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Uwimbabazi Francine
Applied Biology, University of Rwanda-College of Science and Technology (former KIST) PO. Box 3900 Kigali, Rwanda.

Uwimana Jeannette
Applied Biology, University of Rwanda-College of Science and Technology (former KIST) PO. Box 3900 Kigali, Rwanda.

Rutanga Jean Pierre
Applied Biology, University of Rwanda-College of Science and Technology (former KIST) PO. Box 3900 Kigali, Rwanda.

Correspondence:
Rutanga Jean Pierre
Vrije Universiteit Brussel, Interuniversity Programme Molecular Biology (IPMB), Pleinlaan 2-Building E-1050 Brussels, Belgium.

Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*

Uwimbabazi Francine, Uwimana Jeannette, Rutanga Jean Pierre

Abstract
The present study was carried out to screen and evaluate antimicrobial activity of leaf and bark extracts of *Azadirachta indica.* Ethanol and aqueous extract of leaves and barks of *A. indica* (Neem) were tested against *Escherichia coli* and *Staphylococcus aureus* which are known to be resistant to various antibiotics. Neem materials which were used during this study were collected from Bugesera district precisely at Nyamata in Murama sector whereas the bacterial strains were isolated from microbiology laboratory of CHUK (Centre Hospitalier Universitaire de Kigali) and transported under safe conditions to be kept in KIST laboratory under favorable conditions for each of them. The efficiency of the extracts was studied and determined by applying different extract concentrations onto the two cultured bacterial strains using the disc diffusion method.
In this study, we examined the in vitro effect of extracts of different neem (*Azadirachta indica)* plant (leaf and bark) on *Staphylococcus aureus* and *Escherichia Coli*. Ethanol and aqueous extracts were prepared from both dry and fresh neem's leaves and barks. The susceptibility of tested bacteria to both extracts was determined by measuring the diameter of inhibition zones formed around plates. With the statistical test analysis, the comparison done showed that, Fresh Neem materials was found to be the most showing much effect on both *Escherichia coli* and *Staphylococcus aureus*. Comparing Neem leaves and Neem barks, always their fresh extracts were found more efficient than dry extracts; the same as ethanol extracts were more effective than aqueous extracts in all cases, *S.aureus* was the only bacterium susceptibly affected by these neem extracts used; while *E.coli* didn't respond to any of them. The results showed that the effectiveness of the extracts was dependent of the concentration used thus the increase of extract concentration increased the inhibition zone.

Keywords: Neem extract, aqueous extract, ethanol extract, antimicrobial activity, dry Neem extract, fresh Neem extract.

1. Introduction
Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health and well-being. Although many drugs that come from trees generally have been replaced by more potent synthetic ones, trees remain a source for some drug ingredients [1].
Medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anemia for a long time now [2], but the potential of higher plants as source for new drugs is still largely unexplored [3]. Systematic screening of them may result in the discovery of novel effective compounds [4].
Neem (*Azadirachta indica*) commonly called 'India Lilac' or 'Margosa', belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. Neem is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species. Various parts of the neem tree have been used as traditional Ayurvedic medicine in India. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Neem oil finds use to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and phthisis [5].
Neem tree has adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth. Neem tree requires little water and plenty of sunlight [6].

The tree grows naturally in areas where the rainfall is in the range of 450 to 1200 mm. However, it has been introduced successfully even in areas where the rainfall is as low as 150 to 250 mm. Neem grows on altitudes up to 1500 m. It can grow well in wide temperature range of 0 °C to 49 °C. In Rwanda it grows in regions with hot weather like Bugesera and Kibungo. Therefore, we will be using neem samples from one of these regions, in order to determine the antimicrobial activity of Azadirachta indica plant extracts against some specific bacteria which cause different infections and diseases on the human being.

Plant treatment is popular in medicine; plants are used traditionally to cure various diseases like it is the case in Rwanda. But in Rwanda, some important medicinal plants are not yet introduced or are rarely used, this makes the Rwandan traditional medicine poor and it needs improvement. Based on medicine, most records of morbidity and mortality occurring as bacterial infections due to fact that most of the bacteria pathogens have developed resistance to antibiotics. Their resistance to antibiotics increases mortality and the length of stay in the hospital, therefore the research and the identification of this uncommon tree against some bacterial pathogens for their microbial activity causing disease will be beneficial as sources of antimicrobial substances.

Our study on Neem *(Azadirachta indica*) was primarily done in order to enable us to trace and witness how it is effective on some pathogens causing diseases especially the ones responsible for intestinal infections (diseases) such as *Staphylococcus aureus* and *Escherichia coli.*

Neem has been described differently worldwide, which has sounded as an answer to many diseases. In Rwanda, it is new among common plants that are used for medicine purposes; as a matter of fact, we decided to do our study on this tree so that we emphasize the efficacy of Neem (*Azadirachta indica*) which is also a contribution to Rwandan traditional medicine.

This study helped us to improve our scientific knowledge in terms of antimicrobial activities of medicinal plants as well.

As this tree was proven to be very important in solving health problems, if found really helpful after this study, people will be aware of its effectiveness.

Generally, medicinal herbs are less expensive than other drugs; this justifies Neem's frequent use in many countries. Therefore, as Rwandan population will get to know it more, they will be interested in it and even research institutions will get encouraged in finding more about it.

The general objective of our study was to determine the antibacterial activity of Neem (*Azadirachta indica*) leaf and bark extracts on *Staphylococcus aureus* and *Escherichia coli.*

Whereas our specific objectives were:

-to determine the antibacterial effect of Neem on *E.coli*; and

-to determine the antibacterial effect of Neem on *S.aureus.*

2. Material and Methods

2.1 Collection of plant materials

The fresh Neem leaves and stem bark were collected from BUGESERA district precisely at Nyamata in Murama sector and safely taken in KIST biology laboratory for further experimental analysis. In all cases, dried and fresh plants materials extractions were used for this study.

2.2 Laboratory equipments

Materials and reagents used in this study are listed in appendices. This research was mainly carried out in Kigali Institute of Science and Technology (KIST), biology laboratory.

2.3 Extract preparation

2.3.1 Preparation of aqueous extracts from fresh Neem plant materials

The extracts from fresh Neem (*Azadirachta indica*) were prepared immediately after sample collection following different steps. 225 grams of fresh leaves and 238 grams of stem bark were collected then they were washed separately in different containers with distilled water then they were cut into small pieces. For preparation, 20 g of small green neem leaves were soaked into 250 ml of water overnight, the same as fresh stem bark then the next day they were filtered. The filtrates were then evaporated using a rotary evaporator; the two samples (leaves and stem bark) were still separated. The obtained extracts were 1.4 g and 1.6 g for leaves and barks respectively, and then they were weighed and stored at 5 °C.

2.3.2 Preparation of aqueous extracts from dried Neem plant materials

The collected leaves and stem bark were weighed 199 grams of fresh leaves and 203 grams of fresh barks then they were washed with distilled water and allowed to dry at 55 °C. The dried plant materials were ground into fine powder. The powder from dry leaves was 70.8 g and for barks was 80.6 g; they were stored in different bottle until needed for use. For preparation of extracts, 20 g of powdered leaves and stem bark were soaked each in 250 ml of distilled water. The mixtures in different containers were kept for 24 hours in shaking water bath fewer than 40 °C. After the mixtures were filtered using a muslin broth. The filtrate was evaporated to get the final extract using a rotary evaporator attached to a vacuum pump. 1.7 and 1.8 g of extracts were obtained from leaves and barks respectively and stored at 5 °C after being weighed.

2.3.3 Preparation of ethanol extract from fresh Neem plant materials

Fresh leaves and stem barks were cut into small pieces using a knife then they were put into different conical flasks, each flask with 25 g of plant material. The flasks were then filled with 50 ml of absolute ethanol, and then allowed to stand for 48 hours for extraction. After the ethanol extracts were separated from residues using muslin broth then the filtrates were evaporated using a rotary evaporator attached to a vacuum pump, so as to get the final extracts. 2.2 g for leaves and 2.4 g for barks were obtained, and then stored in different containers under 5 °C.

2.3.4 Preparation of ethanol extract from dry Neem plant materials

Dried leaves and stem bark were ground into fine particles using an electrical blender. 20 g of the ground stem bark and leaves were separately soaked into 250 ml of ethanol (95 %) in conical flasks. The mixtures were kept in shaking water and allowed to stand for about 48 hours for extraction then the resulting solutions were filtered using a muslin broth. The filtrates were then evaporated in order to get the final extracts. 2.1 g for leaves and 2.4 g barks were obtained and then stored under 5^0 c.

2.4 Bacteria isolation and inoculation

The two bacteria to be used during this study, *E.coli* and *S. aureus* were isolated and identified from CHUK patients, following standard procedures as described by Cowan and Steel (1974) [7] and Cheesbrough (2002) [8].

This was done in CHUK microbiology laboratory. The pure culture were brought in KIST laboratory, and then the two strains were transferred in two test tubes containing Nutrient

broths agar Then, they were labeled and incubated at 37 °C for 24 hrs.

2.5 Preparation of concentrations for antibacterial assay

2.5.1 Serial dilution

Each plant extract was subjected to a serial dilution using sterile distilled water as a diluent.1ml from each crude extract was added into a tube containing 9ml of sterile distilled water, from this tube, a serial dilution was done and covered a dilution range of 10^{-1} to 10^{-7}. This helped to determine the minimum inhibitory concentration (MIC) of each extract on each strain.

2.5.2 Bacteria inoculation and disc-diffusion method

Media specific for each strain were prepared (MSA for S.*aureus* and MacConkey for E.*coli*) and on each labeled plate that contains the medium, was inoculated 40 µl of standardized broth culture of the bacteria. The spreader was used to ensure uniform distribution of the microorganisms on surface of plates.

This method disc diffusion was described by Kirby-Bauer. Normally, this method is used for testing the effect of chemical drugs on bacteria; therefore the same method was used in order to compare the effectiveness of different neem extracts concentrations from stem bark and leaves; obtained by using water and ethanol as solvents.

Filter paper discs were prepared and sterilized, these discs were then soaked in different concentration of extract, and then they were aseptically placed over the media with specific bacteria. The plates were incubated in an upright position at 37 °C for 24 hours. The diameters of inhibition zones were measured in cm.

3. Results

3.1 Antibacterial activity of *Azadirachta indica* leaves on *S.aureus*

The results obtained after experiment showed that *S.aureus* strains responded differently to both ethanol and aqueous leaf extracts. On this strain we used both dried and fresh leaves and the comparison was done based on the inhibition zones obtained after incubation. The table below shows the inhibition zones in cm, formed by aqueous and ethanol extracts from fresh Azadirachta indica leaves.

Table 1. Antibacterial activity of fresh *Azadirachta indica* leaves

Serial dilution concentration levels of leaf extracts	Zones of inhibition (in cm)	
	Water extract from fresh leaves	Ethanol extract from fresh leaves
10^{-1}	2.4	3
10^{-2}	2	2.5
10^{-3}	1.9	2.1
10^{-4}	1.4	1.7
10^{-5}	Growth	1
10^{-6}	Growth	Growth
10^{-7}	Growth	Growth

As shown by the results in the table above, the zone of inhibition for both aqueous and ethanol extract decreases as the dilution factor increases. On water, the MIC is 10^{-4} while on ethanol it is 10^{-5}. These results are interpreted graphically in the figure 1 below:

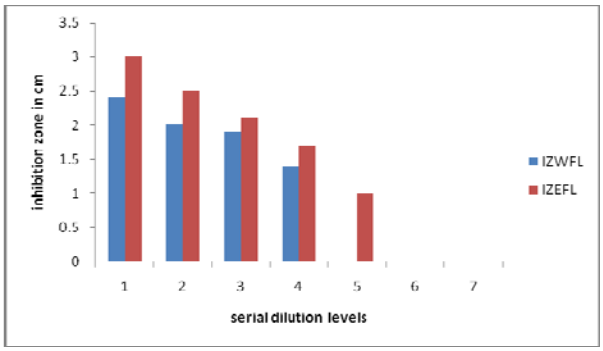

Fig 1: Antibacterial activity of fresh *Azadirachta indica* leaves

IZWFL: Inhibition zone of water extract from fresh leaves
IZEFL: Inhibition zone of ethanol extract from fresh leaves
1, 2, 3, 4, 5, 6 and7 represent 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} respectively.

The table below shows the inhibition zones in cm, formed by aqueous and ethanol extracts from dry Azadirachta indica leaves.

Table 2: Antibacterial activity of dry *Azadirachta indica* leaves

Dilution concentration of leaf extracts	Zones of inhibition (cm)	
	Water extract from dry leaves	Ethanol extract from dry leaves
10^{-1}	2.2	2.7
10^{-2}	1.8	2.2
10^{-3}	1.2	1.5
10^{-4}	Growth	1.1
10^{-5}	Growth	0.84
10^{-6}	Growth	Growth
10^{-7}	Growth	Growth

The inhibition zone decreases as the dilution factor increases. The MIC for water extract from dry leaves is 10^{-3} while that of ethanol extract is 10^{-5}; these results were also interpreted graphically in the Figure 2 below:

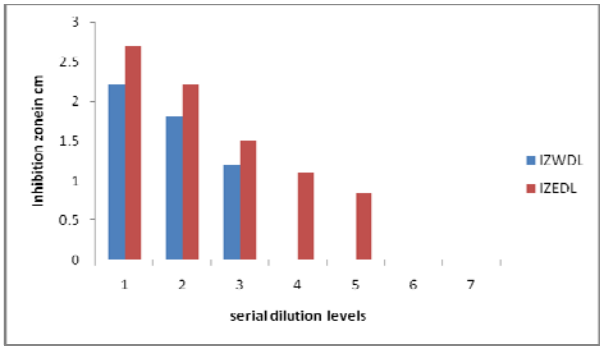

Fig 2: Antibacterial activity of dry *Azadirachta indica* leaves

IZWDL: Inhibition zone of water extract from dry leaves
IZEDL: Inhibition zone of ethanol extract from dry leaves
1, 2, 3, 4, 5, 6 and 7 represent 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} respectively. Using SPSS program means of inhibition zones for water and ethanol extracts, mean difference between water and ethanol extract, t value and p value for both fresh and dry Azadirachta indica leaves were calculated as shown in SPSS tables below:

Table 3: Mean results for fresh leaf extracts

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	IZWFL	1.471	7	1.1828	.4471
	IZEFL	1.100	7	1.0693	.4041

Table 4: Mean results for dry leaf extracts

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	IZWDL	1.1914	7	1.02949	.38911
	IZEDL	.743	7	.9710	.3670

Table 5: Mean difference, t value, and p value for fresh leaf extracts

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZWFL - IZEFL	.3714	.3592	.1358	.0392	.7037	2.736	6	.034

Table 6. Mean difference, t value, and p value for dry leaf extracts

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZWDL - IZEDL	.44857	.40969	.15485	.06967	.82747	2.897	6	.027

The mean comparison between ethanol and water extracts for both fresh and dry Azadirachta indica leaves shows that the two means are significantly different, this is because for both water and ethanol extracts, p value is smaller than 0.05 (significance level). The mean difference for both fresh and dry leaves was calculated by taking ethanol extract mean minus that of water extract. Thus, the ethanol leaf extracts were more effective compared to aqueous leaf extracts in both dry and fresh leaves.

Usually when the p value is less than 5% H1 (alternative – positive hypothesis) should be accepted and reject H0 (null hypothesis).

H0: There is no significant difference between the two means

H1: There is a statistical difference between the two means; therefore as in this case calculated p values were less than 5%, H1 is accepted there is a statistical difference between the two means.

3.2 Antibacterial activity of *Azadirachta indica* stem bark on *S.aureus*

S.aureus was also tested using fresh stem barks extracted with water and ethanol solvents, the results obtained are shown in the table below:

Table 7: Antibacterial activity of fresh *Azadirachta indica* bark

Dilution concentration of bark extracts	Zones of inhibition (cm)	
	Water extract from fresh stem bark	Ethanol extract from fresh stem bark
10^{-1}	3.7	4.4
10^{-2}	3.2	3.8
10^{-3}	1.8	2.5
10^{-4}	Growth	1.2
10^{-5}	Growth	Growth
10^{-6}	Growth	Growth
10^{-7}	Growth	Growth

As shown by the results, the inhibition zone varies in the same way as for leaf extracts. The MIC for fresh stem bark water extract is 10^{-3} while that for fresh stem bark ethanol extract is 10^{-4}. The same results are interpreted in the graph below:

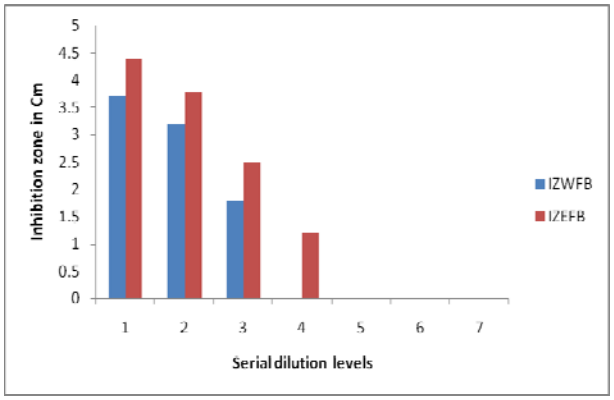

Fig 3: Antibacterial activity of fresh *Azadirachta indica* bark

IZWFB: Inhibition zone of water extract from fresh barks

IZEFB: Inhibition zone of ethanol extract from fresh barks

1, 2, 3, 4, 5, 6 and 7 represent 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} respectively.

Water and ethanol extracts from dry stem bark were also applied on *S. aureus*; the results are in the table below:

Table 8: Antibacterial activities of dry *Azadirachta indica* stem bark

Dilution concentration of bark extracts	Zones of inhibition (cm)	
	Water extract from dry stem bark	Ethanol extract from dry stem bark
10^{-1}	3.5	4.1
10^{-2}	3.1	3.6
10^{-3}	2	2.6
10^{-4}	1.2	1.4
10^{-5}	Growth	Growth
10^{-6}	Growth	Growth
10^{-7}	Growth	Growth

The inhibition zone is also varying in the same way as for other results; it decreases with the increase of the dilution factor. The MIC for both dry stem bark water extract and dry stem bark ethanol extract is 10^{-4}. These results are interpreted in the following graph:

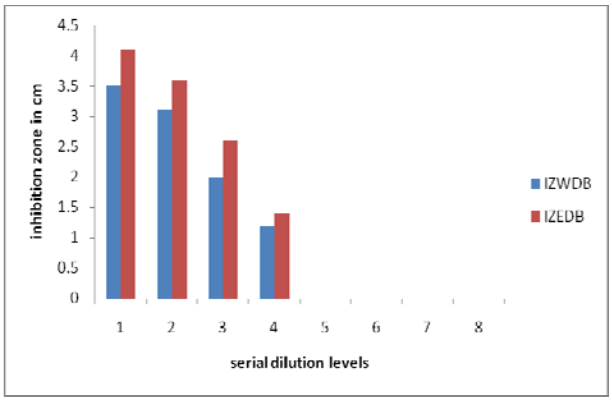

Fig 4: Antibacterial activities of dry Azadirachta indica stem bark

IZWDB: Inhibition zone of water extract from dry barks
IZEDB: Inhibition zone of ethanol extract from dry barks
1, 2, 3, 4, 5, 6 and 7 represent 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} respectively.
The tables below represent the SPSS products of bark extracts

Table 9: SPSS mean results of fresh bark extracts

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	IZWFB	1.700	7	1.8824	.7115
	IZEFB	1.243	7	1.6511	.6241

Table 10: SPSS mean results of dry bark extracts

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	IZWDB	1.671	7	1.7764	.6714
	IZEDB	1.400	7	1.5044	.5686

Table 11: Mean difference, t value and p value for fresh bark extracts

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZWFB - IZEFB	.4571	.4685	.1771	.0238	.8905	2.581	6	.042

Table 12: Mean difference, t value and p value for dry bark extracts

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZWDB - IZEDB	.2714	.2870	.1085	.0060	.5369	2.502	6	.046

The two means are significantly different because for both water and ethanol extracts for both fresh and dry barks, p value is less than the significance level. Their mean difference was calculated by taking ethanol extract mean minus water extract mean. Thus, the ethanol bark extracts were more effective compared to aqueous bark extracts in both dry and fresh barks.

H0: There is no significant difference between the two means
H1: There is a significant difference between the two means
For this case, the p values are less than 5%, therefore H1 is accepted because there is a significant difference between the two means.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZEFB - IZEDB	.0286	.1704	.0644	-.1291	.1862	.444	6	.673

3.3 Comparison between dry and fresh leaf ethanol extracts

The antibacterial effect of Neem on *S.aureus*, by comparing the fresh and dry leaves both extracted with ethanol their mean difference was calculated and is equal to 0.28 as shown in the table below:

Table 13: Mean difference, t value and p value of ethanol extracts for both dry and fresh leaves

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZEFL – IZEDL	.28000	.25060	.09472	.04823	.51177	2.956	6	.025

As the p value is less than the significance level (5 %), the two mean are different and the fresh *Azadirachta indica* leaves showed greater effect compared to the dry ones.H1 is accepted and H0 is rejected.

3.4 Comparison between dry and fresh bark ethanol extracts

The SPPS table below represents the effect of fresh and dry neem bark through p value by considering ethanol extracts:

Table 14: Mean difference, t value and p value for fresh and dry ethanol extracts

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZEFB - IZEDB	.0286	.1704	.0644	-.1291	.1862	.444	6	.673

Differently to fresh and dry leaves results, fresh and dry bark means are the same due to the fact that p value calculated is greater than 5 %; this means that between dry and fresh barks there is no significant difference in terms of effectiveness against *S. aureus.* H1 is rejected and H0 is accepted.

3.5 Comparison between fresh leaves and fresh barks

As we compared neem effect based on the solvents used, the effect of neem parts used was also studied in terms of their effectiveness on *S. aureus.*

The antimicrobial activity of leaf and bark fresh extracts of neem, was determined by considering p value calculated in the table below:

Table 15. Mean difference, t value and p value for fresh bark and fresh leaf ethanol extracts.

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZEFB - IZEFL	.2286	.8845	.3343	-.5895	1.0466	.684	6	.520

Due to the fact that the p value calculated is greater than the significance level (5%), ethanol fresh bark and ethanol fresh leaves effect on *S.aureus* showed no significant difference. H1 is rejected and H0 is accepted.

3.6 Comparison between ethanol extracts from dry leaves and dry barks

Ethanol extracts from dry leaves and dry barks were also compared in order to see which one is more effective according to the calculated p value.

Table 16: Comparison between leaves and barks dry extracts (ethanol extracts)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	IZEDB - IZEDL	.48000	.84782	.32045	-.30410	1.26410	1.498	6	.185

For this case, there was no much difference between the mean for dry leaves and dry barks which gave rise to a p value greater than the significance level (p value= 0.185>0.05). This means that there is no significant difference in their effectiveness on *S.aureus*. H1 is rejected and H0 is accepted.

3.7 Antibacterial activity of *Azadirachta indica* materials (leaves and stem bark) on *E.coli*

As shown in the tables above *Azadirachta indica* (neem) extracts has an antibacterial effect on S.*aureus*, but it was an exception when applied on E.*coli* these extracts showed no effects because no clear zone were formed on both aqueous and ethanol extracts from both fresh and dry neem materials, this strain had resistance that is going to be discussed later.

4. Discussion

Azadirachta indica extracts used in this study had shown an antibacterial effect as presented in different tables and graphs used in results interpretation, but the effect was seen on *Staphylococcus aureus* only, while the *E.coli* was not affected by any of the used extracts. This might be due to the fact that *E.coli* can alter their genetic makeup with astonishing rapidity. In general, gram negative bacteria show resistance to antibiotics because of their cell wall. Resistant bacteria change their cell walls slightly, so the antibiotics cannot attach or they produce enzymes to disable the antibiotics, so the *E.coli* might have done the same and consequently, *Azadirachta indica* (neem) extracts did not show any effect on it. Our results are different to those obtained during a study carried out by Gajendrasinh *et al.,* (2012) whereby *E.coli* was the most susceptible bacterium to aqueous and ethanol extracts of *Azadirachta indica* [9].

Ethanol extract from both dry and fresh bark extracts were more effective against *S.aureus* compared to water extracts. These results are the same as the ones obtained in study carried out by Gajendrasinh *et al.* (2012) [9]. In all cases, ethanol extracts were the best in terms of effectiveness against *S.aureus.* According to the report done by Kirtikar and Basu (1975), [10] whose results are similar to the ones we obtained, they said that the active ingredients are slightly soluble in water and freely soluble in organic solvents such as alcohols, these ingredients include: azardirachtin, 1-maliantriol, salannin, nimbin, nimbdin and others. Our results also confirm the information reported by Ibekwe *et al.* (2001) [11] whereby ethanol extracts were more effective than water extracts.

When comparing between dry and fresh leaf extracts, we considered ethanol extracts because ethanol was found to be more effective than water. Extract from fresh leaves were more effective than that from dry leaves. This inefficiency of dry leaves might be due to the fact that they were exposed to high temperature during the drying period thus there might be loss of some important components which could help in *S. aureus* growth inhibition according to other studies, this may be caused by the slow response of *S. aureus* to extract of dry neem leaf compared to the fresh leaf. this slower response is due to the slower diffusion of active ingredients into the solution from the dry neem leaves relative to the fresh leaves [12]. This was not the case for barks because for both dry and fresh bark extract the effect was the same as shown by p value calculated which was greater than the significance level this implies that there is no difference between their means.

For comparing the effectiveness of barks to that of leaves, ethanol extracts from fresh barks were compared to ethanol extracts from fresh leaves; and there was no difference because the p value was greater than the significance level, this was also the case for ethanol extracts from dry barks when compared to ethanol extracts from dry leaves. This different from the study done by Mamman *et al.* (2001), [13] whereby the stem-bark extracts were the stem bark was found to be more effective than the leaf extract in terms of antibacterial activity.

For all extracts used during this study, the bactericidal activity increased with the increase of the extract concentration, this

means that the inhibition zone was higher on plates that contain extract with low dilution factor, this is also observed in the report done by Esimone *et al*. (1998), [14] which says that extract of plants inhibit the growth of various microorganisms at different concentrations. This is similar to the results recorded in this study where the increase in the concentration of extracts corresponded to the increase of diameter of inhibition zones.

In this study the negative results of antibacterial activity of plants did not mean absence of bioactive constituents nor that is the plant inactive. Active compound(s) may be present in insufficient quantities in the extracts to show activity with the concentration employed [15]. Neem extracts with low concentrations didn't show effect on *S.aureus* but by increasing the concentration of extract, effect was obtained as the dilution level decreases.

5. Conclusion

This research was carried out in order to determine the effect of *Azadirachta indica* crude extracts on the two bacteria strains (S.*aureus* and *E.coli*), as it was shown by the results, Neem effect on these bacteria was determined; *E.coli* showed resistance on all extracts used. Ethanol extracts were more efficient in all cases whether for dry and fresh neem barks and leaves. Fresh leaves are greater in their effectiveness compared to dry leaves. Bark results did not reveal the difference in their effect on *S.aureus.* This information provided by this study on antibacterial effect of *Azadirachta indica,* will make it easier for dosage determination and chemotherapeutic index of the extract if they were to be processed into drugs. Lastly, based on the information got from this study the antibacterial effect of *Azadirachta indica* (Neem) can change depending on Neem parts used, the solvent used, even the state of the material used whether it is dry or fresh, moreover the extract concentration matters a lot because each extract has its minimum inhibition concentration (MIC) which is the highest dilution of a plant extract that still retain an inhibitory effect against the growth of a microorganism.

Though this tree was found to be useful in medicine, it is uncommon and it is found in few areas in Rwanda, thus it will be better if Rwandans get to know it for them to use it as they use other traditional herbs that we are familiar with.

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