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Research Article

A COMPARISON ON PHYTOCHEMICAL AND PHYSICOCHEMICAL EVALUATION OF STEM PART OF PLANTS *KALANCHOE PINNATUM AND KALANCHOE CRENATA* (ADREWS) HAW

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ABSTRACT

Kalanchoe pinnata (Lam.) Pers. and *K. crenata* (Andrews) Haw. of family Crassulaceae, are popularly used in the treatment of many diseases. [1] Both species are often misidentified as the other, because of their similar popular uses and names, and the similar external morphology of the leaves. [1] They show close proximity in usage, habitat, preparation and identification. The external morphological features of *Kalanchoe crenata* resemble that of *Kalanchoe pinnatum*. Ethnobotanically, most often they are prepared and administered the same way.[2] The plant is rich in both macro and micro elements, vitamins, calcium, phosphorus, ascorbic acid, inulin [3] and other compounds like saponin, flavonoids, anthraquinones, xanthones, bryophyllin A and B.[4] Alkaloids and saponins are present in the aqueous and alcoholic extracts of plant parts. [6] The green callus of the plant contains malic acid, quinones and tocopherol. [7] The present research includes phytochemical and physicochemical evaluation of stems of *Kalanchoe* pinnatum & *Kalanchoe crenata.*

KEYWORDS: *Kalanchoe pinnatum*, *Kalanchoe crenata,* phytochemical evaluation, physicochemical evaluation

INTRODUCTION:

Kalanchoe pinnatum and *Kalanchoe crenata* belong to the plant family Crassulaceae. They show close proximity in usage, habitat, preparation and identification. The external morphological features of *Kalanchoe crenata* resemble that of *Kalanchoe pinnatum*. Ethnobotanically, most often they are prepared and administered the same way.[2] *Kalanchoe pinnatum* is used in ethnomedicine generally for the treatment of ear-ache, cough, diarrhoea, dysentery, abscesses, ulcers, insect bites, heart-troubles, epilepsy, arthritis, dysmenorrhea and whitlow. [2] The external applications of *Kalanchoe crenata* are the same as those of *Kalanchoe pinnatum*. The juice obtained by squeezing the plant part that have been passed over fire slightly, is most commonly used for the treatment of headache, general debility, dysentery, smallpox and convulsion. One or two drops of the juice is dropped into the ear for earache. Other works have also shown that this plant possesses analgesic, anticonvulsion, antiinflammatory, antiarthritic and antispasmolytic properti

MATERIAL AND METHODS

Authentication of Plant Material by Morphological Characters:

The stem parts of plants *K. pinnatum* and *K. crenata* were collected from Landran campus mohali (pb) and 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 parts are preserved in the department, Chandigarh College of Pharmacy, Landran (Mohali) for the future reference CCP/HB/MB/02.

Collection and Preparation of Plant Materials:

The stem parts of plants *K. pinnatum* and *K. crenata* were collected in bulk quantity from Landran campus, Mohali (Punjab) in month of July after confirmed authenticity. 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

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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 stems of both plants were determined as given below:

Total Ash

Accurately weighed 2 g of air-dried powder of each sample was 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. [9, 10]

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. [9, 10]

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. [11, 12]

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 soxhlet 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. [9]

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 stems of *K. pinnatum & 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

RESULT & DISCUSSION

Physicochemical Evaluation

Ash Values:

Various types of Ash Values (Total Ash Value, Acid Insoluble and Water Soluble Ash) were found to be highest in stems of *K. crenata* than *K. pinnatum*. The total ash values of stems were found to be 6.4% in case of *K. pinnatum* and 12.8 % in case of *K. crenata* respectively. 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 values of stems of *K. pinnatum* & *K. crenata*

Plants	Total Ash Value (%w/w)	Water Soluble (%w/w)	Acid Insoluble (%w/w)
K. pinnatum (S)	6.4±0.32	1.28±0.24	1.42±0.22
K. crenata (S)	12.8±0.19	4.12±0.13	3.01±0.36

S- Stems

Fluorescence Analysis

Fluorescence analysis of the powder drug and of the different extracts was done by observing under day light, 254nm and 365nm. Various methods were used for the study. The different methods gave different fluorescence, hence could be used as a distinguishing parameter for the plant. The fluorescence characteristic for the plant was due to the combination with many chemical constituents present in it. This parameter can be used to distinguish between different parts (leaves and stems) of plant and its adulteration with other drugs.

Table 2: Fluorescence Analysis of Powdered Stems of *K. pinnatum* & *K. crenata*

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

Table 3: Fluorescence Analysis of Successive Extracts of Stems Extracts of *K. Pinnatum* & *K. crenata*

Sr. No.	Extract	*K. Pinnatum*			*K. crenata*		
		Day light	Short UV 254 nm	Long UV 365 nm	Day light	Short UV 254 nm	Long UV 365 nm
1	Pet. Ether (60-80^0)	Yellowish green	Dark Green	Dark Orange	Yellowish green	Green	Orange
2	Benzene	Dark Green	Dark green	Dark green	Green	Dark green	Dark green
3	Chloroform	Green	Brown	Dark brown	Green	Brown	Dark brown
4	Acetone	Brown	Greenish brown	Dark brown	Light brown	Greenish brown	Dark brown
5	Ethanol	Greenish yellow	Deep Green	Yellow	Greenish yellow	Green	Yellow
6	Water	Light brown	Green	Green	Light brown	Green	Green

Phytochemical Evaluation:

Extraction Yield

Extraction Yield for each of successive solvent extract obtained is weighed and its physical appearance and yield are reported in Table. Figure demonstrate that the water soluble extractive values in stems of both drugs (*K. pinnatum* and *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 4: Percentage Yield of Successive Solvent Extracts of Stems of *K. pinnatum* & *K. crenata*

Sr. No.	Extracts	Stems *K. pinnatum* (%w/w)	Stems *K. crenata*(%w/w)
1	Pet. ether (60-80^0)	2.70	1.56
2	Benzene	0.45	0.17
3	Chloroform	0.27	0.32
4	Acetone	1.89	1.76
5	Ethanol	2.42	2.73
6	Water	8.40	6.87

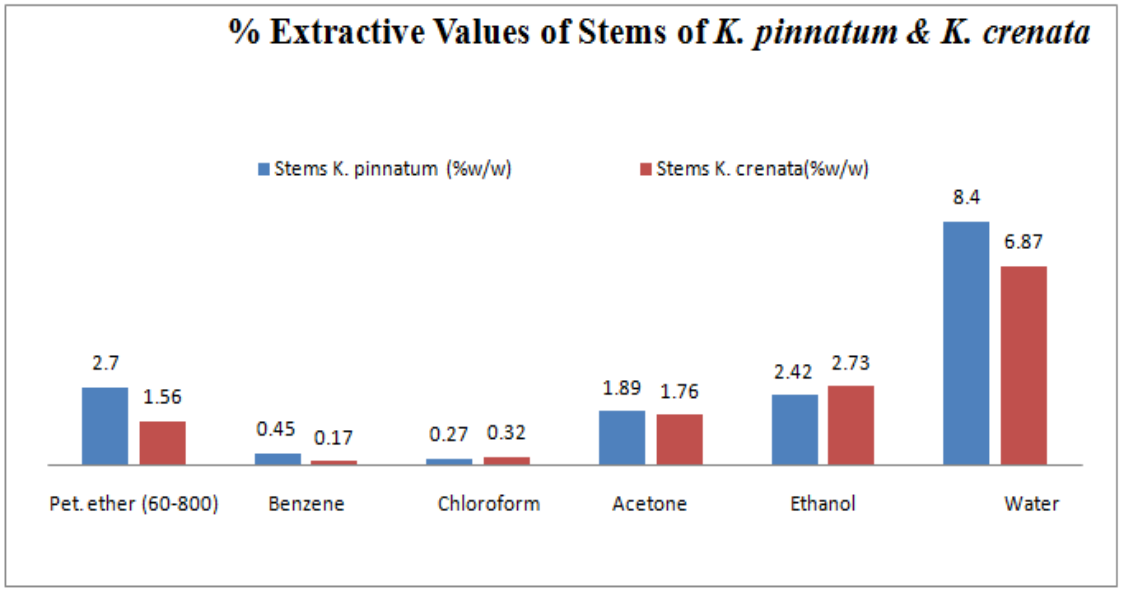

Figure 1: Comparison of Successive Extractive Values of Stems of *K. pinnatum* & *K. Crenata*

Phytochemical Screening:

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

The Phytochemical constituents present in successive extracts of stems of *K. pinnatum* & *K. crenata* are presented in **Tables 5& 6**

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

Sr. 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 6: Phytochemical Screening of Various Extracts of Stems of *K. crenata*

Sr. 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 of *kalachoe crenata* plant. The ash values of *Kalanchoe pinnatum* & *kalachoe crenata* 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 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 (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 stems revealed that the plant contains phytosterols, phenolic acid, flavonoids, tannins, alkaloids, saponins etc.

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