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Chapter-6

Summary & Conclusion

6. SUMMARY AND CONCLUSION

In India number of traditional systems of medicine are practiced since centuries. These include Ayurveda, Siddha, Unani etc. apart from those having ethnobotanical usage of herbs at regional level by the inhabitants or tribes, as medicine. Although these systems possess rich heritage as health care, these were confined to Vaidyas, Hakeems etc. and were not reached to industrial level of manufacture and distribution of different formulations practiced, due to lack of documentation of scientific facts regarding the properties of drugs and medicaments used.

Investigations on plant for their biological utility therefore have become important task throughout the world on a scientific basis. Generally the lead for these investigations in order to select the plant species comes from either the literature available in traditional systems or directly among the folkore practioners. This information provides a basis of screening these natural products or plants following a well accepted protocol in order to eliminate useless ones from useful ones. Modern system of medicine although extremely equipped for combating many disorders by providing effective medicaments, still certain diseases like hepatic disorders, viral infections, rheumatic disorders etc. could only be treated symptomatically. The available agents in alternative systems therefore need to be tapped for obtaining effective medicaments.

A significant number of population in the country suffers from hepatic disorders and rely on herbal products for the cure. Despite the significant popularity of several herbal medicines in general, and for liver diseases in particular, they are still unacceptable as the treatment modalities for liver diseases. The limiting factors contribute to this eventuality are (i) lack of standardization of the herbal drugs; (ii) lack of identification of active ingredient(s)/principles(s); (iii) lack of toxicological evaluation (Radha *et al.*, 2005).

The present study was envisaged to systematically validate two selected medicinal plants designated as hepatoprotective in traditional medicine. Selected plants for study were *Feronia limonia* and *Tecomella undulata*. *Feronia limonia* is reported as 'tonic for liver' in indigenous system of medicine Although the leaves are reported to possess hepatoprotective activity no systematic and detailed studies i.e., *in vitro* and *in vivo*

screening of extracts, fractions and isolated compounds for hepatoprotective potential had been done yet. Similarly the use of *Tecomella undulata* in the indigenous systems of medicine for liver, spleen and abdominal complaints has attracted us to carry detailed study as regard to its hepatoprotective potential.

Hence, the leaves, stem bark and root bark of *Feronia limonia*; leaves and stem bark of *Tecomella undulata* were screened for hepatoprotective activity. The plant parts as above of *Feronia limonia* were collected from the campus of The M.S. University of Baroda, Gujarat, India, and leaves and stem bark of *Tecomella undulata* were collected from village Rajpipla, Vadodara, Gujarat, India. Both the plant materials were authenticated in Botany Department of The M.S. University of Baroda, Vadodara.

The selected plant materials were first evaluated for their correct identity by studying their pharmacognostic features. Various quality control parameters like, foreign matter, ash values, extractive values, loss on drying, elemental analysis was determined as per WHO guidelines. The coarsely powdered plant materials were than sucessively extracted with solvents of increasing polarity viz., petroleum ether, benzene, chloroform, ethyl acetate, methanol and water. The sucessive extracts were then subjected to preliminary phytochemical test for the presence of secondary metabolites. Total phenolic & total flavanoid contents were determined for both the plants. A complete TLC finger print profile of the secondary metabolites comprising of the typical spectra, R_f value, UV absorption maxima and the percentage proportion of the individual components in the extract are recorded and documented. Isolated compounds from both the plants were analysed and quantified by HPTLC and HPLC methods.

In order to derive scientific evidences to the reported traditional claims, *in vitro* cytotoxicity assay (MTT assay) and hepatoprotective activity was performed for various extracts, fractions and isolated compounds. Hepatoprotective activity was determined in cell supernatant by estimating the SGOT, SGPT levels and to further support this, Acridine orange/ Ethidium bromide staining and cell morphology studies were done. Those extracts, fractions or compounds which gave positive results for hepatoprotective activity were further taken for *in vivo* studies in rats against CCl_4 induced hepatotoxicity.

6.1Feronia limonia

Some important morphological features for the identification of *Feronia limonia* and its parts were observed and documented herein. *Feronia limonia* is a deciduous tree with imparipinnate, unipinnate compound leaves which are arranged in alternate manner. Leaves are green, obovate with an obtuse notched or cenulate apex. Stem bark is ridged, fissured and scaly and there are sharp spines: 3/4 to 2 in (2-5 cm) long on some of the zigzag twigs. Root bark is also slightly yellowish brown in colour, ridged and scaly in appearance.

But for proper identification of medicinal herbs, results of morphological studies only , poses unacceptability because the plants are either available in mutilated form or are sold without definite structure of the organ. Hence microscopic evaluation remains indispensable and cost effective tool of conventional analytical pharmacognosy. So, important microscopic features of leaf, stem bark and root bark of *Feronia limonia* have been documented in the present study.

Microscopic features of *Feronia limonia* leaves showed that leaves are relatively hypostomatic. Stomata are restricted to the lower epidermis with the guard cells sunken relative to the other epidermal cells. The vascular bundles are with several xylem elements arranged in radial rows. Phloem lies towards the epidermises and conducting elements of phloem is seen alternating with sclerenchyma cells. Stem bark of *Feronia limonia* appears to be comprised of a ring bark. It has outermost periderm layers composed of 20-25 layers. Vascular elements and starch grains are the characteristic features of stem bark. Cork, cortex with abundant starch grains, phloem elements, sclerenchymatous cells and pericyclic fibres are the characteristic features of FL root bark.

Powder microscopy analysis of leaves, showed the presence of multicellular uniseriate trichomes, stomata, polygonal epidermal cells. Powder of stem bark showed the presence of cork cells, phelloderm cells, periderm cells, xylem, rhomboidal crystals and parenchyma cells. Powder of root bark showed the presence of parenchyma, parenchyma with crystals, starch grains and xylem fibers.

Proximate analysis was performed to help setting certain standards for dried drugs in order to avoid batch-to-batch variation and to judge their quality and purity. Parameters like total ash, acid insoluble ash, water soluble ash, water soluble extractive value and alcohol soluble extractive values, elemental analysis and fluroscence analysis were studied under proximate analysis.

Total ash value was found to be highest in stem bark followed by root bark and leaves. Similarly water soluble extractive values were higher in root bark followed by leaves and stem bark. These determinations provided an idea regarding the probable content of various inorganic metal ions as well as the nature of the constituents present. More ash value indicates the presence of more inorganic matter.

Results of elemental analysis showed that potassium and iron were present in highest quantities in all the three parts viz., leaves, stem bark and root bark followed by sodium content. Apart from this zinc, manganese, magnesium and copper were present in smaller quantities in all the studied parts.

The colour of the plant extract in UV light is mainly due to its chemical composition. The same extract may appear in different colors at different wavelength of light (254 and 366 nm) and these colors are characteristic for the particular drug or different parts of same drug. Therefore, fluorescence studies were done on powdered leaves, stem bark and root bark of *Feronia limonia.*

Successive extracts were prepared by subjecting coarsely powdered plant materials to the solvents of increasing polarity viz., petroleum ether, toluene, chloroform, ethyl acetate, methanol and water. The successive extracts were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents. Results of preliminary phytochemical tests indicate that coumarins and flavanoids were he major group of constituents present in most of the extracts of leaves, stem bark and root bark. Alkaloids, saponins and glycosides were present only in some extracts. Most of the compounds were found to be present in methanol extract, therefore, the same extract had been used for fingerprinting studies.

Thus, in continuation of our studies on qualitative analysis of secondary metabolites present in successive extracts of leaves, stem bark and root bark of *Feronia limonia*, TLC studies were performed. A complete TLC finger print profile of the successive extracts comprises of the typical spectra, R_f value, UV absorption maxima for chemical constituent present in the extracts, were recorded and documented.

Results of HPLC analysis of methanolic extract of *Feronia limonia* leaves, stem bark and root bark (mobile phase, methanol-water; 1:1, flow rate; 2ml/min, detection; 254 nm and 366 nm) shows that various constituents are present in them which is confirmed by the various peaks present in the chromatogram at different retention time (in mins). The reported data may be of great value as a reference standard for evaluation of the plant materials.

The total phenolic content of methanol extract of *Feronia limonia* leaves, stem bark and root bark were found to be 0.0312±0.81; 0.0495±0.75; 0.043±0.70 % w/w respectively, representing the various phenolic compounds like poly phenol, phenolic acid etc. The total flavanoid content determined by $AlCl_3$ method in methanol extract of *Feronia limonia* leaves, stem bark and root bark were found to be 0.0015±0.33, 0.016±0.13, 0.012±0.81 % w/w respectively.

Studied plant parts were extracted with methanol and various fractions were prepared. Identification of major constituents present in the prepared fractions of leaves was monitered by TLC and derivatization with different detecting reagent. Based on the evidence in the literature and coumarin being the major secondary metabolites of *Feronia limonia* leaves, the chloroform fraction (FL-9) of methanol extract (FL-7) was chosen for phytochemical studies. One compound designated as (MR-2) was isolated by column chromatography and preparative TLC of same fractions. MR-2 was characterized by IR, NMR,and Mass spectroscopy as -9-methoxy-furo [3,2-g] chromen-7-one. The % purity of isolated compound found to be 96 %, as determined by analytical HPLC.

Based on the HPTLC fingerprinting pattern of *Feronia limonia* stem bark the chloroform fraction (FSB-9) of methanol extract (FSB-7) was chosen for isolation studies. One compound designated as (MR-1) was isolated by column chromatography and

preparative TLC. MR-1 was characterized by IR, NMR and Mass spectroscopy as marmesin. The % purity of isolated compound marmesin was found to be 100%, as determined by analytical HPLC.

The same compound (MR-1) was found to be present in root bark of *Feronia limonia* as revealed by the Co-TLC.

In order to get qualitative insights into the bioactive constituents of the leaves; stem bark and root bark of *F. limonia* qualitative assays were done. TLC characterization of total tannin (poly phenols) using mobile phase n-butanol- acetic acid- water, 40:1:5 and Ferric chloride reagent from the FL-1, FL-7 and FL-9 revealed 1,10 and 5; stem bark FSB-7 revealed 5; root bark FRB-7 revealed 6, yellowish black zones respectively between the start and solvent front. Separation of flavanoids using mobile phase ethyl acetate– formic acid–acetic acid–water 100:11:11:27 and derivatized with Natural product reagent/PEG it showed number of spots between the start and solvent front .

Marmesin a marker constituent of *Feronia limonia* was quantified in stem bark by validated HPTLC method. Chloroform: methanol (9.5:0.5v/v) was used as mobile phase. Plates were scanned at 238 nm and marmesin content was found to be 0.03412 %w/w in stem bark. HPLC method was also performed to quantify this marker in leaves, stem bark and root bark and it was found to be 4.71± 0.32, 4.91 ±0.69 , 20.86± 1.05 respectively.

In order to provide a scientific basis and also to validate traditional utilization preliminary *in vitro* cytotoxicity of plant extracts and bioassay guided fractions has gained importance in recent times for primary level screening. Additionally, these studies aid to correlate the activity with some components in the plant. Thus biological screening along with chemical profiling provides additional means of standardization of a plant drug.

For assessing hepatoprotective potential of phytoconstituents and bioassay guided fractions, human hepatoma cell line (Hep G2) emerged as a popular and an effective *in vitro* model because of its functional similarity with an intact liver.

Different extracts, fractions and compounds obtained from the various parts of FL, were subjected to MTT assay at different concentrations of 10, 20, 100, 250, 500, 750 and 1000 µg/ml. For FL leaves FL-1, FL-7, FL-9 and MR-2 showed more than 50 % cell

viability at 250 µg/ml concentration. Similarly for stem bark and root bark FSBs and FRBs showed more than 50 % cell viability at 250 µg/ml concentration therefore these compounds and fractions were further investigated for in vitro hepatoprotective potential. However, FL-10 and FL-11 recorded significant cytotoxicity which was characterized by less than 50 % cell viability at the dose studied herein.

Hepatoprotective studies were carried out for FLs, FSBs and FRBs along with 1% CCl_4 as a toxicant. On the basis of SGOT, SGPT levels and % cell viability studies on these samples, it was found that only FL-7, FL-9 and MR-2 from FLs; FSB-7 and MR-1 from FSBs; FRB-7 and MR-1 from FRBs showed good hepatoprotective effect and so only these samples were taken forward for in vivo hepatoprotective effect in rats.

Acridine orange (AO) / Ethidium bromide (EB) staining was done as supportive study to the hepatoprotective potential on control and treated groups (FLs, FSBs ,FRBs and CCl_4 groups). Results revealed that CCl_4 treatment accounted for maximum number of EB positive cell whereas the control group recorded AO positive cells. Also more number of AO positive cells were recorded in FLs, FSBs, FRBs and silymarin treated groups.

As per the guideline no. 423 prescribed by OECD the *in vivo* acute toxicity studies were performed. No mortality was recorded in animals that were orally administered upto 5000mg/kg, 2000mg/kg and 1000mg/kg extracts, fractions and compounds respectively.

For *in vivo* hepatic lipid peroxidation study and *in vivo* antioxidant (SOD, CAT, GSH, AA and LPO) activity was performed for FLs, FSBs, FRBs and Silymarin, at different doses. Pre- treatment with FLs (FL-7, FL-9 and MR-2); FSBs (FSB-7); FRBs (FRB-7 and MR-1) and silymarin was able to prevent CCl_4induced depletion of hepatic antioxidants at higher dose.

Finally, CCl_4 induced hepatotoxicity study and protective effect of FLS, FSBs and FRBs on CCl_4 treated group was studied. Various parameters of liver injury like AST, ALT, ALP, total bilirubin and total protein were estimated. Results showed that, CCl_4 treated group recorded significantly elevated levels of AST, ALT, ALP and total bilirubin whereas the total protein content in plasma significantly reduces. However, FLs (FL-7, FL-9 and MR-2); FSBs (FSB-7); FRBs (FRB-7 and MR-1) treated group prevented CCl_4

induced decrement in plasma protein. Higher doses of all samples were the most efficient in mitigating CCl_4 induced hepatotoxicity.

6.2 *Tecomella undulata*

Tecomella undulata seem. (Bignoniaceae) is a deciduous medium sized tree, which is commonly known as *Rohitaka, Rohira* and *Rakta-Rohida* in India. Some important morphological features for identification of *Tecomella undulata* and its parts are documented herein. It is a deciduous and nearly evergreen tree of arid and semi-arid regions. The leaves are elongated, alternate, rounded at the tips; lamina elliptic-oblong to elliptic-lanceolate or linear-oblong. Stem bark of *Tecomella undulata*, occurs in flat or slightly curved pieces of about 6-8 mm in thickness. The outer surface of bark is grayish brown with occasional small dark patches.

Microscopic features showed that *Tecomella undulata* leaves are isobilateral with mesophyll tissue comprising of palisade layers on either side of the spongy tissue. Both epidermises are covered with thick cuticle. Multicellular stalked glands are intermittently found deeply sunken in both epidermises. Large cresceutic vascular bundles lies on the abaxial side with phloem towards the abaxial side and xylem elements arranged radially toward the adaxial side. Young stem barks showed a single layer thick walled epidermis, some of its cells have non- glandular and glandular hairs.

Powder microscopy analysis of leaves showed the presence of anisocytic stomata with guard cells and surrounding 5-6 subsidary cells with arched walls were seen embedded intermittently between epidermal cells. Fragments of the multicellular glands and multicellular trichomes wre also observed. Powder microscopy analysis of *Tecomella undulata* showed the presence unicellular glandular trichomes, periderm cells and scleraids/ scleratic cells.

Proximate analysis was done to help setting certain standards for dried drugs in order to avoid batch-to-batch variation and to judge its quality. Parameters like total ash, acid insoluble ash, water ssoluble ash, water soluble extractive value and alcohol soluble

extractive values, elemental analysis and fluroscence analysis were studied under proximate analysis.

Total ash value of *Tecomella undulata* leaves and stem bark showed relatively higher ash value indicating high quantity of carbonates and oxide. Low acid insoluble ash values in leaves and stem bark are indicative of presence of less silicious material like earth sand.

The higher water soluble extractive values of *Tecomella undulata* leaves (21.49±0.65) and stem bark (31.31±0.54) indicate the presence of very high quantity of polar constituents than non polar constituents.

Results of elemental analysis shows that iron is present in highest quantities in both plant parts viz., leaves and stem bark whereas zinc, copper and manganese are present in minor quantities. The colour of the plant extract is mainly due to its chemical composition. The same extract may appear in different colors at different wavelength of light (254 and 366 nm) and these colors are characteristic for the particular drug or different parts of same drug. Therefore, fluorescence studies were done on leaves and stem bark of Tecomella *undulata.*

Thus, in continuation of our studies on qualitative analysis of secondary metabolites present in successive extracts of leaves and stem bark of *Tecomella undulata*, TLC studies were performed. A complete TLC finger print profile of the successive extracts comprises of the typical spectra, R_f value, UV absorption maxima and UV absorption maxima for chemical constituent present in the extracts, were recorded and documented.

Results of HPLC analysis of *Tecomella undulata* leaves and stem bark methanol extract in mobile phase, acetonitrile-water; 75:25v/v at flow rate of 1ml/min, detection at 254 nm and 366 nm showed that various constituents were present in them which was confirmed by the various peaks present in the chromatogram at different retention time (in mins). The reported data may be of great value as a reference standard for evaluation of the plant materials.

The total phenolic content of methanol extract of *Tecomella undulata* leaves and stem bark was found to be 0.125±0.20 and 0.173±0.18 % w/w respectively, representing the various phenolic compounds like poly phenol, phenolic acid etc. The total flavanoid

content determined by $AlCl_3$ method in methanol extract of *Tecomella undulata* leaves and stem bark was found to be 0.0139±6.66, 0.0021±8.81 respectively.

Leaves of *Tecomella undulata* were extracted with petroleum ether and methanol to produce unsaponifible fraction from pet ether extract and chloroform fraction was prepared by liquid -liquid partition from methanol extract. Identification of betunilic acid present in the prepared fractions of leaves was checked by HPLC (mobile phase, acetonitrile-water; 85:15v/v, flow rate; 1ml/min, detection; 205 nm, retention time; 7.613). The chromatogram of methanol extract of leaves showed many peaks with retention time (in mins) and chromatogram of marker compound shows single major peak at retention time 7.613. Retention time of marker constituent was overlaid with one of the constituent present in leaves methanolic extract, reconfirmation of identity of marker in leaves was done by adding standard solution in leaf methanol extract sample.

Based on the HPTLC fingerprinting pattern of *Tecomella undulata* stem bark the acidic fraction (TSB-2) obtained from petroleum ether extract was chosen for phytochemical studies. One compound designated as (MS-1) was isolated by preparative TLC from TSB-2. MS-1 was characterized by overlaid IR spectra as lapachol. The % purity of isolated compound lapachol was found to be 97 %, as determined by analytical HPLC.

Another compound (MS-2) was isolated from ethyl acetate fraction (TSB-9) of methanolic extract of *Tecomella undulata* stem bark.TSB-9 was subjected to column chromatography and preparative TLC to obtain MS-2. MS-2 was characterized by overlaid IR spectra and UV spectra as betunilic acid. The % purity of isolated compound was found to be 98 %, as determined by analytical HPLC.

Lapachol a marker constituent of *Tecomella undulata* and it was quantified in stem bark by validated HPLC method. Acetonitrile: 0.25% acetic acid in water (50:50 v/v) was used as mobile phase. Detection was done at 262 nm and lapachol was content was found to be 2.015 $\pm$ 0.08 mg per 100 gm powder.

In order to derive scientific evidences and also to validate traditional utilization, in recent times *in vitro* cytotoxicity of plant extracts and bioassay guided fractions has gained importance for primary level screening. Additionally, these studies aid to correlate the

activity with some components in the plant. Thus biological screening along with chemical profiling provides additional means of standardization of a plant drug. For assessing hepatoprotective potential of phytoconstituents and fractions, human hepatoma cell line (Hep G2) emerged as a popular and effective *in vitro* model because of its functional similarity with an intact liver.

Different extracts, fractions and compounds obtained from leaves and stem bark of *Tecomella undulata*, were subjected to MTT assay at different concentrations of 10, 20, 100, 250, 500, 750 and 1000 µg/ml. For *Tecomella undulata* leaves only TL-1, TL-2 and TL-7 showed more than 50 % cell viability at 250 µg/ml concentration. Similarly for stem bark TSB-1, TSB-7, TSB-9, TSB-10 and MS-2 showed more than 50 % cell viability at 250 µg/ml concentrations therefore these were further investigated for *in vitro* hepatoprotective potential. However, TSB-2 and MS-1 recorded significant cytotoxicity which was characterized by less than 50 % cell viability at the dose studied herein.

The MTT assay was followed by hepatoprotective studies on TLs and TSBs along with 1% CCl_4 as a toxicant. On the basis of SGOT, SGPT levels and % cell viability studies on these samples, it was found that only TL-2 from leaves and TSB-7, TSB-9 and MS-1 showed good hepatoprotective effect whereas TL-1, TL-7 from leaves and TSB-1 and TSb-10 from stem bark has non-linear dose dependent decrement in activity levels of SGOT and SGPT and therefore only TL-2 was taken forward for in vivo hepatoprotective effect in rats.

Acridine orange (AO) / Ethidium bromide (EB) staining was done as supportive study to the hepatoprotective potential on control and treated groups (TLs, TSBs and CCl_4 groups). Results revealed that CCl_4 treatment accounted for maximum number of EB positive cell whereas the control group recorded AO positive cells. Also more number of AO positive cells were recorded in TL-2, TSB-7, TSB-9, MS-1 and silymarin treated groups.

As per the OECD guidelines for acute *in vivo* acute toxicity were performed. No mortality was recorded in animals that were orally administered upto 5000mg/kg, 2000mg/kg and 1000mg/kg extracts, fractions and compounds respectively.

For *in vivo* studies, hepatic lipid peroxidation study and *in vivo* antioxidant (SOD, CAT, GSH, AA and LPO) activity were performed on TLs, TSBs, and Silymarin, at different doses. Pre- treatment with TLs (TL-2); TSBs (TSB-7, TSB-9 and MS-2) and silymarin was able to prevent CCl_4 induced depletion of hepatic antioxidants at higher dose.

Finally, CCl_4 induced hepatotoxicity study and protective effect of TLs and TSBs on CCl_4 treated group was studied. Various parameters of liver injury like AST, ALT, ALP, total bilirubin and total protein were estimated. Results showed that, CCl_4 treated group recorded significantly elevated levels of AST, ALT, ALP and total bilirubin whereas the total protein content in plasma significantly reduced. However, TLs (TL-2) and TSBs (TSB-7, TSB-9 and MS-2) treated group prevented CCl_4 induced decrement in plasma protein. Higher dose of all samples were the most efficient in mitigating CCl_4 induced hepatotoxicity.

Conclusions

- The selected plants *Feronia limonia* and *Tecomella undulata* were successfully evaluated for their correct identity by studying their pharmacognostic features.
- Parameters like, foreign matter, ash value, extractive value, loss on drying, elemental analysis was determined, as per WHO guidelines, for assessing their quality standards.
- Marmesin was isolated from stem and root bark of *Feronia limonia* whereas 9-methoxy-furo [3,2-g] chromen-7-one (MR-2) was first time isolated from leaves of *Feronia limonia*. It was further identified in stem bark of *Feronia limonia* by Co-TLC.
- Lapachol was isolated from the stem bark of *Tecomella undulata* and betunilic acid was first time isolated from stem bark, it was also identified in leaves of *Tecomella undulata* by HPLC.
- Validated HPTLC and HPLC methods were developed for quantification of marmesin in leaves, stem bark and root bark of *Feronia limonia*.

- Validated HPLC method was developed for quantification of lapachol in *Tecomella undulata* stem bark.

The *in vitro* and *in vivo* studies, that the methanolic extract of *Feronia limonia* root bark (FRB-7) showed maximum hepatoprotection followed by leaves and stem bark extracts. These results could be attributable to the relatively high content of flavanoids and coumarins in root bark, which provides maximum conjugation with free radical species generated because of CCl_4 toxicity thereby reducing the number of free radicals and extent of cellular damage by decreasing lipid peroxidation, scavenging super oxide radicals and maintaining level of GSH.

The isolated compounds marmesin (MR-1) and 9-methoxy-furo [3,2-g] chromen-7-one (MR-2) were first time subjected to *in vitro* and *in vivo* hepatoprotective potential and they gave significant hepatoprotective activity at higher dose (100 mg/kg body weight).

From the results of biological studies on *Tecomella undulata*, it was concluded that the unsaponifible fraction (TL-2) obtained from leaves petroleum ether extract and ethyl acetate fraction (TSB-9) obtained from stem bark methanolic extract, possess good hepatoprotective potential.

It was concluded from the *in vitro* cytotoxicity studies that the isolated compound, lapachol (MS-1) was toxic to HepG2 cells and so it was not carried further to hepatoprotective studies. Another isolated compound betunilic acid (MS-2) showed significant hepatoprotective activity at higher dose (150 mg/kg body weight).

Hence, through the present studies on both the selected plants, *Feronia limonia* and *Tecomella undulate*, their hepatoprotective claims could be validated to a reasonably accepted level.