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% 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 diapocaroten-6-oate, methyl (9z-6 –oxo-6,5′-diapocaroten-6-oate, methyl( 4z-,4,8-dimethyloxo-dodecyl)-2,4,6,8,10 – pentaenoate 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, antisyndentic, 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 Homeopathy, 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 inflammatrive. A decoction is used as a gargle for relaxed sore throat. The principal colouring matter is Lawsone. 2-hydroxy, 1:4-napthaquinone (C10H6)3 (Anonymous 1988) Hither to the physico-chemical standards are lacking hence, the authors have undertaken these study to lay down standards in the Homeopathic 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. Udhagamandalam, 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 CC14 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 Lowasonia 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 taw 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 an 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    |
### EXTRACTIVE VALUES IN DIFFERENT SOLVENTS

| S.No. | SOLVENTS                  | EXTRACTIVE VALUES |
|-------|---------------------------|-------------------|
| 1.    | Acetone                   | 8.25 %W/W         |
| 2.    | Absolute alcohol          | 8.7 %W/W          |
| 3.    | Chloroform                | 11.05 %W/W        |
| 4.    | Methanol                  | 14.05 %W/W        |
| 5.    | Pet. ether (60-80oC)      | 6.95 %W/W         |
| 6.    | D water                   | 19.5 %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                                        |
| 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-CHEMICALSTANDRADISATION OF MOTHER TINCTURES

| S.NO. | PARAMETERS | OBSERVATION |
|-------|------------|-------------|
|       |            | A           | B           |

A: Bixa orellana  
B: Lawsonia inermis
1. Organoleptic properties
   a) Appearance: Non-viscous, liquid
   b) Colour: Light maroon
   c) Odour: Characteristic

2. Sediments:
   3. Wt. Per ml: 0880 gm
   4. Total solids: 1.73 %W/W
   5. Alcohol content: 64 %v/v
   6. pH value: 4.52

A – Bixa orellana
B- Lawsonia inermis

TABLE –IV A
CHROMATOGRAPHIC RESULTS OF BIXA ORELLANA

| 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 |
|        | Chloroform methanol (9:1)v/v | Iodine vapours | 6 | 0.91 |

TABLE – IV B
CHROMATOGRAPHIC RESULTS OF LAWSONIA INERMIS

| 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]               | 1           | 215.4nm         |
| 2.    | Lawsonia inermis 35%                                |             |                 |
| 2.    | Alcohol extract [B]                                 | 1           | 214.6nm         |
