INTRODUCTION

The microbial safety of fruit juices continues to be a major concern to regulatory agencies and food industries around the globe. Fruit juices are popular drinks as they are healthy foods for their low content of sodium, cholesterol, fat and high concentration of vitamin C, polyphenols and antioxidants. They are important part of modern diet in many countries. Consumption of fresh fruit juices helps in preventing many degenerative diseases such as cardiovascular problems and several cancers. In developing country like India, a large population of all income and age groups consume freshly squeezed fruit and vegetable juice such as apple, orange, sweetlime, carrot and pineapple. Microbiologically stability of fruit juices is largely determined by low pH, low oxygen content, pasteurization and preservatives. Fruits which are used as raw material in fruit juices have pH values between 2.0 and 4.5 (ICMSF, 2005; Kumar et al., 2009; Titarmare et al., 2009; Raybaudi-Massilia et al., 2009b; Patrignani et al., 2010; Vantarakis et al., 2011).

Fruit juices create a suitable environment for the growth of acid loving microorganisms. The most commonly encountered bacterial genera are acetic acid bacteria, lactic acid bacteria, Clostridium, Bacillus, members of Enterobacteriaceae family and some heat resistant bacteria such as Alicyclobacillus acidoterrestris and Propionibacterium cyclohexinicum. Fruit juices act as an ideal substrate for yeasts. Candida sp., Torulopsis sp., Hanseniaspora sp. and Saccharomyces sp. are able to grow at low temperature in citrus juices. Saccharomyces cerevisiae and Schizosaccharomyces pombe are most significant spoilage yeasts in fruit juices. Some yeasts are resistant to weak organic acid preservatives for example Zygosaccharomyces bailli which pose a problem in food and beverage industry (ICMSF, 2005; Rawsthorne and Phister, 2006; Stratford, 2006; Walker and Phillips, 2007, 2008 a,b; Tribst et al., 2009; Lawlor et al., 2009; Durak et al., 2010; Fleet, 2011; Danyluk et al., 2011; Smit et al., 2011; Steyn et al., 2011; Tserennamind et al., 2011).

In addition to bacteria and yeasts, moulds such as Penicillium sp., Aspergillus sp., Eurotium sp., Alternaria sp., Cladosporium sp., Paecilomyces sp. and Botrytis sp. have been shown to be involved in the spoilage of fresh fruits and thermally processed fruit products. Spoilage of fruit juices by heat resistant fungi has also been documented in many countries. Neosartorya fischeri, Byssoschlamys nivea, B. fulva, Talaromyces flavus and Eupenicillium sp. are some of heat resistant fungi found in fruit juices (Salomao et al., 2007; Raybaudi-
Several fungi such as **Penicillium expansum**, **P. citrinum**, **Aspergillus carbonarius**, **B. fulva** and **B. nivea** produce mycotoxins in fruit juices and hence are of major concern in public health (Delage *et al*., 2003; Wareing and Davenport, 2005).

There are several reports of occurrence of pathogenic microorganisms in unpasteurized street vended fruit juices (Lewis *et al*., 2004; Mahale *et al*., 2008; Ketema *et al*., 2008; Titarmare *et al*., 2009; Tambaker *et al*., 2009; Sunetetha *et al*., 2011; Rashed *et al*., 2013). **Escherichia coli**, **Salmonella sp.**, **Cryptosporidium parvum** and **Staphylococcus aureus** are responsible for food borne outbreaks associated with consumption of fruit juices (Raybaydi Massilia *et al*., 2009b; Tribst *et al*., 2009; Sospedra *et al*., 2012).

Factors that affect the spoilage and shelf life of fruit juices are intrinsic and extrinsic. Intrinsic factors include pH, organic acids, oxidation reduction potential, reduced water activity, availability of nutrients, the presence of antimicrobial compounds and competing microflora. Extrinsic factors include storage temperature, storage time, relative humidity conditions during storage and packaging material characteristics. The shelf life of juices may be increased by keeping at low temperature, which is attributed to retard the growth of microorganisms by rendering them metabolically inactive (Davidson, 2001; Bates *et al*., 2001; ICMSF, 2005; Lawlor *et al*., 2009; Raybaydi Massilia *et al*., 2009b; Raccach and Mellatdoust, 2007).

Keeping in view the challenge posed by spoilage and pathogenic microorganisms to both the fruit juice industry and public health authorities; several guidelines have been published by national food standard agencies to control the microbiological quality and safety of fruit juices. The main aim of food preservation methods includes inactivation, growth delay and growth prevention of spoilage and pathogenic microorganisms. Low temperature, reduced water activity, acidification, fermentation, modified atmosphere packaging, addition of antimicrobials, compartmentalization in water in oil emulsions are some food preservation methods preventing or slowing down the growth of microorganisms. On other hand pasteurization, sterilization, microwave heating, ionization radiation, high pressure, pulsed electric field and high frequency ultrasound act by inactivating microorganism (Vigil *et al*., 2005; Raybaudi-Massilia *et al*., 2009b; Negi, 2012).
Fruit juices are pasteurized by applying temperature of 85-95\°C for 2 minutes. This pasteurization temperature is only effective against pathogens such as *E.coli* and *Salmonella* but are not effective against ascospores of heat resistant fungi and heat resistant bacteria and produce some undesirable effects on foods such as loss of nutrients and reduction of fresh like flavor (Salomao *et al.*, 2007; Kuldiloke and Eshtiaghi, 2008; Tribst *et al.*, 2009; Corbo *et al.*, 2010; Durak *et al.*, 2010; Kutama *et al.*, 2010; Smit *et al.*, 2011; Danyluk *et al.*, 2011; Steyn *et al.*, 2011; Mosqueda-Melgar *et al.*, 2012).

Instead of pasteurization, other means of preservation techniques are required to prevent spoilage occurring within the shelf life of a product. New technologies such as high hydrostatic pressure (HHP), high pressure homogenization (HPH), pulsed electric field (PEF), ultrasound and irradiations have been developed to maintain nutritional and sensory quality of fruit juices (Mosqueda-Melgar *et al.*, 2008 a,b; Rupasinghe and Yu, 2012). These novel non-thermal technologies have the ability to inactivate microorganisms at ambient or near ambient temperatures, thus avoiding the deleterious effect of heat has on flavour, colour and nutrient value of foods but fail until now, when applied alone, to destroy spores (Ross *et al.*, 2003; Devileghere *et al.*, 2004). Campos and Cristianini (2007) detected 5 log reductions of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in orange juice by applying treatment of high pressure homogenization. Other researchers have also observed same level of inactivation for other microorganisms in several juices and suggested its use for the prolongation of the shelf life of fruit juices (Corbo *et al.*, 2010; Patil *et al.*, 2011; Bevilacqua *et al.*, 2012). Other non-thermal technology such as high intensity pulsed electric field proving to be beneficial to inactivate microorganisms, decrease the activity of enzymes and increase the shelf life of foods. But these emerging non-thermal technologies have been considered too energy expensive and costly to be practical for use in food processing industry. Also the existence of highly resistant microbial populations limits the efficacies of emerging non thermal technologies (Raybaudi-Massilia *et al.*, 2009b; Mosqueda-Melgar *et al.*, 2012).

Chemical preservatives such as benzoic acids, sorbic acids, sulphur dioxide and p-hydroxy benzoic acids are commonly used as food preservatives in fruit juices. However, these chemical preservatives often have side effects on humans for example benzoic acid is converted into benzene in foods and *S. cerevisiae, Pichia anamala* are able to decarboxylate
sorbic acid to 1,3 pentadiene which cause kerosene like off-odour. *Schizosaccharomyces pombe* may produce off flavors in the presence of sulphite. Due to growing evidences about the harmful effects of chemical preservatives, there is continuous pressure to reduce the amount of added preservative in foods (Bates *et al.*, 2001; Burt, 2004; ICMSF, 2005; Bajpai *et al.*, 2008; Shi *et al.*, 2010; Tajkarimi *et al.*, 2010; Tserennadmid *et al.*, 2011). To overcome the negative effects of physical methods and chemical preservatives, the recent emphasis is toward the use of antimicrobials compounds from the natural sources. Antimicrobials are chemical compounds or substances that may delay microbial growth or cause microbial death in a food matrix. The major targets for such antimicrobials are food poisoning (infective agents and toxin producers) and spoilage microorganisms whose metabolic end products or enzymes cause off-odors, off-flavors, texture problems, and discoloration. Antimicrobials are used in foods for two main reasons: 1) to preserve the foods 2) to prevent/control the growth of microorganisms including pathogenic microorganisms. The source of these natural antimicrobials is plants (plant secondary metabolites in essential oils, phytoalexins) microorganisms (bacteriocins and organic acids) and animals (lysozyme, lactoperoxidase) (Rico *et al.*, 2007; Raybaudi- Massilia *et al.*, 2009b; Tiwari *et al.*, 2009; Tajkarimi *et al.*, 2010; Negi, 2012).

Natural antimicrobials from plants have been used as preservative from the ancient times. There are more than 1340 plants with defined antimicrobial compounds and over 30,000 components have been isolated from phenol group containing plant oil compounds and used in food industry. Spices and herbs have been added to foods not only for flavours but also for medicines and food preservatives. Application of both extracts and essential oil of plant origin antimicrobials could be potential alternative to chemical preservatives. Plant essential oil is gaining a wide interest in food industry for their potential as decontaminating agents as they are generally recognized as safe (GRAS) (Burt, 2004; Bajpai *et al.*, 2008; Tajkarimi *et al.*, 2010; Lucera *et al.*, 2012).

The first scientific evidence regarding the preservation potential of spices was reported in *Cinnamomum zeylanicum* which showed the antimicrobial potential against the endospore producing heat resistant bacteria. Clove was used as a preservative to disguise spoilage in meat, syrups, sauces and sweetmeats. In the 1910s, cinnamon and mustard were shown to be effective in preserving applesauce. Since then other spices, such as allspice, bay
leaf, caraway, coriander, cumin, oregano, rosemary, sage and thyme, have been reported to have significant bacteriostatic properties (Tajkarimi et al., 2010). Plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for control of microbial growth owing to their chemical diversity. Besides antimicrobial several plants are being used in different areas of human health such as traditional medicine, functional foods, dietary supplements and recombinant protein manufacturing. Several studies have been conducted around the globe to prove efficacy of plant products and various compounds isolated from these plants are secondary metabolites which possess antimicrobial and medicinal properties (Cowan, 1999; Beuchat, 2001; Friedman et al., 2002, 2004; Burt, 2004; Mosqueda- Melgar et al. 2008a; Raybaudi-Massilia et al., 2009b; Tiwari et al., 2009; Tajkarimi et al., 2010; Negi, 2012). Plants have their own antimicrobial activities due to the presence of alkaloids, phenols, glycosides, steroids, essential oil, coumarines and tannins (Raskin et al., 2002; Ramawat et al., 2009; Skrinjar and Nemet, 2009).

Consumers demand for fresh and healthy fruit juices without pasteurization and chemical preservatives, hence there is need of searching new preservatives from plants. Hence the present work aims at the following objectives:-

- Study of association of microbes with common fruit juices sold in the retail market of Kurukshetra.
- Study of effect of temperature on the shelf life of fruit juices.
- Antimicrobial study of crude plant extracts and chemical food preservatives on the selected spoilage microorganisms of fruit juices.
- Identification of the best bioactive plant antimicrobial and its effect on the shelf life of fruit juices.