14. FUTURE SCOPE OF THE STUDY

- Filing a patent for the effective formulation
- Studying the mechanism of the wound healing at the molecular level.
LIST OF PUBLICATIONS


LIST OF PRESENTATIONS

National Conferences

1. Poster presentation at 2nd IPA-Student congress held at Acharya B. M. Reddy College of Pharmacy, Bangalore on July-9th to 11, 2009.

2. Presented a poster in the Silver Jubilee celebrations at Sri Ramachandra University on September 2010.

3. Presented an oral presentation in the 2nd National Conference on Recent Trends in Industrial Pharmacognosy, at Mother Theresa Post Graduate & Research Institute of Health Sciences, Pudhuchery on 16th March 2013 and won the FIRST PRIZE.

4. Presented an Oral Presentation in the National Seminar on Recent Trends in Nano biotechnology held during 19th to 20th September 2013 at Anna University.

International Conferences

1. Oral presentation in the International Conference on Ayurveda organised by Sai Ram Ayurveda Medical College & Research Centre on December 12th to 13th, 2011.

2. Presented a poster in the 12th International congress of Ethnopharmacology at Jadavpur University, Kolkata during Feb 17th to 19th, 2012.


Abstract:
Restoration of normal cell structures is favoured through the healing of wounds which is depending upon migration and proliferation. Collagen formation, angiogenesis and migration of cells is an intricate process of wound healing through fibroblasts. Currently there is less evidence for the wound healing at cellular level. Alcoholic extract of the aerial parts of Urena Lobata was used to study the fibroblast cell migration and proliferation using scratch wound assay procedure. The present study provides the scientific rationale of the ethanolic extract of plant in the management of wounds.

Keywords: Urena Lobata, Malvaceae, Scratch assay and fibroblast proliferation

Introduction
Wounds are dermal changes in which opening or breaking of the skin may be resulted due to accidental or intentional trauma. Anatomical stability may be restored through the proper healing of and the skin functional status. There may be a sequence of events for the repairing of the tissues such as inflammation, proliferation and migration of cell types [1]. Healing is also completed by knitting of collagen. The treatment and cure of various diseases in Indian traditional medicine is based on the herbal origin [2]. Management of wound by herbal medicine involve debriment, providing moist environment and disinfection [3]. Urena Lobata also known as Caesar weed is an erect, branched shrub of 0.6m to 2.5m high. It is very variable and more or less hairy. Its stem is often with reddish branches. The leaves are pale beneath, heart shaped at the base, toothed or somewhat lobed or angled, [4]. The flowers are pink or purplish, about 1.7mm in diameter and borne singly in the axil of the leaves, [5]. The fruits are rounded, but flattened and about 7mm in diameter with carpels covered with barbed spines, [6]. Traditionally the leaves are used as demulcent, vulnerary, against gonorrhoea, as a haemostat, contraceptive, for liver disease and applied as a poultice to ulcers. Flowers are expectorant. Bark juice is used against dysentery. Seeds are potent vermifuge. Stems furnish a textile fibre. [7] The present study evaluates the wound healing effect of the aerial parts of Urena Lobata by 3T3 fibroblast cell migration and proliferation by scratch assay which mimics in vivo migration cells.
**Urenalobata** is one of the useful medicinal plants that is used extensively in ethno medical practices It is an erect, branched shrub of 0.6m to 2.5m high. It is very variable and more or less hairy. Its stem is often with reddish branches. The leaves are pale beneath, heart shaped at the base, toothed or somewhat lobed or angled. [8]. The flowers are pink or purplish, about 1.7mm in diameter and borne singly in the axil of the leaves. [9]. The fruits are rounded, but flattened and about 7mm in diameter with carpels covered with barbed spines. [10]. Different extracts of the leaves were used in herbal medicine to cure diverse ailments. The medicinal potentials of this shrub as claimed by traditional medical practitioners range from the cure of rheumatism, gonorrhea, toothache and wounds to fungal infections (ringworm) expulsion of worms, intestine cleansing. In South Africa it is used for high blood pressure, fever, asthma, syphilis; [11, 12].

**MATERIALS AND EQUIPMENTS**

1. Ethanolic extract of *Urenalobata*
2. Human Keratinocyte Cell Line (HaCaT)
3. Mouse fibroblast 3T3 cells
4. Dulbecco’s Modified Eagle Medium, DMEM (Invitrogen #10313-021)
5. Fetal Bovine Serum (ATCC #30-2020)
6. PBS (Invitrogen #14190-144)
7. BD Falcon 24-well tissue culture plate (Fisher #08-772-1H; BD #353226)
8. Rainin pipet tips, 1 ml (Rainin #GPS-L1000)
9. Glutaraldehyde (Sigma-Aldrich #G6257)
10. Ethanol (Sigma-Aldrich #459836)
11. Crystal violet (Sigma-Aldrich C3886)

Cell culture incubator: 37 °C and 5% CO2.

**PLANT MATERIAL**

Aerial parts of the plant *Urenalobata* was collected from Thirunelveli district during the month of February 2010 and authenticated by the Dr.Chelladurai,Taxonomist.

**PREPARATION OF ETHANOLIC EXTRACT OF URENA LOBATA**

2.5 kg of shade dried powder of the plant was extracted using ethanol and concentrated in rotary evaporator at 50°-60°C under reduced pressure leaving a dark brown residue which was stored in air tight container at 4°C till further use.

**PROCEDURE**

**Wound healing Scratch assay**

For the in vitro scratch assay [13,14], HaCaT cells were seeded in six-well plates (Nunc, Wiesbaden, Germany) in a density of 5 10⁵ cells/well in growth medium. Cells were grown until they had reached a confluence of about 80%. Then a scratch was made through each well using a sterile 200-lL pipet tip. Cells were washed twice with PBS pH 7.4 and the medium was changed. As a positive control TGF-β1 (1 ng/ml) was added to the medium; the test ethanolic extract of *Urenalobata*, which was added in a concentration of 50μg/ml. Scratches were documented under the microscope (x150) immediately after the wounding procedure and once more when kept at 37 LC, 5% CO2 for 48 h. Pictures were taken exactly at the same position before and after the incubation to document the repair process. The experiments were repeated twice and representative pictures are shown.

**Cell proliferation assay**

Fibroblast 3T3 (103) cells were seeded in a 96-well culture plate in a humidified 5% CO2 atmosphere. Cells were serum starved for 24 hours and then incubated with 5, 10, 25 and 50μg/ml of test ethanolic extract of *Urenalobata* and further incubated for 24 hours. After 24 hours 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added and formazan product was dissolved in 100μl of DMSO. Absorbance at 570 nm was measured with microtiter plate reader and cell viability was determined by tryphan blue exclusion method.

**RESULTS**

**Cell migration assay**

Test extract treatment enhanced the cell migration of HaCaT cells when tested by scratch wound assay. After 12hr of treatment with 50μg of test extract, the migratory nature of the fibroblast could be seen by microscopic examination. Complete covering of the wound was observed within 24 hours of treatment, similar to that of EGF. This response was not recorded either in the cell culture plate maintained as untreated control or placebo treatment (Figure 1).

**Fibroblast proliferation assay**

An increased proliferation of fibroblast in response to treatment with test extract was observed. The rate of proliferation was directly proportional to the concentration. However, concentration above 50μg/ml did not greatly influence the proliferation rate. 30% increase in the proliferation of fibroblast in response to test extract at a concentration of 50μg/ml was demonstrated, whereas in the positive control, EGF showed 70% increase in cell proliferation. Cell proliferation was insignificant in untreated and placebo treated control (Table 1 and Figure 2).

**DISCUSSION**

The present study suggests that test extract of *Urenalobata*enhances the cell proliferation and migration of fibroblasts. Besides the keratinocytes the 3T3 fibroblasts play a role in healing process. Wound healing effect of the test extract was evidenced by enhanced cell proliferation and cell migration of fibroblast.
Fig1: Cell Migration Assay

a. Control- 12hours, b. Control- 24hours, c. Test Extract-12hours, d. Test Extract-24hours, e. EFG-12hours, f. EFG-24hours

Table 1

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Concentration (μg/ml)</th>
<th>% Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>4.</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>5.</td>
<td>EGF 3</td>
<td>69</td>
</tr>
</tbody>
</table>

Figure 2
CONCLUSION
Management of wounds can be possible by using the ethanolic extract of *Urena lobata* which promotes the cell proliferation and migration.

ACKNOWLEDGEMENTS
I profusely thank the Management of Sri Ramachandra University, for providing me the facilities to carry out the research. Also, my heartfelt thanks to Dr. Anuradha Dhanasekaran, Assistant professor, Center for Biotechnology, Anna University, Who helped me in carrying the In-vitro cell culture studies.

REFERENCES
Case Report

Pharmacognostical, phytochemical and Anti oxidant studies of the aerial parts of *Urena lobata* L.

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The present study is to determine the pharmacognostical phytochemical and antioxidant studies of *Urena lobata* Linn. In Pharmacognostical studies microscopical characteristics of leaf and stem, analytical parameters such as ash values extractive value, analysis of major elements and anti oxidant studies were carried out. The ethanolic and ethyl acetate extracts showed significant DPPH (71.35%, 69.74%) activity compared to chloroform extract (53.85%) at 200 mcg. A biphasic response with ethanolic and ethyl acetate extract (81.94%, 78.30) and a dose dependent antioxidant activity with chloroform extract (42.58%) at 1000 mcg by nitric oxide scavenging.

Keywords: *Urena lobata*, Malvaceae, pharmacognostic, phytochemical analysis and antioxidant studies.

INTRODUCTION

Plants are the only economic source of a number of well established and important drugs; in addition they are the sources of some chemical intermediates needed for the production of a number of drugs. The popularity of natural drugs all over the world in recent years is an indication of significant contribution of Pharmacognosy in modern medicine. The present work intends to study the Pharmacognostical, Phytochemical and antioxidant studies of *Urena lobata* Linn.

*Urena lobata* Linn. of Malvaceae family is of medicinal value. It is a shrub of 60-250cm or more height and basal diameter of 7cm (Pharmacognosy of Ayurvedic drug. 1962). This medicinal plant is useful in many diseases, in the form various extracts of leaves and roots. Traditionally the plant is used as diuretic, febrifuge and rheumatism. It is useful for wounds, toothache, gonorrhea and food for animals as well as humans (Mazumder et al., 2001). Aerial parts of *Urena lobata* is reported to contain Mangiferin and Quercetin and roots having imperatonin and furanocoumarin (Keshab 2004).

MATERIALS AND METHODS

Plant material

The plant material *Urena lobata* Linn (Malvaceae) were collected from the Thirunelveli district, Tamilnadu, during the month of February in the year 2010 and authenticated by Dr. V. Chelladurai, Taxonomist, Thirunelveli. A specimen voucher was deposited in the college herbarium for future reference. Fresh drug obtained were shade-dried and coarsely powdered and passed through sieve 100 mesh sizes and stored in air-tight containers for further use.

Pharmacognostical Studies

The histology of the aerial parts and roots of *Urena lobata* was carried as per the standard procedures (Khandelwal et al., 1995). Analytical parameters like ash values,
extractive values, were studied as per the Indian Pharmacopoeia (Indian Pharmacopoeia, 1996).

The total ash were dissolved in the mixture of 5ml of HNO₃ and 5ml of HCl and made up to 100 ml using HPLC Grade water using certified standard supplied by Merck and Thermo, filtered and analyzed with ICP-AES system.

**Standards used**

Certified standards supplied by Merck and Thermo.

**Preparation of the extracts**

The entire plant material of *Urena lobata*, were collected, shade dried and powdered and extracted for 24, 48 and 72 hours continuously using solvents of increasing polarity namely Chloroform, Ethyl acetate and ethanol. All the extracts were filtered through Whatmann filter paper and evaporated on a water bath and finally dried under vacuum.

**Phytochemical screening**

Preliminary phytoconstituents analysis of the various extracts was carried out using standard procedures and specific reagents (Kokate and Purohit. 1999). Also the quantitative estimation of flavonoids and the tannins were estimated using the standard procedures (Rajpal. 2002; Rajpal. 2002).

**Determination of Lipid peroxidase activity**

The formation of malondialdehyde is the basis for the well known TBA method used for evaluating the extent of lipid per oxidation. At low pH and high temperature (100°) malondialdehyde binds with TBA to form a red complex that can be measured at 532 nm. TBA method was used to measure the carbonyl compound obtained by linoleic acid oxidation at later stage of lipid per oxidation. Absorbance of supernatant was measured at 532nm (Gutteridge 1995). The results were tabulated in table 5.

**DPPH Radical Scavenging Activity**

Radical scavenging activity was determined with a DPPH as a free radical by using various concentrations (50-1000 mcg/ml) of the extracts (viz Chloroform, Ethyl acetate and ethanol extracts). The decrease in absorbance was measured at 517 nm after 30 minutes of incubation at room temperature. Quercetin was used as standard. Antiradical activity is defined as the amount of inhibitor (Phenolic compound) necessary to decrease the initial DPPH radical concentration by 50 % (EC 50) (Hiruma 1999). The results were tabulated in table 6 and figure 1.

**Nitric Oxide (NO) Radical Scavenging Activity**

Sample of various concentration were used to determine their effect on the NO radical scavenging activity using sodium nitroprusside generating NO system compared

Figure 1. In vitro DPPH radical scavenging activity of U. lobata L

Table 7. Invitro Nitric oxide Scavenging Activity of U. lobata. L

<table>
<thead>
<tr>
<th>Concentration (mcg/ml)</th>
<th>Percentage Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol extract</td>
</tr>
<tr>
<td>50</td>
<td>21.45</td>
</tr>
<tr>
<td>100</td>
<td>45.23</td>
</tr>
<tr>
<td>200</td>
<td>72.69</td>
</tr>
<tr>
<td>400</td>
<td>78.25</td>
</tr>
<tr>
<td>800</td>
<td>80.56</td>
</tr>
<tr>
<td>1000</td>
<td>81.94</td>
</tr>
</tbody>
</table>

Significant p<0.001 level. Each value represents mean ± S.D. of 3 observations

Figure 2. Invitro Nitric oxide scavenging activity of U. lobata. L

RESULTS

The microscopical characters of leaf showed stellate trichomes with lignified epidermal cells of trichomes and rosette calcium oxalate crystals. The stem showed periderm, wide secondary phloem with dilated funnel shaped phloem rays, conical bands phloem and phloem fibers along with xylem elements.
Table 1. Estimation of the Percentage of Ash values

<table>
<thead>
<tr>
<th>Ash Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>8.31%w/w</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>2.48%w/w</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>2.10%w/w</td>
</tr>
</tbody>
</table>

Table 2. Extractive values

<table>
<thead>
<tr>
<th>Extractive Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water soluble extractive</td>
<td>10.88%w/w</td>
</tr>
<tr>
<td>Alcohol soluble extractive</td>
<td>7.2%w/w</td>
</tr>
</tbody>
</table>

Table 3. Analysis of major elements

<table>
<thead>
<tr>
<th>Elements</th>
<th>% ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>19.86</td>
</tr>
<tr>
<td>Potassium</td>
<td>14.37</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.41</td>
</tr>
<tr>
<td>Lead</td>
<td>25.4</td>
</tr>
</tbody>
</table>

Table 4. Qualitative Chemical Examination of various extracts.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Chloroform extract</th>
<th>Ethyl acetate extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannin and Phenolics</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

The leaf constants such as stomatal number as 63.33-73.33; stomatal index as 20.5 and the palisade ratio as 3, total ash, acid insoluble ash and water insoluble ash were found to be within the limits prescribed in Ayush guidelines and recorded in Table 1 and extractive values are tabulated in Table 2.

Major elements determined from the ash of *Urena lobata* (entire) are Ca, K, Na, Pb. The amount of elements present is noted in Table 3.

The preliminary Phytochemical work on *Urena lobata*, showed that it contains sterols, tannins in the ethanolic extract and flavonoids in Chloroform extract, phenols and tannins in ethyl acetate extract. The amount of flavonoids was found to be 1.15%w/w and tannins as 0.45%w/w. The qualitative chemical analysis results were recorded in Table 4.

Both the ethanolic and ethyl acetate extracts showed significant antioxidant activity in all invitro free radical scavenging models when compared to chloroform extract. Invitro DPPH radical scavenging activity, the percentage inhibition was 71.35%, 69.74% and 53.85% in ethanol, ethyl acetate and chloroform extracts at 200 mcg/ml when compared with curcumin at 62 mcg/ml showed only 84.7% inhibition [14]. The ethanol and ethyl acetate extracts showed a biphasic response where as the chloroform extract showed a dose dependent increase in the activity. In case of nitric oxide scavenging activity, the ethanol extract showed 81.94%, ethyl acetate extract showed 78.30 % and the chloroform extract showed 42.58 % inhibition at 1000mcg/ml compared with 88.02 % inhibition by Quercetin [14]. Both the extracts showed a dose dependent increase in activity. A dose dependent increase in total antioxidant activity was shown by ethanolic extract. Thus the antioxidant potential of the extract of *U.lobata* L may be due to the presence of polyphenolic compounds, which needs further analysis.

CONCLUSION

The present study was carried out to establish the identification of *Urena lobata*. Phytochemical screening showed the presence of sterols, tannins, flavonoids. The presence of flavonoid like compounds in *Urena lobata* can be used as antioxidant drug. The present work can be concluded that, this traditional herb may represent new source of antioxidant, immunomodulatory, wound healing as well as antimicrobial with stable biologically active components that can establish a scientific base for the use of plants in modern medicines.
ACKNOWLEDGEMENT

The authors thank the management of Sri Ramachandra University for providing necessary facilities and encouragement.

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Pharmacognosy of Ayurvedic drug (1962). Department of Pharmacognosy, University of Kerala. 5:108-112.