Chapter 6.

DISCUSSION
6.1 Extractive yield

The extractive yield depends on solvents, time and temperature of extraction as well as the chemical nature of sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the most important factors (Shimada et al., 1992). The traditional healers or practitioners make use of water primarily as a solvent but there are many reports where organic solvents showed better activity as compared to aqueous extracts (de Boer et al., 2005; Parekh and Chanda, 2006b). In the present study, extraction was done in water (aqueous) and organic solvents of different polarity. In all the screened plants, the extractive yield was maximum in aqueous extract as compared to organic solvents. This may be because the phytoconstituents present in these plants are more extracted in water. Similar results were also reported by (Lii et al., 2009; Beevi et al., 2010; Chanda et al., 2011b; Kaneria et al., 2011). The organic extracts showed varied levels. The petroleum ether extract had more yield in 11 plants, while in four plants, acetone extract had more yield, suggesting that there is no universal criteria for maximum yield in a particular solvent. It varies from plant to plant because of the nature of secondary metabolites present in them and their proportion also varies.

6.2 Antimicrobial screening

For a long period of time, plants have been a valuable source of products to treat a wide range of medical problems, including ailments caused by microbial infection. Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern. The clinical efficacy of many antibiotics is threatened by the emergence of multi-drug resistant pathogens. Therefore, researchers are turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia et al., 2004). Plant based antimicrobial compounds have dual advantage i.e. enormous therapeutical potential and least side effects that are often associated with synthetic antimicrobials.
Bacillus species are common microbes found in most natural environment, including soil, water, plant and animal tissues. Most Bacillus species are regarded as having little pathogenic potential, but both B. cereus and B. subtilis are known to act as primary invaders or secondary infectious agents in a number of diseases and have been implicated in some cases of food poisoning (Kumar et al., 2006). S. epidermidis is a predominant pathogen of skin diseases (Johannes et al., 2001). S. aureus is a known pathogen commonly associated with respiratory and diarrheal diseases (Viljoen et al., 2005). S. aureus expresses surface proteins that promote attachment to host proteins forming part of the extracellular matrix on epithelial and endothelial cell surfaces, as well as is a component of blood clots (Gnan and Demello, 1999; Baie and Sheikh, 2000). S. aureus and P. aeruginosa are common pathogens causing serious infections (Gnanamani et al., 2003). Of the two million nosocomial infections each year, 10% are caused by P. aeruginosa. Infections caused by P. aeruginosa are among the most difficult to treat with conventional antibiotics (Gnan and Demello, 1999; Bail and Sheikh, 2000; Kumar et al., 2006). P. aeruginosa is the most prevalent pathogen capable of causing life threatening illnesses and has been implicated in several infections of human and animals. Due to multi resistancy of P. aeruginosa and lack of active antibiotics against this bacterium, increasing incidence of nosocomial infections and high mortality have been reported (Giamarellos-Bourboulis et al., 1999). E. coli is an opportunistic pathogen and is the common cause of travelers diarrhea and other diarrhoeagenic infections in human (Ogueke et al., 2007).

Over the last two decades, fungal infections with special references to Candida sp. have become important public health concerns (Pfaller and Diekema, 2004). C. albicans is the most common cause of candidiasis, other Candida species particularly C. glabrata have emerged as important cause of oropharyngeal candidiasis and candidemia (Fan et al., 2008; Kliemann et al., 2008). C. albicans is responsible for infections in people and can cause vulvovaginitis, oral thrush, nosocomial infection and candidiasis (Jarvis, 1995; Eggimann et al., 2003). All the plants investigated possessed remarkable antimicrobial activity against pathogenic microorganisms tested, therefore it can be stated that different solvent extracts of screened plants can be used as an antimicrobics.
In the present study, Gram negative bacteria were more susceptible than Gram positive bacteria towards plant extracts, which contradict the previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Parekh and Chanda 2007c; Kaneria et al., 2009; Bajpai et al., 2010; Tenore et al., 2011). The reason for this difference in sensitivity may be ascribed to the differences in morphological constitutions between these microorganisms. The Gram negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components, which makes the cell wall impermeable to antimicrobial chemical substances. On the other hand, Gram positive bacteria have only an outer peptidoglycan layer which is not an effective permeability barrier. The susceptibility of Gram negative bacteria towards plant extracts is reported by Chanda et al. (2010b).

The plant extracts showed better antibacterial activity as compared to antifungal activity. Similar results are also reported by Khan et al. (2008), Stefanovic et al. (2009), Faiza et al. (2011) and Vaghasiya et al. (2011b). Resistance of fungi to plant extracts can be explained by chitinous structure of the cell wall, which disallows easy penetration of bioactive substances (Ogundare et al., 2006). The Gram positive bacteria were more sensitive than Gram negative bacteria and fungi, which is similar to the previous reports (Russell, 2003; Chanda and Nair, 2010; Trabelsi et al., 2010; Kaneria and Chanda, 2011; Miceli et al., 2011).

Amongst 16 screened plants, *Psidium guajava* demonstrated significant antimicrobial activity against both bacteria (Gram positive and Gram negative) and fungi. *P. guajava* extracts possess a broad spectrum of activity against a panel of microbes responsible for the most common infectious diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds.

### 6.3 Quantitative phytochemical analysis

Polyphenols are secondary plant metabolites that are ubiquitously present in plant products and are reported to have several biological activities including antioxidant activity (Chandini et al., 2008). The biological activities are believed to be
due to their redox properties which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Rice-Evans et al., 1996; Itagaki et al., 2009). In the present work, among the sixteen plants investigated, acetone extracts of \textit{P. guajava} and \textit{M. zapota} had considerably greater phenolic content and acetone extract had maximum phenolic content as compared to other solvent extracts, as also reported by Sun et al. (2006), Kaneria and Chanda (2011), Kaneria et al. (2011) and Musa et al. (2011). Phenolic content is a good indicator of the antioxidant capacity of a plant since there are many reports that there is a direct correlation between phenol content and antioxidant activity (Perez et al., 2011; Rakholiya et al., 2011; von Staszewski et al., 2011). However, this is not always true. In some plants the phenolic content and antioxidant activities do not show positive correlation (Yu et al., 2002; Chanda and Dave, 2010; Chanda and Nagani, 2010; Locatelli et al., 2010).

Naturally occurring alkaloids are nitrogenous compounds that constitute the pharmacogenically active basic principles of plants. Alkaloids have been divided into 3 major classes depending on the precursors and the final structure. The true alkaloids are derived from amino acids, are basic and contain nitrogen in a heterocyclic ring for example, nicotine. Common alkaloid ring structures include the pyridines, pyrroles, indoles, pyrrolidines, isoquinolines, and piperidines. A benzylisoquinoline alkaloid, papaverine was shown to have inhibitory effect on several viruses and indoquinoline alkaloids from \textit{Cryptolepsis sanguinolenta} displayed activity against a number of gram negative bacteria and yeast (Silva et al., 1996). In the present work, among the sixteen plants investigated, the highest total alkaloid content was in \textit{A. scholaris} followed by \textit{A. squamosa}.

6.4 Antioxidant activities

Antioxidant compounds act by several mechanisms such as, inhibition of generation and scavenging activity against reactive oxygen species (ROS), superoxide anion radical scavenging, hydroxyl radical scavenging activities, inhibition of oxidative enzymes and reducing capacity assessment. Free radicals and other reactive species cause the oxidation of biomolecules (proteins, amino acids, lipid, and DNA)
and loss of function of enzymes, which leads to cell injury and eventually necrotic cell death or apoptosis (Ghaisas and Navghare, 2008).

Antioxidant activity is a dependent system and the characteristic of a particular system can influence the outcome of the analysis. Owing to the complex reactive facets of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by any single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity (Schlesier et al., 2002; Chanda and Dave, 2009). Therefore in the present work the antioxidant activity was determined by DPPH free radical and superoxide anion radical scavenging activity in all the solvent extracts of screened plants.

6.4.1 DPPH free radical scavenging activity

Free radicals are the primary cause of oxidative damage of biological molecules in the human body, and they are related to many diseases and disorders. DPPH method is one of the most popular methods in natural antioxidant studies because it is easy, rapid and convenient (Argolo et al., 2004; Roginsky and Lissi, 2005). When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form diphenylpicrylhydrazine (non radical) with the loss of this violet colour (Molyneux, 2004). The actual reaction that is taking place between the DPPH stable radical and the antioxidant (AH) is DPPH$^-$ + (AH)$_n$ → DPPH-H + (A$^\cdot$)$_n$. The radical that is formed (A$^\cdot$) in general is less reactive, depending on the structure of the molecule, or it can follow a radical-radical interaction to create a stable molecule (Fang et al., 2002; Briante et al., 2003; Huang et al., 2005b). The antioxidants are able to reduce the stable DPPH radical to yellow-colored and the antioxidant power is indicated by the degree of discoloration which could be determined by discoloration which could be determined by measuring of a decrease in the absorbance at 517 nm. Because of the ease and convenience of this reaction; it has now widespread use in the free radical scavenging activity assessment. DPPH radical scavenging activity of the extracts was concentration dependent and a lower IC$_{50}$ value reflects better protective action. The acetone extract of *P. guajava*, *P. granatum* and *M. zapota* had lowest IC$_{50}$ value which was less than that of standard.
ascorbic acid. Incidentally these plants had maximum phenol content thus supporting the general view that phenol content is a good indicator of antioxidant capacity.

### 6.4.2 Superoxide anion radical scavenging activity

Superoxide anion radical is biologically quite toxic and is deployed by the immune system to kill invading microorganisms. It is an oxygen-centered radical with selective reactivity. It is produced by a number of enzyme systems in autooxidation reactions and by non-enzymatic electron transfers that univalently reduce molecular oxygen. The biological toxicity of superoxide is due to its capacity to inactivate iron-sulfur cluster-containing enzymes, which are critical in a wide variety of metabolic pathways, thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radical. It can also reduce certain iron complex such as cytochrome c (Gulcin et al., 2010). Generally, superoxide anions are converted to oxygen and hydrogen peroxide by superoxide dismutase (SOD), or they react with nitric oxide (NO) to form peroxynitrite. Hydrogen peroxide can be converted into water and oxygen by catalase (Fang et al., 2002). Therefore, superoxide scavenging capacity in the human body is very important as the first line of defense against oxidative stress. Scavenging of superoxide anion radical is important for protection against early events in oxidative damage (Fu and Mao, 2008). Under oxidative stress, the concentration of this species increases dramatically in all cells, inducing several pathophysiological processes, due to its transformation into more reactive species (Gulcin et al., 2007).

The lowest IC$_{50}$ value was in acetone extract of *P. guajava*, *T. catappa*, *S. cumini*, *P. granatum* and *M. zapota* which was even better than that of the standard gallic acid. The data of the present study suggests that acetone extract of *P. guajava*, *T. catappa*, *S. cumini*, *P. granatum* and *M. zapota* are strong superoxide anion quenchers; the constituents of acetone extract of *P. guajava*, *T. catappa*, *S. cumini*, *P. granatum* and *M. zapota* are capable of scavenging reactive species such as superoxide via a mechanism of electron/hydrogen donation and should be able to prevent oxidative damage of the major bio-molecules: proteins and lipids as also suggested by Kaur et al. (2006b).
6.5 Pharmacognostic study

Indian systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant and ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly an essential prerequisite. 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 (WHO, 2002).

Pharmacognosy studies help in identification and authentication of the plant material. Pharmacognosy has multidisciplinary characters to identify the drugs, its origin, morphology and microscopic studies, to determine the quality of the drug, its chemical compositions, therapeutic effects, etc. (Jarald and Jarald, 2007). The process of standardization can be achieved by stepwise pharmacognostic studies. The standardization of a crude drug is integral part of establishing its correct identity. Before any crude drug can be in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established (Abere et al., 2007). Therapeutic efficacies of medicinal plants depend upon the quality and quantity of chemical constituents. It has been established that chemical constituents of a plant species vary with regard to climate and season (Mallavarapu et al., 1995).

*Psidium guajava*, widely used in traditional medicines has tremendous medicinal potential owing to its multifaceted biological functions. The salient diagnostic characteristics of leaf were arc shaped vascular bundles, animocytic stomata, xylem vessels, prism and cluster type of calcium oxalate crystals. These characters can be used for standardization of drugs and also used for preparation of plant monographs. Similar study is reported by various workers in other plants like *Actinodaphne hookeri* Meissn (Prajapati et al., 2008), *Oxystelma esculentum* (L.f.) R.br. Ex Schltes (Poornima et al., 2009), *Datura fastuosa* Linn (Nivedhitha et al., 2010), *Manilkara hexandra* (Roxb.) Dubard (Chanda et al., 2010a), *Polyalthia longifolia* var. pendula (Dave et al., 2010), *Vitex trifolia* Linn. (Thenmozhi et al., 2011), *Punica granatum* L. (Bapodara et al., 2011) and *Citrus paradisi* Var. Duncan (Gupta et al., 2011).
The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of dry powder of *P. guajava* leaves was not very high, hence it would discourage bacteria, fungi or yeast growth. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash is particularly important in the evaluation of purity of drugs; i.e. the presence or absence of foreign inorganic matter such as metallic salts or silica. Acid insoluble ash measures the amount of silica present, especially sand. Water soluble ash is the water soluble portion of the total ash (Dave *et al.*, 2010). Less amount of these three parameters indicate that the inorganic matter and silica was less in *P. guajava* leaves. The extractive values are useful to evaluate the chemical constituents of crude drug (Thomas *et al.*, 2008).

### 6.6 Antimicrobial activity of fractions

FS-I and FS-II exhibited potent inhibitory activity against all the 25 tested bacterial and fungal pathogens (Fig. 17) though FS-II showed better inhibitory activity than FS-I. This broad spectrum of antimicrobial activity of both fractions may be due to the presence of antimicrobial constituents, especially in FS-II. Both the fractions showed better antimicrobial activity than acetone extract.

Both fractions showed concentration dependent activity. Gram negative bacteria were more susceptible than Gram positive bacteria which contradict the results of crude extracts. It is noteworthy that isolated fractions showed activity against *C. freundii* and *E. aerogenes* which were not inhibited by crude acetone extract.

The basis of sensitivity of test organisms may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds presente in the crude extracts. The weak activities of crude extracts may probably could have resulted from antagonistic interactions of compounds from different chemical constituents in them. Cowan (1999) showed that several classes of polyphenol such as phenolic acids, flavonoids and tannins serve as plant defense mechanism against pathogenic microorganisms. In fact, the site and number of hydroxyl groups on the
phenol components increased the toxicity against the microorganisms. Tsuchita et al. (1996) linked the antimicrobial effects of flavonoids to their capacity to form complexes with extracellular and soluble proteins and with the cell wall.

6.7 Antioxidant activity of solvent extracts and fractions

6.7.1 DPPH free radical scavenging activity

The DPPH assay provides basic information on the antiradical activity of the extracts. During DPPH free radical test the capacity of the samples to donate hydrogen atom and/or electron to this blue/purple stable radical and converting it to yellow diphenyl picryl hydrazine molecule was measured (Tepe et al., 2005). This reaction is used for measuring the ability of the extracts or pure molecules (such as ascorbic acid) to scavenge free radicals. The IC\(_{50}\) value of FS-II was lower than FS-I (Table 14). Low IC\(_{50}\) value indicates high antioxidant activity, therefore, FS II possesses better DPPH free radical scavenging activity than FS I.

6.7.2 Superoxide anion radical scavenging activity

Superoxide radicals are known to be very harmful to cellular components since they are the precursors of many ROS, for example, when in presence of metal ions, the superoxide radical would further produce a highly reactive hydroxyl radical (MacDonald-Wicks et al., 2006). Additionally, the superoxide radical can be decomposed to form a stronger oxidative species such as singlet oxygen and hydroxyl radicals (Wu et al., 2008). In the present work, better superoxide anion radical scavenging activity was shown by FS II than FS I (Table 14; Fig. 19). The IC\(_{50}\) value of FS-II was less than that of the standard gallic acid, indicating FS II possess a good superoxide anion scavenging capacity.

6.7.3 Hydroxyl radical scavenging activity

The hydroxyl radical (\('\text{OH}'\)) is an extremely reactive free radical formed in biological systems, can react with almost all the biomacromolecules functioning in
living cells (Lai et al., 2010). Hydroxyl radical can easily cross cell membranes, readily react with most biomolecules including carbohydrates, proteins, lipids and DNA in cells and cause tissue damage or cell death (Yuan et al., 2008b). This radical has the capacity to join nucleotides in DNA and can cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity (Moskovitz et al., 2002; Manian et al., 2008). The hydroxyl radical is a dangerous molecule capable of damaging almost each and every molecule found in living cells (Lee et al., 2002). Thus, removing hydroxyl radical is important for the protection of living systems (Sun et al., 2010). The scavenging effect of acetone extract on the hydroxyl radical was more efficient than other solvent extracts and fractions (Table 14). These results suggest that the extracts of *P. guajava* are capable of scavenging hydroxyl radicals and could help prevent or ameliorate oxidative damage.

### 6.7.4 ABTS radical cation scavenging activity

The ABTS radical cation decolourisation test is another method widely used to assess antioxidant activity. The ABTS\(^{\bullet+}\) formed from the reaction ABTS-e → ABTS\(^{\bullet+}\) reacts quickly with the electron/hydrogen donors to form colourless ABTS. Reduction in colour indicates reduction of ABTS radical (Adedapo et al., 2008). All four extracts and two fractions showed ABTS radical cation scavenging activity but at to a different level. IC\(_{50}\) values ranged from 3.25 to 125 µg ml\(^{-1}\). The lower IC\(_{50}\) values of acetone extract and FS II than that of the standard ascorbic acid indicate the strong ABTS radical cation scavenging activity.

### 6.7.5 Reducing capacity assessment

Many studies have demonstrated that the reducing power in natural plant extracts is strongly correlated with their antioxidant activities (Chanda and Kaneria, 2011; Huang et al., 2011). Therefore, the reducing capacity may be used as an indicator of the potential antioxidant activity. In general, the reducing properties are associated with the presence of reductones, which have been shown to exert antioxidant action by donating a hydrogen atom (Wu et al., 2008).
The reducing power of the extracts was determined using a modified $\text{Fe}^{3+}$ to $\text{Fe}^{2+}$ reduction assay, whereby the yellow colour of the extract changes to various shades of green and blue, depending on the reducing power of the extract. The presence of antioxidants in the samples causes the reduction of the $\text{Fe}^{3+}$/ferricyanide complex to the $\text{Fe}^{2+}$ form, and $\text{Fe}^{2+}$ can be monitored by measurement of the formation of Perl’s Prussian blue at 700 nm (Prasad et al., 2010). Increasing absorbance of the reaction mixture at 700 nm indicates an increase in the reducing power of the extract. Amongst all the different solvent extracts and fractions, reducing capacity of acetone extract was the best and was comparable with that of standard ascorbic acid. Amongst all the different solvent extracts and fractions, acetone extract had maximum phenol content therefore it can be stated that there was a direct correlation between phenol content and reducing capacity assessment in $P.\ guajava$. The extracts of $P.\ guajava$ showed a strong, concentration dependent reducing capability (Fig. 23).

6.7.6 Ferric reducing antioxidant power

FRAP assay is commonly used for the routine analysis of single antioxidant and total antioxidant activity of plant extracts (Xu et al., 2009; Kaneria et al., 2011). Antioxidative activity has been proposed to be related to its reducing power. Therefore, the antioxidant potential of different solvent extracts and fractions were estimated for their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II). FRAP assay was used by several authors for the assessment of antioxidant activity of various samples (Kaneria et al., 2011; Sowndhararajan et al., 2011; Tai et al., 2011). The FRAP assay treats the antioxidants contained in the samples as reductants in a redox linked colorimetric reaction, and the value reflects the reducing power of antioxidants. The procedure is relatively simple and easy to standardise. The antioxidant potentials of different samples were estimated by their ability to reduce the TPTZ–Fe(III) complex to the TPTZ–Fe(II) complex. At low pH (optimum pH 3.6) a ferric salt, $\text{Fe(III)}(\text{TPTZ})_2\text{Cl}_3$ (TPTZ) (as an oxidant), is reduced by antioxidants to its intense blue colored form $\text{Fe}^{2+}$-TPTZ complex ($\text{Fe}^{2+}$ tripyridyltriazine) with maximum absorbance at 593 nm (Benzie and Strain, 1996). In the present study, higher FRAP values were measured in acetone extract as compared to other extracts and fractions of $P.\ guajava$ (Fig. 24). Halvorsen et al. (2006) suggested that most of the secondary
metabolites are redox-active compounds that will be picked up by the FRAP assay. Higher FRAP values, together with increased concentrations of antioxidant compounds, have also been reported by Kaneria et al. (2011), Nencini et al. (2011) and Rajkumar et al. (2011).

6.7.7 Correlation of the different activities with the total phenol and flavonoid content

Among the extracts analyzed in this study, TPC was highest for PAC, as well as PAC showed considerable antioxidant capacities. PTO had a lower concentration of phenolics and also showed lower antioxidant capacities. The $r^2$ values indicated a high degree of correlation between TPC and various antioxidant activities like DPPH, OH, SO, ABTS, RCA and FRAP, whereas the antioxidant activity showed a weak correlation with FC (Table 15). This confirms that the phenolic compounds are responsible for the antioxidant capacity of *Psidium guajava*.

According to these results, there is good relationship between total phenolic content and antioxidant activity, which is also supported by the work of others (Perez et al., 2011; Rakholiya et al., 2011; von Staszewski et al., 2011). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. They also have a metal chelation potential (Rice-Evans et al., 1995).

6.8 Pharmacology

6.8.1 Acute toxicity

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells. This interaction can vary depending on the chemical properties of the toxicants and the cell membrane, as it may occur on the cell surface, within the cell body, or in the tissues beneath as well as at the extracellular matrix. Toxicology testing is an essential requirement for the development of modern pharmaceutical compounds. Administration of PGA at various dose levels produced no clinical signs, adverse effects, or abnormal behavior on tested animals.
The absence of change in body weight suggested that there was no major negative impact on the general metabolic status of the animals. Similar results were also reported by Delaney et al. (2008), Rasekh et al. (2008) and Takami et al. (2008). Consumption of feed, water and body weight was not affected by administration of PGA suggesting that it did not induce appetite suppression and had no deleterious effects on health status, growth or development of the animals.

Hematological parameters provide vital information regarding the status of bone marrow activity and intravascular effect such as hemolysis. Blood is an important index of physiological and pathological status in human and animals and the parameters usually measured are hemoglobin, packed cell volume, white blood cell count, platelets count (Kumar et al., 2007b). There was no significant change in the levels of most parameters at all doses of PGA tested. The small transient of values observed in blood hematology did not show any dose responsiveness. Thus the slight changes of PGA on hematological parameters can be considered as insignificant.

Generally, the reductions in organ weights are simple and sensitive indices of toxicity after exposure to toxic substances (Tan et al., 2008b). Organ weight revealed that PGA, at the doses used, did not produce organ swelling, atrophy and hypertrophy. An increase in this parameter is an indication of inflammation while decrease may be due to cell constriction. The non-significant changes in the organ weight ratios of organs demonstrate that the PGA may not cause any inflammation or cellular constriction of these organs.

6.8.2 Antiulcer activity

The gastric mucosa is constantly exposed to potentially noxious stimuli of endogenous (acid, pepsin, bile) and exogenous (alcohol, drugs) origin. It is commonly believed that the tolerance of the gastric mucosa to damage, originates from continuously operating defensive mechanisms, which include mucosal blood flow, mucus and bicarbonate secretion, and gastric mucosal potential difference. It is generally accepted that it results from an imbalance between aggressive factors (such as acid, pepsin) and the maintenance of the mucosal integrity through the endogenous defense mechanism (Piper and Stiel, 1986). To regain the balance, different
therapeutic agents including plant extracts are used to inhibit the gastric acid secretion or to encourage the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells, or interfering with the prostaglandin synthesis.

Even though many products are available in the market for the treatment of gastric ulcers, including antacids, proton pump inhibitors, anticholinergics and histamine $H_2$-antagonists, most of these drugs produce several adverse reactions such as gynecomastia, hematopoietic changes, acute interstitial nephritis (Ra and Tobe, 2004), thrombocytopenia (Zlabek and Anderson, 2002), anaphylaxis reactions (Gonzalez et al., 2002), nephrotoxicity and hepatotoxicity (Fisher and Le Couteur, 2001). Therefore there is need for newer, natural drugs free of side effects and adverse reactions. Medicinal plants are one of the most attractive sources of new drugs, and have been shown to give promising results in treatment of gastric ulcer.

6.8.2.1 Ethanol induced gastric ulcer

Ethanol induced gastric lesions are thought to arise as a result of stasis in gastric blood flow, which contributes to the development of the hemorrhagic and necrotic aspects of tissue injury (Guth et al., 1984). Occurrence of these ulcers, which is predominant in the glandular part of the stomach, was reported to stimulate the formation of reactive oxygen species (Mizui et al., 1987), resulting in damage to rat gastric mucosa (Peskar et al., 1986). Ethanol induced damage to gastric mucosa is multi factorial. One of the major factors is its rapid penetration into the gastric mucosa, which leads to an increased mucosal permeability and the release of vasoactive factors, which cause vascular damage and gastric injury (Suleyman et al., 2001; Narayan et al., 2004). The high production of free radicals is another factor associated with gastric injury and is due to increased lipid peroxidation and damage to gastric surface. Accumulation of activated neutrophils in the gastric mucosa may be a source of this free radical generation (Vanisree et al., 1996). The obtained results suggest that PGA had remarkably restricted ethanol-induced depletion of gastric wall mucus which may be because of its strong antioxidant and free radical scavenging potential.
6.8.2.2 Ethanol/HCl-induced gastric ulcer

The HCl/ethanol method of inducing gastric lesions is a rapid and convenient way of screening plant extracts for antiulcer potency, which is assessed in terms of the absence or reduction in macroscopically visible lesions (Hara and Okabe, 1985; Tan et al., 2000). The injury caused by ethanol/HCl confers a direct topical effect on gastric mucosa, which is an undesirable effect and a good model to investigate products with a possible cytoprotective activity (Mizui and Doteuchi, 1983). A number of mechanisms that include enhanced gastric mucosal defense through increased mucus and/or bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity (Antonio et al., 2004), can mediate the gastric mucosal protection against HCl/ethanol. In addition, ethanol also induces solubilization of the mucus constituents, decreases the difference of potential in mucosa thus increasing the flow of Na\(^+\) and K\(^+\) to the lumen and pepsin secretion, and also increases H\(^+\) ions and histamine (Szabo and Vattay, 1990). The presence of HCl solution accelerates (Sun et al., 1991) and intensifies the lesion, impairing the protection of the mucosa by chemical agents that need to be more effective.

The PGA reduced the volume of gastric juice, pH, free acidity, total acidity significantly and hence ulcer index showing the anti-secretory mechanism (Goel and Bhattacharya, 1991). HCl-Ethanol induced gastric damage ranging from endothelial microvascular damage to development of macroscopic gastric mucosal lesions, is attributed mainly to the inhibition of biosynthesis of cytoprotective prostaglandins (PG) resulting in overproduction of leukotrienes and other products of the 5-lipoxygenase pathway (Nasuti et al., 2006). These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H\(^+\) and Na\(^+\) ions reducing the transmucosal potential difference and induce formation of erosions and ulcers. In this model, PGA was able to produce a significant reduction of the gastric mucosal damage, indicating a probable local increase in PG synthesis (Rainsford, 1987).

HCl-ethanol induced gastric damage in rat is possibly through leukotrienes production and also involvement of 5-lipoxygenase in the formation of ulcer lesion. Prostaglandins also play a role in ethanol–induced ulcer. Thus the protective effect of the PGA against the gastric damage might be due to protection against 5-
lipooxygenase or leukotriene pathway. The cytoprotective action possibly stimulates the prostaglandin synthesis, which in turn is involved in cytoprotection of the gastric mucosa (Malairajan et al., 2007b). On the other hand, some triterpenes are known as antiulcer drugs and their action has been suggested to be due to: (I) activation of cellular protection (Hara and Okabe, 1985), (II) reduction of mucosal prostaglandins metabolism – cytoprotective action (Konturek, 1986), (III) reduction of gastric vascular permeability (Wagner, 1982). At the both doses used, the animals had no depressive, excitatory or sleepiness symptoms, suggesting that probably centrally acting components involved in antiulcer action are present in PGA.

The present study demonstrated that PGA protects the mucosa of the stomach of the rats even at low doses. In both ulcer models, PGA was effective in reducing ulcer lesions and this was in a dose dependent manner. This effect was better than that of the standard. PGA may inhibit the release of gastric hydrochloric acid and afford protection against gastric mucosal damage.