Plumbago zeylanica (Plumbaginaceae)
(Synonym: P.viscosa)

English:White leadwort, Ceylon leadwort, plumbago India:Chitrak, chitramol
German: Bleiwurz, Zahnkraut
African vernacular names:
Arabia: Ensain, enkin Chagga: Osuhure Kilongo: mzura Ndebele: matsisa
Swahili: Sanza Tswana: Mosikomabe
Pharm. definition: Herba Plumbaginis

The plant
Plumbago zeylanica is native in Southeast Asia and is growing in Africa, too. It is a branched evergreen shrub reaching up to 2 meters. Leaves are dark-green, ovate 30 cm long and 15 cm wide. The flowers are white in dense racemes, individuals around 1cm across, flowering all the year long. In Europe Plumbago capensis is cultivated as an ornamental shrub with blue flowers.
It was featured as the Plant of the Week November 1-7, 2002.

Plant parts used
The whole plant, the roots, powder of the roots

Constituents
A raw phytochemical overview with thin layer chromatography of **crude extracts** showed the presence of alkaloids, phenols and flavonoids (2).
From the **roots** of P. zeylanica were isolated:
The naphthoquinones plumbagin, composed naphthoquinones, like plumbagin, 3-biplumbagin, chloroplumbagin, chitranone, elliptone
The coumarins seselin, 5-methoxyseselin, suberosin and xanthyletin
Other compounds were 2,2-dimethyl-5-hydroxy-6-acetylchromene, plumbagin acid, ß-sitosterol, ß-sitosteryl-glucoside, bakuchiol, 12-hydroxyisobakuchiol, saponaretin, isoorientin, isoaffinetin, psorealen.
Between all these compounds plumbagin is the major ingredient (5-hydroxy-2-methyl-1,4-naphthoquinone, ($C_{11}H_8O_3$),with 1% in the **whole plant**, but with higher percentages in the **root**, crystallising as slender orange coloured needles, soluble in organic solvents, less soluble in water, volatile with steam (21).
The **stem** brings only a trace and the **leave**s bring no plumbagin.

Quantative determination of naphthoquinones by HPLC
Three methods can be recommended:
1) Reversed phase column chromatography with a gradient mobile phase water-methanol, detection at 254 nm (29)
2) A HPLC method with a Waters apparatus, 10µ Spherogel column (Altex Scientific Berkeley CA, USA), mobile phase n-hexane-chloroform-2-propanol (30:70:2), detection at 267 nm, flow rate 1 ml/min, detection time within 15 min (10).
3) Zorbax Extend C18 column chromatography: Column 150 x 4.6 mm ID, 5 µm, mobile phase water-methanol (10+90), coupled with a tandem mass spectrometer (11).

For the determination of the **root extract** the first method seems to be the best one.
A bioassay-guided fractionating of the dichloromethane extract of **aerial parts** from P. zeylanica led to the isolation of ß-sitosterol, ß-sitosteryl-3ß-glucopyranoside-6`-O-palmitate, lupenone, lupeol acetate, plumbagin, and trilinolein (17).

Traditional uses

In the Ayurvedic and Sidda medicine P. zeylanica has been assigned medical properties and is used in formulations for Ayurvedic medicines:
In India against fever and malaria, against diarrhoea, dyspepsia, piles, and skin diseases including leprotic lesions (10), in Nepal as an antiviral medicine, in Taiwanese folk medicine for anti-Helicobacter activity, in Assam for family planning and birth control and permanent sterilization (26,13); In Northwest-Ethiopia for treatment of gastro-intestinal complaints (9), in South-Western Nigerian folk medicine against parasitic diseases, scabies and ulcers (10).
In Madras (India), Amrita Bindu, a salt-spice-herbal mixture, is used as an antioxidant (16).

Results of experimental studies

Antibacterial and antimycotic activity
Alcoholic crude extracts of P. zeylanica were investigated for their ability to inhibit the growth of multiresistant (16-23 ß-lactam antibiotics) strains of E. coli and Shigella. Compared with other plant extracts they showed high activity with MIC value of 0.64-10.24 mg/ml. After fractionating the ethyl acetate fraction exhibited the highest potency (with a lower MIC). The plant extracts tested in vitro for haemolysis in sheep erythrocytes had no activity (2).
Antibiotic resistant strains of E. coli and Staphylococcus aureus, inoculated in an antibiotic (streptomycin, rifampicin) medium showed a delayed growth due to the resistance. However, the growth was completely prevented when the bacteria were grown in the medium with antibiotic and plumbagin (7).
Infections with Helicobacter pylori were inhibited in vitro by ethyl acetate extracts of P. zeylanica with a minimum bactericidal concentration (5.12 - 20.48 mg/ml). The bactericidal activity appeared in a dose-dependent manner (29). In rats and mice roots of P. zeylanica so called chitrak, added to the feed increased the growth of coliform bacteria for 5 %, significantly, similar to mexaform (Geigy).
Authors call chitrak an intestinal flora normalizer (12).
When tested against the resistant strain of Mycobacterium tuberculosis (H37RV) the inhibitory activity of Plumbagin was <12.5 µg/ml. The antimycotic activity of isonicotin acid hydrazide against Mycobacterium intracellurum, M. smegmatis, M. xenopei and M. chelonei combined with plumbagin was lowered from a MIC value of 1.25-2.5 to 0.15-0.3 µg/ml (15).
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80 ethanolic extracts from 47 Indian plant species were tested in vitro for antiplasmodial activity.31 extracts were found to be active, five of them, together with P. zeylanicum, are of special interest for further antimalarial studies (22).

Cytotoxicity
Beta-sitosterol and plumbagin isolated by a bioassay guided fractionation of the dichloromethane extract from aerial parts of P. zeylanica were toxic against the cancer cell lines:
ß-sitosterol against MCF7 and Bowes cancer cell lines (IC50 113µM and 152µM) and inhibited Bowes cell growth with IC50 36.5 µM).
Plumbagin was toxic against MCF7 and Bowes cells (IC50 1.28µM, and 1.39µM) (17).
In Swiss albino mice the activity of plumbagin was investigated on mouse bone marrow cells by a micronucleus assay. The LD50 for the animals was 16 mg/kg.
The mice (25-30g) were given orally 16, 8, 4 mg/kg body weight in 1% carboxymethyl cellulose for 5 consecutive days.
Plumbagin induced micronuclei at all these doses and proved to be toxic for mouse bone marrow cells. Glutathion S-transferase activity did not change with a plumbagin dose 4 mg/kg, but was significantly inhibited by the higher doses of 8 and 16 mg/kg (24).
Biochemical effects
In rat liver mitochondria antioxidant effects of the aqueous and alcoholic root extracts were tested, corresponding to medicinal preparations. In the method using ferric reducing/ antioxidant powder boiled ethanolic extracts were the most effective ones. In the method with 2`,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid, boiled aqueous extracts were most efficient. In further investigations were a lot of effects on lipid metabolism. In conclusion, the extracts and plumbagin have significant antioxidant abilities which may explain reported therapeutic effects (25).
According to clinical experiences (no further informations) the authors investigated P. zeylanica against acute promyelocytic leukaemia. The following methods were used:
MTT colorimetric assay for cell inhibitory rates, light microscopy and transmission electron microscopy for morphologic changes, DNA gel electrophoresis and flow-cytometry for cell apoptosis
The results were:
2-15 µM plumbagin inhibited the proliferation of NB4 cells, dose-dependently. Chromosome condensation and apoptotic body formation was followed by blockade of NB4 cells in G2/M of the cell cycle. The conclusion was, plumbagin can inhibit cell proliferation, blocks cell cycle and induces apoptosis of APL cell line NB4 (30).

In Madras (India), Amrita Bindu, a salt-spice-herbal mixture, is a traditional folk medicine. It consists of Piper nigrum, Piper longum, Cyperus rotundus, Zingiber officinale and Plumbago zeylanica.
Because Amrita Bindu is said to bear antioxidant potential experiments with two lines of rats were performed:
In the experiment **I** rats were fed with normal diet.
In the experiment **II** rats were given feed mixed with Amrita Bindu for 3 weeks (4g/kg of feed).
Both experimental groups were challenged against a single intraperitoneal injection of phenylhydrazine (7.5 mg/kg body weight). After 24 and 72 h the blood was analyzed for free radicals and antioxidant levels.
Amrita Bindu pre-treated rats showed significantly lower levels of free radicals, lipid peroxidation and protein carbonyls with significantly higher levels of antioxidants, when compared with rats without Amrita Bindu pre-treatment on extract administration. Amrita Bindu seems to have an antioxidant potential against oxidative damages (16).
Pharmacological effects

Swiss Albino mice pre-treated with an alcoholic root extract of P. zeylanica (250 and 500 mg/kg body weight) showed protection against cyclophosphamide-induced genotoxicity, reduced the frequency of micronucleated polychromatic erythrocytes, and increased the normochromatic erythrocyte ratio in the bone marrow (23).
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In male Wistar rats plumbagin (4mg/kg bodyweight) from P. zeylanica reduced the growth in 3-methyl-4-dimethyl aminoazobenzine (3MeDAB) induced hepatoma. In hepatoma bearing rats levels of hexokinase, phosphoglucoisomerase and aldolase increased ($p<0.001$),but they decreased in only plumbagin treated rats to near normal levels. In tumour hosts glucose-6-phosphatase and fructose-1,6-diphosphatase decreased ($p<0.001$).
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In hyperglycaemic rats the effects of ethanol extracts from the roots of P. zeylanica were studied on key enzymes of glycolysis and other biochemical parameters. Thigh muscle hexokinase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase activities were significantly ($p<0.05$) reduced by 12.7 %, 51.02 %, 24.32 %, and 25.16 % in the treated animals (18).
In rats the effects of 50% ethanol extract from P. zeylanica roots were tested on locomotoric behaviour and central dopaminergic activity. The extracts (single doses orally 100, 200, 300 mg/kg body weight) significantly raised the spontaneous motility of the animals. The ambulatory and rotatory behaviour was higher in the treated group than in the control group ($p < 0.05$). In the ambulatory behaviour marked differences were between the values of 100 and 300mg/kg. The responses were stimulatory and dose-depending. The root extracts enhanced the spontaneous ambulatory activity without inducing stereotypic behaviour. The authors assume stimulatory properties in the root extract mediated by dopaminergic mechanisms in the rat brain (4).
Albino rats treated with P. zeylanica root powder during first 7 days of pregnancy abolished uterine proteins (13,000 – 75,000 Da) resulting in pre-implantationary loss (6)

Allergic and modulatory effects

The modulatory ability of plumbagin from P. zeylanica was studied on peritoneal macrophages of BALBC mice. The functions of macrophages are antibactericidal activity, against Staphylococcus aureus, and release of hydrogen peroxide and superoxide anions. In low doses plumbagin exerted a constant increase in the antibactericidal activity. The higher the dose the higher the response was, observed up to six weeks. But in the next two weeks a considerable decline in the bactericidal activity was noticed comparable to low dose. This indicates that plumbagin augments the macrophage antibactericidal activity at low concentrations and inhibits it at higher concentrations (1).
Allergic reactions of ethanolic extracts (70 %) from P. zeylanica stems were investigated. The extracts (500, 1000mg/kg orally) inhibited dose-dependently systemic anaphylactic shocks, induced by compound 48/80 in mice, reduced homologous passive cutaneous anaphylaxis and skin reactions, induced by histamine or serotonin in rats. Significant differences were observed at the dose of 1000mg/kg. In vitro the extracts (5.0-50 µg/ml) dose-dependently

reduced histamine release from rat peritoneal mast cells, caused by the compound 48/80 as antigen. The same extract (50 µg/ml) markedly increased intracellular cAMP content of rat mast cells (5).
Plumbagin, derived from P. zeylanica modulates cellular proliferation, carcinogenesis and radioresistance. All these reactions should be regulated by the activation of the transcription factor NF-kappa B activation pathway. Plumbagin inhibits NF-kappa B activation induced by TNF, other carcinogens and inflammatory stimuli like phorbol myristate acetate. In certain tumour cells plumbagin suppresses the constitutive NF-kappa B activation, over all.
The authors believe that plumbagin is a potent inhibitor of the NF-kappa B activation pathway. This leads to the suppression of gene products. This may explain the effects described above (20).

Results of clinical studies

There is only one obscure reference of a Chinese author who speaks of previous clinical experiences with P. zeylanica. In the literature there is no reference about experimental or clinical trials (30).

Toxicity

LD50 for Swiss albino mice: 16 mg/kg (23)

Evaluation

Both in vivo and in vitro studies of Plumbago zeylanica root extracts and plumbagin indicate that these compounds exhibit high levels of bioactivity such as antimicrobial, antitumour, antihepatatomic, antimodulatory and antimacrophage ones. Higher doses change this useful activity into a toxic one
One can hypothesize that these results are caused mainly by plumbagin. Other compounds like coumarins in the plant intensify the effects. The modulatory effects may be explained by the naphthoquinone structure of plumbagin, comparable to a redox substance.

Because of the toxic effects neither the plant extract nor plumbagin can be recommended for the use with men.

Plumbago zeylanica

No treatment of men

References Plumbago

1. Abdul KM, Rachender RP (1995) Modulatory effect of plumbagin of macrophage functions in Balb/c mice. I. Potentiation of macrophage bactericidal activity Immunpharmacol 30, 3: 231-6 Pub Med 8557 523
2. Ahmad I, Aquil F (2006) In vitro efficacy of bioactive extracts of 15 medicinal plants against Esbetal-producing multidrug-resistant enteric bacteria Microbiol Res 2006
3. Aquil F, Ahmad I, Owais M (2006) Evaluation of anti-methicillin-resistant Staphylococcus aureus (MRSA) and synergy of some bioactive plant extracts Biotechnol J 1(10):1093-1102
4. Bopaiah CP, Pradhan N (2001) Central nervous system stimulatory action from the root extract of Plumbago zeylanica in rats Phytother Res 15(2): 153-6
5. Dai Y, Hou LF, Chan JP et al. (2004) Inhibition of immediate allergic reactions by ethanol extract from Plumbago zeylanica stems Biol Pharm Bull 27(3): 429-32

6. 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 J Exp Biol 29(6): 521-2
7. Durgar R, Sridhar P, Polasa H (1990) Effects of plumbagin on antibiotic resistance in bacteria Indian J Med Res 91: 18-20
8. Gebre-Mariam T, Neubert R, Schmidt PC et al. (2006) Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders J Ethnopharmacol 104(1-2):n182-7
9. Giday M, Teklehaymanot T, Animut A et al. (20069 Medicinal plants of the Shinasa, Agewawi and Amhara people in Northwest Ethiopia J Ethnopharm 2006 Oct 20
10. Gupta MM, Verma RK, Uniyal GC et al. (1993) Determination of plumbagin by normal-phase high performance liquid chromatography J Chrom 209-12
11. Hsieh YJ, Lin LC, Tsai TH (2005) Determination and identification of plumbagin from the roots of Plumbago zeylanica by liquid chromatography with tandem mass spectrometry J Chromatogr A 1083 (1-2):141-5
12. Iyengar MA, Pendse GS (1966) Plumbago zeylanica L. (Chitrak).A gastrointestinal flora normaliser Planta Med 14 (3): 337-51
13. Kamboj VP, Dhawan PM (1982) Research on plants for fertility regulation in India J Ethnopharmacol 6 (2): 191-226
14. Lin LC, Yang LL, Chou CJ (2003) Cytotoxic naphthoquinones and plumbagic acid glycosides from Plumbago zeylanica Phytochem 62 (4): 619-22
15. Mossa JS, El-Feraly LS, Muhammad I (2004) Anti-mycobacterial constituents from Juniperus procera, Ferula communis and Plumbago zeylanica and their in vitro synergistic activity with isonicotinic acid hydrazide Phytother Res 16(11): 934-7
16. Natarajan KS, Narasimhan M, Shanmugasundaram KR et al. (2006) Antioxidant activity of a salt-spice-herbal mixture against free radical induction J Ethnopharmacol 105 (1-2): 76-83
17. Nguyen AT, Malonne H, Duez P et al. (2004) Cytotoxic constituents from Plumbago zeylanica Fitoterapia 75 (5): 500-4
18. Olagunju JA, Jobi AA, Oyedapo OO (1999) An investigation into the biochemical basis of the observed hyperglycaemia in rats treated with ethanol root extract of Plumbago zeylanica Phytoter Res 13(4): 346-8
19. Parimala R, Sachdanandam P (1993) Effect of plumbagin on some glucose metabolising enzymes studied in rats in experimental hepatoma Mol Cell Biochem 125 (1): 59-63
20. Sandur SK, Ichikawa H, Sethi G et al. (2006) Plumbagin suppresses NF-kappaB activation and NF-kappaB-regulated gene products through modulation of p65 and kappaB alpha kinase activation J Biol Chem 281 (25): 17023-33
21. Sharma I, Gusain D, Dixit VP (1991) Hypolipidaemic and antiatherosclerotic effects of plumbagin in rats Indian J Physiol Pharmacol 35(1):10-14
22. Simonsen HT, Nordskjold JB, Smitt UW et al. (2001) In vitro screening of Indian medicinal plants for antiplasmodial activity J Ethnopharmacol 74 (2): 195-2004
23. Sivakumar V, Niranjali DS (2006) Protective effect of Plumbago zeylanica against cyclophosphamide-induced genotoxicity and oxidative stress in Swiss albino mice Drug Chem Toxicol 29(3):279-88
24. Sivakumar V, Prakash R, Murali MR et al. (2005) In vivo nucleus assay and GST activity in assessing genotoxicity of plumbagin in Swiss albino mice Drug Chem Toxicol 28 (4): 499-507
25. TilakJC, Adhikari S, Devasagayam TP (2004) Antioxidant properties of Plumbago zeylanica, an Indian medicinal plant and its active ingredient, plumbagin Redox

Rep 9 (4):219-27
26. Tiwari K, Majumder R, Bhattacharjee S (1982) Folklore information from Assam for family planning and birth control Int Crude Drug Res 20 (3): 133-7
27. Vander Veijver LM, Lötter AP (1971) The constituents in the roots of Plumbago auriculata Lam.and Plumbago zeylanica L. responsible for antibacterial activity Planta Med 20: 8-13
28. Vijayakumar R, Senthilvelan M, Ravindran R, Devi RS (2006) Plumbago zeylanica action on blood coagulation profile with and without blood volume reduction Vascul Pharmacol 45(2): 86-90 Pub Med 1653 1123
29. Wang YC, Huang TL (2005) High-perfomance liquid chromatography for quantification of plumbagin an anti-Helicobacter pylori compound of Plumbago zeylanica J Chromatogr A 1094:99-104
30. Zhao YL; Lu DP (2006) Effects of plumbagin on the human acute promyelocytic leukaemia cells in vitro Zhongguo Shi Yan Xue Ye Xue Za Zhi 14(2): 208-11 Pub Med 1663 8181

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Authors conclude an anticarcinogenic property of Plumbagin against hepatoma in rats (19).
In hyperglycaemic rats the effects of ethanol extracts from the roots of P. zeylanica were studied on key enzymes of glycolysis and other biochemical parameters. Thigh muscle hexokinase, phosphofructokinase, pyruvate kinase and lactate dehydrogenase activities were significantly ($p<0.05$) reduced by 12.7 %, 51.02 %, 24.32 %, and 25.16 % in the treated animals (18).
In rats the effects of 50% ethanol extract from P. zeylanica roots were tested on locomotoric behaviour and central dopaminergic activity. The extracts (single doses orally 100, 200, 300 mg/kg body weight) significantly raised the spontaneous motility of the animals. The ambulatory and rotatory behaviour was higher in the treated group than in the control group ($p < 0.05$). In the ambulatory behaviour marked differences were between the values of 100 and 300mg/kg. The responses were stimulatory and dose-depending. The root extracts enhanced the spontaneous ambulatory activity without inducing stereotypic behaviour. The authors assume stimulatory properties in the root extract mediated by dopaminergic mechanisms in the rat brain (4).
Albino rats treated with P. zeylanica root powder during first 7 days of pregnancy abolished uterine proteins (13,000 – 75,000 Da) resulting in pre-implantationary loss (6)

Allergic and modulatory effects

The modulatory ability of plumbagin from P. zeylanica was studied on peritoneal macrophages of BALBC mice. The functions of macrophages are antibactericidal activity, against Staphylococcus aureus, and release of hydrogen peroxide and superoxide anions. In low doses plumbagin exerted a constant increase in the antibactericidal activity. The higher the dose the higher the response was, observed up to six weeks. But in the next two weeks a considerable decline in the bactericidal activity was noticed comparable to low dose. This indicates that plumbagin augments the macrophage antibactericidal activity at low concentrations and inhibits it at higher concentrations (1).
Allergic reactions of ethanolic extracts (70 %) from P. zeylanica stems were investigated. The extracts (500, 1000mg/kg orally) inhibited dose-dependently systemic anaphylactic shocks, induced by compound 48/80 in mice, reduced homologous passive cutaneous anaphylaxis and skin reactions, induced by histamine or serotonin in rats. Significant differences were observed at the dose of 1000mg/kg. In vitro the extracts (5.0-50 µg/ml) dose-dependently

reduced histamine release from rat peritoneal mast cells, caused by the compound 48/80 as antigen. The same extract (50 µg/ml) markedly increased intracellular cAMP content of rat mast cells (5).
Plumbagin, derived from P. zeylanica modulates cellular proliferation, carcinogenesis and radioresistance. All these reactions should be regulated by the activation of the transcription factor NF-kappa B activation pathway. Plumbagin inhibits NF-kappa B activation induced by TNF, other carcinogens and inflammatory stimuli like phorbol myristate acetate. In certain tumour cells plumbagin suppresses the constitutive NF-kappa B activation, over all.
The authors believe that plumbagin is a potent inhibitor of the NF-kappa B activation pathway. This leads to the suppression of gene products. This may explain the effects described above (20).

Results of clinical studies

There is only one obscure reference of a Chinese author who speaks of previous clinical experiences with P. zeylanica. In the literature there is no reference about experimental or clinical trials (30).

Toxicity

LD50 for Swiss albino mice: 16 mg/kg (23)

Evaluation

Both in vivo and in vitro studies of Plumbago zeylanica root extracts and plumbagin indicate that these compounds exhibit high levels of bioactivity such as antimicrobial, antitumour, antihepatatomic, antimodulatory and antimacrophage ones. Higher doses change this useful activity into a toxic one
One can hypothesize that these results are caused mainly by plumbagin. Other compounds like coumarins in the plant intensify the effects. The modulatory effects may be explained by the naphthoquinone structure of plumbagin, comparable to a redox substance.

Because of the toxic effects neither the plant extract nor plumbagin can be recommended for the use with men.

Plumbago zeylanica

No treatment of men

References Plumbago

1. Abdul KM, Rachender RP (1995) Modulatory effect of plumbagin of macrophage functions in Balb/c mice. I. Potentiation of macrophage bactericidal activity Immunpharmacol 30, 3: 231-6 Pub Med 8557 523
2. Ahmad I, Aquil F (2006) In vitro efficacy of bioactive extracts of 15 medicinal plants against Esbetal-producing multidrug-resistant enteric bacteria Microbiol Res 2006
3. Aquil F, Ahmad I, Owais M (2006) Evaluation of anti-methicillin-resistant Staphylococcus aureus (MRSA) and synergy of some bioactive plant extracts Biotechnol J 1(10):1093-1102
4. Bopaiah CP, Pradhan N (2001) Central nervous system stimulatory action from the root extract of Plumbago zeylanica in rats Phytother Res 15(2): 153-6
5. Dai Y, Hou LF, Chan JP et al. (2004) Inhibition of immediate allergic reactions by ethanol extract from Plumbago zeylanica stems Biol Pharm Bull 27(3): 429-32

6. 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 J Exp Biol 29(6): 521-2
7. Durgar R, Sridhar P, Polasa H (1990) Effects of plumbagin on antibiotic resistance in bacteria Indian J Med Res 91: 18-20
8. Gebre-Mariam T, Neubert R, Schmidt PC et al. (2006) Antiviral activities of some Ethiopian medicinal plants used for the treatment of dermatological disorders J Ethnopharmacol 104(1-2):n182-7
9. Giday M, Teklehaymanot T, Animut A et al. (20069 Medicinal plants of the Shinasa, Agewawi and Amhara people in Northwest Ethiopia J Ethnopharm 2006 Oct 20
10. Gupta MM, Verma RK, Uniyal GC et al. (1993) Determination of plumbagin by normal-phase high performance liquid chromatography J Chrom 209-12
11. Hsieh YJ, Lin LC, Tsai TH (2005) Determination and identification of plumbagin from the roots of Plumbago zeylanica by liquid chromatography with tandem mass spectrometry J Chromatogr A 1083 (1-2):141-5
12. Iyengar MA, Pendse GS (1966) Plumbago zeylanica L. (Chitrak).A gastrointestinal flora normaliser Planta Med 14 (3): 337-51
13. Kamboj VP, Dhawan PM (1982) Research on plants for fertility regulation in India J Ethnopharmacol 6 (2): 191-226
14. Lin LC, Yang LL, Chou CJ (2003) Cytotoxic naphthoquinones and plumbagic acid glycosides from Plumbago zeylanica Phytochem 62 (4): 619-22
15. Mossa JS, El-Feraly LS, Muhammad I (2004) Anti-mycobacterial constituents from Juniperus procera, Ferula communis and Plumbago zeylanica and their in vitro synergistic activity with isonicotinic acid hydrazide Phytother Res 16(11): 934-7
16. Natarajan KS, Narasimhan M, Shanmugasundaram KR et al. (2006) Antioxidant activity of a salt-spice-herbal mixture against free radical induction J Ethnopharmacol 105 (1-2): 76-83
17. Nguyen AT, Malonne H, Duez P et al. (2004) Cytotoxic constituents from Plumbago zeylanica Fitoterapia 75 (5): 500-4
18. Olagunju JA, Jobi AA, Oyedapo OO (1999) An investigation into the biochemical basis of the observed hyperglycaemia in rats treated with ethanol root extract of Plumbago zeylanica Phytoter Res 13(4): 346-8
19. Parimala R, Sachdanandam P (1993) Effect of plumbagin on some glucose metabolising enzymes studied in rats in experimental hepatoma Mol Cell Biochem 125 (1): 59-63
20. Sandur SK, Ichikawa H, Sethi G et al. (2006) Plumbagin suppresses NF-kappaB activation and NF-kappaB-regulated gene products through modulation of p65 and kappaB alpha kinase activation J Biol Chem 281 (25): 17023-33
21. Sharma I, Gusain D, Dixit VP (1991) Hypolipidaemic and antiatherosclerotic effects of plumbagin in rats Indian J Physiol Pharmacol 35(1):10-14
22. Simonsen HT, Nordskjold JB, Smitt UW et al. (2001) In vitro screening of Indian medicinal plants for antiplasmodial activity J Ethnopharmacol 74 (2): 195-2004
23. Sivakumar V, Niranjali DS (2006) Protective effect of Plumbago zeylanica against cyclophosphamide-induced genotoxicity and oxidative stress in Swiss albino mice Drug Chem Toxicol 29(3):279-88
24. Sivakumar V, Prakash R, Murali MR et al. (2005) In vivo nucleus assay and GST activity in assessing genotoxicity of plumbagin in Swiss albino mice Drug Chem Toxicol 28 (4): 499-507
25. TilakJC, Adhikari S, Devasagayam TP (2004) Antioxidant properties of Plumbago zeylanica, an Indian medicinal plant and its active ingredient, plumbagin Redox

Rep 9 (4):219-27
26. Tiwari K, Majumder R, Bhattacharjee S (1982) Folklore information from Assam for family planning and birth control Int Crude Drug Res 20 (3): 133-7
27. Vander Veijver LM, Lötter AP (1971) The constituents in the roots of Plumbago auriculata Lam.and Plumbago zeylanica L. responsible for antibacterial activity Planta Med 20: 8-13
28. Vijayakumar R, Senthilvelan M, Ravindran R, Devi RS (2006) Plumbago zeylanica action on blood coagulation profile with and without blood volume reduction Vascul Pharmacol 45(2): 86-90 Pub Med 1653 1123
29. Wang YC, Huang TL (2005) High-perfomance liquid chromatography for quantification of plumbagin an anti-Helicobacter pylori compound of Plumbago zeylanica J Chromatogr A 1094:99-104
30. Zhao YL; Lu DP (2006) Effects of plumbagin on the human acute promyelocytic leukaemia cells in vitro Zhongguo Shi Yan Xue Ye Xue Za Zhi 14(2): 208-11 Pub Med 1663 8181