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# Preclinical Studies of *Annona Muricata* Leaf Extract by BSLT Method

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**ABSTARCT:** The existing literatures show that the *Annona muricata* extract can be consumed in the form of decoction to treat wound disorders. But the limitation of this excellent therapy is that the dose is not accurate. Hence an attempt has been made to prepare Ethanol extract preparation containing *Annona muricata* leaves extract as per literature formulation were prepared and to improve the therapeutic efficiency and patient compliance few toxicity study were carried out. However, no scientific record is available for the wound healing, and also for toxological studies of leaves of the plant *Annona muricata*. Hence the present study was designed to evaluate these activities of Ethanolic extracts of *Annona muricata* using BSLT method. The wound healing activity of ethanolic extract of leaves part of *Annona muricata* was investigated by Brine shrimp lethality for toxicity and experiment was carried out. From the study it can be concluded the plant *Annona muricata* could be considered as a treatment for the wound healing activity. The activities may probably due to the presence of alkaloids, terpenoids, flavonoids, carbohydrates and anthraquinones. The present study supports the traditionally used medicinal plant *Annona muricata* can be currently used in gel form for treatment of wound.

**KEYWORDS:** *Annona muricata*; BSLT study.

## I. INTRODUCTION

Wounds are generally classified as, wounds without tissue loss and wounds with tissue loss, such as burn wounds, wounds caused as a result of trauma, abrasions or as secondary events in chronic ailments e.g.: venous stasis, diabetic ulcers or pressure sores and iatrogenic wounds such as skin graft donor sites and derma abrasions. Wound healing involves complex series of interactions between different cell types, cytokine mediators and the extra cellular matrix. The phases of normal wound healing include haemostasis, inflammation, proliferation, and remodeling. *Annona muricata* is a rhizomatous plant from the genus Oxalis, common in most of Europe and parts of Asia. The binomial name is *Annona muricata*, because of sour taste. The plant has reported for antioxidant, diuretic, antiscorbutic, refrigerant action and on decoction. It gives relief from fever, haemorrhage, urinary disorders, the juice of leaves is used as gargle and is remedy for ulcers in the mouth, nutritional disorder. The previous studies reported the antioxidants influences wound healing activity. Hence the present study is undertaken to evaluate the wound healing activity of *Annona muricata*.

*Annona muricata* is an amazing wild edible that has leaves and flowers that are best eaten raw. Commonly called sour grass, and often confused with “yellow clover”, Wood Sorrel is a sour treat that will quench your thirst, while you enjoy it as a trail side nibble.

The familiar acid of the Wood Sorrels is refreshing in warm weather and the leaves have long been popular with trampers and mountain climbers for their mildly tonic and refreshing properties. In small quantities the foliage is a wholesome addition to a salad, but, on account of the abundance of oxalic acid contained in the plant, it is unwise to eat the foliage in very large quantity.

## II. METHODOLOGY

### Plant Survey & collection & Authenticating Plant:

The leaves of *Annona muricata* was collected from the local region, Karnataka, Talkadu region and authenticated by Taxonomist. The herbarium of the plant was been kept in our institution. The leaves were dried in the shade for 7 days at room temperature (28°C).

#### Extraction of Plant materials:

The crisp plant materials were washed with running faucet water and shade dried. The examples were squashed to coarse powder by processor. These coarse powders (25g) were then exposed to defatted with oil ether and after that separated with ethanol till weariness by utilizing Soxhlet apparatus. The gathered concentrates were saved at that point taken up for further examinations. The DMSO (Dimethylsulfoxide) is go about as broken up solvents for these concentrates.

#### Preliminary Qualitative Biochemical Tests for detection of chemical constituent:

Preliminary phytochemicals analysis was carried out for ethanol extract as per standard methods.

#### Detection of Alkaloids:

The powder of ethanol extract was dissolved in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

##### *Mayer's test:*

Filtrate was treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

##### *a) Wagner's test:*

Filtrate was treated with Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

#### Detection of Flavonoids

*Lead acetate test:* Ethanol extract powder was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

*a) Sulphuric acid test:* Extract was treated with few drops of sulphuric acid. Formation of orange color indicates the presence of flavonoids.

#### Detection of Steroids

2ml of acetic anhydride was added to 0.5g of the powder ethanol extract, with 2ml of  $H_2SO_4$ . The color changed from violet to blue or green in sample indicates the presence of steroids.

#### Detection of Terpenoids

##### *Salkowski's test*

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown coloration of the inner face indicates the presence of terpenoids.

#### Detection of Anthraquinones

##### *Borntrager's test*

About 0.2g of the powdered extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of  $CHCl_3$  was added to the filtrate. Few drops of 10%  $NH_4$  were added to the mixture and heated. Formation of pink colour indicates the presence of anthraquinones.

##### Detection of Phenols

*a) Ferric chloride test:* Extract was treated with few drops of ferric chloride solution.

Formation of bluish black color indicates the presence of phenol.

*b) Lead acetate test:* Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

#### Detection of Saponins:

About 0.2g of the ethanol powder extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy mass of small bubbles) shows the presence of saponins.

#### Detection of Tannins:

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

#### Detection of Carbohydrates:

Extract was dissolved in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

**Detection of Oils and Resins:**

Test solution was applied on filter paper. It develops transparent appearance on the filter paper. It indicates the presence of oils and resins

**To evaluate the cytotoxic studies by leaf extract****Brine Shrimp Lethality Assay (BSLA)**

(Artemiasalina) eggs were hatched in artificial sea water prepared from commercial sea salt 38g/L. A lamp was placed above the open side of the tank to attract the hatched shrimps close to the tank wall. After 24 hours, the shrimps matured as nauplii (Artemiasalina) and were ready for the assay. The brine shrimp lethality bioassay was carried out on the ethanol extract using the standard. Twenty milligrams of the extract was dissolved in 1 ml of Propylene glycol/Tween 80/water (4:1:4) to give a crude extract concentration of 20 mg/mL. A two-fold serial dilution was carried out with salt water to obtain a test solution in the range of 0.1-10 mg/mL. Each concentration was tested in triplicate. A test tube containing propylene glycol/Tween 80/water (4:1:4) in 5 ml of salt water was used as the negative control. Ten milligrams of potassium dichromate (as positive control) was dissolved in propylene glycol/Tween 80/water (4:1:4) and serially diluted, to obtain test concentrations ranging from 0.01 to 5 mg/mL. A suspension of larvae (0.1 ml), containing about 10-15 larvae, was added into each test tube and incubated for 24 hours. The test tubes were then examined and the number of dead larvae in each bottle. The total number of shrimps in each bottle was counted and recorded. The death percentage and lethal concentration ( $LC_{50}$ ) were determined using statistical analysis.

Equation:

Percentage of Death (%):  $(\text{Total naupii} - \text{Alive naupii}) \times 100\% / \text{Total naupii}$

**III. RESULTS**

Plant extracts:

The percentage yield of the ethanolic leaf extract of *Annona muricata* was found to be 68.78 % obtained by soxhlet extraction process. Both powder as well as extract of *Annona muricata* were used in quantitative analysis and verified

Qualitative phytochemical analysis of *Annona muricata* leaves.

**Table 1:** Phytochemical characteristics of ethanolic leaves extract & powder of *Annona muricata* leaves.

Sl. No	Phytochemical Test	Ethanolic leaf extract of <i>Annona muricata</i>	Ethanolic leaf powder of <i>Annona muricata</i>
1	Carbohydrates	+	+
2	Alkaloids	-	-
3	Saponins	+	-
4	Phenolics	-	-
5	Flavonoids	-	-
6	Steroids	+	+
7	Tannins	+	+

**Brine Shrimp Lethality Test:**

**Table 2:** Effect of ethanolic leaves extract of *Annona muricata* on brine shrimp lethality assay.

Sl No	Time (hours)	Conc	No of Nauplii	Total no of Surviving nauplii	Total No of dead nauplii in (%)		
					<i>Annona muricata</i> Leaf extract	<i>Annona muricata</i> Leaf powder	Mortality %
1	2	0.1%	25	18	07	07	72%
2	4	0.2%	25	15	10	10	60%
3	6	0.4%	25	10	15	15	40%
4	8	0.8%	25	06	09	09	36%



As shown in the Table 2, Ethanolic leaves extract of *Annona muricata* was subjected to Brine Shrimp lethality bioassay for possible cytotoxic action was carried out against at different concentrations of 1 µg, 10 µg, 100 µg and 1000 µg. The suspensions of larva were examined after 08 hours of the incubation period. At 100 µg/ml concentration of leaves extract of *Annona muricata* showed 50 percent of mortality rate and 1000 µg/ml concentration of leaves extract of *Annona muricata* showed 100 percent of mortality rate. In this study, ethanolic leaves extract of *Annona muricata* was found to be toxic to Brine Shrimp nauplii, with LC<sub>50</sub> value of 100 µg/ml.

#### IV. CONCLUSION

Ethanolic leaf extract of *Annona muricata* been effective shown active live Brine shrimp's for its toxicity test. In this study ethanolic leaf extract of *Annona muricata* was found to be toxic to Brine shrimp nauplii with LC<sub>50</sub> value of 100 µg/ml. This study will be carry forwarded for further analytical studies.

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