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Antibacterial Activity of Neem (*Azadirachta indica*) on Staphylococcus Aureus

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Abstract

Plants have many bio-active compounds which are potential medicinal agents. Herbal medicines are popular in developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world. Plants provide an alternate source for the search of neem treatments. There are plenty of plants in traditional medicine which have protective and therapeutic properties. Most likely plants will continue to be valued source of neem molecules which may after possible chemical manipulation; provide new and improved drugs. Bacteria resistance to antibiotics is a major problem for clinicians and therapeutic industry and great efforts are been made to screen wide varieties of medicinal plants from the traditional system of medicine with the hope of getting some newer, safer and more effective agents that can be used to fight infectious diseases. Different parts of the neem plant have medicinal properties. The leaf, bark, oil, flower, fruit and seed exhibit great properties which include antiallergic, antifungal, antibiotic, antidermatic, antibacterial, anti-inflammatory, insecticidal, larvacidal, antimalarial, anti-ulcer and other biological activities. The neem leaf extracts where gotten by solvent extraction method while the anti-bacterial activity was tested using the Kirby Bauer sensitivity test. The ethanolic extract exhibited better and stronger anti-bacterial activity than the aqueous extract. This shows that neem plant has medicinal properties.

Keywords: neem plant, anti-bacterial activity, ethanolic extract, medicinal properties.

Introduction

Plants have many bio-active compounds which are potential medicinal agents. Herbal medicines are popular in developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world (Mogal et al., 2018). Plants provide an alternate source for the search of Neem treatments. There are plenty of plants in traditional medicine which have protective and therapeutic properties (Alzohairy, 2016). Most likely plants will continue to be valued source of neem molecules which may after possible chemical manipulation; provide new and improved drugs (Pauler et al., 2019).

Bacteria resistance to antibiotics is a major problem for clinicians and therapeutic industry and great efforts are been made to screen wide varieties of medicinal plants from the traditional system of medicine with the hope of getting some newer, safer and more effective agents that can be used to fight infectious diseases (Zingne et al., 2019).

Different parts of the Neem plant have medicinal properties. The leaf, bark, oil, flower, fruit and seed exhibit great properties which include antiallergic, antifungal, antibiotic, antidermatic, antibacterial, anti-inflammatory, insecticidal, larvacidal, antimalarial, anti-ulcer and other biological activities (Campos et al., 2016). Herrera- Calderon et al. (2019) reported that neem has great antimicrobial activity and it contains 35 biological active compounds. They also stated that the leaf juice and things are usually used to clean the teeth and also used as a tonic. It has been in report that people of India place the leaves on their beds, books, cupboards to control bugs (Banerjee et al., 2012).

Antimicrobial activity of Neem plant

The neem plant extracts and its different constituents play important roles in the inhibition of several microbes which include bacteria, fungi and viruses. Divya Kumari et al. (2019) tested the antibacterial activity of the Neem plant extracts of methanol, hexane and chloroform on *Escheria coli, proteous vulgaris, klebsiella pneumoniae, Bacillus subtilis, Micrococcus luteus, Streptococcus, faecalis and Enterococcus faecalis.* They are reported that the methanol extract was most effective chloroform was reasonable effective and hexane showed the least antibacterial activity. According to Mafou-Sonhafouo et al. (2019), the stem and back of the Neem plant showed tremendous antibacterial activity against *Klebsiella, Serratia* species and Streptococcus. Shah et al. (2019) reported in their work that the Neem plant methanolic extracts showed antibacterial activity against *vibro cholerae* while chloroform extracts had activity against *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis* and *Streptococcus faecalis*. The seed extracts minimum inhibitory concentration (MIC) was lower compared to the leaf extracts when they were tested against species of *Trichophyton* and *Epidermatophyton floccosum*. The antifungal activity of the neem plant has been tested on different pathogens with various neem plant part extracts with different solvents such as ethanol, water and ethyl acetone, the organisms include *Microsporum gypsum, Aspergillus terrus, Candida albicans, Aspergillus niger, Aspergillus fumigatus*, and *Aspergillus flavus* by using different concentrations of the extracts, the results showed that the leaf extracts had the best activity on the tested pathogens as shown in table 1 below. This antifungal activity increased with increase in concentration (Kalid et al., 1989; Blum et al., 2019; Ziladi et al., 2019; Singaravelu et al., 2019; Jerobin et al., 2015; Bodiba et al., 2018; Mistry et al., 2015).

Table 1: Antibacterial activity in *vitro of Azadirachta indica* reported in

Azadirachta indica	**Microorganism**	**MIC**	**MBC**
oil	Hclicobacter pylori	25 -51 ug/ml.	43-68 tig/ml.
Leaves ethanolic extract	Methicillin-resistant *Staphyhfoccus aureus* *Staphylococcus aureus* Enterococcus faecalis	31.25- 125mg/mL	250-500 mg/mL
Bark extract	Pseudomona aeruginosa Pseudomona mirabilis	500- 1000 ug/ml.	n.d.
Neem oil nanoemulsion	*Vibrium vulnificus*	6 mg/mL	n.d
Leaves ethanolic extract	*Streptococcus mutans* *Streptococcus mutans*	6.25 mg/mL 125 ug	n.d. 250 ug
Leaves methanol extract	*Enterococcus faecalis* *Staphylococus aureus* *Candida albicans*	500 ug 250 ug n.a.	1 ing 500 ug n.a.

MIC: minimum inhibitory concentration; MBC.: minimum bactericidal concentration; n.d.: not determined; n.a: no activity

Adapted from Herrera-Calderon *et al* (2019)

Biological Compounds of Neem Plant

The plant contains different bio-active compounds which exhibit different activities. These phytochemicals are listed in table 2 below.

Table 2: Bioactive compounds *of Azadirachta indica* reported in scientific literatures

No.	Compound Name	Source	Biological activity
1	Nimbidin	Seed oil	Anti- inflammatory
2	Azadirachtin	Seed oil	Antimalarial
3	Nimbin	Seed oil	Spermicidal.
4	Mahmoodin	Seed oil	Antibacterial
5	Margolone, mergolonone and isomargolonone	Bark	Antibacterial
6	Cyclic trisulphide and cyclic tetrasulphide	Leaf	Antifungal
7	Gedunine	Seed oil	Antifungal
8	Polysaccharides	Bark	Anti-inflammatory.
9	NB-2 peptidoglucan	Bark	Immunamodulatory.

Adapted from Herrera-Calderon et al. (2019)

Medicinal Properties of Neem Plant

The research work carried out by Chaudan et al. (2015) to assess the antibacterial action of the leaf, seed, bark, and fruit extracts of Azadirachta indica (Neem plant) on microbes obtained from the month of adults, showed that the leaf and bark extracts had antibacterial activities against all the bacteria isolated and tested while the fruit and seed extracts showed antibacterial actions only at greater concentrations. Dua et al. (1995) affirmed that the leaf extracts of the neem plant had the best antibacterial activity which confirmed the presence and strength of the bioactive compounds and also proved the usage of the plant in major health maintenance.

Antifungal Activity of Neem plant

Jerobin et al. (2015) tested the antidermatophytic activity of the neem leaf ethanol and aqueous extracts on dermatophytes from 88 clinical cases by using the agar dilution technique. The results showed the ethanolic extract had better activity compared to the aqueous extracts. The methanolic and the acetonic extracts of the neem plant were also tested for their antifungal activities against two fungal strains, namely; Aspergullis fumigates and Aspergillus niger by Kelmanson et al. (2000). The results showed that the methanolic extract gave a better antifungal activity compared to the acetonic extracts. They also reported that the neem plant exhibit great medicinal properties. Parts are like the leaf, flower, bark, fruit and seed contain different active chemicals which perform different functions (Alzohairy, 2016; Biswas, et al., 2002).

Some of the medicinal properties of the Neem plant are listed in table 3 below.

Table 3. Tradional uses from *Azadirachta indica* reported in research articles.

Leaf	Leprosy, diuretic, malaria, piles, pyrexia, chicken pox, smallpox and remove toxins, cleanse blood.
Root	Used as a disinfectant, antimicrobial and provocative diseases.
Seed	Mosquito coils, Rheumatism, anthelmintic, antileprotic
Seed oil	Used as an Antiseptic for ulcers and useful for skin diseases like ringworm and scabies, fever and leprosy, and for antibacterial use.
Fruit	Fruit extracts of neem beneficial for Insecticidal, diabetes, constipation and anthelmintic
Bark	Use as a cure for fever
Stembark	Anti-cancerous
Flower	Cough and non-toxic
Young branch	Used for tooth diseases

Adapted from Herrera-Calderon et al (2019)

Mechanism of action of the Bio-active compounds

Different parts of the neem plant exhibit antimicrobial activities against microbes through inhibiting the growth of the microbes by breaking down their cellular. It has also been shown that the free radical hunting action of neem is due to the presence of *Azadirachtin* and *nimbolide* (Claube et al., 2014).

Methodology

The leaf extracts were obtained as described by Ugwu et al., (2017), while Kirby Bauer sensitivity test was used for the antibacterial test activity of the neem plant. Traditional cultural techniques were used to culture, isolate and identify the bacterial isolates. Freshly collected neem leaves were allowed to dry under room temperature for 5 days in the laboratory after which 50grams of the dry leaves were placed in 50ml of ethanol and sterile distilled water respectively for 24hours. These were then filtered with filter paper to remove the residue. The filtrate was then placed in the water bath distillate it in order to increase the concentration of the extracts. Media used for this study is nutrient agar, it was used to culture the test organism (*Staphylococcus aureus*) obtained from the saliva samples of eight (8) volunteers. The saliva samples were collected with sterile glass bottles. After the preparation of the culture medium, it was dispensed into petri dishes containing an aliquot of the diluent sample which were then incubated for 24 hours at 37^{o}C. The pour plate method was used for culturing the bacterial species. The pure cultures were obtained by streaking the individual colonies on fresh nutrient agar plates. Morphological and biochemical tests were then carried out to identify the organism.

Filter paper discs were prepared from sterile filter paper and soaked in the neem plant leaf extracts of ethanol and sterile distilled water for 24hrs. Thereafter, fresh plate of pure cultures were prepared again to test for the antibacterial activity of the neem plant leaf extracts. The soaked filter paper dishes were placed on the agar containing the pure cultures and then incubated for 24hrs at 37°C.

Results and Conclusion

Table 4: Biochemical Test and sensitive test on Staphylococcus species

Sample	Catalase Test	Coagulase Test	Aqueous extract	Ethanol extract (Zone of inhibition) cm
A	+	-	Non	19
B	+	-	Non	23
C	+	-	Non	17
D	+	-	Non	21
E	+	-	Non	24
F	+	-	Non	2.5
G	+	-	Non	22
H	+	-	Non	25

From the results obtained from this study, it was clear that the antibacterial activity of the neem plant leaf extracts of ethanol exhibited higher zones of inhibition compared to aqueous leaf extracts which had little or no effect on the organisms. The alcohol extracts had stronger effect on the bacterial isolates. This further confirms that the neem plant contains medicinal bio-active compounds.

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