CHAPTER - V

SUMMARY AND CONCLUSION
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Immune recognition is remarkable for its specificity. Once the foreign molecules are recognized by the immune system enlists the participants to mount an appropriate response to eliminate or neutralize the invader. Exposure to the same foreign organism at a later stage induces a memory response. All these studies come under the science of immunology. And to enhance or to reduce the power of immunity we switch on to immunomodulators.

The nature that has gifted the health has also provided numerous natural resources to maintain it. Man's life has always been extensively connected with plants and the nature surrounding him. There is no single aspect of human life, where plants do not play any direct or indirect role. For every basic need like food, fuel, shelter, clothing and medicines etc., man is dependent on plants. Ethno medicine has been used to define the medicinal uses of plants in relation to human being and it also gives the initial information about a particular plant.

The various extract and compounds obtained from plants were named as plant products or natural products. India has a long traditional user of plant products especially in the field of Ayurvedic, Siddha, Unani and Homeopathic systems of medicine. The primitive man used raw materials and raw extracts of plants to alleviate sickness and ailments without any scientific knowledge of their active ingredients.
In order to increase our life span and to live healthy life in the present polluted world we have to improve our immunity. To enhance the immunity of our body we have to use some immunostimulators, which are either chemical or of herbal origin. Chemical immunostimulators are not very beneficial because they cause lots of side effects as well as are very expensive also. On the other hand herbal immunostimulators are not so expensive and are very abundant in nature. Nowadays many drugs are obtained almost exclusively from wild plants. As the wild plant species are insufficient to meet the ever-growing demand so we have to cultivate them and search more plants having Ethanomedicinal properties.

In some cases like in organ transplantation, foetus erythroblastosis etc. we have to suppress our immune system and agents that suppress the immune responses are known as immunosuppressants. Some plants, have been used by villagers to cure for various ailment and are very abundant, are as follows: *Boerhavia diffusa*, *Calotropis procera*, *Careya arborea*, *Costus speciosus*, *Ocimum basilicum* and *Luffa acutangula*, *Cleome gynandra*, *Cocculus hirsutus*, *Lantana camara* etc.

*Cleome gynandra* is a member of the family Cleomaceae and has been used to heal wounds, tumors, ulcers, pain, earache etc. Cocculus hirsutus belongs to family Menispermaceae folkloric information is that it cures malarial fever, jaundice, kidney problems, and headache. *Lantana*
camara of family Verbenaceae is very common herb and is used to cures cancer, tumor, fever, cold, chicken pox and to treat sores. Decoctions were applied externally for leprosy and scabies.

The plants Cleome gynandra, Lantana camara and Cocculus hirsutus were collected from Pant Nagar locality of District Sagar (M.P.), during the month of August-September. The collected plants were stored in Department of Zoology, Dr. Hari Singh Gour University, Sagar (M.P.) Aerial parts of the plants were shade dried for thirty days and then dried plant material was reduced to coarse powder and stored in airtight containers. Powdered plant material is subjected to ethanol extraction using Soxhlet and aqueous extraction using the process of maceration.

Percent yield of aqueous and ethanolic extract of Cleome gynandra were calculated and found to be 28.6% and 16.8% respectively. Percent yield for aqueous and ethanolic extract of Cocculus hirsutus were 25.5% and 21.25% respectively and percent yield of aqueous and ethanolic extract of Lantana camara was found to be 20.3% and 17.2%.

In toxicity studies all the extracts of all three plants i.e. Cleome gynandra, Cocculus hirsutus and Lantana camara, did not showed any symptom of toxicity and mortality up to 500mg/kg b. wt.

Qualitative analysis of aqueous extract of Cleome gynandra showed the presence of carbohydrates, glycosides, saponins and proteins; whereas in ethanolic extract alkaloids, carbohydrates, glycosides, fats, saponins and flavonoids are present. Protein was absent probably due to
denaturation during the process of Soxhlet extraction. *Cocculus hirsutus* showed the presence of carbohydrates, glycosides, saponins and protein in aqueous extract where as alkaloids, carbohydrates, glycosides, fats were present in ethanolic extract. Carbohydrates, glycosides, saponins, protein and flavonoids are present in the aqueous extract of *Lantana camara* and alkaloids, glycosides, fats and flavonoids were giving their presence in ethanolic extract of *Lantana camara*. As flavonoids are found in both the extract hence, it gives a potent immunostimulatory activity.

Thin layer chromatographic studies were performed for aqueous, ethanolic extracts and their fractions. Aqueous extract of *Cleome gynandra* have shown best separation in Acetone: Water (90:10) to give four spots (Rf value - 0.856, 0.721, 0.423 and 0.261) Ethanol soluble fraction have shown three spots (Rf value - 0.811, 0.621, 0.251) in Chloroform: Methanol: Water (65: 35:10) and Ethanol insoluble fraction have also shown three spots (Rf value - 0.541, 0.432, 0.110) in Acetone: Water (90: 10) and spots were visualized by H₂SO₄. Ethanolic extract of the drug best separates in Benzene: Acetone (80: 20) and give five spots (Rf value - 0.810, 0.65, 0.517, 0.362, 0.224) Water-soluble fraction gives four spots (Rf value - 0.62, 0.45, 0.33, 0.15) in nButanol: Acetic Acid: Water (60:20:20), H₂SO₄ is used to visualization. Water insoluble fraction separates in Ethanol: Ethylacetate (20: 80) and give five spots and spots were visualized by Dragendorff reagent (Rf value - 0.91, 0.85, 0.75, 0.65 and 0.60). Above results showes that *al*kaloid, saponin, sugars and its
derivatives were found in aqueous and ethanolic extract of *Cleome gynandra*.

Aqueous extract of *Cocculus hirsutus* have shown best separation in Acetone: Water (90:10) and give five spots (Rf values are 0.912, 0.856, 0.721, 0.626 and 0.321) Ethanol soluble fraction give best separation in Chloroform: Methanol: Water (65: 35: 10) and four spots were visible having Rf values 0.600, 0.432, 0.312 and 0.222. Ethanol insoluble fraction gave four spots of Rf values 0.900, 0.812, 0.550 and 0.300 in Chloroform: Ethanol (100: 2). H₂SO₄ was used for visualization. Ethanolic extract of *Cocculus hirsutus* showed best separation in Benzene: Acotone (80: 20) and give six spots (Rf values 0.92, 0.91, 0.89, 0.87, 0.11 and 0.08) spots visualized in Daylight. On the other hand water-soluble fraction separates in Ethanol: Ethylacetate (20: 80) and gave four spots (Rf values 0.86, 0.65, 0.43 and 0.15) Spots were visualized by Dragendorff reagent. Water insoluble fraction gave five spots (Rf values 0.95, 0.70, 0.44, 0.22 and 0.11) in Cyclohexane: Chloroform: Diethyl amine (50: 40: 10) spots visualized in Daylight.Both the extract of *Cocculus hirsutus* gave different types of saponin, alkaloids and carbohydrates.

Aqueous extract of *Lantana camara* gave best separation in Acetone: Water (90:10) and gave four spots (Rf value 0.67, 0.60, 0.32 and 0.22) Ethanol soluble fraction gave three spots in the solvent Chloroform: Methanol (60:40), Rf value 0.55, 0.213, 0.11 and Ethanol insoluble
fraction give three spots of R$_f$ value 0.70, 0.65 and 0.45 for solvent system Chloroform: Methanol (94: 6). H$_2$SO$_4$ used for spot detection. Ethanolic extract of *Lantana camara* gives four spots in Cyclohexane: Chloroform: Di ethylamine [50: 40: 10] (R$_f$ value 0.77, 0.63, 0.52 and 0.30) and were detected in daylight. Water-soluble fraction gives three spots (R$_f$ value 0.70, 0.67 and 0.43), in Acetic acid: Water: n-Butanol (20:20:60), Water insoluble fraction gives three spots in Benzene: Acetone (80: 20) (R$_f$ value: 0.72, 0.51 and 0.45) both the above fractions were visualized by H$_2$SO$_4$.

Immunomodulatory activity of different plant extract and their fraction were evaluated by determining the:

1. Phagocytosis by carbon clearance method.
2. Drug Induced Myelosuppression test.
3. Delayed type hypersensitivity test. (Paw edema)
4. SRBC agglutination Test.

Albino rats (Sprouse dawley) of either sex and weighted between 100-125 grams were used for the assessment of immunomodulatory activity of ethanolic & aqueous extracts and their fractions.

Crude Aqueous extract of *Cleome gynandra* increases the phagocytic index in a dose dependent manner. Ethanol soluble fraction of the extract did not mark any significant change whereas ethanol insoluble fraction significantly increases the phagocytic index and this
indicates that crude aqueous extract and ethanol insoluble fraction are potent immunostimulators.

Crude ethanolic extract showed a slight decline in phagocytic index when compare with control. Water-soluble fraction did not show any remarkable change whereas reduction in the phagocytic index was observed in water insoluble fraction.

Crude aqueous and ethanolic extract of Cocculus hirsutus enhances the rate of phagocytosis and hence, increases the phagocytic index. All the fractions of aqueous and ethanolic extract increase the phagocytic index but water-soluble fraction of ethanolic extract did not show any significant change.

Phagocytic index increases in a dose dependent manner by the administration of crude aqueous and ethanolic extracts and water-soluble fraction of ethanolic extract showed irregular trend in the values of phagocytic index.

From the above results it is inferred that the aqueous extract of Cleome gynandra, Cocculus hirsutus, Lantana camara and ethanolic extract of Cocculus hirsutus, Lantana camara enhances the process of phagocytosis. It is possible that these extracts may influence the role of neutrophils and various enzymes that take part in the process of phagocytosis. The extract may also influence the role of monocytes, fixed tissues macrophages in spleen. All the above extract contains glycosides, saponins and polysaccharides and these compounds activate the
reticuloendothelial system, which results in a significant increase in the phagocytic index.

Ethanolic extract of *Cleome gynandra* did not show immunostimulant properties but water soluble fraction showed the presence of immunostimulant property, this suggest that the immunostimulating substances mainly are water soluble in nature and were present in low concentration in the ethanolic extract, however, their concentration becomes high in aqueous fraction, which is responsible for the slight immunostimulating properties of water soluble fraction of *Cleome gynandra*.

Humoral immune response was studied by measuring agglutination titre against SRBC at various serum dilutions. Aqueous extract of *Cleome gynandra* significantly increase the humoral immune response to SRBC when animals were compared with control. Agglutination titre to SRBC was increased significantly at the serum dilution up to X: 160. Ethanol soluble fraction did not showed any significant effect where as ethanol insoluble fraction enhanced the agglutination titre to SRBC at the serum dilution up to X: 80 and X: 160 in a dose dependent manner.

Ethanolic extract suppresses the humoral immune response. There was a decrease in the agglutination titre to SRBC and agglutination occur only at X: 20 at the dose of 150mg/kg b.wt. Water-soluble fraction
showed similar results as in the case of control but decline in the level of agglutination could be seen in the water insoluble fraction.

Aqueous and ethanolic extracts of *Cocculus hirsutus* showed increase in agglutination titre at the serum dilution up to X: 160 and Ethanol soluble fraction of aqueous extract and water soluble fraction of ethanolic extract did not show any remarkable difference.

Aqueous and ethanolic extracts of *Lantana camara* showed agglutination titre up to serum dilution of X: 160 in dose dependent manner. Water soluble and low doses of water insoluble fractions of ethanolic extract did not show any increase in the agglutination titre when compared with control.

Studies suggest that the aqueous extract of *Cleome gynandra*, *Cocculus hirsutus*, *Lantana camara* and ethanolic extract of *Cocculus hirsutus*, *Lantana camara* influence the role of immunoglobulins resulting in activation of B-cells and so there is an increase in the secretion of antibodies, which gives the higher agglutination titre against SRBC cells. Antibodies function as the effect of humoral response by binding to antigen and neutralizing it forming clusters that are more readily ingested by phagocytic cells.

In other experiment drug induced myelosuppression is observed. In this test myelosuppression was produced by administration of cytotoxic drug, cyclophosphamide, in an experimental animal. Cyclophosphamide is known to produce significant suppression of
humoral and cellular as well as non-specific and specific cellular immune response. Cyclophosphamide treatment resulted in significant lowering of hemoglobin concentration, RBC count, platelet count, total WBC count, lymphocyte etc. It was observed that the suppressive effect of cyclophosphamide is reduced by the administration of crude aqueous extract of *Cleome gynandra*. Ethanol soluble fraction did not show any significant increase in the various hematological parameters whereas ethanol insoluble fraction protects the effect of cyclophosphamide in a dose dependent manner.

In the case of ethanolic extract of *Cleome gynandra* treated animals the level of Heamoglobin, RBC & WBC counts and platelet was increased slightly. Crude aqueous extract gives a slight decrease in WBC and platelet number when compare with the group, which received only cyclophosphamide. This shows that ethanolic extract did not show any remarkable protective effect. Water-soluble fraction of ethanolic extract did not show any significant effect whereas water insoluble fraction of ethanolic extract affected negatively the values of Heamoglobin, RBC, WBC count, lymphocyte, platelet etc and it did not show any protective effect against cyclophosphamide.

When Crude aqueous extract of *Cocculus hirsutus* is administered then the hematological parameters increase in a dose dependent manner. In ethanol soluble fraction hematological parameters increase but a significant change was not observed. Ethanol insoluble fraction showed a significant change at all doses (50, 100, 150mg/kg b.wt.).
Ethanolic extract of *Cocculus hirsutus* gave protection against cyclophosphamide with increasing doses. Slight protection was observed in water-soluble fraction at all doses (50, 100, 150mg/kg b.wt). Water insoluble fraction gradually increases the heamoglobin percentage in a dose dependent manner.

Aqueous extract of *Lantana camara* gives protection against cyclophosphamide. Ethanol soluble fraction also showed a positive effect over various parameters of blood. Ethanol insoluble fraction showed a significant increase in various blood parameters in a dose dependent manner. Crude extract and ethanol soluble fraction showed a same pattern.

Ethanolic extract of *Lantana camara* increases the percentage of heamoglobin and protect the animal from the adverse effect of cyclophosphamide. Water-soluble and water insoluble fraction elevate the heamoglobin concentration, but water-soluble fraction did not make any remarkable change.

On the basis of above studies it can be observed that the aqueous and ethanolic extracts of *Cleome gynandra, Cocculus hirsutus* and *Lantana camara* possess stimulatory properties and increase the protective effects in immunosuppressed animals. The significant increase in WBC, Eosinophil count, Lymphocytes, monocytes and platelets by these drug extracts suggest the positive effect on immune system.

Effect of Aqueous and ethanolic extract of *Cleome gynandra* on cell-mediated immune response was studied by DTH to sheep red blood
erythrocyte [SRBC] and paw volume is observed after 24, 48, 72 and 96 hrs and compared the treated animals with the control animal. In aqueous extract paw volume is significantly increased at 24 and 48 hrs and then significantly decreased at 72 and 96hrs when studies performed with the doses of 100, 150mg/kg b.wt. The ethanol soluble fraction of aqueous extract did not show any significant results but the ethanol insoluble fraction more significantly increased the paw volume at 24 and 48hrs and reduced the edema more significantly at 72 and 96 hrs as compared to control.

Ethanolic extract also shows the decrease in the paw volume during the increasing time period but paw volume is increased as compare to the control volume. Water-soluble fraction did not show any remarkable change in paw volume at different doses (50, 100, 150mg/kg b.wt.) as compare to control. Water insoluble fraction at the doses of 100 and 150mg/kg b.wt exerts a remarkable decrease during the increase in the time period.

The Cocculus hirsutus increases the paw edema in all the studies. Aqueous extract increases the paw volume at 24 hrs and 48 hrs and then reduce it significantly at all the doses i.e. 50, 100 and 150mg/kg b.wt. Slight reduction in paw volume is observed with ethanol soluble fraction. Ethanol insoluble fraction significantly increases the paw volume at 24 and 48 hrs.

Ethanolic extract of Cocculus hirsutus enhances the paw volume significantly in the case of crude ethanolic extract and water insoluble...
fraction. And water-soluble fraction did not make any remarkable change. Crude ethanolic extract of dose 50, 100, and 150mg/kg body wt. showed a significant increase and paw edema after 24 hrs. Water insoluble fraction at 50-mg/kg b. wt. reduces paw volume in 24 hrs to 96 hrs

*Lantana camara* increases the edema in a very remarkable way in 24 hrs while at 96 hrs the paw edema reduces significantly. Ethanol soluble fraction of aqueous extract of 50, 100, and 150mg/kg b. wt did not show any remarkable change. Slight reduction was observed in all these cases. Ethanol insoluble fraction also shows similar results as the crude extract exhibited.

Crude ethanolic extract gives a gradual increase in paw volume at 24 and 48 hrs and reduces the paw edema significantly in 72 and 96 hrs. This indicates that the extract modulates the delayed type hypersensitivity reaction *i.e.* the extract influences the cytotoxic T-cells, release of macrophage migration factor MMF plus other hormones and various enzymes, which result an early increase in paw edema and better reduction of edema in later hrs *i.e.* 72 and 96 hrs

Result of the present study demonstrate that all the three plants have potent immunomodulatory activity and because they are abundant in nature, easily available and have low toxic index hence these plants are routinely used by folks. In the case of *Cleome gynandra* it was observed that immunostimulant activity is absent in the ethanolic extract while the aqueous fraction has water-soluble immunostimulating
substances their low concentration present in the ethanolic extract, however, their concentration become high in aqueous fraction, of ethanolic extract which is responsible for the slight immunostimulant activity.

*Cocculus hirsutus* showed immunostimulant property in both the extracts. In some cases fluctuation in immune response was observed and it suggest that active constituent activates the system to some limit and then started to decrease and this type of observation was not dose dependent. Aqueous extract is more potent immunostimulant as compare to ethanolic extract yet water-soluble fraction of ethanolic extract did not invoke immune processes, it seems that the content are soluble in ethanol but not in water.

*Lantana camara* is also a good immunomodulator and showed stimulant activity at all the levels of study. Ethanolic extract in some of the cases show a slight stimulation where as aqueous extract and its fraction showed an immunostimulant property.

Studies on immunomodulatory activity of *Cleome gynandra*, *Cocculus hirsutus* and *Lantana camara* reveals that all these plants were capable to influence the innate as well as the adaptive immune systems and the present studies reveals that these plants have certain immunomodulatory compounds which can be used as immunomodulators to cure various ailments. In the present study the ethanol insoluble fraction of aqueous extract of each plant showed more potent immunostimulant properties and the possible reason behind it
these fractions contains polysaccharides and proteins, those are very big in their molecular weights and size and contains so many antigenic sites on their surfaces and the more effective antigenicity of these compounds makes them more effective immunostimulant. The ethanolic extract and their fractions showed less potent activity as compare to aqueous extracts and the low solubility of polysaccharides and other high molecular weight compounds and also the denaturation of proteins in these extracts minimizes their immunostimulant properties. Present studies supports the possible use of these plants in various ailments but before that further more studies of their active fractions and active constituents required to establish the plant products of these plants as a potent immunomodulators.