6. Summary and Conclusion
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6.1. Summary

Many of the medicinal plants and herbs we use today have been studied by modern science for application in today's medical field. These studies have shown that our ancestral folk used of these medicinal plants and herbs were very much correct in their applications. With this aim, the present work focused on the validation of use of plant Clerodendrum infortunatum frequently employed in traditional medicine for skin problems and tumor.

The plant Clerodendrum infortunatum has been widely reported to have several medicinal properties. Leaves and roots employed externally for tumors and certain skin diseases. Leaves are used as tonic for chiretta antiper and febge in malaria especially of children, snake bite, scorpion stings and sprouts (Chopra et al., 1956). In ayurveda bark is used for diabetes (Yoganarasimhan, 1996).

In order to understand the factors responsible for the pharmacological activities, phytochemical analysis is quintessential. Phytochemical analysis of different solvent extracts of leaves and roots of C. infortunatum showed the presence of steroids, triterpenoids, flavonoids, tannins, glycosides, carbohydrates and absence of alkaloids.

In light of traditional claim of the plant, preliminary pharmacological experiments were designed to screen the crude extracts (petroleum ether, chloroform and ethanol /ethyl acetate fraction) of leaves and roots of C. infortunatum for antioxidant, hepatoprotective, wound healing, antitumor and anti-mutagenic activities.
The extract/fraction was screened for pharmacological activities. As a result of this the crude extracts of only petroleum ether and ethanol from leaf and root samples of C. infortunatum were found to be more active in exhibiting various beneficial properties.

The active extracts were further selected for fine phytochemical analysis which was carried out by isolating and characterizing the active components using spectroscopic techniques such as, IR, $^1$H NMR, $^{13}$C NMR and Mass (FAB* and EIS) spectral studies. The characterized compounds were found to be clerosterol, quercetin, betulinic acid cleroamine and lupeol acetate.

All the extracts and isolated constituents were screened for DPPH free radical activity. Among the extracts the ethanol extract of both leaf and root exhibited highest scavenging activity. The results are interesting because the inhibition showed by the ethanol extracts of both leaf and root is almost equivalent to the results of BHT (Butylated hydroxyl toluene) which served as the standard.

The isolated constituents from the active extracts of roots and leaves were also screened for DPPH radical scavenging activity which is the preliminary study to screen the antioxidant potential. Among the isolated constituents tested in this study, Quercetin and cleroamine had the strong radical scavenging activity 94.84 and 68.64% at a concentration of 250 μg/mL respectively. While the clerosterol, betulinic acid and lupeol acetate was found to be insignificant against DPPH radical.

The scavenging of hydroxyl, superoxide anion and nitric oxide radical by ethanol extract of leaf and root increased in a dose-dependent manner. This activity exhibited by the ethanol extract of leaf and root proved to be stronger in offering scavenging activity on O$^-$ and NO radicals (IC$^{50}$ 0.173, 0.221 mg/mL and 0.148, 0.216 mg/mL respectively) when compared to both the standards BHT and α-tocopherol.
The effect of ethanol extracts of leaves and roots were also assessed on in vivo model of oxidative stress; CCl₄ induced hepatotoxicity. In the CCl₄ alone intoxicated animals serum AST, ALT and ALP were significantly increased compared to controls, where as in animals administered with ethanol extract of leaves and roots along with CCl₄ showed significant (p<0.001) decrease of all the above serum enzymes.

The effect of ethanol extracts of leaves and roots were also assessed for antioxidant enzymes and molecules. In CCl₄- intoxicated rats there was a significant (p<0.001) increase in the level of MDA compared to the control group. Treatment with ethanol extract significantly (p<0.001) prevented this raise in level. CAT, SOD and Peroxidase content have significantly increased, in extract treated groups whereas CCl₄-intoxicated group has shown significant decrease in enzyme levels compare to control group. Ethanol extract of leaf at the dose of 200mg/kg.b.w. and root ethanol extract at the dose of 250 mg/kg b.w has shown maximum protection.

The histopathological studies of liver sections of normal control animals showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins. The liver sections of CCl₄-intoxicated rats showed massive fatty changes, necrosis and ballooning degeneration and the loss of cellular boundaries. The liver section of ethanol extract (leaf and root) treated rats showed more or less fatty changes, necrosis and lymphocyte infiltration in the liver sections which is similar to the observations made in control groups.

In light of traditional claims of the plant in skin diseases all the three solvent extracts of leaves and roots and the isolated compounds were assessed for the wound healing activity in rats using three standard models viz., excision, incision and dead space wound. The commercial Nitrofurazone ointment was used as a reference standard. Among the crude extracts treated animals the petroleum ether and ethanol extract of leaf, root chloroform and ethanol extract treated ones showed significant wound healing activity in all the three wound models comparable with the standard nitrofurazone.
In case of isolated constituent clerosterol and lupeol acetate showed significant (p<0.001) wound healing activity in all the three models compared to controls.

Histological observations of the granuloma tissue also evidenced the wound healing property of *C. infortunatum* extracts and isolated constituents. The sections of granuloma tissue obtained from the animals administered petroleum ether and ethanol extract of leaf, root chloroform and ethanol extract. On the other hand, animals treated with the clerosterol and lupeol acetate supports the wound healing exhibited by the extracts and constituents.

In order to investigate the effects of *C. infortunatum* leaf and root extracts against EAT cell death. EAT cells were incubated in the absence and presence of increasing concentrations of petroleum ether, chloroform, ethanol extract of leaf and root and also ethyl acetate fraction of root (50-1000μg) for three hours and the viability was measured by trypan blue exclusion method. Among the tested extracts the petroleum ether extract and ethyl acetate fraction of root was most potent in inducing cytotoxicity against EAT cells.

In case of isolated constituents betulinic acid and lupeol acetate was most potent in inducing the cytotoxicity against EAT cells, therefore, we can pinpoint that the presence of betulinic acid and lupeol acetate could be main reason for the antitumor activity of the *C. infortunatum*.

Based on the *in vitro* cytotoxicity results, *in vivo* experiments were performed in EAT-bearing mice, the preliminary results have indicated that the petroleum ether extract, ethyl acetate fraction of root and its isolated constituents betulinic acid and lupeol acetate showed a significant decrease in the total body weight, ascitic tumor cell number and total ascetic fluid, it also exhibited the effects on mean survival time and increase in life span of EAT bearing mice and blood cell count compared to the controls.

Effect of extracts of *C. infortunatum* on the hematological parameters of EAT cells bearing mice group was also studied. The total WBC counts in EAT bearing mice found increased when compared with normal controls. The differential count
of WBC showed that the percentage of neutrophils significantly increased while that of lymphocytes, eosinophils significantly decreased in EAT cells bearing group as compared to normal mice.

- Among the treated extracts/ fraction the petroleum ether extract and ethyl acetate fraction significantly reduced the total WBC count and also strongly inhibited the decrease in lymphocytes and eosinophils. The reversal total WBC count and differential count of WBC by the petroleum ether extract and ethyl acetate fraction treatment towards the values of the normal group clearly indicated that petroleum ether extract and ethyl acetate fraction possessed protective action on the haemopoietic system.

- Isolated constituents namely, betulinic acid and lupeol acetate showed significant in vivo antitumor activity.

- It is also established that chromosome damage is always associated with cancer cells. There are several reports indicating the antimutagenic and anticarcinogenic properties of medicinal plants. Hence, antimutagenic activity of petroleum ether and ethyl acetate fraction of roots and its isolated constituents were checked by using in vivo system of Swiss albino mice. A known mutagen ethyl methanesulfonate (EMS) was used as standard mutagen (positive control). Maximum frequency of chromosomal aberrations were observed in bone marrow cells of EMS treated animals where as in combined treatment of extracts as well as isolated constituents along with EMS treated animals a significant (p<0.01) decrease in chromosomal aberrations were noticed.

6.2. Conclusion

There is a growing interest in correlating phytochemical constituents of a plant with its pharmacological activity. Scientists have even started correlating the botanical properties of plants with their pharmacological activities. In future, more co-ordinated multidimensional research aimed at correlating botanical and phytochemical properties to specific pharmacological activities is expected. The present studies provide the scientific
evidence for the presence of several beneficial medicinal properties in the plant material *C. infortunatum* belonging to family Verbenaceae. The phytochemical studies have clearly demonstrated that the plant *Clerodendrum infortunatum* is a rich source of phenolics and flavonoids. Therefore, the presence of these compounds in the plant extracts has exhibited strong antioxidant and pharmacological activities.

The results of antitumour activity have demonstrated that *C. infortunatum* extracts and isolated constituents possess antitumor activity on Ehrlich ascitic tumor cells. It is presumed that the presence of the antioxidant and anticancer properties together could also be attributed to the presence of anti-mutagenic property of the plant. Hence, these findings clearly tells that the plant *C. infortunatum* extracts does not contain phytochemical constituents with mutagenic property. The crude extracts of *C. infortunatum* leaf and root extracts exhibited potent biological properties. These activities might be due to the presence of the clerosterol, quercetin, betulinic acid, cleroamine and lupeol acetate. However, there could be many more compounds in the petroleum ether and ethanol extracts which needs a thorough phytochemical characterization. When isolated compounds have also exhibited the potent biological properties with various parameters, it is opined that the plant *C. infortunatum* do possess the medicinal properties. Thus, the studies carried out provide a strong supportive scientific evidence for the medicinal use of *C. infortunatum* against various diseases, thereby justifying its use in the Indian traditional system of medicine. This may further clarify the specific properties of the plant. It is believed that this plant could be exploited as a better economical and abundant bioresource of phenolics and flavonoids for cosmetics and pharmaceutical industry.