Anti-agrobacterium Tumefaciens Activity of *Kalanchoe Pinnata* (Lam.) Pers. Leaves (Ywet Kya Pin Pauk)

Thinn Thinn Swe[1]

Abstract

Myanmar medicinal plants constitute an effective source of traditional and modern medicines. Herbal medicine is a major component in traditional practice. The present study was carried out to evaluate the pharmacology activity of *Kalanchoe pinnata* (Lam.) Pers. It belongs to the family Crassulaceae and widely distributed and grows in throughout the tropics the world and in Myanmar. It was collected from Dagon University, East Dagon Township and Yangon Region. It is locally known as "Ywet Kya Pin Pauk". The collected plants were classified and identified with the help of literatures for morphological characters. In the morphological study, the plant is a succulent perennial herb; stem robust; leaves are opposite and decussate; inflorescences are terminal, paniculate cymes; flower hypogynous. Anti-agrobacterium tumefaciens activity of 95% ethanol and aqueous extracts of *K. pinnata* (Lam.) pers. leaves were tested on crown gall produced by bacteria, *Agrobacterium tumefaciens* was isolated from *Sandoricum koetjape* (Burm.F) Merr. (Thitto, Family-Meliaceae) leaves by using Potato Crown Gall (PCG) test. Anti-agrobacterium tumefaciens were tested with the various doses of 95% ethanol and aqueous extracts of *K. pinnata* (Lam.) Pers. leaves. Both extracts prevented the crown-gall formation with 1.0 g/disc. But crown-gall formation was not prevented with 0.25g/disc and 0.5g/disc. Medicinal plants were the potent pharmaceutical products that show causing potent pharmacological effects on the human beings.

Keywords: Crown gall formation, Anti-agrobacterium tumefaciens activity

Introduction

Medicinal plants have been used as traditional treatment for numerous human diseases for thousands of years.*Kalanchoe pinnata* (Lam.) Pers. glabrous stem; leaves petiolate, simple or 3-partite, leaflets oblong or elliptic crenate or subincised-crenate; calyx long, purplish green; corolla globose-octagonal at the base, green, constricted in the middle, the exerted parts reddish-purple; hypogynous scales subquadrate, free or slightly adherent to the carpels; fruit enclosed in the persistent papery calyx and corolla; seeds small oblong-ellipsoid, smooth, longitudinally obscurely striate (Hooker, 1879).

Microbes are ubiquitous and some of them cause disease in humans, hence they are quite important of medical or clinical point of view. Microorganisms are present at almost all the body surface both externally and internally. The indigenous flora of human body contains pathogen (Dubey and Maheshware, 2002).

Crown gall is a neoplastic disease of plants which occurs in more than 60 families of dicotyledons and many gymnosperms. These diseases are characterized by the transformation of normal plant cells into autonomous tumor cells in a short period of time. Once initiated the tumor processes the capacity for autonomous growth independent of normal control mechanism of host (Galsky and Wilesy, 1979).

Agrobacterium tumefaciens is a Gram-negative soil bacterium, rod-shaped and motile. Colonies are non-pigmented and voluminous, simply appearance (Collin, 2001). *Agrobacterium* has invaded the plants it sustains a long-term associated with the plant cell. These stages can be roughly defined as time points when *Agrobacterium* come into close contact with the plant cell at initiation of infection, the T-DNA is transferred into the plant cell and the encoded oncogenes are expressed, and morphological changes indicate the development of a tumor. Furthermore, translocation of nutrients and signaling molecules from the host into the tumor still can take place and influence the physiological state of tumor (Lee *et al*., 2009).

[1] Associate Professor, Dr, Department of Botany, Monywa University

Duke (2002) reported that *Kalanchoe pinnata* (Lam.) Pers. has activities such as analgesic, antibacterial, anticancer, antiedemic, anti-inflammatory, antiplaque antiseptic, antispasmodic, emollient and fungicide, 10 g leaf applied to forehead for headache. It must not for used pregnant, puerperal, or lactating mothers and small children.

Khare (2007) mentioned that *Kalanchoe pinnata* (Lam.) Pers. leaf disinfectant antibacterial (used for boils, insect bites, swelling burns, wounds). Leaves are also eaten to control diabetes. Leaves yield glycosides of quercetin and kaempferol, and fumaric acid. Plants extracts effected on antifungal diseases

The aim of this research is extensively application of plants in Myanmar traditional medicine and promoting the Myanmar traditional medicine scientifically. The objective is to do the experiment of anti-agrobacterium tumefaciens activity on potato crown gall was tested by Potato Crown Gall (PCG) method.

Material and Methods

Collection and identification of *Kalanchoe pinnata* (Lam.) Pers.

The plant specimens were collected from Dagon University campus, East Dagon Township, Yangon Region, from June 2011 to April 2012. For morphological study, the plant specimens were recorded in detail for taxonomic description and identified in the Botany Department, Dagon University with the help of literature such as Hooker (1879), the collected specimens such as habitat, leaves, inflorescence and flowers were recorded by digital camera.

Anti-agrobacterium tumefaciens activities of 95% ethanolic and aqueous extract of *Kalanchoe pinnata* (Lam.) Pers. leaves on Potato Crown Gall (PCG)

The plant gall producing bacteria, *Agrobacterium tumefaciens* was obtained from the isolation of leaves gall from *Sandoricum keojape* (Burm.f.) Merr. (Thitto, Familly- Meliaceae). All of these strains have been maintained as solid slants under refrigeration. For inoculation of the potato discs, 48 hours broth cultures containing $5x10^7$ - 5 x 10^9 cells/ml were used at Central Research and Development Centre (CRDC).

Material

Petri dish, conical flask, fresh free diseases potato, beaker, knife, cork borer.

Test sample

95% ethanolic and aqueous extracts of *Kalanchoe pinnata* (Lam.) Pers. leaves.

Chemical

95% ethanol, sodium hypochlorite (Clorox), dimethyl sulphoxide (DMSO), agar powder, I_2KI (Lugol's solution)

Methods

Preparation of nutrient broth culture

Meat extract (0.5 g), peptone (0.5 g) and sodium chloride (0.25 g) were dissolved in distilled water and the pH of the resulting solution adjusted to 7.2. Then the nutrient broth medium was put into sterilized conical flask and was plugged with cotton wool and autoclaved at 121°C for 15 min. After that, the broth medium was allowed to cool and a loopful of isolated bacteria (*Agrobacterium tumefaeciens*) was inoculated into the broth medium near the flame of a spirit burner and then shaked at 27°C for 48hr on the shaker.

Procedure

Fresh, free disease potato were obtained from Hledan market, Kamarywet Township in Yangon and were used within 48 hours before transfer to the laboratory.

Tubers of moderated size were surface-sterilized by immersion in 50% sodium hypochlorite (Clorox) for 20 min. The ends were remove and soaked for 10 min more in Clorox. A core of the tissue was extract from each tuber by using surface-sterlized (ethanol and flame) 1.0 cm wide cork borer and 2 cm pieces were removed from each end and discarded and the remainder of the cylinder is cut into 0.5 cm thick disc with a surface-sterilized cutter. The discs were then transferred to agar plates (1.5 g of agar was dissolved in 100 ml distilled water, autoclaved for 20min at 121°C, 20ml poured into each petri dish). Each plate contained three potato discs and was used for each simple dilution.Sample (0.25 g, 0.5 g and 1.0 g) were respectively dissolved in dimethylsulphoxide (DMSO) (2ml) and filtered through millipore filters (0.22 µm) into sterile tube. This solution (0.5 ml) was added to sterile distilled water (1.5 ml)

and 2ml broth culture of *Agrobacterium tumesfaciens* strain. Controls were made in this way 0.5 ml of DMSO and 1.5 ml of sterile distilled water were added to the tube containing 2 ml of broth culture of *Agrobacterium tumesfaciens*.

One drop (0.05 ml) from these tubes were used to inoculate each potato disc by using a sterile disposable pipette spreading it over the disc surface. After inoculation, petri dishes were sealed by paraffin and incubated at 27-30°C for 14 days. Tumors were observed on potato discs after 14 days under stereo-microscope followed by staining with Lugol's solution (10% KI and 5% I_2) after 30 minutes and compared with control. The anti-agrobacterium tumefaciens activity was examined by observation of crown gall produced or not.

Procedure for screening of anti-agrobacterium tumefaciens activity by Potato Crown Gall (PCG) test

Surface sterilization of potato in clorox solution

Immersion of potato cutting both ends in another clorox solution

Extraction of potato core with cork borer

Figure 1. Test for anti-agrobacterium tumefaciens activity by Potato Crown Gall (PCG)

Results

Morphological characters of *Kalanchoe pinnata* (Lam.) Pres.

Scientific name - *Kalanchoe pinnata* (Lam.) Pers.
Family - Crassulaceae
English name - Air plant, life plant
Myanmar name - Ywet Kya Pin Pauk

Perennial unbranched herbs, succulent, Leaves opposite and decussate, simple and 3-partite leaflets, lower leaves usually simple, opposite and decussate; upper trifoliate compound leaves, dark-purple maroon margin, all leaves thickly flashy. Inflorescences terminal, paniculate, cymes. Flowers tubular, pendulous, reddish-purple. Sepals (4), persistent, inferior. Petals (4), synpetalous, constricted, above pink to reddish-purple, below constricted part subglobose, persistent, inferior. Stamens 4+4, 2-series, introse, basifixed, longitudinal dehiscence, inferior. Carples 4, fused at the base, ovoid-oblong, axile placentation, numerous ovule in each locule, styles 4, equal slender; stigma simple, scale-like disk at base of each ovary wall, bilobed, yellow, hypogynous. Fruits follicles avoid oblong, enclosed in the papery calyx and corolla. Many seeded, small (Figure 2).

Figure 2. Morphological characters of *Kalanchoe pinnata* (Lam.) Pers.

A. Natural habit B. 3- Partite Leaflets C. Simple leaves
D. Infloresence E. L.S of flower F. C.S of Ovary

Anti-agrobacterium tumefaciens activity of 95% ethanolic and aqueous extracts of *Kalanchoe pinnata* (Lam.) Pers. leaves on Potato Crown Gall (PCG)

The anti-agrobacterium tumefaciens activity of the 95% ethanolic and aqueous extracts of *Kalanchoe pinnata* (Lam.) Pers. leaves was investigated by using Potato Crown Gall (PCG) test with *Agrobacterium tumefaciens*. The tested samples were dissolved in dimethylsulphoxide (DMSO), diluted and mixed with bacterial culture, the bacterial suspension was inoculated 14 days, at room temperature. After that, the crown gall were appeared on potato discs and checked by knob with Lugol's (I_2-KI) solution. In the control, the formation of white knob on the blue background indicated that the presence of crown galls cells because there is no starch in crown gall cells. The active test samples did not form any crown gall on the potato discs and its surface was remained as blue as shown in Table 1, Figure 3 and 4.

From this experiment, it was found that 95% ethanolic and aqueous extracts of *K. pinnata* (Lam.) Pers. leaves were good in preventing the crown gall formation with the dose of 1.0 g/ disc *in vitro* potato disc assays. But crown gall formation was not prevented by dose of 0.25 g/ disc and 1.0 g/disc of this plant leaves.

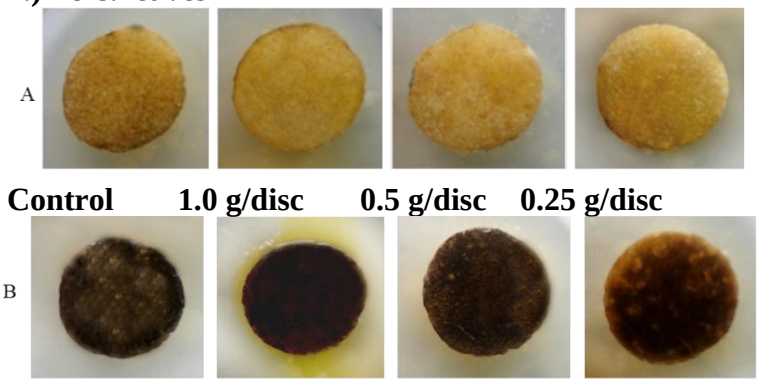
Therefore, it can be concluded that 95% ethanolic and aqueous extracts of *K. pinnata* (Lam.) Pers. leaves of the dose of 1.0 g/didc has anti-agrobacterium tumefaciens activity considerably but the dose of 0.25 g/disc and 05 g/disc did not show anti-agrobacterium tumefaciens activity.

Table.1 Anti-agrobacterium tumefaciens activity of 95% ethanolic and aqueous extracts of *Kalanchoe pinnata* (Lam.) Pers. leaves through *Agrobacterium tumefaciens* infection using potato disc bioassay

No.	Test sample	Dosage (g)	Crown gall formation
1	Control	1.0 g/disc	+
2	95% ethanol extract	1.0 g/disc	-
		0.5 g/disc	+
		0.25 g/disc	+
3	Aqueous extract	1.0 g/disc	-
		0.5 g/disc	+
		0.25 g/disc	+

(+) crown gall formation, (-) not crown gall formation

Anti-agrobacterium tumefaciens activity of 95% ethanolic extract of *Kalanchoe pinnata* (Lam.) Pers. leaves

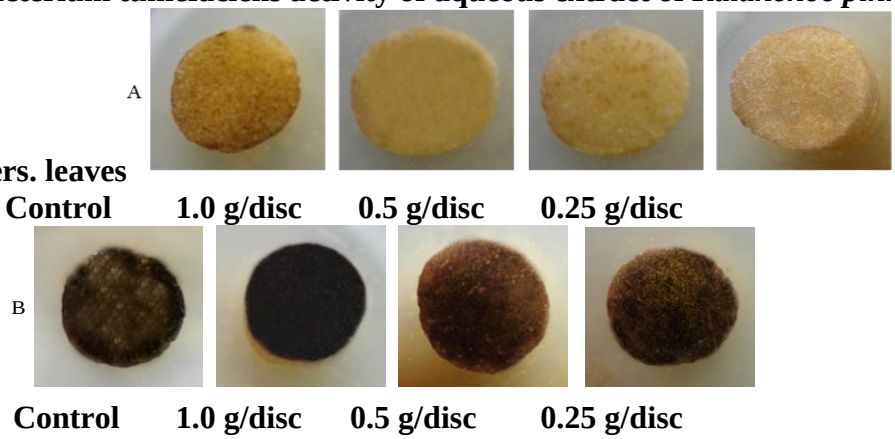


A - Before staining with Lugol's solution

B - After staining with Lugol's solution

Figure. 3 The crown gall formation with the doses of 1.0 g/disc, 0.5 g/disc and 0.25 g/disc of 95% ethanolic extract of *Kalanchoe pinnata* (Lam.) Pers. leaves

Anti-agrobacterium tumefaciens activity of aqueous extract of *Kalanchoe pinnata* (Lam.) Pers. leaves



A - Before staining with Lugol's solution

B - After staining with Lugol's solution

Figure. 4 The crown gall formation with the doses of 1 g/disc, 0.5 g/disc and 0.25 g/disc of aqueous extract of *Kalanchoe pinnata* (Lam.) Pers. leaves

Discussion and Conclusion

In this study, *Kalanchoe pinnata* (Lam.) Pers. is perennial unbranched herbs, stem robust, erect woody at the basal part. Leaves are opposite and decussate, lower leaves simple and upper trifoliate compound, tip obtuse, bicrenate, dark purple marron margin. After of leaf, produces new young plants. Inflorescence is paniculated cymes with pendulous flowers. Below the corolla tube constricted. Carpels 4, scale-like disk present at the base of each ovary wall. Above these characters are in agreement with Hooker, 1979.

In this experiment, it was found that both 95% ethanolic and aqueous extracts of *K. pinnata* (lam.) Pers. leaves were prevented the crown gall formation with 1.0 g/disc. Therefore it can be found that 95% ethanolic and aqueous extracts (doses of 1.0 g/disc) have anti-agrobacterium tumefaciens activity but doses of 0.25 g/disc and 0.5 g/disc of both extracts did not have anti-agrobacterium tumefaciens activity. Both 95% ethanolic and aqueous extracts were prevented the crown gall formation with 1.0/disc. But crown-gall formation not prevented with 0.25 g/disc and 0.5 g/disc.

This plant is important source of potentially useful structures for the development of new chemotherapeutic agents. Therefore, the *K. pinnata* (Lam.) Pers. could be assumed to be beneficial for human health, especially in Myanmar traditional medicine.

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