CHAPTER – VIII
GC-MS ANALYSIS OF PHYTOCOMPONENTS IN THE
METHANOLIC LEAF EXTRACT OF
Pimenta dioica

8.1 INTRODUCTION

Plants and fruits are one of the main sources of biologically active compounds. An estimated of the World Health Organization (WHO) states that around 85 - 90% of the world’s population are consuming traditional herbal medicines (WHO, 2002). From many decades, plants have been utilized throughout the world as sources of treatment for various health problems. The indigenous knowledge of various medicinal plants has been gaining worldwide recognition. Medicinal plants contain various physiologically active compounds, which over the years have been utilized in trado-medical practice for the treatment of many diseases (Adebanjo, 1983).

From before four thousand years, the medical knowledge of the Indian subcontinent has been termed as “Ayurveda”. The actual meaning of Ayurveda is “science of life,” which remains as an imperative system of medicine as well as drug therapy in India. The disease evolved in the body due to exogenous factors has been reported in Ayurveda medicine. It also covered all aspects of diseases, pharmacy and therapeutics in Sanskrit literature (Ramar, 2008). Today
the pharmacologically active compounds of various Ayurvedic medicines are being identified and their effectiveness in drug therapy are being identified. Medicinal plants have provided to be a potent source of inspiration for the production of various novel drug compounds, as plant derived medicines have been making big contributions to human health from historic ages, whose phytochemical compounds have the ability to exert multiple biological properties such as anti-oxidant, free radical scavenging potential, anti-inflammatory, anti-carcinogenic, anti-ulcer, anti-fertility, etc. (Miller, 1996).

A knowledge about the phytochemical constituents of plants is important not only for the discovery of therapeutic agents, but also because such knowledge may be of great importances in disclosing new sources of novel phytocompounds for the synthesis of chemical substances for therapeutic uses as well as for discovering the significances of folkloric remedies (Milne et al., 1993). Hence, a complete validation of the herbal drugs has evolved as a new branch of science which, emphasize and prioritizing the standardization of the natural drugs and products as several of the phytochemicals have complementary as well as overlapping mechanism of action. Mass spectrometry along with chromatographic separation procedures such as gas chromatography (GC-MS) is normally utilized for the complete
analysis of components present in traditional medicines as well as medicinal plants. In recent years GC-MS studies have gained increasing application for the analyzing constituents in medicinal plants as this technique is proved to be a benificial method for the analysis of non-polar components and essential oil, fatty acids, lipids and alkaloids (Betz et al., 1997).

Chromatography is the term employed to distinguish a separation technique in which a mobile phase carrying a mixture is made to move in contact with a selectively absorbent stationary phase. It also plays a primarily role as an analytical technique for quality control and standardization of standard therapeutics (Andrew, 2007). The principle of gas chromatography is adsorption and partition and is one of the most extensively used techniques and has grown to be one of the most important tools for the separation of volatile compounds. The combination of speed, sensitivity and high resolving power in gas chromatography provides a very adequate technique for the breakup of complex samples. Moreover, the coupling to spectrometric methods such as mass spectrometry (MS) for the direct identification of unknown compounds is easy to install.

Gas chromatography has a really broad sphere of applications. But, its first and main area of use is in the separation as well as the
analysis of multicomponent mixtures such as essential oils, hydrocarbons and solvents. Basically, the use of the flame ionization detector and the electron capture detector, gas chromatography can quantitatively determine materials present at very low concentrations in the sample under analysis. Due to its simplicity, sensitivity and effectiveness in separating components of mixtures, gas chromatography have become one of the most important tools in chemistry and biology. It is extensively utilized for quantitative and qualitative analysis of mixtures, and for the determination of such thermo chemical constants.

*Pimenta dioica*, commonly known as Allspice has been utilized by early Central American civilizations as a flavoring for chocolate. The 17th century Spanish explorers gave Allspice its name Pimenta, due to its peppery flavor. As a medicine, Allspice has almost the same usage, as cloves and their oils. It is used as a digestive adding agent, has an antiseptic and slightly anaesthetic action (Ridley, 1983). The botanical name of allspice is *Pimenta dioica* (L.) Merr. and it belongs to the Myrtaceae family. It possesses an aromatic taste and the flavor resembles a mixture of cinnamon, cloves and nutmeg, hence the name as allspice is given to this plant (Neal, 1965; Weiss, 2002). Important plant metabolites reported to be present in this plant are glycosides,
gum, minerals, quercetin, resin, sugar, tannin and vitamins (A, C, niacin, riboflavin and thiamine).

*P. dioica* is traditionally used as a spice as well as condiment, but it is industrially used for tanning purposes, as flavouring agent and perfuming agent in soaps, tonics and for appetizing medicines. Different plant parts have been used to relieve dental and muscle aches, as well as used to treat rheumatic pains, colds, menstrual cramps, indigestion, flatulence, diabetes, viral infections, sinusitis, bronchitis, depression, nervous exhaustion, hysterical paroxysms, arthritis and fatigue (Nakatani, 1994; Christman, 2004). The extensive medicinal properties and wide structural as well as biological diversity of the active constituents present, such as phenolic acids, flavonoids, catechins, galloyl glucosides have initiated the screening of more pharmacologically active phytoconstituents present in *P. dioica* (Kikuzaki et al., 2000). Hence the present study was aimed to identify the phytoconstituents present in *Pimenta dioica* leaf methanolic extract using GC-MS analysis.

**8.2 MATERIALS AND METHODS**

Gas chromatography-mass spectrometry (GC-MS) (Niessen, 2001) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances
with a test sample. Application of GC-MS include drug detection, fire investigation, environment analysis, explosives investigation and identification of unknown samples.

GC-MS sample was prepared by dissolving about 1 mg of sample in 5 ml of methanol. Active extract was dissolved in HPLC grade methanol and subjected to GC and MS JEOL II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography) equipped with secondary elector multiplier. The column (HP5) was used with fused silica 50x 0.25 mm. I.D. Analysis conditions were 20 minutes at 100°C and 235°C for column temperature, 240°C for injector temperature, in which helium was the carrier gas used and split ratio was 5:4. The sample (1μl) was evaporated in a split less injector at 300°C. Run time was 22 minutes. The components were identified by gas chromatography coupled with mass spectrometry.

**Mass spectrum**

Interpretation of mass spectra of GC-MS was done using the database of National Institute of Standard and Technology (NIST) library search which is having more than 62,000 drug formulation. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST-08 and Wiley-
libraries. The name, molecular weight and structure of the components of the test materials were validated.

**8.3 RESULTS**

The identified compounds of the *Pimenta dioica* leaf methanolic extract based on the retention indices, area percentage and chemical structure are given in the Table 1. The results showed the presence of phenol, 2-methoxy-3-(2-propenyl)- (90.233%), trans, cis-1,8-dimethylspiro[4.5] decane (1.011%), hentriacontane (0.851%), hentriacontane (0.555%), hentriacontane (2.910%), 2h-1-benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, acetate, [2r-]{(1.462%), octasiloxane1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15 - hexadecamethyl-(0.613%), and hop-22(29)-en-3.beta.-ol (2.365%). The spectrum profile of GC-MS confirmed the presence of 8 major components with retention time of 11.212, 16.469, 25.028, 25.678, 26.318, 26.758 28.869 and 29.330 h respectively. The individual fragmentation of components is illustrated in figure (29 – 37)
Fig. 29. GC-MS chromatogram of the methanolic leaf extract of 
_Pimenta dioica_
Fig. 30. Mass spectrum of phenol, 2-methoxy-3-(2-propenyl)-
Fig. 31. Mass spectrum of trans,cis-1,8-dimethylspiro[4.5]decane
Fig. 32. Mass spectrum of hentriacontane
Fig. 33. Mass spectrum of hentriacontane
Fig. 34. Mass spectrum of hentriacontane
Fig. 35. Mass spectrum of 2h-1-benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2- (4,8,12-trimethyltridecyl)-, acetate, [2r-]
Fig. 36. Mass spectrum of octasiloxane,

1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- hexadecamethyl-
Fig. 37. Mass spectrum of hop-22(29)-en-3.beta.-ol
8.4 DISCUSSION

Plants are rich in a variety of phytochemicals such as phenolics and flavonoids, which provide health benefits. Phytochemical is a natural bioactive compound found in plants that acts as a defense system against diseases more accurately and protect against such diseases (Krishnaiah et al., 2009). Significantly, many studies suggest that these compounds are important antioxidant substances that act as reducing agents, singlet oxygen quenchers or electron donors with chelating properties (Hanasaki, 1994). Plant polyphenols are secondary metabolism products and they constitute one of the most numerous and widely distributed groups of natural antioxidants. Polyphenols act as reducing agents and antioxidants in-vitro, via several mechanisms including the scavenging of free radicals, chelation of transition metals, as well as the mediation and inhibition of enzymes. (Cao, 1997; Rice-Evans 1997; Chang, 2007).

In addition to the activity of plant products as free radical scavengers, they also function as inhibitors of lipid peroxidation (Xie et al., 1993; Formica and Regelson, 1995). The antioxidant properties of phenolic compounds and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen (Rice-Evans et al., 1996). When the phytochemical compounds react
with a free radical, delocalization of the gained electron over the phenolic antioxidant and the aromatic nucleus that prevents the continuation of the free radical chain reaction. This is referred to as free radical scavenging. But polyphenolic compounds inhibit peroxidation through a variety of mechanisms (Cuvelier et al., 1992). The compounds identified by GC-MS in methanolic extracts are medicinally valuable and possess various pharmaceutical applications. The identified phytocomponents needs further research on toxicological aspects to develop new and safer drugs (Priya et al, 2011).

The more exact composition of phytochemical constituents by qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS) (Cong et al., 2007). GC-MS is one of the excellent techniques to identify the phytoconstituents present in volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters, etc. It helps reveal the presence of such phytochemical constituents that could be of an important contribution to the medicinal quality of the plant under study. The identification of the phytochemical compounds would be confirmed based on the peak area, retention time and molecular formula (Semakkani and Thangapandian, 2012).
For quantitative determination, gas chromatography with flame ionization detector (GC-FID) and GC-MS are preferred (Lee et al., 2005; Lampronti et al., 2006; Haznagy Radnal et al., 2007). The essential oil yield was 1.3% (v/v), which is higher than that found in a study of the leaves of sun dried P. dioica specimen that were grown in the southern region of Bahia (Oliveira et al., 2009). Marongu et al. (2005) found that the essential oil of P. dioica leaves from Australia constituted 77.9% eugenol. The allspice leaves extract showed good protection in cyclophosphamide induced myelosuppression which could be because of the existence of total phenolic compounds. The eugenol is found to be the major component in the existence of leaves that may be responsible for the potential activity. The total radical scavenging property could also be reasonable for the myelostimulant property (Yogendra Nayak and Abhilash, 2008).

In India, Pimenta are used to flavoring of rice, which gives a distinct aroma. The allspice berry powder is considered as a very significant spice in the meat industry for tenderizing of meat (Sharma, 2003; Seidemann, 2005). The essential oil of berries of Pimenta dioica has been previously reported to contain the following components such as limonene, 1,8 cineole, terpinolene, β-caryophyllene, β-selinene and methyl eugenol. The Jamaican Pimenta leaf oil contains eugenol,
methyl eugenol, myrcene and β-caryophyllene (Tucker et al., 1992). Another study carried out on *Pimenta* leaf oil in Jamaica revealed the presence of eugenol, methyl eugenol and β-caryophyllene as the main compounds whereas myrcene was found in trace levels. The leaf oil is thought to be inferior to the berry oil (Jirovetz et al., 2007). In the previous reports the therapeutic properties of the allspice berry oils are listed as anesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acaricidal, carminative, muscle relaxant, rubefacient, stimulant and tonic.

The chemical composition of *Pimenta* spp. leaves investigated from various countries has been reported (Tucker et al, 1992; Bello et al, 1995; Pino and Rosado, 1996; Bello et al., 1998; Pino et al., 2002; Jirovetz et al., 2007). The major component of leaf oil *P. dioica* was the phenyl propanoid eugenol ranging from 54.3 to 79.2 % (Pino and Rosado, 1996). *P. jamaicensis* leaf oils were dominated by eugenol (61.8%), limonene (10.4%) and β-caryophyllene (5.8%). *P. racemosa* leaf oil was mainly composed of phenylpropanoids (44.4 to 68.9%), chavicol (15.5%) and methyl eugenol (11.9%) (Tucker et al., 1992). This composition is similar to the same variety cultivated in Benin (Aydoun et al., 1996) and North India (Pragadheesh et al., 2013). The leaf oil from *P. adenoclada* was dominated by the sesquiterpenes
caryophyllene oxide (15.4%) murolol (9.4%) and humulene epoxide
(7.6%) (Pino et al., 2002). *Pimento pseudocaryophyllus* was
predominated by chavibetol (70.9%) and methyl eugenol (20.7%)
(Marques et al., 2010).

The leaf oil of *Pimenta dioica* from Cuba contained a major
amount of eugenol (54.3%) (Pino and Rosado, 1996). *P. dioica* from
Jamaica contained 76% of eugenol and 7.1% of methyl eugenol
(Jirovetz et al, 2007). The leaf oil of *Pimenta guatemelensis* was rich in
the phenyl propanooid eugenol (72.8%) (Carlos and Jose, 2015). The
most common ingredients tested in the chemical extraction of the
leaves of *P. dioica* are polyphenols, lignins and terpenoids (Zhang and
Lokeshwar, 2012). The most important component isolated from
*P. dioica* with strong antioxidant activity is eugenol, which comprises
60-90% (Kamatou et al., 2012). Eugenol exhibits antibacterial,
antifungal, anti-inflammatory, antioxidant and antiproliferative
activity.

The major compound of most of the *Pimenta racemosa* oil is
eugenol (70 – 80 %) (Lawrence, 1997; BACIS-Boelens 2000;
Lawrence 2000). In bay leaf oil of *P. racemosa* var. *terebinthia*, \(\alpha\)-terpinyl acetate, \(\alpha\)-terpineol and methoxy eugenol as well as in *P.
racemosa* var. *grisea*, 4-methoxy-isoeugenol and 4-methoxy eugenol
were found as dominating constituents (Saenz, 2004). In the present study, the GC-MS analysis of the methanolic leaf extract of *P. dioica* revealed the presence of eight components. In terms of percentage occurrence, phenol, 2-methoxy-3-(2-propenyl)-, hop-22(29)-en-3.beta.-ol and hentriacontane were predominant in the extract. Thus this type of GC-MS analysis are found to be the first step towards understanding the nature of active principles located in the medicinal plant, *P. dioica*.