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Analysis of Bioactive Constituents (HPLC) of Chloroform Leaf Extract from *Kalanchoe pinnata* in Takum, Taraba State Nigeria

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Abstract

HPLC techniques were employed to analyze the bioactive components of the chloroform leaf extract of *Kalanchoe pinnata* leaf from Takum in Taraba State. A botanist from Taraba State University's Department of Biological Sciences in Jalingo identified the plant leaves, which were then gathered, cleaned, and processed. Using cold maceration, extraction was done by weighing 1500g of the powdered leaf into 1 liter of ethanol and distill water, respectively. Until further investigation, the extracts were stored in a refrigerator in a firmly closed container. The injection valve was used for the HPLC analysis, and the conditions were 20 µl, a UV variable wavelength detector (set at 300 nm), reprosol 100 C8mn, 5 µm 4.6 x 150mm column (30°C), and sensitivity of 0.001. Phosphate buffer (v/v) was used as an aqueous solvent (A) and CH3CN as an organic solvent (B) in HPLC. Gradient elution of the analytes occurred at a flow rate of 1 milliliter per minute. Software generated chromatograms. Operating temperature of the HPLC instrument was room temperature (23 ± 2°C). After injecting 20 µl of each diluted extract into the HPLC three times, the average peak areas were produced and utilized for quantification. Based on the HPLC analysis, the chloroform leaf extract of *Kalanchoe pinnata* contained the following bioactive constituents: quercetin, gallic acid, and chlorogenic

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acid. Additionally, the presence of vitamins B1 and B3 was also noted. The different bioactive constituents eluted at different retention times, and their respective amounts were also detected.

Keywords: Analysis, Bioactive, Constituents, Chloroform, Leaf Extract, Kalanchoe Pinnata

INTRODUCTION

The primary dietary source is plants. They have an abundance of nutrients. They are also abundant in substances with anti-inflammatory and restorative properties (Awuchi, 2019). Without any awareness of the substances found in them or how they worked, people have been using plants to treat illness since ancient times. Societies all across the world have created unique traditions over the ages to understand medicinal plants and their applications. The prevalence of natural products with medicinal qualities has led to an increase in the usage of herbal treatments and health care preparations made from widely used traditional herbs and medicinal plants (Thenmozhi et al., 2010).

Phytochemicals and plant extracts have established antibacterial qualities, therefore using them in medical interventions can have a significant impact. A multitude of research undertaken in many nations have demonstrated its efficiency in the past few years. The antibacterial properties of many plants have been exploited; these properties are a result of chemicals that are produced during the secondary metabolism of the plant.

The succulent perennial herb *Kalanchoe pinnata,* sometimes referred to as the "miracle leaf" or "life plant," is a member of the Crassulaceae family. Due to its adaptability and therapeutic qualities, it originated in Madagascar and has since spread to tropical and subtropical regions of the world (Mitra et al., 2018).

This plant has been used traditionally in folk medicine for a very long time to cure a wide range of illnesses, such as burns, wounds, infections, gastrointestinal issues, and inflammation (Yadav et al., 2020). Researchers are looking into the phytochemistry and pharmacological properties of *Kalanchoe pinnata* due to its potential as a treatment.

Kalanchoe pinnata has a variety of bioactive components, including flavonoids, alkaloids, terpenoids, phenolic compounds, and polysaccharides, according to phytochemical research (Bhowmik et al., 2020). These phytoconstituents are in charge of the pharmacological actions and contribute to its therapeutic qualities. However, variables

including geographic location, environmental circumstances, and plant maturity can affect the precise makeup and concentration of bioactive components (Nguyen et al., 2019).

MATERIALS AND METHODS

Study area

This study was carried out in Takum local government area of Taraba state, it borders the republic of Cameroon in the south, Ussa local government to the west Donga local government to the north.

Apparatus and Equipment

Beakers, measuring cylinder, conical flask, filter paper, funnel, wash bottles, spatula, analytical weighing balance, hand gloves, crucible, and water bath.

Reagents and Solvents

Chloroform (95%)

Materials: mature and healthy parts of *Kalanchoe pinanta* was harvested from Takum, identification was carried out by a botanist in the department of biological sciences to ascertain the validity of the plant

Methods

Preparation of *kalanchoe pinnata* leaves: the leaves of *Kalanchoe pinnata* was thoroughly washed with water to remove any contaminant which may be attached to the leaves, it was transferred into a clean mortal and was pounded into small fine particles using pestle.



Kalanchoe pinnata plant

Extraction process

Hands et al. (2008) used a cold maceration technique to extract bioactive compounds from *Kalanchoe pinnata*. The technique involved measuring 1000ml of chloroform and transferring it to 1500g pounded leaves in a conical flask. The mixture was sealed to prevent evaporation and contamination and left at room temperature for 72 hours (3 days) for efficient extraction.

The mixture was passed through a filter paper to remove the liquid extract from the residue (plant materials). The filtrate was then placed in an evaporating dish and placed in a water bath set at a low temperature (300) to evaporate the liquid and leave behind the solid extract.

Preparation of extract and determination protocol

The methodology for extraction and determination was prepared using the adjusted techniques of Nalini and Anuradha (2017).

The experiment utilized powdered whole plant leaves of *Kalanchoe pinnata*. The ground leaf was water-soaked, filtered, and refrigerated in a tightly-sealed container pending additional examination.

High performance Liquid Chromatography

The preparation of the sample involved dissolving 10 mg of dry crude extract in 10 ml of extraction solvent (HPLC grade) to get a 1 mg/ml solution, filtering through 0.45 µm Millipore, and injecting the resulting 20µL into the waters HPLC system. The preparation of the standards involved taking 1.2 mg of standard (Flavone) in 5 ml of chloroform (HPLC grade) and creating a standard curve from it.

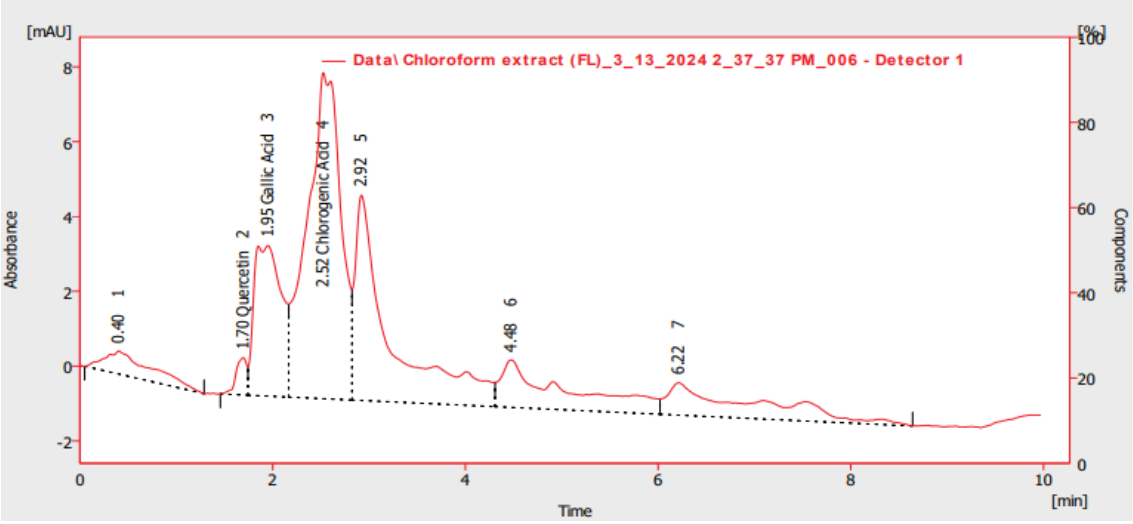
Protocol:

HPLC analysis was performed using a 20 µl injection valve and a UV variable wavelength detector (tuned to 300 nm). The sensitivity was 0.001, with a reprosol of 100 C8mn and a 5 µm column measuring 4.6 x 150mm at 30°C. The HPLC solvents were phosphate buffer (v/v), an aqueous solvent (A), and CH3CN, an organic solvent (B). The analytes were eluted in a gradient at a flow rate of 1 ml/min. Chromatograms were created using software. The HPLC instrument ran at room temperature (23 ± 2°C). Each diluted extract (20 µl) was injected into the HPLC three times, and the average peak area was reported and quantified.

RESULTS

Results of HPLC for chloroform extract of *Kalanchoe pinnata* leaf

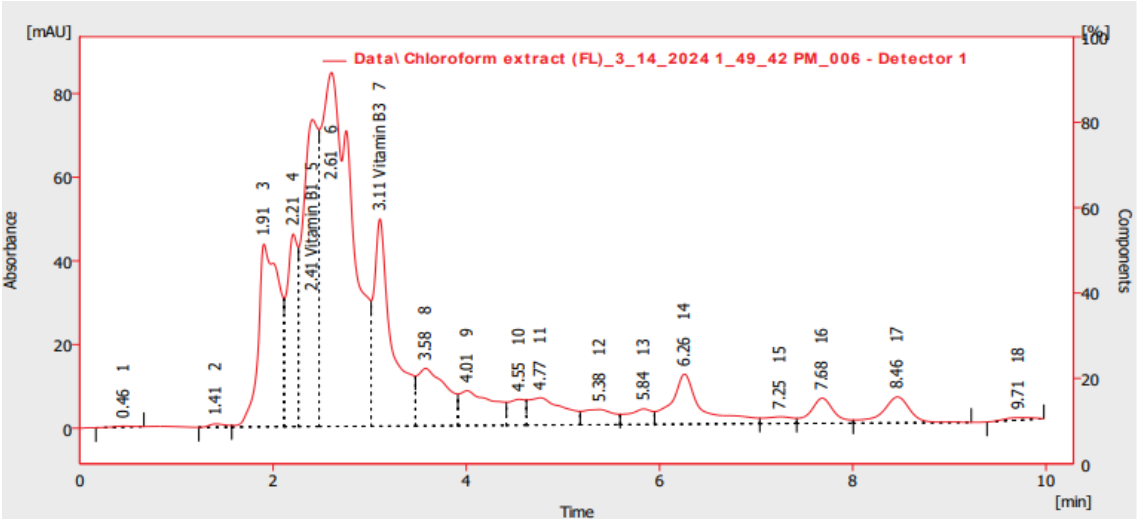
Retention Time (min)	Response	Amount (ppm)	Amount (%)	Peak type	Coupound Name
1.695	7.704	0.130	1.0	Ordnr	Quercertin
1.952	79.976	0.052	0.4	Ordnr	Gallic Acid
2.525	212.357	12.198	98.5	Ordnr	Chlorogenic Acid



Graph 1: Chromatogram of HPLC for chloroform extract for Quercertin, Gallic Acid and Chlorogenic Acid

Results of HPLC for chloroform extract of *Kalanchoe pinnata* leaf for vitamin b1 and b3

Retention time (min)	Response	Amount (mg)	Amount (%)	Peak type	Compound Name
2.408	804.328	544.210	23.8	Ordnr	Vitamin B1
3.107	662.938	1743.615	76.2	Ordnr	Vitamin B4



Graph 2: Chromatogram of HPLC for chloroform extract for Vitamin B1 and B3.

DISCUSSION

From Table 1, Quercetin, which was eluted at a retention time of 0.402 minutes. The amount detected is 0.130 ppm, which corresponds to 1.0% of the total amount, Quecertin

Quercetin is important due to its wide-ranging health benefits and therapeutic potential. It possesses antioxidant, anti-inflammatory, antiviral, and anticancer properties, making it a valuable compound for human health (Boots *et al.*, 2008). Quercetin's ability to modulate various cellular pathways and its favorable safety profile contribute to its importance as a natural remedy for numerous health conditions. Gallic Acid, which was eluted at a retention time of 1.695 minutes. The amount detected is 0.052 ppm, which corresponds to 0.4% of the total amount; Gallic acid holds significance due to its diverse therapeutic potential and beneficial effects on human health. It exhibits antioxidant, anti-inflammatory, anticancer, antimicrobial, and neuroprotective properties (Salehi *et al.*, 2019). Its ability to modulate various molecular pathways makes it valuable in the prevention and treatment of various diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. and also, Chlorogenic Acid which also was eluted at a retention time of 1.952 minutes. The amount detected is 12.198 ppm, which corresponds to 98.5% of the total amount; Chlorogenic acid is notable for its diverse health benefits, including antioxidant, anti-inflammatory, and neuroprotective properties. It has been studied for its potential in managing various conditions such as obesity, diabetes, cardiovascular diseases, and neurodegenerative disorders (Ong *et al.*, 2018). Its ability to modulate multiple cellular

pathways makes it a promising natural compound for promoting human health. Analysis of flavonoids in chloroform extract of plant shows 3 compounds.

(Table 1 and Graph 1) after comparing with standard retention time of various flavonoids, it shows peak at 7.4000 which is closer to myricetin

Table 2 shows the presence of Vitamin B1, also known as thiamine, Vitamin B1, is crucial for energy metabolism, nerve function, and maintaining a healthy nervous system. It plays a vital role in carbohydrate metabolism, converting food into energy that the body can use. Thiamine deficiency can lead to serious health problems, including neurological disorders like beriberi and Wernicke-Korsakoff syndrome. and Vitamin B3, also known as niacin, is essential for various bodily functions, including energy metabolism, DNA repair, and cell signaling. It is crucial for maintaining healthy skin, proper digestion, and nerve function. Niacin deficiency can lead to a condition called pellagra, characterized by symptoms such as dermatitis, diarrhea, dementia, and death if left untreated.

Both vitamin B1 and vitamin B3 are water-soluble vitamins, which means they are not stored in the body and need to be obtained through dietary sources regularly. They are commonly found in foods such as whole grains, legumes, nuts, seeds, lean meats, and green leafy vegetables.

CONCLUSION

Preliminary phytochemical screening of chloroform extracts of *K.Pinnata* shows the presence of Quercetin, Chlorogenic Acid and gallic Acid respectively using HPLC.

From these above phytochemical investigation it was concluded that chloroform extract of *Kalanchoe pinnata* leaf contains various flavonoids and steroids. This will lead to further pharmacological investigation of this plant.

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