Chapter 5

Discussion

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The records of medicinal use of plants goes back to early civilization .Thus, it is not surprising to know that the pteridophytes, constituting fern and fern allies have been known to man for more than 2000 years for their medicinal values (Kirtikar and Basu, 1935; Nayar, 1957; Chopra *et al.*, 1958; Kumar and Roy, 1972; Watt, 1972; Sharma and Vyas, 1985). With increasing reports of adverse effects of synthetic drugs there has been an alarming rise in the search of safe and natural drugs. Plants have been providing an important contribution to health care regardless of enormous development achieved in modern medicines. Various metabolites present in the plant contribute to its medicinal value as it is known to produce some specific physiological action on the human body (Hill, 1952).

Recently, fern and fern allies have been reported as a potential group of plants for developing valuable drugs (Shil and Choudhury, 2009). Consequently, across the globe pteridological research has been initiated to verify its biological efficacy.

Thus, in the present study effort has been made to screen eight locally available fern samples collected from various areas of Darjeeling district, North Bengal region, for the presence of diversified phytochemicals along with evaluating the biological activities like antioxidant, antimicrobial and antidiabetic (*in vitro* and *in vivo).* In the present study, phytochemical screening of the powdered frond samples of *Nephrolepis cordifolia*, *Cyclosorus dentatus*, *Dicranopteris linearis, Drynaria quercifolia, Phymatosorus cuspidatus*, *Microsorum punctatum*, *Pteris biaurita* and *Pteris vittata* revealed the presence of phenol, flavonoid, tannin, terpenoid carbohydrates, reducing sugar and protein. Similarly, Irudayaraj and Senthamarai (2004) had reported the presence of phenolics like catechin and tannin in the rhizome of *D. quercifolia* (L.) J. Smith. Further, tannin was detected in the fronds of *D. quercifolia* (Mithraja *et al.*, 2012). Flavonoids and tannins have also been detected in hydro-alcoholic extract of *Equisetum arvense* L. by Santos Jr. *et al.* (2005a, 2005b). Our findings was in accordance to a study conducted by Britto *et al.* (2012) where they have also detected the presence of phenolic compounds, flavonoids and tannins in *P. vittata* and *P. biaurita.* Further, flavonoid (mainly rutin) terpenoids and tannins have also been reported in *P. vittata* (Singh *et al.*, 2008). *D. linearis* along with many other ferns studied by Mithraja *et al.* (2012) revealed the presence of tannin. *C. dentatus, N. acutifolia* and *M. punctatum* showed the presence of total phenol and flavonoids (Chai *et al.*, 2015).

Phenolic compounds are ubiquitously present in all the plants and plant parts at varying concentrations and contribute immensely towards the medicinal properties of the plants. Plant phenolics are effective vasodilators, help in reducing inflammation and are anticancerous, antioxidant, antidiabetic and antimutagenic agents. Further, its preventive role in various neurodegenerative disease are encouraging (Padilla *et al.,* 2005; Mohanlal *et al*., 2013; Zhang *et al*., 2011; Jin *et al*., 2006; Kusirisin *et al*., 2009; Scalbert *et al*., 2005; Luo *et al.*, 2002; Parekh and Chanda, 2007). Despite the beneficial health effects of phenolics, it is only in the recent years that enormous attention has been paid towards the role of phenolics (Manach *et al.,* 2004). Currently, structures of more than 8000 plant phenolics have been elucidated.

Plant phenolics are the most abundantly distributed secondary metabolites bearing a common aromatic ring with one or more hydroxyl groups (Chirinos *et al.,* 2009). Naturally occurring phenols are soluble in water and may occur in combination with a sugar molecule, as glycoside (Harbone, 1998). In plants, it is synthesized mainly during physiological or environmental stresses such UV radiation, injuries or pathogen attack. Few years back, Quideau *et al.* (2011) proposed that only those secondary metabolites that are produced through shikimate/ phenylpropanoid pathway or 'polyketide' acetate/malonate pathway should be termed as "Plant phenolics". Depending on the number of phenol units in the molecule polyphenols are classified as simple phenols, phenolic acids and flavonoids, lignins, lignans, coumarins, condensed and hydrolysable tannins (Soto-Vaca *et al.*, 2012).

Flavonoids are the chief compounds among other polyphenols and are known to possess antiviral, antibacterial, antifungal, anticancer, anti-allergic and anti inflammatory activities (Di Carlo *et al.,* 1999; Montro *et al.,* 2005). Structurally, it has a flavan nucleus with 15 carbon atom. The carbon atoms are arranged in a ring of three as C6-C3-C6 which are labeled as A, B and C. They are known to possess an inherent effective ability to scavenge most of the harmful oxidizing molecules or reactive oxygen/nitrogen molecules involved in various life threatening diseases (Bravo, 1998). Flavonoids have been also reported to exhibit protection against various cardiovascular diseases and cancers. It represses the development of cancer by inhibiting the enzymes involved in estrogen production. For example, flavonoids inhibit estrogen synthetase involved in coupling estrogen to its receptor (Okwu and Omadamiro, 2005). They have also been designated as "biological response modifiers" (Cushnie and Lamb, 2005).

Tannins have been shown to possess antiviral, antibacterial, antiparasitic, antiinflammatory, antiulcer and antioxidant activity. Since, tannin can precipitate the proteins from the exposed tissues forming a protective layer, it is widely used in the treamtment of burns. Other medicinal

applications are in treating gonorrhoea, leucorrhoea, piles, inflammation and even used as an antidote. Studies also revealed the HIV replication inhibitory activity of tannin. Most of the drugs containing tannin are used as an astringent and diuretic in medicine (Lu *et al.,* 2004; Akiyama *et al.,* 2001; Kolodziej *et al.,* 2005).

Terpenoids have been used in pharmaceutical industries since long years as antibiotics, antiseptic, insecticidal and anthelmintic (Duke, 1992; Parveen *et al*., 2010). Terpenoids have been detected in many of the ferns studied by Rajurkar and Kunda (2012), Britto *et al.* (2012) and Abraham and Aeri (2012). These natural compounds are muti-cyclic in structures and are derived from 5-hydrocarbon isoprene units (CH_2=C(CH_3)-CH=CH_2) (Elbein *et al.,* 1999; Langenheim, 1994). These natural lipids are ubiquitously present in all forms of living things (Elbein *et al.,* 1999). Commercially, terpenoids are used to add flavours and fragrances in cosmetics, foods and agricultural products (Harborne *et al.,* 1991).

Carbohydrates are one of the important and abundantly available biomolecules. It forms the basic elements of glycosylated natural products that are employed as anticancerous and antimicrobial drugs, for instances, nojirimycin (imimosugars), streptomycin (aminoglycosides) etc (Dewick, 2001; Asano, 2003). Many of the polysaccharides isolated and purified from medicinal herbs of China have been reported to act as an effective immunomodulatory (Jayabalan *et al.*, 1994). Furthermore, proteins have been identified as a potent antioxidant. Our findings was in accordance to Muraleedharannair *et al.* (2012) who reported presence of carbohydrates in *D. quercifolia* and proteins in the acetone extract of *A. caudataum.* However, they could not detect protein in any of the extracts of the rhizome of *D. quercifolia*, while we were able to detect protein in the powdered frond sample of *D. quercifolia.* Oloyede *et al.* (2013) in their study too reported the presence of carbohydrates in *N. cordifolia.*

Anthraquinones are the naturally occurring aromatic compounds and are known to possess antioxidant, antimutagenicity and antitumor activities (Demirezer, *et al*. 2001; Lee *et al.*, 2005; Kimura *et al.*, 2008). In our study, none of the ferns studied showed the presence of anthraquinones which was in contrast to the findings of Britto *et al.* (2012) who have reported presence of anthraquinones in petroleum ether, chloroform amd methanol extract of *P. biaurita.* Likewise, Owoyale *et al.* (2005) and Makinde *et al.* (2007) had also reported its presence in their study. On the other hand, Effiong and Sanni (2009) reported the absence of anthraquinones along with saponins, alkaloid and flavonoid in *Lemna pauciscostata.*

Alkaloid was not detected in any of the samples studied except in *P. biaurita.* Absence of alkaloid has been reported by Aiyegoro and Okoh (2010), Gopinath *et al.* (2012) and Yadav *et al.*

(2014) in their study. Zakaria *et al.* (2011) and Rodzil *et al.* (2013) had also reported the absence of alkaloid in *D. linearis*. Conversely, alkaloid was detected in *P. vittata* along with *P. biaurita* in a study performed by Gracelin *et al.* (2013). Alkaloids are the natural compounds bearing mostly basic nitrogen atoms and are known to have anticonvulsant, hypotensive, antiprotozoal, antimalarial and antimicrobial activities (Singh and Kapoor, 1980; Ali and Ghatak, 1975; Quetin-Lacqlercq *et al.,* 1995; Frederich *et al.,* 2002). Further, cardiac glycosides test showed positive results for all the plants studied except *M. punctatum* and *N. cordifolia.* Presence of cardiac glycosides has been reported by Rancon *et al.* (2001), Aiyelaagbe and Osamudiamen (2009), Hima *et al.* (2011) and Agrawal *et al.* (2014). Cardiac glycosides are effective antifungal agents (Abbassy *et al.,* 2007). Likewise, Na^+/K^+ pumps are inhibited by cardiac glycosides causing a rise in the Na^+ and Ca^{2+} levels in the myocytes. This aids in cardiac muscular contractions and reduces the chances of a cardiac arrest. Cardiac glycosides have also been used to increase the proficiency of heart under a controlled dosage since both toxic and therapeutic doses are very close to each other (Denwick, 2002).

Saponins belong to the group of glycosides and possess a property of foaming like soap in an aqueous solution, thereby making them an efficient foaming and surface active agent. Besides industrial applications, saponins have been known to precipitate and coagulate RBCs (Okwu, 2004; Sodipo *et al.,* 2000) and exhibit antifungal (Aboaba *et al.,* 2001), antihyperglycemic (Sauvaire *et al.,* 1996; Vats *et al.,* 2003), antioxidant (Gulcin *et al.,* 2004), antimicrobial (Mandal *et al.,* 2005) and anti-inflammatory activities (Gepdireman *et al.,* 2005). In the present study, saponin was detected only in *N. cordifolia, C. dentatus, D. quercifolia, P. cuspidatus, D. linearis* and *P. vittata.* Plant extracts rich in saponins have been reported by Falodun *et al.* (2006), Sani *et al.* (2007) and Govindappa *et al.* (2011).

Steroid was detected in *N. cordifolia, C. dentatus* and *P. biaurita* amongst all the ferns studied. It has been reported to reduce cholesterol, regulate immune responses and are pharmacologically important because of their association with various sex hormones (Shah *et al.,* 2009; Santhi *et al.,* 2011). Further, biological activities like antibacterial, anti-inflammatory, cardiovascular, hepatoprotective and antitumor was also described by many authors (Bermejo *et al.,* 2000; Guisalberti, 1998; Emam *et al.,* 1997). Our finding was in agreement with that of Britto *et al.* (2012) where they could detect the presence of steroid in *P. biaurita* and other ferns in their study. Similarly, other workers like Mohammed *et al.* (2010), Korwar *et al.* (2010), Aiyegoro and Okoh (2010) and Runa *et al.* (2013).

Following phytochemical screening, quantification of some of the bioactive components was performed in the present study. Amongst the plants studied highest amount of phenol was observed in *N. cordifolia* and lowest in *M. punctatum*. Our finding was in agreement with that of Gracelin *et al.* (2013) where similar amount of phenolic content was reported in *P. vittata* while slight difference was observed in the phenolic content of *P. biaurita.* Lai and Lim (2011) had reported very high amount of phenol in *D. quercifolia* and *D. linearis* than obtained in the present study. Phenolic content of *P. vittata* and *M. punctatum* was similar to that of present investigation. In their study, one of the species belonging to *Nephrolepis i.e N. biserrata* was observed to have moderate amount of phenol whereas in our study very high amount has been quantified in the species of same genus *Nephrolepis (N. cordifolia).* Likewise, very high phenol content was reported by Paul and Banerjee (2013) in aqueous and ethanolic extract of *P. vittata.* In our study, phenolic content of *D. quercifolia* was not in concordance to the findings of Anuja *et al.* (2014) who reported high total phenolic content in the fertile fronds of *D. quercifolia* than in the present study.

Various environmental stresses have been known to be responsible for the production of phenolic compounds in plants; for example light has been shown to greatly influence the synthesis of flavonoids. Increased levels of flavonoids (antioxidants) have been reported in the plants available in higher altitudes as many prevailing stresses such as decreased pressure, low atmospheric temperature, exposure to higher UV rays etc augments its synthesis (Chanishvili *et al.,* 2007). This possibly could be one of the reasons behind higher phenolic content in *N. cordifolia.*

Further, there are reports suggesting the protective role of phenolics against UV-B damage and successive cell death. Phenolics are known to protect DNA against dimerization and breakage which in turns protects the cell death that may arise through UV damages (Strack, 1997).

Differences in the phenolic content between the intraspecific/interspecific samples studied by different workers may be attributed to the method used for determination. Presence of diverse natural phenolics in the plant material and other oxi3dized substrates prevalent in the extract may interfere in the measurement of total phenols either by inhibiting, enhancing or adding the content (Singleton and Rossi, 1965; Singleton *et al.,* 1999).

Protein content was found to be highest in *N. cordifolia* and lowest in *P. vittata.* Almost all the ferns were observed to have considerable amount of protein in them. In addition to antioxidant property, use of proteins or its hydrolysates in cosmetics and food may add on to its functional

and nutritional ability (Moure *et al.,* 2006). Thus, presence of appreciable amount of protein in these ferns may possibly promote health benefits if consumed after thorough investigation.

Further, quantification of total flavonoid revealed it to be highest in *N. cordifolia* and lowest in *M. punctatum*. Slightly higher total flavonoid content has been reported by Gracelin *et al.* (2013) in *P. biaurita* while for *P. vittata* the flavonoid content was almost similar. Further, higher amount of flavonoid in the study conducted by Paul and Banerjee (2013) and Chai *et al.* (2012) have been reported. Flavonoids are the nutritionally important phenolic compounds (Manach *et al*., 2004) and their therapeutic applications are well established.

Tannins are another group of widely available and important polyphenols in the plant kingdom with numerous pharmacological applications. *N. cordifolia* was found to be highest in tannin content while *M. punctatum* had the lowest content. Our finding for *P. biaurita* was at par with that of Gracelin *et al.* (2013), on the other hand, lesser amount of tannin than in the present investigation has been obtained in *P. vittata*. Chang *et al.* (2007) reported higher amount of tannin in the aqueous extract of the ferns studied.

In the present study, total sugar content was found to be highest in *N. cordifolia* while reducing sugar was highest in *P. biaurita.* Carbohydrates are abundantly available biomolecules and find its application in various natural products that needs to be glycosylated, many of which have been used as anticancerous and antimicrobial drugs. For example, iminosugars like nojirimycin, aminoglycosides like streptomycin etc are some commonly used glycosylated natural products (Dewick, 2001; Asano, 2003).

Further, carbohydrates have been reported to exhibit antioxidative activity. Basu *et al.* (2012) reported Pusa Basmati polished seeds containing higher sucrose and starch content revealed better superoxide and hydroxyl scavenging capacity.

Photosynthetic pigments like chlorophylls have been known to possess several beneficial properties for example chlorophyllin (water soluble analogue of chlorophyll) was proven to be more efficient than the parent compound (Negishi, 1997; Dashwood *et al,* 1998). Numerous reports have suggested strong antioxidant activity of chlorophyllin and its usage to treat number of human diseases without evident harmful effects (Sato *et al*., 1984; Sato *et al.,* 1985; Kamat *et al*., 2000; Kumar *et al.,* 2001). In the present study highest amount of total chlorophyll and chlorophyll a was found in *P. biaurita* while chlorophyll b in *P. cuspidatus*. On the other hand, lowest level of total chlorophyll and chlorophyll a was found in *M. punctatum* and chlorophyll b in *D. quercifolia*. Considerable amount of chlorophyll was found in almost all the ferns studied.

Lipid content was found to be highest in *Drynaria quercifolia* and lowest in *N. cordifolia.* However, lipid content found in the present study in all the ferns was much higher than that reported in *Prunella vulgaris* by Rasool *et al.* (2010) in their study. Biologically lipids are important for energy storage, signaling and cell membrane development. Further, carotenoid content was found to be highest in *P. biaurita* and lowest in *M. punctatum.* Jadhav *et al.* (2011) reported appreciable amount of carotenoid for vegetative and fertile frond of *P. vittata*. Carotenoids are also known to exhibit anti-carcinogenic, anti-oxidative, antihypertensive, antimicrobial and anti-mutagenic activities (Kähkönen *et al.*, 1999; Yen *et al.,* 2002).

In the present study vitamins such as vitamin C and E was quantified spectophotometrically. Vitamins are organic compounds which cannot be produced *in vivo* and are required in a very small quantity for performing several biochemical functions. Thus, it is essential to obtain from the diet or consume as the supplement (Peter, 1990). Vitamin C is known for its antioxidative activity and for its potential to prevent arteriosclerosis (Addo, 2004). Similarly, vitamin E or tocopherols (α, β, γ, and δ) are the potent free radical, lipid peroxy and superoxides scavengers and an anti-hyperglycemic agent (Diplock, 1989; Baydas *et al.*, 2002; Celik *et al.*, 2002). Comparative analysis revealed that *N. cordifolia* contained the highest amount of ascorbic acid while the least was obtained in *Phymatosorus cuspidatus*. Lesser amount of vitamin C in *Nerium indicum* than compared to all the ferns studied except *P. cuspidatus* was reported by Dey *et al.* (2012). Vitamin E was found to be highest in *P. cuspidatus* and lowest in *D. linearis*. Considerable amount of vitamin E has been reported by many workers in their respective studies (Franke *et al.,* 2010; Mohd Adzim Khalili *et al.,* 2010).

In the present study, both qualitative and quantitative analysis revealed the presence of varied bioactive components in a varying amount in all the ferns studied. Though, in many cases differences in the finding with that of other workers was evident which may be attributed to numerous factors like age of the plant, time and percentage of humidity of the harvested plant samples, geographical differences, varying distribution of biological compounds in the plant organs and method used for determination. Hence, taking into the consideration of beneficial role of these phytochemicals (both primary and secondary metabolites) in human health, these ferns may be regarded as the potential source of CAM (complementary alternative medicines).

In recent times, natural products of plant origin such as flavonoids, phenols, terpenoids, steroids etc. have gained substantial attention due to its diverse application in therapeutics/nutraceuticals mainly because of its antioxidant activity (Takeoka and Dao, 2003; DeFeudis *et al.,* 2003) Antioxidants are the substances that are involved in inhibiting and scavenging free radicals

thereby protecting humans against various infection and dreadful diseases. Moreover, antioxidant compounds may be extracted using different solvents from the plant samples.

Thus, for determining the antioxidative potential of these ferns, the samples were first extracted in three different solvents namely ethanol, methanol and hot water. As it is evident that differences in the polarities of the solvent plays an important role in extraction, as antioxidant compounds with different chemical characteristics may have solubility difference in solvents (Turkmen *et al.,* 2006). In the present study, the highest extraction yield was observed in methanolic extract of *N. cordifolia* and lowest in ethanolic extract of *D. linearis.* However, in general methanol showed higher extractive potential followed by hot water and ethanol. Higher extraction yield in methanol trend was reported by Hasmida *et al.* (2014) in their study. In our study, the higher extraction yield observed in methanol and hot water than in ethanol may be because of the higher solubility potential of proteins and carbohydrates in methanol and water than in ethanol (Zeliński and Kozlowska, 2000).

Plant derived natural antioxidants has recently received considerable attention because of its multifaceted activity in improving or correcting numerous human disorders/diseases. Moreover, in comparison to the synthetic drugs, ingestion of natural antioxidants are generally accepted to be safer. Presence of diverse kind of antioxidant compounds makes it rather difficult to claim the role of single compound which can be attributed towards the antioxidant potential of the plants. Thus numerous methods varying in the chemistry i.e in terms of target molecules or difference in the generation of radicals have been developed in the measurement of total antioxidant activity. Many reports have been published time to time for revealing the antioxidative potential of ferns (Bora *et al.,* 2005; Gayathri *et al.,* 2005; Wang *et al.,* 2007; Mimica-Dukic *et al.*, 2008; Hort *et al.*, 2008; Lai *et al.,* 2010; Paulsamy *et al.*, 2013; Valizadeh *et al.,* 2015).

In the present study, methods such as DPPH radical scavenging, hydorgen peroxide scavenging, nitric oxide scavenging, superoxide scavenging and ferric reducing antioxidant activity have been employed.

DPPH radical scavenging activity is one of the commonly used methods to determine the antioxidative potential of the extracts. The method is based on scavenging the free stable DPPH radical (2,2'-diphenyl-1-picrylhydrazyl radical) by some of the antioxidant substances present in the plant extracts. DPPH has a deep purple coloration with a characteristic absorption at 517nm which turns into yellow colour after being exposed with the proton donating compounds or antioxidant present in the solution. Higher the colour change, greater is the potential of an antioxidants. A sharp decrease in the absorbance of the reaction mixtures in the present study

revealed considerable antioxidative activity of the plant samples under study. Almost all the samples were able to scavenge DPPH radical irrespective of the solvent used. However, highest activity was observed in the hot water extract of *N. cordifolia* and lowest in the ethanolic extract of *M. punctatum*. Higher DPPH activity in aqueous extracts has been reported by Chai and Wong (2012) in *Selaginella willdenowii* and in *Davallia solida* by Chen *et al.* (2008). However, it was in contrast to the finding of Zakaria *et al.* (2011) where methanolic extract of *Dicranopteris linearis* revealed higher DPPH activity when compared to the aqueous extract and chloroform extract.

Superoxide anion radicals are highly reactive free radicals that are immediately produced into the living cells when oxygen is being taken up by the cells. It is also known to be produced endogenously by xanthine oxidase which is involved in the conversion of hypoxanthine to uric acid. Being highly reactive it has harmful effects on various cellular components leading to innumerable diseases and are effectively involved in peroxidation of lipids (Attarde *et al*., 2011). Generally, biological system is endowed with the power of eliminating its toxic effect by superoxide dismutase (Chung *et al.,* 2005). Interestingly, in the present study all the samples showed very good superoxide radical scavenging activity. Highest activity was revealed by the methanolic extract of *N. cordifolia* and lowest by the ethanolic extract of *M. punctatum.* Our findings was in agreement with that of Saeed *et al.* (2012) who observed higher superoxide activity in methanolic extracts than the other extracts studied. Similarly, methanolic extract of *D. linearis* was observed to exhibit higher activity than aqueous and chloroform extract (Zakaria *et al.*, 2011).

In nature, hydrogen peroxides are present ubiquitously (food, air, water, human body, plants and microorganisms) at low concentrations. It rapidly gets decomposed into oxygen and water molecules which may further lead to the production of hydroxyl radicals through Fenton reaction. This in turn initiates lipid preoxidation and damages DNA (Sahreen *et al.,* 2011). Almost all the samples were able to efficiently scavenge the H_2O_2 in the present study. Methanolic extracts of all the samples showed better H_2O_2 scavenging activity followed by water and ethanolic extract. Amongst the plants *N. cordifolia* showed the highest activity while the lowest was exhibited by ethanolic extract of *M. punctatum*. Attarde *et al.* (2011) had reported methanolic extract of *Limonia acidissima* to exhibit higher hydrogen peroxide activity than the chloroform extract.

Nitric oxide can be generated from sodium nitroprusside at physiological pH. This interacts with oxygen to form nitric ions which can be estimated with Griess reagent at 540nm. Nitric oxide scavenger thereby competes with oxygen causing decrease in the formation of nitric ions. Nitric

oxide (NO) is another very reactive free radical that is associated with various pathological conditions (Chen *et al.*, 2008; Ebrahimzadeh *et al.*, 2009). Thus, scavenging nitric oxide by the extracts may be regarded beneficial for health as it can avoid the harmful effects of excessive NO generation. In the present study, sharp decrease was observed in absorbance with the increase in the concentration. Methanolic extracts of all the samples showed higher activity when compared to the other solvents. *N. cordifolia* showed the highest scavenging activity amongst the plants studied. Similarly, Attarde *et al.* (2011) also reported higher activity in methanolic extract compared to petroleum ether and chloroform extract in their study.

The ferric reducing properties of the extract depends on the ability of an antioxidant compound to donate an electron to Fe (III) (ferricyanide complex) thereby reducing it to Fe^{2+} (ferrous complex).The antioxidants present in the extracts was able to reduce Fe^{3+} to Fe^{2+} which was evident by the increase in the absorbance of the extracts with increase in the concentration at 700nm. Though all the samples irrespective of the solvents revealed considerable reducing power, methanolic extracts of *N. cordifolia* showed the highest while ethanolic extract of *M. punctatum* showed the lowest reducing activity.

Lai and Lim (2011) has also observed similar DPPH activity of methanolic extract of *M. punctatum* while for ferric reducing ability the value was lesser than that of our study. However, our IC_{50} values for *D. quercifolia* and *D. linearis* was higher than their findings. Zakaria *et al.* (2007) reported *D. linearis* to exhibit high DPPH and superoxide scavenging activity. Conversely, Chang *et al.* (2007) observed higher antioxidant activities in water extracts than ethanol extracts. Thus it is rather difficult to choose one suitable solvent system responsible for better antioxidant activity. Hence, it is mandatory to perform thorough study using different solvents for extraction following antioxidant activity as different solvent may have different potentials in extracting antioxidants from the plants.

Phenolic compounds of plants with their hydroxyl groups have been known to play important roles in scavenging the free radicals. In our study too, *N. cordifolia* and *C. dentatus* with higher phenolic contents (total phenol, flavonoid and tannin) with respect to other plants showed higher antioxidative activity. However, it may not be the sole compound responsible for the antioxidant activity as other compounds (like proteins, carbohydrates, lipids, carotenoids etc) may have synergistically contributed towards the activity.

Thus, to understand the contribution of each phytochemical towards the antioxidant activity in individual plants principle component analysis was performed. PCA, in many instances have been employed to examine the relationship between the antioxidant activity and the phenolic

contents of various kind products like medicinal plants and teas (Komes *et al*., 2011; Deetae *et al.*, 2012). In the present study, the contents of various phytochemicals varied in each plant which consequently influenced the antioxidant activity in a different manner. Besides other factors studied, the most positively influencing factors were total flavonoid, total phenols, tannin and lipids as they showed high positive correlation with all the antioxidant activities in almost all the plants. Pereira *et al.* (2014) had reported that the variability in the total phenols and flavonoids contents in the different brands of tea bags revealed difference in the antioxidant activities such as DPPH and ABTS. They observed that green and white teas with higher levels of total phenols and flavonoids exhibited better free radical scavenging activity. Likewise, positive correlation between total phenolic compounds, such as phenolic acids and flavonoids and antioxidant activities such as DPPH, FRAP and ABTS have been reported by many authors Vasco *et al*. (2008), Rufino *et al*. (2010) and Almeida *et al*. (2011). Studies by Chai *et al*. (2013) observed that *Phymatopteris triloba* containing higher flavonoids and hydroxycinnamic revealed positive correlation with antioxidant activities. Further, Alessandra *et al.* (2013) has reported that high loading was observed in principal component 1 for fatty acids in their studies revealing high positive contributions from monounsaturated fatty acids and negative contributions from polyunsaturated fatty acids towards the antioxidant activities. Our findings was similar to that of Khomdram and Singh (2011) who observed high positive correlation of tannin with the free radical scavenging activity in the seeds of eight plants such as *Elsholtzia blanda, E. communis* var. purple flower, *E. communis* var. white flower, *E. stachyodes, Hyptis suaveolens, Ocimum americanum, O. basilicum* and *Perilla frutescens.*

Further, HPLC analysis was performed to identify and quantify different phenolics present in the samples. Amongst the various phenolics identified in the samples, prevalent ones were caffeic acid, ferulic acid and salicyclic acid.

Caffeic acid content was found to be highest in *N. cordifolia* while *C. dentatus* revealed highest ferulic acid and salicyclic acid. Wojdyło *et al.* (2007) had reported higher value of ferulic acid and caffeic acid in thirty two different spices while Proestos *et al.* (2006) had reported lower values of ferulic acid and caffeic acid in aromatic plants than in *C. dentatus* and *N. cordifolia* respectively.

Presence of catechin was recorded in all the investigated plant samples except *C. dentatus* with highest concentration in *N. cordifolia* and lowest in *P. biaurita*. These values were higher than that was reported in *Origanum vulgare* L. by Proestos and Komaitis (2013).

The HPLC chromatogram revealed the presence of gallic acid in *N. cordifolia, C. dentatus* and *M. punctatum* amongst all the other samples studied. Highest concentration was found in *N. cordifolia* and lowest in *C. dentatus*. Our values were higher than those reported by Proestos and Komaitis (2013) in greek aromatic plants. Further, ferulic, vanillic, five chlorogenic acid isomers, 3,4- dihydroxybenzoic acid (DHBA), 4- hydroxybenzoic acid, 4- hydroxycinnamic acid, 4-hydroxycinnamoyl-quinic and caffeic acid has been detected in *Polypodium leucotomos* (Garcia *et al.* 2006). Further, Syafni *et al.* (2012) had isolated 3,4-dihydroxybenzoic acid and 3,4-dihydroxybenzaldehyde from the ethyl acetate fraction of tree fern (*Trichomanes chinense*).

Numerous studies have reported that phenolic in the extracts may have contributed to their biological activities. Wang (2003) reported inhibition of photo-peroxidation of linoleic acid by ferulic acid at higher concentrations. Further, Kim and Lee (2004) reported that the antioxidant activity of *E. purpurea, T. vulgare, Achillea millefolium, Hypericum performatum and O. vulgare* was mainly because of the phenolic acids, mostly caffeic and p-coumaric acid. In their study, they reported that the higher antioxidant activity of caffeic acid than p-coumaric acid was basically because of the 3,4-position of dihydroxylation in caffeic acid. Morever, additional conjugation in the propenoic side chain might have contributed towards higher antioxidant activity of caffeic acid thereby facilitating the delocalization of electrons between the aromatic ring and propenoic group. Similarly, molecules like gallic acid, hydrolysable tannins and flavonols are known to exhibit potent radical scavenging activity as it contains many hydroxyl groups, especially ortho-dihydroxy groups (catechol structure). Further, two phloroglucinol derivatives from *Dryopteris crassirhizoma* has been reported to exhibit antioxidant activity (Lee *et al.,* 2003).

Thus, amongst many other bioactive compounds that would have contributed towards the antioxidative potential of the ferns studied, phenolics like caffeic, ferulic and salicyclic acids may have further added to the antioxidative potential. Major phenolics that might have contributed significantly to the highest antioxidant activity in *N. cordifolia* are phloroglucinol, catechin and gallic acid besides the prevalent ones. However, ferulic and salicylic acid may be the major compounds involved in *C. dentatus* whereas in *D. quercifolia* it may be ferulic, salicylic catechol and catechin while in *P. cuspidatus* and *D. linearis*, catechin and 3,4- Dihydroxybenzoic acid (DHBA) respectively would have been involved in the antioxidant activity. Phenolics like pyrogallol along with phloroglucinol, catechin, ferulic and salicylic acid may have attributed towards the antioxidant activity in *P. vittata* whereas for *P. biaurita* ferulic and salicylic acid may be the main contributors. Catechin, DHBA and gallic acid besides ferulic, caffeic and salicylic acid may have added to its antioxidative reports. However, other phenolics present at the lower

concentrations as well would have contributed towards the antioxidant potential of the ferns under study.

Antimicrobial potential of ferns have been reported time to time by many authors (Banerjee and Sen, 1980; Singh *et al.,* 2008; Dalli *et al.,* 2007; Lai *et al.,* 2010; Ganguly *et al.,* 2011). In this line of work, attempt has been made in the present study to demonstrate the antimicrobial activity of three ferns selected on the basis of their activities (*N. cordifolia, C. dentatus* and *M. punctatum*) extracted in methanol and hot water. Prominent antibacterial activity against both gram positive and gram negative bacteria was observed by all the plant extracts. Methanolic extracts in all the cases exhibited higher antimicrobial activity. Among the plant samples, *N. cordifolia* showed highest inhibition zones for all the bacteria tested and lowest by *M. punctatum*. However, it was in contrast to the finding of Rani *et al.* (2010) where aqueous extracts of all the three plant samples (*Psilotum nudum, Nephrolepis biserrata* and *Nephrolepis cordifolia*) were found to be effective in inhibiting the growth of both bacteria and fungi at varying degree. Nonetheless, irrespective of the concentrations used the zone of inhibition against *B. cereus* by the water extract of *N. cordifolia* was similar to our findings. Conversely, Kandhasamy *et al.* (2008) has reported higher antibacterial activity of methanolic and ethanolic extracts of *D. quercifolia* in their study. Kumar and Kaushik (2011) had observed antibacterial potential of ethanolic and chloroform extract of *Christella dentata* Frosk. (*C. dentatus*) against *Bacillus subtilis, Escherichia coli, Salmonella typhi and Staphylococcus aureus.* However, zone of inhibition was much lesser in comparison to our findings.

Since, in the present study all the extracts were able to inhibit the growth of both gram positive and gram negative bacteria, these ferns may be a potential source of broad spectrum antibacterial agents. However, the mechanism as to how they control the activity is to be further examined.

Likewise, higher antifungal activity was recorded for methanolic extracts of *N. cordifolia* which was evident by both its potential to inhibit the spore germination and radial growth of all the three fungi used in the present study. The finding was not in agreement with of Rani *et al.* (2010) where water extract of *N. cordifolia* revealed higher inhibition zones than the other solvents used. Likewise, antifungal activity of different fractions of *P. biaurita* has been reported by Dalli *et al.* (2007) where highest inhibition of radial fungal growth was observed by the fraction III obtained by hydrolysates further extracted with ethyl acetate.Similar to the present study, Kanan and Al-Najar (2008) have also reported comparatively higher antifungal activity by the methanolic extracts of the plants under study. They observed that the growth of *Penicillium digitatum* was more effectively inhibited by the methanolic extract of cinnamon bark than the other fractions.

Likewise, complete inhibition was observed only by the methanolic fraction of garlic. On the other hand, the most effective fraction of sticky fleabane leaves and harmal seeds was the methanolic and hexane fractions. On the contrary, besides methanolic fraction, the aqueous fraction of nightshade leaves revealed the most effective inhibitory activity.

Presence of various bioactive compounds which would have been better extracted in methanol may have contributed towards the antimicrobial activity. The inhibitory activity of catechin has been reported by Chunmei *et al.* (2010). Similarly, components like 3,4- dihydroxybenzoic acid, isolated terpenoid like Friedelin have been effective against both gram positive and gram negative bacteria (Chiozem *et al.*, 2009; Khan *et al.*, 2007). Additionally, compounds like cinnamaldehyde, eugenol and cinnamic acid have been shown to exhibit to antifungal activities (Inouye *et al*., 2000; Gill and Holly, 2004).

Diabetes is one of the prevalently occurring diseases characterized by the change in glucose, lipids, carbohydrates and protein metabolism (Bussa and Pinnapareddy, 2010). Diabetes has now taken the form of a global human epidemic. Synthetic drugs used in the treatment have been reported to show adverse effects. Thus, in the recent years medicinal plants with antihyperglycemic activity have been greatly explored (Priyadarsini *et al.*, 2010; Mary and Gayathri, 2014; Gupta *et al.*, 2015).

Since the previous experiments proved that the selected ferns possess significant phytochemicals as well as antioxidant properties, it was considered worthwhile to determine whether any of these ferns antihyperglycemic activity. For this part of study, methanolic and hot water extracts of *N. cordifolia, C. dentatus* and *M. punctatum* were selected. In the beginning, *in vitro* activity was tested by α-Amylase inhibition studies.

α-Amylase is one of the key enzymes present in the small intestine which is responsible for carbohydrate metabolism breaking down polysaccharides to monosaccharides, thus increasing the postprandial blood glucose level. Inhibition of α-amylase activity postpones digestion of carbohydrate and glucose absorption in turn leading to the reduction of postprandial hyperglycemia (Bressler and Johnson, 1992). Among the extracts, methanolic extract of *N. cordifolia* and hot water extract of *M. punctatum* revealed the highest and the lowest α-amylase inhibitory activity respectively in a dose-dependent manner. Similar inhibitory activity has been reported in *Thespesia populnea* by Sangeetha and Vedasree (2012) in which ethyl acetate and methanol extracts showed comparatively better activity than the petroleum ether and chloroform extract. However, our finding was in contrast to that of Jiju *et al.* (2013) who reported higher α-

amylase activity by the aqueous leaf extracts of *Carica papaya* followed by ethyl acetate and alcohol.

Further, considering the hazard that may be caused from methanol consumption, aqueous extracts of two samples (*N. cordifolia* and *C. dentatus*) showing better α-amylase inhibitory activity was chosen for further *in vivo* antidiabetic activity.

Initially, diabetes was induced partially in rats by streptozotocin. Streptozotocin (STZ) leads to β-cells necrosis by selectively causing β-cells cytotoxicity through generation of various free radicals which in turn leads to impairment in glucose metabolism (Papaccio *et al.*, 2000). Severe decrease in blood glucose levels and body weight are the characteristic features of STZ-induced diabetic rats. Due to improper glucose utilization breakdown of fat and protein is accelerated in hyperglycemia. Moreover, structural proteins are involved in maintaining the body weight. Since in hyperglycemia, rate of protein catabolism far exceeds its production, there occurs continuous decrease in the body weight of the untreated diabetic rats (Ramesh and Pugalendi, 2006). In this study, treatment of diabetic rats with the *C. dentatus* and *N. cordifolia* could prevent the loss in body weight. This may be possibly because of the improvement in glucose metabolism due to increase in insulin level by the extracts. Though both the extracts showed protection against body loss, however, maximum protective effect against body loss was observed in the rats treated with *C. dentatus* and minimum by the extracts of *N. cordifolia*. Likewise, diabetic rats treated with *C. dentatus* were able to exhibit maximum decrease in the blood glucose level. Treatment by the extracts may have prevented β-cells from further damages by STZ, in turn increasing the insulin release. Our result was in concordance to that of Tanzin *et al.* (2013) who reported the dose-dependent reduction in the blood glucose levels of diabetic rats by the methanolic extracts of *Christella dentata.*

Further, alteration in lipid profile has been encountered in diabetes mellitus increasing the risk of cardio-vascular diseases (Maghrani *et al.*, 2005). Increased cholesterol and triglycerides level with the decrease in HDL-cholesterol are commonly reported in hyperglycemic condtion (Uttra *et al.*, 2011). In the present investigation, amongst all the groups, those receiving *C. dentatus* extract at higher concentration resulted in the maximum reduction of serum cholesterol and triglyceride level and an increment in the HDL-cholesterol compared to the untreated diabetic rats. Similar effects of aqueous and ethanolic extract of *Adiantum philippense* Linn. has been reported by Paul *et al.* (2012).

Additionally, elevation in the levels of liver marker enzymes (SGOT and SGPT) is common in the diabetic patients leading to hepatoxicity (Ghosh and Suryawanshi, 2001). In the present study

administration of extracts to the group of diabetic rats was able to bring down the level of transaminases (hepatic biomarkers like SGPT and SGOT) to near normal which correlates with the findings of Dey *et al.* (2015). They reported the efficacy of *Nerium oleander* leaf extracts to decrease in the liver markers enzymes in alloxanized animals. Thus, it could be suggested that both *C. dentatus* and *N. cordifolia* had the ability to restore the normal liver functions.

Furthermore, increased levels of serum urea and creatinine are regarded as chief markers of nephropathy in diabetic rats (Idonije *et al.*, 2011). Thus, the reduction in urea and creatinine levels of diabetic rats after treatment with the *C. dentatus* and *N. cordifolia* indicated that these extracts may have prohibited further kidney damages in the diabetic treated rats.

An interesting finding was that higher concentration of *N. cordifolia* showed lesser *in vivo* activity which correlates with the findings of Kasabri *et al.* (2011) who observed that the aqueous extract of *Sarcopoterium spinosum* and *Pistacia atlantica* at lower concentration caused higher reduction in postprandial glucose level than higher concentration. Moreover, auto-inhibitory activity of the extracts has been reported at higher concentrations of the extracts (Prince *et al.,* 1998). In addition to that, plant extracts may contain some hyperglycemic compounds, besides hypoglycemic components (Murthy *et al.,* 2003).

Moreover, tocopherol, phenolics like catechin, epicatechin, gallic acid and 4-hydroxybenzoic acid has been reported to modulate the glucose metabolism in various ways. Thus, presence of these bioactive components may have contributed towards the antidiabetic activity of these plants. However, the exact mechanism for *in vivo* antidiabetic activity of these plants still remains unclear.

It was observed that while both the extracts showed both *in vitro* and *in vivo* antidiabetic activities, there was difference among the two. While *N. cordifolia* had shown better *in vitro* activity, *C. dentatus* showed better *in vivo* activity. It is thus clear that while *in vitro* tests can be considered as an indication of the activity, body responses may vary, due to several internal factors which may be interacting. Hence, for proper understanding such studies should be based not only on *in vitro tests*, but also on *in vivo* tests which can validate the *in vitro* results.

Understanding the chemical nature of the extracts following the isolation and characterization of lead compounds responsible for biological activities is important for pharmaceutically exploiting these compounds, thereby leading to the discovery and development of newer drugs (Mariswamy *et al.,* 2011).Thus, we have made an attempt to partially purify and characterize the active compounds underlying the various activities shown by *C. dentatus* and *N. cordifolia* extract.

Column chromatographic technique using solvents at different ranges from non-polar to polar (Hexane to methanol) was employed in the present study. It is evident from the previous records that the solvent system with varying partition coefficient (K) for each target compound is necessary for successful separation of the biomolecules (Ito, 2005). Highest yield (in gram) was obtained in Hexane:EtOAC (1:1) solvent system of *C. dentatus* (*Cd*B) and in EtOAC:MeOH (1:1) of *N. cordifolia* (*Nc*D) extracts giving an indication that *C. dentatus* may be rich in non-polar compounds while *N. cordifolia* may be partially polar.

All the fractions revealed higher antioxidant (DPPH activity) and antidiabetic (α-amylase inhibitory activity), however highest antioxidant activity was shown by *Cd*B of *C. dentatus* while *Nc*E of *N. cordifolia.* On the contrary, *Cd*C and *Nc*C fractions of respective plants were shown to exhibit higher α-amylase inhibitory activity. Kumar *et al.* (2011) had observed higher antioxidant and α-amylase inhibitory activity in the methanolic (100%) eluted fraction of *Asystasia dalzelliana* leaves.

Interestingly, all the eluted fractions revealed higher antioxidant (DPPH activity) and antidiabetic (α-amylase inhibitory activity) than the crude extracts. This can be expected, since fractionation and purification leads to concentration of the active compounds in these specific fractions. Crude extracts would be containing a large number of other compounds and hence the active compounds would be in lesser amounts or its activity may be inhibited by other compounds.

Further, characterization of these eluted fractions through GC-MS revealed an array of compounds comprising of terpenoids, fatty acids, phytosterols, phenylpropanoids and phenolics. *Cd*B fraction was identified to have sixteen different compounds with 2,2,7.7-tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one showing highest relative abundance. Likewise, *Cd*C with Cycladopentanone,2,5- dicyclopentylidene-l, *Cd*D with 2(1H)Naphthalenone, 3,5,6,7,8,8a- dimethyl-6-(1-methylethenyl)- and CdE with cyclotetracosane also showed high relative abundance.

Similarly, *Nc*B was identified with thirteen compounds, *Nc*C with eleven, *Nc*D with ten and *Nc*E with seven different compounds. Highest relative abundance in *Nc*B was of nonadecane, *Nc*C was of pentadecanoic acid, 13- methyl, methyl ester, *Nc*D was of pentadecanoic acid, 13- methyl, methyl ester and *Nc*E was of 1- Oxycyclopentadecan-2- one,15,15-dimethyl.

Our finding was in agreement with the previous studies, as most of the identified compounds in our study have been reported in some other plant extracts as well. For instance, compounds like hexadecanoic acid methyl ester, pentadecanoic acid, 14- methyl- methyl ester, hexadecane,

nonadecane, eicosane, phytol, docosane and 1,2-benzenedicarboxylic acid, mono(2- ethylhexyl) ester- was identified in the leaves of *Azadirachta indica* (Akpuaka *et al.,* 2013). Compounds like 10-Octadecenoic acid, methyl ester and phytol has been identified in *Jatropha curcas***,** *Psidium guajava* and *Andrographis paniculata* by (Rahman *et al.,* 2014) while Phenol 2,4-Bis(1,1-Dimethylethyl)- in *Pinus granatum* rind extract by Prakash and Suneetha (2014). Tetracosane, tricosane, eicosane and octadecane was identified in *Salvia palaestina* and *S. ceratophylla* besides numerous other essential oils (Gürsoy *et al.,* 2012).

Furthermore, almost all the compounds revealed in the present study have been reported to possess various biological activities. Hexadecanoic acid, methyl ester and 10-Octadecenoic acid, methyl ester have been reported to exhibit antifungal and antimicrobial activity (Hema *et al.,* 2011; Asghar *et al.,* 2011). Similarly, eicosane is known to possess antifungal, antibacterial, antitumor and cytotoxic activity (Hsouna *et al.,* 2011). Likewise, 1,2- Benzenedicarboxylic acid, mono(2-ethylhexyl) ester are known for its antifungal, antiretroviral, antitumor, antidiabetic, anti cancer, antioxtioxidant, anti scabies, anti inflammatory and antimicrobial potentials (Syeda *et al.,* 2011; Balachandran *et al.,* 2012; Bagavathi and Ramasamy, 2012). Phytol is known for its antioxidant and antimicrobial activity Rani *et al.* (2011). Oleic acid has been known to regulate blood pressure, lipid levels, immune and inflammation responses (Meechaona *et al.,* 2007). Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- is reported to show antidiabetic acitivity (Adeneye and Agbaje, 2008; Dannemann, 2008). Heptadecanoic acid, 16-methyl, methyl ester has been known to exhibit antioxidant and antimicrobial activity (Lalitharani *et al.,* 2010).

The compounds identified in these samples may act as potential ligands or lead compounds against antidiabetic target proteins/enzymes. Morevover, both the extracts had revealed appreciable results in an *in vivo* antidiabetic study; thus, we have made an *in silico* molecular approach to understand some of the possible mechanisms of action behind the antidiabetic activity of *Nc* and *Cd* and to select the potential antidiabetic components by docking some of the compounds against target proteins/enzymes involved in glucose metabolism such as glucokinase (IV4S), fructose 1,6- bisphosphatase (2JJK), 11β-Hydroxysteroid dehydrogenase type 1 (2BEL) and peroxisome proliferators activated receptor gamma (3DZY).

Glucokinase (GK/ IV4S) plays an important role in glucose metabolism and is expressed only in liver and pancreatic cells. In pancreatic β-cells, glucokinase is the rate limiting enzyme and is involved in the determination of rate of glucose-induced secretion of insulin whereas in liver it catalyzes the first step in glucose metabolism and determines the rate of utilization and synthesis

of glucose and glycogen respectively (Ferre *et al.*, 1996; Grimsby *et al.*, 2003). The activation of glucokinase activity may cause reduction in the glucose level as it may enhance the release of pancreatic insulin thereby improving the glucose metabolism in liver cells. Diabetes leads to the decrease in the activity of several enzymes including glucokinase either due to total or partial deficieny of insulin causing disruption in glucose metabolism which in turn leads to impaired utilization of glucose and increased hepatic glucose production. Besides many other compounds showing affinity with glucokinase, corynan-17-ol, 18,19- didehydro-10-methoxy-acetate (ester) and propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- possess better interaction with the IV4S, which may lead to the activation of glucokinase thus increasing the utilization of glucose and leading to decreased blood sugar level. Chandramohan *et al.* (2008) reported that diabetic rats treated with 3-hydroxymethyl xylitol (3-HMX) active principle from *Casearia esculenta* (Roxb.) efficiently increased the glucokinase activity.

Fructose 1,6- bisphosphatase (2JJK) and glucose-6-phosphatase are the primary enzymes involved in the regulation of gluconeogenic pathway. The activity of 2JJK in liver is known to increase during diabetic condition leading to the increased production of glucose which may be because of the increased enzymes synthesis (Pederson *et al.*, 2005; Mitra *et al.*, 1995). Insulin can normally suppress the activity of fructose 1,6- bisphosphatase and glucose-6-phosphatase thereby causing inhibition of hepatic glucose production (Wiernsperger and Bailey, 1999; Chen *et al.* 2000). In our study, many of the compounds docked with 2JJK showed good binding affinity, however, the best fit was revealed by corynan-17-ol, 18,19- didehydro-10-methoxy-acetate (ester) and propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-. Mahendran *et al.* (2014) based on their docking studies reported the inhibition of 2JJK activity by the compounds 1,2,8-trihydroxy-6-methoxy xanthone and 1,2-dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl isolated from *Swertia corymbosa.*

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is the enzyme involved in the conversion of inert inactive 11 keto-products (cortisone) to active cortisol or vice versa which is one of the major cause for obesity and metabolic syndrome (Seckl and Walker, 2001; Anagnostis *et al.*, 2009). The possible involvement of 11β-HSD 1 in this conversion to cortisol which in turn may be involved in a pathogenic role in Type 2 diabetes has been reported by earlier workers (Morgan *et al.*, 2009). It is thus possible to inhibit the binding of cortisol to glucocorticoid receptor and thereby converting active cortisol to the inactive form could be an alternate method for treating diabetes and obesity. Recently, there has been an increase in the number of patents for 11β -HSD 1 inhibitors with the reports of total of 15 applications registered by the US Patent office for 11β -

HSD 1 inhibitors, out of which, 10 bioactive compounds (most of them in phase I clinical trials) against 11β -HSD 1 are being employed in the development of treatment for Type 2 Diabetes mellitus (Research and markets.com, 2012). In the present study, compounds corynan-17-ol, 18,19- didehydro-10-methoxy-acetate (ester) and propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- possess greater binding affinity with 11β-HSD 1. Ramirez-Espinosa *et al.* (2013) has reported e *in silico* inhibition of 11β-HSD 1 by compounds morolic, moronic, urosolic and oleanolic acid.

Some other factors associated with diabetes are carbohydrate metabolism and differentiation of 3T3-L1 adipocytes. Peroxisome proliferators activated receptor gamma (PPAR-γ) plays an important role in 3T3-L1 differentiation (Po-Jung *et al.*, 2005). It is the nuclear receptor family protein and is ligand-activated transcription factor that is expressed most abundantly in adipose tissue. PPAR-γ is involved in mediating the antidiabetic activity of the drugs belonging to the thiazolidindione (Campbell, 2005). PPAR-γ being one of the key transcriptional factors plays an important role in the regulation of adipogenesis, insulin sensitivity and glucose homeostasis (Willson *et al.*, 2000). In our study, the best fit with PPAR- γ (3DZY) was revealed by corynan-17-ol, 18,19- didehydro-10-methoxy-acetate (ester) and propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-. Deepa *et al.* (2013) reported compounds 5-hydroxy-7,8-dimethoxyflavanone and 17,19,20-trihydroxy-5beta, 8α H, 9beta H, 10 α-labd-13-En-16,15-olactone identified in *Andrographis* sp to interact with receptors C/EBP-α and PPAR- γ with maximum fitness score.

Based on the molecular docking studies compounds corynan-17-ol, 18,19- didehydro-10-methoxy-acetate (ester) and propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)- may be considered as potential antidiabetic agents as hit structures for designing potent and specific drugs. These compounds along with other similar ones may have concomitantly helped to exhibit the antidiabetic activity by either activating glucokinase or inhibiting the activity of other proteins/enzymes involved in glucose metabolism. However, further validation of the compounds through *in vivo* studies, toxicity studies, clinical trails and pharmacokinetic studies are required before these compounds find their application in pharmaceuticals.

Presence of diversified types of bioactive components may have attributed to the antioxidant, antimicrobial and antidiabetic activities exhibited by these ferns. Moreover, GC-MS analyses of *N. cordifolia* and *C. dentatus* revealed the presence of compounds efficient in antimicrobial activity which may be one of the reasons behind prominent antimicrobial activity. There is no

previous report of the identification of such compounds from these ferns or their biological activities of this region.

These ferns could be considered as the potential source for exploiting their applications in pharmaceuticals. However, further detailed investigations are required for the isolation and characterization of respective active compounds along with elucidating the mechanisms involved in biological activities.