International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-1, 2014 ISSN No: 2277 - 7873

REVIEW ARTICLE

Review on Morphology, Chemical Constituents and Phytopharmacological Activities of *Plumbago zeylanica* Linn.

Arunbala*, Babita Gautam, Anshu Bala, Ajay Kumar, Chetan kr. Dubey

Department of Pharmacy, Rameshwaram Institute of Technology and Management, Lucknow-227202, Uttar Pradesh, India.

Manuscript No: IJPRS/V3/I1/00094, Received On: 22/02/2014, Accepted On: 26/02/2014

ABSTRACT

Plants are exploited as food and medicine since ancient periods. The therapeutic use of medicinal plants has gained a considerable momentum in the past few decades. But the emergence and use of more and more synthetic drugs resulting in higher incidence of adverse drug reactions have inspired mankind to go back to nature for safer remedies because they contain numerous active constituents of immense therapeutic response. In this particular review, I have made some attempted to focus on the work carried out on Plumbago zeylanica as it is not much common plant and it is one of the threatened species, still I have choose this due to its tremendous pharmacological properties with least side effects as it will produce natural products. So that further work must be carried out before we lose it. It's commonly known as 'chitraka', and mostly identified in different traditional system of medicine for curement of various skin diseases, intestinal worm and infections in the form of powder and paste. Plumbago mainly contains napthoquinones, binapthoquinones, coumarins, steroidal compound and difuranonapthoquinones.

KEYWORDS

Plumbago zeylanica Linn. Morphology, Pharmacological activity, Chemical constituents

INTRODUCTION

The plant plumbago zeylanica is commonly known as white leadwort, chitraka. Plumbago zeylanica Linn (plumbaginaceae) is semi-climbing, sub-scandant shrub of warm temperature tropical region of the world[1] and grows wild in India (especially in Bengal, uttar Pradesh, Andhra Pradesh) and Sri Lanka[2] the family plumbaginaceae consist of 10 genera and 280 species. Its genus include 3 species, namely P.indica, P.capensis and P.zeylanica which are distributed in several parts of India, as wild or in cultivation due to its more therapeutic uses.[3] Plumbago zeylanica is very popular in India and Asia as a remedy for skins diseases, inflammation and intestinal worm.[4] All parts of the plant used, but the roots have impressed the chemist and biologist due to tremendous pharmacological activity the aerial parts or pulped roots are used as abortifacient, laxative, expectorant, appetizer, while powered bark of root or leaves are employed for syphilis, gonorrhea, tuberculosis, rheumatic pain, swellings and wound healing.[5,6] Root decoction with boiled milk for inflammation in the mouth, throat or chest.[7]. The leaves are used as aphrodisiac, dysentery and scabies.[8] Its roots and its constituents are credited with potential therapeutic properties for ailments like inflammation, muscular pain[4] in traditional system of medicine like Ayurvedic and Unani

***Address for Correspondence:**
Arunbala
Rameshwaram Institute of Technology and Management, Lucknow-227202, Uttar Pradesh, India.
E-Mail Id: sarojarunbala@gmail.com

system, it also reported to possess anticancer[9,10], anti-tumor[11], antioxidant[12,13], antimycobacterial[14] hepatoprotective[15] neuroprotective, cardiotonic and central nervous system stimulating[16,17] and anti-fertility[18], analgesic[8], anti-convulsion[19], wound healing[20], anti-bacterial properties[21]. The goal of reviewing this plant is to explore the benefits for human health. Plumbago zeylanica is being as a medicinal herb in India around 1500B.C. it has been traced in Vedic times. The aim of this article is to compile all the scientific data generated for better basis for therapeutic use as it has steroidal compounds, natural coumarins and napthoquinones.

Botanical Classification[22]

Kingdom: Plantae

Order: Caryophyllales

Family: Plumbaginaceae

Genus: Plumbago

Species: P.zeylanica

Vernacular Name[2]

Arabia	Shitaraj, Enkin
Assamese	Agiyachit, Agnachit
Bengali	Chita, Safaid-chitrak
Burma	Kanchopphiju, Kinkhenphiu
Chinese	Pai Hua T'eng
English	White flowered leadwort, Ceylon leadwort
French	Dentalaire
German	Bleiwurz, Zahnkraut
Gujarati	Chitrakmula
Hindi	Chira, Chitrak

Macroscopy

Plumbago zeylanica is an evergreen small perennial shrub with semi woody stems and numerous branches, which grown to a height of 3-5 feet. Its leaves are simple, alternate, oblong, spirally arranged, hairy margin, thick and flashly.[23] The flowers are white in color, 10-20cm, bracts ovate to lanceolate, flower bisexual, pentamerous. Calyx is green. Corolla white tube is long and slender. The flower calyx has glandular hairs which help in trapping and killing insects by secreting sticky mucilage.[24] Fruit an oblong capsule, apex acute with 5 furrows, one seeded. Seeds oblong, 5-6mm long, reddish brown to dark brown. Its roots are stout, cylindrical and are irregular bent having transverse, blackish red in color, with a pungent odour.[25,26]

Microscopy

A. Roots[26]

- **Cork:** T.S. of roots showed outermost layer cork with 5-7 rows of light brown cells, cubical to rectangular in shape.
- **Secondary cortex:** 2-3 rows of thin walled rectangular secondary cortex, light brown cells.
- **Cortex:** Simple or compound cortex cells round with intercellular spaces, filled with starch grains.
- **Phloem:** Small, polygonal and thin walled, well developed with phloem fibres, lignified with pointed ends and narrow lumen, yellow contents absent.
- **Cambium:** Single-layered, tangentially elongated.
- **Xylem:** Light yellow to whitish, xylem vessels arranged in single or radial rows with a length of 190.87µm, width 67.09µm, and wall thickness 5.6µm, fibres had a length of 318.71µm, width of 30.09µm and wall thickness 4.32µm, tracheids are also filled with starch grains, medullary rays are narrow.

- **Medullary ray**: Single to multilayered loaded with simple to compound starch grains, radially elongated cells absent.

B. Powder (root)

Fragments of thin walled parenchyma cells with simple starch grains, spiral to helical elongated xylem vessels, phloem fibers, simple to compounded starch grains, cork cells.[27]

C. Leaves

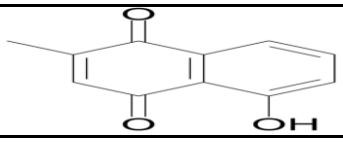
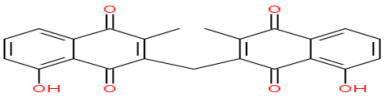
Dicotyledonous organization, irregular in shape.

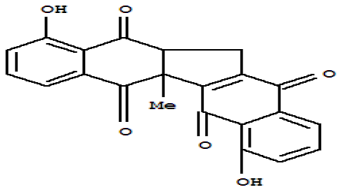
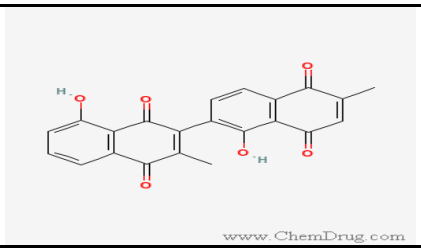
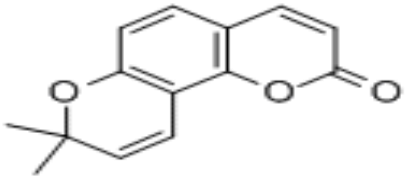
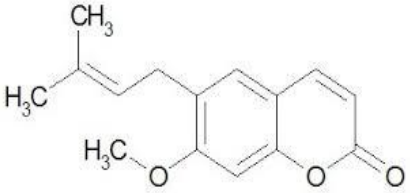
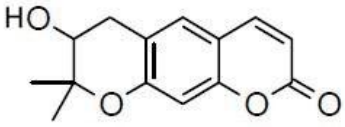
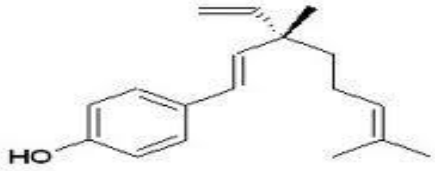
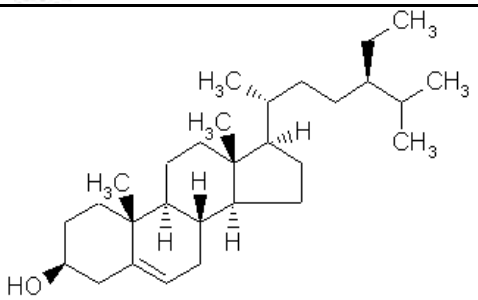
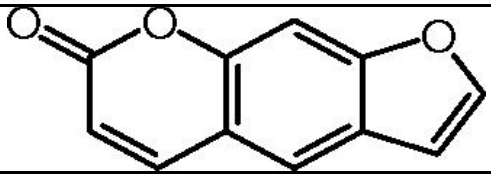
- **Lamina**: One palisade layer, two to three spongy layers.
- **Mesophyll**: Idioblast cells were occasionally found in intercellular spaces containing lesser amount of tannins than *P.indica.*
- **Vascular bundles**: horizontal.
- **Trichomes**: not found an exception to that of P.indica which contains non-glandular trichomes in the outermost layer of stems.[28]

Traditional Uses

- **Africa**: Cold infusion of the root is used for influenza and black water fever.[29]
- **Nepal**: as anti-viral medicine.[33]
- **East Africa**: Paste of powdered root or the root sap is used for tattooing by different tribes.[29]
- **Zimbabwe:** Plumbago zeylanica root is cooked with meat in soups as an aphrodisiac. Bark used to stop bleeding, cure baldness and treat diarrheas.[30]
- **Ethiopia**: Powdered bark, root or leaves are used to treat gonorrhea, syphilis, tuberculosis, rheumatic pain, and swelling.[30]
- **Tanzania**: Roots and bark mixed with poisonous insects are burned and ashes applied to incision as a cure for tumors. In water are used as ear drops to treat carache.[31]
- **Nigeria**: Roots powered with vegetable oil are applied to rheumatic swellings.[32]
- **Madagascar**: Roots applied a vesicant.[32]
- **Mauritius& Rodriguez**: Root decoction is used to treat diarrhea and dyspepsia.[32]
- **India**: against fever, malaria, diarrhea, piles and skin diseases. In Assam for family planning and birth control and permanent sterilization.[29]

Phytochemistry

Sl.no.	Constituents	Structures	Reference
1.	**Napthoquinones**		
1.1	Plumbagin		[34]
1.2	Biplumbagin		[34]
1.3	3-chloroplumbagin		[34]

1.4	Zeylanone		[35]
1.5	Chitranone		[36]
2.	**Coumarins**		
2.1	Seselin		[35]
2.2	Suberosin		[37]
2.3	Xanthyletin		[36]
3.	**Other compounds**		
3.1	Bakuchiol		[37]
3.2	βsitoserol		[38]
3.3	Psoralen		[39]

Pharmacological Activities

Anti-bacterial Activity[21]

The antibacterial activity of plants leaves extracts against five different organisms of *streptococcus aureus, staphylococcus aureus, bacillus subtiles, Pseudomonas aeruginosa, E.coli* using disc diffusion method. The anti-bacterial activity of methanol and chloroform extract showed positive result against the entire organism. The methanol extract inhibit *streptococcus aureus, staphylococcus aureus, bacillus subtiles, Pseudomonas aeruginosa, E.coli* at 10µL concentration indicated by the zone of inhibition around the disc in the culture plate except that the plate containing *pseudomonas aeruginosa.* But in 20µL concentration the plant showed positive results against all the five tested samples.

Chloroform leaf extract showed anti-bacterial activity against *streptococcus aureus, staphylococcus aureus, bacillus subtiles, Pseudomonas aeruginosa, and E.coli.* Its inhibition was moderate and lower.

The obtained results indicates both methanol and chloroform extracts has anti-microbial activity against organism especially methanol extract than chloroform extract. The result showed that the methanol extract of Plumbago zeylanica showed more inhibitory effect than the other plant extracts. This tends to show that the active ingredients of plant parts are better than extracts with methanol than chloroform.

The methanol extracts contains alkaloids, coumarins and tannins.[40] coumarins and tannins have anti-bacterial properties.[41]

Anti-fertility Activity[18]

Acetone and ethanolic extracts of the leaves of *Plumbago zeylanica* were effective in interrupting the estrous cycle of the rats ($p<0.05$) at dose levels 200 and 400 mg/kg. The animals exhibited prolonged diestrous stage of the estrous cycle corresponding to a temporary inhibition of ovulation. The anti-ovulatory activity was reversible on discontinuation of treatment.

Anti-diabetic activity[42]

Oral administration of ethanolic root extract of *Plumbago zeylanica* (100mg, 200mg/kg/p.o), tolbutamide (250mg/kg/p.o) increased the activity of hexokinase and decreased the activity of glucose-6-phosphate ($p<0.001$) in streptozotocin in treated diabetic rats.

Hepatoprotective Activity[43]

Hepatoprotective activity of aerial parts of *Plumbago zeylanica* Linn against carbon tetrachloride induced hepatotoxicity in rats. Rats treated with CCl_4 showed a significant hepatic damage[44] as observed from elevated serum level of hepato specific enzymes as well as severe alteration in different liver parameters SGPT, SGOT, ALP and total bilirubin in serum were increased in carbon tetrachloride intoxication control animals. Treatment with the methanolic extracts of *Plumbago zeylanica* caused significant protection against CCl_4 induced increased in serum enzymes level and bilirubin in a dose responsible manner. The degree of protection observed much with higher dose of the extract (70 mg/kg).

The triterpenes and steroids found in *Plumbago zeylanica* were effective against Paracetamol-induced hepatic toxicity. The blood sample taken from animals treated with petroleum ether root extract of the plant at the dose 300 mg/kg body weight has a significant protection against Paracetamol-induced hepato cellular injury.[15]

Larvicidal Activity[45]

Patil et al., (2010) studied the methanolic extract of *Plumbago zeylanica* roots possess Larvicidal activities against two mosquito species, *Aedes aegypti* and *anopheles stephensi* was found to be 169.61±7.99 mg/lit and 222.34±8.65 mg/lit.

Immunosuppressive[46]

The aqueous root extract of *Plumbago zeylanica* (1mg) with ovalbumin (10µg i.p.) exhibited the significant suppression of ovalbumin specific IgG antibody response (0.025 O.D at 4nm) in BALB/c mice determined by the enzyme-linked immunosorbent assay (ELISA) with dilution

factor 1:400 for 56 days ($p<0.05$). The extract (2 mg) with ovalbumin (10µg i.p.) also suppressed the anti-ovalbumin antibody response in dose dependent manner (0.3 O.D at 490nm).

Anti-oxidant Activity[47]

Nile *et al.*, (2010) studied the isolation and spectral data foe new flavonoid 2-(2, 4-Dihydroxy-phenyl)-3, 6, 8 trihydroxy chromen-4-one from the roots of *Plumbago zeylanica* were determined and the antioxidant activity was studied by free radical scavenging and superoxide radical scavenging methods. The antioxidant activity by DPPH was found to be 96µg/ml and by NBT as 4.6µg/ml which were greater than that of standard by DPPH and 10µg/ml by NBT assay.

Blood Coagulation Profile Activity[48]

Vijaykumar *et al.*, (2006) founded that the structure of active principal of the plant is similar to that of vitamin K. The plant extract (2mg/kg body weight) and napthoquinones (2mg/kg body weight) given to individual groups were screened for its effect on bleeding time, clotting time, prothrombin time, platelet count and platelet adhesion in albino rats 1-day, 15-day, 31-day treatment. The platelet adhesion was significant decreased after plant extract and napthoquinones treated animals in both with and without blood volume reduction after 15th as well as 31st day. Even at a dosage level (2mg/kg body weight), the chronic *P.zeylanica* administration prolongs the bleeding time by altering platelet adhesiveness and the coagulation.

Anti-Allergic Activity[49]

Dai *et al.*, (2004) studied that the 70% ethanol extract of *Plumbago zeylanica stems* show ability to inhibit mast cell-dependent immediate allergic reaction. This is evidenced by the fact that the extract could inhibit systemic anaphylactic shock induced by compound 48/80 in mice, reduced homologous passive cutaneous anaphylaxis and skin reaction induced by histamine or serotonin in rats. In vitro, the extract could reduce histamine release from rat peritoneal mast cells caused by compound 48/80 and antigen. It also markedly increased intracellular c AMP contents of rat mast cells.

Anti-Cancer Activity[50]

Hiradeve *et al.*, (2010) studied the anticancer evaluation property of *Plumbago zeylanica* L. leaves against Ehrlich Ascites carcinoma was done in animal model.

Administration of the ethanolic extract of the leaves at concentration 200mg/kg reduced the tumor volume (3.42±0.082), packed cell volume (1.05±0.092) and viable tumor cell count $\%10^7$ cells/ml (4.85±0.23) in a dose dependent manner.

Memory-Inducing Activity[8]

Mittal *et al.*, (2010) studied the effect of *P.zeylanica* roots were employed on scopolamine induced amnesia for learning and memory of mice. The chloroform extract of plant at a dose 200mg/kg has showed promising enhancing effect in mice. The extract significantly reverses the amnesia induced by scopolamine (0.4mg/kg i.p.).

Anti-Fungal Activity[51]

Plumbagin, the chief constituent found in the plant, at the concentration of 100µg inhibits the growth of fungal pathogens *viz.*, *cladosporium cladosporioides* (28.2 cm^2), *Alternaria tunis* (12.5cm^2), *Botrydiplodia theobromae* (12.5 cm^2), *Aspergillusniger* (12.5 cm^2), *Fusariumoxysporum* (7.1 cm^2), *colletotrichum gloeosporioides* (5.4 cm^2) and *cercospora nicotinae* (5.3 cm^2).

Abortifacient Activity[52]

Devarsh *et al.*, (1991) studied that the treatment with powered root of *P.zeylanica* during the first 7 days of pregnancy resulted in per implantation loss together with loss of uterine of uterine proteins of 13,000; 19,000 and 26,000 and 75,000 Da molecular weights. For those given root from day 6 to 17 of pregnancy proteins of molecular weights 55,000 and 65,000 Da was absent. This shows those proteins of 13,000; 19,000; and 75,000 Da influence implantation while those of 55,000 and 65,000 Da are required for maintenance of pregnancy.

Lipid Metabolism Activity[53]

It is reported that Plumbagin reduce serum cholesterol and LDL-cholesterol by 53%-86% and 61%-91% respectively; lower cholesterol/phospholipids ration by 45.8%; elevate decreased HDL-cholesterol significantly in rabbits. It was also observed that plumbagin could prevent accumulation of cholesterol and triglycerides in liver and aorta and regressed atheroma plaques of thoracic and abdominal aorta. The treated rabbits excreted more faecal cholesterol and phospholipids.

CONCLUSION

In present review article, we briefly describe the traditional knowledge, pharmacological and therapeutic application of the plant *Plumbago zeylanica* Linn as it has different class of chemical constituent such as Plumbagin, Coumarins, Napthoquinones and Flavonoid. It has been used in ancient times also. So this is an attempt to compile and document information on different aspects of *Plumbago zeylanica* and highlight the need for research and development.

ACKNOWLEDGEMENT

The satisfaction and pleasure that accompany the successful completion of my review article is incomplete without expressing gratitude to the people who did their level lest by contributing in their own ways. I am deeply indebted my esteemed guide Dr. Pushpendra Kumar Tripathi and co-guide Mr. Chetan Dubey for their valuable and meticulous guidance. I also liked to thank my collage Rameshwaram Institute of Technology and Management, Lucknow.

REFERENCES

1. Mandavkar, Y. D., & Jalalpure, S. S. (2011). A comprehensive review on Plumbago zeylanica Linn. *African journal of Pharmacy and Pharmacology*, *5*(25), 2738-2747.
2. Kirtikar, K. P., Basu, B. D. (1975). Indian Medicinal plants. 2nd ed-4.New Delhi: Jayyed Press, 532.
3. Chetty, K. M. (2006). Pharmaceutical Studies and Therapeutic Uses of Plumbago Zeylanica L. Roots (Chitraka, Chitramulamu). *Ethnobotanical Leaflets*, 10, 294-304.
4. Tilak, J. C., Adhikari, S., & Devasagayam, T. P. (2004). Antioxidant properties of Plumbago zeylanica, an Indian medicinal plant and its active ingredient, plumbagin. *Redox report*, *9*(4), 219-227.
5. Thakur, R. S., Puri, H. S., & Husain, A. (1989). *Major medicinal plants of India* (p. 585). RSM Nagar, Lucknow: Central Institute of medicinal and aromatic plants.
6. Bhattacharjee, S. K. (1998). *Handbook of Medicinal Plants*. Pointer Publishers, p. 274.
7. Teshome, K., Gebre-Mariam, T., Asres, K., Perry, F., & Engidawork, E. (2008). Toxicity studies on dermal application of plant extract of Plumbago zeylanica used in Ethiopian traditional medicine. *Journal of ethnopharmacology*, *117*(2), 236-248.
8. Mittal, V., Sharma, S. K., Kaushik, D., Khatri, M., & Tomar, K. (2010). A comparative study of analgesic activity of Plumbago zeylanica Linn. Callus and root extracts in experimental mice. *Res. J. Pharm. Biol. Chem. Sci*, *1*(4), 830-836.
9. Nguyen, A. T., Malonne, H., Duez, P., Vanhaelen-Fastre, R., Vanhaelen, M., & Fontaine, J. (2004). Cytotoxic constituents from Plumbago zeylanica. *Fitoterapia*, *75*(5), 500-504.
10. Xu, K. H., & Lu, D. P. (2010). Plumbagin induces ROS-mediated apoptosis in human promyelocytic leukemia cells in vivo. *Leukemia research*, *34*(5), 658-665.
11. Yang, S. J., Chang, S. C., Wen, H. C., Chen, C. Y., Liao, J. F., & Chang, C. H. (2010). Plumbagin activates ERK1/2 and Akt via superoxide, Src and PI3-kinase in 3T3-L1 cells. *European journal of pharmacology*, *638*(1), 21-28.
12. SivaKumar, V., Prakash, R., Murali, M. R., Devaraj, H., & Niranjali Devaraj, S. (2005). In vivo micronucleus assay and GST activity in assessing genotoxicity of plumbagin in

Swiss albino mice. *Drug and chemical toxicology*, *28*(4), 279-88.

13. Sivakumar, V., Devaraj, S. N. (2006). Protective Effect of Plumbago Zeylanica against Cyclophoshamide-induced Genotoxicity and Oxidative stress in swiss albino mice. *Drug and chemical toxicology*, 29, 279-88.
14. Mossa, J. S., El-Feraly, F. S., & Muhammad, I. (2004). Antimycobacterial constituents from Juniperus procera, Ferula communis and Plumbago zeylanica and their in vitro synergistic activity with isonicotinic acid hydrazide. *Phytotherapy Research*, *18*(11), 934-937.
15. Kanchana, N., & Sadiq, A. M. (2011). Hepatoprotective effect of plumbago zeylanica on paracetamol induced liver toxicity in rats. *International Journal of Pharmacy & Pharmaceutical Sciences*, *3*(1), 151-4.
16. Bopaiah, C. P., & Pradhan, N. (2001). Central nervous system stimulatory action from the root extract of Plumbago zeylanica in rats. *Phytotherapy Research*, *15*(2), 153-156.
17. Mallikadevi, T., Paulsamy, S. (2010). Plumbago Zeylanica: a potential plant for antimicrobial activity. *Plant Arch*, 10, 547-550.
18. Edwin, S., Joshi, S. B., & Jain, D. C. (2009). Antifertility activity of leaves of Plumbago zeylanica Linn. in female albino rats. *European J. of Contraception and Reproductive Healthcare*, *14*(3), 233-239.
19. Vishnukanta, Rana, A. C. (2010). Evaluation of anti-convulsant activity of Plumbago Zeylanica Linn leaf extracts. *Asian J. Pharma. Clin. Res*, 3, 76-78.
20. Devender Rao Kodati, Shashidher Burra, and Kumar Goud P. (2011). Evaluation of wound healing activity of methanolic root extract of Plumbago Zeylanica Linn in wistar albino rats. *Asian J. Plant Sci. Res*, 1 (Suppl 2), 26-34.
21. Sudha Devi, M., Thenmozhi, M. (2011). Antibacterial activity of Plumbago Zeylanica leaf extracts. *International J. Res. Biomed. Biotech*, 1 (Suppl 1), 1-4.
22. Satyavati, G. V., Gupta, A. K., Tondon, N. (1987). Medicinal plants of India. Indian Council of Medical Res 1st ed. (vol.2), New Delhi.
23. Geltz, N. R., & Russell, S. D. (1988). Two-dimensional electrophoretic studies of the proteins and polypeptides in mature pollen grains and the male germ unit of Plumbago zeylanica. *Plant physiology*, *88*(3), 764-769.
24. Schlauer J. (1997). New data relating to the evolution and phylogeny of some carnivorous plant families. *Carnivorous plants Newsletter*, 26, 34-38.
25. Chen, Z. F., Tan, M. X., Liu, Y. C., Peng, Y., Wang, H. H., Liu, H. G., & Liang, H. (2011). Synthesis, characterization and preliminary cytotoxicity evaluation of five Lanthanide (III)–Plumbagin complexes. *Journal of inorganic biochemistry*, *105*(3), 308-316.
26. Gupta, S., Ahirwar, D., Jhade, D. *et al*. (2010). Pharmacognostic standardization, physic and phytochemical evaluation of Plumbago Zeylanica Linn root. *Drug Invention Today*, 2 (Suppl 9), 408-410.
27. Girija, T. P., Sereenam K., Unnikrishnan, K. P., Remashree, A. B. (2005). Pharmacognostic and Phytochemical Studies on the Raw Drug Chitraka. *Life Sciences*, 03-22.
28. Nak-iam, V., Rattanalangam, C., Komputsa, Y. *et al.* (2000). Comparative structural organization of stems and leaves of Plumbago Zeylanica Linn and Plumbago Indica Linn. 26th congress on Science and Technology of Thailand.
29. Tiwari, K. C., Majumder, R., & Bhattacharjee, S. (1982). Folklore information from Assam for family planning and birth control. *Pharmaceutical Biology*, *20*(3), 133-137.

30. Giday, M., Teklehaymanot, T., Animut, A., & Mekonnen, Y. (2007). Medicinal plants of the Shinasha, Agew-awi and Amhara peoples in northwest Ethiopia. *Journal of Ethnopharmacology*, *110*(3), 516-525.

31. Neuwinger, H. D. (2000). *African traditional medicine: a dictionary of plant use and applications. With supplement: search system for diseases*. Medpharm. 589.

32. Gupta, M. M., Verma, R. K., Gupta, A. P. (1993). A chemical investigation of Plumbago Zeylanica. *Curr Res Med Aromat plants*, 17, 161-164.

33. Kamboj, V. P., & Dhawan, B. N. (1982). Research on plants for fertility regulation in India. *Journal of Ethnopharmacology*, *6*(2), 191-226.

34. Chen, Z. F., Tan, M. X., Liu, L. M., Liu, Y. C., Wang, H. S., Yang, B., Peng, Y., Liu, H. G., Liang, H., Orvig, C. (2011). Cytotoxicity of the traditional Chinese medicine (TCM) plumbagin in its copper chemistry. *Afr. J. Pharm. Pharmacol.* 28, 10824-10833.

35. Michael, A. S., Thompson, C. G., & Abramovitz, M. (1956). Artemia salina as a test organism for bioassay. *Science*, *123*, 464.

36. Wang, Y. C., & Huang, T. L. (2005). High-performance liquid chromatography for quantification of plumbagin, an anti-Helicobacter pylori compound of Plumbago zeylanica L. *Journal of chromatography A*, *1094*(1), 99-104.

37. Nguyen, A. T., Malonne, H., Duez, P., Vanhaelen-Fastre, R., Vanhaelen, M., & Fontaine, J. (2004). Cytotoxic constituents from Plumbago zeylanica. *Fitoterapia*, *75*(5), 500-504.

38. Zhang, Q. R., Mei, Z. N., Yang, G. Z., Xiao, Y. X. (2008). Chemical constituents from the aerial parts of Plumbago zeylanica L. *J. Chinese Pharmaceutics Sci*., 17, 144-147.

39. Anonymous. In: The Welth of India: A Dictionary of Indian Raw Materials and Industrial products. New Delhi, India CSIR 1989; 2: 163-4.

40. Okamoto, I., Doi, H., Kotani, E., Takeya, T. (2001). The aryl-aryl coupling reaction of 1-napthol with snCl4 for 2, 2'-binapthol synthesis and its application to the biomimetic synthesis of binapthoquinones isolated from Plumbago Zeylanica. *Tetrahedron letter*, 42, 2987-2989.

41. Hedberg, I., Hedberg, O., Madat, P. J., Mshigeni, K. E., Mshiu, E. N., & Samuelsson, G. (1983). Inventory of plants used in traditional medicine in Tanzania. II. Plants of the families Dilleniaceae—Opiliaceae. *Journal of Ethnopharmacology*, *9*(1), 105-127.

42. Zarmouh, M. M., Subramaniyam, K., Viswanathan, S., & Kumar, P. G. (2010). Cause and effect of Plumbago zeylanica root extract on blood glucose and hepatic enzymes in experimental diabetic rats. *African Journal of Microbiology Research*, *4*(24), 2674-2677.

43. Kumar, R., Kumar, S., Patra, A., & Jayalakshmi, S. (2009). Hepatoprotective activity of aerial parts of Plumbago zeylanica linn against carbon tetrachloride-induced hepatotoxicity in rats. *International journal of pharmacy and pharmaceutical sciences*, *1*(1), 171-5.

44. Slater, T. F. (1965). *Biochemical mechanism of liver injury*. London: Academic Press.

45. Patil, S. V., Patil, C. D., Salunkhe, R. B., & Salunke, B. K. (2010). Larvicidal activities of six plants extracts against two mosquito species, Aedes aegypti and Anopheles stephensi. *Trop Biomed*, *27*(3), 360-365.

46. Poosarla, A., & Athota, R. R. (2010). Immunosuppressive properties of aqueous extract of Plumbago zeylanica in Balb/c mice. *J Med Plants Res*, *4*(20), 2138-2143.

47. Nile, S. H., & Khobragade, C. N. (2010). Antioxidant activity and flavonoid derivatives of Plumbago zeylanica. *J Nat Prod*, *3*, 130-3.

48. Vijayakumar, R., Senthilvelan, M., Ravindran, R., & Devi, R. S. (2006). Plumbago zeylanica action on blood coagulation profile with and without blood volume reduction. *Vascular pharmacology*, *45*(2), 86-90.

49. Dai, Y., Hou, L. F., Chan, Y. P., Cheng, L., & But, P. P. H. (2004). Inhibition of immediate allergic reactions by ethanol extract from Plumbago zeylanica stems. *Biological & pharmaceutical bulletin*, *27*(3), 429-432.

50. Hiradeve, S., Danao, K., Kharabe, V., & Mendhe, B. (2010). Evaluation of anticancer activity of Plumbago zeylanica Linn leaf extract. *International Journal of Biomedical Research*, *1*(2), 01-09.

51. Adikaram, N. K., Karunaratne, V., Bandara, B. R., Hewage, C. M., Abayasekara, C., & Mendis, B. S. S. (2002). Antifungal properties of plumbagin. *Journal of the National Science Foundation of Sri Lanka*, *30*(3-4), 89-95.

52. Devarshi, P., Patil, S., & Kanase, A. (1991). Effect of Plumbago zeylanica root powder induced preimplantationary loss and abortion on uterine luminal proteins in albino rats. *Indian journal of experimental biology*, *29*(6), 521-522.

53. Sharma, I. N. D. U., Gusain, D. E. E. P. A. L. I., & Dixit, V. P. (1991). Hypolipidaemic and antiatherosclerotic effects of plumbagin in rabbits. *Indian journal of physiology and pharmacology*, *35*(1), 10-14.