CHAPTER II

PHYTOCHEMICAL AND GC - MS ANALYSIS OF THE ETHANOL FRUIT EXTRACT OF OPUNTIA ELATIOR
1. Introduction

Medicinal plant products have a long history of indigenous use in India as well as other countries. Phytotherapy has a very long tradition, although proper scientific explanation is relatively new. The status of herbal medicine has been fast growing all over the world during the last few decades (Kaur et al., 2011). Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago. From that point the development of traditional medical systems incorporating plants as a means of therapy can be traced back only as far as recorded documents of their likeness. The number of higher plant species (angiosperms and gymnosperms) on this planet is estimated at 250,000, with a lower level at 215,000 and an upper level as high as 500,000. Of these, only about 6% have been screened for biological activity, and the reported 15% have been evaluated phytochemically. Plants because of their long term use (often hundreds or thousands of years) lay human have an advantage in this area. One might expect any bioactive compounds obtained from such plants to have low human toxicity (Fabricant and Farnsworth., 2001). It is extremely difficult to assess the value of any approach to the use of higher plants to develop new drugs. There are advantages and disadvantages of using plants as the starting point in any drug development program. If one elects to use information suggesting that specific plants may yield useful drugs based on long-term use by humans (ethnomedicine) one can rationalize that any isolated active compounds from the plants are likely to be safer than active compounds from plants with no history of human use. Also, plants are a renewable source of starting material in many but not all cases. According to World Health Organization (2002:7), “Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual
techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being” (Payyappallimana., 2009).

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. Modern medicine has been obtained from folk medicine and traditional system through chemical and pharmaceutical screening.

In recent years, secondary plant metabolites (phytochemicals) previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents. Plant-based foods, such as fruits, vegetables, and whole grains, which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the risk of chronic diseases. It is estimated that more than 5000 individual phytochemicals have been identified in fruits, vegetables, and grains (Liu., 2007). There is increasing evidence that fruits and vegetables may protect against numerous chronic diseases, including cancer, cardio, cerebro vascular, ocular and neurological diseases (Block et al., 1992; Steinmetz and Potter., 1996; Ness and Powles., 1997; Youdim and Joseph., 2001). The protective effect of fruit and vegetables has generally been attributed to their antioxidant constituents including vitamin C, vitamin E, carotenoids, glutathione, flavonoids and phenolic acids as well as other unidentified compounds (Sies and Stahl., 1995; Kuti., 2004).

Phenols help in the natural defense of plants against pests and diseases, while the plant sterols and policosanols are mostly components of wax and plant oils. The phytochemicals have gained increased interest due to their antioxidant activity,
cholesterol lowering properties and other potential health benefits. (Awika and Rooney., 2004)

There are reports that plants rich in tannins have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane. Alkaloids are commonly found to have antimicrobial properties (Christudas et al., 2012). Presently, the United States is the largest market for Indian botanical products accounting for about 50% of the total exports. Global trend leading to increasing demand of medicinal plants for pharmaceuticals, phytochemicals, nutraceuticals, cosmetics and other products is an opportunity sector for Indian trade and commerce (Raghavendra et al., 2009).

The cactus family is unusual among tropical plants. Cactus pear, produced by perennial Opuntia cactus, belongs to the Cactaceae family and is well adapted to arid and semiarid climates, where water shall be a limiting factor in cultivation (Benson., 1982; Kuti., 2004). Cacti have been exploited as a cheap, alternate source of food suitable for humans and feed for animals and are cultivated as ornamental crops. Cacti exhibit many characteristics of a suitable crop model to achieve productivity and sustainability with minimal ecological or environmental impact to meet the growing demands for food and as a new source for meeting nutritional health requirements and thus deserves further investigations (Estrada – Luna et al., 2008; Shebdakar et al., 2010). Due to agricultural problems linked to increasing arid zones and declining water resources, Opuntia sp. is gaining importance as an effective food source (Stintzing et al., 2001; Figueroa et al., 2010). Many species of Opuntia cacti produce edible highly flavored fruit known as “Cactus pear”. Cactus pear fruit is a many seeded berry with a thick peel, enclosing a delicately flavored seedy pulp (Barbera et
Cactus pear fruits are rich source of nutrients and vitamins (Saway et al., 1983; Teles et al., 1984) and are eaten fresh, dried or preserved in jams, syrups or processed into candy-like products (Hoffman., 1980; Kuti., 2004).

The *O. elatior* complete plant is considered as a medicinal plant in Ahmednagar regions of India. The information was collected from different ethnic groups, villagers, traditional healers/vaidyas who use the plants for medicinal purpose (Aher et al., 2011).

Prickly pear antioxidant actions could protect mammalian cells and organs and slow the aging process, illness and disease (Kuti., 2004). It could also lower low density lipoprotein (LDL) and cholesterol levels helping to check high blood pressure (Fernandez et al., 1992). Concentrated juice in combination with the fruit is reported to be an aphrodisiac and also believed to be of value in spermatorrhoea and gonorrhoea (Shebdakar et al., 2010).

The phylloclade of the *O. elatior* has been used as a galactogogue to enhance the lactating ability of the nursing mothers of Warli tribes of Maharashtra. Exact procedure has not been scientifically proven but the supporting data indicate that the high content of flavonoid affect the endocrine system and hormone function, which promotes the flow of milk (Sayed et al., 2007). The Opuntia species is used for the treatment of hypoglycemia, diabetes, high blood cholesterol and obesity (Hegwood., 1990).

In Chinese medicine, cactus fruits are considered as weak poisons and used for the treatment of inflammation, pain and as detoxification agents for snake bites (Wang., 1988; Shebdakar et al., 2010). As the use of natural sweeteners other than sucrose is a priority area for the food industry (Shebdakar et al., 2010), cactus fruits
have been used as a raw material in the creation of natural sweeteners (Saenz et al., 1998). Similar to other fruits cactus pears also have the nutritive value with high protein and fiber content (Saenz and Sepulveda., 2001).

Hence screening of fruit extracts for various enzymes such as arylamidases, lipases, proteinases and glucosidases has been carried out. Among these enzymes, proteinases are the most important plant enzymes used in food, pharmaceuticals, detergent, leather and wood industries (Mantell and Mac Kee., 1985; Shebdakar et al., 2010).

Generally, cactus pear fruit contains about 85% water, 11 – 14% fermentable sugar, 1.8% crude fiber, 0.1% lipids, 0.21 – 1.5% protein and 0.4 – 1.6% ash (Salim et al., 2009). It also contains calcium and vitamin C upto 60 and 30 mg/100g and vitamin A 50 IU which are the main dietary essentials (Salim et al., 2009; Shebdakar et al., 2010).

The phytochemical analysis of the Opuntia fruit showed the presence of carbohydrates, flavonoids, phenolics, betalains and color pigment betacyanin as an active principle and high amount of sugar content. The low acidity of fruit makes it very sweet and delicious. The fruit juice also showed a hematinic, analgesic and anti-asthmatic activity (Chauhan., 2010). However, no information is available for total fruit extract components and its toxicity effect.

The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti–infective agents, from plants. The literature study reveals that till today there is only one record of phytochemical composition and pharmacological study of the fruit juice of
*O. elatior* (Chauhan., 2010). Thus the present study helps to further investigate about the phytochemical screening of the complete fruit that paves the way to know the pharmaceutical, nutraceutical and pharmacological applications of *O. elatior* Mill fruits. Hence, the focus of this study is on detection of phytochemical compounds present in the fruits of *O. elatior* Mill.

**Opuntia elatior Mill:**

They are commonly known as Prickly pears, because of their edible fruits. The prickly pears are said to have been accidentally introduced into India and other eastern countries by early European travelers, who used to carry these plants for use as vegetable to prevent scurvy during their long voyages. Prickly pears typically grow with flat, rounded cladodes (also called platyclades) that are armed with two kinds of spines; large, smooth, fixed spines and small, hairlike prickles called glochids, which easily penetrate skin and detach from the plant. The scientific classification of the plant is as follows (Evans., 2005):

**Kingdom:** Plantae

**Division:** Magnoliophyta (Angiosperms)

**Class:** Magnoliopsida (Dicotyledons)

**Subclass:** Archichlamydeae

**Order:** Caryophyllales (Cactales)

**Family:** Cactaceae

**Subfamily:** Opuntioideae

**Tribe:** Opuntieae

**Genus:** *Opuntia*

**Species:** *elatior* Mill.
**Synonyms:**

*English:* Prickly pear, Slipper Thorn,

*Hindi:* Hathathairoa, Nagphana, Nagphani

*Kannada:* Paapaskalli

*Sanskrit:* Bahudugdhika, Bahushala, Dondavrikshaka, Guda, Gula, Kandarohaka,

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2. **Review of Literature**

Both the aqueous and methanolic extracts of seeds of *Aegle marmelos* contain alkaloids, carbohydrates, proteins, glycosides and phenolics qualitatively, after the preliminary phytochemical screening (Sharma *et al*., 2011). The hydroalcoholic leaf extract of Terminalia arjuna is found to contain many phytochemical constituents after the preliminary phytochemical screening (Nema *et al*., 2012). The crude extract of *Mangifera indica* contains many phytochemical constituents of pharmaceutical importance (Aiyelaagbe and Osamudiamen., 2009). Phytochemical studies of 12 medicinal plants revealed the presence of phytochemical components that possess antibacterial effect (Parekh and Chanda., 2007). Fifty medicinal plants of Nigeria possess phytochemical compounds which are responsible for antibacterial effect (Kubmarawa *et al*., 2007). The preliminary studies of the *Piliostigma thonningii* seeds found to possess many phytochemical compounds after the phytochemical analysis (Jimoh and Oladiji., 2005). Phytochemical studies of *Cnidoscolus aconitifolius* revealed the presence of phytochemical components which possess antibacterial effect (Awoyinka *et al*., 2007). Phytochemical studies of *Amorphaphallus paeoniiifolius* tuber revealed the presence of phytochemical components which are of biologically
importance (De et al., 2010). Phytochemical studies of *Dendrophthoe falcata* revealed the presence of phytochemical components which are of biologically importance (Vinod et al., 2010). The ethanolic bark extract of *Bahunia racemosa* revealed the presence of many phytochemical constituents of biological importance (Kumar et al., 2010). The phytochemical screening of Alafia barteri revealed the presence of many biologically important constituents which has antiplasmodial activity (Lasisi et al., 2012). The phytochemical screening of *Picrorrhiza kurroa* revealed the presence of many biologically active components which has antimicrobial activity (Rathee et al., 2012). Many biologically active constituents were analyzed after the phytochemical screening of *Schotia latifolia* which were used traditionally for oxidative stress ailments in South Africa (Mbaebie et al., 2012). The methanolic extract of some of the medicinal plants revealed the presence of some biologically active components which are found to be antimicrobial in nature (Antonisamy et al., 2012).

The GC – MS analysis of the ethanol extract of *Sarcostemma secamone* showed a wide variety of the phytochemical constituents which are found to possess chemopreventive activity against colon cancer, arthritis (Kumari et al., 2012). The GC – MS analysis of the fungus *Monochaetia kansensis* reveals the presence of alkanes, alkenes and hydrocarbons (Yogeswari et al., 2012). The ethyl acetate fraction of *Lansianthera Africana* revealed the presence of some pharmacologically active compounds after the GC – MS analysis (Okokon et al., 2013). The GC – MS analysis of hexane extract of *Sericocalyx schomburgkii* revealed the presence of many steroids which are a good source of nutraceutical and functional food ingredients (Phuruengrat and Phaisansuthichol., 2006). The GC – MS analysis of ethanol extract of *Grewiaum bellifera* revealed the presence of 16 phytochemical compounds which are a good source of diabetic treatment (Gunasekaran et al., 2013). The ethanol extract of
Polycarpaea corymbosa after GC – MS analysis revealed the presence of 13 compounds which could be used for ailments by traditional practitioners (Balamurugan et al., 2012). The GC – MS analysis of ethanol extract of Stylosanthes fruticosa confirmed the presence of 33 phytochemical compounds which may be of prime importance in the pharmaceutical sector (Peter and Raj., 2012). Twelve biologically active compounds have been identified after the GC – MS analysis of the methanolic extract of cones of Cycas beddomie (Ravikumar et al., 2012). Twelve biologically active compounds have been identified after the GC – MS analysis of the methanolic extract of Canthium parviflorum Lamk (Prabhu et al., 2013). The methanolic extract of five plants i.e., Bauhinia recemosa Lam., Caryota urens L, Commelina benghalensis L, Garcinia indica (Du Petit- Thou.) Choisy. and Gmelina arborea Roxb confirmed the presence of biologically active compounds present in the plant sample after the GC – MS analysis (Mahadkar et al., 2013). Sixteen bioactive compounds were identified after the GC – MS analysis of the methanolic extract of rhizome of Tectaria coadunate (Dubal et al., 2013). Seven bioactive compounds were identified in the ethanol extract of Rauwolfia densiflora after the GC – MS analysis (Shunmughapriya and Kalavathy., 2012). The ethanol extract of Andrographis paniculata revealed the presence of hydrocarbons, alcohol, aromatics after the GC – MS analysis (Eddy et al., 2011). The GC-MS analysis of methanolic extract of root and aerial part of Polycarpaea corybosa led to the identification of 30 and 24 compounds respectively (Sindhu and Manorama., 2013). Cinnamaldehyde present in the methanolic extract of Cinnamon zylanicum was responsible for the anti microbial activity, and was confirmed by GC – MS analysis (Uma et al., 2009). The methanolic extract of Enicostemma littorale confirmed the presence of ether compound which acts as an antifungal agent after the GC – MS analysis (Ambikapathy et al., 2011).
The methanol extract of *Thymus vulgaris* confirmed the presence of biologically active compounds after the GC – MS analysis (Al Hashmi *et al*., 2013). The aqueous bark extract of *Mimusops elengi* confirmed the presence of nine biologically active components after the GC – MS analysis (Ruikar *et al*., 2009). The GC – MS analysis revealed the presence of 32 biologically active compounds present in the aerial parts of the *Caralluma umbellate* (Jeyakumar *et al*., 2013). Six compounds have been identified from the GC – MS analysis of the ethanol extract of *Odina wodier* which are biologically important compounds (Saravanan *et al*., 2013). The GC – MS analysis determined the presence of 13 biologically active compounds from the ethanolic extract of *Andrographis paniculata* (Kalaivani *et al*., 2012). The ethanolic extract of *Merremia hederacea* reveals the presence of seven phytochemical components after the GC – MS analysis (Charles *et al*., 2012). Biologically active components were identified after the GC – MS analysis of the ethanol extract of *Vitex negundo* (Kumar *et al*., 2010).

### 3. Materials and Methods

#### 3.1 Experimental materials

**Collection and identification of the plant material:** The fruits of *O.elatior* Mill were collected from the field, Mysore district and authenticated by the Department of Studies in Botany, University of Mysore, Manasagangothri, Mysore.

#### 3.2 Methods

**Preparation of the plant extract:** The ripened fruits were shade dried and powdered using an electric grinder. This powder was dissolved in 500 ml of ethanol with continuous stirring for about 5–6 h. Then suspension filtered using Whatmann filter paper (No 1). This filtered solvent was subjected to the flash evaporator until the
contents of ethanol completely evaporated and collected in receiving flask in the form of crude extract. Finally the ethanolic extract of total fruit was kept in an incubator for drying at 38°C for 48 h. The resultant fruit crude extract was stored at 4°C until further use.

**Phytochemical screening:** Screening was done to identify the following phytochemicals in the extracts: alkaloids, carbohydrate, fat and oils, flavonoids, phenolics, saponins, steroids and tannins (Harbourne., 1973; Trease and Evans., 1983).

**Test for alkaloids:** 0.5 g of crude defatted with 5% ethyl ether for 15 minute. The defatted sample was extracted for 20 minute with 5 ml of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for 10 minute at 3000 r.p.m. A volume of 1 ml of the filtrate was treated with few drops of Mayer’s reagent and a second 1 ml was treated with Dragendorff’s reagent and turbidity was observed.

**Test for carbohydrates:** A quantity of 0.5 g extract was shaken with Molish's reagent, add conc. H$_2$SO$_4$ through sides of the test tube. Purple color appears between the two liquids. This is the indication of the presence of carbohydrates.

**Tests for fats and oils:** a) A portion of the crude extract should be placed on a blotting paper. Appearance of stains after drying will be an indication for the presence of fats and oils.

b) To 0.5 g of extract few drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein was added and heated on a water bath for 1-2 h formation of soap represents the presence of fats and oils.
Test for flavonoids: A portion of crude powder was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate and was shaken with 1 ml of dilute ammonia solution later yellow coloration was observed.

Test for saponins: A quantity of 0.5 g of crude powder was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponins.

Test for steroids: A quantity of 0.5 g of crude powder was dissolved in 5 ml of methanol. A volume of 1 ml of the extract was treated with 0.5 ml of acetic acid anhydride and cooled on ice. This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulphuric acid was then added carefully by means of a pipette. At the separation level of the two liquids, a reddish – brown ring was formed, as an indication for the presence of steroids.

Test for tannins and phenolic compounds: A quantity of 0.5 g of the crude powder was stirred with 10 ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue- black precipitate was taken as evidence for the presence of tannin and phenolic compounds.

Gas chromatography-mass spectrometry

The volatile extract of the fruits was analyzed on a gas chromatograph (HP6890; Agilent Technologies USA Ltd) directly linked to a HP5973 mass selective
detector (Agilent Technologies) operated in electron impact mode (source temperature 230°C; transfer line 250°C). The HP-5 MS phenyl methyl siloxane non polar capillary column (30 m x 0.25 mm x0.25 µ.) max 350°C (Agilent part No 190915 – 433) was used for the separation of fractions. The mobile phase was Helium 99.999% purity (Praxair India Ltd) passed through the universal trap for removing the contaminants. The split inlet was used with split ratio of 50:1 and inlet temperature of 280°C. The oven temperature program was set at 70°C min⁻² with 2 minutes hold and a ramp of 10°C min⁻¹ till 260°C and held for 5 minutes with column flow of 1 ml/minute. The mass spectral detector was maintained at a temperature of 280°C with the interface temperature of 230°C. The mass spectra created using the MS was compared with the Wiley mass spectral library.(Wiley W9N11.L and NIST 2.0 version)

4. Results

4.1 Phytochemical screening

The preliminary phytochemical screening of the fruit extract of O. elatior revealed the presence of alkaloids, carbohydrate, fat and oils, flavonoids, phenolics, saponins, steroids and tannins (Table 1) for the first time in ethanolic extract.

4.2 GC – MS analysis

The components present in the ethanol extract of the O.elatior fruit extract were identified by GC – MS analysis (Figure 1). The active principles with retention time, molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of whole fruit of O.elatior has been mentioned in Table 1. The GC – MS analysis of the ethanol fruit extract of O.elatior revealed the presence of 14 active components. One of the compound out of 14 compounds were found to be 5-
hydroxymethylfurfural with an unidentified functional group, the retention time was and the relative percentage was 9.20 minutes and 29.41% respectively. The other 13 compounds present were as follows, 2-furancarboxaldehyde (14.70%), 2,5-furandione,3-methyl (12.25%), 2,5-furandicarboxaldehyde (3.67%), 2,3 dihydro, 3,5-dihydroxy 6 methyl 4H pyran -4-one (4.90%), 2,5-Furandione, dihydro-3- (2-methyl-2-propenyl)- (2.45%), pentanoic acid, 2,2-dimethyl- ethyl ester (4.90%), 2-Furancarboxylic acid, 1-methylethyl ester (6.86%), maltol propionate (1.22%), 4H-Pyran-4-one, 3,5-dichloro-2,6-dimethyl (9.80%), 4H-Pyran-4-one, 5-(acetyloxy)-2-((acetyloxy)methyl) (1.96%), 2-Tetrazene, 1,1,4,4-tetrakis(1-methylethyl)- (1.47%), 2-Furancarboxaldehyde, 5-(ethoxymethyl)- (1.47%), 5-hydroxymethylfurfural with an unidentified functional group (29.41%), 5,5’-Oxy – Dimethylene –bis ( 2 – Furaldehyde) (4.90%).

5. Discussion

Medicinal plants due to their therapeutic values have long been used to address human diseases. In view of serious side effects posed by the medicinal derivatives from chemical origin natural products with high medicinal values are gaining much importance in recent years (Sreekanth et al., 2007). Hence the present study was carried out to assess the important phytoconstituents present in the fruits of O. elatior, in support of traditional and folkloric use.

The preliminary phytochemical screening of the fruit extract of O. elatior revealed the presence of alkaloids, carbohydrate, fat and oils, flavonoids, phenolics, saponins, steroids and tannins (Table 1) for the first time in ethanolic extract. Whereas, the methanol extract of some flowers of medicinal plants belongs to
different families also showed the presence of phytochemicals (Gracelin and Britto., 2011) as reported in the present study.

Alkaloids with the aid of their defense mechanism act as phytoprotective agent against invading microorganism (Lewis and Elwin Lewis., 1977; Lata and Dubey., 2010) due to analgesic, antisplasmodic and bactericidal activity (Lata and Dubey., 2010; Stray 1998; Okwu and Okwu., 2004). In addition, alkaloids are heterocyclic compounds that have proved to have pharmacological properties such as hypotensive activity, anticonvulsant activity, antiprotozoal, antimicrobial and antimalarial activities (Ali and Ghatak 1975; Singh and Kapoor., 1980). Some alkaloids are known to be useful in correcting renal disorders and most effective for managing hypertension and also providing protection for the heart (Konkwara., 1976; Lata and Dubey., 2010).

The *O. elatior* fruit with high sugar content make it highly delicious and sweet in taste. The sugar pattern in the fruit pulp is very simple and contains glucose and fructose virtually in equal amounts as has been reported by Feugang et al (2006) in all cactus pears. However, directly absorbed high glucose concentrations in cactus fruits represents an energy source instantly available for brain and nerve cells, while ructose being sweeter may enhance the fruit’s flavor (Feugang et al., 2006; Guizani et al., 2012). The cactus mucilages are composed of polysaccharides such as arabinose, galactose, shammose and galactouronic acid. Thus these mucilages have a high water holding capacity, so they could serve as thickening or emulsifying agents and form viscous or gelatinous colloids (Piga., 2004). In addition, the use of natural sweeteners other than sucrose is a priority area for the food industry. Thus *O. elatior* one of the
Cactus fruits can be used for large scale production of natural sweeteners as has opined by Shebdakar et al. (2010).

Cactus pear is a new source of fruit oils as seed and pulp oil consists of significant amount of neutral lipid (87% of total lipids) and polar lipids (52.9% of total lipids) respectively. The fat-soluble vitamins associated with the cactus fruit seed and pulp oils helps in preventing the lipid fractions from oxidative damage (Feugang et al., 2006). Further study revealed that supplementation with cactus pear oil/ cactus pear seeds are useful in reducing the serum cholesterol level (Shetty et al., 2011) as they contained mainly unsaturated fatty acids and an appreciable level of fat soluble vitamin, wherein, unsaponifiable content found higher than sunflower, which is useful for the consumer and also for the producer of oils. Hence, the unsaponifiable fraction of vegetable oil has applications in cosmetics and pharmacology due to its biological properties (Guizani et al., 2012). O. elatior shall also be used as a potential material.

Flavonoids are recognized as a very important phytochemical mainly for their antioxidant activity, metal chelating properties and beneficial role in variety of cellular processes (Dreosti., 2000). According to Gupta et al., (2002) a methanolic extract from Opuntia dillenii exerted antispermatic effects in animal tests on rats, due to the presence of flavones derivatives, vitexin and myricetin. Also, flavonoids extracted from the seeds of Vitex negundo showed not only a significant drop in sperm count but also decreased motility up to 30% (Das et al., 2004). Thus, flavanoids reported from the O. elatior has some pharmaceutical applications.
Epidemiological studies have shown that consumption of food and beverages rich in phenolic content can reduce the risk of heart disease by slowing the progression of the atherosclerosis by acting as antioxidants toward LDL (Steinburg., 1997; Chang et al., 2008; Subhashini et al., 2010). As, the nutritional and health benefits of cactus fruits are associated with their antioxidant properties related to phenolic compounds (Feugang et al., 2006; Yahia and Jacobo., 2011) the presence of phenolic compounds in the O. elatior prove to be the potential plant material in the natural medicinal products. The O. elatior being one of the members of the cactus pear family has the total antioxidant activity that found to be two fold higher compared with that of a pear, apple, tomato and grape and similar to that of a red grape raisin, orange and grapefruit (Livrea and Tesoriere., 2006; Yahia and Jacobo., 2011). Thus the presence of phenolic compounds in the fruit extract of O. elatior might have a positive effect on the redox balance to reduce LDL levels (Yahia., 2010; Yahia and Jacobo., 2011).

Saponins extracted from Albizia lebbeck and Cestrum parqui reduced the sperm concentration of testes and decreased sperm motility significantly (Gupta et al., 2005; Souad et al., 2007). Thus saponins present in the O. elatior fruit extract might also exhibit antispermatogenic effect.

Steroidal compounds observed in O. elatior have importance and interest in pharmacy. Because the leaves of C. rutidosperma are used as a vegetable for expectant or breastfeeding mothers to ensure their hormonal balance, steroidal structure could serve as potent starting material in the synthesis of these hormones (Edeoga et al., 2005). Steroids in modern clinical studies have revealed their role as
anti-inflammatory and analgesic agents (Singh., 2006; Majaw and Moirangthem., 2009).

Together, alkaloids, flavonoids, phenolics, saponins and tannins have been linked or suggested to be involved with antibacterial and antiviral activity, while tannins and flavonoids are thought to be responsible for antidiarrheal activity. Investigations on the mode of action indicate that tannins and flavonoids increase colonic water and electrolyte reabsorption and other phytochemicals act by inhibiting intestinal mobility, while some components have been shown to inhibit particular enteropathogens (Enzo., 2007; Majaw and Moirangthem., 2009).

The GC – MS analysis revealed the presence of 14 compounds out of which one is found to be novel. 5-hydroxymethylfurfural has been identified as a probable compound with a novel side group. The relative percentage of the novel compound (29.4117) is higher compared with all other compounds. 2 – furancarboxy aldehyde is found to be one of the compound with high relative percentage next to novel compound. 2 – furancarboxy aldehyde is found to be mutagenic for mammalian somatic cells, and it may have the possible toxic effects on the reproductive system of male. The LD$_{50}$ of this compound is found to be 400 – 500 mg/ kg bw for mice. (Material safety data sheet).

The GC - MS analysis of the fruit sample revealed the presence of 14 compounds. Out of 14 compounds one is considered to be 5-hydroxymethylfurfural with an unidentified functional group. The retention time is 9.200 minutes and the relative percentage is found to be 29.4117%. The relative percentage of 5-
hydroxymethylfurfural with an unidentified functional group is found to be high compared to all other 13 compounds. Thus the role of the unidentified compound for the health benefits and pharmaceutical uses is still unaware and further investigation on the structural details and beneficial medicinal properties of the unidentified compound is required. Seven compounds from the remaining thirteen compounds are found to be the derivatives of furans, namely, 2-furancarboxyaldehyde (14.7058%), 2,5-furandione,3-methyl (12.2549%), 2-5-furandicarboxyaldehyde (3.6764%), 2,5-furandione,dihydro-3-(2-methyl-2-propenyl)- (2.4509%), 2-furancarboxylic acid,1-methyl ethyl ester (4.9019%), 2-furancarboxaldehyde,5-(ethoxymethyl)- (1.4705%), 5,5′-Oxy-Dimethylene-bis(2-furaldehyde) (4.9019%). It is evident from the result that the relative percentage of the furan derivatives are high (46.3231%) when compared with other non furan derivatives (24.2643%). In general, the furan and its derivatives are said to be genotoxic, mutagenic and carcinogenic in its activity depending on the mode of administration. At higher dosage these compounds cause lethality in respective animal models. Some of the results reveals that carbofurans are reproductive toxic agents in rats (Pant et al., 1995). It is investigated that furan derivatives, benzofuran derivatives, naphthofuran derivatives and furobenzopyran derivatives, show antifertility activity (Chaudhary et al., 2008). Aroylbenzodifurans are checked for anti implantation activity in albino rats gave a positive results (Krishnamurthy et al., 1999). Nitrofuran compounds inhibits the spermatogenesis process and causes atrophy in the testis (Miyaji et al., 2008).

Thus, the presence of unidentified compound initiated further investigation on the fruits of O.elatior. Further analysis of the fruit extract is being done for the NMR
studies for structural analysis of the compound. Thus the present study confirms the presence of a novel compound with a novel group.

Thus the presence of alkaloids, flavonoids, phenolics, saponins, steroids, tannins, and the presence of 5-hydroxymethylfurfural with an unidentified functional group in the fruit extract of *O. elatior* open ample scope for further investigations on anticancer, antibacterial, antiviral, antidiarrheal and anti-inflammatory activities.
Table 1: Phytochemical components present in the whole fruit extract of *Opuntia elatior*

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compounds</th>
<th>Present/ Absent</th>
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<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Present</td>
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<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>Present</td>
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<tr>
<td>3.</td>
<td>Fats and oils</td>
<td>Present</td>
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<td>4.</td>
<td>Flavanoids</td>
<td>Present</td>
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<tr>
<td>5.</td>
<td>Phenolics</td>
<td>Present</td>
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<td>6.</td>
<td>Saponin</td>
<td>Present</td>
</tr>
<tr>
<td>7.</td>
<td>Steroids</td>
<td>Present</td>
</tr>
<tr>
<td>8.</td>
<td>Tannins</td>
<td>Present</td>
</tr>
</tbody>
</table>
Table 2: Components detected in the whole fruit of *Opuntia elatior*

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Retention time</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Relative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.125</td>
<td>2- furancarboxaldehyde</td>
<td>C₅H₄O₃</td>
<td>112</td>
<td>14.7058</td>
</tr>
<tr>
<td>2.</td>
<td>4.605</td>
<td>2,5-Furandione, 3-methyl</td>
<td>C₅H₄O₃</td>
<td>112</td>
<td>12.2549</td>
</tr>
<tr>
<td>3.</td>
<td>4.864</td>
<td>2,5-Furandicarboxaldehyde</td>
<td>C₆H₄O₃</td>
<td>124</td>
<td>3.6764</td>
</tr>
<tr>
<td>4.</td>
<td>5.099</td>
<td>2,3 dihydro, 3,5- dihydroxy 6 methyl 4H pyran-4-one</td>
<td>-</td>
<td>-</td>
<td>4.9019</td>
</tr>
<tr>
<td>5.</td>
<td>5.540</td>
<td>2,5-Furandione, dihydro-3- (2-methyl-2-propenyl)</td>
<td>C₈H₁₀O₃</td>
<td>154</td>
<td>2.4509</td>
</tr>
<tr>
<td>6.</td>
<td>6.192</td>
<td>Pentanoic acid, 2,2-dimethyl-ethyl ester</td>
<td>C₉H₁₈O₂</td>
<td>158</td>
<td>4.9019</td>
</tr>
<tr>
<td>7.</td>
<td>6.765</td>
<td>2-Furancarboxylic acid, 1-methylethyl ester</td>
<td>C₈H₁₀O₃</td>
<td>154</td>
<td>6.8627</td>
</tr>
<tr>
<td>8.</td>
<td>7.226</td>
<td>Maltol propionate</td>
<td>C₇H₁₀O₄</td>
<td>182</td>
<td>1.2254</td>
</tr>
<tr>
<td>9.</td>
<td>7.766</td>
<td>4H-Pyran-4-one, 3,5-dichloro-2,6-dimethyl</td>
<td>C₇H₆Cl₂O₂</td>
<td>192</td>
<td>9.8039</td>
</tr>
<tr>
<td>10.</td>
<td>8.389</td>
<td>4H-Pyran-4-one, 5- (acetyloxy)-2 (acetyloxy)methyl</td>
<td>C₁₀H₁₀O₆</td>
<td>226</td>
<td>1.9607</td>
</tr>
<tr>
<td>11.</td>
<td>8.506</td>
<td>2-Tetrazene, 1,1,4,4-tetrakis (1-methylethyl)</td>
<td>C₁₂H₂₈N₄</td>
<td>228</td>
<td>1.4705</td>
</tr>
<tr>
<td>12.</td>
<td>8.889</td>
<td>2-Furancarboxaldehyde, 5- (ethoxymethyl)</td>
<td>C₈H₁₀O₃</td>
<td>154.06</td>
<td>1.4705</td>
</tr>
<tr>
<td>13.</td>
<td>9.200</td>
<td>5 – Hydroxy methyl furfural with an unidentified functional group</td>
<td>-</td>
<td>-</td>
<td>29.4117</td>
</tr>
<tr>
<td>14.</td>
<td>18.082</td>
<td>5,5’-Oxy – Dimethylene –bis (2 – Furaldehyde)</td>
<td>C₁₂H₁₀O₅</td>
<td>234.05</td>
<td>4.9019</td>
</tr>
</tbody>
</table>
Fig 1: GC – MS chromatogram of the ethanol extract of whole fruit of *Opuntia elatior*
Fig 2(a), (b), (c): Mass Spectrum of 2,5-Furandione, 3-methyl (2a), 2,5-Furandicarboxaldehyde (2b), 2,5-Furandione, dihydro-3-(2-methyl-2-propenyl)-(2c)
Fig 3(a), (b), (c): Mass spectrum of Pentanoic acid, 2,2-dimethyl-ethyl ester (3a), 2-Furancarboxylic acid, 1-methylethyl ester (3b), Maltol propionate (3c)
Fig 4(a), (b), (c): Mass spectrum of 4H-Pyran-4-one, 3,5-dichloro-2,6-dimethyl (4a), 4H-Pyran-4-one, 5-(acetyloxy)-2-((acetyloxy)methyl) (4b), 2-Tetrazene, 1,1,4,4-tetrakis(1-methylethyl) (4c)
Fig 5(a), (b), (c): Mass spectrum of 5-Hydroxymethylfurfural (5a), 2,3:4,5-Bis(2-butylene)tetrahydrofurfural (5b), 2-Furancarboxylic acid, 1-cyclopentylethyl ester (5c)
Fig 6(a), (b), (c): Mass Spectrum of 2- Furanethanol, β-ethoxy- (6a), 2-Furanacetic acid, a-hydroxy- (6b), 2-Furanmethanol, a-(2-nitropropyl)-, (R*,R*) (6c)
Fig 7: Mass Spectrum of 2-Furanacetic acid, a-hydroxy-