FORMULATION AND EVALUATION OF *AILANTHUS EXCELSA* SYRUP FOR HEPATOPROTECTIVE ACTIVITY

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ABSTRACT

This study explores the formulation of a syrup containing Ailanthus excelsa bark extract for potential use in the treatment of jaundice. The syrup was evaluated for physicochemical properties, phytochemical content, antimicrobial activity, and hepatoprotective effects on HepG2 cells. The ethanolic extract exhibited significant flavonoid and phenolic content, as well as strong antimicrobial and cytoprotective activity, suggesting potential as a supportive therapy for liver-related disorders.

Keywords: *Ailanthus excelsa*, jaundice, syrup formulation, hepatoprotective, antimicrobial, MTT assay

INTRODUCTION

BACKGROUND STUDIES

The incidence of jaundice is approximately 40,000 per 100,000 individuals of patients. is more common among Asian and mixed Asian/white infants than white infants. mainly from commonly affects young patients. mainly from and liver commonly affects older patients. The 10 year- of cirrhosis is approximately 34-66 percent, largely dependent on the cause of cirrhosis. jaundice, particularly from commonly affects young

patients. Male are more commonly affected by and liver cancer than female. Female are more commonly affected by jaundice.

JAUNDICE

Jaundice is a condition characterized by the yellowing of the skin, sclera (the whites of the eyes), and mucous membranes due to elevated levels of bilirubin in the blood. Bilirubin is a yellow-orange pigment produced by the breakdown of red blood cells in the body. Typically, this substance is processed by the liver and excreted in bile, but when there's an imbalance between its production and elimination, jaundice occurs.

TREATMENT

Antiviral drugs - Entecavir, Tenofovir, Lamivudine, Adefovir and Telbivudine, Ursodeoxycholic acid (UDCA), Cholestyramine, N-acetylcysteine (NAC), Corticosteroids or immunosuppressants.

These synthetic drugs might not be as effective for treating jaundice because jaundice itself is often a symptom of an underlying condition rather than a standalone illness. It's typically caused by an excess of bilirubin in the blood, which can result from issues like liver disease, obstruction of the bile ducts, or excessive breakdown of red blood cells. The complexity of jaundice often requires addressing the root cause, which synthetic drugs might not effectively target. Treatment usually involves addressing the underlying condition causing the elevated bilirubin levels rather than directly treating jaundice itself. For instance, in cases caused by liver disease, treatment would focus on managing the liver condition rather than solely targeting jaundice symptoms.

Natural remedies or holistic approaches can sometimes complement medical treatments.The global herbal supplements and remedies market exhibited robust growth over the last decade, with little or no significant decline on account of the recent economic recession.Escalating prices, tighter budgets and high healthcare and lifestyle costs, have actually driven consumers towards the more economical, healthier and safer option of alternative medicine and dietary supplements, to get relief from physical and mental disorders. Presently, the market for herbal supplements varies by region based on factors such as consumer awareness, product availability, and forms of delivery, product acceptance, and regional regulations. In the US and Europe herbal medicines represent a major share of the pharmaceutical market and are included in the regular medical practice. In terms of growth rate, the Asia-Pacific market, led largely by China and India is set to pave the way with the highest CAGR of 10.5% through 2017. In terms of the product segments, Multi Herbs

dominates as the largest segment, capturing a significant share of the overall herbal supplements and remedies market worldwide.

Traditionally used herbs for Jaundice :

Curcuma longa, Silybum marianum, Taraxacum officinale, Emblica officinalis, Zingiber officinale, Phyllanthus niruri, Ailanthus excelsa

METHODOLOGY

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The plant of *Ailanthus excelsa* were vendor from local area of Dharapuram. They were authenticated from The Tamil Nadu Agriculture University, Coimbatore. The plant was authenticated by Dr.M. U Shariff [Scientist and Head of Botanical Department, The Tamil Nadu Agriculture University, Coimbatore].

EXTRACTION OF PLANT MATERIAL AND PREPARATION OF EXTRACT

The commonly employed technique for separation of active substance from crude drug is called as ‘Extraction’ which involves the use of different solvents. The plant material used for extraction should be properly authenticated or identified. The choice of the plant material for extraction depends upon its nature and the components required being isolated. The dried powdered plant material is commonly used for extraction. The solvent used for extraction is called menstrum and the residue is known as marc.

METHODS FOR PLANT EXTRACTION

SOXHLET EXTRACTOR

The Ailanthus excelsa stem bark was blended into coarse powder with the help of a mixer-grinder. Round bottom flask was cleaned, dried and fixed in the Soxhlet apparatus.Weighed accurately about 25g powder which was packed in muslin cloth and fixed into the thimble.

PHYTOCHEMICAL SCREENING OF EXTRACTS

The various extract obtained after extraction were subjected for phytochemical screening to determine the presence of following various phytochemical present in the extracts.

DETERMINATION OF HEAVY METALS

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution. it is used for substances that yield clear, colourless solutions with sodium hydroxide solutions.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique used in chemistry to separate, identify, and quantify components in a mixture. It's a form of column chromatography that uses a liquid mobile phase to move a sample through a chromatographic column packed with a stationary phase.

FORMULATION OF HERBAL SYRUP

66.7 g of Sucrose was weighed and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 100 ml. Herbal syrup prepared in three different concentration of herbal extracts of 20ml, 30ml, 40ml namely F1, F2, F3 respectively. Filter the herbal extract before add into the sucrose solution. Add the herbal extract into the sucrose solution.

S.NO	INGREDIENTS	WORKING FORMULA		
		F1	F2	F3
1	Extract	20 ml	30 ml	40 ml
2	Sucrose solution	78 ml	68 ml	58 ml
3	Flavouring agent	2 ml	2 ml	2 ml

EVALUATION OF HERBAL SYRUP

PHYSICOCHEMICAL PARAMETERS

The herbal syrup was evaluated for various physicochemical parameters such as physical appearance (colour, odour, taste), pH.

a) Color examination: Five ml final syrup was taken into watch glasses and placed against white back ground in white tube light. It was observed for its color by naked eye.
b) Odour examination: Two ml of final syrup was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.
c) Taste examination: A pinch of final syrup was taken and examined for its taste on taste buds of the tongue.
d) Determination of pH: Placed an accurately measured amount 10 ml of the final syrup in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.
e) Determination of Specific gravity: Clean thoroughly the specific gravity bottle with chromic or nitric acid. Rinse the bottle at least two to three times with purified water. If required, rinse the bottle with an organic solvent like acetone and dry. Take weight of empty dry bottle with capillary tube stopper. Fill the bottle with distilled water and place stopper; wipe out excess liquid from side tube using tissue paper (w2). Weight bottle with stopper and water on analytical balance (w2). Repeat the procedure for liquid under test by replacing the water after emptying and drying as mentioned in step 4 to 6. Weight bottle with stopper and liquid under test on analytical balance (w3).

Formula for specific gravity:

Specific gravity of liquid under test (syrup) = weight of liquid under test /weight of water = *w5/w4.*

IN-VITRO STUDIES

ANTI-BACTERIAL ACTIVITY

MICROBIAL STRAINS

The ethanolic extract of Ailanthus excelsa and aqueous Ailanthus excelsa extract were individually tested against different microorganisms including 2 Gram positive bacteria (Bacillus subtilis ATCC6633, Staphylococcus aureus CIP 7625) and 2 Gram-negative bacteria (klebsiella pneumoniae CIP 8291, Escherichia coli ATCC10536). The bacterial strains were cultured on Mueller-Hinton agar for 48h at 37°C before use. All microorganisms were regenerated twice before use in the manipulations.

DISC DIFFUSION ASSAY

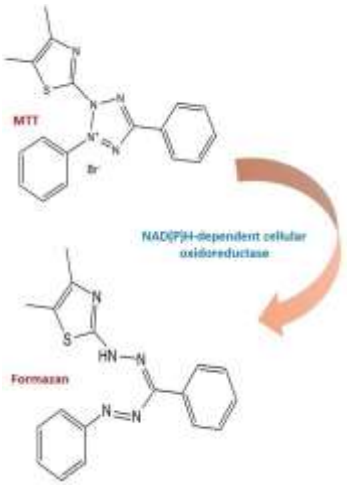
Disk-diffusion method was employed for the determination of antimicrobial activity of the ethanolic extract of Ailanthus excelsa. Microbial suspensions were prepared in sterile 0.9 % saline and adjusted as inoculum to a final concentration. A volume of 20 mL of Mueller-Hinton agar and Sabouraud, respectively, for bacterial strains was inoculated with 20 µL of microbial suspension and then poured into a Petri dish. The plates were left at room temperature for 30 min to allow the culture media to solidify. Each paper disk of 6 mm diameter was impregnated with 0.5, 1, 1.5, 2.5 µg respectively, by syrup formulation (in methanol) and then applied manually on the surface of the agar plates inoculated with microorganisms. Syrup of Ailanthus excelsa (25 µg/disk) were used as positive reference standards to determine the sensitivity of Gram-positive and Gram-negative bacteria species, respectively. The plates were kept at 4 °C for 2 h to allow diffusion, and then incubated for 24 h at 37 °C for bacteria. The antimicrobial activity was determined by measuring with a zone reader, for the diameters of inhibition zones, including disk diameter.

Determination of minimum inhibitory concentration

The minimal inhibitory concentration (MIC) test is performed using Mueller Hinton Agar (MHA), which is the best medium for routine susceptibility tests because it has good reproducibility, low in sulfonamide, trimethoprim, and tetracycline inhibitors, and gives satisfactory growth of most bacterial pathogens. The inoculum is prepared using a suitable nutrient broth.

CYTOTOXIC ACTIVITY ON HEPG2 CELLS USING MTT ASSAY

The MTT calorimetric assay determines the ability of viable cells to convert a soluble tetrazolium salt [3–(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) into an insoluble formazan precipitate. Tetrazolium salts accept electrons from oxidized substrates or appropriate enzymes, such as NADH and NADPH. In particular, MTT is reduced at the ubiquinone and cytochrome b and c sites of the mitochondrial electron transport system and is the result of succinate dehydrogenase activity. This reaction converts the yellow salts to blue-colored formazan crystals that can be dissolved in an organic solvent whose concentration can be spectrophotometrically determined. Owing to the many advantages of the assay, it is today considered a significant advance over traditional techniques. In fact, it is rapid, versatile, quantitative, and highly reproducible with a low intra-test variation between datapoints (±15% SD); it is useful in a large-scale, antitumor drug-screening program. Moreover, the test can also be used for floating cells, such as leukemias and small cell lung carcinoma, and always allows sufficient time for cell replication, drug-induced cell death, and loss of enzymatic activity, which generates the formazan product from the MTT substrate.



CELL CULTURES AND VIABILITY

Hepatocellular carcinoma HepG2 cell line was obtained from the NCCs, Pune, India. The HepG2 were grown in RPMI-1640 and DMEM supplemented with 10% FBS, streptomycin (100 U/mL), penicillin (100 µg/mL) and sodium bicarbonate (3.7 g/mL). The culture was maintained in 100-mm dishes at 37°C in 5% CO2 humidified incubator (3111, ThermoForma, Ohio, USA). The cell viabilities of samples in HepG2 cells damaged by CCl4 were measured by the MTT assay. Briefly, cells were plated at a density of 2 ×104 cells per well in a 96-well flat-bottom microtiter plate at various concentrations (µg/mL) of samples. After 24-h incubation, the culture media were replaced with media containing CCl4 (10 mM) and incubated for 2h. At the end of the incubation, 25µL of MTT solution (5.0 mg/mL) was added to each well and incubated for 4h at 37°C. The cells were then lysed with DMSO (200 µL per well), and the reduced intracellular formazan product was quantified in a Bio-Rad enzyme-linked immunosorbent assay microplate reader (680, Bio-Rad, Hercules, CA, USA) at 540nm. Cell viability was expressed as the percentage of control absorbance at 540 nm. The data are presented as the mean of triplicate samples ± SD.

ENZYMATIC ANALYSIS

The cells were washed with phosphate-buffered saline (PBS) and exposed to fresh medium containing CCl4 (100mM) at various concentrations (µg/mL) of samples or medium alone. After 6h of CCl4 treatment, GOT and GPT levels in the medium were measured as described in the assay kits. After removal of the medium, cells were washed twice with ice-cold PBS and used for western blot analysis.

RESULT AND DISCUSSION

Traditional remedies are widely used to treat jaundice as they are more available and affordable than synthetic drugs.

EXTRACTION

The percentage yield of aqueous Soxhlet extract of Ailanthus excelsa was found to be 10%. Ethanolic extract of Ailanthus excelsa was found to be 14%. It was revealed that, high percentage yield was found in ethanol extract of Ailanthus excelsa. The percentage yield of various extract were presented in the table.

S.NO	TYPE OF EXTRACT	% YIELD (W/V)	COLOUR OF EXTRACT
1	Aqueous Soxhlet extract Of *Ailanthus Excelsa*	10%	Light brown
2	Ethanolic Soxhlet extract Of *Ailanthus Excelsa*	14%	Dark brown

PHYTOCHEMICAL SCREENING

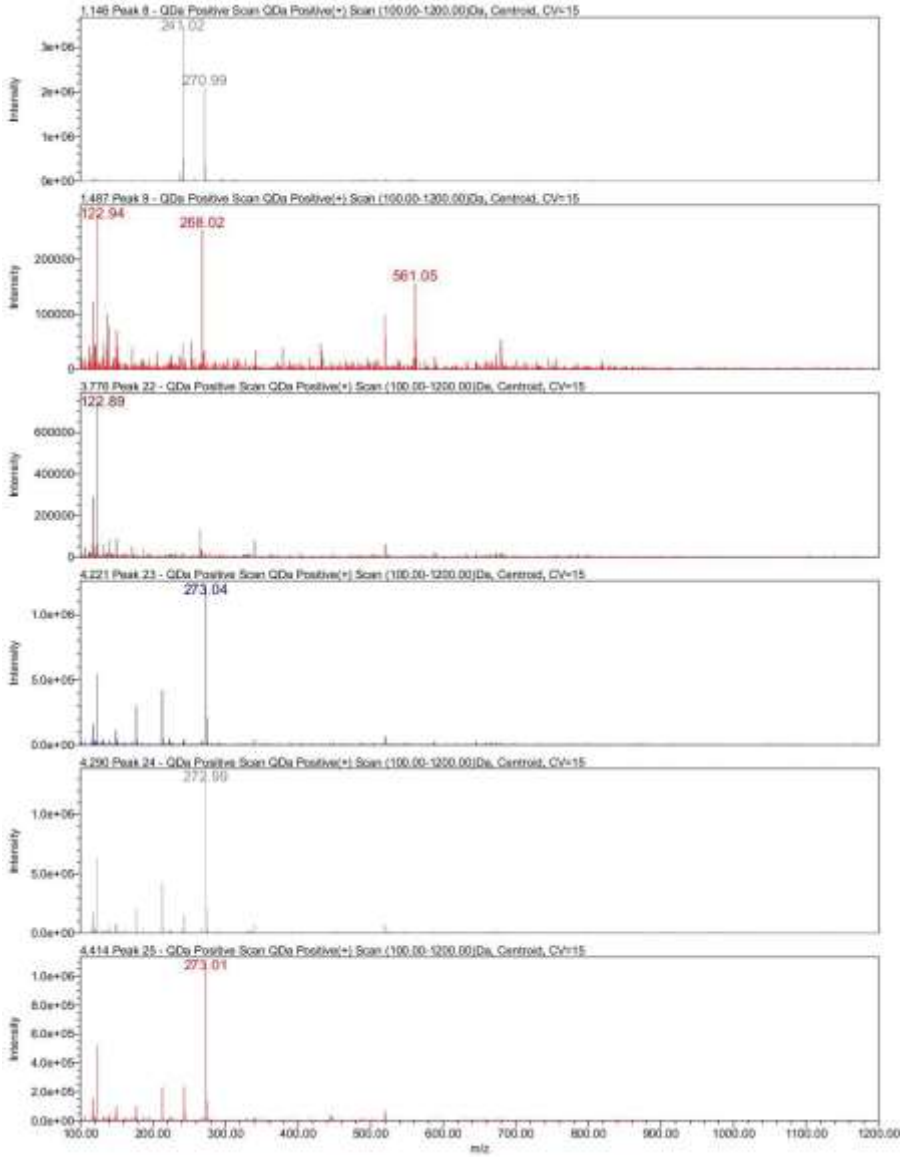
Ailanthus excelsa bark powder are extracted by Soxhlet extractor by two different solvents (Ethanol and Aqueous) based on reported literatures. In that, alkaloid was found to be absent in both ethanolic and aqueous extract. Saponins were found to be present in both ethanolic and aqueous extract. Flavanoids were found to be present in ethanolic extract and absent in aqueous extract. Tannin was found to be present in absent in aqueous extract and present in ethanolic extract. Phenols were found to be present in both aqueous and ethanolic extract.

DETERMINATION FOR HEAVY METALS

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution. it is used for substances that yield clear colourless solutions with sodium hydroxide solutions.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Extract of A. excelsa showed the presence of phenol (Gallic Acid) in ethanolic extract when analyzed by HPLC method. The RT value of standard phenol was found to be 1.071 and also it showed the presence of flavonoid (Quercetin) and its RT value is found to be 3.933.



OPTIMASATION OF λ-max (WAVELENGTH)

Formulation	Solvent used	Zone of Inhibition(mm)			
		BC	EC	KP	SA
F20 (A)	Ethanol	10	0.5	2	0.5
F30 (B)	Ethanol	13	9	13	0.5
F40 (C)	Ethanol	12	12	14	4
Control (D)	Ethanol	17	17	16	17

It was observed in the range of 400-800nm.

FORMULATION OF SYRUP

Three different concentration of herbal syrups were formulated using ethanolic A. excelsa extract namely F20, F30, F40.

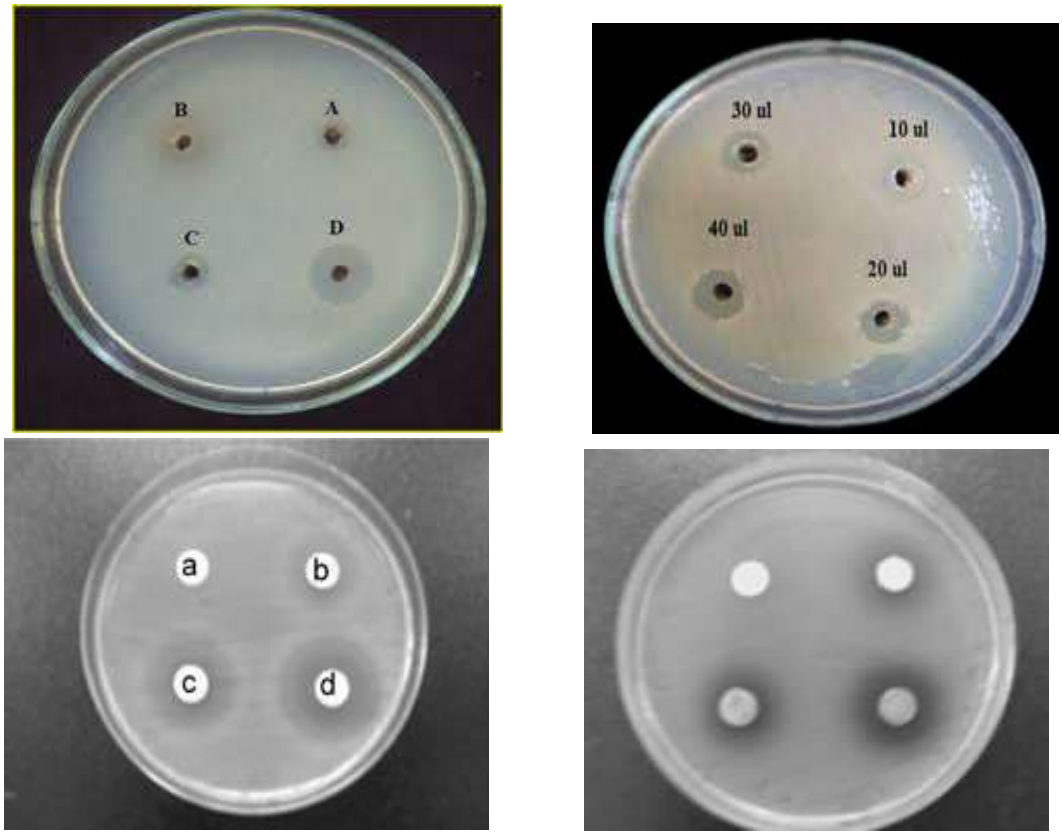
EVALUATION OF SYRUP

S.NO	EVALUATION PARAMETERS	RESULT
1	Color	Pale yellow
2	Odour	Aromatic
3	Taste	Sweet pungant taste
4	Appearance	Translucent
5	pH	6.01
6	Specific gravity	0.52
7	Viscosity	17 cP at 0.5 torque

ANTI - MICROBIAL ACTIVITY

The average zone of inhibition of Ailanthus excelsa was found to be 15mm.The ethanolic extracts show good anti bacterial activity than the aqueous extract. As concentration increases the zone of inhibition also increases in the Ailanthus excelsa.

Control: Cepodoxime, BC: Basilus subtillis, EC: Escherichia coli, KP: Klebsiella pnemoniae, SA: Staphylococcus aureus



Control: Cepodoxime, BC: *Basilus subtillis*, EC: *Escherichia coli*, KP: *Klebsiella pnemoniae*, SA: *Staphylococcus aureus*

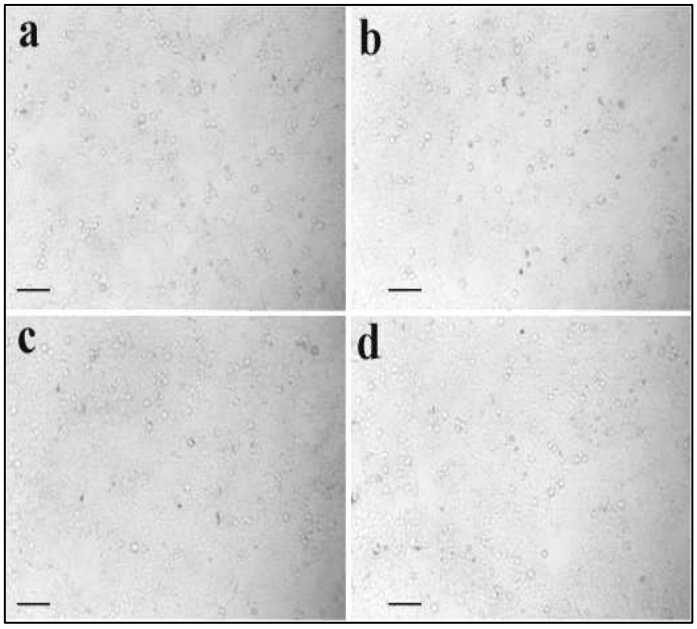
HEPATOPROTECTIVE ACTIVITY

Hepatoprotective activity of samples (µg/mL) are done by using MTT assay. Only 10 to 20% of compounds were extracted in Aqueous due to polarity in nature whereas 60% of compounds were extracted in Ethanol due to non-polarity in nature. Comparatively, Ethanolic extract shows less Minimum Inhibitory Concentration **(IC_{50})** than aqueous extract.

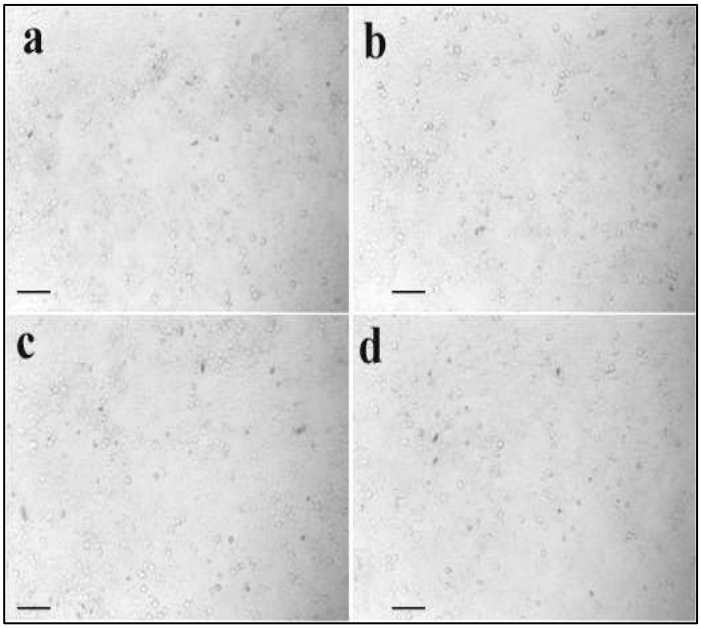
SAMPLES	HEPG2 CELLS (IC_{50})
AE	60±0.5
EE	19 ±1.3
F20	56 ±1.0
F30	60 ±1.5
F40	80 ±0.5

When cell undergoes stress, the pre- inflammatory markers such as GPT (Glutamate Pyruvate Transaminase) and GOT (Glutamic Oxaloacetic Transaminase) are released that causes inflammation on hepatic cells. The cell stress was induced by using chloroform and leads to hepatitis and the following concentration of different extracts and formulations were administered to the HEP G2 cells.

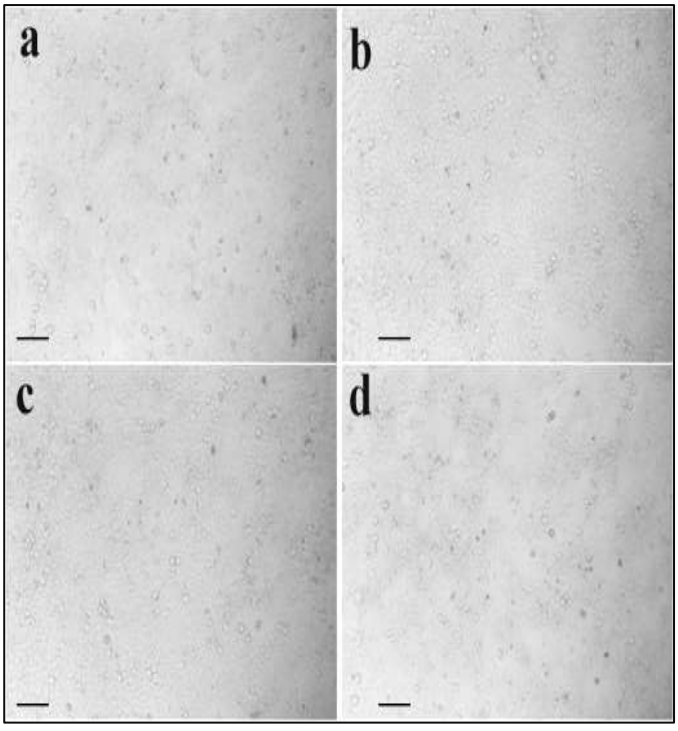
a- Untreated; b-25 µg/ml; c- 50 µg /ml; d- 100 µg /Ml.



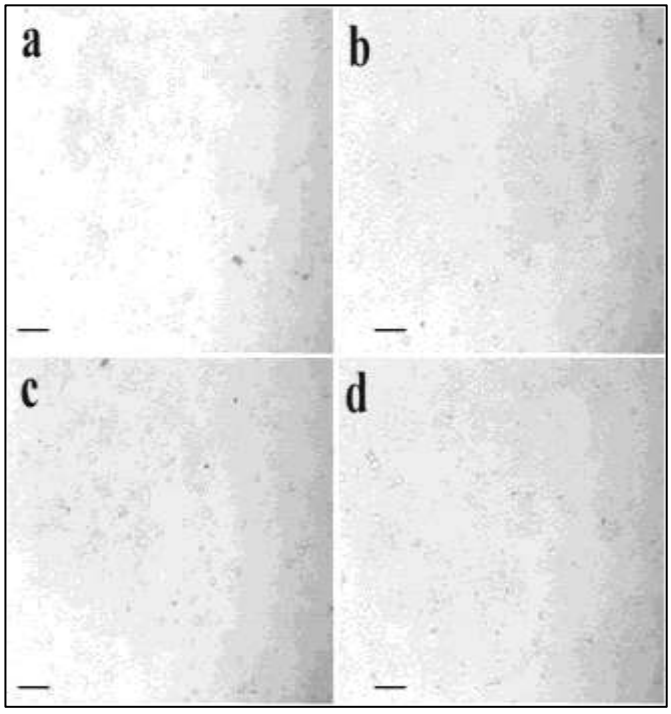
AQUEOUS EXTRACT



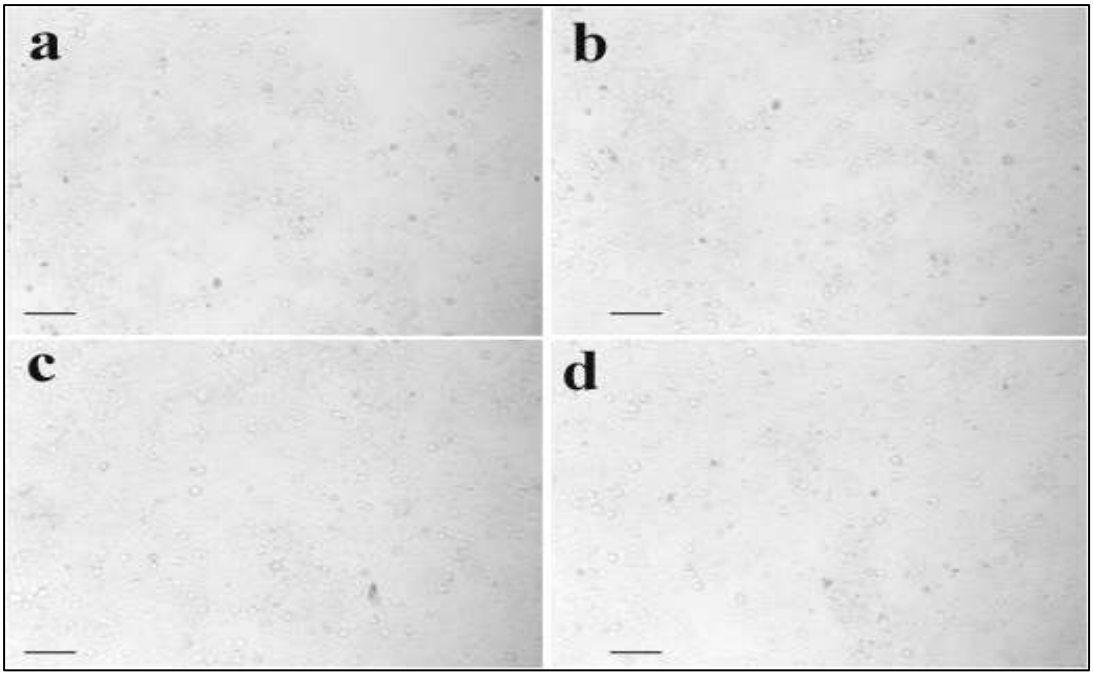
ETHANOLIC EXTRACT



F20

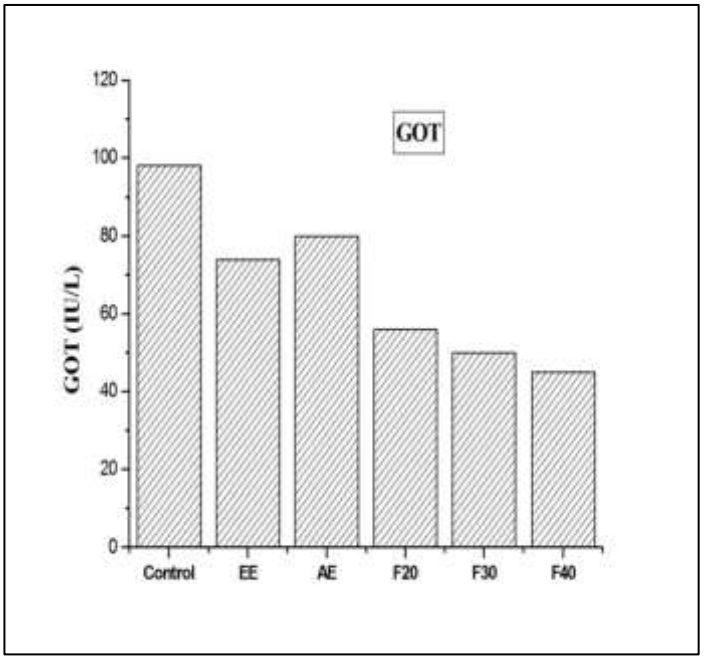


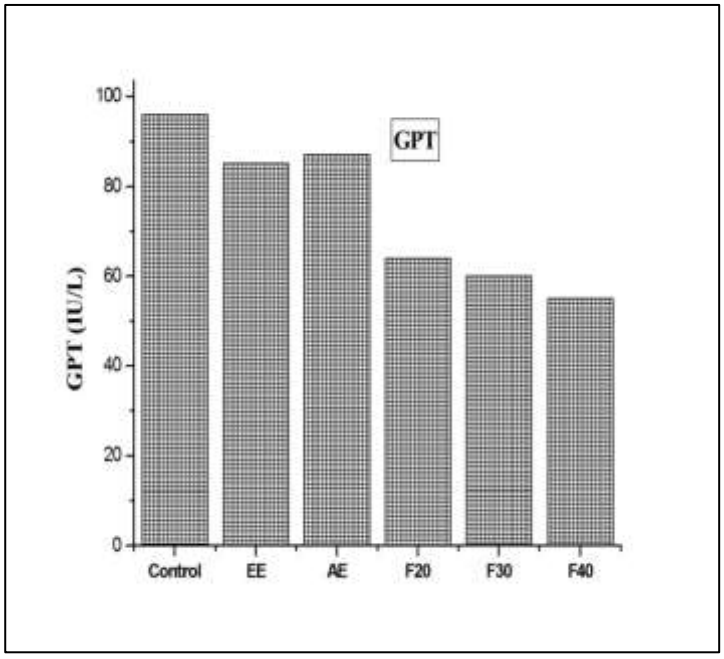
F30



F40

GPT			
control	97.4	97.5	97.7
EE	71.3	71.5	71.6
AE	83.4	83.6	83.7
F20	59.3	59.4	59.6
F30	52.5	52.6	52.7
F30	49.2	49.5	49.7
GOT			
Control	97.4	97.5	97.7
EE	71.3	71.5	71.6
AE	83.4	83.6	83.7
F20	59.3	59.4	59.6
F30	52.5	52.6	52.7
F40	49.2	49.5	49.7





HEPATO PROTECTIVITY AGAINST INFLAMMATED HEP G2 CELLS

RESULT INTERPRETATION

In the present study, different formulations were evaluated for their hepatoprotective activity. Good potencies were displayed by F20. Formulation of F30 & F40 showed amazingly better response against inflammation of hepatic cells. These formulations were found to be non- toxic upto concentration of 100 µg/m.

CONCLUSION

The present study successfully formulated and evaluated a herbal syrup using Ailanthus excelsa bark extract, demonstrating promising hepatoprotective potential. The ethanolic extract showed superior phytochemical content, antimicrobial activity, and cytoprotective effects compared to the aqueous extract, likely due to higher concentrations of flavonoids and phenolics. The syrup formulations, especially F30 and F40, exhibited notable efficacy in vitro against HepG2 liver cells damaged by CCl_4, with significant reduction in hepatic markers (GPT and GOT). These findings provide a scientific basis for the traditional use of *Ailanthus excelsa* in liver-related disorders and support further preclinical studies to confirm its safety and therapeutic potential in managing jaundice and associated hepatic dysfunctions. This is the first study to report with the scientific evidence for the herbal formulation for hepatoptotectivity activity. The phenol and flavonoid present in both the formulations may be responsible for hepatoprotectivity avtivity. Preclinical study has to be carried out to confirm hepatoprotectivity activity.

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