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In-vitro Evaluation of Antibacterial Activity of *Azadirachta indica* and *Cinnamomum zeylanicum* Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Antibiotics have saved countless lives and continue to be a mainstay of therapy for bacterial infections. However, disease-causing microbes that have become resistant to antibiotic therapy are increasing problem in animals and public health. Plant extracts are of novel attention as antimicrobial agents in medicine, *Azadirachta indica and Cinnamomum zeylanicum* are famous herbs with a number of ethno-medicinal purposes. An experimental study was conducted from November, 2017 to March, 2018 with the objective of investigating in-vitro antibacterial activity of *Azadirachta indica and Cinnamomum zeylanicum* against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*. The leaves of *A. indica* and bark of *C. zeylanicum* were extracted using two different extractors, 70% ethanol and 70% methanol, antibacterial activity of these plants were performed at different concentrations (100, 150 and 200mg/ml) by Kirby-Bauer disc diffusion method. The mean diameters of zones of inhibition were calculated and analyzed using analysis of variance (ANOVA). Differences between means were also evaluated at $p<0.05$. Overall, the results of this study showed that the active compounds of *A. indica* (against *S. aureus* only) and *C. zeylanicum* extracts have an anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Except for *A. indica,* that showed no activity against *E. coli*. The highest antibacterial inhibition against *Staphylococcus aureus was* (16 ± 0.76mm) and (13.86 ± 1.29mm) revealed by ethanol crude extract of *A. indica and C. zeylanicum* at 200mg/ml concentration respectively while least inhibition zone (9.08 ± 0.8mm) was obtained from methanol crude extract of *C. zeylanicum* at 100mg/ml concentration against *E. coli*. *Azadirachta indica* had no effect for *E. coli* in both extractors**.** Hence, further study is suggested on identification of active compound to analyze the active ingredients responsible for this effect at an in-vitro and in-vivo levels and toxicity test to assure safety and effectiveness.

Key words: *Azadirachta indica* · Antibacterial activity · *Cinnamomum zeylanicum* · *Escherichia coli* · *Staphylococcus aureus*

INTRODUCTION

Plants have not only nutritional value but also, in the eyes of the local people, they have medicinal and ritual or magical values [1]. The term medicinal plant includes useful plants for primary health care and as an injury and plants used traditionally for foods and drinks which are believed that they are good for health; the medicinal plants include foods, drinks, herbs and spices [2]. These medicinal plants are considered as a rich source of ingredients which can be used in drug development and synthesis [3].

Traditional medicinal plants have important contributions in the health care system of local communities as the main source of medicine for the majority of the rural population. These medical systems are heavily dependent on various plant species and plant based products. Since time immemorial, plants have been crucial sources of both preventive and curative traditional medicine preparations for human beings and livestock [4].

The various literature available show the significant role of medicinal plant in primary health care delivery in Ethiopia where 70% of the human and 90% of livestock population depend on traditional medicine similar to many

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developing countries particularly that of Sub-Saharan African countries. The traditional health care is culturally deep rooted with oral and written pharmacopoeias. Ethiopian plants have shown very effective medicinal value for some ailments of human and domestic animals thus medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs throughout the country [2].

In Ethiopia, ethno veterinary surveys conducted in different parts of the country show the use of different medicinal plants for treatment of various infectious diseases of humans and livestock by traditional healers [5-9]. A number of medicinal plants with significant antimicrobial activity have also been reported by Giday and Ameni [5].

Infectious diseases are the principal cause of death worldwide. Antibiotic resistance has become a global concern [10].The clinical efficacies of many existing antibiotics are being threatened by the emergence of multidrug-resistant pathogens [11]. More over the new generation antibiotics are less available and are expensive for resource poor communities [12]. Hence, the rapid development of multi-drug resistant strains of bacteria increased the occurrence of bacterial infections that cannot be treated with conventional antimicrobial agents [13].

Diseases need be prevented and controlled in order to attain a sustainable and viable production of livestock. Diarrhea is the most common pathology in young calves and *Escherichia coli* represents one of its main etiological agents [14, 15]. Mastitis, caused by the Staphylococcus SPP (Mainly *Staphylococcus aureus*) is the most important disorder in cows and leads to reduced milk production and increased production costs [16, 17]. These bacteria have revealed multi-resistance to antimicrobials in different continents and present a public health risk [15, 18 & 19].

Plants have been a source of herbal remedies throughout the history of mankind. Various medicinal plants have been used for years in daily life to treat diseases all over the world [20]. Plant-based antimicrobials represent a vast unused source of medicines and are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [21]. Herbs are also invaluable source of modern drugs. More than 30% of modern drugs are derived from plants [22]. Because of the above-mentioned reasons; lots of efforts have been made to discover new antimicrobial agents from various sources such as plants, micro-organisms and animals [23].

The medicinal plant used in this study was leaves of *Azadirachta Indica* (Neem is a common name in English and locally called nim or limo) [24] the botanic family of *Meliaceae* [25]. The *Azadirachta indica* is a very useful traditional medicinal plant in the African sub-continent and every part of the tree has been used as traditional medicine for household remedy against various human and animal illnesses [26, 27]. Most of the parts of the plant contain compounds with proven antiseptic, antiviral, antipyretic; anti-inflammatory, antiulcer insecticidal and antifungal properties. Moreover; it possesses antibacterial activity [28]. And also in Ethiopia, *A. indica* (Limo) is used as an insecticide or insect repellant [24]. Plant parts like root, bark, seed and leaves have been an important source of medicine since thousands of years. In recent studies a predominant interest has been observed in evaluating different plant extracts for their antimicrobial properties against bacteria [29].

The second medicinal plant used in present study was bark of *Cinnamomum zeylanicum* (Kerefa is the common name in Amharic and cinnamon in English). It is a spice obtained from the inner bark of trees the genus *Cinnamomum* that is used in both sweet and savory foods. Cinnamon bark is one of the most popular spices in use in every home. It has a delicate fragrance and a warm agreeable taste. It is extensively used as an apices or condiments in the form of small pieces or powder. The cinnamon bark smells like cinnamon and tastes like camphor, which it yields on distillation. Cinnamon leaves, when bruised, smell spicy and have a hot taste; the berry tastes a little like juniper and when ripe, bruised and boiled it gives off an oily matter which cools and solidifies as “cinnamon suet”. Cinnamon is used in traditional medicine, fights tooth decay, soothes upset stomach and Clears up urinary-tract infections, diabetes, etc. [30].

Regardless of this, the medicinal plants, *Azadirachta indica* and C*innamomum zeylanicum* having antibacterial activities, in Ethiopia there are a limited studies or publications about the antimicrobial actions of those plants (Are you sure?? sure). And also due to excessive consumption of synthetic drugs, drug resistance rate of pathogenic bacteria is increasing and the need to find new compounds is necessary.

Therefore, the main objective the study was,

- To evaluate antibacterial activity of *Azadirachta indica* and *Cinnamomum zeylanicum* against *Staphylococcus aureus* and *Escherichia coli.*

MATERIALS AND METHODS

Study Area and Study Period: The present study was conducted in Mekelle town, Tigray region, northern Ethiopia, from November, 2017 to March, 2018.Geographically, it is located at 39° 29`E and 13° 30` N at an altitude of 2000 m. a. s. l. The climate of the study area conforms to that of Ethiopian Highlands. The mean annual rainfall is 619mm, which is bimodal with short rainy seasons occurring from March to May and from mid- July and August. The annual minimum and maximum temperature is 11.8°C and 29.9°C, respectively [31]. Evaluation of the antimicrobial activities of the plant extracts was carried out in pharmacology and microbiology laboratory at college of veterinary medicine, Mekelle University, Ethiopia.

Study Design: An in-vitro experimental study design was employed to evaluate the antibacterial activity of two medicinal plants, *A. indica* and *C. zeylanicum*. The two plants were classified into two treatment groups; each of the group was subjected to 70% ethanol and 70% methanol for extraction of its active ingredients then diluted to produce 100,150 and 200 mg/ml concentrations. Then, each plant with their concentrations was tested against Gram positive *S. aureus* and Gram negative *E. coli.* Moreover, as control group, Sulfamethoxazole trimethoprim (25µg/disc) and Dimethyl sulfoxide (DMSO) were used as positive and negative control respectively (Fig. 1).

Preparation of Plant Extracts: The method involved in extraction of these medicinal plants was maceration. In which it is extraction technique involved in the separation of medicinally active portions of plant part as described by Ncube *et al*. [32] by using a solvent (In this study 70 % ethanol and methanol).

Collection of Plant Materials: Plant parts used in study were collected from different areas of Mekelle; leaves of *Azadirachta indica* were collected from around the college of veterinary medicine and bark of *Cinnamomum zeylanicum* was purchased from Mekelle market.

Size Reduction: Leaves of *Azadirachta indica* were washed thoroughly in running tap water and left to dry at room temperature for 2 days. Air dried leaves of *Azadirachta indica* and properly washed and dried barks of cinnamon were grounded into powder.

Soaking: Powdered plant materials were measured by sensitive balance. 250 grams of the powder of *A. indica* and *C. zeylanicum* were mixed separately with 1,000 ml ethanol and 1,000 ml methanol in 4 separate conical flasks. The flasks containing extracts were shaken by orbital shaker for 10 minutes 4hr interval for one day and placed at room temperature for 3 days. The procedure was repeated two times to obtain more crude extracts.

Filtration and Concentrations Making: After three days of the first and two days second maceration the supernatant containing extracts of *A. indica* and *C. zeylanicum* were then filtered by double layer cheesecloth and sieved into a beaker. The content in the beaker was again filtered through Whatman filter paper to remove all debris.

The filtrates were transferred into pre-weighed clean and dry beakers and were placed on water bath at 42°C to obtain ethanol and methanol free extract residue of *A. indica* and *C. zeylanicum*. Lastly, the extracts were placed

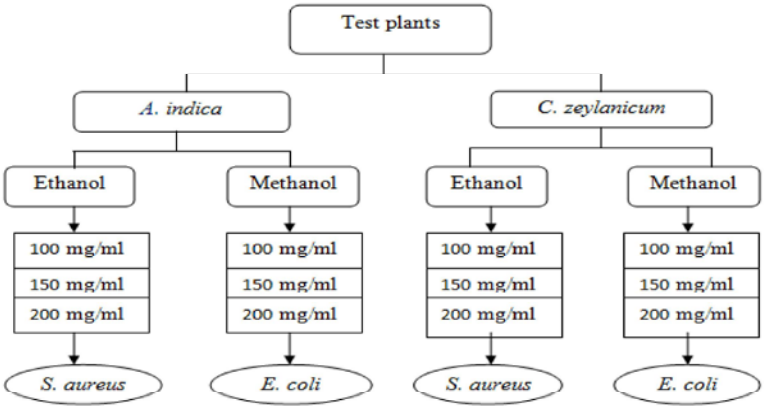

Fig. 1: Diagrammatic Representation of the Experimental Design

in a hot oven to remove the rest solvent and extracts were dried. Stock solution of 100mg/ml, 150mg/ml and 200mg/ml were prepared in Dimethyl sulfoxide (DMSO) vortexed well, labeled and the extracts were kept in tightly stoppered bottle stored at 4°C in the refrigerator until used for the anti-microbial testing.

Test Organisms Used: Microbial strains were obtained from the molecular laboratory in the college of veterinary medicine, Mekelle University, Ethiopia. Two bacterial strains were tested against the above-mentioned plant extracts in which one was gram positive *Staphylococcus aureus* and the other was gram negative namely, *Escherichia coli*.

Determination of Antibacterial Activity of the Plant Extract by Disc Diffusion Method: Disc diffusion method described by Kirby Bauer *et al.* [33] was used to assay antibacterial activity of the two plant extract against two bacterial strains.

Preparation of Test Bacterial Suspensions: From a pure bacterial culture, four to five colonies of *S. aureus* and *E.coli* were taken with a sterilized wire loop separately. It was added to 5 ml of 0.85% saline in the test tube and more colonies were added until it achieves the turbidity of 0.5 McFarland standards (prepared by adding 0.5 ml of 1.75% (w/v) barium chloride BaCl2 to 99.5 ml of 1% (v/v) sulfuric acid (NH_2SO_4) [34]. The turbidity of the test bacterial suspension was compared with 0.5 McFarland (Vigorously shaken before use) against a white background with contrasting black line under adequate light. Increased turbidity was reduced by adding sterile saline solution. It is thought that Standardized inoculums have a concentration of 1-2 × 10^8 (cfu) colony forming unit [35].

Medium Preparation: 3.8g of Muller Hinton Agar was added to 100 ml distilled water and autoclaved at 121° C for 15 minutes at 15 lbs. and poured in sterile plates up to a uniform thickness of approximately 4mm and the agar was allowed to solidify and used.

Inoculation of Plates: Sterilized cotton swab was dipped into the standardized bacterial suspension of *S. aureus* and *E. coli* in separate tests tubes. Excess inoculums were removed by lightly pressing the swab against the tube wall at a level above that of the liquid. It was inoculated on the Mueller Hinton agar by streaking with the swab containing the inoculums. The Plate was rotated by 60° and the rubbing procedure was repeated three times; to ensure an even distribution of the inoculums. It was allowed for 5 minutes to dry the surface of the medium; to remove excess moisture [35].

Paper Disc Preparation, Impregnation and Application on the Inoculated Plate: Discs that are used to impregnate plant extract were prepared from Whatman No.1 filter paper by punching into 6mm disc form using paper puncher and were sterilized. Each of the sterile paper discs (Those without antibiotics) were incorporated and soaked individually with 20µl of stock solutions (100mg/ml, 150mg/ml and 200mg/ml) of both extracts using micropipette in separate plates and allowed to dry. Using sterile forceps; impregnated discs were placed on the surface of the inoculated plate. Immediately, it was pressed down gently with the forceps to ensure complete contact between the paper disc and the agar surface. The plates were allowed to dry in air for 5 minutes to remove excess moisture of the media [36].

Positive control discs with antibiotics (Sulfamethoxazole Trimethoprim) and negative control discs containing neat solvents (DMSO) were run parallel in the same plate. The plates were incubated in an inverted position at 37°C for 24hrs and the antibacterial activities of both plants with two extractors against two test bacteria were analyzed by measuring the diameter of the zones of inhibition in mm. Generally, 5 discs were placed on 100mm plate (3 extract concentration, one positive control and negative control).

Data Collection and Statistical Analysis: All the experimental results were performed three times and the inhibition zones were measured using digital caliper. The data was entered and managed in a Microsoft Excel spreadsheet and analyzed using (SPSS) Statistical Package for Social Sciences. The difference in risk factors such as bacterial strain, concentration of crude extract and types of extractor used with inhibition zone were analyzed by using analysis of variance (One way ANOVA) technique and value of $p<0.05$ was considered as significant. The results were expressed as mean ± Standard Deviation (SD) for two isolates of the bacterium.

RESULTS

Yield of Crude Extracts: In this study, two extractors (Ethanol and methanol) and two plants (*A. indica* and *C. zeylanicum*) were used. From the two extractors used maximum yield of crude extract of *A. indica* was obtained

Table 1: Percentage yield of crude extracts from test plants with different extractors

		Yields	
Test plant	Parts used	Ethanol	Methanol
Azadirachta indica	Leave	12%	16.4%
Cinnamomum zeylanicum	Bark	7.2%	8%

Table 2: Mean zones of inhibition of *A. indica* leave and *C. zeylanicum* bark with ethanol and methanol extract at different concentrations against *S. aureus* and *E. coli.*

Con. in Zone of inhibition in mm (Mean±SD)			Total		
Plant used Extractor (mg/ml)	S. aureus	*p*-value	*E. coli*	*p*-value	*P*-value
A. indica Methanol	100	10.66±1.45		0.00	0.00
	150	12±1.23	0.0096	0.00	0.00
	200	14.18±1.77		0.00	0.00
Ethanol	100	12.82±1.98		0.00	0.00
	150	14.44±1.05	0.01	0.00	0.00
	200	16±0.76		0.00	0.00
C. zeylanicum Methanol	100	9.61±1.21		9.08±0.8	0.44
	150	11±1.25	0.011	10±1.17	0.0030.23
	200	12.86±1.71		11.72±0.9	0.23
Ethanol	100	11.41±0.94		10.18±0.66	0.04
	150	12.05±1.05	0.009	11.16±1.33	0.0090.27
	200	13.86±1.29		13.37±1.82	0.62
S×T (+VE control)	25µg	19.8±2.44	0.00	24.94±1.8	0.00
DMSO (-VE control)	0.00		0.00		

S×T Sulfamethoxazole Trimethoprim
DMSO Dimethyl Sulfoxide
+VE Positive
-VE Negative
Con. Concentration

by using methanol extractor which was 16.4% followed by ethanol with the yield of 12%. In case of bark of *C. zeylanicum* methanol has yielded 8% while ethanol yielded 7.2% (Table: 1).

Antibacterial Screening: The tested plant extracts against *S. aureus* and *E. coli* showed significant antibacterial activity with different concentrations. Of the two crude extracts tested; ethanol crude extract of *A. indica* showed the maximum zones of inhibition followed by methanol for *S. aureus* and except for *E. coli* both ethanol and methanol extract of *A. indica* has no inhibition zone. *C. zeylanicum* had also antibacterial activity against both test organisms. Zones of inhibition in the entire instance increased with an increase in the concentration (Table 2).

The result indicated that both medicinal plants showed statistically significant action ($p<0.05$) against *S. aureus* at all the tested concentrations (100mg/ml, 150mg/ml and 200mg/ml). *C. zeylanicum* also showed statistical significance for both test bacteria (*S. aureus* and *E.coli*) at all concentrations. In this study, the highest zone of inhibition 16±0.76mm and 13.86±1.29mm were obtained for *A. indica and C. zeylanicum* ethanol and methanol extract at 200 mg/ml against *S. aureus* respectively. The least zone of inhibition was revealed by *C. zeylanicum* methanol extract, 9.08±0.8mm against *E. coli* at 100mg/ml. When compared between crude extract, leaves of *A. indica* have more zone of inhibition than *C. zeylanicum* bark for *S. aureus* in both extractors (Table 2).

DISCUSSION

Azadirachta indica leave and *C. zeylanicum* bark extracts used in this study had shown an antibacterial effect in both ethanol and methanol extractors as presented in table 2. But *A. indica* showed the effect on *Staphylococcus aureus* only, while the *E. coli* was not affected by this plant.

The present study correlates with the findings of Uwimbabazi *et al*. [37] who reported that bark and leaves of *A. indica* with aqueous and ethanol extract has antibacterial activity against *S. aureus* and found

ineffective against *E. coli.* They reasoned that it 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* extracts did not show any effect on it.

This study, also agrees with report of Maragathavalli *et al.* [38] where *E. coli* was not affected by methanol and ethanol extract of leaves of *Azadirachta indica* at varying concentration of each extract, 200mg/ml, 150 mg/ml, 100mg/ml, 50mg/ml, 25mg/ml prepared by using disc diffusion method. It is in line with this study in that leaves of *A. indica* ethanol and methanol extract showed antibacterial activity against *staphylococcus aureus.* This might be due to the phytoconstituents alkaloids, glycosides, flavonoids and saponins [39].

On the other hand in present study the results clearly revealed difference to those obtained during a study carried out by Gajendrasinh *et al.* [40] who reported that *E. coli* was the most susceptible bacterium to ethanol and aqueous extracts of *A. indica*. This might be due to the variation in the plant part used; where in the present study leaves of *A. indica* with methanol and ethanol extract were used; instead of fruit of the plant and aqueous extract unlike that of the previous study. And also the difference in antibacterial activity of this plant might be due to geographical difference on their distribution in which the plants were collected that varied the concentration of the active ingredients.

Report of Effiong *et al*. [41] also differs from present study, whereby ethanol stem bark extract of *A. indica* had activity against *E. coli* at concentration of 12.5 mg/ml and 6.25mg/ml. This might be again due part of the plant used that lead to have antibacterial activity. However, it is in line with this study where ethanol leave extract had no activity against *E. coli* at these concentrations.

Previous studies showed that different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent ethanol and methanol extracts. Both methanol and ethanol were proved to be good solvents in extracting inhibitory substances from medical plants. Those solvents have ability to extract secondary metabolites which have antimicrobial activity and capability to degrade inert part of the plant which is inactive [28].

According to the previous report ethanol and methanol might have higher solubility for more phytoconstituents, consequently the highest antibacterial activity and the two extracts of *A. indica* with (ethanol and methanol) were more active against the Gram positive bacterial strains (*Staphylococcus aureus*) than the Gram-negative bacterial strains (*E. coli*); which correlate with present study. The higher resistance of Gram-negative bacteria to plant extracts of *Azadirachta indica* might have an effective permeability barrier, comprised of thick murein layer in this outer membrane, which restricts the penetration of amphipathic compounds and multidrug resistance pumps that extrude toxins across the barrier. It is possible that the apparent ineffectiveness of plant antimicrobials is largely due to the permeability barrier [28].

Ethanol extract of leaves of *A. indica* was effective against *S. aureus* as compared to methanol extracts. This result is the same with study carried out by Venkata *et al*. [28] and Maria *et al*. [42] who reported that neem methanol extracts showed a decreased zone of inhibition when compared to the ethanol extracts in *Staphylococcus aureus* and *E. coli*. They reasoned that it might be due to the presence of different secondary biologically active phytochemicals were present in the ethanol extracts of *Azadirachta indica*; like alkaloids, flavanoids, resins, bitter, tannins, cardiac glycosides, reducing sugar and triterpenes, volatile oils, saponins and steroids in the ethanol extracts found by previous study. However, the present study is not in line with previous findings who reported *A. indica* had shown activity against *E. coli* in both extractors.

For the second plant earlier studies suggested that ethanol extract of *C. zeylanicum* was the most effective as an antibacterial agent against *S. aureus* and less effective against *E. coli.* Previous study correlates with present study but, with less zone of inhibition in both test organisms. The antibacterial activity of this plant has been attributed to the presence of some active constituents in the extract. The previous study revealed that cinnamaldehyde to be the major constituent of cinnamon oil [43].

Similarly, Rana *et al.* [44] reported that gram +ve bacteria (*S. aureus*) were more prone to inhibition by *C. zeylanicum as* compare to gram –ve bacteria (*E. coli*) which associate with the present study. It might be due to volatile action of cinnamon oil and due to absence of lipopolysaccharide layer in gram positive bacteria that might function as an effective barrier against any incoming bio-molecule [45].

There might be another possibility that essential oils may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to death of bacterial cells after massive ion leakage [46]. The present study also correlates with the study of Shahbaa [47] who reported that the extracts of the cinnamon inhibited the gram-positive bacteria better than the gram negative. Generally, plant extracts are usually more active against gram positive bacteria than gram-negative bacteria.

Furthermore, Sana and Ifra [48] reported that the ethanol extract of cinnamon has shown antibacterial activity against *E. coli* at all concentrations (10, 20, 60 and 80 mg/ml). This finding agrees with the present study but, higher concentrations were used (100, 150 and 200mg/ml) in this study. The previous study against *E. coli* with cinnamon showed highest inhibition zone with low concentrations than the present study. This might be due to geographical difference on their distribution in which the plants were collected that varied the concentration of the active ingredients.

CONCLUSION AND RECOMMENDATIONS

The experimental evidence of the present investigation showed that both *A. indica* leave and *C. zeylanicum* bark extracts produced marked growth inhibitory effect on *S. aureus* using ethanol and methanol extractors. Besides, *C. zeylanicum* had inhibitory effect on *E. coli* but, *Azadirachta indica* did not show antibacterial effect on *E. coli* in both extractors. However, the pattern of inhibition varied with the bacterial strain, concentration, plant extract and solvent used. *A. indica* revealed the most potential antibacterial activity followed by *C. zeylanicum for S. aureus*. Therefore, from this finding the following recommendations' are forwarded:

- The phytochemical components of *indica* leave and *C. zeylanicum* bark should be studied.
- Further study *on the antimicrobial activities of Azadirachta indica* should be studied against *E. coli*.

List of Abbreviations:

- *A. indica*
  Azadirachta indica
- *C. zeylanicum*
  Cinnamomum zeylanicum
- DMSO
  Dimethyl sulfoxide
- SD
  Standard Deviation

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REFERENCES

1. Abbink, J., 1995. Medicinal and ritual plants of the Ethiopian southwest: an account of recent research. Indigenous Knowledge and Development Monitor, 3: 6-8.
2. Endashaw, B., 2007. Study on Actual Situation of Medicinal Plants in Ethiopia Prepared for Japan Association for International Collaboration of Agriculture and Forestry: 1-73 available at:https://www.scribd.com/doc/83507057/Actual-Situation-of-Medicinal-Plants-in-Ethiopia (Accessed on May 2018).
3. Rasool, H., 2012. Medicinal Plants (importance and Uses). Pharmaceutics Anal Acta 3: e139. doi:10.4172/2153-2435.1000e139
4. Dery, B., R. Otsyina and C. Ngatigwa, 1999. Indigenous knowledge of medicinal trees and setting priorities for their domestication in shinyanga rejoin, Tanzania. International center for research in agroforestry, nairobi. Cambridge university press, Britain.
5. Giday, M. and G. Ameni, 2003. An ethinobotanical survey on plants of veterinary importance in two woredas of southern Tigray, northern Ethiopia. SINET Ethiopian Journal of Science, 26: 123-136.

6. Sori, T., M. Bekana, G. Adunga and E. Kelbesa, 2004. Medicinal plants in Ethno veterinary practices of Borana pastoralists, Southern Ethiopia International Journal of Applied and Research of Veterinary Medicine, 2: 220-225.
7. Yineger, H., E. Kelbessa, T. Bekele and E. Lulekal, 2007. Ethno veterinary medicinal plants at Bale Mountains National Park. Ethiopian Journal of Ethno pharmacology,112: 55-70.
8. Lulekal, E., E. Kelbessa, T. Bekele and H. Yineger, 2008. An ethno botanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia. Journal of Ethno biology and Ethno medicine, 4: 1-10.
9. Wondimu, T., Z. Asfaw and E. Kelbessa, 2007. Ethno botanical study of medicinal plants around 'Dheeraa' town, Arsi Zone, Ethiopian Journal of Ethno pharmacology, 112: 152-161.
10. Westh, H., C. Zinn and V. Rosdadahl, 2004. An International multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. Microbial Drug Resistance, 10: 169-176.
11. Bandow, J., H. Brotz and L. Leichert, 2003. Proteomic approach to understanding antibiotic action. Antimicrobial chemotherapy, 47: 948-955.
12. Jones, F., 1996. Herbs useful plants: Their role in history and today. Eur. Journal of Gastonterol. Hepatol., 8: 1227-1231.
13. Sieradski, K., R. Roberts, S. Haber and A. Tomasz, 1999. The development of vancomycin resistance in a patient with methicillin resistant staphylococcus aureus infection. N. Engl. Journal of Medicine, 340: 517-523.
14. Salvadori, M., G. Valadares, D. Leite, J. Blanco and T. Yano, 2003. Virulence factors of Escherichia coli isolated from calves with diarrhea in Brazil. Brazil Journal of Microbiology, 34: 230-5.
15. Duse, A., K. Waller, U. Emanuelson, H. Unnerstad, Y. Persson and B. Bengtsson, 2015. Risk factors for antimicrobial resistance in fecal Escherichia coli from pre weaned dairy calves. Journal of Dairy Science, 98: 500-16.
16. Su, X., A. Howell and D. Souza, 2012. Antibacterial effects of plant-derived extracts against methicillin-resistant Staphylococcus aureus. Foodborne Pathogenic Disease, 9: 573-8.
17. Saidi, R., D. Khelef and R. Kaidi, 2013. Bovine mastitis: prevalence of bacterial pathogens and evaluation of early screening test. African Journal of Microbiology Research, 7: 777-782.
18. Toyang, N., J. Wanyama, M. Nuwanyakpa and S. Django, 2007. Ethno veterinary medicine: a practical approach to the treatment of cattle diseases in sub-Saharan. 2nd ed. Africa Roosendaal. Netherlands: Agromisa Foundation and CTA. pp: 3-88. Samoilova, Z., G. Smirnova, N. Muzyka and O. Oktyabrskya, 2014. Medicinal plant extracts variously modulate susceptibility of Escherichia coli to different antibiotics. Microbiology Research, 169: 307-13.
19. Nisri, L., M. Meqdam and A. Alkofahi, 1999. Antibacterial activity of Jordanian medicinal plants. Pharmacological Biology, 37(3): 196-201.
20. Parekh, J., D. Jadeja and S. Chanda, 2005. Efficacy of aqueous and methanol extracts of medicinal plants for potential antibacterial activity, Turk. Journal of Biology, 29: 203-210.
21. McCorkle, C., 1995. Back to the future: Lessons from ethno veterinary research, development and extension for studying and applying local knowledge on Agricultural Food. Human Values Soc., 22: 52-80.
22 Tomoko, N., A. Takashi, T. Hiromu, I. Yuka, M. Hiroko, I. Munekazu, T. Totshiyuki, I. Tetsuro, A. Fugio, I. Iriya, N. Tsutomu and W. Kazuttito, 2002. Antibacterial activity of extracts prepared from tropical and sub-tropical plants on methicillin resistant Staphylococcus aureus. Journal of Health Science, 48: 273-289.
23. Mesfin, K., G. Tekle and T. Tesfay, 2013. Ethnobotanical Study of Traditional Medicinal Plants Usedby Indigenous People of Gemad District, Northern Ethiopia. Journal of Medicinal Plants Studies, 1(4): 32-37.
24. Mohammed, H., A. Fadhil, H. Tart and A. Omer, 2015. Antibacterial Activity of Azadirachta indica (Neem) Leaf Extract against Bacterial Pathogens in Sudan. American Journal of Research Community, 3(5): 246-251.
25. Abdullah, A., S. Shahed, A. Farzana, K. Sajal, C. Sreedam and C. Sitesh, 2011. Evaluation of brine shrimp lethality and antimicrobial activity of A. indica leaf extract on some drugs resistance bacteria in Bangladesh. Pharmacognosy Journal, 20(3): 66-71.
26. Mahapatra, S., M. Holmes, C. Young, J. Cheville and M. Kohli, 2011. Novel molecular targets of A. indica associated with inhibition of tumor growth in prostate cancer. American Association of Pharmaceutical Science Journal, 13(3): 365-377.

27. Venkata, N., E. Raju, G. Divakar, B. Hari, T. Sadan, R. Rudra, F. Maheshwara and C. Reddy, 2013. In-VitroAntibacterial Activity of Azadirachta Indica against Human Pathogens. International Research Journal of Pharmacology and Applied Science, 3(1): 81-84.
28. Prabhat, A., B. Navneet and A. Chauhan, 2010. Medicinal plants against dental pathogens. RepOpin, 2: 37-42.
29. Tomar, R. and V. Shrivastava, 2015. In-Vitro Efficacy Evaluation of Ethanolic Extract of Cinnamomum zeylanicum (Cinnamomum) as Potential Antimicrobial Agent against Clinical Isolates. International Journal of Pharmaceutical Science and Research, 6(5): 2201-04.
30. Bureau of Finance and Economic Development of Tigray Regional State, 2008. Mekelle Ethiopia. Bulletin, 1: 35-45.
31. Ncube, N., A. Afolayan and A. Okoh, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology, 7(12): 1797-1806.
32. Bauer, A., W. Kirby, J. Sherries and M. Turckp, 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical. Pathology, 45: 493-496.
33. Villanova, P., 2013. Clinical Laboratory Standards Institute (CLSI-formerly NCCLS), Performance Standards for Antimicrobial Disk Susceptibility Tests, 6th ed., M2-A11, 33(1).
34. Tendencia, E., 2004. Disk diffusion method. In Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquatic animals and environment Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center, pp: 13-29.
35. Jan Hudzicki, 2009. Kirby Bauer Disk Diffusion Susceptibility laboratory Test Protocol. American Society for Microbiology. pp: 1-23. Available at: http://www.asmscience.org/content/education/protocol/protocol.3189 (Accessed on May, 2018).
36. Uwimbabazi, F., J. Uwimana and J.P. Rutanga, 2015. Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli*, Journal of Medicinal Plants Study, 3(4): 85-91.
37. Maragathavalli, S., S. Brindha, N. Kaviyarasi, B. Annadurai and S. Gangwar, 2012. Antimicrobial Activity in Leaf Extract of Neem (Azadirachta Indica). International Journal of Science and Nature, 3(1): 110-113.
38. Hafiza, R., 2000. Peptides antibiotics, Lancet, 349: 418-422.
39. Gajendrasinh, P., M. Bhavika, S. Rohit, A. Hetal and P. Prajapati, 2012. In vitro Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (Azadirachta indica) and Tulsi (Ocimum sanctum L.). International Journal of Current Research Biology and Medicine, 3(3): 582-586.
40. Effiong, E., A. Gwana, M. Halima, S. Umeh, B. Bagudu and C. Okoli, 2016. Phytochemical Analysis and Antimicrobial Activity of Methanolic, Ethanolic and Acetonic Extracts of Stem Bark and Leaf of Neem Plant (Azadirachta indica). Journal of Diseases and Medicinal Plants, 2(3): Pp. 14-25.doi: 10.11648/j.jdmp.20160203.11
41. Maria, C., M. Romilly and Z. Mende, 2017. phytochemical screening and the antimicrobial activity of the leaves of Azadirachta indica, a. Juss. International Journal of Scientific Engineering and Research, 8(5): 2229-5518.
42. Seenivasan, P., J. Manickkam and I. Savarimuthu, 2006. In vitro antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine, 6: 39.
43. Rana, I., S. Aarti and G. Rashmi, 2011. International Journal of Pharmacology and Pharmaceutical Science, 3(4): 376-380.
44. Inouye, S., T. Takizawa and H. Yamaguchi, 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogen by gaseous contact. Journal of Antimicrobial Chemotherapy, 47(5): 565-573.
45. Walsh, S., J. Maillard, A. Russel, C. Catrenich, D. Charbonneau and R. Bartolo, 2003. Activity and mechanism of action of selected biocidal agents on Gram-positive and negative bacteria. Journal of Applied Microbiology, 94(2): 240-247.
46. Shahbaa, M., 2014. Preliminary screening of the antibacterial activity of Cinnamomum zeylanicum (Cinnamon) barks. Journal of Pharmacy and Biological Sciences, 9(5): 30-34.
47. Sana, M. and G. Ifra, 2012.Antibacterial Activity of Aqueous and Ethanolic Extracts of Garlic, Cinnamon and Turmeric against Escherichia Coli ATCC 25922 and Bacillus Subtilis Dsm 3256. International Journal of Applied Biology and Pharmaceutical Technology, 3(2):131-136

Note: currently we are using window 2007 and 2010. There is no widow 2003. It's known that, it will create spacing problem when opened in window 2003. Excuse for that.

Table below shows summary of the present findings. It is to clarify the above explanation.

	Antibacterial activity	
Ethanol and methanol plant extract	*S. aureus*	*E. coli*
A. indica	yes	no
C. zeylanicum	yes	yes