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A comparative pharmacognostical and phytochemical analysis of *Kalanchoe pinnata* (Lam.) Pers. leaf extracts

Bhavsar Shruti, Dhru Bhavita, Zaveri Maitreyi and Chandel Divya

Abstract

Kalanchoe pinnata (Lam.) Pers. has many pharmacological properties such as anticancer, antidiabetic, insecticidal, antimicrobial, anti-urolithiatic, etc. The plant leaves are used as ethno medicine traditionally. Various methods of medicinal preparations are used but most effective, scientific and safe preparation is not known yet. In the present study, four types of extracts of *K. pinnata* leaves were prepared using different methods (Extract 1 – fresh leaf juice, Extract 2 – cold aqueous extract of fresh leaves, Extract 3 – decoction of fresh leaves and Extract 4 – decoction of dried leaf powder). The extracts were investigated for its macroscopical, microscopical, physicochemical and phytochemical properties and the comparative statistical analysis was done. All the extracts showed significant results for their phytochemical study, and Extract 1 showed highest diversity of compounds which places it as best extracts. This study provides important information for the selection of best extract as folkloric preparation.

Keywords: *Kalanchoe pinnata*, *Bryophyllum pinnatum*, pharmacognostical analysis, phytochemical analysis, leaf extracts, comparative

Introduction

Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects [1]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. Large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care [2]. During past years public interest has greatly increased in public interest. Medicinal plants are plants in which one or more of their organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. World Health Organization consultative group that formulated this definition stated also that, such a description makes it possible to distinguish between medicinal plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study [3]. Such plants should be investigated to better understand their properties, safety and efficacy.

Extensive use of plant belonging to *Kalanchoe* species in complementary and alternative therapy has been widely reported. It is one of the Pashanbhed (meaning 'stone breaker') plant mentioned in ancient Ayurvedic literature. *Kalanchoe pinnata* (Lam.) Pers. (Synonyms: *Bryophyllum pinnatum*, *Bryophyllum Calycinum*) belongs to family *Crassulaceae*, and is commonly known as life plant, air plant (Mexican), love plant, Canterbury bells, Cathedral bells etc. It is a perennial herb growing widely and used in folkloric medicine in tropical India, Africa, China, Australia and tropical America [4].

It is a succulent glabrous and ornamental herb, which can grow in houses and garden. It is 0.3-1.2 m high with stems obtusely four angled stem and variable, decussate leaves. The lower leaves are usually simple or occasionally compound, the upper usually 3-5 or sometimes 7 foliolate, long petioled (United by a ridge around the stem). Leaflets ovate or elliptic, crenate or serrate. Flowers pendent, in large spreading panicles with opposite stout branches; pedicels slender. Calyx 2.5-3.8 cm. long, striated red, and green at the base, pale green above; teeth triangular, Corolla swollen and octagonal at the base, constricted in the middle, reddish purple; lobes triangular. Filaments green at the base, pinkish below the anther, anthers hastate, black. Hypogynous scales subquadrate, free or slightly adherent to the carpels, Styles green. Fruit enclosed in the persistent papery calyx and corolla. Seeds small, oblong-ellipsoid, smooth, scarcely striate [5-7].

The plant contains alkaloids, phenolics, tannins, macro elements (magnesium, calcium, potassium, phosphorus, sodium), microelements, (iron, zinc), vitamins (ascorbic acid,

riboflavin, thiamine, niacin) [18]. Leaves contain astragalol, rutin, kaempferol, quercetin [9]. Fresh leaves of plant contain three new constituents, bryophyllol, bryophollone and bryophollone. Three new compounds, bryophynol, two phenanthrene are also present [10]. Two insecticidal bufadienolides were isolated from methanolic extract of leaves, bryophyllin A and bryophyllin C [11]. Leaf contains amino acids i.e. thiamine, pyridoxine, ascorbic acid, glycine, cysteine, casein, nicotinamide. Food contents are also present i.e. carbohydrates, protein, lipids; minerals i.e. sodium, calcium, potassium, phosphorus, magnesium, ferrous, copper, zinc and sugars i.e. raffinose, lactose, sucrose, glucose [12]. Identified active ingredients include alkaloids bufadienolides, flavonoids, glycosides, steroids and organic acids etc [13-17].

This plant has diverse pharmacological activities such as toxic to cattle [18], cytotoxicity [19], antihistaminic [20], immunosuppressive [21], antimutagenic [22], anticancer [23, 24], antihypertensive [25], hepatoprotective [26, 27], wound healing activity [28], uterine contractility [29], antidiabetic [30, 31], antinociceptive and anti-inflammatory [30, 32], analgesic [33], insecticidal, fungitoxic and phytotoxic [34], antileishmanial [35], neuropharmacological [36], nephroprotective [37], antiulcer [38], antimicrobial [39, 40], tracheal antispasmodic [41], anti-allergic [42], antioxidant [39, 43, 44], antidepressant [45], anti-urolithiatic [46], gastroprotective [47], anthelmintic [48], etc. The studies reported are variable and many have not been repeated and confirmed.

Locally in Gujarat, India area, the leaves are consumed by various methods of preparations like, drinking leaves juice directly by squeezing them, chewing fresh leaves or eating dried leaf powder followed by drinking water, as a hot decoction of whole or crushed fresh leaves in water and as a hot decoction of dried leaf powder in water etc. Fresh or dried plant materials can be used as a source for the extraction. Tiwari (2005) has reported about plant extract preparation from the fresh plant tissues [49]. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, the plants are usually air dried to a constant weight before extraction [50-52]. But no scientific, most effective and safe method for this plant has been proven for use. So, in the present study, 4 types of *Kalanchoe pinnata* leaf extracts were analyzed, prepared according to folkloric methods for the comparison of their properties. Objectives were to establish the best extract preparation for use, to evaluate the diversity of phytoconstituents present in all four extracts and compare their quality and quantity for better analysis and to use this data for further toxicological studies.

Materials and Methods

Collection and authentication plant material

The leaves of the plant were collected from Gandhinagar, Gujarat, India during the month of March and authenticated by Dr. M. N. Zaveri, Head, Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Gandhinagar. The voucher specimen PH/14/009 was deposited in K.B.I.P.E.R, Gandhinagar, Gujarat, India.

Pharmacognostical study

Fresh leaves were sun dried and pulverized in a mechanical grinder to obtain coarse powder and passed through sieve (60#), stored in airtight amber coloured bottle and used for present work.

Macroscopical analysis [7]

Macroscopical examination of the plant was done by the observation of morphological characters and comparison to the reported literature.

Microscopical analysis [53,54]

Microscopical examination of the herbal raw material was done by the standard methods like, leaf surface preparation, preparation of sections, powder study etc. and observed under microscope.

Physicochemical evaluation [55-57]

1. Determination of moisture content/ Loss on drying

To determine the amount of moisture content, 10gm of leaf powder was dried in pre weighed porcelain dish at 105 °C in hot air oven and then weighed again. Percentage was calculated with reference to initial weight of leaf powder.

2. Determination of extractive values

Extractive values of leaves of *Kalanchoe pinnata* were determined by the following methods:

a) Determination of water soluble extractive value:

4 gm of dried leaf powder was soaked in 100 ml of water for 1 hr. and mixed properly. The mixture was boiled (100 °C) on water bath and then filtered. Filtrate was evaporated in pre weighed porcelain dish and dried at 105 °C. Water soluble extractive value was calculated.

b) Determination of alcohol soluble extractive value:

4 gm of powdered material was macerated with 100 ml of alcohol in shaking condition and allowed to stand for 16 hr. and filtered. Filtrate was then evaporated in pre weighed porcelain dish and dried at 105 °C. Alcohol soluble extractive value was calculated.

3. Determination of Ash values

Ash values like total ash, acid insoluble ash and water soluble ash of leaf of *Kalanchoe pinnata* was determined by following methods:

a) Determination of Total ash

2 gm of leaf powder was taken in pre weighed silica crucible and incinerated in a muffle furnace at 500 °C- 600 °C till carbon free ash was obtained. Percentage of ash was calculated with reference to initial weight of dried powder.

b) Determination of acid insoluble ash

Ash obtained from total ash was boiled for 5 min. with 25 ml of 1 N HCl and filtered using ashless filter paper to collect insoluble matter. The filter paper was transferred into a pre weighed silica crucible and incinerated at 650 °C in muffle furnace until free from carbon. Percentage of acid insoluble ash was calculated with reference to dried powder.

c) Determination of water soluble ash value

Ash obtained from total ash was boiled for 5 min. with 25 ml of water. Soluble matter was collected on an ashless filter paper. The filter paper was transferred into pre weighed silica crucible and incinerated at 450 °C in muffle furnace. Percentage of water soluble ash was measured with reference to dried powder.

Extract preparation

Four types of extracts were prepared from the leaves of

Kalanchoe pinnata in accordance with the local traditional methods of consuming it.

1. Fresh leaf juice (Extract 1)

50 gm of leaves were washed thoroughly with distilled water and were crushed without any solvent in mechanical grinder. Pulp was squeezed with muslin cloth and filtrate was again filtered through Whatman filter paper No.1. Measured quantity of extract was used for evaporation to dryness to calculate the percentage yield of extract.

2. Maceration (Extract 2)

Extract 2 was prepared by stirring pulp of 50 gm of fresh leaves with 500 ml of distilled water without heat. Exhaustive extraction was done by changing solvent. It was filtered every time with muslin cloth and Whatman filter paper No.1 respectively. Filtrate was evaporated to dryness in pre weighed porcelain evaporating dish. The percentage yield was calculated with reference to the difference of initial weight of leaves taken.

3. Decoction of fresh leaves (Extract 3)

Pulp of 50 gm of leaves was refluxed in 500 ml of distilled water at 100 °C. Solvent was changed for exhaustive extraction and filtered with muslin cloth and Whatman filter paper No. 1 respectively. Filtrate was evaporated to dryness in pre weighed porcelain dish and the percentage yield was calculated with reference to the difference of initial weight of leaves taken.

4. Decoction of dried powdered leaves (Extract 4)

50 gm of dried leaf powder was boiled with 500 ml of distilled water at 100 °C. Solvent was changed for exhaustive extraction and filtered through muslin cloth and Whatman filter paper No. 1 respectively. Filtrate was evaporated to dryness in pre weighed porcelain dish. The percentage yield was calculated with reference to initial weight of dried leaf powder.

Phytochemical analysis of *Kalanchoe pinnata* leaves

Extracts were reconstituted in distilled water for the phytochemical testing. In some tests dried extracts were treated directly as per the protocol.

1. Qualitative phytochemical screening ^[58]

Qualitative phytochemical tests were carried out for Carbohydrates, Proteins and amino acids, Alkaloids, Glycosides, Flavonoids, Tannins, Phenolics, Steroids, Anthraquinone, Saponin, Triterpenoids and Phlobatannins according to standard procedure.

2. Quantitative Phytochemical Screening

a) Estimation of total phenolic content ^[59]

Estimation of total Phenolic content was done as per the method of Singleton and Rossi, with slight modifications. The absorbance was recorded at 765 nm after 30 mins. Gallic acid was used as a standard compound.

b) Estimation of total flavonoid content (by AlCl₃ method) ^[60]

Estimation of total flavonoid content was done as per the method of Zhishen *et al.*, with slight modifications. The amount of total flavonoid was determined with the AlCl₃ reagent. Quercetin was used as a standard compound. Absorbance of pink chromogen was measured at 510 nm vs. blank.

c) Estimation of total alkaloid content ^[61]

Estimation of total alkaloid content was done as per the method of Narasimhan and Mehrotra, with slight modifications. Extract was dissolved in dilute HCl and water was added. This was extracted with chloroform in separating funnel. The aqueous layer was neutralized with Na₂CO₃ (10% w/w) and buffered with NH₃ to pH 9-10. Neutralized aqueous layer was extracted with chloroform. The chloroform layer was evaporated up to dryness. To this 0.1 N HCl was added and titrated against 0.1 N NaOH. [Factor: 1 ml of 0.1 N NaOH = 0.0188 gm of alkaloid].

d) Estimation of total saponin content (by determining foaming index) ^[62]

Estimation of total saponin content was done as per the method of Mukherjee, with slight modifications. Foaming index was calculated using the following formula.

Foaming index = 1000/A. Where, A= the volume in ml of the decoction used for the dilution in the tube where foaming to a height of 1 cm is observed.

Results

Pharmacognostical study

Plant was authenticated by detailed observation of macroscopical and microscopical characters.

Macroscopical characters

Kalanchoe pinnata is a succulent glabrous herb which found 0.3-1.2 m, sometimes 2 m in height; stems of plant were obtusely four angled. The older was light colored while the younger was reddish and speckled with white (Figure a). Leaves of plant were variable and decussate. The lower were simple or occasionally compound in different individual plants while the upper were three to five or sometimes seven foliolate with long petiole which were united by a ridge around the stem. Leaflets were found ovate or elliptic and crenate or serrate in plant (Figure b). Flowers were pendent and hanged in large spreading panicles with opposite stout branches where pedicels were slender. Calyx were also present with 2.5-3.8 cm. length, red coloured striated, and green at the base while pale green above; and triangular teeth. Corolla of the flower was swollen and octagonal at the base. Lobes of were triangular. It was constricted in the middle with reddish purple colour (Figure c and d). The leaves were often found produced on their crenatures at the extremities of the lateral nerves where buds were furnished with root, stem and leaves which drop off and at once become new plants (Figure e).

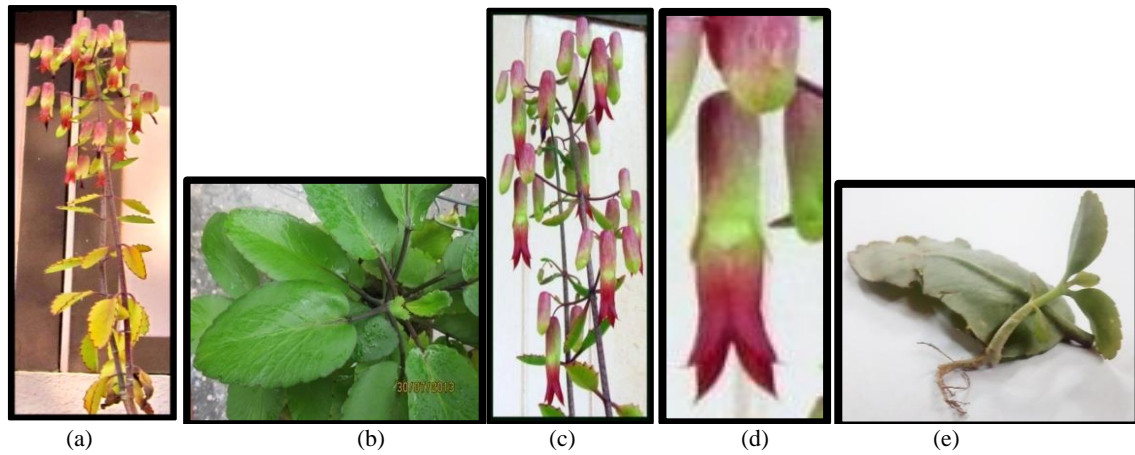


Fig 1: Plant morphology – macroscopical characters

Microscopical characters

In the transverse section of leaf, Lamina showing a layer of epidermis, hypodermis, palisade cell and meristele and in transverse section of leaf passing through midrib, showing collenchymatous tissue, meristele, upper epidermis, lower epidermis and vascular bundle (Figure 2: a, b, c, d and e). Circular outline with anisocytic stomata were observed in the

leaf surface preparation (Figure 3: a, b). Longitudinal cut fragments of leaf petiole shows prismatic crystals of calcium oxalate embedded in parenchymatous cells, and annular and spiral vessels were also observed (Figure 4: a, b). In the powder study, part of vascular bundle, epidermis, annular and spiral xylem vessels were observed (Figure 5: a, b and c).

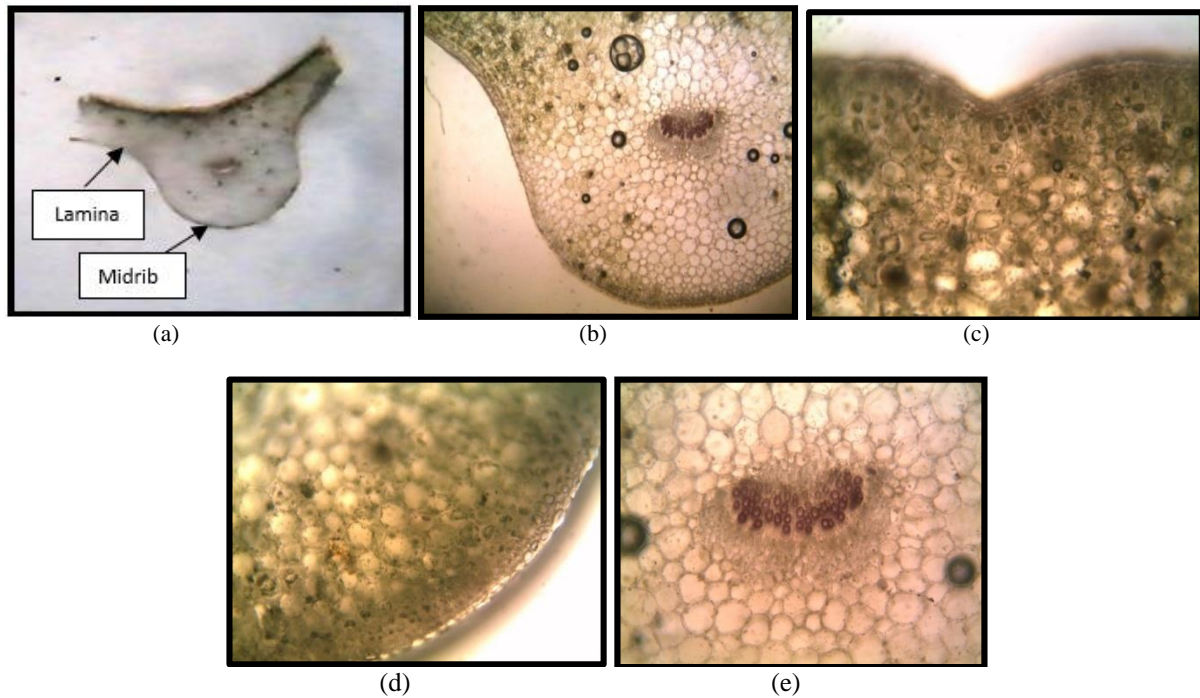


Fig 2: T. S. of *Kalanchoe pinnata* leaf passing through midrib. (a) Whole section in dissecting microscope (4X), (b) Midrib, (c) Upper epidermis, (d) Lower epidermis, (e) Vascular bundle

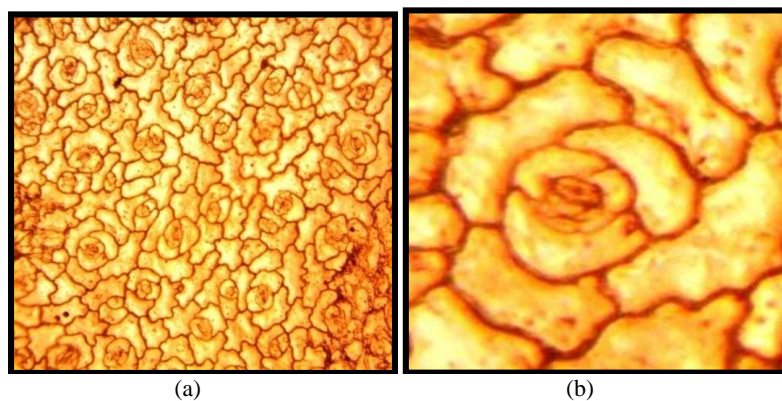


Fig 3: Anisocytic stomata in leaf surface preparation. (a) View in 10X, (b) Magnified image

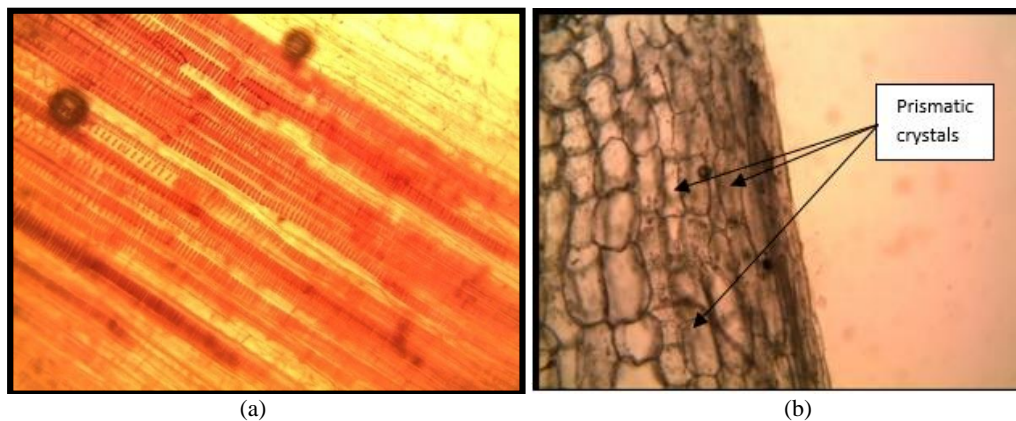


Fig 4: L.S. of leaf Petiole. (a) Annular and spiral vessels, (b) Parenchymatous cells

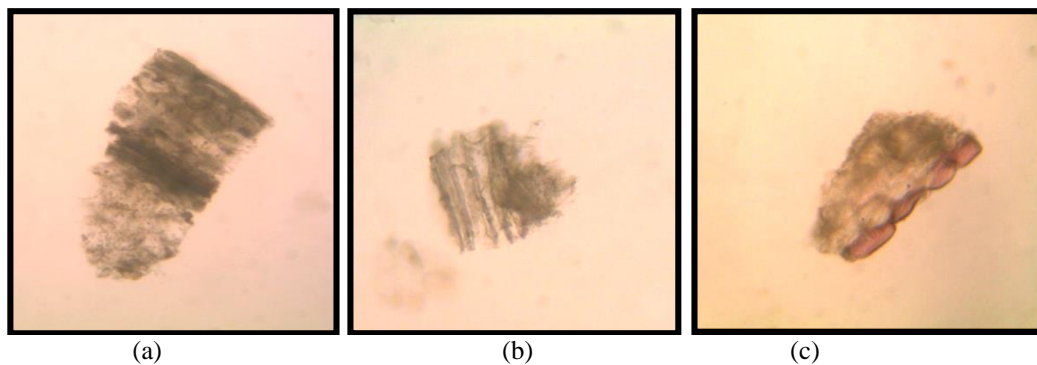


Fig 5: Powder study. (a) Epidermis, (b) Xylem vessel, (c) Vascular bundle

Physicochemical evaluation

For the proper identification of plant, physicochemical parameters (moisture content (LOD), extractive values and ash values) provide useful information.

Moisture content was found to be 1.31% in *Kalanchoe pinnata*. Water soluble extractive value was found greater (35%) than alcohol soluble extractive value (16%) which means water soluble extract contains more components. Total ash obtained was 9.5% in which 0.5% of ash was acid insoluble where as 2.25% of ash was water soluble.

Table 1: Showing results of physicochemical parameters of *Kalanchoe pinnata* leaf powder

Parameters	Value (%)
LOD	1.31
Water soluble extractive value	35
Alcohol soluble extractive value	16
Total ash	9.50
Acid insoluble ash	0.50
Water soluble ash	2.25

Percentage yield of extracts prepared

When the percentage yield of all the extracts was compared with each other, Extract 1 was significantly high when compared to Extract 2, 3 and 4 ($p < 0.001$), Extract 2 was low

when compared to Extract 3 and 4 ($p < 0.001$), Extract 3 was non-significant when compared to Extract 4. Extract 1 showed highest percentage yield (31%) when compared to other extracts.

Table 2: Showing percentage yield of extracts

Extract No.	1	2	3	4
% yield of extracts	31	1.75 ^{***}	4.45 ^{***, ###}	4.50 ^{***, ###, ns}

* Extract 1 vs. 2, 3, and 4; *** ($p < 0.001$)

Extract 2 vs. 3 and 4; ### ($p < 0.001$)

+ Extract 3 vs. 4; ns = non-significant

Phytochemical analysis of *Kalanchoe pinnata* leaves

1. Qualitative phytochemical analysis

The phytochemical analysis was performed for Carbohydrates, Proteins and amino acids, Alkaloids, Glycosides, Flavonoids Tannins, Phenolics, Steroids, Anthraquinone, Saponins, Triterpenoids, and Phlobatannins. As per results shown in Table 3, majority tests were positive for the extract 1 which means it contains highest Phytoconstituents. Liebermann-Burchard test in Steroids group is important for the analysis of Cardinolides (Bufadienolides) which are active components of the plant. The test showed positive results for both the Extract 1 and 3.

Table 3: Showing comparative results of qualitative phytochemical screening of *Kalanchoe pinnata* leaf extracts

Sr. No.	Class of drugs & their tests	Extract 1	Extract 2	Extract 3	Extract 4
1	Carbohydrates				
	Molisch's test	+	+	+	+
	Fehling's test	+	+	-	-
	Benedict's test	+	+	+	+
	Iodine test	+	-	-	-
2	Proteins & Amino acids				
	Ninhydrin test	+	+	+	-

	Xanthoproteic test	+	+	+	+
	Precipitation test	+	+	+	+
3	Alkaloids				
	Dragendorff's test	+	+	+	+
	Wagner's test	+	+	+	+
	Hager's test	-	+	-	-
	Tannic acid test	-	+	+	-
4	Glycosides				
	Salkowski's test	+	+	+	+
	Keller killiani's test	+	+	-	+
	Legal's test	+	-	-	-
5	Flavonoids				
	Shinoda test	+	+	+	+
	Alkali test	+	+	+	+
	Lead acetate	+	-	+	+
	Harborn's test	+	+	+	+
6	Tannins				
	Ferric chloride test	+	+	+	+
	Gelatin-salt test	+	+	-	-
7	Phenolics				
	Ferric chloride test	+	+	+	+
8	Steroids				
	Salkowski's test	+	+	+	+
	Liebermann-Burchard test	+	-	-	+
9	Anthraquinone				
	Modified Borntrager's test	+	-	-	-
	Ciulei test	-	-	-	+
10	Saponins				
	Foam test/ Froth test	+	+	+	+
11	Triterpenoids				
	Liebermann-Burchard test	+	+	+	+
	Salkowski reaction	+	+	-	+
12	Phlobatannins				
	Turbidity test	+	+	+	+

2. Quantitative Phytochemical screening

1) Total Phenolic content

Total Phenolic content was calculated from standard of Gallic acid (Figure 7). Concentration of Total phenolic content for all the extracts was compared (Table 4). Extract 1 was significantly high when compared to Extract 2, 3 and 4 ($p < 0.001$), Extract 2 was significantly low when compared to Extract 3 and 4 ($p < 0.001$) and Extract 3 was significantly low when compared to Extract 4 ($p < 0.001$). The maximum phenolic content was found in Extract 1 (232.9 $\mu\text{g/ml}$).

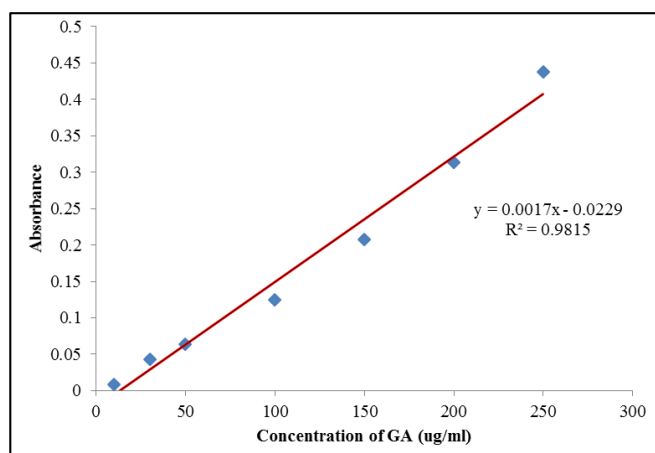


Fig 6: Standard graph of Gallic acid

2) Total Flavonoid content

The results were determined from the standard graph (Figure 7) and were expressed as Quercetin equivalent. Concentration of Total flavonoid content for all the extracts was compared

(Table 4). Extract 1 was significantly high when compared to Extract 2, 3 and 4 ($p < 0.001$), Extract 2 was significantly low when compared to Extract 3 and 4 ($p < 0.001$) and Extract 3 was significantly high when compared to Extract 4 ($p < 0.001$). The maximum flavonoid content was found in Extract 1 (54.96 $\mu\text{g/ml}$).

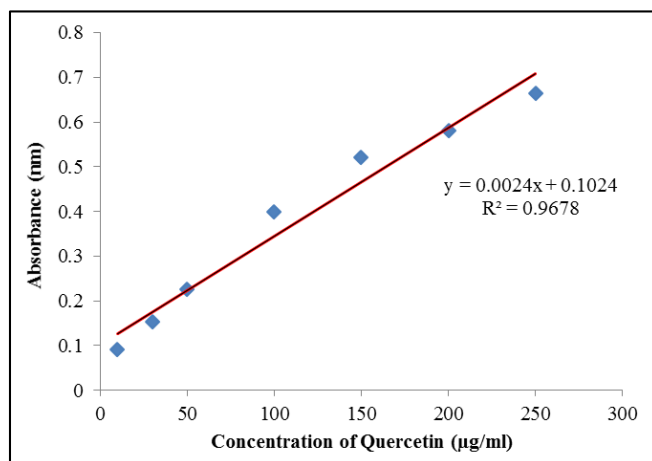


Fig 7: Standard graph of Quercetin

3) Total alkaloid content

Total alkaloid content in the extracts expressed in % leaf material. Concentration of Total alkaloids content for all the extracts was compared (Table 4). Extract 1 was significantly high when compared to Extract 2, 3 and 4 ($p < 0.001$), Extract 2 was significantly low when compared to Extract 3 and 4 ($p < 0.001$) and Extract 3 was significantly high when

compared to Extract 4 ($p < 0.001$). The maximum alkaloids content was found in Extract 1 (18.8 mg/10 ml).

4) Total saponin content (Foaming index)

Total Saponin content was expressed in terms of Foaming index (Table 4) and the total Saponin content for all the extracts was compared (Table 4). Extract 4 showed low Saponin content when compared to Extract 1. Extract 2 and 3 showed Saponin content less than 100 which was also low compared to Extract 1 and 4. The maximum Saponin content found in terms of foaming index was in Extract 1 (400).

Table 4: Showing Comparative concentrations of Phytoconstituents present in different extracts of *Kalanchoe pinnata*.

Phytoconstituents	Extract 1	Extract 2	Extract 3	Extract 4
Total Phenolics ($\mu\text{g/ml}$)	232.9	63.01***	98.93***,###	91.52***,###,+++
Total Flavonoids ($\mu\text{g/ml}$)	54.96	3.86***	42.49***,###	27.02***,###,+++
Total Alkaloids (mg/10 ml)	18.8	17.3***	18.4***,###	17.7***,###,+++
Total Saponins (Index)	400	<100	<100	125

* Extract 1 vs. Extract 2, 3 and 4; *** ($p < 0.001$)

Extract 2 vs. Extract 3 and 4; ### ($p < 0.001$)

+ Extract 3 vs. Extract 4; +++ ($p < 0.001$)

Discussion

Any medicinal plant requires detailed study prior to its use because; the therapeutic efficacy is absolutely dependent on the quality of the plant material used. The original and basic approach towards pharmacognosy includes study of morphological system, study of the cell structures and organization and study of tissue system, which still holds a key in the identification of the correct species of the plant and also to help us to differentiate between closely related species of the same genus. It is also first step to standardize a drug, which is the need of the day [63].

The leaf of *Kalanchoe pinnata* (*Craculaceae*) was studied for its Pharmacognostical and comparative phytochemical parameters in the four different extracts which were prepared as per the traditional procedures popular locally.

Pharmacognostical studies on the microscopical characters, physicochemical constants, powder study are a valuable source of information and provide suitable standards for the identification of the plant material for the investigation. In the present study, the diagnostic characteristics of the leaf sections (T.S., L.S., and Surface preparation) and powdered leaf were studied for the identification and authentication of the plant and the results were coordinated with the standards [54].

In physicochemical constants, determination of ash values is useful for detecting adulterated or low grade products, exhausted drugs and excess of sandy or earthy matter [64]. Different ash figures include total ash, acid insoluble ash, water soluble ash, sulphated ash, carbonated ash etc. Total ash is useful to include drugs which have been coated with chalk, lime or calcium sulphate to improve the appearance. Acid insoluble ash gives evidence of the presence of silica and excessive earthy matter. Water soluble ash detects the presence of material that has been exhausted with water. The determination of extractive values are used as a means of evaluating drugs, the constituents of which are not readily estimated by any other chemical or biological assay method. In the present study, both the acid insoluble ash and water soluble ash were present where value of water soluble ash was greater than acid insoluble ash. Total ash value also showed notable number (9.50 %). Water soluble extractive value was more than alcohol soluble extractive value which proves water a better solvent and traditionally also the leaves are

consumed with water. LOD parameter is useful to check the quality of dried plant product related to moisture content. The moisture content of the dried leaf powder in the present study was only 1.31% which can be neglected.

Phytochemical analysis includes qualitative and quantitative analysis, useful for the investigation of the presence and quantification of primary and secondary components of the plant. The investigation should be followed by the standardized extraction procedures, isolation of the secondary components and other studies like efficacy, toxicity etc. The purpose of the present study was to identify the extraction procedure which has the optimum therapeutic portions of the crude drug of the medicinal plant *Kalanchoe pinnata*. Effect of extracted plant phytochemicals depends on: the nature of the plant material, its origin and degree of processing, moisture content, and particle size. The variation in different extraction methods that will affect quantity and secondary metabolite composition of an extract depends on: type of extraction, time of extraction, temperature, nature of solvent, solvent concentration and polarity [65]. The percentage yield (mass of extract/ mass of dry matter) was used as an indicator of the effects of the extraction conditions. Extract 1 showed maximum percentage yield probably because no solvent was used here.

The best extract should be selected for the further quality standard analysis as per highest diversity of compounds present in it. The present study showed phytochemical variations in results of qualitative and quantitative analysis for all the four extracts. Tests were showing results for the presence of secondary metabolites as per their type and quantity. The reactions showed variation in colour intensity also, due to the variation in quantity of components present in all the extracts. In the present study, though, all the extract showed variation for the same tests, they were prepared in same solvent although method of extraction was different. Extract 1 showed highest diversity of compounds because it was extracted in pure form and no temperature related processes had been done for the extraction.

The quantitative phytochemical analysis results also showed variations in the quantity of particular components for all the extracts, and the Extract 1 showed highest content of total phenolic, total flavonoids, total alkaloids and total saponin (foaming index). Hence, according to this study the fresh leaf juice (Extract 1) is most suitable for medicinal use. The study should be further explored for the selection of best extraction method for folkloric use and Ayurvedic preparation. The extracts should be investigated for Cytotoxicological and Genotoxicological parameters for safety standards.

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