Chapter 5

General Discussion
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*Pajanelia longifolia* (Willd.) K. Schumann belonging to the Family Bignoniaceae is an important ethnomedicinal plant reported from Southern Assam part of North East India. In ethnomedicine, the plant has been attributed to have antimicrobial and hepatoprotective activity (Dutta Choudhury, 1999; Choudhury, 2007). However, the plant has never been studied for isolation and characterization of natural products those can be useful in developing drugs against microbial infections and hepatic damage.

The analysis of the crude extracts of leaves and bark of *P. longifolia* against various pathogenic micro-organisms showed that the plant possesses immense anti-microbial activity. The ethyl acetate and 70% ethanolic crude extract of the leaves were effective against almost all the microbes under investigation. The effect of the bark was also observed to be significant on the micro-organisms. On comparison with several standard antibiotics, the therapeutic efficacies of the plant extracts were highly significant.

The therapeutic potentialities of medicinal plants have been increasing. Ethnomedicinal plants in recent times have been a very good source of
new potential drugs. Several medicinal plants have been demonstrated to have potential anti-microbial activity, however, no such reports have so far been published where the *Pajanelia longifolia* have been studied. The crude extracts obtained during this study, are mixtures of multiple components, thus the data obtained were considered and interpreted in an appropriate context. The extraction procedure employed in obtaining the crude extracts from leaves and bark are highly specific and emphasis was given on isolating crude fractions based on their polarity. In most of the studies, the isolation procedure of the crude extracts is not specific (Jain *et al.* 2008). In the experiment, the ethyl acetate extract of the leaves have demonstrated high level of anti-microbial activity. The extract was most effective against *Streptococcus* sp. and *Staphylococcus* sp. followed by *Candida* sp., *Proteus* sp., *E. coli*, *Salmonella* sp., *Bacillus* sp. and *Klebsiella* sp. On the other hand, the petroleum ether has no significant anti-microbial activity. The 70% ethanolic extracts of the leaves though have anti-microbial activity, there was not much variation. This suggests that in the leaves, ethyl acetate extract containing medium polar components is the most bioactive component of the leaves and bark. The non polar crude fractions i.e., the petroleum ether extracts have no significant anti-microbial activity.
The present investigation though observes many similar results as reported in case of many other plants (McCutcheon et al., 1994; Jones et al., 2000; Thirach et al., 2003; Sader et al., 2004; Webster et al., 2008) and some differences were also noted. This discrepancy in findings may be the results of different microbial strains used. In addition, a single extract has varying degree of activity against different strains. Such differences may also be due to the extraction procedure used. The petroleum ether extract contains mostly non-polar components. The ethyl acetate and ethanolic extracts facilitates more complete extraction of components with wide degree of polarity (Evans, 1996).

In the present study, the petroleum ether, ethyl acetate and 70% ethanolic extracts of the bark were used to evaluate the hepatoprotective activity. CCl₄ is being used extensively to induce hepatic damage in many experimental animals (Bhatal et al. 1983). The liver damage is assessed by biochemical parameters like Serum Bilirubin content, SALP, SGPT, SGOT, etc. CCl₄ produces an experimental damage that histologically resembles viral hepatitis (James and Pickering, 1976). Toxicity begins with the endoplasmic reticulum, which resulted in the loss of metabolic enzymes located in the intracellular structures (Recnagal, 1983). The effect of CCl₄ on the experimental mice taken during the investigation were observed by the
increase in the serum bilirubin content. In the control group the bilirubin content was measured to be 1.28 mg/dl, which increased to 6.92 mg/dl upon CCl₄ treatment. Upon application of the standard drug Silymarin, the bilirubin content came down to 3.73 mg/dl. The effect of plant extract was significant. The ethyl acetate extract was most effective followed by the 70% ethanolic extract. This indicates that the components present in the ethyl acetate and 70% ethanolic extracts of the bark are effective in providing defense against hepatic damage. It has been observed in many previous investigations that CCl₃ radical is produced upon CCl₄ treatment, which reacts with oxygen to give trichloromethoxy radical by a reaction catalyzed by Cytochrome P450 2E1 (Jain et al., 2008). The level of the enzymes like SALP, SGPT and SGOT showed interesting results. The level increased upon CCl₄ treatment, which indicated hepatic damage. The reductions in the level of the enzymes after plant extract treatment indicated the stabilization of the plasma membrane and repair of the CCl₄ induced damage of hepatic tissues. Thabrew et al. (1987) reported that the serum level of transaminase returns to normal once the hepatic cell damage is repaired. Alkaline Phosphatase is a prototype of these enzymes that reflects the pathological alterations in the biliary flow (Ploa and Hewitt, 1989). The CCl₄ induced elevations in the enzymatic activity in the serum in line with high level of bilirubin content. The ethyl acetate and
70% ethanolic extracts induced the suppression of the increased SALP activity with concurrent decline in the serum bilirubin content suggests stabilization of the biliary dysfunction in the liver due to CCl₄.

Four compounds were isolated and characterized from *Pajanelia longifolia*. The preliminary phytochemical analysis showed the presence of various secondary metabolites in various crude extracts. This indicates that both leaf and bark of this plant can provide wide range of compounds may be with high bioactivity. The gas chromatography analysis of the crude extracts of the plant provided useful information. Various peaks were observed in the GC chromatogram, showing the presence of various isolable fractions in the crude extracts and eventually these were taken up for further phytochemical characterization. In course of the experiment, several fraction were isolated through column chromatography, four compounds were finally isolated in pure form, viz., longifolate, pajanal, 2-propylbenzaldehyde and 2-(4, 5-dihydro-3-methyl-5-oxo-1-phenyl-4-pyrazolyl)-5-nitrobenzoic acid. Hundreds of plants were previously investigated for isolation and characterization of natural products, however, till date only one active principle, an alkaloid Pajaneelin was reported (Anonymous, 1948 - 1976).
Virtual screening is a computational method that reduces the time and effort associated with lead identification. Analysis of drug likeliness of a compound is an important step. The isolated compounds were analyzed using online server for their drug likeliness. Compound 1, 2 and 4 showed drug likeliness. Considering the Lipinski’s rule, Compound 1, 2 and 4 possesseesd good chemical characteristics to be a drug.

Molecular docking techniques dock small molecules into protein binding site. Success of the docking depends on the 3D target protein with or without a ligand bound. In the present investigation, Compound 1 and Compound 4 were docked against DNA polymerase of E. coli and RNA-dependent RNA polymerase genotype 2a of hepatitis C virus. The docking results did not showed any potential binding of the ligands (compounds) with the target, indicating that 3D targets are not specific. However, no proper specific 3D target could be found. Further investigation is thus needed to identify a proper drug targets for these compounds in order to understand exact molecular mechanism of the drug molecules as they have shown positive results in the wet lab studies.