

## Full Length Research Paper

# Screening of biologically active constituents from leaves of *Aloe elegans* and their antimicrobial activities against clinical pathogens

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The flourishing of the war b/n pathogens and antibiotics is contingent on the diligent trial to find effective antibiotics; so that to lessen the fear faced by resistant microbes. The screening carried out manifested the presence of saponins, glycosides, tannins, reduced sugars, terpenoids, flavonoids and phenols. Alkaloids and steroids were not traced from any of the extracts. Aqueous and solvent extracts from leaves of *Aloe elegans* were inspected for antimicrobial activity against pathogens namely *Klebsiella pneumonia*, *Salmonella thypharium*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* by well and disc diffusion method. Disk diffusion method showed a better result than well diffusion method. The highest poisoning against all the microbes was seen by ethanol extract. The extract showed unsurpassed antifungal activity than antibacterial activity. Minimum inhibitory concentration (MIC) assay was ascertained for this extract against bacteria and fungi. Ethanol extract bespoke maximum toxicity against *S. aureus* at 0.78 mg/ml concentration succeeded by *E. coli* at 1.56 mg/ml concentration. The upshots provide justification for the possibility to utilize plant extracts to treat sundry infectious diseases.

**Key words:** *Aloe elegans*, minimum inhibitory concentration (MIC), antimicrobial, phytochemicals.

## INTRODUCTION

Plant materials remain a crucial source to fight stringent diseases in the world. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Edeoga et al., 2005). The widespread use of a limited number of antimicrobial agents concomitantly with the reduced

arsenal of drugs with antimicrobial function, has led to the development of resistance to drugs that oppose both fungal and bacterial infections, which has been an increasing problem (Zida et al., 2016). The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of

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phytochemical constituents (Nostro et al., 2000). They are naturally occurring in the medicinal plants, leaves, and roots that have defense mechanisms and protect against various pathogens. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoids, alkaloids, and phenolic compounds (Krishnaiah et al., 2007). terpenoids exhibit various important pharmacological activities, that is, anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities (Mahato and Sen, 1997). Alkaloids are used as anesthetic agents and are found in medicinal plants (Herouart et al., 1988). Ergo, it is momentous to take action for the screening of different plants by considering their different floral parts in order to affirm their use as medicinal valued material and to bespeak the active principles by compartmentalization and characterisation of their constituents. In our investigation of *Aloe elegans*, an aqueous extract and three solvent extracts of leaves of *A. elegans* were screened for their phytochemical constituents and their possible antimicrobial activity.

Antimicrobial study of plants has gained prevalence and many researchers (Anzabi, 2014; Mariana et al., 2017) have shown that plants could be used as alternative medicines to combat the problem presented by resistant pathogens. *Aloe vera*, one of the species of Aloes, has been well studied in every aspect, it could be harnessed and has been used in the cosmetic industry. The successful and many uses of *A. vera* has propelled researchers to explore other plants with rigours. *Aloe elegans* is a regionally endemic species of the Aloeacea family which is only found in Eritrea and Ethiopia (Demissew and Nordal, 2010). To the best of our knowledge for this species, few or incomplete studies have been carried out in the struggle to find an alternative drug from plants. The present study investigated the potential use of *A. elegans* as a better source of antimicrobial agents.

*Aloe* species have been used for a long time as folk medicine for the treatment of constipation, burns and dermatitis. Recently, some species of *Aloe*, have been used in a wide range of skin and hair care products, and also form the basis of health drinks and tonics. The slimy gel inside the leaves consists of a complex mixture of polysaccharides, amino acids, minerals, trace element and other biologically active substances, such as enzymes. *Aloe* species have been the source of laxative drugs, the main purgative principle being an anthrone-C glycoside, *aloin*, which occurs at levels of between 18 and 30% of the dried product (British Pharmacopoeia, 1993, United States Pharmacopoeia, 1995). Recent research has indicated that aloe might kill the bacteria responsible for tuberculosis, *Mycobacterium tuberculosis*, and also the herpes virus causing herpes genitalis. Research has further shown that aloe inhibits growth of many common organisms such as yeasts, fungi, and

bacteria infecting wounds (Demissew and Nordal, 2010).

## MATERIALS AND METHODS

### Plant preparation

*A. elegans* leaves were gleaned from subzone of Mai-Nefhi, 20 km north east of Asmara. *Aloe elegans* was authenticated by a taxonomist in the Department of Botany, College of Mai-Nefhi, Prof. Gebrehiwet Medhanie. The plant material was washed to remove dirt and adhering materials and was sliced. The gel was obtained simply by rubbing using knife, and then dried in a shed. After being dried in a shade, the plant materials were ground in electric grinder. The powder was packed in plastic bag until further use.

### Preparation of extracts

For water extraction, Parekh and Chanda (2006) method was followed with little modification. Air dried leaves of *A. elegans*, which was packed in plastic bag as a powder was summed to 500 ml of distilled water. This mixture was boiled with very low heat for about 120 min. After that it was cooled and filtered through 8 layers of muslin cloth for a sterility purpose, and finally centrifuged at 5000 g for 10 min and the supernatant was gleaned. This was done twice. Lastly, the supernatant was concentrated to make the final volume, one fourth of the original volume using a rotavapour and the crude extract was dissolved in distilled water and stored at 4°C for further usage (Parekh and Chanda, 2006).

For solvent extraction, Parekh and Chanda (2006) method was followed with some little modification. Air dried leaves of *A. elegans* (100 g), which was packed in plastic bag as a powder; was mixed with 500 ml of organic solvent (petroleum ether, ethyl acetate and ethanol) in 500 ml flask with cotton wool as a lid and then kept on rotary shaker at 220 rpm for 72 h. Succeeded by collecting the supernatant and filtered through 8 layers of muslin cloth for a sterility purpose and finally the solvent was evaporated to make the final volume one fourth of the original volume using a rotavapour and the crude extract was dissolved in dimethylsulfoxide (DMSO) and stored at 4°C (Parekh and Chanda, 2006).

### Qualitative phytochemical screening

Phytochemical screening by standard methods were implemented to the newly groomed extracts to assure the absence or the presence of the proceeding phytochemicals such as tannins, steroids, alkaloids, glycosides, phenols, reduced sugars, alkaloids, saponins and flavonoids (Parekh and Chanda, 2006).

### Test microorganisms

Microorganisms were given by National Health Laboratory of Eritrea. Two gram-positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus*; three gram-negative bacteria: *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and a fungi: *Candida albicans* were investigated.

### Preparation of inoculum

Some colonies from nutrient agar of pure growth of each tested organism were transferred to 5 ml of Muller Hinton Broth for bacteria and Sabouraud dextrose broth for fungi. Both broths were incubated overnight at 37°C. The suspension was diluted with

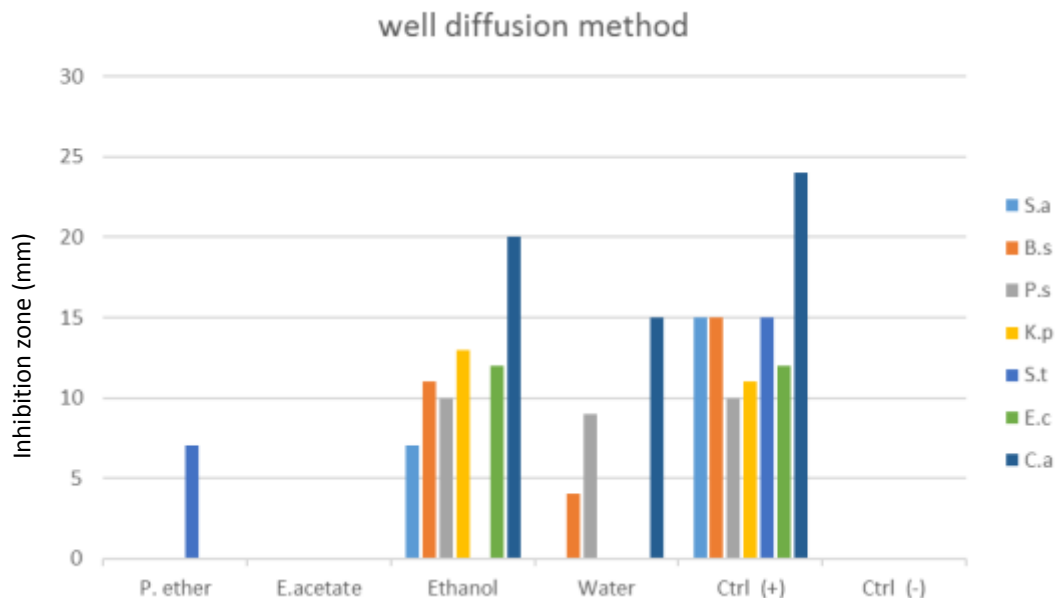


Figure 1. The results for well diffusion method.

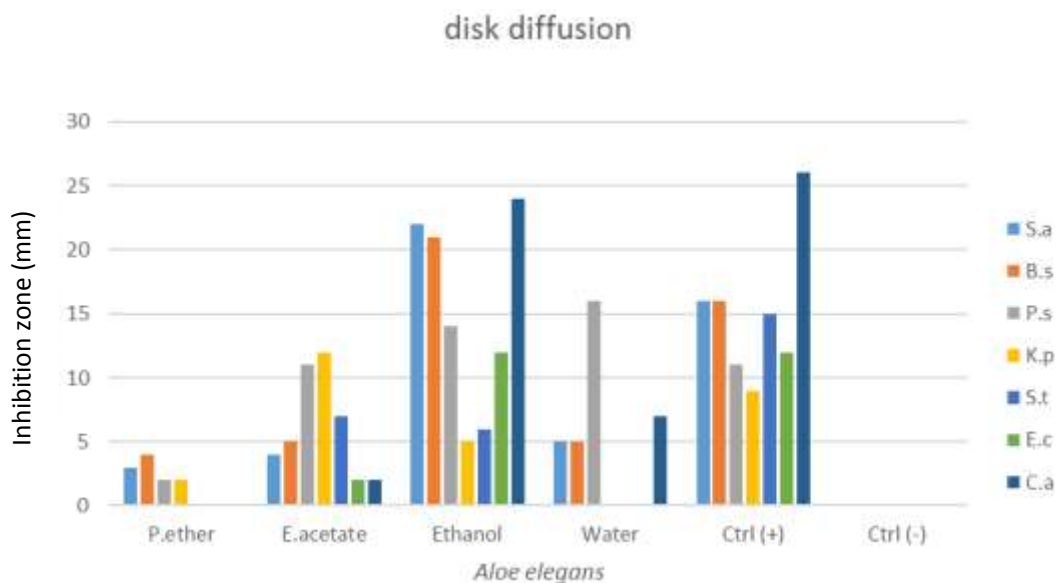


Figure 2. Results for the results of disk diffusion method.

sterile distilled water to obtain approximately 106 to 108 CFU/ml (McFarland, 1907).

**Preparation standard concentrations**

One gram of each solvent and aqueous extract were dissolved into 5 ml of dimethylsulphoxide and 5 g of sterilized water, respectively. This harvested in 200 mg/ml of stock solution as a standard concentration of both types of extracts. Solvent extracts were pasteurized at 62°C while water extract was sterilized by using membrane filters.

**Antimicrobial assay**

The antimicrobial assay was implemented by dual procedures, namely, disk and well diffusion methods (Bauer et al., 1966; Perez et al., 1990). A 100 µl of inoculum (108 CFU) was inoculated with the Muller Hinton Agar and was made to be poured into sterile petri plates (HiMedia).

The well diffusion procedure was done by pipetting 100 µl of our test compound into the wells; which was built by sterilized borer of 0.6 cm. Similarly, the diffusion procedure was performed by pipetting our experiment compound (100 µl) onto the disc (0.6 cm) which was stuck in the agar. The plates were allowed to be

**Table 1.** The phytochemicals of *Aloe elegans*.

Phytochemicals	Solvent extracts			
	Petroleum ether	Ethyl acetate	Ethanol	Distilled water
Flavonoids	-	-	-	+
Glycosides	-	+	-	-
Phenols	-	-	+	-
Reduced sugars	-	-	-	-
Saponins	+	+	+	+
Tannins	-	-	+	-
Terpenoids	-	-	+	-
Alkaloids	-	-	-	-
Steroids	-	-	-	-

Presence of phytochemicals = "+", Absence of phytochemicals = "-".

incubated for 24 h at 37°C. Gentamycin (10 µg/disc) and nystatin discs (100 µg/disc) were used as positive controls for the bacteria and fungus, respectively. Negative control was prepared using respective solvent. Microbial growth was determined by measuring the diameter of zone of inhibition. The experiment was done in triplicate form and the mean values with their standard deviation are presented.

#### Minimum inhibitory concentration (MIC) assay

MIC procedure was exercised to an extract which was efficient in inhibiting the pathogens by both the well and disk diffusion method. MIC of ethanol extract was ascertained by INT calorimetric assay (a rapid p-Iodnitrotetrazolium chloride) (Eloff, 1998). Concisely, the test solvent sample was dissolved in DMSO which in turn was dissolved in MHB. The yielded solution was diluted serially by two fold in 96 well microtiter plate. An inoculum of 100 µl, which was calibrated to 1.5×10<sup>6</sup> CFU/ml groomed in MHB which was finally added. Plates with inoculums were sealed with a sterilized lid and mixed with a shaker to blend the composition, and was allowed to be incubated at 37°C for about 18 h. The amount of DMSO was made to be <2.5%, which means DMSO could not make an impact to the growth of microorganisms. A negative control was experimented parallel to the experiment, which contained a well with a 100 µl of inoculum+MHB+DMSO. Gentamycin was taken as a reference. After 18 h of incubation with 37°C, proceeded by addition of 40 µl INT (0.2 mg/ml), and incubated at 37°C for 30 min. The MIC of the ethanol extract was disclosed. Those microbials which were viable changed this yellow dye to pink. MIC is defined as it is the lowest concentration that precludes this mutability and results complete inhibition of the growth of microbes.

## RESULTS AND DISCUSSION

Phytochemical constituents such as phenols, flavonoids, tannins, alkaloids, saponins and many other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against predation by many microorganisms, insects and other herbivores (Bonjar et al., 2004). The phytochemical result is depicted in Table 1. The present study evinced the presence of medicinally active constituents of saponins, glycosides, tannins, terpenoids, flavonoids and phenols. Steroids and

alkaloids were not present in the plant extracts studied. It is not surprising that ethanol extract of *A. elegans* showed significant inhibition of all tested bacterial and fungal organisms. Almost all the phytochemicals that fight against microbes have been contained in the ethanol extract. The bioactive compounds that are present in plants act by peculiar mechanism and exert antimicrobial activity. The antimicrobial activity of the extract may be due to the presence of phenols, tannins, terpenoids, and flavonoids. Phenols and tannins act their antimicrobial activity by binding to adhesins, enzyme inhibition, substrate deprivation, complex with cell wall, membrane disruption, and metal complexion. Terpenoids act their antimicrobial activity by membrane disruption. Flavonoids act in a way by binding to adhesins and complexing to cell wall of the microbes (Prashant et al., 2011). Next to ethanol extract, water extract showed an inhibition which cannot be underestimated; this might be due to the presence of flavonoids.

Out of the four extracts tested, ethanol extract was the best inhibitor of microbes. It showed inhibition to all organisms followed by an aqueous extract in both methods. The outcome for the antimicrobial results is depicted in Tables 2 and 3 as well as Figures 1 and 2, respectively. In this study, disk diffusion showed better inhibition than well diffusion method. The reason could be due to the plant extract added, which was able to diffuse in the bottom of the plate and thus be far from bacteria grown on the surface. Petroleum ether and ethyl acetate were the weakest extracts to show minimum inhibition in this study which might be caused by being weak solvents to dissolve the active components of the plant. Out of the five bacteria, *S. aureus* was the most susceptible organism which inhibited up to 22 mm, whilst *Salmonella thypharium* and *E. coli* proved to be the most resistant strain. Antifungal result was very promising; it showed up to 24 mm zone of inhibition in ethanol extract. The positive control for fungus showed its highest inhibition zone of up to 26 mm. Further study should be done to determine what would be their combined effect. In

**Table 2.** Well diffusion method.

Extract	Microorganism (zone of inhibition (mm in diameter) (Mean $\pm$ SD) (n=3))						
	S.a	B.s	P.s	K.p	S.t	E.c	C.a
P.ether	-	-	-	-	7 $\pm$ 0.25	-	-
E.acetate	-	-	-	-	-	-	-
Ethanol	7 $\pm$ 0.28	11 $\pm$ 0.78	10 $\pm$ 0.25	13 $\pm$ 0.90	-	12 $\pm$ 0.58	20 $\pm$ 0.21
Water	-	4 $\pm$ 0.42	9 $\pm$ 0.82	-	-	-	15 $\pm$ 0.12
Ctrl (+)	15 $\pm$ 0.36	15 $\pm$ 0.12	10 $\pm$ 0.22	11 $\pm$ 0.10	15 $\pm$ 0.11	12 $\pm$ 0.12	24 $\pm$ 0.24
Ctrl (-)	-	-	-	-	-	-	-

*Escherichia coli* (E.c), *Klebsiella pneumonia* (K.p), *Pseudomonas aeruginosa* (P.s), *Salmonella thypharium* (S.a), *Bacillus subtilis* (B.s), *Candida albicans* (C.a), Control(ctrl.) ; diameter of inhibition zone less than 6mm is considered as negative(-). (+) control= gentamycin (10  $\mu$ g/disc) for bacteria and nystatin discs (100  $\mu$ g/disk), (-) control= DMSO for the solvent extracts and Distilled water for the aqueous extract.

**Table 3.** Disk diffusion method.

Extract	Microorganism (zone of inhibition (mm in diameter) (Mean $\pm$ SD) (n=3))						
	S.a	B.s	P.s	K.p	S.t	E.c	C.a
P.ether	3 $\pm$ 0.06	4 $\pm$ 0.08	2 $\pm$ 0.09	2 $\pm$ 0.21	-	-	-
E.acetate	4 $\pm$ 0.22	5 $\pm$ 0.21	11 $\pm$ 0.12	12 $\pm$ 0.05	7 $\pm$ 0.23	2 $\pm$ 0.50	2 $\pm$ 0.23
Ethanol	22 $\pm$ 0.36	21 $\pm$ 1.0	14 $\pm$ 1.0	5 $\pm$ 0.85	6 $\pm$ 0.91	12 $\pm$ 0.89	24 $\pm$ 0.20
Water	5 $\pm$ 3.6	5 $\pm$ 0.88	16 $\pm$ 0.06	-	-	-	7 $\pm$ 0.56
Ctrl (+)	16 $\pm$ 0.36	16 $\pm$ 0.12	11 $\pm$ 0.22	9 $\pm$ 0.10	15 $\pm$ 0.11	12 $\pm$ 0.23	26 $\pm$ 0.8
Ctrl (-)	-	-	-	-	-	-	-

*Escherichia coli* (E.c), *Klebsiella pneumonia* (K.p), *Pseudomonas aeruginosa* (P.s), *Salmonella thypharium* (S.a), *Bacillus subtilis* (B.s), *Candida albicans* (C.a), Control(ctrl.) ; diameter of inhibition zone less than 6mm is considered as negative(-). (+) control= gentamycin (10  $\mu$ g/disc) for bacteria and nystatin discs (100  $\mu$ g/disk), (-) control= DMSO for the solvent extracts and Distilled water for the aqueous extract.

**Table 4.** Minimum Inhibitory Concentration of *A. elegans*.

Microorganism	Efficacious ethanolic extract	
	<i>A. elegans</i> (mg/ml)	Gentamycin/Nystatin ( $\mu$ g/ml)
<i>E. coli</i>	1.56	12.5
<i>K. pneumonia</i>	12.5	100
<i>P. aeruginosa</i>	6.25	1.5
<i>S. thypharium</i>	12.5	100
<i>S. aureus</i>	0.78	1.5
<i>B. subtilis</i>	25	12
<i>C. albicans</i>	6.25	1.5

(+) control= gentamycin for bacteria and nystatin for fungus.

general, Gram negative bacteria were resistant strains compared to gram positive strains; the reason is simple; lipopolysaccharide (LPS) layer of gram-negative bacteria in outer membrane have a high hydrophobicity which acts as a strong permeability barrier against hydrophobic molecules. Hydrophobic molecules can pass through cell wall of gram positive bacteria easier than the gram-

negative bacteria, because cell wall of the gram-positive bacteria contained only peptidoglycan (Ababutain, 2011).

Ethanol extract showed the highest activity against pathogens which ultimately became the reason to be chosen for MIC test. Outcomes for MIC are shown in Table 4. Ethanol extract showed maximum toxicity against *S. aureus* at 0.78 mg/ml concentration which is

succeeded by *E. coli* at 1.56 mg/ml. Poor toxicity was seen against *B. subtilis* at 25 mg/ml.

## Conclusion

The upshots provides justification for the use of ethanol extract as a medicine to treat sundry infectious diseases and *A. elegans* might be a replacer plant for *A. vera* usage after more researches are done on this important plant.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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