



Anti-Microbial Comparative Analysis Of *Azadirachta Indica* (Neem) And *Morinda Lucida* (Oruwo) Leaves For Morbidity Control In Selected Bacterial Isolates

Owonibi Sustain Kehinde, Olufade Ikeolu Idowu, Bolaji Ayokunle Samuel, Attah Titilola
Olubunmi, Lawal Isiaka Abife.

Department of Science Laboratory Technology, The Federal Polytechnic, P.M.B. 231, Ede, Nigeria.

*Correspondence author: E-mail: owonibisk@gmail.com (S.K. Owonibi), Tel: +2348038191951

Abstract - The use of plants and their extracts to treat infections is a practice in the olden days. Ethanolic extract of leaf of *Azadirachta indica* known as neem plant and *Morinda lucida* were investigated for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Pseudomonas originosa* using agar diffusion method. The effectiveness of the plant extracts on bacterial isolates was investigated. The zone of inhibition of *Azadirachta indica* against four bacterial isolates was measured ranging from (1-9mm). It has effect on *Escherichia coli* and *Staphylococcus*, and least effect on *Streptococcus pyogenes* and *Pseudomonas originosa*. The zone of inhibition of *Morinda lucida* on four bacterial isolates was measured ranging from (1-8mm). It has more performance on *Escherichia coli* and *Staphylococcus aureus* *Streptococcus pyogenes*. Both plant extracts were found to be effective against the selected pathogens especially *Escherichia coli* and *Staphylococcus aureus* and least performance on *Streptococcus pyogene*. Hence, both plants were found to possess antimicrobial effect that can be credulous in the treatment of diseases especially those caused by the four pathogens.

1.0 INTRODUCTION

Medicinal plants are plants used in treating and preventing specific ailments and diseases that affect animals and human beings. Medicine from plant sources have been in use throughout ages and thus play significant role among the rural dwellers. About 80% of the people in the developing countries rely on the use of botanicals for their primary health care (Muthu *et al.*, 2006). There had been an increased interest in various disciplines on the importance of medicinal plants and the contribution of phytomedicine to the well-being of great number of the world's population (Bimpa *et al.*, 2007).

Finding healing powers in plants is an ancient thought. Plant derived substances have recently become of great interest owing to their versatile applications (Baris *et al.*, 2006, Das *et al.*, 2010). Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases that are generally considered to be harmful to humans (Anselem, 2004). Wild vegetables in particular, play significant roles in the livelihood of many communities in the developing countries of the world as food and for medicinal purposes (Arowosegbe, 2013). Many of these vegetables are underutilized because of inadequate scientific knowledge of their nutritional potentials (Awobajo *et al.*, 2010) as well as their medicinal uses (Jimoh *et al.*, 2010).

Man depends on plants not only for food, but also to cure his various ailments since several thousand years. Some plant decoctions are of great value in the treatment of diarrhea or gastrointestinal disorder, urinary tract infections, skin infections, infertility, and wound (Ergene *et al.*, 2006). Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant (Abreu *et al.*, 2012). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Duraipandiyan *et al.*, 2006, Djeussi *et al.*, 2013).

•

Microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs (Ahmad *et al.*, 1998). Furthermore, antibiotics are sometimes associated with side effects (Cunha, 2001), whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature (Vermani and Garg, 2002).

The desire to capture the wisdom of traditional healing systems has led to a resurgence of interest in herbal medicines (Tyler, 2000), particularly in Europe and North America, where herbal products have been incorporated into so-called 'alternative', 'complementary', 'holistic' or 'integrative' medical systems. Recently the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants (Bisignano *et al.*, 1996; Lis-Balchin and Deans 1996; Moaz and Neeman, 1998; Hammer *et al.*, 1999).

For herbal products in common use, evidence of efficacy may be based upon traditional use, testimonials, clinical studies, both controlled and uncontrolled, and randomized, double blind, and placebo-controlled trials. The aim of this research work is to find out antibacterial effect of *Azadirachta indica* and *Morinda lucida* extracts on the bacteria isolates bridging the gap between assumption and facts provided by scientific testing.

2.0 MATERIALS AND METHODS

2.1. Sample collection

The medicinal plants used in this study were the leaves *Azadirachta indica* and *Morinda lucida*, which were duly identified to avoid collection of wrong samples. They were obtained from the different communities in Ede Local Government Area, State of Osun, Nigeria. The samples were immediately transported to the laboratory for use. The test organisms were obtained from the Microbiology Department, Federal Polytechnic Ede, Osun State. The collected plant materials were washed with distilled water to reduce microbial load to a large extent. They were further air dried and after sufficiently dried, a warring industrial blender was used to crush the leaves to powder and then weighed.

2.2. Extraction of leaves material

The extraction method described by Agbafor (2004) was adopted using ethanol as the extraction solvent.

2.3. Collection and Maintenance of Test Organisms

Pure clinical isolates of *Escherichia coli*, *Pseudomonas originosa*, *Streptococcus pyrogenes* and *Staphylococcus aureus* were collected from the department of Microbiology, Federal Polytechnic, Ede, Osun State, Nigeria. The isolates were stored in refrigerator at 4°C in the laboratory.

2.4. Phytochemical screening

Preliminary phytochemical analysis was carried out on the plant extracts which was done with method of Harbone (1998) and Parekh and Chanda (2007)

2.5. Evaluation of the Antibacterial Effects

0.1 ml of inoculums were introduced into the molten nutrient agar in a sterile Petri-dish and were evenly spread with swab stick, sterile 6 mm cork borer was used to bore 3 holes and 1 ml, 2 ml, 3 ml of the prepared extracts were introduced into the holes accordingly, the inoculated plates were allowed to stand for sometimes for proper diffusion of the extracts into the medium and were incubated at 37°C for 24

hours and examined for zone of inhibition. Each zone of inhibition was measured in millimeter with a ruler.

3.0 RESULT AND DISCUSSION

3.1 RESULTS

TABLE 1- Zone of Inhibition of Ethanolic Extracts of *Azadirachta indica* and *Morinda lucida* against the selected pathogens in (mm)

Extract	Solvent	Volume (mL)	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pyrogenes</i>	<i>Pseudomonas originosa</i>
<i>A. indica</i>	Ethanol	1	5mm	4mm	-	-
		2	6mm	7mm	1mm	2mm
		3	9mm	8mm	3mm	6mm
<i>M. lucida</i>	Ethanol	1	2mm	2mm	-	-
		2	5mm	4m	-	-
		3	8mm	5mm	2mm	1mm

(-): no antimicrobial activity.

TABLE 2 – Result for Photochemical screening

S/N	Phytochemicals	<i>Azadirachta indica</i>	<i>Morinda lucida</i>
1.	Flavonoids	++	++
2.	Saponins	+	+
3.	Tannin	+	+
4.	Glycosides	-	-
5.	Phlobatannins	+	-
6.	Anthraquinones	+++	++
7.	Steroid	++	+
8.	Alkaloids	+++	++
9.	Terpenoids	-	-

(+) = Present in minute concentration

(++) = Present in moderate concentration

(+++)= Present in high concentration

(-) = Not present

3.2 DISCUSSION

From table 1, *in vitro* test of leaf extract of *Azadirachta indica* and *Morinda lucida* at different concentrations against four pathogenic bacterial strains, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, and *Pseudomonas originosa*, is shown which were assessed in terms of zone of inhibition of bacterial growth. The ethanol extract of *Azadirachta indica* and *Morinda lucida* revealed an inhibitory effect against the four bacterial isolates with a zone of inhibition ranging from (1-9mm). The extract of *Azadirachta indica* had effect on *Escherichia coli* and *Staphylococcus aureus* at any concentration. The zone of inhibition increased as the amount of extracts was increased. It also had effect on *Streptococcus pyrogenes* and *Pseudomonas spp* when the amount of extract added was increased. The extract of *Azadirachta indica* showed better antibacterial activity compared to the extracts of *Morinda lucida*.

Table 2 shows the phytochemical constituents of ethanolic extract of *Azadirachta indica* leaves to contain steroid, anthraquinone in relatively high concentration, flavonoids, phlobatannins, tannins, alkaloids and saponins in medium and low concentration while glycoside and terpenoid are not present. Flavonoids, saponins, tannins, anthraquinone, steroid and alkaloids were present in *Morinda lucida* which show that it is highly medicinal and good for human consumption. The presence of flavonoid in both extract, which

are strong antioxidant, make them good candidates for medicinal use. The beneficial medicinal effect of plant materials basically results from the secondary products present in the plant and is not usually attributed to a single compound but a combination of the metabolites (Parekh *et al.*, 2005).

At present, most pathogenic bacteria develop antibiotic resistance. Thus to overwhelm this frightening problem, it is an urgency to discover a number of novel active compounds. Extracts from these plants which are biologically active compounds can be applied for the synthesis of potent drugs (Chabuck *et al.*, 2018).

4.0 CONCLUSION

The results presented from the phytochemical screening of *Azadirachta indica* and *Morinda lucida* leaf extracts revealed the presence of anthraquinone, flavonoids, saponins, tannins, steroid and alkaloids at different concentration confirming the great potential of these bioactive compounds and its usefulness in treatment of various diseases. The antimicrobial activity of *Azadirachta indica* and *Morinda lucida* on the test organisms indicates that the leaves contain good antibacterial activity thereby pointing to their importance as a source of alternative medicine. Thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro should be subjected in vivo testing to evaluate the efficacy in controlling the incidence of disease in crops, plants, and humans.

References

- Abreu, A.C., A.J. Mcbrain and M. Simeos. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. *Natural Product Report*, no.29, pp. 1007-1021.
- Adesogan, E.K. (1973). Anthraquinones and anthraquinols from *Morinda lucida*: The biogenic significance of Oruwal and Oruwalol. *Tetrahedron*, 29: 4099-102.
- Agbafor, K.N. (2004). The effect of aqueous and organic extracts of fresh leaves of *Baphia nitida* on tissue acetylcholinesterase in guinea pigs. *Journal of Sci. Technol.*, 10: 1-8
- Ahmad, I., Mehmood, Z., and Mohammad, M. (1998). Screening of some Indian medicinal plants for their microbial properties. *Journal of ethnopharmacology*. DOI: 10. 1016/S0378-8741(98)00055-5.
- Anselem, A. (2004). Herbs for healing pax herbals Edo State, Nigeria
- Arowosegbe, S. (2013). Preliminary domestication and cultivation efforts on some medicinally important wild vegetables in Ado-Ekiti, Nigeria. *Bio-Sci. Res. Bull.* 29(2): 101 -107.
- Awobajo, P.O., Olatunji-Bello, I.I., Obilade, T.T. and Odugbemi, T.O. (2010). Knowledge of the nutritional and medicinal use of some vegetables among a cross section of market women in two major food markets in Lagos State, South West Nigeria. *Pak. J. Nutr.* 9(3): 216,221.
- Baris, O., Gulluce, M., Sahin, F., Ozer, H., Kilic, H., Ozkan, H., Sokmen, M., Ozbek, T. (2006). Biological activities of the essential oil and methanol extract of *Achillea Biebersteinii* Afan. (Asteraceae). *Turk. J. Biol.* 30: 65-73.
- Bimpa, P.N.f., Agbor, G.A., Oben, J.E., and Ngogang, J.Y. (2007). Phytochemical studies and Antioxidant properties of four Medicinal plants used in Cameroon. *Afr. J. Trad. CAM* 4(4): 495-500.
- Bisignano G, Germanó MP, Nostro A Sanogo R ((1996). Drugs used in Africa as dyes: antimicrobial activities. *Phytother. Res.* 9: 346-350.
- Cunha, B.A. (2001). Antibiotics side effect. *Medical Clinics of North America*, 85(1):149-85.
- Chabuck, Z., Hadi, B., and Hindi, N. (2018). Evaluation of Antimicrobial Activity of Different Aquatic Extracts Against Bacterial Isolates from UTI in Babylon Province, Iraq. *Journal of Pure and Applied Microbiology*, vol. 12(2), p. 693-700.
- Das, K., Twari, R.K.S., and Shrivastava, D.K. (2010), Techniques for evaluation of medicinal plant products as antimicrobial agent: current methods and future trends. *Journal of Medicinal Plants Research*, vol. 4, no. 2, pp. 104-111.
- Djeussi, E.D., Noumedem, J.A., Seukep, J.A., Fankam, A.G., Voukeng, I.K., Tankeo, S.B., Nkuete, A.H., and Kuete, V. (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complementary and Alternative Medicine*, 13:164
- Dureipandigan, V., Ayyanar, M., and Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine*, 6:35
- Ergene, A., Guler, P., Mrici, S., Hamzaoglu, E., and Duran, A. (2006). Antibacteria and antifungal activity of

- Heracleum sphondilium* subsp. *Artvinense*. *African Journal of Biotech.* vol. 5 (11), pp. 1087-1089.
- Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* 86(6): 985.
- Harborne, J.B, (1998). Classes and functions of secondary products from plants, In N.J. Walton and D.E. Brown (Eds). *Chemicals from Plants*, London: Imperial College Press, pp. 1-25.
- Jimoh, P.O., Adedapo, A.A., AHero, A.A., Koduru, S. and Afolayan, A.J. (2010). Evaluation of the polyphenolic, nutritive and biological activities of the acetone, methanol and water extracts of *Amaranthus asper*. *The Open Complementary Medicinal Journal*, Vol. 2, 7-14.
- Lis-Balchin M, Deans SG (1996). Antimicrobial effects of hydrophilic extracts of *Pelargonium* species (Geraniaceae). *Lett. Appl. Microbiol.* 23: 205-207.
- Maoz M, Neeman I (1998). Antimicrobial effects of aqueous plant extracts on the fungi *Microsporum canis* and *Trichophyton rubrum* and on three bacterial species. *Lett. Appl. Microbiol.* 26: 61-63.
- Muthu, C., Agyanar, M., Raja, N. and Ignacimuthu, S. (2006). Medicinal used by traditional healers in Kanchaepuram District of Tamil Nadu. India. *J. Ethnomed.* 2: 43.
- Nweze, E.L., Okafor, J.L., and Njoku O. (2004). Antimicrobial Activities of Methanolic extracts of *Trume guineensis* (Scchumn and Thorn) and *Morinda lucinda* used in Nigerian Herbal Medicinal practice. *Journal of Biological Research and Biotechnology.* 2(1): 34-46.
- Tyler, V.E. (2000). Herbal medicine: From the past to the future. *Public Health Nutr.* 3:447-452.
- Udeinya, I.J. (1993). Antimalarial Activity of Nigerian Neem Leaves. *Trans. Royal Soc. Trop. Med. Hyg.* -87:471.
- Parekh, J., Jadeja, D., Chanda, S. (2005). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turk. J. Biol.* 29: 203-210.
- Parekh, J., and Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, 10: 175-181.
- Vermani, K., Garg, S. (2002). Herbal medicines for Sexually Transmitted Diseases and AIDS. *Journal of Ethnopharmacology*, 80: 49-66.