6. Discussion

Indian systems of medicine uses medicinal plants as a major raw material for treatment. Standards need to be laid down to check the identity of the plant. Standard for plant quality also done using pharmacognostic and anatomical methods. A detailed pharmacognostic evaluation therefore is highly an essential prerequisite. Organoleptic evaluation play a vital role in quality checking of plant drugs. Sensory features of the crude drug are one of the important events in diagnostic characters (Kokate, 1986). According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken. In this way, organoleptic features obtained for MISK powder could be considered as a quality standard (Table 5.1). The results of the present study could be considered as a standard for this plant as this is the prime work on this seed kernel. Higher percentage of water extractive indicated (Table 5.2) the presence of high molecular weight substance (Anonymous, 2006). These physiochemical constituents were found to be within the standard prescription of ayurvedic pharmacopeia of India as well as WHO (Anonymous, 1998).

*Mangifera indica* seed kernel is used in traditional system of medicine. It has tremendous medicinal potential owing to its multifaceted biological functions. Present study on pharmacognostical characters of *Mangifera indica* seed kernel provide useful information to check the proper identity and also help to differentiate the genuine drug source from the closely related species. The total ash value is particularly important for the detection of metal, salts and silica (Musa *et al.*, 2006). Very low ash value indicated the drug used in this study free from foreign matter. Acid Insoluble ash is a part of total ashes which mainly consist of silica and indicate contamination with earthy material Ash values were used to determine the quality and purity of crude drug. An inorganic element present in the raw drug indirectly indicates the water soluble ash (Kokate, 1986). The extractive
values were useful to evaluate the chemical constituents present in the crude drug (Kokate, 1986). Water-soluble extractive value was higher, indicating the presence of highly polar chemicals such as flavonoids, protein, carbohydrates, etc. Alcoholic extractive values indicated that the plant showed higher polar compounds like phenolic compounds etc. Presence of phenolic compounds and tannins in this plant part supports chemically the anti diarrhoeal activity and antimicrobial activity, which was in agreement with various earlier reports (McDonald et al., 2001). Behavior of drug materials under UV radiation and visible light exhibited different colour depending upon the various chromophores present in the material. The same extract may appear different at different wavelength of light. Seed kernel powder exhibited black colouration when treated with sulphuric acid. The results revealed the presence of phenols, alkaloids, terpenoids, flavonoids, tannins, lignin’s, saponins and carbohydrates in seed kernel.

Microbial limit assay is essential to check microbial load and to assess pathogenic contaminations. The results revealed that there is no pathogenic microbial flora in the MISK powder. Other aerobic bacterial flora were also within the limit and within the acceptable limits as per international standards of medicinal plant raw material as well as finished products (Anonymous, 1998).

Quality control of plant materials in relation to microorganisms is highly essential as virulent microbial agents may cause infection or intoxication. If the fungal count exceeds the normal, then it may lead to accumulation of mycotoxins in the crude powder, which in turn may cause severe problem. Increase in bacterial load may also lead to intoxications (Anonymous, 2006). The MISK showed the presence of fewer microorganisms that is within the limits of international and Indian pharmacopoeia standards. Pharmacognostical analysis confirmed purity and quality of the MISK and would be considered as a standard for future assessment.
Five different bacterial strains were isolated from various clinical samples. Identification was done based on XLD agar, EMB agar, Blood agar, Hektoen enteric agar, Baired parker agar and Cetrimide agar (Table 5.5). Isolates were subjected to microscopic methods and also subjected to biochemical characterization. Biochemical reactions and colour of the colony on different medium indirectly indicates the genomic nature of the isolates. These features were compared with standard and the test organisms were identified as *E. coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonasaeruginosa* (Plate VIII, IX, X, XI and XII).

Siedelman *et al.*, (2012) and Walters *et al.*, (2012) reported that 76.5% of the community acquired infections are due to multidrug resistant microorganisms. They reported that pathogens like *E. coli*, *Salmonella*, *Vibrio* and *Staphylococcus aureus* were 100% resistance to ciprofloxacin, cephalosporin, cefodoxime and are 98% resistant to erythromycin and bacitracin and 93% to novobiocin & tetracycline. Most of the pathogens showed multiple virulent factors (Tchesnokova *et al.*, 2011). The clinical isolates used in this study were also found to be multidrug resistant isolates (Table 5.6). Medicinal plants are considered as the best alternative source of medicine and used to overcome antibiotic resistance and highly virulence of the pathogens.

Drug resistance in microbes becomes a big problem along with the emergence of new infectious diseases. Microbes acquire resistance against the available antibiotics by the production of drug degrading enzymes, having resistant plasmids, alteration of metabolic pathway, etc., (Bender *et al.*, 1990; Lanski, 1998; Raghunath, 2008 and Kenneth *et al.*, 2002).

In the present study, *MISK* extracts exhibited good antimicrobial activity (Table 5.7 and 5.8), which could be due to the phytochemicals present in the extracts. One of the previous studies indicated the presence of steroids, terpenoids, flavonoids, phenolic compounds in both extracts (Rajan *et al.*, 2011b). They reported that tannins were only
present in alcoholic extracts along with other reported chemicals. This is also evidenced by Rajeshwari & Ramachandramurty (2013). Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and are thought to be responsible for coagulating the wall proteins of pathogenic organisms. Thus, MISK extract containing this compound may serve as a potential source of bioactive compounds in the treatment of infectious diseases. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase (Li et al., 2003). They also serve as health promoting compounds as a result of their anion radicals (Hausteen, 1983).

*Mangifera indica* phenolic extract was subjected to antimicrobial activity assay. Results revealed that the extract produced at least 10mm of zone of inhibition against all the organisms tested at 200µg/disc concentration. Basically, the MIC value indicates the potential of each extract to inhibit the microbial growth at lowest concentration. Based on the initial antimicrobial screening assay, the strains which show high positive results against the tested *M.indica* extracts were selected for further studies to determine the MIC value and the MIC value are as shown in Table 5.9. From these results, it was appeared that, there are substantial differences between the MICs of both aqueous and phenolic seed kernel extracts.

Most of the identified components with antimicrobial activity extracted from plants are aromatic or saturated organic compounds which are more soluble in polar solvents such as water and organic solvents. However water extracts were less potent. This can be attributed to the presence of water-soluble compounds such as polysaccharides and polypeptides, which are commonly more effective as inhibitors of pathogen adsorption and have no real impact as antimicrobial agents (Ncube et al., 2008). The antibacterial activity demonstrated by water extract provides the scientific bases for the use of water extracts in traditional treatment of diseases. There are also reports in literature that organic solvent is a better solvent for consistent extraction of antimicrobial substances for medicinal plants.
(Elloff, 1998). This may be attributed to two reasons, firstly, the nature and potentiality of biologically active components (alkaloids, steroids, flavonoids, essential oils, biterpenoids), which could be enhanced in the presence of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number or amount of active constituents responsible for antibacterial activity (Jeyachandran et al., 2010). This is also proved in this study in which phenolic extracts exhibited the highest antibacterial activity against all clinical isolates tested (Table 5.7 and 5.8).

There are many reports available that plants have been evaluated In Vitro for their antibacterial potency against some important human pathogenic bacteria (Kulkarni, 1999; Hiremath et al., 1993; Srivastava and Lal, 1997; Adelakun et al., 2001; Verma and Dohroo, 2003; Singh and Singh, 2005). Gram positive bacteria were more susceptible than that of gram negative bacteria in response to the MISK extract observed in the present study. It is in line with the previous reports (Patni et al., 2005; Karou et al., 2005). Scherrer and Gerhardt (1971) reported that gram positive bacteria have outer peptidoglycan layer which is not an efficient barrier. The gram negative bacteria have an outer phospholipidic membrane that makes the cell wall impermeable to lipophilic solutes, while the prune constitute a selective barrier to hydrophilic solutes with an exclusion limits of about 600 Da. Many results confirmed these observations that most plant extracts were found to be more active against gram positive bacteria than gram negative ones (Nikaido and Vaara, 1985; Kelmanson et al., 2000). Similar type of results were reported by Masika and Afolayan, (2002).

Antimicrobial property of a plant depends on its biologically active phytoconstituents. A wide range of antiinfective actions have been assigned to tannins (Haslam, 1996). Some authors have found that more highly oxidized phenols are inhibitorier (Scalbert, 1991; Urs and Dunleavy, 1975). Flavonoids are synthesized by plants in response to microbial infection (Dixon et al., 1983). Terpenoids are active against bacteria (Ahmed et al., 1993), fungi (Ayafor et al., 1994), viruses (Fujiokea and Kashiwada, 1994) and protozoa (Ghoshal et al., 1996). Hence, the plant which was subjected to this investigation reveals the presence of
active phytochemicals, which exhibits many beneficial properties. MISK extracts inhibited the growth of all types of microbial population (Table 5.7 and 5.8). Tannins and phenolic compounds found in MISK, which precipitates cell wall proteins of microorganisms and also suppress prokaryotic DNA replication.

In traditional societies nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes. The consumption of non-cultivated botanicals plays a central role in the diet, but very few ethno pharmacological and phytopharmacological studies have dealt exhaustively with the potential health benefits of such diets. In olden days, MISK is consumed as a food during starvation. It contains starch along with other secondary metabolites (Plate V). In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. ROS produced In Vivo include superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hypochlorous acid (HOCl). H$_2$O$_2$ and O$_2$ can interact in the presence of certain transition metal ions to yield a highly-reactive oxidizing species, the hydroxyl radical (OH) (Aruoma and Halliwell, 1987). Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, sclerosis, aging and neoplastic diseases (Roy and Burdon, 1994). They are also involved in autoimmune disorder like rheumatoid arthritis etc. (Rao et al., 2004). Antioxidant plays a vital role in protecting the cells against free radicals. They scavenge free radicals thereby protecting the cells from metabolic disorders.

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract (Bhuiyan et al., 2009). Scavenging of DPPH radical is related to the inhibition of lipid peroxidation (Rekka and Kourounakis 1991). DPPH is usually used as a substance to evaluate the antioxidant activity (Tara et al., 2012). Antioxidants either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character (Pan et al., 2008). DPPH test, which is based on the ability of DPPH, a stable free radical, to decolourize in the presence of antioxidants, is a direct and reliable
method for determining radical scavenging action (Raquibul *et al.*, 2009). The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search the In Vitro general antioxidant activity of pure compounds as well as plant extracts (Koleva *et al.*, 2002). The reducing capacity of compounds could serve as indicator of potential antioxidant property (Meir *et al.*, 1995). In the present study, the percentage of scavenging effect on the DPPH radical was concurrently increased with the increase in the concentration of both aqueous and phenolic extracts of *M. indica* seed kernel from 20 to 100 μg/ml and their IC$_{50}$ values (the concentration required to inhibit radical formation by 50%) ranged from 90 to 97 μg /mL (Table 5.10). The medicinal effects of plants are often attributed to the antioxidant activity of phytochemical constituents mainly phenolic, flavonoids and flavonols (Miliauskas *et al.*, 2004). It is claimed that phenolic compounds are powerful chain breaking antioxidants (Shahidi and Wanasundra, 1992). The scavenging activity of phenolic group is due to its hydroxyl group (Hateno *et al.*, 1987). This indicates that *M.indica* possesses hydrogen donating capabilities for both aqueous and phenolic of seed kernel extracts and does scavenging free radicals. This indicates that *M.indica* extract has high amounted of phenolic compound. It could be considered as good potential source of natural antioxidants.

Tanaka *et al.*, (1988) have observed a direct correlation between antioxidant activity and reducing power of certain plant extracts. Reducing power activity is often used to evaluate the ability of natural antioxidant to donate electron (Yildirim *et al.*, 2000, Dorman *et al.*, 2003). The reducing properties are generally associated with the presence of reductones (Duh *et al.*, 1999), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Ferric reducing power indirectly indicated the free radical scavenging power of the extracts. Here also percentage of inhibitance increased with increased concentration of the extracts. About 93.33±0.003% free radical scavenging power was exhibits by Phenolic extract at 100μg/mL concentration with 15.13±0.03μg/mL IC$_{50}$ value which is 5 times lower than that of standard (Table 5.11). The reducing power of extract of *M.indica* was found
remarkable and the reducing power of the extract was observed to rise as the concentration of the extract gradually increased.

Superoxide anion is oxygen centered iron complexes such as cytochrome. Superoxide radicals are known to be very harmful to cellular components as a precursor of oxygen species (Halliwell & Gutreridge, 1999). Superoxide anion is the first electron products of oxygen. Superoxide anions damage the cell directly and indirectly by forming hydrogen peroxide, hydroxide or singlet oxygen during pathological condition. Free radical scavenging powers of the both aqueous and phenolic extracts of *M. indica* seed kernel were depends on the hydrogen or electron donating power. Flavanoids like phenolic compounds greatly donate hydrogen and electron and had high antioxidant power. Both aqueous and phenolic extracts showed IC$_{50}$ values as higher concentration for both aqueous and phenolic extract and standard (Table 5.12).

Nitric oxide (NO), being a potent pleiotropic mediator in physiological processes and a diffusible free radical in the pathological conditions, reacts with superoxide anion and form a potentially cytotoxic molecule, the 'peroxynitrite (ONOO-)'. Its protonated form, peroxynitrous acid (ONOOH), is a very strong oxidant (Malinski, 2007). The main route of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. Under physiological conditions, peroxynitrite also forms an adduct with carbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins in living systems (Saumya and Mahaboobbasha, 2011). Table 5.13 showed the significant decrease in the NO radical is due to the scavenging activity of the both the extracts.

ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants (Sreejayan, 1997). In the total antioxidant activity, ABTS is blue chromophores produced by the reaction between ABTS and potassium per sulfate and in the presence of the *M.indica* seed kernel extracts. The extracts efficiently scavenged ABTS radicals generated by the reaction
between 2, 2’-azinobis (3- ethylbenzothiazolin-6-sulphonic acid) (ABTS) and ammonium per sulfate. Table 5.14 showed ABTS antioxidant capacity of MISK extract. The IC\text{50} value high in phenolic extracts when compared to the aqueous extract.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe\text{2+} and possibly Cu\text{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects (Kumaran and Karunakaran, 2007). H\text{2}O\text{2} act as a recipient and receive the electrons from \textit{M.indica} extracts of antioxidant compound. \textit{M. indica} extracts showed good antioxidant power with potential IC\text{50} values (Table 5.15). It indicates the \textit{M.indica} extracts donate the electron in H\text{2}O\text{2} and it can scavenge the radicals.

The antioxidative activities observed can be attributed to either the different mechanisms exhibited by different phenol and polyphenolic compounds that is, tocopherols, Gallic acid, tannins, flavonoids and other organic acids and to the synergistic effects of different compounds. Many studies have shown that many phenolic compound contribute significantly to the antioxidant activity (Demla \textit{et al.}, 2012) and act as highly effective free radical scavengers which is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Table 5.28 showed the presence of phenol and polyphenolics compound like flavonoids, tannin etc in both the extracts.

Biological and chemical research in life science evidenced that free radicals and reactive oxygen species can be involved in a high number of diseases (Jain and Agarwall, 2008). Numerous physiological and biochemical processes in the human body may produce oxygen centered free radical and other reactive oxygen species and byproducts. Over production of such free radical cause oxidative damage to biomolecules leading to many chronic diseases (Halliwell, 1974). Plants are the important source for free radical
scavenging molecules. Intake of natural antioxidants has been associated with reduced risk of cancer; cardiovascular diseases, diabetes and other diseases associated with ageing.

Antioxidant is one of the most essential ingredients of today’s menu/therapy because the antioxidative system protects the animal against reactive oxygen species (H₂O₂, superoxide, OH, singlet oxygen & nitrogen species) induced oxidative damage. Various synthetic antioxidants (BHT) are on the use, but they are suspected to be carcinogenic (Singh et al., 2002). Natural antioxidants, therefore, have gained importance. Aqueous and phenolic extracts of MISK has been studied for its antioxidant properties using different In Vitro antioxidant methods. Flavonoids, phenolic acids, tannins, steroids are found in the MISKPE. MISK extracts showed good antioxidant effect, which could be due to the available phytoconstituents. In this respect, polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer (Sreeram et al., 2005), antiproliferative, antimicrobial, wound healing (Nasr et al., 1996) and antibacterial (Das et al., 1999) activities including antioxidant activity (Gil et al., 2000).

The antioxidant activity of MISK might be due to inactivation of free radicals or complex forming with metal ions or combination thereof. The results of preliminary phytochemical screening of aqueous and alcoholic extracts of MISK revealed the availability of multiple polar and non-polar chemical constituents (Table 5.29). Steroids, terpenoids, flavonoids, phenolic compounds, lignin, fat and oil, inulin, proteins, carbohydrates were found in both extracts. Alkaloids were present only in alcoholic extracts whereas saponins and cardiac glycosides were present only in aqueous extract. Flavonoids and tannins are a major group of compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

In the traditional medicine system, Mangifera indica is used in the management of diarrhoea. Our results showed that the phenolic extract of M. indica seed kernel inhibited significantly (p < 0.05) castor oil-induced diarrhoea in rats. Diarrhoea may be characterized as the abnormally frequent defecation of faeces of low consistency
which may be due to a disturbance in the transport of water and electrolytes in the intestines. Despite the multiplicity of aetiologies, the four major mechanisms responsible for the pathophysiology in water and electrolytes transport are (i) increased luminal osmolarity (osmotic diarrhoea), (ii) increased electrolytes secretion (secretory diarrhoea), (iii) decreased electrolytes absorption, and (iv) deranged intestinal motility causing a decreased transit time (Gaginella et al., 1978). These include inhibition of intestinal Na⁺, K⁺ ATPase activity, thus reducing normal fluid absorption (Capasso et al., 1994), activation of adenylate cyclase or mucosal cAMP-mediated active secretion (Galvez et al., 1993), stimulation of prostaglandin formation (Pinto et al., 1992) and platelet activating factor (Mascolo et al., 1996). Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil (Ammon et al., 1974). Castor oil causes diarrhoea due to its active metabolite, ricinolic acid. It leads to changes the electrolyte permeability of intestine and also it stimulates the release of prostaglandin and peristaltic activity of small intestine. In this study, the phenolic extract of Mangifera indica exhibited a significant antidiarrhoeal activity. The results were comparing to that of the standard drug loperamide with regard to the severity of diarrhoea (Table 5.16, 5.17 and 5.18).

This report revealed that the phytochemicals present in MISKPE reduces the release of prostaglandins and therefore considered to delay castor oil induced diarrhoea. Prostaglandins are considered as a good diarrhoeogenic agent. It promotes vasoilation, smooth muscle contraction and mucous secretion. Plant extracts not only reduces castor oil induced diarrhoea but also reduces microbial burden of the intestine and reduces the toxigenic effect created by the microorganisms.

Phytochemical analysis of MISKP revealed the presence of tannins, flavonoids, phenolic compounds (Table 5.28). These compounds act on the castor oil induced diarrhoea in different mechanisms. Flavonoids exerts antidiarrhoeal activity by inhibiting the release of autocooids and prostaglandins, by inhibiting contractions caused by spasmogens, by stimulates normalization of the deranged water transport across the mucosal cells and also by inhibiting
GI release of acetylcholine. Phenolic compounds makes intestinal mucosa more resistant and reduces secretion, stimulates normalization of deranged water transport across the mucosal cells and reduction of the intestinal transit, blocks the binding of B subunit of heat-labile enterotoxin to GM1, resulting in the suppression of heat-labile enterotoxin-induced diarrhea, astringent action, increases supply of digestible proteins by animals by forming protein complexes in rumen, interferes with energy generation by uncoupling oxidative phosphorylation, causes a decrease in G.I. metabolism (Meyer et al., 1997). Steroids enhance intestinal absorption of Na+ and water.

Results of this study is in line with earlier studies, but their experiment were done using different plant (Meyer et al., 1997; Cerutti, 1994; Roy et al., 2002). Earlier studies showed that antidysenteric and antidiarrhoeal properties of medicinal plants were due to steroids, terpenoids, flavonoids, phenolic compounds, tannins, lignin, carbohydrates and proteins. Hence, tannins, reducing sugars and sterols may be responsible for the mechanism of action of antidiarrhoeal activity. Antidiarrhoeal activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistant and hence, reduce secretion (Cerutti, 1994). This can be due to the fact that the extracts increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. Loperamide, apart from regulating the gastrointestinal tract, is also reported to slow down transit in the small intestine, reduce colon flow rate and consequently any effect on colonic motility (Roy et al., 2002).

Furthermore, the phenolic extract was significantly reduced intestinal transit as evidenced by the decrease in the distance traveled by charcoal meal. Table 5.17 showed that the M.indica seed kernel extract suppressed the propulsion of charcoal meal thereby increasing the absorption of water and electrolytes and also it increase the weight of the intestine when compare to the standard drug loperamide. Antidiarrhoeal properties of medicinal plants were found to be due to phenol, tannins, flavonoids, alkaloids, saponins, reducing sugar, sterols and/or terpenes (Venkatesan et al., 2005). The
antidiarrheal activity of flavonoids has been described to their ability to inhibit intestinal motility and hydro-electrolytic secretions (Rao et al., 1997 & Di Carlo et al., 1993) which are altered in this intestinal condition. In vitro and in vivo experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandins E2 (Sanchez de Medina et al., 1997). In addition, flavonoids present antioxidant properties (Su et al., 2000) which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (Mora et al., 1990).

Antiulcer activity of phenolic extract of *Mangifera indica* seed kernel was evaluated using acid alcohol induced ulceration model. Ulcers are thought to be due to an imbalance between offensive factors such as acid and pepsin and defensive factors such as mucin secretion, cell proliferation, prostaglandins, etc (Rao et al., 2001). Acid induces histamine, which is a potent stimulator of acid secretion. Ethanol causes disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucous depletion and free radical production (Sakatan and Juvekar, 2009). This is attributed to the release of superoxide anion and hydroperoxy free radicals cause acute and chronic ulceration in the gastric mucosa (Jude and Paul, 2009). Ethanol also induced gastric lesion formation, which contributes to the development of the haemorrhage and necrotic tissue injury (Soll, 1990; Surendra, 1999). *M.indica* seed kernel phenolic extract significantly reduces mean ulcer count. It may be due to antisecretory effect of *M.indica* seed kernel, which significantly reduces the formation of ulcers (Table 5.19 and Plate XVI). Plate XVI showed the phenolic extract of *M.indica* seed kernel is gradually increase the ulcer healing process in HCl : alcohol induced animals. It clearly indicates the 400 µg of extracts act as antiulcer drugs. It also increases the pH, reduce the acidity of stomach and also it reduces the protein content in ulcer affected animals (Table 5.20). Phytochemical constituents of the *M.indica* seed kernel phenolic extracts were highly responsible for reducing ill effects of acid alcohol by preventing mast cell activity and normalize the H2 secretion in the stomach. Rajan et al., (2012) revealed the presence of flavonoids, phenolic compounds, tannins; alkaloids in phenolic extracts of *M. indica* seed kernel. Phenols, Tannins, flavonoids were responsible for antiulcer activity. Flavonoids prevents ulcer by free radicals scavenging
mechanisms. In Vivo antioxidant assay (LPO, SOD and GSH) revealed that *M.indica* seed kernel could be considered as an effective antioxidant compound and prevents ulcer and its related complications (Table 5.21). Tannins have been reported to possess antioxidant, wound healing, antimicrobial and antiulcer activity. *M.indica* seed kernel phenolic extract could be considered as a good antiulcer agent may be due to the green chemicals found on the plant materials. Polyphenolics substance of this plant also may responsible for antioxidant and antiulcer activity (Wu *et al.*, 2015).

Stomach of group II animals (P. Fig. 63) showed marked reddening of stomach which is due to cellular infiltration. Studies suggested that the ethanol damage to the gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Szabo *et al.*, 1995). Ethanol produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and production of mucus (Marhuenda *et al.*, 1993). Exposure to ethanol increases the extension of cellular damage in a dose-dependent way (Mutoh *et al.*, 1990).

The present study demonstrated the potentials of MISKPE significantly reduced gastric ulceration as indicated by the reduction in ulcer index in the acid alcohol induced animal models. These results suggested that both extracts possesses anti-secretory potency as well as acid neutralizing effect. Furthermore, based on findings by Ubaka *et al.*, (2010) the anti-secretory effect is suggested to be one of the mechanism through which the extracts was able to protect the stomach mucosa from acid and alcohol induced damage. Abdulla *et al.*, (2010) demonstrated that the reduction of neutrophil infiltration into ulcerated gastric tissues helped to prevent gastric ulcers in rats. Wasman *et al.*, (2010) showed that the oral administration of plant extract prior to ethanol administration significantly decreased neutrophil infiltration into the gastric mucosa. Ethanol causes extensive damage to the gastric mucosa and leads to increased neutrophil infiltration into this tissue.
Level of catalase, SOD and GPx were high among the tissues treated with herbal extracts and ranitidine. Variable antioxidant enzyme level was noted in Gp II. Other group (Gp III, IV, V, VI and VII) animals regained the nature of antioxidant enzymes as normal. SOD converts superoxide to hydrogen peroxide (H$_2$O$_2$), which is transformed into water by catalase in the lysosomes or by glutathione peroxidase in the mitochondria (Johansen et al., 2005). MDA is the final product of lipid peroxidation and is used to determine lipid peroxidation levels (Dursun et al., 2009). Lipid peroxidation causes a loss of membrane fluidity, impaired ion transport and membrane integrity and ultimately a loss of cellular function. Oxidative stress plays an important role in the pathogenesis of various diseases including gastric ulcer, with antioxidants being reported to play a significant role in the protection of gastric mucosa against various necrotic agents (Trivedi and Rawal, 2001). Antioxidants could help to protect cells from damage caused by oxidative stress while enhancing the body’s defensesystems against degenerative diseases. Administration of antioxidants inhibits ethanol-induced gastric injury in rat (Ligumsky et al., 1995). In addition, phenolic compound are the major contributors to the antioxidant activities of MISKPE.

Kobayashi et al., (2001) reported that terpenoids exerts a protective effect against mucosal lesions through inhibition of neutrophils infiltration in the ulcerated gastric tissue and Shimizu et al., (2000) demonstrated that the reduction of neutrophils infiltration into ulcerated gastric tissue promotes the healing of gastric ulcers in mice. Present study revealed the presence of terpenoids in the extracts of MISKPE.

Antioxidant enzymes like SOD are the first line of defense against peroxidation. They are highly specific in their catalytic mode of action and decrease the gastric mucosal damage against free radicals. MISKPE offers gastric protection against acid alcohol induced ulcer by significantly blocking lipid peroxidation which is proved by the reduced levels of lipid peroxide in plant extract treated animal groups. The above results confirmed the antioxidant and antiulcer activity of MISK (Renuka Devi et al., 2007).
Normal gastric mucosal glands with normal cellular architecture were observed in MISKPE treated animal groups. Peptic ulcers are caused when the natural balances between the aggressive factors of acid, pepsin, defensive mechanisms of mucus, bicarbonate, mucosal turnover and blood supply (mucosal barrier) are disturbed (Piper and Stiel, 1986). Baron et al. (1980) suggested that acid and pepsin are relatively less important as causative agents and that a defect in the defensive mechanism of gastric mucosa is the first step towards ulcer formation (Marhuenda et al., 1993). Although in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism (Piper and Stiel, 1986).

Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucous barrier (Sanyal, 1983). Prostaglandins are important cyclo protective agents in the gastrointestinal track because they increase mucous secretion, bicarbonate secretion and mucosal blood flow. Hydrophobic surfactant like phospholipid secretion in the gastric epithelial cells is also stimulated by the prostaglandin (Aly, 1987). They also stabilize mucosal mast cells, lysosomal membranes and they inhibit free radical production. Aspirin is a potent inhibitor of prostaglandin synthesis through its irreversible acetylisation of cyclooxygenase. This inhibition is one of the main reasons for mucosal injury in the stomach and duodenum. Aspirin also breaks the gastric mucosal barrier by non prostaglandin dependent mechanisms leading to a reduction in mucosal potential difference and back diffusion of hydrogen ions (Hawkey et al., 1991).

The above experiments and results prove the gastroprotective activities of MISKPE in acid alcohol induced ulcerated rats. Various literature on preliminary phytochemical studies of MISKPE revealed the presence of flavonoids (Achari et al., 1984). The
antiulcerogenic and gastroprotective activity of flavonoids have been widely reported (Parmar and Shikha, 1998). This indicates that the gastroprotective role of MISKPE observed in the present investigation may be due to the polyphenols present in it.

Cancer is often associated with increased risk of death and the toxic side effects caused by the modern medicine, many cancer patients seek alternative and complementary methods of treatment such as usage of phytomedicine (Sridharan et al., 2012). The result of the present study on a phenolic extract of *M.indica* seed kernel against EAC cells indicates the cytotoxic activity increases with concentration. The criteria for judging the value of any anticancer drug are the prolongation of life span, inhibition of gain in average body weight and the decrease in WBC. Phytochemical studies indicate that the presence of phenols, tannins, flavonoids, saponins amino acids, etc. Polyphenolic compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells. The mechanism of action of anticancer activity of phenols could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenols reduce the amount of cellular protein and mitotic index and colony formation during cell proliferation of cancer cells (Zhao et al., 2007). The phenolic extract of *M.indica* seed kernel has high amount of phenol and polyphenolic compounds it regulate the above mechanism in EAC induced albino rats in present study. The rapid increase in ascitic fluid volume was observed in tumour bearing mice, ascitic fluid is the direct nutritional source for tumors growth it meets the nutritional requirements of tumor cells (Raju et al., 2011). Table 5.22 indicates the increase of life span of tumor bearing mice indicates reduction of nutritional fluid volume and seization of the tumor growth is a positive result and further determines the antitumor effect of *M.indica* seed kernel. Furthermore, the reduced volume of EAC and increased survival time of the mice suggest that the extracts might have exerted a delay in vascular permeability to the cells (Bhist et al., 2010). While the standard drug, 5-fluorouracil as a pyrimidine analogue, is transformed inside the cell into different cytotoxic metabolites
which are then incorporated into DNA and RNA, eventually inducing cell cycle arrest and apoptosis by inhibiting the cell’s ability to synthesize DNA. The standard drug activity thus indicates a gene expression mechanism, which is indirect cytotoxicity. The phenolic extract *M. indica* seed kernel has followed this mechanism.

Ascitic tumor implantation promotes local inflammatory reactions leading to increase in vascular permeability, and results in intense edema formation, cellular migration and progressive ascitic fluid formation. Ascitic fluid is essential to tumor growth, since it constitutes the direct nutritional source for tumour cells. It highly decreased the viable cell count and also increases the non viable cell count respectively 2.96% (Table 5.25). It may be due to the absorption of EAC induced rats in phenolic extract *M.indica* seed kernel by viable cells which leads the lysis of the cells through the activation of macrophages or some cytokinnin production. The cytokines produced in the body by the lymphocytes are known as interleukins and they mediate cytotoxic actions through the cell surface receptors in relevant target cells. Interleukins stimulate the growth and activity of immune cells, which target cancer cells. It acts as an antitumor agent by increasing the cytolytic activity of antigen-specific cytotoxic T lymphocytes and natural killer (NK) cells and by increasing the gene expression responsible for encoding the lytic component of cytotoxic granules, that is, perforin and granzymes (Chatterjee et al., 2011). Reduction in viable cell count and increased non-viable cell count towards normal in tumor host suggested that *M.indica* seed kernel stimulate the growth and activity of immune cells by the production of Interleukins, which target tumor cells and cause lysis of the tumor cells by indirect cytotoxic mechanism. Table 5.26 shows the *M.indica* seed kernel it induce the glucose and protein level in cancer treatment animals. It indicates the *M.indica* extract was directly induced the host metabolism and synthesis the energy and enzymes for metabolic process.
The induction of the EAC to the experimental animals causes the oxidative stress which facilitated the increased production of free radicals. Excessive production of free radicals damage the macro molecules such as lipids and it can induce the lipid peroxidation (Fenninger and Mider, 1954). The end product of the lipid peroxidation was reported to be higher in cancer tissue. The *M.indica* seed kernel is effectively reduce the the lipid peroxidise in EAC bearing animals and also it have the ability to scavenge or inhibit the production of the free radicals (Table 5. 26). SOD and CAT are present in oxygen metabolising cells and their function is to provide a defense against the potentially damaging free radicals such as superoxide dand hydrogen peroxidae. A decreased activity of SOD in EAC inoculated animals due to the loss of Mn+ in SOD system and loss of mitochondria (Sun *et al.*, 1989). The inhibition of SOD activity is due to the tumour progression. The administration of phenolic extract of *M.indica* seed kernel in different doses it increase the SOD activity. The *M.indica* seed kernel has prevented the mitochondrial damage caused by the free radicals which facilitated the increased activity of SOD.

Catalase is a heme containing enzyme, it play a vital role in oxidative stress condition. The disease controlled animals were the low level of the CAT when compared to the normal group. It causes the inactivation of superoxide free radicals through the converting to the ferroxy and ferryl state of the enzyme (Kono *et al.*, 1982) iron has an important role in catalase activity. In tumour condition the iron level is highly increased it reduce the catlase activity because the iron binding and transferring activity of iron it directly decrease the catalase activity in EAC induced animals. The experimental animals are orally administered to the phenolic extract *M.indica* seed kernel, it increase the catalase enzyme. The increase amount of catalase the conversion of ferrous and ferryl formations have highly decreased and increase the binding capacity of iron with transferring which facilitated the increased synthesis of catalase.
Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia (Price and Greenfield, 1958; Hogland 1982; Rajeshwar et al., 2005). The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage, and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions (Rajeshwar et al., 2005; Sarada et al., 2012). Treatment with phenolic extract of Mangifera indica seed kernel brought back the hemoglobin (Hb) content, RBC and WBC count more or less to normal levels. This clearly indicates that M.indica seed kernel possess protective action on the hemopoietic system (Table 5.27). In the last few years, the identification and development of phenolic compounds or extracts from different plants has become a major area of health- and medical-related research.

Reduction in the viable cell count and increased dead cell count towards normal in tumour host indicated that the extracts stimulate the growth and activity of immune cells by the production of interleukins. The reduced volume of EAC and increased survival time of the animal suggests that the extract might have exerted a delay in vascular permeability to the cells (Bhist et al., 2010), while the standard drug 5-Fluourouracil as a pyrimidine analogue is transformed inside the cell into different cytotoxic metabolites which are then incorporated into DNA and RNA eventually inducing cell cycle arrest and apoptosis by inhibiting the DNA synthesis. The standard drug activity thus indicates a gene expression mechanism is indirect cytotoxicity. The antitumour activity of the extract appears to also follow this mechanism (Marklund et al., 1982).

Myelosuppression and anaemia are the major problem in cancer (Maseki et al., 1981; Badami et al., 2003). The anaemia encountered in the tumour bearing mice is due to the reduction in the RBC count or haemoglobin percentage and this may occur due to iron deficiency or due to haemolytic or myelopathic conditions. Treatment with MISKPE restored the haematological profiles as compared to EAC mice. This indicated that MISKPE possess protective action on the haemopoietic system. EAC tumour bearing mice (Gp II) showed a significant decrease in the RBC count, haemoglobin and a significant
increase in the WBC count as compared to the normal control. Treatment with extracts significantly decreases the lymphocyte count with an increase in the neutrophil counts. Treatment with 5-FU (Reference drug) also restored normal haematological profile (Table 5.27).

Cell proliferation, a characteristic feature of cancer. It is inversely proportional to oxidative stress on the host. Several studies have demonstrated that tumour bearing animals can experience a systemic change of antioxidant enzymes in organs distant from the tumour. The tumour cells produce substantial amount of hydrogen peroxide, which may be released into circulation and then transported to the liver for detoxification (Devasena et al., 2002), which is also evidenced in our report. Further, growing tumours sequester essential antioxidants from the host tissues and meet their demand (Bhuvaneswari et al., 2004).

Free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells. The SOD and catalase level was decreased in EAC bearing mice leads to decrease in total SOD activity in liver. The inhibition of SOD and CAT activity results in tumour growth. The administration of MISKPE at different dose (100mg/kg and 200mg/kg) increased the SOD and CAT level, which may indicate the antioxidant and free radical scavenging property.

Authentication, quality control, and stability testing of crude plant extracts are generally achieved and well established through NMR and capillary GC, using specific detectors such as UV–Vis, RI, and coupled systems (MS), which allow the qualitative and quantitative determination of the composition of the markers or active substances.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of
FTIR peak values and functional groups were represented in Table 5.30. The FTIR spectrum profile was illustrated in the Plate XXI. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. Hence, the phenolic extracts subjected to UV-VIS and FTIR analysis for the identification of chemical constituents present in *M. indica*. In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method.

The current pioneering study suggests that phenolic extract is a potent therapeutic agent. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity. This was substantiated from the FTIR studies. The Characterstc peak showed notable functional groups for phenolic compounds.

An IR spectrum of the plant extract shows the presence of OH group and UV-VIS spectrum of *M. indica* extracts has absorption bands at 324 and 290 nm. These absorption bands are characteristic for phenols and its derivatives. The phenols spectra typically consist of two absorption maxima in the ranges 230-290 nm and 300-350 nm which was in synchronous with our results P. Fig. 86. The precise position and relative intensities of these maxima give valuable information on the nature of the phenols.

Analysis of the extract under FTIR and UV-VIS spectroscopic technique showed that the presence of phenolic compound which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Further research will be needed to find out the structural analysis of flavonoid compound by use of different analytical methods such as NMR and GC-Mass spectrophotometer.

Phenolics can enhance the body's immune system to recognize and destroy cancer cells as well as inhibiting the development of new blood vessels (angiogenesis) that is necessary for tumour growth. They also attenuate adhesiveness and invasiveness of cancer
cells thereby reducing their metastatic potential. Augmentation of the efficacy of standard chemo- and radiotherapeutic treatment regimes and the prevention of resistance to these agents is another important effect of plant phenolics that warrants further research. Plant phenolics appear to have both preventative and treatment potential in combating cancer and warrant further, in-depth research (Dai and Mumper 2010).

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. The structure diversity is a result of the variation in hydroxylation pattern, stereochemistry at the three chiral centers. This result was substantiated with the present NMR results where, the peaks showed aromatic rings with hydroxyl groups.

Quantification of phenolic compounds in plant extract is influenced by the chemical nature of the analyse, as well as assay method, selection of standards and presence of interfering substances. Because of the heterogeneity of natural phenolics and the possible interference from other readily oxidized substances in the plant materials, it is not surprising that several methods have been used for determination of total phenolics. The chemical structures of the purified phenolic acids were confirmed by GC-MS and NMR (Khoddami et al., 2013). Phenolic extract of M. indica shows the phenol and poly phenolic compound such as mangiferin (1, 6, 7-trihydroxy-2-(3, 4, 5 – trihydroxy – 6 – hydroxymethyl – tetrahydro – pyran – 2 - yl) - xanthone – 9 - one) and poly phenol (Benzene -1, 3, 5 - triol). In present study both the compounds was carried out antioxidant, antiulcer, antidiarrhoeal and anticancer activities.

Flavonoids, such as quercetin, are antioxidants. They scavenge damaging particles in the body known as free radicals, which damage cell membranes, tamper with DNA and even cause cell death. Antioxidants can neutralize free radicals and may reduce or even help prevent some of the damage they cause. They also help in keeping LDL ("bad") cholesterol under control, which scientists think may contribute to heart disease. Flavonoids act like an antihistamine and an antiinflammatory drug and may help protect against heart disease and cancer. Quercetin can also help stabilize the cells that release
histamine in the body and thereby has an antiinflammatory effect (Boots et al., 2008; Boots et al., 2007; Cai et al., 2000 and Chan et al., 2000). Quercetin is said to have a number of uses, but most of these are based on early findings from laboratory studies. Some early studies have suggested quercetin has antihistamine properties and it is often promoted to help control allergies and asthma (Schabath et al., 2005; Shoskes et al., 1999 and Volate et al., 2005).

Polyphenolic compounds might inhibit cancer cells by xenobiotic metabolizing enzymes that alter metabolic activation of potential carcinogens, while some flavonoids could also alter hormone production and inhibit aromatase to prevent the development of cancer cells. The mechanism of action of anticancer activity of phenols could be by disturbing the cellular division during mitosis at the telophase stage. It was also reported that phenols reduce the amount of cellular protein and mitotic index and colony formation during cell proliferation of cancer cells (Zhao et al., 2007). The Elrich tumour cells are one of the rapidly growing carcinoma with very aggressive behavior and are able to grow in almost all strains of mice (Segura et al., 2000). Ascitic tumour implantation promotes local inflammatory reactions leading to increase in vascular permeability and results in intense edema formation, cellular migration and progressive ascetic fluid formation. Ascetic fluid is essential for tumour growth. Since it was constitutes the direct nutritional source for tumour cells. Thus MISK extracts decrease the tumour volume, tumour weight and packed cell volume of solid tumour. Reduction in the viable cell count and increased dead cell count towards normal in tumour host suggest antitumour effect against EAC cells in mice (Bala et al., 2010).

Flavonoids are most dominantly known for their antioxidant activity and reduce LPO not only by preventing or slowing the onset of cell necrosis, but also by improving vascularity. Phenolic compounds are commonly known for their antioxidant, anti-inflammatory and antimicrobial activities, while, tannins have been reported to possess antioxidant, wound healing and antimicrobial activities. Phytochemical study of AME indicated the presence of phenolic compounds, flavonoids, tannins, saponins and
phytosterols, etc., which have got important pharmacological effects. Recent research has also shown that, through overlapping or complementary effects, the complex mixture of phytochemical in fruits and vegetables provides a better protective effect on health than single phytochemical. The protective effects of MISKPE may therefore, be attributed to the complex mixture of phytochemical present in MISKPE, which have been reported to have antidiarrhoeal, antiulcer, anti-inflammatory, immunomodulatory and antimicrobial properties (Ghatule et al., 2014).

Overall biopotential study revealed that MISKPE showed better results. It may be due to the presence of higher concentrations of phytochemicals like tannins, phenolic compounds and flavonoids in phenolic extracts than aqueous extract.