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Evaluation of Anti- diarrheal activity of Dried leaves of Kalanchoe Pinnata Lam.

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ABSTRACT

Background:- Diarrheal disease is a major cause of morbidity and mortality throughout the world, particularly in developing countries. Currently available drugs are linked with adverse effects, contraindications, and risk of resistance. Traditionally, the Dried leaves concoction of *Kalanchoe Pinnata Lam.* is claimed to be used for diarrhea. However, the safety and efficacy of the leaf extract have not been scientifically approved yet. Therefore, the study was conducted to validate its antidiarrheal activity and safety profile in mice.

Method:- The extract was obtained by the maceration technique in 95% Ethanol and water. Phytochemical screening tests were done for secondary metabolites by using standard tests. The antidiarrheal activity of the test extract at the doses of 150 mg /kg and 250 mg /kg was evaluated by using castor oil-induced models in mice.

Result:- In an acute toxicity study, there were no visible signs of toxicity and mortality following a single oral administration of 2000 mg/kg. Phytochemical screening tests revealed the presence of of Ethanol extract showed presence of Alkaloids, Proteins, Carbohydrate, Phenols and Tannins, Flavonoids, Saponins, Glycosides, Triterpenoids. And Aqueous extract showed presence of Alkaloids, Proteins, Carbohydrate, Phenols and Tannins, Flavonoids. Saponins, Glycosides, Triterpenoids. The extract significantly prolonged the onset of diarrhea and reduced the weight of wet and total feces at Group of mice treated with castor oil (0.3 ml) are called disease control group showed a significant ($P<0.05$) increase the biochemical parameters. Group of mice which pre-treated with Loperamide (2 mg/kg orally) was standard group, expresses significant *($P<0.05$)* inhibition of increase the biochemical parameters group of mice which were pre- treated with Ethanolic Extract (150 and 250 mg/kg orally) demonstrate significant *($P< 0.05$)* effect of Ethanolic Extract on biochemical parameters.

Conclusion:- The study demonstrated that the test extract showed promising antidiarrheal activity.

Keywords: *Kalanchoe Pinnata* Lam., diarrhea, Herbal Medicine, Loperamide.

INTRODUCTION

HERBAL MEDICINE:- Herbal Medicine sometimes referred to as herbalism or botanical medicine is the use of herbs for their medicinal value. An herb is a plant part valved for its medicinal aromatic or qualities herb plants produce and contain a variety of chemical substance that act upon the body.[(1)] Herbal Medicine is the oldest form of Medicine known to mankind. It was the main stay of many early civilizations and still the most widely practiced form of medicine in the world today.[(2)] Herbs had been used by all cultures throughout history. It was an integral part of plant available to him. The plants provide food. Clothing, shelter and medicine much of the medicinal use of plants seems to have been developed through observations of wild animals and by trial and error.

As time went on, each tribe added themedicinal power of herbs in their area to its knowledge base. They methodically collected information on herbs in their area to its knowledge base. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, will into the 20th century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native people. The World Health Organization (WHO) defines traditional medicinal plants as natural plant materials which are used at least or in the absence industrial processing for the treatment of disease at a local or regional scale. Traditional herbal medicine has been used in developing and developed countries for thousands of years because it is natural and causes comparatively fever complications. Early medical history is consistent with the history of herbal medicine. The first books written about medicine were the first books written about plants, including the texts of the Ebers Papyrus, written 1500 BC, in which the names of many plants have been appeared. Different types of traditional drugs are widely used in Asia, Africa and Latin America to meet basic health needs. This use is growing rapidly in industrialized countries, which is often referred to as complementary and alternative medicine (CAM) to cover health systems, practices, and products are not presently considered to be part of conventional medicines. Across the world, among all the various traditional medical systems, traditional Chinese medicine (TCM) is currently the most popular, followed by Indian medicine. In Western countries, Oriental Medicine refers to Chinese, Japanese, and Korean medicines selected by immigrants from Korean, while "Asian medicine" often includes TCM, India (Ayurveda) and Tibetan medicine. Among all treatments in traditional medicine systems, medicinal plants are most frequently used.[3]

The bark of the cinchona tree contains quinine, which today is a widely prescribed treatment of for Malaria especially in countries that cannot afford to purchase the more expensive anti-Malarial Drugs produced by the pharmaceutical industry.[4]The essential difference between herbalism and conventional medicine is that, while in conventional medicine the most active constituent is extracted from the plant and then Synthesized in the laboratory to make the Drug, in herbal medicine extracts from the whole plant are used. The herbalist believes that the sum of the action of the whole plant is more balanced that then of any one of its main constituents within the plant will limit it. Where the active Constituent is less powerful. Other constituent will increase its effectiveness, or ensure that it reaches the part of the body that requires treatment. Herbal Medicines consist of natural plant material and not synthesized, chemical herbal remedies are less likely to cause unpleasant side effects then orthodox drug. However, it should be borne in mind that plant remedies are very powerful and can be dangerous if prescribed dosages are exceeded. Some plant phytochemical is toxic, pyrrolizidine alkaloids are hepatotoxic and large dosages can cause conclusionsand rapid death. Herbal remedies should not be taken alongside conventional drugs or in pregnancy without consulting a qualified herbal practitioner or your doctor. All herbal remedies are derived

From plants they can be given internally as:-

Tablets

Infusions:-these are produced by preening boiling, water over leaves or flowers

Decoctions: -these are produced by boiling barks or roots in water for a period of a time.

Tinctures: -these are made by steeping plant parts in a mixture of alcohol and water.

Elixirs: -these are the alcoholic extracts of herbs, usually with an ethanol percentage of 12 -38%.

Herbal medicines can also be administered externally, ointments and poultices. For herbal medicinal products that have been proposed for non – traditional indications or are modified from their traditional form (e.g., highly processed or special extracts), a full license is required in most cases, and efficacy has to be proven by clinical studies. In several countries, such products are not used. The quality of imported medicinal plants and their preparations is assessed differently in different Member States. In some cases, no specific regulations exist concerning the control of raw materials or crude drugs, particularly for products that enter the market as foodstuffs or other products that are not controlled in the same way as medicinal products. Finished products are often treated as new chemical entities with full proof of quality, safety and efficacy being required.[5]

DIARRHEA

Diarrhea is a condition characterized by an increase in the number of liquidity of a person's stool. Water 60-90% (>90%) is called diarrhea. The frequency of elimination and consistency of stools vary from person to person. It's among the leading death causing diseases especially in developing countries. Approximately millions of people die every year in third world countries. Children are more susceptible to this disease hich is accounted as the leading death causes in children especially under 5years. The signs of dehydration often begin with loss of irritable behaviour and the normal stretchness of the skin which can progress to decreased urination, loss of skin colour, a faster heart rate and a decrease in responsiveness as it becomes more severe. It can be acute or chronic and can range in severity from mild to life-threatening and it has many causes and pathophysiologic mechanisms.[6]

Deposit the efforts of international organization to control this disease; still the in cadence of diarrhea is very high. Some antibiotics are used as anti-diarrheal drug, but those drugs sometimes show some adverse effects and microorganism are tend to develop resistance towards them.[7] Therefore the search for safe and more effective agents from plant origin has continued to be an important area of active research. Plants have long been a very important source of new drugs. Many plant species have been screened for substance with therapeutic activity. For the treatment of diarrhea, medicinal plants are a potential source of anti-diarrheal drugs.[8] Moreover, many international organizations is including WHO have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practice.[9]At present, around 25% of drug isolated from plants and there are numerous evidences available about the use of medicinal plants including their pharmacological and biochemical properties.[10]

➢ Classification and Etiological Factor of Diarrhea

- **Classification according to disease condition**

❖ **The Mild:-**without the symptom of desiccation and toxicosis
❖ **The moderate:-**with mild symptom desiccation and toxicosis
❖ **The severe:-**with severe symptom of desiccation and toxicosis

- **Classification according to etiological factor**

Diarrhea is categorized into acute or chronic and infectious or non-infectious based on the duration and type of symptom.

1. Infectious: many types of infectious diarrheal disease:-

A. Vibrio species: vibrio are gram negative bacilli that grow naturally in estuarine and marine environments worldwide, and can survive in contaminated waters with increased salinity and temperature (up to 37°C). There are 12 species of Vibrio that have been implicated in human infections, with the most prominent being Vibrio cholera, and vulnificus.V.Cholera, the etiologic agent of cholera, is sub classified based upon somatic O antigens.v.Cholera O1 and O139 are responsible for causing classic cholera which can occur in epidemics or worldwide pandemics.

B. Shigella species: Shigella are gram negative bacilli that have four recognised species: S. sonnei, S. flexneri, S.dysenteriae, S.boydiim, S.sonnei enteritis an occurs predominantly in industrialized countries while S. flexneri occurs in developing countries. Sdysenteriae results in the most severe infections and S. boydii is infrequently isolated. Humans are the only known reservoirs for Shigella. Shigellosis is transmitted by the fecal– oral route, primarily by people with contaminated hands and less commonly through contaminated water or food.

C. Salmonella species:- Salmonella are gram negative bacilli that have more than 2400 unique serogroups, but are broadly classified into typoidal species (S. typhi and S. paratyphi) and non typhoidal species (S. enteritidis and S. typhimurium are the most common isolates). S. typhi and S. paratyphi has no reservoirs other than humans and can cause disease with a very low inoculums. S. typhi produces a febrile illness Calles typhoid fever. After passing through the intestinal cell lining, S. typhi is engulfed by macrophages, where it is able to replicate, and get transported to the liver, spleen and bone marrow.[11]

2. Non-infectious:-

a) Dietary diarrhea:- That is dyspepsia diarrhea caused by Mal dietary, for example diarrhea of the infant who take cow's milk instead of mother's milk or caused by adding food

b) Symptomatic diarrhea:- That is diarrhea complicated other disease. For this kind of diarrhea, stool culture is negative, for example pneumonia and tracheitis are complicated with simultaneously diarrhea classified.

- **Classification according to diarrhea**

1. Acute diarrhea: the most common, acute diarrhea is loose watery diarrhea that lasts one to two days. This type doesn't need treatment and it usually goes away after a few days**.** Acute diarrhea can be classified

A. Infectious diarrhea:

- **Viral diarrhea:** -the most common causes of acute viral diarrhea in infants and toddlers are rotavirus, astrovirous and lorovirous.
- **Bacillary diarrhea:-** it is a gastrointestinal disease caused by bacterial infections. Symptoms include severe diarrhea, fever, stomach pain, nausea and vomiting. The condition is most common in developing countries with poor sanitation. Some cases can be life threatening and require antibiotics and hospitalization.
- **Fungi diarrhea:-** signs and symptoms of gastrointestinal fungal infections include diarrhea, vomiting, menela, hemorrhage, abdominal pain, and fever, and are often similar regardless of the time of fungus involved.

B. Acute poisoning: - same poisoning caused by biotoxin and chemical toxin.

C. Intestinal disease: - acute hemorrhagic enteritis necroticans, acute episode by chronic nonspecific ulcerative colitis, croon's disease and partial intestinal obstruction etc.

D. Generalized disease:- uremia hyperthyroidism, acute systemic injection such as typhus pare typhoid fever, hematospecsis and laptospirois etc.

E. Drug diarrhea:- the common drugs which cause diarrhea are as followed:catharries agents, cholinergic drugs or cholinesterase inhibition, digitoram, diuretics, anticancer, chemotherapy drugs.

2. Persisting diarrhea: - The course is more than 2 weeks but less than 2 months.

3. Chronic diarrhea:- Diarrhea that lasts for more than four weeks or comes and goes regularly over a long period of time is called chronic diarrhea. the course is more 2 months persisting diarrhea and chronic diarrhea can be classified as followed:-

- **Intestinal infection:-** Several types of bacteria can enter your body through contaminated food or water and cause diarrhea. Common bacteria that cause diarrhea include Campylobacter link, Escherichia coli link (E. coli**),** Salmonella link, and Shigella link.
- **Intestinal malabsorption:-**

A. Pancreatic diarrhea:- caused by pancreas lesion such as chronic pancreatitis, cancer of pancreas, cystic fibrosis.

B. Lack of conjugated cholerio acid: Severe hepatopathy such as biliary cirrhosis, chronic obstruction of biliary tract and ilea- disease such as crohn′s diseases.

C. Bacterial hypertrophy: Blind loop syndrome multiple intestinal constriction such as crohn′s disease, instestinal tuberculosis, radioactive enteritis, diverticulum of jejunum, gastro colic fistula, partial intestinal obstruction, systemic cirrhosis and so on.

D. Intestinal mucosa congestion: Portal hypertension obstruction of portal vein and hepatic, right cardiac insufficiency.

E. Primary intestinal mucosal cell abnormality:- Disaccharide and monosaccharidose deficiency, β-lipoprotein insufficiency.

F. Small intestine mucous membrane lesion: Such as celiac disease, eosinophil gastroenteritis, intestinal tractamyloid degeneration, radioactivity enteritis and whipple disease etc.

G. Lymph obstruction: intestinal tract lymphoma, tuberculosis of mesenteric glands, tumor transfer etc.

H. Intestinal tract lesion: Chronic nonspecific ulcerative colitis, crohn′s diseases, radioactivity enteritis, diuretic colitis of colon, familial polyposis coli and partial intestinal obstruction.

I. Generalized Disease: Such as uremia systemic lupus erythematosus. Systemic cirrhosis hyper thyroidism and nicotinic acid deficiency.

J. Drug diarrhea: Take thyroxin sodium, anta- acid agent digitoram, sidero- agent and diuretic etc.[12]

➢ **Symptoms of diarrhea**

Diarrhea refers to watery stools, but it may be accompanied by other symptoms. These include:
Stomach pain, Abdominal cramps, Bloating, Weight loss, Fever, Body aches, Chills.
Diarrhea is also a symptom of other conditions, some of which can be serious. other possible symptoms are:
Blood or pus in the stool, Persistent vomiting, Dehydration

➢ **Transmissions:** Diarrhea is caused by infectious organism including viruses, bacteria, protozoa and helminthes that are transmitted from the stool of one individual to the mouth of another termed fecal oral transmission. They differ in the route from the stool to the mouth and in the number of organism needed to cause infection and illness. Among bacteria, the ability to survive stomach acid is an important determinant of the in culums size required to cause illness for example Shigella bacteria are resistant to low PH and a few thousand organism suffix, which are readily transferred by direct person to person contact or through contamination of inanimate objects, such as a cup. In contrast, bacteria readily killed by acid, such as vibrio cholera require millons of organism to cause illness, and therefore must first multiply in food or water to an infectious dose. Some pathogens such as rotavirus, display a sharp host. Species inference and others have a broad host range. Among salmonella bacteria, certain bio serotypes are adapted to infect animals and pose no threat to humans and others are adapted to humans and do not infects animals. The majority, however are not adapted to a specific host and can infect either humans or domestics animals, thus facilitating transmission of these organism to humans less than a dozen of the more than 2,500 individual salmonella cause the majority of human infection reflecting the requirement for genes that encode essential virulence factors. The ability to identify virulence genes and their products has led to new molecular approaches to epidemiology and diagnosis andundoubtebly will lead to new measure to prevent and treat diarrhea.[13]

➢ **Causes of diarrhea:**

Diarrhea may be caused by many things, including:

- A bacterial infection
- A virus
- Trouble digesting certain things (food intolerance).
- Food allergy (such as celiac disease, gluten allergy).
- Parasites that enter the body through food or water.
- A reaction to medicines.
- An intestinal disease, such as inflammatory bowel disease.
- A problem with how your stomach and bowels work (functional bowel disorder), such as irritable bowel syndrome.
- A result of surgery on the stomach or gall bladder.
- Recent antibiotic use.
- Metabolic conditions such as thyroid problems.
- Other less common reasons such as damage from radiation treatments or tumors that make too many hormone.

➢ **Diagnosis of diarrhea**

These may include:

- **A full blood count:** Anemia may suggest malnutrition, bleeding ulceration, or IBD.
- **Liver function tests:** These will include testing albumin levels.
- **Tests for malabsorption:** These will check the absorption of calcium, vitamin B-12, and folate. They will also assess iron status and thyroid function.
- **Tests for antibodies:** These may detect celiac disease.
- **Stool tests:** doctors can identify parasites, bacteria, and a few viruses in stool cultures. Stool tests can also reveal microscopic blood, white blood cells, and other clues for diagnosis.[14]

➢ **Treatment of diarrhea**

Symptoms of settle within a few days or so as your immune system usually clear the infection. Rarely admission to hospital is needed if symptoms are severe, or if complications develope.
The following diarrhea treatments are commonly advised until symptoms ease.

A. Drink lots of water

The aim is to prevent lack of fluid in the body (dehydration), or to replace lost fluids in the body.

- As a rough guide, drink at least 200 ml after each bout of diarrhea (after each watery stool (faeces).
- This extra fluid is in addition to what you would normally drink. For example, an adult will normally drink about two liters a day but more in hot countries. The above advice of 200 mls after each bout of diarrhea in addition to this usual amount that you drink.
- If you have been sick (vomited), wait 5-10 minutes and then start drinking again but more slowly. For example, a sip every 2-3 minutes but making sure that your totally intake is as described above.
- You will need to drink even more if you are dehydrated. A doctor will advise how much to drink if you are dehydrated. For most adults, fluids drunk to keep hydrated should mainly be water.

➢ **Rehydration drinks**

Rehydration drinks are recommended for people who are frail, or over the age of 60, or who have underlying health problems. They are made from sachets that you can buy from pharmacies (e.g., Dioralyte). (The sachets are also available on prescription.) You add the contents of the sachets to water.

Rehydration drinks provide a good balance of water, salts and sugar. They do not stop or reduce diarrhea. However, the small amount of sugar and salt helps the water to be absorbed better from the gut into the body.

In children with gastroenteritis, dilute fruit juice (especially apple juice) can be useful, and may be as good as rehydration drinks in some cases. Apple juice can be diluted 50:50 with water. Children often prefer the taste of this to water or rehydration drinks, and so it can be easier to get them to take small amounts regularly.

➢ **Salt/sugar mixtures:-** Home-made salt/sugar mixtures are used in developing countries if rehydration drinks are not available but they have to be made carefully, as too much salt can be dangerous. Rehydration drinks are cheap and readily available in UK and are the best treatment.

B. Anti-secretory medicines:- These are designed to be used with rehydration treatment. They reduce amount of water that is released into the gut during an episode of diarrhea. They can be used for young children who are older than 3 months of age, and for adults.

C. Eat as normally as possible:- It is used to be advised to not eat for a while if you had infectious diarrhea. However, now it is Advised to eat small, light meals if you can be guided by your appetite. You may not feel like Food and most adults can do without food for a few days. Eat as soon as you are able – but don't stop drinking. If you do feel like eating, avoid fatty, spicy or heavy food at first. Plain foods such as whole meal bread and rice are good foods to try eating first.[15]

***Kalanchoe pinnata* Lam.:- Plant Profile:-**

- **Parts used:-** Leaves
- **Botanical Name:-** *Kalanchoe pinnata* Lam.
- **Family :-** Crassulaceae
- **Taxonomical Classification:-**

Kingdom	plantae
Order	Saxifragales
Family	Crassulaceae
Class	magnoliopsida
Genus	*kalanchoe*
species	*Kalanchoe pinnata* Lam.

Table No. 1:- Taxonomical status of *Kalanchoe pinnata* Lam.

- **Vernacular Name:-**

English name	Christmas kalanchoe
Tamil	Malaikkalli
Malayalam	Llamulacci
Hindi	Jakh me hayat
Sanskrit	Parnabijah
Bengal	Koppata

Table No. 2:- Vernacular Name of *Kalanchoe pinnata* Lam.

Fig No.1:-Plant of *Kalanchoe Pinnata* Lam.

Fig. No.2:-Leaves of *Kalanchoe Pinnata* Lam

➢ **Geographical Profile:-** *kalanchoe pinnata* Lam. is native to Madagascar and has become naturalized in tropical and subtropical areas, in habiting warm and temperate climates from sea level to 2,600m (8,500 ft), occupying sites on rock in tropical evergreen and dry deciduous forests, as well as Montana forests.[16]

➢ **Distribution :-** *Kalanchoe pinnata* Lam. has become naturalized in temperate regions of India, Asia, Australia. New Zealand, West indies, Macaronesia, Melanesia, Polynesia and Hawaii. It is also widely distributed in the Philippines and it is known as Katakatakaorkataka- taka which is also adjective meaning astonishing or remarkeable.[17]

➢ **Chemical constituent:-** *Kalanchoe Pinnata* Lam. is rich in alakaloids, triterpenes, glycosides, flavonoids, cardienolides , steroids, bufadienolides and lipids. The leaves contain a group of chemicals called bufadienolids which are very active.[18]

➢ **Pharmacological activity:-**

♦ **Anti- diarrheal activity**

Herbal Tonic:- The plant is good sources of ascorbic acids, riboflavin, thiamine and niacin. Natural ascorbic acid is vital for the body performance i.e. normal formation of intercellular substance throughout the body, including collagen, bone matrix and tooth dentine. The plant used in herbal medicine for the treatment of common cold and other diseases like prostate cancer.[19]

Neuro-pharmacological activity:-*Kalanchoe Pinnata* Lam. has been used since 1921 in traditional medicine as an antipsychotic agent. Salahdeenet al showed that the aqueous leaf extract possesses depressant action on CNS.

Antihypertensive activity:- Herb possesses hypotensive activity and lend credence to the folkloric use of the herb in the management of hypertension. The plant commonly used in the management of all the types and grades of hypertension by some yorubas of western Nigeria *kalanchoe pinnata* has been recorded in Trinidad and Tobago as being used as a traditional treatment for hypertension.

Analgesic, Anti-inflammatory, **Wound healing activity:-**These properties bestow high medicinal activities on the extracts from Kalanchoe Pinnata *Lam.* Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes.

Anticancer activity:- *Kalanchoe Pinnata* Lam. compounds have marked anticancer therapeutic value against cancer cells.[20]

➢ **Parts of plant:-**

Leaves:- leaves variable decussate the lower usually simple or occasionally compound,8-12 cm in size ,the upper usually 3-5 or sometimes 7-folio late, long pointed, the petioles united by a ridge round the stem.

Stems:- Stems obtusely four angled the older light colored, younger parts reddish speckled with white.[21]

Flower:- Reddish purple, pendent in large spreading panicles with opposite stout branches.

Fruit:- Enclosed in the persistent papery calyx and corolla.

Seeds:- Seeds are Small smooth oblong ellipsoid, scarcely striate, smooth.[22]

MATERIAL AND METHODS

Collection of Plant:- From Ladvi Nursery of Gandhinagar, (Mandleshwar)
Authentication of Plant :- By Professor Mr. GIRISH SHIV (Asst. Professor of Botany) Govt. P. G. Collage, Khargone (M. P.)
Preparation of extract:- By using Ethanol and Distilled water.
Experimental Animals:- Albino mice weighing between (25- 30 gm) were procured from institute of Animal Health & Veterinary Biological Rasalpura Mhow (M. P.) and are available in the department of pharmacology of Charak Institute of Pharmacy**,** Mandleshwar. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and supervision study was performed in Charak Institute of Pharmacy, Mandleshwar. CPCSEA Registration No. CIP/ 22-23/ 003/ 2242 CPCSEA.
Acute Toxicity Test:- LD_{50} of prepared extracts was evaluated by OECD guideline no. 425.
List of Instruments and Chemicals:-
Glass Wares:- Round Bottom flask, Beakers, Glass rod, Heating Mental, Measuring Cylinder, Test Tube, Test Tube stand, Funnel, Specula, Weighing balance, etc.
Chemicals:- Ethanol **Drug:-** Loperamide, Castor oil

Name of Instruments	Manufacture
Heating Mantle	Lab Hosp Corporation Mumbai
Refrigerator	Videocon
Weighing Balances	Weight PAD Series Digital Scale

Table No. 3- List of Instruments No. 3 List of Instrument

Name of Glasswares	Volume of Glasswares	Quantity of Glasswares
Beaker	500ml	3
Glass rod	20mm	1
Measuring cylinder	50ml	1
Pipette	2ml	1
Round bottom flask	250ml	2
Test tube	10ml	14
Volumetric flask	100ml	1

Table No. 4: List of Glassware's

Statistical Analysis:- All results are expressed as mean standard error of mean (SEM). All statistical analyses were performed by one way ANOVA followed by the Tukey HSD post- hoc test where $P< 0.05$ was regarded as statistical significant.

EXPERIMENTAL WORK AND RESULTS:-

Collection of Plant:- The leaves of *Kalanchoe pinnata* Lam. were collected from Ecocenter (Ladvi) Nursery Gandhinagar, (Mandleshwar). The leaves were collected in the dried form. It were completely cleaned and made dust free. The leaves were grinded by an electric mill to produce coarse powder. Powdered material was kept in air tight containers.
Authentication of plant:- The collected material was compared with the published description of the drug and with authentic specimen collected Leaves material was identified by acknowledged expert. The drug was identified and authenticated by professor.
Preparation of extracts:- Extract was prepared by Maceration process About 200 gm. of powdered leaves was taken to prepare extract. The powder was macerated with 95% Ethanol and water. The extraction continued for 7 days. Extracts was filtered by what man filter paper and evaporated to dryness at low temperature (<40 ^{0}c). Extract was collected in a beaker which was covered with Aluminium foil. The dark brownish sticky solution was occurred. The percent yield obtained from Ethanolic extract was 15% and that of Aqueous extract was 13%. Extract were preserved in refrigerator for phytochemical screening, acute oral toxicity study and evaluation of Anti- diarrheal activity.[(64)]

Plant Name	Part Used	Method	Ethanol (95%)
Kalanchoe Pinnata Lam.	Leaves	Maceration	15%

Table No. 5 :- Extractive value of *Kalanchoe pinnata* Lam.

Phytochemical Screening:- The extract was tested for the presence of bioactive compound by using following standard method[65, 66]:-

Test for Alkaloids

Dragendroffs Test:- To 1 ml of extract , 1 ml of Dragendroff reagent was added (Potassium Bismuth iodide solution).

Observation:- An Orange Red color precipitate was observed.

Result:- Indicates the presence of Alkaloids.

Mayers Test:- To 1 ml of extract, 1 ml of mayers reagent was added (Potassium Mercuric Iodide solution).

Observation:- Whitish yellow or cream coloured precipitate was observed.

Result:- Indicates the presence of Alkaloids.

Wagners Test:- To 1 ml of the extract, 2 ml of wagners reagent was added (Iodine Potassium Iodide).

Observation:-Reddish brown colored precipitate was observed.

Result:- Indicates the presence of Alkaloids.

Test For Protein′s

Millons Test:- To 1 ml crude extract was mixed with 2 ml of millons reagent.

Observation:- White precipitate was appeared which turned red upon gentle heating.

Result:- Indicates the presence of protein**.**

Test for Carbohydrate

Fehlings Test:- Equal volume of Fehling A & Fehling B reagent was mixed together and 2 ml of it was added to 1 ml crude extract and gently boiled.

Observation:- A brick red precipitate appeared at the bottom of the test tube.

Result:- Indicated the presence of carbohydrates.

Benedicts Test :- To 1.5 ml Crude extract was with 2 ml of benedicts reagent and boiled.

Observations:- A reddish brown precipitated was observed.

Result:- Indicated the presence of the carbohydrate.

Molisch Test:-To 2 ml crude extract was mixed with 2 ml of Molisch reagent and the mixture was shaken properly, after that 2 ml of concentrated H_2SO_4 was poured carefully along the side of the test tube.

Observation:- A violet ring at the interphase was observe.

Result:- Indicate the presence of the carbohydrate.

Test for Flavonoids

Shinoda Test:- To 1 ml crude extract was mixed with few fragments of magnesium ribbon and concentrated HCL was added drop wise.

Observation:- Pink scarlet color appeared was observed.

Result:- Indicates the presence of flavonoids.

Alkaline Test:- To 2 ml crude extract was mixed with 2 ml of 2% solution of NaoH.

Observation:- An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid.

Result:- Indicated the presence of flavonoids**.**

Test for Saponin′s

Foaming Test:- To 3 ml crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously.

Observation:- Formation of stable foams was observed.

Result:- Indicated the presence of saponins.

Test for Glycosides

Salkowski Test:- Crude extract was mixed with 2 ml of chloroform then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently.

Observation:- A reddish grinish yellow colourwas observed.
Result:-Indicate the presence of Glycoside.
Killer- Killani Test:- To 1 ml of crude extract mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2 % solution of $Fecl_3$. The mixture was then poured into another test containing 2 ml of concentrated H_2SO_4.
Observation:- A Brown color precipitate was observed.
Result:- Indicate the presence of Glycoside.
Test For Triterpenoids
Liebermann Buchard Test:- To 2 ml Crude extract was mixed with few drops of acetic anhydride boiled and cooled concentrated sulphuric acid was then added from the side of the test tube and observed for the formation of a Brown ring at the junction of two layers.
Observation:- Upper layer and Formation of deep red color in the lower layer were observed.
Result:- Indicate the presence of Triterpenoids.

S.No.	Test	Ethanol Extract	Aqeous Extract
1	**Alkaloids Test**		
	Dragendroff Test	+	+
	Mayer Test	+	+
	Wagner Test	+	+
2	**Proteins Test**		
	Millon Test	–	+
3	**Carbohydrates Test**		
	Fehling Test	+	+
	Benedict Test	+	+
	Molish Test	–	–
4	**Phenol and Tannin Test**	+	–
5	**Flavonoids Test**		
	Shinoda Test	–	+
	Alkaline Test	+	+
6	**Saponins Test**		
	Foaming Test	+	+
7	**Glycosides Test**		
	Salkowski Test	+	+
	Killer- Killani Test	-	+
	Triterpenoids Test		
	Liebermann Buchard Test	+	+

(+) Presence of Chemical Constituents, (–) Absence of Chemical Constituents

Table No. 6:- Phytochemical screening of leaves of *Kalanchoe Pinnata* Lam.

Determination of LD_{50} of The Ethanolic and Aqueous of *Kalanchoe Pinnata* Lam. extract in Mice by acute Toxicity studies

Dose Fixation:- Acute Oral toxicity study done according to OECD guideline (AOT 425) on albino mice. A dose of 2000 mg/kg was selected. One animal was administered a dose of 2000 mg/kg on first day. The animal was observed for 24 hours. The animal showed no signs of discomfort or symptoms so, the same dose was repeated on same animal. The animal survived without any symptom. Based on the above observation, LD_{50} of the compound was confirmed to be greater than 2000 mg/kg for the prepared Ethanolic extract. Any dose below 2000 mg/kg could be used as a dose animals.

Extraction Process The total yield obtained from extraction was found to be Ethanolic extract 15% and Aqueous Extract 13%.

Acute Toxicity Study

Observation:- All the animals survived without any symptoms or toxicity during the observation upto 24 hrs. based on the above observation. LD_{50} of the compound was confirmed to be greater than 2000 mg/kg for the test compound.

Inference:- Any dose below 2000 mg/kg could be used as a dose for animals the biological evaluation of Anti-diarrheal activity was carried out at dose of 150, 250 mg/kg body weight.

Anti-diarrheal Studies:-

I. Castor oil- induced diarrhea

Castor oil- induced diarrhea

Test Compounds:- The Ethanolic and Aqueous Extract of Leaves of *Kalanchoe Pinnata* Lam. and Standard drug Loperamide were used.

Chemicals and Reagent:- Castor oil, Loperamide

Experimental Animal:- Albino mice (25- 30 gm) used in the preset study. The animals were fed with pellet diet and water ad labium all the animals were acclimatized for a week before use.

Castor oil induced model

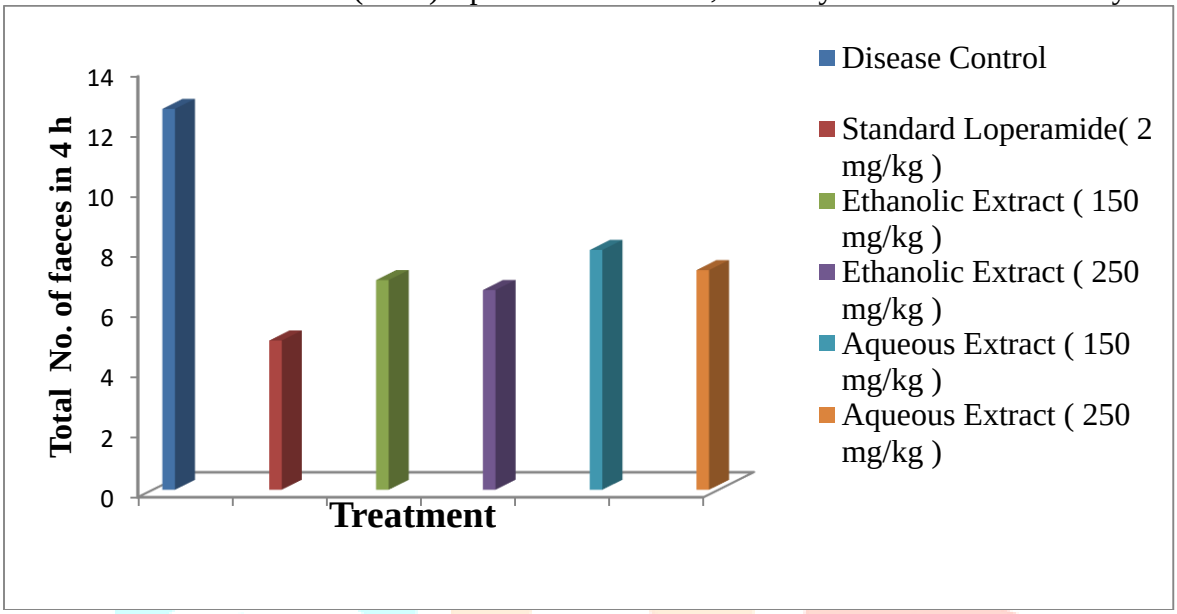
The mice were randomly divided into six groups with 5 animals in each. Group I- mice received castor oil orally. Group II- mice received Standard drug Loperamide (2 mg/kg) orally + castor oil. Group III- Ethanolic Extract (150 mg/kg b.w.) + Castor oil Group IV- Ethanolic extract (250 mg/kg b. w.) + castor oil. Group V- mice received Aqueous extract (150 mg/kg b. w.) + castor oil and Group VI- mice received Aqueous extract (250 mg/kg b. w.) + castor oil. All animals of each group giving 0. 3 ml castor oil orally 45 minutes. After the drug extract to produce diarrhea. all mice placed into individual cages and floor and walls are covered with blotting paper. Blotting paper was changed every hour. Observation time of 4 hour during this time the total No. of faeces and the No. of wet faeces were noted.[67]

Treatment	Dose mg/ kg	Total no. of faeces in 4 hour	Total No. of wet Faeces in 4 hour
Disease control	–	12.67±1.20	15.66±0.88
Loperamide	2 mg / kg	5.00±0.58**	7.76±1.15**
Ethanolic Extract	150 mg /kg	7.00±1.50	9.67±0.29
Ethanolic extract	250 mg / kg	6.67±0.59*	8.00±1.00*
Aqueous Extract	150 mg/ kg	8.00±0.52	9.00±1.00

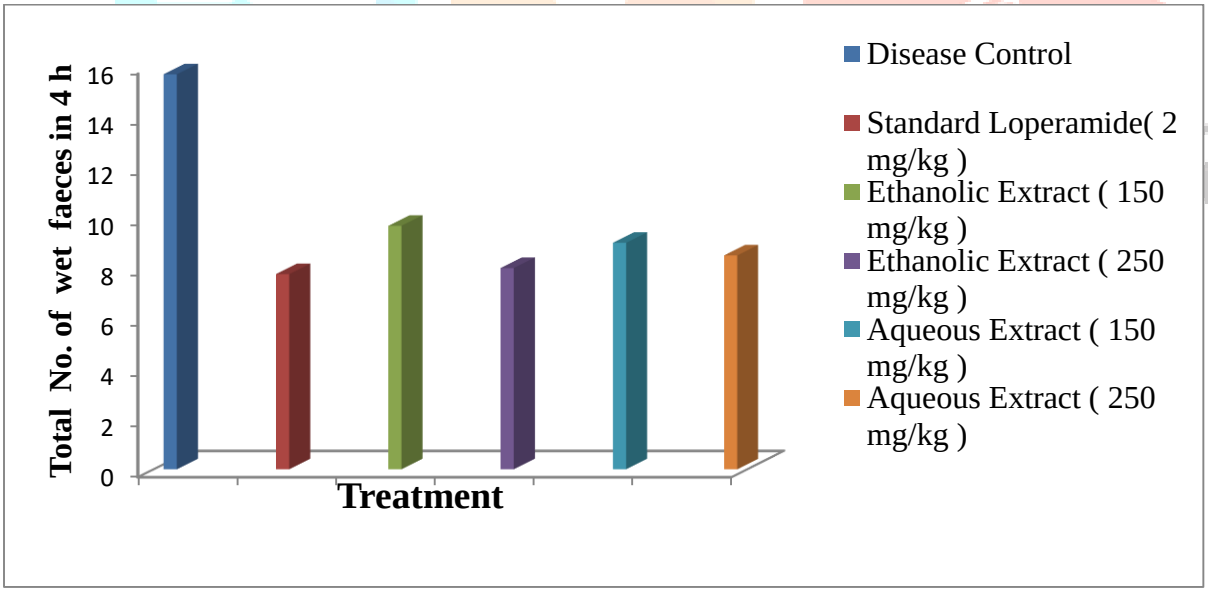
Aqueous Extract	250 mg/ kg	7.33±1.04	8.50±1.25

Table No. 7:- Castor oil- induced diarrhea model

Values are mean ± S.E.M. (n = 6)** p< 0.05 vs. control, one way ANOVA followed by Dunnett's Test.



Graph No.1: Comparison of Total No. of faeces in 4 h



Graph No. 2: Comparison of Total No. of wet faeces in 4 h

DISCUSSION

The Leaves of *Kalanchoe Pinnata* Lam. belonging to family is a widely growing plant through India. The plant also has many valiable medicinal properties:-

Extractive Value

The powder leaves (200 gm) was taken for extraction and the extraction was proceeding with two solvents using Ethanol and Water extraction process. From among the extracts Ethanolic extract afforded maximum yield (15%) and Aqueous extract yield (13%).

Phytochemical Screening

Investigation on the preliminary phytochemical screening of Ethanol extract showed presence of Alkaloids, Proteins, Carbohydrate, Phenols and Tannins, Flavonoids, Saponins, Glycosides, Triterpenoids, etc. Aqueous extract showed presence of Alkaloids, Proteins, Carbohydrate, Phenols and Tannins, Flavonoids. Saponins, Glycosides, Triterpenoids, different solvents were prepared by Ethanol and Aqueous extract using standardized procedure and also subjected to Anti- diarrheal activity *Kalanchoe Pinnata* Lam. exhibited significant Anti-diarrheal activity with respect to control.

Acute Toxicity Study

The Ethanolic and Aqueous Extract of leaves of *Kalanchoe Pinnata* Lam. administered orally to found that both the test compound at a limit test one dose level for a dose 2000 mg/ kg.

Anti- diarrheal Activity:-

The present study showed that the Ethanolic extract and Aqueous Extract of *Kalanchoe Pinnata* Lam. posses Anti- diarrheal activity as evidenced by its significant effect by castor oil- induced diarrhea in mice.

Castor oil- induced diarrhea in experimental mice:-

Group of mice treated with castor oil (0.3 ml) are called disease control group showed a significant ($P<0.05$) increase the biochemical parameters. Group of mice which pre- treated with Loperamide (2 mg/kg orally) was standard group, expresses significant *($P<0.05$)* inhibition of increase the biochemical parameters group of mice which were pre- treated with Ethanolic Extract (150 and 250 mg/kg orally) demonstrate significant *($P< 0.05$)* effect of Ethanolic Extract on biochemical parameters. Data were analyzed as mean ± S.E.M. statistical analysis were done by using one way ANOVA followed by Dunnett′s test (Disease control group was Compared with the test group). Group Loperamide, Ethanoli Extract- 150 mg/ kg and 250 mg/ kg and Aqueous Extract- 150 mg/ kg and 250 mg/ kg were compared with the diseases control group by using one way ANOVA and followed by Dunnett′s test. The Ethanolic extract showed significant inhibition against biochemical parameters induced by castor- oil as compared to disease control. Among the 150 mg and 250 mg dose showed significant *($P< 0.05$)* effect of Ethanolic Extract on castor oil- induced diarrhea. group of mice which were pre- treated with Aqueous Extract (150 mg/kg and 250 mg/kg) demonstrate significant *($P < 0.05$)* effect of Aqueous Extract on castor-oil induced diarrhea.

SUMMARY

The literature survey reveals that many important properties shown by *Kalanchoe Pinnata* Lam. Were used in folk medicine. Its Anti- diarrheal potential is one of them. None of any available scientific literature present study Anti- diarrheal activity of leaves part of this plant.

In view of this, the present study was undertaken to investigate the Anti- diarrheal potential of the leaves of *Kalanchoe Pinnata* Lam. In this study first, extract was prepared using maceration process in ethanol and aqueous. The total yield obtained from extraction was found to be 15% and 13%. Then preliminary phytochemical screening was carried out for identification of flavonoids, alkaloids, tannins and saponins by using different types of chemical tests. The result of phytochemicals screening shows that Ethanolic extract contained Alkaloids, Carbohydrate, and Tannin. The Acute Oral toxicity (AOT) study for determine of LD_{50} was done by dose fixation method according to OECD guideline (AOT 425). This was performed in albino mice. The observation of this study confirmed that, LD_{50} of the compound was greater than 2000 mg/ kg for the test compound. Any dose below 2000 mg/ kg could be used as a dose for animals and evaluation of Anti- diarrheal activity was carried out at doses for animals and evaluation of Anti- diarrheal activity was carried out at doses of 150 and 250 mg/ kg body weight by using castor- oil induced diarrhea. statistical analysis carried out by one way ANOVA and followed by Dunnett′s test using prism software. The Ethanolic and Aqueous extract showed significant inhibition biochemical parameters induced by castor-oil as compared to disease control.

CONCLUSION

The Leaves of plant *Kalanchoe Pinnata* Lam. belonging to family *Crassulaceae*, were studied for phytochemicals & pharmacological studies.

The present work demonstrate that the *Kalanchoe Pinnata* Lam. extracts posses Anti- diarrheal activity in castor oil induced diarrhea in experiment animal i.e. mice in present study. From the above observation it was concluded that Ethanolic and aqueous extracts of *Kalanchoe Pinnata* Lam. posses Anti- diarrheal activity in both the dose levels which are comparable with the standard. The Ethanolic and Aqueous extract of *Kalanchoe Pinnata* Lam. (respectively, 150 mg/ kg, 250 mg/ kg), showed Anti- diarrheal action but Ethanolic extract 150 mg/ kg and Aqueous extract 250 mg/ kg which showed markedly effective as a Anti- diarrheal action compared with diseases control. The Ethanolic and Aqueous extract of selected plant identifies the phytochemicals constituents responsible for the observed Anti- diarrheal activity. It is suggested and assumed that a further exploration of the present research work is needed to come up with Anti- diarrheal agent.

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