In the Western Ghats, the plant diversity is mostly dependent on the seasonality in the region (Davidar et al., 2005). More than 27 species of orchids have been discovered in the Western Ghats of India in past fifty years (Aravind et al., 2007). In our study we found ten different species of *Dendrobium* in various hotspots and spill over regions in Karnataka. *Dendrobium* has been floristically less explored in these regions of Western Ghats. In the present study it was observed that the requisite factors required for thriving of *Dendrobiums* are relative humidity, temperature, rainfall light, altitude, and the phorophyte. Most of the species varies in respect to the requirement from their habitat to the tolerance level to the fluctuations in the environmental conditions. Ecological amplitude is the spectrum of requirements and the following range of tolerance of a species (Sharma., 2005). There is specific ecological amplitude for each species for successful growth.

During the critical conditions, the plant adapts itself by producing several structural, chemical and physiological characteristics. The production of secondary metabolites is also a part of plant defense mechanism against pests, herbivores and pathogens. A tremendous range of secondary metabolites are produced from shikimic acid pathway or aromatic acid. The main class of secondary metabolites that are produced by the plants: flavonoids, polyphenolic compounds, terpenoids, alkaloids, sulphur containing compounds, etc., (Theis and Lerdau, 2003; Zhao et al., 2005).

Phenolic compounds like tristin, gigantol, confusarin and moscatilin are regarded as reference phenol and were studied in *Dendrobium* species (Yang et al., 2004, 2006; Zhang et al., 2005, Thomas et al., 2016). The phytochemical analysis of the species confirmed the presence of several potent phenolic compounds. These
secondary metabolites are produced as a metabolic byproduct in the plant system and is reported to have medicinal properties (Mantle et al., 2000). It serves as a defense molecule against insects, pathogens and animals (Grover et al., 2002). To the best of our knowledge, this is the first study reporting the presence of moscatilin, resveratrol, gigantol, tristin and confusarin in Dendrobium species (hybrids and wild) of the Western Ghats of Karnataka, India. Furthermore, the results obtained showed that Dendrobium species collected are a good source of stilbenes like moscatilin and other reference phenolic compounds annually. From the phytochemically enriched Dendrobium, there is a possibility of developing more value-added products and nutraceuticals to increase the health benefits.

The DNA isolation protocol adapted for the study was found to be very rapid and yielded high quality DNA which is a pre-requisite for any downstream molecular process. Similar observations were made by Ramirez-Parra et al. 2003 (Ramirez-Parra et al., 2003). Bentley & Bassam 1996 were of the opinion that >1mM magnesium chloride concentration was needed for plants which is true in our study (Bentley and Bassam, 1996). However, concentrations above 2mM resulted in poor or no amplification. This could be attributed to the non-specific binding of Taq at higher concentrations resulting in poor or no amplification. BSA was found to be a better adjuvant than DMSO in the present study which is lined with the observations of Swain & Sarkar 2013 and Kreader 1996 (Kreader, 1996; Swain and Sarkar, 2013). Also increasing concentrations of DMSO was found to be inhibitory. Highest polymorphism of 100% and lowest of 80% with an average of 91.07% polymorphic marker per primer was seen. Similar results were obtained by Zha et al., although, decamers and species included were different (Zha et al., 2009).
The dendrogram obtained can be co-related with their habitat and morphological features. Species in the first cluster (*D. heterocarpum, D. barbatulum, D. aqueum, D. herbaceum*) are from around Madikeri which is situated at an altitude of >5000ft above sea level. Their position in the tree also suggests that their evolution happened sometime in the recent past. Their leaves are leathery, large and the stem morphologies resemble one another. The placement of the three plant types (*D. ovatum, D. crepidatum, and D. macrostachyum*) in the second cluster was expected, which corroborates the barcoding data. The plants in this group are found at altitudes <1000ft below sea level and restricted to regions of Karkala, Udupi, Kudremukh and Kollur. Leaves are generally oblong and stem is long and slender in all the three species. Before the flowering season, leaves are deciduous and all the three species look similar in appearance making it hard to distinguish them. Results of RAPD and DNA barcoding analysis showed polymorphism in the studied species. The placement of the seven species into two distinct clusters is appropriate and highlights the utility of the RAPD as an efficient dominant molecular marker. RAPD and DNA barcodes allowed assessing the genetic variability among the collected *Dendrobium* species. The DNA barcodes assessed variability in several regions dispersed throughout the genome (Zietkiewicz *et al.*, 1994). The RAPD markers, used in this study are extensively used for analyzing genetic diversity in other plants (Sureja *et al.*, 2006; Guerra Jr. *et al.*, 2010).

The DNA in this instance was extracted from two dissimilar looking plants at different stages of their lifecycle. This indicates that identification of congeneric species solely on their morphology could prove to be a pitfall. Similarly, *D. ovatum* and *D. barbatulum* were not placed in separate clades but included in the same group.
with zero genetic distance. Both the plants were morphologically very suggesting very less variance and hence the placement. Very low levels of nucleotide substitution in chloroplast genome have been reported and the placement could be ascribed to this reason (Wolfe, 1987). Also, uniparental inheritance of chloroplast genomes even after speciation is a plausible explanation. All the other plants were correctly resolved and identified which shows that matK is a reliable barcode for species identification. Our results are in concordance with Lahaye et al., 2008, where matK was proven to rightly identify >90% of the orchids included in their study.

After Majumder and Sen isolated moscatilin from D. moscatum, it was Chen et al who extracted it from D. loddigesii (Majumder and Sen, 1987; Chen et al., 2000). Modern studies have shown that compounds extracted from Dendrobium orchids have potential anticancer properties (Chanvorachote et al., 2013; Kowitdamrong et al., 2013). To understand the anticancer property of moscatilin, its inhibitory activity on cell migration and proliferation was evaluated in the present study. This is the first report evaluating the effect of moscatilin extracted from wild Dendrobium collected from Western Ghats of Karnataka.

To determine the inhibitory property of moscatilin on cancer cell migration, cytotoxicity assay was performed using methanol as solvent and was compared to results of Mitomycin C drug. According to the present data, breast carcinoma cells (MCF7) is the most sensitive cells when compared to the other cancer cells used in the study. The osteosarcoma cells (Saos2) and hepatocarcinoma cells (HepG2) had similar sensitive pattern to moscatilin. The results showed that solvent control did not have any significant effect on the viability on the cells. It was found that the proliferation of
the cancer cells by moscatilin was inhibited in a dose-dependent manner. A bibenzyl compound similar to moscatilin termed TDB, extracted from *D. ellipsophyllum*, was also a potent anticancer compound against lung cancer (Chaotham *et al.*, 2014). Moscatin, an active principle isolated from *D. loddigesii* also showed similar results as moscatilin. Moscatin showed antiplatelet properties by inhibiting collagen-induced platelet aggregation (Chen *et al.*, 2000). The cell viability was examined using MTT assay. In the recent decade, it was unraveled that moscatilin causes early DNA double stranded break which results in moscatilin-induced apoptosis in colorectal cancer cells (Chen *et al.*, 2008).

Our study on protoplast cross-culturing showed the rare combinations of metabolites and phytochemical assemblage *in vitro*. It is important to standardize the yield, variability, and growth maintenance medium for live and dividing protoplast to achieve a consistent yield of rare and low produce natural products. Selection medium allows in the assessment of the division rate of atypical protoplast colonies from heterokaryons and their biomass growth. This technique of protoplast fusion may serve as an exemplary method to accumulate compounds which are constrained to specific tissue or species. It also stimulates new approach for biosynthesis related studies. Novel hybrids of *Dendrobium* species can be generated through this technique. It is very vital to formulate a technique to increase the efficiency of interspecific crosses and also to investigate their potency (Pati *et al.*, 2008). Production of many high yielding crop varieties can be facilitated through protoplast fusion (Liu *et al.*, 2007; Jiang *et al.*, 2013). Maximum protoplast density was obtained from *D. ovatum* leaves which could be due its slender texture compared to
hybrid leaves. In protoplast isolation, optimization of the enzyme concentration is the utmost critical step. The enzyme concentration is essential in generating increased biomass of healthy and viable protoplasts.

It also maintains generated protoplasts in an osmoticum-based growth medium for further experiments. Cellulosic fibres are nested in the plant cell wall matrix. The plant cell wall is made up of hemicelluloses and pectin which has to be digested for the generation of protoplasts. Hence, the appropriate proportion of the cellulase and pectinase was efficient for dissolution of the cell wall. Similar studies on winged bean and crown gall cell line of *Parthenocissus tricuspidata* showed that protoplast regeneration was successfully done with Percoll gradient when compared to sucrose-mannitol gradient system (Fakhrai *et al*., 1988). Being osmotically inert, Percoll gradient showed increase in viscosity level above 20%. Due to which Percoll gradient was more preferred than sucrose or mannitol gradient. After the protoplast were generated. The cells were stained using a panel of fluorescent dyes to understand the vigor prior to the fusion protocol.

The use of dyes helped in identifying the heterofusants and atypical microcolonies. There was only limited success in producing interspecific hybrids in orchids due to incompatibility sexually. Protoplast fusion has enabled to promote genetic traits in plants (Melchers *et al*., 1992; Belarmino *et al*., 1996; Jarl *et al*., 1999), mushrooms (Sunagawa M, 1992; Zhao and Chang, 1995, 1996) and many fungi (Kiyohara *et al*., 1990; Kirimura *et al*., 1997) for many years. It is vital to remove the homokaryons and unfused protoplast from the culture medium but it was retained in our study as metabolite profiling was to done. The rise and fall of stilbenes were
analyzed with flavonoid pathway as the pathway for moscatilin has not been identified yet. The levels of natural products in vitro varied mostly because of the substrate utilization and diverse enzyme activity.

Thus, the study demonstrated that natural product assemblage can be achieved through protoplast fusion which is usually inhibited in tissue or species specific manner. Chemical fingerprinting studies, for example, this is noteworthy as they would help in recognizing key chemotaxonomic markers and biosynthetic pathway of stilbenes in family Dendrobium.