CHAPTER 14

Antimicrobial and Phytochemical Properties of Neem Leaf and Bark Extracts on Selected Microorganisms

Ohalete, C. N[1] and Anyanwu, G. O.[2]

1.Department of Microbiology, Imo State University, Owerri2. Department of Biology/Microbiology,Federal Polytechnic Nekede, OwerriEmail: ohaletechinyere@gmail.com: +2348036699179

Abstract

This study evaluated the antimicrobial and phytochemical properties of neem leaf and bark extracts on selected microorganisms. Neem plant parts (bark and leaf) were gotten from neem plant tree at School of Business Technology, Federal Polytechnic Nekede, Owerri, Imo State. The plant parts were dried, ground and extracted using ethanol. Qualitative phytochemical screening was adopted in the determination of the phytochemical constituents of the extracts while agar well diffusion technique was adopted in the determination of the antimicrobial activities of the extracts against *Staphylococcus, Salmonella, Escherichia, Pseudomonas* and *Candida* species. Tube dilution technique was adopted in the minimum inhibitory concentration of the extracts. The presence of saponins, anthranoids, anthraquinones, alkaloids, phenols, tannins and phlobatannins and cardiac glycosides were detected from the leaf and stem bark extracts. The zones of inhibition recorded ranged with 18mm to 24mm with leaf extract and 16mm to 20mm with stem bark extract. Inhibitory concentrations recorded ranged from 125mg/ml to 250mg/ml with both leaf and stem bark extracts. Bactericidal effect was recorded at 250mg/ml and 500mg/ml. The leaf extract of neem showed better antimicrobial activity compared to the stem bark extract. Similarly, *Salmonella* and *Staphylococcus* species were the most susceptible bacteria compared to the other test organisms. The results of this study therefore justify the use of these plant parts in alternative medicine.

Keywords: Neem plant, phytochemical, bactericidal, extract, inhibition.

INTRODUCTION

Background to the study

Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too. Plants

provide an alternative strategy in search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties (Kayode & Kayode, 2011). It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved drugs (**Uwimbabazi, Uwimana & Rutanga, 2015**).

*Azadirachta indica commonly known as; neem, nimtree or Indian lilac (Dogoyaro)*is one of such medicinal plants belonging to the Meliaceae family (Abalaka, **Oyewole & Kolawole**, 2012). *Azadirachta indica*, commonly known as neem, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex (**Uwimbabazi et al., 2015**). More than 140 compounds have been isolated from different parts of neem. All parts of the neem tree- leaves, flowers, seeds, fruits, roots, and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders (Sharma, Pankaj & Mamun, 2011; Ogbuewu, Odoemenam, Obikaonu, Opara, Emenalom & Uchegbu, 2011; Subbalakshmi, 2012).

The medicinal utilities of neem products have been described especially for neem leaf. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, anti-inflammatory, anti-hyperglycaemic, anti-ulcer, anti-malarial, anti-fungal, anti-bacterial, antiviral, antioxidant, anti-mutagenic and anti-carcinogenic properties (Jyotsna & Saonere, 2011; Gajendrasinh, Bhavika, Rohit, Hetal & Prajapati, 2012; Mamman, Mshelia, Susbatrus & Sambo, 2013; **Sonalkar, Nitave, Sachin & Kagalkar, 2014**). 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 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 (Wiratno et al., 2009).

The extracts from neem plant have been used as antimicrobial agents in the inhibition of the growth of pathogenic microorganisms. Uwimbabazi et al. (2015) reported zones of inhibition with neem leaf extracts ranging from

12mm to 18mm with aqueous extract and 14mm to 27mm with ethanol extract. Similarly, Mishra, Neema & Niketa(2013) reported zones of inhibition with neem leaf and seed extracts to range from 8mm to 24mm against *Staphylococcus aureus* and *Escherichia coli.*

MTERIALS AND METHODS

Collection of samples

Neem plant parts (bark and leaf) were gotten from neem plant tree at School of Business Technology, Federal Polytechnic Nekede, Owerri, Imo State. The bark was cut using sharp knife while the leaves were plugged out from the branches. The plant parts were identified by a plant Taxonomist and were taken to the laboratory for drying, grinding and further processing.

Extraction of active ingredients from neem bark and leaf

Twenty grams (20g) of ground leaf and bark powders were subjected to extraction using soaking method in ethanol. Twenty grams (20g) each of the powder was poured into 95% ethanol in a beaker. The content of the beaker was allowed to soak overnight. The content was filtered using muslin cloth and stored in a sterile container (**Uwimbabazi et al., 2015**).

Collection of test organisms

Two Gram positive bacteria; *Staphylococcus species, Salmonella* speciesand two Gram negative bacteria; *Escherichia species*and *Pseudomonas aeruginosa and fungi Candida species* was used in this study. The test organisms were isolated from urine samples from students within Federal Polytechnic Nekede Medical Center.

Standardization of inocula

0.5 M McFarland's standard solution of the test organisms were used for the study. The method described by CLSI (2010) was adopted in the standardization of inocula.

Antimicrobial screening of neemleaf and bark extracts

The agar well diffusion techniques as described by **Uwimbabazi et al. (2015) was** adopted for this study to evaluate the antimicrobial activity of the leaf and bark extracts.A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland's turbidity standards on the surface of already prepared and dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. Two wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. The extracts were then poured into the wells and the plates were allowed to stand for about 30 minutes for proper

diffusion of the solutions before being incubated at 37°C for 24 hours (CLSI, 2010). After 24 hours, antibacterial activity was evaluated by measuring the diameter of the zones of inhibition produced by the extracts against the test organisms in millimeters.

Tests for minimum inhibitory concentrations (MIC)

For the MIC tests, about two milliliters (2ml) each of the leaf and bark extracts were added in four milliliters (4ml) of peptone water; this gives 500mg/ml. Thereafter, two-fold serial dilutions were carried out from the 500 mg/ml concentration by transferring 2 ml of the 500 mg/ml concentration to 4 ml of peptone water contained in a test tube and homogenized properly. This procedure of transferring 2 ml of the tube to 2 ml of peptone water contained in the subsequent tube was continued until the eighth tube. The following concentrations were thereafter obtained, 500mg/ml, 250 mg/ml, 125 mg/ml, 62.50 mg/ml, 31.25 mg/ml, 15.62 mg/ml, 7.81 mg/ml and 3.90 mg/ml. Having obtained the different concentrations and dilutions, three drops of overnight broth cultures of the test organisms were inoculated into the dilutions in each case of the test organisms. The tubes were then incubated at 37^0C for 24 hours. The lowest concentration of the extracts that inhibited the growth of the test organisms was recorded as the MIC.

Test for minimum bactericidal concentrations (MBC)

Tubes showing no visible growth from the MIC test was sub-cultured onto sterile nutrient agar plates and incubated at 37^0C for 24 hours. The lowest concentration of the leaf extract yielding no growth was recorded as the minimum bactericidal concentrations.

Qualitative phytochemical screening of the leaf extracts

The method described by Ekeleme et al. (2017) was adopted in the determination of the preliminary qualitative phytochemical constituents of the extracts. The phytochemicals that were determined include saponins, tannins, alkaloids, anthraquinones, cardiac glycosides, phenols and phlobatannins.

Test for saponins

Ten milliliters (10ml) of distilled water were added to about two milliliters (2ml) of each of the extracts in a test tube and shaken vigorously. Persistent frothing even after heating is an indication of the presence of saponins.

Test for anthranoids

Two milliliters (2ml) of each of the extracts, five milliliters (5ml) of 0.5M

potassium hydroxide was added and mixed properly. Then 6 drops of acetic acid were added followed by 2ml of toluene. To the upper layer formed, 2ml of 0.5M potassium hydroxide was added. A change in colour of the mixture was an indication of a positive test while no colour change was an indication of a negative test.

Test for anthraquinones

To about 2ml of each of the extracts, 5ml of 10% ammonia was added and shaken vigorously. 2ml of benzene was thereafter be added. A colour change was an indication of a positive test while none was an indication of a negative test.

Test for phenol

5ml of each of the extracts was mixed with 8ml of distilled water in a test tube and 6ml of Ferric chloride was added to the mixture. A colour change to light brown is an indication of a positive test while none indicates a negative test.

Test for alkaloids

To about 2ml of each of the extracts, 5ml of 1% aqueous hydrochloric acid was added and placed in a water bath for 3 minutes and thereafter 3 drops of Mayer's reagent was added. A white precipitate was an indication of a positive test while none was an indication of a negative test.

Test for tannins

To about 1ml of each of the extracts, 2ml of 1% ferric chloride was added. A colour change was an indication of a positive test while none was an indication of a negative test.

Test for phlobatannins

To about 2ml of each of the extracts, 2ml of 1% aqueous hydrochloric acid was added and boiled. The presence of white precipitate was an indication of a positive test while none was an indication of a negative test.

Test for cardiac glycoside

The Salkowski test was employed in this test. To 1ml of the extracts, 2ml of chloroform was added and then 2ml of concentrated tetraoxosulphate (VI) acid was added to form a lower layer. A reddish brown colour at the interphase was an indication of a positive test while none was an indication of a negative test.

RESULTS AND DISCUSSION

RESULTS

The results of this study are shown in Tables 1 to 4. Table 1 showed the result of the phytochemical constituents of the leaf and stem bark extracts. The presence of saponins, anthranoids, anthraquinones, alkaloids, phenols, tannins and phlobatannins and cardiac glycosides were detected with the leaf and stem bark extract.

Table 2 showed the result of the antimicrobial susceptibility pattern of the extracts against the test organisms. The zones of inhibition recorded ranged from 18mm to 24mm with leaf extract and 16mm to 20mm with stem bark extract.

Table 3 showed the result of the minimum inhibitory concentration of the leaf and stem bark extracts against the test microorganisms. Inhibitory concentrations recorded ranged from 125mg/ml to 250mg/ml with both leaf and stem bark extracts.

Table 4 showed the result of the minimum bactericidal concentration of the root and stem bark extracts against the test microorganisms. Bactericidal effect was recorded 250mg/ml and 500mg/ml.

Table 1: Phytochemical screening of the leaf and stem bark extracts

Phytochemical constituents	Plant parts/Result	
	Leaf extract	Stem bark extract
Saponins	+	+
Anthranoids	+	-
Anthraquinones	+	-
Phenols	+	+
Alkaloids	+	+
Tannins	+	+
Cardiac glycosides	+	+
Phlobatannins	+	+

Key: - = Absence of phytochemicals + = Presence of phytochemicals

Table 2: Antimicrobial susceptibility pattern of the extracts against the test microorganisms

Test organisms	Plant parts/Zone of inhibition (mm)	
	Leaf extract	Bark extract
Pseudomonas species	18	18
Salmonella species	24	16
Staphylococcus species	20	20
Candida species	18	16

Key: mm = Millimeter

Table 3: Minimum inhibitory concentrations of the extracts against the test microorganisms

Test organisms	Plant parts/Zone of inhibition (mm)	
	Leaf extract	Bark extract
Pseudomonas species	250	250
Salmonella species	125	250
Staphylococcus species	125	125
Candida species	250	250

Key: mg/ml = Milligram per milliliter

Table 4: Minimum bactericidal/fungicidal concentrations of the extracts against the test microorganisms

Test organisms	Plant parts/Zone of inhibition (mm)	
	Leaf extract	Bark extract
Pseudomonas species	500	500
Salmonella species	250	500
Staphylococcus species	250	ND
Candida species	500	ND

Key: mg/ml = Milligram per milliliter

DISCUSSION

Plants are known of their ability to maintain good health since antiquity. Nowadays, the interest in natural products as antimicrobial agents has greatly increased due to the gradual collapse of antibiotics caused by the emergence of multi-drug-resistant pathogens (Abdallah & Koko, 2017). This study evaluated the antimicrobial and phytochemical properties of neem leaf and bark extracts on selected microorganisms. Table 1 showed the result of the phytochemical constituents of the leaf and stem bark extracts. The presence of saponins, anthranoids, anthraquinones, alkaloids, phenols, tannins and phlobatannins and cardiac glycosides were detected with the

leaf and stem bark extract. Abalaka et al. (2012) reported the presence of saponins, phlobatannins, tannins and cardiac glycosides from neem leaf extracts. Tiwari et al. (2012) reported that the different composition of the active components in plants give medicinal plants an edge as better therapeutic agents.

Table 2 showed the result of the antimicrobial susceptibility pattern of the extracts against the test organisms. The zones of inhibition recorded ranged from 18mm to 24mm with leaf extract and 16mm to 20mm with stem bark extract. Uwimbabazi et al. (2015) reported zones of inhibition with neem leaf extracts ranging from 12mm to 18mm with aqueous extract and 14mm to 27mm with ethanol extract. Similarly, Mishra et al. (2013) reported zones of inhibition with neem leaf and seed extracts to range from 8mm to 24mm against *Staphylococcus aureus* and *Escherichia coli.*

Leaf extract neem showed higher zones of inhibition compared to the stem bark extract. These results are the same as the one obtained in the study carried out by Sanjeet et al. (2015) who reported inhibitory zones from 12 to 28mm after 90 hours of incubation against *Aspergillus niger, Proteus vulgaris, Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*.

Table 3 showed the result of the minimum inhibitory concentration of the leaf and stem bark extracts against the test microorganisms. Inhibitory concentrations recorded ranged from 125mg/ml to 250mg/ml with both leaf and stem bark extracts. Jabeen et al. (2013) reported that aqueous extracts of various parts of neem such as neem oil against *Alternaria solani* Sorauer and results confirmed that ethyl acetate fraction was found most effective in retarding fungal growth with MIC of 0.19 mg and this fraction was also effective than fungicide (metalaxyl + mancozeb) as the fungicide has MIC of 0.78mg.

Table 4 showed the result of the minimum bactericidal concentration of the root and stem bark extracts against the test microorganisms. Bactericidal effect was recorded 250mg/ml and 500mg/ml. Antimicrobial properties recorded with neem leaf and stem bark extracts could be attributed to the presence of phytochemicals as shown in Table 3. Phytochemicals are chemical compounds that occur naturally in plants. Alkaloids are reported to be present in this plant that has pharmaceutical applications (Ekeleme etal., 2017). Phenols are chemical compounds that occur ubiquitously as natural

colour pigments responsible for the colour of fruits of plants. They play important role in plant defense against pathogens, and this applied in the control of human pathogen infection. Saponins are also important therapeutics as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for activity of cardiac glycosides. Tannins are used as antiseptic, and this activity is due to the presence of the phenolic group. They are used as wound healing agents (Sarker & Nahar, 2017).

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

The results of this study revealed that leaf and stem bark extracts of neem had antimicrobial activities against some pathogenic microorganisms. The leaf extract of neem showed better antimicrobial activity compared to the stem bark extract. Similarly, the *Salmonella* and *Staphylococcus* species were the most susceptible bacteria compared to the other test organisms. The antimicrobial activities of the neem extracts could be attributed to the presence of phytochemical constituents of the plant parts. The results of this study therefore justify the use of these plant parts in alternative medicine.

RECOMMENDATIONS

1. The planting and propagation of this plant should be encouraged in the society to be always it available for people.
2. There should be study carried out to investigate the toxicity level of these plant parts to justify their antimicrobial activity.
3. There should be enlightenment program geared towards educating people on the importance of medicinal plants such as neem to humans.

REFERENCES

Abalaka, M., Oyewole, O.A., & Kolawole, A.R. (2012).Antibacterial activities of *Azadirachta Indica* against some bacterial pathogens. *Advances in Life Sciences*, 2(2), 5-8

Abdallah, E.M., & Koko, W.S. (2017). *Medicinal plants of antimicrobial and immunomodulating properties.* Spain: Formatex Research Center.

Anjali, K., Ritesh, K., Sudarshan, M., Jaipal, S.C., & Kumar, S. (2013). Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. *The Bioscan*, **8**, 671–674.

Clinical Laboratory Standard Institute (2010). *Performance standards for antimicrobial susceptibility testing: Nineteenth Informational Supplement.* Clinical and Laboratory Standards Institute, Wayne.

Efferth, T., & Koch, E. (2011). Complex interactions between Phytochemicals. TheMulti-Target Therapeutic concept of Phytotherapy. *Current Drug Targets*, **12**(1): 122–132.

Ekeleme, K., Tsaku, P., Nkene, I., Ufomadu, U., Abimiku, R., & Sidi, M. (2017). Phytochemical analysis and antibacterial activity of *Psidium guajava* L. leaf extracts. *GSC Biological and Pharmaceutical Sciences*, **1**(2), 13-19.

Gajendrasinh, P.R., Bhavika, M.K., Rohit, S., Hetal, A., & Prajapati, P.K. (2012). *In vitro* Antibacterial study of two commonly used medicinal plants in Ayurveda: Neem (*Azadirachta indica L.*) and Tulsi (*Ocimum sanctum L.*). *International Journal of Pharmaceutical and Biological Association,* **3**(3), 582-586.

Ghimeray, A.K., Jin, C.W., Ghimire, B.K., & Che, D.H. (2009). Antioxidant activity and quantitative estimation of *Azadirachtin* and Nimbin in *Azadirachta indica*. *African Journal of Biotechnology,***54**(2), 1684–5315.

Ghonmode, W.N., Balsaraf, O.D., Tambe, T.N., Saujanya, M.P., Patil, A.K., & Kakde, D.D. (2013). Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *E. feacalis*—an *in vitro* study. *Journal of International Oral Health*, **5**(6), 61–66.

Girish, K. and Shankara, B.S. (2008). medicinal properties of Neem (*Azadirachtaindica*). *Electronic Journal of Biology,***4**(3):102-111

Hossain, M. A., Shah, M.D., & Sakari, M. (2011). Gas chromatography–mass spectrometry analysis of various organic extracts of *Merremia borneensis* from Sabah. *AsianPacific Journal of Tropical Medicine*, **4**(8), 637–641.

Hossain, M.A., Al-Toubi, W.A.S.A., Weli, M., Al-Riyami, Q.A., & Al-Sabahi, J.N. (2013). Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *Journal of Taibah University for Science*, **7**(4), 181–188.

Jabeen, K., Hanif, S., Naz, S., & Iqbal, S. (2013). Antifungal activity of *Azadirachta indica* against *Alternaria solani*. *Journal of Life Sciences and Technologies*, **1**(1), 89–93.

Jyotsna, A. and Saonere, S. (2011). Neem - natural contraceptive for male and female – an overview. I*nternational Journal of Biomolecules and Biomedicine,***1**(2), 1-6.

Kayode, A.A.A., & Kayode, O.T. (2011). Some medical values of *Telfairia occidentalis: A review. American Journal of Biochemistry and Molecular Biology,* **1**, 30-38.

Kiranmai, M., Kumar, C.B.M., & Ibrahim, M.D. (2011). Free radical scavenging activity of neem tree (*Azadirachta indica A.* Juss Var., Meluaceae) root barks extract. *Asian Journal of Pharmaceutical and Clinical Research,* **4**(4), 134-136.

Mamman, P.H., Mshelia, W.P., Susbatrus, S.C., & Sambo, K.W. (2013). Antibacterial effects of crude extract of *Azadirachtaindica* against *Escherichia coli, salmonella speciesand Staphylococcus aureus. InternationalJournalMedicalScience*, **5**(1), 14-18.

Mishra, A., Neema, M. and Niketa, P.P. (2013). Antibacterial effects of crude extract of *Azadirachta indica* against *Escherichia coli* and *Staphylococcus aureus. International Journal of Science, Environment and Technology*, **2**(5), 989 – 993.

Mohammad, M.A. (2016). Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Evidence-Based Complementary and Alternative Medicine,*6, 1-11.

Ogbuewu, I.P., Odoemenam, V.U., Obikaonu, H.O., Opara, M.N., Emenalom, O.O., & Uchegbu, M.C. (2011). The Growing Importance of Neem (*Azadirachta indica* A. Juss) In Agriculture, Industry, Medicine and Envirnment: A Review. *Research Journal of Medicinal Plant*, 5(3): 230-245.

Paul, R., Prasad, M., & Sah, N.K. (2011). Anticancer biology of *Azadirachta indica* L (neem): a mini review. *Cancer Biology and Therapy*, 12(6), 467–476.

Priyadarsini, R.V., Manikandan, P., Kumar, G.H., & Nagini, S. (2009). The neem limonoids azadirachtin and nimbolide inhibit hamster cheek pouch carcinogenesis by modulating xenobiotic metabolizing enzymes, DNA damage, antioxidants, invasion and angiogenesis. *Free Radical Research*, 43(5), 492–504.

Sanjeet, K., Upadhay, J.P., & Kumar, S. (2005). Evaluation of plant extracts for control of *Alternaria* leaf spot of *Vicia faba*. *Annals of Plant Protection and Science*, 13, 258–259.

Sarker, E., & Nahar, R. (2017). Ethnopharmacology and alternative medicine. *Current Science*, 90, 1552-1554.

Sarmiento, W.C., Maramba, C.C., & Gonzales, M.L.M. (2011). An *in vitro* study on the antibacterial effect of neem (*Azadirachta indica*) leaf extracts on methicillin-sensitive and methicillin resistant *Staphylococcus aureus*. *PIDSP Journal*, 12(1), 40–45.

Sharma, L., Pankaj, K. & Mamun, L. (2011). Review on neem (*Azadirachtaindica*): Thousand problem one solution. *International Research Journal of Pharmacy*, 2(12): 97-102.

Shrivastava, D.K., & Swarnkar, K. (2014). Antifungal activity of leaf extract of neem (*Azadirachta indica* Linn). *International Journal of Current Microbiology and Applied Sciences*, 3(5), 305–308.

Sonalkar, M., Nitave, Y., Sachin, A., & Kagalkar, A.A. (2014). Review on Neem plant. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(4), 590-598.

Sonalkar, M., Nitave, Y., Sachin, A., & Kagalkar, A.A. (2014). Review on Neem plant. *World Journal of Pharmacy and Pharmaceutical Sciences*, 3(4): 590-598.

Subbalakshmi, L. (2012). Neem products and their agricultural applications. *JournalofBiopesticides*, 5, 72-76.

Tiwari, V., Darmani, N.A., Yue, B.Y., & Shukla, D. (2012). *In vitro* antiviral activity of neem (*Azardirachta indica* L.) bark extract against herpes simplex virus type-1 infection. *Phytotherapy Research*, 24(8), 1132–1140.

Uwimbabazi, F., Uwimana, J., & Rutanga, J.P. (2015). Assessment of antibacterial activity of Neem plant (*Azadirachta indica*) on *Staphylococcus aureus* and *Escherichia coli. Journal of Medicinal Plants Studies*, 3(4), 85-91.

Wiratno, D., Taniwiryono, H., Vanden-Berg, J.A.G., Riksen, I.M.C.M., Rietjens, S.R., Murk, A.J. (2009). Nematicidal Activity of Plant Extracts against the Root-Knot Nematode, *Meloidogyne incognita. The Open Natural Products Journal, 2,* 77-85.

Yerima, M.B., Jodi, S.M., Oyinbo, K., Maishanu, H.M., Farouq, A.A. & Junaidu, A.U. (2012). Effect of neem extracts (*Azadirachta indica*) on bacteria isolated from adult mouth. *Journal of Basic and Applied Sciences*, 20, 64–67.

Zong, A., Cao, H., & Wang, F. (2012). Anticancer polysaccharides from natural resources: a review of recent research. *Carbohydrate Polymers*, 90(4), 1395–1410.