



PRELIMINARY PHYTOCHEMICAL STUDIES OF *KALANCHOE GASTONIS-BONNIERI*

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ABSTRACT

Kalanchoe gastonis-bonnierei is a succulent plant native of Madagascar. The leaves have been used in traditional system of medicine for the treatment of ovarian cysts, urinary tract infections, vaginal infections, uterine fibroids. Unfortunately very few works related to the phytochemistry of this species is reported in literatures. The present investigation was carried out to reveal the important phytochemicals present in the leaf extract. The preliminary screening showed the presence of many phytochemicals such as alkaloids, flavonoids, terpenoids, saponoin, fixed oils and fats. High Performance Thin Layer Chromatography analysis was carried out with optimized solvent system consisting of ethyl acetate, formic acid, glacial acetic acid and water in the ratio of 8:1:1:2. The densitometric scanning of the chormatograms of hydroaclcoholic extract showed 7peaks at 254 nm 8 peaks at 366 nm. The phytochemicals detected in the present study justifies the therapeutic uses of the leaves in the traditional medicines.

KEY WORDS: *Kalanchoe gastonis-bonnierei*, Ethnomedical study, Phytochemical screening, HPTLC Chromatogram.



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INTRODUCTION

Kalanchoe gastonis-bonnieri Raym.-Hamet and H. Perrier is one of the 125 species of the genus *Kalanchoe* belonging to Crassulaceae family. It is a succulent herb, native of Madagascar from where it was subsequently introduced into tropical countries¹. They are called "Donkey ear plants" as they have leaves resembling the shape of a donkey's ear. Leaves grow in opposite pairs along stems and are lance- or spatula-shaped (15-38 cm long), smooth-surfaced, with wavy or scalloped leaf edges. Flowers grow in rounded clusters at the tops of stems and are tubular and flask-like in shape with yellowish to reddish 4-lobed petals². The ethnomedical study reported the use of this plant in the treatment of infections, inflammation, menopausal hot flashes, menorrhagia, menstrual cramps (dysmenorrhea), ovarian cysts, pain, urinary tract infections, Vaginal infections^{3,4}. Latino healers in the New York City frequently use the leaves of this plant in the treatment of uterine fibroid³. The Leaf decoction of this plant is effective in treating uro-genital problems⁵. The leaf juice is reported to be having antifertility and contraceptive properties⁶. The present study was conducted to reveal the important phytochemicals present in leaves of the plant so as to correlate the therapeutic properties of the leaf extracts with the secondary metabolites present.

MATERIALS AND METHODS

Collection of samples

The leaves of *Kalanchoe gastonis-bonnieri* were collected from plants grown and maintained by the Bappalal Vaidya Botanical Research Centre, Dept. of Biosciences of Veer Narmad South Gujarat University.

Preparation of plant extract

The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was sequentially extracted with

Petroleum ether, Ethanol, Hydroalcohol (water and 50% ethanol in 1:1 proportion) and water.

Hot Aqueous extraction

20gms. of dried powdered leaf was taken in a conical flask and was filled with 200ml of distilled water. The mixture was heated on a hot plate under continuous stirring at 30°-40°C for 30 minutes. Then the water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis. The water extract was kept in refrigerator at 4° C when not in use.

Solvent extraction

About 20gm of powdered leaf was uniformly packed into a thimble in a soxhlet apparatus and extracted with 200ml Petroleum ether, 95% Ethanol (V/V) and Hydroalcohol. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 3-4 hours. The excess solvent was evaporated and the dried extract was kept in refrigerator at 4°C for their future use in phytochemical analysis.

Phytochemical screening

Chemical tests were carried out with all the solvent fractions using standard procedure described by Siddiqi and Ali⁷, Sofwara⁸ and Sazda et al.⁹.

Alkaloid

Few ml of solvent free extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was and the filtrate was divided in to two equal portions. One portion was treated with few drops of Mayer's reagent and the other with equal amounts of Dragendroff's .Turbidity of the resulting precipitate in the both reagents was taken as evidence for the presence of alkaloids.

Flavonoid

Few ml of extract solution was treated with dilute sodium hydroxide solution which turned the solution to yellow and then it was treated with 5N hydrochloric acid which turned the

solution colourless for the presence of flavonoids and Orange color for flavones.

Glycoside

To the few ml of the extract 2ml of distilled water is added followed by addition of 2ml of 5% ferric chloride. The solution is allowed to heat on water bath for 15 minutes, cooled and then benzene is added and shaken to separate the benzene layer. Formation of pink or red colour is observed after addition of a few drops of ammonia if glycoside is present.

Terpenoid and steroid

A small portion of extract was dissolved in 1ml of chloroform and filtered. To the filtrate on ice, 1 ml of acetic acid was added and then a few drops of conc Sulphuric acid were run down the side of the test tube. The appearance of a pink or pinkish-brown ring / colour indicates the presence of terpenoids. The appearance of blue, bluish-green or a rapid change from pink to blue colours indicates the presence of steroids and a combination of pink and these colours indicates the presence of both steroids and terpenoids¹⁰

Saponins

20ml water is added to the solvent free extract and shaken vigorously; layer of foam formation indicates the presence of saponin.

Tannins

1 ml of 5% ferric chloride to solvent free extract is added. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

Fixed oils and fats

To solvent free extract, a few drops of 0.5 N alcoholic KOH along with a drop of phenolphthalein is added, mixture is heated on water bath for 2 hours, formation of soap indicates the presence of fixed oils and fats.

Reducing sugar

To 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

TLC (Thin Layer Chromatography) and HPTLC profile (High Performance Thin Layer Chromatography)

TLC and HPTLC studies were carried out following the method of Harborne¹¹ and Wagner et.al¹²

Developing solvent system

A number of solvent systems consisting of different ratios of polar and non polar solvents like Toluene, Ethylamine, Diethylamine, ethyl acetate, formic acid, glacial acetic acid, water, n-butanol etc were tried.

TLC: 10ul of sample was loaded on pre-coated silica gel 60 F 254 aluminium plate and developed on solvent system comprising of ethyl acetate: formic acid: glacial acetic acid : water in the ratio of 8:1:1:2, 7:2:1:1, 8:2:1:1 and 8:1:2:1 and observed under visible light after spraying with Anisaldehyde Sulphuric acid.

HPTLC

Sample application

10ul of Sample were applied on 5 x 10 cm, pre-coated silica gel 60 F 254 aluminium plate (MERCK) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed WIN CATS software.

Development of chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with same solvent system used in the TLC procedure for 15 min.

Detection of spots

The air-dried plates were viewed in ultraviolet radiation and also under visual light. The chromatograms were scanned by UV densitometer at 254 nm and 366 nm after derivatization with anisaldehyde-sulphuric acid reagent. The retention factor (Rf) values and finger print data were recorded by WIN CATS software.

RESULTS AND DISCUSSIONS

The preliminary screening of Hydroalcoholic and ethanolic extracts of leaf of *Kalanchoe gastonis-bonnierii* showed the presence of various phytoconstituents like Alkaloids, glycoside, flavonoid terpenoids, saponin and Fixed oils and fats. The presence of Fixed oils and fats was not detected in the aqueous extract. The petroleum ether extract showed the presence of fixed oil and fats only. Steroid, tannin and reducing sugar were absent in all the solvent extracts (Table 1). TLC and HPTLC were performed with all the extracts using different solvent system. The hydroalcoholic extract showed the best result. The phytoconstituent showed good separation on system comprising of ratios of 8:1:1:2 of ethyl acetate, formic acid, glacial acetic acid and water after spraying with anisaldehyde sulphuric acid. UV Densitometric Scanning of the HPTLC plates showed 7 well separated peaks at 254nm (Fig. 2a) with the Rf values ranging from 0.05 to 1.00. Maximum concentration (42.23%) of the phytoconstituent was found to be at Rf value 1.00 (Table 2). At 366nm wavelength (Fig.2b) 8well separated peaks were obtained with the Rf ranging from 0.14 to 1.00. The phytoconstituent separated at peak No.6

showed a maximum concentration of 36.19% Rf value 0.9 (Table 3). The Corresponding HPTLC plates at 254nm, 366nm and at visible light are presented in Fig. 1a, 1b, and 1c. Very inconspicuous scientific study regarding the phytochemicals present in this medicinal species of *Kalanchoe* is reported in literature . Dr. Duke's ethnomedical database ¹³ and Germosén-Robineau ¹⁴ briefly reported phytochemicals like catechol tannins, coumarins, flavones, saponins, sterols and triterpenes to be present in this plant but detailed study is absent. In the present study the phytochemicals showed separation after spraying with anisaldehyde sulphuric acid hence they might belong to the category of terpenoids, phenol or sugar ¹². In the preliminary screening also, terpenoids was found to be present. Hence the phytochemicals detected in the HPTLC analysis might belong to terpenoid group of metabolites. Previous studies with *Kalanchoe pinnata*, one of the most studied species of this genus showed that terpenoids are one of the main constituents of this species¹⁵. Plant terpenoids consists of an enormous group of substances based on isoprene molecule with a carbon skeleton. They play a role in traditional herbal remedies ¹⁶.

Table 1
Detection of different types of phytochemicals in different solvent

Constituents	Petroleum Ether	95%Ethanol	Hydroalchol	Water
Alkaloid	-	+	+	+
Flavonoid	-	+	+	+
Glycoside	-	+	+	+
Terpenoid	-	+	+	+
Steroid	-	-	-	-
Saponin	-	+	+	+
Tanin	-	-	-	-
Fixed oil and Fats	+	+	+	-
Reducing sugar	-	-	-	-

The different phytochemicals detected in different solvent extracts is shown in the above table.
(*+ shows the presence of component and '-' shows the absence)

Table 2
HPTLC Densitometric analysis of Hydroalcohol fraction at 254 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	16.0	0.01	71.5	7.76	0.05	13.1	1474.2	4.34
2	0.23	12.4	0.38	40.0	4.34	0.41	35.9	2812.6	8.28
3	0.43	35.0	0.50	46.6	5.05	0.53	42.7	2430.6	7.15
4	0.59	41.1	0.74	99.8	10.83	0.75	98.8	6335.8	18.65
5	0.76	98.9	0.80	140.1	15.20	0.83	132.7	5771.0	16.99
6	0.84	132.9	0.85	134.5	14.59	0.91	107.0	5777.1	17.00
7	0.92	107.3	0.97	389.1	42.23	1.00	6.5	9373.8	27.59

7 well separated peaks with the Rf values ranging from 0.05 to 1.00, is shown. Maximum concentration (42.23%) of the phytoconstituent was found to be at Rf value 1.00

Table 3
HPTLC Densitometric analysis of Hydroalcohol fraction at 366 nm

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	3.2	0.12	13.8	1.01	0.14	10.0	418.5	1.04
2	0.27	25.9	0.40	47.3	3.44	0.41	46.6	3065.2	7.59
3	0.50	42.6	0.54	45.2	3.29	0.60	28.6	2220.9	5.50
4	0.60	28.7	0.68	89.9	6.54	0.73	63.8	4889.4	12.11
5	0.73	63.9	0.81	384.8	28.02	0.83	256.8	12903.5	31.97
6	0.84	258.1	0.86	497.0	36.19	0.90	82.7	11280.6	27.95
7	0.90	82.7	0.93	91.8	6.68	0.95	77.3	2222.7	5.51
8	0.95	78.8	0.97	203.5	14.82	1.00	1.8	3362.5	8.33

8 well separated peaks were obtained with the Rf ranging from 0.14 to 1.00. The phytoconstituent separated at peak No.6 showed a maximum concentration of 36.19% Rf value 0.9

HPTLC plates at different wavelength

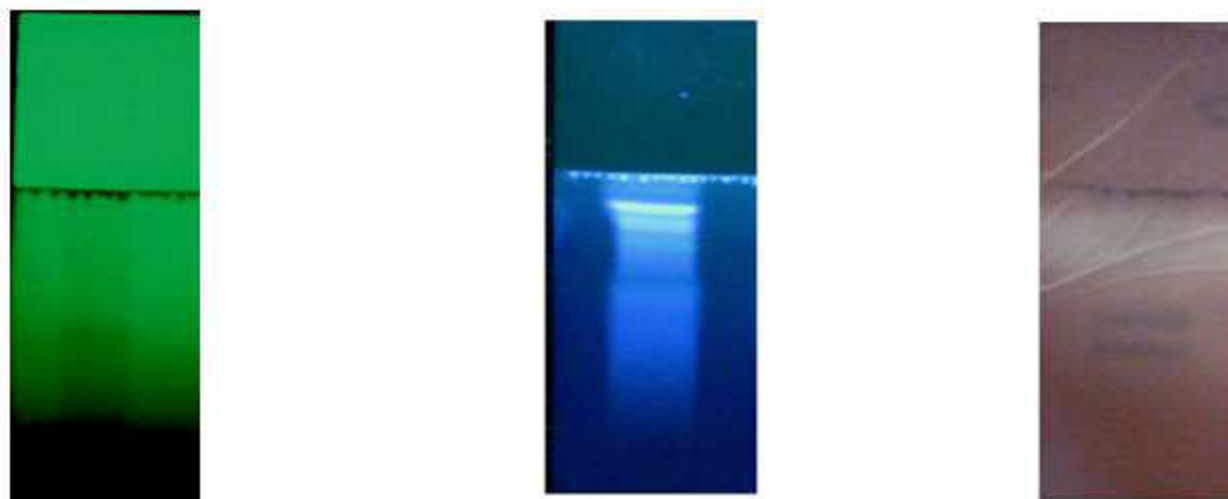
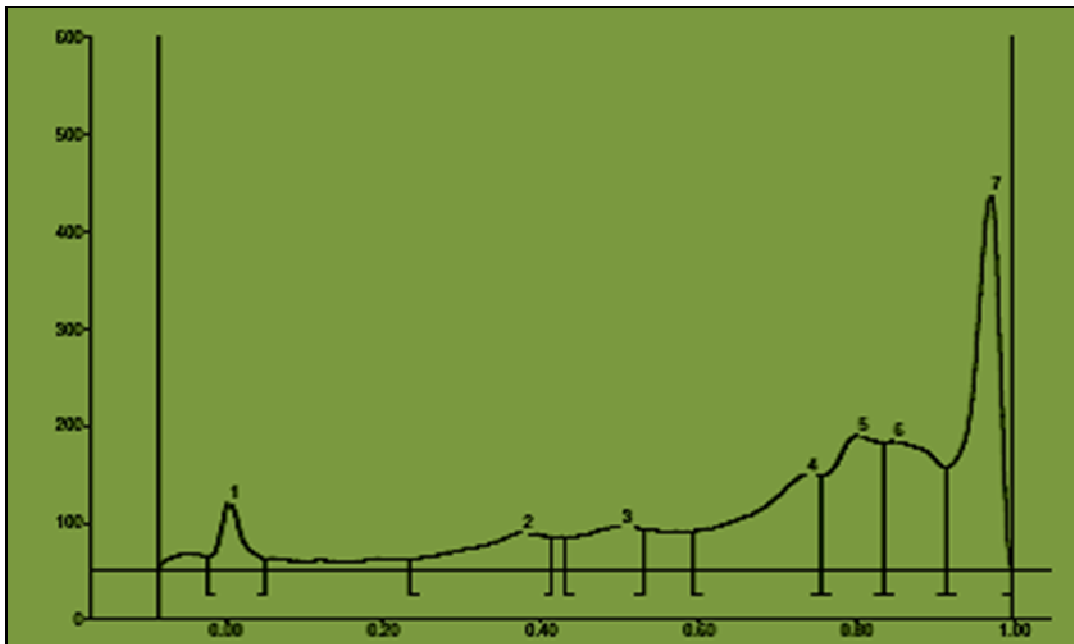


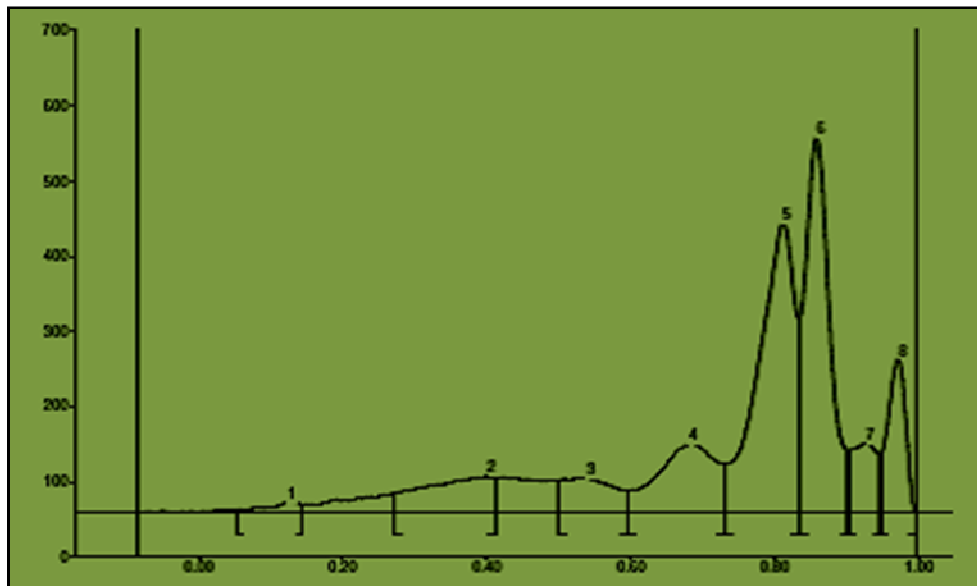
Figure 1a
HPTLC Plate at 254nm **Figure. 1b.HPTLC Plate at 366nm** **Figure.1c HPTLC Plate at visible**

Figure 2a
HPTLC Densitometric scan 254nm



7 well separated peaks at 254nm were observed.

Figure 2b
HPTLC Densitogram at 366nm



8 well separated peaks at 366nm were observed.

CONCLUSION

The traditional medicinal practice of using plants is highly recommended and at the same time emphasis is laid to characterize the active constituents responsible for the activity of these

plants¹⁷The leaf of *Kalanchoe gastonis-bonnieri* is very much used in traditional medicine. This present work attempts to reveal the phytochemicals of the leaf of *Kalanchoe*

gastonis-bonnierii. All the solvent fractions, Petroleum ether, 95% ethanol, Hydroalcohol and water were subjected to the preliminary screening. HPTLC analysis confirmed the presence of the medicinally valuable secondary metabolite, most probably belonging to the terpenoid group. The chromatograms obtained in the present study could be used as the reference standard for further separation and isolation of the active compounds from this plant. Further work to characterize other chemical constituents and compounds is necessary.

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