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Comparative Antimicrobial Activities of the Gel, Leaf and Anthraquinone Fractionates of Four *Aloe* Species (*Aloe camperi*, *Aloe elegans*, *Aloe eumassawana* and *Aloe scholleri*)

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Abstract

This study aimed to determine the medicinal significance of traditionally used *Aloe* plants by testing their crude extracts and Anthraquinone fractionates for antimicrobial activity against standard stock organisms. The significance of this study is to promote the discovery of new alternative to drugs currently being used. The gel and leaf cover of these four plants were extracted using cold ethanol extraction method and Anthraquinones were fractionated from the leaf cover using the liquid-liquid extraction technique. The extracts obtained were then screened in different concentrations for their antimicrobial activity against stock organisms *Staphylococcus aureus* (*ATCC*[1] 6538) and *Escherchia coli* (*ATCC* 25922) to access the activity of the *aloe* species against gram positive and gram negative bacteria. It was determined that the crude extracts of the gel and leaf part of the four *aloe* species had antimicrobial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Escherchia coli*) bacteria and *Escherchia coli* showed the least sensitivity towards the four plant extracts while *Staphylococcus aureus* was highly susceptible.

Keywords

Aloe camperi, *Aloe elegans*, *Aloe eumassawana*, *Aloe scholleri*, Antimicrobial Activity, Gel, Leaf, Anthraquinones

[1]American Type Culture Collection (ATCC).

1. Introduction

Traditional medicines, which include herbal remedies, acupuncture and spiritual therapies, have been used for millennia by various peoples to treat acute and chronic illnesses. In many developing countries, they remain the most accessible and most commonly used form of medical care while in developed countries pharmaceutical medicines are used [1]. The ancient people used the juice of *Aloe* as a remedy for the disturbing symptoms of ingestion of rancid or stale food and for intestinal gas. And also to cure wounds, stomach problems, constipation, headaches, alopecia (balding), skin irritations, oral problems and many other disturbances [2].

In Eritrea, there are nine *aloe* species, in which *Aloe neosteudneri* and *Aloe scholleri* are locally found whereas *Aloe camperi*, *Aloe elegans*, *Aloe eumassawana*, *Aloe adigratana*, *Aloe macrocarpa*, *Aloe percrassa* and *Aloe trichosanta* are regionally endemic. For this study *Aloe camperi*, *Aloe elegans*, *Aloe eumassawana* and *Aloe scholleri* are selected. The four *aloe* species are categorized under the family of *Aloaceae* and genus *Aloe*. Traditionally in Eritrea they are used for abdominal discomforts, anti-dandruff, TB treatment, digestion problems, skin infections and skin moisturizer [3].

Aloe eumassawana inhabits near the graveyards of Hirgigo, consisting leaves of 14 - 18 per rosette, tip is shallowly curved with reddish brown color. It blooms during December and January with drought and frost tolerant characteristics. *Aloe camperi* occupies areas from north Nakfa to Ethiopian borders. Its leaves are 12 - 16 per rosette and has brownish red margin with 6 - 7 spines. This plant blooms during late winter or early & mid spring and as every *aloe* species it is drought tolerant and requires sunny to partial shade. *Aloe elegans* flowers red, orange or pale yellow during November to January. Its Leaves are 16 - 20 per rosette, usually curved with 60 cm long and composed of marginal spines of 4 - 7. Its growth habitant is highlands of Eritrea to north of Ethiopia. *Aloe scholleri* is only known from the Kohaito plateau in Eritrea, with dense Leaves 25 - 35 per rosette, spreading triangularly 40 - 52 cm long, 9 - 14 cm broad at the base. This plant blooms March to April [3].

Unlike the other *Aloe* species such as *Aloe Vera* with almost worldwide distribution and well documented studies indicating its antimicrobial activity and its phytochemical constituents, there is no much research done on the four Eritrean endemic *Aloe* species included in this study and hence no much is known about their phytochemical and bioactivity. Therefore the study is the first one to be done on antimicrobial activity of the four Eritrean endemic *aloe* species and will help as a data source for further researches.

2. Methods and Materials

2.1. Study Design

This research was an experimental study. Four *Aloe* species (*A. camperi*, *A. elegans*, *A. scholleri*, *A. eumassawana*) were screened for their *in vitro* antimicrobi-

al activity against known gram positive (*Staphylococcus aureus* ATCC 6538) and gram negative (*Escherichia coli ATCC* 25922) bacteria.

2.2. Sample Collection

The four *aloe* species selected for this study were collected from a botanical garden of Asmara Collage of Health Sciences and were authenticated by a botanist Dr. Ghebrehiwet Medhanie. Gathering of the leaves was made through a sterile technique by the members of the group in which a clean cut was made at the base of each leaf using a sterile knife and transferred into a plastic bag to avoid direct sunlight.

2.3. Sample Processing

The processing of the plant material began within the first hour of collection and excessive accumulation of the product was avoided which could create oxidation of the plant or destruction of the plant constituents. The leaf was properly washed and separated from its gel. The leaf cover and the gel were then dried and followed their respective extraction procedure.

2.4. Gel Extraction

Cold extraction method was used for the extraction of the gel part of the four *aloe* species. The gel was oven dried at 50˚C - 60˚C for 24 hrs and grinded using a mortar and pestle. Powdered gel was then soaked in 70% ethanol in 1:5 ratios for extraction. The extracts were concentrated using a Rotary evaporator and used for antimicrobial assay.

2.5. Leaf Extraction

The leaf cover was extracted using cold extraction method. The leaf cover was oven dried at 45˚C - 50˚C for 24 hrs and grinded. Powdered leaf was then soaked in 70% ethanol in 1:5 ratios for extraction. The extracts were concentrated using a Rotary evaporator and used for antimicrobial assay.

2.6. Anthraquinone Fractionation

For the fractionation of Anthraquinone glycosides from the leaf part of the four plant species liquid-liquid extraction technique was used. First 30 grams of a well dried and grinded leaf of each plant species was soaked in 100 ml of tap water over night and was boiled until foams showed. Then fluid part was then separated through a funnel with gauze overlaid on it. Then was centrifuged for 10 minutes at 4000 rpm, supernatant was mixed well with 1.5 ml hydrochloric acid as well 225 ml of chloroform. This mixture was then shaken vigorously three times in separating funnel apparatus. At this point the chloroform was separately placed in a clean glass with a tightly closing lid and a 1.5 g sodium bicarbonate was added to the aqueous layer mixed for 3 minutes subsequently followed by centrifugation for 20 minutes at 4000 rpm. Afterwards the supernatant was

mixed with 500 ml of 10.5% hydrated ferric chloride then heated for 20 minutes. The hot solution was then heated again with 25 ml hydrochloric acid for 20 minutes. After the hot solution was cooled, using 300 ml ether the aqueous layer was shaken forcefully three times through separating funnel, ether layer that was itself promptly washed three times using water. Lastly the Anthraquinones were extracted from the ether rich layer and the other active compounds were extracted from chloroform rich layer using a separating funnel. Both the layers were evaporated separately using a Rotary evaporator. The extracts were then screened for their antimicrobial activity.

2.7. Media Preparation

For the bioassay three media were prepared namely; Brain heart infusion broth, nutrient agar and Muller Hinton agar media according to the manufacturer instructions. Brain heart infusion was used for the preparation of suspension of the Stock organism and Muller Hinton agar media was used for the sensitivity test.

2.8. Inoculum Preparation and Standardization

The stock organisms were inoculated on nutrient agar media and incubated for 24 hrs. Cultures that were grown on this media were used to take 3 - 5 colony forming units (CFU) for preparation of suspension which was further checked for turbidity equilibration against 0.5 McFarland turbidity standards.

2.9. Antimicrobial Sensitivity Test

Streak well method was used for the sensitivity test of the cold extracts (gel and leaf cover) of the four *Aloe* species. Wells were made using 8 mm sized cork borer. Ethanol dissolved extracts of 100 ul were delivered using a sterile pipette to each well following inoculation of the media with the inoculum standardized against 0.5 McFarland turbidity standard. After 24 hrs of incubation (37˚C) inhibition zone was measured.

In disc diffusion method, 6 mm discs were made from Whatman no-1 filter paper using a puncture, sterilized in an autoclave and were impregnated with 10 ul of ether and chloroform dissolved extracts. The discs were allowed to dry and placed on inoculated Muller Hinton media with the inoculum standardized against 0.5 McFarland turbidity standard. After 24 hrs of incubation, the inhibition zone produced was measured in terms of millimeter.

2.10. Quality Control

All the equipment used for bioassay was autoclaved/heat sterilized prior to each procedure. The three media prepared were autoclaved at 121˚C for 15 minutes along with autoclave strip of Bacillus sterothermophilus as a quality control for sterilization process. 10% of the prepared media were incubated at 37˚C for 24 hours for sterility check. Performance check was done for the media using known stock organisms and standard drugs (Gentamycine, Ciprofloxacin) which showed

results within accepted zones of inhibition. The solvents used for extraction without the extracts were used as a negative control.

2.11. Data Analysis

Each experiment of the plant extract for each pathogen was repeated three times. Statistical analysis was performed using SPSS software version 20. Values were presented as means ± standard deviation (SD). Factorial analysis of variance (ANOVA) was used for comparison of the differences between means. Where appropriate, Post hoc analysis was undertaken using Tukey b or Dunnet test. A $p < 0.05$ was considered significant.

3. Results

3.1. Yield of the Extracts

Cold extracts of the gel

The percentage yield of each plant in cold extraction using 70% ethanol was calculated and summarized in Table 1. From the Gel extracts of the four *Aloe* species, *Aloe eumassawana* gave highest percentage yield (%w/w) and *Aloe elegans* gave the least.

Cold extracts of the Leaf

The percentage yield of each plant in cold extraction using 70% ethanol was calculated and is presented in Table 2. The highest percentage yield was produced be *Aloe scholleri* and lowest by *Aloe elegans.*

Liquid-liquid extracts of the Anthraquinone

Percentage yield of each solvent of the four *aloe* plants was calculated and is presented in Table 3. *Aloe elegans* and *Aloe eumassawana* gave the highest percentage yield in both Chloroform and ether extract. *Aloe scholleri* gave the lowest percentage yield in chloroform and ether extracts.

3.2. Antimicrobial Activity of the Four Plant Extract

1) GEL

All the gel extract of the *Aloe* species have antimicrobial activity against the two pathogens and there is significant connection between the extract and concentration Except in *Aloe elegans* against *S. aureus* (P = 0.279) and *Aloe camperi* (P = 0.288) *and Aloe eumassawana* (P = 0.67) against *E. coli* refer Table 4.

Table 1. Percentage yield (w/w) of cold extract of the gel part of the four *Aloe* species.

Plant Material (GEL)	Total Weight of the material (g)	Weight used for the extraction(g)	Weight of the Extract (g)	Yield % (w/w)
Aloe camperi	15	12	8.0	66.7
Aloe elegans	29	12	6.2	51.6
Aloe schoelleri	18	12	9.3	77.5
Aloe eumassawana	12	12	10.0	83.3

g = grams.

Table 2. Percentage yield (%w/w) of cold extract of the leaf cover of four *Aloe* species.

Plant Material (LEAF)	Total Weight of the material (g)	Weight used for the extraction(g)	Weight of the Extract (g)	Yield (% w/w)
Aloe camperi	123	60	18	30.0
Aloe elegans	214	60	11	18.3
Aloe scholleri	138	60	25	41.7
Aloe eumassawana	91	60	13	21.7

g = grams.

Table 3. Percentage yield of AQs extracts from 30 grams each of the leaf cover of the four *Aloe* species.

Plant material (AQs)	Solvent used	Weight of the Extract (g)	Yield (w/w) %
Aloe camperi	Ether	0.228	0.76
	Chloroform	0.828	2.76
Aloe elegans	Ether	0.250	0.83
	Chloroform	0.959	3.20
Aloe scholleri	Ether	0.173	0.58
	Chloroform	0.592	1.97
Aloe eumassawana	Ether	0.285	0.95
	Chloroform	1.35	3.20

g = grams.

Table 4. Antimicrobial activity of gel extract of the four *Aloe* species with different concentration, each value represents the means in mm ± SD.

Pathogens	*Aloe camperi*			*Aloe elegans*			*Aloe scholleri*			*Aloe eumassawana*		
	250 mg/ml	500 mg/ml	P. value	250 mg/ml	500 mg/ml	P. value	250 mg/ml	500 mg/ml	P. value	250 mg/ml	500 mg/ml	P. value
S. aureus	30 ± 1	34 ± 1	0.008	33 ± 1	34 ± 2	0.279	22 ± 1	26 ± 1	0.008	30 ± 1	36 ± 2	0.013
E. coli	10 ± 1	11 ± 1	0.288	12 ± 0.6	18 ± 2	0.013	10 ± 0	13 ± 1	0.007	8 ± 1	9.6 ± 0.5	0.67

Against *S. aureus*

In pairwise comparison of the plant species, at 250 mg/ml concentration most of the plant species have significant difference between them ($p < 0.05$) except between *Aloe camperi* and *Aloe eumassawana* ($p = 1.000$). And in 500 mg/ml concentration except in between *Aloe camperi*, *Aloe elegans* and *Aloe eumassawana* who showed no significant difference ($p > 0.05$) *Aloe scholleri* have significant difference at that concentration with the other *Aloe* species (**Figure 1**). In addition the positive controls used have significant difference with the plant extracts ($p < 0.05$) against *S. aureus* in both concentrations except in between *Aloe scholleri* in 500 mg/ml concentration and ciprofloxacin ($p > 0.05$).

Against *E.coli*

In pairwise comparison of the plant species, at 250 mg/ml concentration most of the plant species have significant difference between them ($p < 0.05$) except

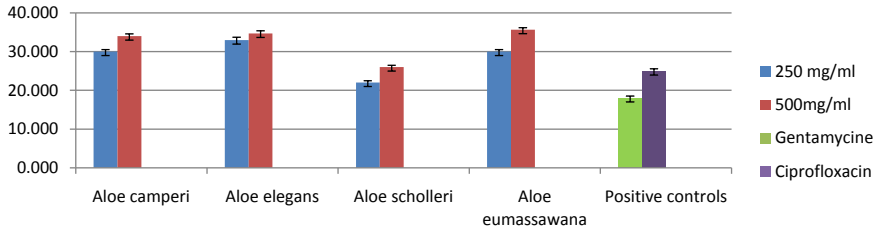

Figure 1. Antimicrobial activity of gel extract of the four *Aloe* species against *S. aureus*.

between *Aloe camperi* and *Aloe scholleri* ($p > 0.05$). And in 500 mg/ml concentration except in between *Aloe camperi* and *Aloe eumassawana* who showed no significant difference ($p > 0.05$) all the other *aloe* species have significant difference at that concentration in between each other. In addition the positive controls used have significant difference with the plant extracts ($p < 0.05$) against *E. coli* in both concentrations except in between *Aloe elegans* in 500 mg/ml concentration and Gentamycine ($p > 0.05$) **Figure 2**.

2) LEAF

All the leaf extract of the *Aloe* species have antimicrobial activity against the two pathogens and there is significant connection between the extract and concentration Except in *Aloe scholleri* against *S. aureus* ($P = 0.288$) suggesting it is not influenced by concentration refer **Table 5**.

Against *S. aureus*

In pairwise comparison of the plant species, Both in 250 mg/ml and 500 mg/ml concentration all the plant species have significant difference between them ($p < 0.05$). More over Ciprofloxacin used as a positive control have no significant difference with the plant extracts of *Aloe camperi* and *Aloe eumassawana*in 250 mg/ml ($p > 0.05$) and Gentamycine have no significant difference with *Aloe scholleri* in both concentrations ($p > 0.05$) **Figure 3**.

Against *E. coli*

In pairwise comparison of the plant species, at 250 mg/ml concentration most of the plant species have significant difference between them ($p < 0.05$) except between *Aloe camperi* and *Aloe scholleri* ($p > 0.05$). And in 500 mg/ml concentration except in between *Aloe camperi*, *Aloe scholleri* and *Aloe eumassawana* who showed no significant difference ($p > 0.05$) *Aloe elegans* have significant difference with the other *Aloe* species. In addition the positive controls used have significant difference with all the plant extracts ($p < 0.05$) against *E. coli* in both concentrations **Figure 4**.

3) ANTHRAQUINONE

All the Anthraquinone extracts of all the *Aloe* species have antimicrobial activity against the two pathogens and there is significant connection between the extract and the solvent and as Anthraquinones are extracted in the ether layer most of the *Aloe* species have higher activity in the ether layer Except in *Aloe scholleri* which showed higher activity for the two organisms in the chloroform layer refer **Table 6**.

Table 5. Antimicrobial activity of leaf extract of the four *Aloe* species with different concentration, each value represents the means in mm ± SD.

Pathogens	*Aloe camperi*			*Aloe elegans*			*Aloe scholleri*			*Aloe eumassawana*		
	250 mg/ml	500 mg/ml	P.value	250 mg/ml	500 mg/ml	P. value	250 mg/ml	500 mg/ml	P. value	250 mg/ml	500 mg/ml	P. value
S. aureus	26 ± 1	32 ± 1	0.002	27 ± 0	30 ± 1	0.007	18 ± 1	19 ± 1	0.288	24 ± 0.5	34 ± 0.5	0.000
E. coli	10 ± 0	13 ± 0.5	0.001	14 ± 0.5	18 ± 0	0.001	10 ± 0	12 ± 1	0.026	8 ± 0.5	12 ± 0.5	0.001

Table 6. Antimicrobial activity of Anthraquinone extract of the four *Aloe* species with ether and chloroform solvents, each value represents the means in mm ± SD.

Pathogens	*Aloe camperi*			*Aloe elegans*			*Aloe scholleri*			*Aloe eumassawana*		
	Ether	Chloroform	P. value	Ether	chloroform	P. value	Ether	chloroform	P. value	Ether	chloroform	P. value
S. aureus	23 ± 1	19 ± 0.5	0.007	42 ± 0	17 ± 2	0.0	11 ± 0.5	25 ± 0.5	0.0	30 ± 1	23 ± 0.5	0.0
E. coli	15 ± 1	10 ± 0	0.001	29 ± 2	14 ± 0.5	0.0	10 ± 1	11 ± 0.5	0.06	30 ± 0	12 ± 2	0.0

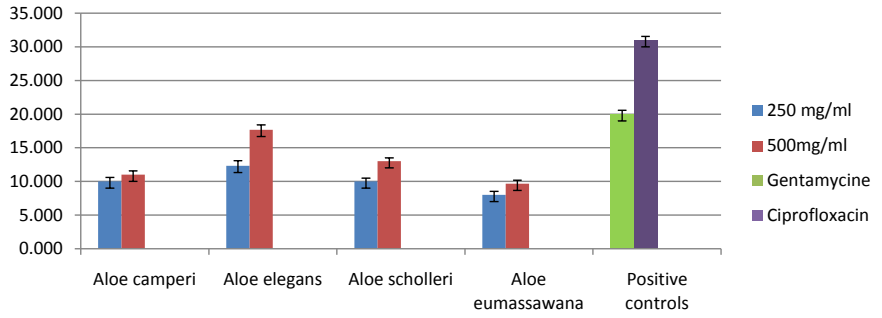

Figure 2. Antimicrobial activity of gel extract of the four *Aloe* species against *E. coli.*

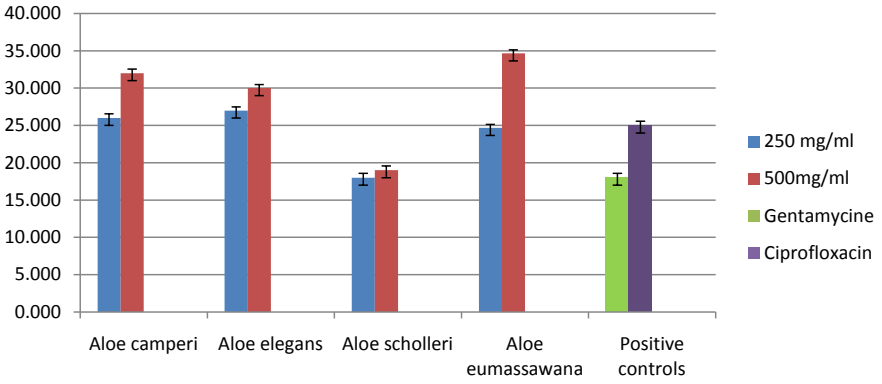

Figure 3. Antimicrobial activity of leaf extract of the four *Aloe* species against *S. aureus.*

Against *S. aureus*

In pairwise comparison of the plant species, Both in ether and chloroform extracts all the plant species have significant difference between them ($p < 0.05$). More over Ciprofloxacin used as a positive control have no significant difference with the plant extracts of *Aloe scholleri* in chloroform extract ($p > 0.05$) and Gentamycine have no significant difference with *Aloe camperi* and *Aloe elegans* in chloroform extract ($p > 0.05$) **Figure 5**.

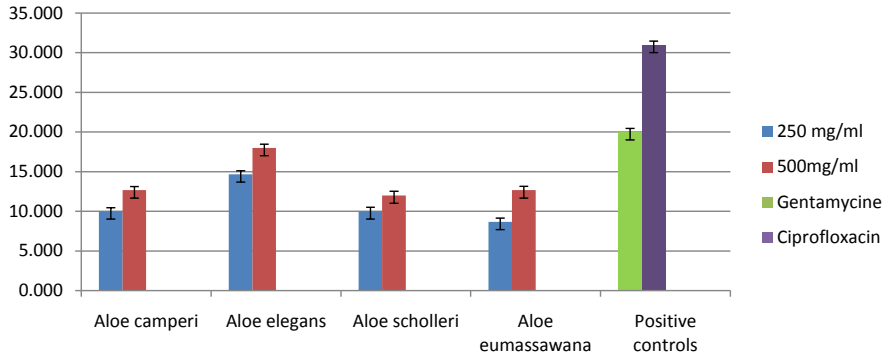

Figure 4. Antimicrobial activity of leaf extract of the four *Aloe* species against *E. coli.*

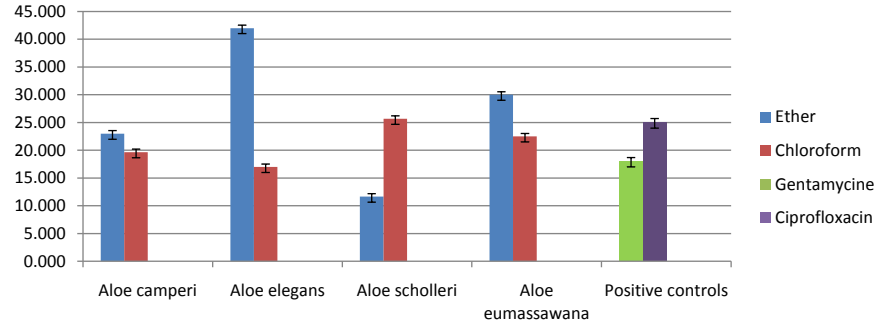

Figure 5. Antimicrobial activity of Anthraquinones of the four *Aloe* species against *S. aureus.*

Against *E. coli*

In pairwise comparison of the plant species, Both in ether and chloroform extracts of the most plant species have significant difference between them ($p < 0.05$) except in between *Aloe elegans* and *Aloe eumassawana* in ether extract ($p > 0.05$) and *Aloe scholleri* and *Aloe eumassawana* in chloroform extract($p > 0.05$). In addition Ciprofloxacin used as a positive control have no significant difference with the plant extracts of *Aloe elegans* and *Aloe eumassawana* in ether extract ($p > 0.05$) **Figure 6**.

4. Discussion

In Eritrea there are nine *aloe* species among which four are included in this study. The gel part is mostly used by the society for the treatment of digestive problems, skin infections, anti-dandruff etc., without any knowledge on the chemical constituents and the presence or absence of the plant activity.

In the current study the gel, of the four *aloe* species showed antimicrobial activity against two selected pathogens making this study similar with the study done by BUKHARI S *et al.* which shows antimicrobial activity of *Aloe vera* gel against uropathogens. But also In contrast to our study, the study done by BURHARI *et al.* showed almost equal anti-bacterial activity against both pathogens while our study showed higher activity against *Staphylococcus aureus* than *Escherchia coli* [4] [10].

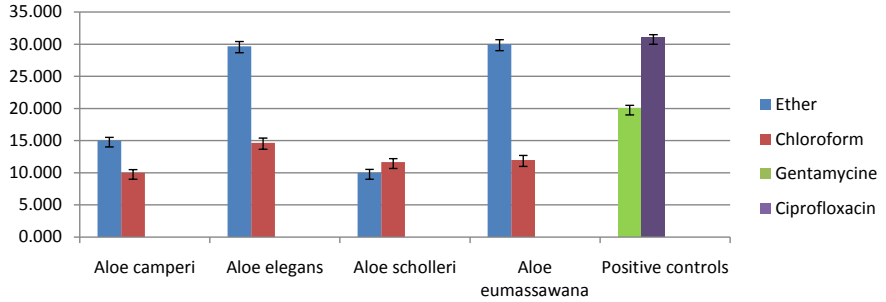

Figure 6. Antimicrobial activity of Anthraquinones of the four *Aloe* species against *E. coli.*

The study on antimicrobial Properties of *Aloe vera*, *Aloe volkensii* and *Aloe secundiflora* gets lower zones of inhibition than the current study this can be due to the difference in the *Aloe* species used for the test [5].

In case of the leaf extract *Aloe eumassawana* had the highest activity in 500 mg/ml concentration while *Aloe scholleri* had the lowest against *Staphylococcus aureus*. While *Aloe elegans* showed the highest activity against *Escherchia coli*. So with respect to the leaf cover extract, our findings were different from the study done by Hagir Omer Musa Abakar *et al.* [6]. Which can be attributed to the difference in the concentration used for the extraction, solvent used that in which they used methanol and other solvents while this study used ethanol for extraction and most importantly the *Aloe* species were different [7].

As for the Anthraquinone fractionates the ether and chloroform extracts of the four *aloe* species also showed different inhibitory effect against the two stock organisms (*Staphylococcus aureus*, *Escherchia coli)* similar with the study done by Pooja Agrawal *et al.* suggesting that the polarities of the solvent play a vital role in extraction which in turn influence the antimicrobial potential of the extracts [7]. And, the result indicates that the active constituents of *Aloe elegans*, *Aloe eumassawana* and *Aloe camperi* are best extracted in the ether layer as the highest inhibitions against the organisms were seen with the ether extracts while the active ingredients of *Aloe scholleri* are best extracted in the chloroform layer suggesting further investigation to find its active constituents.

When the ether and chloroform extracts are compared ether extracts shows the highest activity as a result of higher content of Anthraquinones. This can be backboned by the study done in Portugal by M. Malmir *et al.* suggesting that anthraquinones as potential antimicrobial agents [8]. Highest effects were also scored by ether extracts of *Aloe elegans* and *Aloe eumassawana* but *Aloe scholleri* showed lowest effect in the ether extract against the two stock organisms.

Moreover antimicrobial activity of the gel extracts of all *Aloe* species against the two pathogens improved when the concentration of the extracts was increased from 250 mg/ml to 500 mg/ml except for *Aloe elegans* against *Staphylococcus aureus* ($P = 0.279$), *Aloe camperi* and *Aloe eumassawana* against *Escherchia coli* ($P = 0.288, 0.67$) respectively. The antimicrobial activity of the leaf extracts of all *Aloe* species improved against the two pathogens when the concentration of the extracts was increased from 250 mg/ml to 500 mg/ml except for

Aloe scholleri against *Staphylococcus aureus* (P = 0.288). And the antimicrobial activity of the Anthraquinones of three *Aloe* species showed higher activity against the two pathogens in the ether extract except for *Aloe scholleri.* This findings are related with the study done by Renisheya Joy Jeba Malar T. *et al.* [9].

Generally in this study *Escherchia coli* showed the least sensitivity towards the four plant extracts while *Staphylococcus aureus* was highly susceptible which is in contrast with the study done by Renisheya Joy Jeba Malar T. *et al.* in which *Staphylococcus aureus* was the least sensitive from the microorganisms [9] [10].

5. Conclusions

It was determined that the crude extracts of the gel and leaf part of the four *aloe* species had antimicrobial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Escherchia coli*) bacteria. Suggesting that all the four *aloe* species can be used as an alternative to chemicals in medications for the treatment of food poisoning and infections caused by the microbes.

Borntragers Reaction used for monitoring the ether extract gave positive result, indicating the presence of anthraquinones. The result of the ether extract subjected to Antimicrobial Sensitivity Test showed antimicrobial activity against the two microbes. The highest inhibition produced in ether extract was by *Aloe elegans* against *Staphylococcus aureus*. Thus we can conclude Anthraquinones can be the active constituent present in the leaf. Since the chloroform extract also showed activity against the stock organisms, further investigation is required to identify these active constituents responsible for this activity.

In sum, this investigation shows that both the gel and the leaf parts of the four *Aloe* species are useful and that they can complement one another in their medicinal capabilities and more work should be carried out on the leaf and gel part of the four *aloe* species to reveal its potentials.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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