3.1 INTRODUCTION

Polyphenols are secondary metabolites of plants and are widely distributed in beverages and plant-derived foods. Human consumption studies indicate 1 g of total polyphenols is frequently consumed per day and it is not anticipated that any acute or lethal toxicity would be observed through the oral intake route (Scalbert and Williamson, 2000). Phenolic compounds have the capacities to quench lipid peroxidation, prevent DNA oxidative damage, scavenge free radicals (Cao and Cao 1999), and prevent inhibition of cell communication (Sigler and Ruch 1993), all of which are precursors to degenerative diseases. Free radicals cause depletion of the immune system antioxidants, change in gene expression, and induce abnormal proteins resulting in degenerative diseases and aging.

Antioxidant nutrients and phytonutrients inhibit the oxidation of living cells by free radicals by protecting the lipids of the cell membranes through free radical scavenging, blocking the initiators of free radical attack, neutralizing or converting free radicals into less active, stable products thus breaking the chain reaction and assisting in salvaging oxidized antioxidants enabling them to continue to be of benefit (Halliwell *et al*., 1992). There are 2 main antioxidant defense mechanisms developed by living organisms: enzymatic and nonenzymatic components defense systems. An array of small molecules including polyphenols falls under the later system (Rice-Evans *et al*., 1997). Polyphenols have the ability to scavenge free radicals via hydrogen donation or electron donation (Shahidi *et al*., 1992). A phenolic molecule is often characteristic of a plant species or even of a particular organ or tissue of that plant. The antioxidant activity of polyphenols is governed by the number, reactivity, and location of their aromatic hydroxyl groups (Chen *et al*., 1996).

The main classes of polyphenols are defined according to the nature of their carbon skeleton and they are: phenolic acids, flavonoids, stilbenes, and lignans (Lee *et al*., 2003). Other dietary polyphenols are not well-defined chemical entities and result from the oxidative polymerization of flavonoids and phenolic acids (Santos-Buelga and Scalbert 2000). The means of extracting polyphenols from plants is crucial as some polyphenols can be denatured by heat and lost by some solvents. Besides, some solvents are toxic and render the extracts unsafe for human consumption. Decoction is an extraction method of choice due to the absence of any

organic solvent, as is the case with the industrial production of *Vimang*, a mango stem bark extract in Cuba. Specific polyphenolic compounds can be determined and quantified by chromatographic techniques, while total phenols can be estimated by reduction of the Folin–Ciocalteu reagent (Singleton and Rossi, 1965). Besides these, antioxidative capacity assays of plant extracts can also be used to predict their polyphenolic quantity and/or activity.

The importance of phenolic compounds in juice or wine is related to their antioxidant activity. Epidemiological studies have demonstrated that the consumption of phenol–rich foods and beverages contributes to the reduction of coronary heart disease mortality (Cul *et al*., 2002), the inhibition of chronic inflammation and thrombosis (Kinsella *et al*., 1993), and the prevention of certain types of cancer and that the benefits are greater with a diet rich in a particular class of phenols named flavonoids (Bell *et al*., 2000). Phenolic compounds contribute to wine colour, taste, structure and make the product suitable for ageing. Red wines are rich in simple and complex phenolic compounds mainly represented by phenolic acids, flavonols, monomeric catechins, and tannins (Katalinic *et al*., 2004). The grape phenol composition and content are affected by several factors such as variety, ripening time, climate, soil and place of growing. In addition, wine–making technologies together with oenological practices and ageing modify both the phenolic composition and the antioxidant activity. Several analytical methods are available for assessing the antioxidant activity of foods and beverages but, in absence of a standardized method, their results depend on the experimental conditions applied.

Natural and synthetic antioxidants can inhibit or delay the process of lipid oxidation. Antioxidants refer to any substances present at low concentration in foodstuffs and able to significantly prevent oxidation mediated by prooxidants. Prooxidants, considered as synonymous with reactive oxygen species, refer to any substances that when being with low concentration in foods can cause or promote an oxidative reaction. An antioxidant may play a role in antioxidation as a free radical scavenger, reducing agent, chelator, and/or singlet oxygen scavenger. Numerous synthetic antioxidants have been registered, but only a few species are permitted as food additives by the law because of their toxicity effects and other side-effects. Typical antioxidants permitted as the food additives are butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), pueraria glycoside (PG), and tertiary-butylatedhydroquinone (TBHQ). Recently, public's concern about the problems of human health caused by food

additives has once more evoked food scientists to the enthusiasm of seeking natural antioxidants from various sources. So far one of the understandings on this issue is that natural antioxidants are primarily plant phenolic compounds occurring in all parts of plant bodies. Common phenolic antioxidants from plant sources include flavonoid compounds, cinnamic acid derivatives, coumarins, tocopherols, carotenoids, and polyfunctional organic acids (Shahidi and Wansaundara, 1992).

The overproduction of reactive oxygen and nitrogen species (ROS and RNS, respectively) by phagocytes causes oxidative damage to membrane lipids, DNA, proteins and lipoproteins. These reactions have functional consequences, which may be deleterious to cells and tissues. Thus, the inhibition of ROS and RNS production is a popular target for the attenuation of many inflammatory diseases (Shen *et al.* 2002). Dietary polyphenols with antioxidative effects from fruit and vegetables play an important role in a prevention of the oxidative stress (Mojzisova and Kuchta 2001). Another important source of polyphenolic antioxidants is wine, particularly red wine. It has been demonstrated that polyphenols from wine have not only antioxidative but also anti-inflammatory effects (Oak *et al.* 2005) and that they can prevent cardiovascular diseases (Babal *et al.* 2006). It is furthermore suggested that they prevent free radical mediated lipid peroxidation of low density lipoproteins (LDL), which is associated with cell aging and chronic disesases such as atherosclerosis (Oak *et al.* 2005; Rajdl *et al.* 2007). It is postulated that the antioxidant and free radical scavenging properties of phenolic compounds, present in red wine, may partly explain the ”French paradox”, i.e. the fact that French people have low incidence of coronary heart disease, despite having a diet high in fat and being heavy smokers (Aruoma, 1994). The main polyphenolic compounds in red wine belong to two major classes: flavonoids and stilbenes. Of the flavonoids, (+)-catechin, (–)-epicatechin and quercetin and of the stilbenes, trans-resveratrol, are the most abundant polyphenols in wine. Exact mechanisms by which flavonoids protect against oxidative stress-mediated diseases (such as atherosclerosis) are still a matter of debate (Benito *et al.,* 2004).

3.2 REVIEW OF LITERATURE

Fruits and vegetables are important constituents of human diet and are rich sources of nutrients such as vitamin C, polyphenolic compounds, provitamin A carotenoids, and minerals. More than 5000 individual phytochemicals have been identified in fruits, vegetables, and grains (Liu, 2003). Some of these contribute to the antioxidant potential of fruits and vegetables. Ascorbic acid and phenolics are known as hydrophilic antioxidants, whereas carotenoids are known as lipophilic antioxidants (Halliwell, 1996). Oxidative stress caused by free radicals is involved in the aetiology of a wide range of chronic diseases, such as cancer, cataract, cerebral pathologies, rheumatoid arthritis, age related macular degeneration and other degenerative diseases. This happens because free radicals are chemically highly reactive and could cause oxidative damage to important cellular macromolecules, such as nucleic acids, proteins and lipids (Clifford, 1995). Epidemiological studies have indicated that frequent consumption of natural antioxidants could lower the risk of chronic diseases (Temple, 2000). Thus, the natural antioxidants present in fruits and vegetables could scavenge free radicals and provide radioprotective ability to these foods. Lifestyle-related diseases such as cancer, coronary diseases and hypertension have increased recently. These diseases are closely related to the change in dietary habits like increased intake of animal protein or fats and a decreased intake of dietary fiber. As a result, various fruit and vegetable juices, as a functional food have gained popularity to reduce the incidence of these diseases (Song *et al*., 2006).

3.2.1 Polyphenolic compounds and antioxidant properties in mango: In the past few years, there has been increasing interest in the study of mango phenolics from mango fruits, peels, seeds, leaves, flowers, and stem bark due to their antioxidative and health promoting properties that make consumption of mangoes and derived products a healthy habit. Bioactive compounds found in the mango fruits, among other plants and herbs, have been shown to have possible health benefits with antioxidative, anticarcinogenic, antiatherosclerotic, antimutagenic, and angiogenesis inhibitory activities (Cao and Cao, 1999).

3.2.1.1 Polyphenolic composition of mango pulp: Mangiferin, gallic acids (*m*-digallic and *m*-trigallic acids), gallotannins, quercetin, isoquercetin, ellagic acid, and *β*-glucogallin are among the polyphenolic compounds already identified in the mango pulp (Schieber *et al*., 2000). Gallic acid has been identified as the major polyphenol present in mango fruits, followed by 6 hydrolysable tannins and 4 minor compounds, *p*-OH-benzoic acid, *m*-coumaric acid, *p*-coumaric acid, and ferulic acid (Kim *et al*., 2007). Schieber *et al*. (2000) found 6.9 mg/kg of gallic acid and 4.4 mg/kg of mangiferin in mango pulp. In a polyphenol screening of 20 mango varieties, Saleh and El-Ansari (1975) reported the co-occurrence of mangiferin, isomangiferin, and homomangiferin in mango fruit pulp. Mangiferin has been shown elsewhere to be the main compound of leaves and stem bark with great medicinal values. It has been reported that phenolic compounds and their associated antioxidant capacity decrease as fruit ripens (Kim *et al*., 2007). Gallotannins represent the major components of unripe fruits and seeds. According to Prabha and Patwardhan (1986) gallic acid is the substrate of polyphenol oxidase in the fruit pulp, whereas ellagic acid is the predominant substrate in mango peel.

3.2.1.2 Polyphenolic composition of mango peel: During mango fruit development, the total phenols have been found to be higher in the peel than in the flesh at all stages (Lakshminarayana *et al*., 1970), with an estimated total polyphenol content in mango peel of 4066 mg (GAE)/kg (dry matter) (Berardini *et al*., 2005b). Generally, ripe peels contain higher total polyphenols than raw peels (Ajila *et al*., 2007). The polyphenolic constituents of mango peel include mangiferin, quercetin, rhamnetin, ellagic acid, kaempferol, and their related conjugates where it can be seen that the 2 main polyphenols in mango peel are mangiferin and quercetin 3-0-galactoside. Berardini *et al*. (2005b) found that, while mangiferin contents slightly decreased at elevated temperatures, the contents of the other xanthone derivatives significantly increased. The observed changes may be attributed to the formation of xanthones from benzophenone derivatives, which were recently identified in mango peels (Berardini *et al*., 2004) and which are considered precursors of xanthone C-glycosides (Larrauri, 1999). Anthocyanins have also been identified in the mango peel and estimated to range from 203 to 565 mg/100 g (dry matter) depending on variety and stage of maturity (Berardini *et al*., 2005b). In their study on the antioxidative activity of mango peel extract, Berardini *et al*., (2005b)

established that the antioxidative capacity of the extract was higher than that of standard mangiferin and quercetin 3-Oglucoside, thus indicating that the antioxidative capacity of the peel extract cannot be attributed to a single component but to the synergistic effect of all the compounds present.

3.2.1.3 Polyphenolic composition of mango seed kernels: Besides the pulp and the peel, mango seed kernels are equally rich in polyphenols with potent antioxidative activity, but ironically the seeds are always discarded as waste during processing and consumption of the mango fruit. As an example, in India about 300000 metric tons of mango seed kernels are discarded every year (Char and Azeemoddin, 1989). Ahmed *et al*. (2007) identified and quantified various polyphenolic compounds in the mango seed kernel: tannin 20.7 mg/100 g, gallic acid 6.0 mg/ 100 g, coumarin 12.6 mg/100 g, caffeic acid 7.7 mg/100 g, vanillin 20.2 mg/100 g, mangiferin 4.2 mg/100 g, ferulic acid 10.4 mg/100 g, cinnamic acid 11.2 mg/100 g, and unknown compounds 7.1 mg/100 g. The total polyphenolic content of the mango seed kernel extract was estimated to be 112 mg (GAE)/ 100 g (Ahmed *et al*., 2007). Soong and Barlow (2004) assayed the antioxidant activity of a variety of fruit seeds, namely, mango, jackfruit, longan, avocado, and tamarind and found that the antioxidant activity of the mango seed kernel was the highest, a fact attributed to its high polyphenolic content. These points to a reason to industrially utilize the mango seed kernel as a functional food ingredient.

3.2.1.4 Antioxidant capacity of mango: The antioxidant capacity of mango fruit appears to be largely influenced by the polyphenol and flavonoid contents. Highly significant linear correlations were observed between antioxidant capacity of the mango fruit samples and their polyphenol and flavonoid contents. These data are in agreement with other reports in the literature, phenolic acids and flavonoid compounds have been the main phytochemicals responsible for the antioxidant capacity of fruit, and the antioxidant capacity of fruits and vegetables is due primarily to nonvitamin C phytochemicals (Prior *et al*., 1998). Flavonoids are the most common and widely distributed group of plant phenolic compounds, which usually are very effective antioxidants (Yanishlieva-Maslarova, 2001). Several studies also revealed that the phenolic content in the plants are associated with their antioxidant activities, probably due

to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Chang *et al*., 2001). In general, phenolic and flavonoid content of mango fruits are major sources of natural antioxidants. Thus the antioxidant capacity of mango appears to be largely influenced by the total polyphenols levels. There are a few previous studies on the antioxidant capacity of mango fruits. The antioxidant potential of commonly grown tropical, subtropical fruit has been rated in the order of guava >mango > papaya > lemon (Torunn *et al*., 2009). Some other studies also demonstrated the total antioxidant capacity of mangoes (Berardini *et al*., 2005a; Ajila *et al*., 2007; Corral-Aguayo *et al*., 2008). During mango processing, the peels emerge as a byproduct and are usually discarded as waste. A number of valuable antioxidant compounds are contained in this residue (Ajila *et al*., 2007). For this reason, there are studies addressing the use of natural antioxidants obtained from mango agro-industry residues as food preservers in substitution to artificial antioxidants (Abdalla *et al*., 2006).

3.2.2 Polyphenolic compounds and antioxidant properties in wine: Wine is one of the functional fermented foods that have many health benefits like reduction in coronary heart disease, development of healthier blood vessels in elderly people, decreasing ovarian cancer risk in women and making the bones stronger, and improvement of lung functions (Altenburg and Zouboulis, 2008). Wine phenolics were considered to scavenge reactive oxygen species (ROS), and to inhibit human low-density lipoprotein (LDL) oxidation. During the last few decades, an attempt to interpret the 'French paradox' the low incidence of coronary heart disease in France, in spite of high fat intake, placed wine in the centre of the scientific investigation. Its protective action against cardiovascular diseases is attributed not only to ethanol, but also to a variety of micro-constituents. White and especially, red wines are considered as rich sources of antioxidant polyphenolic compounds that exert both anti-inflammatory and antioxidant actions (Marianna *et al*., 2010). Flavonoids in wine exist either in free form or bound to other flavonoids, sugars, non-flavonoids or combinations of these compounds. Flavonols and anthocyanidins originate predominately from the skin, whereas catechins and leucoanthocyanins originate mainly from the seeds and stems. Non-flavonoids

partly originate through yeast metabolism and from the wood of oak barrels (Kumar *et al*., 2012).

Wine consumption reduces the susceptibility of LDL to oxidation which is important for the prevention of arteriosclerosis development. A moderate wine consumption also increases serum antioxidant capacity (Cooper *et al*., 2004). A favourable influence on the reduction of cancer incidence and on chronic inflammatory diseases, the development of both being associated with oxygen free radical, is probable as well (Scalbert *et al*., 2005). Moreover, the presence of native antioxidants in wines at sufficient levels can significantly reduce the need for exogenous additives (ascorbic acid, SO_2 etc.) that can be linked to allergic effects occurring during wine consumption in more than 15% consumers.

Among alcoholic beverages, red wine has been reported to be more protective against coronary heart disease than other alcoholic beverages (Gronbaek *et al*. 1995). Different wines have different quantities and spectra of native antioxidants and therefore different health benefits. Wine composition, including the contents of phenolic compounds, varies markedly depending on the grape cultivar, soil, nutrition, climatic conditions, weather, winemaking procedure, and conditions of maturation and storage. Over 500 different compounds, of which 160 are esters, have been identified in different wine types. These include water (74–87%, w/w), ethanol (10–14%), saccharides (0.05–10%), organic acids (0.05–0.7%), phenols (0.01–0.2%), and glycerol (Soleas *et al*., 1997a). Phenolic compounds have long been considered to be basic components of wines and over 200 compounds have been identified. The concentration of total phenolic compounds in commercially available red wines is rarely above 2.5 g/L (Singleton, 1982). Two primary classes of phenolics that occur in grapes and wine are flavonoids and nonflavonoids. Flavonoids commonly constitute > 85% of the phenolics content (≥ 1 g/L) in red wines. In white wines, flavonoids typically comprise < 20% of the total phenolics content (≤ 50 mg/L). Their dietary intake has been shown to be inversely related to coronary heart disease mortality (Knekt *et al*., 1996). The most common flavonoids in white and red wines are flavonols, catechins (flavan-3-ols), and anthocyanidins, the latter being found only in red wine. Small amounts of free leucoanthocyanins (flavan-3,4-diols) also occur. Flavonoids exist free or bound to other flavonoids, sugars, nonflavonoids, or combinations of these compounds. Flavonols and anthocyanidins originate predominately from the skin,

whereas catechins and leucoanthocyanins originate mainly from the seeds and stems. Nonflavonoids partly originate also from yeast and the wood of oak barrels. The phenolic composition and the extractability of grapes largely depend on the grape variety and the winemaking process conditions (Soleas *et al*., 1997b). The amount of flavonoids extracted during vinification is influenced by many factors, including temperature, mixing, the parameters of the fermentation vessel, the duration of skin maceration, ethanol concentration, SO_2, yeast strain, pH, and pectolytic enzymes.

The concentration of phenolic compounds in wine increases during skin fermentation and subsequently begins to decrease as phenols bind with proteins and yeast hulls (cell remnants), and precipitate. During fining and maturation, the concentrations of phenolic compounds continue to decrease. Their concentration is further substantially decreased in aging. Aging in oak-wood barrels (barrique wines) can also increase the contents of particular phenolic compounds (Matejicek *et al*., 2005). Some phenols in wine arise by the activity of micro-organisms as secondary aromatic compounds in the course of the degradation of phenolic acids or lignin as well. They arise as by-products of lactic and alcoholic fermentations. Such a compound is e.g. ferulylalcohol (or 4-vinylguajacol) and other similar alcohols. The taste and other sensory characteristics are primarily due to a few compounds that occur individually at concentrations above 100 mg/L. Lower phenolic acids account for flat flavor while larger polyphenols contribute to bitterness and astringency. Tannins present in red wine are rarely found in white wines in significant amounts.

3.2.2.1 Measurement of polyphenolics in wine: The presence of phenolics and tannins in the wine product has a definite impact on wine flavor and overall quality. This effect is often hard to characterize chemically and sensorially, but the impact is great. Phenolics influence taste, odor, color and clarity. These organic acids, phenolics and mineral salts are less than 2% of the wine product itself, but have a dramatic influence on sensory quality. We now have access to formidable tools for measuring phenolic levels and correlating them with sensory quality. In the past, gas chromatography was used exclusively to measure volatile constituents in wine. Besides gas chromatography, we can now utilize high pressure liquid column chromatography to measure monomeric and polymeric phenols including all major components in wine (Price,

1997). Monomeric and polymeric anthocyanins are important for wine coloration and their presence influences the solubility of other phenolics present in wine. Polymeric phenols and tannins influence mouthfeel and their presence can be easily measured by HPLC as well. Price (1997) has developed a complete analysis of wine and polyphenolic constituents including analysis for wine color and flavor components. Spider diagrams can be utilized to evaluate presence of certain important constituents in a wine product and these results can be compared to sensory panel results from the same wine product.

The types and concentrations of the phenolic compounds in wines depend on grape cultivars, ripening and climatic conditions (Goldberg *et al*., 1996). High performance liquid chromatography (HPLC) technique has been generally used in order to determinate the phenolic compounds in wine samples (Revilla and Ryan, 2000; Lopez *et al*., 2001). In the determination of the wine phenolics, different extraction methods including solid-phase extraction with C18 or strong anion exchange anionic cartridges, liquid-liquid extraction with different organic solvents have been used. In this study, wine samples injected directly to HPLC. So, the method permitted the determination of phenolic compounds in wines without any prior purification. A direct RP-HPLC injection technique without sample preparation was used by Revilla and Ryan (2000), Lopez *et al*., (2001), and Suarez *et al*., (2005). Recently Kumar *et al*. (2012) used LC-MS technique for determination of phenolic composition in Cv. *Alphonso* wine and identified 19 phenolic compounds.

3.2.3 Mango wine and γ-irradiation: During the past many centuries grapes have been the major source for the wine production; however, researchers found that other fruits were also suitable for wine production such as banana, apple, mango, acerola and cocoa. The choice of fruit depends upon several factors like availability of raw material, market demand, production facilities and sound economic reasons (Kumar *et al*., 2009). In India, the total production of wine was 8.35 million bottles per year (Gill *et al*., 2009), indicating a wide scope for wine production from indigenous fruits such as mango. Diets rich in fruit and vegetables decreases oxidative DNA damage, and supplementation of this diet with wine further decreased the damage. This protective effect of wine was more significant when subjects consumed a high fat and high calorific diet (Leighton *et al*., 1999). Epidemiological evidence suggests that moderate

consumption of wine (20-30 g alcohol per day) is associated with reduced risk of death from cardiovascular diseases and to a lesser extent cancer (Greenrod *et al*., 2005).

Recently, many researchers have attempted to apply radiation technology for enhancing biological activities of various foods (Lee *et al*., 2011). Microbial decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process. Radiation treatment in dose range of 2-7 kGy, depending on physical state of food, can effectively eliminate potentially pathogenic bacteria. Radiation decontamination of dry ingredients, herbs and enzyme preparations with doses of 3–10 kGy has proved to be a viable alternative to fumigation and aided in reducing microbial load. Radiation treatment at doses of 1.5–5.0 kGy under specific conditions has also been shown to be feasible for control of many foodborne parasites, thereby making foods safe for human consumption (Farkas, 1998). Similarly, irradiation of pre-packaged vegetables and ready to eat meals and fruits at doses between 2.0 to 3.0 kGy reduced the total viable cells to levels below the detection limit of 100 cells/g and counts did not increase significantly during storage at 5ºC (Patterson and Stewart, 2003). Treatment with ionizing radiation such as γ-rays also has the advantage that it improves the total polyphenolic and flavonoid content thus enhancing antioxidant activities of various natural products while making them microbe-free (Lee *et al*., 2009; Mishra *et al*., 2011).

Hence, this study was aimed to investigate the effects of γ-irradiation on the total poly phenolic compounds, flavonoid content and antioxidant activity of mango juice and wine from eight locally available mango cultivars, and also to assess the *in vitro* protective activity of mango juice and wine against radiation induced DNA damage.

3.3 MATERIALS AND METHODS

3.3.1 Chemicals and phenolic standard solutions: All the reagents and solvents used in this study were of analytical grade. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), N,N-dimethyl-p-phenylendiamine (DMPD), N-(1-naphtyl) ethylenediamine dihydrochloride, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic) acid, and acetic acid were procured from Sigma chemical Co., St. Louis, Mo., U.S.A; HPLC grade methanol from Merck (India); 2,6-dichlorophenol indophenol, sodium nitroprusside (SNP) and sulphanilamide were

from Himedia, Mumbai, India; Folin-Ciocalteu phenol reagent, $AlCl_3$, $FeCl_3 \cdot 6H_2O$, Na_2CO_3 and other chemical solvents were obtained from S.D**.** Fine Chemicals Ltd., Mumbai, India. Purified pUC19 plasmid DNA was procured from Bangalore Genei Pvt. Ltd., Bangalore, India. The water used in the analysis was obtained from a Milli-Q water purification system, Millipore (Bedford, MA). All solvents used as the mobile phase were previously filtered through 0.45-µm membranes (Millipore) and degassed prior to use.

Gallic acid, protocatechic acid, *p*-OH-benzoic acid, vanillic acid, syringic acid, ellagic acid, caffeic acid, *p*-coumaric acid, *m*-coumaric acid, ferulic acid, synapic acid, rutin and quercetin standards were purchased from Sigma-Aldrich (Steinheim, Germany) and (+)-catechin and chlorogenic acid were supplied from Fluka (Buchs, Switzerland). Stock solutions of all the phenolic standards were prepared in methanol.

3.3.2 Mango fruit processing: Eight cultivars (Cv.) of ripe mango fruits viz., *Alphonso*, *Banginapalli*, *Mulgoa*, *Neelam*, *Raspuri*, *Rumani*, *Sindhura* and *Totapuri* grown in Andhra Pradesh, South India, were procured from the local market. All fruits were of ideal in ripeness for consumption and were uniform in size. Fresh edible puree of the fruits was processed according the methodology described in section 2.3.2.

3.3.3 Wine yeast and preparation of inoculam: Inoculam was prepared according to the methodology described in section 2.3.3.

3.3.4 Mango wine making: Mango wine fermentation was performed according to the methodology described in section 2.3.4.

3.3.5 γ-Irradiation: This was performed according to the methodology described in section 2.3.5.

3.3.6 Determination of ascorbic acid (AA) and dehydro ascorbic acid (DHAA) content of mango juice: The ascorbic acid and dehydro ascorbic acid content of mango juice samples was determined by the method of Vinci *et al*. (1995), with minor modifications. 10 mL of each

mango juice samples were stirred in ultra pure water (15 mL) for 5 min with a micro grinder. The mixture was filtered and the total final volume was adjusted to 25 mL with ultra pure water and centrifuged at 1000 × g for 15 min. 2 mL of the each supernatants were filtered in a 0.45 μm Millipore membrane and a 30 μL aliquot was immediately used for HPLC analysis. The HPLC system (Model PU 980; JASCO International Co. Ltd., Tokyo, Japan), equipped with a C-18 reverse phase stainless steel column (250 mm × 4.6 mm, Thermo Hypersil-Keystone; Thermo Fisher Scientific Inc., Waltham, MA), which was kept at 25 °C with a PDA detector. The chromatographic conditions were: ultra pure water with metaphosphoric acid at pH 2.2 as mobile phase; detection at 238 nm and flow rate of 1.0 mL/mim.

Reduction of dehydroascorbic acid (DHAA) was performed with dithiothreitol (DTT). An aliquot (1.0 mL) of the each sample supernatant was mixed with 0.1 ml phosphate buffer pH 7.4 to obtain a final pH of 6.0, and 100 mM DTT was added to obtain a final concentration of 8.0 mM. The mixture was kept in the dark for 10 min to convert DHAA into AA, followed by sample analysis for total vitamin C content under the same chromatographic conditions described for the ascorbic acid analysis. DHAA was calculated from the difference in contents between total vitamin C and AA before conversion, using a L-ascorbic acid standard curve. The peaks of interest were identified by comparing the retention time of ascorbic acid standards and samples; and mainly through the absorption spectrum, since a diode array detector was used. All determinations were performed in triplicates.

3.3.7 Determination of the total polyphenolic content (TPC): The total polyphenolic content of mango juice and wine samples was determined by the method of Singleton and Rossi (1965) using Folin-Ciocalteu reagent and modified by Varakumar *et al*. (2011). Briefly 1000 μL of diluted mango juice and wine was mixed with 1800 μL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and incubated at room temperature (RT) for 5 min followed by the addition of 1200 μL of 20% Na_2CO_3. The mixture was incubated for 90 min at RT and absorbance against prepared reagent blank was measured at 765 nm using spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The total polyphenolic content of the mango juice and wine was compared with gallic acid standard curve and expressed as mg gallic acid equiv. /L (mg GAE/L).

3.3.8 Determination of the total flavonoid content (TFC): The total flavonoid content of mango juice and wine samples was measured using colorimetric method described by Chang *et al*. (2002) with some modifications. Briefly 1000 µL of mango juice and wine sample were mixed with 1500 µL distilled water, to which 100 µL aluminum chloride (10% w/v), 100 µL potassium acetate (1 M) and 2800 µL distilled water were added. The mixed solutions were vortexed and allowed to stand for 30 min at RT, and then absorbance of the reaction mixture was recorded at 415 nm using spectrophotometer. The total flavonoid content of the mango juice and wine samples was estimated from the standard curve prepared using rutin and expressed as mg rutin equiv. /L (mg RE/L).

3.3.9 Extraction and characterization of mango juice polyphenolic compounds by HPLC: The mango juice samples (50 mL) were adjusted to pH 7.0 with 2 N NaOH and extracted with 50 mL ethyl acetate at 30 °C by stirring for 5 min, using a casing vessel with a reflux condenser. The mixture was then centrifuged at 5000 rpm for 5 min. The organic layer was transferred, and the extraction process was repeated twice with 50 mL ethyl acetate. The three organic layers were pooled, and evaporated to dryness at 35 °C by means of a helical gas flow (nitrogen) at 1.8 bars by vortexing action. The resulting residue was redissolved in 2 mL methanol. Individual polyphenolic compounds were characterized and quantified by HPLC system (Model PU 980; JASCO International Co. Ltd., Tokyo, Japan), equipped with a C-18 reverse phase stainless steel column (250 mm × 4.6 mm, Thermo Hypersil-Keystone; Thermo Fisher Scientific Inc., Waltham, MA), which was kept at 25 °C with a PDA detector as previously described by Schieber *et al*. (2000) with some modifications. The mobile phases were (A) 2% (v/v) acetic acid in water and (B) 0.5% acetic acid in water and acetonitrile (50:50, v/v). The detection was carried out at 280 and 320 nm at the flow rate of 1 mL/min. The gradient elution was as follows: 10-55% B (50 min), 55-100% B (10 min), 100-10% B (5 min). The injection volume for all samples was 20 µL. Peaks were identified by comparing their retention times (RT) with that of authentic standards injected under analysis conditions.

Gallic acid, *p*-OH-benzoic acid, ellagic acid, *m*-coumaric acid and quercetin were dissolved in methanol (1mg/mL), and dilute solutions (1:5) from these stock solutions were used to prepare calibration curves of standards. Three replicates of each sample were used for

HPLC analyses. All samples and standards were injected in triplicate and mean ± S.D were used. Standard graphs were prepared by plotting peak area against concentration using above standards.

3.3.10 Extraction of mango wine polyphenols and HPLC analysis: Extraction of mango wine polyphenols and HPLC analysis was carried out according to the method reported by Bonerz *et al*. (2008) with some modifications. An aliquot of 10 mL of mango wine was extracted twice with 8 mL of ethyl acetate for 20 min. The ester phase was concentrated on a rotary evaporator under 30 °C and then redissolved in 5 mL of 1:1 (v/v) methanol/water. Individual polyphenolic compounds were characterized and quantified by HPLC system (described in section 3.3.9). The mobile phase consisted of 2% acetic acid in water (solvent A) and 1% acetic acid in methanol (solvent B) by applying the following gradient: 0-4 min: 10% B, 4-45 min: 10-60% B, 45-55 min: 60-95% B, 55-60 min: 95% B, 60-63 min: 95-10% B and 63-65 min: 10% B. The detection was carried out at 280 and 320 nm at the flow rate of 1 mL/min. The injection volume for all samples was 10 µL. Peaks were identified by comparing their retention times (RT) with that of authentic standards injected under analysis conditions.

Standard graphs were prepared according to the methodology described in section 3.3.9.

3.3.11 Assays of antioxidant activities

3.3.11.1 DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay: The DPPH free radical scavenging activity of the control and irradiated mango juice and wine samples was measured according to the procedure described by Brand-Williams *et al*. (1995) and modified by Kumar *et al*. (2012). DPPH is a stable free radical having λmax at 517 nm and the assay is based on the discolouration of the compound when reduced by a free radical scavenger. The stock reagent solution was prepared by dissolving 24 mg of DPPH in 100 mL methanol and stored at –20 °C until use. The working solution was obtained by mixing 10 mL of stock solution with 45 mL methanol to obtain an absorbance value of 1.1 ± 0.02 at 517 nm, using a spectrophotometer. Different volumes (20, 60 and100 µL) of mango juice and wine samples were allowed to react with DPPH solution in a final reaction volume of 3 mL. The mixture was shaken vigorously and allowed to stand for 30 min in the dark at RT. The decrease in

absorbance of the resulting solution was then measured at 517 nm against methanol as a blank. A control sample without mango wine was also analyzed and the percentage radical-scavenging activity (% RSA) was calculated using the relationship:

% RSA = $[(A_{control} - A_{sample}) / A_{control}] \times 100$

Where A = absorbance at 517 nm.

3.3.11.2 FRAP (ferric reducing/antioxidant power) assay: FRAP assay is a direct measure of antioxidant capacity and measures the change in absorbance at 593 nm owing to the formation of a Perl's prussian blue coloured Fe^{II} tripyridyltriazine compound from colourless oxidized Fe^{III} form by the action of electron donating antioxidants. This assay was performed according to the method described by Benzie and Strain (1996) and modified by Kumar *et al*. (2012). Briefly the working FRAP reagent was prepared by mixing 10 volumes of 300 mM acetate buffer, pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. Prior to use, freshly prepared FRAP reagent was heated for 10 min at 37 °C in a water bath. Different volumes (100, 300 and 500 µL) of mango juice and wine samples were allowed to react with 3.0 mL of the FRAP reagent and the final volume was made up to 4.0 mL with distilled water. The absorbance of the coloured end product (ferrous tripyridyltriazine complex) was then measured after 8 min at 593nm using UV-visible spectrophotometer. A standard curve was prepared using different concentrations (100–1000 µM/L) of $FeSO_4.7H_2O$. The antioxidant efficiency of the sample solution was calculated with reference to the standard curve given by a Fe^{2+} solution of known concentration. Ferric reducing power was expressed in mM Fe^{2+}/kg and mM Fe^{2+}/L for mango juice and wine samples, respectively.

3.3.11.3 Nitric oxide (NO) scavenging assay: Nitric oxide (NO), owing to its unpaired electron is classified as a free radical and displays significant reactivity with certain types of proteins and other free radicals. *In vitro* inhibition of NO radical is also a measure of antioxidant activity and is based on the inhibition of NO radical generated from sodium nitroprusside (SNP) in buffer saline and measured by Griess reagent. The NO scavenging capacity was measured according to method described by Sreejayan and Rao (1997). Briefly

2.0 mL of test mixture contained 10 mM SNP (1500 µL) in phosphate buffer saline and different volumes (100, 300 and 500 µL) of mango juice and wine samples. After 150 min of incubation, 1.5 mL was withdrawn and remaining test mixture was mixed with 1.5 mL of Griess reagent [1.0% sulphanilamide, 2.5% H_3PO_4 and 0.1% N-(1-naphtyl) ethylenediamine dihydrochloride]. The absorbance of the nitrite with sulphanilamide and subsequent coupling with N-(1-naphtyl) ethylenediamine dihydrochloride was measured at 546 nm using UV-Vis spectrophotometer. A control sample with no added mango wine was also analyzed and the percent of NO scavenging capacity was calculated using the relationship:

NO scavenging capacity (%) = $[(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$

Where Abs = absorbance at 546 nm.

3.3.11.4 ABTS (2, 2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic) acid) radical cation-scavenging assay: The ABTS radical cation-scavenging assay was performed according to Giao *et al*. (2007). ABTS (7 mM) was dissolved in water and the blue/green ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowed to stand in the dark at RT for 16 h before use. To obtain an absorbance of 0.700 ± 0.005 at 734 nm, the stock solution was diluted with Millipore water as necessary. Different volumes (20, 60 and 100 µL) of mango juice samples in PBS were added to 900 µL of this diluted solution, and the absorbance at 734 nm was determined after 2 min initial mixing. The antioxidant solution reduced the radical cation to ABTS, which reduced the color. The extent of decolorization was calculated as the % reduction in absorbance.

3.3.11.5 DMPD (N, N-dimethyl-p-phenylendiamine) scavenging assay: This assay is based on the reduction of buffered solution of DMPD in acetate buffer and ferric chloride. This involves measurement of decrease in absorbance of DMPD in presence of scavengers at its absorption maxima of 505 nm. The DMPD assay was performed according to method described by Fogliano *et al*. (1999). The pink $DMPD^{\bullet+}$ was prepared by mixing 1 mL of 200 mM DMPD solution, 0.4 mL of 0.05 M ferric chloride (III), and 100 mL of 0.1 M sodium acetate buffer (pH 5.25). According to the studies carried out, it reached stable absorbance values at 505 nm with values around 0.900 ± 0.100 after a period of 18 and 21 h after its preparation. The DMPD

radical cation was stable up to 12 h at RT. Different volumes of mango wine (20, 60 and 100 µL) samples were added to 2.0 mL of $DMPD^{\bullet+}$ solution and the total volume was adjusted to 3.0 mL with distilled water. After 10 min of incubation, the absorbance of the reaction mixture was recorded at 505 nm using UV-Vis spectrophotometer. The buffer solution was used as a blank sample. The DMPD•+ scavenging capacity was calculated using the following equation.

$DMPD^{\bullet+}$ scavenging capacity (%) = $[(A_0 - A_1) / A_0] \times 100$

Where A_0 is the absorbance of the initial concentration of DMPD; A_1 is the absorbance of the remaining concentration of DMPD.

3.3.12 Evaluation of *in vitro* radioprotective effect of mango juice: The *in vitro* radioprotective potential of mango juice was evaluated using the method described earlier by Saxena *et al*. (2011). A 15 µL aliquot of clear mango juice was mixed with 15 µL of ultra purified (using caesium chloride density gradient) pUC19 plasmid DNA (~400 ng) (Bangalore Genei, India) in a sterile microfuge tube (Eppendorf, 1.5 ml). The samples were radiation treated at different doses (0.5, 1 and 3 kGy), and subjected to agarose gel (1%) electrophoresis at 4 V/cm, using Tris–acetate–EDTA (TAE) buffer (pH 8.0). Then the gel was stained with ethidium bromide (0.5 µg/mL) and visualised using a UV-transilluminator.

3.3.13 Effect of mango wine on H_2O_2 and UV irradiation induced DNA damage: Protective effect of mango wine on H_2O_2 and UV radiation induced DNA damage was investigated *in vitro* using pUC19 plasmid DNA (Bangalore Genei Pvt. Ltd., India). The plasmid DNA was oxidized in presence of H_2O_2 and further irradiated with UV in presence or absence of mango wine according to Jagtap *et al*. (2011). In brief, the experiments were performed in a volume of 15 µL in an eppendorf tube containing 400 ng of plasmid DNA, H_2O_2 was added to final concentration of 100 mM with and without 10 µL of mango wine. The reaction mixture was exposed to UV irradiation for 5 min on the surface of UV mini transilluminator with intensity of 150 W at 312 nm at ambient temperature. After irradiation, the mixture was incubated at RT for 15 min. To the mixture, gel loading dye was added and the fragments were separated by electrophoresis. Untreated plasmid DNA was used as a control in each run of gel electrophoresis along with UV and H_2O_2 treatments.

3.3.14 *In vitro* protective effect of mango wine on γ-irradiation induced DNA damage: The radio protective effect of mango wine was evaluated using the *in vitro* method of Jagtap *et al*. (2011) with some modifications. In brief, wine from different mango cultivars (10 µL) was mixed with pUC19 plasmid DNA (~400 ng) at final volume of 15 µL in a sterile 1.5 mL eppendorf tube. The samples were irradiated with 0.5 kGy dose of γ-irradiation. The irradiated samples were mixed with 6X gel loading dye and the fragments were separated by electrophoresis. Untreated plasmid DNA was used as a control.

3.3.15 Agarose gel electrophoresis: Electrophoresis was carried out on 1% agarose in 1X TAE buffer (2 M Tris, 1M sodium acetate, 50 mM EDTA, and pH 8.0) at RT using mini electrophoresis system. Subsequently, the gel containing 20 µL of ethidium bromide (10 mg/mL) was observed under UV light with transilluminator and photographed.

3.3.16 Statistical analysis: All values obtained were from juice and wine samples prepared from three separate lots of mango for each variety and results were expressed as mean ± standard deviation. Linear correlations between various antioxidant parameters and phenolic content were calculated using the Pearson's correlation coefficient analysis by the SPSS statistical software (version 12, SPSS Inc., USA). One-way analysis of variance (ANOVA) was done to confirm the variability of data and validity of results. Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments.

3.4 RESULTS AND DISCUSSION

3.4.1 Effect of γ-irradiation on the ascorbic acid (AA) and dehydro ascorbic acid (DHAA) contents of mango juice: Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. AA is reversibly oxidised to form L-dehydroascorbic acid (DHAA), which also exhibits biological activity. Since DHAA can be easily converted into AA in the human body it is important to measure both AA and DHA in fruits and vegetables to know vitamin C activity (Lee and Kader, 2000). It is well known that vitamin C is the most sensitive of all water-soluble

vitamins to an irradiation (Kilcast, 1994). However, it has been noted that when reporting vitamin C levels in irradiated food, many workers have not taken into consideration the fact that ionizing radiation can cause a partial conversion of AA to DHAA (Kilcast, 1994). Since both compounds have a vitamin C activity in the body it is important that both should be measured and considered. The effects of γ-irradiation on AA and DHAA contents of mango juice samples are shown in Table 3.1. The AA content range of the control (0 kGy) mango juices was between 14.43 and 24.37 (mg/100 mL), the lowest AA was found in *Rumani* and highest was in *Neelam* cultivars, respectively (Table 3.1). A significant ($P \leq 0.05$) reduction in AA was observed in all the irradiated mango juice samples with increase in irradiation dose. The lowest content of AA was found in the juice samples treated at 3 kGy. A similar finding was also reported by Hussain *et al*. (2010) in peaches. This significant decrease could be due to the partial oxidation of AA to DHAA (Song *et al*., 2007). AA is also highly sensitive to oxidation. Further, γ-irradiation can cause radiolysis of water in juice producing free radicals such as $H^{\bullet}$, $OH^{\bullet}$, eaq^{-}. Among these, $OH^{\bullet}$ is a strong oxidizing agent (Diehl, 1995) that has the ability to oxidize AA. Harder *et al*., (2009) have reported that irradiation at doses of 1.0 and 2.0 kGy induced a 50% reduction in AA content in nectar of kiwi fruit. Jo *et al*., (2012) also found a significant reduction in AA content when exposed to dose of 3 and 5 kGy in fresh ashitaba and kale juices. Thus, in order to compensate the loss of ascorbic acid during irradiation, exogenous supplementation may be helpful to maintain the normal levels.

The DHAA content range of the control (0 kGy) mango juices was between 0.44 and 0.83 (mg/100 mL), the lowest DHAA was found in the *Rumani* and highest was in *Neelam* cultivars, respectively (Table 3.1). The DHAA content was stable in irradiated mango juice samples of all cultivars studied. Similarly Song *et al*. (2007) reported that the contents of total ascorbic acid, including DHAA, were stable up to 3 kGy of irradiation.

Table 3.1 Effects of γ-irradiation on ascorbic acid (AA) and dehydroascorbic acid (DHAA) content in different cultivars of mango juice

Juice variety	AA (mg/100 mL)				DHAA (mg/100 mL)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	18.92 ± 0.08^{d}	11.03 ± 0.05^{c}	5.43 ± 0.06^{b}	3.67 ± 0.04^{a}	0.66 ± 0.03^{a}	0.72 ± 0.02^{b}	0.74 ± 0.04^{b}	0.75 ± 0.03^{b}
Banginapalli	20.24 ± 0.11^{d}	12.57 ± 0.06^{c}	6.61 ± 0.04^{b}	4.23 ± 0.05^{a}	0.74 ± 0.02^{a}	0.79 ± 0.04^{ab}	0.83 ± 0.03^{bc}	0.85 ± 0.02^{c}
Mulgoa	15.56 ± 0.05^{d}	9.72 ± 0.04^{c}	4.92 ± 0.05^{b}	2.53 ± 0.07^{a}	0.48 ± 0.04^{a}	0.52 ± 0.03^{ab}	0.55 ± 0.02^{b}	0.57 ± 0.03^{b}
Neelam	24.37 ± 0.12^{d}	16.25 ± 0.09^{c}	10.36 ± 0.07^{b}	6.85 ± 0.06^{a}	0.83 ± 0.02^{a}	0.86 ± 0.02^{a}	0.88 ± 0.04^{a}	0.88 ± 0.03^{a}
Raspuri	16.67 ± 0.09^{d}	10.86 ± 0.07^{c}	5.54 ± 0.03^{b}	4.02 ± 0.04^{a}	0.55 ± 0.03^{a}	0.61 ± 0.04^{b}	0.63 ± 0.03^{b}	0.64 ± 0.02^{b}
Rumani	14.43 ± 0.06^{d}	8.39 ± 0.06^{c}	4.18 ± 0.04^{b}	1.97 ± 0.02^{a}	0.44 ± 0.05^{a}	0.49 ± 0.03^{ab}	0.51 ± 0.04^{ab}	0.53 ± 0.03^{c}
Sindhura	19.35 ± 0.07^{d}	12.27 ± 0.08^{c}	6.35 ± 0.06^{b}	3.86 ± 0.04^{a}	0.71 ± 0.02^{a}	0.77 ± 0.05^{ab}	0.79 ± 0.03^{b}	0.81 ± 0.04^{b}
Totapuri	15.74 ± 0.10^{d}	9.83 ± 0.04^{c}	5.07 ± 0.05^{b}	2.61 ± 0.05^{a}	0.52 ± 0.04^{a}	0.56 ± 0.03^{ab}	0.59 ± 0.02^{bc}	0.62 ± 0.02^{c}

Values are given as mean ± S.D (n = 3); Values not sharing a common superscript in a row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range test (DMRT).

3.4.2 Effect of γ-irradiation on total polyphenolic content (TPC) and total flavonoid content (TFC) of mango juice: Various polyphenolic compounds in fruits and vegetables have been reported to exhibit antioxidant activities because of the reactivity of the phenolic moiety, scavenging free radicals, *via* hydrogen donation or electron donation and a strong correlation has been demonstrated between TPC and antioxidant activities (Jayaprakasha and Patil, 2007). The effect of γ-irradiation on TPC of different cultivars of mango juice samples is shown in Table 3.2. TPC in all cultivars of control mango juice ranged from 32.81 to 54.22 (mg GAE/100 mL), the lowest TPC was found in *Mulgoa* and highest was in *Banginapalli* cultivars respectively. There was a significant ($P \leq 0.05$) increase in the TPC in all cultivars of irradiated mango juice samples when compared to non-irradiated controls. At an irradiation dose of 3 kGy, highest TPC was found in juices from *Banginapalli* (67.75 ± 0.19), *Alphonso* (63.54 ± 0.22), and *Sindhura* (62.75 ± 0.24 mg/100 mL), the lowest values were found in juices from *Raspuri* (48.61 ± 0.14), *Rumani* (46.37 ± 0.13) and *Mulgoa* (45.86 ± 0.13 mg/100 mL) (Table 3.2). Similarly Harrison and Were (2007) found significant increase in TPC of irradiated almond skin extracts at dose level of 4 kGy and above and found that this increase could be attributed to their release from glycosidic precursors and the degradation of polymeric phenolic compounds to smaller units during γ-irradiation. Polyphenolic compounds found in mango like mangiferin, isomangiferin, homomangiferin (xanthones), quercetin, kaempferol (flavonols) and anthocyanins; phenolic acid like gallic, protocatechuic gallic, protocatechuic, 4-caffeoylquinic acids, are known to be potent antioxidants. The flavonols (quercetin, kaempferol, and rhamnetin) are present mostly as O-glycosides, whereas mangiferin is a C-glycoside and occurs both in its non-esterified form or conjugated with gallic acid (Ribeiro and Schieber, 2010). The increase in TPC in irradiated samples could thus be accounted for the degradation of these conjugated phenolic compounds. Similar results were also reported in 'Triphala', in which degradation of high molecular weight complex polyphenolic compounds like tannins into simple phenolic compounds like phenolic acids facilitated the release of active ingredients, which contributed to the increased total phenolic content (Kumari *et al*., 2009). In this study, a strong correlation has been demonstrated between increments in TPC and increments in antioxidant activities. The increase in TPC was also reported in soybeans and spices; irradiated soybean samples at dose of 0.5 to 5 kGy showed an increase in free (aglycone) phenolic content

Table 3.2 Effects of γ-irradiation on total polyphenolic content (TPC) and total flavanoid content (TFC) in different cultivars of mango juice

Juice variety	TPC (mg GAE/100 mL)				TFC (mg RE/100 mL)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	50.45 ± 0.16^{a}	53.68 ± 0.19^{b}	58.22 ± 0.17^{c}	63.54 ± 0.22^{d}	14.45 ± 0.04^{a}	14.78 ± 0.07^{a}	15.43 ± 0.11^{b}	16.56 ± 0.08^{c}
Banginapalli	54.22 ± 0.18^{a}	56.45 ± 0.15^{b}	62.34 ± 0.21^{c}	67.75 ± 0.19^{d}	14.32 ± 0.08^{a}	15.41 ± 0.05^{ab}	15.75 ± 0.07^{b}	16.48 ± 0.13^{c}
Mulgoa	32.81 ± 0.12^{a}	38.23 ± 0.14^{b}	43.61 ± 0.18^{c}	45.86 ± 0.13^{d}	12.57 ± 0.06^{a}	13.16 ± 0.11^{ab}	13.98 ± 0.04^{b}	14.12 ± 0.06^{bc}
Neelam	38.76 ± 0.15^{a}	42.16 ± 0.17^{b}	46.44 ± 0.14^{c}	50.28 ± 0.16^{d}	13.76 ± 0.05^{a}	14.09 ± 0.08^{a}	15.22 ± 0.09^{b}	16.14 ± 0.08^{c}
Raspuri	36.54 ± 0.13^{a}	40.37 ± 0.12^{b}	45.09 ± 0.15^{c}	48.61 ± 0.14^{d}	12.98 ± 0.07^{a}	13.27 ± 0.10^{a}	14.56 ± 0.06^{b}	15.24 ± 0.07^{c}
Rumani	34.93 ± 0.17^{a}	36.88 ± 0.15^{b}	42.53 ± 0.18^{c}	46.37 ± 0.13^{d}	13.43 ± 0.11^{a}	13.78 ± 0.06^{a}	14.43 ± 0.05^{b}	14.81 ± 0.11^{b}
Sindhura	49.36 ± 0.19^{a}	52.44 ± 0.17^{b}	56.97 ± 0.16^{c}	62.75 ± 0.24^{d}	14.28 ± 0.06^{a}	15.24 ± 0.12^{ab}	15.61 ± 0.08^{b}	16.33 ± 0.05^{c}
Totapuri	37.87 ± 0.16^{a}	41.65 ± 0.14^{b}	46.08 ± 0.12^{c}	51.19 ± 0.18^{d}	13.29 ± 0.09^{a}	13.47 ± 0.07^{a}	14.15 ± 0.12^{b}	14.62 ± 0.09^{bc}

Values are given as mean ± S.D (n = 3); Values not sharing a common superscript in a row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range test (DMRT).

(Variyar *et al*., 2004). The present study is in agreement with the earlier reports on fresh vegetable juice (Song *et al*., 2006), and ready-to-use tamarind juice (Lee *et al*., 2009).

Flavonoids have been credited with diverse key functions in plant growth and development and also considered as multifunctional bioactive compounds with a wide range of biological activities including antioxidant, anti-inflammatory, antibacterial, antiviral, antimutagenic, antiallergic, and hepatoprotective effects (Middleton and Kandaswami 1994). The protective effects of flavonoids in biological systems were ascribed to their capacity to neutralize free radicals by the transfer of electrons, chelate metal catalysts, activate antioxidant enzymes, and inhibit oxidases (Cos *et al*., 1998). The effect of γ-irradiation on TFC of different cultivars of mango juice samples is shown in Table 3.2. TFC in all cultivars of control mango juice ranged from 12.57 to 14.45 (mg RE/100 mL), the lowest TFC was found in *Mulgoa* and highest was in *Alphonso* cultivars, respectively. There was a significant ($P \leq 0.05$) increase in the TFC in all cultivars of irradiated mango juice samples when compared to non-irradiated controls. At an irradiation dose of 3 kGy, higher TFC was found in juices from *Alphonso* (16.56 ± 0.08), *Banginapalli* (16.48 ± 0.13), and *Sindhura* (16.33 ± 0.05 mg/100 mL), lower values were found in juices from *Rumani* (14.81 ± 0.11), *Totapuri* (14.62 ± 0.09) and *Mulgoa* (14.12 ± 0.06 mg/100 mL) than the controls (Table 3.2). Our results were in agreement with the earlier studies of Mishra *et al*. (2011), who reported that the flavonoid content marginally increased during low temperature storage for 30 days in irradiated and control sugarcane juice samples. Hussein *et al*. (2011) also reported that two types of irradiated Malaysian honey exhibited a significantly ($P < 0.05$) higher content of flavonoids than their non-irradiated counterparts.

3.4.3 Effect of γ-irradiation on the content of individual polyphenolic compounds in mango juice: The effect of γ-irradiation on the individual polyphenolic compounds in all eight cultivars of mango juice samples is shown in Table 3.3. γ-Irradiation significantly altered, either decreased or increased the concentration of the polyphenolic compounds studied. The HPLC chromatogram of the polyphenolic compounds identified by comparing the spectra and retention time with that of respective authentic standards is shown in (Fig. 3.1a) for control and (Fig. 3.1b) for irradiated (3 kGy) mango juice (Cv. *Banginapalli*) and 15 compounds were identified within 65 min of run and were numbered consecutively. However, two major

unidentified peaks were observed before gallic acid and after quercetin peaks at RT of 3.34 and 64.29 min respectively. The major polyphenolic compounds identified in the present study consist of different classes such as phenolic acids and flavanoids (Fig. 3.1). There were significant ($P \leq 0.05$) differences noted in the concentration of individual polyphenolic compounds between non-irradiated and irradiated mango juice samples (Table 3.3). At lower doses i.e at 0.5 and 1 kGy, changes in the concentration of polyphenolic compounds either increased or decreased was similar to that of the trend observed at the dose of 3 kGy, hence the discussion was provided for the same because of its clear-cut impact on polyphenolic compounds. There was a significant ($P \leq 0.05$) increase in the concentration of flavanoids with increase in irradiation dose, irrespective of the cultivars studied and the higher found in *Raspuri* (44.3 ± 1.3), *Mulgoa* (50.4 ± 1.3) and *Mulgoa* (47.0 ± 1.2 µg/mL) for rutin, catechin and quercetin respectively. Similar increase in flavanoids was also observed in strawberries up to 3 kGy (Breitfellner *et al*., 2003). There was also significant ($P \leq 0.05$) increase in the concentration of majority of the phenolic acids studied with exception ferulic and synapic acids wherein a significant ($P \leq 0.05$) decrease in the concentration with increase in irradiation dose was noted. The % increase or decrease of polyphenolic compounds identified in all cutivars of irradiated (3 kGy) mango juice samples are shown in Table 3.3. The increase in phenolic compounds in irradiated mango was found to be proportional to the radiation dose used (0.5-1.5 kGy) (El-Samahy *et al*., 2000). A significant increase in the content of gallic, chlorogenic, *p*-coumaric acids, rutin, and quercetin as a result of γ-irradiation was also reported in two types of Malaysian honey (Hussein *et al*., 2011). Significant increase in phenolic acids was also observed in soybean samples treated with γ-irradiation in the range of 50 to 150 Gy (Variyar *et al*., 2004).

Reports suggest that the main phenolic compounds of mango pulp are gallic acid, mangiferin, quercetin glycosides, and many identified and uncharacterized hydrolyzable tannins, called gallotannins (Schieber *et al*., 2000). In most mango varieties, free gallic acid, 3,4,5-trihydroxybenzoic acid, is the predominant compound present and has been shown to possess a high antioxidant capacity with numerous implications to overall human health (Shanrzad and Bitsch 1998). Gallic acid units possess three hydroxyl groups and an acid group which allow the compound to link with another gallic acid to form an ester, digallic acid

(Masibo and He, 2008). Pinn *et al*. (1993) have reported a dose dependent decrease in tannin content of Brazilian beans upon γ-irradiation up to a dose of 20 kGy. Similar results were also observed by Karmazin *et al*. (1988) during decontamination of tannin containing drugs exposed to radiation doses up to 10 kGy. In the present study, increase in some of the phenolic compounds observed in γ-irradiatiated juice samples might be due to the degradation of hydrolyzable tannins, glycosides and gallic acid esters present in the mango juice samples.

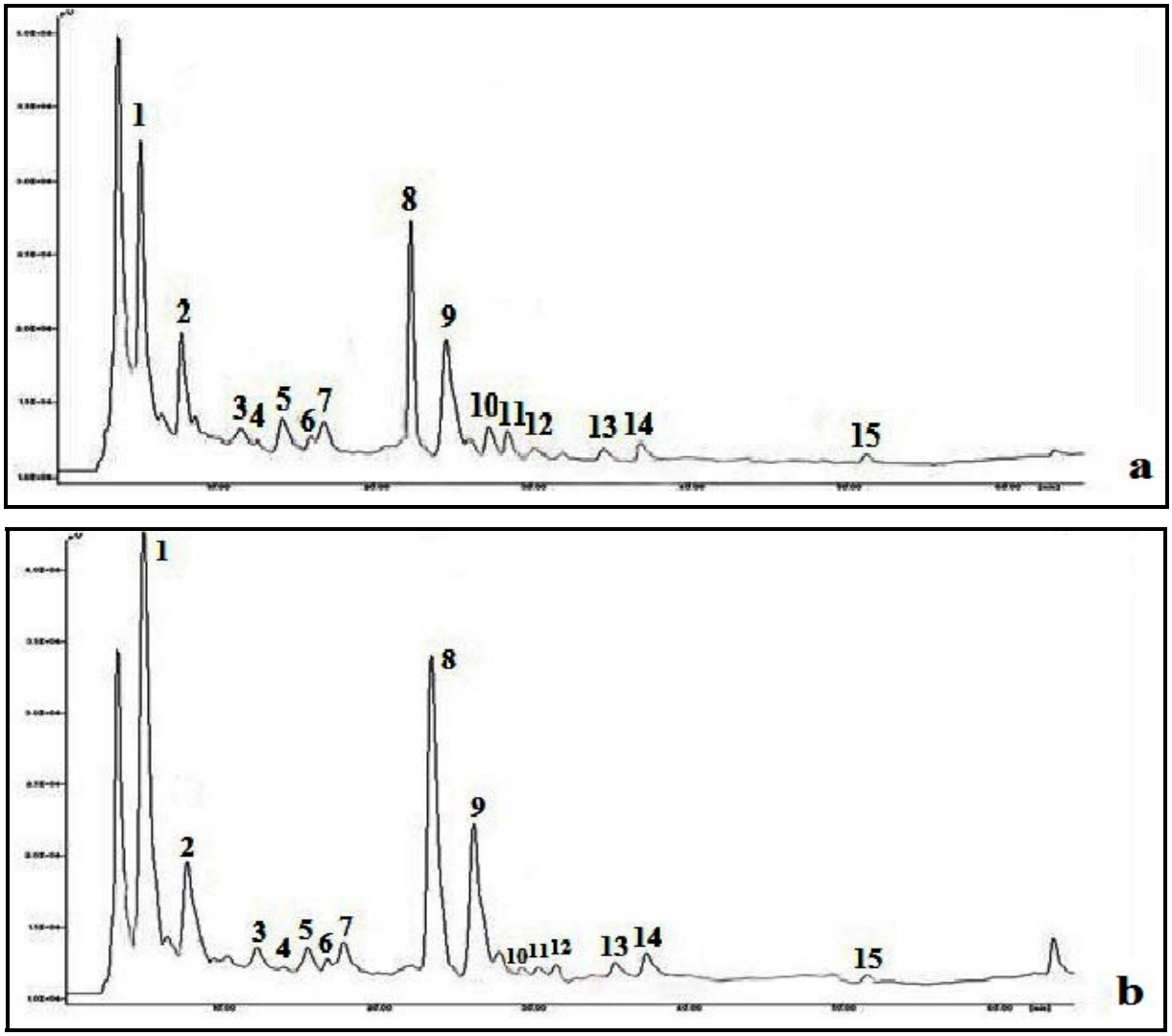

Fig. 3.1 A typical HPLC chromatogram of (a) non-irradiated and (b) γ-irradiated mango juice (Cv. *Banginapalli*). Major mango polyphenols identified and quantified were: (1) gallic acid, (2) protocatechic acid, (3) P-OH benzoic acid, (4) vanillic acid, (5) chlorogenic acid, (6) syringic acid, (7) caffeic acid, (8) p-coumaric acid, (9) m-coumaric acid, (10) ferulic acid, (11) synapic acid, (12) ellagic acid, (13) rutin, (14) (+)-catechin, (15) quercetin.

Table 3.3 Effect of γ-irradiation (3kGy) on individual polyphenolic compounds of mango juice from different cultivars

Polyphenolic compound (µg/mL)	RT (min)	*Alphonso*			*Banginapalli*			*Mulgoa*		
		Control	Irradiated	% Increase	Control	Irradiated	% Increase	Control	Irradiated	% Increase
Gallic acid	5.11	106.3 ± 5.3	376.1 ± 9.9***	253.8	175.9 ± 5.4	563.3 ± 11.9***	220.2	74.4 ± 3.3	175.2 ± 4.4***	135.5
Protocatechuic acid	7.45	17.4 ± 0.9	29.7 ± 1.2**	70.7	77.0 ± 2.6	87.2 ± 3.2*	13.3	14.9 ± 0.5	32.2 ± 1.1***	116.1
P-OH-benzoic acid	11.89	24.3 ± 0.8	43.1 ± 1.3**	77.4	19.8 ± 0.9	36.2 ± 1.0**	82.8	2.2 ± 0.2	6.0 ± 0.3**	172.7
Vanillic acid	15.01	14.7 ± 0.7	30.3 ± 1.2***	106.1	13.7 ± 0.5	14.0 ± 0.4‡	2.2	12.5 ± 0.4	35.6 ± 1.2***	184.8
Chlorogenic acid	15.34	20.1 ± 1.0	40.7 ± 1.3***	102.5	20.1 ± 0.9	45.9 ± 1.1***	128.4	12.2 ± 0.6	41.7 ± 1.4***	241.8
Syringic acid	16.77	3.4 ± 0.3	13.5 ± 0.5**	297.06	3.6 ± 0.2	8.9 ± 0.3***	147.2	3.3 ± 0.2	6.9 ± 0.3**	109.1
Caffeic acid	17.16	34.5 ± 1.1	77.2 ± 3.1***	123.8	35.8 ± 1.1	39.2 ± 0.9*	9.5	8.4 ± 0.5	26.8 ± 0.8***	219.1
p-Coumaric acid	24.41	105.3 ± 5.2	142.3 ± 6.4**	35.1	120.7 ± 6.6	151.8 ± 7.2**	25.8	19.1 ± 0.8	28.4 ± 0.7**	48.7
m-Coumaric acid	26.26	76.7 ± 4.6	82.6 ± 5.0*	7.7	82.1 ± 5.1	88.4 ± 5.8*	7.7	7.3 ± 0.4	59.7 ± 1.3***	717.8
Ferulic acid	28.33	40.3 ± 1.1	18.4 ± 0.9**	-54.3†	45.4 ± 1.3	10.5 ± 0.8***	-76.9†	21.4 ± 0.7	6.9 ± 0.3**	-67.7†
Synapic acid	29.25	35.4 ± 0.9	13.7 ± 0.6**	-61.4†	37.8 ± 1.0	14.1 ± 0.7**	-63.1†	54.9 ± 1.4	15.8 ± 0.6***	-71.2†
Ellagic acid	33.91	9.3 ± 0.5	21.12 ± 1.0***	127.1	8.9 ± 0.6	11.3 ± 0.6*	27.0	4.3 ± 0.3	5.4 ± 0.2*	25.6
Rutin	34.54	6.4 ± 0.6	12.4 ± 0.4*	93.8	15.1 ± 0.4	18.4 ± 0.4*	21.9	6.6 ± 0.5	12.3 ± 0.6**	86.4
(+)-Catechin	37.11	6.0 ± 0.3	12.2 ± 0.3*	103.3	16.2 ± 0.9	16.4 ± 0.8‡	1.2	48.3 ± 1.2	50.4 ± 1.3‡	4.3
Quercetin	51.83	9.4 ± 0.4	9.6 ± 0.3‡	2.1	13.4 ± 0.8	13.7 ± 0.9‡	2.2	45.6 ± 1.1	47.0 ± 1.2‡	3.1

Table 3.3 continued…

Polyphenolic compound (µg/mL)	RT (min)	*Neelam*			*Raspuri*			*Rumani*		
		Control	Irradiated	% Increase	Control	Irradiated	% Increase	Control	Irradiated	% Increase
Gallic acid	5.11	79.3 ± 3.8	182.9 ± 5.2***	130.6	58.7 ± 2.1	61.9 ± 2.2‡	5.5	26.0 ± 0.8	31.4 ± 0.9**	20.8
Protocatechuic acid	7.45	11.2 ± 0.5	12.5 ± 0.6*	11.7	27.2 ± 0.9	30.2 ± 1.0‡	11.1	17.2 ± 0.7	17.3 ± 0.6‡	0.6
P-OH-benzoic acid	11.89	23.0 ± 0.9	27.6 ± 1.0*	20.0	11.3 ± 0.5	11.5 ± 0.4‡	1.8	41.2 ± 1.1	46.6 ± 1.2*	13.1
Vanillic acid	15.01	9.1 ± 0.4	13.4 ± 0.5*	47.3	8.5 ± 0.4	8.7 ± 0.5‡	2.4	7.6 ± 0.4	9.0 ± 0.3‡	18.4
Chlorogenic acid	15.34	45.6 ± 1.2	65.5 ± 1.6**	43.6	24.5 ± 0.8	26.4 ± 0.7‡	7.8	36.9 ± 1.0	49.1 ± 1.3**	33.1
Syringic acid	16.77	6.3 ± 0.5	7.4 ± 0.3‡	17.4	3.6 ± 0.2	6.5 ± 0.3*	80.5	4.1 ± 0.3	4.2 ± 0.2‡	2.4
Caffeic acid	17.16	28.5 ± 0.9	36.8 ± 1.1**	29.1	11.7 ± 0.4	20.3 ± 0.9**	73.5	35.8 ± 0.9	40.7 ± 1.0*	13.7
p-Coumaric acid	24.41	79.6 ± 3.6	132.1 ± 4.3***	65.9	34.3 ± 1.0	34.9 ± 1.1‡	1.7	11.2 ± 0.4	13.6 ± 0.5*	21.4
m-Coumaric acid	26.26	87.7 ± 3.7	91.2 ± 3.9*	4.0	13.7 ± 0.6	25.1 ± 0.7**	83.2	63.1 ± 3.5	63.2 ± 3.4‡	0.2
Ferulic acid	28.33	30.6 ± 0.8	30.7 ± 0.9‡	0.3	38.5 ± 1.1	21.9 ± 0.6**	-43.1†	26.0 ± 0.9	24.3 ± 0.8‡	-6.5†
Synapic acid	29.25	31.7 ± 0.9	45.2 ± 1.2**	42.6	14.0 ± 0.5	19.8 ± 0.7*	41.4	24.8 ± 0.8	21.1 ± 0.7‡	-15.0†
Ellagic acid	33.91	5.8 ± 0.2	6.9 ± 0.5‡	19.0	5.8 ± 0.3	4.0 ± 0.2‡	-31.0†	12.6 ± 0.5	8.4 ± 0.3*	-33.3†
Rutin	34.54	12.4 ± 0.5	35.3 ± 1.1**	184.7	13.2 ± 0.6	44.3 ± 1.3***	235.6	9.8 ± 0.4	12.7 ± 0.5*	29.6
(+)-Catechin	37.11	12.3 ± 0.6	10.0 ± 0.5‡	-18.7†	10.9 ± 0.4	11.1 ± 0.5‡	1.8	5.7 ± 0.3	33.9 ± 0.9***	494.7
Quercetin	51.83	9.4 ± 0.4	9.6 ± 0.3‡	2.1	14.5 ± 0.6	14.6 ± 0.6‡	0.7	6.1 ± 0.4	7.1 ± 0.2‡	16.4

Table 3.3 continued…

Polyphenolic compound (μg/mL)	RT (min)	*Sindhura*			*Totapuri*		
		Control	Irradiated	% Increase	Control	Irradiated	% Increase
Gallic acid	5.11	42.5 ± 1.1	144.9 ± 4.2***	240.9	154.3 ± 4.7	175.9 ± 5.2*	14.0
Protocatechuic acid	7.45	4.8 ± 0.3	12.7 ± 0.6**	164.6	17.0 ± 0.7	37.2 ± 0.9**	118.8
P-OH-benzoic acid	11.89	20.1 ± 0.8	43.3 ± 1.1***	115.4	13.8 ± 0.5	19.7 ± 0.7*	42.7
Vanillic acid	15.01	5.8 ± 0.2	15.9 ± 0.5**	174.1	11.7 ± 0.3	37.4 ± 1.1**	219.7
Chlorogenic acid	15.34	56.7 ± 1.4	67.6 ± 1.6**	19.2	72.6 ± 3.2	101.1 ± 4.3**	39.3
Syringic acid	16.77	16.3 ± 0.5	17.4 ± 0.4‡	6.7	3.9 ± 0.2	8.6 ± 0.4**	120.5
Caffeic acid	17.16	35.9 ± 0.9	37.1 ± 0.8‡	3.3	29.4 ± 1.1	36.2 ± 1.3*	23.1
p-Coumaric acid	24.41	52.6 ± 1.3	60.3 ± 1.5**	14.6	73.2 ± 3.2	122.5 ± 5.0***	67.4
m-Coumaric acid	26.26	60.6 ± 1.4	124.8 ± 6.2***	105.9	26.1 ± 0.9	95.8 ± 3.8***	267.1
Ferulic acid	28.33	50.8 ± 1.2	38.0 ± 0.8**	-25.2†	75.7 ± 3.4	34.0 ± 1.1**	-55.1†
Synapic acid	29.25	37.1 ± 1.1	30.8 ± 1.2*	-17.0†	38.2 ± 0.8	14.1 ± 0.5**	-63.1†
Ellagic acid	33.91	11.4 ± 0.4	5.2 ± 0.2**	-54.4†	5.3 ± 0.2	5.7 ± 0.3‡	7.5
Rutin	34.54	12.7 ± 0.5	27.1 ± 1.1**	113.4	11.5 ± 0.3	12.1 ± 0.4‡	5.2
(+)-Catechin	37.11	9.8 ± 0.3	9.99 ± 0.2‡	1.0	31.9 ± 0.9	35.6 ± 1.2*	11.6
Quercetin	51.83	6.0 ± 0.2	6.2 ± 0.3‡	3.3	10.3 ± 0.4	10.4 ± 0.3‡	1.0

RT: Retention time; ***, **, * significant when compared to that of control (non-irradiated) at $P \leq 0.0001$, 0.001 and 0.01 respectively.
‡ = not significant ($P \leq 0.05$); †= minus (-) value indicate % Decrease.

3.4.4 Effect of γ-irradiation on *in vitro* antioxidant capacities of mango juice: Free radicals are extremely reactive species and are known to damage proteins, cause breakdown of DNA strands and initiate the peroxidation of various compounds thus leading to many health problems and degenerative diseases such as cancer, inflammation, atherosclerosis and accelerated aging. Plant tissues synthesize a wide variety of phenolic compounds which include flavonoids, isoflavones, flavones, anthocyanin, catechin, etc. These compounds exhibit free radical scavenging activity and have received increasing attention for their potential role in the prevention of human diseases as well as in food quality improvement (Khattak *et al*., 2009). The antioxidant capacities of mango juice can be measured using various *in vitro* methods based on different principles since these methods are based on different mechanism of action.

3.4.4.1 DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay: DPPH is a stable free radical, decreases significantly on exposure to proton radical scavengers. This assay is a widely used method to evaluate the free radical scavenging activity in foods, biological systems and in natural antioxidant extracts. DPPH radical scavenging activity can be observed by antioxidants resulted from donating proton to the lone pair electron of the radicals by a rapid fading in its purple colour (Kumar *et al*., 2012). It is generally accepted that the polyphenolic compounds, flavanoids and ascorbic acid are major antioxidants in fruits and vegetables (Cao *et al*., 1996). It was earlier shown that mango wine contains carotenoids (Varakumar *et al*., 2011) and polyphenolic compounds (Kumar *et al*., 2012) possessing various *in vitro* antioxidant activities. There was a significant ($P \leq 0.05$) increase in % RSA in all the cultivars of irradiated mango juice samples when compared to their controls (Fig. 3.2). The percentage increase in radical scavenging activity was found to be prominent up to 1 kGy irrespective of the cultivar studied. However, at 3 kGy dose, the activity slightly decreased in *Alphonso*, *Totapuri* and *Neelam* juice samples. The maximum % RSA was found in juice from *Sindhura* (97.39) followed by *Banginapalli* (95.51) and lowest in *Mulgoa* (84.85%) at 100 µL and 3 kGy dose. However in control samples at the same treatments, the maximum % RSA was observed in *Sindhura* (92.64) followed by *Banginapalli* (89.41) and the lowest were found in *Mulgoa* (80.83%). This increase in % RSA of irradiated samples could be attributed to the increase in TPC during irradiation. A good correlation [$r = 0.992$ and $r = 0.988$ at 1 and 3 kGy (100 µL)]

was found between DPPH radical scavenging activity and total polyphenolic content, indicating a direct relation between them.

As observed in this study, an increase in DPPH scavenging activity due to irradiation has also been reported in carrot juice (Song *et al*. 2006), and ready-to-use tamarind juice (Lee *et al*. 2009). Jo *et al*. (2003) reported that green tea leaves extracts irradiated at 10 and 20 kGy significantly increased the scavenging ability of DPPH radicals. Variyar *et al*. (2004) reported a dose dependent increase in radical scavenging ability of extracts from γ-irradiated soybean exposed to doses between 0.5 and 5 kGy. It can be postulated that the increase in antioxidant activities following γ-irradiation could be due to the degradation of some high molecular weight polymeric phenolic compounds to simple phenols thus enhancing their solubility in the test solvent resulting in greater interaction with the reagents. Hussein *et al*. (2011) reported increased antioxidant activities, resulting from higher polyphenol and flavonoid content in γ-irradiated honey samples.

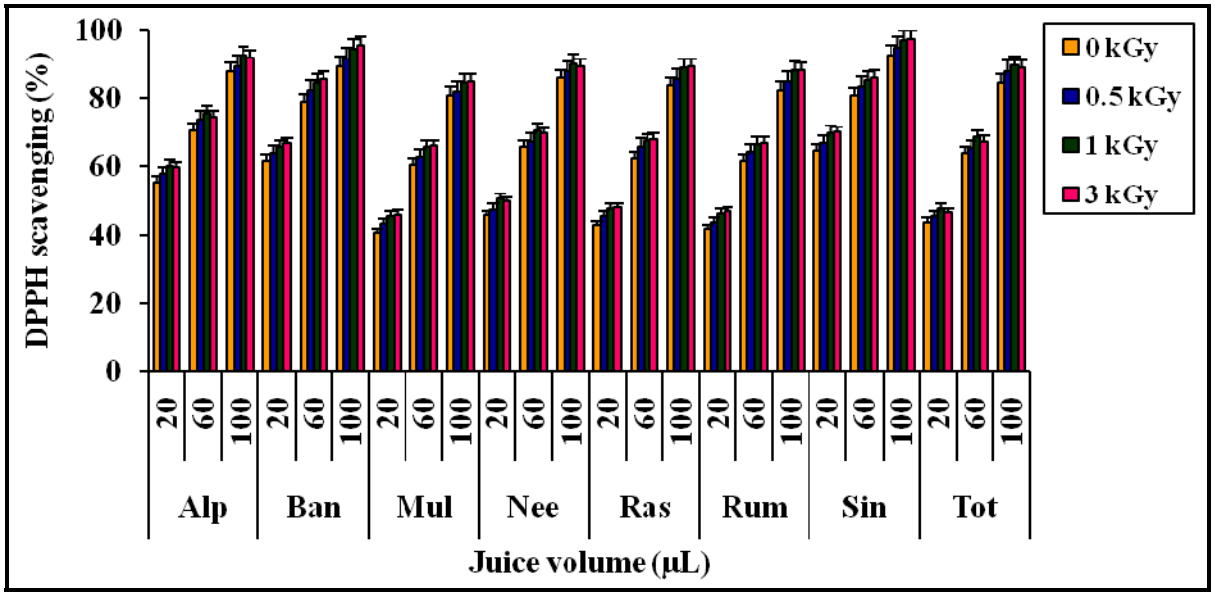

Fig. 3.2 Effect of γ-irradiation on DPPH radical scavenging activity of different cultivars of mango juice (cultivars were abbreviated with their first three letters).

3.4.4.2 FRAP (Ferric reducing antioxidant power) assay: The FRAP assay is routinely used analytical method for evaluation of antioxidant capacity, since it was simple, sensitive and inexpensive. The reducing capacity of a compound might serve as a significant indicator of its

potential antioxidant capacity. This method is based on the reduction of colorless ferric complex (Fe^{3+} -TPTZ) at low pH to a blue colored ferrous complex (Fe^{2+} -TPTZ) by the action of electron-donating antioxidants and the activity can be monitored by measuring the change of absorbance at 593 nm (Benzie and Strain, 1996). In the present investigation, there was a significant ($P \leq 0.05$) increase in the FRAP of all cultivars of irradiated mango juice when compared to non-irradiated juice samples, tested at 0.5 to 1 kGy (Fig. 3.3). However, no significant differences in FRAP activity was observed between 1 and 3 kGy in juice samples from *Banginapalli* and *Sindhura* and a negligible decrease in *Mulgoa* juice samples at 3 kGy irradiation dose. The FRAP activity of the mango juice were in the order: *Banginapalli* > *Sindhura* > *Rumani* > *Alphonso* > *Totapuri* > *Neelam* > Raspuri > *Mulgoa* at a dose of 3 kGy and concentration of 500 µL. In this study also, FRAP activities of mango juice were in good correlation with total phenolic content [r = 0.984 and r = 0.978 at 1 and 3 kGy (500 µL)].

Similar increase in activity has been reported in Chinese cabbage (Ahn *et al*., 2005), ready-to-use tamarind juice (Lee *et al*., 2009) and peach fruits (Hussain *et al*., 2010) as a result of radiation treatment. The increase in total antioxidant activity could be as a result of high phenol accumulation by the radiation treatment as explained in earlier sections above. The polyphenolic compounds act as reducing agents, hydrogen donators and singlet oxygen quenchers, and based on the antioxidant properties, it can be suggested that bioactive compounds present in mango juice have strong scavenging and ferric reducing power.

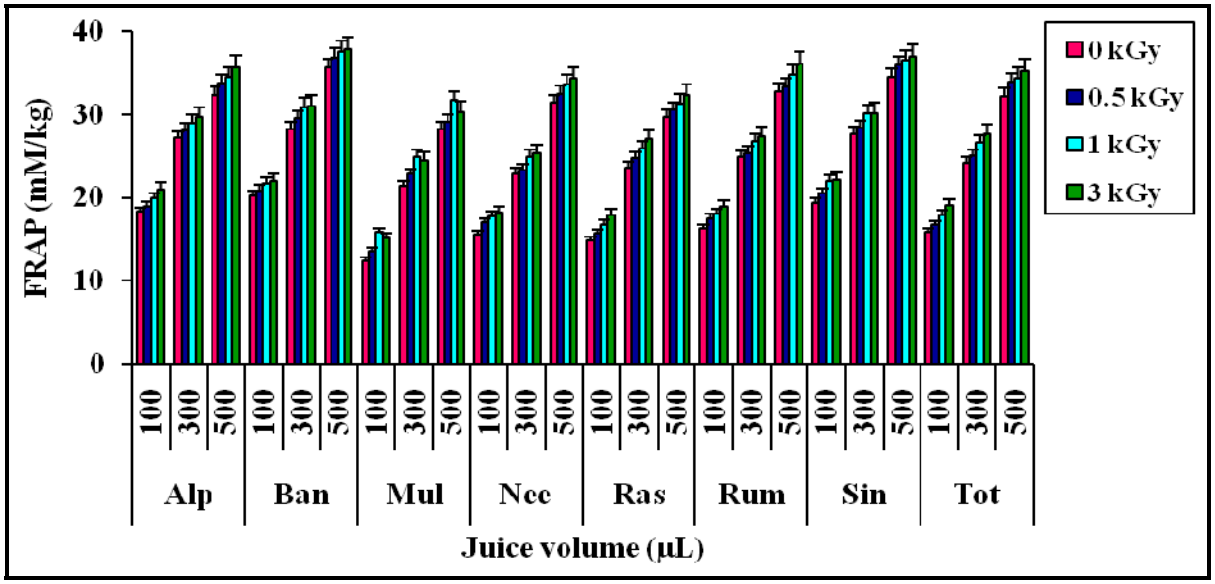

Fig. 3.3 Effect of γ-irradiation on FRAP assay of different cultivars of mango juice.

3.4.4.3 Nitric oxide (NO) scavenging assay: NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effector molecule in various diverse biological systems (Hagerman *et al*. 1998). Hence the screening of various health foods in scavenging of NO radical is warranted. Mango juice possesses several phenolic compounds which showed significant NO scavenging activity. The potential of NO scavenging capacity of irradiated and non-irradiated mango juice samples is shown in Fig. 3.4. There was a significant ($P \leq 0.05$) increase in NO scavenging capacity of irradiated mango juice samples when compared to their non-irradiated controls. The NO scavenging capacity was significantly ($P \leq 0.05$) increased with increase in irradiation dose as well as with concentration tested in all juice samples up to 1 kGy. There was no significant difference in the scavenging activity between 1 and 3 kGy in all the samples except in *Totapuri* and *Mulgoa*, wherein a slight decrease at 3 kGy dose was observed. The highest NO radical scavenging capacity was found in *Banginapalli* (90.62%) followed by *Alphonso* (88.07%) and lowest in *Mulgoa* (79.48%) at higher irradiation dose (3 kGy) and concentration (500 µL). A positive correlation [r = 0.985 and r = 0.978 at 1 and 3 kGy (500 µL)] was observed between total phenolic content and NO radical scavenging capacity of all cultivars of irradiated mango wine. It has been reported that polyphenolic compounds have higher NO scavenging effects under low pH conditions (Mishra *et al*., 2011). Mango juice is rich in polyphenolic compounds and possesses a low pH, and thus showed significant NO scavenging activity.

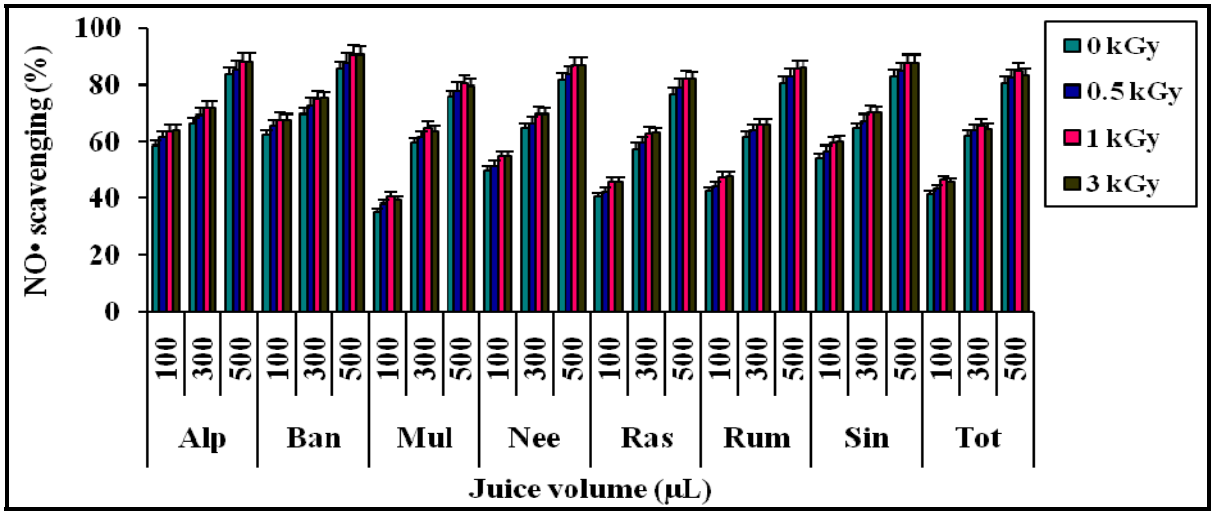

Fig. 3.4 Effect of γ-irradiation on NO scavenging activity of different cultivars of mango juice.

3.4.4.4 ABTS scavenging assay: ABTS is frequently used to measure the antioxidant capacities of foods and this method determines the antioxidant activity of hydrogen donating antioxidants and of chain breaking antioxidants. The ABTS assay is applicable to both lipophillic and hydrophilic compounds (Sasidharan and Menon, 2011). An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process that is associated with a change in absorption. In the present investigation, there was a significant ($P \leq 0.05$) increase in the ABTS scavenging activity of all cultivars of irradiated mango juice when compared to non-irradiated juice samples, tested at 0.5 to 1 kGy (Fig. 3.5). However, no significant differences in ABTS scavenging activity was observed between 1 and 3 kGy in juice samples from *Malgoa*, *Raspuri* and *Totapuri* and a negligible decrease in *Alphonso*, *Banginapalli* and *Neelam* juice samples at 3 kGy irradiation dose. The ABTS scavenging activity of the mango juice were in the order: *Sindhura* > *Banginapalli* > *Alphonso* > *Rumani* > *Totapuri* > *Raspuri* > *Neelam* > *Mulgoa* at a dose of 3 kGy and concentration of 100 µL. In this study also, ABTS scavenging activities of mango juice were in good correlation with total phenolic content [r = 0.986 and r = 0.981 at 1 and 3 kGy (100 µL)]. The increased activity with dosage and concentration could have synergistic effects of polyphenols and other compounds with antioxidant activity. Most fruits tested with high antioxidant capacity in the DPPH model, also showed a high antioxidant capacity in ABTS model.

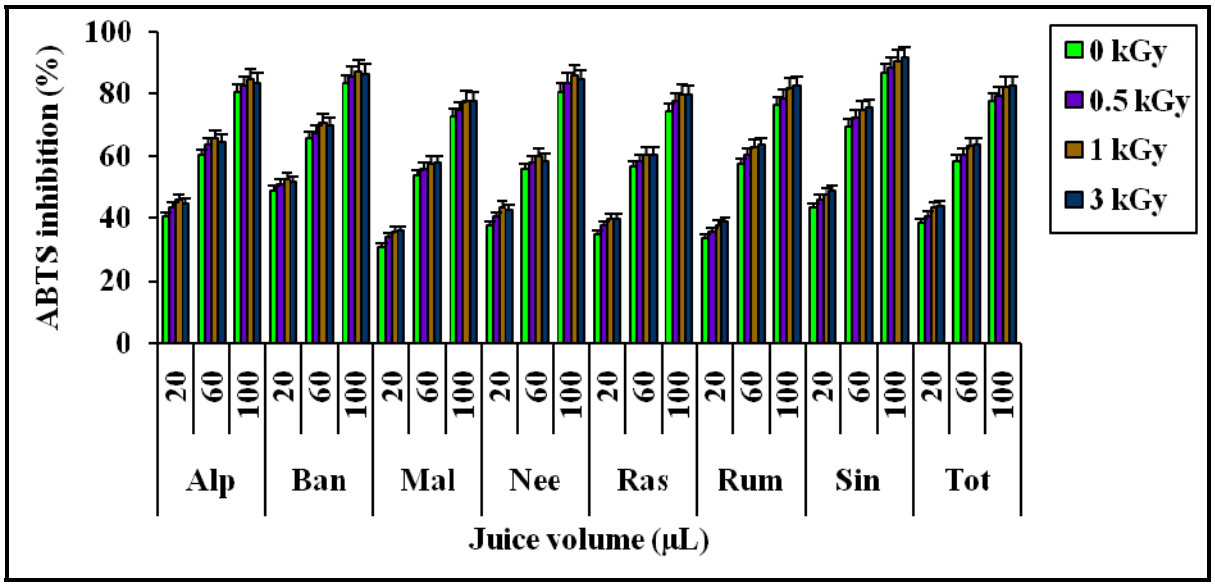

Fig. 3.5 Effect of γ-irradiation on ABTS scavenging activity of different cultivars of mango juice.

3.4.5 *In vitro* radioprotective effect of mango juice on plasmid DNA damage: *In vitro* radioprotective effect of mango juice against γ-irradiation induced DNA (pUC19 plasmid) damage was shown in Fig. 3.6. The non-irradiated plasmid DNA is highly super coiled about 80% (Fig. 3.6, lane 1). When exposed to different doses of γ-irradiation (0.5, 1, and 3 kGy), there was complete degradation of plasmid DNA (Fig. 3.6, lanes 5, 6, 7), whereas in presence of mango juice, the degradation was significantly prevented (Fig. 3.6, lanes 2, 3, 4), thus indicating radioprotective activity of mango juice.

Radiation treatment, as such can produce a variety of lesions in DNA resulting in both single or double strand breaks, alteration of bases, destruction of sugar moiety, cross-linking, and formation of dimers. The formation of open circular form of plasmid DNA from supercoiled plasmid DNA is an indication of single stranded breaks (SSBs), whereas the formation of linear form is indicative of double stranded breaks (DSBs) (Saxena *et al*., 2011). In the present study, mango juice offered protective action against radiation induced DNA damage. Similarly litchi juice was earlier shown to have a protective role against DNA damage (Saxena *et al*., 2011). Aqueous extracts of chili, black pepper, and turmeric have been reported to have similar protective effects (Sharma *et al*., 2000). The protective action of mango juice could be due to the synergistic action of various bioactive compounds in mango juice.

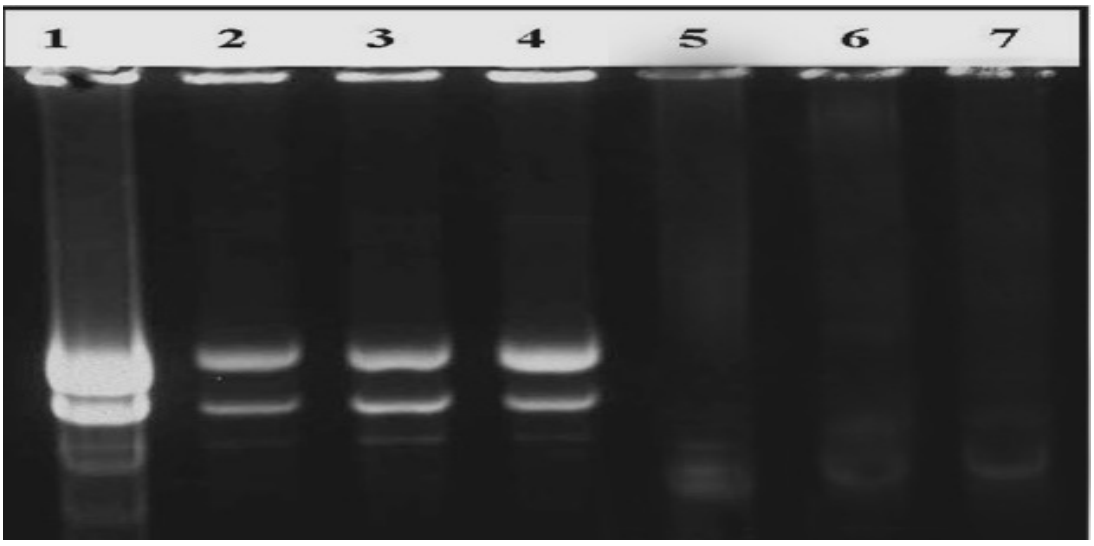

Fig. 3.6 Agarose gel electrophoresis showing radioprotection of pUC19 plasmid DNA by mango juice; Lane 1: Non-irradiated (Control DNA); Lane 2-4: Protection against DNA damage by mango juice at irradiation doses 1, 3 and 5 kGy respectively; Lane 5-7: Degradation of DNA at 0.5, 1 and 3 kGy irradiation doses respectively.

3.4.6 Effect of γ-irradiation on total polyphenolic content (TPC) and total flavonoid content (TFC) of mango wine: Polyphenolic compounds are one of the most important quality parameters of wines, since they contribute to wine organoleptic properties such as colour, astringency, bitterness and possess several biological properties including antioxidant activities. The effect of different irradiation doses on the TPC of mango wine obtained from different cultivars is shown in the Table 3.4. There was a significant ($P \leq 0.05$) increase in the TPC in all the irradiated mango wine when compared to non-irradiated controls. At an irradiation dose of 3 kGy, highest TPC was found in wines from *Banginapalli* (555.3 ± 1.8), *Alphonso* (532.4 ± 2.2), and *Sindhura* (508.7 ± 1.6 mg/L), the lowest values were found in wines from *Raspuri* (335.8 ± 2.4), *Rumani* (304.7 ± 1.9) and *Mulgoa* (226.8 ± 3.1 mg/L). The increase in TPC as a result of γ-irradiation could be due to radiolysis of phenolics (eg. gallic acid, caffeic acid, *etc*.) in aqueous solution that led to their radiolytic degradation as a result of hydroxyl radical attack (Breitfellner *et al*., 2003), which is in accordance with the previous studies on Indian spices, clove and nutmeg (Variyar *et al*., 1998), almond skin extracts (Harrison and Were, 2007), soybean seeds (Stajner *et al*., 2007), ready-to-use tamarind juice (Lee *et al*., 2009), and Malaysian honey (Hussein *et al*., 2011). In contrary to these reports, no significant differences were observed in irradiated cumin when compared to that of control (Kim *et al*., 2009) and a significant reduction in polyphenolic content was observed with increase in dose of γ-irradiation in minimally processed Chinese cabbage (Ahn *et al*., 2005).

Flavonoids, due to their metal-chelating capability together with radical scavenging property act as natural plant antioxidants and are also reported to inhibit lipid peroxidation and to retard the progress of many chronic diseases (Kinsella *et al*., 1993). There was a significant ($P \leq 0.05$) dose dependent increase in the TFC in all irradiated mango wine samples when compared to non-irradiated controls (Table 3.4). The highest TFC was found in wine from *Sindhura* (165.1 ± 1.3), and *Alphonso* (156.8 ± 1.2 mg/L) and lowest in wine from *Raspuri* (89.7 ± 1.4) and *Mulgoa* (68.6 ± 1.6 mg/L) at 3 kGy. These results were in agreement with previous studies, who reported a significant increase in flavonoid content in minimally processed arugula (Nunes *et al*., 2008) and two types of Malaysian honey (Hussein *et al*., 2011).

Table 3.4 Effect of γ-irradiation on total polyphenolic content (TPC) and total flavanoid content (TFC) in different types of mango wine

Wine variety	TPC (mg GAE/ L)				TFC (mg RE/L)			
	0 kGy	0.5 kGy	1 kGy	3 kGy	0 kGy	0.5 kGy	1 kGy	3 kGy
Alphonso	518.4 ± 1.8^{a}	520.7 ± 2.4^{a}	525.5 ± 1.6^{b}	532.4 ± 2.2^{c}	152.7 ± 0.9^{a}	153.5 ± 1.3^{a}	154.3 ± 1.1^{a}	156.8 ± 1.2^{b}
Banginapalli	542.6 ± 2.3^{a}	543.9 ± 2.5^{ab}	547.5 ± 1.9^{b}	555.3 ± 1.8^{c}	146.3 ± 1.2^{a}	147.8 ± 0.9^{ab}	149.5 ± 1.4^{bc}	151.4 ± 1.1^{c}
Mulgoa	214.7 ± 3.2^{a}	216.2 ± 1.9^{a}	221.4 ± 2.4^{b}	226.8 ± 3.1^{c}	63.8 ± 1.1^{a}	65.1 ± 1.5^{ab}	66.9 ± 1.3^{bc}	68.6 ± 1.6^{c}
Neelam	370.3 ± 2.3^{a}	371.8 ± 1.8^{a}	376.5 ± 2.1^{b}	382.4 ± 2.4^{c}	109.2 ± 1.0^{a}	110.7 ± 1.2^{ab}	111.5 ± 0.9^{bc}	113.4 ± 1.4^{c}
Raspuri	323.4 ± 1.9^{a}	325.2 ± 2.2^{a}	331.7 ± 2.1^{b}	335.8 ± 2.4^{c}	85.6 ± 1.3^{a}	86.8 ± 1.1^{ab}	88.2 ± 1.2^{bc}	89.7 ± 1.4^{c}
Rumani	292.6 ± 2.4^{a}	293.9 ± 1.6^{a}	299.3 ± 2.1^{b}	304.7 ± 1.9^{c}	104.2 ± 1.2^{a}	106.4 ± 1.0^{b}	107.3 ± 0.9^{bc}	108.8 ± 1.1^{c}
Sindhura	495.2 ± 2.1^{a}	497.4 ± 1.8^{a}	502.3 ± 1.9^{b}	508.7 ± 1.6^{c}	160.4 ± 0.9^{a}	162.4 ± 1.4^{ab}	163.7 ± 1.5^{bc}	165.1 ± 1.3^{c}
Totapuri	345.5 ± 2.5^{a}	347.2 ± 2.6^{a}	352.6 ± 1.7^{b}	356.8 ± 2.8^{b}	95.4 ± 1.4^{a}	96.8 ± 1.2^{a}	97.6 ± 1.0^{ab}	99.7 ± 1.3^{b}

Values are given as mean ± S.D (n = 3); Values not sharing a common superscript in a row differ significantly at $P \leq 0.05$ according to Duncan's Multiple Range test (DMRT).

3.4.7 Effect of γ-irradiation on the individual polyphenols in mango wine: The effect of γ-irradiation on the content of individual polyphenolic compounds in all eight cultivars of mango wine is shown in Table 3.5. γ-Irradiation significantly altered, either decreased or increased the concentration of the polyphenolic compounds studied. The HPLC profile of polyphenolic compounds identified in both the control (a) and γ-irradiated (3 kGy) (b) mango wine (Cv. *Banginapalli*) is shown in Fig. 3.7. The major polyphenolic compounds identified in the present study consist of different classes such as phenolic acids and flavanoids (Fig. 3.7). At lower doses i.e at 0.5 and 1 kGy, changes in the concentration of polyphenolic compounds either increased or decreased was similar to that of the trend observed at the dose of 3 kGy, hence the discussion was provided for the same because of its clear-cut impact on polyphenolic compounds. There was significant increase in the concentration of flavanoids with increase in irradiation dose, irrespective of the cultivars studied and the highest was found in *Raspuri* (56.1 ± 3.3), *Totapuri* (58.0 ± 1.1) and *Totapuri* (56.0 ± 2.3 µg/mL) for rutin, catechin and quercetin, respectively. Similar increase in flavanoids was also observed in strawberries up to 3 kGy (Breitfellner *et al*., 2003). There was significant increase in the concentration of majority of the phenolic acids studied with exception of ellagic acid, which remained unaltered and that of ferulic and synapic acid, wherein a significant decrease in the concentration with increase in irradiation dose was noted (Table 3.5). A significant increase in the content of gallic, chlorogenic, *p*-coumaric acids, rutin, and quercetin as a result of γ-irradiation was also reported in two types of Malaysian honey (Hussein *et al*., 2011). Significant increase in phenolic acids was also observed in soybean samples treated with γ-irradiation in the range of 50 to 150 Gy (Variyar *et al*., 2004). The increase in phenolic acids could be due to the higher extractability as a result of depolymerization and dissolution of cell wall polysaccharides during γ-irradiation (Siddhuraju *et al*., 2002). The increase in phenolic compounds in irradiated mango was found to be proportional to the radiation dose used (0.5-1.5 kGy) (El-Samahy *et al*., 2000). However, in contrast to the current findings, a significant reduction in phenolic content of fresh pomegranate fruits exposed to γ-irradiation was reported (Shahbaz *et al*., 2014). It was also reported that low dose of γ-irradiation on citrus peels could enhance the synthesis of total phenolic compounds and the total phenolic content was correlated to the activity of certain enzymes such as phenylalanine ammonia-lyase during storage (Oufedjikh *et al*., 2000). Thus γ-irradiation may

modify the levels of some of the enzymes resulting in enhanced or decreased synthesis of phenolic acids. Further, irradiation can exert its effects via direct or indirect mechanisms. Thus, radiolysis of water results in the production of radicals such as hydrated electrons, hydroxyl radicals and hydrogen atoms (Hussein *et al*., 2011). These radicals as a part of the indirect effect may break the glycosidic bonds of phenolic compounds that are present in wine samples, leading to the formation of new compounds. Thus increase in phenolic compounds in γ-irradiated samples could be attributed to the release of phenolic compounds from glycosidic components as well as via the degradation of the larger phenolic compounds into smaller ones by γ-irradiation (Variyar *et al*., 2004; Harrison and Were, 2007).

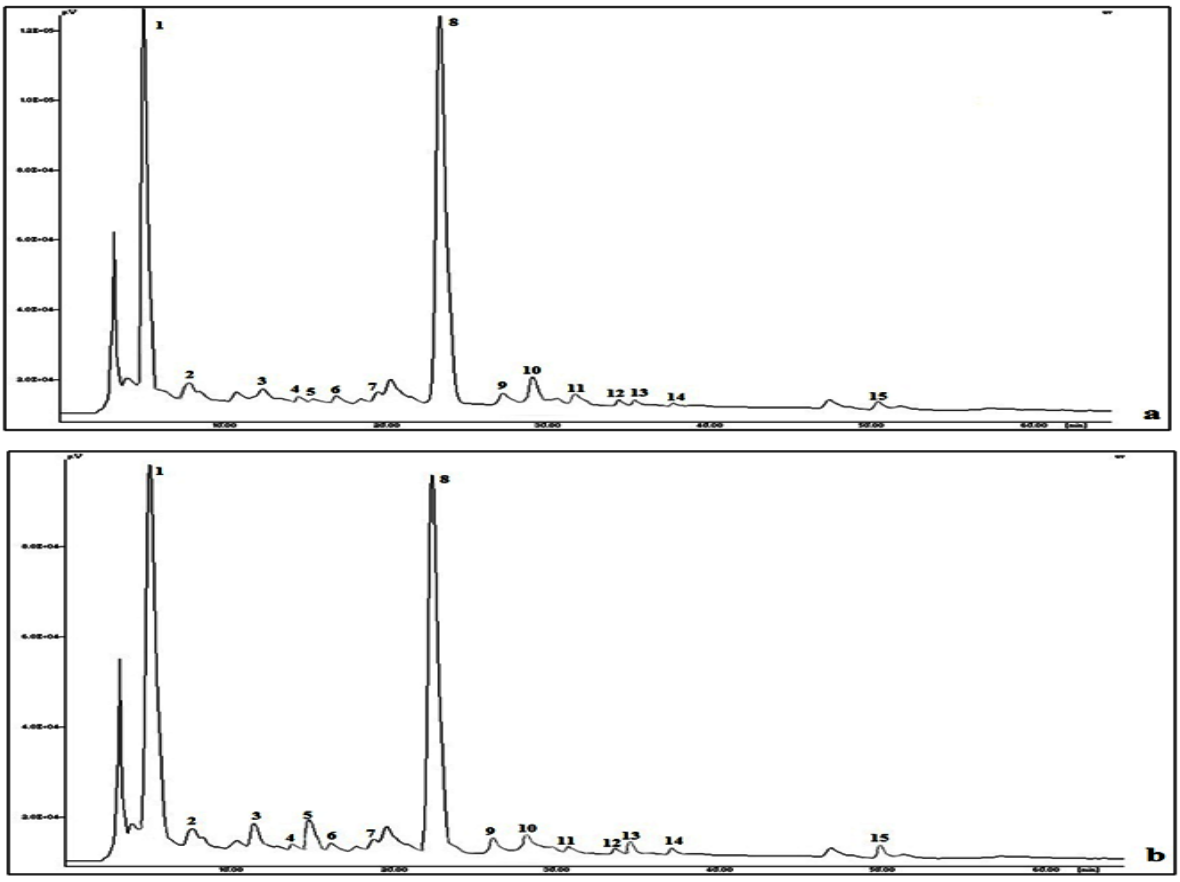

Fig. 3.7 A typical HPLC chromatogram of (a) non-irradiated and (b) γ-irradiated mango wine (Cv. *Banginapalli*). Major polyphenolic compounds identified and quantified were: (1) gallic acid, (2) protocatechic acid, (3) P-OH benzoic acid, (4) vanillic acid, (5) chlorogenic acid, (6) syringic acid, (7) caffeic acid, (8) p-coumaric acid, (9) m-coumaric acid, (10) ferulic acid, (11) synapic acid, (12) ellagic acid, (13) rutin, (14) (+)-catechin, (15) quercetin.

Table 3.5 Effect of γ-irradiation (3kGy) on the individual polyphenolic compounds in different types of mango wine

Polyphenolic compound (μg/mL)	RT (min)	*Alphonso*			*Banginapalli*			*Mulgoa*		
		Control	Irradiated	% Increase	Control	Irradiated	% Increase	Control	Irradiated	% Increase
Gallic acid	5.32	199.1 ± 7.1	201.9 ± 8.6‡	1.41	176.7 ± 6.2	392.3 ± 10.3***	122.01	147.4 ± 2.2	154.3 ± 3.9*	4.68
Protocatechuic acid	7.68	20.1 ± 0.9	27.6 ± 1.8**	37.31	18.0 ± 0.9	19.9 ± 1.0*	10.56	3.9 ± 0.4	12.4 ± 0.8***	217.95
P-OH-benzoic acid	11.44	8.2 ± 0.7	10.6 ± 0.6*	29.27	21.0 ± 1.9	44.2 ± 2.2***	110.48	4.5 ± 0.7	9.3 ± 0.9**	106.67
Vanillic acid	15.09	9.5 ± 0.7	20.4 ± 1.9***	114.74	8.3 ± 0.8	10.2 ± 0.5*	22.89	5.5 ± 0.5	12.6 ± 0.8***	129.09
Chlorogenic acid	15.86	20.1 ± 1.0	37.2 ± 1.2***	85.07	18.8 ± 1.1	59.6 ± 2.4***	217.02	12.4 ± 0.7	27.4 ± 1.7***	120.97
Syringic acid	16.82	13.6 ± 0.8	23.4 ± 0.9*	72.06	11.3 ± 0.7	21.5 ± 0.9*	90.27	19.5 ± 1.1	26.6 ± 1.3*	36.41
Caffeic acid	17.74	37.4 ± 1.7	47.2 ± 2.0**	26.20	16.7 ± 0.9	17.7 ± 0.8‡	1.80	13.1 ± 0.9	13.6 ± 0.8‡	3.82
p-Coumaric acid	23.48	176.9 ± 7.0	180.4 ± 6.4*	1.98	235.4 ± 8.4	291.6 ± 7.7**	23.87	50.6 ± 2.4	59.2 ± 2.6*	17.00
m-Coumaric acid	27.21	129.7 ± 5.7	140.5 ± 6.1**	8.33	20.0 ± 0.9	28.9 ± 1.5*	44.50	10.6 ± 0.9	57.8 ± 3.3***	445.28
Ferulic acid	28.93	21.5 ± 1.0	19.3 ± 1.1‡	-10.23†	35.2 ± 1.4	22.1 ± 0.9***	-37.22†	18.5 ± 1.3	10.5 ± 0.6**	-43.24†
Synapic acid	31.75	26.2 ± 0.8	16.5 ± 2.1**	-37.02†	29.1 ± 1.8	12.8 ± 1.0***	-56.01†	47.8 ± 3.0	12.8 ± 1.4***	-73.22†
Ellagic acid	34.46	12.7 ± 0.6	13.9 ± 0.9‡	9.45	13.1 ± 0.9	13.8 ± 0.8‡	5.34	2.8 ± 0.4	3.5 ± 0.5‡	25.00
Rutin	36.01	13.5 ± 0.9	44.3 ± 1.2***	228.15	12.9 ± 1.1	32.9 ± 1.4***	155.04	14.6 ± 0.8	30.9 ± 1.2***	111.64
(+)-Catechin	39.37	11.4 ± 0.8	18.3 ± 0.9**	60.53	11.3 ± 0.8	18.9 ± 1.1**	67.26	12.7 ± 1.8	17.1 ± 0.7**	34.65
Quercetin	50.65	14.5± 0.7	18.5 ± 0.7**	27.59	17.5 ± 0.9	28.1 ± 1.5***	60.57	9.1 ± 0.8	16.0 ± 1.4**	75.82

Table 3.5 continued…

Polyphenolic compound (μg/mL)	RT (min)	*Neelam*			*Raspuri*			*Rumani*		
		Control	Irradiated	% Increase	Control	Irradiated	% Increase	Control	Irradiated	% Increase
Gallic acid	5.32	183.8 ± 8.4	213.4 ± 10.2*	16.10	111.0 ± 2.9	115.8 ± 3.7*	4.3	100.4 ± 5.0	103.5 ± 4.8‡	3.1
Protocatechuic acid	7.68	8.2 ± 0.6	6.3 ± 0.5*	-23.17†	10.8 ± 0.8	12.9 ± 0.7*	19.4	11.6 ± 1.0	18.7 ± 1.1**	61.2
P-OH-benzoic acid	11.44	6.9 ± 0.7	8.2 ± 0.4*	18.84	11.3 ± 0.7	12.0 ± 0.8‡	6.2	14.5 ± 0.7	27.8 ± 1.2***	91.7
Vanillic acid	15.09	11.6 ± 0.8	12.2 ± 0.9‡	5.17	8.4 ± 0.6	10.3 ± 0.7*	22.6	10.4 ± 0.8	11.0 ± 0.9‡	5.8
Chlorogenic acid	15.86	33.0 ± 1.8	37.8 ± 1.7*	14.55	32.2 ± 1.9	34.3 ± 2.2‡	6.5	19.5 ± 1.1	31.6 ± 2.8**	62.1
Syringic acid	16.82	11.6 ± 0.7	18.3 ± 1.0***	57.76	6.3 ± 0.5	3.2 ± 0.3***	-49.2†	5.8 ± 0.3	20.6 ± 1.1***	255.2
Caffeic acid	17.74	20.0 ± 1.9	29.8 ± 1.1**	49.00	21.4 ± 1.1	28.4 ± 1.5**	32.7	10.7 ± 0.9	18.9 ± 1.0***	76.6
p-Coumaric acid	23.48	125.6 ± 5.0	125.7 ± 5.2‡	0.08	48.8 ± 2.6	48.8 ± 2.8‡	0.0	31.0 ± 2.1	42.1 ± 3.2**	35.8
m-Coumaric acid	27.21	44.8 ± 2.7	61.7 ± 3.5**	37.72	44.7 ± 2.7	52.6 ± 1.6**	17.7	18.1 ± 1.2	56.0 ± 2.5***	209.4
Ferulic acid	28.93	27.9± 1.3	27.0 ± 1.1‡	-3.23†	14.5 ± 0.9	8.9 ± 0.5***	-38.6†	20.2 ± 1.3	10.7 ± 0.8***	-47.0†
Synapic acid	31.75	26.2 ± 1.1	25.4 ± 3.5‡	-3.05†	26.3 ± 1.1	14.7 ± 0.7***	-44.1†	18.2 ± 1.1	17.6 ± 2.2‡	-3.3†
Ellagic acid	34.46	7.5 ± 0.5	8.4 ± 0.7‡	12.00	6.8 ± 0.9	8.0 ± 0.6‡	17.6	10.2 ± 1.0	9.4 ± 0.7‡	-7.8†
Rutin	36.01	27.3 ± 2.0	47.2 ± 2.7***	72.89	41.6 ± 2.8	56.1 ± 3.3**	34.9	27.6 ± 1.1	32.7 ± 1.5**	18.5
(+)-Catechin	39.37	28.0 ± 1.2	36.5 ± 1.8**	30.36	24.3 ± 1.0	32.1 ± 1.5**	32.1	18.4 ± 2.1	29.2 ± 0.9***	58.7
Quercetin	50.65	14.5 ± 0.8	31.2 ± 1.3***	115.17	8.4 ± 0.9	36.5 ± 1.5***	334.5	28.4 ± 1.4	38.1 ± 1.9**	34.2

Table 3.5 continued…

Polyphenolic compound (µg/mL)	RT (min)	*Sindhura*			*Totapuri*		
		Control	Irradiated	% Increase	Control	Irradiated	% Increase
Gallic acid	5.32	164.3 ± 6.7	275.4 ± 10.1***	67.6	165.4 ± 5.7	286.8 ± 9.0***	73.4
Protocatechuic acid	7.68	6.4 ± 0.7	19.5 ± 1.3***	204.7	10.7 ± 1.1	24.8 ± 1.2***	131.8
P-OH-benzoic acid	11.44	24.0 ± 1.1	25.6 ± 1.0*	6.7	32.3 ± 2.2	39.5 ± 1.1*	22.3
Vanillic acid	15.09	7.9 ± 0.6	9.1 ± 0.9‡	15.2	9.3 ± 0.7	15.2 ± 0.9**	63.4
Chlorogenic acid	15.86	24.8 ± 2.4	47.5 ± 3.1***	91.5	13.1 ± 0.8	14.8 ± 0.6*	13.0
Syringic acid	16.82	20.7 ± 1.3	27.7 ± 1.6**	33.8	7.7 ± 0.5	12.3 ± 0.9**	59.7
Caffeic acid	17.74	15.6 ± 0.9	55.0 ± 3.2***	252.6	34.6 ± 2.3	42.3 ± 2.1***	22.3
p-Coumaric acid	23.48	235.9 ± 7.9	236.2 ± 6.1‡	0.1	36.4 ± 1.8	36.3 ± 1.9‡	-0.3†
m-Coumaric acid	27.21	132.4 ± 5.2	145.2 ± 4.7*	9.7	23.5 ± 1.7	35.8 ± 1.1**	52.3
Ferulic acid	28.93	30.7 ± 1.8	30.5 ± 1.9‡	-0.7†	35.2 ± 1.9	21.5 ± 1.2***	-38.9†
Synapic acid	31.75	36.7 ± 2.7	22.4 ± 1.4**	-39.0†	41.1 ± 2.9	31.4 ± 2.2**	-23.6†
Ellagic acid	34.46	10.5 ± 0.9	11.4 ± 1.1‡	8.6	9.0 ± 0.5	10.3 ± 1.0‡	14.4
Rutin	36.01	14.6 ± 0.6	18.0 ± 0.8**	23.3	44.3 ± 3.1	55.2 ± 3.6**	24.6
(+)-Catechin	39.37	17.6 ± 1.2	27.8 ± 1.1***	58.0	28.7 ± 2.3	58.0 ± 1.1***	102.1
Quercetin	50.65	16.1 ± 1.0	31.2 ± 1.3***	93.8	45.8 ± 3.2	56.0 ± 2.3**	22.3

RT: Retention time; ***, **, * significant when compared to that of control (non-irradiated) at $P \leq 0.0001$, 0.001 and 0.01 respectively.
‡ = not significant ($P \leq 0.05$); †= minus (-) value indicate % Decrease.

3.4.8 Effect of γ-irradiation on *in vitro* antioxidant capacities of mango wine: Several studies have demonstrated the antioxidant abilities of various foodstuffs using different methods which are based on the transfer of electrons or hydrogen atoms or the detection of lipid and protein oxidation activities. There has been an increasing interest in identifying the most satisfactory method to quantify the antioxidant profile of beverages such as wine to correlate with their possible health benefits (Rivero–Perez *et al*., 2007). However due to the complex nature of various phytochemicals in wine, no single method can be applied to evaluate the total antioxidant capacity. Therefore, antioxidant capacity of different cultivars of mango wine was determined by different *in vitro* methods to get an appropriate data.

3.4.8.1 DPPH radical scavenging assay (% RSA): Electron acceptors, such as molecular oxygen reacts readily with free radicals and are converted to reactive oxygen species (ROS) that include superoxide anions ($O^{\bullet}_2{}^{-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$). These reactive species are known to induce oxidative damage to lipids, proteins and nucleic acids eventually causing atherosclerosis, ageing, cancer, diabetes, inflammation and several degenerative diseases in humans (Choi *et al*., 2002). The relatively stable organic radical DPPH is widely used to determine the scavenging activity of naturally occurring compounds, such as polyphenols and anthocyanins in crude mixtures. The purple DPPH radical is reduced by antioxidants through the donation of a proton resulting in change in colour to yellow. There was a significant ($P \leq 0.05$) increase in % RSA in all the cultivars of irradiated mango wine when compared to their controls (Fig. 3.8). The percentage increase in radical scavenging activity was found to be prominent up to 1 kGy irrespective of the cultivar studied. However, at 3 kGy dose, the activity slightly decreased in *Alphonso*, *Totapuri* and *Neelam*. The maximum % RSA was found in wine from *Sindhura* (97.14) followed by *Banginapalli* (95.06) and lowest in *Mulgoa* (83.64%) at 100 µL and 3 kGy dose. However, in control samples at the same treatments, the maximum % RSA was observed in *Sindhura* (90.5) followed by *Banginapalli* (89.2) and the lowest were found in *Mulgoa* (78.4%). This increase in % RSA of irradiated samples could be attributed to the increase in TPC during irradiation. A good correlation [$r = 0.923$ and $r = 0.914$ at 1 and 3 kGy (100 µL)] was found between DPPH radical scavenging activity and total polyphenolic content, indicating a direct relation between them. Similar increase in DPPH

radical scavenging activity due to irradiation was also reported in soybean (Variyar *et al*., 2004; Stajner *et al*., 2007), ready-to-use tamarind juice (Lee *et al*., 2009) and peach fruit extracts (Hussain *et al*., 2010).

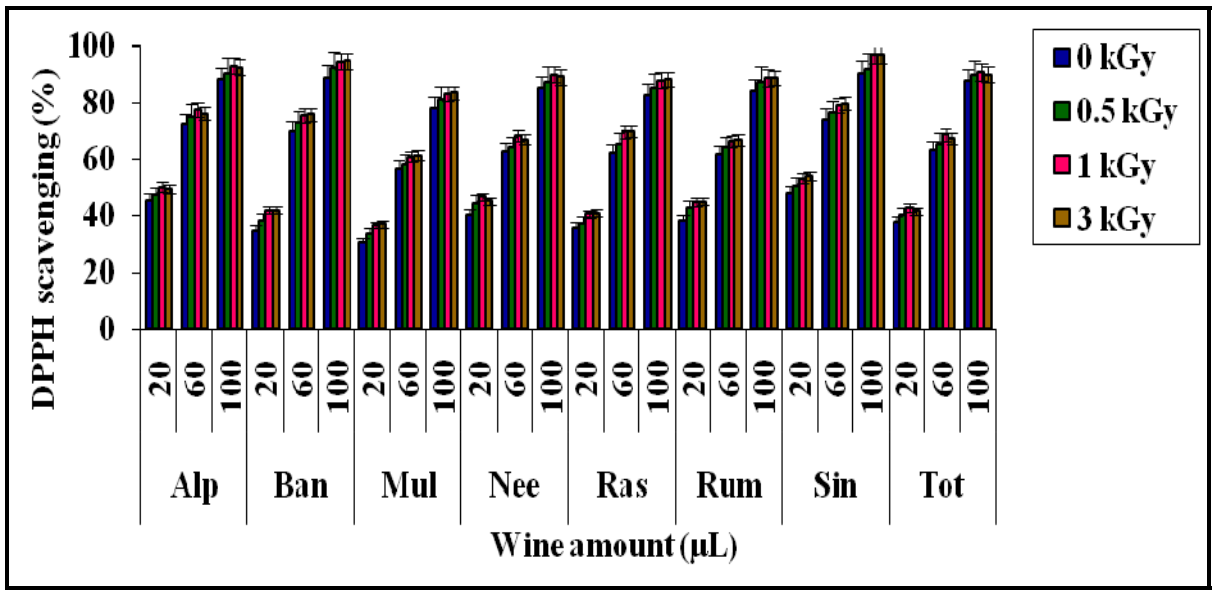

Fig. 3.8 Effect of γ-irradiation on DPPH radical scavenging activity of different types of mango wine (cultivars were abbreviated with their first three letters).

3.4.8.2 FRAP (Ferric reducing antioxidant power) assay: The reducing capacity of a compound might serve as a significant indicator of its potential antioxidant capacity. Generally, the reducing properties are associated with the presence of compounds that exert their action by breaking the free radical chain through the donation of a hydrogen atom (Kumar *et al*., 2012). The FRAP assay is based on the fact that at low pH (optimum pH 3.6) Fe^{3+}-TPTZ complex is reduced by antioxidants to its intense blue coloured form Fe^{2+}-TPTZ which has maximum absorbance at 593 nm (Lee *et al*., 2009). In the present investigation, there was a significant ($P \leq 0.05$) increase in the FRAP of all cultivars of irradiated mango wine when compared to non-irradiated wine samples (Fig. 3.9), tested at 0.5 to 1 kGy. However, no significant differences in FRAP activity was observed between 1 and 3 kGy in wine from *Banginapalli* and *Sindhura* and a negligible decrease in *Mulgoa* wine samples at 3 kGy irradiation dose. The FRAP activity of the wine were in the order: *Banginapalli* > *Alphonso* > *Sindhura* >*Neelam* > *Totapuri* > *Rumani* > *Raspuri* > *Mulgoa* at a dose of 3 kGy and concentration of 500 µL. In this study also, FRAP

activities of mango wine were in good correlation with total phenolic content [r = 0.966 and r = 0.966 at 1 and 3 kGy (500 µL)]. In a similar study, the antioxidant activity of jackfruit wine by FRAP assay was found to be correlated well with that of total phenolic content (Jagtap *et al*., 2011). The irradiation induced increase in total antioxidant activity as determined by FRAP assay could be due to the degradation of some high molecular weight polymeric phenols, leading to more phenolic compounds with increased solubility in the test solvents (Hussain *et al*., 2010). Similar increase in FRAP activity, as a result of irradiation has been reported in some foodstuffs such as ready-to-use tamarind juice (Lee *et al*., 2009), soybeans (Stajner *et al*., 2007), Chinese cabbage (Ahn *et al*., 2005), and peach fruit extracts (Hussain *et al*., 2010).

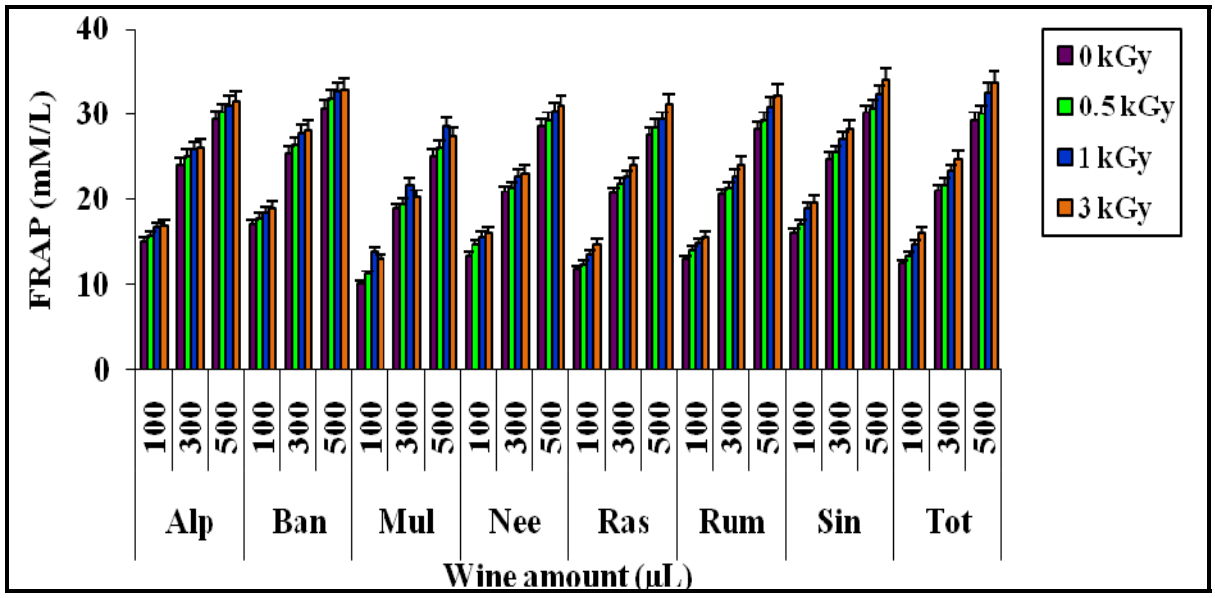

Fig. 3.9 Effect of γ-irradiation on FRAP assay of different types of mango wine.

3.4.8.3 Nitric oxide (NO) scavenging assay: Oxidative stress caused by the production of excess NO during infection or inflammation has been implicated in the pathogenesis of several diseases, including cancer, diabetes and renal disease (Jagtap *et al*., 2011). Sodium nitroprusside in aqueous solutions at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of NO compete with oxygen leading to reduced production of NO (Sreejayan and Rao, 1997). It has been reported that phenolic compounds have higher NO scavenging effects

under low pH conditions (Jagtap *et al*., 2011). Mango wine possesses phenolic compounds which showed significant NO scavenging activity. The potential of NO scavenging capacity of irradiated and non-irradiated mango wine is shown in Fig. 3.10. There was a significant ($P \leq 0.05$) increase in NO scavenging capacity of irradiated mango wine samples when compared to their non-irradiated controls. The NO scavenging capacity was significantly ($P \leq 0.05$) increased with increase in irradiation dose as well as with concentration tested in all wine samples up to 1 kGy. There was no significant difference in the scavenging activity between 1 and 3 kGy in all the samples except in *Totapuri* and *Mulgoa*, wherein a slight decrease at 3 kGy dose was observed. The highest NO radical scavenging capacity was found in *Banginapalli* (88.2%) followed by *Sindhura* (87.6%) and lowest in *Mulgoa* (74.44%) at higher irradiation dose (3 kGy) and concentration (500 µL). A positive correlation [r = 0.881 and r = 0.883 at 1 and 3 kGy (500 µL)] was observed between total phenolic content and NO radical scavenging capacity of all cultivars of irradiated mango wine.

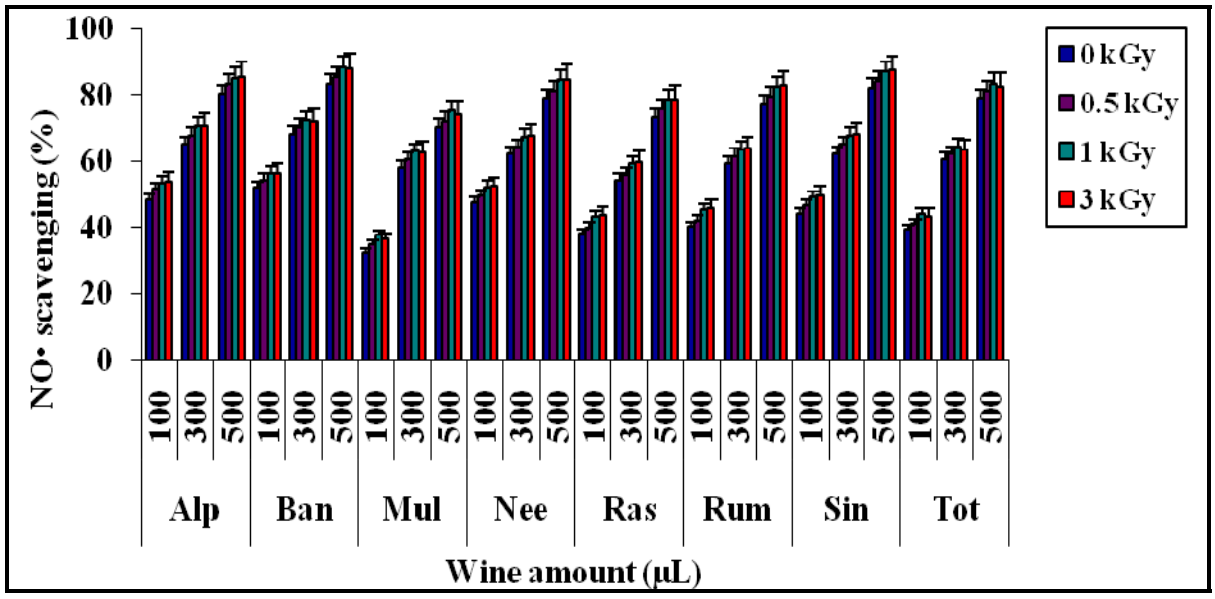

Fig. 3.10 Effect of γ-irradiation on NO scavenging activity of different types of mango wine.

3.4.8.4 DMPD scavenging assay: This assay has some advantages such as high stability of the coloured end product, rapid reaction, cost effectiveness and is less cumbersome. The basic principle of the assay is that DMPD can form a stable and coloured radical cation ($DMPD^{\bullet+}$) at

an acidic pH and in the presence of a suitable oxidant solution ($FeCl_3$). The λ-max of DMPD•$^+$ is at 505 nm and the antioxidant compounds, which could donate a hydrogen atom to DMPD•$^+$, quench the red colour and produce a discolouration of the solution proportional to their concentration (Fogliano *et al*., 1999). As shown in Fig. 3.11, mango wine was an effective DMPD•$^+$ radical scavenger which was found to be dependent on both the sample concentration and radiation dose. The DMPD•$^+$ radical scavenging capacity was significantly ($P \leq 0.05$) increased in all the irradiated mango wine when compared to non-irradiated controls. However, this increase in activity was significant ($P \leq 0.05$) only up to 1 kGy. There was a slight decrease in activity at 3 kGy in *Alphonso*, *Banginapalli* and *Neelam* cultivars while no significant difference was noted in *Raspuri*, *Totapuri*, and *Mulgoa* cultivars at this dose. The highest activity was found in *Banginapalli* (95.27%), followed by *Sindhura* (90.87%) and lowest in *Mulgoa* (77.8%) at higher irradiation dose (3 kGy) and concentration (100 µL). A positive correlation [r = 0.865 and r = 0.850 at 1 and 3 kGy (100 µL)] was observed between total phenolic content and DMPD•$^+$ radical scavenging capacity of all the irradiated mango wine.

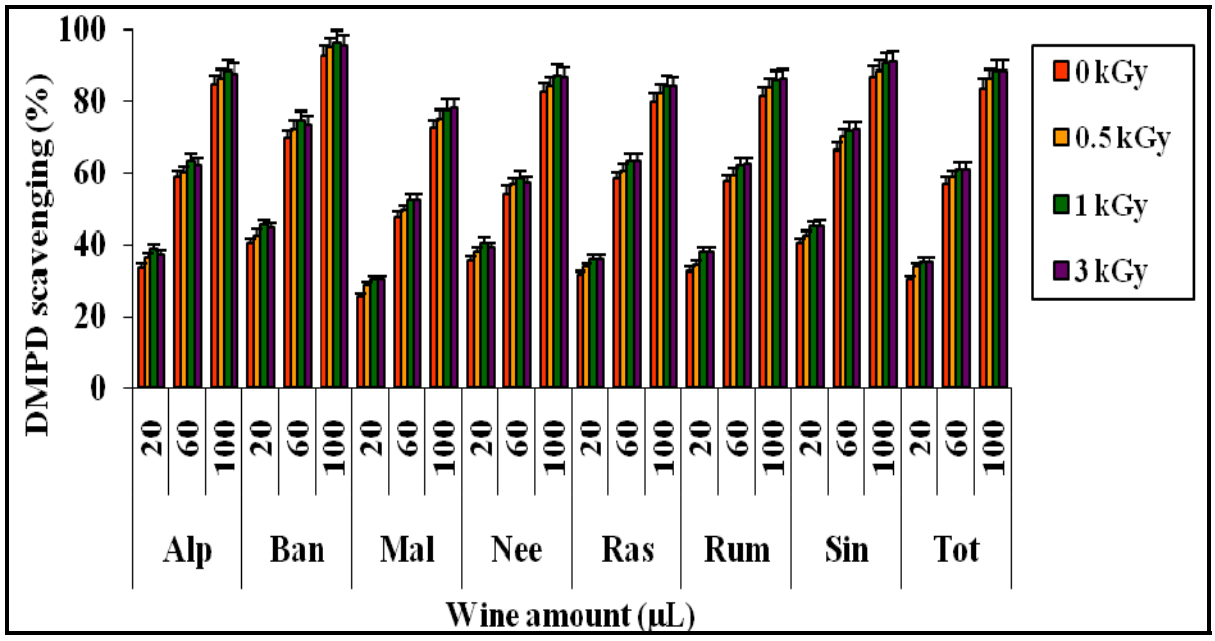

Fig. 3.11 Effect of γ-irradiation on DMPD scavenging activity of different types of mango wine.

3.4.9 *In vitro* protective effect of mango wine on DNA damage induced by H_2O_2-UV photolysis: The protective effect of mango wine against DNA damage induced by hydroxyl radicals ($OH^{\bullet}$) was evaluated on pUC19 plasmid DNA. The Fig. 3.12 shows the electrophoretic pattern of plasmid DNA after UV-photolysis of H_2O_2 (100 mM) in the absence or presence of different cultivars of mango wine (10 µL). DNA derived from pUC19 plasmid showed two bands on agarose gel electrophoresis (Lane C), the faster-moving band corresponding to the native form of supercoiled circular DNA (ccc) and the slower-migrating band being the open circular form (oc). The $OH^{\bullet}$ generated by UV-photolysis of H_2O_2 produced DNA strand scission and breakage. It is now well accepted that the extremely reactive $OH^{\bullet}$ radical derived from $O_2^{\bullet-}$ and H_2O_2 is a cause of DNA strand scission in cellular damage (Wiseman and Halliwell, 1996). Neither H_2O_2 nor UV alone induces DNA damage (Choi *et al*., 2002), but their combination resulted in almost complete degradation of plasmid DNA (Lane A). This damage was reduced in DNA treated with UV and H_2O_2 in the presence of different types of mango wine (Lanes AW-TW). Several flavonoids exhibited relatively strong DNA-protecting capacity at concentrations between 1 and 100 µM (Melidou *et al*., 2005). Mango wine contains good amount of polyphenols, flavonoids and carotenoids, those offered significant protection against H_2O_2-UV radiation induced DNA damage. Similar DNA protective role of red wines (Rivero–Perez *et al*., 2007) and jackfruit wine (Jagtap *et al*., 2011) was also reported earlier.

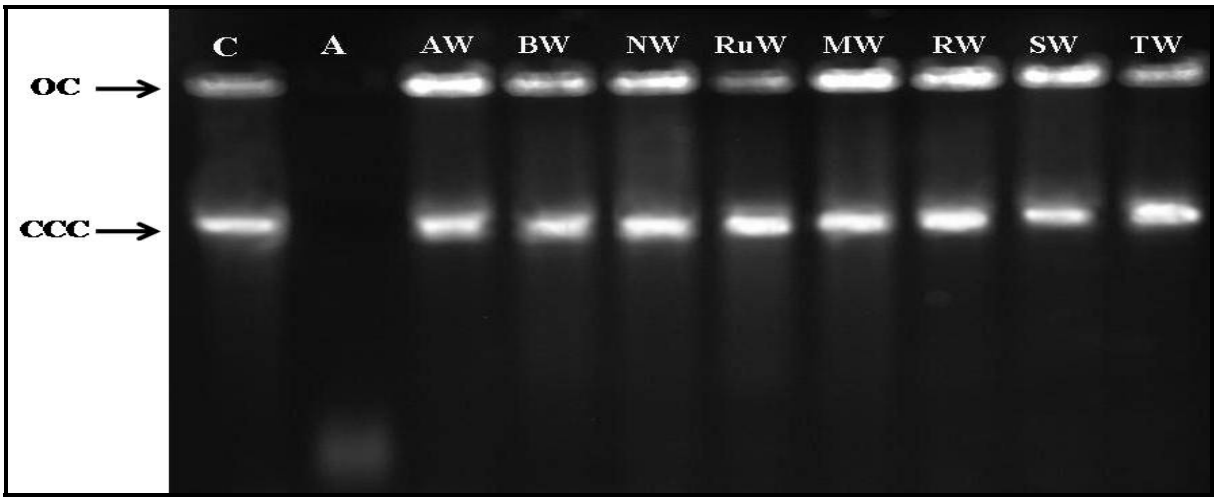

Fig. 3.12 Agarose gel electrophoretic pattern of pUC19 plasmid DNA after UV-photolysis of hydrogen peroxide (H_2O_2) in the presence and absence of different types of mango wine; Lane C: control DNA; Lane A: DNA + H_2O_2 + UV; Lane AW - TW: DNA + H_2O_2 + UV + different types of mango wine (10µL/assay). (AW=*Alphonso*, BW=*Banginapalli*, NW=*Neelam*, RuW=*Rumani*, MW=*Mulgoa*, RW=*Raspuri*, SW=*Sindhura*, TW=*Totapuri* wines).

3.4.10 Radiation protection of plasmid DNA by mango wine: The potential of mango wine to prevent DNA damage was investigated using pUC19 plasmid DNA as the major site of radiation-induced damage. The plasmid DNA showed two bands on agarose gel electrophoresis (Lane C, Fig. 3.13). Exposure to low dose of γ-radiation (0.5 kGy) resulted in production of strand breaks as a result of which the supercoiled (ccc) form of plasmid DNA was converted to open circular (oc) form or linear form. The disappearance of ccc form of DNA can be taken as an index of DNA damage induced by the radiation exposure. Exposure of plasmid DNA to 0.5 kGy in absence of mango wine results in near disappearance of the ccc form (Lane A, Fig. 3.13). This damage was reduced in the presence of different types of mango wine (10 µL) (Lanes AW to TW, Fig. 3.13). Exposure to ionizing radiation produces a variety of lesions in DNA such as single strand breaks, double strand breaks, DNA-DNA and DNA protein cross-links together with damage to nucleotide bases. The evaluation of these lesions forms an essential step in the examination of the sequence of events leading to mutagenic, carcinogenic and lethal effects of radiation (Saxena *et al*., 2011). In this study the presence of mango wine, reduced degradation of the ccc form during irradiation indicated *in vitro* protection of DNA damage by mango wine. Similar work on the radiation protection of plasmid DNA was reported by jackfruit wine (Jagtap *et al*., 2011), litchi juice (Saxena *et al*., 2011), apple polyphenols (Chaudhary *et al*., 2006), and gallic acid (Gandhi and Nair, 2005).

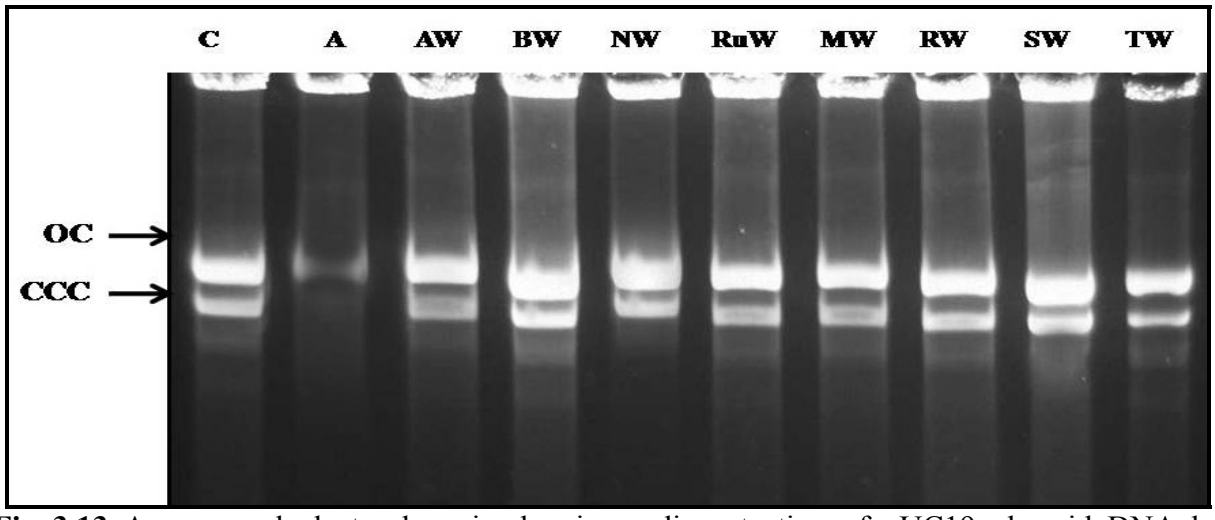

Fig. 3.13 Agarose gel electrophoresis showing radioprotection of pUC19 plasmid DNA by different types of mango wine; Lane C: control DNA; Lane A: DNA irradiated without wine; Lane AW to TW: Irradiated DNA with different types of mango wine (10µL/assay). (AW=*Alphonso*, BW=*Banginapalli*, NW=*Neelam*, RuW=*Rumani*, MW=*Mulgoa*, RW=*Raspuri*, SW=*Sindhura*, TW=*Totapuri* wines).

3.5 CONCLUSIONS

Irradiation doses used in this study resulted in increase in total polyphenolic and flavonoid content and antioxidant activities with a decrease in ascorbic acid content in all cultivars of mango juice samples. Mango juice was found to be rich source of antioxidants and possessed strong *in vitro* radioprotective ability, even after exposure to dose of 3 kGy. γ-Irradiation is also significantly increased the total polyphenolic compounds and flavanoids, leading to increased free radical scavenging activities in all mango wine samples. The increased activities observed could be due to the synergistic action of antioxidant compounds occurring in mango wine such as polyphenolic compounds, flavanoids and carotenoids. Further, mango wine was also shown to protect DNA damage induced by hydroxyl radicals and γ-irradiation. Hence it can be concluded that application of γ-irradiation to mango juice and wine has manifold benefits. To the best of our knowledge this is the first report on the applicability of γ-irradiation in improving the quality of mango juice and wine.