SUMMARY & CONCLUSION

Summary

In India, many Ayurvedic practitioners are using various indigenous for the treatment of different types of diseased conditions. Although the application of these medicaments has a sound tradition and a rational background according to the Indian system of medicine, perhaps it is essential to investigate the rationality of their use in modern scientific terms. The scientific studies to work out the actual efficacy and other limitations to the drugs would definitely widen their scope for further use if they come out to be really effective. The presently available drugs provide only symptomatic relief and are not free from side effects. The greatest disadvantage of this synthetic drug lies in their toxicity and reappearance of symptoms after discontinuation. The target was to discover newer drugs from plant kingdom, which may provide therapeutic cure and would be acceptable by developing nation like India. Scientists in Central Drugs Research Institute, Lucknow have studied nearly two thousand Indian medicinal plants for their various pharmacological properties.

The thesis entitled “Phytopharmacological investigation on Elephantopus scaber, Celosia argentea and Catharanthus pusillus” embodies the work on the Phytopharmacological studies of the different fractions of Elephantopus scaber, Celosia argentea and Catharanthus pusillus. This has been presented in eight chapters and a chapter wise summary is given below.

Chapter 1: Introduction

This chapter comprises of an introduction to the topic and put light on the traditional medicine of ancient India, a brief discussion on the plant metabolites and the diversity of natural products in their biological properties and overview of main molecular targets that are modulated by plant medicine.

Chapter 2: Review of literature

A structure representation and careful review of available literature throws light on the efforts made by eminent researchers were concluded for the three species along with the identification of the compounds from various parts of Elephantopus scaber, Celosia argentea and Catharanthus pusillus. And different activities were carried out for the three species are described in this chapter.
Chapter 3: Preliminary Phytochemical studies

Preliminary Phytochemical analysis for the three plants *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* and there fractions has been carried out by using standard procedures and Percentage of soluble extractive values were recorded. The total phenolic and total alkaloid content estimated by using Gallic acid and Atropine total equivalents respectively.

Chapter 4: Acute toxicity studies

The selected twelve fractions of three plants showed neither visible sign of toxicity nor mortality. The results clearly indicated non-toxicity of the extracts at a dose of 2000 mg/kg. Hence, there is no LD$_{50}$ and all the extracts tested are considered safe and nontoxic.

Chapter 5: In vitro Anti-oxidant activity

This chapter deals with the introduction of free radicals, antioxidants and list of plants with antioxidant activity. The experimental protocols for in vitro antioxidant activity of the fractions of selected plants *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* and known antioxidant ascorbic acid in scavenging the free radicals superoxide, hydroxyl and DPPH activity, and their results. All these aspects have been discussed in an elaborate manner.

Super oxide radical scavenging activity

The different fractions of *Elephantopus scaber* were produced concentration dependent percentage inhibition of superoxide radical and produced maximum activity at a concentration of 160 µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. Among the four types of *Elephantopus scaber* fractions, the methanol fraction showed better activity than other fractions at 160 µg concentrations.

The different fractions of *Celosia argentea* were produced concentration dependent percentage inhibition of superoxide radical and produced maximum activity at a concentration of 160 µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. Among the four fractions of *Celosia argentea*, the hydro alcoholic fraction showed better activity than remaining extracts at 160 µg concentrations.

The different fractions of *Catharanthus pusillus* were produced concentration dependent percentage inhibition of superoxide radical, produced maximum activity at a concentration of 160 µg, and there after the percentage
inhibition were raised gradually to its maximum level with higher concentrations. Among the four fractions of *Catharanthus pusillus* the hydro alcoholic fraction showed better activity than remaining extracts at 160 µg concentrations.

**Hydroxide radical scavenging activity**

The different fractions of *Elephantopus scaber* were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC₅₀ values for hydroxyl radical of hydro alcoholic, methanol, ethyl acetate and hexane fractions *Elephantopus scaber* were found to be 197µg, 194µg, 245µg and 392µg respectively. The mean IC₅₀ value of ascorbic acid was found to be 66µg.

The different fractions of *Celosia argentea* were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC₅₀ values for hydroxyl radical of hydro alcoholic, methanol, ethyl acetate and hexane fractions of *Celosia argentea* were found to be 196µg, 205µg, 243µg and 413µg respectively. The mean IC₅₀ value of ascorbic acid was found to be 66µg.

The different fractions of *Catharanthus pusillus* were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC₅₀ values for hydroxyl radical of hydro alcoholic, methanol, ethyl acetate and hexane fractions of *Catharanthus pusillus* were found to be 260µg, 198µg, 319µg and 786µg respectively. The mean IC₅₀ value of ascorbic acid was found to be 56.5µg.

**DPPH radical scavenging activity**

In the present study, the different fractions of *Elephantopus scaber* were found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC₅₀ values for DPPH radical of hydro alcoholic, methanol, ethyl acetate and hexane fractions *Elephantopus scaber* were found to be 147µg, 112µg, 313 µg and 602µg respectively. The mean IC₅₀ value of ascorbic acid was found to be 16µg.

The different fractions *Celosia argentea* were found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC₅₀ values for DPPH radical of hydro alcoholic, methanol, ethyl acetate and hexane fractions of *Celosia argentea* were found to be 104µg, 144µg, 535µg and 607µg respectively. The mean IC₅₀ value of ascorbic acid was found to be 16µg.

The different fractions of *Catharanthus pusillus* were found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC₅₀ values for hydroxyl radical of hydro alcoholic, methanol, ethyl acetate and hexane
fractions of *Catharanthus pusillus* were found to be 200µg, 197µg, 246µg and 375µg respectively. The mean IC\textsubscript{50} value of ascorbic acid was found to be 72.1µg.

**Chapter 6: Screening for Hepatoprotective activity**

This chapter includes that the selected plant fractions were screened hepatoprotective activity (CCl\textsubscript{4} induced hepatic damage in rats)

Hepatoprotective activity of different fractions of *Elephantopus scaber* reveals that the ethanol fraction shows good percentage protection than compared to the other fractions. In our phytochemical studies, we found the ethanol fraction has more phenolic and alkaloid content 30.42±0.52 (mg/gm), 23.26±0.24 (mg/gm) respectively and in the anti oxidant activity also Ethanol fraction shows significant free radical scavenging activity than other fractions.

Hepatoprotective activity of different fractions of *Celosia argentea* reveals that the ethanol fraction shows good percentage protection than compared to the other fractions. In our earlier studies in laboratory, we found the ethanol fraction has more phenolic and alkaloid content 38.56±0.31 (mg/gm), 26.34±0.18 (mg/gm) respectively and in the anti oxidant activity also ethanol fraction shows significant free radical scavenging activity than other fractions.

Hepatoprotective activity of different fractions of *Catharanthus pusillus* reveals that the methanol fraction shows good percentage protection than compared to the other fractions. In our earlier studies in laboratory, we found the more phenolic and alkaloid content 31.69±0.56 (mg/gm), 30.13±0.26 (mg/gm) respectively and in the anti oxidant activity also methanol fraction shows significant free radical scavenging activity than other fractions.

Overall study on different fractions of *Elephantopus scaber, Celosia argentea and Catharanthus pusillus* reveals that the ethanol and methanol fraction of these three plants shows good percentage protection than compared to the other fractions. In our earlier in vitro studies in the phenolic and alkaloid content of these fractions are more than other fractions, in anti oxidant activity also ethanol and methanol fractions shows significant free radical scavenging activity than other fractions.

**Chapter 7: Assessment of Anti-inflammatory potential**

This chapter describes about the anti-inflammatory activity of different fractions of  *Elephantopus scaber, Celosia argentea* and  *Catharanthus pusillus* fractions in carrageenan induced paw oedema in rats. Acute oral toxicity studies
reveal that the different fractions up to 2000 mg/kg have not produced any mortality in experimental animals.

The ethanol fraction of *Elephantopus scaber* exhibited good anti-inflammatory activity when compared to the extracts of *Celosia argentea* and *Catharanthus pusillus*. Pharmacological investigations clearly indicated that anti-inflammatory activity in many plants have been attributed to their flavanoid and sterol contents (Mansour *et al.*, 1990). Several flavanoids isolated from the medicinal plants have been discovered to possess significant anti-inflammatory activity (Duke, 1992).

Our phytochemical studies report the presence of flavanoids, triterpenoids, phytosterols and alkaloids in *Elephantopus scaber, Celosia argentea* and *Catharanthus pusillus*. These compounds may be responsible for the anti-inflammatory activity. The results clearly indicated that the pretreatment with the selected plant extracts and indomethacin suppressed the increase in paw oedema produced by the phlogistic agent. Among all the four fractions of selected plants, the ethanol fraction produced significant reduction of paw oedema.

Presence of higher ratio of the above-mentioned compounds in *Elephantopus scaber* and in other two plants *Celosia argentea* and *Catharanthus pusillus* may be responsible for potent activity.

Ethanol, methanol fractions from the three selected species had produced significant activity at the lower to higher doses, where as the ethyl acetate and hexane extracts produced good reduction at the maximum dose. The ethanol fraction of *Elephantopus scaber* produced significant (P<0.001) reduction at 125, 250 & 500 mg/kg when compared to drug vehicle treated control group.

Ethanol (125, 250 and 500 mg/kg) and methanol fractions of *Celosia argentea* at three different doses produced significant (P<0.05) reduction at 125,250 and 500 mg/kg when compared to drug vehicle treated control group. The ethyl acetate and hexane extracts of *Celosia argentea* tested, at the doses 125,250 and 500 mg/kg were exhibited significant (p<0.001) activity, where as the dose 500 mg/kg exhibited a highly significant (p<0.001) effect when compared to drug vehicle treated control group.

Ethanol (125, 250 and 500 mg/kg) and methanol fractions of *Catharanthus pusillus* produced significant (P<0.001) reduction at 125,250 and 500 mg/kg when compared to drug vehicle treated control group. The ethyl acetate and hexane...
extracts of *Catharanthus pusillus* tested, at the doses 125,250 and 500 mg/kg were exhibited significant (p<0.001) activity, where as the dose 400 mg/kg exhibited a highly significant (p<0.001) effect when compared to drug vehicle treated control group.

The percentage inhibition of the maximal paw oedema during 6 h for the ethanol fraction of *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* at 500 mg/kg were in the following order *Elephantopus scaber > Catharanthus pusillus > Celosia argentea*.

The percentage inhibition of the maximal paw oedema during 6 h for the methanol fraction of *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* at 500 mg/kg were in the following order *Celosia argentea > Catharanthus pusillus > Elephantopus scaber*.

The percentage inhibition of the maximal paw oedema during 6 h for the ethyl acetate fraction of *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* at 500 mg/kg were in the following order *Celosia argentea > Catharanthus pusillus > Elephantopus scaber*.

The percentage inhibition of the maximal paw oedema during 6 h for the hexane fraction of *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* at 500 mg/kg were in the following order *Catharanthus pusillus > Celosia argentea > Elephantopus scaber*

**Chapter 8: Investigation of in vitro Anti-microbial activity against**

**Microorganisms responsible for Septic Arthritis**

The different fractions of *Elephantopus scaber* were inhibit the growth of microorganisms. The ethanol fraction shows more zone of inhibition than compared other fractions. The zone of inhibition of ethanol fraction is less then or equal to the positive controls (for bacteria Refampcin50µg/100 µl and for fungi Griseofulvin50µg/100 µl).

The different fractions of *Celosia argentea* were inhibit the growth of microorganisms. The ethanol fraction shows more zone of inhibition than compared other fractions. The zone of inhibition of ethanol fraction is less then or equal to the positive controls (for bacteria Refampcin 50µg/100 µl and for fungi Griseofulvin 50µg/100 µl).

The different fractions of *Catharanthus pusillus* were inhibit the growth of microorganisms. The methanol fraction shows more zone of inhibition than
compared other fractions. The zone of inhibition of methanol fraction is less then or equal to the positive controls (for bacteria Refampcin50µg/100 µl and for fungi Griseofulvin50µg/100 µl).
CONCLUSION

The present study suggest that the fractions of *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* showed neither toxicity nor mortality. Hence the extracts tested are considered safe and nontoxic. The preliminary phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the different fractions. Several such compounds are known to possess potent antioxidant activity, some of these constituents have already been isolated from this plant. Hence, the observed antioxidant activity may be due to the presence of any of these constituents. The plant exhibited strong anti-inflammatory, hepatoprotective, antimicrobial activities.

Some of the plant fractions of *Elephantopus scaber*, *Celosia argentea* and *Catharanthus pusillus* showed good anti-inflammatory activity against carrageenan induced rat paw oedema. Similarly some of the fractions of these plants showed good antimicrobial activity against microorganisms responsible for infectious arthritis.

Hexadecanoic acid (Wang et al., 2004), Phytol (Wang et al., 2004), Elephantopin (Wang et al., 2004), Isodeoxyelephantopin (Ichikawa et al., 2006), Deoxyelephantopin (Ichikawa et al., 2006), Scabertopin (Liang QL and Min ZD, 2006), Elescaberin (Liang et al., 2008), 8Nor-22(R) With a 2,6,23-trienolide (Daisy et al., 2009) etc. were previously isolated from *Elephantopus scaber*.

Celogentin A (Jun’ichi Kobayashi et al., 2001), Cristatin (Jun’ichi Kobayashi et al., 2001), Celogentin C (Jun’ichi Kobayashi et al., 2001), β-sitosterol (Qian Xue et al., 2011), Stigmasterol (Qian Xue et al., 2011), Palmitic acid (Patel et al., 2010) etc. were previously isolated from *Celosia argentea*.

Lochnerine (M. Tin-wa, et al., 1968), Ajmalicine (Rajeev and Nair, 2006), N-Benzoyl-L-phenylalaninol (Battersby and Kapil, 1965), Vindorosine (Rafael Zarate, et al., 2001), Yohimbine (Rafael Zarate, et al., 2001), Catharanthine (Rafael Zarate, et al., 2001) etc. were previously isolated from *Catharanthus pusillus*.

One or more of the above mentioned constituents may be responsible for the observed anti-infectious arthritic activity. The specific compound(s) responsible for this observed activity are unknown. On the other hand, these compound(s) may be responsible for observed significant antioxidant and hepatoprotective activity.
activities. Anyhow further research has to be done to identify the specific compounds responsible for the observed pharmacological activities.