

Standardization, Phytochemical evaluation and Anthelmintic activity of *Kalanchoe marnieriana* (Crassulaceae)

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Abstract: Background: *Kalanchoe marnieriana* (Crassulaceae), commonly known as succulent's flowery plant, is regarded as extremely useful in traditional medicine. Because of its analgesic and anti-inflammatory qualities, this succulent is frequently used to treat a variety of illnesses. *Kalanchoe* is also used by many because of its ability to promote wound healing and strengthen the immune system in general. Its stems, leaves, and flowers are used to treat a variety of illnesses. This study aims to determine the quality standardization, evaluation of the phytochemical and anthelmintic activities on earthworms using an extract of *Kalanchoe marnieriana*.

- Determining the flavonoid concentration of *Kalanchoe marnieriana* extracts is the key objective of standardization. The result can often be achieved by using rutin as a reference chemical using the AlCl₃ technique to measure absorbance in a spectrophotometer with ultraviolet-visible wavelengths. The results are then expressed in the substance equivalents.
- Phytochemical evaluation was done by some standard tests.
- In vitro anthelmintic activity was assessed by the earthworm method.

The phytochemical test revealed that microgreens extract of *K. Marnieriana* contains alkaloids, reducing sugar, glycosides, terpenoids, saponins, carbohydrates, and protein.

Results & Discussion: The accurate validation and description of a phytotherapeutic product's or herbal preparation's properties, such as extracts and crude pharmaceuticals, is crucial to its quality. The creation of uniform goods is the final outcome of this procedure, improving their medium- and long-term safety and effectiveness. The plant material and extract were thus characterised, offering crucial details regarding their physicochemical characteristics as well as scientific backing for possible therapeutic uses of the extract or an isolated compound.

Conclusion: Ethnomedicine has long used species of the genus *Kalanchoe* for therapeutic purposes because of its exceptional healing qualities. When employed in traditional medicine, the chemical and physical similarities between several species can cause confusion. The purpose of this review is to give a summary and discussion of the *kalanchoe* species' documented traditional usage, botanical characteristics, chemical components, and pharmacological potential.

Keywords: *Kalanchoe marnieriana*; standardization; flavonoids, Medicinal plants, succulent's flowery plants; traditional use; chemical components; pharmacological activities; natural products; *Kalanchoe*; Crassulaceae etc..

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Introduction

Overview

Nature is always a fantastic way to show the everyday events of life. Natural remedies for human ailments are originating from minerals, plants, and animals. Nowadays, the use of therapeutic herbs is becoming more and more popular. Plants unquestionably

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offer vital services that significantly affect ecosystems. Without plants, humans and other living things cannot exist as they overview should (Jamshidi-Kia et al., 2018). All of man's requirements, including clothing, food, shelter, scents, and, last but not least, medicines, have been met by plants. Numerous advanced traditional medical systems, including Chinese, Unani, and

Ayurvedic, have their roots in plants (GuribFakim, 2006). The Chinese book "Pen T'Sao," which is about roots and grasses, lists 365 medications, which are dried pieces of medicinal plants, which was written by Emperor Shen Nung around 2500 BC. Many of these drugs are still used today, including ephedra, ginseng, podophyllum, jimson weed, camphor, Theae folium, and the great yellow gentian. The Vedas, the nation's sacred writings, reference the use of herbs for treatment, which is widespread in India. Cloves, pepper, and nutmeg are just a few of the Indian spices that are still in use today (Dal Cero et al., 2022).

Known as "the father of pharmacognosy," Dioscorides was the most well-known writer on plant remedies in antiquity and worked as a pharmacologist and military doctor for Nero's Army. He researched medicinal plants wherever he travelled with the Roman Army. He composed "De Materia Medica" about the year 77 AD. The therapeutic herbs that served as the basis for materia medica until the late Middle Ages and the Renaissance are extensively covered in this well-known ancient history literature, which has been translated several times. 657 of the 944 medications that have been reported are plant-based, and their appearance, location, method of collection, production of the medicinal formulations and therapeutic impact are all described. Together with a description of the plant, various language names and the locations where they are found or cultivated are also given. Although alkaloids or other substances with severe effects are mentioned, the majority of plants are mildly effective (fragrant hellebore, false hellebore, henbane, jimson weed, poppy, buttercup and deadly nightshade) (Petrovska, 2012).

Medicinal Plant

As per the World Health Organisation, a medicinal plant is "any plant that contains chemical substances that can be applied to therapeutic purposes or that are precursors for chemopharmaceutical semi synthesis in one or more of its organs." The designation "medical" for a plant denotes whether it has therapeutic benefit, is an active ingredient in a medicinal mixture, or can be used as a medicine. Consequently, the term "medical plants" describes a group of plants that possess special qualities or characteristics that enable them to be employed as medicinal products and therapeutic agents in healthcare. The traditional knowledge of humanity includes the use of medicinal plants by indigenous physicians from pre-Hispanic times (Heinrich, Ankli, Frei, Wiemann, & Sticher, 1998). Some medicinal plants are a significant source of components for antitumor, antivirals, antiepileptic, antibiotics, anti-inflammatories, and antinociceptives, among other medications (Alonso-Castro et al., 2011; Le Rhun, Devos, & Bourg, 2019; Sharma, Flores-Vallejodel, Cardoso-Taketa, & Villarreal, 2017; Wang et al., 2019). The secondary metabolites of medicinal plants have pharmacological properties that make them useful.

Herbal Medicine

Herbal medicine has grown in popularity as a medical treatment. Although herbal medicine and traditional pharmacological therapies differ in a number of ways, conventional trial technique can be used to evaluate the effectiveness of herbal medicine.

For particular ailments, a number of particular herbal extracts have been shown to be effective. Despite the widespread misconception that all natural remedies are safe, there are hazards associated with using herbal remedies. In the end, it is important to determine whether herbal medicines are more detrimental than beneficial for

a given ailment. The usage of medicinal herbs has existed since the dawn of time. More than 80% of people utilise products made from medicinal plants worldwide, according to data from the World Health Organisation (Gupta, 2015). Phytotherapy is based on the use of herbal items and medicines for both prevention and treatment. Rational phytotherapy is a modern concept in herbal medicine that makes use of standardised botanical extracts. According to Pharmacopoeia guidelines and other relevant regulations, herbal medicines and herbal isolates (tinctures, extracts, and essential oils) are subjected to quality control (Djordjevic, 2017).

"The production company manufactured commercially that contain active ingredients which are completely and naturally original, not chemically altered plant substances, and are responsible for the overall therapeutic effect of the product" is how herbal medicines (HMs) are defined. The belief that HMs are "safe" or "safer" than traditional pharmaceuticals due to their "natural" nature has led to customers turning to plant-derived remedies (Alostad et al., 2020).

The role of botanical therapy in modern science

The country's constitution protects the medicinal plants and the related Bhutanese traditional medicine (BTM), which is supported by the government and accepted by the general people. Although the BTM describes more than 1000 therapeutic plants, only 300 species are currently gathered for BTM formulations every day. These health care

Bhutan's "Gross National Happiness (GNH)" and bio discovery initiatives have been fuelled in part by plants. To present, there isn't a review that addresses the systematic assessments of the contributions made by the BTM and medicinal plants to bio discovery and GNH. Chopra et al. (1982). The attributes of biological resources, including plants, animals, and microorganisms, are confusing; their entire components or derivatives can be employed for traditional medicine, science, chemotherapeutics, social, economic, and environmental objectives. In traditional medicines (TM), which serve as primary healthcare for 80% of the world's population, plants are utilised as bulk ingredients. In addition to their vital role in primary healthcare, medicinal plants have been a major source of new drugs in the modern era. This is mostly because a new drug lead candidate's hit rate is increased by their extensive clinical usage history. According to Fabricant and Farnsworth's research of the origins of the medications created between 1981 and 2001, 80% of the 122 medications produced from plants had ancestral ethno pharmacological usage. Between 2000 and 2005, about five therapeutic plant-based medications were released onto the US market, and seven more plant-derived medicines are presently undergoing clinical studies worldwide. An estimated 50,000 plant species are utilised in traditional medicine (TM) globally, with Asian remedies accounting for the majority of these uses. New medication discoveries may be aided by the ethnobotanical knowledge found in these medicinal plants. A list of increased plant availability and medical plant use by the chosen nations is provided in Table 1. According to reports, Indians use no more than 20.0% of their plant flora for medicinal purposes, followed by Sri Lanka (16.5%), China (18.9%), Vietnam (17.1%), and Thailand (15.5%). Despite having a large number of plant species, countries like the USA, Australia, Indonesia, and Malaysia do not use many of them as medicines. According to Wangchuk P. et al. (2011), Aboriginal people use 7.8% of Australia's higher plant flora as therapeutic plants.

Table1: Number of plant flora and the medicinal plants reported from selected countries

Country	Higher Plant Species	Medicinal Plant Species	Percentage of Medicinal Plant
Africa	45,000	5000	11.1
Australia	19,324	1,511	7.8
China	26,092	4,941	18.9
Bhutan	5,603	600	10.7
India	15,000	3,000	20.0
Indonesia	22,500	1,000	4.4
Malaysia	15,500	1,200	7.7
Nepal	6,973	700	10.0
Pakistan	4,950	300	6.18
Philippines	8,931	850	9.5
Srilanka	3,314	550	16.5
Thailand	11,625	1,800	15.5
U.S.A	21,641	2,564	11.8
Vietnam	10,500	1,800	17.1

Homeopathy Medicinal System

The Greek terms *hómoios* (similar) and *páthos* (suffering) are the roots of the English word homeopathy. This indicates that homeopathy uses ingredients that treat natural ailments.

create consequences that are comparable to the pain. This medicinal approach's central tenet is *similia similes curentur*. The German physician Dr. Christian Friedrich Samuel Hahnemann, who lived from 10 April 1755 to 2 July 1843, developed the system. The fundamental idea of homeopathy is comparable. By considering the sick person's physical, emotional, social, and spiritual needs, the individualisation principle also promotes a holistic approach to health, illness, and therapy. This idea is currently becoming more and more popular in biomedicine as personalised medicine, or theranostics, is becoming a key component of long-term disease diagnosis and treatment. The minimum dose theory refers to the practice of prescribing medications that contain a remarkably little amount of the original therapeutic components. Nano pharmacology is a new field that is evolving. Using only medications whose therapeutic efficacy has been reliably confirmed on humans is another important contribution of Hahnemann. This was dubbed Drug Proving. The scientific community is beginning to accept this more widely, and

it is now known as a Human Pathogenic Trial. HPT with the development of research, the scientific correctness of the ideas put

out by Hahnemann in the early 18th century is thus being confirmed.

Unani Medicine System

Greek pioneers established the Unani System of Medicine, which Arabs later refined into a sophisticated medical system based on the teachings of Galenos (Galen) and Buqrat (Hippocrates). Ever since, Unani medicine has been portrayed as Greco-Arab medicine. The four attributes of the live human body—hot, cold, wet, and dry—as well as the Hippocratic idea of the four senses of humor—blood, phlegm, yellow bile, and black bile—form the basis of this concept. The Arabian doctor interpreted the Greek concepts as seven principles (Unmoor-e-Tabbiya), which included elements (Arkan), temperament (Mizaj), senses of humour (Akhlat), organs (Aaza), spirit (Arwah), capacities (Qowa), and functions (Afaal). They are symbolised as earth, water, fire, and air. According to this approach, the constitution of the body, its health, and illnesses are all caused by these principles (1). The Unani System of Medicine (USM) has been acknowledged by the World Health Organisation (WHO) as a substitute system to address the medical requirements of the general public. Alternative medicine is practiced all around the world. The ancient traditional medical systems of China, Egypt, India, Iraq, Persia, and Syria are all incorporated into Unani, one of the most well-known forms of traditional medicine. Another name for it is Arab medicine. In several East Asian and Arab nations, unani is still widely used. In many nations with easy access to

modern treatment, the usage of unani medicine and herbal products is steadily increasing. India has granted it formal status and acknowledged it as one of the alternative health care systems.

Herbal Medicine System

The use of herbal medicine dates back to ancient cultures. It entails the therapeutic application of plants to cure illnesses and improve overall health and wellness.

Because they contain strong components, some plants should be used with the same caution as prescription drugs. A lot of pharmaceutical drugs are synthetic versions of naturally occurring substances that are found in plants. Digitalis, for example, was a plant that was used to treat cardiac problems. (Chopra and others, 1982).

Herbal remedies and active ingredients:

Herbal remedies include active ingredients. Many herbal medicines have unidentified active components. A single active ingredient that comes from a plant source serves as the foundation for some pharmacological drugs. Herbal medicine practitioners think that if an active ingredient is used alone, it may lose its effectiveness or become less safe.

For example, the plant meadowsweet contains salicylic acid, which is used to manufacture aspirin. While meadowsweet naturally includes various components that reduce irritation from salicylic acid, aspirin can induce bleeding in the stomach lining.

Uses of particular plants in medicine:

The goal of herbal medicine is to restore the body to its natural equilibrium so that it can heal itself. Herbs have varying effects on the body's systems.

Herbs with traditional applications and frequent usage in herbal therapy include:

- Echinacea: to help the body fight infection and boost the immune system. used to cure conditions like herpes, fever, and boils.

- Garlic: Reduces blood fats and cholesterol, a form of blood fat, which lowers the risk of heart disease. Garlic is also used to treat sinusitis, colds, and other respiratory diseases because of its antiviral and antibacterial qualities.
- Ginger: Several studies have demonstrated the effectiveness of ginger in alleviating nausea, particularly morning sickness and motion sickness.
- Ginkgo biloba is frequently used to treat tinnitus, or ringing in the ears, and poor blood circulation.
- Ginseng is typically used to alleviate weariness, such as during the healing process after an illness. Although excessive ginseng use has been linked to elevated blood pressure, it is also utilised to lower cholesterol and blood pressure.
- Dong Quai (dang GUI): used to treat gynaecological issues such period discomfort, menopause symptoms, and premenstrual stress. Dong quai has been shown in several tests to reduce blood pressure.

Ayurveda Medicine System

India is renowned for its Ayurvedic, Siddha, and Unani ancient medical systems. Even the ancient Vedas and other texts make reference to medical systems. In India, the Ayurvedic philosophy emerged and evolved between 2500 and 500 BC [1]. Ayurveda literally means "science of life," as the ancient Indian medical system was centred on understanding human nature and ailment. It has been noted that people in good health have a well-balanced metabolism. Ayurveda provides a comprehensive method for living a long, healthy life, which is why it is also known as the "science of longevity." It provides food and nutrition-based regimens to revitalise the body. Many common ailments, including food allergies, for which there are currently few effective treatments, can be cured with its help. But it's important to remember that Ayurvedic nutrition is not a "magic bullet" approach; rather, it depends on the patient's complete cooperation. The system is interactive, easy to use, and instructive.

Table 3. Use of medicinal plants in the world

Drug/Chemical	Action/Clinical Use	Plant Source
Acetyldigoxin	Cardiotonic	Digitalis lanata
Aesculetin	Anti-dysentery	Frazinusr hychophylla
Agriumphol	Anti-helminthic	Agriomnia supatoria
Ajmalicine	Circulatory Disorders	Rauvolfias epentina
Allantoin	Vulnerary	Several plants
Anabesine	Skeletal muscle relaxant	Anabasis sphylla
Andrographolide	Baccillary dysentery	Andrograp his paniculata
Anisodamine	Anticholinergic	Anisodus tanguticus
Anisodine	Anticholinergic	Anisodustanguticus
Arecoline	Anthelmintic	Areca catechu
Atropine	Anticholinergic	Atropa belladonna
Benzyl benzoate	Scabicide	Several plants

Berberine	Bacillary dysentery	Berberis vulgaris
Bergenin	Antitussive	Ardisia japonica
Betulinic acid	Anti-cancerous	Betula alba
Borneol	Antipyretic	Several plants
Codeine	Analgesic, Antitussive	Papaver somniferum
Cocaine	Local Anaesthetic	Erythroxylum coca
Caffeine	CNS stimulant	Camellia sinensis
Deserpidine	Antihypertensive	Rauvolfiacanescens
Deslanoside	Cardiotonic	Digitalis lanata
L- Dopa	Anti-parkinsonism	Mucunasp
Digitalin	Cardiotonic	Digitalis purpurea
Danthron	Laxative	Cassia species
Ephedrine	Sympathomimetic	Ephedra sinica

PLANT REVIEW



Figure: 1

A genus of over 125 tropical, succulent plants in the stonecrop family Crassulaceae, Kalanchoe (also known as "kalanchoe" or "kalanchoe") is primarily indigenous to Madagascar and tropical Africa. In 1979, a Kalanchoe species was used as a resupply to the Soviet Salyut 1 space station, making it one among the first plants to be launched into space.

Some kalanchoes cannot withstand the 6–8 hours of sunlight that most of them need each day and can thrive in strong shade or bright, indirect sunlight. There are currently 36 genera in the family Crassulaceae J. St.-Hil. This family's species are typically found in Africa and Asia mainly in Madagascar and Arabia, but also in Australia (Figure2).

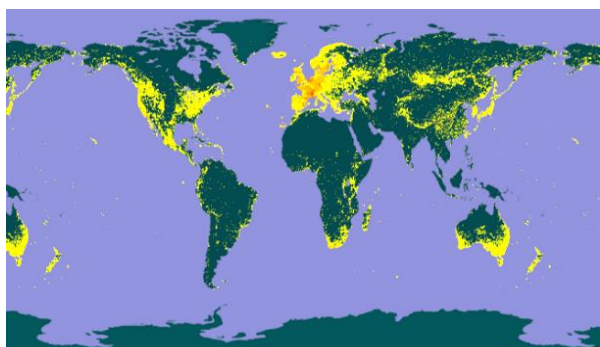


Figure: 2

There are 179 acknowledged plant species in the genus Kalanchoe Adans, which is a member of the Crassulaceae family. Heterotypic synonyms include Baumgartenia Tratt., Bryophyllum Salisb., Crassuvia Comm. ex Lam., Geaya Costantin and Poiss., Kitchingia Baker, Meristostylus Klotzsch, Physocalycium Vest, and Vereia Andrews. Table 1 lists the synonyms for the designated species as determined by Plants of the World Online, which has been confirmed by the Royal Botanic Gardens, as well as the

number of occurrences worldwide as determined by the Global Biodiversity Information Facility.

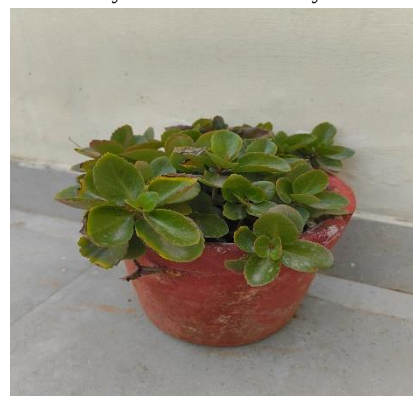


Figure: 3

Different Regions have different name of Kalanchoe marnieriana

Plant Name: Kalanchoe Marnieriana

English name: Marnier's Kalanchoe

Hindi : Rasila podha

Malayalam: Kalanco marniyarana

Tamil: Kalanchoe marnieriana

Marathi: Panfuti

Sanskrit: Parnabija

Indonesia: Kalanchoe marnietriana

Malaysia : Kalanchoe marnietriana

Cambodia: Malacoma Marina

Scientific Classification of kalanchoe marnieriana

Kingdom : Plantae (Plants)

Phylum: Tracheophyta (Vascular Plants)
 Class: Magnoliopsida (Dicots)
 Order: Saxifragales
 Family: Crassulaceae (Stonecrop Family)
 Genus: Kalanchoe
 Species: Kalanchoe marnieriana

Taxonomy and Morphology

Kalanchoe is a perennial succulent plant belonging to the Crassulaceae family. Succulent plants, which have thick and fleshy parts (especially leaves), are resistant to arid climate and soil conditions and present high water holding capacity. These plants can easily store water in plant parts such as leaves or stems. Succulent plants include around 60 different plant families such as Cactaceae, Agavoideae, Aizoaceae and Crassulaceae (Eggli, 2003; Royal Horticulture Society, 2021). Nowadays, it is accepted that there are 33 genera and nearly 1400 species belonging to the Crassulaceae family. This great diversity contains many morphological features such as different forms of plant, leaf and flower structures.

Kingdom : Plantae

Subkingdom: Viridiplantae

Infrakingdom: Streptophyta

Super division: Angiosperms

Division: Magnoliophyta

Subdivision: Magnoliidae

Class: Magnoliopsida

Superorder: Saxifraganae

Order: Saxifragales

Family: Crassulaceae

Genus: Kalanchoe

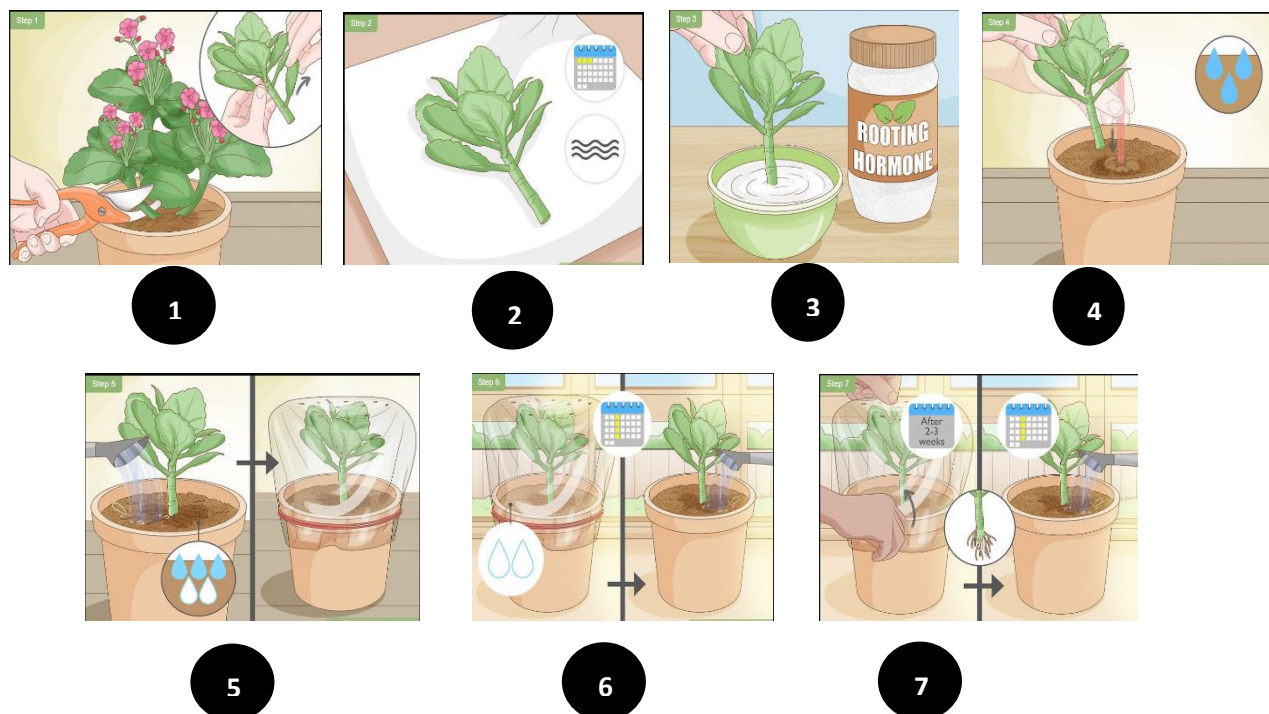
Species: Kalanchoe marnieriana

Kalanchoe marnieriana Cultivation

Bright and cheery succulents, kalanchoes have vibrant flowers and lush green leaves. As long as they are in a reasonably warm environment and receive sunlight, they are quite easy to maintain and may be grown both indoors and outdoors. Kalanchoe, like many other succulents, require very little watering and may even suffer from over watering. Kalanchoe make excellent low-maintenance houseplants or even seasonal presents because they are very easy to care for and resistant to many illnesses that affect more delicate plants.

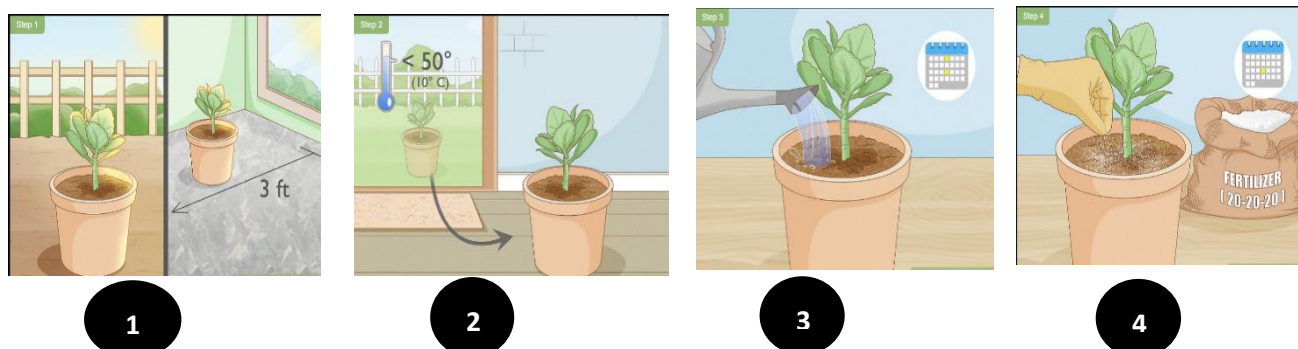
Planting Kalanchoe

- Make a root cutting from an existing plant.
- Let the cutting dry for 3 days
- Dip the end of the cutting in rooting hormone
- Place the cutting in the soil
- Wet the soil and cover the pot with a plastic bag
- Place the pot in bright, indirect sunlight and water occasionally
- Take the bag off the top after 2-3 weeks



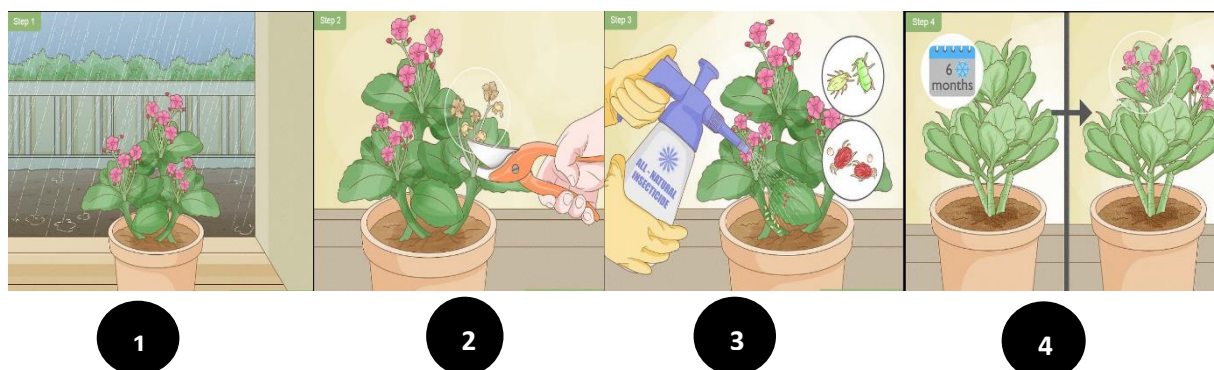
Place the cutting in the soil.

- Wet the soil and cover the pot with a plastic bag
- Place the pot in bright, indirect sunlight and water occasionally.
- Take the bag off the top after 2-3 weeks



Protecting and Pruning Your Kalanchoe Plant

- Keep your plant out of the rain.
- Clip off dead flower stalks
- Use all-natural insecticide alternatives to combat bugs



Botanical Source and Description

Family Crassulaceae, sometimes known as the orpine or stonecrop family (synonym: Sedaceae), is a large dicotyledon family that primarily consists of succulent herbs, while some genera and species tend to be small shrubs or trees. The majority of the family's members are notable for their xeromorphic structure, especially the presence of water-storing tissue in the stem and leaf. It is thought that certain plants can directly take water from the atmosphere through adventitious roots, unique hairs, or epidermal cells. Although they are employed in horticulture, members of this family are not regarded as significant crop plants. Many of them are remarkably beautiful and hardy, usually requiring little maintenance.



Figure: 5

Succulent glasshouse subshrubs or herbs with eye-catching blossoms and foliage. Usually sturdy, upright plants with opposite, fleshy, sessile, or stalked leaves that range in shape from whole to crenate to pinnatifid, and yellow, purple, or scarlet flowers. Palisade tissue is frequently infrequent, opposite, or alternate,

exstipulate, and the leaf is typically centric or intermediate between dorsiventral and centric. Typically, rare, hairs come in a variety of forms, including bladder-like hairs that are occasionally referred to as epidermal cells, glandular hairs with short or long stalks that occasionally secrete mucilage, three-armed, pointed hairs, biserial hairs that give the leaf a cobweb-like surface, and switches between these and glandular shaggy types.

Traditional Uses:

- *Kalanchoe marnieriana* has been used topically to treat cuts, wounds, boils, and ulcers.
- Numerous infections have been treated with the herb.
- Rheumatism, coughs, fevers, and other inflammatory ailments have all been treated with *Kalanchoe* in the past.
- Marnier's *Kalanchoe* is a stress-relieving and air-purifying plant that enhances wellbeing.
- Additionally, it has been used to treat skin disorders, vomiting, diarrhoea, dysentery, and in certain situations, hypertension.

Medicinal Uses:

- According to studies, *Kalanchoe* species including *K. marnieriana* may have anti-inflammatory qualities in their extracts.
- Traditionally, *K. marnieriana* has been used to help heal cuts and wounds, just like other *Kalanchoe* species.
- *Kalanchoe* species may have antibacterial qualities, according to some study, which could help treat illnesses.
- Although studies are still being conducted, some research indicates that parts of *Kalanchoe* species, especially *K. marnieriana*, may have anti-cancer potential.

- *K. marnieriana* should not be kept near pets since, like other *Kalanchoe* species, it contains substances that can be harmful to animals.

Nutritive Values:

- In addition to having a high water content, *Kalanchoe marnieriana* lacks the key nutrients such as proteins, carbs, and vitamins that would make it a substantial food source.
- *Kalanchoe marnieriana* may also be dangerous, and certain *Kalanchoe* species contain substances that might be harmful if consumed.
- A common houseplant or outdoor adornment, *Kalanchoe marnieriana* is prized mainly for its visual attractiveness and low maintenance requirements.

Literature Review

Chemical Review

The succulent plant *Kalanchoe marnieriana*, often called Marnier's *Kalanchoe*, is a member of the Crassulaceae family. Although there are few particular studies on the chemical makeup of *K. marnieriana*, studies on related *Kalanchoe* species indicate that it might include bufadienolides, which are bioactive substances with anticancer and cardio tonic effects.

Alkaloids: The leaves contain alkaloids, which are used in a variety of traditional applications.

Flavonoids: Flavonoids are a significant class of secondary metabolites found in *kalanchoe* that are recognised for their antibacterial, anti-inflammatory, and antioxidant qualities.

Glycosides: These include cardiac glycosides, such as bufadienolides, which have been identified in species of *Kalanchoe*.

Bufadienolides: Bufadienolides are a class of compounds that have significant action, resembling cardiac glycosides such as digoxin.

Other substances: Extracts from *kalanchoe* have also been shown to include lipids, sterols, and fatty acids. Additionally, found in leaves, triterpenes contribute to a variety of pharmacological characteristics.

Conclusion: The existence of bufadienolides in closely related *Kalanchoe* species raises the possibility that *K. marnieriana* also contains similar substances, despite the lack of direct chemical tests of the plant. The precise components of *K. marnieriana* must be confirmed, and their possible biological activities and safety profiles must be evaluated, through more phytochemical research.

Biological activity

Research on *Kalanchoe* species has uncovered a range of biological activity.

Anthelmintic activity: According to reports, *Kalanchoe marnieriana*'s mature bark extracts, both aqueous and ethanolic, have anthelmintic properties against roundworms, tapeworms, and earthworms.

The anthelmintic, thrombolytic, and cytotoxic properties of the *Kalanchoe marnieriana* stem methanolic extract. The study employed anthelmintic activity using aquarium worm *Tubificoides*, thrombolytic activity utilising human red blood cells, and cytotoxic activity using brine shrimp lethality.

Anticancer Activity: The anticancer activity of *kalanchoe* is substantial. Oesophageal, breast, and colon cancers are among the

cancer types for which it is utilised. Cancer is a phrase used to describe a condition where aberrant cells have a tendency to multiply uncontrollably and, in certain situations, spread. To develop treatments for cancer, a lot of research has been done.

Analgesic properties: *Kalanchoe marnieriana* has long been used as a pain reliever. In Swiss albino mice, the anti-nociceptive response of *Kalanchoe marnieriana* bark methanol extract demonstrated a notable decrease in the quantity of writhing brought on by intraperitoneal acetic acid injection and had protective effects on heat-induced pain. In a similar vein, the ethanolic extract of *Kalanchoe marnieriana* leaf demonstrated notable analgesic activity in Wistar albino rat animal models of acetic acid-induced pain (0.6%, 10 ml/kg) as well as the latency to flick the paw or jump from the hot plate kept at 55° C or the time required to remove the tail from hot water (55° C).

Cardio tonic Effects: Research has demonstrated that bufadienolides found in *kalanchoe* species raise intracellular calcium ion concentration and inhibit the Na⁺/K⁺-ATPase pump, which increases the heart's contractile force.

Impact on Immunomodulation: Flavonoids such as quercetin have demonstrated immunomodulatory properties by reducing the proliferation of lymphocytes and altering the generation of cytokines.

The antibacterial and antiviral properties of extracts derived from *Kalanchoe* species indicate that they may find use in therapeutic settings.

Antibacterial and Antiviral Properties: Although there aren't many direct investigations on *Kalanchoe marnieriana*'s antibacterial activity, the fact that similar *Kalanchoe* species contain antimicrobial components raises the possibility that *K. marnieriana* has antibacterial qualities as well. To verify these possible advantages and evaluate *K. marnieriana*'s safety and effectiveness for medical usage, more investigation is necessary. Similar plants of *Kalanchoe* show the antimicrobial and antiviral properties such as given below:

***Kalanchoe laciniata*:** Using an n-hexane extract, the biggest zone of inhibition (52 mm) was seen against *Escherichia coli*, demonstrating the strong antibacterial activity of an ethanolic extract of *K. laciniata*.

***Kalanchoe pinnata*:** Similar to the common antibiotic amoxicillin, ethanolic leaf extracts shown antibacterial efficacy against *Staphylococcus aureus*.

***Kalanchoe crenata*:** Squeezed leaf extracts showed efficacy against a range of species, with at least 8 mg/ml to 512 mg/ml exhibiting the least inhibitory doses.

Conclusion: Despite the paucity of particular research on *Kalanchoe marnieriana*'s biological activity, the plant may share bioactive chemicals and biological activities with other *Kalanchoe* species that are known to have therapeutic benefits. To validate these possible advantages and evaluate *K. marnieriana*'s safety and effectiveness for medical usage, more investigation is necessary.

Present Study Protocol

Protocol-1

A modified variant of the 1989 protocol proposed by Doležal and associates (1) was used in the first the FCM method protocol attempt with *Kalanchoe*, utilising the lysis buffer LB01 and the

fluorochrome PI. The following were components of the lysis buffer:

0.1% (v/v) Triton X-100, 80mM KCl, 6-20mM NaCl, 0.5mM spermine tetrahydrochloride, 15mM Tris-EDTA, 2mM EDTA, and 0.1% KCl. Increased LB01 volume to 5ml and the addition of roughly 0.4g to 0.5g of fresh, immature *Kalanchoe* leaf material were the first modifications (49) made. Before the sample was chopped, 15µl of βmercaptoethanol and 5µl of 1µg ml⁻¹ RNase A were added to the LB01 buffer, even though the original LB01 contained 15mM of βmercaptoethanol. Each sample were cut with a sharp razor blade for about a minute in a glass petri dish that was continuously maintained cold on an ice block.

Homogenates were then passed through a 70µm nylon mesh filter (Spectra/Mesh, Lot No. 3308327), stained with 100µl of PI, and left to incubate for 10 minutes at 4°C in the dark. Using the Guava® easyCyte™ 8HT Benchtop Flow Cytometer (made by Luminex), samples were centrifuged at 400 rpm and 4°C

Protocol-2

Protocol 2 included further modifications to Protocol 1, such as: (i) adding 2% PVP-40 (w/v) to the LB01 buffer composition (8,46); (ii) adding an hour to the PI incubation period; and (iii) filtering the homogenate using a 30µm nylon mesh (Spectra/Mesh, Lot No. 3309326). The remaining buffer ingredients and sample preparation procedures were identical to those in protocol 1.

Protocol-3

The third protocol used the same buffer composition as protocol 2 (LB01 with 2% PVP-40), but it also used a 400 times centrifugation speed and sample preparation that was adapted from the published work using dragon fruit (*Hylocereus* spp. Cactaceae) (7).

g. All sample preparation amounts were reduced from protocol 2 to roughly 0.15g for each of the internal standards and 0.2g fresh young leaf for all *Kalanchoe* species. Additional modifications to protocol 2 included using 1 ml of LB01 buffer, adding 3 µl of βmercaptoethanol and 1 µl of RNase A, and using 5 µl of PI. Leaf samples were chopped on ice in a glass petri dish for approximately one minute. Transferring the liquid homogenate and cut leaf pieces to sterile tubes marked S1, K1, and SK1 (S for standard, K for *Kalanchoe*, and SK for both standard and *Kalanchoe*) was done. Prior to being placed vertically on ice for an additional half hour, all tubes were positioned horizontally on ice and shaken for half an hour on an orbital platform (Labline 3-D Rotator). A clean 1.5 ml centrifuge tube labelled S2, K2, and SK2 received five hundred microlitres of the supernatant from S1 and K1 and one millilitre from SK1. The tubes were then stained with five microlitres (S2 & K2) and ten microlitres (SK2) with propidium iodide. The samples underwent a 15-minute incubation period at 4°C in the dark. Following filtration through a 30µm nylon mesh into a sterile 1.5 ml tube with the labels S3, K3, and SK3, the samples were centrifuged for one minute at 400 x g and 4°C. Otherwise, more centrifugation stages were carried out in 15-second increments at the same speed until a pellet was evident.

The study protocol consisted of the following steps:

- Collection and Identification
- Drying and Grinding
- Extraction
- Filtration and Drying

- Leaves Extract has been found
- Extraction of the Material parts (Leaf)

Solvent: Methanol

Phytochemicals Screening

- Saponins
- Tannins
- Flavanoids
- Alkaloids
- Proteins
- Resins
- Starch
- Phenol
- Carbohydrates

Aim and Objectives

Aim:

- Standardization, Phytochemical evaluation and Anthelmintic activity of *Kalanchoe marnieriana*

Objectives

- Collection, identification and procurement of plant material.
- Pharmacognostical evaluation of plant material.
- Preliminary phytochemical evaluation of plant material.
- Evaluation of anthelmintic activity.

Methodology

Materials & Methods

Plant Collection and Authentication: The leaves of *Kalanchoe marnieriana* was collected from the garden of Om Sterling Global university, Hisar-125001, Haryana and authenticated by Botany department of FRI, Haryana, India. The leaves of *Kalanchoe marnieriana* and plant materials were washed thoroughly 2-3 times with running water and once with sterile distilled water.



Figure:6

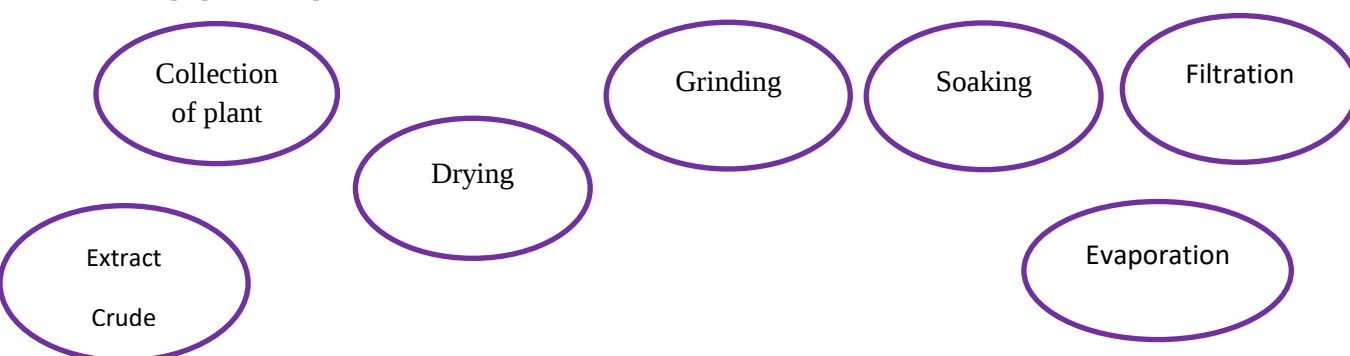
Drying and Grinding of plants leaves: Plant pieces or unwanted elements were removed from the leaves. After thoroughly cleaning the leaves to get rid of any debris, they were left to dry in the shade for 10 days. The leaves that were exposed to direct sunshine were kept shade-dried to prevent deterioration. After that, these were dried in an oven set to a significantly lower temperature for six hours in order to improve grinding. Using a blender, the dried plants were mixed into a coarse powder.



Figure:7

Storage and Preservation of the plants leaves: Before moving on to the next step, the dried powder was kept in an airtight container protected from oxidation, re-absorption of moisture, excessive heat or humidity, the growth of mould and bacteria, and insect and rat

Extract preparation steps-



Microscopic Study and Macroscopy Study

Morphology



Figure: 9

Kalanchoe marnieriana belongs to the plant family Crassulaceae and genus *Kalanchoe*. *Kalanchoe marnieriana* is a succulent subshrub that is distinguished by its unique leaves and trailing habit. Its blue-green, flat, spherical leaves can turn crimson at the tips when exposed to intense light or colder temperatures. They often have a powdered bloom and are oppositely placed on the stalks.



Figure: 10

Macroscopy of plant material: The morphological and organoleptic parameters viz. texture, shape, size, colour, odour etc. of the whole

infestation. Drugs must be stored properly to preserve their high level of quality and shield them from the aforementioned deteriorating agents and causes.

Extraction of plants leaves: Shade-dried plant leaves chopped into small pieces by using mortar and pestle, grinded into powdered form. The powdered plant material was subjected to sequential solvent extraction by soxhlet extraction method. The extraction was done with different solvents in their increasing order polarity such as hexane chloroform and methanol. All the extracts were evaporated using rotary evaporator and the percentage yield was thus recorded. Dried extracts were stored in airtight containers for further studies. Concentrated extracts were subjected to various chemical tests in order to detect the various phytoconstituents.

plant material were noted mainly by naked eye observation and with the help of simple microscope Olympus OIC DM.

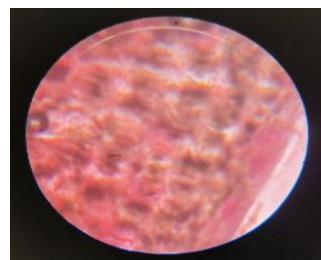


Figure: 11

Powder microscopy of cytomorphological features: Fine dried whole plant powdered samples (~ 2 g) were separately treated with different solutions i.e. aqueous saturated chloral hydrate (for maceration), 50% glycerine, phloroglucinol in conc. HCl (for staining lignified tissues) and 0.02N iodine reagent (for starch grains), mounted on slides with 50% glycerine following a standard Protocol and observed under the binocular compound microscope (Olympus OIC07964) at 10× and 40× magnifications. The camera Lucida drawing of cytomorphological features were prepared using mirror type attachment with the same microscope. Photomicrographs of different cellular structures and inclusions were taken using Magcam DC14 camera attached to an Olympus CX21i trinocular compound microscope.

Preliminary Phytochemical Screening Of Aqueous Extract Of *Kalanchoe Marnieriana* (*Kalanchoe*)

Aqueous extract of *Kalanchoe Marnieriana* (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification

of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, aminoacids, sterols as per Harborne 1998.

Extraction of crude drug

- Take 50 gm of powdered crude drug and macerate it with 500 ml of water for 24 hrs.
- Then occasionally shake with 6hr time period and allow it to stand for 18 hr.
- After filtration evaporate the filtrate to dryness in a tare flat bottom shallow dish.
- Preparation of test solution
- Take 500 mg of extract and dissolve it in 100 ml of water. stir the solution till the extract is completely soluble in water.
- The sample solution is then subjected to various qualitative tests to reveal the presence or absence of common phytopharmaceuticals.

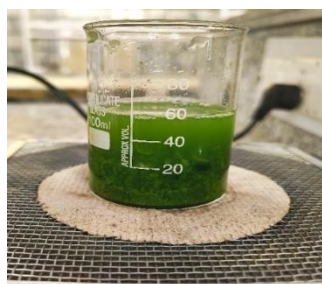


Figure: 12

Test for Alkaloids

About 2 gm of the powdered material was mixed with 1gm of calcium hydroxide and 5 mL of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200 mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was evaporated. To this 5 mL of dilute hydrochloric acid was added followed by 2 mL of each of the following reagents.

- Mayer's Test: A small quantity of the extract was treated with Mayer's reagent. Cream color precipitate indicates the presence of alkaloids.
- Dragendorff's Test: A small quantity of the extract was treated with Dragendorff's reagent. Orange brown precipitate indicates the presence of alkaloids.
- Wagner's Test: A small quantity of extract was treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.
- Hager's Test: A small quantity of extract was treated with Hager's reagent. Yellow precipitate indicates the presence of alkaloids.

Test for Carbohydrates

- Molisch's Test: The extract of the powdered drug was treated with 2-3 drops of 1% alcoholic α naphthol and 2mL of concentrated
- sulphuric acid was added along the sides of the test tube. A purple color indicating the presence of carbohydrates.

- Fehling's Test: The extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugar.
- Benedict's Test: The extract of the powdered leaf was treated with equal volume of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugar.

Modified Born Trager's Test

About 0.1 g of the powdered drug was boiled for 2 minutes with dil. HCl and few drops of FeCl_3 solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dil. NH_3 solution was added to the benzene extract. No pink color was observed in ammonia Cal layer showing the presence of glycosides.

Test for Phenols

Ferric Chloride Test: Add ferric chloride to the extract. A blue or greenish colour indicates the presence of phenolic compounds.

Test for Sterols: The powdered drug was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

Salkowski's Test: A few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in color indicating the presence of sterols.

Test for Liebermann- Burchard's: To the chloroform solution a few drops of acetic anhydride and 1 mL of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

Test for Saponins

Froth Test: 0.1g of powder was vigorously shaken with 5ml of distilled water in a test tube for 30 seconds and was left undisturbed for 20 min, persistent froth indicated presence of saponins.

Test for Tannins

Ferric Chloride: Small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

Gold Beater Skin Test: Add 2 % hydrochloric acid to all small piece of g old beater's skin, rinses it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a 1% ferrous sulphate solution. A brown or black color on the skin indicates presence of tannin.

Test for Phenolic Compounds

Ferric Chloride: A small quantity of the powdered drug was extracted with water. To the alcoholic extract few drops of ferric chloride solution was added. Bluish black color was produced indicating the presence of tannins.

Test for Folin Coicalteu Reagent: To a drop of methanolic extract of a few drops of Folin Coicalteu reagent was added, development of bluish green color showed presence of phenol.

Test for Flavonoids

Shinoda's Test: Little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red color was obtained indicating the presence of flavonoids.

Alkali Test: To the small quantity of test solution 10% aqueous sodium hydroxide solution was added. Yellow orange color was produced indicating the presence of flavonoids.

Lead Acetate: To the test solution add a mixture of 10 % lead acetate in few drops added. It gives white precipitate.

Test for Acid: To the small quantity of test solution, few drops of concentrated sulphuric acid were added. Yellow orange color was obtained indicates the presence of flavonoids.

Test for Proteins and Amino Acids

Millon's Test: Small quantity of acidulous – alcoholic extract of the powdered drug was heated with Million's reagent. White precipitate turned red on heating indicate the presence of proteins.

Biuret Test: To one portion of acidulous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution and one

Fluorescence Analysis

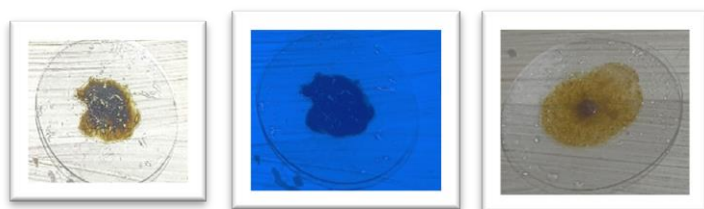


Figure: 13

The coarse drug powder (~ 0.5 g each) was treated with different (18 in number) reagents (5 ml each) such as, acids and alkaline solutions along with other solvents (including distilled water) inside clean test tubes, which were shaken well and allowed to stand for about 24 hours. The individual solutions were observed under normal daylight and UV (254 nm and 365 nm) light for their characteristic colours and compared with the standard colour chart.

Evaluation of Physicochemical



Figure: 14

The physicochemical constant like ash values, Swelling Index, Foaming Index, loss on drying and Extractive values of the plant material were determined by using finely divided powder as per standard guidelines. Extractability was studied with different solvents like hexane, acetone, chloroform, ethyl acetate, methanol, ethanol, water and with equivolume aqueous ethanol. Extractions were performed by conventional cold and hot extraction method and microwave assisted extraction was also done.

Ash Value

drop of dilute copper sulphate solution were added. Violet color was obtained indicating the presence of proteins.

Ninhydrin Test: To the test solution add Ninhydrin solution, boil, violet color indicates presence of amino acid.

Test for Sulphur Containing Amino Acid: 5 ml test solution is mixed with 2 ml 40 % sodium hydroxide and 2 drops of 10% lead acetate solution. Then boil the solution turned black or brownish due to PLS formation.

Test for Terpenoid's: Little of the powdered drug was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionyl chloride. Pink colour solution appeared which indicated the presence.

Preliminary of Phytochemical

The finely powdered plant materials were subjected to soxhlet extraction for 1 h, separately with petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol, water and with equivolume aqueous ethanol. The individual extracts were evaporated to dryness and used for screening the presence of secondary metabolites.

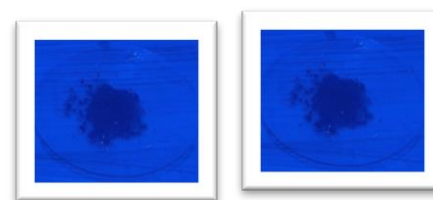


Figure: 15

The determination of ash values (total ash, acid-insoluble ash, and water-soluble ash) is a fundamental aspect of pharmacognostic evaluation for medicinal plants like *Kalanchoe marnieriana* plant. These values help assess the purity and quality of the plant material and identify adulteration or contamination with inorganic matter.

Determination of Ash Values

- Accurately weigh 2–3 g of the powdered of *Kalanchoe Marnieriana* dried extracts.
- Place it in a crucible and incinerate at about 500–600°C in a muffle furnace until free from carbon (i.e., white ash).
- Cool in a desiccator and weigh.
- Calculate the percentage of total ash with reference to the air-dried sample.

- Total Ash: 9.51 %

Determination of Total Ash: About 2g of powdered drug was weighed accurately and placed in tarred silica crucible and incinerated at 450°C in muffle furnace until free from carbon. Crucible cooled, kept in a desiccator and weighed. Same procedure was repeated to arrive at constant weight. The % of total ash obtained was calculated with reference to the air-dried drug.



Figure: 16

Total Ash value of powdered crude drug was recorded.

Total Ash value of sample = $100 (Z - X) / Y$

Z = Weight of total dish + ash (after complete incineration)

X = Weight of the empty dish

Y = Weight of the drug taken

Foaming Index

The foaming index of *Kalanchoe Marnieriana*, serves as an indicator of its saponin content, compounds known for their ability to produce foam when agitated in aqueous solutions. While specific numerical values for the foaming index of *Kalanchoe Marnieriana* are not consistently reported across studies, qualitative assessments confirm the presence of saponins in the plant.

While exact foaming index values for *Kalanchoe marnieriana* are not uniformly documented, qualitative tests consistently demonstrate the presence of saponins in the plant. These findings support the traditional use of *Kalanchoe Marnieriana* in herbal medicine, particularly for its potential expectorant properties.

Determination of Loss on Drying

The process of drying a sample at a particular temperature or employing a chemical titration method to remove water from it provides the basis for determining the moisture content. The moisture content of the sample is reflected in the weight loss that was noted. Drying the sample at (105°C to 110°C) until a consistent weight is reached is the Loss on Drying (LOD) method, which is the most widely used technique for herbal medications.

Procedure

Initially weigh the empty china dish. Then weight of powdered *kalanchoe marnieriana* sample (1gm accurately) is taken in a moisture balance or a pre weighed crucible. The sample is heated in a hot air oven at (105°C to 110°C) for a specific duration (usually 2-2.5 hours with 25 minutes' interval) or until a constant weight is observed. The dried sample is cooled in a desiccator (with calcium chloride pellets) and reweighed of constant value.

Note the reading of weighed sample. Observed and calculate the value of %LOD with the using formula and determined it.

$$\% \text{LOD} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where: $W_2 - W_3$ = Weight loss due to drying and $W_2 - W_1$ = Initial weight of the sample

Weight of empty china dish (W_1) = 18.76 gm.

Weight of sample of *kalanchoe marnieriana* sample = 4gm

Weight of empty china dish + Weight of sample before drying (W_2) = 22.76 gm.

Weight of empty china dish + Weight of sample after drying (W_3) = 22.58 gm.

$$\% \text{LOD} = \frac{W_2 - W_3}{W_2 - W_1}$$

$$= \frac{22.76 - 22.58}{22.76 - 21.76} \times 100$$

$$= 0.18/1 \times 100 = 18\% \text{ Moisture content}$$

$$100 = 18\%$$

Moisture content The calculated moisture content was to be determined 18% from the given *Kalanchoe marnieriana* sample drug herbs.



Figure: 17

Determination of Swelling Index

The **swelling index** measures the volume increase of plant material upon contact with water, which indicates the presence of mucilage, gums, or pectins. In *Kalanchoe Marnieriana*, this index helps to evaluate its mucilaginous or demulcent properties, useful in treating respiratory or gastrointestinal conditions.

Materials Required:

Coarse powdered *Kalanchoe Marnieriana*, 25 mL glass stoppered graduated cylinders, Distilled water, Shaker or vortex (optional), Stopwatch or timer.

- **Sample Preparation:** Weigh exactly **1.0 g** of coarsely powdered of *Kalanchoe Marnieriana*. Transfer to a **25 mL graduated cylinder**.
- **Add Water:** Add **25.0 mL** of distilled water. Shake thoroughly every 10 minutes for **1 hour** (or place on a shaker). **Standing Time:** the cylinder to stand undisturbed for **3 hours** at room temperature.
- **Measurement:** After 3 hours, record the final volume occupied by the swollen material, including any mucilage.

- **Repeat:** Perform the test in **triplicate** for accuracy. Example Result: Studies on *Kalanchoe Marnieriana* report swelling indices ranging from **2.0 to 4.5 mL/g**, depending on the plant part used and its mucilage content.

Thin layer chromatography profiling

TLC (Thin Layer Chromatography): TLC was carried out following the method of Harborne¹¹ and Wagner et.al

Developing solvent system: A number of solvent systems consisting of different ratios of polar and non-polar solvents like Toluene, Ethylamine, Diethylamine, ethyl acetate, formic acid, glacial acetic acid, water, n-butanol etc. were tried.



Figure: 18

Thin Layer Chromatography: 10ul of sample was loaded on pre-coated silica gel 60 F 254 aluminium plate and developed on solvent system comprising of ethyl acetate: formic acid: glacial acetic acid: water in the ratio of 8:1:1:2, 7:2:1:1, 8:2:1:1 and 8:1:2:1 and observed under visible light after spraying with Anisaldehyde Sulphuric acid.

Development of chromatogram: After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with same solvent system used in the TLC procedure for 15 min.

Detection of spots: The air-dried plates were viewed in ultraviolet radiation and also under visual light. The chromatograms were scanned by UV densitometer at 254 nm and 366 nm after derivatization with anisaldehyde-sulphuric acid reagent. The retention factor (Rf) values and finger print data were recorded by WIN CATS software.

Result and Discussions: The preliminary screening of ethanolic extracts of leaf of *Kalanchoe marnieriana* showed the presence of various phytoconstituents. The presence of Fixed oils and fats was not detected in the aqueous extract. TLC was performed with all the extracts using different solvent system. The hydro alcoholic extract showed the best result. The phytoconstituent showed good separation on system comprising of ratios of 8:1:1:2 of ethyl acetate, formic acid, glacial acetic acid and water after spraying with anisaldehyde sulphuric acid. Maximum concentration (42.23%) of the phytoconstituent was found to be at Rf value 1.00. At 366nm wavelength 8well separated peaks were obtained with the Rf ranging from 0.14 to 1.00. The phytoconstituent separated at peak showed a maximum concentration of 36.19% Rf value 0.9. The Corresponding TLC plates at 254nm, 366nm and at visible light are presented. Very inconspicuous scientific study regarding the phytochemicals present in this medicinal species of *Kalanchoe* is reported in literature.

In- Vivo evaluation of Anthelmintic activity on Earthworms

Material Used: In the present investigation of Anthelmintic activity, Carboxy Methyl Cellulose (CMC), Ethanol, Saline was used. All the material was used in laboratory grade.



Figure: 19

Worm Collection: The Indian adult earthworm *Pheretima posthuma* were collected from water logged areas and washed with water to remove all kinds of dirty water from them. They have physiological resemblance with the intestinal round worm parasites of human beings 18, 19, 20.



Figure: 20

Preparation of Plant Extract: The leaves of *Kalanchoe marnieriana* was shade dried and crushed in an electrical blender into powder and sieved to get a coarse powder. The powder was subjected to Soxhlet extraction using ethanol for 72 hours. The solvent was evaporated using rotary evaporator then the extract was used for the evaluation of anthelmintic activity.

Preparation of Concentrations: The ethanolic leaf extract of *Kalanchoe marnieriana* was made into four different concentrations such as 20 mg/ml, 40 mg/ml, 80 mg/ml by dissolving in normal saline. Albendazole was used as reference drug. 0.5% w/v Carboxy Methyl Cellulose (CMC) used as a suspending agent.



Figure: 21

Anthelmintic Assay: The anthelmintic activity was carried according to the standard method 21, 22, 23. Adult Indian earthworm *Pheretima posthuma* has an anatomical and physiological resemblance to the intestinal roundworm parasites of human beings. Indian earthworms were placed in a Petridish containing different concentrations (20 mg/ml, 40 mg/ml, 80 mg/ml) of ethanolic leaf extract of *Kalanchoe marnieriana* and standard drug Albendazole. Each Petri dish contains earthworms and observed for time of paralysis as well as time death. Time of paralysis recorded when no movement of any sort could be observed, except when the worm was shaken vigorously as well as the time of death was recorded after ascertaining that worms

neither moved when shaken. Finally, the test results were compared with standard reference compound Albendazole.

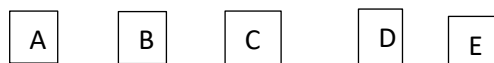


Figure: 21

1. Standard (Albendazole 20mg/ml) (A)
2. Control (Distilled Water) (B)
3. Test Sample 20 mg/ml (C)
4. Test Solution-40 ml (D)
5. Test Solution-80 ml (E)

Result and Discussions: The ethanolic leaf extract of *Kalanchoe marnieriana* shows potent Anthelmintic activity on *Pheretima posthuma*. This activity was concentration dependent. As the concentration increases the extract produce maximal effect. Higher concentrations of ethanolic leaf extract of *Kalanchoe marnieriana* produce a paralytic effect earlier and time taken for death was shorter.

It shows maximum efficacy at 80 mg/ml concentration than the standard drug (Albendazole).

Conclusion: It can be concluded that the ethanolic leaf extract of *Kalanchoe marnieriana* has shown more significant anthelmintic activity when compared to Albendazole against Indian earthworm *Pheretima posthuma*. The product of *Kalanchoe marnieriana* is used as an Anthelmintic agent. Further, the active constituents responsible for Anthelmintic activity can be explored.

Results and Discussion

Morphological characters:

Even though some are shrubs and a few are annuals, the majority of kalanchoe species are perennial herbaceous plants. Usually waxy or hairy, the thick leaves come in a wide variety of shapes. Typically, they are carried down the stems in different directions. The components of the orange, red, or yellow flowers are multiples of four. Clonal plantlets are produced by certain species from the base of the plant or along the outer edges of the leaves. The most popular species, prized for their unique foliage, are the devil's backbone, also known as mother of thousands (*K. daigremontiana*), velvet leaf, or felt bush (*K. beharensis*), penwiper plant (*K. marmorata*), and panda plant (*Kalanchoe tomentosa*).

Figure: 21



Derived from *K. blossfeldiana*, a variety of eye-catching potted plants, known as florist's kalanchoe and distinguished by their vibrant flowers, are frequently marketed in the winter for their flowers, which can last up to eight weeks. With crops that produce [seed](#) freely and come true closely enough for the purposes in view, growing from seed usually is the cheapest and most satisfactory method of [plant propagation](#). Many types of seeds may be sown in open ground and, barring extreme wetness or extreme aridity, germinate well enough for practical purposes. Some species of plants, in their [cultivated](#) forms, do not produce seed—e.g., banana, pineapple, and sugarcane. In a great number of cultivated species, seedlings vary so much that the desired traits are found in only a small proportion. For these and other reasons, horticulturists resort to asexual propagation—i.e., the division or separation and indefinite subdivision of the original plant having the desired traits.

Fluorescence analysis

The fluorescence analysis of coarse dried whole plant powders treated with different reagents reveals the presence of chromophoric compounds in them (Table 1). Different shades of pink fluorescence with 50% KOH, acetic acid, chloroform, ethanol, toluene and light shades of orange and peach coloured fluorescence with 1N HCl, 1N NaOH and Ammonia respectively under 366 nm UV light. No fluorescence was observed under normal daylight and short UV (254 nm) light, indicating small amount of chromophores in the sample.

Table1: Florescence analysis of *Kalanchoe marnieriana* whole plant powder:

Fluorescence Reagents	Visible/Day Light	Short UV (254 nm)	Long UV (366 nm)
1N Hcl	Pink		Faintly fluorescent very light orange
1N NaOH	Greenish brown	Light lemon green	Fluorescent peach
1N NaOH + Methanol	Leafy green	Very faint lemon green	Fluorescent pale bluish green
50% KOH	Yellowish brown	Light green	Fluorescent very light pink
50% H2SO4	Black	Grey	Dark grey
Conc. H2SO4	Black	Grey	Dark grey
Conc. HNO3	Yellow straw	—	Fluorescent grey
Acetic acid	Light green	Very faint lemon green	Faintly fluorescent pink

50% HNO ₃	Light green	–	–
Iodine solution	Reddish violet	Light green with grey centre	Dark grey
Distilled water	Opaque solution	–	Faintly fluorescent no colour
Chloroform	Rusty green	Very light yellowish green	Faintly fluorescent pink
Acetone	Green	Light green	Pink
Ammonia	Reddish brown	–	Fluorescent light peach
Ethanol	Light green	Faint light green	Fluorescent light pink
K ₂ Cr ₂ O ₇	Rusty brown	Lemon green	Bluish grey
K ₂ Cr ₂ O ₇	Blackish brown	Light green	Dark grey

Determination of Ash Value

Accurately weigh 2–3 g of the powdered *Kalanchoe marnieriana*. Place it in a crucible and incinerate at about 500–600°C in a muffle furnace until free from carbon (i.e., white ash). Cool in a desiccator and weigh. Calculate the percentage of total ash with reference to the air-dried sample.

Determination of Total Ash: About 2g of powdered drug was weighed accurately and placed in tarred silica crucible and incinerated at 550°C in muffle furnace until free from carbon. Crucible cooled, kept in a desiccator and weighed. Same procedure was repeated to arrive at constant weight. The final weight of the Ash was 0.951gm.

The % of total ash obtained was calculated with reference to the air-dried drug.

Total ash value of powdered crude drug was recorded.

Ash Content (%) = (Weight of Ash Residue / Weight of Original Sample) * 100

Total Ash: ~9.51%

% ASH = ((ashed wt.) - (crucible wt.)) x 100/ ((crucible and sample wt.) - (crucible wt.))

Z = Weight of total dish + ash (after complete incineration)

X = Weight of the empty dish Y = Weight of the drug taken

Table 2: Physico-chemical evaluation of *Kalanchoe marnieriana*

Physicochemical Parameters	Percentage
Loss on drying (LOD)	18%±0.14
Total ash value	9.51±0.54
Water soluble ash value	2.63±0.32
Acid insoluble ash value	3.33±0.26
Sulphated Ash	3.43±0.38
pH value (10% aq. suspension)	6.55±0.76

Table 3: Preliminary Phytochemical analysis of leave of *kalanchoe marnieriana*

Phytochemical constituents	Leave Extract		
	Ethanol	Chloroform	Hexane
Alkaloids	Positive	Positive	Negative
Carbohydrates	Positive	Positive	Positive
Flavanoids	Positive	Positive	Positive
Saponins	Positive	Negative	Negative
Terpenoids	Positive	Positive	Negative
Tannins	Negative	Positive	Negative

The results of this investigation's phytochemical screening and qualitative assessment of *Kalanchoe marnieriana* leaves revealed the presence of terpenoid, alkaloid, flavonoid, tannins, and carbohydrate in chloroform. Terpenoids, alkaloids, flavonoids, and

carbohydrates are all present in the ethanol extract of *Kalanchoe marnieriana* leaves. The *Kalanchoe marnieriana* leaf extract showed flavonoids in the hexane extract.

Think Layer Chromatography

Table 4:

Sample	Number of bands	Rf values
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<i>Kalanchoe marnieriana</i>	4	0.132, 0.256, 0.834, 0.976
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TLC profile results are shown in Table 4. After sulphuric acid staining and when viewed under UV light, the number of bands formed was counted, and the R_f (Refractive) values were calculated.

In- Vivo Anthelmintic activity on Earthworms

Kalanchoe marnieriana leaf extract in ethanol exhibits strong anthelmintic action against *Pheretima posthuma*. This activity required focus. The extract produces its maximum effect as the concentration rises.

Table 5: Anthelmintic activity on Earthworms

Extract	Concentration	Paralyzed	Death
Ethanolic Extract	20mg/ml	53.5min±0.52	59min±0.06
	40mg/ml	42.2min±0.58	44.1min±0.32
	80mg/ml	33.7min±0.23	36.7min±0.27
Albendazole Standard	20mg/ml	41.34min±0.41	46min±1.33

Conclusion

Phytochemical analysis and antimicrobial research of any chosen plant species are important techniques to prove that the chosen plant species could be utilised as powerful medications. We decided on the *Kalanchoe marnieriana* plant, which is widely available on campus, for the moment our research. It has a unique reputation and is a well-known medication for a variety of illnesses. It can also be used to purify the air and add aesthetic value. It is a well-liked option for indoor as well as outdoor gardening because of its fascinating leaves, resistance to drought, and persistent flowers. The previous information makes it extremely readily apparent that the plants under study may be a good source of beneficial bioactive substances. This investigation revealed that the plant's leaf extract includes a tiny amount of alkaloids and flavanoids in addition to other chemical elements, making the plant's leaves a valuable source of phytochemicals. We discovered that our chosen plant exhibits strong anthelmintic activity against earthworms based on our antimicrobial investigation. In comparison to albendazole, the ethanolic leaf extract of *Kalanchoe marnieriana* has demonstrated notable anthelmintic action against the Indian earthworm *Pheretima posthuma*. *Kalanchoe marnieriana*'s substance is utilised as an anthelmintic. It is also possible to investigate the active ingredients that give anthelmintic action. Therefore, the analysis of our findings makes it abundantly evident that we may utilise our plants as naturally occurring antibacterial medications. The identification and characterisation of the active principles behind bio-efficacy and bioactivity will be the focus of future research.

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