Chapter 8

Discussion
As per the WHO guideline, standardization is the initial steps required to diagnose the organized or unorganized crude drugs with its actual quality and property and also to identify the adulterant \(^1\). In recent years herbal formulations are occupying global economy and the use of herbal therapy is gaining maximum interest rather than the modern synthetic medicines. WHO stated that about 70-80 percent of world population have faiths on herbal medicines because of less side effects and are significantly effective in treating disease ailments \(^2\). Natural products, which have evolved over millions of years, have a unique chemical diversity, which results in diversity in their biological activities and drug-like properties. Those products have become one of the most important resources for developing new lead compounds and scaffolds \(^3\). Natural products will undergo continual use toward meeting the urgent need to develop effective drugs, and they will play a leading role in the discovery of drugs for treating human diseases, especially critical diseases \(^4\).

As both the plants were ethnomedicinally documented in many literatures and is also utilized by the villagers of North East as well as Eastern part of India for their primary health care system, the aim of this present study is to set up a standard monograph profile of the tuberous root of *Mirabilis jalapa* and leaf of *Physalis minima* by pharmacognostically, physicochemically, phytochemically and pharmacologically standardize methods.

Standard methods were followed for the collection and processing of the plants and their useful parts. The parts of plants were authenticated by Dr. P. P. Baruah (HOD, Department of Botany) Gauhati University, India. The plant parts were subjected to successive extraction by cold maceration process and the processes were done by standard protocols and the universally accepts methodologies.
A range of information on ethno pharmacological uses, phytochemical analysis of *Mirabilis jalapa* and *Physalis minima* have reported in **Chapter 2**. Apart from this, special attention has been made to find out the recently developed methods of different phytochemical analysis and pharmacological screening model like flash chromatography technology, cardioprotective effect and immunostimulatory effect in the literature review. Review of literature helped to established and reported the unexploited parameters of these plants which can be beneficial for the development of another site of therapeutic value.

The aim and objectives were discussed in **Chapter 3** of the present research. The scope of the present research lies in the isolation and identification of some bio-constituents for immunodeficiency disorder and cardiovascular diseases from *Mirabilis jalapa* and *Physalis minima*. As per our literature survey, no such work has been reported in the research field. Such compounds may be proven to be potent molecules and will contribute towards the strengthening and development of the phytomedicine era.

The pharmacognostical and phytochemical studies of the plants were covered in **Chapter 4**. Macroscopic features like size, shape, colour, taste, odour of tuberous root of *Mirabilis jalapa* were evaluated. The macroscopical studies revealed that the roots are fleshy, branched, occasionally presence of bud on the surface, externally brown to dark grey and internally whitish to buff in colour, slightly sweetish in taste and without any odour. Similarly, microscopically, the tuberous root has shown the important diagnostic characters of Nyctaginaceae family which was characterized by the presence and arrangement of collateral closed vascular bundles embedded in parenchymatous ground tissue, lignified xylem elements with spiral thickenings, uniseriate medullary rays and acicular calcium oxalate crystals$^5$. 
On the other hand the macroscopical studies of leaf of *Physalis minima* revealed that the dentate margin, presence of 4-6 veins on each side of midrib, lateral veins are run towards marginal teeth, veins are reticulate in shape, base asymmetrical and long petioles. Microscopically studies has shown the important diagnostic characters of Solanaceae family which is characterized by presence of dorsiventral leaves, anomocytic stomata, grandular or uniseriate trichomes and cluster crystal of calcium oxalate. The physicochemical parameters were evaluated to determine the presence of adulteration as well as to measure the purity of crude drugs in powder form. Ash value of crude drugs was performed to measure the presence of inorganic matters in it and which makes the formulation impure or adulterated. The result of total ash indicates the presence of salt of carbonate, phosphate, silicates of sodium, calcium and magnesium as impurities, while acid insoluble ash was performed to detect the presence of silica, water soluble ash was estimated to detect the amount of inorganic substances exhausted by water, where as sulphated ash represented the amount of salt present in powder drug. WHO said that the presence of inorganic matters in the powder form of crude drugs should be very minimum. So experimental analysis proved that the above mentioned parameters such as total ash (12.2% w/w), acid insoluble ash (3.3% w/w), water soluble ash (6.4% w/w), sulphated ash (2.1% w/w) were found to be less quantity of in the powder form of tuberous roots of *Mirabilis jalapa*. In case of powder form of leaf of *Physalis minima* the above understudied parameters were also found to be less quantity such as total ash (11.4% w/w), acid insoluble ash (2.2% w/w), water soluble ash (6.1% w/w), sulphated ash (2.4% w/w) respectively as mentioned in WHO guidelines. Loss on drying was estimated to determine the presence of moisture content present in the drugs. The presence of moisture may lead
to the microbial growth as well as deterioration of the nature of active constituent in crude drug \(^6\). Loss on drying for tuberous roots of Mirabilis jalapa and leaf of Physalis minima were found to be 6.66% w/w and 8.23% w/w respectively. The result shown the lower level of loss on drying reduced the chance of microbial contamination. Extractive values were estimated to examine the solubility of drug in different solvents and quantity as well as the nature of bio constituents present in the crude drugs \(^7\). In the tuberous roots of Mirabilis jalapa the alcoholic, water and ether extractives were found to be 15 % w/w, 10.6% w/w and 2.1% w/w respectively. In leaf of Physalis minima the alcoholic, water and ether extractives were found to be 10.4% w/w, 9.5% w/w and 1.8% w/w respectively. The results of extractive values from different plant extracts of both plants were confirmed that the maximum bio-constituents were soluble in alcohol in the both plant extract. Swelling factor indicated the presence of mucilages, gums, pectin, hemicelluloses in the plant sample \(^8\). From the experimental analysis the swelling factor found to less than 1ml in both plant parts. So it was proved that the present the presence of mucilages, gums, pectin and hemicelluloses in the plant sample in low quantity. Foaming index was determined to understand on the presence of a remarkable quantity of saponins in the crude drugs \(^8\). In both plant it was found to be below 100. So it was indicated that the presence of saponin is also in low quantity. WHO recommended limitation of presence of heavy metal in crude drugs are as follows: Lead (10 ppm), Cadmium (0.3 ppm), Zinc (10 ppm), Mercury (1 ppm) so presence of heavy metals (Pb, Ca, Hg and Zn) in powder drug of tuber and leaf were found to be within the prescribed limit, signifying that the plant is safe for consumption and devoid of harmful metals. Fluorescence analysis with powdered drugs in presence of different reagents was performed to establish a qualitative identification of
crude drug in which few compound present in the drug sample have shown special character in terms of exhibiting fluorescence in day light and under UV lights ⁹.

In phytochemical study of powdered drug of tuberous root of *Mirabilis jalapa* was initiated for successive extraction process by cold maceration technique on increasing polarity basis. The preliminary phytochemical study of methanolic extract of *Mirabilis jalapa* have shown the presence of alkaloids, carbohydrate, flavonoids and glycosides. Further quantitative phytochemical study was undertaken to determine different percentage different bioconstituents present in the part of the plant. From the result it has concluded that the percentage of flavonoid is higher than the other constituents. In addition the preliminary under studied pharmacological activities were carried out by taking different fraction of crude extract. The ethyl acetate fraction has shown significant potentiality compare to other fractions (Data not present). The TLC study was performed with different ratio of solvent systems among those ethyl acetate and methanol (7:3) is confirmed the presence of flavonoid in tuberous root of MEMJ. Hence it has been decided to isolate the flavonoid contained in the part of the plant by taking ethyl acetate and methanol as solvent systems in advanced flash chromatographic method. The isolated flavonoid was further purified and recrystallised by using methanol.

Isolated compound of tuberous root of MEMJ was obtained as flavonoid glycoside which was light yellow colour crystal, soluble in methanol and give reddish pink color with Mg + HCl (Shinoda reagent). The compound was subjected to acid hydrolysis to obtained aglycone part. The chemical structure of isolated compound was further characterized from its physical parameters and spectral (UV, IR, Mass, ¹H-NMR, ¹³C-NMR) data. The melting point of compound determined by DSC and it was found to be 317⁰C. The UV absorption
spectrum of isolated compound of tuberous root of MEMJ showed strong absorption at 365nm in its spectrum, which implied the presence of phenolic aromatic rings. The methanolic solution of compound exhibited typical UV absorption characteristics after the addition of various shifting reagents. The UV spectrum showed absorption bands reagents shifts of the compound to be a 7-substituted derivatives. The absence of free 7-hydroxyl group in the compound was observed in lack of shift of Band II in the presence of NaOAc. IR spectrum of the compound confirmed the presence of -OH group (3253 cm\(^{-1}\)), conjugated carbonyl group (1661 cm\(^{-1}\)) and aromatic C-C (1605 cm\(^{-1}\)). Mass spectrum showed the presence of base peak at m/z 301.12 indicating the molecular weight of compound 301. NMR spectra were performed by DMSO-\(d_6\) solvent. The \(^1\)H-NMR spectrum of the compound was exhibited that the singlet at 2.50 ppm indicates the presence of 1-H at C-6 position. The singlet peak at 3.53 ppm indicates the presence of 1-H at C-8 position. The presence of lone pair electron on the oxygen atom of OH ion make the OH proton deshielded as a result the OH peak appeared at down field. The double peak at 6.19 ppm is due to the C-OH proton at C-3 position (\(J=1.03\)MHz) again two doublet at 6.42 ppm and 6.90 ppm are due to presence of OH at C-5 and C-\& position (\(J=1.06\) and 1.04 respectively). The multiplet at 7.56 ppm is due to 2’ 1H of the phenyl ring substituted at 2 position of the coumarin moiety (\(J=1.00\) MHz). The doublet at 7.68 ppm is due to one proton present in the substituted phenyl ring at C-5’ position. Singlet at 9.32 ppm is due to the 1H at C-6’ position. The singlet peak at 10.81ppm and 12.48 ppm are due to presence of C-OH proton at C-3’ and C-4’ position. \(^13\)C-NMR spectrum of the compound revealed the presence of 15 carbons. A signal at δ 175.9 in the \(^13\)C-NMR spectrum indicates the presence of conjugated carbonyl group in the compound. The prominent peak at 93.33,
98.15 correspond to the carbon atom at position 2 and 6 respectively. The peak at 102.98 corresponds for C (C=O) at position 4, the peak at 135.68, 145 and 146.76 corresponds to the carbon atom (C-OH) at position 3, 5, 7 respectively. The prominent peak at 115.03 corresponds to the carbon atom at position 8. The peak at 115.57 and 119.97 are due to the fused carbon between the two rings of chromen nucleus at position 9 and 10. The prominent peak at 163.82 and 175.78 is due to the C atom (C-OH) at position 3’ and 4’ of substituted phenyl ring. Peak at 121.94 depict the C atom at 1’ position. Finally peak at 174.64, 156.11 and 160.57 is due to the carbon atom (CH) at position 2’, 5’, 6’ respectively. On the basis of the results obtained from chemical test, physical properties and spectroscopic data, structure of compound was identified as 2-(3’, 4’ dihydroxy phenyl) 3,5,7- trihydroxy Chromen 4-one and in this study the compound considered as compound 1 represented in fig. 8.1.

![Fig. 8.1 Structure of 2 - (3’, 4’ dihydroxy phenyl) 3, 5, 7- trihydroxy Chromen 4-one](image)

Similarly the phytochemical study the powdered drug of leaf of *Physalis minima* was initiated for successive extraction process by cold maceration technique on increasing polarity basis. Preliminary phytochemical analysis has shown good result on the presence of alkaloids, steroids, tannin, flavonoids and protein. Among all extracts, methanolic and
chloroform extract have shown the positive test for steroid in crude drug. The content of bio-constituent and preliminary pharmacological studies encourages to isolate the steroid presence in the part of the plant. TLC study was performed with different ratio of solvent system among those chloroform and methanol (9:1) is confirmed the presence of steroid in leaf of MEPM. The bioactive compound has been isolated through flash chromatography with same solvent system used in TLC. The isolated compound further purified and recrystallised with methanol.

Isolated Compound of MEPM leaf was obtained as phytosterol which was white amorphous compound soluble in chloroform and give green color with Libermann Burchard reaction. The compound was subjected to acid hydrolysis to obtained aglycone part. The chemical structure of isolated compound was further characterized from its physical parameters and spectral (UV, IR, Mass, $^1$H-NMR, $^{13}$C-NMR) data. Melting point of the compound was determined with DSC and it was found to be 135.31°C. UV spectroscopy of the compound depicts $\lambda_{\text{max}}$ at 298nm. IR spectrum of the compound confirmed presence of OH stretching at 3418.29; C-H stretching at 2934.62 and 2860.19; tri-substituted double bond at 1646.31; presence of CH$_2$ at 1457.86; OH bending at 1372.75; C-O stretching at 1054.46. CHNS analysis revealed that percentage of carbon found to be 73.562%, percentage of hydrogen found to be 11.178% and oxygen found to be 15.260%. Mass spectrum showed the presence of base peak at m/z 414.54 indicating the molecular weight of compound 414. $^1$H NMR spectra depicts the presence of a doublet at 2.28 ppm and 2.29ppm (J value 2.08) which corresponds to the 1H at C3 position. Further the presence of OH group at position 3 also gives a singlet towards down field at 5.37 ppm due the presence of electronegative O atom. Further the signals at downfield was observed
at 7.28 ppm due to the one proton of C=C at C6 position. The singlet at 1.16 ppm and 0.984 ppm is for the two angular methyl proton at C 18 and C 19 position respectively. The appearance of signals as two doublet signals at 0.8409 ppm and 0.8455 ppm (J value 3.28 and 1.02) is due to the presence of 3 methyl proton at C21 and C26 respectively. A triplet at 0.8236 ppm (J value 5.32) is due to 1 H at C26 position. Doublet at 0.8634 ppm (J value 4.69) is due to the presence of 3 H at C27 position. The singlet at 0.6984 ppm and 1.0004 ppm is due to the presence of 3 H at C 28 and C29 respectively. $^{13}$C NMR Shows signal at 140.76 ppm and 121.73 ppm indicated the presence of a double bond between (C-5) and (C-6) in the compound. The value 36.51 ppm and 19.84 ppm corresponds to the angular methyl group at C18 and C19 position respectively. Spectra shows 29 carbon signal including six methyl groups at (position C18, C19, C21, C26, C2 and C29) respectively, eleven methylene groups at (position C1, C2, C4, C7, C11, C12, C15, C16, C22, C23 and C28) respectively, nine methane conjugates at (position C3, C6, C8, C9 C14, C17, C24 and C25) and three quaternary carbons at (position C5, C10 and C13). On the basis of the results obtained from chemical test, physical properties and spectroscopic data, structure of compound was identified as 17- (5-Ethyl-6-methylheptan-2-yl) -10, 13-dimethyl -2, 3, 4, 7, 8, 9, 11, 12, 14, 15, 16, 17-dodecahydro -1H – cyclopenta – phenanthren – 3 - ol. The compound considered as compound 2 represented below fig. 9.2.
Determination of acute toxicity has covered in Chapter 5 of the present studies. In the acute toxicity studies it was found that there was no mortality at the dose of 2000 mg/kg bw as well as 5000 mg /kg bw of MEMJ tuberous root and MEPM leaf. So the drugs were found as safe. Therefore dose optimization was done (i.e. 1/20th, 1/10th and 1/5th of 2000 mg /kg bw) so 100 mg/kg, 200 mg/kg and 400 mg/kg p.o. were selected for the experimental study. For the oral dose optimization of isolated compound of MEMJ tuberous root and MEPM leaf, the dose were calculated based on the individual percentage yield of isolated compound of MEMJ (2.78% w/w) and isolated compound MEPM (3.05% w/w) calculated in terms of the minimum effective dose of MEMJ tuber (200 mg/kg, p.o.) and MEPM leaf (200 mg/kg, p.o.) that produced significant immunomodulatory activity. Therefore, in the present study, dose for the oral administration of isolated compound of MEMJ tuber and MEPM leaf were selected at (5.56 mg/kg, p.o. and 6.1 mg/kg, p.o. respectively) for the experimental study. Similarly for cardioprotective activity the minimum effective dose of MEMJ tuber (200 mg/kg bw) and MEPM leaf is (400 mg/kg, p.o.) that produced significant cardioprotective activity. Therefore, in the present study, dose for the oral administration of isolated compound of MEMJ and MEPM leaf
were selected at (5.56 mg/kg, p.o. and 12.2 mg/kg, p.o. respectively) for the experimental study.

The details of Pharmacological parameters were discussed in Chapter 6 and 7. In this study the traditional claim of Mirabilis jalapa and Physalis minima for its immunostimulatory activity was proved scientifically in Chapter 6. From literature it was found that the neutrophils play the vital role in innate immune system. It circulates in the blood around the body and they signal if an infectious agent is present, they are the first cells to move to the site of the infection to start to kill the microorganism by formation of oxygen radicals \(^{11, 12}\). In the present study, methanolic extract and isolated compound of Mirabilis jalapa tuber inducing a significant increase in neutrophils to nylon fibres, which correlates the increase in percent neutrophils at high dose (at 200mg/kg bw and 400mg/kg bw and isolated compound 1 at 5.56 mg/kg bw). This may potentially help in increasing immunity of body against microbial infections. The initial focus of the greater part of the immunostimulatory compound is accepted to be macrophages which have a major role in modulating the immune system \(^{13}\). To evaluate the effect on reticuloendothelial cell mediated phagocytosis, the carbon clearance assay was performed \(^{14}\). At the point when colloidal ink containing carbon particles are infused specifically into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is coordinated by an exponential condition is known as phagocytic index \(^{15}\). Methanolic extract of Mirabilis jalapa tubers at dosing 200 and 400mg/kg and the isolated compound 1 from the plant at dose 5.56mg/kg bw enhanced the phagocytic capacity by showing the improvement in the clearance rate of carbon by the cells of the RES. Heamagglutination antibody titer was determined to establish the humoral response against SRBC. Antibody particles, a result of
B lymphocytes and plasma cells, are fundamental to humoral insusceptible reactions; IgG and IgM are the significant immunoglobulins which are included in the supplement enactment, opsonization, balance of toxins and so forth. Humoral immunity to SRBCs increased by administration of methanolic extract as well as active bio-constituents of plant, which is proved by rising up the antibody titre in animals also signify the role of T and B lymphocyte in the antibody synthesis. The high values of haemagglutinating antibody titre obtained in the case of all the extract as well as isolated compounds have shown that increase in humoral immune response is directly proportionate to immunostimulation. Cell-mediated immunity (CMI) involves effect or mechanisms carried out by T lymphocytes and their products (lymphokines). CMI reactions are vital to defense against infectious agents, infection of foreign matters and delayed-type hypersensitivity reactions. Delayed hypersensitivity is a major mechanism of defense against various intracellular pathogens, including mycobacteria, fungi, and certain parasites, and it occurs in transplant rejection and tumor immunity. Therefore, enhance the DTH response in mice in light of immune system microorganism subordinate antigen uncovered the stimulatory impact of methanolic extract at high dose of 200 and 400 mg/kg bw and isolated compound 1 of *Mirabilis jalapa* tuber at 5.56 mg/kg bw on white blood cells. As the isolated compound found to be flavonoid so this immunomodulatory effect of the the plant part could be due to the presence of flavonoids only. The review of literature also supports the immunomodulatory property of flavonoids.

Similarly immunostimulatory effect of methanolic extract of *Physalis minima* (MEPM) leaf and isolated compound 2 of MEPM leaf also exploited. In this study, MEPM leaf inducing a significant increase in neutrophils to nylon fibres, which represents the increase
in percent of neutrophils at high dose (at 400mg/kg bw and isolated compound 2 at 6.1 mg/kg bw). So it was proved to improve immunity power of body against microbial infections. Methanolic extract of Physalis minima leaf at dose 400mg/kg bw and isolated compound 2 at 6.1 mg/kg bw enhanced the phagocytic capacity by showing the improvement in the clearance rate of carbon by the cells of the RES. The high values of haemagglutinating antibody titre obtained in the case of extract at dose 200 mg/kg bw, 400mg/kg bw and isolated compound 2 at 6.1 mg/kg bw have shown that increase in humoral immune response is directly proportionate to immunostimulation. An increase in DTH response at high dose of 200 and 400 mg/kg bw and isolated compound 2 at 6.1 mg/kg bw indicates that the methanolic extract of Physalis minima and its isolated compound 2 have a stimulatory effect on lymphocytes and accessory cell types required for the expression of the reaction. As the isolated compound of methanolic extract was found to be phytosterol. So the immunostimulatory effect of this plant may be due the presence of that isolated compound only. The review of literature also supports the immunostimulatory property of phytosterol. 

In chapter 7 the present research work was also designed to evaluate the cardioprotective activity of MEMJ tuberous root and isolated compound 1 (flavonoid) as well as MEPM leaf and isolated compound 2 (Phytosterol) respectively in doxorubicin-induced cardiotoxicity in rats. Literature review suggested that doxorubicin intake generates the free radicals like superoxide and hydrogen peroxide in heart tissue results myocardial oxidative stress. This free radical generation plays an important role in the doxorubicin induced cardiotoxicity. Movement of free radical in the heart tissue decreases the activity of detoxifying agents like SOD, CAT and GSH. Additionally, doxorubicin also has a high
attraction in favor of the phospholipid component of the mitochondrial membrane in cardiac myocytes, directing to presence of doxorubicin in the heart tissue \(^2^2\). The doxorubicin-induced mitochondrial injury is serious to the heart as it has severe unfavorable effects on the contractile functioning of the cardiac myocytes by producing alterations in the energy metabolism \(^2^3\).

Treatment with MEMJ tuberous root as well as isolated compound 1 from MEMJ tuberous root able to significantly reduce the doxorubicin-induced cardiotoxic manifestations in multiple ways. Increase in the level of plasma triglycerides, total cholesterol and low density lipoproteins in the doxorubicin-treated group indicate doxorubicin may be interfering with metabolism or the biosynthesis of lipids. Treatment with test drugs MEMJ at the dose level of 200 and 400 mg/kg bw as well as isolated compound 1 of MEMJ tuberous root at 5.56 mg/kg bw have shown the reduction in serum lipid profile levels in a dose related fashion. The lipid lowering effect of plant extract and its isolated compound may be due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor mediated catabolism of LDL cholesterol and an increase in the uptake of LDL from blood by the liver \(^2^4\).

A deficit of oxygen supply or glucose may injure the myocardial cells and the cell membrane becomes porous as a result the enzymes from cardiac tissues are come out to the blood. It has been reported that doxorubicin induced free radical generation generates membrane degradation and disruption of cardiac myocytes, which can lead to elevations of LDH and CPK in the serum \(^2^5,^2^6\). In the present study, an increase in the activities of LDH, CPK, AST, ALP and ALT were observed in doxorubicin-treated rats. Treatment with MEMJ and isolated compound 1 of MEMJ tuberous root (at dose 200mg/kg, 400 mg/kg bw
and 5.56 mg/kg bw respectively) decreased the enzyme activities in serum and restored the same in the heart. This could be due to a protective or membrane-stabilizing effect of extract and its isolated compound on the myocardium, reducing the cardiac damage, and thereby restricting the leakage of these enzymes 27, 28.

Cardioprotective activity of MEMJ as well as isolated compound 1 from MEMJ tuberous root were further supported by increased myocardial antioxidant enzyme activity and decreased extent of lipid peroxidation with high dose at 200 mg/kg bw and 400 mg/kg bw and isolated compound 1 at 5.56 mg/kg bw. Cellular GSH depletion is closely related to the lipid peroxidation and disturbance of Ca\(^{2+}\) influx induced by toxic agents. Over production of oxidative free radicals enhances lipid peroxidation. Lipid peroxidation is known to cause cellular damage and is primarily responsible for reactive oxygen species induced organ damage. Increased level of MDA and decreased levels of GSH, SOD and CAT were observed in heart tissue in doxorubicin treated animals.

On the other hand the treatment with MEPM leaf as well as isolated compound 2 from MEPM leaf also able to significantly reduce the doxorubicin induced cardiotoxic manifestations in multiple ways. Test drug MEPM leaf at 400 mg/kg bw and isolated compound 2 of MEPM leaf at 12.2 mg/ kg bw have shown the reduction in serum lipid profile levels in a dose related fashion. Therefore the extract and isolated compound 2 both have lipid effect reducing property. This was happened due to inhibition of hepatic cholesterol biosynthesis, increased fecal bile acid secretion and stimulation of receptor mediated catabolism of LDL cholesterol and an increase in the uptake of LDL from blood by the liver 24.
Doxorubicin increases the free radical movement in the cardiac tissue which leads to damage the tissue and elevations of LDH and CPK in the serum. In the present study, an increase in the activities of LDH, CPK, AST, ALP and ALT were observed in doxorubicin-treated rats. Treatment with MEPM leaf and isolated compound 2 of MEPM leaf (at dose 400 mg/kg bw and 12.2 mg/kg bw respectively) decreased the enzyme activities in serum and restored in the heart. This could be due to a defensive or membrane-stabilizing effect of extract and isolated compound on the myocardium.

Cardioprotective activity of MEPM leaf as well as isolated compound 2 from MEPM leaf were further supported by increased myocardial antioxidant enzyme activity like GSH, SOD and CAT and decreased the MDA level with high dose at 400mg/kg bw and isolated compound 2 at 12.2 mg/kg bw.

The present study has shown that administration of the both extracts MEMJ tuberous root and MEPM leaf at high doses and isolated compound 1 and 2 respectively from both extract efficiently counteracted the doxorubicin induced cardiac tissue damage by significant decrease in MDA and elevated the GSH content to near-normal levels, which prevented degradation of cellular macromolecules and thus cell disruption, probably by decreasing the Ca\(^{2+}\) influx and also increase in GSH, SOD and CAT levels. These results indicated the protective effect of MEMJ tuberous root and MEPM leaf and the isolated compounds from both extracts on doxorubicin-induced cardiotoxicity by boosting the endogenous non-enzymatic and enzymatic antioxidant systems, which entailed scavenging of oxidative free radicals.

Histopathological examination of different cardiac sections revealed that doxorubicin caused anomalous histological changes in the cardiac tissue like loss of myofibrils,
vacuolization of the cytoplasm and swelling of mitochondria. However, treatment with MEMJ tuberous root and MEPM leaf as well as isolated compounds 1 and 2 from both extracts respectively prevented the changes and maintained the histological structure almost similar to that of normal control. Therefore from the result it can be stated that the cardioprotective activity of both plant parts *Mirabilis jalapa* tuber and *Physalis minima* leaf were due to the presence of flavonoid and phytosterol respectively. This finding is in accord with the reported cardiac protective properties as per the literature survey and ethnomedicinal practice of both the plants respectively.\textsuperscript{31,32}
References:


