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A COMPARATIVE STUDY ON THE EXTRACTS OF *KALANCHOE PINNATA* (LINN.) PERS. USING CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT: *Kalanchoe* genus is known for its therapeutic values. *Kalanchoe pinnata* (Linn.) Pers. originating from African continent is known to exhibit wound-healing, antioxidant, anticancer and hepatoprotective activities. Traditionally, it is used to treat stones of the gall bladder. Hence, determination of the phytoconstituents of the plant that are responsible for treatment of various ailments became the area under study. The plant samples were collected from Kanchipuram District, Tamil Nadu, India, to carry out various research analyses. In this study, the aqueous and solvent extracts of the plant were prepared and screened for the presence of phytocompounds by thin layer chromatography (TLC). A comparative study on both the extracts based on results obtained after TLC was made to choose the better amongst the extracts. A gas chromatographic mass spectroscopy (GC-MS) analysis of the chosen extract was performed to determine the compounds present in it. The GC-MS results obtained gave a new dimension to our research studies.

INTRODUCTION: Plants are the abundant sources of various therapeutic agents that are used to treat different kinds of ailments and diseases. *Kalanchoe* is one such genus which has many species with medicinal properties^{1, 2}. *Kalanchoe pinnata* (Linn.) Pers. (synonym: *Bryophyllum pinnatum*) commonly known as “Miracle leaf” or “Ranakalli” in Tamil belongs to the Crassulaceae family. This plant has high wound-healing properties³ (hence, the name) and various other medicinal uses (reason why many studies have been made). It is a succulent, perennial plant that grows about 3 to 5 feet tall.

The leaves are fleshy, distinctively scalloped. The stems are tall and hollow bearing pendulous bell-like flowers. This plant is most commonly found on plains and temperate regions. It can be cultivated in any type of region ranging from high altitudes to low-lying areas. The typical growth-supporting temperature being 25°C, it grows in extreme or arid conditions as well. Such supporting climatic conditions are prevalent in southern regions of India, Kanchipuram district, Tamil Nadu, which marks an abundant source of this plant. When the plant grows under stress or high temperature, appearance of a pinkish layer or region is observed near the leaf margins. This is due to chlorosis (chlorophyll degradation) or low water content in the leaves. Hence, the climatic conditions play a vital role for the growth of this plant. And the soil in which the plant is raised is another factor to consider, as the nutrients supplemented to the plant determine the healthy, thick, greenish appearance of the leaves. When optimum environmental conditions are provided, the plant grows at a

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quicker rate. New plantlets that arise from the leaf margins can be cut off from the parent and cultivated separately on pots or barren lands.

This plant is known to possess a wide variety of activities including antioxidant^{4,5}, antinociceptive⁶, hepatoprotective⁷, antimicrobial^{8,9}, anticancer¹⁰ and anthelmintic¹¹ properties. It is also used to treat gall stones¹². It acts as an anti-inflammatory⁶ agent and can be used for treating oedema. The presence of the phytochemicals that are the causatives for the above mentioned activities could be detected and analysed by chromatographic techniques such as thin layer chromatography (TLC), gas chromatography mass spectroscopy (GC-MS) and high performance thin layer chromatography (HPTLC).

In this study, the aqueous and solvent extracts of the plant were subject to TLC analysis. A comparative study on the aqueous and solvent extracts was carried out based on the results obtained. Further, GC-MS analysis was carried out to determine the phytochemicals present in the extract that yielded better results.

MATERIALS AND METHODS:

Plant Collection:

Fresh and healthy leaves that were hand-picked from the farms near, Chengalpet, Kanchipuram District, Tamil Nadu, India, have been identified and authenticated by Dr. Murugeswaran, Regional Research Institute of Unani Medicine, Royapuram, Chennai (**Fig. 1**).



FIG. 1: HERBARIUM SHEET OF THE PLANT *KALANCHOE PINNATA* (LINN.) PERS.

Preparation of Plant Extract:

Fresh leaves of *K. pinnata* (1 Kg) were washed to remove any impurities present. After shadow drying, the leaves were crushed to fine paste in a mixer followed by the extraction process. The paste was divided into two portions. One portion was used to obtain aqueous extract and the other was soaked in the solvent Diethyl ether (DE) (SRL, Mumbai, Maharashtra, India) to obtain the DE extract.

Chromatographic Analysis:

TLC analysis: About 5 to 10 μ L of each plant extract taken using a 3" capillary tube (Top Tech Lab Equipments, Thane, Maharashtra, India) was spotted on F₂₅₄ Silica gel 60 TLC plates

(size=20 \times 20 cm; thickness \leq 30 μ m) (Merck, Mumbai, Maharashtra, India) with toluene (Rankem, Gujarat, India) and ethyl acetate (Merck) as mobile phase in the ratio 9:1.

GC-MS analysis: The sample extract was injected into the GC column for further analysis. The GC-MS instrument 5975 inert XL MSD (Agilent Technologies, CA, USA) is equipped with J&W DB-5ms capillary column (30 m \times 0.25 mm; film thickness 0.25 μ m). The initial temperature was set at 40 $^{\circ}$ C which increased to 150 $^{\circ}$ C at the rate of 10 $^{\circ}$ C/min. The temperature was again increased to 230 $^{\circ}$ C at the rate of 5 $^{\circ}$ C/min. The process continued till the temperature reached 280 $^{\circ}$ C at the rate of 20 $^{\circ}$ C/min which was held for 8 minutes.

The injector port temperature remained constant at 280°C and detector temperature was 250°C then. Helium was used as the carrier gas with a flow rate of 1 mL/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively.

RESULTS AND DISCUSSION:

TLC Analysis:

The DE extract moved swiftly to form bands, while there was no band formation when the aqueous extract moved over the TLC plates (Fig. 2). The R_f values for the bands obtained were 0.14, 0.2, 0.22, 0.28, 0.33, 0.42, 0.6, 0.66 and 0.76.

GC-MS Analysis:

The analysis of DE extract showed major peaks at retention time 12.924, 18.448, 18.565, 18.668, 19.022 and 19.359 minutes (Fig. 3). And the corresponding compounds identified were butylated hydroxytoluene, 2-hexadecene, 3, 7, 11, 15 - tetramethyl - [R-[R*,R*-(E)]] -, Bicyclo [3.1.1]heptane,2,6,6-trimethyl-, cyclohexanol, 1-ethynyl- and 9-octadecyne. Some smaller peaks were also obtained and the compounds pertaining to those peaks were n-hexadecanoic acid and 9,12-octadecadienoic acid.

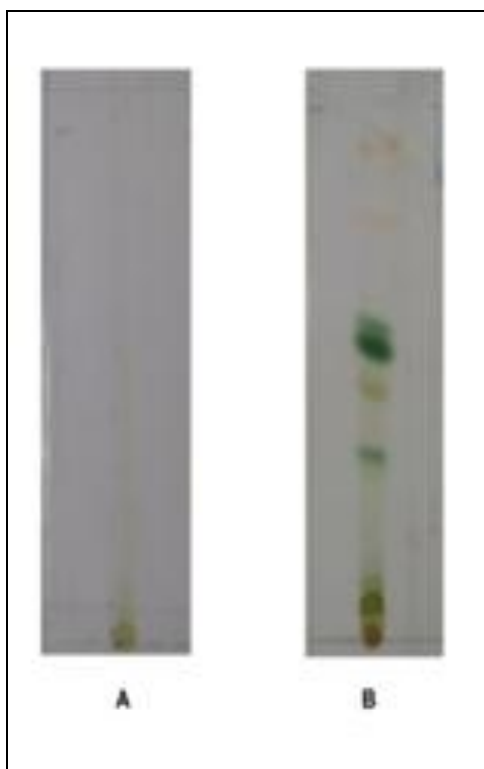


FIG. 2: TLC PLATES OF (A) AQUEOUS EXTRACT AND (B) DE EXTRACT.

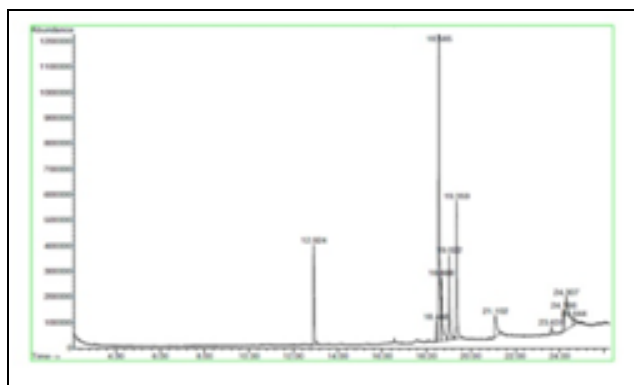


FIG. 3: GC-MS ANALYSIS REPORT OF DE EXTRACT.

CONCLUSION: Based on TLC analysis, it was inferred that the solvent extract of the plant yielded better results than aqueous extract. From the GC-MS report, different compounds present in the solvent extract were identified. Further to this, research studies are being carried out to determine the therapeutic value of the compounds present in the extract.

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REFERENCES:

1. Abdel-Raouf HS: Anatomical traits of some species of *Kalanchoe* (Crassulaceae) and their taxonomic value. *Annals of Agricultural Science* 2012; 57(1):73-79.
2. Milad R, El-Ahmady S and Singab AN: Genus *Kalanchoe* (Crassulaceae): A review of its ethnomedicinal, botanical, chemical and pharmacological properties. *European Journal of Medicinal Plants* 2014; 4(1):86-104.
3. Khan M, Patil PA and Shobha JC: Influence of *Bryophyllum pinnatum* (Lam.) leaf extract on wound healing in albino rats. *Journal of Natural Remedies* 2004; 4(1):41-46.
4. Phatak RS and Hendre AS: Total antioxidant capacity (TAC) of fresh leaves of *Kalanchoe pinnata*. *Journal of Pharmacognosy and Phytochemistry* 2014; 2(5):32-35.
5. Chandra Mohan S, Balamurugan V, Elayaraja R and Prabakaran AS: Antioxidant and phytochemical potential of medicinal plant *Kalanchoe pinnata*. *International Journal of Pharmaceutical Sciences and Research* 2012; 3(3):881-885.
6. Ferreira RT, Coutinho MA, Malvar D do C, Costa EA, Florentino IF, Costa SS and Vanderlinde FA: Mechanisms underlying the antinociceptive, antiedematogenic, and anti-inflammatory activity of the main flavonoid from *Kalanchoe pinnata*. *Evidence-Based Complementary and Alternative Medicine* 2014; 2014:429256.

7. Yadav NP and Dixit VK: Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. Journal of Ethnopharmacology 2003; 86:197-202.
8. Akinpelu DA: Antimicrobial activity of *Bryophyllum pinnatum* leaves. Fitoterapia 2000; 71(2):193-194.
9. Raj V, Kumar A, Singh V, Kumar P and Kumar V: *In vitro* antimicrobial activity of *Kalanchoe pinnata* leaf. International Journal of Current Pharmaceutical Research 2012; 4(3):70-73.
10. Mahata S, Maru S, Shukla S, Pandey A, Mugesh G, Das BC and Bharti AC: Anticancer property of *Bryophyllum pinnata* (Lam.) Oken. leaf on human cervical cancer cells. BMC Complementary and Alternative Medicine 2012; 12:15.
11. Majaz QA, Nazim S, Asir Q, Shoeb Q and Bilal GM: Screening of *in vitro* anthelmintic activity of *Kalanchoe pinnata* roots. International Journal of Research in Ayurveda and Pharmacy 2011; 2(1):221-223.
12. Raj A, Gururaja MP, Joshi H and Shastry CS: *Kalanchoe pinnatum* in treatment of gallstones: An ethnopharmacological review. International Journal of PharmTech Research 2014; 6(1):252-261.

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