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Efficacy of *Aloe secundiflora, Azadirachta indica* and C*innamomum verum* against *Escherichia coli* and *Salmonella typhi*

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Abstract

The study was aimed to examine the antimicrobial potential crude extracts of *Aloe secundiflora, Azadirachta indica* and *Cinnamomum verum* against the selected pathogens *Escherichia coli* and *Salmonella typhi.* The bacteria were identified and confirmed by conventional microbiology procedure. Antimicrobial study was carried out by diffusion method against the pathogens by using the crude extracts of *Aloe secundiflora, Azadirachta indica* and *Cinnamomum verum*. The antibacterial activity has been observed crude extracts of *Aloe secundiflora*, *Cinamommum verum* and *Azadirachta indica*against *E. coli and S. typhi* with varied activity. The maximum inhibition zones of *A. secundiflora* were 30.00mm for *E. coli* and 30.00mm for *S. typhi*, that of *A. indica* 24.00mm for *E. coli* and 22.00mm for *S. typhi*, and that of *C. verum* 30mm for *E. coli* and 16.00mm for *S. typhi* were observed. It is hoped that this study would lead to the establishment of some herbs that could be used to formulate new and alternative potent antimicrobial drugs of natural origin.

Key-Words: *Aloe secundiflora, Azadirachta indica, Cinnamomum verum.Salmonella typhi, Escherichia coli,* zone of inhibition.

Introduction

Background Study

Traditional medicine has been in practice for many centuries by a substantial proportion of the population. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases. Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20% of the plants are found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources (Mothana and Linclequist, 2005).Whereas the use of several chemicals in food and several antibiotic medicines has made some bacteria to develop resistance in their population.

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Spices have got excellent antibacterial properties, in many countries people use these along with boiled food preparations which actually reduce its antibacterial properties (Atefl, D.A. and Erdo Urul, O.T, 2003). Spices such as garlic, ginger, clove and *Cinamommum verum* has been used traditionally for both culinary and medicinal purposes (Pankaj Sah et al, 2012).

Aloe secundiflora,is a succulent from the Aloe family (400 different species) with its origin in African continent. Its thick leaves contain the water supply for the plant to survive long periods of drought (Foster, 1999). The leaves have a high capacity of retaining water also in very warm dry climates and therefore this plant can survive very harsh circumstances where most other vegetation disappears.

When a leaf is cut, an orange-yellow sap drips from the open end, this is called the gel.

Aloe secundiflora gel consists of 99.3% water. The remaining 0.7% is made up of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product (Kamble Kaveri M, et al, 2013).

Azadirachta indica (*Azadirachta indica*) tree is a fast growing evergreen popular tree found commonly in

India, Africa and America. It is perhaps the most useful traditional medicinal plant available all year round. The herb is known to exert anti-cancer, antioxidant, wound-healing, and anti-microbial properties are also known to be one of these plants from which almost every part is used (Herbcyclopedia, 2014).

Cinamommum verum is the bark of the evergreen tropical *Cinamommum verum*. It may be in the form of quill or ground powder. It is known for its anti-inflammatory, anti-oxidant, antimicrobial, anti-diabetic and anti-tumor properties. It not only adds aroma and taste to food but also has profound health benefits. It is of various types including the Chinese (cassia) *Cinamommum verum*, Ceylon *Cinamommum verum* and the verum species (Seyed Fazel Nabavi, et al 2015).

Escherichia coli, abbreviated as *E. coli* are bacteria found in the environment, foods, and intestines of people and animals. *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick

Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses.

The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons. *E. coli* consists of a diverse group of bacteria. Pathogenic *E. coli* strains are categorized into pathotypes. Six pathotypes are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli*. The first type is Shiga toxin-producing *E. coli*which may also be referred to asVerocytotoxin-producing *E. coli or* enterohemorrhagic *E. coli.* This pathotype is the most commonly associated with foodborne outbreaks.The others are Enterotoxigenic *E. coli* , Enteropathogenic *E. coli*, Enteroaggregative *E. coli*, Enteroinvasive *E. coli* and Diffusely adherent *E. coli(CDC, 2015).*

Salmonella typhi is a Gram-negative bacterium which grows in the intestines and blood (Wain, J et al, 2015).It causes an infection called typhoid. Typhoid fever is a disease that remains an important public health problem in developing countries. In 2000, it was estimated that over 2.16 million episodes of typhoid occurred worldwide (WHO, 2008).

Statement of the Problem

Bacterial infections from *E. coli* and *S. typhi* causes vast of diseases which may hamper the normal functioning of the human body.There have been contradicting reports that herbs which have been used for quite a long time in treating these diseases have not been effective; there is lack of existing information on which herbs are the most effective and at what concentration are they effective.

The objectives were to compare the antimicrobial activities of *Aloe secundiflora, Azadirachta indica,* and *Cinamommum verum* against *Escherichia coli* and *Salmonella typhi*, and to ascertain the best concentration for which each herb can act more effectively on the test organisms.

Material and Methods

Collection of Plant Material

Abeaker was used to collect *Aloe secundiflora*leaves from the nature conservancy, and plastic bags used to collect *Azadirachta indica* plant leavesand *Cinamommum verum* from Kisumu. Confirmation of the plant materials were done at Universty of Eastern Africa, Baraton Department of Biological Sciences.

Extraction of Plant Material

Aloe secundiflora leaves were washed with distilled water and the gel extracted by crushing the leaves using a sterilized mortar and pestle and then put in asterile beaker (Kaveri et al, 2013).Volume to volume concentration of 100%, 80% and 60% in normal saline were made. *Cinamommum verum* bark and *Azadirachta indica* leaves were washed with distilled water and air dried. *Cinamommum verum* was grinded using a blender and the powders transferred to a clean sterile beaker while *Azadirachta indica* was crushed using a sterile mortar and pestle then sieved into a clean sterile beaker.For each herb, three concentrations of 50mg/ml, 100mg/ml and 150mg/ml were made and left for 96hours.

Key for *Cinamommum verum* and *Azadirachta indica*

2.5 g dissolved in 50 ml normal saline to obtain 50mg/ml

5g dissolved in 50ml normal saline to obtain 100mg/ml

7.5g dissolved in 50ml normal saline to obtain 150mg/ml

These were then filtered using sterile filter papers to give the crude *Cinamommum verum* and *Azadirachta indica*.

Isolation of Test organisms

Escherichia coli

Samples were collected using sterile swabs from toilet seats and immersed on nutrient broth for 6 hours.Using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile nutrient agar plates.The growth was transferred using a sterile inoculating needle and streaked for isolation onto MacConkey agar plate and incubated in at 37°C for 24 hours. On MacConkey the *E.coli* appeared pink in color *(*Alonso, J.L.et al, 1999).

Salmonella typhi

Fecal samples were collected from Baraton Clinic. The samples were diluted in distilled water then inoculated on XLD media.The black colonies on XLD were isolated and further tested with IMVIC sensitivity test and gram staining.

Antibacterial activity of *Aloe secundiflora, Azadirachta indica* and *Cinnamomum verum*

Sterile Nutrient agar plates were prepared. Durham's were sterilized using ethanol and a flame, cooled and used to make wells on the media. The bacterial test organisms- *Escherichia coli* and *Salmonella typhi* were spread over the agar plates respectively using separate sterile swabs.Clean pipettes were sterilized with ethanol and rinsed in distilled water, then used to put the different concentrations in the wells with each well clearly labeled for its respective concentration.

The plates were left on the sterile surface for 5 minutes, and then incubated at 37°C. After 24 hours, the diameter of the minimum zone of inhibition was measured in mm and results recorded. For each test, three trials were performed for better analysis of the results.

Statistical Analysis

The data obtained by measuring the zones of inhibition was subjected to ONE WAY ANOVA test to determine whether there was any significant difference between the herbs and their different concentrations.

Results and Discussion

The objectives were to compare the antimicrobial activities of *Aloe secundiflora*, *Azadirachta indica*, and *Cinamommum verum* against *Escherichia coli* and *Salmonella typhi*. The successive *Aloe secundiflora*leaf extracts using normal saline solution at a concentration of100 %, 80 % and 60 % showed significance difference amongst all concentrations that is between 60% and 80%, 80% and 100% and 60% and 100% for both *Escherichia coli* and *Salmonella typhi*. Therefore the activity increases with concentrations (Table 1 and 2).

Table 1: Antimicrobial activity of *Aloe secundiflora*on *E. coli*

Multiple Comparisons

Dependent Variable: ZI
Games-Howell

(I) CONCENTRATION OF AV	(J) CONCENTRATION OF AV	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100%	80%	26.00000*	3.05505	.025	8.0034	43.9966
	60%	26.00000*	3.05505	.025	8.0034	43.9966
80%	100%	-26.00000*	3.05505	.025	-43.9966	-8.0034
	60%	.00000	.00000	.	.0000	.0000
60%	100%	-26.00000*	3.05505	.025	-43.9966	-8.0034
	80%	.00000	.00000	.	.0000	.0000

*. The mean difference is significant at the 0.05 level.

Multiple Comparisons

Table 2: Antimicrobial activity of *Aloe secundiflora* on *S. typhi*

Dependent Variable: ZI
Games-Howell

(I) CONCENTRATION OF AV	(J) CONCENTRATION OF AV	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100%	80%	29.33333*	.66667	.001	25.4062	33.2605
	60%	29.33333*	.66667	.001	25.4062	33.2605
80%	100%	-29.33333*	.66667	.001	-33.2605	-25.4062
	60%	.00000	.00000	.	.0000	.0000

60%	100%	-29.33333*	.66667	.001	-33.2605	-25.4062
	80%	.00000	.00000	.	.0000	.0000
*. The mean difference is significant at the 0.05 level.						

The successive *Azadirachta indica* leaf extracts using normal saline solution at a concentration of 2.5 mg/ml, 5 mg/ml and 7.5 mg/ml. There was no significant difference between concentrations 2.5 mg/ml and 5 mg/ml or 5 mg/ml and 7.5 mg/ml for both *Escherichia coli* and *Salmonella typhi*. However, there was significant difference between 2.5mg/ml and 7.5 mg/ml therefore 7.5 mg/ml is the best concentration that can be used on these test organisms (Table 3 and 4).

Table 3: Antimicrobial activity of *Azadirachta indica*on *E. coli*

Multiple Comparisons							
Dependent Variable: ZI							
	(I) Various concentration of *AZADIRACHTA INDICA*	(J) Various concentration of *AZADIRACHTA INDICA*	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	2.5	5	-2.00000	1.44016	.214	-5.5240	1.5240
		7.5	-5.33333*	1.44016	.010	-8.8573	-1.8094
	5	2.5	2.00000	1.44016	.214	-1.5240	5.5240
		7.5	-3.33333	1.44016	.060	-6.8573	.1906
	7.5	2.5	5.33333*	1.44016	.010	1.8094	8.8573
		5	3.33333	1.44016	.060	-.1906	6.8573
*. The mean difference is significant at the 0.05 level.							

Table 4: Antimicrobial activity of *Azadirachta indica* on *S. typhi*

Multiple Comparisons							
Dependent Variable: ZI							
	(I) Various concentration of *AZADIRACHTA INDICA*	(J) Various concentration of *AZADIRACHTA INDICA*	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	2.5	5	-4.00000	2.30940	.134	-9.6509	1.6509
		7.5	-6.00000*	2.30940	.041	-11.6509	-.3491
	5	2.5	4.00000	2.30940	.134	-1.6509	9.6509
		7.5	-2.00000	2.30940	.420	-7.6509	3.6509
	7.5	2.5	6.00000*	2.30940	.041	.3491	11.6509
		5	2.00000	2.30940	.420	-3.6509	7.6509
*. The mean difference is significant at the 0.05 level.							

The successive *Cinnamomum verum* leaf extracts using normal saline solution at a concentration of 2.5 mg/ml, 5 mg/ml and 7.5 mg/ml. There was no significant difference between concentrations 2.5 mg/ml and 5 mg/ml or 5 mg/ml and 7.5 mg/ml for both *Escherichia coli* and *Salmonella typhi*. However, there was significant difference between 2.5 mg/ml and 7.5 mg/ml therefore 7.5 mg/ml is the best concentration that can be used on these test organisms (Table 5 and 6).

Table 5: Statistical Analysis of *Cinnamomum verum* on *E.coli*

Multiple Comparisons							
Dependent Variable: ZI							
	(I) Various concentration of *CINAMOMUM VERUM*	(J) Various concentration of *CINAMOMUM VERUM*	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound

LSD	2.5	5	-10.66667*	1.08866	.000	-13.33305	-8.00028
		7.5	-16.66667*	1.08866	.000	-19.33305	-14.00028
	5	2.5	10.66667*	1.08866	.000	8.00028	13.33305
		7.5	-6.00000*	1.08866	.001	-8.6639	-3.3361
	7.5	2.5	16.66667*	1.08866	.000	14.00028	19.33305
		5	6.00000*	1.08866	.001	3.3361	8.6639

*. The mean difference is significant at the 0.05 level.

Table 6: Statistical Analysis of *Cinnamomum verum* on *S.typhi*

Multiple Comparisons

Dependent Variable: ZI

	(I) Various concentration of *CINAMOMMUM VERUM*	(J) Various concentration of *CINAMOMMUM VERUM*	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	2.5	5	-1.33333	1.53960	.420	-5.1006	2.4339
		7.5	-4.66667*	1.53960	.023	-8.4339	-.8994
		2.5	1.33333	1.53960	.420	-2.4339	5.1006
		7.5	-3.33333	1.53960	.074	-7.1006	.4339
	7.5	2.5	4.66667*	1.53960	.023	.8994	8.4339
		5	3.33333	1.53960	.074	-.4339	7.1006

*. The mean difference is significant at the 0.05 level.

For *Cinamommum verum* and *Azadirachta indica*, there was no significant difference between concentrations 2.5 and 5 or 5 and 7.5 for both *E.coli* and *S. typhi.* However, there was significant difference between 2.5 and 7.5 therefore 7.5 is the best concentration that can be used on these test organisms.

Concluion and Recommendation

From the study, there is no significant difference between concentrations of 50mg/ml and 100mg/ml for *Cinamommum verum* and *Azadirachta indica*. However, there was a significant difference between 50mg/ml and 150ml and thus the best concentration that can be used against the two test organisms' is150mg/ml. For *Aloe secundiflora*, there was significance in all percentages used from the data analyzed, *Azadirachta indica* has high efficacy on *E. coli* and *Salmonella typhi* compared to *Aloe secundiflora* and *Cinnamomum verum* and therefore we reject the null hypothesis. This study recommends that solutions containing these herbs made and be used in laboratories for wipe-downs; also further studies should be done to investigate the side effects of these herbs.

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Table 1.1: Antimicrobial Activity of Antibiotics on *E. coli* and *S. typhi*

Antibiotics (Positive control)	Zone of Inhibition (mm)	
	E. coli	*S. typhi*
1st Trial Ciprofloxacine 50mg/ml	48mm	42mm
2nd Trial Ciprofloxacine 50mg/ml	44mm	40mm

Table 1.2: Antimicrobial Activity of Normal Saline on *E. coli* and *S. typhi*

Normal Saline (Negative control)	Zone of Inhibition (mm)	
	E. coli	*S. typhi*
1st Trial	00mm	00mm
2nd Trial	00mm	00mm

Table 1.3: Antimicrobial Activity of *Aloe secundiflora* on *E. coli and S. typhi*

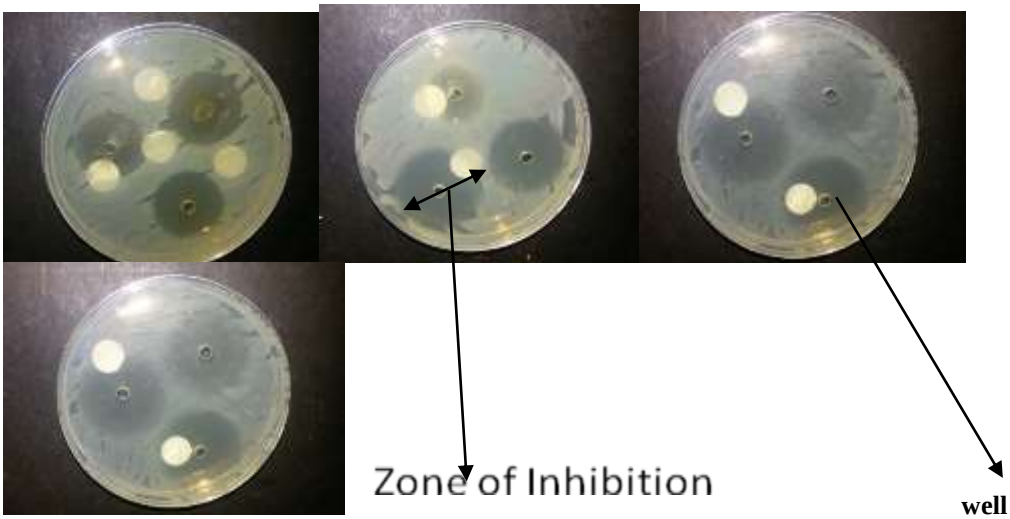
Concentrations (v/v)	Zone of Inhibition (mm)	
	E. coli	*S. typhi*
1st Trial		
100%	20mm	28mm
80%	00mm	00mm
60%	00mm	00mm
2nd Trial		
100%	28mm	30mm
80%	00mm	00mm
60%	00mm	00mm
3rd Trial		
100%	30mm	30mm
80%	00mm	00mm
60%	00mm	00mm

Table 1.4: Antimicrobial Activity of *Azadirachta indica* on *E. coli and S. typhi*

Concentrations	Zone of Inhibition (mm)	
	E. coli	*S. typhi*
1st Trial		
150mg/ml	24mm	22mm
100mg/ml	22mm	20mm
50mg/ml	22mm	18mm
2nd Trial		
150mg/ml	24mm	26mm
100mg/ml	22mm	24mm
50mg/ml	20mm	22mm
3rd Trial		
150mg/ml	28mm	24mm
100mg/ml	22mm	22mm
50mg/ml	18mm	14mm

Table 1.5: Antimicrobial Activity of *Cinnamomum verum* on *E. coli and S. typhi*

Concentrations	Zone of Inhibition (mm)	
	E. coli	*S. typhi*
1st Trial		
150mg/ml	30mm	16mm
100mg/ml	24mm	12mm
50mg/ml	14mm	14mm
2nd Trial		
150mg/ml	30mm	20mm
100mg/ml	24mm	16mm
50mg/ml	12mm	14mm
3rd Trial		
150mg/ml	30mm	18mm
100mg/ml	22mm	16mm
50mg/ml	16mm	12mm



Plant extracts

IMVIC Test- *S. Typhi*

IMVIC Test- *E. coli*

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