

PHYSICO-CHEMICAL PROFILE OF SOME COLOURING PLANTS USED IN HOMOEOPATHY

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ABSTRACT: *The objective of this paper deal with the physico chemical aspects of certain colouring plants namely. **Bixa orellana** Linn. (Leaves) and **Lawsonia inermis** Linn (Leaves). The determined data under the physico chemical, chromatographic and spectrophotometric studies can be taken as a pharmacopoeial standards.*

INTRODUCTION

Bixa orellana, family-Bixaceae, it is commonly known in Hindi-Latkan, Senduria, Sanskrit-Shonapushpa. Tamil-jafframaram, English-Annatto, Lipstick tree In Latin it is **Bixa orellana** Linn. Seeds are source of orange red dye, annatto. A shrub or a small tree 2.5 min height it is found throughout the hotter parts of India. It is cultivated for seeds to some extent in Orissa A.P., Maharashtra. It is grown as ornamental hedge. The tree is found to thrive at elevation of 600-900 m where annual rain fall ranges from 125-175 cm. The seeds, which are dry, hard, bright red and have a characteristics smell are considered good for dyeing. They form 30-40% k of the total weight of fruits. The red pulpy seed coat (testa) is the source of the dye. Annatto also called orlean. A carotenoid, bixin, is the principle colouring matter present in the seeds. A water soluble yellow dye, orellin, methyl

Bixin, β -carotene, cryptoxanthin lutein and zeaxanthin are also present. From the seed coat of **Bixa orellana** fruits, six minor carotenoids and one C14, carotenoid viz. Dimethyl (9z,9'z)-6,6'- diapo carotene-6,6'-dioate, methyl (9z-10' -oxo-6,10'-

diapocaroten-6-oate, methyl (9z-6 -oxo-6,5'-diapocaroten-6-oate, methyl(4z,-4,8-dimethyl-12-oxo-dodecyl-2,4,6,8,10 - pent aenoate are new compound (Mercandante, A.Z: (1997). Bixin can also be used as an absorption indicator. Annatto dye has been proved non-carcinogenic. The dye is extensively used for colouring butter, ghee, cheese, ice-cream, chocolate, meat, cereals, spices ect. The pulp surrounding the seeds is widely employed in medicine as a haemostatic, astringent, antidysentric, diuretic, laxative, digestive, epilepsy and skin diseases. In central America and Mexico, the oil pressed from seeds is used for treating leprosy (Anonymous 1988). In Homoeopathy, **Bixa orellana** is recommended for leprosy, eczema and elephantiasis [Boericke 1024, 1991 (Reper.Ed.)] Extracts of **Bixa orellana** also exhibited platelet antiaggregant activity [Vollar R (1997)] while **Lawsonia inermis** Lin. Family-Lythraceae is commonly known in Sanskrit-Mendika, Hindi-Mehndi, Tamil-Maruthani. It is a glabrous, much branched Shrub, with grayish brown bark, leaves opposite sup-sessile, elliptic, entire, acute or obtuse, it is widely cultivated as an

ornamental and dye plant in many tropical and warm temperate regions

Henna has long been used in India and Middle East countries for colouring palms of hands, soles of feet and finger nails, It is also used for dyeing hair beard and eyebrows for personal adornment, which is also used for colouring leathers and skin. It act as a substantive dye to keratin and imparts an orange-red colour. It is harmless and causes no irritation of skin (Anonymous. 1988) it produces agreeable aroma and enhances attractiveness. Among the natural aids to beautification, henna has tenaciously maintained its popularity form ancient times to beyond memory [Hannan. A (Hakim) 1997].

Henna leaves are used as a prophylactic against skin diseases. They have astringent properties. They are used externally in the form of a paste or decoction against boils, burns, bruises and skin inflammative. A decoction is used as a gargle for relaxed sore throat. The principal colouring matter is Lawsone. 2-hydroxy, 1:4-napthaquinone (C₁₀H₆O₃) (Anonymous 1988) Hither to the physico- chemical standards are lacking hence, the authors have undertaken these study to lay down standards in the Homoeopathic system of medicine.

MATERIALS AND METHODS

The air dried (shade dried) sample of seeds of **Bixa orellana** Linn and leaves of **Lawsonia inermis** Linn were supplied by survey of Medicinal Plants and Collection Unit. Udthagamandalam, Tamilnadu, the dried samples were comminuted to obtain a coarse powder (10/44) which are then used for determination of moisture content (Loss on drying at 105oC) extractive value in different solvents (varying polarity) and ash value, acid insoluble ash, water soluble ash.

The above parameters have been determined in accordance with procedures given in H.P.I and I.P. The mother tinctures were prepared as per the H.P.I (1971) in this method 100gm of **Bixa orellana** Lin in coarse powder was kept with 687 ml of 95%of alcohol and 313 ml of purified water for 24 hours. After that it was filtered and made upto 1000 ml using the same solvent ratio. In this way 100 gm of **Lawsonia inermis** Linn. was kept with 633 ml purified water and 367 ml of 95% alcohol for 24 hours after that it was filtered and made upto 1000 ml using the same solvent ratio and technique.

All chemicals and solvents used were of analytical grade silica gel-G of E-merck was used of thin layer chromatography and work was carried at room temperature. U.V. spectra were recorded on Shimadzu model 160A.

The above alcoholic extracts (mother tinctures) were studied for the

- A. Physico-chemical constants
- B. Chromatography
- C. U.V. absorbance

PHYSICO-CHEMICAL COMSTANTS

Physico-chemical parameters viz. organoleptic properties, wit per ml, total solids alcohol content, PH value were determined as per the procedure laid down in the Homeopathic pharmacopoeia of India.

CHROMATOGRAPHY

For thin layer chromatography 25ml of alcoholic extract (**Bixa orellana Q**) was evaporated on water bath to remove alcohol, the remaining aqueous part was extracted with 25ml of chloroform (three times). All the three fraction were combined and concentrated to 2 ml and 15 µl was applied

on TLC plate and it was developed using chloroform: methanol (9:1) v/v as mobile phase and visualised with antimony trichloride in CCl₄ Iodine vapours, while 25ml mother tincture of **Lawsonia inermis** was evaporated on water bath to remove alcohol. Concentrated extracts was prepared in the foregone way and 15µl was applied on TLC plate and it was developed using benzene: methanol (40:1:1) v/v as mobile phase and 1% methanolic KOH Solution used for visualisation.

U.V. ABSORBANCE

For U.V. absorbance the mother tinctures were diluted to suitable level with menstruum (i.e. Bixa orellana 65% and Lawsonia inermis 35% alcohol respectively). The spectrum are recorded at the range of 200-400 nm for the both drug. The peak of maximum absorption are given in the Table5.

RESULTS AND DISCUSSION

Bixa orellana Linn and Lawsonia inermis Linn. have been allotted for Drug Standardisation programme at Homoeopathic Drug Research Institute. Lucknow by Central Council of Research in Homeopathy, New Delhi to lay down standard for raw drug and finished product in homoeopathic system of Medicine. The raw drug studies shows that moisture content total ash content of powdered drug, water soluble ash, extractive value if different solvents like acetone, alcohol, chloroform, methanol, petroleum ether and distilled water have also been determined to supplement the analytical data for laying down the standard for these drug. The above results are presented in Table1.

Formulation of the mother tinctures have been done on the basis of Maximum Extractive Value (M.E.V) determined by using various strength of alcohol (Table 2) and percolation method have been used for the preparation of the tinctures. Physico-chemical standardization studies of the mother tinctures namely wt per ml, total solids, alcohol content, pH value, which have been summarized in Table 3.

It is evident from the TLC studies that is chloroform extract of the Bixa orellana Q, two prominent spots appeared in antimony trichloride reagent and six prominent spots appeared when the plate was kept in Iodine vapours whereas that mother tincture of Lawsonia inermis shows four prominent spots in methanolic KOH reagent. The results are depicted in Table 4A & 4B. Suitable diluted mother tincture (Bixa orellana) when scanned under the U.V. Visible spectra in the range of 200-400 nm shows one distinct peak (maximum absorbance) at 215.4 nm whereas the mother tincture (Lawsonia inermis) exhibits one distinct peak at 214.6 nm.

CONCLUSION

The parameters determined in standardization of crude drugs and their mother tinctures can be taken as pharmacopoeial standards of these drugs in Homoeopathic system of medicine.

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TABLE -1
STANDARDISATION OF RAWDRUGS

S.No.	PARAMETERS	QUANTITATIVE A	VALUES B
1.	Moisture content (L.O.D. at 105oC)	12.03%W/W	10.74%W/W
2.	Total ash content of Powdered drug	4.0730%W/W	6.0730%W/W

3.	Water soluble ash	1.97%W/W	2.45%W/W
4.	Acid insoluble ash	0.14%W/W	0.550%W/W

EXTRACTIVE VALUES IN DIFFERENT SOLVENTS

S.No.	SOLVENTS	EXTRACTIVE	
		A	B
1.	Acetone	8.25 %W/W	13.1 %W/W
2.	Absolute alcohol	8.7 %W/W	24.58 %W/W
3.	Chloroform	11.05 %W/W	5.58 %W/W
4.	Methanol	14.05 %W/W	31.55 %W/W
5.	Pet. ether (60-80oC)	6.95 %W/W	2.8 %W/W
6.	D water	19.5 %W/W	23.5 %W/W

A – Bixa orellana

B- Lawsonia inermis

TABLE – II
DETERMINATION OF MEX USING DIFFERENT RATIO OF ALCOHOL AND WATER

S.No.	Strength of alcohol %v/v	Mean Extractive Value		Remarks
		A	B	
1.	30	20.575	33.44	A-65% of alcohol used for preparation on mother tincture on the basis of MEV
2.	35	21.705	37.65	
3.	40	20.183	33.63	
4.	45	19.7	32.02	
5.	50	20.25	30.92	
6.	55	19.9	32.80	
7.	60	19.375	35.03	
8.	65	22.115	34.73	
9.	70	19.85	34.43	
10.	75	17.05	31.55	
11.	80	15.16	20.63	
12.	85	13.02	30.13	
13.	90	14.37	28.80	
14.	95	9.79	25.00	
15.	99.5	8.70	24.58	

A – Bixa orellana

B- Lawsonia inermis

TABLE –III
PHYSICO-CHEMICAL STANDARDS OF MOTHER TINCTURES

S.NO.	PARAMETERS	OBSERVATION	
		A	B

1.	Organoleptic properties a) Appearance b) Colour c) Odour	Non-viscous, liquid Light maroon Characteristic	Non-viscous, liquid Brown Pleasant
2.	Sediments	Absent	Absent
3.	Wt. Per ml	0880 gm	0.95 gm
4.	Total solids	1.73 %W/W	3.4 %W/W
5.	Alcohol content	64 %v/v	34 %v/v
6.	pH value	4.52	5.2

A – Bixa orellana

B- Lawsonia inermis

TABLE –IV A
CHROMATOGRAPHIC RESULTS OF BIXA ORELLANA

EXTRACT : Chloroform extract of mother tincture

ADSORBENT : Silica Gel-G

LAYER THICKNESS : 0.5 mm on wet condition

S. No.	Solvent system	Detecting agent	No. of spots	R f values
1.	Chloroform methanol (9:1)v/v	Antimony trichloride in CC14	2	0.29 0.91
2.	Chloroform methanaol (9:1)v/v	Iodine vapours	6	0.89 0.83 0.78 0.64 0.28 0.12

TABLE – IV B
CHROMATOGRAPHIC RESULTS OF LAWSONIA INERMIS

EXTRACT : Chloroform extract of mother tincture

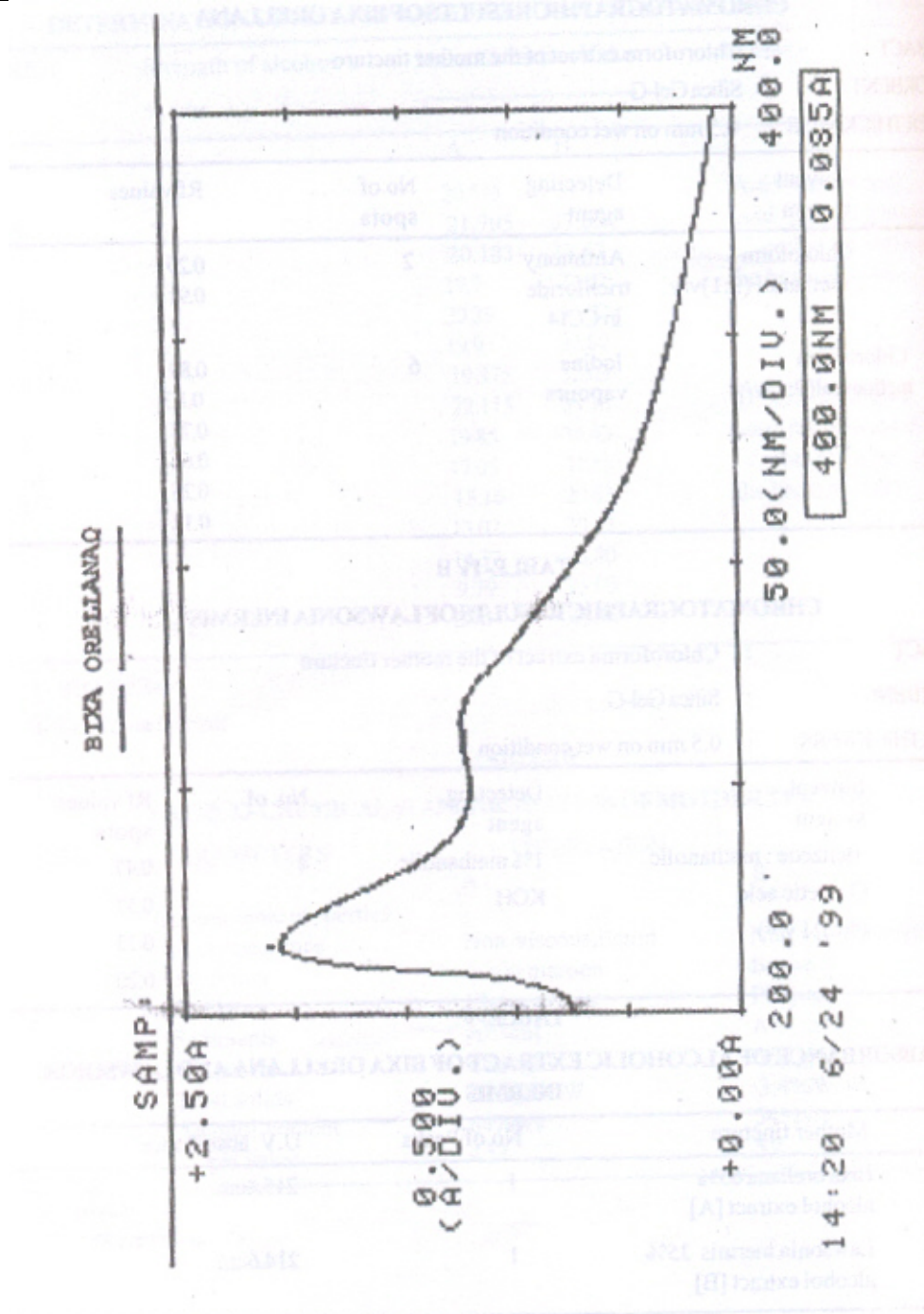
ADSORBENT : Silica Gel-G

LAYER THICKNESS : 0.5 mm on wet condition

S. No.	Solvent system	Detecting agent	No. of spots	R f values
1.	Benzene: methanolic G acetic acid (40:1:1 v/v)	1% methanolic KOH	4	Spots 0.47 0.37 0.13 0.20

TABLE – V
U.V ABSORBANCE OF ALCOHOLIC EXTRACT OF BIXA ORELLANA AND
LAWSONIA INERMIS

S.No.	Mother tincture	No.of Peaks	U.V. absorbance
1.	Bixa orellana 65% alcohol extract [A] Lawsonia inermis 35%	1	215.4nm
2.	Alcohol extract [B]	1	214.6nm



LAWSONIA INERNSO

SAMP:

+2.00A

(A/DIV.)

+0.00A

200.0

50.0(NM/DIV.) 400.0 NM

12:20 6/18 '99

400.0NM 0.0004A