Effect of Neem (*Azadirachta indica*) Plant Extract on Microorganism and the Importance of its Bioactive Compounds on Human Health.

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DECLARATION

I hereby Declare that this project work title (Effect of Neem (*Azadirachta indica*) Plant Extract on Microorganism and the Importance of it Bioactive Compounds on Human Health) is the product of my research efforts undertaken under the supervision of Dr. Salim F. Bashir and has not been presented elsewhere for the award of degree or certificate. All sources have been duly acknowledged.

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CERTIFICATION

This is to certify that the research work for this project/thesis/dissertation titled (Effect of Neem (*Azadirachta indica*) Plant Extract on Microorganism and the Importance of it Bioactive Compounds on Human Health) by David Yusuf Hassan (SPS/MCB/PMB/22/0002) was carried out under my supervision.

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Signature & Date

APPROVAL

This project/thesis/dissertation titled (Effect of Neem (*Azadirachta indica*) Plant Extract on Microorganism and the Importance of it Bioactive Compounds on Human Health) by David Yusuf Hassan (SPS/MCB/PMB/22/0002) has been examined and approved for the award of PGD in Microbiology.

Supervisor	Signature & Date
Head Of Department	Signature & Date
P.G. School Representative	Signature & Date

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Hassan David Yusuf

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DEDICATION

This work is dedicated first and foremost to the Almighty GOD who sustained my life during the period of the study, and to my beloved parents and the whole members of the family, I really appreciate you all.

Table of Contents

1.0 Introduction

Plants for many years are mainly the raw resource concerning the production of antibiotics which are currently in use (Viji *et al*. 2010). Plants are mines of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Samie *et al*., 2010). 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 *et al.,* 2015).

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). *Azadirachta indica*, commonly known as neem, neem tree or Dogon yaro in Hausa. It is typically grown in tropical and subtropical regions. Neem belongs to family *meliaceae.* It is also called "village pharmacy of south Asia" (India) because of its enormous medicinal properties and people use it most of the time without knowing it multiple advantages (Khan *et al.,* 2021).

Neem grows up to 20–23 m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are compound, imparipinnate, with each comprising 5–15 leaflets. Its fruits are green in color which turn golden yellow on ripening in the months of June–August. Taxonomic position of *Azadirachta indica* (neem) is classified in:

Table 1: Taxonomic position of *Azadirachta indica* (neem) (Girish & Neem).

Order	Rutales
Suborder	Retinae
Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	Azodirachta
Species	Indica

Adapted from Girish, (2008)

Neem elaborates a vast array of biologically active compounds that are chemically diverse and structurally complex (Uwimbabazi *et al.,* 2015). Different parts of the Neem plant have medicinal properties. The leaf, bark, oil, flower, fruit and seed exhibit great properties which include anti-allergic, antifungal, antibiotic, anti-dermatic, 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 is 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).

2. Antimicrobial and Pharmacological Efficacy of Neem Plant Extract

Studies have indicated that neem has important role in inhibition of bacterial growth. It is effective against number of pathogenic bacteria, fungi and viruses (Biswas *et al.,* 2000).

2.1 Antibacterial Activity

The anti-bacterial activity of the bark, leaf, seed, and fruit extracts of *Azadirachta indica* (neem) on bacteria isolated from adult mouth had revealed that bark and leaf extracts showed antibacterial activity against s*taphylococcus aureus*. Furthermore, seed and fruit extracts showed antibacterial activity only at higher concentrations (Dhanya & Sidhu 2011). A study alsoshows inhibitory zones from 12 to 28mm after 24 hours of incubation against *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus* (Gupta *et al.,* 2001). Neem plant extracts of methanol, hexane and chloroform tested against *Escherichia coli, proteous vulgaris, klebsiella pneumoniae, Bacillus subtilis, Micrococcus luteus, Streptococcus, faecalis and Enterococcus faecalis* showed that the methanol extract was most effective, chloroform was reasonable effective and hexane showed the least antibacterial activity Divya Kumari *et al.,* (2019). The stem and back of the Neem plant also showed tremendous antibacterial activity against *Klebsiella, Serratia* species and *Streptococcus* Mafou-Sonhafouo *et al.,* (2019). The extracts from neem plant have been used as antimicrobial agents in the inhibition of the growth of pathogenic microorganisms, a study by Mishra, Neema & Niketa (2013) indicate zones of inhibition with neem leaf and seed extracts to range from 8mm to 24mm against *Staphylococcus aureus* and *Escherichia coli.*

2.2 Antiviral Activity

Results demonstrated that neem bark (NBE) extricate essentially blocked HSV-1 passage into cells at fixations extending from 50 to 100 μg/mL. Besides, blocking movement of NBE was seen when

the concentrate was preincubated with the infection yet not with the objective cells recommending an immediate hostile to HSV-1 property of the neem bark. Leaves concentrate of neem (*Azadirachta indica* A. Juss.) (NCL-11) has demonstrated virucidal action against coxsackievirus infection B-4 as proposed by means of infection inactivation and yield decrease measure other than meddling at an early occasion of its replication cycle (Govindachari *et al.,* 1997).

2.3 Antifungal Activity

Examination was made to assess the adequacy of different concentrates of neem leaf on seed borne fungi *Aspergillus* and *Rhizopus* and results affirmed that development of both the contagious species was fundamentally hindered and controlled with both alcoholic and water extricate. According to a study, alcoholic concentrate of neem leaf was best when contrasted with fluid concentrate for impeding the development of both parasitic species (Lessa *et al.,* 2010). Another finding demonstrated the antimicrobial part of watery concentrates of neem cake in the hindrance of spore germination against three sporulating organisms, for example, *C. lunata, H. pennisetti,* and *C. gloeosporioides f. sp.mangiferae* and aftereffects of the examination uncovered that methanol and ethanol concentrate of *Azadirachtaindica* showed development restraint against *Aspergillusflavus, Alternaria solani,* and *Cladosporium* (Kher & Chaurasia 1997).

2.4 Antioxidant Activity

The main culprits for generation of many diseases are free radical and oxygen species. Therefore, the important step in prevention of diseases is neutralization of activity of free radical. Antioxidant deactivates the reactive oxygen species/free radical. It also stabilizes the reactive oxygen species before they attack on any biological system. They activate the antioxidative enzyme. This ant oxidative enzyme prevents the damage due to reactive oxygen species and free radicals. *Azadirachtaindica* (neem) have been reported to have anti-oxidant activity. The leaves, seeds, bark and oil of neem are rich source of antioxidants. A study in literature showed that in vitro antioxidant activity in different crude extracts of the leaves of *Azadirachta indica* (neem) and antioxidant capacity of different crude extracts was as follows: chloroform >butanol> ethyl acetate extract > hexane extract > methanol extract (Biswas *et al.,* 2002). The chloroform crude extracts of neem could be used as a natural antioxidant. Other results revealed that azadirachtin and nimbolide showed concentration-dependent antiradical scavenging activity and reductive potential in the following order: nimbolide>azadirachtin>ascorbate. According to a study, administration of azadirachtin and nimbolide inhibited the development of DMBA-induced HBP carcinomas

through prevention of procarcinogen activation and oxidative DNA damage and upregulation of antioxidant and carcinogen detoxification enzymes (Prashant *et al.*, 2007).

2.5 Hepatoprotective Effect of Neem

A study was performed to investigate the hepatoprotective role of azadirachtin-A in carbon tetrachloride (CCl4) induced hepatotoxicity in rats and histology and ultrastructure results confirmed that pretreatment with azadirachtin-A dose-dependently reduced hepatocellular necrosis. Furthermore, results of the study show that pretreatment with azadirachtin-A at the higher dose levels moderately restores the rat liver to normal (Singh & Sastry 1997).

2.6 Anticarcinogenic activity

Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7,12 dimethylbenzene anthracene (DMBA), as revealed by reduced incidence of neo-plasm (Arivazhagan *et al.,* 2000). Neem may exert its chemo preventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes. That neem leaf extract exerts its protective effect in *M*ethyl- *N*ı-nitro-*N*-nitrosoguanidine (MNNG) (a carcinogenic material)-induced oxidative stress has also been demonstrated by the reduced formation of lipid peroxides and enhanced level of antioxidants and detoxifying enzymes in the stomach, a primary target organ for MNNG as well as in the liver and in circulation (Arivazhagan *et al.*, 2000).

2.7 Neem and Biofilm-Forming Pathogens

Biofilms, or communities of bacteria composed of biofilm-associated cells and extracellular polymeric substance (EPS) components (e.g., proteins, polysaccharides, and extracellular DNA), are notably recalcitrant to outside stressors, including those from the immune system and from drugs (Bae and Jeon, 2013; Yonezawa *et al.*, 2019; de Vor *et al.*, 2020).

Significant biofilm-associated human infections are caused by species such as *S. aureus*, *E. faecalis*, and *P. aeruginosa* (Vestby *et al.,* 2020). A. indica has shown activity against biofilm-forming strains of some of these pathogens. For example, a neem leaf ethanolic extract was found to inhibit *S. aureus* and methicillin-resistant *S. aureus* (MRSA) biofilm adherence at 62.5 and 125 μg/ml, respectively (Quelemes *et al.,* 2015). Additionally, Guchhait et al. found that ripe neem seed extracts had antibiofilm activity against *S. aureus* and *Vibrio cholerae*. The minimum biofilm inhibitory concentrations (MBIC) and minimum biofilm eradication concentrations (MBEC) for this extract were 100 and 300 μg/ml, respectively, against *S. aureus* and 300 and 500 μg/ml, respectively, against *V. cholerae* (Guchhait *et al.,* 2022). Furthermore, using a mouse model of *V. cholerae* infection, Thakurta et al. showed that administration of methanolic neem leaf extract at

100–1800 mg/kg body weight reduced intestinal fluid secretion by 27.7%–77.9% and doses ≥300 mg/kg inhibited Vibrio-induced hemorrhage in the murine intestine without signs of toxicity (Thakurta *et al.,* 2007). Overall, evidence indicates that neem has great potential to be used as a therapeutic for resistant bacterial infections. However, future research that utilizes animal models will be crucial to determine whether neem-derived products fit in with established antibiotic regimens and/or work alone to eradicate biofilms in vivo.

2.8 Influenza and Neem Phytochemicals

Flu leads to an estimated 290,000-650,000 deaths annually (WHO, 2020). Due to these consistently high levels of associated morbidity and mortality around the world, influenza is one of the most intensely researched viruses. Molecular docking experiments identified a total of four neem phytochemicals that interact with conserved residues of either the nucleoprotein or the non-structural (NS1) protein of influenza. Though requiring further testing, this may indicate the ability to act as a universal drug against the flu virus. This recent evidence indicate taht *A. indica* may represent a robust source of novel drugs against viruses such as influenza. (Ahmad *et al.,* 2015; Ahmad *et al.,* 2016).

3.0 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
1	Nimbidin	Seed oil
2	Azadirachtin	Seed oil
3	Nimbin	Seed oil
4	Mahmoodin	Seed oil
5	Margolone, mergolonone and isomargolonone ,0	Bark
6	Cyclic trisulphide and cyclic tetrasulphide	Leaf
7	Gedunine	Seed oil
8	Polysaccharides	Bark
9	NB-2 peptidoglucan	Bark

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

3.1 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 mouth 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.

Table 3. Traditional 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)

3.2 Toxicity Studies of *Azadirachta indica* Plant Leaves

A few in vivo models have been implemented to more accurately reflect human infection and disease testing; these models include intraperitoneal or intravenous injection, or oral or gastric administration of neem oil-related drugs in mice, rats, guinea pigs, and rabbits. These published animal studies indicate that the acute toxicity level of neem greatly depends on the plant component and solvent used to make the extract, as well as on the treatment route and species used in the model (Braga *et al.,* 2021). As an example, oral administration of an ethanolic neem leaf extract less than 2000 mg/kg body weight did not cause mortality in mice (Kanagasanthosh *et al.*,

2015). Conversely, the ethanolic extract of neem stem bark given to rats at 50–200 mg/kg altered the biochemical markers of toxicity and may have consequential effects on organ function (Ashafa *et al.,* 2012). Although the available in vivo data will need to be further developed before neem oil extracts and phytochemicals are applied in a clinical setting, the United States Environmental Protection Agency has stated that cold-pressed neem oil should have "no unreasonable adverse effects to the US population and the environment" (Agency U.S E.P., 2012). For neem-derived products, nonaqueous extracts are generally the most toxic, while unprocessed materials and pure phytochemicals from the neem tree have relatively low toxicities (Boeke *et al.,* 2004). Given the currently available information summarized here, an important goal of future antimicrobial testing of neem oil and its products should be the standardization of extracts and administration methods.

Conclusion

Azadirachta indica plant extract had shown a broad-spectrum antimicrobial activity. This confirms the presence and strength of bioactive compounds present in the plant. Dua *et al.,* (1995) affirmed that the plant extract had the best antibacterial activity and also proved the usage of the plant in health maintenance. In recent years, there are several groups who have incorporated neem into novel materials and technologies that have broad implications for human health. Specifically, green synthesized copper or silver nanoparticles and hydrogels, nanocellulose films, chitosan-copper oxide biopolymers, and hydroxyapatite have all been constructed to include neem extracts and have substantial antimicrobial activity, including against multidrug-resistant bacterial species. Both in vitro and in vivo data suggest that these composite materials represent a growing industry of creative antimicrobial technologies that have the potential to revolutionize infectious disease treatments and biomedical science as a whole. (Nagaraj and Samiappan, 2019; Revathi and Thambidurai, 2019; Algebaly *et al.*, 2020; Asghar and Asghar, 2020; Lakkim *et al.*, 2020).

Recommendation

In order to develop realistic A. indica-based treatment regimens that could be used in humans, there are clearly many intriguing areas for future investigation. Undoubtably, future experiments will need to elucidate the mechanisms of action of neem and the associated phytochemicals. Given the available data summarized in this review, some of the most promising areas of investigation moving forward appear to be:

1) The application of individual neem phytochemicals and derivatives thereof as antimicrobial agents alone or used in combination with existing treatments.

2) The inclusion of hundreds of available medicinal plant products, extracts, and phytochemicals in screens for potential inhibitors of emerging and resistant infectious diseases.

It is also important to note that to gain maximal utility from these areas of research, close attention should be paid to the types of extracts (including both the particular part of the plant and the solvent) that have already been tested against which organisms. Some level of standardization should be considered so that comparisons can be made and patterns can be recognized across multiple studies.

REFERENCE

Alzohairy, M.A. (2016). Therapeutic role of *Azadirachta indica* (Neem) and their active constituents in diseases prevention and treatment. *Evid Based Complement Alternate Med*; 7382506.

Ahmad, A., Ahad, A., Rao, A. Q., and Husnain, T. (2015). Molecular Docking Based Screening of Neem-Derived Compounds with the NS1 Protein of Influenza Virus. Bioinformation 11 (7), 359–365. doi:10.6026/97320630011359

Ahmad, A., Javed, M. R., Rao, A. Q., and Husnain, T. (2016). Designing and Screening of Universal Drug from Neem (Azadirachta indica) and Standard Drug Chemicals against Influenza Virus Nucleoprotein. BMC Complement. Altern. Med. 16 (1), 519. doi:10.1186/s12906-016-1469-2

Algebaly, A. S., Mohammed, A. E., Abutaha, N., and Elobeid, M. M. (2020). Biogenic Synthesis of Silver Nanoparticles: Antibacterial and Cytotoxic Potential. Saudi J. Biol. Sci. 27 (5), 1340–1351. doi:10.1016/j.sjbs.2019.12.014

Asghar, M. A., and Asghar, M. A. (2020). Green Synthesized and Characterized Copper Nanoparticles Using Various New Plants Extracts Aggravate Microbial Cell Membrane Damage after Interaction with Lipopolysaccharide. Int. J. Biol. Macromol. 160, 1168–1176. doi:10.1016 j.ijbiomac.2020.05.198

Ashafa, A. O., Orekoya, L. O., and Yakubu, M. T. (2012). Toxicity Profile of Ethanolic Extract of Azadirachta indica Stem Bark in Male Wistar Rats. Asian Pac J. Trop. Biomed. 2 (10), 811–817. doi:10.1016/S2221-1691(12)60234-2

Arivazhagan, S., Balasenthil, S. and Nagini, S., *Cell Biochem.Funct*., **2000**, 18, 17–21.

Biswas, K., Chattopadhyay, I., Banerjee, R.K., Bandyopadhyay, U. 2002. Biological activities and medicinal properties of neem (Azadirachtaindica) Current Science, 82 (11), 1336–1345.

Braga, T. M., Rocha, L., Chung, T. Y., Oliveira, R. F., Pinho, C., Oliveira, A. I., et al. (2021). Azadirachta indica A. Juss. In Vivo Toxicity-An Updated Review. Molecules 26 (2). doi:10.3390/molecules26020252

Biswas, K., Chattopadhyay, I., Banerjee, R.K., Bandyopadhyay, U. 2002. Biological activities and medicinal properties of neem (Azadirachtaindica) Current Science, 82 (11), 1336–1345.

Banerjee, S; Kim,. Shariff, M; Khatoon, H., & Yusoff, S.M.(2012). Antibacterial activity of Neem (Azadrachta indica). Leaves of Vibrio spp. Isolated from cultured shrimp. Asian J. Animal Veterinary ADV, 3923: 1-7.

Bae, J., and Jeon, B. (2013). Increased Emergence of Fluoroquinolone-Resistant Campylobacter jejuni in Biofilm. Antimicrob. Agents Chemother. 57 (10), 5195–5196. doi:10.1128/AAC.00995-13

Boeke, S. J., Boersma, M. G., Alink, G. M., van Loon, J. J., van Huis, A., Dicke, M., et al. (2004). Safety Evaluation of Neem (Azadirachta indica) Derived Pesticides. J. Ethnopharmacol. 94 (1), 25–41. doi:10.1016/j.jep.2004.05.011

Campos, E.V; de Oliveira, J.L; pascoli, M, de lima, R., & Fraceto, L.F. (2016). Neem oil and grop protection from now to the future. Front plant Sci;7: 1494.gtg

Chaube, S.K., Shrivastav, T.G. Timari, M., Prasad, S., Tripathi, A., & Pandey, A.K. (2014). Neem (Azadirachta indica) leaf extract deteriorates oocyte quality by inucing Ros-mediated apoptosis in mammals. Springerplus. 3:464.

Dhanya Kumar, N.M., Sidhu, P. 2011. The antimicrobial activity of AzadirachtaIndica, Glycyrrhizaglabrat, Cinnamumzeylanicum, Syzygiumaromaticum, Acacia nilotica on Streptococcus mutans and Enterococcus faecalis: An in vitro study. Endodontology, 23, 18–25.

Divya Kumya, P., Khijimatgar, S., Shenoy, S.M., Chomdhury, A., Lynch, E., And Chomdhury, C.R.(2019). Antibacterial activity of new atraumatic restorative treatment materials Incorporated with Azadirachta indica (Neem) against Streptococcus mutans. J Oral Bio Ganiofac Res. 9(4), 321-5.

Dua, V.K., Nappal, B.N., & Sharma, V.P. (1995). Repellent action of Neem cream against mosquitoes. Indian J Malarial. 32(2), 47-53.

De Vor, L., Rooijakkers, S. H. M., and van Strijp, J. A. G. (2020). Staphylococci Evade the Innate Immune Response by Disarming Neutrophils and Forming Biofilms. FEBS Lett. 594 (16), 2556–2569. doi:10.1002/1873-3468.13767

Govindachari, T.R., Suresh, G., Gopalakrishnan, G., Banumathy, B., Masilamani, S. 1997. Identification of antifungal compounds from the seed oil of Azadirachtaindica. Phytoparasitica, 26 (2), 109–116.

Gupta, S.K., Prakash, J., Srivastava, S. 2001. Validation of traditional claim of Tulsi, Ocimum sanctum Linn. as a medicinal plant. Indian Journal of Experimental Biology, (40), 765–73.

Girish, K., Neem, S.B.S. 2008. A green treasure. Electronic Journal of Biology, 4, 102–111.

Jerobin, J. Makwana, P., Suresh Kumar, R.S., Sudaramoorthy, R, Mukherjee, A. & Achandrasekaran N. (2015). Antibacterial activity of Neem nanoemulsion and its toxicity assessment of human lymphocytes in vitro. Int J Nanomedicine. 10(1), 77-86.

Kher, A., Chaurasia, S.C. 1997. Antifungal activity of essential oils of three medical plants. Indian Drugs, (15), 41–42.

Kelmanson, J. E., Jager, A.K., & Van Stadan, J., (2000). Zulu medicina plants with antibacterial activity. J Ethanpharmscol. 69(3), 241-6.

Lessa, F.C., Nogueira, I., Vargas Fda S., Spolidorio, D.M., Hebling, J., García-Godoy, F. 2010. Direct and transdentinal antibacterial activity of chlorhexidine. American Journal of Dentistry, 23: 255–9.

Khan MA, Yaqoob S, Ahmad S. Antimicrobial activity of azadirachta indica, against target pathogens and its utility as a disinfectant and floor cleaner. J Evolution Med Dent Sci 2021;10(25):1899-1903, DOI: 10.14260/jemds/2021/392

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.

Mafou-Sonhafouo z V., Kana, J.R., & Dongmo, K. (2009). Effects of graded leaves of Azadirachta indica seed oil on growth performance and biochemical profiles of broiler chickens. Vet Med Sci. 5(3), 442-50.

Nagaraj, A., and Samiappan, S. (2019). Presentation of Antibacterial and Therapeutic Anti-inflammatory Potentials to Hydroxyapatite via Biomimetic with Azadirachta indica: AnIn Vitro Anti-inflammatory Assessment inContradiction of LPS-Induced Stress inRAW264.7 Cells. Front. Microbiol. 10, 1757. doi:10.3389/fmicb.2019.01757

Prabhat, Ajaybhan, Navneet, Chauhan, A. 2010. Evaluation of antimicrobial activity of six medicinal plants against dental pathogens. Report and Opinion (2), 37–42.

Prashant, G.M., Chandu, G.N., Murulikrishna, K.S., Shafiulla, M.D. 2007. The effect of mango and neem extract on four organisms causing dental caries: Streptococcus mutans, Streptococcus salivavius, Streptococcus mitis, and Streptococcus sanguis: An in vitro study. Indian Journal of Dental Research, (18), 148–51.

Quelemes, P. V., Perfeito, M. L., Guimarães, M. A., dos Santos, R. C., Lima, D. F., Nascimento, C., et al. (2015). Effect of Neem (Azadirachta indica A. Juss) Leaf Extract on Resistant Staphylococcus aureus Biofilm Formation and Schistosoma Mansoni Worms. J. Ethnopharmacol. 175, 287–294. doi:10.1016/j.jep.2015.09.026

Revathi, T., and Thambidurai, S. (2019). Cytotoxic, Antioxidant and Antibacterial Activities of Copper Oxide Incorporated Chitosan-Neem Seed Biocomposites. Int. J. Biol. Macromol. 139, 867–878. doi: 10.1016/j.ijbiomac.2019.07.214

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.

Singh, N., Sastry, M.S. 1997. Antimicrobial activity of Neem oil. Indian Journal of Pharmacology, (13), 102–106.

Samie, A., Tambani, T., Harshfield, E., & Green, E. (2010). Antifungal activities of selected venda medicinal plants against *Candida albican*s, *Candidi akrusei* and *Cryptococcus neoformans* isolated from South African AIDS patients. *African Journal of Biotechnology*, *9,* 265-276.

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.

Vestby, L. K.,Grønseth, T., Simm, R., andNesse, L. L. (2020). Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiot. (Basel) 9 (2). doi:10.3390 antibiotics9020059

WHO (2020). Burden of Disease. Available: https://www.who.int/teams/globalinfluenza programme/surveillance-and-monitoring/burden-of-disease.

Yonezawa, H., Osaki, T., Hojo, F., and Kamiya, S. (2019). Effect of Helicobacter pylori Biofilm Formation on Susceptibility to Amoxicillin, Metronidazole and Clarithromycin. Microb. Pathog. 132, 100–108. doi:10.1016/j.micpath.2019.04.030

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

Zingue, S., Kamga Silihe, K., & Fouba Bourfane, I. (2019). Potential of regular consumption of Cameroonian Neem (*Azadirachta indica*) oil for prevention of the 7,12-Dimethylben (a) anthracene induced breast cancer in high-fat/ sucrose-fed wistar rats, *Evid Based Complement Alternate Med*. 2031460.