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Article

Antimicrobial Activity of Kalanchoe Pinnata: A Review

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***Abstract*— *Kalanchoe pinnata* (synonym to *Byrophyllum pinnatum*) or commonly known as "Setawar" is a medicinal plant belongs to the Crassulaceae family. It is also known as "life plant" and "resurrection plant" due to its multiple roles in traditional medication. The therapeutic values of *K. pinnata* mostly lie on the presence of phytochemicals or plant active compounds which possess high potential as a natural antimicrobial agent source. Given the global health threat of antimicrobial resistance towards synthetic drugs, uncovering the natural sources as a novel drug is of crucial need. In this regard this review highlights the antimicrobial property of *K. pinnata* extract, the bioactive compound analysis of this plant extract and their mode of action against pathogenic microorganisms. The potent bioactive compounds extracted from *K. pinnata* plant could be further explored as an alternative medicine to the current synthetic antimicrobial drugs.**

***Keywords*— Kalanchoe pinnata; antimicrobial activity; bioactive compounds; natural drugs**

I. INTRODUCTION

The search for potent antimicrobial agents has been shifted to plants. Some plants are medicinally practical in treating disease as the efficiency of antimicrobial property of certain plants is astonishingly great in most cases [1]. Many plants have prevailing antimicrobial activity since they contain bioactive compounds such as alkaloids, flavonoids, terpenoids and tannins. Medicinal plants could be a key source in discovering new drugs because they have pronounced potential to inhibit or kill both spoilage and pathogenic microorganisms. Natural medicines are more acceptable to the human body than modern synthetic drugs [2].

A myriad of modern drugs has been derived from natural sources based on their uses in traditional medication. In addition to offering diverse therapeutic outcomes, plant-based medicines also provide a comparative advantage in terms of lesser side effects and better cost efficiency than conventional drugs [3]. Notably, medicinal plants would have bioactive molecules alternative to synthetic antibiotics with higher safety and efficiency, which will play a key role in maintaining human health [4]. Microbial resistance to conventional antibiotics and their rapid progression has raised severe concerns in treating infectious diseases. A significant increase in the pathogenic resistant strains has been reported, which steered the novel multi-resistant organisms against the current synthetic drugs. Indiscriminate and intense use of the current antimicrobials agent results in selective pressure and makes the bacteria less susceptible to presently available antibiotics [5].

In addition, diseases caused by resistant bacteria often fail to respond to conventional therapy, which causes prolonged illness and amplify the risk of death. This severe issue spurs a greater need for more potent plant-mediated antimicrobial agents, alongside uncovering the active ingredients that can act as a template to generate new antimicrobial drugs. *Kalanchoe pinnata*, locally known as "Setawar", is one of such plants with auspicious antimicrobial properties. The presence of bioactive compounds within this plant indicates its antimicrobial activity against pathogenic microorganisms and holds various therapeutic benefits, making it a good alternative for the current synthetic antibiotics [6].

This review paper focuses on the antimicrobial activity of *K. pinnata* bioactive molecules in terms of antibacterial, antifungal and antiviral properties. Also, it presents the analysis of active compounds directly involved in antimicrobial activities. Finally, the mechanism of action of the bioactive compounds against pathogens is briefly discussed.

II. BACKGROUND OF *KALANCHOE PINNATA*

Kalanchoe pinnata is one of the succulent, perennial and *Kalanchoe pinnata* is a succulent, perennial and corpulent vegetable with glabrous and tuberous stems. This species can reach up to 150 cm in height. It is a popular houseplant and is often grown as an indoor ornamental because of its attractive and unique appearance. Additionally, this type of plant is easy to grow since it can easily be propagated through a leaf or stems cutting and requires minimal care [6]. It is characterised by fleshy, thick green leaves distinctively scalloped, tall hollow stems and bell-like pendulous flowers. The word "succulent" is derived from the Latin word "sucus", which means sap or juice. *K. pinnata* (Fig. 1a) is synonymous with *Byrophyllum pinnatum*, which is also known as "miracle plant", "air plant", "life plant", and "wonder of the world" that belongs to the Crassulaceae family. The different names arise according to the differences in the regions and countries. For instance, it is called "Hoja del aire" in Spain, "Lao di sheng gen" and "Oliwa-ku-kahakai" by Hawaiians, and "Katakataka" or "Kataka-taka", which mean remarkable in Philipines. *K. pinnata* is also renowned as the "mother of thousands" because of the abundance of miniature plantlets that arise along the notches of the leaf margins (Fig. 1b), which can be detached from the mother and cultivated on pots or barren lands separately. It is widely distributed in Asia, Australia, Macaronesia, New Zealand, Mascarenes, West Indies, Melanesia and Hawaii [7].

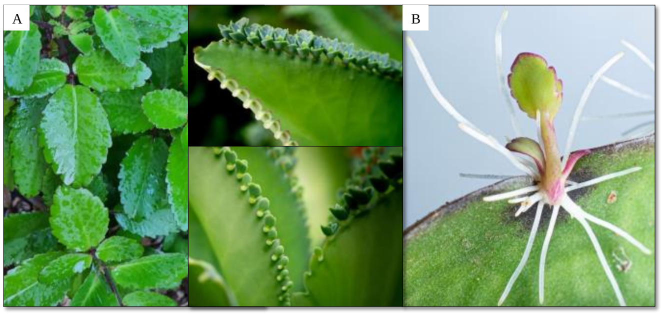

Figure 1 (A) *K. pinnata* leaves and (B) plantlets (adapted from: internet source)

K. pinnata is a homoeopathic plant widely used in traditional medicine to treat various diseases and infections. It has been used in ethnomedicine to treat abscesses, burns, diarrhoea, earache, insect bites, rheumatism and ulcers [7, 8]. It is also used mainly in the ayurvedic system of medicine as an analgesic, astringent, carminative and to treat nausea and vomiting. According to Sahin *et al.*, various complex chemical substances such as alkaloids, bufadienolides, cardenolides, flavonoids, glycosides, triterpenoids, steroids, and lipids has given the plant different healing properties [9]. These healing properties have been shown to possess various pharmacological benefits such as antimicrobial [10], antioxidant, antiviral [11], anti-allergic [12] and analgesic [13]. Table 1 below lists the bioactive compounds encompassed in the *K. pinnata* species.

III. ANTIMICROBIAL ACTIVITY

Plants used in traditional medicine are loaded with bioactive compounds and have a high potential for new antimicrobial compounds [17-18]. According to Sarker *et al.*, around 75% of the population in developing countries still depend on the plant for medical treatments, including wound healing and antimicrobial agents [19]. Secondary metabolites or bioactive compounds produced by plants have been discovered to inhibit microbial growth and virulence. *K. pinnata* has shown such antimicrobial activity and proven to treat the wound. For example, plant extracts can cure cutaneous leishmaniasis caused by trypanosome protozoa [22].

Antimicrobial activity can be described as any natural, semi-synthetic or synthetic origin used to kill or inhibit the growth of microorganisms [21]. In the study to determine the effects of extraction solvents, methanol, chloroform, petroleum ether, acetone and ethyl acetate on *K. pinnata* antimicrobial activity against several pathogens, methanolic extract from leaves part revealed the highest activity toward tested microorganisms. Table II below lists the antimicrobial properties of *K. pinnata* extracts on the tested microorganisms.

TABLE I. PLANT PART, TYPES OF EXTRACT AND THE BIOACTIVE COMPOUNDS RELATED TO *K. PINNATA* SPECIES

Plant Part	Types of Extract	Bioactive Compounds	References
Leaves	Methanolic	Bufadienolides	[20]
Leaves	Ethanolic	Alkaloids, Flavonoids Glycosides, Phenolics, Steroids, Terpenoids, Tannins	[21]
Fresh leaves	Aqueous	Flavonoids	[18]
Dried leaves	Aqueous, Ethanolic, Diethyl ether and 20% Acetic acid	Alkaloids, Flavonoids, Phenols, Tannins, Vitamins, Minerals	[22]
Powdered plant	Ethanolic	Flavonoids	[9]
Shoots	Petroleum ether	Steroid (stigmasterol)	[16]
Roots	Aqueous, Chloroform, Ethanol, Ether	Alkaloids, Flavonoids, Glycosides, Steroids, Saponins, Tannins, Carbohydrates, Proteins, Amino acids	[13]

TABLE III. TYPE OF K. PINNATA EXTRACTS, METHODS USED, TEST STRAINS AND OBSERVATION

Extracts and Methods	Test Strains	Results	References
• leaf extract • ethyl acetate and methanol • *in vitro*: broth microdilution • *in vivo*: Swiss mice's guts	• *Helicobacter pylori*	• Extract showed anti-*helicobacter* activity with minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of 32 and 256 µg/ml, respectively. • Bacterial load of gastric mucosa is reduced.	[31]
• leaf extract of wild-type and transgenic *K. pinnata*. • aqueous • *in vivo*: directly applied to bacteria-infected wounds	• *Pseudomonas aeruginosa* • *Staphylococcus aureus*	• Both transgenic and wild type plant extracts showed a significant anti-microbial activity.	[32]
• leaf extract • 60% methanol • 95% ethanol • aqueous • *in vitro*: agar well diffusion	• *Escherichia coli* • *S. aureus* • *P. aeruginosa* • *Candida albicans*	• Presence of zone of inhibitions with MIC values of 30 mg/ml for *S. aureus*. • Methanol extract showed the most effective antimicrobial properties. • Leaf extracts showed an antifungal activity.	[33]
• methanolic extract • ethanolic crude extract • partitions extract (in ethyl acetate, hexane) • *in vitro*: broth microdilution	• *Salmonella typhi* • *P. aeruginosa* • *S. aureus* • *C. albicans* • *Cryptococcus neoformans* • *Candida parapsilosis*	• Crude extract showed a significant antibacterial activity and antifungal properties. • Ethyl acetate fraction showed a significant antimicrobial activity. • Isolated flavonoids have the strongest effect.	[34]
• ethanolic extract • *in vitro*: agar-diffusion	• *E. coli* • *P. aeruginosa* • *S. typhi* • *Shigella dysenteriae* • *Vibrio cholera* • *Bacillus subtilis* • *Bacillus megaterium*	• Bacteria growth was inhibited except for *S. typhi* and *V. cholera*. • High antimicrobial activity was observed against *E. coli*.	[8]
• root extract • chloroform, petroleum ether, methanol and water • *in vitro*: disk diffusion	• *E. coli* • *P. aeruginosa* • *S. aureus* • *C. albicans*	• Methanolic extract showed the most effective antimicrobial activity against all bacteria except *C. albicans*	[13]

A. *Antibacterial activity of K. pinnata*

Bacterial infections are among the harmful infectious diseases that could threaten human health. The antibacterial properties of medicinal plants are attributable to different bioactive compounds in the extracts. The previous study suggested that the phenolic compounds extracted from the plant possess antibacterial activity. The plant extract is effective and capable of treating typhoid fever and other infections caused by harmful bacteria. The leaf juice of K. pinnata has demonstrated significant antibacterial activity via in vitro tests towards E. coli, Staphylococcus, Bacillus, Shigella, Pseudomonas, including several strains of multi-drug resistant bacteria [27]. Akinpelu [10] demonstrated inhibitory activity of 60% methanolic leaf extract (25 mg/extract) against E. coli, B. subtilis, P. vulgaris, S. aureus and S. dysenteriae. Okwu and Nnamdi [7] reported that two flavonoids and alkaloids isolated from ethanolic extract of K. pinnata showed antimicrobial activity. The active compounds can effectively inhibit the growth of several Gram-positive and Gram-negative bacteria species.

Meanwhile, another study tested three bioactive compounds extracted from K. pinnata leaves against respiratory infection-causing bacteria. The findings showed a significant effect of antibacterial activity correlated with the traditional use to treat respiratory tract infections, including pneumonia [22-23]. Another study by Etim et al. revealed that leaf extract possesses higher antibacterial activity against Staphylococcus sp. and Streptococcus sp. isolated from the respiratory tract of infants. The findings confirmed the usefulness of the plant extract in the treatment of infant respiratory infections and as a promising antibacterial agent in the pharmaceutical industry [28]. Górniak et al. suggested that bioactive compounds from plant extract have antibacterial properties since they can constrain bacterial DNA replication, disrupt bacterial cell walls, inhibit biofilm formation and reduce the production of bacterial toxins to the host [29].

B. *Antifungal activity of K. pinnata*

A previous study on antifungal activities of petroleum ether and aqueous extract of K. pinnata stated that both extracts show an almost similar effect to the commercially available standard drug, Griseofulvin [30]. The result from another study revealed that the methanolic extract of K. pinnata effectively inhibits 76% and 51% growth of two fungal strains, which are Aspergillus niger and Aspergillus flavus, respectively. It is reported that the transgenic K. pinnata produces Cecropin P1 (CecP1) antimicrobial peptides. K. pinnata extract enriched with CecP1 have shown effective and immediate elimination of fungal pathogen C. albicans from infected wound compared to the commercial fungicide [31].

C. *Antiviral activity of K. pinnata*

Supratman *et al*. suggested that bufadieonalides isolated Supratman et al. suggested that bufadienolides isolated from leaf extract of K. pinnata successfully inhibit the activation of Epstein-Barr virus early antigen that effect the B-lymphocytes of humans and suppress the tumour formation [14]. Another study by Cryer et al. reported that two tested compounds, namely KPB-100 and KPB-200 isolated from K. pinnata roots, exhibit antiviral activity against human alpha-herpesvirus (HHV) 1 and 2 and vaccinia virus (VACV) [32]. Besides, the chloroform extract of the plant shows anti-HPV (human papillomavirus) properties by inhibiting the expression of viral protein [11].

IV. BIOACTIVE COMPOUND ANALYSIS

According to their functional role, bioactive compounds are categorised into primary and secondary metabolites [33]. The critical stages of acquiring the quality bioactive molecule are appropriate solvent selection, extraction, fractionation methods, and identification techniques. K. pinnata extracts contain bioactive compounds such as flavonoids, alkaloids, phenols, saponins, tannins, triterpenoids, glycosides, bufadienolides, carbohydrates and organic acids [3].

A. *Extraction method*

Extraction is the essential initial step in the analysis of the medicinal plant. This stage will extract the desired bioactive compounds before further purification and characterisation treatments. Extraction is a process that involves the separation of medicinally active constituents or secondary metabolites of the plant from inactive or inert components through the use of selective solvents. Solvents are diffused into plant material and solubilised components with similar polarity during the process. This technique is controlled by various parameters such as plant part used as starting sample material, the solvent used for extraction procedure, temperature, particle size and solvent to plant material ratio [34]. Ncube *et al*. [35] described that the time taken for the extraction could be shortened by grinding the plant material into a more refined form as it will increase the contact of sample surface area with the solvent system. The increment may facilitate the rate of extraction while shaking the plant material-solvent mixture will also accelerate the rate of the extraction process.

The type of solvent is the fundamental parameter that needs to be considered. The successful analysis of plant biologically active constituents relies on the properties of solvent used in the extraction procedure [35]. Good solvent properties include minimal toxicity, ease of evaporation at low heat, preservation action, promotes rapid physiological absorption of the extract, and preventing the dissociation of potentially active components during plant extract preparation. Solvent selection for the extraction of plant phytochemicals is based on the polarity of the solute of interest. The underlying mechanism is that solvents with similar polarity to the solute will disperse into plant material and simply dissolve the target [36].

B. *Purification method*

Fractionation or purification is a process that involves the separation of plant extracts into various fractions. The fractions will be separated into several segments comprising different compounds. The process will resume until pure compounds are completely isolated [34]. Plant extracts are rich in complex phytochemicals, making the separation techniques challenging. An alternative step to troubleshoot this problem is utilising several mobile phases to intensify the polarity and obtain a high-value result. The most common method to separate and purify bioactive compounds is column chromatography. This technique is convenient and offers assorted stationary phases. Sophisticated instruments such as Thin Layer Chromatography (TLC) and High-Pressure Liquid Chromatography (HPLC) have been developed to accelerate the purification process of the bioactive molecules [36].

C. Identification and characterization method

Compound identification is a technique that involves detection of the functional group, carbon and hydrogen arrangement and structural elucidation of bioactive constituents from plant extracts. The pure bioactive compounds can be detected by using different kinds of spectroscopic techniques such as Nuclear Magnetic Resonance (NMR), Infrared (IR), UV-visible and mass spectroscopy (MS). The Tandem Mass Spectrometry technique provides valuable data on the phytochemical compounds' structural elucidation. The combined application of HPLC and MS will offer quick and precise recognition of bioactive compounds in plant extracts. Additionally, liquid chromatography coupled with mass spectrometry (LC/MS) is also a powerful technique to identify bioactive compounds. A study by Shruti *et al*. investigated the phytochemical analysis of *K. pinnata* leaves. The result indicates that the fresh leaf juice contains the highest phenolic, flavonoids, alkaloids, and saponin content, making it the most suitable extract for medical purposes [36].

V. MECHANISM OF ACTION

Bioactive compounds such as flavonoids, alkaloids, phenolic compounds are responsible for inhibiting bacterial growth. Gyawali *et al*. [37] reported that such phytochemical substances would disrupt the cell enzyme systems and modify the genetic material of bacterial cells by attacking the phospholipid bilayer of the cell membrane. The phytochemical compounds, particularly flavonoids, have the ability to penetrate the cell membrane of bacteria, which will damage the membrane and alter the intracellular pH. In addition, flavonoids and saponins exhibit antibacterial properties due to their ability to form a complex with soluble protein, extracellular protein and bacterial cell wall [38].

Meanwhile, bioactive compounds such as phenols and aldehyde are believed to have antifungal properties. Ergosterol is the primary fungi's sterol derivatives found in the fungal cell membrane. Its vital roles are regulating cell permeability, preserving cell function, and safeguarding the membrane-bound enzymes' activities [33]. Generally, fungicides will attack the fungal cells by forming a complex with ergosterol to interrupt its essential functions or inhibit their biosynthesis. According to Ansari *et al*., phenols and aldehydes have strong hydrophobicity strength to diffuse the cell membrane phospholipid bilayer and subsequently act towards ergosterol in the fungal cell. Interestingly, they can also penetrate and invade the fungal nucleus, regulating the ergosterol biosynthesis. This mode of action will modify the fatty acid profiles, cell membrane and osmotic imbalance to ultimately result in permanent damage to the hyphae membrane, conidiophores and cell death [39].

Furthermore, the presence of quercetin in plant extracts is responsible for its antiviral properties. Quercetin is a natural flavonoid that has been shown to exhibit anti-hepatitis C virus activity (HCV) [40]. It can significantly reduce the viral genome replication, the synthesis of infectious HCV particles, and the infectivity rate of the new viral particles produced [41]. Non-structural protein 3 (NS3) is a protein encoded by the HCV genome, essential for viral growth and replication considered a potential anti-HCV drug target. Additionally, quercetin suppressed HCV via inhibition of NS3 protease activity. Bachmetov *et al.'s study* reported that flavonoid quercetin has significantly suppressed HCV production and replication by inhibiting NS3 protease activity, essential for HCV gene expression [42].

VI. CONCLUSION

The present review briefly discusses the wide variety of bioactive compounds in the *K. pinnata* that exhibit antimicrobial activity against several pathogens and their modes of action. Collectively, this review concludes that the plant does possess not only antibacterial activity but also contain antifungal and antiviral activity as well. Several studies have successfully proved the antimicrobial action of the *K. pinnata* plant extract, marking them as a valuable and promising drug in the pharmaceutical field. These collective studies indicate that this plant could be a source of the potent antimicrobial agent as an alternative to the currently available antibiotic. However, only a few studies have been focused on the antifungal and antiviral activity of the *K. pinnata* extract and the extraction, purification and identification of bioactive compounds and their specific modes of action. Future work will concentrate on this missing link, and *in silico* study would be beneficial to predict the molecular docking of these bioactive compounds towards specific protein and specific locations in the cell. Furthermore, limited clinical trials have been conducted on this plant, which further accentuates the essentiality of *in vivo* study to evaluate the plant extracts' efficacy, sensitivity, and safety for widespread commercialisation.

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