5.1 Introduction

Secondary metabolites

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (Fatima et al., 2006). Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi et al., 2008). Bisht and Kamal (1994) observed that there is strong need to investigate the chemical composition of many plants to determine their ability to be used as fungicides, bactericides or insecticides. According to a report by Walker, the medicinal properties of plants could be seen in their response to attacks from insect predators and disease organisms. This is achieved by the accumulation of phytochemicals at the sites of infection of plants, several of which are insecticidal, anti-bacterial, antifungal, etc (Walker, 1975; Ameen et al., 2005).

Thin Layer Chromatography

Thin layer chromatographic technique is a useful analytical tool for the isolation of organic compounds. TLC is widely used in natural product extract analysis, stability tests of extracts and finished products, and in sample quality control. Characteristic features of TLC include: analysis of many samples and comparison of their phytochemical profiles on the same plate (Cimpoiu, 1996). The identification of separated components can be achieved on the basis of retention factor (Rf) values and color spots. In relation to other chromatographic
methods, TLC offer the simplest and cheapest means of detecting natural product constituents, requiring little sample clean-up and equipment (Nyiredy and Glowniak, 2001).

**High Performance Liquid Chromatography (HPLC)**

High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques. It is widely applied for separations and purifications in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries. Chromatographic process can be defined as separation technique involving mass-transfer between stationary and mobile phase. HPLC utilizes a liquid mobile phase to separate the components of a mixture. As a result, HPLC acquires a high degree of versatility not found in other chromatographic systems and it has the ability to easily separate a wide variety of chemical mixtures (Skoog *et al.*, 1998).

**Gas Chromatography - Mass Spectrometry**

GC can separate volatile and semi volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. Therefore, the combination of the two techniques was suggested (Ronald, 1999).

This chapter deals in detail the phytochemistry of selected potential fern *P. biaurita* which was analyzed through qualitative preliminary phytochemical analysis, quantitative secondary metabolites, TLC, HPLC and GC- MS.
5.2 Review of Literature

Balasubramanian et al. (2008) screened the fern C. parasitica endemic to Western Ghats of Tamil Nadu for its phytochemical constituents and evaluated its pesticidal properties against two lepidopteran pests viz., Spodoptera litura (Fab.) and Helicoverpa armigera (Hub). The results of the preliminary phytochemical analysis revealed that all the fractions except AF contains steroids. Among the other groups of phytochemicals, saponins and santhoprotiens were recorded in CE, EE and AF fractions and tannins and flavonoids in EE and AF. HPLC results of the phytoecdysone characterization revealed that C. parasitica contains two major phytoecdysteroids α and –ecdysteroids. It shows -ecdysone in CE and -ecdysone in EE and CEF fractions.

Bharat et al. (2011) evaluated the phytochemical analysis such as chlorophylls, carotenoids and polyphenols contents in the eleven species of ferns from the Satara district of Maharashtra. The different species showed variation in their photosynthetic pigments. The different species shows increase in photosynthetic pigment during vegetative stage and decreases during mature stage. The decrease in phytochemicals during reproductive stage may be due to sporangium formation which may create a stress during the maturation and increasing the amount of phenols.

Bonadies et al. (2004) performed Gas chromatographic (GC) analyses for analyzing the Mass spectrometry of ptaquiloside, the toxic sesquiterpene from bracken fern Pteridium aquilinum, var. latiusculum. They successfully analyzed the spectrum of ptaquiloside.
**Bresciani et al. (2003)** described the phytochemical analysis and analgesic activity of a non polar fraction obtained from *Adiantum cuneatum* grown in Brazil. The results showed that the hexane fraction as well as two pure compounds, identified as filicene (1) and filicenal (2), given intraperitoneally, exhibited potent analgesic activity when evaluated in two models of pain in mice, writhing test and formalin-induced pain.

**Chen et al. (2008)** isolated three new compounds: 2R,3R-pteratin L 3-O-β-D-glucopyranoside β-Dxylopyranosyl (1→2)-7-O-benzoyl-β-D-glucopyranoside and 4-O-benzoyl-β-D-xylopyranosyl (1→2)-7-O-benzoyl-β-D-glucopyranoside together with nine known compounds from the ethyl acetate extract of *Pteris ensiformis*. 5-[2-Hydroxyethylidene]-2(5H)-furanone which had been synthesized, was isolated from natural sources for the first time. The structures of all isolated compounds were determined on the basis of mass and spectroscopic evidence.

**Dalli et al. (2007)** characterized the antimicrobial compounds from a common fern, *Pteris biaurita*. The ethyl acetate fraction of methanol extract of the plant was analyzed and the active fraction was detected using TLC plate bioassay. The active fraction was scraped and subjected to UV- spectrophotometric analysis such as HPLC and GC-MS analysis. It revealed that extract contain a mixture of eicosences and heptadecanes.

**Hayat et al. (2002)** isolated two new triterpenes, adininaneone and adininaonol were from *Adiantum incisum* and also isolated three known triterpenes, adiantone, isoadiantone and 23-hydroxyfernene for the first time from the methanolic extracts of *A. insicum*. The structures were elucidated with the help of modern spectroscopic techniques.
Hima et al. (2012) screened the phytochemical compounds of eight different organic solvents extracts of *Hemionitis arifolia*. Phytochemical screening showed the presence of flavonoids, steroids and glycosides along with reducing sugar in all the extracts.

Kumudhavalli and Jaykar (2012) evaluated petroleum ether, chloroform, acetone, ethanol and aqueous extracts of *Hemionitis arifolia* for preliminary phytochemical screening. The ethanolic and aqueous extracts showed the presence of flavonoids, carbohydrates, phenolic compounds and sterols were the major phyto constituents.

Mradu et al. (2012) carried out the qualitative and quantitative HPLC analysis of twelve phenolic compounds namely Ellagic acid, Catechol, Gallic acid, Quercetin, Resorcinol, Tannic acid, Vanillin, Salicylic acid, Acetyl Salicylic acid, Benzoic acid, Phloroglucinol and Ascorbic acid. The objective of the research was to determine the standard HPLC chromatograms of twelve prominent phenolic compounds found in medicinal plants using four mobile phases having different elution gradients and run times. Four HPLC methods using different mobile phases were used.

Muraleedharannair et al. (2012) examined the phyto-constituents of *Adiantum caudataum*, *Adiantum latifolium*, *Adiantum lunulatum*, *Christella dentate* and *Christella parasitica*, to provide chemical marker and inter-specific variation between the medicinally important genuses. A total of five plants and 30 extracts were examined for the phytochemical screening. The crude extracts of *A. caudataum*, *A. latifolium*, *A. lunulatum*, *C. dentata* and *C. parasitica* showed varied degree of phyto-constituents with reference to solvents of the plant extracts.

Nilesh et al. (2011) carried out a gas chromatography-mass spectrometry analysis for the identification of compounds from ethanolic extract of *Polypodium decumanum*. The
following compounds were identified for the first time in the sample: 3,4-dihydroxybenzoic acid, linoleic acid, chlorogenic acid, 4- hydroxybenzoic acid and ferulic acid. The main type of the compound identified was fatty acids derivatives.

Rajurkar and Kunda (2012) screened *Adiantum capillus - veneris* for phytochemicals and metal content. It was observed that it contains 8.3 % moisture, 11.44 % ethanol extractable matter and 24.00 % water extractable matter. The Soxhlet extraction of *Adiantum capillus veneris* showed the presence of phenolics and terpenoids (2.73 %), fats and waxes (0.20 %), alkaloids (0.53 %), quaternary and Noxides (26.33 %) and fiber (67.23 %).
5.3 Materials and Methods

Qualitative analysis of phytochemical (Preliminary analysis)

The condensed five solvents such as petroleum ether, benzene, chloroform, methanol and aqueous extracts of *P. biaurita* were used for preliminary screening of phytochemicals such as alkaloids, steroids, reducing sugars, catechins, anthraquinones, flavonoids, terpenoids, sugars, phenols, saponins, tannins and amino acids. The presence of phytochemicals from methanol extract of all the samples were qualitatively determined by adopting standard method of Brindha *et al.* (1981) (Table 5.1).

Quantitative analysis of phytochemicals (secondary metabolites)

The phytochemicals which are present in the five solvents extracts of *P. biaurita* were determined and quantified by following standard procedures.

Determination of total phenolic compounds

The determination total phenolic content of crude drug extract was done using Folin-Ciocalteu reagent with some modifications (Hagerman *et al.*, 2000). For total phenolic estimation, 100 mg of the each extracts of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na$_2$CO$_3$ solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve.
obtained from various diluted concentrations of Gallic acid (Fig.5a). The amount of total phenolic compound was calculated by following formula,

\[ C = \frac{c \cdot V}{m} \]

Where,

\[ C = \text{total content of phenolic compounds mg/g plant extract (GAE)} \]

\[ c = \text{concentration of Gallic acid established from the calibration curve} \]

\[ V = \text{volume of extract} \]

\[ m = \text{weight of pure plant extract} \]

**Determination of total flavonoids**

For the determination of the total flavonoid content, the aluminium chloride method is incorporated using rutin as the standard (Kumaran and Karunakaran, 2006). The method is based on the formation of the flavonoids - aluminium complex which has an absorptivity maximum at 415nm. 100µl of the plant extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions (Fig.5b). All determinations were carried out in triplicates. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:
\[ X = \frac{(A_0 \cdot m)}{(A \cdot m)} \]

Where,

\( X \) = total flavonoids content, mg/g plant extract in RE

\( A \) = absorbance of plant extract

\( A_0 \) = absorbance of standard rutin

\( m \) = weight of plant extract

\( m_0 \) = weight of rutin in the solution

**Determination of total alkaloids**

The total alkaloid content in the two ferns was determined using Harborne (1973) method. 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

**Determination of total tannins**

The total tannin content was determined by Van-Burden and Robinson (1981) method. 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml
volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl$_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Fig.5c). The tannin content was calculated by following formula,

\[ y = ax + b \]

Where,

\[ y = \text{the rutin equivalent (mg/g)} \]

\[ x = \text{the absorbance} \]

**Determination of total saponins**

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 cm$^3$ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponins content was calculated as percentage.
Thin Layer Chromatography (TLC)

10 ml of sample was taken and evaporated; the paste of the evaporated extracts was used for TLC. A combination of Hexane, Methanol and Ethyl acetate in the ratio 8:1:1 was used as solvent mixture. The compounds in extracts were isolated by TLC using Merck silica pre-coated aluminium plates of 200 μm thickness with above solvent systems of different polarities. The extracts to be analyzed were spotted on the plate. The plates were placed in TLC chamber and the chromatogram was developed with the solvent mixture. The TLC plates were taken out and visualized in visible light, UV light (265 nm & 365 nm) and iodine chamber and spots were marked. The migration pattern was recorded and the Rf value of each spot was calculated using the formula and tabulated.

Data analysis

The Rf values of different spots were calculated using the formula

\[
\text{Rf value} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}
\]

Gas Chromatography and Mass Spectroscopy (GC –MS) analysis

Preparation of extract

Among the five solvents extracts, methanol extract showed maximum amount of phytochemicals. Hence methanol extract was selected for spectroscopic analysis. 2 µl of the methanol extract of *P. biaurita* was employed for GC/MS analysis (Merlin *et al*., 2009).

Instruments and chromatographic conditions

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS)
instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

**Identification of components**

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

**High Performance Liquid Chromatography (HPLC)**

**Preparation of standard solutions**

Standards 6- Pyridazinedione, 1,2- dihydro-4-methyl and 1- Propyne-3-chloro were procured from Sigma Aldrich, Bangalore. Standard stock solutions (500 µg/ml) were prepared by dissolving 50 µg of 3,6- Pyridazinedione, 1,2- dihydro-4-methyl and 1- Propyne-3-chloro in 5 ml of warm methanol and was made up to 100 ml with distilled water and sonicated for 20 minutes. Standard solutions were prepared by diluting the stock solution with 50 % methanol to obtain the desired concentration.
Preparation of sample solutions

The methanol extract of sample was used for HPLC analysis. All extracts were filtered through a Whatman no.1 filter paper. 30 ml filtered extract was evaporated by a rotatory vacuum evaporator. The evaporated residues with constant weight were stored prior to analysis in dark at 4°C. 200 mg of extract was dissolved with 4 ml methanol, sonicated at 35 °C for 15 minutes and filtered through 0.45 µm filter and applied (50 µl) on to HPLC column.

Apparatus and chromatographic conditions

The HPLC analyses were carried out on a Shimadzu, LC-10 AT VP, consisting of SCL-10Avp system controller, degassing unit DGU-14A, low-pressure gradient flow control valve FCV-10ADvp, auto injector SIL-10ADvp with 500 µl loop, column oven CTO-10AC, a UV detector SPD-10Avp using a 254 (5µm). The temperature was maintained at 25°C with injection volume of 200 µl and flow rate of 1ml/min. Active compounds were separated using reverse-phase LiChrosorb C-18 column with the methanol: water mobile phase and detected at 210 nm. HPLC conditions for analysis of samples were achieved by varying mobile phase composition 80% methanol in a gradient with 100 % methanol. The chromatography system was equilibrated by the mobile phase. When same retention times and peak areas for repetitive injections of standard were observed, separation of sample could then be carried out.

Data analysis

Calibration data calculated from peak area and height at different retention time were compared with standard. The peak area of each sample was plotted against the concentration
to obtain the calibration graph. The amount of active compound was calculated using the following formula (Scott, 1996),

\[
CP (s) = \frac{AP(s) \times CP (st)}{AP (st)}
\]

\(CP (s)\) = the concentration of the solute in the mixture (µg/g)

\(AP (s)\) = the area of the peak for the sample

\(AP (st)\) = the area of the peak for the standard

\(CP (st)\) = the concentration of standard used for injecting in HPLC
5.4 Results and discussion

Qualitative analysis of phytochemical (Preliminary analysis)

The bactericidal properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, saponins, steroids etc. The preliminary phytochemical screening of the five solvents extracts of *P. biaurita* revealed the presence and absence of secondary metabolites (Plate 14).

In qualitative analysis of five solvents such as petroleum ether, benzene, chloroform, methanol and aqueous extracts of *P. biaurita* exhibited positive results for eleven phytochemical tests. 11 phytochemical tests were positive in methanol extract of the fern. In benzene extract 8 tests were positive. In chloroform and petroleum ether extracts of the plant showed positive for 7 and 6 tests respectively. 5 tests were positive in aqueous extract of the fern. Maximum tests were positive in methanol extract followed by benzene, chloroform and petroleum ether extracts of the fern. Minimum tests were positive in aqueous extract of the fern (Table 5.2).

Based on the results of preliminary screening the methanol extract of potential fern contains maximum amount of phyto constituents (Fig.5d). Hence the selected fern extract exhibited efficient bactericidal activities. According to the results it was clearly confirmed that the bactericidal activity of fern extract completely caused by the presence of phytochemicals. All the extracts were subjected to further analytical tests for the quantification of phytochemical compounds.
Muraleedharannair et al. (2012) examined the phyto-constituents of *Adiantum caudatum*, *Adiantum latifolium*, *Adiantum lunulatum*, *Christella dentate* and *Christella parasitica*, to provide chemical marker and inter-specific variation between the medicinally important genuses. A total of five plants and 30 extracts were examined for the phytochemical screening. The crude extracts of *A. caudatum*, *A. latifolium*, *A. lunulatum*, *C. dentata* and *C. parasitica* showed varied degree of phyto-constituents with reference to solvents of the plant extracts. In the present investigation five solvents extracts of *P. biaurita* was examined for the phytochemical screening and various phytochemicals were found. Kumudhavalli and Jaykar (2012) evaluated the petroleum ether, chloroform, acetone, ethanol and aqueous extracts of the fern *Hemionitis arifolia* for preliminary phytochemical screening. The ethanolic and aqueous extracts showed the presence of flavonoids, carbohydrates, phenolic compounds and sterols were the major phyto constituents. The methanol extract of *P. biaurita* showed the presence of alkaloids, flavonoids, phenolic compounds, steroids etc in the present study. Alkaloids and flavonoids are the source of antimicrobial activities. Tannins may have the potential values as cytotoxic agents (Aguinaldo et al., 2005). Saponins have been implicated as bioactive antibacterial agents (Mandal et al., 2005). In the present study the above four compounds were identified in methanol extract of *P. biaurita*. Hence it was confirmed that the methanol extract of *P. biaurita* exhibited significant antibacterial activity against *Xcpvc*.

**Quantitative analysis of phytochemicals**

The amount of phytochemicals which found in the ferns extract was quantitatively determined by standard procedures (Plate 14 & Table 5.3). The presence of maximum amount of phytochemicals determined the maximum efficiency of bactericidal activity.
All the extracts of *P. biaurita* showed different amount of phytochemicals (Fig.5e). Among the five components alkaloids content was highest in all the four solvents extracts followed by flavonoids and phenolic compounds except in methanol extract. In methanol extract flavonoids content was highest among the five components. Hence the methanol extract of the selected fern *P. biaurita* exhibited significant antibacterial activity among the other solvent extracts. The amount of tannins and saponins was very low in all the extract. It was clearly showed that the antibacterial activity of fern extract was caused by the presence of highest amount of flavonoids, alkaloids and phenolic compounds in it.

Pragada *et al.* (2011) carried out preliminary phytochemical analysis and quantification of total phenols, *in-vitro* antioxidant and antibacterial activities of the hydro alcoholic (70% ethanol) extract of *Acalypha indica*. Totally five compounds such as alkaloids, flavonoids, phenolic compounds, saponins and tannins were quantified in ferns extracts. Rajurkar and Kunda (2012) screened *Adiantum capillus-veneris* for phytochemicals and metal content. The Soxhlet extraction of *Adiantum capillus-veneris* showed the presence of phenolics and terpenoids (2.73 %), fats and waxes (0.20 %), alkaloids (0.53 %), quaternary and Noxides (26.33 %) and fiber (67.23 %). But in the present study, highest amount of alkaloids (17.33mg/g), flavonoids (18.85mg/g) and phenolics (13.53mg/g) were quantified in the methanol extract of *P. biaurita*.

**Thin Layer Chromatography**

The data of quantitative separation of secondary metabolites from five solvents such as petroleum ether, benzene, chloroform, methanol and water extracts of potential fern *P. biaurita* by Thin Layer Chromatography was tabulated (Table 5.4-5.7). Rf values obtained by TLC patterns are useful to establish their identity and purity of the herbs. The plates were
first exposed to visible light then viewed through UV (365nm & 265nm) and in Iodine chamber to observe the variously coloured bands (Plate 14).

TLC chromatogram of five solvent extracts of *P. biaurita* under visible light revealed 6 bands in petroleum ether extract, 9 bands in benzene extract, 7 bands in chloroform extract, 10 bands in methanol extract and 6 bands in aqueous extract. In all the extracts the highest Rf value was 0.96 with green coloured bands except aqueous extract. The least Rf value was 0.14 with bluish green coloured bands in all the extracts (Table 5.4).

Under UV light (365 nm) chromatogram revealed 8 bands in petroleum ether extract, 11 bands in benzene extract, 8 bands in chloroform extract, 14 bands in methanol extract and 7 bands in aqueous extract. In all the extracts the highest Rf value was 0.96 with reddish brown coloured bands except aqueous extract. The least Rf value was 0.04 in chloroform extract with dark blue coloured bands (Table 5.5).

Under UV light (265 nm) the chromatogram revealed 11 bands in petroleum ether extract, 13 bands in benzene extract, 10 bands in chloroform extract, 17 bands in methanol extract and 9 bands in aqueous extract. The highest Rf value was 0.98 with light green coloured band and the least Rf value was 0.02 with pale orange coloured band in all the extracts (Table 5.6).

When the chromatogram was exposed to iodine vapour, it revealed 6 bands in petroleum ether and chloroform extracts, 7 bands in benzene extract, 9 bands in methanol extract and 5 bands in aqueous extract. In all the extracts the highest Rf value was 0.92 with dark orange coloured bands. The least Rf value was 0.02 in petroleum ether extracts with reddish brown coloured bands (Table 5.7). The results of quantitative and qualitative phytochemical analysis and TLC revealed that among the five solvents extracts, methanol
extract of *P. biaurita* showed maximum amount of phytochemicals. Hence methanol extract was selected for spectroscopic analysis.

**Gas Chromatography and Mass Spectroscopy**

GC-MS chromatogram of the methanol extract of *P. biaurita* showed 15 peaks indicating the presence of fifteen phytochemical constituents (Fig. 5f). On comparison of the mass spectra of the constituents with the NIST library fifteen phyto constituents were characterized and identified (Table 5.8). The various phytochemicals contribute to bactericidal and other medicinal activity of the plant. Three major phytochemical constituent’s mass spectra are presented in Figures 5g-5i. They were identified as 1-propyne, 3-chloro (6.19%), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (27.07%), and 3,6-Pyridazinedione, 1,2 – dihydro-4-methyl (50.73%).

**High Performance Liquid Chromatography (HPLC)**

The standard 1-Propyne, 3-chloro- graph showed 32.4% height and 30.2% area (Fig. 5j). The amount of active compound 1-Propyne, 3-chloro- was 25.6353µg/g in the fern extract (Fig. 5k). Likewise the standard 3,6- Pyridazinedione, 1,2 – dihydro-4-methyl graph showed 67.6% height and 69.7% area. The total amount of active compound 3,6-Pyridazinedione, 1,2 – dihydro-4-methyl was 245.9719µg/g in the fern extract (Fig. 5l). Among the two compounds 3,6- Pyridazinedione, 1,2 – dihydro-4-methyl was found maximum amount in the potential fern compared with other compound (Table 5.9). Hence it was confirmed that the efficient antibacterial activity was caused by the presence of the active compounds 1-Propyne, 3-chloro- and 3,6-Pyridazinedione, 1,2 – dihydro-4-methyl.

Dalli *et al.* (2007) characterized the antimicrobial compounds from *Pteris biaurita*. The ethyl acetate fraction of methanol extract of the plant was analyzed and the active
fraction was detected using TLC plate bioassay. The active fraction was scraped and subjected to UV- spectrophotometric analysis such as HPLC and GC-MS analysis. But in the present study crude methanol extract was subjected to TLC, HPLC and GC-MS analysis for characterizing the antimicrobial compounds. Bonadies et al. (2004) performed Gas chromatographic (GC) analyses for analyzing the Mass spectrometry of ptaquiloside, the toxic sesquiterpene from bracken fern _Pteridium aquilinum, var. latiusculum_. They successfully analyzed the spectrum of ptaquiloside. Nilesh et al. (2011) carried out a gas chromatography-mass spectrometry analysis for the identification of compounds from ethanolic extract of _Polypodium decumanum_. Gopalakrishnan and Vadivel (2011) identified the twenty phytochemical constituents from the ethanolic extracts of _Mussaenda frondosa_ with the aid of GCMS technique. They also suggested that isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. In the present study 15 compounds including two major compounds such as 1-Propyne, 3-chloro- and 3,6- Pyridazinedione, 1,2 – dihydro-4-methyl were identified in methanol extract of _P. biaurita_ using GC-MS analysis. Hence based on the detailed phytochemical, chromatographical and spectroscopical studies it was concluded that _P. biaurita_ contains various bioactive compounds.
5.5 References


investigation of aerial parts of *Gmelina asiatica* Linn by GC-MS. *Pharma. Res.*, 1(3): 152-
156.

Phenolic Compounds Present in Medicinal Plants, *Int. J. Pharm. Phytochem.Res.* 4(3); 
162-167.

Inter-specific variation studies on the phyto-constituents of *Christella* and *Adiantum* using 

24. Nilesh, K., Kshirsagar, M.D and Vipin, S. 2011. GC-MS analysis of ethanolic extract of 


26. Obdoni, B.O. and Ochuko, P.O. 2001. Phytochemical studies and comparative efficacy of 
the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global. 

investigation and *in vitro* anti oxidant, anti microbial activity of different fractions of 

374.


