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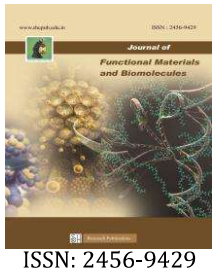

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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOL LEAF EXTRACTS OF KALANCHOE PINNATA

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Abstract

The present study investigates the phytochemical constituents and evaluates the antibacterial activity of aqueous and ethanol leaf extracts of *Kalanchoe pinnata*, a medicinal plant traditionally used in various therapeutic applications. The results of phytochemical screening of aqueous and ethanol leaf extracts of *Kalanchoe pinnata* revealed the presence of some secondary metabolites like alkaloids, carbohydrates, flavonoids, steroids, terpenoids, tannins, quinones, phenols and absence the bioactive compounds namely saponins and glycosides. The antibacterial activity was assessed using the agar well diffusion method against selected Gram-positive and Gram-negative bacterial strains, including *Streptococcus pneumonia* and *Escherichia coli*. Results indicated that both aqueous and ethanol extracts exhibited significant antibacterial activity, with the ethanol extract demonstrating a broader and more potent zone of inhibition compared to the aqueous counterpart. These findings suggest that *Kalanchoe pinnata* possesses valuable phytochemicals with promising antibacterial properties, making it a potential source for the development of natural antimicrobial agents.

Keywords: Kalanchoe pinnata, phytochemicals, antibacterial activity, and bioactive compounds.

1. Introduction

Medicinal plants have been rediscovered in the plant kingdom recently. The plants are highly accessible, relatively inexpensive, and have little side effects. The plants used for medicinal purposes over the millennia are most likely to discover therapeutically useful anticancer and antihepatic compounds. Medicinal plants are the best choice for a wide variety of drugs. According to the world health organization traditional medicines are used by over 80% of individuals in developing countries [1]. Medicinal plants have long been discovered scientifically for their many therapeutic benefits, including antimicrobial, anti-inflammatory, antifungal, contraceptive traits of medicinal plants[2].

The plants, which are nutritious chemicals that protect human's center diverse diseases, are responsible for the phytochemicals medicinal activity. The primary and secondary phytochemicals are classified into two categories depending on their function in plant metabolism. The most important components are carbon, amino, protein and chlorophylls, and alkaloids, saponins, steroids, flavoids, tannins are seconary metabolites [3]. Antibiotic resistance is currently a major global health concern.

Multidrug-resistant bacteria are emerging and spreading rapidly, threatening our ability to treat common infectious diseases. Indiscriminate use of commercial antibiotics is considered to be one of the main reasons for

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drug-resistance. According to the World Health Organization (WHO), drug-resistant diseases could cause 10 million deaths each year by 2050 and damage the global economy significantly. Thus, it is very crucial to identify products with antimicrobial properties that could be utilized to develop novel and efficient antibiotics [4].

Kalanchoe is a genus that has many species most of which are used as agents to treat various ailments. Plants belonging to this genus have been traditionally known for their pharmaceutical value and have been studied by scientists for a very long time. Kalanchoe pinnata commonly known as “Ranakalli” “Miracle leaf”, “Mexican Love plant”, “Katakataka”, “Cathedral Bells”, “Air plant”, “Life plant”, “Goethe plant”, “Wonder of the World” and so on belongs to the Crassulaceae family. It is also known as “Mother of thousand” as new plantlets arise from the leaf margins which can be cut off from the parent and cultivated separately on pots or barren lands [5]. No systemic scientific report available on the entitled “Phyochemical Analysis and Antibacterial Activity of Aqueous and Ethanol Leaf Extracts of Kalanchoe pinnata”. They are the following objective such as to assess the preliminary phytochemical analysis and antibacterial activity of aqueous and ethanol extract leaf of extract of Kalanchoe pinnata.

2. MATERIALS AND METHODS

2.1. Collection of Plant material

Fresh Kalanchoe pinnata leaves collected from Tirupattur, dried and converted into a powder using an electric blender. The dried powders were used for further analysis. Take 5 grams of Kalanchoe pinnata leaf + 50 ml of distilled water and 5 g of Kalanchoe pinnata leaf + 50 ml of ethanol was placed in a thimble and extracted for 8 cycles in a Soxhlet apparatus separately. After 8 cycles, extract was filtered by whatman no.1 filter paper. The Fig. 1 shows the Kalanchoe pinnata Leaf and its extraction are below,

2.2. Phytochemical Analysis

The plant extract solutions were assessed for the existence of the phytochemical analysis by using the following standard methods. [6]

2.2.1. Test for Anthraquinone

10 ml of benzene was added in 6 g of the Ephedra powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

2.2.2. Test for Tannin

10 ml of bromine water was added to the 0.5 g plant extracts. Decoloration of bromine water showed the presence of tannins.

2.2.3. Test for Saponin

5.0 ml of distilled water was mixed with plant extracts in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins

2.2.4. Tests for Flavonoid

Shinoda Test

Pieces of magnesium ribbon and HCL concentrated were mixed with aqueous plant extract after few minutes and pink color showed the presence of flavonoid.

Alkaline Reagent Test

2 ml of 2.0% NaOH mixture was mixed with plant extracts; concentrated yellow color was produced, which became colorless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

2.2.5. Tests for Glycoside

Liebermann’s Test

Added 2.0 ml of acetic acid and 2 ml of chloroform with whole plant extracts. The mixture was then cooled and we added H2SO4 concentrated. Green color showed the entity of glycine, steroidal part of glycosides.

Keller-Kiliani Test

A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl3 mixture was mixed with the 10 ml plant extracts and 1 ml H2SO4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides [7].

Salkowski's Test

Added 2 ml H2SO4 concentrated to the whole plant extracts. A reddish brown color formed which indicated the presence of steroidal a glycone part of the glycoside.

2.2.6. Test for Terpenoid

Added 2.0 ml of chloroform was added with the 5 ml plant extracts and evaporated on the water path and then boiled with 3 ml of H2SO4 concentrated. A grey color formed which showed the entity of terpenoids.

2.2.7. Test for Steroid

Added 2 ml of chloroform and concentrated H2SO4 were added with the 5 ml plant extracts. In the lower chloroform layer red color appeared that indicated the presence of steroids.

2.2.8. Alkaloid

The solvent free extract (50mg) was stirred with one ml of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids. Mayer's Test: To the filtrate, a drop of Mayer's reagent was added along the sides of the test tube. A white precipitate indicates the test as positive.

2.2.9. Carbohydrate

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

2.3. Antibacterial Activity

Principle

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters [8].

Reagents

1. Muller Hinton Agar Medium (1L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml of distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

Procedure

Petriplates containing 20ml of Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20µl of the plant extracts were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

3. RESULTS AND DISCUSSION

In plants the naturally occurring chemical compounds are phytochemicals. They give organoleptic properties and color to the plant. In many places, as a dietary accessory they are comfortably approachable but

dormant health advantages of phytochemicals are only reachable from the utilization of whole plant. Phytochemicals are beneficial to boost up immunolatory responses and also provide "immunity against many diseases. Some phytochemicals are known to reveal medicinal and physiological activities which are phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids and phytosterols were respectively [9].

3.1. The Preliminary Phytochemical Analysis of AQKp and EKp

The results of phytochemical screening of aqueous and ethanol leaf extracts of Kalanchoe pinnata revealed the presence of some secondary metabolites like alkaloids, carbohydrates, flavonoids, steroids, terpenoids, tannins, quinones, phenols and absence the bioactive compounds namely saponins and glycosides. The Table 1 and Fig 2 shows the Preliminary phytochemical analysis of aqueous and ethanol extracts of Kalanchoe pinnata as follows,

Table 1: The Preliminary Phytochemical Analysis

Phytochemical Constituents	*Kalanchoe pinnata Leaf*	
	Aqueous	Ethanol
Carbohydrates	+	+
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	+
Terpenoids	+	+
Tannins	+	+
Quinones	+	+
Phenols	+	+
Saponins	-	-
Glycosides	-	-

Indicated as: + means Presence, - means Absence

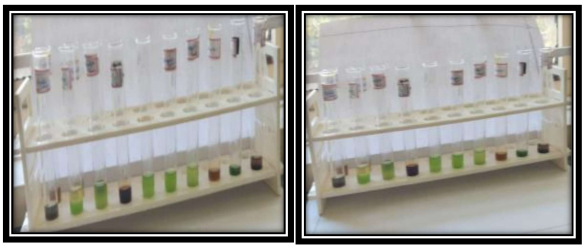

Fig 2: Preliminary phytochemical analysis of AQKp and Ekp

3.2. The Antibacterial activity of Kalanchoe pinnata

The Antimicrobial are commonly practice at least 2000 yrs ago used for Egyptians and Greeks specific mold in plant extract to treating various infections. The antimicrobial is an agent may be killed the microorganism called microbicide and stops the growth means bacteriostatic agent. The antimicrobial medicine to cure infection means antimicrobial chemotherapy while to prevent infection known as antimicrobial prophylaxis [10]. The antibacterial activity of aqueous and ethanol leaf extract of Kalanchoe pinnata against both Gram positive and Gram negative bacteria such as Streptococcus pneumonia and Escherichia coli tested by disc diffusion method. The Table 2 and Fig.3 shows the Antibacterial activity of aqueous and ethanol leaf extract of Kalanchoe pinnata as follows,

Table 2: The Antibacterial activity of Kalanchoe pinnata

The ethanol leaf extract of Kalanchoe pinnata showed maximum zone of inhibition namely Escherichia coli (14mm, 16mm and 18mm) followed by Streptococcus pneumonia (12mm, 14mm and 16mm)". "When compared to positive control ranges between Escherichia coli (22mm) and Streptococcus pneumonia (18mm) and Aqueous extract leaf extract of Kalanchoe pinnata showed maximum zone of inhibition namely Escherichia coli (12mm, 14mm and 16mm) followed by Streptococcus pneumonia (12mm, 13mm and 13mm)". When compared to positive control ranges between Escherichia coli (22mm) and Streptococcus pneumonia (17mm) were respectively.

List of Microorganisms	Concentration (µg/ml)	Zone of Inhibition (mm)	
		Aqueous	Ethanol
Streptococcus pneumonia	50	12	12
	100	13	14
	150	13	16
	PC	17	18
Escherichia coli	50	12	14
	100	14	16
	150	16	18
	PC	22	22

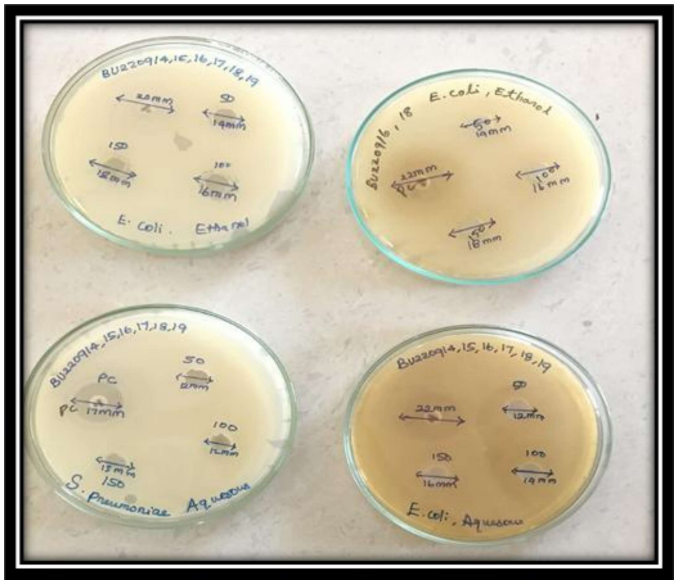

Fig. 3: Antibacterial activity of AQKp and Ekp

4. CONCLUSION

The study on the phytochemical analysis and antibacterial activity of aqueous and ethanol leaf extracts of Kalanchoe pinnata reveals that both extracts are rich in various phytochemicals, including flavonoids, alkaloids, and tannins. The ethanol extract, in particular, exhibited stronger antibacterial activity against selected bacterial strains compared to the aqueous extract, highlighting the influence of the solvent on the extraction efficacy of bioactive compounds. These findings validate the traditional use of Kalanchoe pinnata in herbal medicine for treating infections and underscore its potential as a source for developing new antibacterial agents. Further research is recommended to isolate the specific compounds responsible for this activity and to understand their mechanisms of action.

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Conflict of Interest: Nil

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