PHARMACOGNOSTIC AND PHYTOCHEMICAL ANALYSIS OF OXALIS LATIFOLIA KUNTH AND THEIR ANTIULCER ACTIVITY ON ALBINO RATS

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ABSTRACT
The objective of the present study was to evaluate the pharmacognostic, phytochemical analysis and antiulcer potential of methanol extracts of *Oxalis latifolia kunth* in different experimental induced ulcer models in rats. In the present study different extracts, methanol (200 mg/kg and 400 mg/kg) extract of leaves of the plant were examined in Pylorus ligation and Indomethacin induced gastric ulcer in rats. Various parameters like volume of gastric acid secretion, pH, total acidity, ulcer index and antioxidant parameters were determined and compared between extract treated, standard and vehicle control group animals following ulcer induction. Among different dose of alcoholic extract, high dose showed significant antiulcer activity in Pylorus ligation and Indomethacin induced ulceration. The result of present study concluded that the alcoholic extract of leaves of the plant of *Oxalis latifolia kunth* has antiulcer activity in Pylorus ligation and Indomethacin induced gastric ulcer model in rats. The extract containing flavonoidal component *latifolin A* is as luteolin-6″-(E-p-hydroxycinnamoyl) 4’-O-β-D-glucopyranoside is isolated, analysed by various spectral analysis and formulated as a capsule. This latifolin A capsule is main responsible for the anti ulcer activity.

KEYWORDS: Pylorus ligation, Indomethacin, Total acidity, *Latifolin A*, *Oxalis latifolia* etc.
INTRODUCTION

Peptic ulcer disease is a serious gastrointestinal disorder. The formation of peptic ulcers depends on the presence of acid and peptic activity in gastric juice plus a breakdown in mucosal defenses. There are two major factors that can disrupt the mucosal resistance to injury: non-steroidal anti-inflammatory drugs NSAID like e.g. aspirin and Helicobacter pylori (H. pylori) infection.\(^{[1]}\) Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer.\(^{[2]}\) But most of these drugs exhibit serious side effects like arrhythmias, gynaecomastia, impotence, arthralgia, hypergastrinemia and haemopoietic changes.\(^{[3]}\) Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors.\(^{[4]}\)

The plant *Oxalis latifolia kunth* belonging to Oxalidaceae family is a stem less herb of cosmopolitan distribution found abundantly in agricultural farms, gardens, lawns etc. Leaves dark green, slight and characteristic odour, sore and astringent taste. Leaves are digitately 3-foliate, leaflets, obcordate, chartaceous, pilose base, cuneate, margin entire, apex, emarginiate, Pseudoumbels, axillary, 1-6flowered, bracts two, linear, bracteole, Sepals, five lanceolate, petals oblanceolate apex, emarginated. The plant contains such as carbohydrates, saponins, phenol, flavanoid, Flavonol glycosides\(^{[5]}\) cardiac glycosides, phytosterol, fixed oils and fats, gums and mucilage in *Oxalis latifolia kunth*. The Oxalis species are reported to cure various disorders such as paralysis, stomach disorder, antiulcer, anti-inflammatory, antioxidant and it also acts as thirst reliever.\(^{[6]}\)

MATERIALS AND METHODS

Plant material

*Oxalis latifolia* kunth plant was collected from in and around deciduous forest of Talakona Forest in the state of Andhra Pradesh, India, during September 2019. Plant was authenticated by Dr. K. Madhava Chetty, Plant Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The voucher specimen (2019/1214) of the plant was deposited at the college for further reference.
Pharmacognostic study of *oxalis latifolia* kunth

**Macroscopical study**

The macroscopical description of plant include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. were studied. Macroscopic characters of the plant *Oxalis latifolia* kunth (Oxalidaceae) was studied directly in the field, and photographed under original environment.\(^7\)

**Microscopic study**\(^8\)

**Transverse section of crude drug (Leaf)**

Microscopical examination of the plant drugs is essential to study the adulterants also indispensable in identification. Microscopical evaluation of the plant drugs helps to identify the organized drugs by their known histological characters and used to confirm the structural details of the drugs from plant origin.

**Powder microscopy**

The shade dried leaves of *Oxalis latifolia* kunth had been powdered and the powders that passes through sieve number 60\# individually and then afflicted by powder analysis.

**Preparation of the extract**

The plant leaves were dried on filter paper sheets under shade at room temperature until changing of color of filter papers and milled into coarse powder. 200 g of powder material placed was extracted with 70% methanol in a Soxhlet apparatus for 8-12 h. Solvent were removed at temperature below 50°C in an oven. The residue (extract) of respective plant material was stored at 4°C for further experimental studies. Aqueous extract were prepared by taking 100g of the powdered plant material with 500 ml of distilled water in a Soxhlet apparatus for 8-12 h. The filtrate was then concentrated and the extract was stored at 4°C for further experimental studies.

**Preliminary phytochemical screening**

Methanolic extract of *Oxalis latifolia* kunth were subjected to preliminary phytochemical screening for the detection of various plants constituents like Tannins, carbohydrates, Saponins, Flavanoids, Glycosides, Proteins, Alkaloids and Phenols.\(^9\)
Isolation of active constituent

5 gm of Methanolic extract Oxalis latifolia kunth (Oxalidaceae) was subjected to silica-gel (100–200 mesh) column (length 100 cm and diameter 3 cm) chromatography and The elution started with hexane followed by hexane- Ethyl acetate (EtOAc) mixtures (9 : 1, 8 : 2 : 7 : 3, 6 : 4, 5 : 5, 4 : 6 : 3 : 7, 2 : 8, 1 : 9), EtOAc (100%), EtOAc-methanol (MeOH) mixtures (9 : 1, 8 : 2 and 7 : 3), and ended with MeOH (100%). A total of 55 fractions, 100 mL each, was collected from the tubes; those with similar thin layer chromatography (TLC) profiles were combined fractions as F1 1-6 (Pl); F2 7-12 (P2); F3 13-18 (P3), F4 19-32 (P4), F5 33-45 (P5); F34-43.

From the five pooled fractions (Pl to P5) eluted with hexane-EtOAc mixtures (9:1) eluted two compounds, further this fraction subjected to column chromatography with hexane: chloroform mixture (9:1, 5:5, 1:9). In this fraction, white yellow crystal powder (164 mg) was eluted from hexane: chloroform (9:1) with melting point 65°C.10,11,12

Ethyl acetate fraction

The column chromatography of the EtOAc soluble sub-fraction (90g) over silica gel was performed using n-hexane/EtOAc (in increasing order of polarity) as solvent system, which afforded six major sub-fractions A-F.

Sub-fraction D

It was subjected to further column chromatography over silica gel using n-hexane-EtOAc (3.5:6.5) and n-hexane-EtOAc (4:6) solvent systems. Which afforded compound 2 (6 mg) and compound 4 (21 mg) respectively.

Sub-fraction E

Further column chromatography of this fraction over silica gel eluting with dichloromethane/methanol solvent system was carried out. It afforded two pure compounds. Compound 3 (8 mg) was obtained from solvent system dichloromethane/methanol (9.5:0.5). Similarly dichloromethane/methanol (9:1) solvent system gave compound 1 (25 mg).

INSTRUMENTS AND MATERIALS

The UV and IR spectra were recorded on a Hitachi UV-3200 and JASCO 302-A spectrometers. 1H NMR and 13C NMR and two-dimensional COSY, NOSEY, HMQC, and HMBC, were recorded on the Bruker AV-400 spectrometer (400 MHz for 1H and 100 MHz for 13C) in C5D5N with TMS as internal stander. Chemical shifts δ are shown in ppm.
relative to TMS as internal standard and scalar coupling are reported in Hz. The HR-FAB-MS were recorded on a JEOL JMS-HX-110 mass spectrometer. Analytical and preparative TLC were carried out on pre-coated Silica gel 60 F254 plates (E. Merck, Darmstadt, Germany), and visualized under UV radiation light and by spraying with the ceric sulfate solution. Silica gel (230-400 mesh, E. Merck) was used for column chromatography.

Formulation development of isolated compound

Preparation of latifolin A granules\(^{(13)}\) (Jyothi D et al., 2017)
Latifolin A granules were prepared by wet granulation method.
Granules were also prepared containing sodium starch glycollate (SSG) as super disintegrant.
SSG is incorporated at different concentration (2%, 3%, 5%) separately and granulations were carried out similar way as described above.

Evaluation of latifolin A granules\(^{(14)}\) (Assadpour E et al., 2017)
Prepared Latifolin A granules were subjected for determination of bulk density, tapped density, Hausner ratio, Carr’s index, angle of repose in order to assess the flow property of granules.

Formulation of latifolin A capsules
Prepared granules were packed into hard gelatin capsule (size 1) using hand operated capsule filling machine such that each capsule contains 400 mg of granules. Latifolin A capsules without SSG were labelled as F1 and capsules containing 2%, 3% and 5% of sodium starch glycolate (SSG) were labelled as F2, F3, and F4 respectively.

Estimation of drug content (Latifolin A) in capsules\(^{(15)}\)
Granules from 10 capsules were mixed and weight of powder equivalent to 5 mg of Latifolin A and extracted with the phosphate buffer of pH 6.8 for 30 min. These solutions were filtered, suitably diluted and absorbance was measured at 208 nm against blank solution (phosphate buffer pH 6.8) using a UV spectrophotometer.

Determination of uniformity of weight
Twenty capsules were selected. Each capsule was weighed on an analytical balance, carefully emptied of its content, the shells reweighed and the weight of content determined. The collective weight of content, average weight of content per capsule and the deviations (%) of individual content weights from the mean were calculated.
**Determination of disintegration time**\[16\]
Disintegration times for capsules were determined by disintegration apparatus. Six capsules were placed in six tubes of the basket and the apparatus was operated using water as release medium maintained at 37 ± 2°C. The capsules were observed and the times taken for complete disintegration of all capsules were determined.

**In vitro dissolution study of capsules**

*In vitro* dissolution study of all the prepared capsule formulations was done using USP Type II paddle dissolution apparatus (Electrolab USP dissolution tester TDT-08L) using 900 ml phosphate buffer pH 6.8 at 100 rpm and results were compared with drug release of *Latifolin A* from capsule formulation F0. An aliquot amount of the sample was withdrawn at regular time intervals and the same volume of pre-warmed (37±0.5°C) fresh dissolution medium was replaced. The samples were filtered, suitably diluted and *Latifolin A* in each sample was analyzed by using Shimadzu UV-spectrophotometer at 208 nm.

**Acute toxicity studies**

The acute toxicity of MEOL was determined as per the OECD guideline no. 423 (Acute toxic class method). It was observed that the rats were not mortal even at 2000 mg/kg dose of MEOL. Hence, 1/5th (400 mg/kg) and 1/10th (200 mg/kg) of MEOL were selected as high dose and low dose respectively for this study.\[17\]

**Animals used**

Experimental animals Albino wistar rats of both sexes weighing between 150-250 g were used. The experimental protocol was approved from Institutional Animal Ethics Committee. Animals were housed under standard conditions of temperature (24±2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle.

**Model I: Pylorus ligation induced gastric ulceration in rats**

Pyloric ligation of the stomach was done according to method of Shay *et al.* with slight modification. Albino rats of either sex were divided into six groups of six animals each.

Animals were fasted for 24 h before the study, but had free access to water.

Animals in the control group received only 0.1% of Tween 80 (10 ml/kg orally). Methanolic extracts of *Oxalis latifolia kunth* at 200 and 400 mg/kg, (p.o.) for each extract were given to the animals in the treatment group. Omeprazole (10 mg/kg) was used as a standard. After 1h
of drugs treatment, they were anaesthetized with the help of anaesthetic ether; the abdomen was opened by a small midline incision below the liroid process. Pyloric portion of the stomach was slightly lifted out and ligated according to method of Shay et al. avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anaesthetic ether after four hours of pylorus ligation. The abdomen was opened, the stomach was removed, and its content drained into a graduated centrifuge tube and centrifuged at 3000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, and total acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity.[18]

**Determination of pH**

An aliquot of 1 ml gastric juice was diluted with 1 ml of distilled water and pH of the solution was measured using pH meter.

**Determination of total acidity**[19]

An aliquot of 1 ml gastric juice diluted with 1 ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01 N NaOH until a permanent pink colour was observed. The volume of 0.01 N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following formula: Acidity = (Vol. of NaOH×N×100)/0.1

**Determination of free acidity**[20]

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01 N NaOH until canary yellow colour was observed. The volume of 0.01 N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

**Macroscopic evaluation of stomach**[21,22]

The stomachs were cut open along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. Number of ulcers was counted and was given scores based on their intensity as follows: 0= no ulcer, 0.5= red coloration, 1= superficial mucosal erosion, 1.5= hemorrhagic streak, 2= deep ulcer or transmural necrosis, 3= perforated or penetrated ulcer.
Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows: Ulcer index (UI) was measured by using following formula:

$$\text{UI} = \text{UN} + \text{US} + \text{UP} \times 10^{-1}$$

Where, UI = Ulcer Index; UN = Average number of ulcers per animal; US = Average number of severity score; UP = Percentage of animals with ulcers.

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \left( \frac{\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Test}}}{\text{Ulcer index}_{\text{Control}}} \right) \times 100$$

**Model II**

**Indomethacin induced ulcer**[23]

Albino rats of either sex were divided into six groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water. Animals in the control group received only vehicle 10 ml/kg orally. Methanolic extracts of *Oxalis latifolia kunth* at 200 and 400 mg/kg, were administered orally for each extract were given to the animals in the treatment group. Omeprazole (10 mg/kg) orally was used as a standard. Indomethacin (25 mg/kg body weight) was administrated orally to all animals 10 min prior to treatment. After 6 h of drugs treatment, rats were sacrificed by an over dose of anaesthetic ether and their stomach was removed. The contents of the stomach were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, and total acidity. 10% v/v Formalin was injected into the totally ligated stomach for storage overnight. The next day, the stomach were opened along the greater curvature, then washed in warm water, and examined under a 3 fold magnifier. The ulcer index was determined as described above.

**Statistical analysis**

Values were expressed as mean ± SEM from 6 animals. Statistical differences were evaluated using a One-way analysis of variance (ANOVA) followed by Dunnet's t-test. Results were considered to be statistically significant at $P<0.05$.

**RESULTS**

**Macroscopic characters of the *oxalis corniculata* L**

*Oxalis latentia kunth* belonging to Oxalidaceae family is a stem less herb of cosmopolitan distribution found abundantly in agricultural farms, gardens, lawns etc. Leaves dark green, slight and characteristic odour, sore and astringent taste. Leaves are digitately 3- foliate, leaflets, obcordate, chartaceous, pilose base , cuneate, margin entire, apex, emarginiate,
Pseudoumbels, axillarly, 1-6 flowered, bracts two, linear, bracteole, Sepals, five lanceolate, petals oblongulate apex, emarginated.

**Fig no. 1:** Macroscopic characters of the *oxalis corniculata* L.

**Microscopical evaluation of Oxalis corniculata L.**

Anatomy of the leaf

Microscopic features

1. Leaflet

The leaflet is thin with less prominent and lateral veins. The mid rib is shallow concave on the adaxial side and slightly projecting on the abaxial side. The mid rib is about 200µm thick. The adaxial epidermis in the midrib portion consists of much dilated circular, thin walled cells are 70µm in height. The abaxial epidermal cells are also dilated and thin walled. The vascular strand consists of a cluster of narrow, angular thin walled. Xylem elements are 8µm wide. These are of phloem elements occurs on the lower end of the xylem strand, the palisade tissue is transcurrent across the vascular bundle and beneath the adaxial epidermis.

Anatomy of the lamina

T.S of the lamina

Lamina

The leaf blade is thin, dorsiventral with thick epidermal layers. The lamina part is about 100 µm wide. Both adaxial and abaxial epidermal layers are quite wide and have large, thin
walled circular cells, measuring 25µm in thickness. The mesophyll tissue consists of a narrow adaxial zone of short, then cylindrical palisade cells and the four layers of small, lobed spongy parenchyma cells.

T.S of the leaf margin
The leaf margin is slightly narrow leaflet and posses circular thin walled cells. They are 25 µm in diameter the mesophyll tissues are as in the middle portion of the lamina.

Epidermal morphology
Epidermal cells and stomata
The epidermal cells are thin walled; their anticlerical walls are highly wavy, so that the cells appear amoeboid in outline. Stomata occur only the lower epidermis and they are absent on the upper epidermis.

Abaxial epidermis with stomata
The stomata do not possess distinct subsidiary cells. The guard cells are elliptical with slit like stomatal pores.

Adaxial epidermis
The guard cells are 15×20µm in size. The adaxial epidermal cells are similar to the abaxial cells in shape and size; but it is apostomatic (without stomata).

Paradermal section showing venation pattern and crystal distribution
Venation pattern
The lateral veins and vein islets are uniformly thin comprising of one or two spiral xylem elements, the veins are straight. They form wide, rectangular on many sided vein islets; the vein islets have well defined vein terminations. Which are long, slender unbranched or branched once or twice.

Crystals in the mesophyll tissue
Crystals
Calcium oxalate crystals are frequently seen in the mesophyll tissue. The crystals are mostly druses or sphere crystals. They are diffuse in distribution and are located in ordinary mesophyll cells. The crystals are up to 20 µm wide.
Powder microscopy of the whole plant

Leaf powder

Leaf powder are seen fragments lamina, with venation and trichomes, isolated trichomes and epidermal peeling fragments of lamina show epidermal trichomes along the leaf margin, as well as on the lamina surface.

Non-glandular covering trichome in the leaf powder

The trichomes are non-glandular type covering trichomes; they are unicellular, unbranched and pointed at the tip. They are mostly curved and wavy. Their walls are fairly thick and smooth. They are up to 300 µm long and 20 µm thick. Epidermal peeling in the powder exhibit thin walled lobed cells. The stomata are anomocytic type.

[AdS – adaxial side; MR – Midrib; La – Lamina]

Figure 2: T. s. of through midrib with lamina.

[AdE-adaxial epidermis; Ph-Pholem; X-Xylem]

Figure 3: T. s. of midrib with lamina enlarged
[AbE-Abaxial epidermis; AdE-Adaxial epidermis; MR-Midrib; PM-Palisade mesophyll; Ph-Phloem; X-Xylem]

Figure 4: T. s. of midrib with lamina enlarged.

[AbE-Abaxial epidermis; AdE-Adaxial epidermis; PM-Palisade mesophyll; SM-Spongy mesophyll]

Figure 5: T. S. of the lamina.

[Ec-epiderma cells; LM-Leaf margin; PM-Palisade mesophyll; SM-Spongy mesophyll]

Figure 6: T. s. of the leaf margin.
Figure 7: Abaxial epidermis with stomata.

Figure 8: Adaxial epidermis with stomata

Figure 9: Adaxial epidermis
Figure 10: vein islets and vein termination.

Figure 11: Vein islets and vein termination.

Figure 12: Crystals in the mesophyll tissue.
Figure 13: Cleared leaf showing vein islets and vein termination.

Figure 14: Cleared leaf showing vein islets and vein termination enlarged.

Figure 15: One vein islets and vein termination enlarged.
Figure 16: Fragment of adaxial epidermis cells with covering trichome.

Figure 17: A covering trichome enlarged.

Figure 18: Non-glandular covering trichome in the leaf powder.
Table no 1: Preliminary Phyto-chemical screening.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Pet. ether</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Fixed Oils &amp; fats</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds &amp; Tannins</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins &amp; Amino acids</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gums &amp; mucilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

"+" = Indicates Positive Result
"−" = Indicates Negative Results

Tab no. 2: Physicochemical parameters of *Oxalis latifolia kunth*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (Loss on drying)</td>
<td>6.52±1.34</td>
</tr>
<tr>
<td>Total ash</td>
<td>6.80±1.52</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>3.52±0.72</td>
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<tr>
<td>Water soluble ash</td>
<td>2.02±0.55</td>
</tr>
<tr>
<td>Petroleum ether soluble extractive value</td>
<td>0.86±0.05</td>
</tr>
<tr>
<td>Chloroform soluble extractive value</td>
<td>2.56±0.06</td>
</tr>
<tr>
<td>Ethyl acetate soluble extractive value</td>
<td>3.65±0.82</td>
</tr>
<tr>
<td>Alcohol soluble extractive value</td>
<td>8.12±1.22</td>
</tr>
<tr>
<td>Water soluble extractive value</td>
<td>10.02±2.51</td>
</tr>
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</table>

Tab no 3: Fluorescence analysis of *Oxalis latifolia kunth*.

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>Visible light</th>
<th>UV light 254nm</th>
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<tbody>
<tr>
<td>Water</td>
<td>Buff</td>
<td>Brown</td>
</tr>
<tr>
<td>NaOH</td>
<td>Dark Brown</td>
<td>Brown</td>
</tr>
<tr>
<td>HCl</td>
<td>Reddish brown</td>
<td>Black</td>
</tr>
<tr>
<td>HNO₃</td>
<td>Brown</td>
<td>Green</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>Dark green</td>
<td>Light green</td>
</tr>
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</table>
Fig no 19: TLC fingerprinting of Methanolic extract of Oxalis latifolia kunth with compound on TLC Silica gel Kiesel gel 60 F254.

Tab no 4: Physical properties of isolated compound.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameter</th>
<th>Observation</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Colour</td>
<td>Yellowish</td>
</tr>
<tr>
<td>2</td>
<td>Shape</td>
<td>Crystalline solid</td>
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</tbody>
</table>

Tab no 5: Chemical tests of isolated compound.

<table>
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<tr>
<th>S. No</th>
<th>Test</th>
<th>Result</th>
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<tbody>
<tr>
<td>1</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
</tbody>
</table>

(Luteolin-6′′-(E-p-hydroxycinnamoyl) 4′-O-β-D-glucopyranoside)

Structure 1: Chemical structure of Latifolin A
Fig. no. 20: Mass spectrum of isolated compound (Positive).

Fig. no. 21: IR spectra of isolated compound.
Fig. no. 22: GCMC-Chromatogram of methanolic extract of *Oxalis latifolia kunth*.

Fig. no. 23: $^1$H NMR spectra of isolated constituent.
Fig. no. 24: $^{13}$C NMR spectra of isolated constituent.

Table no. 14: Formulation of capsules.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity/Capsule (mg)</th>
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<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Latifolin A</td>
<td>5</td>
</tr>
<tr>
<td>Lactose Monohydrate</td>
<td>150</td>
</tr>
<tr>
<td>Starch Paste (5%)</td>
<td>50</td>
</tr>
<tr>
<td>Microcrystalline Cellulose</td>
<td>179</td>
</tr>
<tr>
<td>Sodium Starch Glycollate</td>
<td>-</td>
</tr>
<tr>
<td>Talc (2%)</td>
<td>8</td>
</tr>
<tr>
<td>Magnesium Stearate (2%)</td>
<td>8</td>
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</table>

Tab. no. 13: Evaluation of *latifolin a* granules.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Evaluation Parameters</th>
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<tbody>
<tr>
<td></td>
<td>Bulk Density (g/ml)</td>
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<tr>
<td>F1</td>
<td>0.81±0.02</td>
</tr>
<tr>
<td>F2</td>
<td>0.78±0.05</td>
</tr>
<tr>
<td>F3</td>
<td>0.76±0.02</td>
</tr>
<tr>
<td>F4</td>
<td>0.75±0.03</td>
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Tab no. 14: Physical characterization of Latifolin A capsules.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Evaluation Parameters</th>
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<tbody>
<tr>
<td></td>
<td>Weight variation (mg)</td>
</tr>
<tr>
<td>F1</td>
<td>398±0.4</td>
</tr>
<tr>
<td>F2</td>
<td>400±0.5</td>
</tr>
<tr>
<td>F3</td>
<td>401±0.2</td>
</tr>
</tbody>
</table>

Tab. no. 15: Dissolution profile of latifolin a capsules.

<table>
<thead>
<tr>
<th>Time (Mins)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>5</td>
<td>6.53±0.56</td>
<td>14.25±0.18</td>
<td>26.58±0.34</td>
<td>42.15±0.23</td>
</tr>
<tr>
<td>10</td>
<td>20.22±0.42</td>
<td>29.15±0.34</td>
<td>48.22±0.45</td>
<td>73.21±0.34</td>
</tr>
<tr>
<td>15</td>
<td>35.53±0.21</td>
<td>60.23±0.21</td>
<td>78.56±0.53</td>
<td>97.21±0.65</td>
</tr>
<tr>
<td>30</td>
<td>72.25±0.56</td>
<td>92.32±.43</td>
<td>96.78±0.75</td>
<td>98.23±0.43</td>
</tr>
</tbody>
</table>

Fig. no. Release profile of latifolin a from capsules

It is observed that with increasing the concentration of sodium starch glycolate, the rate and extent of drug release from the formulation F2, F3, F4 were also increased. Thus, the release characteristics were significantly influenced by the concentration of super disintegrants used. The formulation F4 is selected as a better formulation for further studies.
Fig. no. 1: Macroscopic evaluation of stomach ulcer in pyloric ligation induced ulceration in rats.

Table 2: The Effect of methanolic extracts of *Oxalis latifolia kunth* on gastric pH, Gastric Volume, Free Acidity and Total Acidity in Pylorous Ligation Model.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg b.w.)</th>
<th>Gastric pH (ml)</th>
<th>Gastric Volume (ml)</th>
<th>Free Acidity (meq/ltr)</th>
<th>Mean Total Acidity (meq/ltr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1 ml</td>
<td>2.9±0.10</td>
<td>8.6±0.28</td>
<td>6.56±0.07</td>
<td>70.77±0.25</td>
</tr>
<tr>
<td>II</td>
<td>MEOL 200</td>
<td>3.8±0.05**</td>
<td>6.3±0.06**</td>
<td>5.65±0.19**</td>
<td>50.15±0.08**</td>
<td>44.8±0.05***</td>
</tr>
<tr>
<td>III</td>
<td>MEOL 400</td>
<td>4.9±0.17***</td>
<td>5.2±0.18***</td>
<td>4.24±0.29***</td>
<td>44.8±0.05***</td>
<td>34.4±0.14***</td>
</tr>
<tr>
<td>IV</td>
<td>Omeprazole 10</td>
<td>5.7±0.09***</td>
<td>4.5±0.29***</td>
<td>3.43±0.08***</td>
<td>34.4±0.14***</td>
<td></td>
</tr>
</tbody>
</table>

All the values are mean±SEM n=6. ***P<0.001, **P<0.01, compare vs. control, data was analysed using one way ANOVA followed by Tukey multiple comparison test.

Graph 1: Effect of Methanolic extracts of *Oxalis latifolia kunth* on total acidity and % inhibition of ulcer in pyloric ligation induced ulceration in rats.
Fig. 2: Macroscopic evaluation of stomach ulcer in Indomethacin induced ulceration in rats.

Table 3: Effect of methanolic extracts of Oxalis latifolia kunth on pH, total acidity, Ulcer index and % inhibition of ulcer in Indomethacin induced ulceration in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Gastric pH</th>
<th>Mean Total Acidity (meq/ltr)</th>
<th>Ulcer Index</th>
<th>% Inhibition of Ulceration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (1 ml)</td>
<td>1.8±0.10</td>
<td>70.73±2.25</td>
<td>10.69±2.51</td>
<td>--</td>
</tr>
<tr>
<td>II</td>
<td>MEOL (200mg/kg)</td>
<td>3.4±0.05**</td>
<td>55.15±4.68**</td>
<td>4.69±0.51</td>
<td>60.05%</td>
</tr>
<tr>
<td>III</td>
<td>MEOL (400mg/kg)</td>
<td>4.9±0.17***</td>
<td>43.68±2.55***</td>
<td>2.32±0.51***</td>
<td>78.29%</td>
</tr>
<tr>
<td>IV</td>
<td>Omeprazole (10 mg/kg p.o.)</td>
<td>5.7±0.09***</td>
<td>32.41±2.14***</td>
<td>2.36±0.16**</td>
<td>77.29%</td>
</tr>
</tbody>
</table>

Significant values were compared with* P <0.05, ** P <0.01 and *** P <0.001 Indomethacin control Vs treated groups using One way ANOVA followed by Dennett’s test.
Graph 3: Effect of Methanolic extracts of *Oxalis latifolia kunth* on Mean pH and Ulceration index of ulcer in Indomethacin induced ulceration in rats.

**DISCUSSION AND CONCLUSION**

The plant *Oxalis latifolia kunth* belonging to Oxalidaceae family is a stem less herb of cosmopolitan distribution found abundantly in agricultural farms, gardens, lawns etc. Leaves dark green, slight and characterestic odour, sore and astringent taste. Leaves are digitately 3-foliate, leaflets, obcordate, chartaceous, pilose base, cuneate, margin entire, apex, emarginiate, Pseudoumbels, axiallary, 1-6flowered, bracts two, linear, bracteole, Sepals, five lanceolate, petals oblanceolate apex, emarginated.

The microscopical characteristic of the leaf blade is thin, dorsiventral with thick epidermal layers. Stomata occur only the lower epidermis and they are absent on the upper epidermis. The stomata are anomocytic type. Calcium oxalate crystals are frequently seen in the mesophyll tissue. The crystals are mostly druses or sphere crystals. Leaf powder are seen fragments lamina, with venation and trichomes, isolated trichomes. The trichomes are non-glandular type covering trichomes; they are unicellular, unbranched and pointed at the tip.

Qualitative phytochemical analysis of the Methanolic extracts of *Oxalis latifolia kunth*, Oxalidaceae showed the presence of carbohydrates, flavonoids, saponins, and tannins. As the extract of *Oxalis latifolia kunth*, showed the positive result for the presence of flavonoids and it has reported that flavonoids have antiulcerogenic activity. So here may be the ulcerogenic
activity reduced because of the flavonoids, which shows significant result as compared to standard drug.

In this present study the Methanolic extracts of *Oxalis latifolia kunth*, Oxalidaceae were investigated for antiulcer activity by using Pyloric ligation in rats, ethanol induced gastric ulcer in rats and Indomethacin induced gastric ulcer in rats.

There are several risk factors that may contribute to formation of ulcer in human beings such as stress, chronic use of anti-inflammatory drugs, continuous alcohol ingestion, *H. pylori* infection, Zollinger Ellison syndrome, etc. Although in most cases the etiology of ulcer is unknown. An effective anti-ulcer drug should act either by reducing the aggressive factors on gastroduodenal mucosa or by increasing mucosal resistance against them. The critical factors which maintain defense and integrity of gastric and intestinal mucosa include normal mucosal blood flow, local prostaglandins, mucous and bicarbonate secretion, epithelial proliferation and repair.

Non-steroidal anti-inflammatory drugs (NSAIDs) like Indomethacin are known to induce gastric damage, particularly due to inhibition of the cyclooxygenase pathway of arachidonic acid metabolism. It is currently believed that the ulcerogenic effects of the NSAIDs are due to inhibition of cyclooxygenase 1 (COX-1) and that its isoforms, cyclooxygenase 2 (COX-2), plays a pathological role in inflammation, pain and fever. Several studies shown that gastric mucosal prostaglandins (PGs), produced mainly by COX-1, play an important role in maintaining gastric mucosal integrity and Indomethacin markedly decrease mucosal PGE2 level.

On the other hand, recent reports show that Indomethacin is a dual inhibitor of COX-1 and COX-2 because both tromboxanes and inflammatory PGE2 synthesis are suppressed, and that inhibition of both isoform of these enzymes is required for the development of gastric erosions after NSAID administration. Indeed, endogenous PG deficiency alone did not induce visible gastric lesions and the pathogenesis of NSAID-induced gastric lesions also involves luminal acid, neutrophils activation and gastric hyper motility.

The treatment of peptic ulcer is mainly aimed at reducing the hydrochloric acid secretion, increasing gastric cytoprotection, eradication of *H. pylori* or curing Zollinger Ellison syndrome. The discovery of potential antiulcer agent from plants is a developing area. So far,
several plants have been screened for antiulcer activity and many formulations have been developed by combining extracts of these plants.

Pylorus ligation induced ulcer was used to study the effect of seed extracts on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach. This increase in the gastric acid secretion causes ulcers in the stomach. The original Shay rat model involves fasting of rats for 36 h followed by ligation of pyloric end of the stomach. The ulcer index is determined 5 h after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach.

Indomethacin is known to cause ulcer especially in an empty stomach and mostly on the glandular (mucosal) part of the stomach by inhibiting prostaglandin synthetase through the cyclooxygenase pathway. Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair. Suppression of prostaglandins by Indomethacin results in increased susceptibility of the stomach to mucosal injury and gastro duodenal ulceration. The extract was observed to significantly reduce mucosal damage in the Indomethacin induced ulcer model, suggesting the possible extracts mobilization and involvement of prostaglandin in the anti-ulcer effect of the extract.

It may act by multiple mechanisms. The activity might be due to increasing the gastric mucosal resistance, local synthesis of cytoprotective prostaglandins and inhibiting the leukotriene synthesis.

It has also been reported that the presence of phyto-constituents tannins, terpenoids, sterols and flavonoids may be responsible for antiulcer activity. Recent reports and extensive literature review indicated that flavonoids and tannins showed cytopro-ective action by increasing mucosal content of prostaglandins and mucous in gastric mucosa.

The Methanolic extracts of Oxalis latifolia kunth, Oxalidaceae showed significant antiulcer activity due to isolated the compound Latifolin A based on Analytical evidence. Developed novel Latifolin A formulation showed significant anti-ulcer properties. Developed formulation was evaluated on Pylorous ligated and Indomethacin induced ulcer in rats. The extract significantly reduces mucosal damage on the rats. However further studies required
to elucidate the exact mechanism of action for develop its as potent antiulcer drug. These herbal drugs will help to develop new drug molecules for antiulcer therapy.

BIBLIOGRAPHY


