EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 3
438-442
Year: 2018

HPTLC FINGERPRINT PROFILE AND PHARMACOGNOSTIC STUDY OF BILVA FRUIT PULPA (AEGLE MARMELOS (LINN.) CORR

[1]Akanksha Mishra, [2]Pradeep Tripathi, [3]SuryaPrakash Gupta and [4*]Manoj Tripathi

[1-3]Faculty of Pharmacy, AKS University, Satna (M.P.).
[4]Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.).

***Corresponding Author: Dr. Manoj Tripathi**
Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.).

Article Received on 15/01/2018 **Article Revised on 04/02/2018** **Article Accepted on 25/02/2018**

ABSTRACT
Bilva *Aegle marmelos* (Linn.) Corr. (Fam. Rutaceae) is a moderate sized tree, 6-12m high, branches armed with straight and growing wild and also cultivated throughout the country. Its fruit pulpa used to cure various ailments such as atisara, Grahani, Raktatisara, Raktarsha, Vibandha, Kasa, Shwasa, Madhumeha, Shukrameha, Garbhashaya shotha, Jwara, Vamana etc. The present paper provides a detailed account of the pharmacognostic studies of Bilva fruit pulpa. The study includes macro and microscopic study, powder microscopic characteristics, High Performance Thin Layer Chromatography fingerprint profile, preliminary phytochemical screening and physicochemical parameters. The information generated by this particulars study provides relevant HPTLC fingerprint profile and physicochemical data needed for proper identification and authentication of Bilva fruit pulpa and root.

KEYWORDS: *Aegle marmelos* (Linn.) Corr**.** Pharmacognostic study, HPTLC fingerprints profile, Physico-chemical.

INTRODUCTION
Bilva *(Aegle marmelos* (Linn.) Corr**.)** A moderate sized tree, 8-12m high, branches armed with straight, sharp, axillary, 2.5cm long spines. Leaves trifoliate, occasionally digitate, five-foliate; leaflets ovate or ovate-lanceolate, acuminate, crenate, lateral sessile, terminal long petioled. Flowers greenish white, in short axillary panicles. Fruit globose, grey or yellowish, rind woody. Seeds many, oblong, embedded in sacs covered with thick orange coloured sweet pulp **(Nishteswar K and Hemadri K 2010)**. It contains number of chemicals *viz.* coumarins (marmesin, imperatorin, alloimperatorin methyl ether, xanthotoxol, scoparone, scopoletin, umbelliferone, skimmin, isopimpinellin, psoralen and marmelide), alkaloids, polysaccharides yielding galactose, arobinose, uronic acid, fatty acids and essential oils (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acids) etc. It is used to treat various ailments like Atisara, Pravahika, Agnimandya, Grahani, Raktatisara, Raktapravahika, Raktarsha, Vibandha, Kamala, Kasa, Shwasa, Ikshumeha, Madhumeha, Shukrameha, Garbhashaya shotha, Shwetapradara, Jwara, Vamana, Unmada, Netrabhishyandu, and also used to preparation of ayurvedic compound formulations like Bilvapanchaka kvatha, Bilvadi churna, bilvadi ghrita, mushikadya taila, bilva taila, bilvamooladi gutika, dashamoolarishta,bilvadi leha, brihat gangadhara churna, dashmoola taila, pippalyadi taila, Madhyama narayana taila, Dhanyapanchaka kvatha, chyavanaprasha, amritarishta **(Anonymous 2001**). Keeping this aim into consideration, the present study was designed to scientific evaluation of Bilva fruit pulpa. The study includes macro and microscopic characters, powder microscopic characteristics, High Performance Thin Layer Chromatography fingerprints, preliminary phytochemical screening and cphysicochemical parameters. The information generated by this particulars study provides relevant pharmacognostical and physicochemical data needed for proper identification and authentication of Bilva fruit pulpa.

MATERIALS AND METHODS
Collection and identification of plant
Fruit pulpa of *Aegle marmelos* was collected from Arogyadham campus at Deendayal Research Institute, Chitrakoot, Satna (M.P.). The plant material was identified and authenticated with the help of Herbarium specimen at Herbarium of Research Lab, Chitrakoot. All chemicals and reagents were used analytical grade.

Macroscopically studies
Fruit pulpa powder was studied for its organoleptic characters such as colour, odour, and taste **(Kokate**

2006).

Microscopically studies

About 1g powdered drug was treated with Chloral hydrate and 2% potassium hydroxide, boiled and cooled. Washed the treated sample with distilled water 5-6 times for Chloral hydrate and 1-2 times for 2% potassium hydroxide. The treated sample were stained with Sudan III and iodine water, mounted with glycerin and observed under the compound microscope at 40 X 10X magnification of the trinocular research microscope fitted with digital Camera Lucida **(Evans WC, 2003).**

Physico-chemical analysis

Physicochemical studies are includes extractive values (alcohol, water & Hexane soluble), total ash value, acid insoluble ash value and loss on drying at 105^0C **(Anonumous, 2007).**

Fluorescence Analysis

The color change of the powdered samples with respect to different chemical reagents on the basis of different chemical constituents was observed in daylight and ultraviolet as per the methods described **(Choudhary *et al.* 2014).**

Preliminary phytochemical analysis

Preliminary phyto-chemical analysis of aqueous and alcoholic extracts of fruit pulpa of Bilva was carried out by employing standard protocols for determining the presence and/or absence of phytochemical **(Tripathi & Sikarwar 2015).**

HPTLC (High Performance Thin Layer Chromatography)

For HPTLC, 2 gm of sample was extracted with 25 ml of methanol on boiling water bath for 20 min. Filtered through Whatman filter paper No. 1 and concentrated. TLC of methanolic extracts of the sample was carried out on silica gel 60 F254 percolated plates (0.2 mm thickness; from Merck India Limited Mumbai). An applicator from Camag Linomat-5 Camag Switzerland: 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints. The mobile phase used Toluene: Ethyl acetate (7:3). The plate was developed over a distance of 8 cm in a saturated development chamber (Twin through chamber 20X10 cm with SS lid and visualized under 254nm, 366nm and visible light. After spraying with 5% methanolic sulphuric acid followed by heating at 110^0C for 10 min **(Tripathi et al. 2015).**

RESULT AND DISCUSSION

Macroscopic characters

Bilva fruit externally greenish when young, yellowish brown when ripe, sub- globose, 5-18 cm in diameter, rind about 1.5 mm-3 mm thick, hard and woody, seeds flat, oblong, fresh pulp of ripe fruit, brown, dried fruit pulp hard and pale to dark red in colour, frequently breaks away from the rind during drying, leaving a thin layer attached to it. Odour, fainty aromatic, taste, mucilaginous and slightly astringent (Fig 1-3).

Microscopic characters

Bilva fruit Pulpa constitutes the mesocarp of fruit. There is almost no tissue differentiation into distinct zones. Vascular bundles are scattered in the ground tissue some of them are anastomosing in different directions. Ground cells around the vascular strands are compact thick walled and smaller. Similar structure is shown by the central axis except that the cells are smaller and thin walled. Vessels contain bordered pits and the tracheids show spiral thickenings. Fibres are long, linear and the cell walls are lignified. Sclereids show discontinuous lignifications in their cell walls (Fig 4&5).

Fig 1: Plant.

Fig 2: Fruit pulpa with rind.

Fig 3: Dried pieces of fruit pulpa

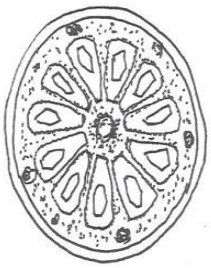

Fig 4: Schematic diagram of fruit in TS.

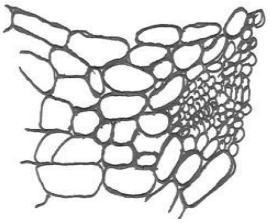

Fig 5: A portion of mesocarp in T.S showing thick walled cells around the smaller.

Table 1: Physico-chemical analysis of *Aegle marmelos* (L.) Corr. Fruit pulpa.

S. No.	Name of tests	Result
1	LOD (%w/w)	6.50%
2	ASE (% w/w)	22%
3	WSE (%) w/w)	66%
4	HSE (% w/w)	4%
5	Total ash (% w/w)	4.1
6	Acid insoluble ash (% w/w)	0.21

Powder microscopic characters

Shows prismatic crystal of calcium oxalate, simple and compound starch grains, oil globules, fragments of isolated vessels of scalariform thickenings, group of parenchymatous cells containing starch grains and calciuim oxalate crystals in surface view, group of parenchymatous cell containing oil globules and starch grains, isolated stone cells, groups of stone cells, fragments of isolated vessels of spiral thickening and fragment of pitted tracheid element (Fig 6-12).

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Physico-chemical results of the drug are given in (Table 1).

Fluorescence Analysis

Fluorescence study was done and results are given in (Table 3).

Preliminary phyto-chemical investigation

Qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. flavonoids, alkaloids, steroids, triterpenoids, essential oil and proteins are present.

Fig. 6: Prismatic crystals of calcium oxalate.

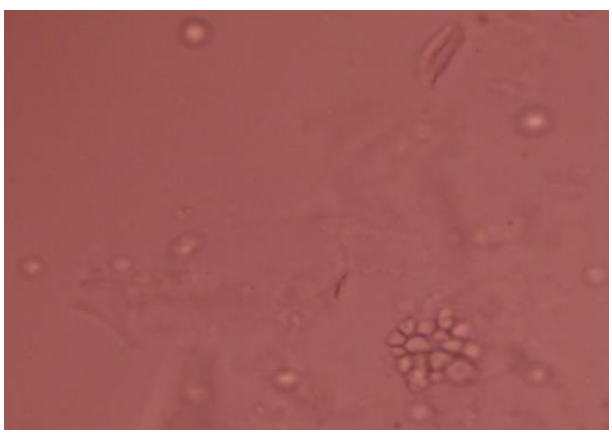

Fig. 7: Starch grains.

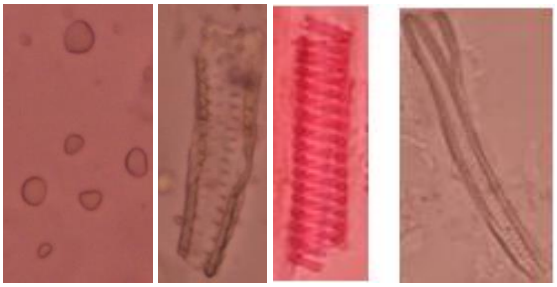

Fig. 8: Oil globule. **Fig.9: Tracheids.**

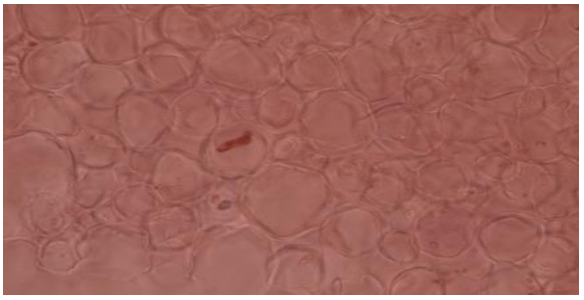

Fig. 10: Parenchymatous cells containing starch grains in surface view.

Fig.11: Fibres.

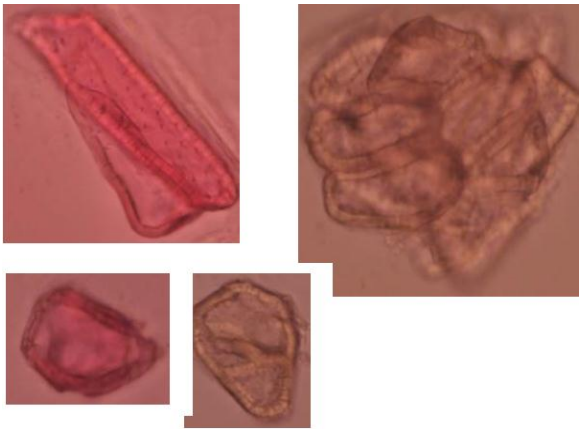

Fig.12: Groups of stone cells.

High performance thin layer chromatography

High performance thin layer chromatography (HPTLC) study of the methanolic extract one spot of the sample extract applied in the Thin Layer Chromatography plate. Major spots Rf values with colour were recorded under, 254nm, 366nm, after derivatization 366nm and UV light. Chromatogram profile and Rf values are given(Fig.13-16 & Table 2). The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify Bilva fruit pulpa. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. High performance thin layer chromatography finger print profile helps in identification of various

phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The High performance thin layer chromatography profile also helps to identify and isolate's important phyto-constituents. These finding could be helpful in identification and authentication.

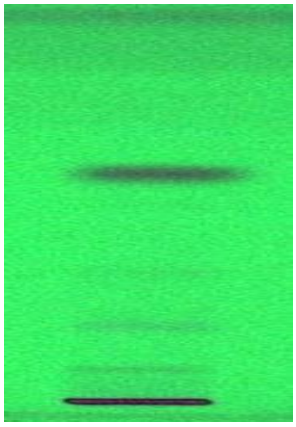

Fig.13: 254 nm before derivatization

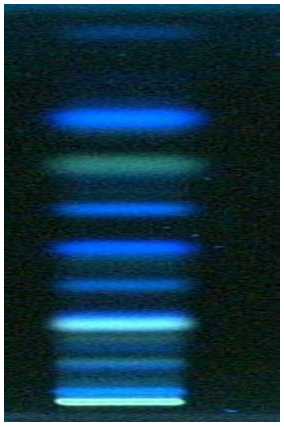

Fig.14: 366 nm before derivatization

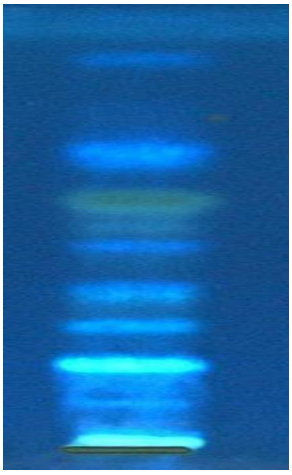

Fig.15: 366 nm after derivatization.

Fig.16: UV light after derivatization.

Table -2: Rf values of HPTLC profile

Rf. values	254 nm	366 nm	366nm After derivatization	UV light After derivatiza tion
Rf_1	0.08 black	0.04 sky blue	0.04 sky blue	0.60 black
Rf_2	0.20 black	0.06 brown	0.08 Sky blue	
Rf_3	0.32 black	0.08 Yellow ish pink	0.20 Sky blue	
Rf_4	0.60 black	0.20 florescence	0.30 blue	
Rf_5		0.30 blue	0.40 blue	
Rf_6		0.40 blue	0.54 blue	
Rf_7		0.54 blue	0.60 yellow	
Rf_8		0.60 yellow	0.74 blue	
Rf_9		0.74 blue		

Table-3: Florescence study.

S. No.	Drug powder+ Chemica l	Observation in UV light light	Observat ion in 366nm
1	Drug powder	Light yellow	Creamish white
2	Powder+ Acetic acid	Light yellow	Light yellow
3	Poder+50 % KOH	Turmeric yellow	Greenish white
4	Powder+1N HCL	Carrot redish	Brown
5	Powder+ 1N NaOH water	Pale yellow	Greenish yellow
6	Powder+ H2SO4	Black	Dark green
7	Powder+ Iodine water	Cream color	Greenish yellow
8	Powder+ 1N NaOH methyl	Yellow	Yellowish green
9	Powder+ 50%H2S O4	Light yellow	Light yellow
10	Powder+ 50%HN O3	Dark yellow	Dark brown

CONCLUSION

Bilva fruit pulpa has numerous uses in traditional medicine to treat several ailment *viz.* in treating stomach disorders, diabetes, cough, intestinal colic, skin diseases, dysentery etc. Due to its wide therapeutic importance it is worthwhile to standardize it for use as drug. The present study reveals HPTLC fingerprint profile and pharmacognostical standardization of drug *Bilva* which would be of immense value in botanical identification and authentication of plant drug may help us in preventing its adulteration.

ACKNOWLEDGEMENT

The authors are grateful to Er. A.K Soni, Chairman, AKS University, Satna (M.P.) for providing necessary facilities.

REFERENCES

1. Nishteswar K and Hemadri K. Dravyaguna – Vijnana, Chaukhamba Sanskrit Pratishthan Delhi, 2010; 23-24.
2. Anonymous *Ayurvedic Pharmacopoeia of India*. Government of India, Ministry of Health & Family Welfare, Department of Indian systems of medicine & Homoeopathy, New Delhi Part, 2001; I(III): 29-30.
3. Kokate CK *Practical Pharmacognosy* Ist ed., Vallabh Prakashan, New Delhi, 2006.
4. Evans WC In: *Trease and Evans Pharmacognosy*, 15th ed., (Saunders, London, 2003; 545-547.
5. Anonymous *Protocol for Testing of Ayurvedic, Siddha & Unani Medicines.* Government of India, Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines Ghaziabad, 2007.
6. Choudhary N, Siddiqui MB and Khatoon S Pharmacognostic evaluation of Tinospora cordifolia (Willd.) Miers and identification of biomarkers, *Indian J Tradit Knowle*, 2014; 13(3): 543-540.
7. Tripathi M and Sikarwar RLS Pharmacognostic Studies on Plaksa (*Ficus virens* Ait.) Stem Bark *Indian Journal of Natural Products and Resources*, 2015; 6(1): 27-32.
8. Tripathi M, Sikarwar RLS, Tiwari A and Dwivedi N, Pharmacognostical identification of ingredients in Laghulaicurna *Indian Journal of Traditional Knowledge*, 2015; 14(4): 531-536.