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Pharmacognostic and Phytochemical Analysis of Oxalis Latifolia Kunth and their Antiulcer Activity in Albino Rats

Jhansi Rani M*[1], Veerasamy Hari Bhaskar[2], Kothapalli Bannoth Chandra Sekhar[3]

[1]Research Scholar, Ratnam Institute of Pharmacy, Pidathapolur (V), Muthukur (M), SPSR Nellore – 524 346, Andhra Pradesh, India
[2]Department of Pharmaceutical Analysis, Sasikanth Reddy College of Pharmacy, Rajupalem, Kodavaluru, SPSR Nellore – 524 316, Andhra Pradesh, India
[3]Department of Chemistry, JNT University, Anantapur, Ananthapuramu – 515002, Andhra Pradesh, India

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ABSTRACT

In a rat model of stomach ulceration caused by Pylorus ligation and Indomethacin, the purpose of the current study was to assess the pharmacognostic, phytochemical analysis, and antiulcer potential of Oxalis latifoliaKunth (OL). The entire OL plant was gathered, dried, and powdered, and its macroscopic and microscopic properties were evaluated. To test for good yield and phytochemical components, powdered OL was extracted using a variety of solvents. Methylated as methanolic *Oxalis latifolia Kunth* (MEOL) was chosen for isolation because it had a high yield and quantity of components. Latifolin A, the active chemical, was isolated from MEOL and subjected to IR, NMR, and mass spectroscopy analysis. Later, Latifolin A was developed as 5 mg/kg capsules and tested for several parameters. Following ulcer induction, a number of measures including gastric acid secretion volume, pH, total acidity, ulcer index, and antioxidant parameters were measured and compared between animals in the negative control, formulation, extract-treated, and standard group. According to the findings of this investigation, Oxalis latifoliaKunth's methanolic extract has a fair yield and a high concentration of phytoconstituents. Latifolia A, a flavonoid component of the extract, is found in luteolin-6′′-(E-p- hydroxycinnamoyl) 4′-O-D-glucopyranoside, which is extracted, evaluated using several spectrum techniques, and then packaged as a capsule. In comparison to MEOL alone, formulation of Latifolia A shown good antiulcer properties in both animals. This could be as a result of other Latifolia A capsule ingredients not interfering. Standard drug is Ranitidine (100mg/kg). Latifolia A is mainly responsible for the antiulcer activity in Pylorus ligation and Indomethacin induced gastric ulcer model in rats.

*Corresponding Author
Name: Jhansi Rani M
Phone: 7981088647
Email: Jhansi.m39@gmail.com

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INTRODUCTION

A dangerous digestive condition is peptic ulcer disease. The presence of acid and peptic activity in gastric juice, as well as a breakdown in mucosal defences, all contribute to the development of peptic ulcers. Non-steroidal anti-inflammatory medicines (NSAID), such as aspirin, and Helicobacter pylori (H. pylori) infection are the two main variables that can compromise the mucosal resistance to damage [1]. Proton pump inhibitors, prostaglandin analogues,

histamine receptor antagonists, and cytoprotective medicines are just a few of the medications that might be used to treat peptic ulcers [2]. However, the majority of these medications have severe adverse effects, including arrhythmias, gynecomastia, impotence, arthralgia, hypergastrinemia, and hemopoeitic alterations. [3] Because of this, herbal remedies are typically employed in situations where medications must be used for extended periods of time. Numerous herbal medications are said to have anti-ulcerogenic properties [4].One of these unstudied plants is Oxalis latifoliaKunth, which is a member of the Oxalidaceae family. Numerous phytoconstituents, including sugars, saponins, phenol, and flavonoids, particularly Latifolin A, were recognised to be present in the plant [5]. According to reports, oxalis species can treat a wide range of illnesses, including paralysis, stomach problems, ulcers, inflammation, and antioxidants. They can also quench your thirst [6].

MATERIALS AND METHODS

Plant Material

The plant known as Oxalis latifolia kunth was found growing in and around the Talakona Forest in the state of Andhra Pradesh. Dr. K. MadhavaChetty, a plant taxonomist from the Department of Botany at Sri Venkateswara University in Tirupati, Andhra Pradesh, India, verified its authenticity. The plant's voucher specimen (2019/1214) was left at the college for future use as a source of information.

Pharmacognostic study of oxalis latifolia

Macroscopic Study

The size, form, nature of the exterior and inner surfaces, types of fracture, and organoleptic characteristics such as colour, scent, taste, etc. were evaluated as part of the macroscopical description of the plant. The plant Oxalis latifoliaKunth (Oxalidaceae) was directly examined in the field and photographed in its natural habitat. [7].

Microscopic Study

Transverse Section of Crude Drug(Leaf)

Examining plant medications under a microscope allows researchers to examine adulterants and identify them. Microscopical analysis of plant medications is used to confirm the structural characteristics of the drugs from plants and to identify the organised drugs by their known histological characteristics [8].

Powder Microscopy

The powdered Oxalis latifolia kunth leaves were shade-dried, and each powder sample was put through sieve number 60# before being subjected to a powder analysis.

Preparation of the Extract

The plant leaves were spread out to dry on sheets of filter paper in the shade at room temperature until the colour of the filter papers changed, and then they were ground into a coarse powder. In a Soxhlet device, 200 g of powdered material were extracted for 8–12 hours with petroleum ether, n-butanol, ethyl acetate, and 70% methanol. In an oven, solvent was withdrawn when the temperature fell below 50°C. For additional experimental studies, the residue (extract) of the relevant plant material was held at 4°C. By mixing 100g of the powdered plant material with 500 ml of distilled water in a Soxhlet system for 8–12 hours, an aqueous extract was created. The extract was then preserved at 4°C for additional experimental research once the filtrate had been concentrated.

Preliminary Phytochemical Screening

To identify different plant elements like tannins, sugars, saponins, flavanoids, glycosides, proteins, alkaloids, and phenols, all of the Oxalis latifolia kunth preparations underwent preliminary phytochemical screening. [9]

Isolation of active constituent

Oxalis latifolia kunth (Oxalidaceae) 5 gm of methanolic extract was exposed to silica-gel (100-200 mesh) column chromatography with a length of 100 cm and a diameter of 3 cm. Hexane was used as the starting solvent for the elution, which was then followed by hexane-ethyl acetate (EtOAc) combinations (9: 1, 8: 2; 7: 3, 6:4, 5: 5, 4: 6; 3: 7, 2: 8, 1: 9); 100% EtOAc; and mixtures of EtOAc-methanol (MeOH) (9: 1, 8: 2 and 7: 3). F1 l-6 (Pl); F2 7-12 (P2); F3 13-18 (P3); F4 19-32 (P4); F5 33-45 (P5); and F34-43 were the combined fractions based on thin layer chromatography (TLC) profiles that were similar to one another. A total of 55 fractions, each measuring 100 mL, were extracted from the tubes.

From the five pooled fractions (Pl to P5), hexane-EtOAc mixtures (9:1) were used to elute.

Instruments and Materials

The UV and IR spectra were recorded on Hitachi UV-3200 and JASCO302-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-for13C) 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 and evaluation of Latifolin A granules

The wet granulation process was used to create latifolin A granules. Additionally, granules containing sodium starch glycollate (SSG) as a super disintegrant were created. Using a hand-operated capsule filling machine, prepared granules were packed into firm gelatin capsules (size 1) so that each capsule contains 400 mg of the granules listed in Table 1. Latifolin A capsule devoid of sodium starch glycolate (SSG) was designated as F1, and capsules containing 2%, 3%, and 5% of SSG were designated as F2, F3, and F4, respectively. Table 1 clearly outlined the formulation process. In order to evaluate the flow property of the granules, prepared Latifolin A granules were submitted to measurements of bulk density, tapped density, Hausner ratio, Carr's index, and angle of repose [10, 11].

Estimation of drug content (Latifolin A) in capsules

Ten capsules' worth of granules were combined, yielding a weight of powder containing 5 mg of latifolin a, which was then extracted in a phosphate buffer at pH 6.8 for 30 minutes. A UV spectrophotometer was used to detect absorbance at 208 nm in comparison to a blank solution (phosphate buffer, pH 6.8) after these solutions had been filtered and appropriately diluted [12].

Determination of uniformity of weight

There were chosen twenty capsules. Each capsule was carefully emptied of its contents before being reweighed on an analytical balance to determine the weight of the material. Calculations were made to determine the total weight of the content, the average weight of the content per capsule, and the individual content weights' percentage deviations from the average.

Determination of Disintegration Time

Disintegration device was used to calculate the duration of capsule disintegration. Six capsules were put into the six basket tubes, and the apparatus was run with water used as the release medium and kept at a temperature of 37 2 °C. The capsules were examined, and the times needed for each capsule to completely dissolve were calculated [13].

In Vitro Dissolution Study of capsules

Utilising a USP Type II paddle dissolution apparatus (Electrolab USP dissolution tester TDT-08L) and 900 ml of phosphate buffer pH 6.8 at 100 rpm, an in vitro dissolution study of all the prepared capsule formulations was conducted. The results were compared with the drug release of Latifolin A from capsule formulation F0. At regular intervals, an aliquot of the material was removed, and the same volume of freshly prepared dissolving media was added. Shimadzu UV-spectrophotometer at 208 nm was used to analyseLatifolin A in each sample after it had been filtered and appropriately diluted.

Invivo evaluation of prepared Latifolia A capsule

Animals

Animal experimentation Both sexes of albino wistar rats weighing between 150 and 250 g were used. The Institutional Animal Ethics Committee gave its approval number IAEC/SJCPS/2019/02 of the study protocol. The animals were kept in normal housing with a 12:12 light: dark cycle, temperature of 24°C, and relative humidity of 30–70%.

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 four groups of six animals each.

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

Group I (Disease control) received saline after gastric pylorus ligation

Group II: Latifolin A (5mg/kg) capsules

Group III: MEOL (200mg/kg/b.w) p.o.,

Group IV: Ranitidine (100mg/kg)as a standard.

The abdomen was opened by a minor midline incision below the lipoid process after they had received medication therapy for one hour. They were then put to sleep with the use of anaesthetic ether. According to the Shay et al. procedure, the pyloric section of the stomach was slightly raised out and ligated to prevent traction on the pylorus or injury to its blood supply. Carefully replacing the stomach, the abdominal wall was then stitched shut. After four hours of pylorus ligation, rats were sacrificed by an overdose of anaesthetic ether.

The stomach was opened, taken out, and its contents were put into a graded centrifuge tube and spun for

10 minutes at 3000 rpm. To measure the pH and total acidity, aliquots (1 ml of each) of the supernatant were collected. Each stomach was checked for lesions in the fore stomach area, and their severity was rated. [14].

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

Two drops of phenolphthalein indicator were added to an aliquot of 1 ml gastric juice that had been diluted with 1 ml of distilled water in a 50 ml conical flask. The mixture was then titrated with 0.01 N NaOH until a persistent pink colour was seen. It was reported how much 0.01 N NaOH was spent. The following formula converts total acidity to mEq/L: Acidity = (Vol. of NaOH$\times$N$\times$100)/0.1 [15]

Determination of free acidity

The Topfer's reagent was applied in place of the phenolphthalein indicator. Gastric juice was added to a sample and titrated with 0.01 N NaOH until a canary yellow tint was seen. It was reported how much 0.01 N NaOH was spent. The same formula used to determine total acidity was used to compute free acidity [16].

Macroscopic evaluation of stomach

The stomachs were sliced open along the larger curvature, cleaned with saline to get rid of any gastric contents or blood clots, and then examined under a 10X microscope to check for ulceration. The number of ulcers was counted, and scores were assigned based on how severe they were: 0 indicates there is no ulcer, 0.5 indicates a red coloration, 1, 1.5 indicates a hemorrhagic streak, 2, and 3 indicate a punctured or pierced ulcer [17, 18]. 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: UI=UN+US+UP X 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: % Inhibition of Ulceration = (Ulcer indexControl–Ulcer indexTest) $\times$ 100/Ulcer index Control.

Model II

Indomethacin induced ulcer

Albino rats of either sex were divided into four groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water.

Group I (Disease control) was indomethacin (40 mg/kg bw) induced ulcer

Group II: Latifolin A (5mg/kg) capsules

Group III: MEOL (200mg/kg/b.w) p.o.,

Group IV: Ranitidine (100mg/kg) as a standard.

Rats were given an overdose of anaesthetic ether to kill them after 6 hours of drug therapy, and their stomachs were removed. The stomach's contents were transferred into a glass tube. The gastric juice was measured and centrifuged for 10 minutes at 2000 rpm. To measure the pH and total acidity, aliquots (1 ml of each) of the supernatant were collected. For storage overnight, 10% v/v Formalin was administered into the completely ligated stomach. The stomach was cut open along its greater curvature the following day, cleaned in warm water, and then examined under a threefold magnification. The ulcer index was calculated as previously mentioned [19].

Statistical analysis

Values were expressed as mean $\pm$ 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<0.01.

RESULTS

Macroscopic Characters of the Oxalis latifolia Kunth L

The stemless plant Oxalis latifoliaKunth, a member of the Oxalidaceae family, is widely distributed around the world and is frequently found in lawns, gardens, and other outdoor areas. Dark green, astringent, painful, and slightly offensive-smelling leaves. Pseudoumbels, axiallary, 1-6 flowered, bracts two, linear, bracteole, Sepals, five lanceolate, petals oblanceolate apex, emarginated, leaves digitately 3-foliate, leaflets obcordate, chartaceous, pilose base, cuneate, margin whole, apex, as shown in Figure 1.

Microscopical Evaluation of Oxalis latifolia L.

Anatomy of the leaf: Microscopic Features

Leaflet

The leaflet is narrow and has lateral veins that are less noticeable. On the adaxial side, the midrib has a shallow concavity, while on the abaxial side, it projects somewhat. The midrib is 200 m thick on average. The adaxial epidermis in the midrib part is made up of 70 m tall, greatly dilated, round cells

Table 1: Formulation of capsules

Ingredients	Quantity/Capsule(mg)			
	F1	F2	F3	
Latifolin A	5	5	5	5
Lactose Monohydrate	150	150	150	150
Starch Paste(5%)	50	50	50	50
Microcrystalline Cellulose	179	171	157	144
Sodium Starch Glycollate	-	8	12	15
Talc (2%)	8	8	8	8
Magnesium Stearate(2%)	8	8	8	8

Table 2: Preliminary phytochemical screening

Tests	Pet.ether	Ethyl acetate	Chloroform	Methanol	Water
Alkaloids	—	-	—	—	—
Carbohydrates	—	-	+	+	+
Glycosides	—	-	—	+	+
Phytosterols	—	-	—	—	—
Fixed Oils & fats	—	-	+	—	+
Saponins	—	-	+	+	+
Phenolic compounds Tannins	—	+	+	+	+
Proteins & Amino acids	—	-	—	—	—
Gums & mucilage	+	+	+	+	+
Flavonoids	+	-	+	+	—

"+"= Indicates Positive; Result "—"= Indicates Negative Results

Table 3: Evaluation of Latifolina granules

Formulation Code	Evaluation Parameters				
	Bulk Density (g/ml)	Tapped Density (g/ml)	Hausner Ratio	Carr's Index (%)	Angle of Repose θ
F1	0.72 ± 0.01	0.69 ± 0.01	1.06 ± 0.03	6.46 ± 0.04	26.47 ± 0.04
F2	0.89 ± 0.04	0.65 ± 0.05	1.07 ± 0.01	7.11 ± 0.01	25.32 ± 0.05
F3	0.87 ± 0.01	0.63 ± 0.03	1.10 ± 0.02	11.32 ± 0.04	22.26 ± 0.06
F4	0.96 ± 0.02	0.63 ± 0.07	1.10 ± 0.01	13.12 ± 0.03	23.43 ± 0.04

Table 4: Physical characterization of Latifolia A capsules

Formulation Code	Evaluation Parameters		
	Weight variation (mg)	Latifolin A content (%)	Disintegration Test (mins)
F1	487 ± 0.3	97.01 ± 0.2	4.4 ± 0.2
F2	311 ± 0.4	98.04 ± 0.1	4.3 ± 0.4
F3	312 ± 0.3	98.01 ± 0.3	3.7 ± 0.4
F4	489 ± 0.4	98.02 ± 0.2	3.1 ± 0.5

Table 5: Dissolution Profile of Latifolia A capsules

Time (Mins)	F1	F2	F3	F4
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
5	7.42 ± 0.47	23.14 ± 0.27	37.47 ± 0.23	33.26 ± 0.14
10	32.11 ± 0.31	38.26 ± 0.43	47.15 ± 0.45	62.21 ± 0.23
15	46.42 ± 0.32	51.35 ± 0.32	69.45 ± 0.44	86.32 ± 0.54
30	80.36 ± 0.45	90.43 ± 0.54	95.67 ± 0.86	98.34 ± 0.41

Table 6: Effect of Latifolia A & MEOL on Gastric pH, Gastric Volume, Free Acidity and Total Acidity in Pylorus Ligation Model

Groups	Gastric pH	Gastric volume (ml)	Free Acidity (meq/ltr)	Mean Total Acidity (meq/ltr)
Negative Control (1ml/b.w)	1.34±0.11	3.36±0.45	42.80±0.154	94.80±17.59
Latifolia A (5 mg/kg)	4.1±0.22**	1.70±0.30**	3.20±0.58**	6.00±0.71**
MEOL (200 mg/kg)	3.30±0.11**	1.86±0.10**	11.80±2.48**	24.80±4.38**
Ranitidine (100 mg/kg)	4.3±0.34**	1.54±0.17**	4.00±0.71**	10.20±1.28**

Values in the results are expressed as mean±SD, (n=6); **P<0.01 significantly different in comparison with Negative control (ANOVA followed by Dunnet's t-test)

Table 7: Effect of Latifolia A & MEOL on Ulcer index and % inhibition of ulcer in Pylorus Ligation Model

Groups	Ulcer index	Ulcer protection
Negative Control (1ml/b.w)	10.69±0.11	—-
Latifolia A (5 mg/kg)	3.425±0.04**	68.38%
MEOL (200 mg/kg)	2.9±0.05**	52.79%
Ranitidine (100 mg/kg)	1.48±0.05**	84.50%

Values in the results are expressed as mean±SD, (n=6); **P<0.01 significantly different in comparison with Negative control (ANOVA followed by Dunnet's t-test)

Table 8: Effect of Latifolia A & MEOL on Ulcer index and % inhibition of ulcer in Indomethacin Induced Ulceration in Rats

Groups	Ulcer index	Ulcer protection
Negative Control (Indomethacin (40mg/kg))	10.75+0.062	—-
Latifolia A (5 mg/kg)	6.54+0.045**	52.61
MEOL (200 mg/kg)	5.41+0.079**	46.63
Ranitidine (100 mg/kg)	4.24+0.074**	70.45

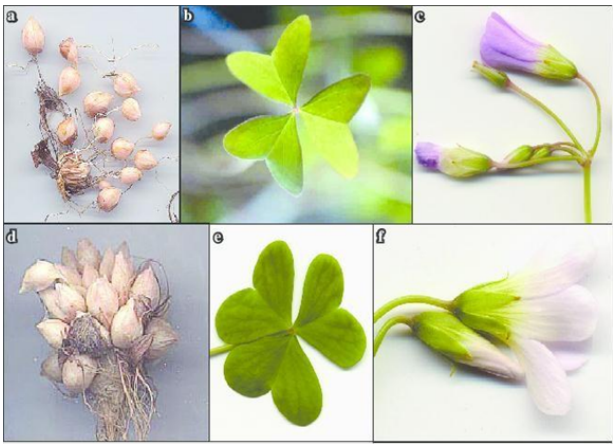

Figure 1: Macroscopic characters of the Oxalis latifolia L

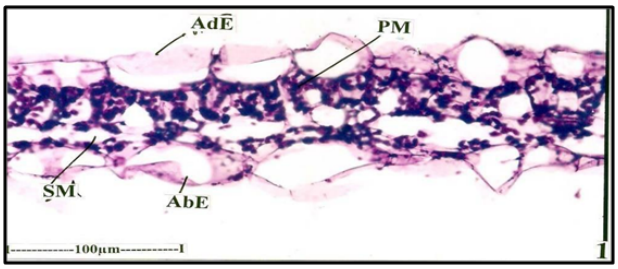

Figure 2: T.S of the lamina

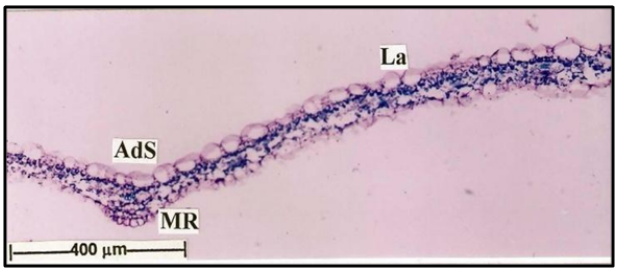

Figure 3: T.S of leaf margin

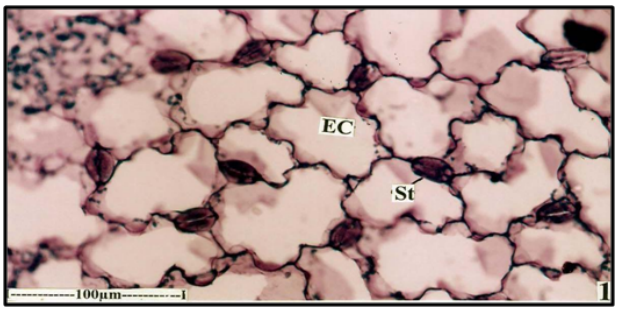

Figure 4: Abaxial epidermis with stomata [Ec-Epidermal cells; St-Stomata]

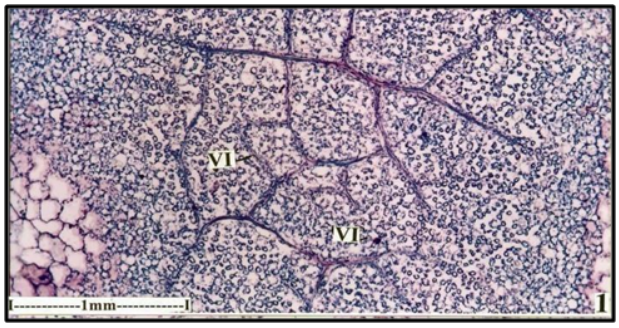

Figure 5: Vein islets and vein termination [VI-vein islets; VT- vein termination]

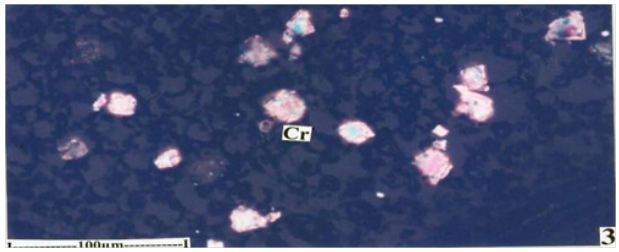

Figure 6: Crystals in the mesophyll tissue [Cr-Crystal]

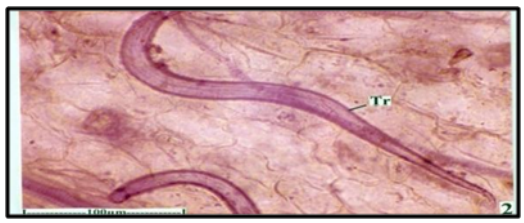

Figure 7: Covering Trichome Enlarged

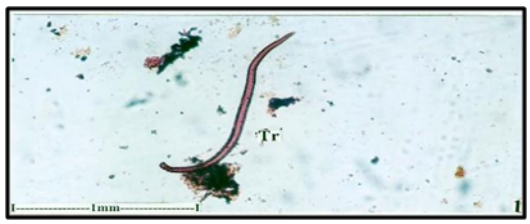

Figure 8: Trichomes in the leaf powder's

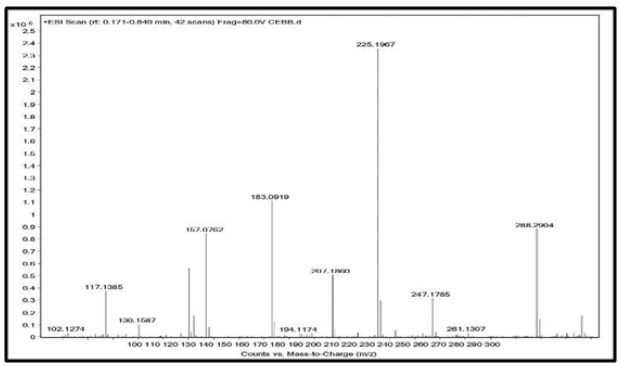

Figure 9: Mass Spectrum of isolated compound(Positive)

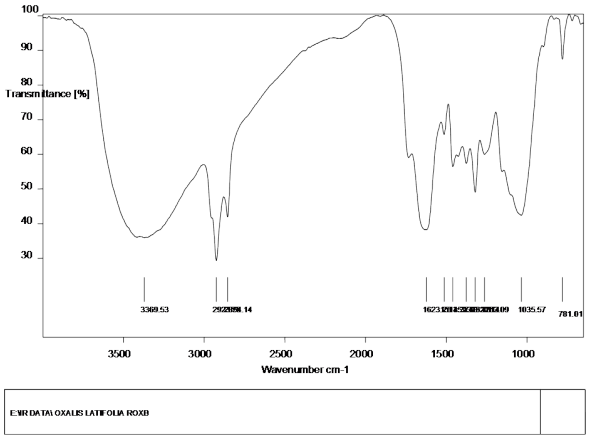

Figure 10: FTIR of Methanolic Extract of OL (MEOL)

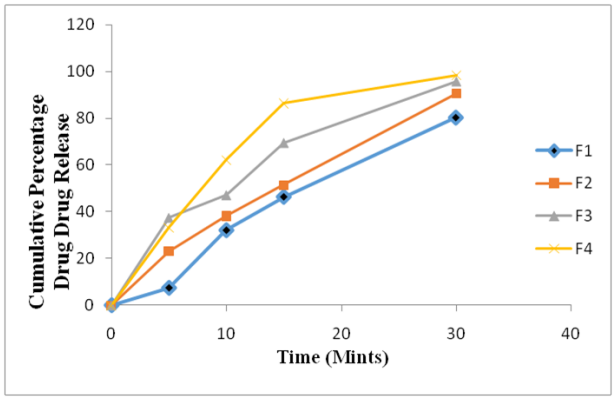

Figure 13: Dissolution Profile of Latifolin A capsules

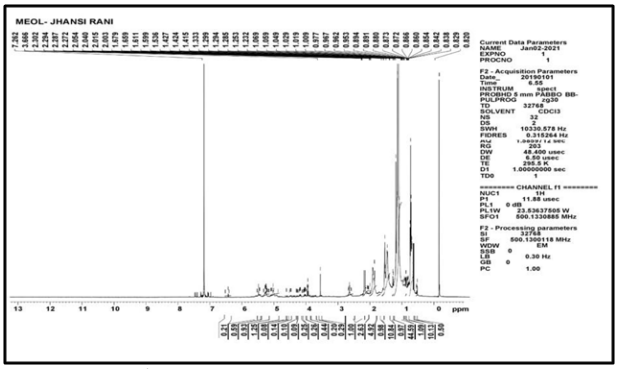

Figure 11: ^{1}HNMR Spectra of Isolated Constituent

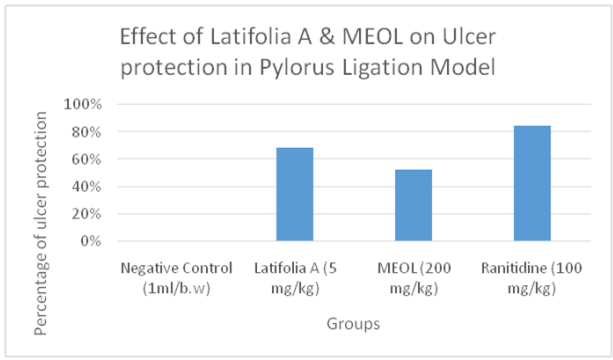

Figure 14: Effect of Latifolin A & MEOL on Ulcer protection in Pylorus Ligation Model

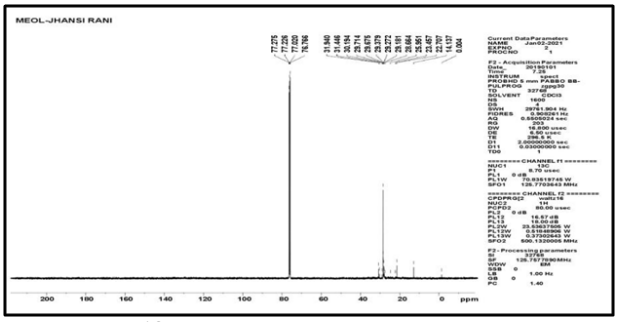

Figure 12: ^{13}CNMR Spectra of Isolated Constituent

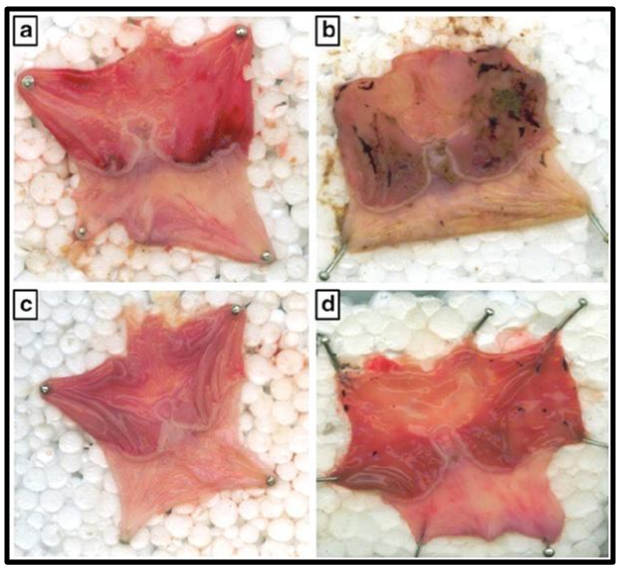

Figure 15: Histological studies of stomach rats in pylorus ligation method (a) Standard (Ranitidine); (b) Negative control; (c) Latifolia A (5mg/kg);
(d) MEOL (200mg/kg)

with thin walls. Additionally dilated and with thin walls are the abaxial epidermal cells. The vascular strand is made up of a collection of angular, thin-walled, narrow vessels. Xylem components have an 8 m width. The palisade tissue is transcurrent over the vascular bundle and beneath the adaxial epidermis, and these phloem parts are found at the lower end of the xylem strand.

T.S of the lamina

The epidermal layers of the leaf blade are thick and

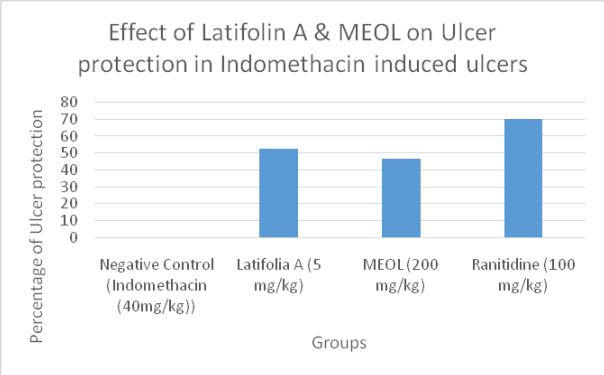

Figure 16: Effect of Latifolin A & MEOL on Ulcer protection in Indomethacin induced ulcers

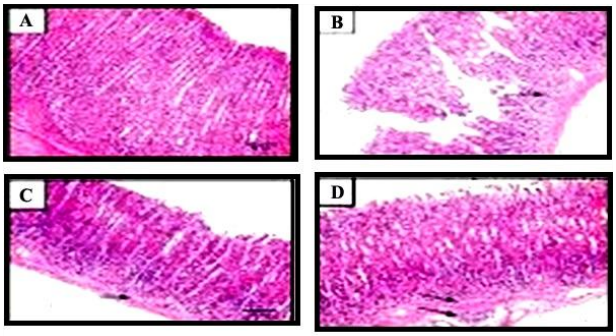

Figure 17: Histological studies of stomach rats in pylorus ligation method (a) Standard (Ranitidine); (b) Negative control; (c) Latifolin A (5mg/kg); (d) MEOL (200mg/kg)

dorsiventrally thin. Lamina is around 100 m wide. The adaxial and abaxial epidermal layers are both quite extensive and contain circular cells with thick walls that measure 25 m in thickness. The four layers of tiny, lobed spongy parenchyma cells observed in Figure 2 and the narrow adaxial zone of short, then cylindrical palisade cells make up the mesophyll tissue.

T.S of the leaf margin

The leaf margin is slightly narrow leaflet and posses circular thin-walled cells.

They are 25μmin diameter the mesophyll tissues areas in the middle portion of the lamina seen in Figure 3.

Epidermal morphology

Epidermal cells and stomata

The anticlerical walls of the epidermal cells are very wavy and have thin walls, giving the cells an amoeboid appearance.

According to Figure 4, stomata are only present in the lower epidermis and absent from the top 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$\times$20μm in size. The adaxial epidermal cells are similar to the abaxial cells in shape and size; but it is apostomatic (without stomata).

Venation pattern

Paradermal section displaying the dispersion of crystals and the venation pattern Venation style.

The lateral veins and vein islets are straight and uniformly thin, made up of one or two spiral xylem components. They develop into broad, rectangular, multi-sided vein islets with clearly marked vein terminations. which, as depicted in Figure 5, are long, slender, and unbranched or only once or twice.

Crystals in the mesophyll tissue

Crystals

The mesophyll tissue frequently contains calcium oxalate crystals. The majority of the crystals are sphere crystals or druses. They are dispersed widely and are seen in typical mesophyll cells, as seen in Figure 6. The crystals can reach a width of 20 m.

Powder microscopy of the whole plant

Leafpowder

Lamina fragments are seen in leaf powder together with venation, trichomes, solitary trichomes, and epidermal peeling. Lamina fragments also display epidermal trichomes on the lamina surface and along the leaf margin.

Non-glandular covering trichome 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 as seen in Figures 7 and 8. The Stomata Are Anomocytic Type.

Non-glandular coating [Tr-Trichome]

Preliminary Phytochemical screening

Powdered OL was extracted with various solvents such as Pet.ether, Ethylacetate, Chloroform, 70% methanol and water and screened for various phytochemical constituents which were briefly depicted in Table 2.

From the preliminary studies, it was evident that methanolic extract of Oxalis latifolia kunth has high phytochemicals. This extract was selected for further studies.

TLC fingerprinting of Methanolic extract of Oxalis latifolia kunth

Mass spectrum of isolated compound

The HR-FAB-MS (positive mode), which is seen in Figure 9, revealed a quasi-molecular ion peak at m/z 595.1459 compatible with the molecular formula C30H26O13, from which the molecular formula was inferred. Further information regarding the structure was provided by the EI-MS fragmentation pattern, which included significant fragments at m/z 286 [M-p-hydroxycinnamoyl-glucose]+ and 486 [M-p-hydroxycinnamoyl]+. Additionally, Diels Alder fragmentation resulted in the existence of pieces at m/z 152 and 442, which confirmed the presence of two hydroxyl groups in ring A and the remaining constituents in ring B.

FTIR of Methanolic Extract of OL

The FTIR of MEOL was shown in Figure 10.

NMR studies of methanolic extract of Oxalis latifolia kunth

The ^{I}H-NMR spectrum of showed a characteristic downfield signal at δ 12.81 assignable to a chelated hydroxyl group at C-5. Two meta coupled hydrogens of ring A were observed as doublets (J=2.0 Hz) at δ 6.25 and 6.53 shown in Figure 11. The three aromatic protons of the tri-substituted ring B resonated at δ 7.81 (1H d, J=2.3 Hz), 7.81 (1H d, J=8.2 Hz) and 7.45 (1H dd, J=8.3, 2.3 Hz).

Thirty signals made up of one methylene, seventeen methines, and twelve quaternary carbons were seen in the 13C-NMR spectra. According to Imperato et al. (1997), the signals at 158.4, 106.1, 178.2, 161.6, and 104.2 corresponded to flavone moiety C-2, C-3, C-4, C-9, and C-10. Additionally, it displayed indications for olefinic carbons at 117.7 and 144.4 as well as an ester carbonyl at 169.1. As illustrated in Figure 12, three oxygenated aromatic carbons resonated at 164.8, 157.2, and 149.2. A hexose moiety's anomeric carbon was measured at 100.3, while its oxymethine and oxymethylene carbons were measured between 75.3 and 62.3.

Latifolin A granules

Latifolia A was assessed using a variety of evaluation criteria, including bulk and tapped density, Hausner ratio, Carr's index, and Angle of repose, which indicate the granules' weight homogeneity and flow characteristics when used to fill capsules. Table 3 showed all of these manufactured capsules' characteristics. The bulk and tapped densities of the granules formed showed little change. Hausner's ratio, which ranged from 1.08 to 1.11, indicated good flow characteristics. Compressibility indexes under 15% are thought to have excellent flowability, whereas those above 25% are thought to have poor flowability. Because all preparations had Carr's indexes 15% lower and angles of repose less than 30, granules had good flow characteristics. The granules' compressibility index ranged from 8.55 to 17.98% and from 27.56 to 29.96o, respectively, in all of the formulations, demonstrating good flow qualities.

Weight variation and disintegration time

Drug content is assessed using a weight variation test to ensure consistent drug distribution. A disintegration test is run to see if capsules dissolve in the allotted amount of time when placed in a liquid medium under test circumstances. I.P. 2010 claims that Latifolia formulations Because the weight deviation of each capsule from the mean was determined to be 7.5%, a move was made to examine weight variance. In more than 85% of the capsule formulations, Latifolia A was present. Disintegration times for Formulations F1, F2, F3, and F4 were 3.5, 3.2, 2.8, and 2.2 mins, respectively, suggesting that as SSG content increased, so did the disintegration rate, which was listed in Table 4.

Dissolution test of Latifolin A capsules

Dissolution tests analyse the degree and pace of solution formation from a dosage form, such as a tablet, capsule, or ointment. These tests are crucial for determining a formulation's bioavailability and efficiency. The amount of SSG, as shown in Table 5 and Figure 13, was found to affect both the rate and amount of drug release from formulations F2, F3, and F4. As a result, the properties of the release were significantly influenced by the concentration of the super disintegrants used. The formulation F4 has been chosen as the best choice for the next study because of its high bioavailability at 5, 10, 15, and 30 minutes.

Acute toxicity studies of MEOL

The aforementioned research were conducted in accordance with OECD- 423 recommendations. OECD recommendations provide for a process that uses three rats at each stage. Three identically aged male albino rats were weighed and given the maximal dose of MEOL extract, or up to 2000 mg/kg/bw orally. The extracts were taken into consideration for further pharmacological testing because the treated animals did not exhibit any toxic symptoms of death up to 2000mg/kg/b.w. Following the administration of the extract, the measured body

weight, behavioural screening, and mortality were recorded for 4 hours continuously, then every 4 hours, then every 24 hours, and ultimately every 14 days. Low dose of extract was produced by p.o., $LD_{50}/10$.

Pyloric ligation induced gastric ulceration

Gastric pylorus ligation alone treated rats increased gastric acid and also resulted in ulcer formation on gastric walls. As a result gastric pH decreased, gastric volume, free & total acidity all increased when compared with Latifolia A & MEOL group ($p < 0.01$) and was provided in Table 6. Contrarily, noteworthy elevation was noted for gastric pH, suppression of gastric volume, free & total acidity was seen after administration of Latifolia A (5mg/kg) & MEOL (200mg/kg) in experimental model after inducing ulcers with gastric pyloric ligation and compared to standard drug (Ranitidine), indicating the efficiency of the Latifolia A which was better than MEOL (200mg/kg) in reducing ulcer index and increase in ulcer protection shown in Table 7 & Figure 14. Further, the above results were supported by histological studies shown in Figure 15.

Indomethacin induced ulceration

Indomethacin alone treated rats increased gastric acid and also resulted in ulcer formation on gastric walls. As a result ulcer index increased and decreased ulcer protection when compared with Latifolin A& MEOL group ($p < 0.01$) and was provided in Table 7. Contrarily, noteworthy decrease in ulcer index and an increase in ulcer protection was seen in Latifolia A, MEOL (200mg/kg) & Ranitidine (100mg/kg) as standard. The efficiency of Latifolin A was better than MEOL (200mg/kg) in reducing ulcer index and increase in ulcer protection shown in Table 8 & Figure 16. Further, the above results were supported by histological studies shown in Figure 17.

DISCUSSION

The stemless herb Oxalis latifolia Kunth, which is a member of the Oxalidaceae family, is widely distributed around the world and can be found in large quantities in lawns, gardens, and other outdoor areas. Dark green, charactaristic-smelling, and astringent-tasting leaves. Pseudo Umbels, axiallary, 1-6 flowered, bracts two, linear, bracteole, Sepals, five lanceolate, petals oblanceolate apex, emarginated; leaves digitately 3-foliate; leaflets obcordate, chartaceous; pilose base; cuneate; margin whole; apex; emarginiate.

The leaf blade has thick epidermal layers with a dorsiventral pattern that is thin and dorsiventral in nature. Only the lower epidermis has stomata; the upper epidermis lacks them. The type of stomata is anomocytic. The mesophyll tissue frequently contains calcium oxalate crystals.

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.

The Oxalis latifoliaKunth, Oxalidaceae, Methanolic extracts contained carbohydrates, flavonoids, saponins, and tannins, according to a qualitative phytochemical examination. The existence of flavonoids in the Oxalis latifolia kunth extract was confirmed, and it has been shown that flavonoids have antiulcerogenic effect. So perhaps the flavonoids' considerable improvement over the usual medication has lowered the ulcerogenic action here.

Oxalis latifolia's methanolic extract (MEOL) was used to isolate Latifolia A. FTIR and NMR tests were performed on latifolin A [17]. All of the criteria for the isolated chemical capsules fell within the acceptable range. Carr's index, bulk and tapped densities, and angle of repose were examined parameters. Four formulations (F1-F4) were also assessed for invitro dissolution and disintegration. In order to evaluate the antiulcer activity against pyloric ligation and indomethacin-induced ulcers, F4 was chosen as the best formulation.

Pylorus ligation increases gastric pH [18]. Upon treatment with Latifolia A, MEOL and Ranitidine gastric pH was reduced including other parameters.

Indomethacin and other non-steroidal anti-inflammatory medicines (NSAIDs) are known to cause stomach damage, particularly when the cyclooxygenase pathway of arachidonic acid metabolism is inhibited. Inhibition of cyclooxygenase 1 (COX-1) is thought to be the cause of the NSAIDs' ulcerogenic effects, while its isoform, cyclooxygenase 2 (COX-2), is thought to play a pathogenic role in inflammation, pain, and fever. Numerous investigations have demonstrated that indomethacin significantly lowers mucosal PGE2 levels and that gastric mucosal prostaglandin (PGs), mostly generated by COX-1, play a crucial role in preserving gastric mucosal integrity [19]. Latifolia A & MEOL were effective in treating ulcers, but Latifolia A is best in both models, i.e., pylorus ligation and indomethacin-induced ulcers. Ranitidine was highest for ulcer protection, followed by Latifolia A capsules, and then MEOL (200mg/kg). This may be an interference with interactions in the isolated

chemical as opposed to the raw MEOL extract.

CONCLUSION

From our study, it was concluded that Oxalis latifolia kunth has an active constituent Latifolia A which can be formulated as a formulation according to patient compliance. Latifolia A to be potent anti-ulcerogenic agent which can be further elucidated for molecular mechanisms involved in treating ulcers.

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Conflict

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