Antimicrobial activity of *Plumbago zeylanica* plant extracts and its application in water and laboratory disinfection

Ayoade A. Adegbite[a], Abdulazeez A, Adebanjo[b] , A.M Yusuf-Babatunde[c] , Obafemi A.Solesi[d] and Risikat A. Sowole[e]

[a]Department of Water Resources Management and Sanitation, Ogun State College of Health Technology, Ilese-Ijebu, P.M.B 2081, Ijebu-Ode Nigeria
aagbite@yahoo.co.uk

[b]Department of Pharmaceutical Techniques, Ogun State College of Health Technology, Ilese-Ijebu, P.M.B 2081, Ijebu-Ode Nigeria
azeezadebanjo@yahoo.com

[c]Department of Pharmaceutical Technology, , Ogun State College of Health Technology, Ilese-Ijebu, P.M.B 2081, Ijebu-Ode Nigeria
yusuf_ademola4u@yahoo.com

[d]Department of Pharmaceutical Techniques, Ogun State College of Health Technology, Ilese-Ijebu, P.M.B 2081, Ijebu-Ode Nigeria
solesi.obafemi@gmail.com

[e]Department of Medical Laboratory Techniques, Ogun State College of Health Technology, Ilese-Ijebu, P.M.B 2081, Ijebu-Ode Nigeria

Abstract.This study was carried out to investigate the antimicrobial activities/ potentials of *Plumbago zeylanica* components (leaf, stem and root) on four bacterial species, *Baccilus subtilis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia* with the aim of using the active part of the plant to be used in water and laboratory disinfection. The plant parts crude extract was concentrated using a rotary evaporator and dried in a freeze drier. Different concentrations of the plant parts were then prepared from the dried plant extract and tested on the four pathogens using agar diffusion methods. The results indicated that active antimicrobial properties are concentrated more in the roots been very effective against *Escherichia coli* even at low concentration. However, at higher concentration all the plant extracts became effective against the bacteria. The study concludes that the roots of *Plumbago zeylanica* possess the highest antimicrobial potentials for disinfection. Then, the root extract was tested on effluent water and the results showed significance reduction level of *Escherichia coli*.

Keywords: antimicrobial, *Escherichia coli*, disinfection, plant extract, *Plumbago zeylanica*, water.

1.INTRODUCTION

Jeyachandran, *et al.*, in 2009 reported and citing different sources, it has been established that over the past few decades there has been much interest in natural materials as sources of new antimicrobial agents. However, the focussed plant *Plumbago zeylanica*, from previous studies established that the plants have active antimicrobial activity. For instance, the plant is of medicinal, pharmaceutical and therapeutic significances have been established in some part of world even in Nigeria (Madhava Chetty, *et al.*, 2006), (Agbaje and Adeniran, 2008), (Dhale and Markandeya, 2011), (Ravikumar and Sudha, 2011), (Manu, *et al.*, 2012) and (Kakad Subhash, Wabale and Kharde, 2013) among others.

Plumbago zeylanica is widely used for its medicinal properties in Africa, and many uses are being confirmed by scientific research (Mungwini, 2006). Although plumbagin may have medicinal potential, e.g. for its antimicrobial and antitumour activity, the use of plumbagin or plumbagin-containing plant material as medicine for humans is dangerous

because of the high toxicity. This can be a limitation for its potential uses for drinking water disinfection (Mungwini, 2006).

Plumbago zeylanica is very popular throughout Africa and Asia as a remedy for skin diseases, infections and intestinal worms, especially leprosy, scabies, ringworm, dermatitis, acne, sores, ulcers of the leg, haemorrhoids and hookworm (Mungwini, 2006) and (Ch.Kethani and Gopala, 2012).

Furthermore, all parts of the plant are used, but the root is considered to have the highest activity. In West Africa the root, or the leaves crushed with lemon juice, are used as a counter-irritant and vesicant. The pulped roots or aerial parts are inserted into the vagina as an abortifacient. This is a dangerous practice as it sometimes results in death (Mungwini, 2006). In Nigeria the roots pounded with vegetable oil are applied to rheumatic swellings. In Ethiopia, powdered bark, root or leaves are used to treat gonorrhoea, syphilis, tuberculosis, rheumatic pain, swellings and wounds. In southern Africa a paste of the root in vinegar, milk and water is used to treat influenza and blackwater fever. *Plumbago zeylanica* root cooked with meat in soup is eaten in Zimbabwe as an aphrodisiac, and it also helps digestion among others (Mungwini, 2006).

The plant has shown antibacterial activity against both gram-positive (e.g. *Staphylococcus*, *Streptococcus*, *Pneumococcus* spp.) and gram-negative (e.g. *Salmonella*, *Neisseria*) bacteria, whereas it is also active against certain yeasts and fungi (*Candida*, *Trichophyton*, *Epidermophyton* and *Microsporum* spp.) and protozoa (*Leishmania*). It has been found to prevent *Escherichia coli* and *Staphylococcus aureus* developing resistance to antibiotics. The plant also has strong antifeedant and moulting inhibiting effects on insects and has nematicidal and acaricidal activities (Mungwini, 2006) and (Ken, 2014).

With these properties of the plant aforementioned, it may serve as a potential disinfectant in the laboratory and for water from different sources that might contains various microorganisms ranging from bacteria, protozoa, worms, fungi that constitute threat to the consumers. Though, there are some previous studies on the use of plants for water treatment. For instance, Suarez, *et al*; (2003) carried out water treatment with seed extracts of Moringa oleifera Lam., a tropical tree that have been proposed as an environment-friendly alternative, due to their traditional use for the clarification of drinking water. However, the precise nature of the active components of the extract is unknown but the plant combining water purification and disinfectant properties (Suarez, *et al*; 2003). Then, Sadgir, *et al*; (2010) developed a plant based substitute for water purification in economical and safe ways against conventional chemical constituents available. The plant *Ocimum sanctum Linn*, was used and it was very effective against *Escherichia coli, Salmonella typhi, Pseudomonas pyocyaneus, Vibrio cholerae, Shigella dysenteriae* and *Proteus vulgaris* within specified contact time. Furthermore, Somani, *et al*., (2011) evaluated the effectiveness of natural herbs for antibacterial activity in water purification where the antimicrobial activity of Tulsi (*Ocimum sanctum*), Neem (*Azadirachta indica*), Wheatgrass (*Triticum aestivum*), Amla (*Phyllanthus emblica*) and Katakphala (*Strychnos potatorum*) were tested by Disc Diffusion Method (Kirby –Bauer Method) after extracting the dried material powder of natural herbs in 50% alcohol (ethanol). An antibacterial activity was observed in all herbs used (Somani *et al*., 2011). In similar way, Harikumar and Manjusha (2013), made an attempt to assess the antibacterial properties of certain selected herbs against different bacteria such as total coliforms, faecal coliforms, *Escherichia coli*, *Bacillus* sp. and *Serratia* sp. *Uiflorum* was also done. After the complete analysis of the antibacterial activity of different herbs, *Ocimum sanctum*, the most efficient herb, was selected and treatment methods based on the herb were developed so that it can be used conveniently in various households.

However, this study focused on evaluation of antimicrobial potentials of *Plumbago zelaynica* for disinfection of water from different sources and laboratory disinfection activities if it is possible to replace the conventional chlorine in water treatment and other disinfectants used in the laboratory.

2 MATERIALS AND METHODS

The study was carried out into two stages: the first stage involved in the preparation of the extracts (leaf, stem and root) and the testing of the extracts on selected microorganisms. The root extract was then further tested on effluent water samples. The analyses were carried out at Pharmaceutical and Water Microbiology laboratories of the College.

2.1 Procedures for Preparation of the Extracts

(a) The fresh *Plumbago zeylanica* plants were collected locally and separated into different parts. The parts were then dried under room temperature conditions not under direct sunlight for a week (7days).
(b) The surface area of the dried plant parts were reduced by bleeding to aid solvent percolation and extraction.
(c) The dried crude drugs were then weighed using a weighing balance and prepared for extraction
(d). Plants parts were extracted using cold Maceration for 48 hours and solvent recovery using rotary evaporator. The concentrated crude extracts were then dried using freeze drier and weight of the dried extract determined.
(e) The dried extracts were stored away for further use.

Table 1: Showing Mass of Powdered Plants Parts and Volume of Ethanol

	Mass of the Powdered parts/g	**Volume of the Ethanol Added/ml**	
		First Extraction	**Second Extraction**
1. Leaf	120	700	200
2. Stem	150	1000	200
3. Root	100	500	400

2.2 Procedures for Preparation of the Yield of Extracts

2.2.1 Yield of Plumbago zeylanica Leaf

Weighing of bottle and cover = 12.8g
Weight of the extracted Leaf part+ Weighing of bottle and cover = 18.7g
Percentage Yield = $\frac{\text{weight of extract}}{\text{Dry Leaf Sample}}$ x 100 = 5.9g/120g x 100 = 4.92%
The yield of the Leaf =4.92%

2.2.2 Yield of Plumbago zeylanica Stem

Weighing of bottle and cover = 12.8g
Weight of the extracted stem part+ Weighing of bottle and cover = 16.3g
Percentage Yield = $\frac{\text{weight of extract}}{\text{Dry Stem Sample}}$ x 100 = 3.5g/150g x 100 = 2.33%
The yield of the stem =2.33 %

2.2.3 Yield of Plumbago zeylanica Root

Weighing of bottle and cover = 12.8g
Weight of the extracted root part+ Weighing of bottle and cover = 23.3g
Percentage Yield = $\frac{\text{weight of extract}}{\text{Dry Root Sample}}$ x 100 = 10.5g/100g x 100 = 10.5%
The yield of the root =10.5 %.

2.3 Preparation of Media

Two different media were prepared (Nutrient agar and Nutrient broth). Nutrient agar-7gram of nutrient agar was dissolved in 250ml of distilled water in a conical flask and covered with foil paper which was then sterilized by autoclaving at 121°C for 15 minutes

(Cheesborough, 2006). After autoclaving, it was allowed to cool to about 45°C and subsequently dispensed into petri-dishes under spirit lamp to avoid contamination.
The plates were allowed to gel (solidified) and about 15minutes from already cooled nutrient agar medium was dispensed in each of the petri-dishes and allowed to gel.

Nutrient broth- 2.5g of nutrient broth medium was weigh into 100ml conical flask and autoclaved at 121°C for 15 minutes. This medium was allowed to cool to about 43°C and dispensed into sterile Mc cartney bottles (Cheesborough, 2006).

2.4 Preparation of Bacteria

Pure culture of the four bacteria species: *Escherichia coli, Klesbiella pneumonia, Staphylococcus aureus and Bacillus subtils* were inoculated into the already prepared dishes containing nutrient broth and left to grow overnight. This process was just to grow homogenous culture of the bacteria for biological assay that followed in the next day.

2.4 Preparation of Biological Assay

14.0g of Nutrient agar medium was weighed into conical flask; distilled water was added to make 500ml solution. This was autoclaved at 121°C for 15 minutes as well. After sterilization, the medium was removed and cooled to about 45°C. The overnight grown broth culture that contained the tested bacteria species with detailed label of all the test bacteria were brought out from incubator. Sterile swab sticks were used each for a bacterium, by rubbing the stick containing the broth culture of the bacteria over the gelled nutrient agar plates separately. The plates were gently and carefully rubbed, to allow the broth to mix totally with the nutrient agar.
The essence of this was to have medium of homogenous growth for each bacterium, so that the plant extracts at different concentrations will have equal access to the same concentration of the bacterium cells.

2.5 Procedures for Preparation of dilution of the Plant Extracts

Different concentrations prepared from the extracts were made with the aid of analytical balance into different plates. Each of the concentration was diluted with 1ml of ethanol to make the following concentrations 25mg/ml, 50mg/ml, 100mg/ml, and 200mg/ml concentrations. The different concentrations of the extracts were inoculated into their respective well on different plates of the bacteria species at the number site under the plates. The plates having inoculated them with the plant extract were incubated for another 24 hours. Observation was made after 24 hours. Inhibitory zones were recorded for those concentrations that inhibited the growth of the bacteria. The measurement of the zones of inhibition was taken as according to WHO/NCCLS (1998).

2.6 Second Stage of the Study

The aim of the experiment is to find out the effect of the *Plumbago zeylanica* root extract on effluent water samples collected from the College hostels. The effluents contain *thermotolerant coliforms* (faecal coliforms with mainly E-Coli) and /or *faecal streptococci* –both are the indicators of contamination of water with human faeces. The experiment was carried out under standard procedures (APHA**,** 2005).

3 RESULTS AND DISCUSSION

The results obtained from the laboratory and microbiology analysis are displayed in the tables and discussed below.

The antimicrobial potentials of ethanolic crude extracts of the stem, leaves and root of *Plumbago zeylanica* was assessed against the test microorganisms *Baccilus subtilis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia*. The aim of the experiment is to detect which part of the plant will be able to inhibit growth of test microorganism in small concentrations suitable for water disinfection. This extract can then be further purified / segregated to detect the active compound or compounds which may be suitable for use in ppm or ppb and non toxic for human consumption.

3.1 Results on the Antimicrobial Effects of *Plumago zeylanica* Extract on four Bacteria Species

Table 2 : Showing Antimicrobial Effects of the stem extract on Four Bacterial Species

	Conc.mg/ml	*Inhibitions by Stem Extract (mm)		
		A	B	AV
Bacillus subtilis	25	-	-	-
	50	9	11	10.0
	100	13	14	13.5
	200	15	16	15.0
Staphylococcus aureus	25	-	-	-
	50	-	-	-
	100	12	10	11.0
	200	14	12	13.0
Escherichia coli	25	14	14	14.0
	50	16	16	16.0
	100	18	19	18.5
	200	20	22	21.0
Klebsiella pneumoniea	25	12	9	10.5
	50	14	13	13.5
	100	16	15	15.5
	200	21	18	19.5

**A (first result) B (second result) AV (average of the two results)*

Table 2 shows the effects of the stem extracts on test microorganisms. Growth was inhibited at all concentration for *Escherichia coli* and *Klebsiella pneumonia* but was not hindered at 25mg/ml for *Baccilus subtilis* and *Staphylococcus aureus.*

Table 3 : Antimicrobial effects of the root extracts on the four bacterial species

	Conc.mg/ml	*Inhibitions by the Root Extract (mm)		
		A	B	AV
Bacillus subtilis	25	11	11	11.0
	50	14	13	13.5
	100	16	15	15.5
	200	18	20	19.0
Staphylococcus aureus	25	10	12	11.0
	50	14	14	14.0
	100	15	16	15.5
	200	23	20	21.5
Escherichia coli	25	25	26	25.5
	50	28	29	28.5
	100	32	33	32.5
	200	38	38	38.0
Klebsiella pneumoniea	25	20	21	20.5
	50	22	23	22.5
	100	25	24	24.5
	200	27	26	26.5

Table 4 : Antimicrobial effects of the leaf extracts on the four bacterial species

	Conc.mg/ml	*Inhibitions by the Leaf Extract		
		A	B	AV
Bacillus subtilis	25	-	-	-
	50	10	11	10.5

	100	13	14	13.5
	200	16	16	16.0
Staphylococcus aureus	25	-	-	-
	50	-	-	-
	100	11	12	11.5
	200	13	14	13.5
Escherichia coli	25	-	-	-
	50	-	-	-
	100	12	13	12.5
	200	14	16	15.0
Klebsiella pneumoniea	25	-	-	-
	50	09	09	9
	100	12	13	12.5
	200	15	15	15.0

Table 4 shows the effects of the leaf extract on test microorganism. The growths of the microorganism were unhindered at 25mg/ml but inhibition of growth was observed at 100mg/ml for all test microorganisms. Growth was observed at 50mg/ml for *Staphylococcus aureus* and *Escherichia coli* but there was inhibition growth at 50mg/ml on plates of *Klebsiella pneumonia* and *Baccilus subtilis*.

Growth was inhibited at all concentrations for all organisms as shown in table 3. The root extracts showed superior inhibitory activities when compared to the extracts from other parts of the plant. This may indicate an accumulation of secondary metabolites with antimicrobial properties in the stem of the plant. The activity of the root crude ethanolic extract was comparable to that of existing antimicrobial agents as shown in table 5 below.

Table 5: Antibiotic Sensitivity on the Test Bacteria Species

	R	CPX	S	SXT	E	PEF	CN	APX	Z	AM
Baccilus subtilis	13	21	15	15	14	15	22	R	20	14
	14	22	14	16	18	18	21	R	18	15
Staphylococcus aureus	-	24	20	18	20	19	20	R	16	R
	-	22	21	16	21	18	21	R	16	R
Escherichia coli	13	21	15	15	14	15	22	R	20	14
	14	22	14	16	18	18	21	R	20	14
Klebsiella pneumonia	10	18	14	14	13	R	16	R	12	R
	11	21	16	15	16	R	18	R	16	R

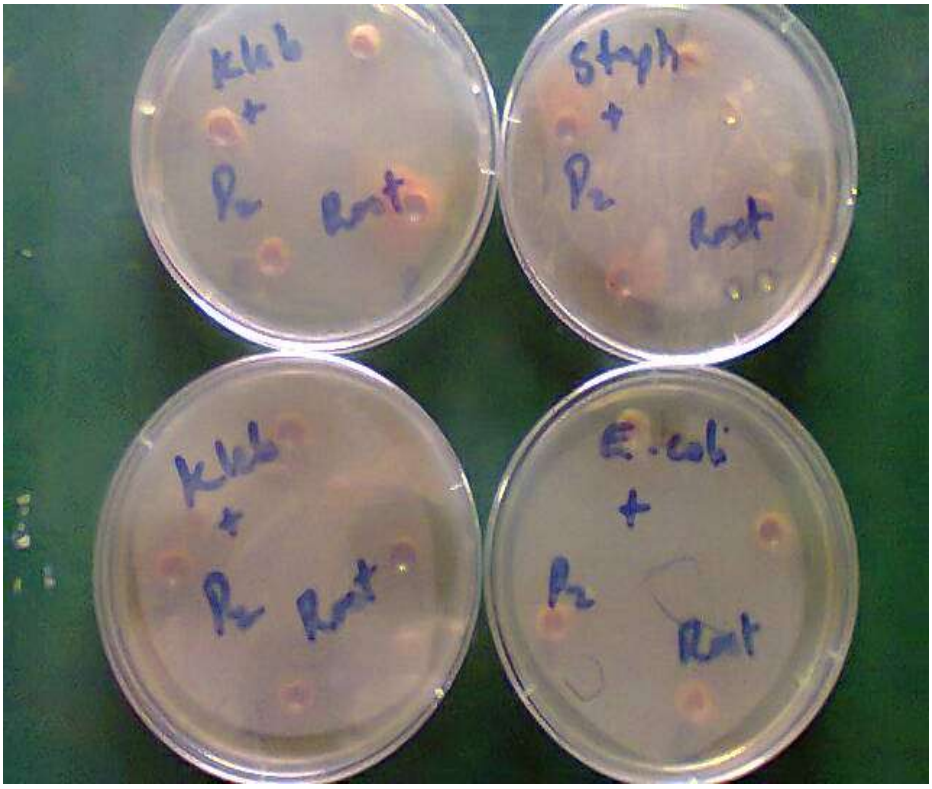

Figure 1: Showing the root zone of inhibitions on the four tested bacteria
Source: (Authors Lab work, 2014)

Figure 2: Showing the stem zone of inhibitions on the four tested bacteria
Source: (Authors Lab work, 2014)

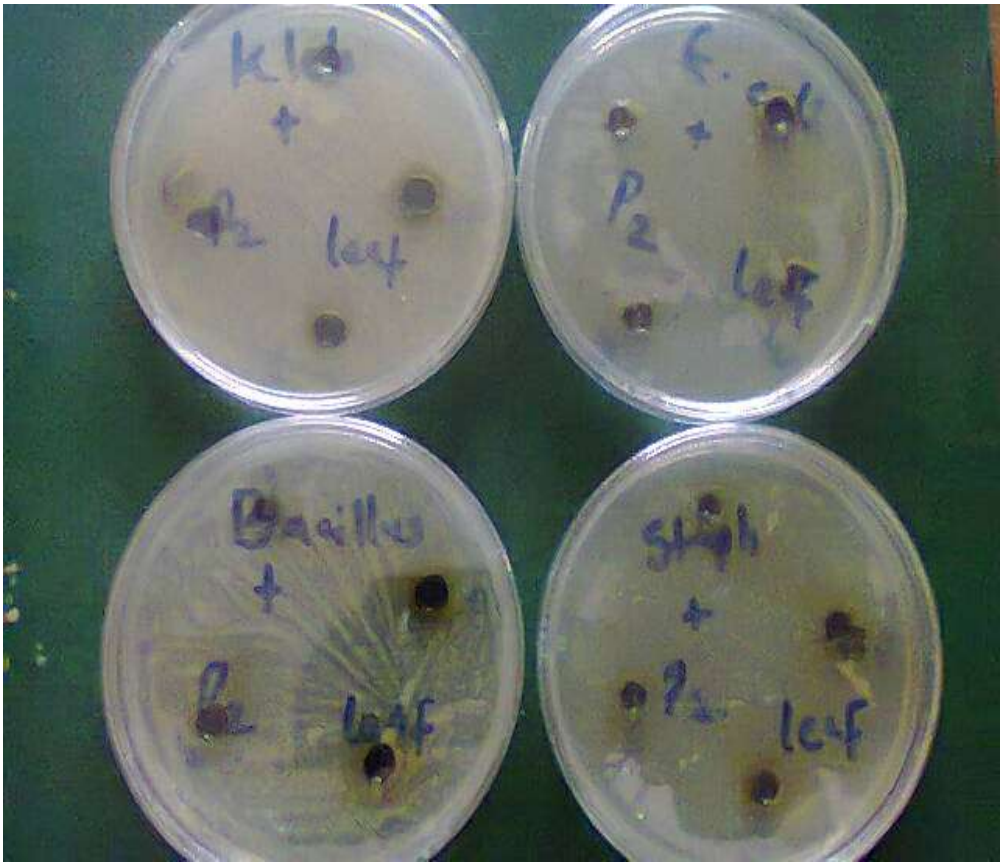

Figure 3: Showing the leaf zone of inhibitions on the four tested bacteria
Source: (Authors Lab work, 2014)

3.2 Results on the Antimicrobial Effects of *Plumago zeylanica* Root on Effluent Water Samples

The presumptive test was carried out on Thermotolerant *Coliforms* and *Faecal streptococcus* using the water effluent with dilution of 10^{-1} Both are positive .Filtration of Membrane Lauryl Sulphate Broth (MLSB) was used for *Thermotolerant Coliforms* which formed yellow colonies and though the result is expected to be, 'Too Numerous To Count' (TNTC) of coliforms, but visually few numbers of *Coliforms* were counted on average value of 5cfu/100ml.

4 CONCLUSION

Plumbago zeylanica was equally found in this study like the previous studies to have antimicrobial effect against the four bacteria species. *Baccilus subtillis, Staphyllococcus aureus, Escherichia coli* and *Klebsiella pneumoniea.* It was found in this study that, the root of *Plumbago zeylanica* possesses the highest anti microbial potentials while the stem and the leaf comparatively showed similar inhibitory reactions. Then the reduction of pathogenic organisms from effluent water samples after incubation by the crude root extract of the plants indicate that it can serve as potential disinfectant in water treatment and laboratory work. The roots extract after bioactivity guided fractionation may show antimicrobial activities similar to chlorine in water treatment. This is an ongoing research to establish this potential of the plant root as a substitute for chlorine in water treatment.

References

American Public Health Association (APHA)**, (**2005**).** Standard methods for the examination of water and wastewater 20th Ed. American water work Association,

Agbaje, E.O., & Adeniran, J. O (2009). Some Gastrointestinal Effects of the Aqueous Root Extract of Plumbago Zeylanica (Lead Wort) African Journal of Biomedical Research, Volume 12, Number 1

Bagla, V.P., McGaw, L.J., & Eloff, J.N (2011). The antiviral activity of six South African plants traditionally used against infections in ethnoveterinary medicine." Vet Microbiol.

Balamurugan, G. & Muthusamy, P.(2009). Antimicrobial studies on *Trianthema portulacastrum linn* extracts. Indian drugs 46 (13):250-252.

Chessborough, M. (2006). District Laboratory Practice in Tropical Countries Tropical Health Technology Cambridge UK 2nd Edition Update

Ch.Kethani, D., & Gopala Krishna, D. (2012). Pharmacognostic, Phytochemical and Biological Study of *Plumbago zeylanica*. International Journal of Natural Products Research 2012; 1(2): 21-23

Dhale, D.A., & Markandeya, S.K (2009). Antimicrobial and Phytochemical Screening of *Plumbago zeylanica* Linn. (Plumbaginaceae) Leaf

Eflora.org (2011) "*Plumbago zeylanica* Linnaeus, Sp. Pl. 1: 151. 1753.". *Flora of North America*. eFloras.org

Jeyachandran, R, Mahesh, A., Cindrella, L., Sudhakar, S,. & Pazhanichamy, K (2009). Antibacterial activity of Plumbagin and Root Extracts of *Plumbago Zeylanica* L. ACTA BIOLOGICA CRACOVIENSIA Series Botanica 51/1: 17–22, 2009

Harikumar, P. S., & Manjusha, C. M. (2013). Study on the antibacterial activity of selected natural herbs and their application in water treatment, Drink. Water Eng. Sci. Discuss., 6, 199-231, doi:10.5194/dwesd-6-199-2013, 2013.

Hostettmann, K. (1991). *Assays for bioactivity*. *Methods in Plant Biochemistry* 6, 360. Academic Press, San Diego.

Kakad S., Wabale, A. S., & Kharde, M. N. (2013). Phytochemical Screening and Antimicrobial Studies on *Plumbago zeylanica* L . Adv. Biores., Vol4 (3) September 2013: 115-117

Ken F (2014). Useful Tropical Plants –The Useful Tropical Plants Database http://tropical.theferns.info/

Kumar, G. & Sharmila, B.G (2013). Ethnomedical and Pharmacological Potentials of *Plumbago zeylanica* L- A Review American Journal of Phytomedicine and Clinical Therapeutics

Manu, P, Ankita L, Swati, R., & Anju, R (2012). *Plumbago zeylanica* L.: A MINI REVIEW International Journal of Pharmaceutical Applications Vol 3, Issue 3, 2012, pp 399-405

Madhava Chetty, K., Sivaji, K, Sudarsanam, G., & Hindu Sekar, P (2006). Pharmaceutical Studies and Therapeutic Uses of *Plumbago Zeylanica* L. Roots (Chitraka, Chitramulamu) *Ethnobotanical Leaflets 10: 294-304. 2006.*

Mungwini, Y.(2006). Plumbago zeylanica L. [Internet] Record from PROTA4U. Schmelzer, G.H. & Gurib-Fakim, A. (Editors). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. <http://www.prota4u.org/search.asp>.

Olagunju, J.A, Jobi, A.A.,& Oyedapo, O.O (1999). An investigation into the biochemical basis of the observed hyperglycemia in rats treated with ethanol root extract of P. zeylanica. Phytother Res 1999; 13:346-348

Oyedapo, O.O (1996). Studies on the bioactivity of the extract of *Plumbago zeylanica*. *Phytotherapy Research* 13:346–348.

Prota 11(1): Medicinal plants/Plantes médicinales Plumbago zeylanica - Protabase Record http://database.prota.org/PROTAhtml/Plumbago%20zeylanica_En.htm

Ravikumar V.R., & Sudha, T. (2011). Phytochemical and Antimicrobial Studies On *Plumbago Zeylanica (L) (*Plumbaginaceae) International Journal Of Research In Pharmacy And Chemistry IJRPC 2011, 1(2)

Somani, S.B., Ingole, N.W., & Patil, S S (2011).Performance Evaluation of Natural Herbs for Antibacterial Activity in Water Purification. International Journal of Engineering Science and Technology (IJEST) Vol. 3 No. 9

Standard Methods for the examination of water and wastewater: Edited by Clescori,L.S, Greenberg,A.E, Trussell,R.R, 1989 17th edition.

Parag, S., Vijyayshree, N., Ranu, N.,& Patil, B.R (2010). Antibacterial activity of Ocimum sanctum Linn. and its application in water purification Res J Chem Environ 14 (3), 46-50.

Suarez, M.,Entenza, J.M, Doerries, C, Meyer, E., Bourquin, L., Sutherland J., Marison I., Moreillon, P.,& Mermod, N. (2003). (*Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities*). Biotechnology and Bioengineering . 5; 81(1):13-20.