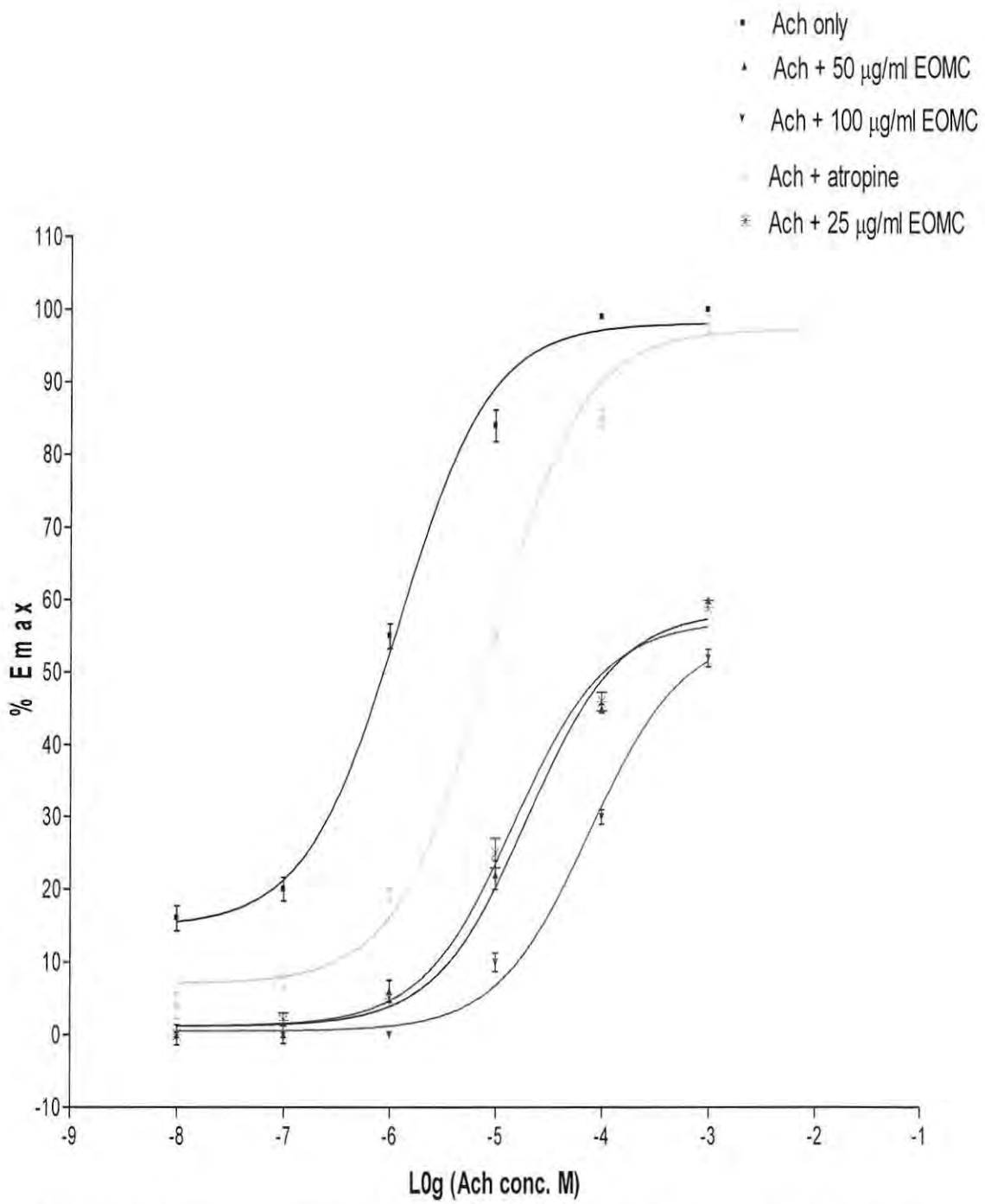


Fig 4: Effect of atropine and increasing concentrations of the essential oil of *Myrtus communis* (EOMC) on the dose response curve of acetylcholine (Ach) on guinea-pig ileum.

Responses were expressed as percent of the maximum contractions (E_{max}) induced by Ach before the addition of oil or control. Each point on the graph is mean ± SEM of the six experiments.

5. DISCUSSION

The aim of the present study was to assess the antidiarrheal and antispasmodic effects of the EOMC using experimental models in mice, rats and guinea pigs. The results obtained from these experimental models indicated that the oil was effective as an antidiarrheal and antispasmodic drug. According to Akah (1988), for a drug to have antidiarrheal activity it should inhibit the production of wet or unformed feces in animals and also inhibit gastrointestinal propulsive action. In the present study, the oil was found to inhibit castor-oil induced diarrhea in mice, normal and castor oil induced gastrointestinal motility in mice, prostaglandin induced enteropooling in rats and acetylcholine induced contraction of guinea pig ileum which are consistent with the above proposition.

5.1 Inhibition of gastrointestinal motility

The oil reduced the intestinal propulsive movement in the charcoal meal treated model significantly. The extent of inhibition was comparable to that produced by the standard drug atropine. The potency difference may be logical since atropine is pure compound compared to the oil which is a mixture of many compounds. The higher dose of the oil produced lesser inhibition compared to the lower once. In fact such observation was also reported by previous authors (Owulade et al., 2004). This effect could be due to the non specific effect of the oil on different systems. This is to mean that at higher dose the oil might produce more than one effect which can oppose the inhibitory effect.

It has been reported that α-pinene has myorelaxant activity on the smooth muscles of the intestine (Camara et al., 2003). So the presence of α-pinene in EOMC (Yadegarinia et al., 2006) may explain the inhibitory effect of the oil on the normal gastrointestinal motility. The mechanism by which atropine inhibits intestinal transit is probably due to its anticholinergic effect (Izzo et al., 1999). So it is possible to deduce that the oil may also inhibit GIT motility by acting on the cholinergic system similar to atropine. The above speculation was further supported by the inhibition of acetylcholine induced ileum contraction by the oil *in ex-vivo* antispasmodic model.

5.2 Inhibition of castor oil-induce diarrhea

Castor oil contains the active component ricinoleate which is converted to ricinoleic acid by the action of lipases in the upper small intestine. The released ricinoleic acid is polar and hence poorly absorbed from GIT lumen. These compounds are known to induce changes in the mucosal permeability, electrolyte transport and intestinal peristalsis, leading to hypersecretory response and diarrhea (Zavala et al., 1998; Yoshio et al., 1999; Sahoo et al., 2006). Ricinoleate inhibits the enzyme sodium-potassium ATPase which increases the permeability of the intestinal epithelium. It causes cytotoxic effect on isolated enterocytes and may also stimulate epithelial cell adenyl cyclase (Munson et al., 1995; Goodman and Gilman, 2006). Ricinoleic acid on the other hand causes local irritation and inflammation of the intestinal mucosa leading to the release of prostaglandins and other mediators. These mediators (prostaglandins and others) increase the permeability of the mucosal cells and alter electrolyte transport, which results in a hypersecretory response (decreasing Na^+ and K^+ absorption), stimulating peristaltic activity and finally leading to diarrhea (Zavala et al., 1998). These all could account for castor oil's antiabsorptive and secretory effect on the mucosa and therefore, it is possible to conclude that castor oil diarrheal model incorporates both secretory and motility diarrhea and can almost accurately mimic the pathophysiology of diarrhea.

Pretreatment of mice with the EOMC caused delay in the onset of copious diarrhea, decreased the frequency of purging (reduction of number of wet stools and total no of stools), and severity of diarrhea (general diarrhea score). Higher dose of the oil produced greater reduction in the amount of stool excreted and the amount of loose stool but the difference was not statistically different compared to the middle and lower dose. This implies that the oil do have good activity even at the lower dose. Simply the oil produced an ADI which is comparable with that produced by the standard antidiarrheal drug loperamide. Loperamide, a drug widely used in the management of diarrheal disorders effectively antagonizes diarrhea induced by castor oil (Tanko et al., 2011).

ADI is a measure of the combined effects of the various components of diarrhea such as onset of diarrheal stools, purging frequency, and intestinal frequency. The fact that the oil produced a significant ADI suggests that it has a strong protective action against diarrhea.

Earlier research works on the EOMC showed that its main constituents are α-pinene and linalool (Monti et al., 2002; Yadegarinia et al., 2006). From other investigation these constituents: α-pinene (Sadraei et al., 2001) and linalool (Sousa et al., 2011) had spasmolytic effect on contraction of GIT smooth muscle. Therefore, inhibition of smooth muscle contraction might be a possible explanation for the antidiarrheal activity of the oil.

Prostaglandins play main role in secretory diarrhea induced by castor oil and hence prostaglandin inhibition could be one of the candidate targets for the antisecretory effect of the oil. This is further supported by the finding that the oil has strong anti-inflammatory activity (Rossi et al., 2009). As mentioned above inflammatory process plays role in both experimental and pathological diarrhea; antisecratory effect may be as a result of the anti-inflammatory effect of the oil and/or direct inhibition of prostaglandin. The spasmolytic and antisecretory effects of the oil may explain the protection against diarrhea induced by castor oil.

5.3 Antienteropooling activity

In this model a prostaglandin analog (misoprostol) was used to induce intestinal fluid secretion. Intestinal fluid secretion induced by castor oil is as a result of castor oil induced prostaglandins (Yoshio et al., 1999). One study also showed that a metabolite of prostaglandin E1 activates a specific chloride channel (ClC-2) in the GIT to enhance intestinal fluid secretion (Lacy, 2006). The above arguments show that the use of prostaglandins as inducer of GIT fluid secretion is appropriate.

The oil inhibited intestinal fluid secretion induced by prostaglandins significantly at all doses used (100, 200, and 400 mg/kg) and the extent of inhibition was comparable with that produced by the standard antidiarrheal drug loperamide. Secretory diarrhea is associated with an activation of chloride channels (CFTR), causing Cl^- efflux from the cell, this efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhea (Field, 2003). The fact that the oil inhibited intestinal fluid secretion seems that it blocks CFTR channels.

The spasmolytic effect produced by the oil might in part explain the fluid accumulation inhibition activity of the oil. That is, when there is decreased motility of GIT, it will give

time for fluid to be absorbed from intestinal lumen. Most recently nitric oxide (NO) has been claimed to contribute to secretory processes which leads to diarrhea via elevation of cGMP and cAMP concentrations (Yu-Chih et al., 2005). And therefore, inhibition of effect of NO could be a possible mechanism for the antisecretory effect of the oil.

5.4 Ex-vivo antispasmodic activity

The antispasmodic activity of EOMC on guinea pig ileum indicates that it inhibits acetylcholine induced contractions. The oil shifted acetylcholine dose response curve to the right and also depressed the maximum response to the agonist acetylcholine, which suggests a non competitive antagonism. However, it is difficult to conclude the antagonistic feature of the oil as non competitive because higher dose of agonist or much lower dose of the oil was not checked. Moreover the presence of several constituents in the oil is also another factor as it is generally difficult to describe the pharmacodynamic property of mixture of compounds due to their multiple actions on different biological systems.

The calculated pD_2 value shows that the oil has strong antagonistic potency even better than atropine. Actually pA_2 value can best describe the antagonistic potency and also used to characterize competitive or non-competitive feature of a compound than pD_2 but pA_2 calculation needs molar concentration of the antagonist. As the oil is mixture of different compounds it is not possible to calculate the molar concentration. Calculation of the molar concentration should be preceded by identification and isolation of the active constituent(s).

The oil was found to be more active in the *ex-vivo* model than the *in vivo* one. This may be because the two systems have physiological differences which can affect the potency of the oil. This is to mean that *in vivo* model is much more complex than the *ex-vivo* model and therefore, the type and the amount of molecules involved and the space to which these molecules diffuse is somehow different in the two systems. In addition to this, the *ex-vivo* model the spasmogen acetylcholine is not continuously produced by the tissue rather added to the medium and hence may not have free access to the tissue site of action which is not the case in the *in vivo* model where the spasmogen is produced continuously at the site of action. Furthermore, the pharmacokinetic parameters can reduce the relative amount of oil reaching at the site of action in the *in vivo* model.

When acetylcholine binds to muscarinic receptors (M_3) in smooth muscles, it causes opening of receptor gated sodium channels. This allows sodium to enter the cells which causes depolarization of the cell membrane. Depolarization in turn opens voltage dependent calcium channels and calcium ions enter the cell to induce the release of calcium from the sarcoplasmic reticulum. The cytosolic calcium thus binds to calmodulin, which results in contraction (Balton, 1979; Rojas et al., 1996). Since the oil blocked the activity of the spasmogen acetylcholine, it might be acting at any point in the common step in the contraction mechanism elicited by this agonist. In short, the anticholinergic activity of the oil could be by blocking M_3 muscarinic receptor and/or by inhibiting post receptor events which leads to smooth muscle contraction.

Data obtained from previous research works indicate that the spasmolytic effect of essential oils obtained from different plants is due to their effects on Ca^{2+} ion channels. For instance, the blockage of L-Type Ca^{2+} channels in the ileum by peppermint oil has been shown by means of electrophysiological technique (Hills and Aaronson, 1991). Different researchers claimed that smooth muscle contraction inhibitory effects of essential oils from various plants with different components are qualitatively similar (Hills and Aaronson, 1991; Hajhashemi et al., 2000). If this is so, all essential oils may have a similar mechanism of action. Therefore, the spasmolytic effect of EOMC could be due to Ca^{2+} ion channel blockage. The above concepts are further supported by another scientific finding on the main constituent of EOMC (α-pinene). This finding showed that the spasmolytic mechanism of action of α-pinene might be blockage of Ca^{2+} channels (Mercier et al., 2009).

From the above speculations the anticholinergic activity of the oil appears to be mediated through inhibition of post M_3 muscarinic receptor events that is, blockage of Ca^{2+} channels rather than directly blocking the receptor itself. However, further work is required before excluding the direct effect of the oil on receptors.

6 CONCLUSIONS AND RECOMMENDATIONS

This study indicates that the essential oil of *M. communis* possesses significant antidiarrheal and antispasmodic activity. The antidiarrheal activity produced by the oil was proved to be highly potent inhibitor of GIT motility and fluid secretion. The oil also possesses antispasmodic activity which is even better than that of atropine when assessed *in ex-vivo* antispasmodic model. Thus, the results obtained confirm scientific basis for the use of *M. communis* in traditional medicine, as an antidiarrhoeal agent and treatment for stomachache.

However, further studies are required to identify the active principle(s). As the oil is a complex mixture of many compounds, the identity of each of these components and the exact mechanism(s) of action of the individual constituents need to be studied. Moreover, the antidiarrheal activity of the plant extract is another area to be covered. At last even though the plant has been used as spices for longer period of time, chronic toxicity should be studied to confirm its long term safety.

REFFERENCES

Adebiyi R, Elsa A, Agaie B, Etuk E. (2006) Antinociceptive and antidepressant like effects of *Securidaca longepedunculata* root extract in mice. *Journal of Ethnopharmacology*, **107**: 234–239.

Ahmad M. (2006) Isolation and Characterization of Secondery metabolites from Myrtus communis L., Delphinium roylel M., and related medicinal Plants. Thesis (PhD) University of Karachi, Pakistan.

Akah P. (1988) Purgative potentials of *Euphorbia heterophylla*. *Fitoterapia,* **60**: 45–48.

Almeida C, Karnikowski M, Foleto R, Baldisserotto B. (1995) Analysis of antidiarrheaic effect of plants used in popular medicine. *Revista de Saude Publica,* **29**: 428–433.

Alzamora R, Omahony F, Harvey J. (2011) Estrogen inhibits chloride secretion caused by cholera and *Escherichia coli* enterotoxins in female rat distal colon. *Steroids,* **76**: 867– 876.

Amole O, Salahdeen H, Onyeahialam A. (2010) Evaluation of the antidiarrhoeal effect of *Lannea welwitschii* Hiern (Anacardiaceae) bark extract. *African Journal of Pharmacy and Pharmacology,* **4**: 165-169.

Appendino G, Maxia L, Bettoni P, Locatelli M, Valdivia C, Ballero M, Stavri M, Gibbons S, Sterner O. (2006) Antibacterial galloylated alkylphloroglucinol glucosides from myrtle (*Myrtus communis*). *Journal of natural product* **69**: 251–254.

Balton T. (1979) Mechanism of action of transmitters and other substances on smooth muscle. *Physiological Reviews*, 59: 649-677.

Barbara G, Joseph T, Terry L, Cindy W. (2009) *Pharmacotherapy Handbook*, 7th ed. New York: McGraw-Hill, Inc.

Bashir S, Memon R, Gilani A. (2011) Antispasmodic and Antidiarrheal Activities of Valeriana hardwickii Wall Rhizome Are Putatively Mediated through Calcium Channel Blockade. Hindawi Publishing Corporation, doi:10.1155/2011/304960.

Bisset W. (2001) Understanding diarrhoea. Current Paediatrics, **11**: 291-295.

Biswas S, Murugesan T, Sinha S, Maiti K, Gayen J, Pal M, Saha B. (2002) Antidiarrhoeal activity of Strychnos potatorum seed extract in rats. *Fitoterapia,* **73**: 43-47.

Bruni A, Ballero M, Poli F. (1997) Quantitative ethnopharmacological study of the Campidano Valley and Urzulei district Sardinia, Italy. *Journal of Ethnopharmacology,* **57**: 97–124..

Camara CC, Nascimento NR, Macedo-Filho CL, Almeida FB, Fonteles MC. (2003) Antispasmodic Effect of the Essential Oil of *Plectranthus barbatus* and some Major Constituents on the Guinea-Pig Ileum. Planta Mededica, **69**: 1080–1085.

Camilleri M. (2004) Chronic Diarrhea: A Review on Pathophysiology and Management for the Clinical Gastroenterologist. *Clinical Gastroenterology and Hepatology,* **2**: 198–206.

Casburn-Jones A. and Farthing M. (2004) Management of Infectious Diarrhea: Recent Advances in Clinical Science. *Journal of Gut*, **53**: 296–305.

Chatterjee T. (1993) *Handbook on Laboratory Mice and Rats.* 1st ed. Department of Pharmaceutical Technology, Jadavpur University, India.

Ching F, Omogbai E, Ozolua R, Okpo, S. (2008) Antidiarrhoeal activities of aqueous extract of *Stereospermum kunthianum. African Journal of Biotechnology*, **7**: 1220-1225.

Deshmukh P P, Fernanbes G, Apul A, Poppo E. (2009) Wound healing activity of Calopropis gigantean root bark in rats. *Journal of Ethanopharmacology*, **125**: 178-181.

El-Yazbi A, Schulz R, Danie E. (2007) Differential inhibitory control of circular and longitudinal smooth muscle layers of Balb/C mouse small intestine. *Autonomic Neuroscience*, **131**: 36–44.

Farnsworth N, Akerle O, Bingle A, Soejarto D, Guo Z. (1985) Medicinal plants in therapy. *Bulletin of the World Health Organization* **63**, 965–98.

Farthing M. (2002) Novel targets for the control of secretory diarrhea. *Gut,* **50**: 15-18.

Farthing M. (2000) Diarrhoea: a significant worldwide problem (Symposium on the treatment of diarrhoeal disease), *International Journal of Antimicrobial Agents*, **14**: 65–69.

Field M. (2003) Intestinal ion transport and the pathophysiology of diarrhea. *Journal of Clinical Investigation*, **111**: 931–943.

Flamini G, Cioni L, Morelli I, Maccioni S, Baldini R. (2004) Phytochemical typologies in some populations of *Myrtus communis* L. on Caprione Promontory. *Journal of Food Chemistry*, **85**: 599–604.

Franca C, Menezes F, Costa L, Niculau E, Alves P, Pinto J, Marçal R. (2008) Analgesic and antidiarrheal properties of Ocimum selloi essential oil in mice. *Fitoterapia,* **79**: 569–573.

Galvez J, Duarte J, de Medina S, Jimenez J, Zarzuelo A. (1996) Inhibitory effects of Quercetin on the Guinea pig ileum contractions. *Phytotherapy Research,* **10**: 66-69.

Goodman and Gilman (2006) *The pharmacological Basic of Therapeutics.* 11thed. New York, Chicago, New Delhi: Mc GrawHill Medical Publishing Division.

Guandalini S. (1997) prolonged diarrhea: etiology and pathogenesis. In: Gracey, M., Walker-smith J., Nestle Nutrition Workshop Series. Philadelphia: Lippincot-Raven publishers, p. 153-170.

Guyton A. and Hall J. (2006) *Text book of medicinal physiology.* 11th ed. Philadelphia: W.B. Saunders.

Hajhashemi V, Sadraei H, Ghannadi AR, Mohseni M. (2000) Spasmolytic and anti-diarrheal effect of Satureja hortensis L. essential oil. *Journal of Ethnopharmacollogy* **71**: 187–192.

Havagiray R, Ramesh C, Sadhna K. (2004) Study of antidiarrheal activity of *Calotropis gigantea* in experimental animals. *Journal of Pharmacology and Pharmaceutical Science,* **7**: 70–75.

Hayder N, Abdelwahed A, Kilani S. (2004) Anti-genotoxic and free-radical scavenging activities of extracts from (Tunisian) *Myrtus communis*. *Mutation Research,* **564**: 89–95.

Heinrich M, Heneka B, Ankli A, Rimpler H, Sticher O, Kostiza T. (2005) Spasmolytic and antidiarrhoeal properties of the Yucatec mayan medicinal plant *Casimiroa tetrameria*. *Journal of Pharmacy and pharmacology*, **57**: 1081-1085.

Herbert L, (2004) New Antibacterial Agents in the Management of Acute Infectious Diarrhea. *Journal of infectious disease*, 7: 1-4.

Hills JM, Aaronson PI. (1991) The mechanism of action of peppermint oil on gastrointestinal smooth muscle: An analysis using patch-clamp electrophysiology and isolated tissue pharmacology in rabbit and guinea-pig. *Gastroenterology*, **101**: 55–65.

Hollenberg D, Zakus D, Cook T, Xu XW. (2008) Re-positioning the role of traditional, complementary and alternative medicine as essential health knowledge in global health: do they still have a role to play? *World Health Population,* **10**: 62–75.

Izzo A, Mascolo N, Capasso R, Germano M, Depasquel R, Capasso F. (1999) Inhibitory effect of cannabinoid agonist on gastric emptying in the rat. *Archieves of Pharmacology*, **360**: 221–223.

Janig W. (2009) Autonomic Nervous System: Central Control of the Gastrointestinal Tract. Christian Albrechts Universit, Germany, Encyclopedia of Neuroscience, 871-881.

Lacy B. (2006) Lubiprostone, a Chloride Channel Activator. *Journal of Clinical Gastroenterology*, **41**: 345–351.

Lecci A, Santicioli P, Maggi C. (2002) Pharmacology of transmission to gastrointestinal muscle. *Current Opinion in Pharmacology* **2**: 630–641.

Leonti M. Casu L, Sanna F, Bonsignore L. (2009) A comparison of medicinal plant use in Sardinia and Sicily. *Journal of Ethnopharmacology* **121**: 255–267.

Lundgren O. and Jodal M. (1997) The Enteric Nervous System and Cholera Toxin–induced Secretion. *Comparative Biochemistry and Physiology*, **118**: 319–327..

Mahboubi M, and GhazianBidgoli F. (2010) In vitro synergistic efficacy of combinationof amphotericin B with Myrtus communis essential oil against clinical isolates of Candida albicans. *Phytomedicine* **17**: 771–774.

Maikere-Faniyo R, Van Puyvelde L, Mutwewingabo A, Habiyaremye F. (1989) Study of Rwandese medicinal plants used in the treatment of diarrhea. *International Journal of Ethnopharmacology,* **26**: 101–109.

Manatsathit S, Herbert L, Farthing M. (2002) Guideline for the management of acute diarrhea in adults. *Journal of Gastroenterology and Hepatology*, **17**: 54–71.

Mekonnen Y. (1999) Effects of Ethanol Extract of *Moringa stenopetala* leaves on Guinea pig and Mouse smooth muscle. *Phytotherapy Research.* **13**: 442-444.

Mercier B, Prost J, Prost M. (2009) The essential oil of turpentine and its major volatile fraction (α- and β-pinenes): A Review. *International Journal of Occupational Medicine and Environmental Health*, **22**: 331–342.

Metges C. (2010) Classical and post-genomic methods to study GIT function with emphasis on the pig. *Livestock Science* **133**: 10–19.

Mimica-Dukic N, Bugarin D, Grbovic S, Mitic-culafic D, Vukovic-Gacic B, Orcic D, Jovin E, Couladis M. (2010) Essential Oil of *Myrtus communis* L. as a Potential Antioxidant and Antimutagenic Agents. *Molecules review*, **15**: 2759-2770.

Mittal P, Gupta V, Kaur G, Garg AK, Singh A. (2010) Phytochemistry and pharmacological activities of *Psidium guajava*: A Review. *International Journal of Pharmaceutical Science and Research*, **1**: 9-19.

Mohammed A, Ahmed H, Goji A, Okpanachi AO, Ezekiel I, Tanko Y. (2009) Preliminary Anti-diarrhoeal Activity of Hydromethanolic Extract of Aerial Part of *Indigofera Pulchra* in rodents. *Asian Journal of Medical Sciences* **1**: 22-25.

Monti D, Chetoni P, Burgalassi S, Najarro M, Saettone MF, Boldrini E. (2002) Effect of different terpene-containing essential oils on permeation of estradiol through hairless mouse skin. *International Journal of Pharmaceutics*, **237**: 209–214.

Mourad F. and Saade N. (2011) Neural regulation of intestinal nutrient absorption, *Progress in Neurobiology*, doi:10.1016/j.pneurobio.2011.07.010.

Munson P. L., Muller R. A., Breese G. R. 1995 *Principles of Pharmacology Basic Concept and Clinical Applications*. New York: Chapman and Hall an International Thomson Publishing company.

OECD, (2001) Guidelines for the testing of chemicals revised draft guideline 423: acute oral toxicity.

Olsson C. and Holmgren S. (2010) Autonomic control of gut motility: A comparative view, *Autonomic Neuroscience*, doi:10.1016/j.autneu.2010.07.002.

Otimenyin S. and Uzochukwu D. (2010) Spasmolytic and Antidiarrhea Effects of the Bark of *Erythrina senegalensis* and Root of *Kigelia Africana. Asian Journal of Pharmaceutical and Clinical Research*, **3**: 12-14.

Owulade M O, Eghianruwa K I, Daramola, F O. (2004) Effects of aqueous extracts of *Hibiscus sabdariffa* calyces and *Ocimum gratissimum* leaves on intestinal transit in rats. *African Journal of Biomedical Research*, **7**: 31-33.

Patil SH, Adkar PP, Shelke TT, Oswal RJ Borase S. (2011) Antidiarrhoeal Activity of Methanol Extract of Leaves of *Calotropis Procera. Journal of Pharmacology and Toxicology*, **1**: 26-30.

Reed K. and Wickham R. (2009) Review of the Gastrointestinal Tract: From Macro to Micro. *Oncology Nursing,* doi: 10.1016/j.soncn.2008.10.002.

Rojas A, Cruz S, Ponce-Monter H, Mata R. (1996) Smooth muscle relaxing compounds from *Dodenaea Viscosa. Planta Medica,* 62: 154-159.

Rossi A, Di Paola R, Mazzon E. Genovese T, Caminiti R, Bramanti P, Pergola C, Koeberle A, Werz O, Sautebin L, Cuzzocrea S. (2009), Myrtucommulone from Myrtus communis Exhibits Potent Anti-Inflammatory Effectiveness in Vivo. *Journal of Pharmacology and Experimental Therapeutics,* **329**: 76–86.

Sadraei H, Asghari GR, Hajhashemi V, Kolagar A, Ebrahimi M. (2001) Spasmolytic activity of essential oil and various extracts of *Ferula gummosa* Boiss. on ileum contractions. *Phytomedicine*, **8**: 370–376.

Sahoo J, Sahu PK, Tripathe DK. (2006) Anti-diarrhoeal activity of *Listea Glutinosa* leaves. *Advances in Pharmacolology and Toxicoogyl*, **7**: 53-55.

Shoba FG. and Thomas M. (2001) Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhoea. *Journal of Ethnopharmacology* **76:** 73–76.

Sibylle K and Stephanie O. (2009) Acute Infectious Diarrhea in Children. *Deutsches Arzteblatt International*, **106**: 539-548.

Sonawane N D, Zhao D, Zegarra-Moran O, Galietta L J, Verkman A S. (2008) Nanomolar CFTR inhibition by pore-occluding divalent polyethylene glycol-malonic acid hydrazides. *Chemistry & Biology,* **15**: 718–728.

Sousa DP, Junior GA, Andrade LN, Batista JS. (2011) Spasmolytic Activity of Chiral Monoterpene Esters. *Records of Natural product*, **5**: 117-122.

Stevens R, Publicover N, Smith T. (2000) Propagation and Neural Regulation of Calcium Waves in Longitudinal and Circular Muscle Layers of Guinea Pig Small Intestine. *Gastroenterology*, **118**: 892–904.

Swamy BM, Sridhar C, Rao TS, Setty T, Ramaiyan D, Veeranna B, Babu A, Lakshminarayanasettry V. (2005) Antidiarrheal Evaluation of *Benincasa hispida* (Thunb.) Cogn. Fruit Extracts. *Iranian Journal of Pharmacology and Therapeutics*, **4**: 24-27.

Tanko Y, Kadiri OT, Mohammed A, Mahdi MA, Musa KY. (2011) Preliminary antidiarrhoeal activity of methanol extract of *Cissus cornifolia* (bak) planch on experimental animals. *Annals of Biological Research*, **2**: 229-237.

Teklehaymanot T. and Gidayy M. (2007) Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, doi: 10.1186/1746-4269-3-12.

Thakurta P, Bhowmik P, Mukherjee S, Hajra TK, Patra A, Baga PK. (2007), Antibacterial, antisecretory and antihemorrhagic activity of Azadirachta indica used to treat cholera and diarrhea in India. *Journal of Ethnopharmacology* **111**: 607–61.

Thomas A. (2006) Gut motility, sphincters and reflex control. *Anaesthesia and Intensive Care Medicine* **7**: 57-58.

Venkataranganna M, Anturlikar S, Gopumadhavan S, Prakash NS, Rafiq M, Murthy GS, Mitra SK. (2002) Antispasmodic Activity of SJ-200 (Himcospaz) An Herbal Preparation. *Journal of Pharmaceutical Biology*, **40**: 416-421.

Vogel H. (2002) *Drug Discovery and evaluation: Pharmacological assays.* 2nd ed. Germany: Springer-Verland.

Wannes A, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B. (2010), Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. *Journal of Food and Chemical Toxicology* **48**: 1362–1370.

Wapnir R, Teichberg S. (2002) Regulation mechanisms of intestinal secretion: implications in nutrient absorption, *Journal of Nutritional Biochemistry,* **13**: 190–199.

World Health Organization, WHO (2009) Diarrheal disease. Available from: http://www.who.int/mediacentre/factsheets/fs330/en/index.htm(Accessed 30/5/ 2011).

World Health Organization, WHO (1996) Scientific working Group: Resistance to antimicrobial agents. Bull. *World Health Organization*, **71**: 335-336.

World Health Organization, WHO (2005) The Treatment of Diarrhoea: a manual for physicians and other senior health workers. *World Health Organization*, **4**: 25-26.

Xue J, Askwith C, Javed N, Cooke H. (2007) Autonomic nervous system and secretion across the intestinal mucosal surface; *Autonomic Neuroscience, Basic and Clinical* **133**: 55–63.

Yadegarinia D, Gachkar L, Rezaei M, B, Taghizadeh M, Astaneh SA, Rasooli I. (2006) Biochemical activities of Iranian Mentha piperita L. and Myrtus communis L. essential oils. *Phytochemistry,* **67**: 1249–125.

Yoshio K, Kazuko S, Bunsyo M, Kazunori H, Atsushi I, Yasuhiro K. (1999) Relationship between antidiarrhoeal effects of Hange-Shashin-To and its active components, *Phytotherapy Research* **13**: 468–473.

Yu-Chih L, Hung-Jung L, Sheng-Hsuan C, Chun-Chin C, Liang- Shung C. (2005) Effect of lipopolysaccharide on diarrhea and gastrointestinal transit in mice: Roles of nitric oxide and prostaglandin E_2. *World Journal of Gastroenterology*, 11: 357-361.

Zanetti S, Cannas S, Molicotti P, Bua A, Cubeddu M, Porcedda S, Marongiu B, Sechi LA. (2010) Evaluation of the Antimicrobial Properties of the Essential Oil *of Myrtus communis* L. against Clinical Strains of Mycobacterium species. doi:10.1155/2010/ 931530.

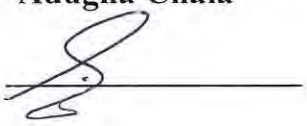
Zavala M, Perez C, Vargos R, Perez M. (1998) Antidiarrhoeal activity of Waltharia americana, Commelina coelestis and *Althernan repens. Journal of Ethnopharmacology* **61**: 41–47.

Ziyyat A, Legssyer A, Mekhfi H. (1997) Phytotherapy of hypertension and diabetes in oriental Morocco. *Journal of Ethnopharmacology* **58**: 45–54.

DECLARATION

I, the undersigned, declare that this thesis work is my original work and has not been presented for a degree in any other University.

Name: Adugna Chala

Signature: 

This thesis has been submitted for examination with my approval as a university advisor

Name: Dr. Ephrem Engidawork, Ph D

Signature: 

Name: Dr. Kaleab Asres, Ph D

Signature 

Place and date of submission: Addis Ababa, Ethiopia, October 2011