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18. Anti- Inflammatory Effects of Extract from *Plumbago Zeylanica*

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Abstract

Plumbago genus (Family-Plumbaginaceae) be one of the most significant medical vegetation which are use for anti-inflammatory, antimicrobial disease. Our work involves the instruction of anti inflammatory and cytotoxic property of Plumbago zeylanica. The root of P. zeylanica extract with methanol was use for formative anti inflammatory effects. The methanol extracts at 350 and 500 mg/kg produced 32.05 and 61.2% inhibition of acute inflammation, in that order, in Carrageenin induce raw paw oedema confirm that P. zeylanica roots are well-organized against acute inflammation. For the assessment of cytotoxicity, the crude dichloromethane remove was subjected to silica gel column chromatography and 120 fractions be composed. The lethal concentration (LC50) price was experiential for crude remove, betasitosterol, gugultetrol-18-ferrulate and it was originate to be 90, 75 and 65 ppm, in that order. The use of Plumbago species as an successful anti inflammatory agent and its cytotoxic belongings have been ascertained and proved. Their structures were elucidate with the help of spectroscopic technique. High presentation fluid chromatography (HPLC) was perform to decide the purity of gugultetrol-17-ferrulate in crude remove and the structure of betasitosterol and gugultetrol-18-ferrulate be identified by means of nuclear compelling character spectroscopy investigation (1H and 13C NMR), Infra red and mass spectroscopy.

Key words: Plumbago zeylanica, high presentation fluid chromatography, anti provocative, cytotoxicity, betasitosterol, gugultetrol-18-ferrulate,.

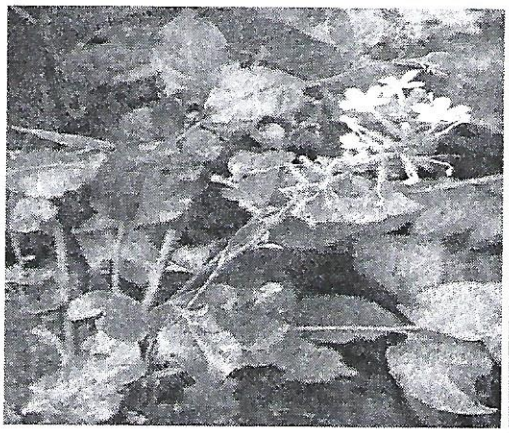
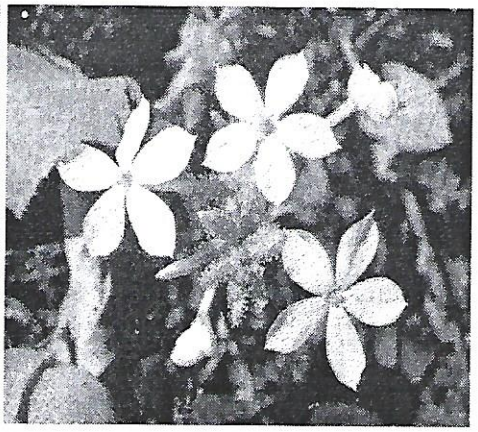
Introduction

Plumbago generally known as chittiramulam, in Tamil as well as white leadwort in English. Plumbaginaceae is scattered as a weed all through the tropical and subtropical country of the world. The family Plumbaginaceae consists of 10 genus and 280 division. These days, Ayurvedic, Hoemoeo and Unani Physicians use plentiful species of medical plants. (Narayana and Thamanna, 1987). Numerous compounds old in today's medication contain a complex

structure and synthesizing these bioactive compound chemically at a low value is not simple (Mujumdar et al., 2000; Madhava, 1998). The intensifying consciousness about side effects of drugs had complete the western pharmaceutical industries to turn towards the plant base Indian in addition to Chinese medicine (Balandrin and Klocke, 1988). Plumbago zeylanica Linn. (Plumbaginaceae) usually called chitrak, is an perennial, subscandant plant found wild in South India and West Bengal. It is also educated in gardens all through India. Its roots are used in established system of tablets to cure various ailments like body pain, headache, fever and swelling . Plumbago zeylanica roots be report to have antioxidant, hypolipidemic, anti artherosclerotic, central anxious system stimulant and anti fertility belongings . It is a endangered plant with possible medical value hence it was measured worthwhile to estimate the analgesic activity of callus obtain from nodal explant and to evaluate its activity with deference to root extract from parent plant. The genus Plumbago include 3 species, specifically Plumbago indica. L, Plumbago rosea. L, Plumbago capensis. L, and Plumbago zeylanica .L, which are dispersed in more than a few parts of India. In P. zeylanica root and bark is an active part old as a conventional herbal medicine to treat more than a few diseases. Compounds isolated from P. zeylanica L. are collected of naphthoquinone, such as plumbagin, chloroplumbagin, chitranone, elliptinone, isoshinanolone and coumarins such as seselin, 5-methoxyseselin, suberosin, and xanthyletin. Other compounds such as 2, 2-dimethyl-5-hydroxy-6- acetylchromene, plumbagin acid have also been isolated and recognized (Yuan-Chuen Wang 2005; Michael, 1956). The entire plant and its root contain used as a folk medicine in Taiwan for the conduct of rheumatic pain, menostasis, carbuncle and damage by bump (Okoli and Akah, 2005). The pharmacological significance of this returning shrub lies in its capability to create a napthoquinone,called plumbagin (Ayo, 2007), mostly in its roots. New discoveries of the tumor inhibitory (Krishnaswami and Puroshothaman, 1980; Roober, 1996) and radio modifying effects (Uma et al., 1999). Pharmacological belongings of plumbagin contain been investigate on anti-cancer, anti-leishmanial, anti-plasmodial, hypoli-pidaemic, antiatherosclerotic, antiallergic, anti-bacterial, anti-fungal, anti-inflammatory, antihyperglycemic, central nervous system stimulatory, cytotoxic and antiinsecticidal Property. (Yuan-chuen et al., 2005; Yen-Ju et al., 2006; Vanisree et al., 2004). Plumbagin be also reported to be an effectual chitin-synthetase inhibitor

(Sleet et al., 1983; Renata, 2001). Plumbagin (2-methyl-5- hydroxy-1,4-naphtho-quinone) is a natural naphthaquinone presentation a wide variety of pharmaceutical activities. The root of

P. zeylanica has been report to be a influential poisonous when given orally or practical to ostium uteri, causes abortion (Azad et al., 1982). The acute toxicity studies of P. zeylanica in albino rats exposed that the oral LD50 of the drug is 65 mg/kg body weight and in the dead animals, the post mortem revealed a profuse bleeding in the viscera (Premakumari et al., 1977).

Materials and Methods

High performance liquid chromatography (HPLC)

(Renata M. S. Celeghini), to check the purity of isolated compound and crude extract of dichloromethane, High resolution HPLC was performed using shimadzu LC –10AT up chromatograph provided with isocratic pump and UV visible detector. The crude dichloromethane extracts were filtered through 2 m -membrane filters and used for analysis. Column of C18 ODS, Gemini 4 µ, 111A of dimensions 251 x 4.6 mm with mobile phase 70:30:2 (methanol : water : acetic acid), was used at flow rate of 0.4 ml / min. The detection wavelength was 254 nm and injection volume was 20 ml.

Plant extraction

The plant P. zeylanica root be composed, shade dried and crushed (Zaheer and Ahsana, 2008). The crushed 1.5 kg of the material was covered with water in solvent dichloromethane (4050 ml) for 48 h And repeat the process for three times to get whole removal. The in the black was detached in a rotary space and store the remove in refrigerator for additional study. Shows the whole plant and root of P. zeylanica. Phytochemical psychoanalysis was done to make sure for the addressees of steroids, alkaloids, flavonoids and terpenoids etc.

Anti-inflammatory activity

The new propose was accepted by the ethical board of central research institute for Siddha, (CCRAS), Chennai – 600 106 (TN). {Ethical approval No. 37/IAEC/Pharma/ CRIS/2006} wister rats were obtained from department of Laboratory Animal Sciences (CPCSEA Registration No: 512/01/a/2001/CPCSEA), (Winter et al., 1962) Animals were housed in groups of three and two in two standard suspended polycarbonate cages with top grill having facilities for feed and drinking water in glass bottles with stainless steel sipper tubes. The environmental conditions were maintained

Thin layer chromatography (TLC)

TLC is perform by silica gel 60 F254 on alumna sheets. The metabolites be useful point wise as unlike a skin circumstance TLC plates and must be eluted with dissimilar solvent scheme. The plate was viewed under ultra violet (UV) lamp at 254 nm. For further clarity the plates were derivatised, using PUNCAL-D solution (A solution of Cerisulphate (1.6 g) and Ammonium hepta molybdate (21.6 g) Conc. Sulphuric acid (50 ml) in 450 ml of water. Spraying the reagent on TLC plate followed by drying and heating did derivitisation at 130°C in a hot air oven. Blue colored spots appear indicates the presence of organic molecules.

Sr. no.	Test	Observation	Result
1	Steroids	Absence pale green color	Absent
2	Carbohydrates	Appearance yellow color	Present
3	Alkaloids	Absence orange color	Absence
4	Tannins	Absence blue color	Absence
5	Flavanoids	Absence yellow color	Absence

Results and Discussions.

This part was checkered for its purity as single spot by HPTLC. To make sure the purity of remote mix and crude take out of dichloromethane the compound be Subjected to HPLC. The consequence obtain by gradient chromatography on C-17 article with UV discovery at 256 nm in addition to eluted with 70:30:1 (methanol: water: acetic acid). Guggultetrol-18-ferrulate contented be examine With the above HPLC method for the 15 dissimilar source of sample of P. zeylanica. The results shows HPLC chromatogram of dichloromethane extract of P. zeylanica as gugultetrol-18-ferrlutate. The preservation time for the 14 different samples in the crude extract was Given with the gugultetrol-18-ferrlutate preservation time as 4.915 min. The mean add to in paw volume in methanol take out of P. zeylanica at 301 and 505 mg/kg and Diclofenac treated groups was 0.41 ± 0.061, 0.23 ± 0.083 and 0.20 ± 0.02 ml, in that order, as compare to 0.58 ±

0.098 in control group. The metabolic take out of P. zeylanica roots were tested for its anti-inflammatory effects at 300 and 500 mg/kg concentration shaped 31.03 and 60.30% ($P < 0.01$) reserve of acute inflamemation, respectively; in carrageen induce oedema proves that methanolic extract of root are next to acute inflammation. Cytotoxicity of dichloromethane extract of Plumbagoz zeylan .The CH peak is assigned to 33.96 - 56.77 ppm. The double bond peaks at 140.76 - 121.72 ppm. The secondary hydroxyl group at 71.81 ppm. IR peaks CH2, CH appears at 2868 cm-1. The molecular peaks at m/e: 414(M)+ and molecular formula is C29H50O, respectively. From the above discussion the structurally possible compound is assigned as beta sitosterol. 1H NMR the CH3 group is assigned to 0.92 ppm all CH2 peaks corresponded to the 1.29 - 1.34 ppm. Secondary hydroxyl group assigned to 3.362 - 3.59 ppm, IR spectrum shows CH3, CH2, at 2925, 2852 cm-1. Aromatic proton at 6.82 - 7.30 ppm, IR peaks at 1466 cm-Methoxy group at 3.52 ppm as a singlet double bond at 5.4 and 5.58 ppm in IR hydroxyl group at 3400 cm-1. At 63.26 - 79.53 ppm. The carboxyl group peaks at 174.59 ppm. The double bond peaks at 149.29 and 101.20 ppm. Methoxy group peaks at 56.76 ppm. The molecular peaks at m/e is 494(M)+ and molecular formula is C28H46O7. From the above discussion the possible compound is assigned as guggultetrol-18-Ferrulate.

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