7. SUMMARY

Traditional medicine employing plant based system of treatment forms an important part of human medicine system. The major use of herbal medicines involve health promotion and therapy for acute and chronic diseases, as well as opposed to the life-threatening conditions. The most common reasons for utilizing traditional medicine are its non-toxic nature, closely resembles to the patient ideology and allays concerns about the adverse effects of chemical (synthetic) medicines, satisfies a desire for more personalized health care, and allows greater public access to health information. Hence, the traditional medicine is most warranted in modern medical system and also in phytotherapeutical drug treatments.

*Passiflora* species is among the significant group of plants known for their rich source of phytoconstituents with known biological values. Since ancient time several species of *Passiflora* have been extensively used in the traditional system of therapeutics in many countries. A strong literature survey revealed that two species of *Passiflora*, namely *P. subpeltata* and *P. leschenaultii* employed traditionally to treat several disorders lacked significant validation. The present study intended to evaluate the pharmacological properties of *P. subpeltata* and *P. leschenaultii* in terms of attenuating oxidative stress, inflammation and hepatic disorders.

The air-dried leaves of *P. subpeltata* and *P. leschenaultii* were extracted with the organic solvents such as petroleum ether, chloroform, acetone, methanol and hot water. Methanol extracts of both plants revealed higher extract yield percentage for *P. subpeltata* (18.8%) and *P. leschenaultii* (17.3%). Quantification assays revealed that acetone extract of *P. leschenaultii* leaf exhibit highest amount of total phenolics (440.24 mg GAE/g extract), tannins (229.29 mg GAE/g extract) and flavonoids (253.33 mg RE/g extract) followed by acetone extract of *P. subpeltata* possessed maximum amount of total phenolics, tannins, and flavonoid (417.65 mg GAE/g extract, 182.91 mg GAE/g extract and 241.33 mg RE/g extract respectively).

The evaluation of *in vitro* antioxidant assays showed that all the extracts of both the selected *Passiflora* species scavenged the radicals and showed the antioxidant
potential. In DPPH scavenging activity, acetone extracts of *P. subpeltata* (27.9 µg/ml) and *P. leschenaultii* (29.14 µg/ml) showed good activity. Indeed, in the ABTS⁺ scavenging activity same acetone extract of *P. subpeltata* (10108.91 μM TEAC/g extract) and *P. leschenaultii* (10509.69 μM TEAC/g extract) expressed significant (*p < 0.05*) activity. With regard to FRAP, metal chelating and phosphomolybdenum activity the acetone extracts of leaves of both *Passiflora* species depicted maximum antioxidant activity. Even in superoxide, hydroxyl and nitric oxide radical scavenging activities, the acetone extracts of *P. subpeltata* (70.80%, 76.74% and 81.09% respectively) and *P. leschenaultii* (71.84%, 83.91% and 79.95% respectively) presented an equivalent level of scavenging activity. Regression correlation (R²) of phenolic and flavonoid compounds were found to positively correlated in all antioxidant/free radical scavenging activities tested.

Based on *in vitro* antioxidant assay and quantification of non-enzymatic antioxidants, leaves acetone extracts of both plant species were selected for the *in vivo* pharmacological investigations. The acetone extracts of *P. subpeltata* and *P. leschenaultii* leaves were evaluated for acute toxicity in Swiss albino mice. The extract did not alter the general behaviour and failed to produce any mortality even at the highest dose (2000 mg/kg) studied and was found to be safe. Based on acute toxicity, two doses *viz.*, 200 and 400 mg/kg were used for further pharmacological studies.

The analgesic activity of acetone extracts of *P. subpeltata* and *P. leschenaultii* leaves was evaluated employing acetic acid induced writhing, formalin induced paw licking and hot plate test models in Swiss albino mice. The injection of acetic acid in control mice resulted in 17.25±0.48 writhes after 30 min, whereas in *P. subpeltata* and *P. leschenaultii* 400 mg/kg extract treated groups, the number of writhes were significantly (*p<0.001*) reduced to 82.73% and 81.43% respectively. In formalin induced paw licking test at a higher dose 400 mg/kg, acetone extracts of *P. subpeltata* and *P. leschenaultii* leaves attenuated the licking response (57.26% and 69.88% respectively). The standard drug morphine and the plant extracts treated groups significantly (*p<0.001*) amplified the latency response without affecting the animal’s capability to perceive the thermal pain onset, in the hot plate test method.
The antipyretic effect of acetone extracts of *P. subpeltata* and *P. leschenaultii* leaves against brewer’s yeast induction was assessed in Wistar albino rats. The results showed that doses of 200 and 400 mg/kg plant extracts and standard drug paracetamol (150 mg/kg) extenuated rectal temperature significantly (*p*<0.001). The animals treated with acetone extract of *P. subpeltata* leaves at dose of 200 and 400 mg/kg showed a reduction of rectal temperature by 1.05°C and 2.55°C respectively after 5 h. Animals treated with acetone extract of *P. leschenaultii* leaf at doses of 200 and 400 mg/kg showed a significant reduction of rectal temperature after 5 h by 1.90°C and 2.42°C respectively.

The anti-inflammatory activity of acetone extracts of leaves of *P. subpeltata* and *P. leschenaultii* were assessed in Wistar albino rats using carrageenan induced acute paw edema, cotton pellet induced granuloma and inflammatory bowel disease models for measuring chronic repair processes. Carrageenan induced paw edema persisted even after 5 h after injection in the induced control group which received no treatment, whereas the oral treatment of acetone extracts from leaves at 400 mg/kg of *P. subpeltata* and *P. leschenaultii* inhibited the edema formation significantly (*p*<0.001) in a dose dependent manner. The treatment with standard indomethacin and plant extracts also suppressed the granulomatous tissue formation in the cotton pellet induced granuloma rat model in a dose dependent manner. Further, it decreased the migration of neutrophils to the peritoneal cavity and reduced the weight of cotton pellets significantly (*p* < 0.001).

Present investigation also demonstrates the protective effect of orally administered leaves acetone extracts of *P. subpeltata* and *P. leschenaultii* in indomethacin (IND) induced inflammation bowel disease in Wistar albino rats. Leaves acetone extracts of both the plants and standard drug prednisolone showed remarkable accumulation and long retention in the proximal duodenum, jejunum and ileum, caecum and colon tissues, and resulted in effective scavenging of ROS and suppression of inflammation in the intestines of IND-treated rats. From the macroscopic observation, serum parameters, enzymatic antioxidant and MPO levels and histological images, it is believed that the oral administration of leaves acetone extracts of *P. subpeltata* and *P. leschenaultii*, could substitute the oral administration of NSAIDs, and could become an imperious attitude for the treatment of intestinal injury in patients continuously captivating NSAIDs.
The current study proves, that pre-treatment with leaves acetone extracts of *P. subpeltata* and *P. leschenaultii* effectively protected against paracetamol induced acute liver injuries in rats. The maximum protection against hepatic damage was achieved at the highest tested dose (400 mg/kg p.o.) in both plant extracts, which exhibited almost an equivalent similar activity to silymarin (25 mg/kg) treatment. The possible mechanisms could be attributed to the fact that acetone extracts of both plants leaves was able to protect the liver against cellular oxidative damage and maintained the intracellular level of antioxidant enzymes. They also significantly (*p* < 0.001) prevented the increase in serum aminotransferases level, reduced oxidative stress and hepatic lesions. Further, the treatment of the Wistar albino rats with the plant extracts elevated haematological parameters and maintained the normal physiological changes from the RBC, Hb and platelet contents increased and the WBC contents decrease in the liver cells.

The leaves acetone extracts of *P. subpeltata* and *P. leschenaultii* contained considerable amount of phytoconstituents. It was found that the major bioactive compounds such as quercetin, gallic acid, apigenin, rutin and catechin were in appreciable amount in the acetone extract of leaves of both species quantified using HPLC analysis. The acetone extracts of *P. subpeltata* leaves were found to contain quercetin (10.59 µg/mg extracts), gallic acid (41.151 µg/mg extract extract), apigenin (10.73 µg/mg extract), rutin (26.956 µg/mg extract) and catechin (9.57 µg/mg extract) while, the leaves acetone extracts of *P. leschenaultii* were found to contain quercetin (40.61 µg/mg), gallic acid (3.93 µg/mg), apigenin (48.23 µg/mg), rutin (42.90 µg/mg) and catechin (3.95µg/mg). Based on the HPLC results the phenolic compounds found in the plant extracts is the reason for the observed pharmacological properties.

LC - MS results of acetone extracts of *P. subpeltata* and *P. leschenaultii* leaves showed the presence of six major likely identified and unknown compounds. In acetone extract of *P. subpeltata* leaves rutin, quercetin, gallic acid, isoflavones and isovitexin were identified. Correspondingly, the acetone extract of *P. leschenaultii* leaves showed the presence of gallic acid, rutin, quercetin, isoorientin, isoschaftoside and schaftoside. These identified compounds in the both plants possess numerous medicinal properties such as
reduction oxidative stress, analgesic, antipyretic, anti-inflammatory, hepatoprotective, anti-cancer, anxiolytic and cardioprotective properties.

As such, the acetone extracts from the leaves of *P. subpeltata* and *P. leschenaultii* could effectively replace the non-steroidal anti-inflammatory drugs and paracetamol toxicity and shadow the adverse effect caused by these synthetic drugs. This is proven from the present pharmacological investigation in addition to scientific validation of the traditional usage.