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Pharmacognostic study and establishment of quality parameters of *Jatropha gossypiifolia* L

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Abstract
Objectives: Today complicated modern research tools for evaluation of the plant drugs are available but microscopic method is one of the simplest and cheapest methods to start with for establishing the correct identity of the source materials.
Material and Methods: Pharmacognostic investigation of leaf and stem of *Jatropha gossypiifolia* Linn. was carried out to determine its macro and microscopic characters. Physicochemical analysis which included parameter like loss on drying, total ash, water soluble ash, acid insoluble ash, sulphated ash, extractive values in different solvents like (petroleum ether, toluene, ethyl acetate, methanol and water) was done. Qualitative phytochemcial analysis and fluorescence analysis was also done.
Results: The leaves possessed a cordate base, sariated glandular margine, sub-acute apex and both surfaces were very rough with rigid hairs on surface. Internally, it showed presence of anomocytic stomata, epidermis, parenchymatous tissue, secretary glands, cluster crystals of calcium oxalate, simple starch grains, glandular and simple covering trichomes scattered as such throughout or attached with the cells of the epidermis. Majority of the glandular trichomes were with 4 to 5 celled uniseriate stalk from petiole region. Phytochemical analysis of the powder revealed the presence of different phytoconstituents like alkaloids, tannins, saponins, phlobatanins, triterpenes and flavonoids; phlobatanins and triterpenes were present in maximum amount. In physicochemical analysis, all the parameters were present within the limits. Maximum extractive value was in polar solvent methanol and water.
Conclusion: The pharmacognostic characters laid down in the present study will work as a tool for identification and standardization parameters of the drug in the crude form and also distinguish the drug from its adulterants.

Keywords: *Jatropha gossypiifolia*; pharmacognosy; phytochemical analysis; physicochemical analysis; macroscopy; microscopy

1. Introduction
Medicinal plants play a key role in maintaining human health and contribute towards well being of human life. Medicinal plants have been widely utilized as effective remedies for the prevention and treatment of variety of disease conditions for millennia by almost every known culture [1]. They are important components of medicines, cosmetics, dyes, beverages, etc. In the present scenario, focus on plant search has increased all over the globe enormously. Many plant species have potential of offering direct therapeutic effect individually or in combinations. Traditionally the plants are being used as medicine since ages simply because they are easily available and affordable by all the people. However, the main drawback is they are prone to adulteration and substitution either deliberately or accidentally. This has to be prevented and the best way is to lay down standardization and quality control parameters for each and every plant under study. Such studies will be finger print for a particular plant and it will ensure quality, purity and identity of the plant under study and will definitely prevent it from adulteration. The therapeutic efficacy will also be maintained.
Jatropha gossypiifolia Linn. is a perennial shrub belonging to family Euphorbiaceae. The family Euphorbiaceae is quite large, comprising 321 genera and 7770 species widely distributed in tropical and sub tropical regions of the world. In India, it is represented by 70 genera and 450 species with common forms being *Acalypha, Croton, Bischofia, Euphorbia, Jatropha, Phyllanthus,* and *Emblica* [2]. The plant parts like leaf and stem are traditionally used to cure toothache, wounds, arthritis, skin disease, ulcer, leprosy, etc. *Jatropha gossypiifolia* is also used for commercial purpose. The plant shows different chemical constituents like 7-keto-beta-sitosterol, beta- amyrin, beta-sitosterol-beta-d-glucoside, linoleic acid, myristic-acid, oleic-acid, stearic- acid, stigmast-5-ene-3-beta-7-alpha-diol, stigmasterol, tannin, taraxasterol, vitexin [3]. The plant shows different biological activities as antibacterial [4], anti alopecia [5], anti abscess [6], antianaemic [7], antioxidant [8], anti-inflammatory [9], anti malaria [10], analgesic [11], antidiabetic [12, 13]. In the present study, pharmacognostic study has been done in this medicinally important plant.

2. Materials and methods

2.1 Plant Collection

The leaf and stem of *Jatropha gossypifolia* L. was collected in August, 2016 from Rajkot, Gujarat, India. The plant was compared with voucher specimen (voucher specimen number PSN691) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The leaves and stem was washed thoroughly with tap water, shade dried and homogenized to fine powder and stored in closed container for further studies. For physicochemical studies, 10 g of dried powder of leaf and stem was extracted by using solvents of different polarities (petroleum ether, toluene, ethyl acetate, methanol and water) by cold percolation method [14]. The solvent was evaporated to dryness and dried crude extract was stored in air tight bottles at 4 ºC. Macroscopic and microscopic characters were studied as described in quality control methods [15]. Photographs at different magnifications were taken by using digital camera.

2.2 Pharmacognostic Studies

Macroscopic studies

Macroscopic studies were carried out by using organoleptic evaluation method. The shape, size, colour, odour, taste, base, texture, margin, apex of leaves and stem were observed [16].

Microscopic studies

Microscopic studies were carried out by preparing thin sections of leaf and stem. The thin sections were further washed with water, stained with congo red, malachite green and mounted in glycerine for observation and confirm its lignifications (10x, 40x). The powder microscopic studies were also carried out and the specific diagnostic characteristic features were recorded [15].

2.3 Physicochemical Analysis

The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, water-soluble ash, sulphated ash and extractive values were determined as per WHO guidelines [17] in dried powder of *J. gossypiifolia*. The details of the procedure followed are as described earlier [18].

2.4 Qualitative phytochemical analysis

The detection of alkaloids, flavonoids, tannins, phlobatanins, saponins, steroids, cardiac glycosides, triterpenes and anthocyanins were carried out following the procedure of Harborne [19]. The details of the procedure followed are as described earlier [20].

2.5 Fluorescence Analysis

Fluorescence study of *J. gossypiifolia* powder was performed as per Chase and Pratt [21]. A small quantity of the powder was placed on a grease free clean microscopic slide and 1-2 drops of freshly prepared reagent solution were added, mixed by gentle tilting of the slide and waited for a few minutes. Then the slide was placed inside the UV chamber and observed in visible light, short (254 nm) and long (365nm) ultra violet radiations. The colours observed by application of different reagents in different radiations were recorded.

3. Results

3.1 Organoleptic and Macroscopic Characteristics of *J. gossypiifolia*

Organoleptic and macroscopic characteristics of *J. gossypiifolia* leaf is given in Table 1 and Fig. 1

Leaves

The leaf was simple palmatipartite and large and they were dark green to brown in colour,

phyllotaxy alternate, the shape was orbicular to brodly ovate in outline, margin sariated glandular, apex sub-acute, leaf base symmetrical, appearance glaborous, venation reticulate, odour was characteristic and taste was acrid. The average leaf size was 3-7 cm in length and 5-7 cm in width. The average size of petiole was 6 cm in length and many stack glands were present on the upper surface (Fig. 1).

Stem

The stem was erect, glandular and brown in colour, cylindrical, up to 40-50 cm in height and 2-7 cm in thickness. The stem consisted of numerous branches, odour was characteristic and taste was acrid. Outer surface was rough and sticky.

Table 1: Organoleptic features of *Jatropha gossypiifolia* L.

Parts	Observation
Part	Leaves
Arrangement	Alternate
Size	7-3 cm long, 5-7 cm wide
Shape	Broadly ovate simple
Colour	Dark green and brown red
Odour	Characteristic
Taste	Acrid
Appearance	Glaborous
Margin	Sariated glandular
Apex	Sub-acute
Base	Symmetrical
Petiole	Long 3-6 cm
Texture	Smooth
Veination	Reticulate veination

Jatropha gossypiifolia L.

Fig 1: Macroscopic characteristics of *J. gossypiifolia*

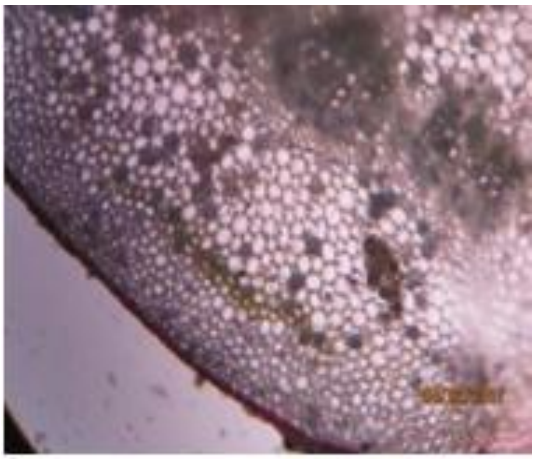
3.2 Microscopic Characteristics

Petiole

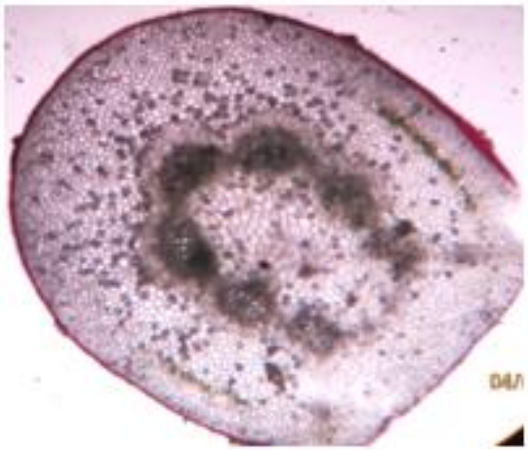
The transverse section of *J. gossypiifolia* petiole is shown in Fig. 2. The petiole was oval shaped towards the distal end to the laminal side. The epidermis was single layered with dark pink coloured cells. The hypodermis was collenchymataous with 5-7 layers (Fig. 2a). The ground tissue was with parenchymatous cells, and vascular bundles were conjoint collateral open type, the size of the vascular bundles varied from centre to margin that is large to small. These were centripetal arranged i.e. xylem was surrounded by the phloem, cluster crystals of calcium oxalate were also observed (Fig. 2b).

Leaf

The transverse section of *J. gossypiifolia* leaf is shown in Fig. 2. The leaf lamina was dorsiventral in nature. The upper epidermis and lower epidermis were single layered, the cuticle was surrounded with dark pink coloured rectangular cells (Fig. 2c). The palisade tissue was single layered, compact with radially elongated cells, the spongy parenchyma were 4-6 layered and many distinct ordinary cut veinlets were seen (Fig. 2d). The mesophyll was large with 6-8 layers, of thick cellulosic cell walled parenchymatous tissue, vascular bundles were present towards the ventral surface. The hypodermis was 4-5 celled with chlorenchymataous tissue; centrally located collateral vascular bundles were conjoint collateral open endarch type (Fig. 2e). Cluster crystals of calcium oxalate and simple starch grains were present (Fig. 2f).



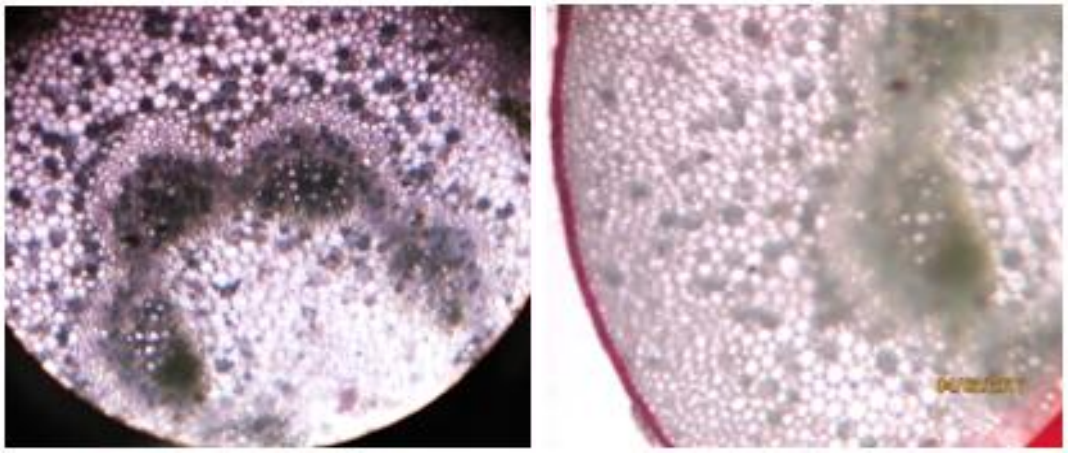
a) T.S of petiole single epidermis



b) T.S of petiole

c) T.S of leaf

d) T.S of leaf with collenchymatous tissue

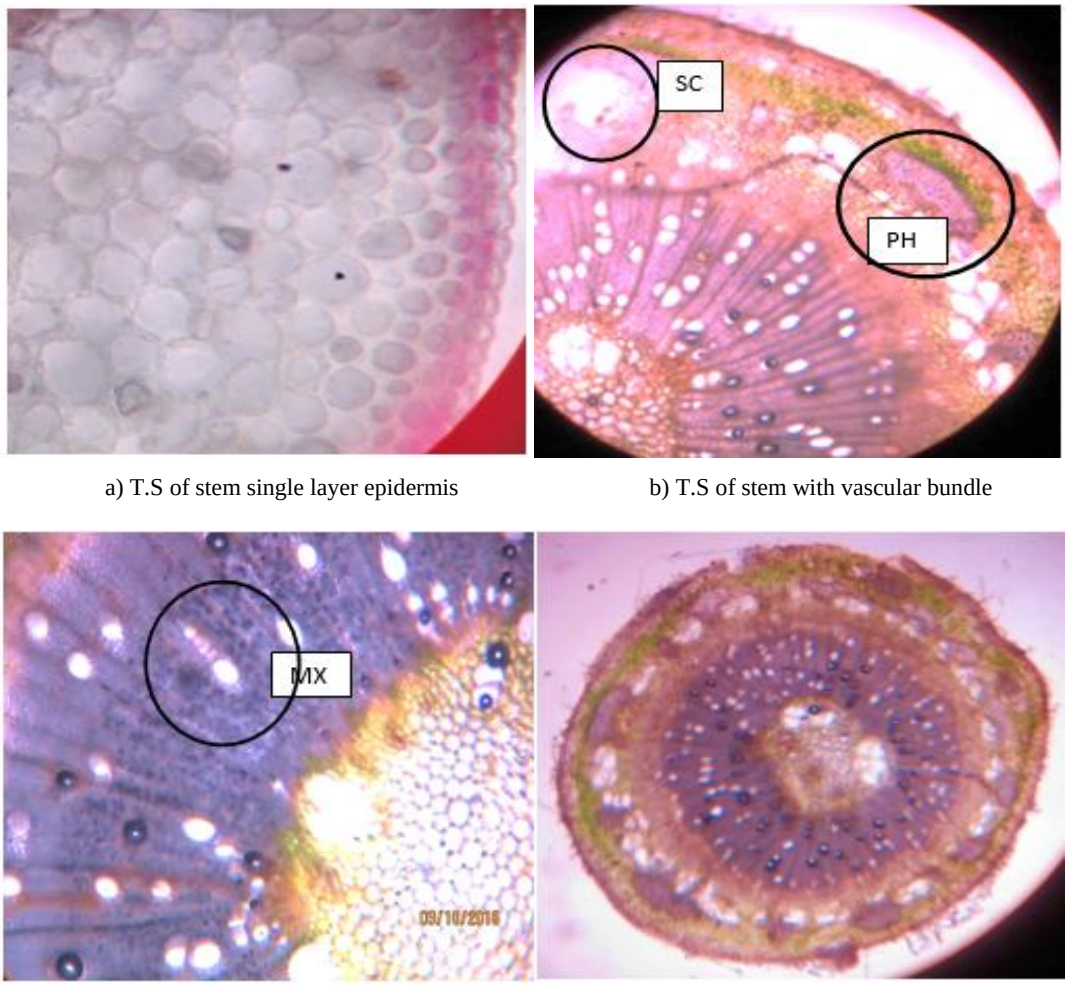


e) T.S of leaf arc shape vascular bundle f) T.S of leaf with cluster crystal calcium oxalate

Fig 2: Microscopic characteristics of *J. gossypiifolia* leaf

Stem

The transverse section of *J. gossypiifolia* stem is shown in Fig. 3. The epidermis was single thick wall layered, the cuticle was surrounded with dark pink coloured oval shape cells (Fig. 3a). The cork cells consisted of 3-5 layers, the vascular bundles were surrounding the polygonal lignified parenchymatous cells. The cortex was of several layers of thin walled tangentially elongated cells; cluster crystals of calcium oxalate and starch grains were present (Fig. 3b). The central pith was very small and vascular bundles were arranged in a ring form (Fig. 3c). The vascular bundle consisted of secondary phloem and secondery xylem; secondary phloem consisted of sieve tubes, companion cells and phloem parenchyma; secondery xylem consisted of lignified trachea, tracheids, fibres and vessels. Xylem fibres were pitted, elongated and moderately thickened; multicellular glandular trichomes were present on epidermis (Fig. 3d).



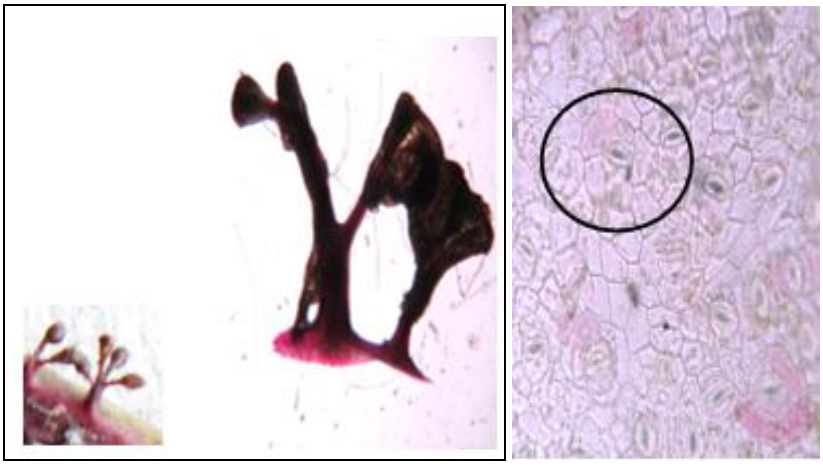
a) T.S of stem single layer epidermis b) T.S of stem with vascular bundle

c) T.S of stem with pith d) T.S of stem with trichomes

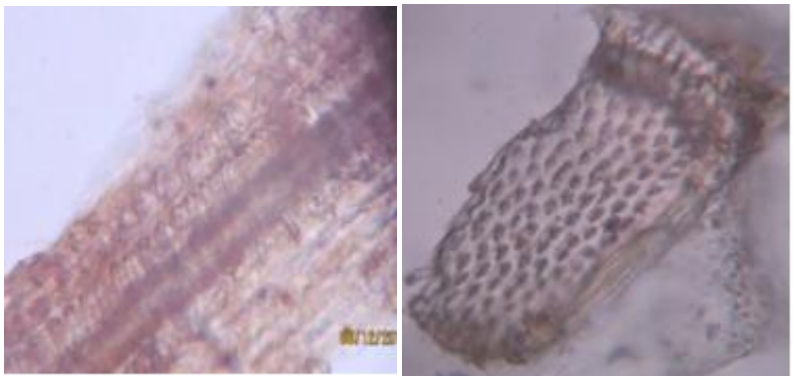
Fig 3: Microscopic characteristics of *J. gossypiifolia* stem

3.3 Powder Microscopy of Plant

The crude powder of *J. gossypiifolia* plant was light brown in colour, fine, odour was characteristics and taste was bitter acrid. The powder microscopy characteristics are shown in Fig. 4. The specific characteristics determined from the powder study under microscopic investigation showed belladonna trichomes with glandular head, paracytic stomata, spiral vessels, pitted vessels, annular vessels, etc.



a) Belladonna with glandular head b) Paracytic stomata



c) Spiral vessels d) Pitted vessels

e) Annular vessels

Fig 4: Microscopic characteristics of powder of *J. gossypiifolia*

3.4 Physicochemical Analysis

The physicochemical analysis of *J. gossypiifolia* plant powder is given in Table 2. The moisture content of dry powder of plant was 92.5%. Hence it would discourage bacteria, fungi or yeast growth. The total ash of plant was 18.75%, while water soluble ash and acid insoluble ash was 3.5% and 2.66% respectively. The sulphated ash of plant was 23.16%. The extractive value of plant is given in Table 2. The maximum soluble extractive value was found in methanol solvent (6.04%) and minimum soluble extractive value was found in petroleum ether solvent (1.17%). The water soluble extractive value of plant was 14.38%.

3.5 Qualitative Phytochemical Analysis

The qualitative phytochemical screening of the crude powder of *J. gossypiifolia* plant is given in Table 3. Phlobatanins and triterpenes were present in maximum amount followed by flavonoids, saponins and tannins (Table 3). Other phytoconstituents were present in trace amount and anthocyanins, steroids and cardiac glycosides were absent.

3.5 Fluorescence Analysis

Fluorescence study of *J. gossypiifolia* crude powder with various reagents revealed characteristic fluorescence at 366 nm and 254 nm wavelength (Table 4). Some constituents show fluorescence in the visible range in daylight. The UV light produce fluorescence in many natural products which do not visible shine in daylight. Hence crude drugs are often evaluated qualitatively in this way and it is a significant parameter for pharmacognostic evaluation of crude drugs [22].

Table 2: Physiochemical parameters of *Jatropha gossypiifolia*

Sr. No.	Parameters	% value(w/w)
1	Loss on drying	92.5
2	Total ash	18.75
3	Water soluble ash	3.5
4	Acid insoluble ash	2.66
5	Sulphated ash	23.16
6	Petroleum ether soluble extractive value	1.17
7	Toluene soluble extractive value	1.75
8	Ethyl acetate soluble extractive value	2.05
9	Methanol soluble extractive value	6.04
10	Water soluble extractive value	14.38

Table 3: Qualitative phytochemical analysis of *Jatropha gossypiifolia*

Sr. No.	Phytochemicals	Observation
1	Alkaloids	
	(1) Mayer's reagent	-
	(2) Dragondroff's reagent	-
	(3) Wagner's reagent	+
2	Flavonoids	++
3	Tannins	++
4	Phlobatanins	++++
5	Saponins	++
6	Steroids	-
7	Cardiac glycosides	-
8	Triterpenes	++++
9	Anthocyanins	-

Note: (++++) very more amount, (+++) more amount, (++) less amount, (+) very less amount (-) absent

Table 4: Fluorescence analysis of *Jatropha gossypiifolia* plant powder

Sr. No.	Treatment	Visible light	Under UV light Short Wave length (254nm)	Under UV light Long Wave length (365nm)
1	1N NaOH (aq)	Dark brown	Black	Dark black
2	1N NaOH (alco)	Brown	Black	Brown
3	Ammonia	Brownish green	Black	Dark brown
4	Picric acid	Dark green	Black	Dark black
5	Petroleum ether	Green	Black	Brown
6	50% HCl	Dark brown	Black	Dark black
7	50%H_2SO_4	Dark brown	Black	Dark black
8	Ethyl acetate	Green	Black	Brown
9	Ethyl alcohol	Dark brown	Black	Dark black
10	Methanol	Dark green	Black	Brown black
11	50% KOH	Brownish green	Dark black	Brown
12	50%HNO_3	Brown	Black	Dark black
13	Acetic acid	Dark green	Black	Brown
14	Iodine in water (1%)	Brownish green	Black	Light brown
15	$FeCl_3$	Black	Black	Dark black

4. Discussion

The complete pharmacognostic analysis of *J. gossypiifolia* plant was attempted which will help in establishing the botanical identity of this plant. The standardization of the herbal medicines is necessary to assure the quality of the drug like the allopathic medicine quality control. This analysis will help to ensure the identity, quality, purity, safety and efficacy of the drug.

Standardization parameters will also help in checking and preventing adulteration and substitution, which is nothing but mixing or substituting the original drug material with other spurious, substandard, defective, spoiled, useless other parts of the same or different plants [23, 24]. Pharmacognostic parameters need to be standardized and established for any plant before it can be included in herbal pharmacopeia. Pharmacognostic study involves organoleptic evaluation, macroscopic characterization, microscopic characterization, powder studies, phytochemical analysis and physiocochemical analysis, etc. The plant showed specific microscopic character as darkly strained epidermis, narrow superficial periderm and a few layers cortex with thick discontinuous cylinders of sclerenchyma cells. Cluster calcium oxalate crystals were common in parenchyma and sclerenchyma. The powder analysed for various phytochemical constituents revealed maximum amount of phlobatanins and triterpenes in plant powder. The physicochemical parameters like ash values, acid insoluble ash, water soluble ash, loss on drying and sulphated ash were found in within limits. Extractive values will give an idea regarding their solubility in a particular solvent which will be useful for further studies and also helps in checking adulteration. In *J. gossypiifolia* plant extractive value was maximum in methanol solvent. The fluorescent analysis under day light and UV light by treatment with different chemical reagents showed different colours. This is attributed to the UV light which produces fluorescence in many natural products that do not visibly fluoresce in daylight. Thus fluorescecne analysis is used for qualitative assessment of crude drugs [25]. The diagnosic features and constant established in this study would be useful for the compilation of a suitable monograph and also helps in proper identification of this plant. Similar work is reported for other plants [26-28].

5. Conclusion

The macroscopic, microscopic, phytochemical, physicochemical and fluroscence parameters established in the present study for *J. gossypiifolia* stem and leaves will be identity of this plant. These parameters will be helpful in maintaining the quality, purity and authenticity of starting material. It will also prevent the crude drug from adulteration and substitution.

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