

Study on Phytochemical Properties, Antibacterial Activity and Cytotoxicity of *Aloe vera* L.

K. Thu, Yin Y. Mon, Tin A. Khaing, and Ohn M. Tun

Abstract—The aim of the study was to investigate phytochemical properties, antimicrobial activity and cytotoxicity of *Aloe vera*. The phytochemical screening of the extracts of leaves of *A. vera* revealed the presence of bioactive compounds such as alkaloids, tannins, flavonoids phenolic compounds, and etc. with absence of cyanogenic glycosides. Three different solvents such as methanol, ethanol and Di-Methyl sulfoxide were used to screen the antimicrobial activity of *A. vera* leaves against four human clinical pathogens by agar well diffusion method. The maximum antibacterial activities were observed in methanol extract followed by ethanol and Di-Methyl sulfoxide. It was also found that remarkable antibacterial activities with methanolic and ethanolic extracts of *A. vera* compared with the standard antibiotic, tetracycline that was not active against *E. coli* and *S. boydii* and supported the view that *A. vera* is a potent antimicrobial agent compared with the conventional antibiotic. Moreover, the brine shrimps (*Artemia salina*) toxicity test exhibited LC₅₀ value was 569.52 ppm. The resulting data indicated that the *A. vera* plant have less toxic effects on brine shrimp. Hence, it is signified that *Aloe vera* plant extract is safe to be used as an antimicrobial agent.

Keywords—*Aloe vera* L., antimicrobial activity, brine shrimp, cytotoxicity, phytochemical properties.

I. INTRODUCTION

A *LOE vera* L. is an important medicinal plant belongs to the family Liliaceae and is also called the magical plant. It is not a cactus; it has thick, tapered, spiny leaves growing from a short stalk near ground level. There are over 250 species of aloe grown around the world. However, only two species are grown today commercially, with *Aloe barbadensis* Miller and *Aloe aborescens* being the most popular. Concentrated extracts of Aloe leaves are used as laxative and as a haemorrhoid treatment. *Aloe* gel can help to stimulate the body's immune system [1].

The *A. vera* plant contains different nutrient contents including vitamins, minerals, enzymes, sugars, phenolic compounds, lignin, saponins, sterol and amino acids. Vitamins namely A, B1, B2, B6, B12, C and E, which the human body

cannot prepare by itself, are available in *A. vera*. Vitamin B complex and C are to play an important role in reducing stress and inflammation [2]. *Aloe* contains the enzymes such as amylase, lipase and carboxypeptidase. Lipase can digest by breaking down fats and sugars. Amylase hydrolyzes starch to liberate dextrin. The activity of serum amylase is increased in acute pancreatitis. The pancreatic carboxypeptidase is metalloenzymes. It inactivates bradykinin and produces an anti-inflammatory effect. During the inflammatory process, bradykinin produces pain associated with vasodilation and its hydrolysis to produce an analgesic effect [3], [4].

Aloe plant contains 25 percent of solid fraction that contain sugars. Sugar acts as immune modulators capable of enhancing and retarding the immune response [5]-[7]. Anthroquinone is a phenolic compound found in the sap. These compounds exert a powerful purgative effect, which are potent antimicrobial agents and possess powerful analgesic effects [8], [9].

Aloe contains saponins which are soapy substances form 3 per cent of the gel and are general cleansers, having antiseptic and anticarcinogen properties [10]. *Aloe* contains Campesterol, F2 Sitosterol and Lupeol [11]. It is an aspirin like compound present in *Aloe* plant possessing anti-inflammatory and anti-bacterial properties. Topically, it has a ketolytic effect which helps to debride a wound of necrotic tissue.

The research studies conducted on *Aloe vera* plant have revealed that through strengthening the T-lymphocyte cells of the blood, it is able to heal the wounds and improve immunity [12]. It contains a compound that neutralizes and binds with FGF-2 receptor, or otherwise alters signaling path-ways for FGF-2 by affecting both GJIC and proliferation of diabetic fibroblast [13].

A. vera gel provides 20 of the 22 necessary amino acids required by the human body. Minerals are defined as natural components formed through geological processes needed in small amounts to regulate body functions. Minerals found in *A. vera* are calcium, zinc, chromium, potassium, etc. Magnesium lactate inhibits histidine decarboxylase and prevents the formation of histamine from the amino acid histidine [14].

A. vera is a very versatile plant that has many different uses. Numerous scientific studies on *A. vera* are demonstrating its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, anti-bacterial, and antifungal properties [15]. A methanolic extract of *A. vera* showed significant *in vitro*

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antibacterial efficacy against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis* and *Shigella flexneri* [16].

Even though, the efficacy of the plant depends on its habitat, soil type, environmental effect and etc. Therefore, it should be needed to screen the activity of medicinal plants before they are used for therapeutic purposes. The present study was emphasized on the determination of the phytochemical components and to validate its antibacterial activity. Moreover, its cytotoxicity was also analyzed.

II. MATERIALS AND METHODS

A. Collection of samples

The leaves of *Aloe vera* plant were collected from Campus of Technological University, Kyaukse, Mandalay Division, and Myanmar.

B. Preparation of Crude Extract

Fresh *A. vera* whole leaves were washed with distilled water, chopped into small pieces, air-dried and grinded into powder. The dried powder was extracted with 95% ethanol for one week. Then it was filtrated through filter paper and the entire extract of *A. vera* then evaporated at 90°C in oven to get a paste form. This concentrated leaf extract was used for further experiments.

C. Preliminary Phytochemical Test

Preliminary phytochemical constituents of *Aloe vera* leaves power components were analyzed qualitatively [17], [18].

D. Examinations of Antimicrobial activity of the *Aloe vera* extracts

The following Gram-positive and Gram-negative bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Shigella boydii*, kindly provided by Department of Biotechnology Laboratory, Mandalay Technological University, Mandalay, Myanmar, were used for antimicrobial assay. All bacterial strains were cultured in Nutrient broth in a water-bath-shaker for 48hr at 37°C. The antibacterial activity of *A. vera* extract was tested using Agar Well Diffusion Technique as described by Reference [19].

Wells of 5 mm diameter were punched out of the solid of respective medium using sterile cork borer and swabbed with an overnight broth culture of the microorganism. Then, the crude leaf extracts were dissolved in Di-Methyl sulfoxide (DMSO), methanol and ethanol. 50 % DMSO was used as negative control. 100 µl of different concentrations of the *A. vera* extracts were filled into each of the wells and then the Petri dishes were incubated at 37 ± 2°C for 24 hrs.

At the end of the incubation period, the antibacterial activity of the *A. vera* extracts with different solvents against the microbes to be assessed by the diameter of the growth inhibition zone formed in mm. The assays were performed in duplicate and inhibition zone was also compared with tetracycline as a reference standard.

E. Toxicity testing against the brine shrimp

(i) Hatching shrimp

Dried cysts of *Artemia salina* (brine shrimp) 0.2 g were hydrated in 400 ml of sterile artificial sea water with salinity about 33 ppt. A 60 watt bulb was positioned upon it to provide a direct light and warmth (25°C) throughout the embryogenesis. Filtered air was passed through the cooled solution to re-oxygenate the medium.

Free swimming nauplii started to appear after 12 hrs and most of the eggs became hatched into free swimming forms by 24 hrs. The nauplii were collected using a Pasteur pipette with a nozzle of at least 1 mm in diameter. The results of brine shrimp toxicity test were determined by the method of Reference [20].

(ii) Bioassay procedure

One ml each of the diluted extract solution (test solution) was added to clean glass vials and ten nauplii were collected with Pasteur pipette from the hatching container and were transferred to each vial carrying over the minimum amount of sea water. The vials with solvent and potassium dichromate solutions were also filled with ten nauplii as controls. Then the remaining bottles were filled with natural sea water as negative control. The vials were restored in the dark room while the temperature was controlled at 25 ± 1°C.

After 24 hrs incubation in the dark room, the vials were taken out for counting of nauplii. Counting of dead nauplii in each vial was made in order to calculate the LC₅₀ (50% Lethal Concentration) by Reed Muench method for each plant extract. Nauplii were considered dead if they lay immobilized at the bottom of the vials.

(iii) Data analysis for toxicity test

A concentration which kills 50% of test organisms, i.e. LC₅₀ values, may be derived from the graphic procedure. This method plots log dosage against percent mortality, and the log dosage at 50% mortality was obtained by intersection. In addition, this method allowed the use of a formula to estimate

$$\overline{SELC}_{50} = \sqrt{(0.79hR/n)} \quad (1)$$

where,

SELC₅₀ = standard error of lethal concentration

h = average of the interval between dosages (log dose)

R = inter-quartile range (LC₅₀ to LC₂₅) from the accumulative percentages

n = number of animals (or average)

If the LC₅₀ or LC₂₅ cannot be obtained from the plot, then the inter-quartile range can be estimated as LC₇₅ to LC₅₀ or twice LC₅₀ to LC₂₅. The 95% confidence limits of the LC₅₀ can be derived from the relationship.

$$\text{Log LC}_{50} \pm 2 \text{ SE LC}_{50} \quad (2)$$

Extracts giving LC₅₀ values greater than 1.0 mg/ml were considered to be nontoxic [21].

III. RESULTS AND DISCUSSIONS

The results from preliminary phytochemical examination were shown in Table I. The phytochemical screening of the extracts of *A. vera* indicated that the presence of alkaloids, glycosides, reducing sugar, phenolic compounds, steroids and terpenoids, carbohydrate, amino acid, flavonoids, tannins and saponin glycosides. Therefore, the most important chemically active (bioactive) constituents: alkaloids, tannin, flavonoid and phenolic compounds were present in the extracts of *A. vera* but cyanogenic glycosides were absent.

TABLE I
PRELIMINARY PHYTOCHEMICAL EXAMINATION OF *ALOE VERA*

| No. | Chemical Constituents | Test reagent | Observation | Results |
|-----|-------------------------|--|------------------------------------|---------|
| 1 | Alkaloids | 1.Mayer's reagent | White ppt | + |
| | | 2.Drangelndroff's reagent | Orange ppt | + |
| | | 3.Sodium picrate solution | Yellow ppt | + |
| | | 4.Wagner's reagent | No, reddish brown | - |
| 2 | Glycosides | 10% Lead acetate solution | White ppt | + |
| 3 | Reducing sugar | Benedict's solution | Reddish brown ppt | + |
| 4 | Phenolic compounds | Ferric chloride solution | Green colour | + |
| 5 | Steroids and terpenoids | Acetic anhydride and conc: H ₂ SO ₄ | Pink colour | + |
| 6 | Carbohydrate | 10% α-naphthol and conc: H ₂ SO ₄ | No purple ring | + |
| 7 | Amino acid | Ninhydrin reagent | Pink colour | + |
| 8 | Flavonoids | Benzene FeCl ₃ (or) conc: HCL, Mg turning | Yellow ppt (or) boiling dissolving | + |
| 9 | Tannins | FeCl ₃ and 10% Lead acetate | White ppt | + |
| 10 | Saponin glycosides | Distilled water | Frothing take place | + |
| 11 | Acidic (or) Basic | Bromocresol green | Blue (base) | + |
| 12 | Cyanogenic glycosides | Distilled water, conc: H ₂ SO ₄ , Sodium picrate paper | No pink colour | - |

+ = Present, - = Absent

TABLE II
ANTIMICROBIAL ACTIVITIES OF *ALOE VERA* EXTRACT DISSOLVED IN DIMETHYL SULFOXIDE

| Organisms | Zone of Inhibition in Diameter (mm) | | | | | | |
|------------------|-------------------------------------|-------|--------|---------|----------|---------|---------|
| | 5mg | 2.5mg | 1.25mg | 0.625mg | 0.3125mg | (+) | (-) |
| | | | | | | Control | Control |
| <i>B. cereus</i> | ND | ND | ND | ND | ND | 18.7 | ND |
| <i>S. aureus</i> | 6 | ND | ND | ND | ND | 13 | ND |
| <i>S. boydii</i> | 12.7 | 9.7 | ND | ND | ND | ND | ND |
| <i>E. coli</i> | 11 | 8.3 | ND | ND | ND | ND | ND |

(+) Control = Tetracycline, (-) Control = 50% DMSO, ND = Not Detected.

TABLE III
ANTIMICROBIAL ACTIVITIES OF *ALOE VERA* EXTRACT DISSOLVED IN ETHANOL

| Organisms | Zone of Inhibition in Diameter (mm) | | | | | | |
|------------------|-------------------------------------|-------|--------|---------|----------|---------|---------|
| | 5mg | 2.5mg | 1.25mg | 0.625mg | 0.3125mg | (+) | (-) |
| | | | | | | Control | Control |
| <i>B. cereus</i> | 15.3 | 13.7 | ND | ND | ND | 17 | ND |
| <i>S. aureus</i> | 11.7 | 9.3 | ND | ND | ND | 14.3 | ND |
| <i>S. boydii</i> | 13.7 | 10.7 | ND | ND | ND | ND | ND |
| <i>E. coli</i> | 14 | 12.3 | ND | ND | ND | ND | ND |

(+) Control = Tetracycline, (-) Control = 50% DMSO, ND = Not Detected.

TABLE IV
ANTIMICROBIAL ACTIVITIES OF *ALOE VERA* EXTRACT DISSOLVED IN METHANOL

| Organisms | Zone of Inhibition in Diameter (mm) | | | | | | |
|------------------|-------------------------------------|-------|--------|---------|----------|---------|---------|
| | 5mg | 2.5mg | 1.25mg | 0.625mg | 0.3125mg | (+) | (-) |
| | | | | | | Control | Control |
| <i>B. cereus</i> | 15.7 | 14.3 | ND | ND | ND | 15 | ND |
| <i>S. aureus</i> | 15 | 12.7 | ND | ND | ND | 12.7 | ND |
| <i>S. boydii</i> | 14 | 12.7 | ND | ND | ND | ND | ND |
| <i>E. coli</i> | 16.3 | 13.7 | ND | ND | ND | ND | ND |

(+) Control = Tetracycline, (-) Control = 50% DMSO, ND = Not Detected.

The methanol extract of *A. vera* showed best antibacterial activity against tested bacteria. Even though, slight activity was shown by DMSO extract, it could inhibit *S. boydii* and *E. coli* compared with standard antibiotics tetracycline. In contrast, the inhibition zone of solvent control 50% DMSO (negative control) was zero so that it was not active against all of the tested microorganisms.

Not all the concentrations of the test solution inhibited the bacteria species with varying degree of sensitivity. The concentrations above 1.25 mg showed good antibacterial activity while the lower concentrations did not show any activity. Moreover, the effect of solvent was dependent on

antibacterial activity. The alcoholic extracts were found to be the better solvents for extraction of anti-microbially active substances compared to DMSO.

The results of the mortality of brine shrimp larvae after 24 hrs of exposure to various concentrations of crude extract were shown in (Table V) and (Fig. 1). The *A. vera* extract exhibited no significant toxicity against brine shrimp with the LC₅₀ value of above 500 ppm. This signified that *A. vera* might not be toxic to human.

The potassium dichromate which used as a positive control exhibited the LC₅₀ values of 33.85 ppm. It was indicated that this value was significantly toxic (LC₅₀ value <1.0 mg/ml (1000 ppm)) against the brine shrimp as shown in Table VI. Since *A. vera* extract was not toxic against brine shrimp therefore it can be used as an antimicrobial agent in known dosage.

TABLE V
MORTALITY OF THE BRINE SHRIMP LARVAE AFTER 24 HOURS OF EXPOSURE TO VARIOUS CONCENTRATIONS OF ALOE VERA

| No | Dose (ppm) | Log Dose | Death | Alive | Accumulated Dead | Accumulated Alive | Ratio Dead:Total | Mortality % |
|----|------------|----------|-------|-------|------------------|-------------------|------------------|-------------|
| 1 | 1600 | 3.2041 | 10 | 0 | 21 | 0 | 21:21 | 100 |
| 2 | 800 | 2.9031 | 6 | 4 | 11 | 4 | 11:15 | 73 |
| 3 | 400 | 2.6021 | 2 | 8 | 5 | 12 | 5:17 | 29 |
| 4 | 200 | 2.3010 | 1 | 9 | 3 | 21 | 3:24 | 13 |
| 5 | 100 | 2 | 2 | 8 | 2 | 29 | 2:31 | 6 |

95% confidence limit of LC₅₀ = 569.52±1.25.

TABLE VI
MORTALITY OF THE BRINE SHRIMP LARVAE AFTER 24 HOURS EXPOSURE OF VARIOUS CONCENTRATIONS OF POTASSIUM DICHROMATE

| No | Dose (ppm) | Log Dose | Death | Alive | Accumulated Dead | Accumulated Alive | Ratio Dead:Total | Mortality % |
|----|------------|----------|-------|-------|------------------|-------------------|------------------|-------------|
| 1 | 50 | 1.6990 | 10 | 0 | 23 | 0 | 23:23 | 100 |
| 2 | 40 | 1.6021 | 9 | 1 | 13 | 1 | 13:14 | 93 |
| 3 | 30 | 1.4771 | 3 | 7 | 4 | 8 | 4:12 | 33 |
| 4 | 20 | 1.3010 | 1 | 9 | 1 | 17 | 1:18 | 6 |
| 5 | 10 | 1 | 0 | 10 | 0 | 27 | 0:27 | 0 |

95% confidence limit of LC₅₀ = 33.85±1.12.

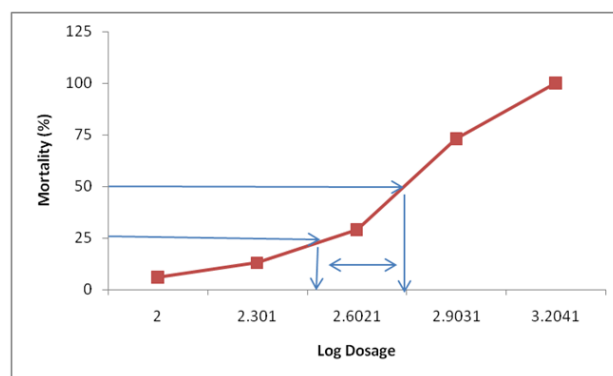


Fig. 1 Estimation of LC₅₀ values for *Aloe vera* Linn. and Standard Error by a Plot of Percent Mortality against Log Dose 95% confidence limit of chronic LC₅₀ = 569.52±1.25

IV. CONCLUSION

This study has revealed that *Aloe vera* gel extract possesses compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in humans. The result of the present study thus explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve microbial infections and underline the importance of ethno botanical approach for the selection of *Aloe vera* in the discovery of new bioactive compounds. This plant could be a source of new antibiotic compounds being nontoxic and less expensive than the allopathic drugs.

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