6. Discussion

DISCUSSION

In last three decades, the scientists are keen and sincere to evaluate many plant drugs used in medicinal plant-lores. It is due to their specific healing properties, healthy action and non toxic effects. Standardization of herbal drugs is very much essential, as they are derived from heterogeneous sources which can lead to variations. These variations can lead to erroneous results in various pharmacological and phytochemical studies.

Thus in recent years there has been an emphasis in standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. According to World Health organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

After present investigation it can be concluded that the pharmacognostical study of Heterophragma adenophyllum yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies.

The results of pharmacognostic study of Heterophragma adenophyllum leaves revealed the presence of secondary metabolites like steroids, terpenoids, glycosides, phenolic compounds and alkaloids. Histochemical study also demonstrates the presence of alkaloid in lamina region of leaves. The results of phytochemical screening shows the presence of alkaloid, steroids and terpenoids as major secondary metabolites in plant, which may be the responsible for biological activity of different extracts and separated fractions.

The isolation of alkaloid is carried out by preparing chloroform fraction after acid base reaction of crude drug. Isolated compound were subjected to IR, NMR and MASS spectroscopy for characterization and structure were proposed as 2-2’-(1-(tert-butoxycarbonyl) pyrrolidine – 3, 4 – diyl) diacetic acid. As alkaloidal compound
was isolated from leaves, biological activity of chloroform extract significantly demonstrated decrease in blood pressure. It’s revelled that; alkaloid was the responsible for anti-hypertensive activity of leaves.

The terpenoidal and steroidal fraction of ethyl acetate extract was subjected to GC-MS analysis. It shows GC-MS experimental data, retention time (RT), and mass fragment of terpenoids and steroids. Individual compound were identified from RT, mass data and by comparison of the data of standard compounds with those of in the literature. Five compounds viz. β amyrin, lupeol, sitosterol, campesterol and palmitic acid were identified in *Heterophragma adenophyllum* leaves.

The identification of β amyrin, lupeol, sitosterol, campesterol and palmitic acid is based on mass spectra and NMR spectra of purified fractions. As the chemical structure of β amyrin and lupeol is based on triterpenoids and sitosterol and campesterol is steroidal. The characteristic m/e fragment of β amyrin and lupeol is 426 were sitosterol and campesterol is 414 and 400 respectively.

The radical-scavenging activity of methanolic extract increased with increasing concentration. DPPH scavenging effect of methanolic extract of *Heterophragma adenophyllum* was found to be 153.52µg/ml and superoxide scavenging activity was 242.20µg/ml. This effect of radical-scavenging may be due to the presence of phenolic compounds in methanolic extract.

Results of antimicrobial activity of terpenoidal and steroidal fraction of *Heterophragma adenophyllum* leaves extract by agar-well diffusion method was shown in table 24. From the results of zone of inhibition it was reveal that the terpenoidal and steroidal fraction possesses an efficient and strong antimicrobial activity against both the gram positive and gram negative bacteria. Results of the antimicrobial activity of terpenoidal and steroidal fraction by micro dilution method were shown in table 25. The results of the minimum inhibitory concentration, it was shows that the almost all tested microorganism was found sensitive towards the terpenoidal and steroidal fraction of *Heterophragma adenophyllum* leaves extract. Phytochemical and preparative thin layer chromatography shows that various compounds of terpenoidal and steroidal nature were present in this fraction.
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Evaluation of anti-hypertensive activity demonstrates that all four extract had shown decrease in systolic blood pressure. As shown in Table 26 ethyl acetate extract (500 mg/kg) significantly (p < 0.001) reduce blood pressure after 14 days of treatment as compared to model group (DOCA salt and 1% NaCl). Chloroform extract and Methanolic extract also shows significant reduction in blood pressure when compared to model control.

As the results of anti-hypertensive activity revelled the significant results was shown by ethyl acetate and chloroform extract, it supports the bioactive constituents was present in respective extract. The pharmacognostic, histochemical and phytochemical results demonstrates the presence of steroid, terpenoid and alkaloid in respective extract.