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Research Article

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EXTRACTION, ESTIMATION, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF KALANCHOE PINNATA BY HPLC AND UV

Maruthi Valluru*, A. Meghana, G. Dinesh,

K. Phanisri and P. Madhuri

VJ's College of Pharmacy # 3- 124, Diwancheruvu,
Rajamahendravaram-533296, Andhra Pradesh, India.

ABSTRACT

Kalanchoe is a succulent perennial plant that grows 3-5 feet tall. Commonly known as 'air plant,' it has tall hollow stems, fleshy dark green leaves that are distinctively scalloped and trimmed in red, and bell-like pendulous flowers. Kalanchoe is botanically classified with two main Latin names which refer to the same plant: Bryophyllumpinnatum and Kalanchoepinnatum (as well as various synonyms of both). This review presents detailed survey of literature on phytochemical and medicinal properties of the plant. The chemicals reported from the plant belong to different classes such as alkaloid, diterpenoidal lactones, glycosides, steroids, phenolics, aliphatic compounds, etc. The notable pharmacological properties include anti-diabetic, anti-neoplastic, antioxidant, immunomodulation, anti-lipidaemic, anti-allergic and many more activities which are yet to be explored.

Keywords: Kalanchoepinnata, Phytoconstituents, Pharamacological activity and anti-oxidant.

INTRODUCTION

Plant chemistry is the basis of the therapeutic uses of herbs. A good knowledge of the chemical composition of plants leads to a better understanding of its possible medicinal value. Primary metabolites include small molecules such as sugars, amino acids, tricarboxylic acids, or Krebs cycle intermediates, proteins, nucleic acids and polysaccharides.

Secondary plant metabolites are numerous chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways. Secondary metabolites have shown to possess various biological effects, which provide the scientific base for the use of herbs in the traditional medicine in many ancient communities. They have been described as antibiotic, antifungal and antiviral and therefore are able to protect plants from pathogens.

Secondary plant metabolites are classified according to their chemical structures into several classes. The classes of secondary plant metabolites include:

- Phenolics
- Alkaloids
- Saponins
- Terpenes
- Lipids

Kalanchoe is a medicinal plant largely used in folk medicine for the treatment of kidney stones, gastric ulcer, pulmonary infection, rheumatoid arthritis etc. Kalanchoe pinnata has become naturalized in temperate regions of Asia, Australia, New Zealand, West Indies, Macaronesia, Mascarenes, Galapagos, Melanesia, Polynesia, and Hawaii. In many of these, such as Hawaii, it is regarded as an invasive species. In French Polynesia, Kalanchoe pinnata has been declared a threat to biodiversity. It is also widely distributed in

the Philippines and it is known as katakataka or kataka-taka which is also an adjective meaning astonishing or remarkable. In India it is cultivated in gardens and wild on the hills of North-Western India, Deccan and Bengal. Medicinal plants have been valued for millennia as a rich source of medicinal substances for the prevention of illnesses and afflictions all over the globe. 1 Kalanchoe pinnata (Crassulaceae) is an erect, succulent perennial shrub that grows to be approximately 1.5 metres tall and reproduces both vegetatively and via seeds. It features tall hollow stems, black bell-like pendulous blooms, and newly dark green leaves that are scalloped and trimmed with red. This plant may be readily propagated by cutting stems or leaves. 2,3 Phytochemicals may protect hominids against a wide range of diseases. Phytochemicals are nonnutritive plant combinations that provide caring, therapeutic, or disease fighting properties. Plants generate these compounds to protect themselves; however, new research shows that certain phytochemicals may protect animals from more than only syndromes. In pods and sages, there are many phytochemicals, each with its own mechanism.

MATERIALS AND METHODS

Collection of plant materials

The plant ***kalanchoe pinnata*** was collected. The leaves of the plant were collected from the tree. The collected leaves were cleaned and were shade dried. After complete dry, the leaves were powdered and were preserved in air tight container when required.

Extraction procedure

The dried leaf powder has been weighed about 25 gm and is subjected to extraction by using solvents like Methanol, ethylacetate and Water individually at temperature 60 C for 48 hours by using soxlet apparatus. The solvent was then recovered using distillation apparatus and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of phytochemical screening and quantification of phytochemicals.

Chemicals

Acetonitrile, Methanol, Water .

Standard solution of the drug

For analysis Rutin weigh 10mg of the standard and is dissolved in 10ml of the diluents and sonicated for one min to dissolve the sample completely. Then it is filtered through 0.2micron meter ultipore filter paper to get a concentration of 1000µg/ml. Further required concentrations were prepared from 1000µg/ml solution by proper dilution.

Preparation of mobile phase

A mixture of Methanol, Acetonitrile and Hplc Water in the ratio of 50:49:1(v/v) was measured accurately. The solution was sonicated till the solvents mixed completely. Then it was filtered through 0.45µm nylon membrane filter paper using vacuum filtration. The final filtrate solution was used as a mobile phase for the estimation of Rutin.

Preparation of sample solution

- Take 0.5gm of sample +10ml of HPLC water/Methanol mix the solution and heat the solution and allow the solution to cool and then filter it
- Take empty beaker and weight the beaker and then filter the sample and transferred to the beaker and evaporate the solvent by using hot pan.
- And the again weight the sample with the beaker and calculate the amount of crude obtained.
- Then take 0.01g of sample and add 10ml of water. This gives 1000mg/ml conc sol then filter the solution.
- Take 1ml of solution from 1000mg/ml which was filtered and to this add 9ml of solvent and this gives 100mg/ml conc.
- Then inject this sample into HPLC by sucking the 100mg/ml sample with injection
- Repeat the same process for all samples.

ANTI-OXIDANT ACTIVITY

DPPH free radical scavenging assay

- DPPH scavenging activity was measured by modified method.

- DPPH scavenging activity was measured by the spectrophotometer.
- Stock solution (6mg in 100 ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance.
- Decrease in the absorbance in presence of sample extract at different concentration (10 to 100 µg/mL) was noted after 15 minutes.
- 1.5 ml of DPPH solution was taken and volume made till 3ml with methanol, absorbance was taken immediately at 517nm for control reading.
- 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3ml with methanol.
- Three test sample were take and each processed similarly. Finally the mean was taken absorbance at zero time was taken for each concentration final decrease in absorbances was noted of dpph with the sample at different concentration after 15 minutes at 517nm.
- The % inhibition of free radical dpph was calculated from the following equation.SS

% scavenging activity = [(A0-A1)/A0] ×100.

A0 is the absorbance of the control.
A1 is the absorbance of the extract.

Dilution Table

S.NO	Concentration	Wavelength	Absorbance	% Inhibition
1	5 µg/Ml	517 nm	2.71	1.08
2	7.5 µg/mL	517 nm	2.17	0.92
3	10 µg/mL	517 nm	1.22	0.4

RESULTS AND DISCUSSION

Results of phytochemical screening plant extracts

1	Steroids	Absent	Absent	absent
2	Tri terpinoids	Absent	Present	absent
3	Saponins	Absent	Present	present
4	Steroidal saponins	Absent	Absent	absent
5	Triterpinoidalsaponins	Absent	Absent	absent
6	Alkaloids	positive	Absent	absent
7	Carbohydrates	Absent	Absent	absent
8	Flavanoids	absent	Present	absent
9	Glycocydes	present	Present	absent
10	Phenoilc compounds	Present	Present	absent

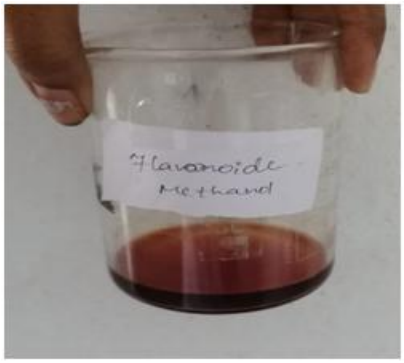
Quantitative estimation

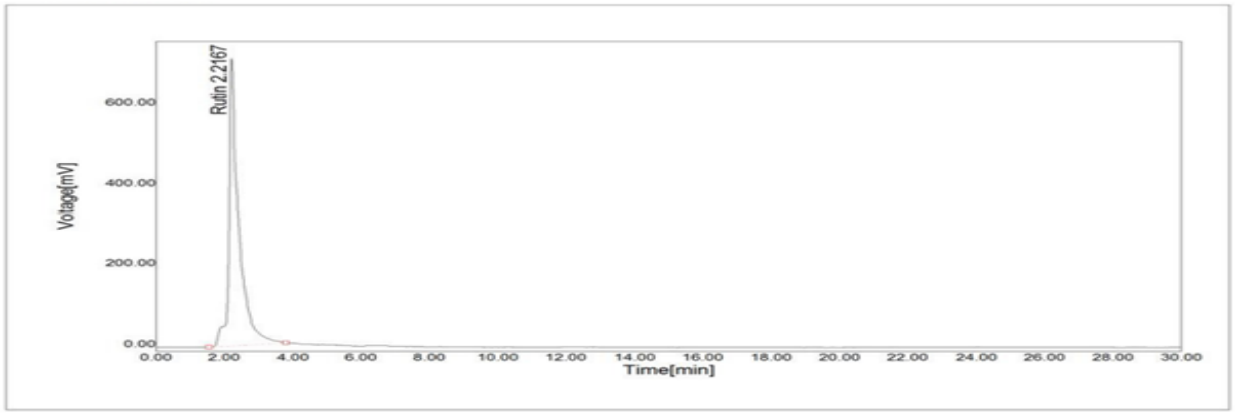
Determination of Flavonoids content

A total of 1 mL of plant extracts were diluted with 200 µL of distilled water separately followed by the addition of 150 µL of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 150 µL of aluminium chloride (10%) solution was added and allowed to stand for 6 min. Then 2 mL of sodium hydroxide (4%) solution was added and made up to 5 mL with distilled water. The mixture was shaken well and left it for 15 min at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

METHANOL (FLAVONOIDS)

HPLC CHROMATOGRAM





Result

No.	Name	RT[min]	Area[mV*s]	Area%	TP	TF	Resolution
1	Rutin	2.2167	14224.9160	100.00	452.9	1.4215	0.0000
Sum			14224.9160				

Result For Rutin in FLAVONOIDS

CONCLUSION

Kalanchoe pinnata plant is considered one of the most promising medicinal properties for the prevention and treatment of many diseases like peptic ulcers, kidney stones. The present study is conducted to investigate the phytochemicals reason for its different medical properties. Different solvents have been used for extraction of the kalanchoe pinnata plant according to their polarity. Majorly Ethyl acetate, Methanol and water solvents are used for extraction and further screening studies were applied to the crude extract. Glycosides, Tannins, Saponins, Terpenoids, Alkaloids, Flavanoids and Phenoilc compounds are have been identified in the plant. Quantitative estimation results confirms the high amounts of various antioxidants are present in the kalanchoe pinnate plant which confirms its presence of various antioxidant chemicals may be reason for its high medicinal properties.

In Methanol extract only rutin was observed. The RT was found to be 2.21 minutes.

The antioxidant activity in KALANCHOE PINNATA leaves was proved and percentage inhibition was found to be 1.08, 0.92, 0.4.

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