SUMMARY AND CONCLUSION
Plants contain thousands of constituents and are a valuable source of new and biologically active molecules. For their investigation, it is important to have the necessary tools at hand. These include suitable chemical and pharmacological screening methods. The methods should be as simple as possible and attempts should be made to have access to a large number of different tests so that many biochemical properties can be screened. Existing procedures, however, are often not reliably predictive for clinical efficiency and care should be taken when interpreting the results.

The pharmacological methods summarized here involve anticancer, antidiabetic, hepatoprotective, antioxidant/radical scavenging and antimicrobial activities. They are most effective when used in conjunction with chemical screening methods. For chemical screening, various chemical tests have been performed for the preliminary information about the content and nature of constituents found in the active extracts.

The plant *Mucuna pruriens* (Family: Fabaceae), commonly known as cow-itch, is an annual climbing legume, indigenous to tropical regions, especially Africa, India and West Indies. The roots and seeds of *Mucuna pruriens* are traditionally used for the treatment of various forms of tumors, hypoglycemic, hypotensive, aphrodisiac, diuretic, analgesic and antipyretic, antioxidant, anti-inflammatory, teratogenic and vermifuge.

The plant *Phyllanthus niruri* (Family: Euphorbiaceae), commonly known as bhui-amarla, is a small, erect, annual herb, indigenous to the rainforests of the Amazon and other tropical areas throughout the world, including the Bahamas, southern India, and China. It is used in traditional medicine for the treatment of various ailments. The whole plant is used hypoglycemic, antihepatotoxic, hypotensive, laxative, stomachic, antibacterial, anti-inflammatory antiinflammatory antinociceptive, carminative, and digestive.

The chemical constituents of the methanol extracts of *Mucuna pruriens* (MEMP) and *Phyllanthus niruri* (MEPN) were primarily identified by qualitative analysis and subsequently confirmed by the thin layer chromatography (TLC) and by the melting points of standard samples. Preliminary phytochemical analysis indicates that MEMP contains alkaloids, steroids, flavonoids, saponins, tannins, terpenoids, lignans and lipids. In the present phytochemical investigation we have isolated 5,6,4\textsuperscript{1}-trihydroxy-7,8,3\textsuperscript{1}-trimethoxy flavone-4\textsuperscript{1}-O-β-D-xylopyranosyl-(1,2)-O-α-L-rhamnopyranoside and flavonoid, Quercetin from *Mucuna pruriens* and 5,6,7,4\textsuperscript{1}-tetrahydroxy-8-(3-methylbut-2-enyl) flavanone-5-O-
rutinoside (nirurin) and acetyl derivative of nirphyllin (nirphylline acetate, 3,3',5,9,9'-pentamethoxy-4-hydroxy-4',5'-methylenedioxy lignan from *Phyllanthus niruri*.

**LD$_{50}$** was performed according to the method of Litchfield and Wilcoxon to determine the relative strength and health hazards of **MEMP** and **MEPN** in Swiss albino mice. The LD$_{50}$ value for **MEMP** and **MEPN** were found to be 1333.52mg/kg b.w. and 1400.27mg/kg b.w., respectively.

**Antitumor activity** of **MEMP** and **MEPN** was demonstrated at the dose of 125mg and 250mg/kg b.w. against EAC cells in mice. A day of incubation was allowed for multiplication of the EAC cells. Fourteen doses of **MEMP** and **MEPN** were injected i.p. from the 1$^\text{st}$ day up to the 14$^\text{th}$ day with 24-h intervals. On day 15, half of the animals were sacrificed and evaluated the tumor volume, packed cell volume, tumor cell count, and viable and nonviable cell count; and remaining animals were observed for the life span of the hosts. At the end of the experimental period, blood was collected and estimated Hb content, RBC, WBC and differential count. The liver was excised and used for the determination of LPO, reduced GSH, SOD and CAT.

The tumor volume packed volume and viable cell count were found to be significantly decreased while nonviable cell count was significantly higher in **MEMP** and **MEPN** treated animals. Further, the median survival time was increased and changes in body weights were decreased by **MEMP** and **MEPN**. It was observed that Hb content and RBC was increased and total WBC count was found to be decreased. In differential count of WBC, the % of neutrophils increased while at the expense of lymphocyte count decreased. The levels of lipid peroxides were significantly increased by **MEMP** and **MEPN**. Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells and antitumor activity in experimental animals. The lowering of lipid peroxidation (LPO) and increase in the levels of reduced GSH, SOD and CAT by **MEMP** and **MEPN** indicate its potential as their inhibitory effect of EAC induced intracellular oxidative stress. All these results clearly indicate that the **MEMP** and **MEPN** have a remarkable capacity to inhibit the growth of tumor induced by EAC cell line by modulating LPO and augmenting antioxidant defense system in EAC bearing mice. It is proposed that the alkaloids, flavonoids, saponins, and lignans present in **MEMP** and **MEPN** are responsible for the potent antitumor activity which can be inferred from the increased life span of EAC tumor bearing mice.
**Antidiabetic activity** of *MEMP* and *MEPN* was evaluated at the dose of 125mg and 250mg/kg b.w. against Streptozotocin (STZ) induced diabetic rats. Diabetes was induced with a single i.p. injection of Streptozotocin (STZ) at a dose of 65mg/kg b.w. The effects of *MEMP* and *MEPN* in diabetic rats were determined by measuring blood glucose levels, food and fluid intake amount and changes in body weights. After 14 days of treatment, the animals were dissected and a drop of blood from the heart was used for the estimation of blood glucose. Tissues (brain, heart, liver, and kidney) were removed and used for the estimation of TBARS, GSH, SOD and CAT activity.

The blood glucose levels were estimated by using One Touch Ultra blood glucose monitoring system. *MEMP* and *MEPN* significantly reduced the blood glucose levels in diabetic rats. It was observed that food and fluid intake was significantly higher and there was no significant difference in the body weights. The levels of TBARS were significantly reduced and GSH, SOD and CAT levels were significantly increased by *MEMP* and *MEPN*. The results reveal that *MEMP* and *MEPN* seem to be effective for reducing oxidative stress and free radical-related diseases including diabetes.

**Hepatoprotective activity** of *MEMP* and *MEPN* was evaluated against the Acetaminophen intoxicated rats at the dose of 125mg and 250mg/kg b.w. Hepatotoxicity was induced by Acetaminophen at the dose of 500mg/kg in rats. The effects of *MEMP* and *MEPN* on Acetaminophen induced rats were estimated by various serum enzymes such as SGOT, SGPT, SALP, bilirubin, total proteins and uric acid. After 7 days of experiment, the animals were dissected and the blood was used for the estimation of above parameters and the liver tissue was excised and used for the estimation of TBARS, GSH, SOD and CAT.

The increased levels of serum enzymes in Acetaminophen induced control rats were restored to normal on administration of *MEMP* and *MEPN* at the doses of 125mg and 250mg/kg respectively. While uric acid levels were increased in *MEMP* and *MEPN* treated rats. It was also observed that the increased liver size in Acetaminophen group rats was decreased in drug treated rats. SGOT, SGPT, SALP, bilirubin, total proteins and uric acid and TBARS antioxidant levels of liver were significantly decreased in Acetaminophen treated rats and GSH, SOD and CAT levels were significantly increased in *MEMP* and *MEPN* treated rats. Further, histopathology of liver tissues indicates the hepatoprotective activity of *MEMP* and *MEPN*. From the above results *MEMP* and *MEPN* can be considered as hepatoprotective agent. Acetaminophen induced liver damage perhaps protected by
MEMP and MEPN by their antioxidant effect on hepatocytes probably by eliminating the deleterious effects of toxic metabolites from Acetaminophen.

The *in vitro* antioxidant and free radical scavenging activities such as lipid peroxidation (LPO), DPPH radical scavenging activity, reducing power, total phenolic content, hydroxyl nitric oxide, superoxide anion and \( \text{H}_2\text{O}_2 \) radical scavenging activities of MEMP and MEPN were studied against various standard drugs.

Non-enzymatic lipid peroxidation was induced by FeSO\(_4\)-ascorbic acid in the rat liver homogenate. The effect of MEMP and MEPN was monitored by the recording the absorbance at 532nm and finally calculated the % inhibition. The generation of lipid peroxidase by Fe\(^{2+}\)-ascorbate in rat liver homogenate was inhibited by MEMP and MEPN at IC\(_{50}\) value of 217.25\(\mu\)g/ml (%inhibition = 64.41) and 62.5\(\mu\)g/ml (%inhibition = 68.88), respectively. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517nm MEMP and MEPN showed the highest activity at all concentrations. IC\(_{50}\) and % inhibition values for MEMP were found to be 38.5mcg and 90.16 and for MEPN the values were 31\(\mu\)g and 74.63, respectively at the concentration of 100\(\mu\)g/ml. The reducing power of MEMP and MEPN were increased with increasing amount of sample. The absorbance for MEMP and MEPN at 1000\(\mu\)g/ml (i.e., 1mg/ml) was found to be 0.821 and 0.735 respectively. The total amount of phenolic content present in MEMP and MEPN were 33.04mg and 45.74mg respectively in 1g of the sample. The MEMP and MEPN were capable of reducing DNA damage at all concentrations (IC\(_{50}\) = 38\(\mu\)g/ml for MEMP and IC\(_{50}\) = 55.25\(\mu\)g/ml for MEPN). The concentration of MEMP and MEPN needed for 50% inhibition of nitric oxide generation was found to be 52.5\(\mu\)g/ml and 228\(\mu\)g/ml respectively. MEMP and MEPN had strong superoxide radical scavenging activity and exhibited nearly equal superoxide radical scavenging activity like Quercetin. The % inhibition for MEMP, MEPN and Quercetin were found to be 59.19 (IC\(_{50}\) = 92.25\(\mu\)g/ml), 68.95 (IC\(_{50}\) = 42.50\(\mu\)g/ml) and 65.19 (IC\(_{50}\) = 42.50\(\mu\)g/ml) respectively. MEMP at 225\(\mu\)g/ml and MEPN at 120.25\(\mu\)g/ml exhibited 50% scavenging activity on \( \text{H}_2\text{O}_2 \) radical. Thus the results of this study show that the MEMP and MEPN can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.
MEMP and MEPN at a concentration of 500µg/ml and 750µg/ml significantly inhibited the growth of some of the tested (Gram positive and Gram negative) microorganisms to various degrees. MEMP and MEPN shown strong antimicrobial activity against Bacillus pumillus 8241, Escherichia Coli 5B and Vibrae Cholera 1353 and 226101. However, their activity against Staphylococcus aureus ML 152 and Vibrae cholera 14035 was found to be significantly less. The antimicrobial activity was compared with the standard Chloramphenicol at a concentration of 10µg/ml.

The chronic toxicity test for MEMP and MEPN at the doses 125mg/kg and 250mg/kg was conducted in Swiss albino mice for 13 weeks. Hematological profile (Hb content, total count of RBC, WBC and differential count of WBC) and biochemical profile (SGOT, SGPT, ALP, cholesterol levels and total bilirubin, etc) remain unaltered by MEMP and MEPN at the dose of 125mg/kg and 250mg/kg b.w. The extracts also did not affect significantly the blood urea level, NPN and serum uric acid content. The results indicated that MEMP and MEPN are not found to be hepato-renal toxic in nature.