STANDARDISATION AND QUALITY EVALUATION OF EUPHORBIN NIVULIA BUCH.HAM.

G.V. R. JOSEPH1 AND VINOD DRANGARI2

1. Regional Research institute (Ayu), JNAMPG&H. Near Gandhi Bhavan, Kothrud, Pune 411029.
2. Poona College of Pharmacy, Bharati Vidyapeeth, Erandvance, Pune 411038.

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ABSTRACT: Present paper deals with the macroscopic, microscopic, preliminary phytochemical and anti-inflammatory activity studies of the leaves of E. nivulia Buch. Ham. Leaves are linear oblanceolate or spathulate in shape with narrowly by cunative base. A pair of spins inserted on flat brown or black corky areas of the stem. In the mid rib non-articulated laticifers are distributed around the vascular bundle. Entire mesophyll consists of thin walled irregular shaped parenchyma cells. At the leaf margin it is replaced with lamellar type collenchyma tissue. Ethanol (50%) extract of the leaf when it was tested for it’s anti-inflammatory activity; to the extent of 39.6%.

Key words – E nivulig Pharmacrgnosy Anti intumation

INTRODUCTION

Euphorbia nivulia Buch.Ham. (Euphorbiaceae), commonly known as “Snuhi” in Indian system of medicine, is a xerophyte grown as a hedge plant. The leaf juice is used internally as a purgative, diuretic and is used acute asthma. It is mixed with neem oil and applied externally in rheumatism. Warm juice is considered to be a good remedy for earache. In Ayurveda latex of E. nivulia (/E., nerifolia) is very well used in the treatment of fistula1-3. Rao and his co-workers4 reported cycloart-25en =3β-ol and cycloadenol from the latex. Satyanarayana and his co workers5 reported the occurrence of cycloaratanepoxy triterpenoid and designated it as a cyclonivuliaol.

Certain Tribes in India use the leaves of E. nivulia for curing the inflammation1. However there is no scientific data available on this aspect. Moreover in Ayurveda both E. nivulia and E. nerifolia are called‘Snuhi’ and there is confusion over the identity of the plant Hence the present investigation is under taken with a view to establish the proper morphological characters and to evaluate the anti-inflammatory activity of the leaves of E.nivulia.

MATERIALS AND METHODS

Collection of the plant material: Leaves of E. nivulia were collected from the vicinity of sardar patel University, V.V. Nagar, Gujarat.

Preparation of the drug.

Morphological study: Fresh leaves were fixed in F.A.A6 and few of them were processed for epidermal structural studies. Epidermal peelings were obtained by scraping of the outer surface of the lamina and the peelings were stained with Delafield’s Hematoxylin7. To study the anatomical details of the leaf customary methods were followed for dehydration, infiltration and embedding6. Sections were cut at 8 to 10 µ thicker and stained with Safranin and Fast green combination. Histochemical tests for carbohydrates, calcium oxalate crystals, tannins, lignins ect. Were carried out8.

Powdered form: Fresh plant material was made into small pieces and shade dried. The dried leaves were powdered through an electric grinder and sieved through a no.60 mesh.
Standard methods were followed for the fluorescence analysis and preliminary phytochemical screening.

Preparation of the extract: 100 g of dried and coarsely powdered leaves of E. nivulia was extracted with three volumes of 50% ethanol by maceration process. The filtered extract were mixed together, concentrated and dried under reduced pressure.

Anti-inflammatory activity: The anti-inflammatory activity of the leaf extract of E. nivulia was studied by using of carrageenan induced rat paw oedema model. The albino rats of the either sex (Wistar strain) weighing 80-120 g were divided into three groups as a control (Group I), standard (Group II) and test drug (Group III). Control group received only vehicle i.e 2% tween 80 solution. Each group consisted of six rats, which were dosed orally, one hour later a sub planter injection of 0.1 ml of 1% solution of carrageenan in distilled water was administered. The volume of the injected foot was measured using plethysmometer, after 24hrs. the formula inhibition was calculated by using the formula-

\[
\text{Percent inhibition} = \frac{1 - (Vd - Vp)}{Vc - Vp} \times 100
\]

Where, Vd-Vp was the difference in paw volume after carrageenan injection and initial paw volume for drug treated animals, while Vc-Vp was the difference in paw volume after carrageenan injection and initial paw volume for control group.

RESULTS AND DISCUSSION

Macroscopy: It is a shrub growing up to 3-9 m height. Stem is hard with straight with often-whorled branches with straight gernate stipulary spines. A pair of spins inserted on flat brown or black corky areas. Leaves are 5-15 cm long, sessile, fleshy. They are linear oblanceolate or spatulate in shape with narrowly by cunative base, only the mid vein is visible in the normal conditions. Secondary and tertiary veins are not visible due to their fleshy nature.

Microscopy: Midrib is semicircular in outline, Epidermis is single layered and they are tangentially elongated with thick cell walls. Cells of ground tissue are thin and spherical to irregular in shape. Towards periphery these cells are more or less spherical in nature, while towards centre they are irregular in shape. Single vascular bundle is located at the centre (Fig 1). Non-articulated laticifers are distributed around the vascular bundle.

Lamina is differentiated into mesophyll, upper and lower epidermis. On surface view both the epidermal layers appear polygonal in shape (fig 4) Lamina is amphistomatic and possessing paracytic type of stomata. Stomata are distributed all over the surface of the lamina but not on the midrib. Non-glandular uniseriate trichomes are seen on lower epidermis. Quantitative values of the lamina are cited in Table I. Stomatal frequency in more on lower epidermis than that of upper epidermis. Mesophyll tissue consists of thin walled parenchyma cells (Fig3) Palisade tissue is found to be absent in the lamina. Moreover the parenchyma tissue of the lamina is not much loosely arranged as seen in the spongy parenchyma of the other dicotyledons. At the margin, parenchyma is replaced by lamellar type of collenchyma tissue (Fig. 2.)

Powder analysis: Fragments of epidermal tissue (lamina) composed of thick walled polygonal cells. Stomata are infrequent. Fragments of collenchyma tissue (Fig. 5) and non-glandular.

Trichomes are found scattered and attached to fragments of epidermis (Fig 7). Vessels show pits on their secondary wall (Fig 9). The cell walls of the fairly abundant fibres are thickened and lignified or only moderately thickened and slightly lignified (Fig 9). Laticifers are long, non-articulated and branched (Fig.8).

For fluorescence analysis, the powdered material was treated with different alkali and acids and they showed different colours under daylight and U.V. light. Results are tabulated in table ii.
Preliminary phytochemical screening of the powdered material has shown the positive results for alkaloids, carbohydrates and flavonoids.

Results of the effect of leaf extract of E. nivulia in carrageenan induced rat paw edema model have been given in Table III. The results of this study indicated moderate anti-inflammatory activity to the extent of 39.6% for 100mg/kg dose of ethanol (50%) extract. Where as the standard prednisolone (5mg/kg) has depicted 54.7% activity.

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Table I
Quantitative Values

| Sr.no | Parameter                      | Observations (Per Square mm.) |
|-------|--------------------------------|-------------------------------|
| 1     | Stomatal index:                |                               |
|       | Upper epidermis                | 6                             |
| 2     | Lower epidermis                | 14                            |
|       | Stomatal frequency:            |                               |
|       | Upper epidermis                | 10                            |
| 3     | Lower epidermis                | 24                            |
|       | Palisade ratio                 | 0                             |
### Table II

Fluorescence Analysis

| Sr.No | Treatment                              | Ordinary light | UV light  |
|-------|----------------------------------------|----------------|-----------|
| 1     | Drug powder (D.P) as such              | Greenish brown | No change |
| 2     | D.P +aq 1N NaOH                        | Yellowish Green| Creamy    |
| 3     | D.P+alco 1N NaOH                       | Light green    | Brick red |
| 4     | D.P +1N HCl                            | Light creamy   | Dirty blue|
| 5     | D.P+50%H2SO4                           | Black          | No change |

### Table III

Anti-inflammatory activity study leaf extract

| Sr.No | Group (Dose) | Initial | Final | Mean | % Inhibition |
|-------|--------------|---------|-------|------|--------------|
| I     | Control (1% Carrageenan) | 0.74    | 1.15  | 0.41 |              |
|       |              | 0.65    | 0.98  | 0.33 |              |
|       |              | 0.90    | 1.48  | 0.58 |              |
|       |              | 0.76    | 1.15  | 0.39 |              |
|       |              | 0.66    | 0.98  | 0.32 |              |
|       |              | 0.86    | 1.29  | 0.43 |              |
| II    | Standard Prednisolone (5mg/kg)         | 0.70    | 0.90  | 0.20 |              |
|       |              | 0.85    | 1.04  | 0.19 |              |
|       |              | 0.80    | 0.98  | 0.18 | 54.7         |
|       |              | 0.96    | 1.14  | 0.18 |              |
|       |              | 0.74    | 0.92  | 0.18 |              |
|       |              | 0.68    | 0.87  | 0.19 |              |
| III   | Plant extract (100mg/kg)               | 0.55    | 0.75  | 0.20 |              |
|       |              | 0.75    | 1.02  | 0.27 |              |
|       |              | 0.69    | 0.90  | 0.21 | 39.6         |
|       |              | 0.60    | 0.87  | 0.27 |              |
|       |              | 0.76    | 1.05  | 0.26 |              |
|       |              | 0.64    | 0.89  | 0.25 |              |
Fig : 1. Schematic diagram of the leaf in T.S.
Fig : 2. Margin of the leaf lamina in T.S. 280 x
Fig: 3 Leaf Lamina in T.S. 72 x
Fig: 4 Lower epidermis in surface view showing paracytic type of stomata 550x

Fig: 5 Collenchyma tissue 500x

Fig: 6 Vessels 500x

Fig: 7 Non glandular trichomes 500x

Fig: 8 Lentleifer tubes 550x

Fig: 9 Fibres 500x