Evaluation of Anti-oxidant and *In-vitro* Hepatoprotective activity of leaf extract of "*Kalanchoe gastonis bonnieri*".

Submitted by

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1. INTRODUCTION

1.1. Medicinal plants

Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. Medicinal plants have undoubtedly been considered by human beings since ancient times. It can be said that before the history and since the early humans recognized and exploited the plants around them for use as fuel, clothing, shelter and food, they became aware of their properties more or less.

The World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Natural products play a pivotal role as a source of drug compounds and, currently, a number of modern drugs which are derived from traditional herbal medicine are used in modern pharmacotherapy.

In Africa, attitudes towards traditional, herbal medicines vary strongly. The use of medicinal plants is sometimes associated with superstition, and therefore rejected by some people in favor of western medicine. On the other hand, there are millions of Africans who prefer traditional methods of treatment. The valuable medicinal properties contained in certain plants are not, however, in doubt. In recent years, for example, the Chinese plant Artemisia annua, has become the essential ingredient in a new generation of anti-malaria drugs.

Governments too need to be thinking about how to promote the benefits that medicinal plants have to offer, which may involve integrating herbal medicine into conventional healthcare systems. This raises important issues, such as regulation of traditional healers and ensuring certain standards are met. Today approximately 80% of the world's population relies on traditional plant-based medicines for primary health care. The

remaining 20% of the world's population also depends on plant products for health care.

In the development of human culture medicinal plants have played an essential role, for example religions and different ceremonies. Among the variety of modern medicines, many of them are produced indirectly from medicinal plants, for example aspirin. Many food crops have medicinal effects, for example garlic. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. The medicinal effects of plants are due to secondary metabolite production of the plants.

Keeping this in consideration there have been increased waves of interest in the field of research in natural product chemistry. This interest can be due to several factors, including therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites, the utility of novel bioactive natural compounds as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents, and advances in solving the demand for supply of complex natural products.

1.2. Plant survey

Plants are important sources of therapeutic drugs and play a significant role in the survival of the tribal and ethnic communities. According to WHO, herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the rural areas of developing countries (WHO, 2001).

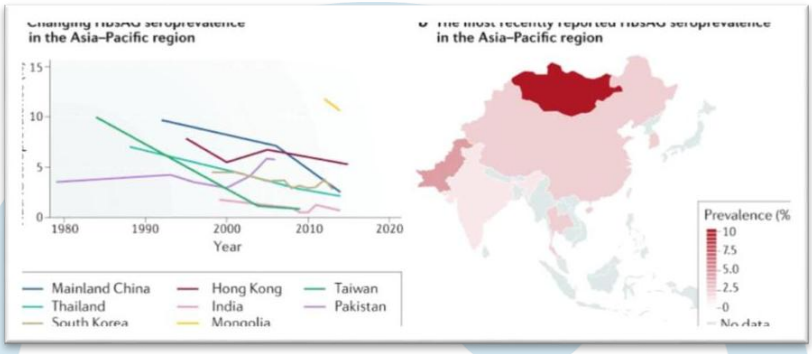
As herbal use becomes more prevalent in the United States, ethnobotanical investigations can provide insight into other medical systems that differ from the biomedical model and could benefit patients. Urban centers, such as New York City, provide study sites that offer access to many diverse ethnic communities and the plants traditionally used in healing.

This ethnobotanical survey is part of an on-going study in New York City investigating Dominican and Chinese healing systems and the herbal and non-herbal treatments used for the following women's conditions: uterine fibroids (benign tumors of uterine smooth muscle); menorrhagia (excessive uterine bleeding); endometriosis (growth of endometrial tissue outside of the uterus); and hot flashes (sudden brief sensations of heat commonly experienced during menopause).

1.3. Epidemiology

The crude annual incidence rate of DILI was 19.1 cases per 100,000 patients per year but there was a very wide range depending on the implicated drug with: – 43 per 100,000 for amoxicillin- clavulanate – 11 per 100,000 for diclofenac – 752 per 100,000 for azathioprine – 675 per
100,000 for infliximab.

An earlier French population-based study suggested a crude annual incidence rate of 13.9 per 100,000 per year and was some 16-fold higher than would be expected by spontaneous reporting to local authorities. A recent study from Korea found a crude rate of 12 per 100,000 per year but only looked at patients that were hospitalized meaning the true incidence of DILI should be higher.



Using general practice databases in the United Kingdom and Spain, the crude incidence rate ranged from 1.35 to 3 cases per 100,000 per year (5, 6) which in part could be explained by only identifying DILI if the patient was hospitalized or referred to a specialist. Similar figures were noted in a hepatology outpatient setting in Sweden of 2.3 per 100,000 per year.

In Japan, an analysis of 1676 DILI cases demonstrated that 10% were related to dietary supplements and 7.1% were related to Chinese herbal drugs and a smaller study in Singapore found more than half of DILI cases were associated with traditional Chinese medicines. New data should start emerging from Latin America with the formation of the Latin DILI Network (LATINDILIN) which encompasses most South American countries along with Mexico.

1.4. Overview of Liver

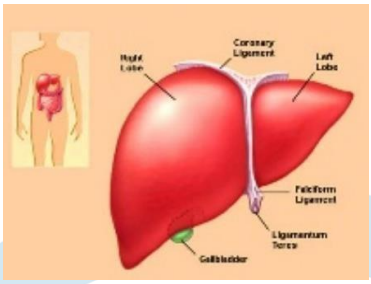
The Liver is the largest gland in a human body, situated in the right side of upper abdominal cavity. The cells of the liver called hepatocytes plays vital functions like;

1. Synthesis of proteins, bile
2. Stores glycogen, vitamins, iron,
3. Metabolises toxic chemicals and drugs.

The capacity of the liver to carry out the several oxidative metabolisms are associated with the high cellular content of cytochrome P450. As liver being the central organ of metabolism, it is highly vulnerable target for injury from drugs and chemicals, the manifestations of which are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure.

Drug metabolism/biotransformation is a process of detoxification in which a substance is chemically modified into a less toxic form under the influence of enzymatic system. The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight

against disease, nutrient supply, energy provision and reproduction.



A healthy liver is a crucial factor for overall health and well-being because liver involves in metabolism, secretion, storage and excretion. Any injury to liver can result in many disorders ranging from transient elevation in liver enzyme to life threatening liver cirrhosis and hepatic failure.

Around 10% of the world's population suffers from liver ailments. This comprises cirrhosis, fibrosis, chronic hepatitis, alcoholic steatosis, and hepatocellular cancer. Liver disease morbidity and mortality, particularly in poorer nations, is a significant public health issue on a global scale. Modern medicine still faces difficulties in treating liver illness.

In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise.Jaundice and hepatitis are two major hepatic disorders that account for a high death rate. Presently only a few hepatoprotective drugs and that too from natural sources (there is not a single effective allopathic medication), are available for the treatment of liver disorders.

1.5. Hepatotoxicity

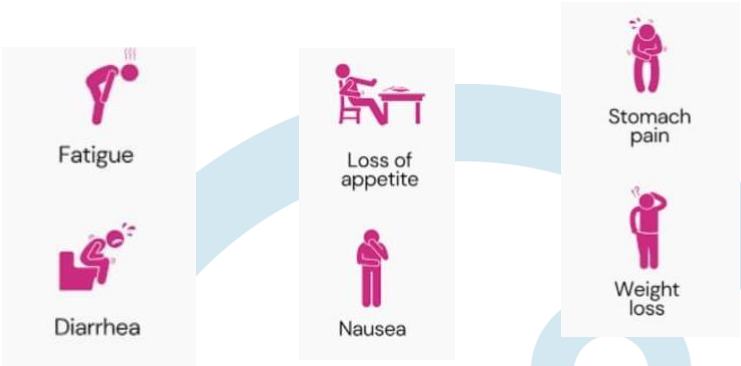
Hepatotoxicity refers to liver damage or dysfunction caused by exposure to toxic substances, such as drugs, chemicals, alcohol, or other harmful agents. It can result in inflammation, liver cell injury, and impaired liver function. Symptoms may include jaundice, fatigue, nausea, and abdominal pain, and in severe cases, it can lead to liver failure.

Drugs:

- Amoxicillin-clavulnate
- Isoniazid
- Ibuprofen

1.6. Symptoms:

Mild forms of toxic hepatitis may not cause any symptoms and may be detected only by blood tests. When signs and symptoms of toxic hepatitis occur, they may include:



- Yellowing of the skin and eyes (jaundice)
- Itching
- Abdominal pain in the upper right portion of the abdomen
- Fatigue
- Loss of appetite
- Nausea and vomiting
- Rash
- Fever
- Weight loss
- Dark or tea-coloured urine

1.7. Risk factors

- Medications: Taking certain prescription drugs or over-the-counter pain relievers, especially in excess
- Liver disease: Having a liver disease, chronic hepatitis B or C, or hepatocellular insufficiency
- Alcohol: Drinking alcohol, especially in excess
- Age: Being older
- Genetics: Having certain genetic mutations or being a carrier of the hepatitis B virus
- Weight: Being underweight or having a low BMI
- Malnutrition: Being malnourished
- HIV: Having HIV infection
- Renal failure: Having severe chronic renal failure
- Immune checkpoint inhibitors: Taking immune checkpoint inhibitors (ICPI)
- Industrial toxins: Working with industrial toxins
- Aflatoxins: Consuming food contaminated with aflatoxins, which are cancer-causing substances produced by a fungus

- Cigarette smoking: Smoking cigarettes

1.8. Hepatoprotective drugs

Hepatoprotective drugs are medications or compounds that help protect the liver from damage caused by toxins, infections, or diseases. These drugs work by preventing liver cell injury, enhancing liver regeneration, and improving liver function. They are commonly used to treat liver conditions such as hepatitis, fatty liver disease, and drug-induced liver damage. Examples include silymarin (from milk thistle), ursodeoxycholic acid (UDCA), and N-acetylcysteine (NAC).

DRUGS:

- Silymarin
- Glycyrrhizin
- N- acetylcysteine
- Adenosylmethionine
- L-Ornithine L-Aspartate

2. LITERATURE REVIEW

1. Kassahun Dires Ayenew *et al.*, 2023. The aim of this study was to evaluate the hepatoprotective effects of methanolic extract of Agave americana leaves on rat liver damage caused by paracetamol. The acute oral toxicity test was conducted in accordance with OECD- 425 recommendations Wistar male rats weighing between 180 and 200 g were used, and six groups with seven animals each were formed. Group I received treatment with gum acacia (2%) at a dose of 2 ml/kg p.o. daily for 7 days. Rats in group II were treated with 2% gum acacia orally daily for seven days along with a single dose of paracetamol (2 mg/kg) p.o. on 7th day. Silymarin (50 mg/kg) was given orally to Group III for 7 days. Plant extract doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg were administered orally to Groups IV -VI for seven days, respectively. All rats in groups III-VI were treated with paracetamol 30 min following extract administration. Blood samples were obtained from the cardiac puncture after paracetamol had been used for 24 h to induce toxicity. No toxicity symptoms or animal fatalities were recorded during the acute toxicity study. The values of AST, ALT, ALP, and total bilirubin were all substantially raised by paracetamol. Pretreatment with A. americana extract reversed these alterations. Results of the methanolic extract of A. americana were comparable to Silymarin.

[Kassahun Dires Ayenew *et al.*,.2023, Hepatoprotective effect of methanol extract of Agave americana leaves on paracetamol induced hepatotoxicity in Wistar albino rats, Ayenew and Wasihun BMC Complementary Medicine and Therapies (2023) 23:99.]

2. Mohammad Saidurrahman *et al.,* **2022.** The designed objective was to evaluate the hepatoprotective activity of ethanolic extract of Pterocarpus marsupium Roxb.Leave(EEPM) against paracetamol-induced liver damage (PILD) in SD rats. Group-I rats were administered with 1 ml of 1% CMC/kg b.wt per day for 8 days. Group-II rats were administered with 1 ml of 1% CMC/kg body wt. per day till 7th day, then 3 g paracetamol/kg body wt. on eighth day. Groups-III, IV and V animals were pretreated with 200 mg EEPM/kg/day,400EEPM/kg/day, 100 mg silymarin/kg/day in that order for seven days and then, administered with a single dose of 3 g paracetamol/kg body wt. p.o. on eighth day. The isolated serum was evaluated for AST, ALT, ALP, TC, ALB, SBL, TP and TG as part of liver function test using regular kit sets .Treatment with 400 mg EEPM/kg/day in group-IV rats showed significant decrease in the levels of ALT, AST, ALP, LDH, total cholesterol, bilirubin, triglyceride, TBARS, liver wt. and

relative liver wt. while significant increase in the levels of final b.wt, TP and ALB, SOD, GSH, CAT compared to group-II rats.Findings indicate that EEPM possesses a potent hepatoprotective activity against paracetamol induced liver damage and protects liver from PILD by preventing paracetamol-induced oxidative stress and altered biochemical markers. This indicates the therapeutic efficacy of Pterocarpus marsupium against liver damage.

[Mohammad Saidurrahman *et al.,* 2022. Evaluation of hepatoprotective activity of ethanolic extract of Pterocarpus marsupium Roxb. leaves against paracetamol-induced liver damage via reduction of oxidative stress, Phytomedicine Plus 2 (2022) 100311.]

3. Hafiz Muhammad Zubair *et al.,* **2022**. Cordia rothii Roem. & Schult. possesses various beneficial effects and is traditionally used in folk medicine against liver diseases but its molecular mechanism remains unclear. Antioxidant and hepatoprotective effects of Cordia rothii methanolic fraction (CRMF) were investigated in CCl4-induced liver injury. The results shows that CRMF significantly increased cell viability against CCl4-induced HepG2 cells. Normal hepatocyte integrity and microstructures were observed in histopathological results. Furthermore, the mRNA level of inflammatory mediators including interleukon (IL)-1β, IL-6, TNF-α, nuclear factor kappa B (NF-KB), IL-10 and nuclear factor-erythroid factor 2-related factor 2 (NrF2) were reverted in CRMF pretreatment groups. Thus, CRMF exhibited strong antioxidant, and hepatoprotective activities, which may involve Nrf2–NFκB pathways.

[Hafiz Muhammad Zubair *et al,. 2022,* Hepatoprotective effect of Cordia rothii extract against CCl4-induced oxidative stress via Nrf2–NFκB pathways .Biomedicine & Pharmacotherapy Volume 156, December 2022, 113840, ELSEVIER.]

4. Shaik abdul Saleem *et al.*,2022. The antimicrobial activity of ethanolic leaf extract of Kalanchoe gastonis bonnieri was tested by Agar well diffusion method. The antimicrobial activity of ethanolic leaf extract of Kalanchoe delagoensis was test by Agar dilution method. The mixtures of Kalanchoe gastonis bonnieri and Kalanchoe delagoensis was tested by Time kill method against six pathogenic bacterial culture.

[Shaik abdul Saleem *et al.*,2022, Evaluation of Anti-microbial activity of Ethanolic extract of Kalanchoe Gastonis-Bonnieri and Kalanchoe Delagoensis. International journel of pharmaceutical research and application volume 7, issue 3 may -june 2022, pp:2069-2076.]

5. Shaik Abdul Saleem *et al.*, 2022. Antimicrobial properties of ethanolic extract (soxhlet apparatus) leaves of Kalanchoe gastonis bonnieri and kalanchoe delagoensis. The antimicrobial activity of ethanolic leaf extract of Kalanchoe gastonis bonnieri was tested by Agar well diffusion method. The MIC endpoint is recorded as the lowest concentration of antimicrobial agent that completely inhibits growth under suitable incubation conditions in agar well diffusion method. The plant extract is collected by using soxhlet extraction apparatus using ethanol as a solvent. The mixtures of Kalanchoe gastonis bonnieri and Kalanchoe delagoensis was tested by Time kill method against six pathogenic bacterial culture. The ethanolic leaf extract of mixture of kalanchoe gastonis bonnieri and kalanchoe delagoensis found to exhibit highest antimicrobial activity when they are tested as a mixture against selected microorganisms.

[Shaik Abdul Saleem et al,.2022, Evaluation of anti-microbial activity of ethanolic extract of *kalanchoe gastonis bonnieri* and kalanchoe delagoensis, International Journal of Pharmaceutical Research and Applications volume 7, Issue 3 May-June 2022, pp: 2069-2076.]

6. Hankhray Boro *et al,*.2021. This study evaluates the hepatoprotective activity of the ethanolic extract of RoMi against the CCl4-induced in-vivo animal model at different dosages (100 and 200 mg/kg BW) in comparison with silymarin as a positive control. Oral administration of eRoMi (200 mg/kg BW) to rats significantly protected serum biochemical parameters (increased ALT, AST, LDH, bilirubin and GGT as well as depletion of antioxidant enzymes and hepatic GSH) and elevation in hepatic lipid peroxidation as compared to CCl4- treated rats. The haematological indices such as erythrocytes, haemoglobin, monocytes and lymphocytes were also normal in eRoMi-treated rats. This study is the first scientific validation for the traditional use of eRoMi to understand its hepatoprotective activity.

[Hankhray Boro *et al.,* 2021, Hepatoprotective activity of the ethanolic extract of Morus indica roots from Indian Bodo tribes. N Applied Sciences (2022) 4:49.]

7. **Antonio Palumbo *et al.,* 2019.** The ethnomedicinal uses of Kalanchoe gastonis-bonnieri (KGB), we evaluated the inhibitory effects on the proliferation of stromal cells from primary benign prostatic hyperplasia (BPH) of four different aqueous extracts from this plant:
underground parts from specimens in flower (TI treatment), leaves from specimens in flower (T2 treatment),

and flowers (T3 treatment) and leaves from specimens not in flower (T4 treatment). T1, T2, T3, and T4 treatments at 250 mg/ml for 72 hours inhibited BPH cells by 56.7%, 29.2%, 39.4%, and 13.5%, respectively, showing that the KGB underground parts extract (T1 treatment) was the most active. The findings shows that the extract of the KGB underground parts (150 and 250mg/ml) stimulates important changes in the BPH cells, modulating crucial processes such as proliferation, viability, and apoptosis. \ The use of the underground parts of Kalanchoe gastonis-bonnieri in condition that causes significant chronic morbidity for men.

[Antonio Palumbo *et al.,* 2019, Potential Therapeutic Effects of Underground Parts of Kalanchoe gastonis-bonnieri on Benign Prostatic Hyperplasia, Antonio Palumbo et al. Evidence-Based Complementary and Alternative Medicine Volume 2019, Article ID 6340757, 10 pages.]

8. Samira L. Abdalla *et al.*, 2017. An aqueous leaf extract of the medicinal species Kalanchoe gastonis-bonnieri (here denominated KGB) has been found to be effective as an antimicrobial agent against canine oral cavity bacteria in in vitro assays. The effect of topic oral administration of KGB on the dried out of dental biofilm in Beagle dog administration an experimental group (0.2% of KGB extract), a negative control group (0.9% of saline solution) and a positive 28 cal group (0.12% chlorhexidine). Each treol group (0.9% of saline solution) cavity daily for 28 days. Similar characteristics and kept under the same management and diet were used (30 beagle dogs). The KGB extract at 0.2% showed efficacy in controlling the formation of plaque compared to the negative control group, and dental calculus in relation to the negative and positive control groups. The treatment with KGB is effective in controlling periodontal disease in dogs, providing new insights into the medicinal properties of this plant.

[Samira L. Abdalla *et al.,* 2017, Efficacy of a Kalanchoe gastonis-bonnieri extract to control bacterial biofilms and dental calculus in dogs. Research gate.]

9. **Sonia Soares Costa *et al.*,2015**. A phytochemical study of leaf juice prepared from specimens collected in the flowering season resulted in the isolation of the new flavonoid quercetin 3-O-α-rhamnopyranoside-7-O-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranoside, as well as the already known 6-C-β-D-glucopyranosyl8-C-β-D-glucopyranosylapigenin (vicenin-2). The structural elucidation was based on 1 H and 13C NMR (HMQC and HMBC) analyses and MS data. This is the first report of a C-glycosyl flavonoid (vicenin-2) in the Crassulaceae family. Additionally, this is the first study in which atropoisomerism has been shown for vicenin-2.

[Sonia Soares Costa *et al.,* A New Triglycosyl Flavonoid Isolated from Leaf Juice of Kalanchoe gastonis-bonnieri (Crassulaceae). Sonia soares costa et al., Nat Prod Commun.2015 Mar,PUBMED.]

10. S.Dasgupta *et al.*,2013. The leaves of Kalanchoe Gastonis-Bonnieri have been used in traditional system of medicine for the treatment of ovarian cysts, urinary tract infections, vaginal infections, uterine fibroids. The preliminary screening showed the presence of many phytochemicals such as alkaloids, flavonoids, terpenoids, saponin, fixed oils and fats. High Performance Thin Layer Chromatography analysis was carried out with optimized solvent system consisting of ethyl acetate, formic acid, glacial acetic acid and water in the ratio of 8:1:1:2. The densitometric scanning of the chromatograms of hydroalcoholic extract showed 7peaks at 254 nm 8 peaks at 366 nm. The phytochemicals detected in the present study justifies the therapeutic uses of the leaves in the traditional medicines.

[S.Dasgupta *et al.*, Preliminary Phytochemical Studies of Kalanchoe Gastonis-Bonnieri. Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 550 -557]

11. **Swarnamoni Das *et al*,.2011.** They evaluate the hepatoprotective activity of Ocimum sanctum and observe whether synergistic hepatoprotection exists with silymarin. Albino rats (150–200 g) were divided into five groups. Groups A and B were normal and experimental controls, respectively. Groups C, D and E received the alcoholic extract of Ocimum Sanctum leaves (OSE) 200 mg/kg BW/day, silymarin 100 mg/kg BW/day and OSE 100 mg/kg BW/day+ silymarin 50 mg/kg BW/day p.o., respectively, for 10 days. Hepatotoxicity was induced in Groups B, C, D and E on the eighth day with paracetamol 2 g/kg BW/day. The hepatoprotective effect was evaluated by performing an assay of the serum proteins, albumin globulin ratio, alkaline phosphatase, transaminases and liver histopathology. Reduction in sinusoidal congestion, cloudy swelling and fatty changes and regenerative areas of the liver were observed on histopathological examination in groups C, D and E, whereas group B showed only hepatic necrosis. The Ocimum sanctum alcoholic leaf extract shows significant hepatoprotective activity and synergism with silymarin.

[Swarnamoni Das *et al.*, Hepatoprotective activity of Ocimum sanctum alcoholic leaf extract against paracetamol-induced liver damage in Albino rats Pharmacognosy Research. January 2011 Vol 3 Issue 1.]

12. Nasrin Aghel *et al.*, 2009. Protective action of Ficus carica leaf ethanolic extract (obtained by maceration) was evaluated in an animal model of hepatotoxicity induced by carbon tetrachloride (CCl4). M ale albino mice were divided into six groups. group I was normal control group; group II received olive oil (CCl4 solvent), groups III-VI received CCl4. After inducing hepatic damage, group III served as control for CCl4; and groups IV- VI received different doses of Ficus carica ethanol extract (200, 400 and 800 mg/kg) prior to intoxication with CCl4. Liver marker enzymes were assayed in serum. Levels of marker enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased significantly in CCl4 treated mice (group III). In groups IV, V and VI, pre-treated with the plant extract and intoxicated with CCl4, decreased activities of these two enzymes were observed. The present observations suggested that the treatment with Ficus carica leaf extract in dose of 200 mg/kg enhanced protection against CCl4 induced hepatic damage**.**

[Nasrin Aghel *et al*,. Hepatoprotective Effect of Ficus carica Leaf Extract on Mice Intoxicated with Carbon Tetrachloride. Iranian Journal of Pharmaceutical Research (2011), 10 (1): 63-68.]

3. AIM AND OBJECTIVE

AIM:

- To evaluate the Anti-oxidant and *In-vitro* Hepatoprotective activity of *Kalanchoe gastonis-bonnieri* leaf extract.

OBJECTIVE:

- Screening of phytochemical constituents from *Kalanchoe gastonis-bonnieri* leaves.
- To evaluate the Anti-oxidant activity from the leaves of *Kalanchoe gastonis bonnieri.*
- To evaluate the and *In-vitro* Hepatoprotective activity from the leaves of *Kalanchoe gastonis bonnieri.*

4. PLANT PROFILE

Classification:

Kingdom : Plantae

Order : Saxifragales

Class : Magnoliopsida

Phylum : Tracheophyta

Family : Crassulaceae

Genus : Kalanchoe

Species : *Kalanchoe gastonis bonnieri*

Synonym:

Bryophyllum gastonis-bonnieri, Bryophyllum adolph-engleri, Kalanchoe Adolph-engleri.

Common Names:

Donkey Ears, Life Plant, Palm Beach bells, Leaf of Life, Miracle Leaf, Giant Kalanchoe, Sprout Leaf Plant, Good Luck Leaf, Sprouting Leaf, Tree of Life.

Botanical Name:

Kalanchoe gastonis-bonnieri

Vernacular Names:

Tamil : Ranakalli

English : Donkey ear **Malayalam** : Mananasilaitra **Portuguese** : Perrier planta-da-vida

Spanish : Oreja de burro, Hojerilla,ojaransin

Biological Source:

It is obtained from the flowering plant of species *Kalanchoe gastonis-bonnieri* .

Biogeography:

The native range of this species is Northwestern Madagascar. Introduced as a Garden plant, it is now naturalized in tropical areas in the Amazon, Africa, Asia, Australia and elsewhere in the native tropics, it is a succulent biennial or subshrub and grows primarily in the seasonally dry tropical biome.

Chemical Constituents:

- Alkaloids
- Flavanoids
- Trepenoids
- Saponins
- Fixed Oils
- Fats

Medicinal Uses:

- Anti-Cancer activity
- Anti-Inflammatory
- Antimicrobial
- Used as Oral Contraceptive
- Traditionally used in the treatment of Urolithiasis
- Used to treat Prostic Hyperplasia
- Used in the treatment of Genital-Urinary and Vaginal infection
- Used as Vaginal Contraceptive

Morphological Characters:

- **Growth form:**

  Succulent herb up to 0.45m tall.

- **Foilage:**

Thick, fleshy leaves (up to 0.3-0.5m long) are grayish-grom with brown to reddish brown spots young leaves are whitish to greyish, while mature leaves are hors green. They a lance-shaped (ovate to lanceolate) with the toothed leaves margin small plantlets often along the margin.

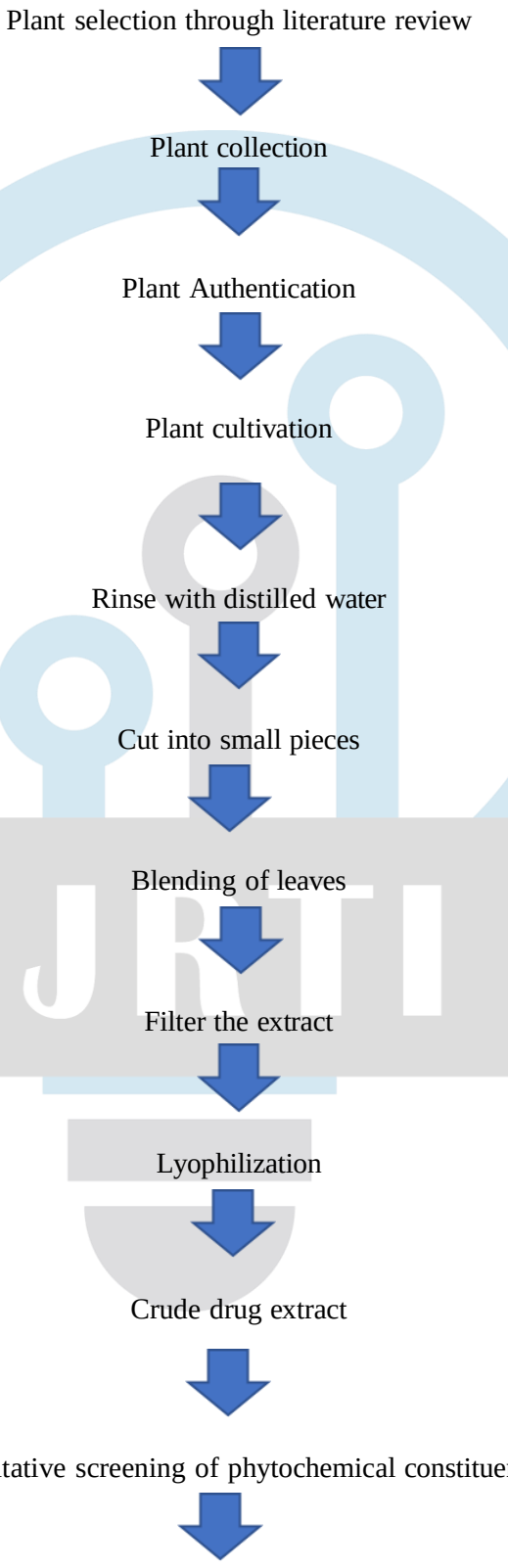
- **Flowers:**

Tubular drooping flowers are reddish to pinkish-orange near the base with yellow, flared tips Flowers are arranged in a branched cluster (known as a terminal inflorescence) and home ins stalk up to 0.62-0.9m tall.

- **Fruit:**

Dry, dehiscent fruits are known as follicles.

5. PLAN OF WORK



6. MATERIALS AND METHODS

6.1. Plant profile

Kalanchoe gastonis bonnieri was collected from Kanyakumari in November 2024 and authenticated by Mrs. Syed Ali Fathima, HOD of Department of Botany, Sadhakathulla Appa College, Tirunelveli. The leaves of the plant material were rinsed with distilled water and blended. After filtering the clear yellow liquid, it was sent for lyophilization. And the received crude product was kept in a container in refrigerator for further use.

6.2. Materials

Distilled water, Cutter, Blender, Cotton, Funnel, Filter paper, Food graded plastic container.

6.3. Procedure

Fresh leaves from *Kalanchoe gastonis-bonnieri* (KGB) (average length of leaves 15 cm) were rinsed with distilled water.

Cut into small pieces, and crushed in a blender.

The extract obtained from the leaves of specimens was filtered

Resulted in a clear yellow liquid.

6.4. Lyophilization process

Lyophilization, also known as freeze-drying, is a preservation method where water is removed from a frozen material under vacuum, causing ice to sublimate directly into vapor without becoming liquid.

6.5. Instrument used: Lyophilizer

6.6. PROCEDURE

- The plant extract is poured into freeze-drying tray.
- It is frozen rapidly at ultra-low temp (-40°C to -80°c) using a deep freezer to form Solid of ice crystals.

Primary drying:

- The frozen sample is placed in a lyophilizer.
- A vacuum is applied to create low pressure allowing ice to sublimate without passing through liquid phase (Solid to vapour)
- The temp is gradually increased to aid sublimation.
- This stage removes about 90% of the water content.

Secondary drying:

- The temperature is further increased to remove any remaining bound water molecules.

- This step ensures complete drying preventing microbial growth and enzymatic degradation.

Storage:

- It is stored at low temperature to maintain stability.
- The powder can be reconstituted with a suitable solvent for further use in antioxidant or pharmacological studies.

6.7. WORK DONE AT

Entrepreneurship Development and Innovation Institute – Anna Business Incubation Research Foundation (EDII – ABIRF) Trichy

7. PHYTOCHEMICAL SCREENING

1. Test for carbohydrate

Experiment	Observation	Inference
a.Molisch's test: To the 1 ml of the extract add 2 drops of Alpha naphthol and mix well. Then add concentrated H_2SO_4 along the sides test tube.	Purple coloured ring or reddish violet colour at the junction of the two liquids.	Presence of carbohydrate
b. Fehling's test: To a small amount of the extract add equal quantity of fehling's solution A and B and heated gently in a water bath for few minutes.	Brick red precipitate is obtained	Presence of carbohydrate
c.Benedict's test: To the few drops of the extract solution add 5 ml of Benedict's reagent and mix well. Then boil the mixture vigorously for 2 minutes and then cool**.**	Red precipitate is obtained	Presence of carbohydrate

d. Barfoed 's test: To a 0.5 ml of extract solution add 5 mil of barfoed's solution and mix well. Then boil the mixture for few minutes.	Red precipitate is obtained.	Presence of carbohydrate

2. Test for phytosterols:

Experiments	Observations	Inference
a.Libermann burchad test: The extract is dissolved in 2 ml of chloroform in a dry test we. To that add 10 drops of acetic anhydride and 2 drops of conc. Sulphuric acid.	The solution becomes red which later turns to bluish green color.	Presence of sterols and steroids.
b. Salkowsi test: The extract was dissolved in chlorofonn and add equal volume of conc.sulphuric acid.	Cherry red or purple colour in chloroform layer and also green fluroscence.	Presence of sterols and steroids.

3. Test for Fixed oils and Fats

Experiments	Observations	Inference
a. Spot test: The extract was pressed on the filter paper.	Permanent oil appearance is produced on the paper.	Presence of fixed oils and fats.
b. Saponification test: Soap solution is formed.	To the extract add potassium B Hydroxide solution.	Presence of fixed oils and fats.

4. Test for glycosides

Experiment	Observation	Inference
a.Legal's test: The extract is dissolved in pyridine sodium nitroprusside solution and made alkaline.	Pink or red colour precipitate is obtained.	Presence of glycosides
b.Baljet test: To the extract add sodium picrate	Yellow to orange colour.	Presence of glycoside

c.Borntrager's test: To the extract solution add few ml of dilute sulphuric acid. Then boil and filter.to the filtrate add ether or chloroform. Organic layer is removed. To which the ammonia solution is added.	Pink or violet colour in organic layer is obtained	Presence of glycosides**.**

3. Test for Flavonoids:

Experiment	**Observation**	**Inference**
a.Shinodas test: A small quantity if the extract was dissolved in alcohol and to this add magnesium metal followed by concentrated hydrochloric acid in dropwise and heated.	Magenta colour is produced.	Presence of flavonoids.
b. Alkaline reagent test: To the extract add sodium hydroxide.	Yellow colour is produced	Presence of flavonoids.
c. Mineral acid reaction test: To the extract add concentrated sulphuric acid.	Orange colour is produced.	Presence of flavonoids

d. Lead acetate test: To a small amount of the extract add a few drops of lead acetate solution.	Yellow precipitate is produced.	Presence of flavonoids

4. Test for Tannins and Phenolic compounds

Experiment	Observation	Inference
a.Ferric chloride test: 100 mg of the extract is boiled 1ml of water and filtered. Taken the filtrate add 1 ml of ferric chloride solution.	Formation of bluish black colour.	Presence of phenolic compounds.
b.Lead acetate test: Take the filtrate in above the test add few drops of lead acetate.	Yellow precipitate is produced.	Presence of phenolic compounds.
c.Potassium dichromate test: Taken the filtrate in above the test add few ml of potassium dichromate	Yellow colour is produced.	Presence of tannins.
d.Potassium ferricyanide test: Taken the filtrate in above the test add potassium ferricyanide and add ammonia solution.	Red colour is produced.	Presence of tannins.

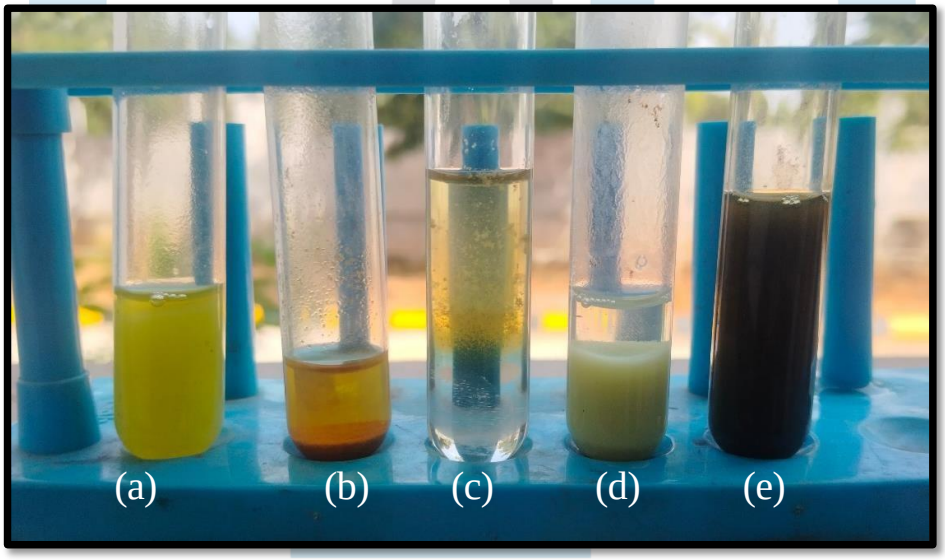
5. Test for Saponins

Experiment	Observation	Inference
a.Frothing test: About 1 gm of extract was boiled with 10 ml of water for 10 mins then it is filtered and cool. Taken the filtrate was shaken vigorously for 2 mins with water.	Frothing was observed	Presence of saponins
b.Emulsification test: Taken the filtrate in above the test and it is shaken vigorously with a few drops of olive oil	An emulsified layer is obtained.	Presence of saponins.

6. Test for Alkaloids

Experiment	Observation	Inference
a.Wagner's test: small quantity of the extract treated with few drops of Hcl and filtered. Then the filtrate is treated with wagner's reagent.	Reddish brown precipitate is formed.	Presence of alkaloids**.**
b. Dragendroff's test: Sample is treated with Hcl and add few drops of dragendroff's reagent.	Orange colour is produced.	Presence of alkaloids.

c.Mayer's test: Sample is treated with Hcl and add few drops of mayer's reagent	Cream precipitate is formed.	Presence of alkaloids
d.Hager's test: Sample is treated with Hcl and add few drops of hager's reagent.	Yellow precipitate is formed	Presence of alkaloids



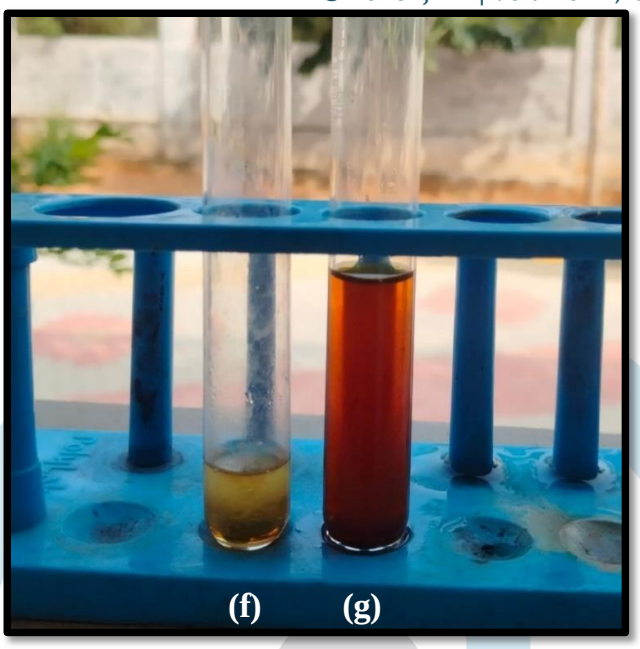
(a) Test for Flavonoids

(b) Test for Carbohydrates

(c) Test for Glycosides

(d) Test for Saponins

(e) Test for Tannins



(f) Test for Phytosterols

(g) Test for Alkaloids

8. EXPERIMENTAL DESIGNN

8.1 Cell Culture Maintenance

- HepG2 (Human hepatocellular carcinoma cell line) was obtained from the National Centre for Cell Sciences (NCCS), Pune, India.
- Cells were maintained in the logarithmic phase of growth in Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin.
- They were maintained at 37°C with 5% CO_2 in 95% air humidified incubator.

8.2 *In vitro* hepatoprotective activity

The hepatoprotective activity of the sample was tested against HepG2 cell line by MTT (3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The cells were seeded in 96-well microplates (1×10^4 cells/well) and incubated at 37°C for 24 h in 5% CO_2 incubator.

After incubation, the cells were exposed to 1% carbon tetrachloride to induce hepatotoxicity in HepG2 cell line and allowed to incubate for 4 h in CO_2 incubator. Then, the CCl_4-induced cells were treated with different

concentrations of sample and incubated for 24 h in CO_2 incubator.

The morphological changes of untreated (control) and the treated cells were observed under digital inverted microscope (20X magnification) after 24 h and photographed. The cells were then washed with phosphate-buffer saline (PBS, pH-7.4) and 20 µl of (MTT) solution was added to each well.

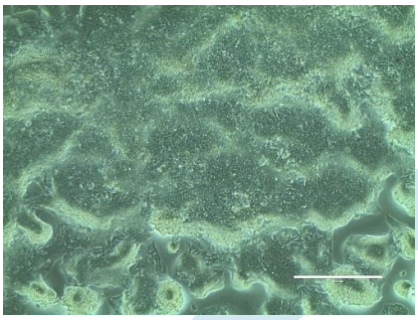
The plates were then stand at 37ºC in the dark for 4 h. The formazan crystals were dissolved in 100µL DMSO and the absorbance was read spectrometrically at 570 nm. Percentage of cell viability was calculated using the formula,

Cell viability (%) = (Absorbance of sample/Absorbance of control) X 100.

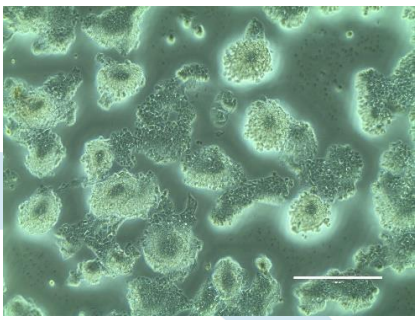
S.NO	Standard drug	Cell viability
1.	Silymarin (50µg/ ml)	57.55
2.	Silymarin (100µg/ ml)	72.77

8.3 MTT ASSAY:

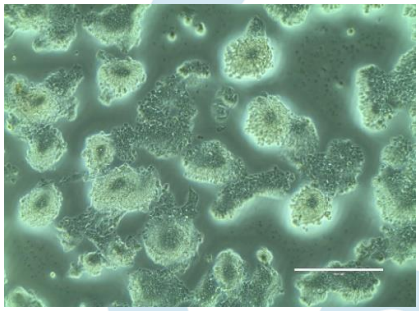
S.no	Treatment	Absorbance		Average	Cell viability (%)
		I	II		
1.	Untreated control	0.886	0.876	0.881	100
2.	CCl_4-induced	0.331	0.315	0.323	36.66288309
3.	CCl_4+25 µg/ml	0.378	0.39	0.384	43.58683314
4.	CCl_4+50 µg/ml	0.467	0.458	0.4625	52.49716232
5.	CCl_4 + 75 µg/ml	0.601	0.618	0.6095	69.18274688
6.	CCl_4+100 µg/ml	0.738	0.726	0.732	83.08740068



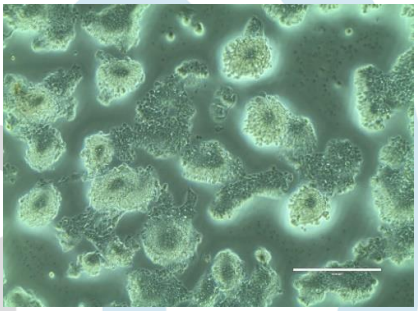
Untreated control



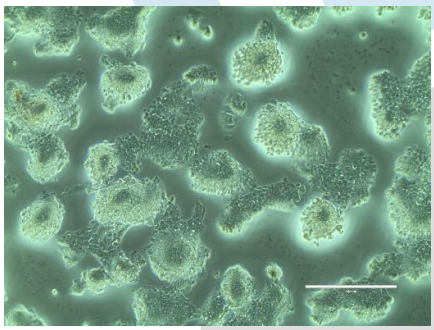
CCl_4-induced



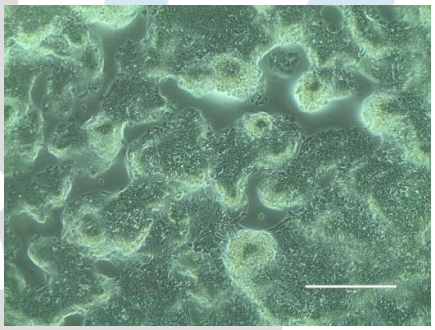
CCl_4+ 25 µg/ml



CCl_4+50 µg/ml



CCl_4 + 75 µg/ml



CCl_4+100 µg/ml

8.4 Anti-oxidant activity:

DPPH radical scavenging assay:

DPPH (2, 2-diphenyl picryl hydrazyl) is a commercially available stable free radical, which is purple in colour. The antioxidant molecules present in the herbal extracts, when incubated, react with DPPH and convert it into di-phenyl hydrazine, which is yellow in colour.

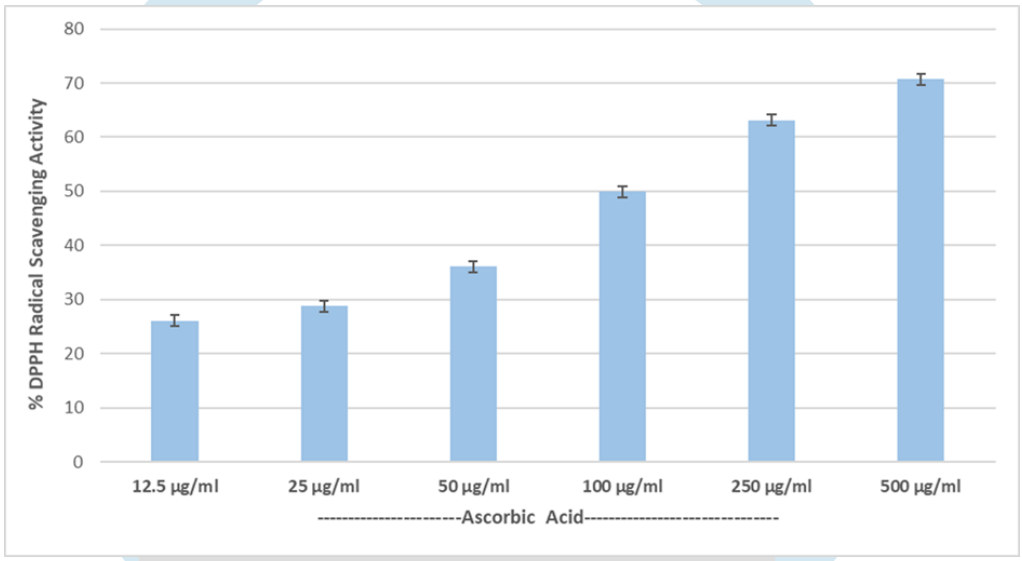
The degree of discoloration of purple to yellow was measured at 520 nm, which is a measure of scavenging potential of plant extracts. 10 µl of plant extract was added to 100 µl of DPPH solution (0.2mM DPPH in methanol) in a microtitre plate.

The reaction mixture was incubated at 25 degree Celsius for 5 minutes, after that the absorbance was measured at 520 nm. The DPPH with corresponding solvents (without plant material) serves as the control. The methanol with respective plant extracts serves as blank.

The DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition.

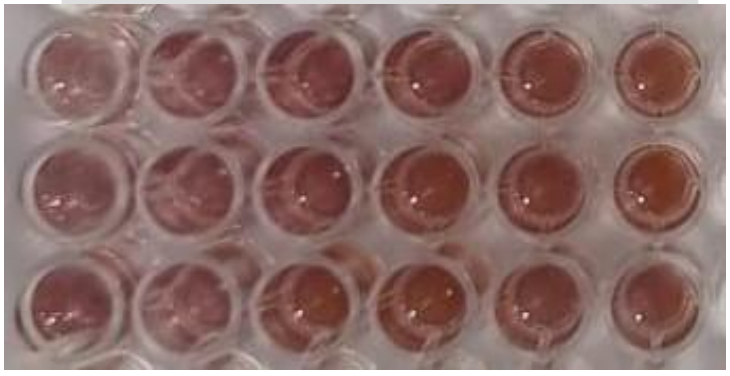
$$\% \text{ Inhibition of DPPH radical} = \frac{\text{Control - Test}}{\text{Control}} \times 100$$

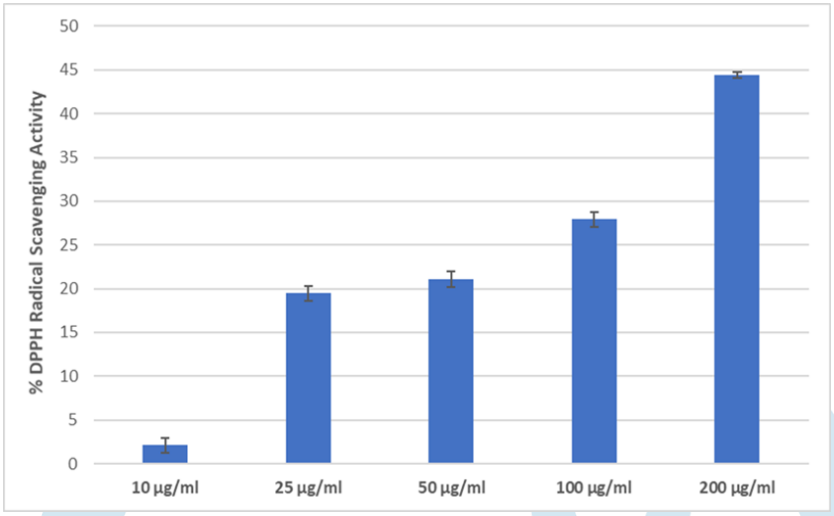
STANDARD



---------- µg/ml ----------

Control 10 25 50 100 200





9. RESULTS AND DISCUSSION

9.1 Preliminary Phytochemical studies of Extract leaves of *Kalanchoe gastonis bonnieri*

S.NO	CHEMICAL TEST	EXTRACT
1.	**Carbohydrates:**	
	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
	Barfoed's test	+
2.	**Phytosterols:**	
	Libermann burchard test	+
	Salkowski's test	+
3.	**Fixed oils and fats:**	
	Spot test	+
	Saponification test	+

4.	**Glycosides:** Legal test Balijet test Borntrager's test	 + + +
5.	**Flavonoids:** Shinoda's test Alkaline reagent test Mineral acid reaction test Lead acetate	 + + + +

S.NO	CHEMICAL TEST	EXTRACT
6.	**Tannins and phenolic compounds:** Lead acetate test Ferric chloride test Potassium ferric cyanide test Potassium dichromate test	 + + + +
7.	**Saponins:** Frothing test Emulsification test	 + +
8.	**Test for Alkaloids:** Mayer's test Wagner's test Hager's test Dragondroff's reagents.	 + + + +

(+) → indicates the presence of active ingredients. (-) → indicates the absence of active ingredients

9.2 *In-vitro* hepatoprotective activity:

S.no	Treatment	Absorbance		Average	Cell viability (%)
		I	II		
1.	Untreated control	0.886	0.876	0.881	100
2.	CCl_4-induced	0.331	0.315	0.323	36.66288309
3.	CCl_4+25 µg/ml	0.378	0.39	0.384	43.58683314
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6.	CCl_4+100 µg/ml	0.738	0.726	0.732	83.08740068

9.3 Anti-oxidant activity:

The free radical-scavenging activity of the leaf extract of Kalanchoe gastonis bonneri was determined using the DPPH method.

The extract was evaluated and compared with standard ascorbic acid. Kalanchoe gastonis bonneri leaf extract showed free radical scavenging activity in a dose-dependent manner through the DPPH method.

11. CONCLUSION

The study successfully evaluated the antioxidant potential of *Kalanchoe gastonis bonnieri*, through phytochemical analysis, *Invitro* assays and antioxidant activity tests.

The results revealed the presence of flavonoids, phenolics and other bioactive compounds, which contribute to its antioxidant potential.

In *Invitro* studies, significant free radical scavenging activity indicating its potential to combat oxidative stress.

In antioxidant assay exhibits anti-oxidant property, which could be beneficial for pharmaceutical and nutraceutical application.

Further studies, including *In vivo* models and clinical trials are recommended to explore its therapeutic applications and safety profile in human health.

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