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Formulation and evaluation of *Phyllanthus niruri* L. leaf extract in the management of hepatotoxicity

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Abstract
Phyllanthus niruri (*Phyllanthus carolinianus* blanco) is commonly known as Bhumi Amla. In Ayurveda *P Niruri* has been extensively used, both as edible (tonic) plants and for its therapeutic potentials. This plant belongs to Euphorbiaceae family. In India it is used as a herbal medicine and called as 'Bhumi Amla'. It is a large genus comprising about 700 species in tropical and subtropical regions. The leaves of *Phyllanthus niruri* collected from botanical garden. The extraction process was carried out using maceration extraction method. The leaves are extracted in ethanol solvent and evaluated for phytoconstitutes present in them. For phytochemical analysis of plant extract chemical test. The plant extract contains vitamins C, Fatty acid, decanoic acid, gallic acid, flavanoid, alkaloids, saponin, phenolic compound. The present study provides that solvent extract of *Phyllanthus niruri* contains medicinally important bioactive compounds and this justifies.
The use of plant species as traditional medicine for treatment of various diseases like anti inflammation antioxidants, antimicrobial agent, analgesic and antipyretic, anti-tumor and hepatoprotective, antiulcerogenic activities. The *Phyllanthus niruri* leaf extract aimed to investigate the protective effect management of hepatotoxicity by formulation of syrup & evaluated their parameters and efficacy and safety foranimal and human. The final and its effectiveness in managing of Hepatotoxicity.

Keywords: *Phyllanthus niruri,* leaf extract, hepatotoxicity, management, hepatoprotective syrup formulation

Introduction
Phyllanthus niruri is a herb that originated in India and is commonly found as a winter weed in warmer regions. It belongs to the Phyllanthus genus, which comprises over 600 species of shrubs, trees, and annual or biennial herbs spread across tropical and subtropical areas. *Phyllanthus niruri*, a member of the Euphorbiaceae family, typically grows up to 60 cm in height. Its name, Phyllanthus, translates to "leaf and flower," reflecting how the flower and fruit appear to merge with the leaf. *Phyllanthus niruri* is a prevalent rainy season weed in cultivated fields and wastelands and has garnered attention from researchers due to its hepatoprotective properties. While there is no specific therapy available for viral hepatitis, *P. niruri* has shown clinical effectiveness in treating Hepatitis B. It is renowned for its liver healing properties and is used in Chinese medicine for treating liver diseases. *Phyllanthus niruri* is a small herb found in tropical and subtropical regions across both hemispheres. It is widely used in folk medicine, with the whole plant, fresh leaves, and fruits being utilized for treating various diseases, particularly hepatitis and other viral infections. The plant contains a diverse array of phytochemicals, including flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins, which have been identified from different parts of *P. niruri*. Extracts of this herb have demonstrated therapeutic effects in numerous clinical studies. It is considered medicinally important for treating various ailments such as dysentery, influenza, vaginitis, tumors, diabetes, diuretics, jaundice, kidney stones, dyspepsia, antihepatotoxic, antihepatitis-B, antihyperglycemic, as well as antiviral and antibacterial purposes. *Phyllanthus niruri* extract has been found to inhibit DNA [1].

Synonym: Niruris annua, diasperus rosellus, Diasperus niruri, *Phyllanthus carolinianus* blanco, bhoomyaamalakee.

Biological source: *Phyllanthus niruri* is a widespread tropical plant commonly found in coastal areas, known by the common name gale of the wind, stone breaker, seed-under- leaf. It belongs to family *Euphorbiaceae* [2].

Geogrophical source

It is a field weed which is found proliferating throughout tropical and subtropical regions of Asia, America, and China. The genus Phyllanthus (L) Murr. Comprises from 600 to 700 species with minor distinguishing features among them. *Phyllanthus niruri* is an annual herb which grows in the wild after first showers of monsoon in Jharkhand, Bihar, Chhattisgarh, etc. states of India. However, it has also been reported to grow commonly in coastal areas. In Indian states it usually grows during second week of June and starts bearing fruits up to mid-July or August. It remains in the wild up to the end of the rainy season. However, under safe conditions it can grow and survive up to mid-winter [3].

General features

Phyllanthus niruri can be commonly found growing profusely alongside crops like gram, wheat, pea, etc. In its natural habitat, it thrives along roadsides, street corners, and areas where building materials are discarded.

Taxonomically, the annual herb *Phyllanthus niruri* belongs to the Phyllanthaceae family of the Malpighiales order within the Magnoliopsida class of the Magnoliophyta Division [4].

***Phyllanthus niruri* is known by different names in various languages**

- **Assamese:** Holpholi, Bengali: Noe.
- **Hindi:** Chalmeri, Bhumyalaki, Konka o Bhuin- avalae, Malayalam-Kijhandli o Marathi: Ray avail, Oria: Narakoli.
- **Sanskrit:** Bhoo datri.
- **Tamil:** Aru. In the Ayurvedic System of medicine, the entire plant of *Phyllanthus niruri* can be utilized for medicinal purposes.

Description

The annual herb typically reaches a height of 30-60 cm and is largely devoid of hair, with stems often branching at the base. Leaves: Abundant, arranged in a distichous manner, often overlapping, and are elliptic, oblong, and obtuse in shape. Stipules are present and sharply pointed. Flowers: Yellowish in color, abundant, and located in the axils of the leaves. Male flowers occur in groups of one to three, while female flowers are solitary. Capsules: Approximately 2.5mm in diameter, flattened globose shape, smooth surface with few or no lobes (Prajapati *et al*.) [5].

Botanical classification

- **Kingdom:** Plantae.
- **Division:** Magnoliophyta.
- **Class:** Magnoliopsida.
- **Order:** Euphorbiales.
- **Family:** Euphorbiaceae.
- **Genus:** Phyllanthus.
- **Species:** Niruri.

Vernacular Name

- **Assamese:** Holpholi; Poram-lokhi.
- **Bengali:** Bhui amla.
- **Hindi:** Chalmeri, Harfarauri, Bhuiaonla.
- **Kannada:** Kirunelli, Nela Nelli.
- **Konkani:** Bhuin-avalae.
- **Telugu:** Ratsavusirike, Nela Usiri.
- **Tamil:** Arunelli, Keela Nelli.
- **Malayalam:** Arinelli, Kizhanelli, Nellipuli.
- **Marathi:** Rayavali, Bhuiavli.
- **Oriya:** Narakoli.
- **Sanskrit:** Amala, Bhumyamlaki, Sukshmadala, Vitunika, Bhoodatri.

Unani Description

- **Unani name:** Bhui Amla, Amlaye Jamen.
- **Botanical name:** *Phyllanthus niruri*.
- **Synonyms:** Amala, Bhumyamlaki, Chalmeri, Harfarauri, Bhuiaonla, Bhoodatri.
- **Mizaz:** Cold & dry in 1st degree.
- **Maza:** Slightly in sour.
- **Boo:** Slightly in sourish smelling.
- **Muzir:** Due to high dosages, it has bad effect on spleen & urinary bladder.
- **Mukhrij:** Expels Safra (Bile).
- **Nafa-e-Khas:** Especially it is effective in hepatic disorders [6].

Chemical constituents

The medicinal plant *Phyllanthus niruri Linn. (Euphorbiaceae*), its wide variety of phytochemicals and their pharmacological properties. The active phytochemicals, flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts of *Phyllanthus niruri*. Extracts of this herb have been proven to have therapeutic effects in many clinical studies [7].

Uses

- Antioxidants.
- Antimicrobial lagent.
- Analgesic and antipyretic.
- Anti-tumor and hepatoprotective, antiulcerogenic activities.
- Haemorrhage, diarrhoea, diabetes.
- Antifungal.
- Antiviral activities

What is hepatotoxicity: Hepatotoxicity is the medical term for damage to liver causes by medicine, chemical or herbal dietary supplement. Hepatotoxicity can be a side effect of HIV medicine [8].

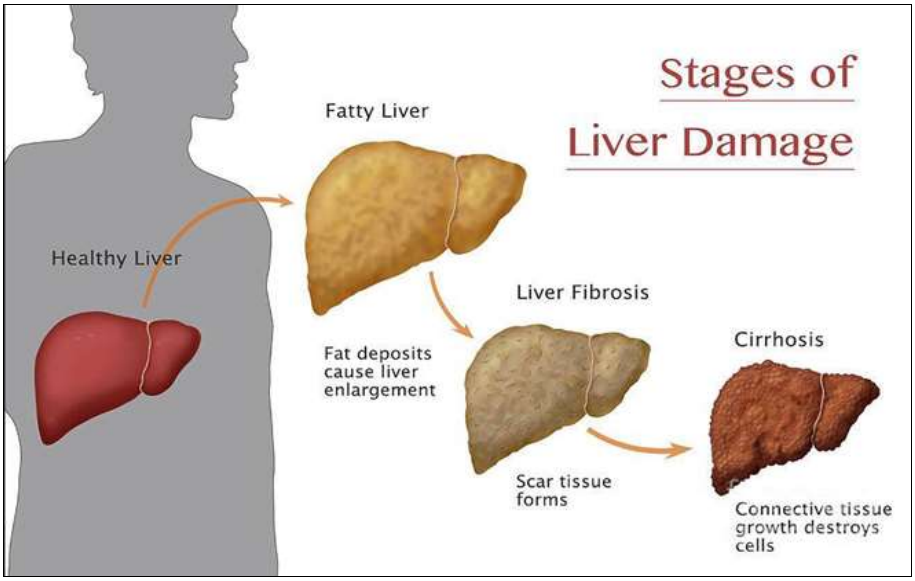


Fig 1: Stages of liver damage

Types of Hepatotoxicity [9]

Drug-Induced Hepatotoxicity: Many medications, including over-the-counter drugs, prescription drugs, and herbal supplements, can cause liver damage. This damage can range from mild elevations in liver enzymes to severe liver failure. Some drugs are directly toxic to the liver, while others may cause immune-mediated liver injury. Common examples include acetaminophen (Tylenol) overdose, certain antibiotics, statins, chemotherapy drugs, and herbal supplements like kava and green tea extract.

Alcohol-Induced Hepatotoxicity: Chronic alcohol consumption is a leading cause of liver damage worldwide. Alcohol metabolism produces toxic by-products that can injure liver cells and lead to inflammation, fatty liver disease, alcoholic hepatitis, fibrosis, and cirrhosis over time. The severity of liver damage correlates with the amount and duration of alcohol consumption

Viral Hepatotoxicity: Hepatitis viruses, including hepatitis A, B, C, D, and E, can cause liver inflammation and damage. These viruses are typically transmitted through contaminated food or water (hepatitis A and E), blood or bodily fluids (hepatitis B, C, and D), or sexual contact. Chronic infection with hepatitis B and C viruses can lead to progressive liver disease, including cirrhosis and liver cancer.

Toxin-Induced Hepatotoxicity: Exposure to certain toxins, chemicals, and environmental pollutants can damage the liver. Examples include industrial chemicals (e.g., carbon tetrachloride), mushroom toxins (e.g., Amanita species), aflatoxins (produced by molds in food), and herbal remedies containing toxic compounds.

Metabolic Hepatotoxicity: Inherited metabolic disorders, such as hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency, and glycogen storage diseases, can cause liver damage due to abnormal accumulation of substances within liver cells. These conditions can lead to inflammation, fibrosis, and cirrhosis if left untreated.

Ischemic Hepatotoxicity: Prolonged periods of reduced blood flow to the liver, such as in ischemic hepatitis (also known as shock liver), can cause liver damage due to insufficient oxygen and nutrient supply. This condition often occurs in critically ill patients with severe shock, heart failure, or hypotension.

Autoimmune Hepatotoxicity: Autoimmune hepatitis is a rare condition in which the immune system mistakenly attacks liver cells, leading to inflammation and liver damage. The exact cause of autoimmune hepatitis is unknown, but genetic, environmental, and immunological factors are believed to play a role.

Causes

Some drugs can cause liver damage as a side effect. This is known as drug induced hepatotoxicity.

Exposure to industrial chemicals or environmental toxins can harm the liver.

Chronic alcohol abuse is a common cause of liver damage, leading to conditions like alcoholic liver disease.

Hepatitis viruses (e.g., hepatitis B and C) can infect the liver and cause inflammation and damage

Symptoms of hepatotoxicity

- Nausea.
- Vomiting.
- Loss of appetite.
- Fever.
- Jaundice (Yellowish of the skin and eyes).
- Dark colour urine.

Literature of review

Antiplatelet and vasorelaxant activity

Masahiro Nagai *et al*. 2006 Methyl brevifolin carboxylate isolated from *P. niruri* exerted vasorelaxant effect on rat aortic rings via inhibition of noradrenaline-induced vasoconstriction mediated by a decrease in calcium ion influx through receptor-operated Ca2+ channels. The same compound also acted as a platelet aggregation inhibitor [10].

Anti-Inflammatory activity

Lim YY, Murtijaya J. *et al*. 2007 The Hexane Extract (HE), the Lignan-Rich Fraction (LRF), or the lignans phyltetralin, nirtetralin, niranthin of *Phyllanthus niruri* when given orally inhibited carrageenan (Cg)-induced paw oedema and neutrophile influx. The HE, the LRF or nirtetralin also

inhibited the increase of IL1-β tissue levels induced by Cg. Bradykinin (BK)-, Platelet Activating Factor (PAF)- and endothelin-1 (ET-1)-induced paw oedema were significantly inhibited by the HE or LRF. Finally, nirtetralin or phyltetralin caused inhibition of paw oedema induced by PAF or ET-1. These results show that the HE, the LRF and the lignans niranthin, phyltetralin and nirtetralin exhibited marked anti-inflammatory properties [11].

Anti-spasmodic activity

Santos AR *et al*. 1994 Research done in Brazil at the Federal University of Santa Catarina in 1984 on *Phyllanthus niruri* revealed an alkaloid (phyllanthoside) in the leaves and stem with strong antispasmodic activity. It served as a relaxing agent for smooth muscles and they concluded that its spasmolytic action probably accounted for the efficacy of *Phyllanthus niruri* in expelling stones [12].

Antioxidant activity

Kamruzzaman HM *et al*. 2016 The Total Phenolic Content (TPC) and antioxidant activity of fresh and dried *Phyllanthus niruri* were evaluated by Folin-Ciocalteau method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assays. Different drying treatments led to significant reduction ($p<0.05$) in antioxidant properties of *Phyllanthus niruri* methanolic extracts, with microwave drying causing the highest decrease in TPC and antioxidant activity exhibited by the reduction in both radical scavenging activity and FRAP, the boiling water extracts appeared to exhibit stronger antioxidant potentials ($p<0.05$) even in dried plant materials. This proves its strong free radical scavenging activity [13].

Antibacterial activity

Mellinger CG, *et al*. 2008 The antibacterial activity of extracts of the root and leaf was assessed against extend spectrum lactamase (ESBL) producing Escherichia coli isolated from the stool samples of HIV seropositive patients using Bauer disc diffusion method. The strains isolated from both HIV sero-positive patients were susceptible to various concentrations of the extracts (5, 10, 20, 40 and 80 mg mL-1). This proves the antibacterial activity of the extract [14].

Wound healing and anti-ulcer properties

Tara, Arul Amuthan *et al*. 2010 A rat study involving the oral administration of ethanolic extracts of the herb showed significant inhibition of the development of indomethacin-induced ulcers. The antiulcer activity has been attributed to gallic acid, beta-sitoterol, ellagic acid and alkaloids-4-methoxy-securinine. Extracts of *P. niruri* also protect against ethanol- induced gastric mucosal ulceration in rats and reverse dexamethasone-suppressed burn wound healing. The exact mechanisms have not been elicited to date [15].

Antihyperuricaemic activity

Cimanga, R. K., *et al*. 2004 Lignans from *P. niruri* were found to be antihyperuricaemic in animals; the effects were comparable with drugs for treating hyperuricaemia and gout-like allopurinol and probenecid. A later study showed that the antihyperuricaemic property of the lignans was due to their uricosur action. This study also showed that *P. niruri* methanol extract had antihyperuricaemic effect primarily attributed to its uricosuric action and partly via xanthine oxidase inhibition [16].

Diuretic Activity

The diuretic, hypotensive and hypoglycemic effects of *Phyllanthus niruri* on human subjects were assessed. Appropriate parameters have been studied in the blood as well as urine samples of the patients. In addition, the physiological profile and dietary pattern before and after the treatment period were assessed. Interestingly, a significant increase in urine volume, urine and serum Na levels was observed after treatment with *Phyllanthus niruri* extract. A significant reduction in systolic blood pressure in non-diabetic hypertensive subjects was noted that further confirmed its diuretic property [17].

Antihyperalgesic

Jeverson Moreira *et al*. 2013 The study evaluated the anti-hyperalgesic activity of corilagin, an atannin from *Phyllanthus niruri*, using chemically and thermally based nociception models in mice. Results showed corilagin's anti-hyperalgesic activity, 20.6 times more potent than acetylsalicylic acid, and significant activity in capsaicin and glutamate models. This suggests corilagin's potential interaction with the glutamatergic system [18].

Cardioprotective

Thippeswamy AH *et al*. 2011 Only one major animal study has been conducted to investigate the attenuating action of *P. niruri* extracts in preventing doxorubicin-associated cardiotoxicity. Pretreatment of rats with *P. niruri* extract significantly protected rat myocardia from doxorubicin toxicity by normalizing cardiac biomarkers, restoring intracellular levels of enzymatic and non-enzymatic antioxidants and decreasing rat cardiac tissue peroxidation [19].

Hepatoprotective

(Meixa *et al*., 1995) *Phyllanthus niruri* has been shown to have significant antihepatitis B virus surface antigen activity in both *in-vivo* and *in-vitro* studies. Hepatitis B infection often leads to a "carrier state" due to the body's inability to clear the virus from liver cells. Detectable levels of various viral antigens, such as HbaAg (the virus's surface antigen) and antibodies to the virus core (HBc antibodies), indicate infection. The hepatoprotective effect of an ayurvedic herbal preparation, HPN-12, containing *Glycyrrhiza glabra, Picrorhiza kurroa, Berberis aristata, Piper longum, Phyllanthus niruri, Solanum dulcamara, Zingiber officinale, Curculigo orchioides, Elettaria cardamomum, Tinospora cordifolia, Desmodium trifolium, and Saccharum officinarum*, has also been documented [20].

Hiv replication

(Naik *et al*., 2003) The aqueous extract of *Phyllanthus niruri* has been reported to possess inhibitory effects on the human immunodeficiency virus (HIV). An investigation was conducted to evaluate the anti-HIV effects of the alkaloidal extract of *Phyllanthus niruri* in human cell lines. The inhibitory effect on HIV replication was assessed by monitoring the inhibition of virus-induced cytopathogenicity in MT-4 cells. The alkaloidal extract of *Phyllanthus niruri* demonstrated a sensitive inhibitory response on the cytopathic effects induced by both strains of the human immunodeficiency virus on human MT-4 cells at the tested concentrations [21].

Lipid lowering

(Chandra *et al*., 2000) The lipid-lowering activity of alcoholic

extracts of *Phyllanthus niruri* was investigated in rats with triton-induced hyperlipidemia. It was observed that administration of triton in rats resulted in a significant increase in serum cholesterol by 3.5- fold, phospholipid by 2-fold, and triglyceride by 1.2-fold. Simultaneous administration of *Phyllanthus niruri* at a dose of 200 mg/kg with triton led to a reduction in the levels of total cholesterol, phospholipid, and triglyceride by 27%, 25%, and 24%, respectively. In another experiment involving cholesterol-fed rats, *Phyllanthus niruri* at a dose of 100 mg/kg effectively lowered the elevated levels of low-density lipoprotein lipids in hyperlipidemic and drug-fed animals [22].

Anti-diabetic

(Raphael *et al*., 2000) An alcoholic extract of *Phyllanthus niruri* was observed to significantly reduce blood sugar levels in both normal rats and rats with alloxan-induced diabetes. In normal rats, administration of *Phyllanthus niruri* at a dose of 200 mg/kg body weight resulted in a 34.5% reduction in blood sugar levels, while at a concentration of 1000 mg/kg body weight, the reduction was 47.4% at 1 hour post-administration. However, by the 6th hour, the values returned to almost normal levels. These findings suggest the potential antidiabetic effects of *Phyllanthus niruri* [23].

Anti-malarial

(Neraliya *et al*., 2004) The antimalarial activity of ethanolic, dichloromethane, and lyophilized aqueous extracts from the root bark of Cassia occidentalis, leaves of Morinda morindoides, and whole plants of *Phyllanthus niruri* was evaluated *in vivo*. Among these extracts, the ethanolic extract of *Phyllanthus niruri* exhibited the highest activity, reducing parasitemia by 73%. The dichloromethane extracts of M. morindoides and *Phyllanthus niruri* showed similar levels of activity, with chemosuppression rates of 74% and 72%, respectively, while the extract from C. occidentalis was slightly less active, with a chemosuppression rate of 60%. However, each lyophilized aqueous extract was less active compared to its corresponding ethanolic extract [24].

Analgesic

(Santos *et al*., 1994) The methanol extract obtained from dried callus tissue, when administered intraperitoneally to mice at a concentration of 10 mg/kg, showed activity against acetic acid-induced writhing and formalin-induced pedal edema. However, at a higher concentration of 50 mg/kg, it was found to be inactive against the tail flick response to radiant heat. Conversely, the ethanol/water (1:1) extract derived from the entire dried plant, when administered intragastrically to male mice at a dose of 50mg/kg, exhibited activity. Additionally, when administered intraperitoneally to male mice at a dose of 0.3mg/kg, the extract also demonstrated antinociceptive effects across five different models of nociception [25].

Hypothesis

The hypothesis is that *Phyllanthus niruri* leaf extract syrup could potentially alleviate hepatotoxicity by its antioxidant properties, which may counteract oxidative stress and reduce liver damage. Additionally, its anti-inflammatory and hepatoprotective effects might aid in restoring liver function.

The formulation aims to provide a safe and effective treatment option, improving liver health and minimizing hepatotoxicity-induced complications. Further research and clinical trials are needed to validate this hypothesis and establish the efficacy of the syrup in hepatotoxicity management.

The *Phyllanthus niruri* leaf extract already studied and conformed of that chemical constituent's present management of hepatotoxicity the aim of investigate the *P. niruri* leaf extract by formulation of syrup and evaluated the safety and efficacy on animal and human

Objective

Due to the conventional treatments for hepatotoxicity and to the traditional use of *niruri* (*Phyllanthus niruri* L.), in addition to beneficial effects shown in recent studies, we evaluated the safety and efficacy of *niruri* syrup for improvement of symptoms hepatotoxicity.

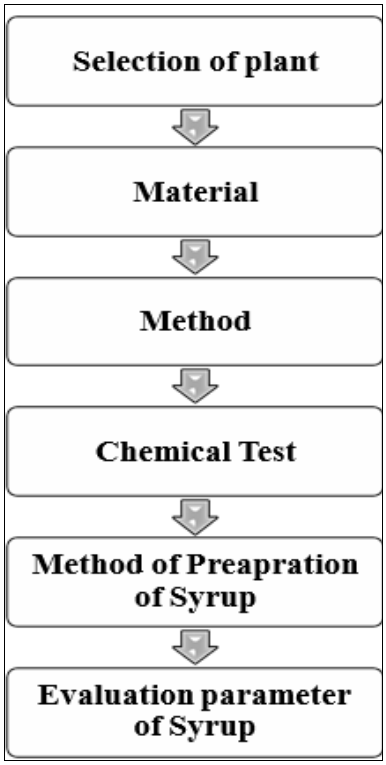
Investigate the protective effect of *Phyllanthus niruri* leaf extract syrup formulation of hepatotoxicity.

The formulation aim to provide a safe and effective treatment options improving liver health minimizing hepatotoxicity - induced

Need of significance

Phyllanthus niruri has been traditionally used in various cultures for its purported medicinal properties, including its potential to support kidney health, liver health, and digestive issues. Formulating products containing this herb allows for standardized doses and easier consumption**.** Formulation ensures that the product contains consistent levels of active compounds, ensuring efficacy and safety for consumers. Evaluation processes help in verifying the quality and potency of the product, ensuring it meets regulatory standards. Formulating *Phyllanthus niruri* into liquid dosage form such as liquid extracts allows for easier consumption and precise dosing, improving patient compliance and convenience. Continuous formulation and evaluation efforts drive research and innovation in the field of herbal medicine, leading to the discovery of new delivery systems, improved formulations, and better understanding of the therapeutic potential of *Phyllanthus niruri*

Proposed plan of work



Material and method of *P. niruri* leaf extract

Collection of plant material Leaves of *Phyllanthus niruri* were collected from the field area of Thengoda, Nashik district in India in January 2024.

Fig 2: Leaves of *Phyllanthus niruri*

Preparation of Phyllanthus leaf extract

The extraction process was carried out using maceration extraction method. The leaves of *Phyllanthus niruri* were collected and washed repeatedly and air dried at room temperature in a cool dry place away from direct sunlight. Then ground to a coarse powder of dried leaf powder take 30 gm of powder was soaked in 300ml of 95% ethanol for 7days. Then the mixture was filtered by using whatmann filter paper.

Fig 3: Powder of *Phyllanthus niruri*

Fig 4: Extract of *Phyllanthus niruri*

Chemical test for *Phyllanthus niruri* leaf extract

Test for Alkaloids (Wagner's reagent): A fraction of extract was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and observed for the formation of reddish brown precipitate (or) coloration.

Test for Flavonoids

To portion of the dissolved extract, a few drops of 10% ferric chloride solution were added. A green or blue colour indicates the presence of phenolic nucleus.

Test for Terpenoids (Salkowski Test)

0.5 gram of each extract was added to 2 ml of chloroform. Concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Tannins

About 0.5 gram of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownishgreen or a blue-black coloration.

Test for Saponins (Foam test)

To 2 ml of extract was added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of Saponins.

Fig 5: Chemical test of *P. niruri* leaf extract

Method and preparation of syrup

Table 1: Formulation1 (F1) for 50 ml

Sr. No	Ingredients	Quantity	Role
1.	*Phyllanthus niruri* leaf extract	25 ml	Treat for hepatotoxicity
2.	Sorbitol	0.25 ml	Stabilizer agent
3.	Methyl paraben	0.020 gm	Preservatives
4.	Amaranth solution	0.10 ml	Colouring agent
5.	Raspberry	0.75 ml	Flavouring agent
6.	Sucrose	33.3%	Sweetening agent
7.	Water	QS	Vehicle

Table 2: formulation 2 (F2) for 50 ml

Sr. No	Ingredients	Quantity	Role
1.	*Phyllanthus niruri* leaf extract	25 ml	Treat for hepatotoxicity
2.	Sorbitol	0.70 ml	Stabilizer agent
3.	Methyl paraben	0.015 gm	Preservatives
4.	Amaranth solution	0.05 ml	Colouring agent
5.	Raspberry	0.70 ml	Flavouring agent
6.	Sucrose	33.3%	Sweetening agent
7.	Water	QS	Vehicle

Table 3: Formulation (F3) for 50ml

Sr. No.	Ingredients	Quantity	Role
1.	*Phyllanthus niruri* leaf extract	25 ml	Treat for hepatotoxicity
2.	Sorbitol	0.65 ml	Stabilizer agent
3.	Methyl wait	0.010 Ka	Preservatives
4.	Amaranth solution	0.07 ml	Colouring agent
5.	Raspberry	0. 5	Flavouring agent
6.	Sucrose	33.3%	Sweetening agent
7.	Water	QS	Vehicle

Preparation of syrup

Weighing sucrose

Weigh the required amount of sucrose in beaker.

Solution preparation

Add purified water to beaker1and heat on water bath (below 70 °C) until a clear solution form.

Sorbitol solution

In beaker 2 combine sorbitol, methyl paraben and *Phyllanthus niruri* leaf extract.

Combining solution

Pour the contents of beaker 2 into beaker 1 (Containing the sucrose solution) and mix thoroughly.

Volume adjustment

Add Colouring agent and flavouring agent to beaker 1 to achieve the desired flavour and colour

In a beaker 3 dissolved additional sucrose in beaker to make up the volume then combine it will beaker 1and pour it suitable container.

Advantages of syrup

1. Helps in cleansing and detoxification liver.
2. Help to improve liver function help in fatty liver.
3. They are more pleasant.
4. It is mask bitter test.

Disadvantages of syrup

1. It is not suitable in the emergency condition.
2. Environmental factor affecting on the stability of the Product.
3. During storage it causes an crystallization of the sugar within the screw cap.

Evaluation parameter for syrup formulation

Colour

1 ml of final syrup was taken in a watch glasses and placed under light and colour is observed by a naked eye.

Odour

1 ml of final syrup was smelled individually and then the odour is detected.

Taste

A pinch of final syrup was placed on taste bud of tongue to identify the taste Determination of viscosity.

Viscosity of herbal syrup is determined by using an ostwald Viscometer. Ostwald viscometer is thoroughly cleaned with chronic acid or acetone. Viscometer should be placed in a vertical position in a suitable stand. Fill the water upto the mark in dried viscometer.

Now note the time required for water to flow from mark A to mark B. Now was the viscometer fill with the herbal syrup, and then note the time required for syrup to flow from mark A to mark B.

Formula of viscosity

Density of test liquid ×time required to flow test required / Density of water × time required to flow water = viscosity of water [22].

Determination of PH

Take a 2 ml of syrup in the test tube and with the help of pH paper checked the pH of syrup.

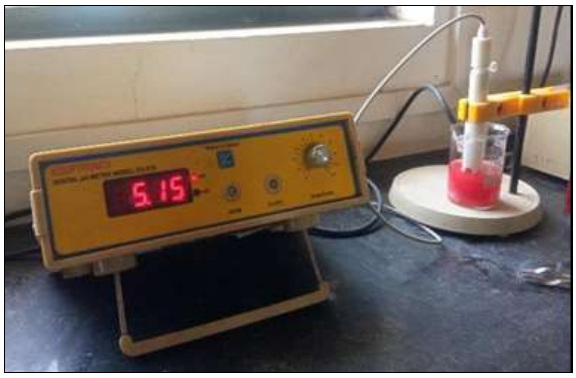


Fig 6: Determination of PH

Determination of density

The density of syrup can be determined by using specific gravity bottle. Clean the specific gravity bottle with nitric acid and rinse with purified water. Note the weight of empty dry bottle (w1). Fill the specific gravity bottle with water and weigh it (w2). Finally note the weight of bottle with a syrup (w3).

Formula for the density

W1: Weight of empty specific gravity bottle. W2: Weight of empty specific gravity bottle + water. W3: Weight of empty specific gravity bottle + syrup Density of syrup=W3-W1/W2-W1 x Density of water.

***In vitro* Anti- Inflammatory test**

Inhibition of albumin denaturation: *In vitro* anti-inflammatory activity was determined by inhibition of protein (egg albumin) denaturation method.

Control Solutions (50 ml): Phosphate buffer saline (28 ml) of pH 6.4 was transferred to freshly prepared egg albumin (2 ml) and distilled water (20 ml) was added to this, to prepared control solution.

Standard Solution (50 ml): Phosphate buffer saline (28 ml) of pH 6.4 was transferred to freshly prepared egg albumin (2 ml) and (20 ml) solution of diclofenac sodium of different concentration ranges from 10 -2000 µg/ml was added to this, to prepared standard solution.

Test Solution (50 ml): Phosphate buffer saline (28ml) of pH 6.4 was transferred to freshly prepared egg albumin (2 ml) and (20ml) solution of F2 formulation of different concentration ranges from 10 -2000 µg/ml was added to this, to prepared test solution.

All the solutions were incubated at 37±2 °C for 15 minute sand it was then heated at 70 °C on a water bath for 5 minutes. The solutions were allowed to cool at room temperature. The absorbance was then measured using UV Visible spectrophotometer at 660 nm using vehicle as blank. The percentage inhibition of protein denaturation was calculated from the control using below under

Formula: Percentage inhibition= (Abstract Control –Abstract Sample) X100 /Abstract control

Fig 7: Anti- inflammatory test

Result and discussion

The final formulation (F3) was obtained is stable than formulations F1, F2 The formulation (F3) was obtained by minimizing the error in formulation F1, F2. The formulation (F3) having antioxidant property hence it will be very helpful for researcher as well as F3 formulations effective for the management of hepatotoxicity and industries to make the similar formulations on large.

Table 4: Phytochemical constitute of the leaf extract of *Phyllanthus niruri*

Tests	Name of tests	In methanol extract
Alkaloids	Wagners reagent	+
Flavonoids	Ferric chloride test	+
Terpenoids	Salkowski test	+
Tannins	Ferric chloride test	+
Saponins	Foam test	+
		+

Table 5: Evaluation of syrup formulation

Formulation	Colour	Odour	Taste	PH	Viscosity	Density
F1	Light pink	Aromatic	Slightly	5	0.014cp	1.45m
F2	Light pink	Aromatic	Slightly	5.1	0.0481cp	1.39m
F3	Light pink	Aromatic	Slightly	5	0.0392cp	1.31.

Anti-inflammatory test

Table 6: Calibration curve of standard sample

Concentration µg/ml	Absorbance
10	0.00498
20	0.00589
30	0.0067
40	0.0075
50	0.0082

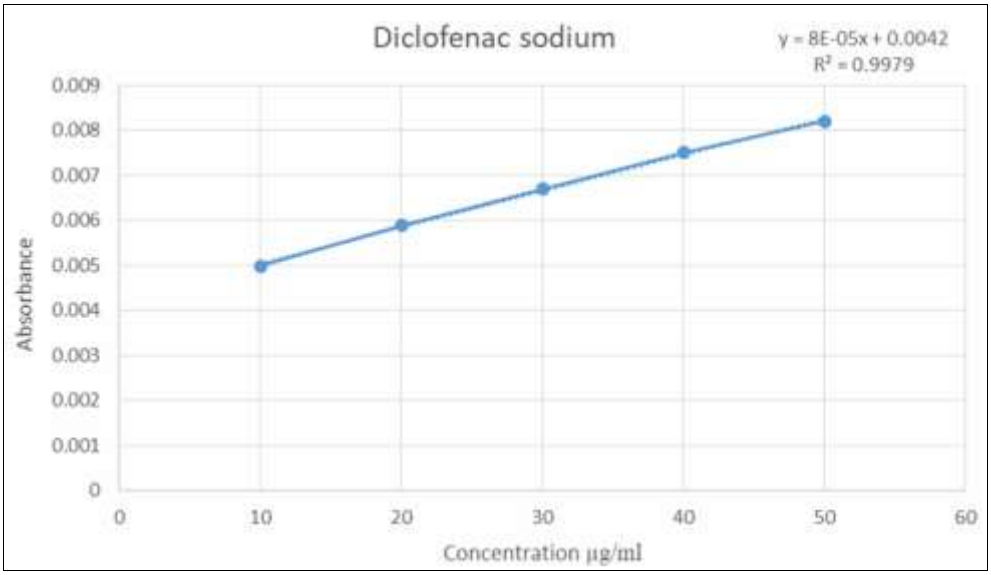


Fig 8: Diclofenac sodium

Table 7: Calibration curve of Test sample in UV

Concentration µg/ml	Absorbance
10	0.0069
20	0.0075
30	0.0082
40	0.0088
50	0.0093

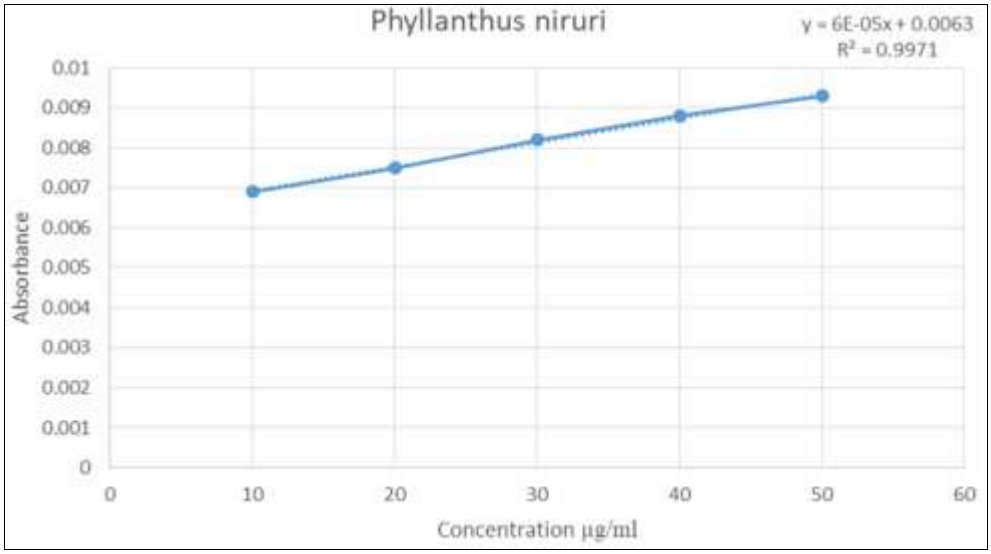


Fig 9: Phyllanthus niruri

Table 8: Percentage inhibition of anti-inflammatory test

Concentration	Absorbance value (Mean)	Standard	Test Sample
	Control		
10ppm	0.036	0.00498	0.0069
20ppm	0.036	0.00589	0.0075
30ppm	0.036	0.0067	0.0082
40ppm	0.036	0.0075	0.0088
50ppm	0.036	0.0082	0.0093

Concentration	Control	% inhibition	% inhibition
		Standard	Test sample
10ppm	0	86.16%	80.73%
20ppm	0	83.63%	79.16%
30ppm	0	81.38%	77.22%
40ppm	0	79.16%	75.55%
50ppm	0	77.22%	74.16%

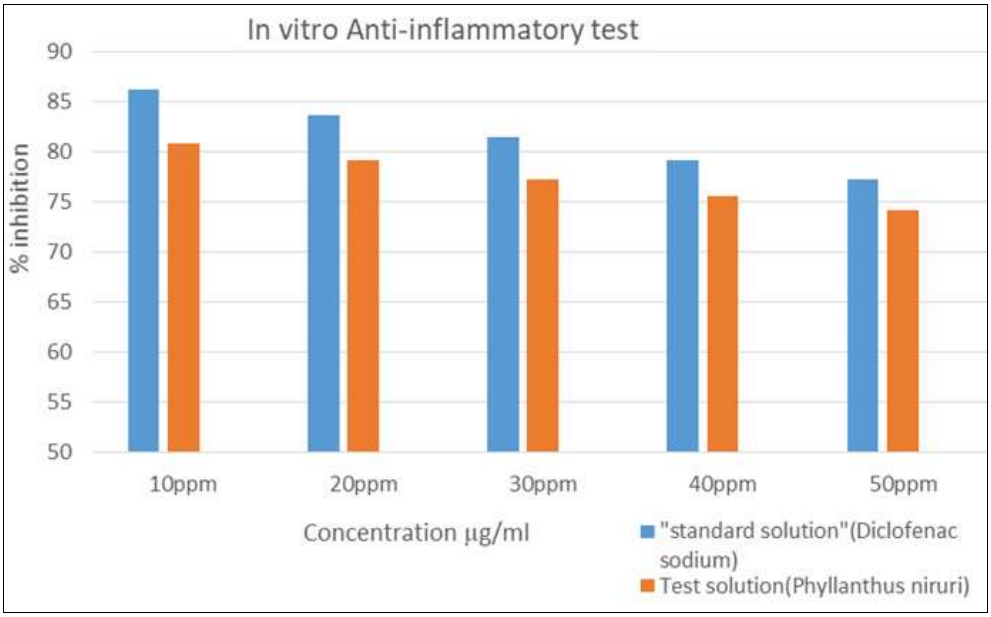


Fig 10: Percentage inhibition graph

According to the procedure of the anti-inflammatory activity, there are the absorbance Of the all samples like Standard solution, Test Solution, Control Solution using the UV Spectrophotometer are measured from that absorbance the above calibration curve of the standard drug (Diclofenac sodium) and Test Sample (*Phyllanthus niruri* leaf extract)& the vitro anti-inflammatory test using egg albumin method. For the anti-inflammatory susceptibility diclofenac sodium is used as standard drug which compare with our sample i.e., *Phyllanthus niruri* leaf extract. The percentage inhibition was calculated by using the formula given in procedure of anti-inflammatory test. The percentage inhibition of both Diclofenac Sodium and *Phyllanthus niruri* leaf extract calculated and compare by applying unpaired 't' test. The unpaired 't' test is applied for the determination of significance difference between two samples. In this test the 'p' value is of < 0.05 shows the significant difference in test and standard samples. The above graph of percentage inhibition shows the significant difference between the percentage of all concentration of test and standard samples.

Conclusion

In conclusion the formulation and Evaluation of *Phyllanthus niruri* Linn leaf extract syrup for the management of Hepatotoxicity show promising potential. This herbal syrup rich in antioxidant and hepatoprotective properties offers a Natural approach to mitigation liver damage caused by various factors. The study finding suggest that this syrup could be a valuable addition to hepatotoxicity Management with Further reaserch and clinical trials needed to validate its effectiveness and safety in real world application overall, *Phyllanthus niruri* Linn leaf extract syrup are safe and effective for Management of Hepatotoxicity.

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