Drug Standardisation

Standardisation of Homoeopathic Drug: "Plumbago Zeylanica Linn.": Physico – Chemical Perspective

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Abstract

Herbal products are being used as drug (the term itself being derived from the French word 'drogue' meaning 'Dry Herbs') from time immemorial in fighting against diseases of different kinds. The whole plant of Plumbago zeylanica Linn. (Plumbaginaceae) is taken for standardization. The crude as well as mother tincture was standardized to lay down standard in homoeopathic system of medicine. Plumbago zeylanica Linn. is having multifarious uses in homoeopathy and other Indian systems of medicine. The whole plant has been used in the form of coarse powder. 50% alcohol was used in preparation of mother tincture. The physico-chemical parameters viz. extractive value, ash value, total solids, alcohol content, pH and analysis by thin layer chromatography (TLC) and U.V. absorbance have been taken as Pharmacopoeial standards.

Introduction

Plumbago zeylanica Linn. (P.zeylanica) belongs to family Plumbaginaceae. It is popularly known in Hindi and Bengali either as Chita or Chitarak. In Tamil it is known as Cithira mulum, a vital drug of Siddha and Ayurveda. It is perennial, sub-scandent shrub, found wild in the forest, of peninsular India, West Bengal and also cultivated throughout India. This species is more widespread and common than Plumbago indica and is possibly indigenous to South East Asia.

The roots of P.zeylanica are said to constitute the original indigenous drug ie, Chitrak or Chitramulam known to Ayurvedic Physicians. The root bark of P.zeylanica contains an orange yellow pigment, Plumbagin (2-methyl-5 hydroxy - 1-4 naphthoquinone ($C_{14}H_8O_3$), free glucose and fructose. Roots are useful in stimulating digestive processes. The root appears to possess abortifacient and vesicant properties. Beside, it is diuretic, caustic and expellant of phlegmatic humours and also useful in rheumatism. It is used against skin irritation, in the treatment of dyspepsia, piles, anasarca, diarrhoea and also various skin diseases. A paste prepared in milk, vinegar or salt and water is used as an external application in leprosy and other skin diseases of an obstinate character. The crude Chitarak is apt to cause abortion. In Africa, a cold infusion of the root is used for influenza and black water fever. A tincture of root bark is antiperiodic. Alcoholic and aqueous extracts of roots showed antibacterial activity[3]. Chloroform extract showed antibacterial activity on some selected microorganism[4]. The Plumbagin active principle of P.zeylanica, is used against cancer under drug delivery system [5]. It is administered intraperitoneally at the dose level of 5 mg/kg and the niosomes of the drug complex showed promising anti-fertility activity [5].The frequent use of poisonous plants in treating several ailments is on the rise. In most of the cases the patients are getting no benefits out of the drugs, but their conditions become worse. Plumbago is meant for use in criminal abortion[6].

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Isolation and structure elicit two new triterpenoids, viz, 1 keto – 3 beta, 19 alpha dihydroxy-urs-12-ene-24,28-dioic acid dimethyl ester, MP 115°C and its 3-0 beta D-arabino pyranosyl derivative (MP 160°C) from the roots of P.zeylanica [11] A total synthesis of P.zeylanica was tried for ethanopharmacomedical interests in the Republics of Fiji and other South Pacific Countries for the development of drug for human life[9].

As there are no physico-chemical data recorded elsewhere to authenticate the drug in Homoeopathic system till date, the author has undertaken this study for the first time.

Materials and Methods

The air dried (shade dried) sample of Plumbago zeylanica Linn, (whole plant) was supplied by Survey of Medicinal Plants and Collection Unit (Homoeopathy), Udhagamandalam, Tamil Nadu. The above sample was coarsely powdered to (10/44) (sieve size) and was subjected for determination of moisture content (loss on drying at 105° C), total ash content, water soluble ash, acid insoluble ash and extractive values in different solvents (varying polarity). The above parameters have been determined in accordance with the procedure given in Homoeopathic Pharmacopoeia of India. Maximum extractive value (MEV) of the drug was determined using different strengths, of alcohol (in order to fix the alcohol content for preparation of mother tincture). Mother tincture was prepared as per HPI [1]. In this method 100 gm of P. zeylanica coarse powder was suspended in 528 ml of 95% alcohol and 472 ml of purified water for 24 hours at room temperature. It was filtered and made up to volume 1000ml using same solvent ratio. Percolation method has been used for the preparation of mother tincture.

The alcoholic extract (Mother tincture) was studied for its:-

- Physico chemical constants
- Chromatography &
- U.V. Absorbance

All chemicals and solvents used were of analytical grade, Silica gel-G was used for thin layer chromatography and all the work were carried out at the room temperature. U.V. spectra was recorded on U.V. spectrophotometer, Shimadzu, Model 160 A.

Physico-chemical Constants

Physico-chemical parameters viz., organoleptic properties, wt. per ml. total solids, alcohol content, pH value were determined as per the procedure laid down in the Homoeopathic Pharmacopoeia of India.

Chromatography

For thin layer chromatography, 25 ml of alcoholic extract was evaporated on waterbath to remove alcohol. The remaining aqueous part was extracted with 25ml of chloroform (three times). All the three fractions were combined and concentrated to 2ml and 15ml was applied on activated silica gel-G coated T.L.C. plate. It was developed using ethyl acetate: petroleum ether (60-80°) (3:7 v/v) as mobile phase and leuco methylene blue solution used for visualization.

U.V.Absorbance

For UV absorbance one part of mother tincture was diluted with 99 parts of menstrum (hydro-alcoholic mixture in a specific ratio that is 50% alcohol.) The spectrum was recorded in the range of 200-400 nm. The tincture exhibits one peak but on making alkaline with a few drops of 1N sodium hydroxide, the tincture gave bathochromic shift. The peaks of maximum absorption are given in table 5.

PLUMBAGO ZEYLANICA

Results and Discussion

Plumbago zelaynica Linn. was allotted under Drug Standardization programme to Homoeopathic Drug Research Institute, Lucknow by Central Council for Research in Homoeopathy, New Delhi to lay down the standards for the raw drug as well as finished product in Homoeopathy system of medicine. The data under the raw drug study, viz. moisture content, ash value, extractive value in different solvents are given as in **Table 1**.

Table 1: Standardization of raw drug

S.No.	Parameters	Quantitative value
1.	Moisture content (Loss on drying at 105°C)	9.68% w/w
2.	Total ash content	6.425 % w/w
3.	Water soluble ash	5.665% w/w
4.	Acid insoluble ash	1.05% w/w

Formulation of the mother tincture has been achieved on the basis of maximum extractive value (MEV) determined by using various fractions of alcohol as mentioned in the **Table 2**.

Extractive Value in different Solvents

S.No.	Solvent	Extractive value
1.	Acetone	0.5% w/w
2.	Absolute alcohol	5.9% w/w
3.	Chloroform	1.5% w/w
4.	Methanol	7.0% w/w
5.	Pet-ether(40-60°)	2.0% w/w
6.	Distilled Water	12.5% w/w

All the estimations were performed using coarse power.

Alcoholic extract gave positive test for Phenolic compound by Ferric chloride. The observed value under the standardization of mother tincture is wt. per ml 0.93g, total solids 1.61 percent w/v, PH 5.2, alcohol content 48% v/v are shown in **Table 3**.

TLC studies of the chloroform extract of the mother tincture reveal two prominent spots at Rf 0.2 (brown) and 0.35 (brown). The results are given in **table 4**.

The diluted mother tincture when scanned under U.V. spectra in the range of 200-400 nm has exhibited one distinct peak with maximum absorbance at 214.8 nm while the tincture on

Table 2: Determination of MEV using different ratio of alcohol and water

S.No.	Strength of Alcohol% v/v	Mean Extractive Value	Remarks
1.	30	16.4	50% of alcohol used for preparation of mother tincture on the basis of M.E.V
2.	35	15.6	
3.	40	17.67	
4.	45	21.8	
5.	50	21.81	
6.	55	15.53	
7.	60	14.6	
8.	65	14.4	
9.	70	11.3	
10.	75	12.2	
11.	80	13.75	
12.	85	11.3	
13.	90	13.4	
14.	95	14.5	
15.	99.5	5.6	

Table 3: Physico – chemical standardization of mother tincture

S.No.	Parameters	Observations
1.	Organoleptic Properties	
	A) Appearance	Hazy, non-viscous liquid
	B) Odour	Characteristic
	C) Colour	Yellowish green
2.	Sediments	Absent
3.	Wt. per ml	0.93 g
4.	Total solids	1.61 % w/v
5.	Alcohol Content	48 % v/v
6.	PH at R.T.	5.2

Table 4: Chromatographic result of Plumbago zeylanica Linn.

Extract : Chloroform extract of mother tincture

Absorbent : Silica gel – G

Layer thickness : 0.5 mm on wet condition

S.No.	Solvent system	Detecting agent	No. of Spots	Rf vlaue	Colour of the spots
1.	Ethyl acetatepetroleum ether (60^0-80^0) –(3:7 v/v)	Leucomethy lene blue	2	0.20.35	BorwnBrown

Table 5: U.V. absorbance of alcohol extract of Plumbago zeylanica Linn.

S.No.	Mother Tincture	No. of peaks	U.V. absorbance
1.	Plumbago zeylanica 50% alcohol extract	1	214.8 nm
2.	After making alkaline	2	369.0 nm 236.5 nm

making alkaline with few drops of 1N sodium hydroxide gave bathochromic shift from 214.8 nm to 236.5 nm and also gave one extra peak at 369 nm which may be due to the presence of phenolic compound viz. naphthoquinone. The peak of maximum absorption is summarized in the **table 5**.

Conclusion

These observed physico-chemical data, Rf value, U.V. absorbance as well as methodology followed in the current study may be taken for identification and standardization of Plumbago zeylanica Linn.

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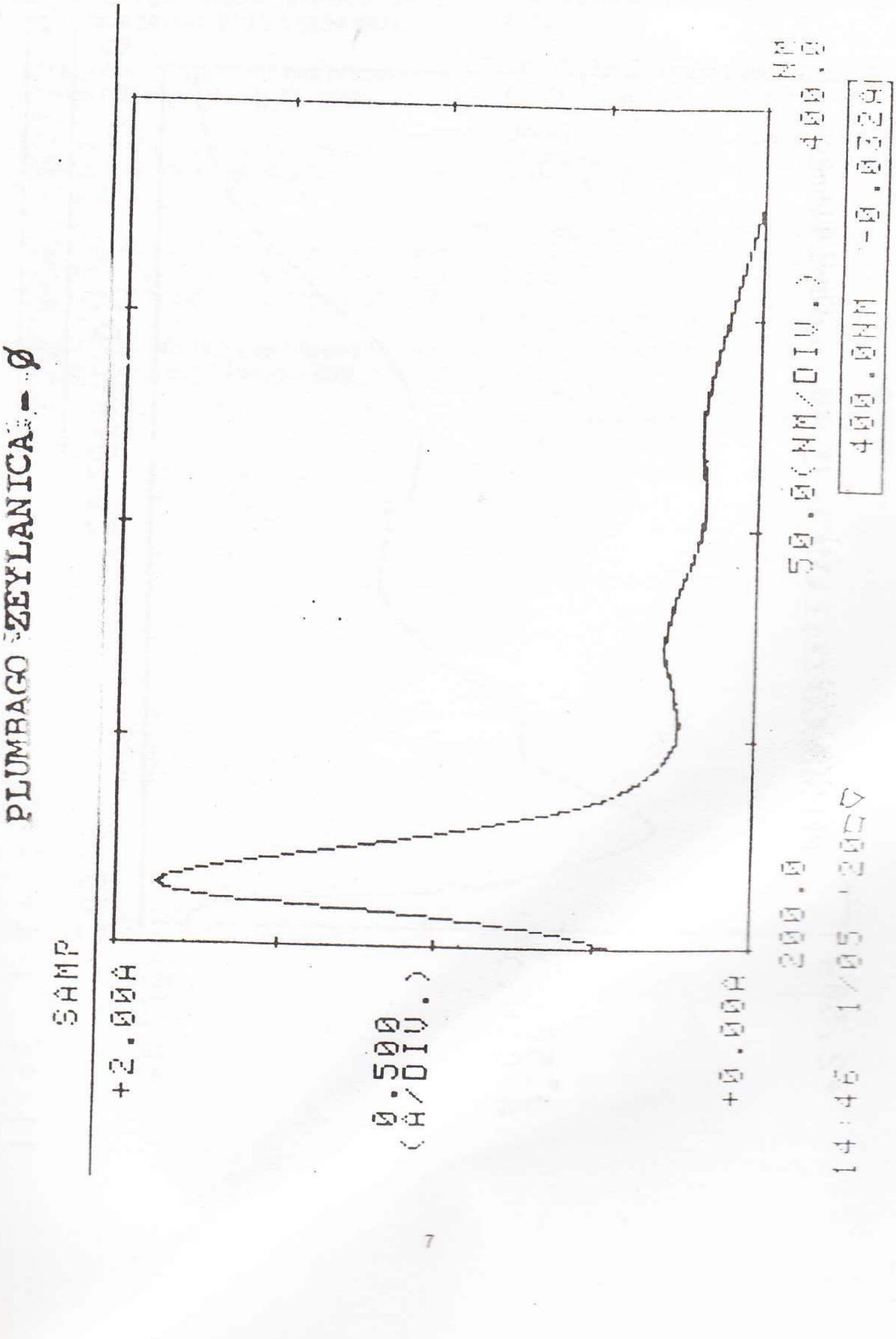
PLUMBAGO ZEYLANICA – Ø
SAMP
+2.00A
0.500
(A/DIV.)
+0.00A
200.0
50.0(NM/DIV.)
400.0
NM
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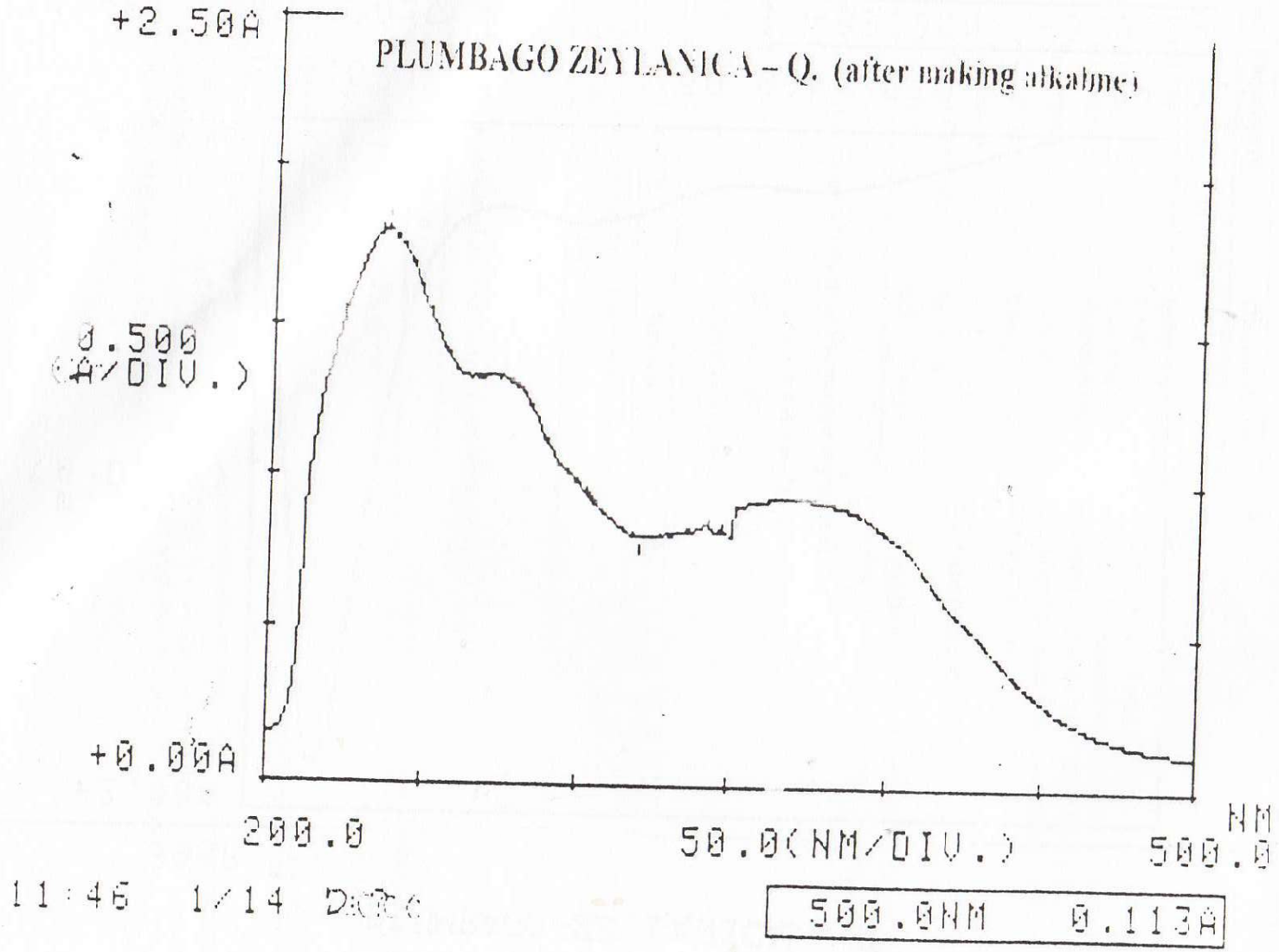
PLUMBAGO ZEYLANICA – Q. (after making alkaline)
+2.50A
0.500
(A/DIV.)
+0.00A
200.0
50.0(NM/DIV.)
500.0
NM
11:46 1/14
500.0NM 0.113A

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