

## Formulation and Evaluation of Hepatoprotective Tonic by Using the Ethanolic Extract of *Tridax Procumbens*

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### ABSTRACT:

Most of herbal tonic was originally derived from plant herbal medicine refers to use extract of aerial parts for medicinal purpose. Along with other dosage from herbal drugs also formulated in the form of tonic. Today tonic is used for treatment of many liver disorders and to overcome symptoms of diseases related to liver. The hepatoprotective tonic is used to treat the liver cirrhosis and alcoholic liver disease condition and other liver injuries in body, by using these tonic the condition is overcome. The extraction of *TridaxProcumbens* is added into sorbitol, it gives flavored to tonic and sodium benzoate and potassium sorbate used as preservative. Two formulation were prepared to observe the variation within the tonic. The density, specific gravity, pH, and organoleptic properties of each produced formulation were assessed. According to the findings, herbal tonic formulation number 2 (F2) is more elegant and stable than F1 formulations.

### I. INTRODUCTION

The liver is the largest gland in the body, plays a central role in metabolic homeostatic, it serves as primary regulatory site for energy metabolism, taking up and processing ingested nutrient for controlled distribution to extra hepatic tissues in addition liver synthesis essential protein, enzymes and co factor required for digestion and normal bodily function. It removes or neutralized poison from the blood, produces immune agents to control infection and remove germs and bacteria from the blood. It produces bile to aid in the absorption of lipids and fat-soluble vitamins, as well as proteins that regulate blood coagulation.<sup>1</sup>

#### Liver disease –

- Primary liver cancer
- Hepatitis A
- Hepatitis B

- Hepatitis C
- Hepatitis D (delta virus )
- Hepatitis E
- Nonalcoholic fatty liver disease
- **Drug induced liver injuries**
- **Liver cirrhosis**
- Autoimmune hepatitis<sup>2</sup>

#### • **Liver cirrhosis –**

Cirrhosis is complication of many livers' disease characterized by abnormal structure and function of the liver. The disease that leads to cirrhosis do so because they injure and kill liver cells. That attempt to replace the cells that have died. After which the inflammation and repair that is associated with the drying liver cells cause the scar tissue to form. There are many causes of cirrhosis including chemicals likes' alcohol, fats, and certain medication.<sup>[3]</sup>

#### • **ALD -Alcohol liver disease –**

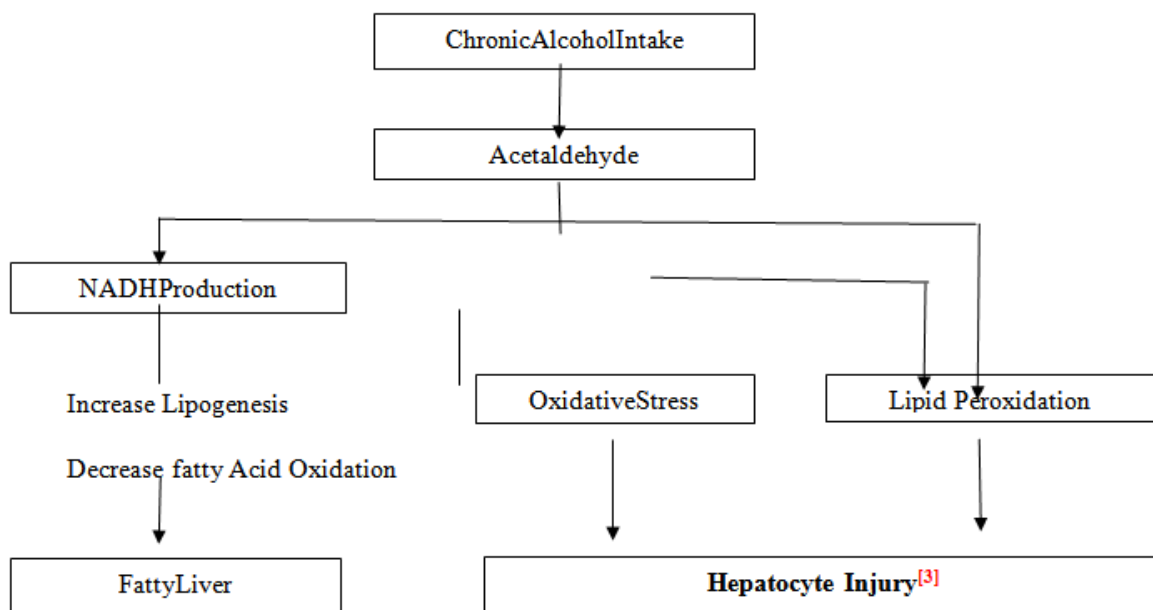
It is result of an over consumption of alcohol that leads to building up fats and scarring of the liver. Cirrhosis of the liver is due to chronic alcoholism. In women two to three drinks per day and in men 4-5 drinks per days. Leads to liver by blocking the normal metabolism of proteins, fats and carbohydrates. Alcohol is metabolized by alcohol dehydrogenase into acetaldehyde then further metabolized by acetaldehyde dehydrogenase into acetic acid, which is finally oxidized in CO<sub>2</sub> and water. This process generates NADH increase the NADH /NAD<sup>+</sup> ratio. A higher NADH concentration induce fatty acid synthesis will a decreased NAD level result in deceased fatty acid oxidation. Subsequently, the higher level of fatty acid signals the liver cell to compound it to glycerol to form triglycerides. These triglycerides accumulate, resulting in fatty liver.<sup>[3]</sup>

• **Fatty liver –**

It is a result of accumulation of fat mainly due to disproportion between fat deposition and removal from liver the accumulation of large quantities of triglycerides results in distension of hepatocytes to several times like fat size. This fatty

liver is a result of changed metabolic activity of changed metabolism. It is usually asymptomatic. The only clinical findings are enlarged liver. <sup>[4]</sup>

- Alcoholic hepatitis –Histological features are present in necrosis of hepatocytes.



**Tridax Procumbens** - A medicinal plant Tridax Procumbens commonly known as coat button or kansari (Hindi) or Ghamara belonging to family Asteraceae. It is a plant that many different communities and traditional Indian medicine use. It is a very promising species that produces secondary metabolites such as alkaloid, steroids, carotenoid, flavonoid (catechins, centaurein and bergeniens), fatty acid, phytosterols, tannins and minerals reported to have a variety of medicinal uses including antioxidant, antibacterial, anti-inflammatory, antimicrobial, vasorelaxant, antileishmanial, anti-anemic, immunomodulatory, hepatoprotective and mosquitocidal activities. <sup>[5]</sup>

**Habitat:**

Tridax Procumbens an annual or perennial herbaceous weed found in tropical and subtropical areas of the world, growing mainly

during the rainy season at meadows, croplands, disturbed areas, lawns, roadside or settled areas. This medicinal herb shows a typical feature of a beneficial weed. <sup>[6]</sup>

**Growth:**

Plants are prostrate or erect, forming patches, with flowering axis 15 to 35cm high.

**Leaves:**

Leaves are opposite, simple, carried by a petiole, 1 to 2 cm long. They are thick, soft and dark green. The lamina is oval to lanceolate, 2 to 6 cm long and 2 to 4 cm wide, base attenuate in the corner and with strongly and irregularly serrated margin. Both sides are hispid, with tuberculate based bristles. Pubescence is most abundant on the underside. <sup>[6]</sup>



**Fig. 1: Aerial Part of TridaxProcumbens**



**Fig. 2: Flower of TridaxProcumbens**

**Table 1. Botanical Classification of *TridaxProcumbens***

<b>Classification</b>	
<b>Division</b>	<b>Classing</b>
<b>Kingdoms</b>	<b>Plantae –plants</b>
<b>Sub kingdoms</b>	<b>Tracheobionta-vascular plant</b>
<b>Division</b>	<b>Spermatopsyta</b>
<b>Subdivision</b>	<b>Magnoliophyta–Flowering plants</b>
<b>Class</b>	<b>Magnoliopsida-Dicotyledons</b>
<b>Subclass</b>	<b>Asteridae</b>
<b>Order</b>	<b>Asterales</b>
<b>Family</b>	<b>Asteraceae-aster Family</b>
<b>Genus</b>	<b><i>Tridax</i> L. –<i>Tridax</i></b>
<b>Species</b>	<b><i>TridaxProcumbens</i> L. -coat buttons<sup>[6]</sup></b>

**TridaxProcumbens Morphology and Pharmacological Activity:**



**Fig. 3. Image during Collection of *T. Procumbens***

**Botanical Morphology:**

Natural habitat:  
 The plant of *TridaxProcumbens* is a perennial herbaceous weed commonly seen in hotter areas of the world. The plant is natively seen in the tropical American region and also native to the tropical areas of Asia, Australia, Africa and India. It is a widely found herb with a distribution throughout the Indian subcontinent. It is found abundantly in the meadows, disturbed areas, lawns, crop land or on the roadside.

The plant has an average height of around 20-60 cm and is branched; Leaves are 4-8 cm long, simple, opposite and stipulate. Inflorescence is

around 12-32 cm, oval shaped and held by peduncle, with ray florets and disc florets. Flowers are daisy like with yellow centred white or yellow petals. Numerous, tubular disc florets are surrounded by a ring of short, strap-shaped ray florets. Fruit is cypsela, black or brown in colour at maturity and surrounded with feathery bristle. The stem is cylindrical and covered with hairs of about 1 num with tap root system. The registered number of chromosomes present in *Tridaxare* 36 (2n). Growth of plant takes place during monsoon season as it requires abundant water for growth and sustenance.<sup>[5]</sup>

Compounds/activity of *T. Procumbens*

Extraction	Compounds/activity	Plant organ
Ethanol-acetic acid	Alkaloids for antimicrobial activity, against human pathogens, antioxidant, Hepatoprotective	Aerial parts. <sup>[5]</sup>

**Table 2. Activity of *T. Procumbens***

**Pharmacological Activity**

*Tridax Procumbens* having various potential therapeutic activities like antimicrobial activity, anti-oxidant, antibiotic efficacies, wound healing activity, insecticidal, anti-inflammatory activity, diarrhea and dysentery. Leaf juice is used to cure fresh wounds, to stop bleeding, as a hair

tonic. In India, *TridaxProcumbens* mainly used for wound healing, as anticoagulant, antifungal and insect repellent. In folk medicines leaf extract were known to treat infectious skin diseases. It is a well-known medicine for liver disorders or hepatoprotective nature besides gastritis and heart burn.<sup>[7]</sup>

**II. MATERIAL AND METHOD**

**List of Chemicals**

Sr. no	Name of chemical	Purchased at
1.	Ethanol	Pharmaceutical laboratory of college
2.	Propylene glycol	Pharmaceutical laboratory of college
3.	Sodium benzoate	Pharmaceutical laboratory of college
4.	Potassium sorbate	Pharmaceutical laboratory of college
5.	Sorbitol	Pharmaceutical laboratory of college
6.	Glycerin	Pharmaceutical laboratory of college
7.	HPMC	Pharmaceutical laboratory of college
8.	SUDAN RED-3	Pharmacognosy laboratory of college

**Table 3. Chemicals required in Research**

**Collection of fresh *TridaxProcumbens* aerial part:**

*TridaxProcumbens* can be found on highways, wastelands, railroads, dykes, river banks, meadows, and dunes. So, we found and identified the aerial part of *TridaxProcumbens* Plant from the college campus of Smt. S. S. Patil College of

Pharmacy, Chopda on 6<sup>th</sup> Jan. 2023 in earlier morning.

The collected *TridaxProcumbens* plant was kept for shade drying in a warehouse for 4 weeks. After the complete drying of plant, the material was grinding with a grinder. Then obtain powder was

passed in a sieve shaker to get uniform size of particles of powder.

#### Pharmacognostical Evaluation of Raw Material: Macroscopic Examination:

As per WHO (1998), the organoleptic and macroscopic properties were estimated based on the colour, size, shape, characteristics of surface, fracture, odour, taste and texture.

**Appearance-** *TridaxProcumbensis* a perennial herb that has a creeping stem which can reach from to 8-30 inches (20-75cm) long.

**Flowers-** *TridaxProcumbensis* flowers have white rays and yellow disc-shaped flowers. It is about 1-1.5 cm in diameter and has a 10-30 cm long stem. Flowering occurs in spring. The plant flowers are looking like daisy. The flower is tubular, yellow centered white or yellow flowers with three to the dry florets. In florescence is capitulum. There are two types of flowers: Ray-like and disk-like florets with tentacles at base. Sometimes the flowers are three-lobed with long drooping heads. Echinoderms black, narrow obconical, 2.0-2.5 mm long, with pinnate papules. Flowering and fruiting all year round.

**Fruits** - The fruits are dark brown to black, oval and 2 mm long pimples, each with 3 to 6 mm long papules. The fruits are hard bruises covered with stiff hairs and have a feathery appearance. At one end there is a white papus-like feather. This plant is invasive in part because it produces so many pimples that each pimples can be caught in the wind in a papule and carried some distance.

**Seeds-** *TridaxProcumbensis* seed germinate at higher temperature (35/25 and 30/20) in the presence of 58 to 78% light. It is highly sensitive to salinity and water stress. Gametes have 36 (diploid) and 18 (haploid) chromosomes. Production is by vapor diffusion and seed production.

**Leaves** - The leaves are irregular to mottled and generally arrowhead shaped. They are simple, ovate, opposite, stipular, lanceolate, 3-7 cm in size. The basal leaves are wedge-shaped, short-stalked and hairy on both sides.

**Stem and Root** - The stem is cylindrical, dense and covered with mm multicellular hairs. Root tuberculosis is a powerful taproot system. The plant stems are 30-50 cm tall, branched, sparsely hairy, and node-rooted.<sup>[6]</sup>

#### Microscopic Examination:

##### Staining Process:

1. Take a clean watch glass and the staining solution Sudan red -3 to it.
2. Transfer the section taken from water to stain solution and keep for 2 to 5 min.
3. Pick up the section after 2-3 min. and transfer into the watch glass containing plain water, so that excess stain is washed away. This section is ready for mounting on a slide.

##### Mounting Process:

1. Take a clean glass on micro slide.
2. On the slide, transfer the section to be mounted, with the help of brush.
3. Add one or two drops of water on the section with the dropper. See that the section is submerged in water.
4. Take a clean cover slip with the help of forceps and needles. Place the coverslip on the section gently.
5. If the any air bubble are seen, slightly lift the coverslip and add a drop of water and replace the coverslip till the air bubble is removed.
6. With the help of blotting paper, wipe off excess water present outside the coverslip. The slide is ready for observation.<sup>[8]</sup>

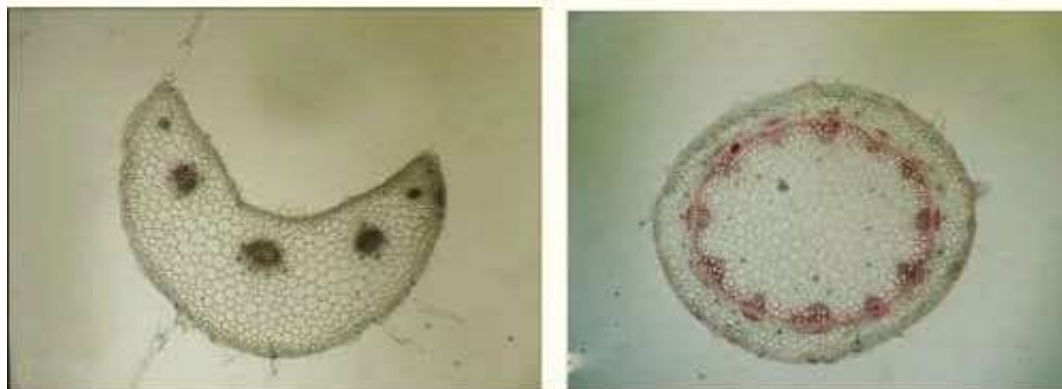


Fig. 4: T. S. of *TridaxProcumbensis*

**Phytochemical Evaluation:**

**1. Foreign Matter:**

**Determination of Foreign Matter**

50 gm of drug of drug sample was taken. It was spread on white paper in a thin layer. The foreign matter was detected with the help of unaided eyes and also by using lens and then foreign matter was separated. Again, the powder is collected and weigh and percentage of foreign matter was calculated.<sup>[8]</sup>

**2. Loss on Drying:**

**Determination of Loss on Drying**

10 gm accurately weighed sample was taken in a dry dish heating at 110°C for 2 hours in oven. Then dish was removed from the oven and cooled and weight was taken. Again, kept in oven for half an hour and weight was noted after cooling. And this procedure repeated till two consecutive weights were equal.<sup>[8]</sup>

$$\text{Percentage of moisture} = \frac{B - C}{B - A} \times 100$$

Here, A is wt. of dry evaporated dish.  
 B is wt. of dry dish + sample.  
 C is constant wt. after heating

**Calculation:** Wt of dry evaporating dish = 25.87gm  
 Wt of dry dish + sample = 25.87 + 10 = 35.87gm  
 Constant wt after heating = 35.11gm

$$\begin{aligned} \text{Percentage of moisture} &= \frac{B - C}{B - A} \times 100 \\ &= \frac{35.87 - 35.11}{35.87 - 25.87} \times 100 \\ &= 7.6\% \end{aligned}$$

The TridaxProcumbens powder contain 7.6% of moisture.

**3. Ash value determination:**

**Determination of Ash Value**

**Procedure:**

- i. Weight and ignite flat, thin, porcelain dish or a tared silica crucible.
- ii. Weight about 2 g of the powdered drug into the dish/crucible
- iii. Support the dish on a pipe-clay triangle placed on a ring of retort stand
- iv. Heat with a burner, using a flame about 2 cm high and supporting the dish about 7 cm above the flame, heat till vapours almost cease to evolved; then lower the dish and heat more strongly until all the carbon is burnt off.
- v. Cool in a desiccator.
- vi. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of the crude drug.<sup>[8]</sup>

**Calculations:**

- i. Weight of empty dish = x = 25.77 g
  - ii. Weight of drug taken = y = 2 g
  - iii. Weight of dish + ash (after complete incineration) = z = 26.02
  - iv. Weight of ash = (z-x) g  
 = 26.02 - 25.77  
 = 0.25
- 2 g of crude drug gives 0.25 g of the ash.

Therefore 100 g of crude drug gives 100/y x (z-x) g of ash

Total ash value of the TridaxProcumbens = 100/2 x 0.25  
 = 12.5 %  
 Total ash value of TridaxProcumbens is 12.5 %.

**4. Determination of Alcohol Soluble Extractive Value**

**Procedure:**

- i. Weight about 4 g of the coarsely powdered drug in a weighing bottle and transfer it to a dry 250 ml conical flask.
- ii. Fill a 100 ml graduated flask to the delivery mark with the solvent (90% ethanol). Wash out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask.
- iii. Cork the flask and set aside for 24 hours, shaking frequently. (Maceration)
- iv. Filter into a 50 ml cylinder. When sufficient filtrate has collected, transfer 25 ml of the filtrate to a weighed, thin porcelain dish, as used for the ash value determinations.

- v. Evaporate to dryness on a water-bath and complete the drying in an oven at 150 ° C for 6 hrs.
- vi. Cool in a desiccator for 30 minutes and weigh immediately
- vii. Calculate the percentage w/w of extractive with reference to the air-dried drug.<sup>[8]</sup>

**Calculation:**

25 ml of alcoholic extract gives = x g of residue  
 100 ml of alcoholic extract gives = 4x g of residue  
 Since 4 g of air-dried drug gives 4x g of alcohol soluble residue.  
 Therefore 100 g of air-dried drug give 80x g of alcohol soluble residue  
 Ethanol soluble extractive value of the sample = 80x %

**Formula:**

$$\text{Percentage of ethanol soluble extractive value} = \frac{\text{dried filtrate (gm)}}{\text{Extract (ml)}} \times 100$$

$$= \frac{25}{40} \times 100$$

$$= 62.5 \%$$

**Extraction of Tridax Procumbens**

**Procedure:**

1. Normally a solid material containing some of the desired compound is placed inside a thimble made

from thick filter paper. Which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser.

2. The solvent is heated to reflux. The solvent vapour travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools and drips back down into the chamber housing the solid material.

3. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

4. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound its concentration in the distillation flask. The advantage of the system is that instead of many portions of warm solvent being passed through the samples just one batch of solvent is recycled.

5. After extraction the solvent is removed typically by means of a rotary evaporator yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.<sup>[8]</sup>



**Fig. 5: Extraction Assembly for T. Procumbens**



**Standardization of extract:**

**Phytochemical Evaluation of prepared extracts**

Examination of phytochemical properties such as pH, heavy metals and microbial contamination was done for the prepared extracts of three crude drug samples.

**1. pH**

The pH of all the extracted solvents were estimated using digital pH meter that had been calibrated and stabilized with buffer tablets. Amount of all aerial part extracts of ethanol were blended in distilled water of 10 ml and stirred for 10 min using a magnetic stirrer.<sup>[9]</sup>

**2. Sample digestion**

Each powdered plant sample of about 1 gm was taken in 250 ml beaker previously contain 80 ml water. Boiled the mixture on hot plate till the volume concentrated to less than 1/4th of total volume (5-10 ml). Placed the mixture in 100 ml volumetric flask on cooling. Rinsed the beaker 3-4 times with distilled water. Transferred the rinse in the 100 ml flask and made the volume using distilled water to 100 ml. The solution of stated amount was pipette out from the above prepared solution and diluted with distilled water to 100 ml. The sample was used for the analysis of copper, zinc, iron and lead.<sup>[9]</sup>

**3. Heavy metal test**

**Copper** – Weighed 286 mg of cupric acetate accurately; added to 1 ml concentrated hydrochloric acid and warmed on water bath. The analytical sample was prepared of concentration 2.86 mg/ml in distilled water. The reading was taken by using copper lamp at 324.8 nm.

**Iron** – Weighed 864 mg of ferric ammonium sulphate accurately and added to 1 ml concentrated hydrochloric acid, warmed on water bath. The sample for analysis was of concentration of 8.64 mg/ml. The reading was taken by using Fe-lamp at 248.3 nm.

**Lead** – The sample of 1mg/ml solution was used to test for presence of lead in nitric acid solution. The reading was taken by using one AMP lamp at 405.8 nm.

**Zinc**–For testing of presence of zinc in sample, the concentration of sample used was 1mg/ml in hydrochloric acid in water. The reading was taken by using copper lamp at 213.9 nm.<sup>[9]</sup>

**4. Microbial contamination**

Standard methods were used to determine Microbial contamination in herbal drugs.<sup>[10-11]</sup>

**Total viable count**

Each powdered sample (10 gm) was weighed accurately and dissolved in 100 ml of sterile nutrient broth, kept in incubator at 37°C for 24 hrs separately. After 24 hrs incubation if culture shows growth of organism, then serial dilution was done. 1 ml sample (plant material + nutrient broth) was added into 9 ml sterile saline. Similarly, dilutions were carried up to 1010 and 1 ml of 106 dilutions were pipetted into each of two sterile nutrient agars. Similarly, 107, 108, 109 and 110 dilutions were pipetted into duplicate plates. Samples were spread on nutrient agar by use of spreader in sterile condition. Plates were further incubated at 37°C for 5 days. Positive and negative control was run. The numbers of colonies were counted and the average for 3 plates was expressed in terms of microorganism per g of plant sample (colony forming units - cfu per gm of plant sample).

**Total Yeast and Mould count**

Each powdered sample (10 gm) was weighed accurately and dissolved in 100 ml of sterile nutrient broth kept in incubator at 37°C for 24 hrs separately. After 24 hrs incubation if culture shows growth of organism, then serial dilution was done. The numbers of colonies were counted the average for 3 plates were expressed in terms of microorganism per g of plant sample colony forming units - cfu per gm of plant sample).

**Preparation of Liver Tonic:**

**Formula:**

Material	Category	Quantity
Ethanolic extract of TridaxProcumbens	API	25
Propylene glycol	Solubilizer	30

Sodium benzoate	Preservative	3
Potassium sorbate	Preservative	2
Sorbitol	Stabilizer and Sweetener	10
Glycerin	Thickener / Diluent and sweetener	15
Water	Diluent	Q.S.

**Table 4. Formulation**

**Procedure of Preparation of Liver Tonic:**

1. Weight and measure all the ingredients accurately.
2. In a clean and dry beaker add 25 g of TridaxProcumbensextract and 30ml of propylene glycol.
3. Heat the beaker over a hot plate at 50 ° to 60 °C while stirring continuously.
4. Once the TridaxProcumbens extract has dissolved in the propylene glycol, add 3gm of sodium benzoate and 2g of potassium sorbate as preservative.
5. Continue stirring the mixture until the sodium benzoate and potassium sorbate have dissolved completely.
6. In a separate beaker mix 15 ml of glycerin and 10 ml sorbitol until dissolved.
7. Add the sorbitol and glycerin mixture to the TridaxProcumbens extract and propylene glycol mixture while stirring continuously.
8. Keep stirring until, all the ingredients are completely dissolved and the mixture has a smooth consistency
9. Make up the volume with water.
10. The tonic is ready. Let it cool down to room temperature and transfer it to clean sterilize bottle.

**Evaluation parameter of liver tonic:**

• **pH of formulation:**

1ml of the oil was weighed in a test tube. 9 ml of water was added. pH of the mixture was determined with the help of a pH meter.

• **Homogeneity**

The formulations were tested for the homogeneity by visual appearance and by touch.

• **Appearance**

The appearance of the formulation was judged by its colour, odour and consistency.<sup>[12]</sup>

• **Procedure to determine density**

- 1) Clean thoroughly the specific gravity bottle with chromic acid or nitric acid.
- 2) Rinse the bottle at least two to three times with distilled water.
- 3) If required, rinse the bottle with an organic solvent like acetone and dry.
- 4) Take the weight of empty dry bottle with capillary tube stopper (w1).
- 5) Fill the bottle with unknown liquid and place the stopper, wipe out excess liquid from outside the tube using tissue paper.
- 6) Weight bottle with unknown liquid on analytical balance (w2).
- 7) Calculate weight in grams of unknown liquid (w3).

Formula for density:

Density of liquid under test (tonic) = weight of liquid under test /volume of liquid under test = w3/v<sup>[12]</sup>

• **Procedure to determine Specific gravity**

- 1) Clean thoroughly the specific gravity bottle with chromic or nitric acid.
- 2) Rinse the bottle at least two to three times with purified water.

- 3) If required, rinse the bottle with an organic solvent like acetone and dry.
- 4) Take weight of empty dry bottle with capillary tube stopper.
- 5) Fill the bottle with distilled water and place stopper; wipe out excess liquid from side tube using tissue paper (w2).
- 6) Weight bottle with stopper and water on analytical balance (w2).
- 7) Repeat the procedure for liquid under test by replacing the water after emptying and drying as mentioned in step 4 to 6.
- 8) Weight bottle with stopper and liquid under test on analytical balance (w3). Formula for specific gravity: Specific gravity of liquid under test (tonic) = weight of liquid under test /weight of water = w5/w4. [12]

• **Procedure to determine Viscosity**

Viscosity was measured with Brookfield digital viscometer at 100 rpm.

- 1) Thoroughly clean the Ostwald viscometer with warm chromic acid and if necessary, used an organic solvent such as acetone.
- 2) Mount viscometer in vertical position on a suitable stand.
- 3) Fill water in dry viscometer up to mark G.
- 4) Count time required, in second for water to flow from mark A to mark B.
- 5) Repeat step 3 at least 3 times to obtained accurate reading.
- 6) Rinse viscometer with test liquid and then fill it up to mark A, find out the time required for liquid to flow to mark B.

- 7) Determination of densities of liquid as mentioned in density determination experiment. [19]  
 Formula for viscosity

$$\text{Viscosity} = \frac{\text{Density of test solution} \times \text{Time required to flow test liquid}}{\text{Density of Water} \times \text{Time required to flow water}^{[24]}}$$

• **Stability Study for Hepatoprotective Tonic:**

Stability testing of the prepared poly herbal formulation was performed on keeping the samples at accelerated temperature conditions. Three portions of the final formulation (A, B and C) were taken in amber coloured glass bottles and were kept at accelerated temperature at 4 °C Room temperature and 47° respectively. The samples were tested for all the physicochemical parameters, turbidity and homogeneity at the interval of 24hr., 48hr. and 72hr. to observe any change. [13]

**III. RESULT AND DISCUSSION**

**Result:**

**Phytochemical evaluation of raw material of TridaxProcumbensaerial part:**

The dried leaves of TridaxProcumbens were evaluated for the different parameters. The 0.11% of foreign matter was detected in the plant matter. Total ash was found to be 12.5 % respectively. Which were in the limits when compared with standard values. The extractive values including extractives soluble in alcohol were 6.25 respectively. Loss of drying is also found to be is 7.6.

Sr. No.	Parameter	Standard value %	Actual value %
1.	Foreign matter	NMT 2	0.11
2.	Total ash	NMT 16	12.5
3.	LOD	Not less than 7	7.6
4.	Alcohol soluble extractive	Not less than 3	6.25

**Table 5. Phytochemical Evaluation Parameters**

**Standardization of Extract:**

The aerial part of TridaxProcumbens was to be extracted in solvent ethanol. All the extract were evaluated for the parameter such as pH, test for heavy metal, microbial contamination and sample digestion.

Sr.NO.	Parameter	Ethanol
1.	PH	6-7
2.	Test of heavy metal	
	Copper	Absent
	Iron	Absent

	Lead	Absent
	Zinc	Absent

**Table 6. Standardization of extract**

**Evaluation of Prepared Formulation:**

TridaxProcumbens tonic was prepared by the combing ethanolic extract of TridaxProcumbensaerial part with other excipients.

The prepared formulations were studied for various parameters including Organoleptic Characters, pH, Viscosity, Density and Specific Gravity given in table.

Sr. No.	Parameter	Formulation	
		F1	F2
1	Organoleptic Characters		
	1. Colour	Yellowish Green	Yellowish Green
	2. Taste	Sweet	Sweet
	3. Odour	Characteristic	Characteristic
	4. Appearance	Clear	Clear
2	pH Determination		
	a) pH paper	Neutral	Neutral
	b) pH meter	5.71	6.63
3	Density	1.06gm	1.06gm
4	Specific Gravity	0.5489	0.5262
5	Viscosity	3.70cp.	3.66cp

**Table 7. Evaluation Parameters of Formulation**

• **Stability study of hepatoprotective Tonic:**

Sample No.	Time (hour)	Temp. (°C)	Physicochemical Parameters					Turbidity/Homogeneity
			Colour	Odour	Taste	pH	Specific Gravity (g/ml)	
A1	24 hr	4 (°C)	NC	NC	NC	6.63	0.5262	No turbidity
B2		R. T.	NC	NC	NC	6.63	0.5262	-
C3		47(°C)	NC	NC	NC	6.63	0.5262	Homogeneity
A1	48 hr	4 (°C)	NC	NC	NC	6.63	0.5262	No turbidity
B2		R. T.	NC	NC	NC	6.63	0.5262	-
C3		47(°C)	NC	NC	NC	6.63	0.5262	Homogeneity
A1	72 hr	4 (°C)	NC	NC	NC	6.63	0.5262	No turbidity
B2		R. T.	NC	NC	NC	6.63	0.5262	-
C3		47(°C)	NC	NC	NC	6.63	0.5262	Homogeneity

**Table 8. Stability Study Data**

The formulation with Hepatoprotective activity was subjected for stability testing to assess the effect of storage at different temperature and humidity. The formulation was found to be stable when subjected to accelerated stability studies at variable temperature and humidity condition. There was no significant change in the physicochemical and organoleptic properties. In the present study, stability studies were carried out during 24hr, 48hr and 72hr of storage formulation was evaluated for physicochemical parameters pharmacological

activity to rule out the significant changes. After 72hr, stored formulation was subjected for physicochemical evaluation along with hepatoprotective activity studies following results were obtained as shown in Table 7

**IV. DISCUSSION:**

In modern times, herbal products are a symbol of safety, in contrast to synthetic drugs that are considered unsafe for humans and the environment. Herbs have been used for centuries for

their medicinal and aromatic properties has been evaluated. It's time to promote them globally. The prepared herbal tonic is having hepatoprotective activity. The presence of 2-O-β-D-glucopyranoside will readily repair the hepatocytes resulting in diagnosis of liver cirrhosis. The tonic is safe for administration and having no side effects. It also used in treatment of fatty liver caused due to overconsumption of alcohol. It helps to promote the normal function of liver.

## V. SUMMARY AND CONCLUSION

### Summary:

Recently in various reports, we saw the public have developing interest of herbal medicines to cure different disorders globally because its accuracy and less side effects. These herbal therapies were employed as treatment, primarily to cure liver cirrhosis disease because of inadequate medication in newly developed medicine useful for cure to liver damage.

The plant has hepatoprotective component helps to reduce of liver damage. The reason for hepatoprotective efficacy of herbal formulation might be useful for cure disease. These herbal medication were usually utilized in folk medicine, the evidence for their effectiveness as well as more safety. In current work, the phytochemical constitution of *TridaxProcumbens* aerial part extracted in ethanolic solvent was studied and show its anti-hepatoprotective activity. The liver cirrhosis is a major lifestyle disorder of the present era because of high amount alcohol consumption, allopathic drugs and there is a wider need and scope for the research of better anti-hepatoprotective tonic.

In the present study, aerial part of *TridaxProcumbens* were evaluated for pharmacognostical, physiochemical characters and documented. The primarily phytochemical study and Quantitative assessment of phytochemical in their plant were performed and documented. The phytochemicals like ash value and extractive value were estimated and documented.

The present study of evaluated plant (extract of aerial part) result in cure of liver cirrhosis and alcohol liver disease. Because conventional medications are typically taken orally, the same course of administration was followed for assessment of the anti-hepatoprotective activity. Liv-pure herbal tonic was used as a treatment for liver cirrhosis and ALD -Alcohol liver disease.

The ethanolic extract of *TridaxProcumbens* aerial part was further used to

formulate the liver tonic showed to have good anti-hepatoprotective property. The plant is localized and easily available. This is a good herbal remedy for liver disease.

### Conclusion:

According to the result of my current study, ethanolic extract showed hepatoprotective activity by inhibiting production of NADH and acetaldehyde. Flavonoids generally show the antioxidant activity, they work as hepatoprotective by reducing the NADH (fatty liver) and galactosamine (liver cirrhosis).

The ethanolic extract of aerial part was further study for hepatoprotective activity. The evaluation of extract shows the presence of 2-O-β-D-glucopyranoside which is help in cure of liver cirrhosis.

The ethanolic extract of aerial part was further developed in the tonic and evaluated for different parameters like appearance, viscosity, pH and homogeneity.

The overall study concluded that the prepared liver tonic was simple, cheap and administration of *Tridax* tonic can reduce or prevent the formation of liver cirrhosis.

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