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DETERMINATION OF THE ANTIBACTERIAL ACTIVITY OF AZADIRACHTA INDICA (NEEM) ON ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

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ABSTRACT

Bacteria are small unicellular organisms, and they can be found abundant in the world. Antibiotics have been used to prevent bacterial infections since their discovery in 1928. Antibiotics resistance is caused when bacteria change the way they respond to the medicines. Due to drug resistance, novel antibiotics are used to treat bacteria resistance. The "Kohomba" or "Neem" leaves are considered antibacterial and fungicidal medicine that is widely used within Sri Lanka. Many parts of the Neem tree are used for various diseases, including the leaves, bark, fruit and flowers. Solvent extraction, Soxhlet extraction, Maceration, Supercritical fluid extraction and Microwave-assisted extraction are commonly used for Neem extraction. This study employed the solvent extraction method using three solvents: water, ethanol and hexane. The main objective of this study is to determine the antibacterial activity of Azadirachta indica (Neem), on Escherichia coli and Staphylococcus aureus strains. The disc diffusion method was done to the antibiotic susceptibility of the plant extract. All three Neem leaf samples were not susceptible to both of the bacteria as they showed no inhibition zones. This could be due to the extraction method or temperature, but the literature has shown strong evidence of antibacterial properties in Neem.

Keywords: Azadirachta indica (Neem), Escherichia coli, Solvent extraction, Staphylococcus aureus

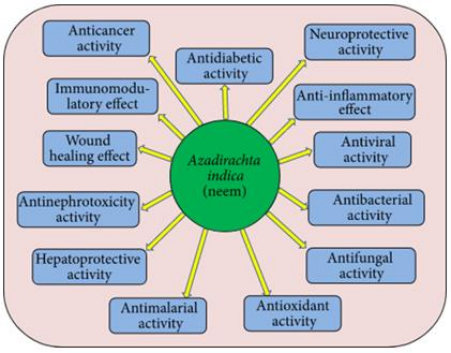
INTRODUCTION

Bacteria are small unicellular organisms, and they can be found everywhere in the world (Graham, 2019). The medicines that are used to prevent and treat bacterial infections are called Antibiotics. Antibiotics are resisted when bacteria change the way they respond to these medicines. The resistance to antibiotics is increasing dangerously at high levels in the world (World Health Organization, 2020). In general, antimicrobial drugs have a critical role in lowering the burden of infectious diseases worldwide.

However, the efficacy of the antibiotics declines when resistant organisms emerge and proliferate. When people discovered that standard antibiotics are being overprescribed and misused, which is leading to the development of germ resistance, the use of plant extracts for medical purposes has gained popularity (Sakha et al., 2018). Due to drug resistance, novel antibiotics are used to treat bacteria resistance. The novel drugs had shown active against gram-positive and gram-negative bacteria (Tacconelli et al., 2018). Due to their reduced adverse effects, natural products and their derivatives are becoming more and more popular in the treatment and prevention of diseases worldwide (Giri, Gangawane and Giri, 2019). In Sri Lanka, the "Kohomba" or Neem leaves are considered a fungicidal and antibacterial medicine that is widely used throughout the country for

various diseases. The scientific name of Neem is Azadirachta Indica and it belongs to the Meliaceae family. It has a role of health-promoting effect due to its rich source of antioxidants (Alzohairy, 2016). Many parts of the Neem tree such as the leaves, bark, fruit, flowers, oil and gum are used for various medical purposes (Islas et al., 2020). Antimicrobial compounds is present in medicinal plants. 140 bioactive compounds are in neem plant (Ali et al., 2021). Asia, Africa, America, and Australia are the places where the Neem plant grows. Neem leaves show antibacterial, antifungal, anti-inflammatory, immunomodulatory, antihyperglycemic, antiulcer, antifungal, antibacterial, antimutagenic, anticancer, antimalarial, antiviral, antioxidant (Seriana et al., 2019). The fuction of neem plant is shown in figure 1. Neem plant has the therapeutic role due to the rich of antioxidant and other active compounds like azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin (Pandey, A. and Pare, P., 2018).

Figure 1 – Function of the neem plant (Alzohairy, 2016)



In Ayurveda, homoeopathic, and Unani medicine Neem plant is widely used (Ghosh et al., 2016). For infectious, metabolic diseases and cancer diseases, the extract and phytochemicals are obtained from the Neem plant (Srivastava et al., 2020). According to the literature, there are several approaches to cure cancer, like activating cellular proliferation, apoptosis, tumor suppressor genes and several other molecular pathways to stop the growth of cancerous cells. According to a study, the active components of neem, flavonoids, are crucial in the prevention of cancer. Figure 2 shows the mechanism of cancer prevention by neem plant (Shareef, and Sohail Akhtar, 2018).

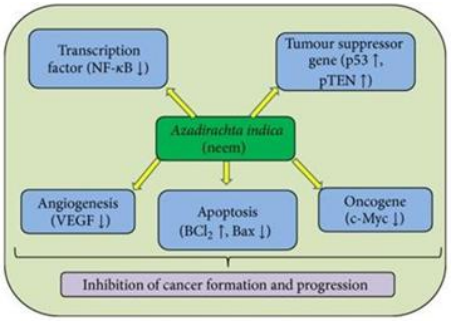


Figure 2 - Neem anticancer pathways (Shareef, and Sohail Akhtar, 2018)

For this experiment mainly two bacteria were selected, namely Escherichia coli (E. coli) and Staphylococcus aureus (S.aureus).

Escherichia coli is a gram-negative bacterium that belongs to the Enterobacteriaceae family. This harmlessly colonizes the human gut, causes intestinal and cause extraintestinal infections (Bonten et al., 2021). The most widely caused disease from E. coli is diarrhoea (Poirel et al., 2018). This bacterium was selected as it was inexpensive and can be easily found anywhere. Many researchers had used this bacterium for their research (Leon et al., 2018). Figure 3 shows E. coli bacteria.

Figure 3 – E. coli bacteria (Ross, 2019)

Staphylococcus aureus is gram-positive bacteria and is cocci-shaped (Taylor and Unakal, 2018). This bacterium caused nosocomial infections and systemic infections (Aggarwal et al., 2019) and it is considered one of the main bacteria that cause diseases in humans (Ansari et al., 2019). Figure 4 shows Staphylococcus aureus bacteria.

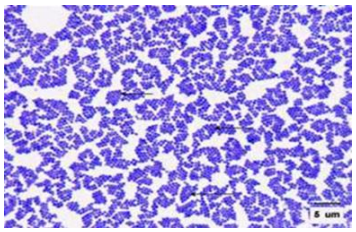


Figure 4 - Staphylococcus Aureus (Taylor and Unakal, 2018)

Antibiotic susceptibility testing (ABST) is used to help determine the best antibiotic to treat a bacterial infection. The disk diffusion method is used for confirming the susceptibility of bacteria. This method was introduced by Bauer and Kirby in 1956. The advantages of this method are simple and cost-effective but the disadvantages are insufficient data availability and poor performance on slow-growing bacteria (Khan, Siddiqui and Park, 2019).

The extraction is a process used to separate the desired natural products from the raw materials. They are many extractions processes that can be used to extract Neem which are solvent extraction, Soxhlet extraction, Maceration, Supercritical fluid extraction and Microwave-assisted extraction (Oshadie et al., 2017). Some of the extraction processes are shown in figure 5. The solvent extraction method was used as the extraction process in this study because it is inexpensive, can collect pure extract, can produce large extract and is easy to use with less effort. There is literature evidence of using other extraction methods however, it is difficult to find literature on the solvent extraction method. Therefore, the solvent extraction method was used in this research. The commonly used solvents are distilled water, ethanol, methanol, hexane, ether and acetone (Sudevan, Vijayarghavan and Arts, 2019). Selectivity, solubility, cost and safety were considered when selecting solvents (Zhang, Lin and Ye, 2018) and Water, ethanol and hexane were used as the solvents.

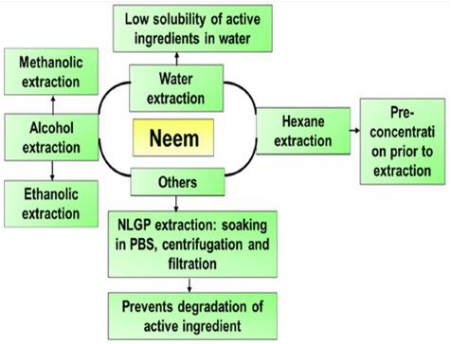


Figure 5 - Extraction methods (Chaudhary, 2017)

Objectives

The general objective of this study is to determine the antibacterial activity of Azadirachta indica (Neem) on Escherichia coli and Staphylococcus aureus.

Specific objectives are:

- Extraction from Neem leaves using water, ethanol and hexane extraction methods.
- Determine the antibacterial sensitivity tests on Escherichia coli and Staphylococcus aureus.
- Determine the antibacterial activity of the Neem leaves.

MATERIALS

Sample preparation

Table 1: *Materials used for sample preparation*

Sample	Scientific name
Neem plant leaves	*Azadirachta Indica*

Preparation of Mueller-Hinton agar and Nutrient broth

Table 2: *Materials used for Mueller-Hinton agar and Nutrient broth*

Reagents	Consumables	Equipment
Mueller Hinton Agar	Conical flasks	Analytical balance
Nutrient Broth	Measuring cylinder	Autoclave
Distilled Water	falcon tubes	
	Petri dishes	
	Spatula	
	Parafilm	
	Foil squares	

Extraction of samples

Table 3: *Materials used for Extraction of samples*

Reagents	Consumables	Equipment
Distilled water	Falcon tubes	Analytical balance
Hexane	Funnel	Refrigerator
100% Ethanol	Measuring cylinder	Mortar and pestle
	Beakers	
	Conical flask	
	Filter paper	

Antibacterial Susceptibility Test

Table 4 - *Materials used for the Antibacterial susceptibility test*

Reagents	Consumables	Equipment
Distilled water	Forceps	Micropipette
Conc. H2SO4	Test tubes	Autoclave
BaCl2	Micropipette tips	Incubator
Gentamicin antibiotic disks	Cuvette	Spectrophotometer
Chloramphenicol antibiotic disks	Measuring cylinder	Refrigerator
	Filter paper discs	
	Cotton swabs	
	Parafilm	
	Foil squares	
	Glass vials	

METHODOLOGY

Extraction of samples

Sample 1 – For Water Solvent: The fresh Neem plant leaves were taken and 10g was measured using an analytical balance. The chopping of Neem leaves was done using a motor and pestle. The chopped leaves were mixed with 10ml of distil water and it was stored in the Falcon tube for one day in the refrigerator. Then it was filtered using Whatman filter paper no 1 into a new falcon tube till further use.

Sample 2- For Ethanol Solvent: Fresh Neem leaves were taken and 10g was measured using an analytical balance. Then it was chopped using a motor and pestle and was mixed with 10ml of absolute ethanol. This sample was stored in the Falcon tube for one day in the refrigerator. Then it was filtered using

Whatman filter paper no 1 into a new falcon tube.

Sample 3 – For Hexane Solvent: Fresh Neem plant leaves were taken and 10g was measured using an analytical balance. Then it was chopped using a motor and pestle. It was mixed with 10ml of hexane and it was stored in the Falcon tube for one day in the refrigerator. Then it was filtered using Whatman filter paper no 1 into a new falcon tube till further use.

These procedures were repeated by changing the concentration of the distilled water, ethanol and hexane to 20ml and 30ml while keeping the Neem leaf mass at 10g.

Antibacterial Susceptibility test

Preparation of discs

For the Antibacterial sensitivity testing (ABST), the Mueller – Hinton agar was made by weighing 38g of Mueller-Hinton agar in an analytical balance and diluting it in 1000mL of distilled water. It was then poured into 500ml conical flasks and sealed with foil. It was autoclaved for 60 minutes at 121°C. After allowing the molten agar to cool slightly, it was poured into sterile Petri dishes set around an open Bunsen flame. It was allowed to cool and used to do the ABST test once set. The surfaces were sterilized and the plates were labelled accordingly with two bacteria. For the ABST test, the set plates were taken and then the two bacteria were added to the Petri plates separately. The Gentamicin antibiotic disks were added to the E. coli plates while the Chloramphenicol antibiotic disks were added to the S.aureus samples. A Petri dish was taken and the autoclaves discs were added. The samples were loaded using the micropipette. Then the Discs were placed and another Petri dish was taken. The discs were kept and the autoclaved water was loaded to the discs.

3.2.2. Preparation of Bacterial dilutions

Bacterial dilutions were required in comparison to the 0.5 MacFarland standard turbidity. To make the 0.5 MacFarland standard, 25 mL of each 1% BaCl2 (aq) and 1% H2SO4 (aq) were prepared with BaCl2(S) and Conc. H2SO4. Then, in a test tube, 0.05 mL of 1% BaCl2 (aq) and 9.95 mL of 1% H2SO4 (aq) were combined to make 10mL of MacFarland standard. The accuracy of the prepared standard was determined by measuring its absorbance at 625 nm with a spectrophotometer. To make the bacterial dilutions, the absorbance range for a MacFarland standard should be between 0.08 and 0.10. 5ml of distilled water was added to a labelled test tube to make the E Coli bacterial dilution. After sterilizing the inoculating loop with the Bunsen flame, loop-fulls of overnight incubated E Coli broth were added to the test tube until the turbidity matched that of the MacFarland standard. To prepare a bacterial dilution of S.aureus, the preceding procedure was repeated.

Disc diffusion method for freshly extracted samples

The surfaces were sterilized. The plates were labelled accordingly with two bacteria. For the ABST test the set plates were taken and then the two bacteria were added to the Petri plates separately. The Gentamicin antibiotic disks were added to the E. coli plates while the Chloramphenicol antibiotic disks were added to the S.aureus samples. A Petri dish was taken and the autoclaves discs were added. Then using the micropipette, the samples were loaded. Then the Discs were placed. Another Petri dish was taken the discs were kept and the autoclaved water was loaded to the discs. It was placed accordingly. It was parafilled and kept in the incubator for one day. Then the results were obtained. Each for E. coli and S.aureus 3 times was repeated. The same procedure was repeated with 10g of chopped Neem leaves and 20ml water,10g

of chopped Neem leaves with 20ml of absolute ethanol and 10g of chopped Neem leaves with 20ml of hexane. The ABST test was repeated.10g of chopped Neem and 30ml of water,10g of chopped Neem and 30ml of absolute ethanol, 10g of chopped Neem and 30ml of hexane was used and the same procedure was used. Finally, the ASBT test was done. In the ABST test, the positive control was the antibiotic, the Negative control was distilling water and the samples were used.

RESULTS

The results obtained from the ABST test are presented below.

Water

The extraction of the Neem was mixed with different ratios of distilled water.

1:1 ratio of the solvent and the sample

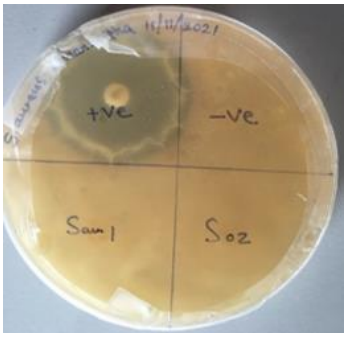
Where 20g of Neem sample and 20ml of distilling water was used. The obtained result for this test is shown in Table 5 - 6 and figure 6.

Table 5 – *Trials for E. coli bacteria in 1:1 ratio water extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Gentamicin	30mm	30mm	30mm
Sample 1 - Blend	0.00	0.00	0.00
Sample 2 – chopped	0.00	0.00	0.00

Table 6 – *Trials for S.aureus bacteria in 1:1 ratio water extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Chloramphenicol	32mm	32mm	32mm
Sample 1 - Blend	0.00	0.00	0.00
Sample 2 – chopped	0.00	0.00	0.00



(A) – S. aureus

(B) – E-Coli

Figure 6 - Results obtained for water 1:1 (S1 -Blend and S2 Chopped) ration blend and chopped samples for both bacteria

1:2 ratio of the solvent and the sample

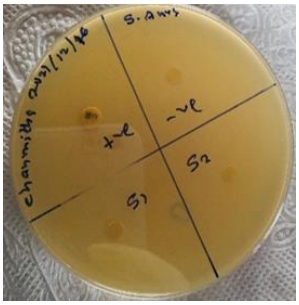
20g of Neem sample and 40ml of distilling water was used and the obtained results for this test are shown in Table 7 - 8 and figure 7.

Table 7 – Trials for E. coli bacteria in 1:2 ratio water extraction method

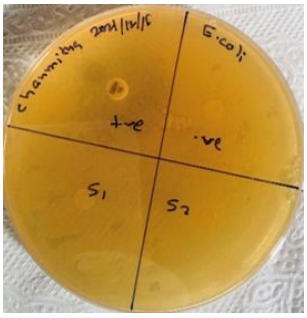
	Trial 1	Trial 2	Trial 3
Negative sample	0.00	0.00	0.00
Gentamicin	20mm	20mm	20mm
Sample	0.00	0.00	0.00

Table 8 – *Trials for S.aureus bacteria in 1:2 ratio water extraction method*

	Trial 1	Trial 2	Trial 3
Negative sample	0.00	0.00	0.00
Chloramphenicol	30mm	30mm	30mm
Sample	0.00	0.00	0.00



(A) – S. aureus



(C) – E-Coli

Figure 7 - Results obtained for water 1:2 (S1) and 1:3 (S2) ration of distilling water samples for both bacteria.

1:3 ratio of the solvent and the sample

20g of Neem sample and 60ml pf distil water were used and the obtained result for this test is shown in Table 9 - 10 and figure 7.

Table 9 – *Trials for E. coli bacteria in 1:3 ratio water extraction method*

	Trial 1	Trial 2	Trial 3
Negative sample	0.00	0.00	0.00
Gentamicin	20mm	20mm	20mm
Sample	0.00	0.00	0.00

Table 10– *Trials for S.aureus bacteria in 1:3 ratio water extraction method*

	Trial 1	Trial 2	Trial 3
Negative sample	0.00	0.00	0.00
Chloramphenicol	30mm	30mm	30mm
Sample	0.00	0.00	0.00

100% Absolute ethanol

The extraction of the Neem was mixed with different ratios of 100% Absolute ethanol.

1:1 ratio of the solvent and the sample

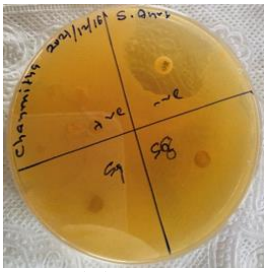
20g of Neem sample and 20ml of 100% absolute ethanol was used. The obtained result for this is shown in Table 11 -12 and figure 8.

Table 11 – *Trials for E. coli bacteria in 1:1 ratio ethanol extraction method*

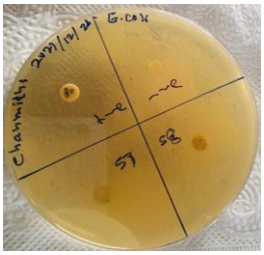
	Trial 1	Trial 2	Trial 3
Negative sample	0.00	0.00	0.00
Gentamicin	30mm	20mm	30mm
Sample 1	0.00	0.00	0.00

Table 12 – *Trials for S.aureus bacteria in 1:1 ratio ethanol extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Chloramphenicol	30mm	20mm	30mm
Sample 1	0.00	0.00	0.00



(A) – S. aureus



(B) – E-Coli

Figure 8 - Results obtained for 1:1(S7) and 1:2 (S8) ration of ethanol samples for both bacteria

1:2 ratio of the solvent and the sample

20g of Neem sample and 40ml of 100% Absolute ethanol was used. The obtained result for this is shown in Table 13 - 14 and figure 8.

Table 13 – *Trials for E. coli bacteria in 1:2 ratio ethanol extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Gentamicin	25mm	30mm	22mm
Sample	0.00	0.00	0.00

Table 14 – *Trials for S.aureus bacteria in 1:2 ratio ethanol extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Chloramphenicol	32mm	32mm	27mm
Sample	0.00	0.00	0.00

1:3 ratio of the solvent and the sample

20g of Neem sample and 60ml of 100% Absolute ethanol was used and the obtained result for this test is shown in Table 15 - 16 and figure 9.

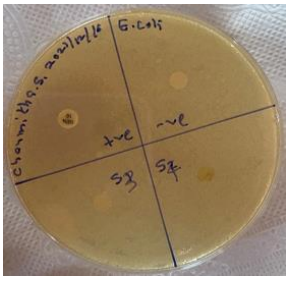
Table 15 – *Trials for E. coli bacteria in 1:3 ratio ethanol extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Gentamicin	30mm	30mm	30mm
Sample	0.00	0.00	0.00

Table 16 – *Trials for S.aureus bacteria in 1:3 ratio ethanol extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Chloramphenicol	30mm	30mm	30mm
Sample	0.00	0.00	0.00

(A) – S. aureus



(B) – E-Coli

Figure 9 - Results obtained for 1:3(S3) Ethanol and 1:1(S4) Hexane ration of both bacteria.

Hexane

The extraction of the Neem was mixed with different ratios of Hexane.

4.3.1. 1:1 ratio of the solvent and the sample

Where 20g of Neem sample and 20ml of Hexane was used. The obtained result for this is shown in Table 17 – 18 and figure 9.

Table 17 – *Trials for E. coli bacteria in 1:1 ratio hexane extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Gentamicin	25mm	26mm	28mm
Sample	0.00	0.00	0.00

Table 18 – *Trials for S.aureus bacteria in 1:1 ratio hexane extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Chloram phenicol	38mm	33mm	35mm
Sample 1	0.00	0.00	0.00

1:2 ratio of the solvent and the product

20g of Neem sample and 40ml of Hexane was used. The obtained result for this test is shown in Table 19 - 20 and figure 10.

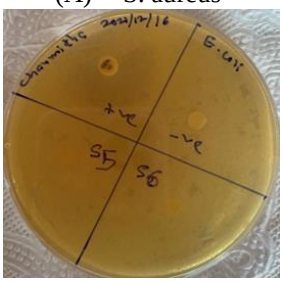
Table 19– *Trials for E. coli bacteria in 1:2 ratio hexane extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Gentamicin	25mm	30mm	22mm
Sample 1	0.00	0.00	0.00

Table 20 – *Trials for S.aureus bacteria in 1:2 ratio hexane extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Chloramphenicol	32mm	32mm	27mm
Sample	0.00	0.00	0.00

(A) – S. aureus



(B) – E-Coli

Figure 10 - Results obtained for 1:2(S5) Ethanol and 1:3(S6) Hexane ration of both bacteria.

1:3 ratio of the solvent and the product

20g of Neem sample and 60ml of Hexane was used. The obtained result for this test is shown in Table 21 - 22 and figure 10.

Table 21 – *Trials for E. coli bacteria in 1:3 ratio hexane extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Gentamicin	30mm	30mm	30mm
Sample	0.00	0.00	0.00

Table 22 – *Trials for S.aureus bacteria in 1:3 ratio hexane extraction method*

	Trial 1	**Trial 2**	**Trial 3**
Negative sample	0.00	0.00	0.00
Chloramphenicol	30mm	30mm	30mm
Sample	0.00	0.00	0.00

DISCUSSION

The antimicrobial activity of Neem plant parts is demonstrated by an inhibitory effect on microbial growth and cell wall breakdown. Active constituents play a role in disease cure by activating antioxidative enzymes, rupturing bacterial cell walls, and acting as a chemo preventive by regulating cellular pathways. Neem plants and their active compounds play a critical role in cancer prevention and progression. (Alzohairy, 2016).

Neem has potent antimicrobial properties. It is made up of 35 biologically active compounds. Neem extracts and their constituents play an important role in the inhibition of a variety of microbes, including viruses, fungi, and bacteria (Herrera-Calderon et al., 2019). Many studies have revealed that the Neem plant contains a diverse range of compounds, several of which have pharmacological potential. Triterpenes are the most commonly used therapeutic compounds among all of these compounds. These potential effects can be attributed to cellular and molecular mechanisms, which include free radical scavenging, detoxification, DNA repair, cell cycle alteration, programmed cell death mitigation and autophagy, immune surveillance, anti-inflammatory, antiangiogenic, and anti-metastatic activities, and the ability to modulate various signaling pathways (Islas et al., 2020).

In the first trial of this research for S. aureus, the antibiotic gentamicin didn't show any inhibition zone. Therefore, it was changed to chloramphenicol antibiotic where the positive control was shown in inhibition zones. None of the Neem plant samples showed any zones for the three solvents which were used. The same procedure was used for three different solvent extraction methods but showed negative results for the antibacterial activity of both bacteria. This finding confirmed the existing literature. Similar research done by Banna, Parveen and Jalaluddin Iqbal showed that the S.aureus bacteria had not shown any zones for various concentrations in the antibacterial susceptibility test. Further, it stated that S. aureus did not show any zone of inhibition even for the highest concentration of extract (Banna, Parveen and Jalaluddin Iqbal, 2014). In this research antibacterial property of Neem

was not confirmed due to the absence of inhibition zones. Previous literature suggests the strongly antibacterial property of Neem ((Mohammed and A. F. A. Omar, 2015), (Chibuzo, 2019) and (Parashar, Sutar and Sanap, 2018). The reason for not having results in this research could be due to the extraction process, not getting optimum plant extract or the temperature.

Water extraction

Water is the most common sustainable solvent, but its high polarity limits non-polar compound solubility. The hydrogen bonding structure of water, as well as related properties such as dielectric constant and solvation power, are altered when it is trapped in hydrophobic pores (Breynaert et al., 2020). The results of this research using the water extraction method were presented in Tables 5-10 and figures 6 and 7 which revealed that the antibiotic for both bacteria had worked in the research but there were no inhibitions zones for any Neem samples. However, when phytochemical screening of water extracts from Neem leaves had shown positive results (Saleh Al-Hashemi and Hossain, 2016). This research done by they have shown positive results for both of these bacteria. In this study they have used neem leaf extract where they have got moderate sensitivity as they have got 16mm and 15mm for E.coli and S.aureus respectively as the inhibion zones(Sadat et al., 2021).

Absolute ethanol extraction

Ethanol is a widely used organic chemical in industrial and consumer goods. The primary industrial applications of this aliphatic alcohol are as an intermediate in the production of other chemicals and as a solvent (Strohm, 2014). The results of this research using ethanol extraction are presented in Tables 11-16 and figure 8 – 9. It was found that the antibiotic for both bacteria had worked in the research but there were no inhibitions zones for any Neem samples. This finding is similar to the existing literature. Maleki et al (2017) had found that there was a resistance of Neem plants to E. coli and S. aureus for ethanol, methanol, and ethyl acetate. The highest activity was found in ethanol and ethyl acetate extracts, which could be attributed to the polarity of these solvents, which could extract more polar and general compounds from this plant, including antimicrobial agents (Maleki et al., 2017). Another research confirmed that the ethanol extract was more effective against E. coli and minimum effectiveness was shown against S. aureus (Sylvia et al., 2019). Because E. coli bacteria can change their genetic makeup so quickly, they can cause no inhibition zones during ethanol extraction on Neem as antibiotic resistance is common in gram-negative bacteria due to their cell wall. Resistant bacteria either modify their cell walls slightly so that antibiotics cannot attach to them, or they produce enzymes that render antibiotics ineffective (Francine, Jeannette and Jean Pierre, 2015).

Hexane extraction

In comparison to ethanol, a polar solvent, N-hexane can extract bioactive compounds as a non-polar solvent suitable for free fatty acid extraction. The extraction process using solvents gives many advantages like its less expensive, can get high yield and high purity (Liauw et al., 2008). The results of this research using hexane extraction are presented in Tables 17-22 and figures 9 – 10. It is found that the antibiotic for both bacteria had worked in the research but there were no inhibitions zones for any Neem samples. However, literature shows positive results for hexane when used in the Soxhlet apparatus and shows low antibacterial activity compared to methanol and chloroform (KOONA and BUDIDA, 2011). Another research

revealed that the zone of inhibition for Neem leaves for the chromatography techniques for hexane (Akpuaka et al., 2013).

CONCLUSION

In this study, Neem leaf extraction was done using the solvent extraction method and the three solvents used were water, absolute ethanol and hexane. The results showed that the antibiotic for both bacteria had worked in the research but there were no inhibitions zones for any Neem samples which are similar to existing literature. However, phytochemical screening of water extracts from Neem leaves had shown positive results. When the Soxhlet apparatus and chromatography technique are used for hexane, there is a zone of inhibition.

Future work

It is recommended to use different extraction methods such as steam distillation, Soxhlet apparatus, Maceration, Supercritical fluid extraction and Microwave-assisted extraction to further investigate the antibacterial activity of Neem leaf. In this research, only three solvents were used. In the future, this can be further done by using other solvents such as methanol, acetone etc. It is also possible to further investigate using other parts of the Neem plant (bark, flowers, seeds, fruits, routes, etc) and their antibacterial activity using other bacteria other than E. coli and S.aureus. To test for antibacterial activity apart from the disc method well diffusion can be used. As in this MBC OR MIC was not conducted as the results were negative but, in the future, if positive results were obtained it can be conducted as well.

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