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A Research Article on Phytochemical and Physicochemical Evaluation of Leaves & Stems of Kalanchoe Crenata (Adrews) Haw

Manisha Bhatti

Chandigarh University Gharuan (Mohali) 140413, India

Abstract : Kalanchoe crenata Andr. (Crassulaceae) is a fleshy herbaceous plant used in the Agrican traditional medicine as remedies against inflammation, earache, headache, asthma, palpitation, abdominal pain, convulsion and general debility [1]. The present research includes phytochemical and physicochemical evaluation of leaves & stems of *Kalanchoe crenata.*
Key Words : *Kalanchoe crenata,* phytochemical evaluation, physicochemical evaluation.

Introduction

*Kalanchoe crenata (*Crassulaceae) is widely used in Africa for the treatment of inflammatory diseases [1]. *Kalanchoe crenata* is probably native to tropical and South Africa naturalized in Brazil, South and South East Asia. In Java it is mostly found in the east on the hot and dry eastern slope of the mountains.

Materials and Methods

Authentication of Plant Material by Morphological Characters:

K. crenata whole plant was collected from Landran campus was subjected to first morphological identification and followed by authentication by Taxonomist Dr. H.B. Singh, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi. A herbarium specimen of these plants is preserved in the department, Chandigarh College of Pharmacy, Landran (Mohali) for the future reference CCP/HB/MB/02.

Collection and Preparation of Plant Materials:

K. crenata complete plant was collected in bulk quantity from Landran campus, Mohali (Punjab) in month of July after confirmed authenticity. Leaves and stems were manually separated. The plant material were washed with water to remove soil, mud, debris and other adhering materials and dried thoroughly in air under shade at room temperature. Coarse powdered of each drug was prepared, passed through sieve no. 40 and stored in air tight containers.

Physicochemical Evaluation:

Determination of Ash values

Ash values for the leaves & stems of plant were determined as given below:

Total Ash

Accurately weighed 2 g of air-dried powder of leaves & stems samples were taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^0C in muffle furnace until free from carbon, cooled in desiccators and weighed. This was then reheated until the difference between two consecutive weighing is not more than 1mg. percentage of total ash was calculated with reference to air- dried drug [2, 3].

Acid Insoluble Ash

The ash so obtained in the procedure for total ash was boiled with 25ml of 2M HCL for 5minutes. The insoluble matter is collected in a crucible or on an ash less filter paper, washed with hot water and ignited. The residue obtained is cooled in a desiccator and weighed. The percentage of acid- insoluble ash was calculated with reference to air-dried drug separately.

Water Soluble Ash

The total ash content obtained was boiled separately for 5min with 25ml of water. The insoluble matter was collected on a crucible or ash less filter paper, washed with hot water and ignites for 15minutes at a temperature not exceeding 450^0C. Weight of the insoluble residue was subtracted from the weight of the ash and the difference in the weight represented the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug separately. [2, 3]

Fluorescence Analysis

Various tests were used to determine the fluorescence characteristics of the powder drug as well as extract using various reagents such as picric acid, wagner's reagent, dragendroff's reagent, nitric acid, fehling's solution, glacial acetic acid, 1M NaOH, 5%$CuSO_4$ and 5%$AgNO_3$ and observe under short UV, long UV and day light. The fluorescence analysis of drug & extracts helps to identify the drug with specific fluorescent colors and also to find out the fluorescent impurities. The study of fluorescence analysis can be used as diagnosis tool for testing adulteration [4, 5].

Phytochemical Evaluation:

Successive Solvent Extraction

100gm of powder drug was packed and then extracted successively using the various solvents in order of increasing polarity in a sox let apparatus for 4-6 hrs. The solvents used for extraction were petroleum ether (60^0-80^0), benzene, chloroform, acetone, ethanol, and water. Each time before extracting with the next solvent the powder material was dried in an air oven below 50^0C. Finally the marc obtained is macerated with water for 48 hours to obtained aqueous extract. The completion of extraction was confirmed by evaporating a few drops of each extract from the thimble on a watch glass and ensuring that no residue remained after evaporation of the solvent [2].

Determination of Extractive Values

The liquid extracts obtained with different solvents were collected separately. The extracts were concentrated by distillation until thick viscous residue remained in distillation flask. The final volumes of extracts were evaporated to dryness below 60^0C, dried in vaccum desiccator and weighed. Extract was taken in a previously tarred evaporating dish. The solvent was completely evaporated and the residue was weighed. The percentage (w/w) of dry extract was calculated on the basis of dried material.

Qualitative Analysis of Extracts:

In the present study, the qualitative chemical tests was performed for all successive extracts of leaves and stems of *K. crenata* to determine the phytoconstituents such as alkaloids, glycosides, tannins, phytosterols, phenolic acids, flavonoids etc. These chemical tests indicate the presence or absence of particular constituents [2, 3, 6].

Results:

Physicochemical Evaluation:

Ash Values:

Various types of Ash Values (Total Ash Value, Acid Insoluble and Water Soluble Ash) were found to be higher in stems than leaves (shown in table 1). The total ash values of leaves & stems were found to be 8% & 12.8 % in case of *K. crenata*. Highest ash value of stems revealed the presence of inorganic materials such as carbonates, silicates and oxalates etc. as heating lost organic materials in the form of CO_2 left behind the inorganic compounds.

Table: 1 Determination of Total Ash value of leaves & stems of *K. crenata*

Plants	Total Ash Value (%w/w)	Water Soluble (%w/w)	Acid Insoluble (%w/w)
K. crenata (L)	8±0.14	2.92±0.16	1.45±0.15
K. crenata (S)	12.8±0.19	4.12±0.13	3.01±0.36

L- Leaves: S- Stems

Extraction Yield

Extraction Yield for each of successive solvent extract obtained is weighed and its physical appearance and yield are reported in **Table 2 and Figure 1** demonstrate that the water soluble extractive values in leaves and stems of *K. crenata* was higher than their respective alcohol soluble extractive values, which revealed that the presence of more water soluble compounds in these drugs.

Table: 2 Percentage Yield of Successive Solvent Extracts of Leaves & Stems of *K. crenata*

S. No	Extract	Leaves (%w/w)	Stems (%w/w)
1	Pet. ether (60-80^0)	5.36	1.56
2	Benzene	0.46	0.17
3	Chloroform	0.77	0.32
4	Acetone	4.32	1.76
5	Ethanol	5.32	2.73
6	Water	11.4	6.87

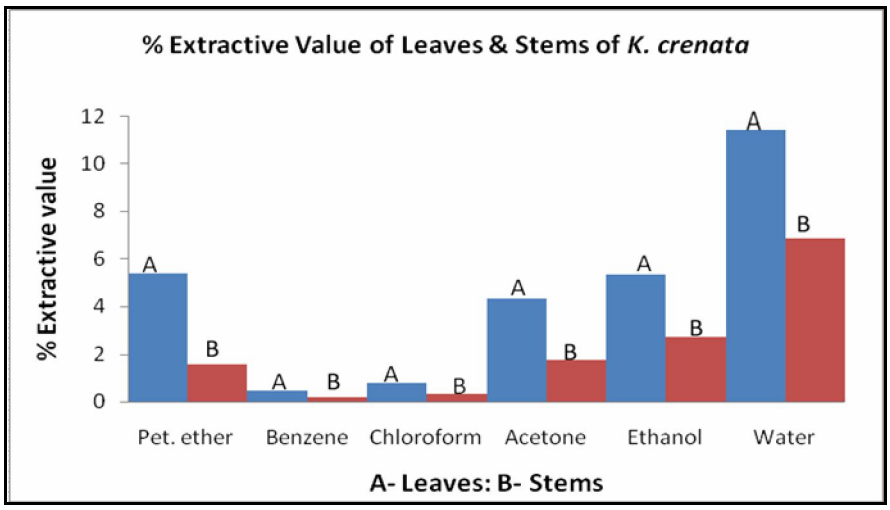

Figure: 1 Comparison of Successive Extractive Values of Leaves & Stems Extracts of *K.crenata*

Table: 3 Fluorescence Analysis of Powdered Leaves & Stems of *K. crenata*

S. no.	Fluorescence Test	Leaves			Stems		
		Day Light	**Short UV 254 nm**	**Long UV 365 nm**	**Day Light**	**Short UV 254 nm**	**Long UV 365 nm**
1	Dry Powder	Light Brown	Brown	Dark brown	Dark Brown	Dark brown	Blackish brown
2	Powder + Conc. HNO_3	Yellow	Light Green	Green	Yellowish Brown	Brown	Dark Brown
3	Powder + Conc. HCl	Light Brown	Green	Brown	Light Brown	Green	Yellowish Brown
4	Powder + Glacial acetic acid	Light Yellow	Green	Brown	Colourless	Colourless	Green
5	Powder + Aq. NaOH	Bluish green	Bluish green	Black	Dark green	Blackish green	Black
6	Powder + Alc. NaoH	Pale yellow	Green	Green	Pale yellow	Green	Green
7	Powder + 5% $AgNO_3$	Brown	Bluish green	Dark brown	Cream	Colourless	Greenish white
8	Powder + Wagner reagent	Dark red	Green	Dark brown	Dark red	Black	Brownish black
9	Powder + 10% Picric acid	Dark yellow	Green	Colourless	Yellow	Yellowish green	Green
10	Powder + Dragondorff reagent	Reddish brown	Black	Brownish black	Reddish brown	Black	Reddish black
11	Powder + Fehling Solution A	Blue	Colourless	Dark blue	Blue	Colourless	Blue
12	Powder + 50% Conc.H_2SO_4	Reddish brown	Greenish black	Black	Reddish brown	Black	Black

Phytochemical Screening:

The active constituents have been identified by performing various qualitative tests in the various successive extracts of leaves and stems of K. *crenata,* are alkaloids, flavonoids, carbohydrates, sterols, phenols and glycosides.

The phytochemical constituents present in successive extracts of leaves & stems of *K. crenata* are presented in **Table 4-5**.

Table 4: Phytochemical Screening of Various Extracts of Leaves of *K. crenata*

S. No.	Type of constituents	Pet. Ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloid	-	-	-	-	+	-
2	Flavonoid	-	-	-	+	+	-
3	Saponins	-	-	-	+	+	+
4	Carbohydrate	+	+	+	+	+	+
5	Phytosterols	+	+	+	+	+	+
6	Tannin	-	-	-	+	+	+
7	Phenolic	-	-	+	+	+	-
8	Coumarin	-	-	-	-	-	-
9	Cardiac glycoside	-	-	+	+	+	+
10	Anthraquinones	-	-	+	+	-	-
11	Essential oil	+	-	-	-	-	-

- + = Present; - = Absent

Table 5: Phytochemical Screening of Various Extracts of Stems of *K. crenata*

S. No.	Type of Constituents	Pet. Ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Alkaloid	-	-	-	-	+	-
2	Flavonoid	-	-	-	+	+	-
3	Saponins	-	-	-	+	+	+
4	Carbohydrate	+	+	+	+	+	+
5	Phytosterols	+	+	+	+	+	+
6	Tannin	-	-	-	+	+	-
7	Phenolic	-	-	-	+	+	
8	Coumarin	-	-	-	-	-	-
9	Cardiac glycoside	-	-	-	+	+	-
10	Anthraquinones	-	-	-	-	-	-
11	Essential oil	-	-	-	-	-	-

- + = Present; - = Absent

After phytochemical screening of different extracts of leaves and stems, it was found that the plant contains phytosterols, phenolic acid, flavonoids, tannins, alkaloids etc. But the leaves also contain cardiac glycosides and essential oil.

Discussion

Total ash value, water soluble and acid insoluble were evaluated. It was found to be highest in stems part of plants. The ash values of leaves & stems were found to be 8% & 12.8 % respectively. This revealed that the presence of inorganic materials such as carbonates, silicates, oxalates etc. The percentage yields for successive extracts were varying to the presence of polar & non-polar components. Extraction yields demonstrate that the water soluble extractive values in leaves and stems of *K. crenata* was higher than their respective alcohol soluble extractive values, which revealed that the presence of more water soluble compounds in these drugs. The plant parts (leaves and stems) and their extracts shows different characteristic colours in day light, short UV, long UV with different reagents, which can used as distinguishing parameters for parts of plant from each other. The different characteristic colours were due to many chemical constituents present in them and it can give an idea of the quality and purity of material. Preliminary phytochemical screening of extracts of leaves and stems revealed that the plant contains phytosterols, phenolic acid, flavonoids, tannins, alkaloids, saponins etc. The leaves contain cardiac glycoside and essential oils also.

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