# Chapter 6

Chapter- 6: DISCUSSIONS ON RESULTS

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Chapter 6

Discussions on Results

The present research described in this was targeted to discover the folklore use of three therapeutic plants *Stylosanthes fruticosa, Indigofera linnae* and *Cinnamomum tamala*. The selected therapeutic plants were screened for various phytochemical and biological evaluation and the scientific data obtained were presented in the earlier chapters. This chapter deliberates discussion on the results acquired for various investigations executed on crude extracts and isolated pure compounds PC/CC/KF3 & PC/CC/KBF.

6.1. Pharmacognostical and Phytochemical analysis

6.1.1. Preliminary Phytochemical investigations

Various crude extracts (alcoholic, aqueous, hydro-alcoholic and hot continuous soxhalation) obtained from *Stylosanthes fruticosa, Indigofera linnae* & *Cinnamomum tamala* were subjected for preliminary phytochemical investigations as described in the earlier chapters. Phytochemical investigation showed total phenolic, flavonoidal content, carbohydrates and glycosides present in the crude extracts of whole plant of *Stylosanthes fruticosa, Indigofera linnae* and bark of *Cinnamomum tamala*. Proteins and amino acids were found as a major source in *Indigofera linnae* and *Cinnamomum tamala*. Phytosterols found minimal amount in all the selected plants. Alkaloids identified as a main source in *Cinnamomum tamala* and *Stylosanthes fruticosa*. Saponin found only in *Stylosanthes fruticosa*. 
The aqueous, Hydro-alcoholic, alcoholic and hot continuous soxhalexate extracts of all the three plants showed flavonoid content.

6.1.2. Transverse section and powder microscopy observations

The selected plants of whole plant of *Sylosanthes fruticosa*, *Indigofera linnae* and barks of *Cinnamomum tamala* were investigated to determine its various tissues present. Leaves and barks of the fresh plant parts were investigated microscopically by performing transverse section of the crude drugs for its identity by determining unique cellular characters present also performed powder microscopic analysis and reported lignified trichomes, fibres and calcium oxalate crystals in *Sylosanthes fruticosa*; parenchyma, tracheids, xylem, fibres were present in *Indigofera linnae*; cortex, xylem and phloem were present in *Cinnamomum tamala*

6.1.3. Proximate analysis

Proximate analysis of dried crude powder of whole plants of *Sylosanthes fruticosa Linn, Indigofera linnae* & bark of *Cinnamomum tamala* were showed that total ash content of 80%, 82.5% and 90%; Acid insoluble ash content of 30%, 03 % and 18.5%; Water soluble ash content of 8%, 12% and 9%; Hot extractable matter of 14%, 19.5% and 29% respectively.

6.1.4. Fluorescence analysis

Fluorescence analysis of dried crude powder of whole plants of *Sylosanthes fruticosa, Indigofera linnae* and dried barks of *Cinnamomum tamala* were observed under UV short and long wave
length by comparing day light and all the plants were explored fluorescent compounds presence.

6.1.5. Powder fineness and sieve size

Powder fineness and sieve size of dried crude powder of whole plants of *Stylosanthes fruticosa* Linn, *Indigofera linnae* & bark of *Cinnamomum tamala* were showing its influence in isolating the bioactive moiety where the coarse powder fineness involves in isolating the phytochemicals.

6.2. In-vitro antioxidant studies

Numerous researches have projected various phytochemicals on their efficient antioxidant activity. Mostly the antioxidant activity screened in natural products (plant kingdom) were commonly seen its potent antioxidant activity in polyphenolic and its related sub class of chemical moiety, alkaloids and steroids. The free radical scavenging effects of all the extracts on hydrogen peroxide radicals were carried out using the procedures discussed in earlier chapter. All the extracts were also screened for their ferric ion reducing power using standard procedures and total antioxidant activity were performed for all the extracts. The results of above mentioned investigations were discussed in this section.

The results obtained for the antioxidant studies carried out for whole plant extracts of *Stylosanthes fruticosa*, *Indigofera linnae* and bark of *Cinnamomum tamala*. Among all the extracts the alcoholic extract and hydro-alcoholic extracts of *Cinnamomum tamala* showed
total antioxidant activity and alcoholic, hydro-alcoholic and aqueous extracts of *Indigofera linnae* showed a potent antioxidant activity in scavenging of hydrogen peroxide models. *Stylosanthes fruticosa* showed moderate antioxidant activity in all the methods performed. Alcoholic extracts by soxhlation of *Stylosanthes fruticosa, Indigofera linnae* and bark of *Cinnamomum tamala* showed moderate activity in reducing power method when compared with the respective reference standard.

### 6.3. Antibacterial activity

The results obtained for the antibacterial activity carried out for the aqueous, alcoholic, hydro alcoholic and alcoholic soxhalet extracts of whole plants of *Stylosanthes fruticosa, Indigofera linnae* and barks of *Cinnamomum tamala* were subjected to a preliminary screening against five standard microorganisms (*Staphylococcus aureus, Proteus vulgaries, Bacillus subtilis, Pseudomonas auriginosa, Escherichia coli*). All the extracts of the selected folklore medicinal plants and its zone of inhibition of *Indigofera linnae* and *Cinnamomum tamala* showed very potent activity than *Stylosanthes fruticosa* extracts.

All these differences in the antibacterial study of the extracts might be due to the chemical composition of the selected plants, the species of the microorganisms used and suitable method of extractions.
6.4. *in-vitro* and *in-vivo* Pharmacological screening

The results of various pharmacological screening performed on the crude extracts of the mentioned above medicinal plants were discussed.

6.4.1. *in-vitro* α-amylase and α-glucosidase inhibition assays for bioactivity guided isolation

*in-vitro* pharmacological activities were carried out by α-amylase and α-glucosidase inhibition assays to determine for whole plant of the aqueous, alcoholic, hydro alcoholic and alcoholic sohxalet extract of *Stylosanthes fruticosa*, *Indigofera linnae* and bark of *Cinnamomum tamala*.

The alcoholic crude extract of *Cinnamomum tamala* was subjected for fractionation with methanol, butanol and water. The methanolic extract showed potent activity for alpha amylase activity; hence the methanolic fraction was subjected for column fractionation for isolation of bioactive compound. The compounds were isolated from the ethyl acetate fractions of *Cinnamomum tamala* alcoholic extract.

From the results acquired the better activities were revealed from alcoholic and aqueous extract of bark of *Cinnamomum tamala* percentage inhibition of aqueous and alcoholic extracts were reported as 97.49% and 93.78% respectively when compared to whole plants of *Stylosanthes fruticosa* aqueous and alcoholic extracts were observed (83.76% and 58.34%) respectively. The aqueous and alcoholic extracts
of *Indigofera linnaei* were shown (62.62% and 83.76%) respectively. Further, bioactivity guided isolation studies were carried out on the most active potent plant extract subjected to isolation of bioactive molecule and obtained fractions of ethyl acetate layer 5.34g (17.80%), butanol layer 9.87g (32.90%) and water layer 12.15g (40.54%) have found. Further, all the partitions were subjected for sub fractionation. Among all the fractionation the compounds were detected by TLC identification showed ideal spots on ethyl acetate fractions while other fractions did not show any major or minor spot on TLC analysis.

Ethyl acetate fractions were further purified with different percentage of pet.ether. The following compounds were isolated and revealed as 50mg of PC/CC/KF1, 90mg of PC/CC/KF2, 35mg of PC/CC/KF3, 49mg of PC/CC/KF5, 1.8g of PC/CC/KF6, 900mg of PC/CC/KF7, 1.0g of PC/CC/KF8 and 0.85g of PC/CC/KBF The compound PC/CC/KF3 was having IC$_{50}$ of 11.69. The isolated caffeine, epicatechin, procatechuic acid and pure compounds PC/CC/KF3 & PC/CC/KBF from methanolic fractions of *C.tamala*.

### 6.4.2. *in-vivo* Antidiabetic activity

Antidiabetic activity of alcoholic extract of dried whole plant of *Indigofera linnaei* 65.03 %, *Stylosanthes fruticosa* 55.03 % and *Cinnamomum tamala* 75.03 % were studied by alloxan induced Folin-Wu Method and among those *Indigofera linnae* and *Cinnamomum tamala* have showed significant decrease in blood glucose level of rabbits were estimated.
6.4.3. *in-vitro* Anthelmintic activity

*In-vitro* evaluation of anthelmintic activity was performed using *Peritima posthuma*, tannins (polyphenolics) have been established to own the anthelmintic activities of natural products. *In-vitro* evaluation of Anthelmintic activity showed potent activity. This clearly shows that aqueous and alcoholic extracts of all the plants have showed related response and good anthelmintic activity when compared with the standard albendazole (500µg/ml) paralysis time and death time were 64.0±0.49 and 94±0.49 respectively. The (100µg/ml, 500µg/ml and 500µg/ml) of SFAQ showed (44.5±0.42 and 95.5±0.42) paralytic time and death time respectively; SFSM showed (28.8±0.47 and 89.1±0.47) paralytic time and death time respectively; CTSM showed (28.8±0.47 and 90.0±0.47) paralytic time and death time respectively.

All the pharmacological results were statistically analysed using student ‘t’-test one-way ANOVA, the P≤ (0.0001) were found to be significant when compared with the reference standard.

6.4.4. Isolation, identification and characterization

This section deals with the discussion of results on the chromatographic and spectral data’s of the isolated compounds. All the isolated compounds spectral data’s were interpreted and compared with the standard compounds. The isolated compounds were purified, identified and characterized to be PC/CC/KF3 (Cinnamic acid), PC/CC/KBR (Epicatechin), procatechuic acid, caffeine and coumarin were compared with the standard compounds by using TLC method, HPLC, IR and NMR (\(^1\)H and \(^{13}\)C) spectroscopy.