Summary and Conclusions
In the present era, rapid occurrence of multi-drug resistance (MDR) and extreme drug resistance (XDR) of fungi has prompted researchers to go for newer and effective drugs. In this quest, medicinal plants and their byproducts prove to be important therapeutics against these opportunistic pathogens with fewer side effects. Hence much attention has been paid to plant derived antifungal compounds based on the knowledge that plants have their own defense system. Hence naturally occurring biologically active plant products are used as an alternative strategy to prevent the spread of diseases; and it has became the part of modern science approach to find newer drugs against pathogenic organisms. Hence, there has been increasing search for new antifungal compounds owing to the lack of efficacy, side effects and or resistance associated with some of the existence drugs. Contrary to synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious disease.

- Seeds of *Dillenia indica*, whole plant of *Nervilia aragoana* and leaves of *Atlantia monophylla* were screened for antifungal activity with different solvents of non-polar to polar (hexane, ethyl acetate, acetone and ethanol). Different fungi like *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae* were used as test organisms in the antifungal studies.

- Disc-diffusion (Zone of inhibition) and Minimum Inhibitory Concentration methods were employed for *in vitro* antifungal activity tests. Hexane extract of seeds of *Dillenia indica* exhibited more antifungal activity than other plant extracts as compared to other solvent extracts. It exhibited 32mm zone of inhibition diameter and 1mg/ml MIC value. It also exhibited antifungal activity on all organisms except on *Cryptococcus neoformans*. Hexane extract of seeds of *Dillenia indica* was selected for further studies as it exerted higher activity on *Candida albicans*.

- Screening for secondary metabolites revealed the presence of phenols, triterpenoids, fatty acids, and carbohydrates in hexane extract of *Dillenia indica*. Carbohydrates and flavonoids are present in the ethyl acetate extract of
N. aragoana. Steroids, triterpenoids, alkaloids and tannins are present in the ethanol extract of A. monophylla.

Gas Chromatography-Mass Spectroscopy revealed the constituents of hexane extract of Dillenia indica. Fatty acids, triterpenoids, esters of carboxylic acid were present as main constituents in the hexane extract of Dillenia indica. Isolation of bioactive fraction from hexane extract of Dillenia indica showed 8:2 fraction exhibited antifungal activity and the constituents were analyzed by GC-MS analysis.

The hexane extract of Dillenia indica at 1 mg/ml concentration decreased the growth rate of Candida albicans at absorbance 540 nm. When hexane extract of Dillenia indica was added at 1 mg/ml concentration, the growth rate was 0.29 O.D at 540 nm, whereas the culture control exhibited 0.58 O.D at 540 nm.

A study on mechanistic action of hexane extract of Dillenia indica on Candida albicans exerts its action on cell wall, which is made up of intact glucans and mannoproteins. It acts by breaking the intact glucan moiety into monosaccharides, as estimated by DNS method. It also exerts its action on proteins showing degradation, as estimated by Bradford method and causing death of cell.

SAP is extracellular proteins, a key virulence factor. Different concentrations (0.5-2 mg/ml) of hexane extract of Dillenia indica were treated with log phase cultures of C. albicans. Velocity of SAP activity was determined by the absorbance at 280 nm. SAP activity gradually decreases with the hexane extract of Dillenia indica treated compared to control cells. When the extracellular proteins were treated with different concentrations of hexane extract of Dillenia indica and by keeping the substrate concentration constant, there was no change observed in all the treated cultures.

It was shown that total SAP was destroyed by hexane extract of Dillenia indica, which was confirmed by SDS-PAGE (10 %) electrophoresis.
Kinetic studies of SAP were determined by Michaelis-Menten, Lineweaver-Burk and Hanes-Woolf plot. $V_{\text{max}}$ was found to be $V_{\text{max}} = 0.0560 \mu M/\text{mg/min}$; and $K_m = 0.1107 \mu M$ in Michaelis-Menten, in Hanes-Woolf $V_{\text{max}} = 0.058 \mu M/\text{mg/min}$; and $K_m = 0.174 \mu M$. From Lineweaver-Burk plot $V_{\text{max}} = 0.06 \mu M/\text{mg/min}$; and $K_m = 0.5 \mu M$.

In vitro antioxidant activity of hexane extract of *Dillenia indica* was carried out by DPPH free radical scavenging activity, screening of TLC-DPPH method, $H_2O_2$ scavenging activity, ABTS radical scavenging activity, reducing power methods, and Superoxide anion scavenging activity.

Toxicity studies of hexane extract of *Dillenia indica* showed no mortality up to 3 g/kg bd.wt. in wistar albino rats. Sub-acute toxicity studies for 21 days also showed no alterations in body weights, organs such as liver and kidney. There was no change in alterations in Serum urea, uric acid, creatinine, protein, bilirubin, triglycerides, cholesterol were estimated in the rats after administration of hexane extract of *D. indica* at doses of 250 and 500 mg/kg bd.wt.

Induced CCl$_4$ toxicity studies were also carried out to check the hepato and nephro protective effect of hexane extract of *Dillenla indica*. The levels of AST, ALT, ALP, bilirubin, urea and creatinine levels were significantly increased and protein content was significantly decreased in CCl$_4$-induced liver damage rats, when compared with control group (P<0.05). On the other hand, the treatment with hexane extract of *Dillenia indica* at doses of 250 and 500 mg/kg.b.wt in CCl$_4$ induced liver damaged rats showed decreased activity of serum enzymes, bilirubin, urea and creatinine and increased the protein content when compared to the control group of rats with CCl$_4$ alone (p<0.05).

Antioxidant enzymes such as Superoxidedismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX), Glutathione Reductase (GR), Glutathione-S-transferase (GST) were estimated in the control, CCl$_4$ group and in
Experimental groups of CCl₄ and hexane extract of Dillenia indica treated rats. There was significant decrease of all these enzymes in CCl₄ alone groups as compared to control (p<0.01), and significant increase in experimental groups of HEDI treated rats. Non-antioxidants such as, Reduced glutathione (GSH), vitamin-C and vitamin-E were estimated in the control, CCl₄ group and in experimental groups of CCl₄ and hexane extract of Dillenia indica treated rats. There was significant decrease in CCl₄ alone groups of all these enzymes compared to control (p<0.01), and caused significant improvement in experimental groups of hexane extract of Dillenia indica treated rats.

- DNA from whole blood was isolated by using triazole kit. Blood DNA damage was analyzed by running agarose gel electrophoresis.

- Efficacy of hexane extract of Dillenia indica was also checked, in vivo on Wistar albino rats. Cutaneous and oropharyngeal candidiasis models were used to study. Experimental models of cutaneous and oral candidiasis in rats have been shown to be simple and highly reproducible in vivo method of studying antifungal efficacy of medicinal plant extracts.

- In cutaneous candidiasis, different groups of rats were taken. Weight loss was observed in all groups and recovery in after treating with hexane extract of Dillenia indica. Increased wound healing activity was observed in all the treated rats. At higher concentration (10 mg/rat), the % of wound healing was 100% where as 80% in lower concentration (5 mg/rat) was observed on 8th day. Erythema was observed in all groups of rats initially, however, decreased in treated groups. Crust formation was increased in treated rats compared to control rats. Coma was not observed in any group of rats.

- Dexamethasone, a corticosteroid was used for immunesupression in rats. In oropharyngeal candidiasis, after treatment with hexane extract of Dillenia indica, a single colony was observed in the treated rats. From in vivo studies it is evident that hexane extract of Dillenia indica could be used for topical application in combating the candidal infections.
Histopathological data showed the presence of *C. albicans* in both skin and dorsum part of the tongue in infected rats and no *C. albicans* growth in treated rats. Quantitative histopathological findings of cutaneous candidiasis showed complete epithelialization in all groups of rats. Macrophages are more in number in *Candida albicans* infected rats.

Histopathological studies were performed to provide direct evidence of the CCl₄ induced toxicity and safety evaluation of hexane extract on *Dillenia indica* on the tissues. Marked disruption of the cell structure was observed in CCl₄ treated rats whereas hexane extract on *Dillenia indica* treated rats showed only minimal disruption of the cell structure in liver and kidney tissues during CCl₄ induced toxicity. This minimal disruption of cells in various tissues provides additional support to the study that hexane extract on *Dillenia indica* has protective effect against oxidative stress.

From the above results it is evident that the hexane extract of seeds of *Dillenia indica* possess good antifungal, wound healing and antioxidant activities.