6. DISCUSSION
Diarrhoea is a frequent medical problem (Farthing, 2000). Intestinal infection is the most common cause of diarrhoea worldwide and is responsible for the deaths of 3–4 million individuals each year, mostly in preschool-age children (Rouf et al., 2003). The major cause of diarrhoea among children in developing countries is malnutrition. In some developing countries, children may suffer repeated attacks of acute diarrhoea, which contribute to the infection–malnutrition cycle and consequent impairment of growth and development. Acute diarrhoea in children leads to significant morbidity and mortality, even in the wealthy industrialized countries. Chronic diarrhoea is also a major problem in some other clinical situations (Syder et al., 1982, Lutterodt, 1989, Mujumdar et al., 2006).

In order to combat the problems of diarrhoea globally, the World Health Organization in Diarrhoeal Disease Control programme has given a special emphasis on the use of traditional medicines in the control and management of diarrhea as medicinal herbs constitute an indispensable component of the traditional medicine practised world wide due to the economical viability, accessibility and ancestral experience (Abdulkarim et al., 2005, Gricilda et al., 2001, Mukhergee et al., 1995, Atta et al., 2005, Vareishang et al., 2004).

Present study was aimed to investigate the anti-diarrhoeal effect and different mechanisms of antidiarrhoeal action of herbal formulations like Mebarid, Enterocin and Kutajarishta. It was also decided to evaluate the possibility of application of pharmacological method in the biostandardisation of Mebarid, Enterocin and Kutajarishta.

Black pepper or kali mirch is a common food ingredient used worldwide and known to one and all due to its day to day use as one of the most popular spices. It stimulates the taste buds in such a way that an alert is sent to the stomach to increase hydrochloric acid secretion, thereby improving digestion. It has long been recognized as a carminative, (a substance that helps to prevent the formation of intestinal gas), a property likely due to its beneficial effect of stimulating hydrochloric acid production. In addition, black pepper has diaphoretic (promotes sweating), and diuretic (promotes urination) properties. It has demonstrated impressive antioxidant properties (Vladimir et al., 2000, Singh et al., 1993). Piperine is an alkaloidal constituent of black pepper recently established as a bioavailability enhancer of drugs and other substances (Kokate et al., 2007). Because of bioavailability enhancer activity of black pepper it can be added in traditional
antidiarrhoeal formulations of different herbs. In the present study antidiarrhoeal activity of black pepper and its mechanisms of action were explored. Combined effect of black pepper with Mebarid, Enterocin and Kutajarishta were also studied in diarrhoea to check its additive effect on antidiarrhoeal effect of Mebarid, Enterocin and Kutajarishta. Antidiarrhoeal activity of piperine was studied to see its contribution as an active constituent in the antidiarrhoeal activity of Black pepper.

Antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta, black pepper and piperine was studied in mice in diarrhoea induced by castor oil model, magnesium sulphate induced diarrhoea model, Charcoal meal test for intestinal motility, castor oil induced intraluminal fluid accumulation test.

Involvement of potassium channels, nitric oxide pathway and $\alpha_2$ adrenergic receptors were studied in mice for antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and black pepper. Effect of Mebarid, Enterocin, Kutajarishta and black pepper on muscarinic receptors, ganglionic receptors, histamine receptors and calcium channels in isolated guinea pig ileum was also studied.

6.1 Antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper.

6.1.1 Antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper in Castor oil induced diarrhoea model

Castor oil induces diarrhea by causing increased secretion of fluid and electrolytes into the lumen of the bowel by intestinal mucosa, resulting in fluid accumulation and a watery luminal content that flows rapidly through the small and large intestines (Mascolo et al., 1994). This is brought about by the irritant effect of ricinoleic acid liberated by pancreatic lipases, which hydrolyse the oil derived from the seeds of *Ricinus communis* (Gaginell et al., 1976, Donowitz et al., 1987, Capasso et al., 1994).

The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins, which stimulates motility and secretion. Ricinoleic acid is also reported to reduce active Na$^+$ and K$^+$ absorption and decrease Na$^+$, K$^+$ ATPase activity in the small intestine and colon (Philips et al., 1965, Nell et al., 1984, Racusen et al., 1979). The castor oil induced diarrhoea demonstrates secretory diarrhoea, since ricinoleic acid, the active ingredient of castor oil, induces
diarrhoea by a hypersecretory response (Uddin et al., 2005, Uddin et al., 2006). The castor oil model therefore incorporates both secretory and motility diarrhea (Rouf et al., 2003, Mujumdar et al., 2005, Simon et al., 1980).

As Mebarid, Enterocin, Kutajarishta and black pepper successfully inhibited the castor oil induced diarrhoea, it can be assumed that the antidiarrhoeal action was exerted by antisecretory mechanism. This was also evident from the reduction of total number of wet faeces in the test groups in the experiment.

6.1.2 Effect of Mebarid, Enterocin, Kutajarishta and Black pepper in magnesium sulphate induced diarrhoea model

Magnesium sulphate induces diarrhoea by increasing the volume of intestinal content through the following steps (Afroz et al., 2006, Zvala et al., 1998):

- Magnesium ions draw water from the surrounding body tissues into the intestinal tract by osmosis.
- The higher quantity of water in the intestinal tract softens and increases the volume of faeces, stimulating nerves in the intestines.
- Magnesium ions also play a role in releasing the peptide hormone cholecystokinin, causing accumulation of water and electrolytes in the intestine and triggering intestinal motility.

Thus, Magnesium sulphate produces the diarrhoea by osmotic properties, preventing reabsorption of water ions, leading to increase in the volume of the intestinal content. It promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water (Afroz et al., 2006, Galvez et al., 1993). Mebarid, Enterocin, Kutajarishta and black pepper reduced the diarrhoea in this model due to increase in the absorption of water and electrolyte from the gastrointestinal tract (Galvez et al., 1993 Zavala et al., 1998, Mujumdar et al., 2005).
6.1.3 Effect of Mebarid, Enterocin, Kutajarishta and Black pepper on gastrointestinal motility.

Gastrointestinal (GI) motility is an integrated process including myoelectrical activity, contractile activity, tone, compliance and transit. These different entities of motility can be generated and modulated by local and circulating neurohumoral substances. Neurohumoral substances and their receptors play a major part in the complex regulation of gastrointestinal motility and have therefore been the predominant targets for drug development. The numerous receptors involved in motility are located mainly on smooth muscle cells and neuronal structures in the extrinsic and intrinsic parts of the enteric nervous system. Within this system, receptor agonists and antagonists interact directly to modify excitatory or inhibitory signals. The intestinal movements are influenced by many drugs either through the nervous mechanism (neurogenic) or through the muscle directly (myogenic) (Bennett, 1992, Waugh et al., 2001, Chaterjee, 1984).

GI motility describes the contraction of the muscles that mix and propel contents in the gastrointestinal tract. The gastrointestinal tract is divided into four distinct parts that are separated by sphincter muscles; these four regions have distinctly different functions to perform and different patterns of motility (contractions). They are the esophagus (carries food to the stomach), stomach (mixes food with digestive enzymes and grinds it down into a more-or-less liquid form), small intestine (absorbs nutrients), and colon (reabsorbs water and eliminates indigestible food residues). Abnormal motility or abnormal sensitivity in any part of the gastrointestinal tract can cause characteristic symptoms (Waugh et al., 2001). An excessive number of high amplitude propagating contractions (rapid transit) can be a cause of diarrhea; it reduces the amount of time food residues remain in the large intestine for water to be reabsorbed. Stimulation of intestinal smooth muscle by drugs (eg, Mg-containing antacids, laxatives, cholinesterase inhibitors, SSRIs) or humoral agents (eg, prostaglandins, serotonin) also can speed transit (Brunton, 2000, Mujumdar et al., 2005, Malik et al., 2010).

Usually the diarrhoea is considered as a consequence of altered motility and fluid accumulation in intestinal tract. Charcoal meal test in mice is a method used to study the effect of drugs on the motility of intestine (Rouf et al., 2003, Fernando et al., 2010). In the present study Mebarid, Enterocin, Kutajarishta and black pepper was found to be the inhibitor of intestinal motility
6.1.4 Effect of Mebarid, Enterocin, Kutajarishta and Black pepper on intraluminal fluid secretion

About 9 liters of fluid pass through the GI system each day and only about 2 liters are ingested, the rest represent secretions from the system itself. About half, 3.5, liters is secreted from the exocrine glands, the salivary glands, the pancreas and the liver, the other half is secreted by the epithelial cells of the of the digestive tract itself. The secretions consist of digestive enzymes, mucous and substantial amounts of fluid and ions. Nearly all this fluid is absorbed. Failures of absorption of the intestinal secretions can thus lead to rapid dehydration and electrolyte imbalance (Chaterjee, 1984, Waugh et al., 2001).

Diarrhoea occurs when the bowels secrete more electrolytes and water than they absorb. Causes of increased secretions include infections, unabsorbed fats, certain drugs, and various intrinsic and extrinsic secretagogues. Infections (eg, gastroenteritis; discussed in Gastroenteritis) are the most common causes of secretory diarrhea. Infections combined with food poisoning are the most common causes of acute diarrhea (< 4 days in duration). Most enterotoxins block Na\(^+\)-H\(^+\) exchange, which is an important driving force for fluid absorption in the small bowel and colon. Unabsorbed dietary fat and bile acids (as in malabsorption syndromes and after ileal resection) can stimulate colonic secretion and cause diarrhea (Guyton et al., 1996). Drugs may stimulate intestinal secretions directly (eg, quinidine, quinine, colchicine, anthraquinone cathartics, castor oil, prostaglandins) or indirectly by impairing fat absorption (eg, orlistat). Various endocrine tumors produce secretagogues, including vipomas (vasoactive intestinal peptide), gastrinomas (gastrin), mastocytosis (histamine), medullary carcinoma of the thyroid (calcitonin and prostaglandins), and carcinoid tumors (histamine, serotonin, and polypeptides). Some of these mediators (eg, prostaglandins, serotonin, and related compounds) also accelerate intestinal transit, colonic transit, or both. Rapid intestinal transit and diminished surface area impair fluid absorption and cause diarrhea. Common causes include small-bowel or large-bowel resection or bypass, gastric resection, and inflammatory bowel disease. Other causes include microscopic colitis (collagenous or lymphocytic colitis) and celiac sprue (Fine, 1998, Kruszka et al., 2002, Das., 2001, Claudia et al., 2009).

The intraluminal fluid accumulation induced by castor oil was blocked by Mebarid, Enterocin, Kutajarishta and black Pepper. Castor oil produces permeability changes in the intestinal mucosa membranes to water and electrolytes resulting in fluid and watery
luminal content that flows rapidly through small and large intestines (Mujumdar et al., 2000). This is brought about by the irritant effect of ricinoleic acid liberated by pancreatic lipases, which hydrolyse the oil derived from the seeds of *Ricinus communis* (Gaginella et al., 1975).

### 6.1.5 Role of potassium channels in antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper

Epithelial cells of the gastrointestinal tract are an important barrier between the "milieu interne" and the luminal content of the gut. They perform transport of nutrients, salts, and water, which is essential for the maintenance of body homeostasis. In these epithelia, a variety of K⁺ channels are expressed, allowing adaptation to different needs. For instance, in gastric mucosa, K⁺ channel function is a prerequisite for acid secretion of parietal cells. K⁺ channels hyperpolarize the membrane voltage, thereby fueling electrogenic transport mechanisms such as Na⁺-coupled reabsorption of nutrients or luminal Cl⁻ secretion. In epithelial cells of small intestine, K⁺ channels provide the driving force for electrogenic transport processes across the plasma membrane, and they are involved in cell volume regulation. Fine tuning of salt and water transport and of K⁺ homeostasis occurs in colonic epithelia cells, where K⁺ channels are involved in secretory and reabsorptive processes. Furthermore, there is growing evidence for changes in epithelial K⁺ channel expression during cell proliferation, differentiation, apoptosis, and, under pathological conditions, carcinogenesis (Poggioli et al., 1995).

Glibenclamide blocks ATP-sensitive K⁺ channels thereby causing membrane depolarization and influx of calcium through voltage-sensitive Ca²⁺ channels. ATP-sensitive K⁺ channels are present in intestinal smooth muscle and epithelial cells, and are opened by drugs like cromokalim and pinacidil and blocked by glibenclamide. K⁺ channel openers can hyperpolarize and relax intestinal smooth muscles, stimulate NaCl absorption in villus cells and they may exert antidiarrhoeal activity (Meisheri et al., 1988, Poggioli et al., 1995, Flavia et al., 1999).

Glibenclamide, a potassium channel blocker has reduced the antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and black pepper. Thus Mebarid, Enterocin, Kutajarishta and black pepper produced the antidiarrhoeal effect by stimulating the potassium channels.
6.1.6 Involvement of nitric oxide pathway in antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper

Nitric oxide (NO) is known to have a protective effect on the gastrointestinal tract. NO is responsible for helping to maintain the integrity of the gastric epithelium and the mucus barrier. NO is a vasodilator and mediates gastric blood flow. In normal physiological states, endogenous NO is proabsorptive as a result of on enteric nervous system, suppression of prostaglandin formation, and opening of basolateral K⁺ channels. However, in some pathophysiological states NO synthase may be produced at higher concentrations that are capable of evoking net secretion and mediating the laxative action of several intestinal secretagogues including castor oil (Izzo et al., 1998, Katzung et al., 2001).

Isosorbide dinitrate (ISDN) is a nitrate used pharmacologically as a vasodilator, e.g. in angina pectoris but also for anal fissure, a condition which is known to involve decreased blood supply leading to poor healing (Rang et al., 2003). It is also used as a direct vasodilator to treat congestive heart failure. Administration of the NO donor, Isosorbide dinitrate leads to a dose-dependent increase in mucus gel thickness in gastric lumen, demonstrating that under certain conditions NO helps to mediate mucus secretion to protect the gastric epithelium (Adeyemi et al., 2009).

NO has been reported to play an important role in castor oil-induced diarrhoea (Uchida et al., 2000). Pretreatment with NO synthase inhibitors from l-arginine prevent castor oil-induced diarrhoea and decrease the intestinal fluid accumulation and Na⁺ secretion induced by castor oil (Adeyemi et al., 2009, Mascolo et al., 1993). Thus, NO is one of the mediators of the intestinal secretion and diarrhoea induced by castor oil (Izzo et al., 1998). We found that the effect of Mebarid, Enterocin, Kutajarishta and black Pepper on castor oil induced diarrhoea was significantly antagonized by ISDN, a NO donor, implying that the antidiarrhoeal effect of Mebarid, Enterocin, Kutajarishta and black Pepper mediated by l-arginine NO pathway. These phenomena indicated a possible NO synthase inhibitor activity of Mebarid, Enterocin, Kutajarishta and black Pepper might reduce NO formation during castor oil-induced diarrhoea.
6.1.7 \( \alpha_2 \) adrenergic receptors in antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper

Sympathetic stimulation has the opposite effect of parasympathetic stimulation. It decreases blood flow to the gut as well as decreases secretions and general gut activity (Burks, 1991). The effect of sympathetic stimulation often only lasts a few minutes, and then an auto regulatory effect takes over restoring the blood supply to normal. However, this shutting off of the blood supply acts to increase flow to other areas of the body in time of stress. Sympathetic stimulation can also cause contraction of the Splanchnic veins, making as much as half a liter of volume available to the general circulation (Waugh et al., 2001, Adeyemi et al., 2009, Mbagwu et al., 2008.).

Stimulation of the enteric nerves by the sympathetic system inhibits GI activity. It does this in a minor way with the direct effect of its secreted norepinephrine, and in a major way by inhibiting action in the enteric plexuses (Akindele et al., 2006).

Yohimbine (\( \alpha_2 \) adrenergic receptor blocker) is an alkaloid with stimulant and aphrodisiac effects found naturally in \textit{Pausinystalia yohimbe} (Yohimbe). It is also found naturally in \textit{Rauwolfia serpentina} (Indian Snakeroot), along with several other active alkaloids. Yohimbine blocks the pre- and post-synaptic alpha-2 adrenoceptors (Paul et al., 2000, Adeyemi et al., 2009).

Yohimbine, a specific \( \alpha_2 \) adrenergic receptor antagonist, did not in any way have significant effect on the observed antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and black Pepper in this study. This precludes interference with this receptor in explaining the effectiveness of Mebarid, Enterocin, Kutajarishta and black pepper in diarrhoea.

6.1.8 Antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper via muscarinic and nicotinic receptors

The effect of parasympathetic stimulation is to increase activity in the entire enteric nervous system. The proximal half of the nervous system is innervated from the cranial parasympathetic nerve fibers via the vagal nerve. The distal half is innervate via the Sacral Parasympathetic nerves. The later gives a rich supply to the Sigmoid colon, rectum and anus, and are important in controlling defecation (Uchiyama et al., 2004).
Parasympathetic stimulation will increase overall blood flow to the gut as well as increasing secretions and general gut activity. Parasympathetic nervous system, mediates its action in the gut by stimulation of muscarinic receptors and nicotinic receptors. Acetylcholine produces its stimulant effect on gastrointestinal tract through muscarinic receptors while nicotine produce stimulant effect via nicotinic receptors (ganglionic receptors) in gastrointestinal tract (Veronica et al., 1999, Lutterdot, 1989, Anwar et al., 2005).

Acetylcholine and nicotine produced contraction of isolated guinea pig ileum (Valiollah et al., 2000). Mebarid, Enterocin, Kutajarishta and Black pepper induced relaxation of spontaneous contractions in isolated guinea pig ileum. Pretreatment with Mebarid inhibited the contractile effect of acetylcholine and nicotine on isolated guinea pig ileum while Enterocin decreased the contractile effect of nicotine only and not the acetylcholine on isolated guinea pig ileum. Stimulant effect of acetylcholine and nicotine on isolated guinea pig ileum was not changed by Kutajarishta and black Pepper. It indicates that antidiarrhoeal effect of Mebarid involve both muscarinic and nicotinic receptors while Enterocin involves nicotinic receptors in its antidiarrhoeal effect. Relaxant effect of Kutajarishta and black pepper is not via muscarinic and nicotinic receptors.

6.1.9 Antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper through histamine receptors

Histamine is synthesized and released from the mast cells in the tissues. It is released as part of the inflammatory processes, increasing capillary permeability, and dilatation. It also causes contraction of smooth muscle of the alimentary tract and stimulates the secretion of gastric juice (Chaterjee, 1984, Rang et al., 2003, Waugh et al., 2001).

$H_1$ and $H_2$ histamine receptors coexist on smooth muscle cells of the gut. $H_1$ receptors mediate contraction and $H_2$ receptors mediate relaxation. The net effect of histamine is contraction, reflecting the dominant influence of $H_1$ receptors. Histamine stimulates contraction by activating $H_1$ receptors (Bodhankar et al., 2010, Arul et al., 2004).

Mebarid, Enterocin and Kutajarishta produced relaxation against histamine induced contraction in guinea pig isolated ileum while Black pepper has not decreased the histamine induced contraction in guinea pig isolated ileum. It shows that antidiarrhoeal effect of Mebarid, Enterocin and Kutajarishta involves the histamine receptors while black pepper did not produced relaxant effect through histamine receptors.
6.1.10 Antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and Black pepper through calcium channels

Cytosolic Ca\(^{2+}\) activity is one of the most important second messengers, e.g., it couples activation of receptors or action potentials to specific cellular effect or mechanisms such as activation of enzymes, exocytosis of transmitter-containing vesicles, muscle contraction, fluid and enzyme secretion, gene transcription, changes of ion conductance, etc (Godfraind et al., 1986, Chaterjee, 1984).

Ca\(^{2+}\) channel is essential for electromechanical coupling and important for pharmaco-mechanical coupling in intestinal smooth muscle. Rhythmic contractions of intestinal smooth muscle are regarded as the result of electromechanical coupling between smooth muscle cells and the interstitial cells of Cajal (ICC). This coupling depends critically on the presence of the Ca\(^{2+}\) in calcium channel. Ca\(^{2+}\) channel acts as both voltage-sensor and executor that initiates and determines contraction in smooth muscle (Chaterjee, 1984, Malik et al., 2010).

The segmental and propulsive movements of intestinal muscle depend on co-ordinated contractions and relaxations of the muscle layers. Intestinal muscle relaxant agonists act through cAMP dependent reduction in intracellular calcium levels, which results mainly in inhibition of intestinal contractions. Reducing levels of intracellular free calcium by calcium channel blockade produces an antidiarrhoeal action (Horowitz et al., 1996, Bart et al., 1995, Gilman et al., 1996, Anwar et al., 2005).

The contractile effect of calcium on isolated guinea pig ileum was attenuated or abolished by Mebarid, Enterocin and Kutajarishta but black pepper has not produced any effect on the calcium induced contraction in isolated guinea pig ileum. It is revealed that antidiarrhoeal effect of Mebarid, Enterocin and Kutajarishta involves the calcium channels while black pepper did not produced relaxant effect via calcium channels.

6.2 Effect of Black pepper on antidiarrhoeal activity of Mebarid, Enterocin, and Kutajarishta.

Black pepper showed the increase in the antidiarrhoeal effect of Mebarid, Enterocin, and Kutajarishta in castor oil and magnesium sulphate induced diarrhoea in mice. Black pepper enhanced the antimitotility effect of Mebarid, Enterocin, and Kutajarishta in mice.
Inhibitory effect of Mebarid, Enterocin, and Kutajarishta on intraluminal fluid accumulation induced by castor oil in mice was increased by black Pepper.

6.3 Antidiarrhoeal effect of Piperine

Piperine has decreased the diarrhoea induced by castor oil and magnesium sulphate. It has also reduced the intestinal motility and intraluminal fluid accumulation induced by castor oil indicating its antisecretory and antimitotility action.

Antidiarrhoeal activity of piperine indicates that it is the chief constituent responsible for antidiarrhoeal effect of black pepper.

6.4 Bio-standardization of antidiarrhoeal herbal formulations

The use of potent chemicals as drugs has increased their potential to cure as well as to cause harm. Serious and life threatening adverse reactions to drugs started making their appearance. The world health organization has supported the use of traditional medicine and has recommended incorporating some of these practices in primary health care in developing countries. The recommendation arouse primarily from the realization that modern health care cannot reach many of these populations and secondly from vindication of some of the traditional practices in the successful treatment of common ailments. Most of the traditional remedies are of plant origin. These remedies usually are crude drugs in modern dosage form but without adequate standards of quality and assurance of safety and efficacy (Ashutosh, 2007).

In recent years, there has been a surge of interest in herbal remedies for a number of ailments. Use of herbal drugs has been an inseparable part of human civilization as many food materials like ginger, garlic, etc. have long been used as medicines (Fernando et al., 2010). There are large numbers of epidemiological and experimental evidence pertaining to world-wide acute diarrhoeal disease, which is one of the principal causes of death in the infants, particularly in malnourished and which is of critical importance in developing countries (Adzu et al., 2003, Lin et al., 2002, Syder, 1982, Lutterodt, 1989). Thus, it becomes important to identify and evaluate commonly available natural drugs as alternative to currently used anti-diarrhoeal drugs which are not completely free from adverse effects (Gilman, 1996).
Mebarid, Enterocin and Kutajarishta are an ayurvedic formulations indicated in amoebic and bacillary dysentery and diarrhoea. The experimental models used in the present investigation are simple and the results have been observed to be reproducible. It appears that biostandardisation of medicinal plant formulations like Mebarid, Enterocin and Kutajarishta used for diarrhoea and dysentery may be possible by including these methods in the battery of tests.

**6.5 Phytochemical analysis:**

Photochemical tests were carried out on Mebarid, Enterocin, Kutajarishta and black pepper in order to ascertain the various chemical compounds inherent in the medicinal plants used in these formulations.

Preliminary phytochemical analysis revealed the presence of carbohydrates, steroids, triterpenoids, alkaloids, flavonoids and tannins in Mebarid and Enterocin. Kutajarishta and black pepper showed the presence of carbohydrates and alkaloids as major constituents (Khandelwal, 2007, Kokate et al., 2007). Presence of piperine in the black pepper used for the study was authenticated by different methods of evaluation like physical properties, chemical tests, melting point, UV spectrophotometry and thin layer chromatography (Harborne, 2005, Agarwal et al., 2007, Kanki et al., 2008).

Antidiarrhoeal activity and antidysenteric properties of medicinal plants were found to be due to the presence of tannins, alkaloids, saponins, flavonoids, steroids and or terpenoids (Gerald et al., 2007, Havagiray et al., 2004, Al-Rehaily et al., 2002). The specific roles of these constituent in the antidiarrhoeal effect of Mebarid, Enterocin, Kutajarishta and black pepper have not been studied but these constituents may be responsible for the in vivo antidiarrhoeal activity of Mebarid, Enterocin, Kutajarishta and black pepper.

Numerous studies have validated the traditional use of antidiarrhoeal medicinal plants by investigating the specific activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption, or reduce electrolyte secretion (Palombo, 2006, Carlo et al., 1994, Rahaman et al., 2002, Angelo et al., 1999). Screening a number of medicinal plants showed that antidiarrhoeal activity of those plants were due to tannins, alkaloids, saponins, flavonoids, sterols, triterpenes, and reducing sugars contained in them (Longana et al., 2000). Some of phytochemicals are present in the active extracts and exhibit antidiarrhoeal abilities. For examples, tannins and
flavonoids are thought to be responsible for anti-diarrheal activity through increasing colonic water and electrolyte reabsorption, or directly inhibiting functions of enterotoxins (2003, Atta et al., 2005). Simple phenolics and proanthocyanidin have been shown to inhibit Cl\(^-\) secretion (Gabriel et al., 1999,). Alkaloids might act by inhibiting intestinal motility (Surva et al., 2010). In addition to these compounds, few studies indicated that triterpenes exhibited anti-diarrheal effects (Bravo, 1998, Abdullahi et al., 2001).

Flavonoids and sugar obtained from the medicinal plants were shown to exhibit antidiarrhoeal properties (Rahaman et al., 1991). The antidiarrhoeal activities of flavonoids have been ascribed to their ability to inhibit intestinal motility, small intestinal transit and hydroelectrolytic secretions which are known to be altered in diarrhoeic conditions (Mbagwu et al., 2008, Attaguile et al., 2004). Flavonoids have been shown to attenuate contraction of guinea pig ileum induced by some spasmogens (Macander, 1986), and inhibit small intestinal transit (Viswanathan et al., 1984, Calzada et al., 2005).

Tannins and tannic acid present in antidiarrhoeal plants denature proteins in the intestinal mucosa by forming protein tannates which make the intestinal mucosa more resistant to chemical alteration and reduce secretion thus showing antisecretory activity (Parimala et al., 2002, Tripathi, 1994, Ashish et al., 1999, Havagiray et al., 2004, Swati et al., 2002).