



# Activity of *Kalanchoe integra* against Selected Pathogenic Bacteria

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## Abstract

*Kalanchoe integra* is a plant that belongs to the Crassulaceae family. In English, the international common name is never die. There are 1500 species of perennial herbs or low shrubs in the genus. Antibiotic resistance is currently at an alarmingly high level. Phytochemicals of *Kalanchoe sp.* were reported as antimicrobial agents. In Rwanda, no study about antimicrobial activity of *K. integra* extracts against bacteria-causing diseases was conducted. Therefore, the aim of the present study was to investigate the antimicrobial effectiveness of *K. integra* phytochemicals against clinically selected pathogenic bacteria (*Shigella sonnei*, *Haemophilus influenza*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pneumoniae*). Maceration technique was used to prepare the leaf and stem extracts using methanol, water and petroleum ether. Phytochemical screening tests revealed that flavonoids, phenolics, and saponins were present in the leaves and stem extracts by using methanol and water. However, tannins were only present in the leaves. No phytochemicals observed by the using petroleum ether. The antimicrobial activity of clinically selected pathogenic bacteria (*S. aureus*, *Haemophilus influenza*, *Streptococcus pneumoniae*, *Shigella sonnei*, *S. typhi*, and *E. coli*) was tested using the agar well diffusion procedure. In order to analyse the data, SPSS was used. The means of inhibition zones of leaf and stem extracts were studied using a two-way ANOVA and both of them showed statistical significant with p-values of 0.03 and 0.04, respectively. Antibacteria activity results showed that *S. typhi* was more sensitive than other tested bacteria. The largest zone of inhibition (20.5 mm) was observed with methanol leaf extract against *S. typhi*, and the smallest inhibition was observed with *H. influenza* (12.5 mm). Leaf and stem extracts using methanol solvent showed activity on all bacteria used in the study except on *S. sonnei*. Leaf and stem water extracts significantly inhibit the growth of *E. coli* with inhibition zone of 14.5 mm and 11.5 mm, respectively, while petroleum ether had no effect on any bacterium. Thus, the present medicinal plant could serve as antibiotics as it showed an important activity against studied bacteria. The study has to continue with other plant parts and other bacteria-causing diseases to make the process cost-effective.

**Keywords:** *K. Integra*; Solvent; Phytochemicals; Clinical Bacteria

## Introduction

Plants absorb water and inorganic compounds with the help of the roots. The leaves then produce nutrients by photosynthesis. Many plants and phytochemicals from them are used to cure diseases caused by microorganisms. It

has been found that medicinal plant has played a vital role in disease management since prehistoric. Most of the population in under development countries utilizes medicinal plants as drug agents. In these countries, conditions like epilepsy, psychosis, pain, etc. are usually treated using herbs. Cholera, gonorrhoea, and typhoid are some of the diseases cured by

phytochemicals of medicinal plants [1]. In these medicinal plants, there is plant called *Kalanchoe integra*. It is a fleshy perennial plant and lives more than two years. It is classified in crassulaceae family. It sometimes includes *Kalanchoe lacinata*. Their common name is Never Die found in tropical to temperate areas. The tannins and flavonoids of *K. integra* were reported to cure the bronchial asthma [2].

The worldwide increase in antibiotic resistance to many Gram negative and positive bacteria is a big problem [3]. The emerging or spreading of new resistance mechanisms of bacteria caused by the less effective of antibiotics are increasing worldwide. This is serious problem since most of the killer diseases such as tuberculosis, pneumonia, septicemia, gonorrhoea, foodborne diseases and diarrhea are caused by those resisting bacteria [4]. *S. aureus* and *S. pneumoniae* are Gram positive bacteria that may cause skin infection, endocarditis and osteomyelitis. These bacteria are resistant to penicillin and other antibiotics related to this. *S. pneumoniae* mainly found in children when colonize nasopharynx can lead to infection by spreading the tissue to cause acute otitis media [5]. Worldwide rate of multidrug resistance of *S. typhi* has increased from 34.2 to 48.5% [6]. This leads to serious diseases in humans especially immune-compromised individuals. It has reported that 30 to 60% of hospitalized patients are because of antibiotic resistance [7].

People in several parts of the world, especially Africa, are utilizing plant extracts to treat themselves from different microbial infections caused by microorganisms like bacteria whenever they became sick or injured in their environment. Although, many studies have been carried out to determine antibiotic susceptibility pattern on different bacteria [8-13], the determination of effectiveness of medicinal plants against different bacteria species is a continuous exercise. Antimicrobial activity of *K. integra* extracts against bacteria that cause diseases was not studied in Rwanda. That why the study of *K. integra* against clinical bacteria is needed. The objective of the present study was thus to assess the antimicrobial effectiveness of *Kalanchoe integra* phytochemicals against *S. sonnei*, *H. influenza*, *S. typhi*, *E. coli*, *S. aureus* and *S. pneumoniae*.

## Materials and Methods

### Chemicals, Plant and Microorganisms

All chemicals and reagents utilized were analytical grade. They were purchased from Sigma-Aldrich (USA) and Hi Media Laboratories (Mumbai, India). *K. integra* leaves and stem were collected from the INES- Ruhengeri botanical garden. The microbes used were *S. typhi*, *S. sonnei*, *H. influenza*, *E. coli*, *S. pneumoniae* and *S. aureus*. They were from Rwanda biomedical center.

### Preparation of *Kalanchoe Integra* Extract

Leaves and stem were washed and dried at room temperature (24°C) for one week. They were then put in hot air oven for 30 min at 100°C for full dryness. 4 kg of *K. integra* leaves and 2 kg of stem were blended differently to form powder using sample mill machine. The three solvent used were 95% methanol, 96% petroleum ether, and distilled water. 20 g of leaves powder was dissolved in 200 ml of each solvent. The same procedure was repeated with stem powder. They were allowed to stand on macerated machine for 5 days until soluble matter was dissolved. Each mixture was sieved by using triple layer of gauze to remove pigments. Haploid machine was used to evaporate solvent [14].

### Screening of Phytochemicals

The qualitative phytochemical analysis of *K. integra* crude extracts was performed to check the presence of alkaloids, saponins, glycosides, resins, phenols, tannins and flavonoids. Phenols were detected by adding 0.5 ml of ferric chloride into stem crude extract and another 0.5 ml of ferric chloride into 1 ml of leaves extract. The presence of phenolic compounds was indicated by the occurrence of a bluish green color. Tannins were detected by pouring 2.5 ml of distilled water into 0.5 g of leaves extract and another into 0.5 ml of the stem extract. After that, the solution was filtered and divided into two parts. 1% lead acetate was added to the first part and the presence of white sediment was a positive test. 1% of ferric chloride solution was added to the second part and the appearance of bluish green color was positive test [15].

Saponins were detected by adding 5 drops of extract into water. After shaking, the mixture was left to stand for 15 min. The presence of saponins was indicated by the foam formation. Flavonoid concentrations were determined by combining 50 mg of powder, 5 mL of distilled water, and 2 mL of NaOH. The presence of flavonoid was indicated by a color change, from yellow to colorless, after adding few drops of HCl. For glycosides, 10 ml of H<sub>2</sub>SO<sub>4</sub> was mixed into 1 ml of extract. Fehling's solution (10 mL) was added. The absence of brick red precipitate indicated a positive test. Alkaloids were detected by mixing 50 mg of powder with 10 ml HCl. The negative test was shown by the absence of turbidity after adding 2 drops of Mayer's reagent [15].

### Sub-culturing of Microorganisms

*H. influenza*, *S. aureus* and *S. sonnei*, *E. coli* were sub-cultured on nutrient agar (NA). *S. pneumoniae* was sub-cultured on blood agar and *S. typhi* was sub-cultured on agar containing xylose, lysine, and deoxycholate. Incubation was performed on the inoculated Petri dishes at 37°C for 24 h.

They were stored at 4°C.

### Antimicrobial Activity of *Kalanchoe integra* Leaves and Stem Extract Against Pathogenic Bacteria

Microbial suspension was prepared by mixing 2 loops full of colony with 4 ml of normal saline in sterile tube. It was then compared with 0.5 McFarland standards for checking the turbidity. Mueller hinton agar (MHA) media were prepared and sterilized. Agar well diffusion method was performed by puncturing the holes of 6 mm in the MHA plates. After that, microbial suspension was inoculated in to punctured media. Prepared extracts were put in all holes according to their concentrations. The positive control used was levofloxacin (5µg/ml) for all the pathogenic bacteria, while solvent without extract was used as negative control. The Petri dishes were incubated for 24 h at 37°C. Inhibition zones were measured in mm.

### Statistical Analysis

All measurements were done in duplicate. For data analysis, SPSS version 22 was utilized. Two-way analysis (ANOVA) and t-test were applied to compare the means of various extract parameters. The statistical analysis was performed at the 95% confidence level and p-values less than 0.05 were considered statistically significant.

### Results and Discussion

#### Screening of Phytochemicals from *Kalanchoe integra*.

In the present study, the activity of *K. integra* against selected pathogenic bacteria was assessed. The plant

parts selected were leaves and stem (Table 1). Similarly, stem [12,13] and leaves [9,10,14,16-19] were also used. However, roots were also utilized by some authors [9,16,17]. Therefore, to extract phytochemicals of *K. integra* that may have antimicrobial activities, any plant part can be utilized. Methanol, petroleum ether, and water were used as extraction solvents (Table 1). Methanol as polar solvent was the solvent of choice in most cases [8-11] followed by non-polar solvent like petroleum ether [9,16,17]. Abdellaoui, et al. [8] and Kolodziejczyk-Czepas, et al. [20] and Stochmal preferred to use ethanol, whereas water was used by Biswas, et al. [9] and Richwagen, et al. [13] Thus, various solvents are tried during screening, and the ones with high yield of phytochemicals are chosen. Many researchers prefer to use polar and non-polar or a mixture of them.

Medicinal plants are the most useful for curing human disease because of phytochemical constituents' presence [21]. After screening, using methanol and water as solvents, the leaves of *K. integra* revealed the presence of flavonoids, tannins, phenols, and saponins. Flavonoids, tannins, and phenols were found in the stem with water and methanol solvents. However, by using petroleum ether, there was no presence of any phytochemical (Table 1). Similarly, flavonoids were reported as chief phytochemical extracted in *Kalanchoe* sp. [8,9,10,16,17,19]. In leaves and stem, saponins [17] and phenols were also seen [8,12,22]. Only Quazi, et al. and Rashmi, et al. [16,17], reported the presence of tannins. Although *K. integra* lacked alkaloids and glycosides (Table 1), alkaloids and glycosides [8,16,17] were present in other *Kalanchoe* sp. Like in the present study, the presence of resins was not reported by any researcher. Thus, *K. integra* can be screened with polar solvents like water and methanol in order to get phytochemicals that may have antimicrobial activities.

Substances	Reagents	Leaves			Stem		
		Methanol	Petroleum ether	Water	Methanol	Petroleum ether	Water
Phenols	Ferric oxide	+	-	+	+	-	+
Tannins	Lead acetate, ferric chloride, distilled water						
Glycosides	Fehling's solution, 50% H <sub>2</sub> SO <sub>4</sub>	-	-	-	-	-	-
Saponins	Distilled water	+	-	+	+	-	+
Alkaloids	Wagner reagent	-	-	-	-	-	-
Flavonoids	Alkaline	+	-	+	+	-	+
Resins	Ethyl alcohol	-	-	-	-	-	-

**Table 1:** Phytochemicals screened from extracts of *Kalanchoe integra* leaves and stem.

+: present, -: absent

### Antibacterial Activity of *Kalanchoe Integra* against Selected Pathogenic Bacteria

Phytochemicals of *Kalanchoe* sp. were reported as antimicrobial, anti-asmathic, anti-inflammatory, antihelmenthic, antioxidant, and anti-tumor agents [9,10,12-14,16,18,19]. The purpose of this study was to show that *K. integra* is effective as an antibacterial agent against *S. aureus*, *E. coli*, *S. pneumoniae*, *S. typhi*, *H. influenza*, and *S. sonnei*. Leaf extracts showed a high inhibition zone compared to the stem extracts. Indeed, leaves methanol extract inhibited all selected bacteria except *S. sonnei*. Tables 2 & 3 show the antimicrobial activities of *K. integra*. The inhibition zones of 12.5 mm on *E. coli* and 18.5 mm on *S. aureus* were recorded with methanol leaf extracts at 100%. Similar inhibition zones of 18, 11, and 12 mm were noted for *S. typhi*, *H. influenza* and *S. pneumoniae*, respectively [16].

The extracts of *K. integra* inhibited also *S. aureus* [11,13,18], *E. coli* [10,11], *Klebsiella pneumoniae* [13], *S. typhi* and *S. pneumoniae* [23]. In contrast, a largest inhibition zone

of 52 mm was observed against *E. coli*, but *S. aureus* showed resistance [18]. The biggest inhibition zone seen could be attributed to high concentration used of leaves powder (300 mg/ml). The Table 4 shows antibacterial activity within three solvents of leaf extracts. Significance was seen within solvents, but not for bacteria. Antibacterial sensitivity results of stem and leaf extracts by agar well diffusion methods showed that leaf extracts were more effective compared to the stem extract. Leaves and stem by using water solvent showed only antimicrobial activity against *E. coli*. (Tables 2-5). Contrastingly, methanol extracts of *K. integra* had no effect *E. coli* [13]. Sarkar, et al. [11] extracted phytochemicals from *K. blossfeldiana* that were active against *E. coli*, *S. aureus*, and *S. pneumoniae* at low concentration of 0.25 mg/ml. In a study conducted by Biswas, et al. [9], root extracts were found to be the most effective antibacterial agents. Therefore, from the present results, methanol leaf extract can be used as antimicrobial agent against the tested bacteria. Table 6 shows comparison of methanol extract of leaves and stem effect on bacteria.

Bacterium	Methanol			Distilled water		
	E1,100%	E2,100%	Mean	E1,100	E2,100	Mean
<i>S. typhi</i>	21	20	20.5	0	0	0
<i>S. aureus</i>	19	18	18.5	0	0	0
<i>H. influenza</i>	16	17	17.5	0	0	0
<i>S. pneumoniae</i>	13	12	12.5	0	0	0
<i>E. coli</i>	12	13	12.5	14	15	14.5
<i>S. sonnei</i>	0	0	0	0	0	0

**Table 2:** Antimicrobial activity of *Kalanchoe integra* leaf extracts, E: experiment.

Bacterium	Methanol			Distilled water		
	E1,100%	E2,100%	Mean	E1,100%	E2,100%	Mean
<i>S. typhi</i>	18	17	17.5	0	0	0
<i>S. aureus</i>	9	10	9.5	0	0	0
<i>H. influenza</i>	13	14	13.5	0	0	0
<i>S. pneumoniae</i>	10	11	10.5	0	0	0
<i>E. coli</i>	9	10	9.5	11	12	11.5
<i>S. sonnei</i>	0	0	0	0	0	0

**Table 3:** Antimicrobial activity of *Kalanchoe integra* stem extracts, E: experiment.

Source of variation	SS	Df	MS	F	P-value	F crit
Bacteria	140.903	5	28.18	0.89	0.52	3.32
Solvents	638.361	2	319.18	10.11	0.003	4.102
Error	315.639	10	31.56			
Total	1094.9	17				

**Table 4:** Effect within three solvents of leaf extracts. Variable rasion (F test) for methanol, petroleum ether and distilled water SS: Sum of square, DF: degree of freedom, MS: mean square, F: crit: F critical.

Source of variation	SS	Df	MS	F	P-value	F crit
Bacteria	88.66	5	17.7	0.9	0.5	3.32
Solvents	344.08	2	172.04	9.01	0.00477	4.1
Error	190.75	10	19.01			
Total	623.5	17				

**Table 5:** Effect within three solvents of stem extracts. Variable ration (F test) for methanol, petroleum ether and distilled water SS: Sum of square, DF: degree of freedom, MS: mean square, F: crit: F critical.

	Variable 1	Variable 2
Mean	13.6	10.08
Variance	56.26	33.84
Observations	6	6
Pooled variance	45.05	
Hypothesized mean difference	0	
Df	10	
t Stat	0.92	
P (T< = t) two-tail	0.37	
t Critical two-tail	2.22	

**Table 6:** Comparison of methanol extract of leaves and stems effect on bacteria.

## Conclusion

The screening of phytochemicals of *K. integra* revealed that leaf and stem consisted of phenols, flavonoids, and saponins. The methanol leaf crude extract of *K. integra* possesses an excellent antibacterial activity against *E. coli*, *S. typhi*, *H. influenza*, *S. pneumoniae*, and *S. aureus*. *S. sonnei* has shown a resistance on both leaf and stem extracts. As the present experimental studies showed a good antibacterial activity of both leaves and stem, the extracts have to be tested to other bacteria-causing diseases. In vivo and toxicity studies have also to be conducted in order to see the possibility of developing a drug from the plant.

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