IJPSR (2012), Vol. 3, Issue 04 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 December, 2011; received in revised form 28 January, 2012; accepted 21 March, 2012

PHARMACOGNOSTICAL, PHYTOCHEMICAL EVALUATION AND ANTIINFLAMMATORY ACTIVITY OF STEM OF *KALANCHOE PINNATA* PERS.

Om Satyam Chaturvedi*, Amit Joshi and Bal Krishna Dubey

Department of Pharmacognosy, TIT-College of Pharmacy, Anand Nagar, Bhopal – 462021, Madhya Pradesh, India

Keywords:
Kalanchoe pinnata pers. stem, Pharmacognostical study, Phytochemical study, Anti-inflammatory activity

Correspondence to Author:

Om Satyam Chaturvedi

Department of Pharmacognosy, TIT-College of Pharmacy, Anand Nagar, Bhopal – 462021, Madhya Pradesh, India

ABSTRACT

Kalanchoe pinnata (Crassulaceae) commonly known as Jakh me hayat. *Kalanchoe pinnata pers* is present naturalized throughout the hot and moist parts of India, particularly common in Bengal. The plant is antibacterial, Anticancer, Antiparasitic, Anti-insecticidal, Anti-allergic. Phytochemical studies had revealed the presence of alkaloids, flavonoids, triterpenes, glycosides, steroids and lipids. Present study was carried out to determine, the morphological, microscopical and phytochemical profiles. Microscopy of stem show endodermis cortex, collateral types of vascular bundle and lignified xylem vessels, etc. The physical parameter such as ash value and extractive value were evaluated. The anti-inflammatory effect of 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4h H chromen-4-one isolated from the medicinal herb *Kalanchoe pinnata pers* stem, was evaluated for its anti-inflammatory activity in the Swiss albino-induced rat paw oedema models of acute inflammation. Flavonoid at doses of 200 and 400 mg/kg, the clinical anti-inflammatory indomethacin at 10 mg/kg, or vehicle were administered orally before injection of the pro-inflammatory compound. The test compound showed significant anti-inflammatory activity against paw edema induced by carrageenin, most notably at the highest test dose of 400 mg/kg. In these assay, the flavonoid compound was more active at 400 mg/kg.

INTRODUCTION: India is one of the richest floristic regions of the world and has been a source of plants and their products, since antiquity, man uses them in different way according to his needs, particularly as food and medicine. Among the entire flora, 35000 to 70000 species have been used for medicinal purposes [1].

Kalanchoe pinnata pers. is a succulent herb upto 1.2 m in height with obtusely 4-angled stems, younger parts reddish speckled with white; leaves opposite, decussate, the lower usually simple,the upper usually 3-7 foliolate, long-petioled, petioles united by a ridge round the stem,crenatures at the extremities of the lateral nerves furnished with rooting vegetative buds; flowers reddish purple, pendent, in large spreading panicles; fruits membranous follicles enclosed in persistent papery calyx and corolla, seed smooth,ellipsed.

The leaves are astringent, sour, and sweet, refrigent, emollient, mucilaginous, haemostatic, vulnerary, depurative, constipating, anodyne, carminative, anti-inflammatory, disinfectant and tonic. They are useful in vitiated conditions of *pitta* and *vata,* heamatemesis, haemorrhoids, menorrhagia, cuts and wounds, discoloration of the skin, boils, sloughing ulcers, ophthalmia, burns, scalds, corn, diarrhea, dysentery,, vomiting and acute inflammation [2].

Organoleptic evaluation of drugs refers to the evaluation of drugs by color, odor, size, shape, taste and special features including touch and texture etc. Organoleptic evaluations can be done by means of organs of special sense which includes the above parameters and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity [3].

Phytochemical evaluation of drug refers to determine quality and purity, such as through Ash value it refers to the adulterants and impurity. Extractive value shows to estimation of specific constituents, soluble in that particular solvent used for extraction. Moisture content the moisture requirements for enzymatic activity and that which microorganisms demand, vary hot only with species, but also with other environmental factor [4].

Inflammation is a physiological reaction to injury or to infectious, allergic, or chemical irritation. Inflammatory processes are complex biochemical phenomena characterized by tissue edema, pain, and leukocyte infiltration [5].

The most common clinical treatments for inflammatory diseases are non-steroidal or steroidal anti-inflammatory compounds [6].

The use of steroidal drugs as anti-inflammatory agents is now controversial, however, due to their multiple side effects [7].

MATERIALS AND METHODS: Stems were collected from local area of Bhopal, Madhya Pradesh, India, in November-December. The plant was identified and authenticated by Dr. ZIia Ul Hasan, HOD**,** Department of Botany, Saifia Science College Bhopal. Prepared herbarium was submitted and the plant was certified as *Kalanchoe pinnata.* Specimen no.-133/Bot/Saifia /11. Croton oil, indomethacin and other material were purchased from PBRI Lab.

Chemicals and instruments: Compound microscope, Camera lucida (mirror type), Stage and eye piece micrometer and other basic equipments and glass wares are used for the present study. Solvents like Methanol, Acetone, Petroleum ether, Water and for activity Croton oil, indomethacin is also used.

Pharmacognostical Studies:

Morphological Studies: The shape, size, color, taste and odour of stems were determined.

Microscopical Studies : Microscopic studies were done by preparing free hand sections of stems. The sections of the stem was cleared with chloral hydrate solution, and stained with safranin and mounted in glycerin. Powders (#60) of the roots were used for observation of powder microscopical characters. The powdered drug was separately treated with Phloroglucinol, Hydrochloric acid solution, glycerin, to determine the presence of lignified cells etc.

Phytochemical Studies: The powder stems of *Kalanchoe pinnata* determined the Ash value, Extractive value, Phytochemical screening [8, 9, 10, 11].

Determination of Ash Values:

Total Ash: To determine the total ash, place about 2 gm of ground air dried drug, accurately weight in a previously ignited and crucible of silica. Spread the material in an even layer and ignite it by gradually increasing the heat to 500-600^{o}C until it is white, indicating the absence of carbon. Cool in a dessicator and weight. Then, we calculated the percentage of ash with reference to air-dried drug.

Acid Insoluble Ash: To determine the acid insoluble ash, boil the ash with 25 of dilute HCL for 5 minutes, collected the insoluble matter in a sintered glass crucible, washed with hot water, ignited, cool in a dessicator and weight. Then, we calculated the percentage of acid-insoluble ash with reference to the air dried drug.

Water Soluble Ash: To determine water soluble ash, total ash, boil with the 25 ml of water for 5 minutes. Insoluble ash was collected in a sintered glass crucible. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^{o}C cool and weight. Then, we calculated the percentage of water soluble ash with reference to the air dried drug.

Determination of Solvent Extractive Values: This method determines the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. For determination of solvent extractive values 5gm of the air dried, coarsely

powdered macerated with 100 ml of water close flask for 24 hours, shaking frequently during first 6 hours and allowing stand for 18 hours. Thereafter, filter rapidly taking precautions against loss of solvent; evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, at 105^{o}C and weight. The percentage of solvent soluble extractive with reference to air dried drug has to be calculated.

Phytochemical Analysis:

Test for Alkaloids: Two grams of ground plant material was treated in a test tube with 10 ml of 1% HCl for 30 minutes in a water bath. The suspension was filtered through cotton into a test tube and was divided into two parts. Five drops of each of Dragendroff's, Wagner's reagent, Mayer's and Hager's reagent were added to the respective parts of the solution and the formation of different color indicated the presence of alkaloids.

Tests for Carbohydrates: Molisch's, Fehling's and Benedict's tests were done to know the presence of carbohydrate.

Molisch's Test: Aqueous or alcoholic solution of the extracted substance was added to 10% alcoholic solution of naphthol. Equal volume of concentrated H2SO4 was added along the side of the tube. A violet ring at the junction of two liquids confirms presence of carbohydrates.

Fehling's Test: 2ml of Fehling's solution A and 2 ml of Fehling's solution B was added to 2ml of extracts. After boiling, if bricked precipitation appears, then reducing sugars are present.

Benedict's Test: 5ml of Benedict's reagent and 3ml of test solution boiled in the water bath. If brick red precipitate appears at the bottom of the test tube then monosaccharides are present.

Test for Cardiac Glycosides:

Keller-Killiani test: To an extract of the drug in glacial acetic acid, few drops of ferric chloride and conc. H_2SO_4 were added. If a reddish brown color is formed at the junction of two layer and upper layer turns bluish green, confirms the presence of cardiac glycosides.

Legal Test: To a solution of glycoside in pyridine, sodium nitroprusside solution and sodium hydroxide solution were added. A pink to red color, confirms presence of cardiac glycosides.

Modified Borntrager's Test: 0.1 g of the extract was added with 5ml of dilute HCl and 5ml of 5% solution of ferric chloride and boiled for 5 minutes, cooled and filtered. This filtrate was shaken with benzene. The benzene layer was separated and an equal volume of dilute solution of ammonia was added. Formation of pink color with the ammonical layer signifies presence of anthraquinone glycosides.

Test for Proteins and Amino Acids:

Biuret Test: 2ml of extract was mixed with 2 ml of 10% NaOH solution and 2 to 3 drops of 1 % Cupper sulphate solution was added. Appearance of violet or purple color indicates the presence of proteins.

Ninhydrin Test: 2 ml of extract was added with 0.5 ml of Ninhydrin solution. The mixture was boiled for 2 minutes and then the solution was cooled. Appearance of blue color shows the presence of amino acid.

Xanthoproteic Test: 2 ml. of extract was added with 1 ml of conc. HNO_3, boiled and cooled. Then 40% NaOH was added drop by drop. Appearance of colored solution indicates the presence of proteins.

Test for Saponins:

Foam test: 1ml of alcoholic and aqueous extract was diluted separately with distilled water to 10 ml and was shaken in a graduated cylinder for 15 minutes and kept aside. One cm layer of foam after standing for 30 minutes indicates the presence of saponin.

Test for Tannins and Phenolic Compounds:

With Ferric Chloride: 5% w/v solution of ferric chloride was added in 90% alcohol. Appearance of blue color is the indication of presence of phenols.

With Lead Acetate: Extracts were mixed with lead acetate. Occurrence of precipitate is the indication of presence of tannins.

With Gelatin Solution: To a solution of tannins (0.5-1%) aqueous solution of gelatin (1%) and Sodium chloride (10%) are added. Appearance of white buff colored precipitated is the indication of presence of phenols.

Test for Steroid and Sterols:

Lieberman Burchard Reagent: To a dry test tube, 2 ml of extract solution was mixed with 2ml of acetic anhydride and 2-3 drops of conc. H_2SO_4 was added. The solution was mixed thoroughly. An emerald green color development is the indication of presence of steroids or sterols.

Salkowski's Test: 5ml of solution of extract was taken in a dry test tube with chloroform. Equal volume of conc. H_2SO_4 was added gently along the sides of the test tube. The acid layer develops a yellow color with a green fluorescence and the chloroform layer will give a play of colors first from bluish red to gradually violet red.

Test for Flavonoids:

With NaOH: The extract were dissolved in water, and then filtered. The filtrate was treated with sodium hydroxide. Yellow color is observed if flavonoids are present

Legal Test: To a solution of glycoside in pyridine, sodium nitroprusside solution and sodium hydroxide solution were added. A pink to red color, confirms presence of cardiac glycosides.

Modified Borntrager's test: 0.1 g of the extract was added with 5ml of dilute HCI and 5ml of 5% solution of ferric chloride and boiled for 5 minutes, cooled and filtered. This filtrate was shaken with benzene. The benzene layer was separated and an equal volume of dilute solution of ammonia was added. Formation of pink color with the ammonical layer signifies presence of anthraquinone glycosides.

Test for Proteins and Amino Acids:

Biuret test: 2ml of extract was mixed with 2 ml of 10% NaOH solution and 2 to 3 drops of 1 % Copper sulphate solution was added. Appearance of violet or purple color indicates the presence of proteins.

Ninhydrin Test: 2 ml of extract was added with 0.5 ml of Ninhydrin solution. The mixture was boiled for 2 minutes, and then the solution was cooled. Appearance of blue color shows the presence of amino acid.

Xanthoproteic Test: 2 ml. of extract was added with 1 ml of conc. HNO3, boiled and cooled. Then 40% NaOH was added drop by drop. Appearance of colored solution indicates the presence of proteins.

Test for Saponins:

Foam Test: 1ml of alcoholic and aqueous extract was diluted separately with distilled water to 10 ml and was shaken in a graduated cylinder for 15 minutes and kept aside. One cm layer of foam after standing for 30 minutes indicates the presence of saponin.

Test for Tannins and Phenolic Compounds:

With Ferric Chloride: 5% w/v solution of ferric chloride was added in 90% alcohol. Appearance of blue color is the indication of presence of phenols.

With Lead Acetate: Extracts were mixed with lead acetate. Occurrence of precipitate is the indication of presence of tannins.

With Gelatin Solution: To a solution of tannins (0.5-1%) aqueous solution of gelatin (1%) and Sodium chloride (10%) are added. Appearance of white buff colored precipitated is the indication of presence of phenols.

Test for Steroid and Sterols:

Lieberman Burchard Reagent: To a dry test tube, 2 ml of extract solution was mixed with 2ml of acetic anhydride and 2-3 drops of conc. H_2SO_4 was added. The solution was mixed thoroughly. An emerald green color development is the indication of presence of steroids or sterols.

Salkowski's Test: 5ml of solution of extract was taken in a dry test tube with chloroform. Equal volume of conc. H_2SO_4 was added gently along the sides of the test tube. The acid layer develops a yellow color with a green fluorescence and the chloroform layer will give a play of colors first from bluish red to gradually violet red.

Anti-inflammatory Activity:

Animals: Male and female Sprague Swiss albino mice, 42 to 45 days old, were obtained from the Laboratory Animal Center, PBRI (Pinnacle Biomedical Research Institute) Bhopal. The animals were quarantined and acclimatized for a week before the treatment. Rats of either sex, weighing 30-40 g, were randomly selected and assigned to treatment and control groups using a randomization process. The animals were housed in

autoclaved polyethylene cages (4 rats in each group per cage) and maintained on a 12:12 h light: dark cycle. The temperature and relative humidity of the animal room were maintained at 23 ± 2°C and 50-70 %.

Acetic acid induced Vascular Permeability: Animals of either sex were divided into four groups of six animals each. The first group (negative control) received the vehicle only (distilled water). Animals of the second were treated orally with the standard drug indomethacine, third and fourth groups were treated orally with the isolated plant extract (stem) at doses of 200 and 400 mg/kg respectively. Thirty minutes after drug administration, edema was induced by the injection of 0.1ml/10kg body weight of 1 % Evans blue solution was injected intravenously in each mouse.

Thirty minutes later, 0.1ml/10g body wt. of 0.7% acetic acid with saline was intraperitoneally injected. Thirty minutes after the administration of acetic acid, the mice were killed by cervical dislocation. The peritoneal cavity was washed with 10 ml of saline and the solution were centrifuged at 1000rpm for 10 min. for eliminate the contaminant. The concentration of Evans blue in the peritoneal cavity was measured by the absorbance at 630 nm in a spectrophotometer. The vascular permeability was represented in terms of the absorbance (A630) which leaked into the cavity. Experiments were performed in triplicate [12].

$$\text{Anti- inflammatory \% inhibition} = \frac{\text{Abs. Control - Abs. Test}}{\text{Abs. Control}} \times 100$$

Croton Oil induced Ear Edema: The experiment was performed using a slight modification of the procedure described by Tonelli *et al***.** An irritant solution was prepared by dissolving 4 parts croton oil in a solvent mixture of 10 parts ethanol, 20 parts pyridine, 66 parts ethyl ether. Stem extract and Indomethacin were dissolved in the same vehicle (irritant).

Group I- served as control group and applied vehicle (irritant) 20 µl topically on inner side of the right ear.

Group II- served as standard, received indomethacin at a dose of 12.5% (w/v) 20 µl topically on inner side of the right ear.

Group III-IV- served as test, received isolated plant extract at a dose of 200 and 400 mg/kg. 20µl topically on inner side of the right ear.

The left ear was kept untreated to serve as control. One hr. later group I & II received croton oil solution. After 4 hours, animals were decapitated. A 8mm cork borer was used to punch out discs from both the treated as well as control ears. The punches were weighed immediately after decapitation and the difference in weight was using to asses the inflammatory response [13].

RESULTS: Macroscopical studies of stems showed that slight green color, characteristic odour, bitter taste, tuberous and glabrous shape.

Microscopical studies showed the T.S. of stem of *Kalanchoe pinnata* and following tissues are observed (**fig. 1**).

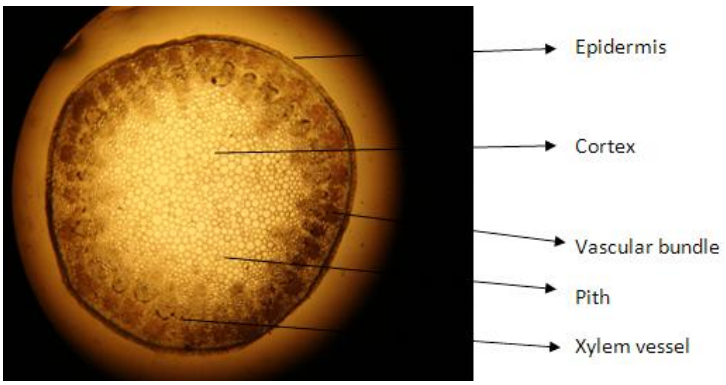

FIG. 1: T.S. OF STEM OF *KALANCHOE PINNATA*

Cortex: Endodermis cortex are present in the T.S. of stem of *Kalanchoe pinnata* (**fig. 2**). **Fig. 3** shows enlarged T.S. of cortex cells.

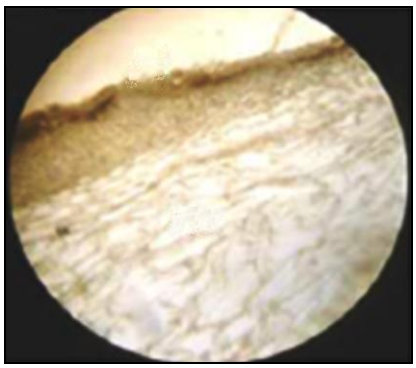

FIG. 2: T.S. SHOWING EPIDERMIS AND CORTEX

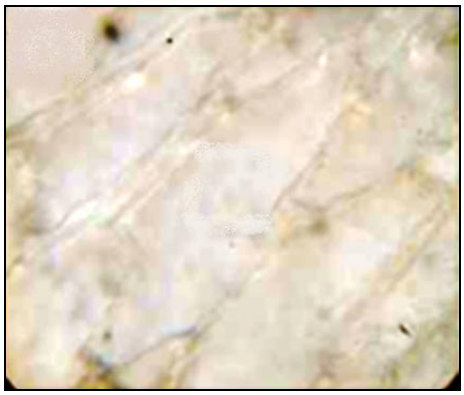

FIG. 3: T.S. ENLARGED SHOWING CORTEX CELLS

Vascular bundles: Collateral types of vascular bundles are present in the T.S. of stem of *Kalanchoe pinnata* (**fig. 4**).

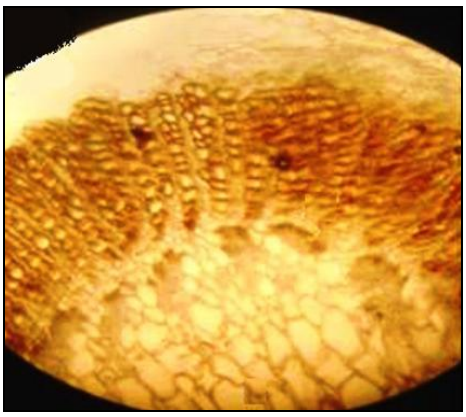

FIG. 4: T.S. SHOWING VASCULAR BUNDLE AND PITH

Xylem vessels: Lignified xylem vessels are present in the T.S. of stem of *Kalanchoe pinnata* (**fig. 5**).

FIG. 5: T.S. SHOWING VASCULAR BUNDLE AND PITH

Further analytical parameters like Ash value (**Table 1),** Extractive values (**Table 2**), phytochemical screening (**Table 3)** were carried out. The above studies enable the identification of the plant material for further investigations and form an important aspect of drug studies.

TABLE 1: ASH VALUES OF STEM OF KALANCHOE PINNATA

Parameter	Determined value % w/w
Total ash	10.5%
Acid insoluble ash	7.1%
Water soluble ash	2.1%

TABLE 2: EXTRACTIVE VALUES IN FOLLOWING SOLVENTS

Parameter	Determined value % w/w
Petroleum ether	11.3%
Methanol	15.2%
Water	10.1%

TABLE 3: PHYTOCHEMICALS IN METHANOLIC, ACETONE, PET. ETHER AND AQUEOUS STEM EXTRACT OF *KALANCHOE PINNATA*

Phytochemicals	Petroleum ether	Acetone	Methanol	Water
Alkaloids	-	-	-	-
Tannins	+	+	+	+
Steroids	-	-	-	-
Flavonoids	+	+	+	+
Glycoside	-	-	-	-
Saponins	-	-	-	+
Carbohydrates	-	-	-	-
Amino acid	-	-	-	-

(+) Present; (-) Absent

Result of Anti-inflammatory Activity: Flavonoids are important constituents of plants that exhibit a variety of beneficial effects on human health. The anti-inflammatory properties of various flavonoid glycosides have been studied in order to establish and characterize their potential utility as therapeutic agents for the treatment of inflammatory diseases [14, 15, 16]. In the present study, four different animal models were employed to evaluate of anti-inflammatory potential of stem extract of *Kalanchoe pinnata pers*.

The anti-inflammatory effect of the Quercetin flavanoid in the acetic acid induced vascular permeability model is shown in **Table 4** and the percent inhibition of inflammation is shown in **Fig. 7 & 8**. Our results revealed that Quercetin flavonoid possessed potent anti-inflammatory activity against the acute phase of inflammation. The experimental group with the highest test dose (400 mg/kg) exhibited a significant anti-inflammatory effect, starting after 3 h ($p < 0.05$); the activity was less than observed for the standard drug indomethacin (10 mg/kg). The experimental groups with lower doses exhibited less inflammation than the negative control but more than the positive control.

TABLE 4: ACUTE ANTI-INFLAMMATORY ACTIVITY OF THE TEST COMPOUND IN THE ACETIC ACID INDUCED VASCULAR PERMEABILITY MODEL % INHIBITION AND EVANS BLUE CONCENTRATION (µg/ml)

Samples	Evans blue concentration (µg/ml)			%Inhibition
Isolated extract (200mg/kg)	22.431±1.426	--	--	48.8
Isolated extract (400mg/kg)	17.321±1.284	--	--	50.86
Standard	--	23.654±0.745	53.39	--
Vehicle	--	50.75±0.618	--	--

Values are expressed as mean ± SD (n = 4). *p<0.05 compared to the negative control

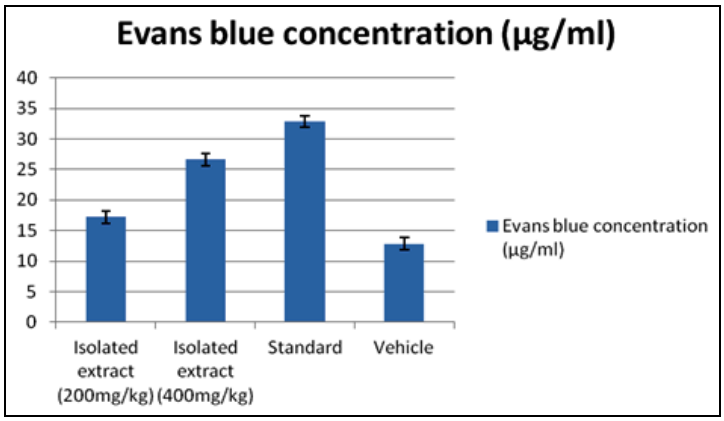

FIG. 6: EVANS BLUE CONCENTRATION

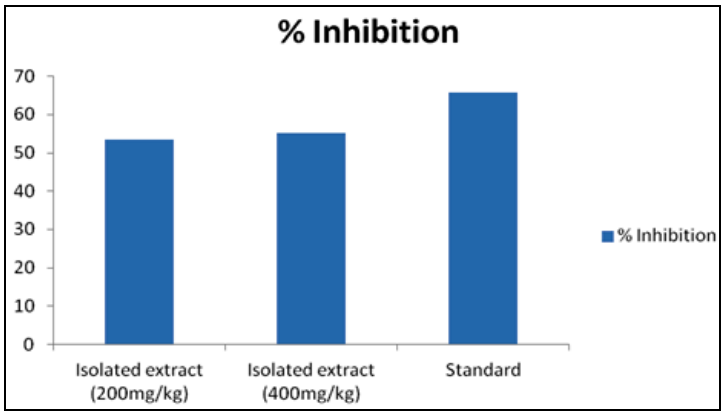

FIG. 7: % INHIBITION OF VASCULAR PERMEABILITY

Following Evans blue solution injection, there was a sudden elevation of vascular permeability compared to histamine and serotonin injection [17], after which increased vascular permeability was maintained by the release of kinins up to 2.30 h. From 2.30 to 6 hr after Evans blue solution, the inflammatory mediators appeared to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site [18]. It had been demonstrated that flavone glycoside is able to inhibit the enzymes responsible for prostaglandin synthesis, as well as other mediators of the inflammatory process. The anti-inflammatory effect of the Quercetin flavanoid in the Acetic acid induced vascular permeability model is shown in Table 4, and the percent inhibition of inflammation is shown in **Table 5**. The experimental group receiving the highest test dose (400 mg/kg) exhibited a significant anti-inflammatory effect, starting 3 h after Evans blue solution injection ($p < 0.05$). Again, the inflammatory response was lower than observed for indomethacin at 10 mg/kg, while the experimental groups receiving lower doses showed less inflammation than the negative control but more than the positive control (**fig. 8 & 9**).

Percentage of inflammation and inhibition at different times for both genders of control and treated rats were not significantly different. The Quercetin flavanoid mediates these anti-inflammatory actions by either inhibiting the synthesis, release, or action of inflammatory mediators involved in inflammation.

TABLE 5: ACUTE ANTI-INFLAMMATORY ACTIVITY OF THE TEST COMPOUND IN THE CROTON OIL INDUCED EAR EDEMA MODEL VOLUME OF EDEMA (mg) AND % INHIBITION

Samples	Dose (mg)	Volume of edema	%Inhibition
Isolated extract	200	3.33±0.816	54.8
Isolated extract	400	2±0.632	63.2
Standard	200	1.5±0.548	81.6
Vehicle	--	9.333±1. 211	--

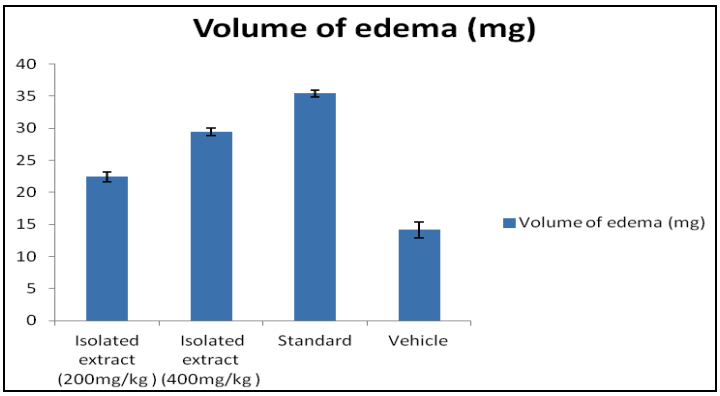

FIG. 8: VOLUME OF EAR EDEMA

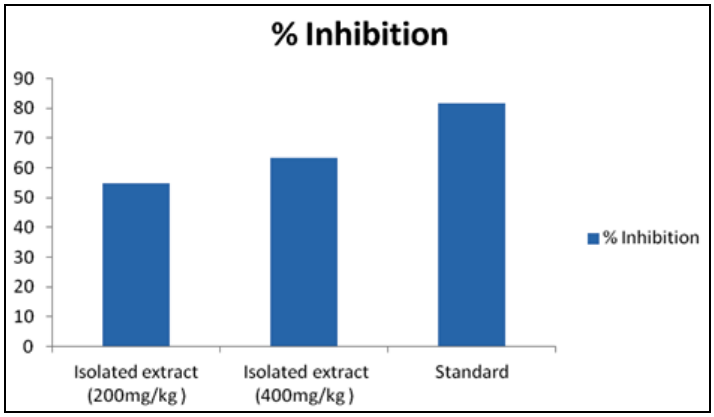

FIG. 9: % INHIBITION OF VOLUME OF EAR EDEMA

In order to assess the test compound's efficacy against the later proliferative phase of inflammation caused by tissue degeneration and fibrosis, we used the Croton Oil Induced Ear Edema. Table 5 shows the effect of the flavone glycoside on Croton Oil Induced Ear Edema formation in rats. Maximal inhibition (63.2 %) of Ear Edema formation was observed at a dose of 400 mg/kg ($p < 0.05$), and this effect was less than that of indomethacin at 10 mg/kg (41.7 % inhibition). Treatment groups with lower doses of the flavone glycoside showed a smaller anti-inflammatory effect than the positive control.

Percentage of inhibition for both genders of control and treated rats were not significantly different. In Croton Oil Induced Ear Edema model, inflammation and granuloma develop over a period of several days. This model tests bio-activity against the proliferative phase of inflammation. This later phase of inflammation involves the proliferation of macrophages, neutrophils, and fibroblast, and multiplication of small blood vessels, which are the basic sources of the highly vascularized reddish mass termed granulation tissue [19].

Hence, a decrease in the granuloma weight indicates a suppression of the proliferative phase mediate by the flavone glycoside.

CONCLUSION: The report of pharmacognostical evaluation of *Kalanchoe pinnata.* The Pharmaco-gnostical studies include macroscopical, microscopical, proximate analysis like ash values, extractive values and other analysis gives valuable information about the plant. It is helpful for correct identification of this plant for the future reference. Taken together, the results strongly suggest that stem extract of *K. pinnata* possesses anti-inflammatory effects.

Further Research Scope: The plants have tremendous potential for further research.

ACKNOWLEDGEMENTS: I wish to extend my sincere thanks to Mr. Amit Joshi for providing encouragement and facilities for carrying out this work and Dr. B. K, Dubey, Director, TIT College of Pharmacy, Bhopal, for his excellent supervision and optimistic approach at every step of my dissertation work.

REFERENCE:

1. Ponnu, S, Santhi, D.K, Jacob, N & Suresh B; 2003; Safety measures with herbs; Indian pharmacist; 2:9-12.
2. Longman O., In: Kalanchoe pinnata in Indian Medicinal Plants, vol. 3, 282.
3. Evans WC. Trease and Evans's Pharmacognosy. 15th edn. London: WB Saunders: 2001. 343,383.
4. Dahanukar, S. A., Kulkarni, R. A. and Rege, N. N., Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.*, 2000, 32, S81–S118.
5. Vickerstaff JM, Bielory L. Type I hyper-sensitivity: atopic allergy. In Understanding Allergy Sensitivity and Immunity: A Comprehensive Guide. New Brunswick: Rutgers University Press, 1990, 126-36.
6. Rainsford KD. Anti-inflammatory drugs in the 21st century. Subcell Biochem 2007;42:3-27.
7. Schäcke H, Döcke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther 2002;96: 23-43
8. Dr. P.K. Mukherjee, Quality Control Herbal Drugs 1st edition reprint 2010 published by Business Horizons. New Delhi. 1342-134, 186-89,287-90.
9. Wallis T.E., 'Text book of Pharmacognosy' Fifth edition, reprint 2005, CBS Publication New Dehli. 578-81.
10. KR Khandelwal, Practical Pharmacognosy Techniques and Experiments, Nirali prakashan, Pune, 2002, 9th ed, 146-160.
11. CK Kokate, Practical Pharmacognosy, Vallabh Vrakashan, Delhi, 2008, 149-156.
12. Whittle B. A. *et al*., Studied on the use of changes in capillary permeability in mice to distinguish between narcotic & non-narcotic analgesics. 2002.
13. Tonelli *et al.* Anti-inflammatory activity of *C. dioscoroida*, received 21 june.
14. Gil B. *et al.* Effects of flavonoids on *Naja Naja* and human recombinant synovial phospholioases A2 and in-flammatory responses in mice. Life Sci 1994;54:PL333-8.
15. Rotelli AE. *et al,*. Comparative study of flavonoids in experimental models of inflammation. Pharmacol Res 2003;48:601-6.
16. Guardia T. *et al,.* Antiinflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Il Farmaco 2001; 56:683-7.
17. Lo TN. *et al*, Dextran and carrageenan evoke different inflammatory response in rat with respect to composition of infiltrates and effect of indomethacin. J Pharmacol Exp Ther 1982; 221:261-7.
18. Brito ARMS, Antonio MA. Oral anti-inflammatory and antiulcerogenic activities of a hydroalcoholic extract and partitioned fractions of *Turnera ulmifolia* (Turnera-ceae). J Ethnopharmacol 1998;61:215-28.
19. S, Nag Chaudhuri AK. Pharmacological studies of the anti-inflammatory profile of *Mikania cordata* (Burn) B.L. Robinson root extract in rodents. Phytother Res 1992;6:252-60.

****