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# PHYTOCHEMICAL INVESTIGATIONS AND PHARMACOLOGICAL EVALUATION OF Mimusopselengi L. BARK FOR HEPATOPROTECTIVE ACTIVITY

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**Abstract:**
*The present study includes phytochemical and pharmacological evaluation of bark. Here the powdered bark was subjected to successive soxhlet extraction using different solvents of increasing polarity. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). Today there is growing interest in chemical composition of plant-based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. Bark of MimusopselengiL. is an evergreen tree, belonging to family Sapotaceae, cultivated in gardens as an ornamental tree. It is traditionally used as cardiotonic, anthelmintic, in the treatment of leprosy and various ailments. The successive methanol and aqueous extracts were subjected for In-vivo hepatoprotective evaluation. The methanolic extract was found more effective. So it was further subjected for preliminary phytochemical and chromatographic examination. Attempt was made to isolate the compounds using isocratic elution technique.*

***Keywords:*** *Elution, Hebalism, Herb, Metabolites, Diagnostic*

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## INTRODUCTION:

Herbal medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Plants produce a variety of chemical substances that act upon the human body.

Many plants bio synthesise chemical substances that are useful for the maintenance of health in humans and other animals. Many are secondary metabolites, of which at least 12,000 have been isolated-a number estimated to be less than 10% of the total. In many cases these substances (particularly the alkaloids) serve as plant defense mechanisms against insects and herbivores. Many of the herbs and species used by humans as season as food yields useful medicinal compounds.

Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). Today there is growing interest in chemical composition of plant-based medicines. Several bioactive constituents have been isolated and studied for pharmacological activity. Herbal medicine is the oldest form of health care known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20$^{th}$ century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound.[1]

## MATERIALS AND METHODS:

### Morphological Characters

Morphology is the study of the form of an object while morphography is the description of that form where the material is known to occur in a particular form. Morphological and organoleptic characters viz. colour, odor, taste, shape and size were observed and evaluated botanically.

### STUDY OF MICROSCOPICAL CHARACTERS

**a. Transverse section of the Bark**

The microtome section of the bark was taken observed under low and high-power magnification of microscope.

**b. Powder microscopy**

The dried bark of *Mimusopselengi*L. were coarsely powdered and boiled with chloral hydrate for 5-10 minutes and then stained with phloroglucinol and HCl in 1:1 ratio, observed under high power (40x), for different diagnostic characters such as cork cells, crystal fibres, medullary rays with attached fibres and cortex cells with starch grains.

**c. Proximate Values**

The following proximate values were determined for the bark powdered *Mimusopselengi*L.

**Extractive values**

Extractive values help to determine the number of soluble constituents in a given amount of medicinal plant material, when extracted with various solvents. The extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of drug and solvent used. The use of single solvent can also be used by means of providing preliminary information of quality of a particular drug sample.

**Alcohol soluble extractive value**

5 g of shade-dried *Mimusopselengi*L. bark powder was macerated with 100ml of 95% ethanol in a closed flask, shaking frequently during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly taking precaution against loss of ethanol. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish, dried at 105$^0$C and weighed.

**Water soluble extractive value**

5 g of shade-dried *Mimusopselengi*L. bark powder was macerated with 100ml of water in a closed flask, shaking frequently during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish, dried at 105$^0$C and weighed. Percentage of extractive values was calculated with reference to the shade-dried barkpowder.

**Petroleum Ether soluble extractive value**

5 g of shade-dried *Mimusopselengi*L. bark powder was macerated with 100ml of Petroleum ether in a closed flask, shaking frequently during the first 6hrs and allowed to stand for 18hrs. Thereafter it was filtered rapidly. Evaporate 25ml of filtrate to dryness in a

tarred flat bottom shallow dish, dried at 105$^0$C and weighed. Percentage of extractive values was calculated with reference to the shade-dried bark powder.

**Moisture content**

An accurately weighed quantity of the shade-dried coarsely powdered *Mimusopselengi*L. bark powder was taken in a tared glass bottle and the initial weight was taken. The crude drug was heated at 105$^0$C in an oven and weighed. This procedure was repeated till a constant weight was obtained. The moisture content of the sample was calculated as percentage with reference to the shade-driedmaterial.

**Ash values**

**Total ash**

3 g of accurately weighed quantity of the shade-dried coarsely powdered bark of *Mimusopselengi*L. was taken in a tared silica crucible and incinerated at a temperature not exceeding 450$^0$C until free from carbon, cooled and weighed. The percentage of total ash was calculated with reference to shade-dried bark powder.

**Acid-insoluble ash**

Total ash obtained was boiled for five minutes with 25 ml of dilute Hydrochloric acid. The insoluble matter was collected on an ash-less filter paper, washed with hot water and ignited, cooled and weighed. The percentage of acid insoluble ash was calculated with reference to shade-dried bark powder.

**Water-soluble ash**

Total ash obtained was boiled for five minutes with 25ml of distilled water, cooled and collected the insoluble matter on an ash-less filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450$^0$C. Subtracted the weight of the insoluble ash. The percentage of water-soluble ash was calculated with reference to shade dried bark powder.

**Sulphated ash**

Silica crucible is heated to redness for 10 minutes; cooled and weighed. 1 gram of air-dried bark powder is placed in silica crucible, moistened with sulphuric acid, ignited gently, again moistened with sulphuric acid and ignited at about 800$^0$C. Cooled and weighed, once again ignited for 15 minutes and weighed. The percentage of sulphated ash was calculated with reference to air-dried barkpowder.

**PHYTOCHEMICALINVESTIGATIONS**

*Mimusops elengi*L. Bark was subjected to following phytochemical investigations:

**Extraction:**

**Successive Extraction:**

The*Mimusopselengi*L. bark was shade dried at room temperature, pulverized, and 250 g of coarse powder each batch was successively extracted with chloroform, methanol and water respectively in increasing order of polarity. The extracts were concentrated under reduced pressure using Rotary flash evaporator and the residues were dried in dessicator over sodiumsulfite.After drying,the respective extracts were weighed and percentage yield was calculated. All the extracts were further subjected for preliminary phytochemical investigations by qualitative chemical tests.

**Preliminary phytochemical analysis:**

All the extracts of *Mimusopselengi*L bark were subjected to qualitative chemical tests to detect the presence of various phytoconstituents.

**Tests for Carbohydrates**

**Molisch's test:**

Treat the extract solution with few drops of alcoholic -napthol. Add 0.2 ml of concentrated $H_2SO_4$ slowly through the sides of the test tube, purple to violet colored ring appears at the junction.

**Benedict's test:**

Treat the extract solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms if reducing sugars are present.

**Fehling's test:**

Equal volume of Fehling's A (Copper sulphate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed along with few drops of extract solution, boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

**Tollen's test:**

To 100mg of extract add 2ml of Tollen's reagent, a silver mirror is obtained inside the wall of the test tube, indicates the presence of aldose sugar.

**Bromine water test:**

It gets decolorized by aldose but not by the ketose, because bromine water oxidizes selectively the aldehyde group to carboxylic group, giving raise to general class of compounds called aldonic acid.

**Tests for Proteins and Aminoacids**

**Millon's Test:**

Extract solution + 2 ml of Millon's reagent (Mercuric nitrate in nitric acid containing traces of nitrous acid) white precipitate appears, which turns red upon gentle

heating.

**NinhydrinTest:**
Amino acids and proteins when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3-trione hydrate), produces violet color.

**Tests forAlkaloids**

**Mayer's test:** Potassium mercuric iodide solution. To the extract/sample solution, add few drops of Mayer's reagent, creamy white precipitate is produced.

**Dragendroff's test:** Potassium bismuth iodide solution. To the extract/sample solution, add few drops of Dragendroff's reagent, reddish brown precipitate is produced.

**Wagner's test:** Solution of Iodine in Potassium Iodide. To the extract/sample solution, add few drops of Wagner's reagent, reddish brown precipitate is produced.

**Hager's Test:** Saturated solution of Picric acid. To the extract/sample solution, add few drops of Hager's reagent, yellow precipitate is produced.

**Tests forTannins**

**Gelatin test:**

Extract solution with 1% gelatin solution containing 10% sodium chloride gives white precipitate.

**Ferric chloride test:** Extract solution gives blue-greencolor precipitate with $FeCl_3$.

**Test for anthraquinone glycosides**

**Modified BorntragerTest:**
To the 5 ml of extract add 5 ml of 5% Ferric chloride & 5 ml dil. HCL. Heat for 5 min in boiling water bath, cool, add benzene or any organic solvent. Shake well. Separate organic layer, add equal volume of dil. Ammonia. Ammonical layer shows pinkish red colour.

**Test for Steroidal glycosides**

**Kedde'stest:**

Extract the leaf powder with chloroform, evaporate to dryness and add one drop of 90% of alcohol and 2 drops of 2% 3,5-dinitro benzoic acid (3,5, dinitrobenzene carboxylic acid - Kedde's reagent) in 90% alcohol. Make alkaline with 20% sodium hydroxide solution. A purple color is produced. The color reaction with dinitrobenzoic acid depends upon the presence of an alpha, beta unsaturated beta lactone in the aglycone.

**PHARMACOLOGICALSCREENING**

**I] In vivo Hepatoprotective activity**

**1) Animal selection**

**2) Acute toxicity study**

**3) Extracts used**

**4) Methods of screening**

**Animal selection:**
The experiment was carried out using Wistar Albino mice of either sex weighing between 25-30gm for acute toxicity study and Wistar Albino rats of either sex weighing around 150-200gm were used for the Hepatoprotective activity. Animals were maintained at normal laboratory conditions and were given standard animal feed.

**Acute Toxicity Studies:**
Albino mice of either sex weighing between 20-30gm were used during investigation. The animals were fasted overnight. The OECD guideline no-423 fixed dose method was adopted and accordingly a dose of Total alcoholic extract was calculated.

As per following the OECD guideline no-423 fixed dose method, the safest dose of the total alcoholic extract is 2000mg/kg body weight. The safe dose was found to be 2000mg/kg body weight; hence $1/10^{th}$ of the dose was taken as effective dose which is found to be 200mg/kg body weight.

**Extract used:**
Successive methanol and aqueous extracts of the bark of *Mimusopselengi*L were screened for hepatoprotective property. The dried methanolic extract & aqueous extract were suspended in distilled water using 1% Tween 80 as an emulsifying agent and were employed to assess hepatoprotective activity. The dose was given orally. Liv-52 syrup was used as positive control and carbon tetrachloride ($CCl_4$) was used as hepatotoxins to induce hepatotoxicity. The Liv-52 dose taken was 5ml/kg body weight by oral route and carbon tetrachloride [$CCl_4$] 1ml/kg body weight by Intra peritonial route.

**Hepatoprotective activity:**
**Carbontetrachloride induced hepatotoxicity model.**
Chronic administration of carbontetrachloride to rats induces severe disturbances of hepatic function together with histologically observable liver disturbances.

Hepatoprotective activity was carried out using male Albino rats (150-180gm). The animals are grouped into five of six animals each & maintained on standard

diet & water ad libitum.

**Group I:**serve as normal control and received 1% Tween-80 (1ml/kg; p.o) daily for 7 days.

**Group II:**serve as toxic control and received $CCl_4$: olive oil (1:1, 2ml/kg of body wt; i.p.) on day 1 and day 7 of theexperiment.

**Group III:**Animals treated with standard drug Liv-52 (5ml/kg; p.o) daily for 7 days and received $CCl_4$: olive oil (1:1, 2ml/kg of body wt; i.p.) on day 1 and day 7, 30 min after administration of Liv-52.

On the 8th day of the experiment, the animals were anaesthetized with mild ether and 1ml blood was collected into the Eppindrof tube by retro-orbital vein puncture. The blood collected in Eppindrof tube was centrifuged to separate serum and used for the estimation of various biochemical parameters like SGOT, SGPT, SALP and Bilirubin (total and direct). Livers were excised and fixed in formalin for assessment of Histopathological studies.

**Assessment of liver function:**

Biochemical parameters such as Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Alkaline Phospatase (SALP) and bilirubin (total & direct) were determined according to the standard procedure prescribed by manufacturer (TransasiaBiomedicals Ltd., Daman, India).

## RESULTS AND DISCUSSION:

**Morphological Characters**

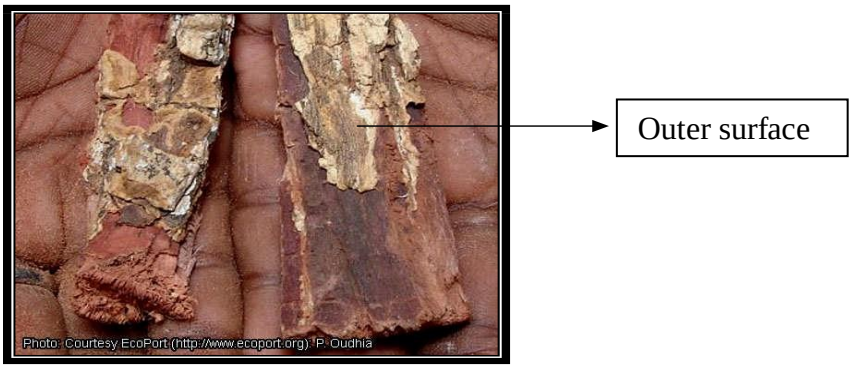


**Figure No. 1 outer surface Bark**

**Table No. 1 Morphological feature of bark**

| S. NO. | FEATURES | OBSERVATION |
|---|---|---|
| 1. | Outer surface | Grey to black. |
| 2. | Inner surface | Reddish brown. |
| 3. | Taste | Bitter. |
| 4. | Odour | Odourless. |
| 5. | Shape | Quilled. |
| 6. | Fracture | Fibrous. |
| 7. | Size | Length: 10-12cm, Bridth: 3-5cm.<br>Thickness : 0.5-1.5cm |

**Microscopic character**

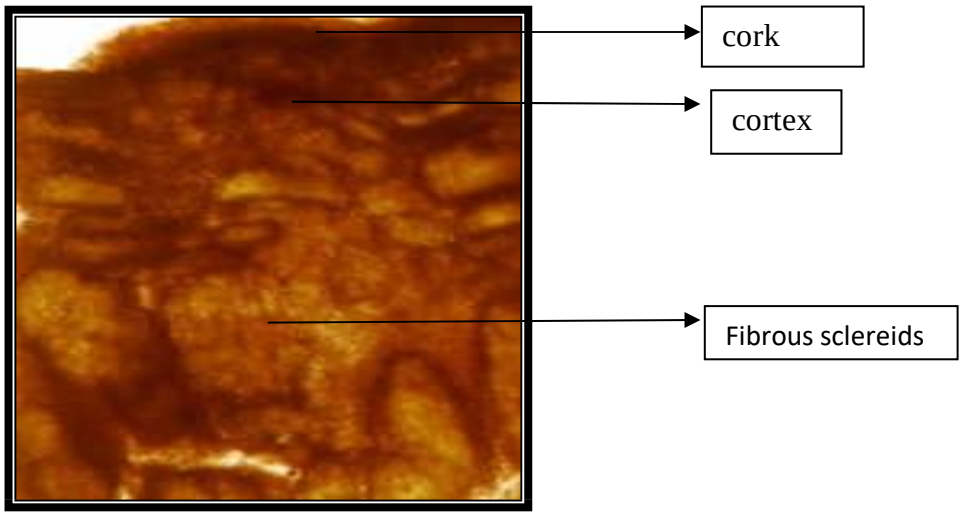


**Figure No: 2 Microscopic character of bark**

**Proximate values of *Mimusopselengi*L bark.**

**Table No: 2 Value of extractive values & ash values of bark**

| S. No. | Parameter | Determined Value % w/w |
|---|---|---|
| A | **Extractive values** | |
| 1 | Alcohol soluble extractive value | 22.00 |
| 2 | Water soluble extractive value | 28.00 |
| 3 | Pet ether soluble extractive value | 1.00 |
| B | Moisture content | 12.75 |
| C | **Ash values** | |
| 1 | Total ash | 6.5 |
| 2 | Water soluble ash | 1.00 |
| 3 | Acid insoluble ash | 1.5 |
| 4 | Sulphated ash | 4.5 |

**PHYTOCHEMICALINVESTIGATIONS**

**Table No: 3 Percentage yield and physical characteristics of various extracts of *Mimusopselengi*L bark.**

| Extract | % Dry wt in gms. | Colour | Odour | Consistency |
|---|---|---|---|---|
| **Chloroform** | **2.86** | **Brownish black** | **Characteristic** | **Sticky mass** |
| **Methanol** | **23.8** | **Brown** | **Characteristic** | **Powder** |
| **Aqueous** | **3.34** | **Reddish brown** | **Characteristic** | **Powder** |

**Methanolic extract was further fractionated with ethyl acetate**

**Table No: 4 Results of ethyl acetate fraction**

| Fraction | % Dry wt in gms. | Colour | Odour | Consistency |
|---|---|---|---|---|
| Ethyl acetate | 4.00 | Reddish brown | Characteristic | Powder |

**Table No: 5 Preliminary phytochemical tests of various extracts of *Mimusopselengi*L bark.**

| Test | Successive Extraction | | | |
|---|---|---|---|---|
| | CHCl3 | MeOH | AQ | EA |
| Alkaloids | +ve | --ve | --ve | --ve |
| Steroids | +ve | --ve | --ve | --ve |
| Carbohydrates | --ve | --ve | --ve | --ve |
| Phenolic compounds | --ve | +ve | +ve | --ve |
| Flavonoids | --ve | +ve | --ve | +ve |
| Glycoside | --ve | --ve | --ve | --ve |
| Triterpenoid | +ve | --ve | --ve | --ve |
| Tannins | --ve | +ve | +ve | +ve |

**Table No: 6 Estimation of Cyanidinin *Mimusopselengi*L. Bark.**

| Sl. No. | Sample | Absorbance at 530nm |
|---|---|---|
| 1 | Ethyl acetate fraction | 0.625 |
| 2 | Methanol extract | 0.406 |

**PHARMACOLOGICAL INVESTIGATIONS**

**Table No: 7 Effect of Methanol and Aqueous extracts on SGPT levels in hepatotoxic rats.**

| Animals | Normal | $CCl_4$ | Standard | Methanol | Aqueous |
|---|---|---|---|---|---|
| 1 | 46.42 | 163.42 | 69.15 | 79.65 | 124.43 |
| 2 | 49.12 | 155.35 | 85.35 | 93.16 | 123.41 |
| 3 | 52.46 | 147.32 | 62.35 | 81.46 | 113.41 |
| 4 | 49.16 | 155.35 | 61.43 | 73.31 | 125.48 |
| 5 | 53.68 | 135.32 | 58.64 | 63.46 | 134.31 |
| 6 | 54.12 | 165.56 | 76.12 | 57.46 | 126.24 |
| Mean | 52.49 | 154.4 | 68.84*** | 73.75*** | 125.5** |
| SEM | 3.269 | 12.57 | 11.26 | 123.97 | 7.485 |
| SD | 1.276 | 4.825 | 3.190 | 5.395 | 3.215 |

## RESULTS AND DISCUSSION:

Present research includes Pharmacognostic, Phytochemical and Hepatoprotective investigations of the bark of
***Mimusopselengi*L.**

**Pharmacognostic Contributions:**

The bark was found to be black to reddish brown in color, odourless, single quilled with rough surface. The Transverse section and powder microscopy of the bark revealed the presence of cork, cortex, medullary rays, phloem fibre, fibrous sclereids, crystal fibres, medullary rays with attached fibres and cortex cells containing starch grains.

**Proximate values:**

**Proximate values for the bark of*Mimusopselengi*L. are as follows: Alcohol soluble**
extractive value (22.00%), Water soluble extractive value (28.00%), Pet ether soluble
extractive value (1.00%), Loss on drying (12.75%), Total ash (6.5%), Acid insoluble ash (1.5%), Water soluble ash (1.00%) and Sulphated ash (4.5%). These values are criterion to put the guidelines of identity and purity of crude drug.

**Phytochemical Investigations:**

Preliminary phytochemical investigations of chloroform, methanol and aqueous extract and ethyl acetate fraction of methanol extract revealed the presence of alkaloids, steroids, triterpenoids, tannins, flavonoids and phenolic compounds. Further, chromatography-based separation of ethyl acetate soluble fraction of methanol extract was carried out. The ethyl acetate fraction was found to contain three spots in TLC then an attempt was made to isolate these compounds by column chromatography with isocratic elution.

**PHARMACOLOGICAL SCREENING:**

**Acute toxicity study:**Acute toxicity studies were performed according to OECD guidelines of Method no: 423 and dose was fixed as 200 mg/kg b.w. of successive methanol and aqueous extracts.

**Hepatoprotective activity:**

The successive methanolic and aqueous extracts of bark of *Mimusopselengi*L. were evaluated for Hepatoprotective studies in Wistar Albino rats by $CCl_4$ induced hepatotoxicity. An increase in levels of SGPT, SGOT, SALP and Bilirubin (Total & Direct) in blood serum was observed. The results indicate that the elevated levels of SGPT, SGOT, SALP and Bilirubin (Total & Direct) in blood serum were significantly reduced in successive methanol and aqueous extracts. The reduction was significant in the following range: successive methanol extract > successive aqueous extract.The Histopathological studies of liver were carried out to support the above activity.

The observations were as follows:
Normal group: liver section showed hepatocytes from central vein andportal traids. $CCl_4$ control group: liver section showed fatty change with lobularnecrosis. Positive control group (Liv-52): liver section showed foci of necrosis.Binucleate hepatocytes seen with regenerativeactivity. Successive methanol extract treated group: sections from liver show minimal hepatocytes necrosis. Good number of binucleate regenerating hepatocytesare alsoseen. Successive aqueous extract treated group: liver section showed dense foci of necrosis. Hepatocytes showing fatty change and few regenerating hepatocytesare alsoseen. From the above studies it is evident that successive methanolic and aqueous extracts of bark of*Mimusopselengi*L. plays a promising role in the treatment of Liver disease and worth for further investigations for isolation of more bioactive phytoconstituents for the above treatment. The overall pharmacognostic, phytochemical investigations and Hepatoprotective research on bark of ***Mimusopselengi*L.** exhibited results to conclude as follows:
Morphological evidences to identify and authenticate the drug are as follows.

Microscopy of bark of *Mimusopselengi*L. Exhibited prominent histological features like cork, cortex, medullary rays, phloem fibre, fibrous sclereids, cork cells, crystal fibres, medullary rays with attached fibres and cortex cells containing starch grains.

Preliminary phytochemical investigations of chloroform, methanol, aqueous extracts and ethyl acetate soluble fraction of methanolic extract have revealed the presence of alkaloids, steroids, triterpenoids, tannins, flavonoids and phenolic compounds.

The successive methanol and aqueous extracts exhibited more promising hepatoprotective activity at the dose of 200mg/kg body weight which is supported by Histopathology.

## CONCLUSION:

The present study was carried out to investigate *Mimusopselengi*L. bark for pharmacognostic, phytochemical and *invivo* hepatoprotective screening.

**Collection and Extraction:**

Authenticated plant material was subjected to

morphological and microscopical analysis. Shade dried and powdered bark was subjected to successive soxhlet extraction with organic solvents of increasing polarity like chloroform, methanol and water. All the extracts were evaluated by qualitative chemical examination for the presence of important phytoconstituents.

**Evaluation for *in-vivo* Hepatoprotective activity:**
To confirm the Hepatoprotective activity of the drug the biochemical studies was carried out on Albino rats and significant reduction of various enzymes like SGOT, SGPT, ALP and bilirubin levels in blood serum of extract treated groups was observed in the following range: successive methanolic extract group > successive aqueous extract group. The above results were supported further by Histopathological studies, which revealed a significant regeneration of the hepatocytes with normal portal vein. The groups treated with above extracts were compared with $CCl_4$ group.

**ACKNOWLEDGEMENT:**
By the grace and mercy of almighty GOD, the most beneficent and merciful, this thesis entitled "**PHYTOCHEMICAL INVESTIGATIONS AND PHARMACOLOGICAL EVALUATION OF MimusopselengiL. BARK FOR HEPATOPROTECTIVE ACTIVITY**" has been a final shape. I extend my sincere thanks **Dr. Alok Pal Jain, Professor & Principal, RKDF college of pharmacy, Bhopal, MP** for his timely help throughout the project as well as providing instrumental facilities for carrying out of my work. Teacher is a guide, philosopher and friend, which I could experience in my respected dear and esteemed guide. I consider myself the luckiest to work under the guidance of **Mrs. Amrita Bhaiji, Associate Professor, Department of Pharmaceutics, RKDF college of pharmacy, Bhopal, M.P.** I take this opportunity to express my deep gratitude, sincere and heart-ful thanks to my reverend guide. It provides me pleasure to convey my graduate to all those who have directly or indirectly contributed to make this work a success.

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