PHARMACOLOGICAL AND PHYTOCHEMICAL EVALUATION OF HEPATOPROTECTIVE POTENTIAL OF CARALLUMA UMBELLATA

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ABSTRACT
The aim of this study is to investigate the detoxification and metabolism of pharmaceuticals and other xenobiotics are carried out by the liver, an important organ. Through pharmacological and phytochemical investigation, this study aims to assess the hepatoprotective properties of Caralluma umbellata. To prepare the sample, one kilogram of Caralluma umbellata leaves were crushed into coarse powder and defatted using Soxhlet's extractor with petroleum ether (65°-85°C). Three milliliters of ethanol were mixed with 150 microliters of DPPH, and the absorbance was measured at 516 nm for the control reading. Test samples with concentrations of 50 g/ml, 100 g/ml, 250 g/ml, 500 g/ml, and 1000 g/ml were obtained. The samples were kept in a dark area for 15 minutes, and after that, the optical density was measured at 516 nm with ascorbic acid serving as the standard and methanol. These results provide that Ethanolic extract (EE) of Caralluma umbellate showed the most efficient hepatoprotective activity against PCM on HepG2 cell lines with silymarin as control compared to Caralluma umbellate extract. bioactive compounds in EE of Caralluma umbellataare further required to evaluate its potential as a potent hepatoprotective agent for liver drug formulation. Further in-vivo and clinical studies are also required to confirm its therapeutic efficacy.
KEYWORDS: Liver, Caralluma umbellata, Ethanol, Phenolic Content, Petroleum Ether.

1. INTRODUCTION

1.1 Liver

In vertebrates, such as humans, the liver is a crucial organ. It is one of the body's largest and most complicated organs and is essential to the upkeep of many physiological functions. Red blood cell decomposition, hormone production, glucose and glycogen synthesis, and carbohydrate metabolism are some of its additional metabolic processes.[1]

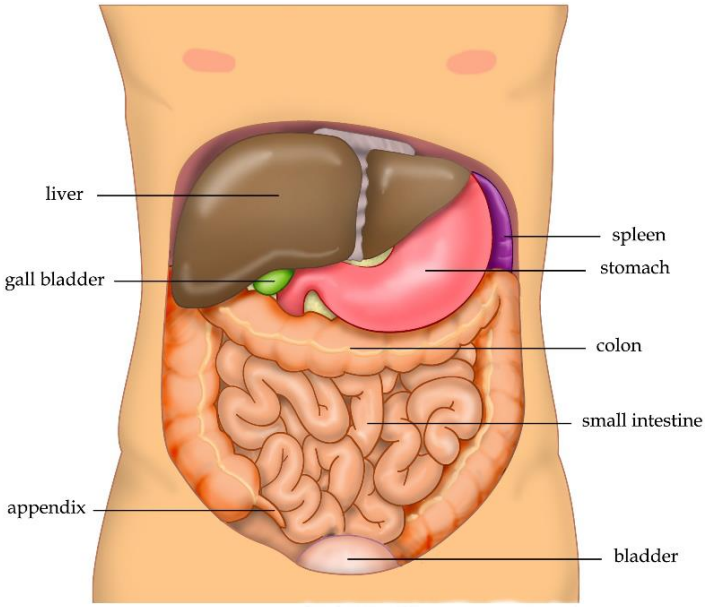

Figure 1.1: Human Liver

Controlling metabolism is one of the liver's main jobs. It breaks down nutrients such as carbs, proteins, and lipids that are taken in by the digestive system. Glycogen is created from glucose by the liver and is then stored for usage when blood sugar levels fall. Additionally, it aids in the synthesis of vital proteins

required for a number of biological processes. The body's detoxification process is carried out by the liver. Drugs, alcohol, and other hazardous chemicals are filtered and neutralized by it, making them less dangerous and easing their elimination from the body. Bile, a greenish-yellow fluid produced by the liver, helps the small intestine break down and absorb lipids. [2]

The gallbladder stores bile, which is released as necessary to aid in the breakdown of dietary lipids into smaller components for simpler absorption. Several vital chemicals are kept in the liver as a storage facility. When necessary, it releases minerals like iron and copper, as well as vitamins like A, D, E, and K, and glycogen that it has stored. The balance of several compounds in the blood, such as the amounts of cholesterol, amino acids, and glucose, is maintained by the liver. Through the production of certain clotting factors, it also contributes to blood clotting. The immune system of the body is significantly influenced by the liver. It assists in clearing the bloodstream of germs and other materials, hence assisting in the prevention of infections. Albumin, which aids in the body's normal fluid balance, and clotting factors, which are necessary for blood coagulation, are two of the significant proteins that the liver is in charge of generating. The liver has a special capacity to partially renew and heal itself. The liver may repair its tissue and regain functioning after harm or injury.[3]

1.2 Structure

The liver has a unique and intricate structure that allows it to carry out its various functions effectively. Here's an overview of the structure of the liver: [4,5,6]

Hepatocytes: The bulk of the liver's tissue is made up of hepatocytes, which are the organ's main functioning cells. Many of the vital processes carried out by the liver, including metabolism, detoxification, and bile synthesis, are carried out by these specialized cells. They are arranged into lobules, which are little structural components with a polygonal form.

The liver is made up of lobules, which are countless microscopic functioning units. There is a central vein at the center of each lobule, which is a cylindrical structure. Hepatocytes round the major vein radially. Small pathways between the hepatocytes known as sinusoids allow blood to circulate through the lobule.

Dual blood supplies are available to the liver. The hepatic artery carries oxygen-rich blood to the liver, whereas the hepatic portal vein carries nutrient-rich blood from the gastrointestinal tract to the liver. The sinusoids within the lobules are created when these blood veins split into smaller branches.

Sinusoids: Within the liver lobules, sinusoids are specialized blood veins. They feature tiny holes called fenestrations that let chemicals from the blood and hepatocytes exchange. As blood flows through the liver, hepatocytes are able to digest and filter it because of this.

Bile is generated by hepatocytes and is collected in a network of tiny tubular structures called bile ducts. The common bile duct, which carries bile to the gallbladder for storage or straight to the small intestine for digesting, is formed when they combine into bigger bile ducts.

Although the gallbladder is not actually a component of the liver, it is directly related to how the liver works. When bile from the liver is required for digestion, it is concentrated and stored there before being released into the small intestine.

Connective tissue and the capsule: The connective tissue capsule that surrounds the liver offers structural support and security. The liver is also divided into lobes and smaller parts by connective tissue. However, the liver's special potential for regeneration enables it to fix.[7]

1.3 Functions of liver

The liver is a crucial organ in the human body that carries out a variety of key tasks, including: [8-11]

- **Metabolism**: The liver plays a crucial role in the metabolism of lipids, proteins, and carbohydrates. Glucose is converted into glycogen for storage and later use. Additionally, it contributes to the breakdown of proteins and fats for energy and produces cholesterol and other essential lipids.
- **Detoxification**: The liver is required for the body's detoxification of toxins, medications, and other hazardous substances. These substances are transformed into less dangerous forms, which are subsequently eliminated through the urine or faeces.
- **Bile production:** The small intestine needs bile from the liver to break down and absorb lipids.
- **Storage:** Iron, copper, as well as the vitamins A, D, E, and K are all stored in the liver as well as other essential vitamins and minerals.
- **Immunological processes:** A crucial part of the immune system is the liver. It produces immunological elements to support the body's defense against infections and purges the circulation of bacteria and other foreign substances.
- **Blood clotting:** The liver produces many essential clotting substances needed for healthy blood coagulation.
- The liver plays a role in regulating the body's levels of various hormones, including insulin and thyroid hormones.

1.4 Hepatotoxicity

Damage to the liver brought on by chemicals is known as hepatotoxicity (from hepatic toxicity). Drug-induced liver injury, specifically acute and chronic liver damage brought on by medications, is the most frequent justification for the recall of a drug after it has been licensed.[12]

The liver is susceptible to the toxicity of these drugs despite its importance in the processing and removal of toxins. Many medications, including halothane when used within therapeutic levels and paracetamol when used excessively, can damage an organism.[13] Other chemical agents, such as those present in industrial and laboratory environments, as well as natural substances

and herbal remedies (two well-known examples being kava, whose mechanism is unknown, and comfrey, whose pyrrolizidine alkaloid concentration causes it), can also induce liver poisoning. The term "hepatotoxin" refers to compounds that affect the liver.[14]

1.5 Causes of Hepatotoxicity

The term "hepatotoxicity" describes liver damage brought on by ingesting drugs or hazardous substances. There are several reasons that might lead to liver damage, including: [15-17]

- Medicines: The liver may be harmed by a number of drugs, including acetaminophen, antibiotics, antifungal medicines, and some chemotherapy treatments.
- Alcohol: Regular drinking can damage the liver and increase the risk of cirrhosis, liver cancer, and alcoholic hepatitis.
- Herbal supplements: A few herbal supplements that have been linked to liver dysfunction include kava, comfrey, and chaparral.
- Chemical exposure: The liver can be harmed by exposure to a number of chemicals, including benzene, vinyl chloride, and carbon tetrachloride.
- Hepatitis viruses, such as those that cause infections with hepatitis A, B, C, D, and E, can damage the liver.
- Autoimmune illnesses: A few autoimmune illnesses, such as autoimmune hepatitis, can damage the liver.
- Metabolic conditions: Conditions including Wilson's disease, hemochromatosis, and alpha-1 antitrypsin deficiency can cause hepatotoxicity.
- Genetic factors: One genetic factor that might raise a person's risk of liver damage is variations in the genes responsible for metabolizing medications.

1.6 Symptoms of Hepatotoxicity

The signs of hepatotoxicity, or liver damage, can vary depending on the severity of the injury and the underlying cause. Several signs and symptoms of hepatotoxicity include: [18-21]

- Jaundice: A yellowing of the skin and eyes caused by a buildup of bilirubin, a waste product of the liver, in the blood.
- Fatigue: Despite getting enough rest, feeling weak and fatigued.
- Vomiting and nausea: Having nausea and vomiting.
- Abdominal pain: Aching in the upper right portion of the abdomen, close to the liver.
- Loss of appetite: A decrease in the desire to eat, which may cause unintended weight loss.
- Dark urine: When bilirubin builds up in the urine, it turns the colour dark.
- White stools: Bile-free, white or clay-colored stools.
- Simple bruising and bleeding: Because the liver produces less blood clotting substances.
- Swelling of the ankles and legs due to fluid retention brought on by liver dysfunction.
- Confusion and disorientation: Serious liver damage can cause confusion, disorientation, and even coma.

1.7 Diagnosis

This remains challenging in clinical praxis since there are no reliable warning indications. Similar clinical and pathological manifestations are produced by a large number of other diseases. To diagnose hepatotoxicity, it is required to demonstrate a causal relationship between the use of the toxin or medicine and subsequent liver damage, but this can be difficult, especially when an idiosyncratic reaction is suspected.[22]Examining the patient's medical history, doing a physical exam, and running a number of diagnostic tests are frequently used to diagnose disorders and illnesses connected to the liver. Here are a few typical techniques for identifying problems of the liver. Gathering data on the patient's medical history, symptoms, and risk factors is the first stage in making a diagnosis. Additionally, they will do a physical examination to check for symptoms of liver illness such abdominal discomfort, enlarged liver, or jaundice (yellowing of the skin and eyes). Blood tests are essential for determining probable liver problems and evaluating liver function. Typical liver function tests include the following:

- Tests for liver enzymes (such as ALT, AST, ALP, and GGT): Elevated values may signify liver inflammation or injury.
- Bilirubin test: High bilirubin levels may be indicative of liver or bile duct issues and may result in jaundice.
- Low amounts of albumin and total protein may indicate liver illness or malnutrition.
- Coagulation tests (such as PT and INR): These measure the liver's ability to produce clotting factors.

In real practice, clinicians are more concerned with how similar or different the patient's biochemical profiles are to the potential toxicity (such as cholestatic damage from amoxycillin-clavulanic acid).[23]

The liver is an essential organ that performs a variety of metabolic tasks, such as detoxification, metabolism, and storage. The health of the liver is seriously threatened by hepatotoxic chemicals and liver conditions. The abundance of hepatoprotective plants that nature has endowed us with, many of which contain bioactive substances that can protect the liver from harm and encourage its regeneration, is a blessing. In this note, we present a comprehensive review of some significant hepatoprotective plants.

A. Milk Thistle (Silybum marianum):

One of the hepatoprotective herbs that has been investigated the most is milk thistle. A compound of flavonolignans called silymarin, which is its active ingredient, includes silybin, silychristin, and silydianin. Silymarin has anti-inflammatory, antioxidant, and antifibrotic characteristics that shield liver cells from toxins, encourage liver cell regeneration, and stop the spread of fibrosis.

B. Picrorhiza kurroa:

Kutki, also known as Picrorhiza kurroa, is a key herb in conventional Ayurvedic therapy. Picrorhizin and kutkin, the two primary bioactive components, have strong

hepatoprotective effects. They support the liver's antioxidant defense mechanisms, lessen inflammation, and help in the process of detoxification.

C. Phyllanthus species:
Several Phyllanthus species, including Phyllanthus amarus and Phyllanthus niruri, have shown hepatoprotective properties. The primary active substances, such as phyllanthin and hypophyllanthin, have antiviral, antioxidant, and anti-inflammatory properties that lessen liver damage brought on by hepatitis viruses and chemical toxins.

D. Andrographis paniculata:
The main hepatoprotective component of Andrographispaniculate, often known as the "King of Bitters," is andrographolide. Antioxidant, anti-inflammatory, and immunomodulatory activities are all present in andrographolide. It lessens the effects of viral infections, oxidative stress, and inflammation on the liver.

E. Curcuma longa (Turmeric):
The hepatoprotective qualities of turmeric's key ingredient, curcumin, have long been acknowledged. In order to prevent liver damage brought on by pollutants, alcohol, and certain disorders, curcumin exerts antioxidant, anti-inflammatory, and antifibrotic properties. It also promotes bile flow and liver cell regeneration.

F. Schisandra chinensis:
Lignans, such as schisandrin, found in the traditional Chinese medicine plant Schisandra chinensis have hepatoprotective effects. The liver is shielded from toxins by Schisandra chinensis, which also lessens inflammation and improves the viability and performance of liver cells. [24]

1.8 Mechanism of Action of Hepatoprotective Activity in Plants

Certain plant chemicals have what is known as "hepatoprotective activity," which refers to their capacity to defend against a variety of hypertensive illnesses, including high blood pressure. A wide variety of bioactive substances that plants generate have therapeutic benefits, including the capacity to control blood pressure. The development of herbal treatments and pharmacological therapies for hypertension can be aided by an understanding of the processes behind the hepatoprotective effect of plants. The key processes behind plants' hepatoprotective action will be examined in this note.

A. Vasodilation:
Vasodilation is one of the main ways by which plants produce their hepatoprotective effects. Plant substances with vasodilatory effects include flavonoids and polyphenols. These substances have the ability to loosen the smooth muscles in blood arteries, which causes them to expand and thus lowers blood pressure. Vasodilatory plant components contribute to hepatoprotective effects by increasing blood flow and lowering peripheral resistance.

B. Antioxidant Activity:
Antioxidants found in many plants include phenolic compounds, vitamin C, and vitamin E. Oxidative stress, which results from an imbalance between the body's antioxidant defenses and the generation of reactive oxygen species (ROS), is linked to hypertension. Plant-based antioxidants scavenge free radicals and lessen oxidative stress, preventing hypertension and associated cardiovascular disorders.

C. Angiotensin-Converting Enzyme (ACE) Inhibition:
The renin-angiotensin-aldosterone system (RAAS), which controls blood pressure, is very important. ACE inhibitors are often recommended drugs to treat hypertension. It's interesting to note that certain plants have natural substances with ACE-inhibitory action. These substances have the ability to inhibit the angiotensin-converting enzyme (ACE), resulting in a reduction in the generation of the powerful vasoconstrictor angiotensin II. Plant-derived substances aid in blood vessel relaxation and blood pressure reduction by blocking ACE.

D. Nitric Oxide (NO) Production:
A crucial function in maintaining cardiovascular health is played by the signaling molecule nitric oxide (NO). It has powerful vasodilator properties and lowers blood pressure. The endothelial cells lining blood arteries have the ability to induce the production of NO in response to certain plant chemicals. Vasodilation is facilitated by increased NO production, which lowers blood pressure and has hepatoprotective action.

E. Diuretic Activity:
Some plants have diuretic qualities, which means they encourage more urine to be produced. Diuretics are frequently given to treat hypertension since they can lower blood pressure and assist reduce blood volume. Plant-derived diuretics can facilitate the excretion of salt and water, which lowers blood pressure by reducing blood volume.

F. Anti-Inflammatory Effects:
Inflammation significantly contributes to the onset and development of hypertension. Chronic low-grade inflammation can result in vascular remodeling, endothelial dysfunction, and elevated blood pressure. The anti-inflammatory characteristics of several plant components can help reduce cardiovascular system inflammation. These substances have anti-inflammatory properties that have hepatoprotective effects.

A complex interaction of many pathways, including vasodilation, antioxidant activity, ACE inhibition, nitric

oxide generation, diuretic effects, and anti-inflammatory qualities, contributes to plants' hepatoprotective efficacy. Plant-derived substances can successfully control blood pressure and guard against hypertension by focusing on these processes. The development of natural treatments and pharmacological interventions for hypertensive diseases will benefit from more study into the discovery and characterization of certain bioactive chemicals found in plants.

1.9 Future Prospects of Hepatoprotective Activity in Herbal Plant Therapy

The term "hepatoprotective activity" describes a substance's capacity to safeguard and advance the health of the liver, a crucial organ involved in metabolism, detoxification, and nutrition storage. For the treatment and prevention of liver problems, many plants and their derivatives have long been used in traditional medical systems, including herbal plant therapy. The subject of hepatoprotective herbal medicine is set for tremendous expansion and bright future possibilities thanks to advancements in scientific study. This brief examines prospective directions for herbal plant treatment research and use in hepatoprotective action.

A. Discovery of Novel Herbal Candidates:

The search for innovative herbal candidates with hepatoprotective qualities among the diversity of plants has enormous promise. The discovery of novel medicinal drugs may result from ongoing attempts to find botanicals, plant extracts, and bioactive substances having hepatoprotective properties. Advanced analytical approaches, bioassay-guided fractionation, and high-throughput screening are technologies that make it possible to find and characterize active substances having hepatoprotective potential.

B. Pharmacological Studies and Mechanistic Insights:

Understanding the mechanisms behind the hepatoprotective action of herbal plants requires in-depth pharmacological research. For the creation of specialized treatments, research into the biochemical pathways, cellular processes, and molecular targets involved in liver protection can be very instructive. Additionally, the intricate interactions between herbal components and liver cells can be revealed via the application of contemporary techniques like omics technologies (genomics, transcriptomics, proteomics, and metabolomics), opening the door for customized therapies.

C. Standardization and Quality Control:

It is essential to create standardized methods for the growth, harvesting, extraction, and formulation of herbal products in order to guarantee the efficacy, safety, and repeatability of herbal plant medicine. To guarantee the consistency and dependability of herbal remedies, quality control procedures are required, including the establishment of botanical reference standards, authentication methods, and marker component identification. Good Manufacturing Practices (GMP) and Good Agricultural and Collection Practices (GACP) application would further improve the quality and dependability of herbal hepatoprotective products.

D. Synergistic Combinations and Formulations:

According to the idea of synergy in herbal medicine, the therapeutic efficiency of a treatment can be increased by combining different plant extracts or components. To create the best possible combination medicines, future study might examine the synergistic interactions of hepatoprotective plants. The development of new formulations, such as drug delivery systems, can increase the bioavailability and targeted transport of active substances to the liver, boosting their hepatoprotective benefits while minimizing potential adverse effects.

E. Clinical Trials and Evidence-Based Medicine:

To evaluate the safety and effectiveness of hepatoprotective herbal formulations, well-designed clinical studies are necessary for the transformation of herbal plant treatment into evidence-based medicine. Long-term follow-ups, pharmacokinetic studies, and randomized controlled trials, among other rigorous clinical research, can offer solid evidence in favor of the use of herbal remedies for liver problems. The incorporation of herbal medicine into conventional healthcare systems will be made easier via cooperative efforts between traditional medicine practitioners, scientists, and regulatory organizations.

F. Combination Therapies with Conventional Medicine:

The management of liver problems may be improved by combining herbal plant therapy with traditional medication. In order to improve the effectiveness of conventional medications, herbal formulations can be utilized as adjuvant treatment. Pharmaceuticals and herbal components may combine synergistically to lower drug doses, minimize side effects, and increase patient compliance. Hepatoprotective action in herbal plant treatment has promise for the future. Our knowledge of the hepatoprotective mechanisms of herbal compounds will grow as a result of ongoing research and development initiatives, as well as technological developments and multidisciplinary partnerships. A huge population worldwide might benefit from safe and effective treatment options for liver problems provided by the integration of herbal medicine into evidence-based healthcare systems. However, to fully realize the promise of herbal plant treatment in hepatoprotection, it is essential to guarantee standardization, quality control, and regulatory monitoring.[25]

1.10 Hepatotoxic agents and their action mechanisms

Hepatotoxins are the molecules that cause liver injury; today, any type of hepatic illness of natural origin may be simulated using various chemicals and medications.

Drugs or compounds that can harm the liver and result in liver toxicity are referred to as hepatotoxic agents. From a little increase in liver enzymes to severe liver damage and, in rare circumstances, liver failure, liver toxicity can take many different forms. It might be easier to prevent or control the negative effects of hepatotoxic substances by being aware of their methods of action. Here are a few typical hepatotoxic substances and their modes of action. [26,27]

A. Carbon tetrachloride (CCl4)

- Metabolism: When carbon tetrachloride is consumed or absorbed, a class of enzymes known as cytochrome P450 enzymes metabolize it largely in the liver. These enzymes change CCl4 into trichloromethyl (•CCl3) and trichloromethylperoxy (•CCl3OO) radicals, two extremely reactive free radicals. [28]

- Trichloromethyl (•CCl3) and trichloromethylperoxy (•CCl3OO) radicals, which are produced during the metabolism of CCl4, are very reactive and unstable. Hepatocytes (liver cells) may experience oxidative stress as a result of these radicals' interactions with numerous biological components.[29]

B. Acetaminophen

- Acetaminophen, usually referred to as paracetamol, is a popular nonprescription painkiller and fever reducer. It is one of the drugs that is most frequently used to treat mild to moderate pain and fever. There are several different ways to take acetaminophen, including pills, capsules, liquid suspensions, and effervescent powders.[30]

Mechanism of Action:
Although the precise mechanism of action of paracetamol is unclear, it is thought to primarily function by blocking an enzyme known as cyclooxygenase (COX). Acetaminophen acts more selectively on COX-2, an enzyme involved in the generation of prostaglandins, than nonsteroidal anti-inflammatory medications (NSAIDs) like aspirin or ibuprofen. Prostaglandins are molecules that encourage pain, fever, and inflammation.[31]

- Pain Relief and Fever Reduction: Acetaminophen inhibits the formation of prostaglandins by inhibiting COX-2, which lowers pain and fever. Acetaminophen has low anti-inflammatory action, in contrast to NSAIDs. It is therefore more efficient at lowering pain and heat than at reducing inflammation brought on by ailments like arthritis.[32]

C. Ethanol

The chemical compound ethanol, sometimes referred to as ethyl alcohol, is a clear, colorless liquid with a somewhat sweet flavor and a distinctive odor. It is among the most extensively used and oldest psychoactive drugs on the planet. Beer, wine, and spirits all include ethanol, which is the sort of alcohol that is present in these liquids.

Production and Sources: Yeast or bacteria transform the sugars in a variety of plant-based materials, such as grains, fruits, and vegetables, into alcohol and carbon dioxide during fermentation, a natural process. Humans have used this procedure to create alcoholic drinks for thousands of years.

- Consumption and Effects: Ethanol is used for its psychoactive properties, which depend on the individual's tolerance levels and the amount drank. Alcohol can lessen inhibitions, promote sociability, and evoke sensations of relaxation and exhilaration when used in moderation. But excessive ethanol use can cause drunkenness, poor balance, slurred speech, and memory problems. It also slows down brain activity and can result in sedation and sleepiness since it is a central nervous system depressant. [33]

- Metabolism and Liver Effects: In the process of metabolizing ethanol, the liver is essential. After consumption, ethanol is broken down by the liver through a series of enzymatic processes. Alcohol dehydrogenase (ADH), one of the key enzymes involved, transforms ethanol into the deadly compound acetaldehyde. Another enzyme called aldehyde dehydrogenase (ALDH) further breaks down acetaldehyde into acetic acid, which is then broken down into carbon dioxide and water to be expelled from the body. [34]

D. D-Galactosamine

Although D-galactosamine by itself does not harm the liver in humans, it has a long history of usage in lab settings to harm the livers of animal models. D-galactosamine impairs protein synthesis in liver cells when given to animals, especially rats, and it also triggers an immunological response that results in liver inflammation and cell death. As a result, D-galactosamine can be used to investigate liver pathophysiology and assess possible liver disease therapies.D-galactosamine has been used in lab studies to better understand the physiology of the liver, liver disorders, and prospective treatment options. It is important to understand, nevertheless, that results from animal research could not always apply to human therapy. Therefore, more investigation and clinical trials are needed to confirm any possible uses in people.[35]

E. Tert-Butyl hydroperoxide (t-BuOOH)

- In the presence of an acid catalyst, tert-butyl alcohol (2-methyl-2-propanol) reacts with hydrogen peroxide to produce tert-butyl hydroperoxide. The substance is only weakly soluble in water but substantially soluble in

organic solvents. It can spontaneously disintegrate with rising temperature or with passing time since it is thermally unstable. [36,37]

- Tert-butyl hydroperoxide is a very reactive substance, hence using it calls for extreme safety measures. Because of its strong flammability and potential for severe reactions, it should be avoided around reducing agents and combustible materials. Due to its sensitivity to heat, stress, and friction, the compound may breakdown violently.
- The right safety gear, including gloves, lab coats, and safety eyewear, should be worn when handling TBHP. It should be kept away from sources of fire and incompatible materials in cold, dry areas.[38]

1.11 LITERATURE REVIEW

Hong et. al.,(2015) reported that "Globally, chronic liver dysfunction or injury is a severe health issue. Numerous liver diseases, such as fatty liver, hepatitis, fibrosis, cirrhosis, and hepatocellular cancer, are all part of chronic liver disease. Current synthetic medications are not very effective at treating chronic liver disease, and they also have unfavorable side effects. In order to treat chronic liver illnesses, a wide variety of medicinal plants and phytochemicals have been researched as complementary and alternative therapies. A systematic study of these herbal medications for chronic liver disease is urgently required because some herbal items have already been used for the management of liver illnesses in some nations or regions. Here, they did a study detailing the potential role, pharmacological research, and molecular mechanisms of treating chronic liver disorders with a number of widely used medicinal herbs and phytochemicals. This review article offers a thorough and methodical examination of what is currently known about the use of traditional medicinal herbs and phytochemicals in the treatment of chronic liver illnesses, as well as any potential hazards that should be addressed in further research."[39]

Latief et. al.,(2017) reported that "A dynamic pathological disorder, liver fibrosis can be slowed down in its early stages. Progressive liver damage may result in cirrhosis, which can develop to liver failure or primary liver cancer, both of which are irreversible disorders, if it is not treated properly clinically. Because of this, the early stages of liver fibrosis should be the focus of treatment. Some supplements and "complementary and alternative medicine (CAM)" merit special notice in this context due to their well-established natural therapeutic methods and durable curative results. Additionally, because CAM has very few adverse effects, it is becoming more and more popular in clinical settings all around the world. Particularly, synthetic pharmaceuticals are being replaced by herbal medicines, which are now viewed as the sources of innovative bioactive chemicals. The effectiveness of phytoproducts as anti-fibrotic, anti-hepatotoxic, and antioxidant agents must be thoroughly assessed in order to create herbal combinations that are effective for treating liver fibrosis. More essential, the chosen plant or substance should have a remarkable propensity to promote the regeneration of hepatocytes."[40]

Rajaratnam et. al.,(2014) reported that "Researchers' interest in herbal medicine has been sparked by the growing number of patients suffering from liver dysfunction as a result of excessive drug and alcohol use. This is due to the limited number of widely used and accessible treatment options for prevalent liver illnesses like cirrhosis, fatty liver, and chronic hepatitis. In the eastern world, herbal remedies have been utilized for many years to treat diseases of the liver and other internal organs. Today, they are a popular therapeutic option for pathological liver illnesses around the world. While many times the processes and modes of action of these plants, as well as their therapeutic efficacy, have not been established, researchers have recently applied scientific methods to analysis the benefits of plants for the treatment of liver disorders. Only a small number of the many plants that have been researched thus far have received in-depth research. The growing popularity of herbal remedies is a reflection of both their alleged efficacy in the treatment and prevention of disease and the notion that they are secure because they are natural."[41]

Amrati et. al.,(2020) reported that "Moroccan traditional medicine uses the medicinal herb Caralluma europaea (C. europaea). Goal of the Research. europaea) is a medicinal plant used in Moroccan traditional medicine. The goal of the current work was to discover the chemical composition and the antioxidant and antiproliferative effects of hydroethanolic and bioactive component classes. Supplies and procedures. Utilizing HPLC, the chemical composition was examined. Both the DPPH and FRAP tests were used to measure the antioxidant capacity. WST-1 was used to inhibit the proliferative activity of malignant cells. The findings indicate that C. europaea) is a plant utilized in Moroccan traditional medicine."[42]

Bellamakondi et. al.,(2014) reported that "A severe health issue that frequently affects people with diabetes is hyperglycemia. Due to the negative effects that might result from using oral anti-hyperglycemic medications to treat hyperglycemia, herbal remedies are becoming more popular due to their effectiveness, low risk of side effects, and affordable price. This investigation aims to assess in vitro investigations of the traditional Indian medicinal plant Caralluma Umbellata Haw's anti-hyperglycemic effect. It was discovered that the methanolic extract significantly absorbed glucose. Additionally, MC was discovered to play a potential effect in suppressing pancreatic lipase and alpha amylase. The findings of the current study indicate that Caralluma Umbellata may have potential anti-diabetic properties, opening up new opportunities for research using animal models and elucidating the mechanism of action."[43]

1.12 PLANT PROFILE (CARALLUMA UMBELLATA)

Succulent plant Caralluma umbellata is a member of the Apocynaceae family. It is abundantly found throughout the country's arid areas, especially in Tamil Nadu, Kerala, and Karnataka, and is referred to as the "umbel star" or "vanyaar-muringa" there. The plant can be an herb or a subshrub, and it has short, fleshy stems that can reach a height of 30 cm. The stems are cylindrical, greenish-brown, and often have 4-6 angles with tiny spines running along the angles.

Figure 1.2: Flowers of Caralluma umbellate.

1.13 Scientific Classification

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Asterids
Order	Gentianales
Family	Apocynaceae
Subfamily	Asclepiadoideae
Tribe	Ceropegieae
Genus	Caralluma

2. MATERIAL AND METHODS

2.1 Chemicals:

"The following items were bought from Hi-Media Laboratories Ltd., Mumbai: foetal bovine serum, phosphate buffered saline, Dulbecco's Modified Eagles Medium, trypsin, ethylenediaminetetraacetic acid, glucose, dimethyl sulfoxide, and hydrogen peroxide. Also utilised was 3-(4,5-dimethyl thiazol-2-yl)-5-diphenyltetrazolium bromide (MTT)."

2.2 Collection of Plant

The whole plant was obtained from a remote location and verified by a botanist. The plant's leaves were separated, completely cleaned, and dehydrated under shady circumstances at room temperature before being subjected to analysis utilizing a variety of criteria. Caralluma umbellata leaves from the neighborhoods were gathered for the study.

2.3 Preparation of extract

One kilogram of Caralluma Umbellata leaves were ground into a coarse powder and defatted using a Soxhlet extractor and petroleum ether (65–85°C) to create the sample. Afterward, ethanol was used to remove the resulting residue. A rotator evaporator was used to further evaporate the obtained liquid ethanol extract. For subsequent examination, the ethanol extract was kept in desiccators. Tests on phytochemicals were conducted. [10-16].

2.4 Phytochemical Parameters [17-23].

2.4.1 Determination of Loss on Drying

5–6 grammes of powder were properly weighed and put into a tarte disappearing dish in order to calculate the loss while drying. After that, the sample was dehydrated at 110°C for 4 hours. The sample was dehydrated after cooling, then weighed hourly until it reached a consistent weight. The sample's moisture content served as the basis for the computation of loss on drying, which was done using the formula below:

$$\text{Loss on Drying} = \frac{\text{weight of powder after drying in geight of empty crucible}}{\text{Initial weight of the powder in g}} \text{x } 100$$

2.4.2 Ash Values

A. Total ash

5 grams of finely ground, air-dried powder was precisely weighed and added in an even layer to a lit crucible (usually made of platinum or silica) to calculate the total ash value. After that, the crucible was steadily heated to 600°C until it became white, signifying that carbon had not been present. The substance was weighed after cooling in a desiccator. The ash was not taken into consideration for additional examination if it contained carbon. In these situations, the deposit was moistened with 2 ml of water or ammonium nitrate solution after the crucible had cooled. It was dried on a water bath before being burnt to a consistent mass once more. After 35 minutes of cooling in a desiccator, the residue was weighed once again. Using the following formula, the total ash value was determined as a percentage of the weight of the dry material:

$$\text{Total ash value} = \frac{\text{weight of empty crucible}}{\text{weight of drug taken}} \text{x } 100$$

B. Acid insoluble ash

The whole ash was combined with 25 mL of HCl and placed in a jar with a glass lid. For 5-7 minutes, the crucible was gently submerged in a water bath. The solution was then added to the container after the glass plate had been washed with 5 mL of warm water. The insoluble material was gathered on an ash-free mesh and rinsed with warm water until the residue turned neutral. The filter paper was then used to transfer the insoluble material to a fresh crucible. A heated plate was used to burn the material in the crucible until a consistent weight was achieved. The surplus was weighed right away after cooling in a desiccator for 30 minutes. As a proportion of the dried plant material, the acid-insoluble ash was computed.

C. Water soluble ash

A silica crucible containing complete ash and 25 mL of filtered water were heated for 5 minutes. After washing it with hot water, the insoluble material was then placed to a sintered glass crucible. The residual solid was then transferred to a new crucible and heated for 15 minutes at 450°C. The surplus was weighed right away after cooling in a desiccator for 30 to 40 minutes. The weight of the deposit was deducted from the overall ash's weight. Calculations were made in relation to the dehydrated plant material to determine the proportion of water-soluble ash.

D. Alcohol extractive value

A conical flask with a stopper was filled with approximately 5.0 g of coarsely powdered, air-dried substance. With intermittent shaking, the powder was macerated in 100 mL of ethanol for 6 hours. After 18 hours, the mixture was immediately filtered while being careful not to lose any solvent. 25 mL of the filtrate was then transferred to a flat-bottomed dish coated with tar and dried by evaporation. The resultant extract was dehydrated for 6 hours at 105 degrees Celsius, chilled for 30 minutes in a desiccator, and weighed right away. It was established what proportion of the air-dried powdered medicinal substance was extractive.

E. Water extractive value

In a closed flask, 5.0 g of the drug material was combined with 100 mL of chloroform, shaken intermittently for the first 6 hours, then left to macerate for an additional 18 hours. Following a quick filtering of the resultant solution, 25 mL of the filtrate was dried on a flat-bottomed plate, dehydrated at 105°C, and weighed. The ratio of the water-soluble extractives to the drug material's dehydrated powder was then computed.

2.5 Qualitative Phytochemical Analysis

To determine the kind of the phytoconstituents, several colour reactions were performed on the dried ethanolic extracts.[17-23].

2.5.1 In-vitro Antioxidant Assay:

A. DPPH Assay

- **Principle**
  DPPH serves as a trap or scavenger for other free radicals. When DPPH is introduced, the molecules involved in a chemical reaction are decreased, which indicates the presence of free radicals. In solution, the DPPH radical exhibits a deep violet hue due to its large 520 nm absorption band. It becomes colourless or light yellow when neutralized. This reaction may be observed visually, and the EPR signal from the DPPH signal or changes in optical absorption at 520 nm can both be used to count the initial radicals.

- **METHOD:**
  A 0.3 mM DPPH solution was made by dissolving 4.3 mg of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) in 6.6 ml of ethanol and then sealing the test tubes with aluminium foil. A control reading was obtained by combining three millilitres of ethanol with 150 microliters of DPPH and measuring the absorbance at 516 nm. The following test samples were obtained: 50 g/ml, 100 g/ml, 250 g/ml, 500 g/ml, and 1000 g/ml. Prior to adding 150 l of DPPH, each sample was diluted in methanol to a level of 3 ml. Following a 15-minute period of darkness, the samples were analyzed for optical density at 516 nm using methanol as the reference and ascorbic acid as the standard:

  "% Antioxidant activity=[(Control Absorbance X Sample Absorbance) / Control Absorbance] x100"

B. Nitric oxide Scavenging Assay:

- **Principle:**
  It was determined how well the nitric oxide radical was scavenged. The Griess-Illosvoy reaction is used to quantify aqueous sodium nitroprusside solution. Nitric oxide and the superoxide anion interact pathologically to form the cytotoxic peroxynitrite. Nitric oxide inhibitors lessen tissue damage and inflammation in inflammatory diseases.

- **Method:**
  "The extract was mixed with 382 mL of 10 mM sodium nitroprusside and 0.5 mL of phosphate buffer saline (pH 7.4). The mixture was then incubated for 150 minutes at 25oC. 1.0 mL of the sulfanilic acid reagent was added to this mixture, and it was let to settle for 5 minutes at room temperature. The absorbance at 540 nm was measured following a further addition of 1.0 mL of naphthyl ethylenediamine dihydrochloride (0.1% w/v) to the mixture. The nitric oxide radicals' capacity to scavenge free radicals was determined using the formula below:"

  S% = [(Acontrol – Asample)/Acontrol ×100
- "Acontrol = absorbance value of the blank control (containing all reagents except the extract solution) and A sample = absorbance value of the test."

C. Hydrogen peroxide Scavenging Capacity:

- **Principle:**
  "The Hydrogen Peroxide Colorimetric Detection test is used to quantify H2O2 quantitatively in a variety of samples, and the outcomes should be read off the standard curve. The samples are mixed with the colorimetric substrate, and the horse radish peroxidase (HRP) is added to begin the reaction. 15 minutes are needed for this process. At 560 nm, the interaction between the substrate and HRP was discovered, which transforms the colourless substrate into a coloured product (pink coloration) when hydrogen peroxide is present. A rise in H2O2 levels causes a linear increase in hue."

- **Procedure:**
  "40 millimoles of hydrogen peroxide solution were produced in phosphate buffer (pH 7.4). Extracts were added to 0.6 mL of a 40 mM hydrogen peroxide solution at concentrations of 50, 100, 250, 500, and 1000 g/mL. At 560 nm, the absorbance was measured after 10 minutes in contrast to a phosphate buffer blank solution. In order to calculate the proportion of hydrogen peroxide scavenging in both ugly fruit extracts and ordinary ascorbic acid, a formula was applied":

  % Scavenged [H_2O_2] = [(AC – AS)/AC] x 100

D. Super oxide dismutase Assay:

- **Principle:**
  "The SOD test inhibits the formation of NADH-phenazine methosulphate-nitro blue tetrazolium formazon. The assay's standard curve, which is generated using a bovine erythrocyte SOD standard, should be used to read all samples. After being exposed to the xanthine oxidase reagent and incubating at room temperature for 20 minutes, a colourless substrate in the detection reagent transforms into a yellow product that can be read at 450 nm. As SOD levels increase in the samples, superoxide levels fall and there is a reduction in the quantity of yellow product. The information is provided as SOD activity per milliliter units."

- **Method:**
  "The test mixture consists of 2.8 ml of distilled water total, 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS, 0.3 ml of NBT, and 0.2 ml of the enzyme preparation. The mixture was incubated at 30°C with NADH (0.2ml) for 90 seconds, and then 1.0ml of glacial acetic acid was added. 4.0 ml of N-butanol was added to the reaction mixture, stirred, and centrifuged after it had been allowed to stand for 10 minutes. The butanol layer's chromogen intensity was measured at 560 nm using a spectrophotometer."

  "% inhibition for the test sample(s): [(Δ A550n/minute) negative control - (Δ A550n/minute) test] x 100 = % Inhibition (Δ A550n/minute) negative control"

2.5.2 In-vitro cell line study:

A. HepG2 cell line

"The human liver hepatocellular carcinoma (HepG2) cell lines were donated by the National Centre for Cell Science (NCCS) in Pune, India. The cells were routinely maintained in DMEM with 10% FBS up until confluence at 37°C in a humidified environment of 5% CO2. The cells were separated using trypsin phosphate versene glucose solution (0.02% trypsin, 0.02% EDTA, and 0.05% glucose in PBS). The stock cultures were cultivated in 25 cm2 culture flasks, and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India). The cell density used for seeding was 1 105 cells/mL. Twice every week, the culture media was replaced."

B. MTT assay

"At a density of 1.0 105 cells/mL, the cells were plated on 96-well flat-bottomed plates and incubated at 37 °C in a humid incubator with 5% CO2. After 24 hours, when a partial monolayer had formed and the monolayer had been washed once, the supernatant was flicked off. The same 500 l was the ultimate volume of each treated and control well. Paracetamol (PCM) (1%), silymarin (100 g/ml), and varying concentrations of Caralluma umbellata ethanolic extract were added to certain wells. The drug silymarin was used as a control. A microscopic inspection was carried out after PCM in toxification for 60 minutes. Cytotoxicity was assessed by calculating the HepG2 cells' percentage viability using the MTT reduction assay. The enzyme-linked immune sorbent assay reader was used to measure the absorbance at 540 and 630 nm [47]. The wells containing medium only served as a blank; the wells containing untreated wells served as a control in the test. There were two runs of the experiment. The formula was used to calculate the proportion of viable cells."

"Percent cell viability= Absorbance of treated cells Absorbance of normal control cells ×100"

C. Statistical analysis

"The results of hepatoprotective activity were expressed as the mean ± SEM."

2.6 LIST OF CHEMICALS USED (Table 2.1)

S.No.	LEGANDS	MANUFACTURED BY
	Sodium hydroxide (NaOH)	Merck
	Cloralhydrate	Spectrochem Pvt. Ltd., Mumbai (India).
	Chloroform	Spectrochem Pvt. Ltd., Mumbai (India).
	Conc. HCL	Spectrochem Pvt. Ltd., Mumbai (India).
	Benzene	Spectrochem Pvt. Ltd., Mumbai (India).
	$NaHCO_3$	Spectrochem Pvt. Ltd., Mumbai (India).
	Ethanol	Spectrochem Pvt. Ltd., Mumbai (India).
	Ethylacetate	Spectrochem Pvt. Ltd., Mumbai (India).
	Conc. H_2SO_4	Himedia (Mumbai).
	$CaCl_2$	Qualigens fine chemicals, Navi Mumbai.
	Sodium Chloride	Qualigens fine chemicals, Navi Mumbai.
	Leadaceatate	Qualigens fine chemicals, Navi Mumbai.
	Conc. HNO_3	Qualigens fine chemicals, Navi Mumbai.
	Hexane	Qualigens fine chemicals, Navi Mumbai.
	Isopropanol	Qualigens fine chemicals, Navi Mumbai.
	Methanol	Qualigens fine chemicals, Navi Mumbai.
	Petroleum ether 60-40 °	Rankem (New Delhi).
	Sodium Sulphate	Rankem (New Delhi).
	Sodium Hydroxide	Rankem (New Delhi).
	Acetone	Rankem (New Delhi).
	Ammonia solution	Loba Chemie (Mumbai).
	Dragendroff Reagent	Loba Chemie (Mumbai).
	Toluene	Loba Chemie (Mumbai).
	Mayar's Reagent	Loba Chemie (Mumbai).
	Millon's Reagent	Loba Chemie (Mumbai).

	Fehling Solution	Loba Chemie (Mumbai).
	Molish's Reagent	Loba Chemie (Mumbai).
	Wagner's Reagent	Loba Chemie (Mumbai).
	Ninhydrin Reagent	Loba Chemie (Mumbai).
	Trichloroacetic acid (TCA)	Merck
	Acetic acid	Merck
	Conc. Ammonia	Merck
	Acetyl acetone	Merck
	1-Butanol	Merck
	Di-ethyl ether	Merck
	Disodium hydrogen phosphate (Na_2HPO_4)	Merck
	Ranitidine	Cadila Health Care
	Formaldehyde solution	Merck
	Formic acid	Merck
	Hydrochloric acid (HCL)	Merck
	Hydrogen peroxide	RFCL Ltd.
	Magnesium chloride	Merck
	Ninhydrin	Merck
	Nitric acid	Merck
	Hydrogen peroxide	Jagsonpal pharmaceutical ltd
	Petroleum Ether	Merck
	3-(4,5-dimethyl thiazol-2-yl)-5-diphenyltetrazolium bromide (MTT)	Merck
	fetal bovine serum (FBS)	Merck
	phosphate buffered saline (PBS)	Merck
	Dulbecco's Modified Eagles Medium (DMEM)	Merck
	Trypsin	Sunchem
	Ethylenediaminetetraacetic acid (EDTA)	Merck
	Glucose	Merck
	Dimethyl sulfoxide	Merck

3. RESULT AND DISCUSSION

3.1 Morphological characters of plant material

For all three plant components, namely the leaves of Caralluma umbellata, the numerous morphological characteristics, such as colour, aroma, taste, size, form, etc., have been researched. All of the plant's components have a distinctive fragrance and a greenish appearance. Leaves have a minty flavor to them. The table below displays the findings of the macroscopical research.

Table: 3.1 Macroscopical features of plant materials

S.No.	Parameters	Parts of plant
1	Colour	Greenish
2	Odor	Aromatic
3	Taste	Mint
4	Size of leaves	2.5-3.5cm
5	Shape	Ovulate

3.2 Extractive Values

For ethanol solutions, the extractive values of the plant were assessed.

Table: 3.2 Extractive Values of the plant extract

S.No.	Name of The Plant Yield	% w/w
1	Leaves extract of Caralluma umbellata	15.61

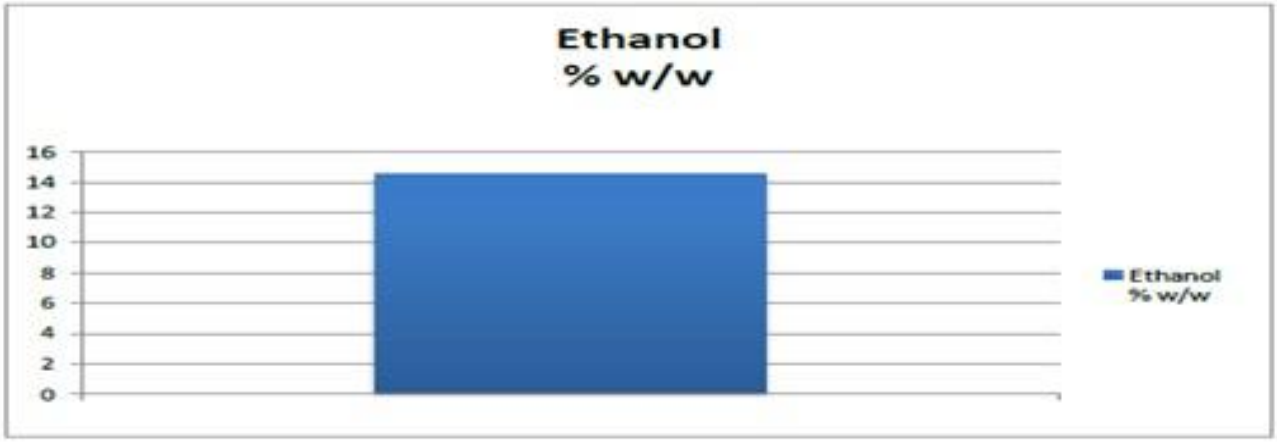

Figure: 3.1 Graph of Extractive Values of the plant extract

3.3 Phytochemical parameters

A. Ash Values:

Caralluma umbellata leaves extract's water-soluble ash content was found to be 5.57%.

B. Extractive Values:

Extractive value (water and ethanol soluble) of Leaves extract of Caralluma umbellatawere discovered to be 15.63 %.

C. Loss on Drying:

The loss on drying of extract of Caralluma umbellatawas discovered to be 5.57 % w/w. All the compiled results are shown in table below.

Table: 3.3 Loss on Drying and Foreign Organic Matter

Crude drugs	Loss on drying (% w/w)	Foreign matter (% w/w)*
Extract of Caralluma umbellate	4.57	1.39

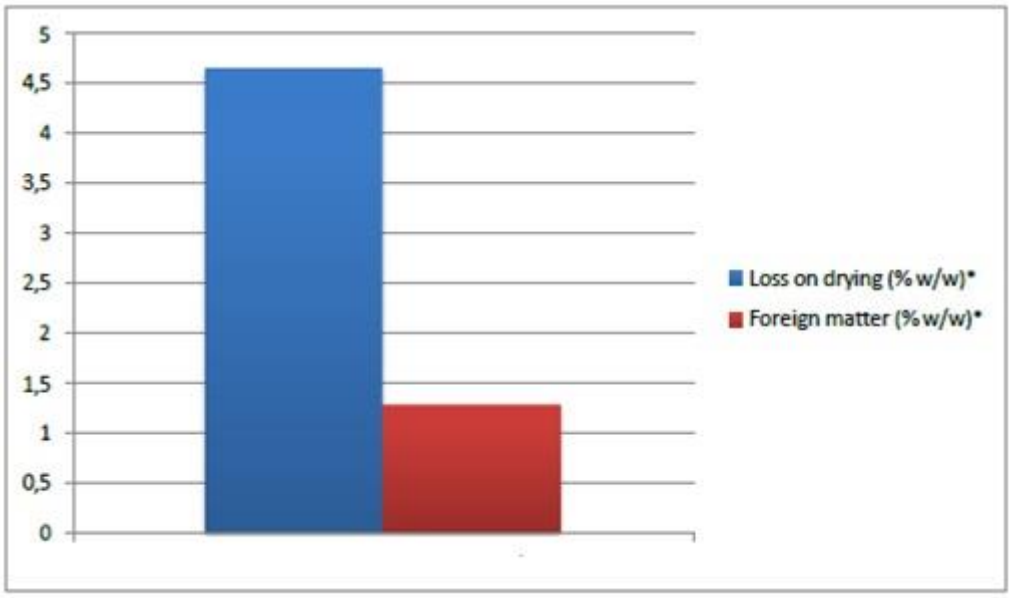

Figure: 3.2 Graph of Loss on Drying and Foreign Organic Matter

Table: 3.4 Total Ash, Acid Insoluble Ash and Water-Soluble Ash Values

Crude drugs	Total ash value* % w/w	Water soluble ash* % w/w	Acid insoluble ash value* % w/w
Leaves extract of Caralluma umbellate	4.10	10.35	6.35

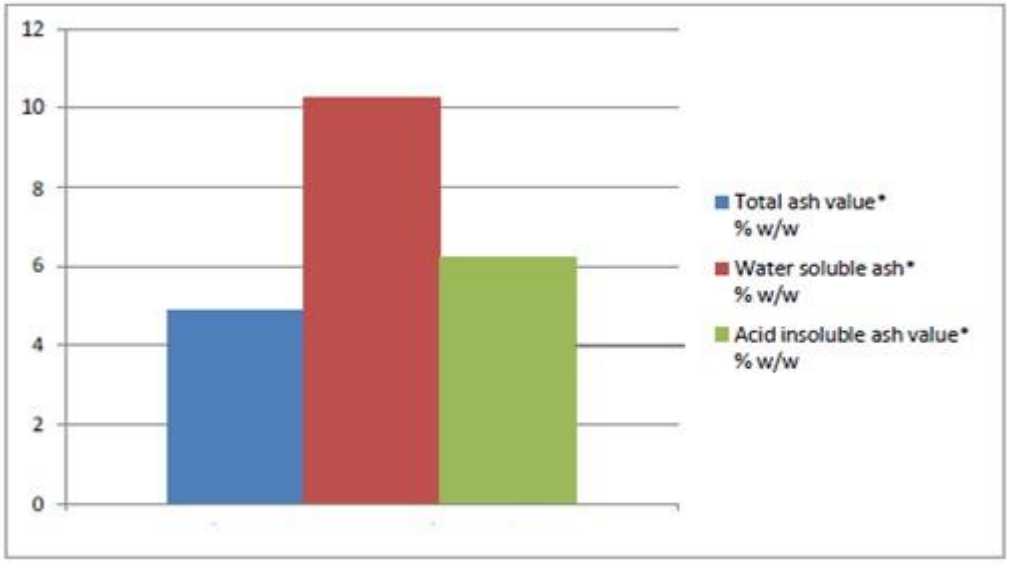

Figure: 3.3 Graph of Total Ash, Acid Insoluble Ash and Water-Soluble Ash Values

3.4 Phytochemical Screening

Concentrates of plant material have demonstrated the presence of saponins, tannins, glycosides, and sugars. The absence of all proteins in the concentrates is not a requirement in and of itself. This analysis shows that the ethanolic separate contains additional components. The leaves' extract was found to have a significant amount of bioactive secondary compounds, including phenols, alkaloids, tannins, glycosides, carbohydrates, and flavonoids, according to a preliminary investigation. This species may have some therapeutic promise because of the presence of these components in it. Furthermore, the presence of a few phytoconstituents in the two distinct concentrates may be the cause of their restorative properties.

Table: 3.5 Phytochemical screening for extract of Leaves extract of Caralluma umbellata

S.No.	Chemical Tests	Ethanolic extract of Caralluma umbellate
1	Steroids and Triterpenoids:	_
2	Saponins:	+
3	Alkaloids:	+
4	Glycosides:	+
5	Tannins and Phenolic compounds:	+
6	Flavonoids:	+
7	Proteins:	-
8	Carbohydrates:	-

3.5 In-vitro antioxidant activity study:

"Ethanolic extract exhibited antioxidant activity at all the concentrations of test solutions. With the increase in concentration of the peel extract (50-1000µg/ml), the percentage of antioxidant activity also increased (79.7-82.4%). Among all maximum antioxidant activity (92.4%) was observed at 1000 µg/ml concentration with IC50 value of 19.8 and ascorbic acid showed 99% antioxidant activity."

Table: 3.6 Results of DPPH Assay

S.No.	Concentration (ug/ml)	%Free radical scavenging activity of extract	%Free radical scavenging activity of standard
1	1000	91.3	98.0
2	500	88.2	92.1
3	250	85.3	87.3
4	100	82.9	84.6
5	50	78.6	80.2

"The nitric oxide scavenging activity of ugli fruit peel extract was evaluated and compared with Ascorbic acid and the results are given in Table 7.7. The IC50 values are calculated from graph and were found to be 47.68 µg/ml (Ascorbic acid) and 486.85 (Plant extract)."

Table: 3.7 Nitric Oxide Assay

S.No.	Concentration (ug/ml)	%Free radical scavenging activity of extract	%Free radical scavenging activity of standard
1	1000	97.2	98.32
2	500	88.69	92.24
3	250	75.97	83.9
4	100	65.86	72.56
5	50	54.56	63.89

"The free radical scavenging ability of ethanolic extracts of ugly fruit on hydrogen peroxide is shown in Table 7.8 and compared ascorbic acid as a standard. The ugly fruit extract was capable of scavenging hydrogen peroxide in an amount dependent manner. 1000, 500, 250, 100, 50µg/ml of the extracts exhibited following % scavenging activity on hydrogen peroxide."

Table: 3.8 Hydrogen Peroxide Assay

S.No.	Concentration (ug/ml)	%Free radical scavenging activity of extract	%Free radical scavenging activity of standard
1	1000	44.5	96.4
2	500	22.1	81.32
3	250	12.5	75.2
4	100	8.9	58.65
5	50	3.8	42.19

"In superoxide dismutase assay, ascorbic acid is used as a standard and the ethanolic extract shows a better antioxidant property. The % free radical scavenging activity of the extract is almost similar to that of the standard values."

Table: 3.9 Superoxide Dismutase Assay

S.No.	Concentration (ug/ml)	%Free radical scavenging activity of extract	%Free radical scavenging activity of standard
1	1000	89.23	99.7
2	500	76.9	86.6
3	250	57.32	75.78
4	100	25.76	64.32
5	50	13.61	59.24

3.6 Pharmacological Study

"The percentage cell viability with respect to the normal control (NC) cell lines (HepG2) at different concentrations of ethanolic extracts of Caralluma umbellatawas determined. The NC cells showed 100±0.03% cell viability in all tested plant samples. The PCM treated HepG2 cells showed 47.7±3.58% cell viability in all extracts of Caralluma umbellata. Out of all, 50 µg/mL concentrations were selected for checking its protective efficacy in presence of PCM. 50 µg/mL ethanolic extract (EE) and PCM showed 132.7±8.48% cell protection,"

The Caralluma umbellata ethanolic leaf extract demonstrated 128–2.74% cell viability at concentrations of 100 g/mL, 50 g/mL, 25 g/mL, and 12.5 g/mL, respectively. Out of them, 50 g/mL concentrations were chosen to test its effectiveness as a protector when PCM was present. 50 g/mL EE and PCM demonstrated cell protection of 167.7 3.58%, whereas 50 g/mL ET and PCM demonstrated cell protection.

3.6.1 Discussion

"Due to their higher tolerability, superior compatibility with the human body, and lower side effects, medicinal plants are vital for meeting the primary and basic health care needs of around 80% of the world's population in both developing and developed countries. The vast activity of many plant components and their therapeutic principles have been studied, and this has led to a rapid increase in the popularity of herbal medications [48]. Caralluma umbellata appears to have a wide range of pharmacological activities when compared to other plant species. According to reports, bioactive substances such saponins, alkaloids, flavonoids, and phenolic compounds are particularly effective against the cytotoxicity of tumor cancer cells. They are frequently employed as anti-asthmatics and antiseptics."

Table: 3.10 Percentage cell viability of HepG2 cell lines with different plant extracts treated groups on HepG2 cell lines

Samples	% cell viability
NC	100±0.03
PCM treated	47.7±3.58
Silymarin+ PCM treated	167.7±3.58
50 µg/mL ET extract+ PCM treated	128±2.74

- The figures show the average of three replicate tests together with their corresponding standard deviations. Cu stands for Caralluma umbellata, NC is for "normal control," and PCM stands for "paracetamol."

- HepG2 cells significantly lost viability after 2 hours of PCM incubation. For the same amount of time, pretreatment with an ethanolic extract of the aforementioned plants' Caralluma umbellata led to a dose-dependent rise in cell viability at concentrations between 12.5 and 50 g/mL, followed by a noticeable drop at 100 g/mL.

- In the test treatment group, this concentration of Caralluma umbellata ethanolic extract was chosen together with PCM since it showed the highest percentage cell viability (167.73.58%). While the extract of Caralluma umbellata had the lowest cell viability (1282.74%), the treated group demonstrated cell viability, demonstrating its hepatoprotective properties. Additionally, when compared to the reference standard, studies have demonstrated that an ethanolic extract of Caralluma umbellata is capable of hepatoprotection and immunomodulation [44]. There have also been encouraging findings in the Wister rat model demonstrating the favourable impact of the ET of Caralluma umbellata in hepatoprotection histologically [55] Our research also demonstrated that Caralluma umbellata has hepatoprotective properties. Phenolic chemicals, flavonoids, and tannins are the primary substances that might effectively combat oxidative damage [56]. Numerous phytochemical analyses showed that, in addition to flavonoids, the ME of the plants was discovered to possess greater concentrations of terpenoids. [57]. Additionally, these extracts can be used in combination therapies both in vivo and in vitro to learn more about their potential synergistic effects on cellular defense. Our research has implications for potential therapeutic uses of Caralluma umbellata plants, either alone or in combination, to treat hepatic diseases.

- An antioxidant5, which can be a natural or synthetic molecule, can prevent the generation of free radicals, which in turn trigger chain reactions and harm to live cells. Its contribution to illness prevention, however, has not been proven. Some common antioxidants include the vitamins C and E, selenium, beta-carotene, lycopene, lutein, and zeaxanthin.

- Free radicals are described as unstable molecules produced by the body during the process of converting food to energy and during physical activity. These molecules cause "oxidative stress," a condition that can result in cell damage. Numerous disorders, including cancer, diabetes, cardiovascular disease, neurological

diseases, cataracts, and age-related macular degeneration, have been linked to oxidative stress. In laboratory tests (such as those involving cells or animals), antioxidant molecules mitigate this oxidative stress.

- In the current work, we assessed the antiradical activity of Caralluma umbellata's ethanolic extracts using the DPPH assay. Ascorbic acid (standard) demonstrated 99% antioxidant activity with an IC50 value at 6.5 g/ml concentration, while the maximal antioxidant activity (92.4%) was recorded at 1000 g/ml concentration with a value of 19.8. The DPPH radical offers a quick and practical way to assess antioxidants and radical scavengers9. The Caralluma umbellata extract's nitric oxide scavenging activity and hydrogen peroxide test were also assessed by comparing it to ascorbic acid and demonstrate successive activity. Increased oxidative stress causes SOD to be used excessively, and peel extract therapy greatly reduced these levels.

- Caralluma umbellata's ethanolic extract has flavonoids and alkaloids, according to phytochemical analysis. Therefore, we may infer that flavonoids were responsible for the antioxidant activity, which necessitates additional investigation to identify and characterize the specific flavonoid.

4. SUMMARY AND CONCLUSION

In comparison to Caralluma umbellate extract, ethanolic extract (EE) of Caralluma umbellata demonstrated the most effective hepatoprotective action against PCM on HepG2 cell lines. According to the aforementioned study, the plants described above have beneficial hepatoprotective properties. Caralluma umbellata's ethanolic extract has flavonoids and alkaloids, according to phytochemical analysis. As a result, we may infer that flavonoids were responsible for the antioxidant activity, which calls for additional investigation to identify and characterize the specific flavonoid. To assess Caralluma umbellata's potential as a powerful hepatoprotective agent for liver medication formulation, these bioactive components in the EE are also needed. To establish its therapeutic effectiveness, more in-vivo and clinical trials are needed.

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