

PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF LEAVES OF  
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**ABSTRACT**

Phytochemical analysis of leaves of *Kalonche pinnata* belonging to the family Crassulaceae was done. The dried leaves were extracted with methanol, and their phytochemical analysis was done. Alkaloids (15.1 mg/100g), Flavonoids (7.2 g CE/100g), Saponins, Cardenolides, Tannins and Phenolic compounds (3.2 g GAE/100g) were found to be present. Physicochemical parameters like water soluble extractive, alcohol soluble extractive, loss on drying, ash content, acid insoluble ash & moisture content were determined.

**KEY WORDS:** *Kalonche pinnata*, methanolic extract, phenolics, flavonoids and alkaloids.**INTRODUCTION**

*Kalonche pinnata*, known as Patharchata in hindi, is a succulent plant belonging to the family Crassulaceae. *Bryophyllum pinnatum* has many pharmacological uses like a suitable treatment in sleep problems in cancer patients<sup>[1]</sup> Also called as 'wonder of life' *Kalonche pinnata* is used to treat diabetic foot infections.<sup>[2]</sup> The plant develops a wound periderm in leaves when exposed to UV-B light and this tissue protects the plant from the stress condition.<sup>[3]</sup> The dichloromethane fraction of the steam distillate of *Kalonche pinnata* leaves has shown excellent insulin secretagogue action and thus can be used in Diabetes mellitus.<sup>[4]</sup> *Bryophyllum pinnatum* is also found suitable in sleep related problems. The results encourage further clinical investigations on the sleep related problems.<sup>[5]</sup> The aqueous leaf extract of *Bryophyllum pinnatum* has demonstrated antihistaminic and expectorant activity in rodent models.<sup>[6]</sup>

The phytochemical analysis of such a vital plant is of prime requirement and with this objective in mind the work was carried forward.

**MATERIAL AND METHODS**

**Material used:** Dried leaves of *Kalonche pinnata*, methanol, phosphate buffer, bromocresol, NH<sub>3</sub>, CHCl<sub>3</sub>, NaOH, HCl, Dragendorff reagent, Hager's reagent, Mayer's reagent, FeCl<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, gelatin, NaCl, lead acetate, AlCl<sub>3</sub>, NaNO<sub>2</sub>, catechin, FolinCiocalteu's reagent, Na<sub>2</sub>CO<sub>3</sub>, gallic acid.

Collection of crude drug: The fresh leaves of *Kalonche pinnata* were collected from the local area, and made free from earthen matter and stored.

**Drying of leaves:** The collected leaves were dried in shade for a few days.

**Comminution:** The dried leaves were subjected to size reduction first by crushing with hands, and then sieved (40 mesh size).

**Extraction:** The coarse powder was subjected to soxhlet extraction continued for 8 cycles (6 hrs) using methanol as a solvent. The extract was filtered and concentrated at reduced temperature on rotary evaporator. The percentage yield was found to be 42.1% w/w and then subjected to preliminary qualitative (6-10) and quantitative (for phenolics, flavonoids and alkaloids).

**Physicochemical tests:** Extractive values. Ash values, moisture content and Loss on Drying were determined using standard procedures. (Table 1).

**Phytochemical tests: (Table 2)**

**Tests of Alkaloids:** The methanolic extract was mixed with ammonia (NH<sub>3</sub>) and then extracted with chloroform (CHCl<sub>3</sub>) solution and dilute hydrochloric acid (HCl) was added. The acid layer was used for chemical tests for alkaloids.

i) Dragendorff's test (Solution of Potassium Bismuth Iodide): Acid layer with few drops of Dragendorff's reagent gave reddish brown precipitate.

ii) Hager's Test (Saturated solution of picric acid): The acid layer with Hager's reagent gave yellow precipitate.

iii) Mayer's test (Potassium Mercuric Iodide): The acid layer with few drops of Mayer's reagent gave a creamy white precipitate.

iv) Wagner's Tests (Solution of Iodine in Potassium Iodide): The acid layer with few drops of Wagner's reagent gave reddish brown precipitate.

#### Tests for steroids

i) Salkowaski Test: Chloroform solution of the extract when shaken with concentrated sulphuric acid ( $H_2SO_4$ ) and on standing showed red colour.

#### Tests for Tannins

**Ferric chloride test:** Extract was mixed with freshly prepared 1 % ferric chloride ( $FeCl_3$ ) solution gave brownish green colour. On adding few drops of 1% solution of gelatin containing 10% sodium chloride gives white precipitate.

#### Tests for Flavonoids

**Lead acetate tests:** Alcoholic solution of the extract when mixed with few drops of 10 % lead acetate gave yellow precipitate.

#### Tests for Saponins

**Foam test:** A small amount of extract was shaken with little quantity of water. The foam was produced that persisted for 10 min.

#### Quantitative determination (Table 3)

##### Determination of total alkaloid content<sup>[7]</sup>

The methanolic extract of the leaves of *Kalonche pinnata* was dissolved in 2 N HCl and then filtered. 1 ml of this solution was then transferred to a separatory funnel and washed with 10 ml chloroform ( $CHCl_3$ ). The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1.0 ml of this solution was transferred to a separating funnel and then 5 ml of bromocresol solution along with 5 ml of phosphate buffer was added. The mixture was shaken and the complex formed was fractionated with chloroform ( $CHCl_3$ ) by vigorous shaking. The fractions were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The procedure was performed thrice.

##### Determination of total flavonoid content<sup>[8]</sup>

The total flavonoid content was measured using colorimetric method. The appropriate amount methanolic extract of leaves of *Kalonche pinnata* was added to a test-tube with distilled water. After 5 minutes 5%  $NaNO_2$  then after 5 minutes 10%  $AlCl_3$  and later after another 5 minutes 1 M NaOH were added to the test tube followed by the addition of distilled water. The absorbance was measured against the blank at 510 nm after 15 minutes. The standard curve was prepared using different concentration of catechin. The flavonoid content was expressed as catechin equivalents (CE) per 100 g of dry weight (dw).

#### Determination of Total Phenolic Content<sup>[8]</sup>

The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method. The appropriate amount of methanolic extract of leaves of *Kalonche pinnata* was oxidized with Folin-Ciocalteu's reagent. The reaction was neutralized with saturated sodium carbonate after 5 minutes. The solution was then immediately diluted to the volume of 50 ml with distilled water. After incubation for 90 minutes the absorbance was measured at 750 nm at room temperature against the blank. Gallic acid was used as the standard. The total phenolic content is expressed as g gallic acid equivalents (GAE) per 100 g of dry weight (dw).

## RESULTS AND DISCUSSION

**Table 1: Physicochemical Parameters of Leaves of *Kalonche pinnata***

S.No.	Physiochemical characters	% content
1	Water soluble extractive	73.3
2	Alcohol soluble extractive	75.5
3	Loss on Drying	5.2
4	Ash content	8.2
5	Acid insoluble ash	1.5
6	Moisture content	2.3

**Table 2: Phytoconstituent Analysis of Methanolic Extract of Leaves of *Kalonche pinnata***

S.No.	Phytoconstituent	Result
1	Alkaloid	+
2	Flavonoid	+
3	Saponins	+
4	Cardenolide	+
5	Tannins	+
6	Phenolic compounds	+

**Table 3: Quantitative Phytochemical Analysis of Methanolic Extract of Leaves of *Kalonche pinnata***

S.No.	Phytochemical	Content
1.	Phenolics (g GAE/100g)	3.2±0.09*
2.	Flavonoid (g CE/100 g)	7.2±0.1*
3.	Alkaloid (mg/100g)	15.1±0.1*

\*Values are expressed as Mean±SD

## CONCLUSION

Phytochemical analysis of leaves of *Kalonche pinnata* was done. Presence of alkaloids, flavonoids, saponins, cardenolides, tannins and phenolic compounds was detected and were found to be present. Quantitative estimation of phenolic, flavonoids and alkaloids was done and were found to be in the concentration 3.2 g GAE/100g, 7.2 g CE/100g and 15.1 mg/100g respectively.

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