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Pharmacognostic and physicochemical standardization of stem of *Duranta Erecta*.

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Abstract

The aim of the present work was the evaluation of pharmacognostic, physicochemical parameters and phytochemical screening of stem of *Duranta erecta*. L for standardization and monograph development. *Duranta erecta* L. is commonly known as pigeon berry belongs to the family Verbenaceae, one of the therapeutically important plants, broadly distributed all throughout the world. It is commonly referred as golden dewdrop, angel whisper, pigeon berry, or skyflower. Macroscopic and microscopical evaluation revealed characters that have diagnostic value useful for identification and authentication of the plant. The Physicochemical analyses reveals values for moisture content, alcohol extractive, water extractive and total ash, acid insoluble ash and fluorescence analysis useful for standardization of plant. Phytochemical analysis shows the presence glycosides, alkaloids, tannins, flavonoids as major type of chemical constituents. Information obtained from these studies can be used as markers in the identification and standardization of this plant as a herbal remedy and also towards monograph development on the plant.

Keywords: *Duranta erecta* L, standardization, pharmacognostic and physicochemical

Introduction

Duranta erecta L belonging to family Verbenaceae is native to clean and open forests. It is used as an ornamental plant in tropical nations ^[1, 2]. It is commonly known as Golden dewdrop, sky blossom, angels-whisper, Katamehedi etc. *Duranta erecta* is an upright to hanging bush that occasionally takes the type of a scrambling bush or once in a while a little tree ^[3, 4]. They mostly occur in tropical and subtropical and few temperate regions. Traditional plants are potential source of natural remedies and remain to be broadly used to treat many diseases. *D. erecta* is used medicinally for the treatment various diseases and ailments. The fruit and leaves have been used as vermifuge, diuretic, in malaria and in intestinal worms. Saponins in the fruits and foliage cause gastro enteric irritation, drowsiness, fever, nausea, vomiting, and convulsions. Dermatitis sometimes occurs from handling the plants ^[5, 6]. Ethyl acetate and aqueous extracts of leaves showed significant antimalarial activity when administered to mice ^[7]. *Duranta erecta* L also exhibits activities such as anti-shigellosis, cytotoxic, antiviral activity, antioxidant, antibacterial, and antimicrobial against human pathogens ^[8]. From the genus *Duranta* several iridoid glycosides as durantosides and lamide, flavonoids and c-alkylated flavonoids and some alkaloids were isolated ^[9]. Establishment of the pharmacognostic profile of the stem of *Duranta erecta* L will assist in standardization which can guarantee quality, purity and identification of plant.



Fig 1: *Duranta erecta* L Plant

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Materials and Methods

Collection and authentication plant materials

Collection of plant specimen was carried out as per standard procedure of collection and WHO guidelines [10, 11]. The plants specimens were collected in morning in the month of October 2017 from Nanded district (M.S.), India. The herbariums were deposited at Botanical survey of India, Pune, for authentication and Dr. Priyanka Ingale identified the plants as *Duranta erecta* L. belongs to family Verbenaceae.

Macroscopic study

Fresh stem of *Duranta erecta* L were taken to study macroscopic features and observed to note organoleptic characteristics.

Microscopic Study

Sections were manually obtained by sectioning with razor blade. It was washed in water and then stained with Phloroglucinol and hydrochloric acid in 1:1 proportion to give some cytochemical reactions. These were mounted on a slide with glycerol to prevent dehydration. All slides were observed under a light microscope.

Physicochemical study

Physicochemical values such as, moisture content, ash values and extractive values were determined according to the official methods and as per WHO guidelines on quality control methods for medicinal plant materials [12, 13].

Determination of water-soluble extractive values

Different extractive values like water soluble and alcohol soluble extractive values were performed by standard method. Five gm of air dried coarsely powdered stem was macerated with 100 ml of chloroform water in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105 °C and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs (12, 13).

Determination of alcohol-soluble extractive value

Five gm of air-dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 105 °C and weighed. Percentage of ethanol soluble extractive value was calculated with reference to air-dried drugs [12, 13].

Determination of total ash values

Accurately weighed 2 g of the air-dried crude drug was taken in a tare silica dish and incinerated at a temperature not exceeding 450 °C until free from carbon, cooled in a desiccator and weight was taken. The process was repeated till constant weight was obtained. The percentage of ash was calculated with reference to air-dried drug [12, 13].

Acid-insoluble ash

The ash obtained as per method described above and boiled with 25 ml of 2 M hydrochloric acid for 5 minutes, filtered, and collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignited and

cooled in a desiccator and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug [12, 13].

Water-soluble ash

The ash, obtained as per the method described above was boiled for 5 minutes with 25 ml of water, filtered and collected the insoluble matter in a Gooch crucible, washed with hot water and the filtrate was ignited for 15 minutes at a temperature not exceeding 450 °C and weight was taken. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug [12, 13].

Determination of moisture content

Accurately weighed dried, glass stopper, shallow weighing bottle. Two gm of sample was transferred to the bottle and covered. Weight was taken and sample was distributed evenly and poured to a depth not exceeding 10 mm. Then loaded bottle was kept in oven and stopper was removed. The sample was dried to constant weight. After drying it was collected to room temperature in a desiccator. Weighed and calculated moisture content in terms of percent w/w [12, 13].

Determination of fluorescence property

Fluorescence analysis of the powdered drug with different chemicals were observed in day light and ultra-violet light (254 nm) [14].

Phytochemical screening

Phytochemical screening was done to determine the presence or absence of secondary metabolites such as tannins, alkaloids, flavonoids, saponins, sterols, and phenolic compounds. This was done according to established procedure [15, 16].

Result and Discussion

Herbarium of the plant specimens includes leaf, stem, flower, capsules were prepared and deposited to Botanical survey of India, Pune. Authentication certificate reference number 'BSI/WRC/100-2/Tech/2017/' was issued.

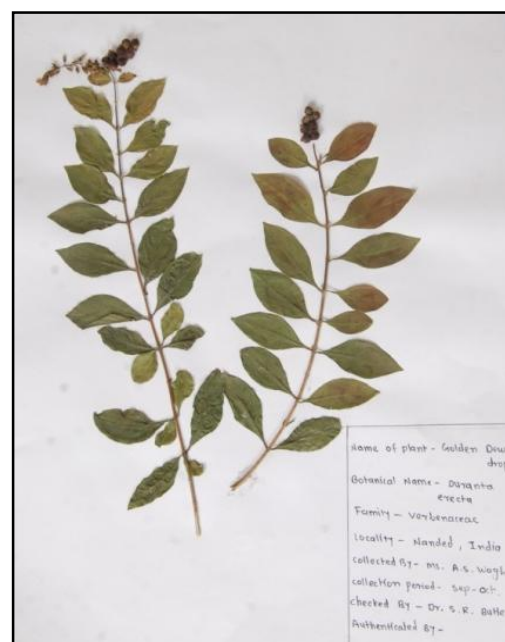


Fig 2: Herbarium Sheet of *Duranta Erecta*

Morphology of *Duranta erecta* L. stem

Table 1: Morphology of Stem

Morphology	Observation
Nature	Branches, Drooping
Pairs	Axillary spines
Surface	Hairy
Colour	Green
Young	Whitish
Mature	
Odour	Odourless
Taste	Bitter



Fig 3: Morphology of *D. erecta* Stem

Microscopy of stem of *Duranta erecta* Stem

The stem is circular in cross-section. The epidermal layer is thin and the cells are circular and thin walled followed by cortex. It includes small angular, thin walled parenchyma cells. The secondary phloem cylinder is thick and comprises sieve elements, parenchyma cells, phloem rays and sclerenchyma masses, located at the peripheral region of the phloem zone. Xylem cylinder consists of outer zone of

secondary xylem elements and inner zone of primary xylem. The pith is parenchymatous. The cells are angular, thin walled and compact.

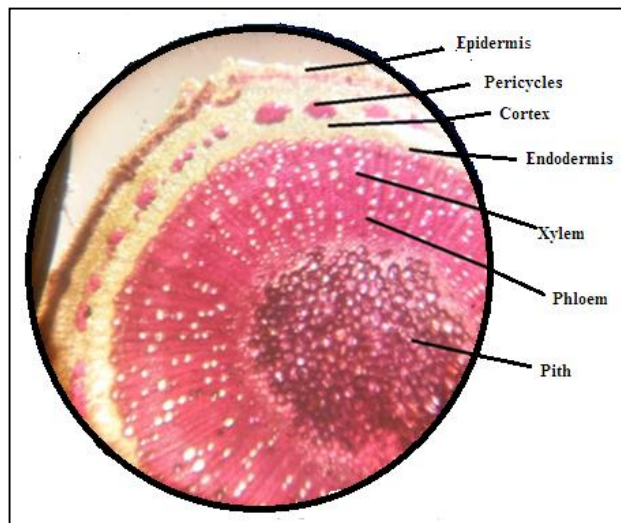


Fig 4: Microscopy of *Duranta erecta* stem

Physicochemical analysis

Physicochemical parameters are presented in Table No.2

Table 2: Physicochemical Study of *Duranta erecta* stem Powder

Sr. No.	Parameter	Value (%w/w)
1	Water soluble extractives	2.73±0.21
2	Alcohol soluble extractives	3.83±0.2.0
3	Total ash value	9.2±0.72
4	Acid insoluble ash value	2.77 ±0.25
5	Water soluble ash value	1.00±0.25
6	Moisture content (Loss on drying method)	3.6±0.51

Values are expressed as average of three readings in percent w/w

Fluorescence analysis

Fluorescence analysis of *Duranta erecta* stems Powder under visible light and UV light (254nm) was given in table no.3

Table 3: Fluorescence analysis of *Duranta erecta* stems Powder

Treatment	Under visible light	Under UV light (254nm)
Powder as such	Brownish yellow	Pale Green
Powder + water	Brownish yellow	Yellow
Powder +Alcohol	Brownish yellow	Yellowish Green
Powder+1N NaOH (Aq)	Pale Yellow	Faint green
Powder+1NNaOH (Alc)	Pale Yellow	Dark Green
Powder + 1N HCl	Brown	Yellow
Powder + conc. HCl	Green	Dark Green
Powder + 50% HNO ₃	Orange	Greenish
Powder + H ₂ SO ₄	Blackish brown	Blackish brown

Phytochemical Screening

Preliminary phytochemical screening of different extracts of stem of *Duranta erecta* revealed the presence of alkaloids, tannins, flavonoids, saponin and steroids (Table 4).

Table 4: Phytochemical screening of extracts *Duranta erecta* Stem

Sr. No.	Test for Phytoconstituents	CHL	EA	ME	AQ
	Steroids				
01	Salkowski test	Positive	Positive	Positive	Negative
02	Liebermann-Buchard test	Positive	Positive	Positive	Negative
	Glycosides				
03	Keller-killani test	Negative	Negative	Negative	Negative
04	Bontrager's test	Negative	Negative	Negative	Negative
05	Modified bontrager's test	Negative	Negative	Negative	Negative
	Alkaloids				
06	Dragendroff's test	Negative	Positive	Positive	Positive
07	Mayer's test	Negative	Positive	Positive	Positive
	Terpenoids				
08	Liebermann-Burchard test	Negative	Negative	Negative	Negative
09	Vanillin-Sulphuric acid test	Negative	Negative	Negative	Negative
	Flavonoids				
10	Shinoda test	Positive	Positive	Positive	Positive
11	Lead acetate test	Positive	Positive	Positive	Positive
	Tannins and Phenolic compounds				
12	Ferric chloride test	Positive	Positive	Positive	Positive
13	Dilute nitric acid test	Positive	Positive	Positive	Positive
	Saponins				
14	Foam formation test	Positive	Positive	Positive	Positive

Note: CHL (Chloroform extract), EA (Ethyl acetate extract) ME (Methanol extract) and AQ (Aqueous extract) of stem powder

Conclusion

Duranta erecta L. was selected for Pharmacognostic investigation includes collection and authentication of plant, morphological and microscopic characteristics and physicochemical study of the powdered drug. Standard procedure is used for collection of plant specimen. Herbarium of the plant was prepared and authenticated by Dr. Priyanka Ingale, Scientist D, Botanical Survey of India, Pune, where a sample specimen has been deposited (Letter no. – Authentication certificate reference number 'BSI/WRC/100-2/Tech/2017/' was issued).

Duranta erecta L. is evergreen shrub or small tree. Stem is usually forms a multi-stemmed herbaceous but woody below, branched solid, green. Flowers tabular with five petals, light blue to violet or purple. Fruits are spherical yellow drupe about borne in showy hanging bunches. These glossy fruit turn from green to orange or yellow in colour as they mature. The stem is circular in cross-section measures. The epidermal layer is thin and the cells are circular and thin walled followed by cortex. It includes small angular, thin walled parenchyma cells. The trichomes are uni-cellular, unbranched, thick and have lignified walls.

The secondary phloem cylinder is thick and comprises sieve elements, parenchyma cells, phloem rays and sclerenchyma masses, located at the peripheral region of the phloem zone. Xylem cylinder consists of outer zone of secondary xylem elements and inner zone of primary xylem. The pith is parenchymatous. The cells are angular, thin walled and compact. Fluorescence analysis of the powdered drug with different chemicals were observed in day light and ultra-violet light (254 nm).

Duranta erecta L. stem showed 2.73±0.21% w/w water soluble extractive values and 3.83±0.2.0% w/w alcohol soluble extractive value. Stem possessed 9.2±0.72, 2.77 ±0.25, and 1.00±0.25 %w/w of total ash, acid insoluble ash and water soluble ash value. Stem powder indicated 3.6±0.51% w/w of moisture content.

Therefore, the result generated from this study would be useful in identification and of the plant material towards quality assurance and also for preparation of a monograph on the plant.

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