

Full Length Research Paper

The antibacterial activity and some trace elemental compositions of the extracts of *Piliostigma thonningii*, a widely used ethnomedicinal tree in Minna, Nigeria

M. B. Etsuyankpa^{1*}, M. M. Ndamitso², M. A. T. Suleman², Jimoh.O.Tijani², S. Idris², Shaba E. Y.² and A. Mohammed³

¹Chemistry Unit, Centre For Preliminary and Extramural Studies, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria.

²Department of Chemistry, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria.

³Department of Micobiology, Federal University of Technology, P.M.B 65, Minna, Niger State, Nigeria.

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The study on antibacterial activity of the extracts of the leaf, stem bark and roots of *Piliostigma thonningii* (Schum) Milne-Redh (Ceasapinaceae) (an underexploited plant in Nigeria) at 25 mg/cm³ each using agar diffusion method, was carried out. The samples were collected from Gidan Kwano Campus of Federal University of Technology, Minna (North Central Nigeria). The extracts of these parts exhibited appreciable activities against the test organisms: *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Salmonella typhi* and *Escherichia coli*. The results obtained in this study corroborated the local medicinal applications of the plant by traditional practitioners in this part of the country.

Key words: *Piliostigma thonningii*, antibacterial activity, elemental composition, *Escherichia coli*.

INTRODUCTION

Generally, persistent contamination of media by pathogenic microorganisms leads to various disease conditions in human life and in recent years, the antimicrobial efficacy of many plant extracts and metals has been investigated against microorganisms including *Staphylococcus aureus*, *Klebsiella* spp., *Escherichia coli*, *Klebsiella* spp., *Streptococcus pyogenes*, *Bacillus subtilis* and *Pseudomonas aeruginosa* (Harris et al., 2002; Kone et al., 2004; Drevensek et al., 2006; Jigna and Sumitra, 2007).

B. subtilis, a ubiquitous rod shaped peritrichous Gram-positive bacterium commonly found in water, soil, air and decomposing plant residues (Ashlea et al., 2008), is used in fermentation processes although it has been reported to produce an extracellular toxin (subtilism) which causes

allergic reactions in individuals repeatedly exposed to it (EPA, 1997). *Proteus vulgaris* on the other hand, is a peritrichous Gram-negative bacillus of the family Enterobacteriaceae which inhabits the intestinal tracts of humans and animals. It is found in soil, water and faecal matters especially those of patients with compromised immune systems. It is a multidrug resistant bacterium that causes urinary and wound infections (Yu et al., 2011). *P. aeruginosa* is a Gram-negative aerobic rod shaped bacterium found in soil, water, skin, flora and most man-made environments (Stojek et al., 2008; Philip et al., 2009). It causes diseases like endocarditis, bacteremia, central nervous system, eye, ear, bone and joint infections. *Salmonella typhi* on the other hand, is a Gram-negative microbe that causes typhoid fever, a disease

*Corresponding author. E-mail: jimohtijani@futminna.edu.ng.

that affects over 16 million people in the world annually (Shangkuan and Lin, 1998; Parkhill et al., 2001; Ganai et al., 2009). It is a multiple drugs resistant bacterium (Cristina and Claudia, 1994). *S. aureus* is a Gram-positive non-spore forming facultative anaerobic bacterium that has high multidrug resistance (Harris et al., 2002; Waters et al., 2011). It has been responsible for causing skin and soft tissue, blood and cardiovascular system diseases. Also, *E. coli* commonly found in the lower intestine of warm blooded organisms is also a gram-negative facultative anaerobic bacterium. It causes uncomplicated infections of the urinary tract (Gabriela et al., 2011) while *Klebsiella pneumonia* is a Gram-negative, non-motile, encapsulated rod-shaped bacterium of the family Enterobacteriaceae which induces septicemia, pneumonia, pneumococcal meningitis and peritonitis (Podschun and Ullmann, 1998) whereas *Streptococcus pyogenes* is responsible for many childhood diseases (Fox and Rohovsky, 1975; CWB Info.com, 1999; Clark, 2000; CDC, 2006; Microbiology Bytes, 2007).

Metals play a wide variety of roles in biological systems acting as cofactors in enzymes and as components of drugs for the treatment of various diseases and conditions (Piedad et al., 2008). It is in this wise that iron plays a key role in the lives of living organisms and a metal that is required for their growth in addition to the critical role it plays in the pathogenesis of infections (Yukihiro et al., 2007). Iron is therefore, used either singly or in combination to combat pathogenic microbial growth in media (Saeed and Farshid, 2008; Scott et al., 2009; Joshi et al., 2011). Studies on the use of various forms of copper as an antimicrobial agent either as a single entity or in combination with other metals or chemical substances have been reported (Yu-Sen et al., 1996; Copper Development Association, 2004; Aisha, 2005; Jayesh et al., 2008; Wheeldon et al., 2008). In these forms, it plays significant role in improving public health functioning at low concentrations as a bacteriostat, fungicide, antiviral, antimould, anti-algae in drinking water and wood preservative (Mike and Craig, 2008; Rabin et al., 2010). It is also one of the relatively small groups of metallic elements essential to human health which is required in trace amounts for the growth and functioning of organisms (Rabin et al., 2010). In addition, magnesium is required for the growth of organisms apart from the fact that it plays an important role in the pathogenesis of infections. Thus, magnesium and most especially its compounds or complexes are employed in chemotherapy for the control of microorganisms (Drevensek et al., 2006; Lellouche et al., 2011). Chromium is a heavy metal that plays a key role in the microbial activity of plant extracts by making these phytochemicals unavailable for antimicrobial activities (Ata-Ullah et al., 2011). At certain concentrations however, this metal could also act as an antimicrobial agent. Zinc plays a key role in the lives of living organisms and is a metal that is required for their growth. It occurs in small amounts in almost all igneous rocks with its principal ores being sul-

phides like sphalerite and wurtzite. However, taking too much of this metal into the body affects human health. This is because it binds to particulate matter and its soluble species are readily available for biological reactions thus making the metal a very toxic one. Therefore, this metal also plays a vital role either alone or in its combined forms in the pathogenesis of infections.

Numerous studies have shown that medicinal plants are the oldest health care products with proven efficacy serving as the basic components of several hard drugs, analgesics, anaesthetics, antibiotics, anti-cancer, anti-parasitic, anti-inflammatory, oral contraceptives and diuretic drugs (Sofowora, 1982; WHO, 1995). It has also been shown that 80% of world's populations rely entirely on local medicine made almost exclusively from plants especially in developing countries like Nigeria where multiple drugs resistance to several orthodox antibiotics is being experienced (WHO, 1995; Akinpelu et al., 2000; Saidu et al., 2000; Adiaratou et al., 2005). For instance, *Abrus precatorius* L. which belongs to the family Fabaceae, has been reported to be used for the treatment of cough, convulsion, rheumatism and as a labour inducer while the fruits and leaves of *Aframomum melegueta* K. Schum of the family Zingiberaceae are used for the treatment of catarrh, small and chicken poxes (Ige, 2011). Furthermore, plants such as *Amaranthus spinosus* and *Annona senegalensis* Pers are used for the treatment of piles, abdominal pains and snake bites (Ige, 2011). Also, the use of *Allium sativum* to reduce the cholesterol levels and boost the immune system of the human body has been reported (Oyeleke et al., 2008), while ginger and garlic have been reported to have inhibitory effects on the growth of coliform bacteria as well as *E. coli*, *Proteus vulgaris*, *S. aureus* and *Salmonella* spp. (Kennedy et al., 2007).

Piliostigma thonningii (Schum) Milne-Redh (Caesalpinaceae) is found growing abundantly as a wild uncultivated small tree in many parts of Nigeria such as Minna, Zaria, Bauchi, Ilorin, Plateau, Lagos and Abeokuta (Keay, 1989). It is a leguminous plant belonging to the family Caesalpinaceae, a family that comprises trees and shrubs. Our attention was drawn to this plant because of several claims on its enormous applications as an ethno-medicinal plant used to cure several diseases including the common malaria, dysentery, fever, infectious respiratory ailment, snake bite, hookworm, hepatobiliary ailments, hydropsy, sterility, rachitis and skin diseases (Tira-Picos et al., 2010).

Although, several countries have already recognized the importance of traditional medical applications in their health care delivery systems (WHO, 1995), traditional healers in Nigeria have not yet been given the position they deserve despite the fact that a large proportion of the population still rely heavily on traditional practitioners, including traditional birth attendants (where traditional birth attendants assist up to 95% of all rural birth), herbalists, bonesetters and a host of local medicinal plants to

satisfy their primary health care needs. These different parts of this plant in order to establish or otherwise debunk the medicinal values of *P. thonningii*.

MATERIALS AND METHODS

Fresh samples of the leaves, roots and stem barks of the plant were randomly taken from the premises of Gidan Kwano Campus of the Federal University of Technology, Minna, Niger State, Nigeria. This plant in the area is found growing freely as a wild small tree. Samples of the root, stem bark and leaves of the plant were collected in three batches between the months of May and July, 2011 and the plant was identified by Professor Z. I. E. Ezenwa of the School of Agriculture and Agricultural Technology, Department of Soil Science, Federal University of Technology, Minna, Nigeria. After sampling, the samples were taken to the laboratory, detached from the twigs, carefully sponged with water containing little detergent and well rinsed with deionized water to remove the surface contaminants. They were then dried at ambient temperature in the laboratory to avoid heat destruction of the active components of the samples. The dried samples were ground into study explored the antibacterial activities of extracts from fine powder by pounding mechanically with clean sterile pestle and mortar to increase the surface area. The ground samples were kept in airtight polyethene bags for further use. When required, the dried ground samples were extracted with four different solvents namely water, petroleum ether, chloroform and methanol. Each sample was separately extracted by using the solvents in turn. 150 g of the powdered sample (in one extraction) was put in a thimble which and introduced into a soxhlet extractor and refluxed for several hours. At the end of all extractions, 9 petroleum ether, 6 methanolic, 3 each of the cold maceration and boiled aqueous extracts of the roots, stem bark and leaves were obtained, respectively. The solvent of each extract was evaporated on the water bath and the residues, after cooling, were covered with aluminium foils for subsequent analysis.

Phytochemical analysis

The crude extracts of the samples were analyzed for their phytochemical constituents using standard methods described by Evans and Trease (1989), Sofowora (1982) and Sofowora (1986).

Antimicrobial analysis

Microorganisms

Antibacterial activity of the obtained plant extracts was evaluated against *S. pyogene*, *P. aeruginosa*, *S. typhi*, *S. aureus*, *E. coli*, *Klebsiella* spp., *P. vulgaris* and *B. subtilis* using agar diffusion method. The strains were obtained from the Department of Microbiology, School of Science and Science Education, Federal University of Technology, Minna, Nigeria and were cultured on a nutrient agar. After activation from stock culture, microorganisms were maintained in the nutrient broth until required for use. Cultures were prepared by growing the strains at 37°C for 24 h in the agar broth and repeatedly sub-cultured in order to obtain pure isolates. These were then Gram stained for proper identification and inoculated into agar slants and stored at 40°C for further use. Before use, each of the test organisms was seeded into 25 cm³ of the nutrient agar to produce a culture whose turbidity was 0.5 on the McFarland scale corresponding to a concentration of 1.0×10^8 colony forming units per cm³ (Saeed and Farshid, 2008). For pre-diffusion, the plates were kept at room temperature for 2 h then incubated at 37°C for 24 h. A total of 60 plates which were randomly divided into

ten test groups with six plates each with each of the test organism tested were used. Under identical incubation and aseptic conditions, positive and negative controls with and without inoculums were prepared.

Agar diffusion test

In this study, nutrient agar which is one of the most widely used *in vitro* media for the evaluation of antibacterial activity that identifies the extracts more likely to have antimicrobial effects on common microorganisms which are highly influenced by the diffusion ability of the extract across the medium was employed. 28.0 g of the powdered commercially prepared nutrient agar was weighed and dissolved in 1 dm³ of sterile distilled water and this was sterilized at 121°C for 15 min. 25 cm³ of this was then dispensed into sterilized single-layered 2 × 10 cm Petri-dishes. Uniform cavities each of 4 mm diameter were drilled at equal intervals in the poured agar by means of a sterile copper loop after 24 h. This was then inoculated with the test bacteria using swab sticks at 37°C for 3 h. The cavities were then filled with the plant extracts immediately after reconstitution (one extract in one hole). This was allowed to stay for 1 h to allow the extracts to be absorbed by the agar. The absorbed nutrient agar was kept in an incubator at 37°C for incubation for 24 h.

Data recording

The diameter of bacterial growth inhibition zones was measured with a millimeter ruler whose accuracy was 0.5 mm. These readings were taken in triplicates in order to minimize error.

Statistical analysis

Statistical analysis was performed using a one-way analysis of variance (ANOVA) for the mean concentrations of the minerals as well as the zones of growth of inhibition among the test organisms using Duncan's multiple range tests and were reported as mean ± SE (standard error) of triplicate values. SPSS 16.0 statistical package was used and the statistical significance of the data obtained was established at $p < 0.05$ or 95% confidence while differences among values were established using post hoc tests.

RESULTS

The antimicrobial activities of the test materials as indicated in Table 1 were determined by the means ± SE (standard errors) of the zones of inhibition in millimeters on all the microorganisms after 24 h. All bacterial strains were inhibited by the test materials with varying degrees of susceptibility. These results revealed that the antimicrobial effect of the aqueous extracts (AE) covered the widest range of microorganisms upon which they were active although the highest zone of inhibition (35 mm) on *E. coli* was exhibited by the petroleum ether extract (PEE) of the roots of this plant. The order of inhibitions of the growth of the test organisms for the root extracts, in decreasing order, was; petroleum ether extract (PEE), methanolic extract (ME), aqueous extract (AE) and chloroform extract (CE) while for the stem extracts, the order was PEE, ME, AE and chloroform extract (CE). The zones of inhibition of growth of organisms by the leaves extracts was in the order PEE = ME > AE > CE. None of

Table 1. The antimicrobial activities (zones of inhibition in mm) of the extracts (100 mg/ml) of the various parts of *P. Thonningii*.

Bacteria	Zones of inhibition (mm)											
	Water			Pet . ether			Methanol			Chloroform		
	Roots	Stem bark	Leaves	Roots	Stem bark	Leaves	Roots	Stem bark	Leaves	Roots	Stem bark	Leaves
<i>Escherichia coli</i>	8.00±0.58 ^a	-	-	35.00±0.72 ^b	-	-	-	-	-	-	-	-
<i>Klebsiella spp</i>	16.00±0.88 ^b	20.00±0.58 ^d	18.00±0.59 ^c	30.00±0.26 ^e	-	-	-	-	-	10.00±0.54 ^a	-	-
<i>Streptococcus pyogene</i>	12.00±0.57 ^a	16.00±0.20 ^b	23.00±0.15 ^c	15.00±0.58 ^b	-	25.00±0.58 ^d	15.00±0.38 ^b	-	-	-	-	-
<i>Staphylococcus aureus</i>	20.00±0.58 ^d	15.00±0.61 ^c	10.00±0.21 ^a	10.00±0.32 ^a	15.00±0.42 ^c	-	13.00±0.54 ^b	23.00±0.48 ^e	-	-	15.00±0.38 ^c	10.00±0.58 ^a
<i>Proteus vulgaris</i>	15.00±0.58 ^b	16.00±0.23 ^b	10.00±0.12 ^a	-	-	-	-	-	-	-	-	-
<i>Salmonella typhi</i>	6.00±0.58 ^a	-	10.00±0.58 ^b	-	34.00±0.32 ^c	-	-	-	-	-	-	-
<i>Bacillus subtilis</i>	5.00±0.00 ^a	6.00±0.24 ^a	10.00±0.24 ^b	-	-	-	17.00±0.28 ^c	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	6.00±0.00 ^a	-	-	-	-	-	-	-	26.00±0.32 ^c	-	10.00±0.24 ^b	-

Values are presented as means±SE of three determinations; values with the same superscript in the same row are not significantly different at $p > 0.05$, - = not active, spp = species, pet. = petroleum.

Table 2. The mineral composition of the extracts of various parts of *P. thonningii* (in mg/kg).

Mineral	Root	Stem bark	Leaf
Copper	0.23±0.02 ^{ab}	0.21±0.01 ^a	0.24±0.01 ^b
Chromium	1.71±0.01 ^a	3.50±0.02 ^b	3.50±0.02 ^b
Magnesium	8.02±0.01 ^a	9.13±0.02 ^c	8.12±0.02 ^b
Iron	2.43±0.03 ^c	1.52±0.02 ^a	1.62±0.02 ^b
Zinc	1.21±0.01 ^b	1.10±0.01 ^a	1.64±0.04 ^c

the stem extracts inhibited the growths of *E. coli* but *B. subtilis* was mildly affected by aqueous extract of the stem bark. Also, for the various leaves extracts, only the aqueous extract inhibited the growth of *S. typhi*. The general order of the sensitivities of these bacteria to the various plant parts extracts in this work was *S. aureus* > *S. pyogene* > *Klebsiella spp.* > *B. subtilis* > *S. typhi* > *P. aeruginosa* > *P. vulgaris* > *E. coli*. The copper contents of the three parts of *P. thonningii* significantly differed from one another (at $p < 0.05$)

although the chromium contents of the stems and leaves were not significantly different (at $p > 0.05$) (Table 2). The magnesium content of the stem-bark of this plant was the highest followed by that of the leaves while the roots had the lowest value. The iron content of the roots was the highest followed by that of the leaves while that of the stem-bark was the lowest ($p < 0.05$). Furthermore, the zinc content of the leaves was the highest while that of the stem bark was the lowest (at $p < 0.05$).

DISCUSSION

In many countries of the world, especially the developing ones, infectious diseases are the leading cause of death and despite the existence of many antibiotics to fight them, antibiotic resistance as well as substandard antibiotics in the developing countries, the control of which has become a global concern (Jigna and Sumitra, 2007). The emergence of these multidrug-resistant pathogens has, in no small measure, affected the clinical effi-

cacy of many of the existing antibiotics. Hence, many infectious diseases are now being treated with herbal remedies throughout the world either as pure compounds or as standardized plant extracts which provide unlimited opportunities for the treatment of ailments caused by the new drug resistant microorganisms. This has thus led the search for more plants that have new antimicrobial components for the treatment of new and re-emerging infectious diseases. Therefore, this research work focused on the folk medical applications of this plant in order to establish its potential antimicrobial activity. Therefore, the survival of these bacteria in different extracts of the plant was compared and from the results, the plant extracts inhibited the growth of various test bacteria at varying degrees which was an indication that the plant possesses active ingredients that can be used to control the growth of these organisms if appropriate concentrations of the extracts are administered. The zones of inhibition of the growths of the microorganisms employed in this work were comparable with those reported for the various extracts of the leaves of *Cassia occidentalis* by Sadiq et al. (2012). In their work, it was observed that 120 mg/ml of the ethanolic and water extracts were not active on the growth of *S. aureus*, various extracts of *P. thonningii* were able to inhibit the growth of this organism in this work. It was also reported that the respective zones of inhibition of the growth of *S. typhi* by the ethanolic and aqueous extracts of *C. occidentalis* leaves were 18 and 17 mm which were higher than the respective 6.0 ± 0.58 and 10.00 ± 0.58 mm obtained in this work for the root and leaves aqueous extracts but lower than the 34.00 ± 0.32 mm obtained for the stem bark PEE. However, the respective 16 and 21 mm zones of inhibition of the growth of *E. coli* by the aqueous and methanolic extracts of the leaves and stem bark of *Ficus capensis* reported by Oyeleke et al. (2008) were higher than the respective 8.00 ± 0.58 and 35.00 ± 0.72 mm obtained for the aqueous and petroleum ether extracts used in this work.

In this study, all the extracts from the three plant parts, except the PEE and ME of the leaves on one hand and CE of the roots on the other hand, were active against the growth of *S. aureus*. In addition, the aqueous extracts of the various parts of *P. thonningii* showed the widest spectrum of growth inhibition on the test organisms probably because of the synergic antimicrobial activities of the total metallic contents of the aqueous extract which was the phase expected to have the highest content of these metals. The result of this study also showed that the aqueous and petroleum ether extracts of the roots inhibited the growth of *E. coli* showing that this plant can likely serve as a good antimicrobial remedy for combating the diseases caused by this bacterium. This thus justified the use of various parts of this plant in ethno-medical preparations in this part of the world for the treatment of such ailments as diarrhoea, abdominal pains, nausea and as a wash, especially for women with urinary tract infections. Furthermore, it was observed that only the

aqueous extracts of the three parts of the plant inhibited the growth of *P. vulgaris*. This indicated that the active components of this plant against this microorganism were likely present in reasonable quantities only in the aqueous extract. Furthermore, since the heavy metals analyzed in this study were present in aqueous phase, they might have had synergic effects on the antimicrobial properties of these extracts especially towards the above mentioned bacterium since some of them have been shown to exhibit antimicrobial activities in various forms (ATSDR, 2011).

The different antimicrobial activities exhibited by the various extracts of *P. thonningii* in this study were in line with the findings of Asuzu et al. (1999), Akinpelu et al. (2000) and Adiaratou et al. (2005) on this plant.

Conclusions

The results of this investigation indicated that *P. thonningii* extracts contained some substantial amounts of phytochemicals that exhibited good antibacterial active-ties and these might have supported the various folk applications of different extracts of this plant to cure ailments from time immemorial. These findings might have also provided research data base for this plant which, hitherto, has been very scanty in this part of the country.

Finally, since some of the extracts employed in this work were able to inhibit the growth of some multiple-drug resistant bacterial strains, *P. thonningii* could be useful in the search for new clinically useful antimicrobial agents.

REFERENCES

- Adiaratou T, Drissa D, Seydou D, Hilde B, Berit SP (2005). Ethnopharmacological survey of different uses of seven medicinal plants from Mali, (West Africa) in the regions Doila, Kolokani and Siby. *J. Ethnobiol. Ethnomed.* 1:7 doi:10.1186/1746-4269-1-7
- Aisha A (2005). Antimicrobial Effects of Copper and Brass ions on the Growth of *Listeria monocytogenes* at different Temperatures, pH and Nutrients. A Dissertation Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College, pp. 4-6.
- Akinpelu DA, Obuotor EM (2000). Antibacterial Activities of *Piliostigmalthonningii* Stem Bark. *Fitoerapia* 71(4):442-443.
- Ashlea ME, Richard L, Roberto K (2008). Ecology and genomics of *Bacillus subtilis*- Review. *Trends Microb.* 16(6): 269-276.
- Asuzu IA, Gray AI, Waterman PG (1999). The antihelmintic activities of D-3 -methylchiroinositol from *Piliostigmalthonningii* Stem Bark. *Fitoerapia* 70:77-79.
- Ata-Ullah, Zahid H, Nida A, Khalid MK, Shahnaz P, Wajid R (2011). The effect of Cadmium and Chromium concentration, on biological activity of *Marsilea minuta*. *J. Chem. Soc. Pak.* 33(6): 874-876.
- ATSDR, Agency for Toxic Substances and Disease Registry (2011). Copper in Drinking Water. Published by the Division of Toxicology, 1600 Clifton Road NE Mailstop F-32 Atlanta, GA 30333. Fax: 1-770-488-4178EPA. Available online at: <http://www.atsdr.cdc.gov/toxfaqs/faq.asp?id=205&tid=37> and retrieved on 20th December, 2011.
- CDC, Centre for Disease Control and Prevention (2006). *Escherichia coli* (O157 : H7). pp.1-6.
- Clark TJ (2000). *Pseudomonas auriginosa*. USMA Health Search Wizard. pp. 1 – 15.
- Copper Development Center (2004). Copper. Available online at: http://www.copper.org.sg/publications/ccca/0309/pg06_japan_research.htm. Accessed on 20th April, 2013.

- Cristina P, Claudia A (1994). In vitro antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi*. J. Ethnopharmacol. 44(1): 41-46.
- Drevensek P, Kosmrlj J, Giester G, Skauge T, Sletten E, Sepčić K, Turel I (2006). X-Ray crystallographic, NMR and antimicrobial activity studies of magnesium complexes of fluoroquinolones - racemic ofloxacin and its S-form, levofloxacin. J. Inorg. Biochem. 100(11):1755-63.
- EPA, US Environmental Protection Agency (1997). Biotechnology Programme under the Toxic Substances Control Acts (TSCA). An Article written on *Bacillus subtilis* Final Risk Assessment. Attachment 1. Final Risk Assessment of *Bacillus subtilis*, February, 1997. Available online at: <http://www2.epa.gov/science-and-technology/substance-and-toxics-science-resources>. Retrieved on 2nd January, 2013.
- Evans WC, Trease GE (1989). *Pharmacognosy*, 12th Edition, ELBS publication, BaillierTindall, East Bourne. pp. 345-359.
- Fox JG, Rohovsky MW (1975). Meningitis caused by *Klebsiella* spp. in two rhesus Monkeys. J. Am. Vet. Med. Assoc. 167: 634 – 636.
- Gabriela H, Michelle C, Nathalie T (2011). Inhibition of *Escherichia coli* CFT073 *fliC* Expression and Motility by Cranberry Materials. Appl. Environ. Microb. 77(19): 6852-6857.
- Ganai S, Arenas RB, Forbes NS (2009). Tumour-targeted delivery of TRAIL using *Salmonella typhimurium* enhances breast cancer survival in mice. Bri. J. Cancer 101: 1683-1691. doi: 10.1038/sj.bjc.6605403
- Harris LG, Foster SJ, Richards RG (2002). An Introduction to *Staphylococcus aureus*, and Techniques for Identifying and Quantifying *S. aureus* Adhesins in Relation to Adhesion to Biomaterials: Review. Eur. Cells Mat. 4:39-60.
- Ige OE (2011). Preliminary Investigations on the Ethnomedicinal Plants of Akoko Division, South West Nigeria. Glob. J. Hortic. Sci. 3(2):84-89.
- Jayesh PR, Arup KC, Siddhartha PD, Suparna M (2008). Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater. 4(3): 707-716.
- Jigna P, Sumitra VC (2007). In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk. J. Bio. 31: 53-58.
- Joshi KT, Pancholi AM, Pandya KS, Thakar AS (2011). Synthesis, Characterization and Antibacterial Activity of Novel Schiff Base Derived From 4-Acetyl-3-Methyl-1-(4'-Methyl- Phenyl)-2-Pyrazolin-5-one and Transition Metal Complexes. Int. J. Res. Chem. Environ. 1(2): 63-69.
- Keay RWJ (1989). Trees of Nigeria. Oxford University Press, New York, pp. 196.
- Kennedy KA, Mensah IG, Christian B, Moses LA (2007). Food and its Preparation Conditions in Hotels in Accra, Ghana: A Concern for Food Safety. Afr. J. Food Agric. Nutr. Dev. 7:5
- Kone WM, Atindehou K, Terreaux C, Hostettamann K, Traore D, Dosso M (2004). Traditional Medicine in Northern Cot D'ivoire: Screening of 50 Medicinal Plants for Antibacterial Activity. J. Ethnopharm. 93:43-49.
- Lellouche J, Friedman A, Lellouche JP, Gedanken A, Banin E (2011). Improved antibacterial and antibiofilm activity of magnesium fluoride nanoparticles obtained by water-based ultrasound chemistry. *Nanomed. Nanotechnology, biology and medicine*. Available online at: <http://www.ncbi.nlm.nih.gov/pubmed?term=>. Retrieved on 28/11/2012.
- Microbiology Bytes (2007). *Staphylococcus aureus* and *Escherichia coli*. pp 1 – 5. Available online at <http://www.microbiologybytes.com/introduction/b3.html>. Retrieved on 19th May, 2007.
- Mike HF, Craig RM (2008). A Comprehensive Review of Copper-Based Wood Preservatives with a Focus on New Micronized or Dispersed Copper Systems. For. Prod. J. 58(11): 6-27.
- Oyeleke SB, Dauda BEN, Boye OA (2008). Antibacterial activity of *Ficus capensis*. Afr. J. Biotechnol. 7(10): 1414-1417.
- Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall KL, Bentley SD, Holden MTG, Sebahia M, Baker S, Basham D, Brooks K, Chillingworth T, Connerton P, Cronin A, Davis P, Davies RM, Dowd L, White N, Farrar J, Feltwell T, Hamlin N, Haque A, Hien TT, Holroyd S, Jagels K, Krogh A, Larsen TS, Leather S, Moule S, O'Gaora P, Parry C, Quail M, Rutherford K, Simmonds M, Skelton J, Stevens K, Whitehead S, Barrell BG (2001). Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature 413: 848- 852.
- Philip DL, Daniel JW, Nancy DH (2009). Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clin. Microb. Rev. 22(4): 582-610.
- Piedad C, Ana MA, Martin C, Octavio P, Katia F, Gino C (2008). Magnetic Behavior and Antibacterial Activity of Iron (III) Complexes. J. Chil. Chem. Soc. 53(2): 1527-1532.
- Podschun R, Ullmann U (1998). *Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods and Pathogenicity Factors. Clin. Microb. Rev. 11(4): 589-603.
- Rabin G, Salam AI, Salma HA, Shahnaz QS, Yosef H (2010). Antimicrobial Activity of Copper Alone and in Combination with Lactic Acid against *Escherichia coli* O157:H7 in Laboratory Medium and on the Surface of Lettuce and Tomatoes. J. Pathol. Vol. 2011, Article ID 650968, 9 pages. doi:10.4061/2011/650968.
- Sadiq IS, Shuaibu M, Bello AB, Tureta SG, Isah A, Izuagie T, Nasiru S, Kamaru MB (2012). Phytochemistry and Antimicrobial Activities of *Cassia occidentalis* Used for Herbal Remedies. J. Chem. Eng. 1(1): 38-41.
- Saeed A, Farshid AK (2008). Antibacterial effects of five different root canal sealing materials. J. Oral Sci. 50(4):469-474.
- Saidu K, Onah J, Orisadipe A, Olusola A, Wambebe C, Gamaniel K (2000). Antiplasmodial, Analgesic and Anti-inflammatory Activities of the Aqueous Extract of the Stem Bark of *Erythrina senegalensis*. J. Ethnopharm. 71: 275 – 280.
- Scott DH, Gerrit JS, Michael WWA (2009). Antimicrobial Activity of the Iron-Sulfur Nitroso Compound Roussin's Black Salt [Fe₄S₃(NO)₇] on the Hyperthermophilic Archaeon, *Pyrococcus furiosus*. Appl. Environ. Microb. 75(7): 1820-1825.
- Shangkuan YH, Lin HC (1998). Application of Random Amplified Polymorphic DNA analysis to differentiate strains of *Salmonella typhi* and other *Salmonella* species. J. Appl. Microb. 85(4): 693-702.
- Sofowora A (1982). Medicinal Plants and Traditional Medicine in Africa: John Willey and Sons, New York, pp. 289.
- Sofowora A (1986). *The State of Medicinal Plants Research in Nigeria*. University Press, Ibadan, Nigeria, p. 86.
- Stojek NM, Szymanska J, Dutkiewicz J (2008). Gram-negative bacteria in water distribution systems of hospitals. Ann. Agric. and Env. Med. 15:135-142.
- Tira-Picos V, Nogueira JM, Gbolade AA (2010). Comparative analysis of leaf essential oil constituents of *Piliostigma thonningii* and *Piliostigma reticulatum*. Int. J. Green Pharm. 4: 67-70.
- Wheeldon LJ, Worthington T, Lambert PA, Hilton AC, Lowden CJ, Elliott TSJ, (2008). Antimicrobial efficacy of copper surfaces against spores and vegetative cells of *Clostridium difficile*: the germination theory. J. Antimicrob. Chem. 522-525.
- WHO, World Health Organization (1995). Traditional Practitioners as Primary Health Workers. A Publication of the World Health Organization Division of Strengthening of Health Services and the Traditional Medicine Programme, Geneva. pp. 3 – 5; 146 pages.
- Yu W, Yong W, Cong-Ming W, Stefan S, Thhangqi S, Wanjiang Z, Qijing Z, Jian-Zhong S (2011). Detection of the staphylococcal multiresistance gene *cfr* in *Proteus vulgaris* of food animal origin. J. Antimicrob. Chem. 66(11): 2521-2526.
- Yukihiko K, Matthew T, Oyeleke O, Bradley EB, Pradeep KS (2007). The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. J. Clin. Invest. 117(4): 877-888.
- Yu-Sen EL, Radisav DV, Stout JE, Victor LY (1996). Individual and combined effects of copper and silver ions on inactivation of *Legionella pneumophila*. Water Res. 30(8): 1905-1913.