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PHARMACOGNOSTICAL STANDERDIZATION, PHYTOCHEMICAL CHARACTERISTICS AND ANATOMICAL STUDIES OF THREE JATROPHA SPECIES FROM INDIA

***Rani B. Bhagat**

PDEA's Baburaoji Gholap College, Sangvi, Pune-411027.

***Corresponding Author: Rani B. Bhagat**
PDEA's Baburaoji Gholap College, Sangvi, Pune-411027.
ORCID ID- 0000-0001-7363-5486

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ABSTRACT

Jatropha, (Gk.- *Jatros*: Doctor, *trophe*: nutrition), which refers to its medicinal usage, is a member of the Euphorbiaceae family, which has 172 species worldwide. *Jatropha*, a member of the Euphorbiaceae family, is well-known for its great economic value. In India it has been reported with eighteen species both in wild and cultivated state. Due to its potential use as biodiesel or biofuel, this Central American native has gained popularity in Africa and Asia. It significantly contributes to the prevention of infectious organisms. It is mentioned in traditional medicine for conditions such as pneumonia, inflammation, dysentery. It has a lot of potential for use in traditional and indigenous medical practices but constraint for its correct identity. Present research work aims to evaluate the three *Jatropha* species namely *J. nana* which is an ephemeral, endemic and threatened species found in Pune, Maharashtra, *J. gossypifolia*, and *J. glandulifera* which are widely occurring throughout India for its phytochemical analysis, Pharmagnostic and anatomical studies. Plant samples were deposited in Agharkar Herbarium of Maharashtra Association (AHMA), Pune and studied for preliminary phytochemical tests, extractive values, crude fiber, qualitative (TLC) and quantitative analysis of phytochemicals using High performance thin layer chromatography (HPTLC), micronutrient analysis using Atomic absorption spectroscopy (AAS), sectioning of leaves and roots of three *Jatropha* species and powder analysis was investigated for its correct identity.

KEYWORDS: AAS, Anatomy, HPTLC, *Jatropha* SPP., Phytochemical, Pharmacognosy, TLC.

INTRODUCTION

Now a days *Jatropha* species has received more attention for use in biodiesel purpose. *Jatropha* has the widest distribution, with species found in Africa, Brazil, India, Mexico, South America, West Indies, Central America, and the Caribbean. *Jatropha* species are used in traditional medicine to cure various ailments in different parts of the world as ornamental plants and energy crops (Sabandar 2013). Several known species from genus *Jatropha* have been reported for their medicinal uses, chemical constituents, and biological activities. In this context the three *Jatropha* species which are found in different states of India are analyzed for phytochemical, pharmacognostic and anatomical studies.

J. nana Dalz. ex Gibs. is a small bushy ephemeral and is endemic to Maharashtra, Pune. It shows absence of glandular hairs. In traditional medicine it is used as antiirritant in opthalmia (Ambasta 1992, Dymock 1972, Biswanath Das, 2000). It has also been reported for phytochemical, antioxidant, antimicrobial and insecticidal properties (Bhagat and Kulkarni 2010). The physico-chemical properties and fatty acid profile was also investigated (Bhagat and Kulkarni 2014).

Jatropha gossypifolia L. originating from South America and used in folk medicine. It is widely distributed in countries of tropical, subtropical, and dry tropical weather and tropical semiarid regions of Africa and the Americas and in different parts of Brazil. It is locally known as lalbherenda, red physic nut or bellyache bush which grows wildly almost throughout India. It is medium sized shrub with palmately 3-5 lobed leaves. Leaf margins, petiole and stem are covered with glandular hairs. It is used in several human and veterinary uses in indigenous systems from different parts (Leaves, stems, roots, seeds and latex) of medicine for the treatment of various ailments, viz. Carbuncles, bathing wounds, blood purifier, diarrhoea, gum infections, stomach ache, eczema, itches, fever, inflammation, skin disease (leprosy) ulcer and veneral diseases (Nadkarni 1976, Dash 2006, Dabur 2007, Labadie 1989). The most frequent reports concern its antihypertensive, anti-inflammatory, antiophidian, analgesic, antipyretic, antimicrobial, healing, antianemic,

antidiabetic, and antihemorrhagic activities. Other uses are also related to this plant, such as biodiesel production, pesticide, insecticide and vermifuge (Félix-Silva 2014**)**. An important feature of *J. gossypiifolia* species is that, due to its important potential medicinal applications, in Brazil, it is included in the National List of Medicinal Plants of Interest to the Brazilian Public Health System. It is also reported with phytochemical constituents like alkaloids, coumarins, flavonoids, lignoids, polyphenols, saponins, steroids, tannins, and terpenoids were detected from different extracts from various parts of this plant (Zhang *et al.*, 2009). The important activities already studied from different plant extracts for this species includes- antihypertensive, antimicrobial, antidiarrhoel, anti-inflammatory, antioxidant, and antineoplasic activities mainly stand out, supporting some of its popular uses (Sabandar 2013, Félix-Silva 2014). Ethno botanical uses of *J. gossypifolia* reported for cancer, diarrhea, dysentery, skin diseases (leprosy), arthritis, ulcer, gum infections and wound healing (Albuquerque 2007). Olaniran *et al*., 2012 studied anatomical features of root and reported diffuse porous wood. Seed oil of *J. gossypifolia* is used in rheumatism and paralytic affections. Whole plant was reported as hypothermic, central nervous system (CNS) depressant and antileukaemic (Akhtar *et al.* 1992). The leaf and root extracts are reported for phytochemical, antioxidant, antibacterial, antidiarrhoel and anti-inflammatory activity (Bhagat *et al.*, 2011, Bhagat and Kulkarni 2014).

J. glandulifera Roxb.is a small bushy shrub found in Peninsular India, Sri Lanka and tropical Africa. It has greenish-yellow flowers with glandular hairs in the axils of leaves. In ayurvedic literature, seeds, leaves, bark and roots for its analgesic properties, inflammation, asthama and bronchitis (Agra *et al*., 2008, Mariz *et al*., 2010). It has also been reported for phytochemical, antioxidant and antibacterial potential. *J. glandulifera* finds frequent use in the Indian traditional medicine. Leaves were used in asthma, analgesic, bronchitis, emmenagogue, inflammation and antidote to scorpion stinge. Stem used in ulcers, to stop bleeding from wounds and cuts. Juice of the plant-used to remove film from the eyes, sinuses, ulcers, foul wound. Root-Brayed with water given to children suffering from abdominal enlargements, purgative, to reduce glandular swellings, in piles (Desai 1927; Chopra and Badhwar 1940, Chopra, 1956, Bapalal 1971, Nadkarni 1976, Kirtikar and Basu 1984, Husain 1992, Sawant 1974). Wound-healing, anti-inflammatory, antimicrobial, antioxidant, antithrombotic and antitumor properties has been extensively documented (Papageorgiou, 2008). Juice is reported to be irritant (Nadkarni 1976). Plant parts has been reported in ulcers, wound healing, ringworm, in rheumatism, as a purgative, in paralysis, itches, in eczema (Seed oil), asthma, analgesic, abdominal, bronchitis, enlargement, swelling, and inflammation (Root) were reported by Chopra and Nayar 1956; Dymock *et al*., 1976; Kirtikar and Basu 1984; Agarwal 1989). In ayurveda and ethno-medicine it has been reported as an analgesic, in asthama, bronchitis and for inflammation (Kirtikar and Basu 1984). The viscid sap (latex) and leaf used in warts, tumors and wound healing (Asolkar, 1965-1981, Daniel 2006). Latex is used as a mouth wash to cure teeth and gum infection (Kadhirvel *et al*., 2010). Ethanol extract of aerial part was effective against Ranikhet disease (Babbar *et al*., 1982 Aswal 1984, Satyavati and Gupta 1987). It is used as antirheumatic, antiparalytic, chronic ulcers, ringworm, stomach disorder, root and oil as purgative. Biological activity of aerial part reported against cancer and viral infections (Husain; 1992). Fatty acid profiling, phytochemical analysis, antimicrobial and insecticidal potential is reported (Bhagat and Kulkarni 2008, 2013; Bhagat *et al*., 2013). The pharmacognostic and anatomical studies has not been presented in any of the earlier studies.

MATERIAL AND METHODS

Collection of plant material

The plant material was collected from the Pune district, Maharashtra and Bellary, Karnataka. Their identity was confirmed using regional floras and voucher specimens were deposited in the Agharkar Herbarium of Maharashtra Association (AHMA), of Agharkar Research Institute (ARI), Pune No. 22478, 24929 and 22484, respectively. The leaves and roots were separated, dried, coarsely powdered passed through sieve no 40 and stored in a closed glass container for further analysis. All reagents used were of analytical grade.

Preliminary phytochemical screening

The shade dried leaves and roots (1 kg) of *J.nana, J. gossypifolia, and J. glandulifera* were first shade dried under control conditions and powdered. The powdered leaves (250 g) were macerated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with Hexane, ethanol, methanol and water respectively for 3 days (1 × 1 L) by cold percolation method and then dried, the solid residue was obtained. The extract was subjected to following preliminary phytochemical screening for the identification of various active constituents. The detection of various phytoconstituents viz. alkaloids, carbohydrates, proteins, fats, starch, tannins, flavonoids, polyphenols, alkaloids, saponins, steroids, lignins and glycosides was carried out with different phytochemical tests by (Paech and Tracey, 1955, Sadasivam and Manickam, 1996, Trease and Evans, 1983, Harborne, 1973, Anonymous, 1989) (Tab. 1).

Phytochemical tests

The plant extracts prepared for the determination of the extractive values were subjected to the various phytochemical tests. However, for some chemical tests specific procedure of extraction was followed.

RESULTS

Ash analysis (Total ash and other values)

Ash is the inorganic residue that remains after the burning of the organic matter. The determination of ash values viz. total ash, acid insoluble ash and water-soluble ash is carried out of the powdered drugs. Total ash value may vary within wide limits due to the differences in the content of calcium oxalate. So it is less significant in the detection of earthy matter adherent to the crude drugs. However, acid-insoluble ash is more significant in the detection of dirt and earthy matter, since, calcium oxalate and other constituents are soluble in hydrochloric acid. The water soluble ash values are used to detect the presence of material exhausted by water. Moreover it may provide an indication whether exhausted material is substituted for genuine one.

The determination of ash values is useful mainly for detection of the inorganic contents (Wallis, 1985). These values help in the assessment of the quality of drug, in the detection of adulteration and dirt as well as earthy matter present in the drug.

Determination of total ash values

For the determination of ash values viz. total ash, acid-insoluble ash and water-soluble ash value, the methods described in 'The Ayurvedic Pharmacopeia of India' (Anonymus, 1989) were followed.

The 2g air-dried leaf and root sample of *Jatropha* species was taken in pre-weighed silica crucibles. These crucibles along with root powder were heated slowly up to 450 °C in muffle furnace for 4 hours to obtain carbon free ash. Then ash was cooled and weighed. This procedure was repeated till constant weight was recorded. The percentage of total ash (carbon free) was calculated with reference to weight of air-dried powder (Graph.1). The same procedure was followed for other *Jatropha* species.

Determination of acid- insoluble ash values

Carbon-free ash, obtained by above procedure was boiled in the crucible with 25 mL of 0.1 N hydrochloric acid for 5 min. The ash was then filtered through ash-less filter paper (Qualigens, Filter paper no.41) and the insoluble matter was collected on it. The crucibles with ash-less filter paper and insoluble residue was kept in furnace at 450 °C for 4 hours. It was then cooled, weighed and the percentage of acid insoluble ash was calculated with reference to the air-dried powder (Graph.1).

Determination of ash values

The ash was boiled for 5 minutes with 25 mL of distilled water. The insoluble matter was collected on the ashless filter paper (Qualigens, Filter paper no.41), washed with hot water (60 °C) and kept in furnace at 450 °C for 4 hours. The weight of the insoluble matter was subtracted from the total weight of ash. The difference in the weight represented the water soluble ash value.

The total ash value, acid- insoluble ash value and water-soluble ash values for the leaves and root were determined by above described procedures. For the determinations of these values the material collected in monsoon season was used. The mean of three readings was calculated.

Observations with respect to total ash value, acid insoluble ash and water-soluble ash of leaves and roots of *Jatropha* are presented along with average mean (Graph. 1).

Extractive values (Anonymous, 1989)

It includes the determination of percentage extractives in different solvents. The water insoluble and acid insoluble extractive values are used as a means of evaluating crude drugs which cannot be readily estimated by any other means. These values indicate the nature and amount of active constituents present in the drugs. The range of percentage extractive for a specific plant species is considered as one of the diagnostic features. Therefore these values are included as one of the parameters in the Pharmacopoeia. The percentage of extractives was determined as per the method of Indian Pharmacopoeia (Graph 2).

The dried plant material was subjected to the size reduction passing it through mesh (# 60). 5 g of material was weighed accurately in a flask and 100 mL of respective solvents were added. The flasks were closed tightly, kept on constant shaker bath for 6 h and allowed to stand still for eighteen hrs. Then the solution was rapidly filtered through filter paper without losing any solvent. The extractives were kept on pre weighed empty beaker and then from the filtrate 25 mL of the solution was evaporated to dryness on water bath. Then the weight of beaker along with the extract was taken. Finally extractive values were recorded by calculating the percentage of extractive and calculated with respect to the weight of air dried drug.

Determination of Crude fibre (Sadasivam and Manickam, 1996)

Crude fiber consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60 to 80% of the cellulose and 4 to 6% of the lignin. Fibre content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality and quantity.

Procedure

2 g of leaf and root samples were boiled with 200 mL of (0.255 ± 0.005) N H_2S0_4 on water bath for 30 minutes. Then filtered through muslin cloth and washed with boiling water until washings were no longer acidic. The residue is taken in beaker with 200 mL of NaOH (0.313 ± 0.005) N solution for 30 minutes. It was filtered through muslin cloth and washed with 25 mL of boiling 1.25% H_2SO_4, thrice with 50 mL distilled water and 25

mL alcohol. Residue was transferred to ashing dish (preweighed dish W_1). It was dried for 2 hours at 130 ± 2ºC temperature in oven. Dish cooled in desiccator and weighed (W_2). It is ignited for 30 minutes at 600 ± 15ºC. Then cooled in desiccator and weighed (W_3). (Table 2)

The percentage of total crude fibre in sample was calculated by using the following formula:

$$\% \text{ Crude fiber} = \frac{\text{Loss in wt. on ignition } (W_2-W_1)-(W_3-W_1)}{\text{Wt. of sample}} \times 100$$

TLC/ HPTLC

Development of HPTLC-Fingerprints

The methods described by Sethi (1996) were followed for the development of fingerprints.

The samples of petroleum ether and methanol extract of leaf and roots were spotted in using spotter (Linomat-IV) on the precoated plate, silica gel Merk-60F 254-aluminium (Merk) of 100 x 100 mm dimensions. The known volume, 10 µl was spotted of each sample on the plates. The plates were developed in the Camag Twin Trough Chamber at 25º C. Densitometric analysis was carried out at 254 nm using CAT's software on Camag III Scanner. Solvents of analytical grade (Qualigens) were employed. The HPTLC pattern for *Jatropha* species are recorded in the form of graphs. The peak numbers and their respective R*f* values in different solvent systems at 254 and 366 nm are tabulated (Tab. 3) See Plate-I-VI.

Micronutrient Analysis by Atomic Absorption Spectrophotometer (AAS)

Micronutrient analysis was carried out using method of (Chapman and Pratt, 1961). Weigh 0.5g of ground dried plant material and into 10 mL crucible. Place the crucibles in a cool muffle furnace. Set furnace temperature to reach 500^{o}C (about 2-3 hours). After 5 hours of muffling at 500^{o}C, turn off the furnace and let it cool. Add 10 mL of 6N HCl into each crucible to dissolve the ash. Transfer all of the solution in the crucible to a glass tube, and dilute to the 50 ml mark with distilled water, and mix thoroughly. Allow suspended materials to settle to the bottom of the tube, or filter with Whatman #42 filter paper. The filtered solution is ready to determine Zn, Fe, Mn, and Cu, with or without further dilution. The samples are read with atomic absorption spectrophotometer (Perkin Elmer, model 2380, USA). using appropriate standards and instrument settings. Set zero with reagent blank, which is 1.2 N HCl solutions (Tab. 4).

Calibration and Standards

1. 1000 ppm Zn, Fe, Mn, and Cu stock solution
2. 100 ppm Zn, Fe, Mn, and Cu working stock solution Dilute 10 ml of 1000 ppm stock solution each to 100 mL with deionized water, respectively.

Calculations

Micronutrient ppm in plant = ppm in reading x 100

Internal Morphology

For anatomical studies free hand sectioning and paraffin embedding methods were employed.

Sections were fixed in FAA (Formalin, 5 mL: 70% ethanol, 90 mL). Sections were dehydrated through ethyl alcohol series and passed through graded xylene. The staining was done with 70% alcoholic light green. The stained sections were mounted in Canada balsam by usual procedure (Johansen, 1940). The sections were photographed under microscope model "Olympus CX 31" With camera attachment having 7.2 x magnifications. Photographs were taken under appropriate magnification. The details of plants, macroscopic characters, microscopic characters viz. powder analysis, leaf and root anatomy have been presented below. Powder analysis of leaves and root powders were sieved (≠ 60 mesh sieve) separately, cleared in chloral hydrate and mounted in glycerin for further observations and photographed under microscope by camera. In the present investigation detailed anatomy of leaf, root and powder analysis of same has been worked out.

***Jatropha nana*-Leaves**

Macroscopic

Leaves simple, petiolate, green, alternate, tri-lobed, shiny above, glabrous below.

Powder Analysis

It is dull green in colour; it shows epidermal cells with stomata, mesophyll cells, palisade cells, vessels with spiral thickenings, few prismatic crystals of calcium oxalate, fibers and raphides. Starch grains are of simple and compound types (Tab. 5 Fig.1)

Microscopic

Epidermis: The epidermal cells are cubical to tangentially elongated on both surfaces. The upper epidermal cells are slightly larger in dimensions, and covered externally with a thin, smooth cuticle than the lower epidermal cells which are relatively smaller and externally covered with slightly striated cuticle. In surface view, the upper epidermal cells are almost polyhedral with more straight walls than those of lower epidermal cells. Trichomes are absent on both the surfaces.

Mesophyll

It is lacunose and composed of single layered palisade cells and narrow zone of spongy tissue. The palisade cells are columnar with scanty intercellular spaces, more or less perpendicular to upper epidermis. Chloroplasts are abundantly present. The spongy tissue consists of loosely arranged parenchyma cells with conspicuous intercellular spaces. Calcium oxalate crystals as rosettes are present in few cells. The cross section through each

vein shows prominent bulge present on the adaxial surface. The epidermal cells, as in marginal region of lamina are cubical and small in dimension with compact cells. It is followed by a wide zone of cortex, the outer layers being collenchymatous cells with well developed angular thickenings. The collenchymatous cells are more i. e. 6-7 layers on adaxial side and less i.e. 4 to 5 layers on abaxial side of bulge. Following parenchymatous tissue lateciferous cells of various sizes are present. A few cells contain calcium oxalate crystals in rosettes. There is a crescent-shaped median vascular bundle with xylem facing the upper and phloem on lower side. The xylem consists of radially arranged vessels which are 3-4 in groups with metaxylem at centre and protoxylem facing on both sides, tracheids, xylem parenchyma and xylem rays. The phloem is represented by sieve tubes, companion cells and phloem parenchyma, which shows few cells of calcium oxalate crystals in rosettes. (Fig. 1)

T.S. of *Jatropha nana* –Root.

Macroscopic
Roots perennial consisting of tap root system, horizontally placed with lateral branches, fibrous. Main root is 80 to 100 cm in length and 4 to 6 cm in diameter, yellowish-white in colour and reddish within; outer surface of the main root is smooth. Root is fleshy and contains reserved food material. (Tab. 5 Fig. 1).

Powder Analysis: It is brownish in colour; it shows presence of cork cells, starch grains, scalariform tracheids, tracheids with pits, rosette crystals of calcium oxalate, fibers and fragments of vessels. (Fig. 1).

Microscopic
TS is circular in outline, showing outermost tissue of the cork consisting phellem, phellogen and phelloderm. It is thin and composed of 6 to 8 or more rows of rectangular or slightly tangentially elongated cells. Inner to the cork is the tangentially elongated parenchymatous cells of cortex. It contains abundant starch grains and rosette calcium oxalate crystals. Cambium is distinct, xylem is with abundant vessels which are scattered throughout and these are single or in groups of two. It is encircled by phloem tissue consists of broad uniseriate medullary rays in continuation with that of xylem rays separated by parenchyma, radiating from centre of the xylem extending up to the cortex. (Fig. 1)

T.S. of *Jatropha gossypifolia* –Leaves.

Macroscopic
Simple, alternate, 3-4-lobed, shiny above, margin glandular, petiole with glandular hairs, reddish in colour.

Powder analysis
It is green in colour; shows vessels with spiral thickenings, mesophyll cells, epidermal cells and a few prismatic crystals of calcium oxalate. (Fig. 2)

Anatomical Characters
Microscopic
Epidermis: The epidermal cells are cubical to tangentially elongated on both surfaces. The upper epidermal cells are slightly larger in dimension, and covered externally with a thin, smooth cuticle than the lower epidermal cells which are relatively smaller and externally covered with slightly striated cuticle. In surface view, the upper epidermal cells are almost polyhedral with more straight walls than those of lower epidermal cells. The trichomes were absent on both the surfaces.

Mesophyll
Mesophyll is lacunose and composed of one layered palisade cells and narrow zone of spongy tissue. The palisade cells are columnar with scanty intercellular spaces, more or less perpendicular to upper epidermis. Chloroplasts are abundantly present. Spongy tissue consists of loosely arranged parenchyma cells with conspicuous intercellular spaces; chloroplasts are profusely present in these cells. Calcium oxalate crystals as rosettes are present in few cells. The cross section through each vein shows a prominent bulge present on the adaxial surface. The epidermal cells, as in marginal region of lamina, are cubical and small in dimension. It is followed by a wide zone of cortex, the outer layers being collenchymatous with well-developed angular thickenings. The collenchymatous cells are more on adaxial side i.e. 7-8 layers and 2-4 layers on abaxial side of bulge. Following parenchymatous cells of various sizes with lateciferous cells are present. A few cells contain calcium oxalate crystals in rosettes. There is a crescent-shaped median vascular bundle with xylem facing the upper and phloem on the lower. Xylem consists of radially arranged vessels which are 4-5 cells in length with metaxylem at centre and protoxylem facing on both sides. Xylem represented with tracheids, xylem parenchyma and xylem rays. The phloem is represented by sieve tubes, companion cells and phloem parenchyma, which shows prominent cells with abundant calcium oxalate crystals in rosettes which forms ring surrounding the xylem cells. (Fig. 2)

T.S. of *Jatropha gossypifolia* -Root
Macroscopic
Roots are perennial consisting of tap root system with lateral branches, fibrous. Main root is 40 to 60 cm in length and 5 to 7 cm in diameter, yellowish-white in colour, outer surface of the main root is smooth. It has characteristic aroma.

Powder Analysis
It is yellowish brown in colour. It shows simple starch grains, scalariform tracheids, rosette calcium oxalate crystals, prismatic crystals and vessels.

Anatomical Description
Microscopic
TS of the root is circular in outline, shows outermost tissue the cork, which is thin and composed of 4 to 6 or more rows of rectangular or slightly tangentially elongated cells. Inner to the cork are tangentially elongated parenchymatous cells of cortex. It contains few starch grains and rosettes calcium oxalate crystals. Cambium is distinct; xylem is parenchymatous with abundant vessels which are isolated or in groups of 2-6. Parenchyma separated by uniseriate medullary rays radiating from centre of the wood extending upto to the cortex. ((Tab. 5 Fig. 2).

e. T.S. of *Jatropha glandulifera* **–**Leaves.

Macroscopic
Simple, green, alternate, 5-lobed, shiny above, glabrous below, margin serrate, long glandular hairs present in the axil of petiole.

Powder analysis
It is green in colour; it shows epidermal cells, mesophyll cells, spiral vessels and a few prismatic crystals of calcium oxalate (Fig. 3)

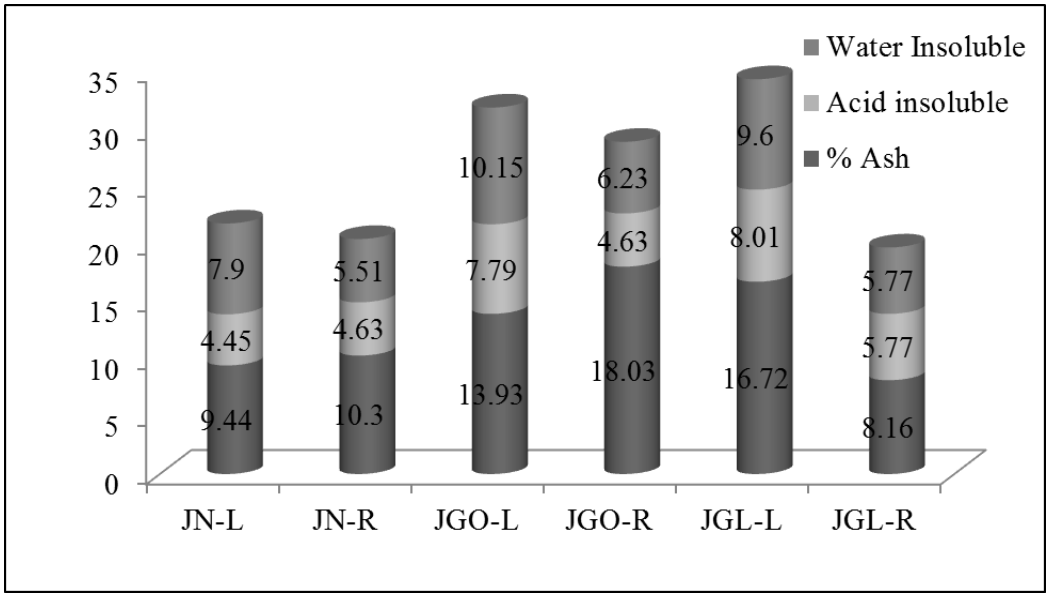
Microscopic
Epidermis: The epidermal cells are cubical to tangentially elongated on both the surfaces. The upper epidermal cells are slightly larger in dimension, and covered externally with a thin, smooth cuticle than the lower epidermal cells which are relatively smaller and externally covered with slightly striated cuticle. In surface view, the upper epidermal cells are almost polyhedral with more straight walls than those of lower epidermal cells. The trichomes absent on both the surfaces.

Mesophyll
The mesophyll is lacunose and composed of one layered palisade cells and narrow zone of spongy tissue. The palisade cells are columnar with scanty intercellular spaces, more or less perpendicular to upper epidermis. Chloroplasts are abundantly present. The spongy tissue consists of loosely arranged parenchyma cells with conspicuous intercellular spaces. Calcium oxalate crystals as rosettes are present in few cells. The cross section through each vein shows a prominent bulge present on the ventral surface. The epidermal cells, as in marginal region of lamina, are cubical and small in dimension. It is followed by a wide zone of cortex, the outer layers being collenchymatous cells with well-developed angular thickenings. The collenchymatous cells develops more on the adaxial side 3 to 10 layers within the bulge than on the dorsal side in the dorsal bulge where they are arranged in 4 to 6 layers. It is followed by parenchymatous cells of various sizes. A number of these cells contain calcium oxalate crystals in rosettes. There is a crescent-shaped median vascular bundle with xylem facing the upper and phloem the lower sides. The xylem consists of radially arranged vessels which are 3-4 in one length, metaxylem at centre and protoxylem facing on both sides, tracheids, xylem parenchyma and xylem rays. The phloem is represented by sieve tubes, companion cells and phloem parenchyma number of which contains calcium oxalate crystals. Epidermis single layered covered with thick cuticle; palisade 1-2 layered; spongy parenchyma 3-5 layered, a few spongy parenchyma cell contains calcium oxalate crystals. Few spongy parenchyma cells get elongated and look like palisade cells. (Fig. 3)

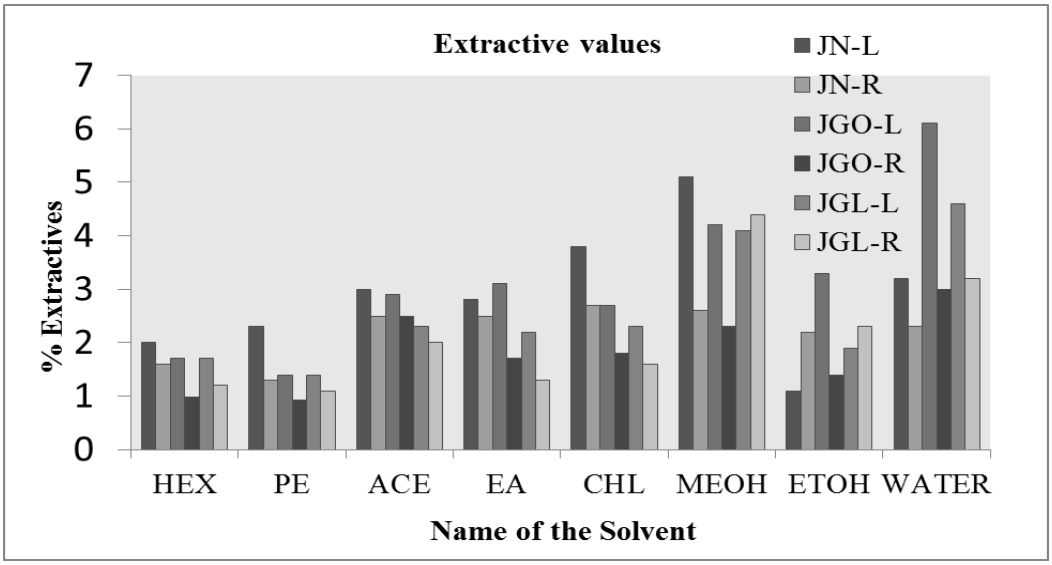
T.S. of *Jatropha glandulifera*- Root
Macroscopic
Roots are perennial consisting of tap root system with few lateral branches, fibrous. Main root 30 to 40 cm in length and 3 to 4 cm in diameter, yellowish-white in colour, outer surface of the main root is smooth. It has characteristic fragrant aroma.

Powder Analysis
It is yellowish brown in colour. It shows simple starch grains, scalariform tracheids, stellate calcium oxalate crystals, prismatic crystals and vessels. (Fig. 3)

Anatomical Description
Microscopic
T.S. of the root is circular in outline, shows outermost tissue the cork, which is thin and composed of 4 to 6 or more rows of rectangular or slightly tangentially elongated cells. Inner to the cork is the tangentially elongated parenchymatous cells of cortex. It contains abundant starch grains and rosettes calcium oxalate crystals, below the cortical region the phloem tissue consist of rows of rosette of calcium oxalate crystals. Cambium is distinct; xylem is parenchymatous with abundant vessels which are scattered throughout often associated with few lignified thin-walled fibers, these are isolated or in groups of two-three. Parenchyma separated by broad uniseriate medullary rays radiating from centre of the xylem extending upto to the cortex. All the rays widen towards periphery. (Tab. 5 Fig. 3)



Graph 1: Quantitative analysis of ash, acid insoluble ash and water insoluble ash.



Hex-Hexane, PE-Pet Ether, ACE-Acetone, EA-Ethyl acetate, CHL-Chloroform, MEoH-Methanol, ETOH-Ethanol

Graph 2: Percent extractive values of three *Jatropha* species.

Tab 1: Qualitative analysis of the selected *Jatropha* species.

Sr. No.	Name of Phytoconstituent	JN-L	JGO-L	JGL-L	JN-R	JGO-R	JGL-R
1	Carbohydrate	+	+	+	+	+	+
2	Proteins	+	+	+	+	+	+
3	Fats	+	+	+	+	+	+
4	Starch	+	+	+	+	+	+
5	Tannins	+	+	+	+	+	+
6	Flavonoids	+	+	+	+	+	+
7	Polyphenols	+	+	+	+	+	+
8	Alkaloids	+	+	+	+	+	+
9	Saponins	+	+	+	+	+	+
10	Steroids	+	+	+	+	+	+
11	Lignins	-	-	-	+	+	+
12	Glycosides	-	-	-	+	+	+

Tab. 2 Total percentage of crude fibre in *Jatropha* species.

Sr. No.	Name of the Plant	Percentage
1	*J. nana*-L	0.13 ± 0.04
2	*J. gossypifolia* –L	0.82 ± 0.01
3	*J. glandulifera*-L	0.07 ± 0.01
4	*J. nana* –R	0.91 ± 0.03
5	*J. gossypifolia* –R	0.273 ± 0.03
6	*J. glandulifera* –R	0.88 ± 0.02

Tab. 3: HPTLC observations for *Jatropha* species Number of peaks with their respective Rf values in different solvent systems at 254 and 366 nm.

Name of Species	Solvent	Total No. of peaks Wavelength (nm) 254	366	Rf values		Solvent system
JN-L	PE	5	6	0.02, 0.21, 0.28, 0.72, 0.92	0.21, 0.31, 0.37, 0.44, 0.50, 0.94	PE: ACE 72:28
	ME	8	5	0.06, 0.14, 0.19, 0.29, 0.40, 0.50, 0.67, 0.97	0.15, 0.27, 0.34, 0.40, 0.48	PE: ACE 70:30
JN-R	PE	8	5	0.11, 0.18, 0.26, 0.31, 0.44, 0.53, 0.74, 0.80	0.15, 0.20, 0.26, 0.53, 0.75	Tolu: EA 80:20
	ME	8	8	0.10, 0.14, 0.17, 0.25, 0.55, 0.74, 0.81, 0.95	0.14, 0.18, 0.29, 0.46, 0.57, 0.75, 0.80, 1.03	PE: ACE 70:30
JGO-L	PE	8	9	0.35, 0.44, 0.51, 0.60, 0.70, 0.75, 0.86, 1.00	0.05, 0.33, 0.37, 0.42, 0.49, 0.55, 0.72, 0.80, 1.00	PE: ACE 72:28
	ME	9	4	0.05, 0.10, 0.15, 0.24, 0.28, 0.32, 0.38, 0.93, 0.96	0.04, 0.10, 0.15, 0.24	PE:ACE 70:30
JGO-R	PE	8	7	0.47, 0.50, 0.57, 0.70, 0.78, 0.82, 0.91, 0.99	0.47, 0.53, 0.62, 0.73, 0.83, 0.89, 0.98	Tolu: EA 80:20
	ME	8	6	0.05, 0.11, 0.21, 0.33, 0.54, 0.76, 0.88, 0.97	0.03, 0.09, 0.17, 0.69, 0.85, 0.99	PE:ACE 74:26
JGL-L	PE	8	4	0.14, 0.24, 0.32, 0.36, 0.63, 0.80, 0.90, 0.95	0.10, 0.34, 0.90, 0.95	PE: ACE 72:28
	ME	8	7	0.10, 0.21, 0.27, 0.39, 0.56, 0.59, 0.62, 0.91	0.05, 0.10, 0.20, 0.24, 0.27, 0.33, 0.41	PE: ACE 70:30
JGL-R	PE	8	5	0.43, 0.51, 0.57, 0.71, 0.79, 0.86, 0.93, 1.00	0.50, 0.58, 0.67, 0.80, 0.87	Tolu: EA 80:20
	ME	8	7	0.04, 0.08, 0.15, 0.22, 0.30, 0.35, 0.46, 0.53, 0.63, 0.86, 0.93, 0.99	0.07, 0.10, 0.16, 0.20, 0.33, 0.45, 0.52	PE: ACE 74:26

PE-Pet Ether, ME-Methanol

Tab.4: Micronutrient Analysis of leaves and roots of *Jatropha* species by Atomic Absorption Spectrophotometer (AAS).

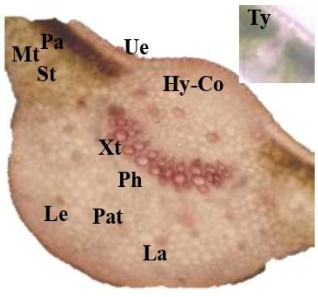
Name of Species	Fe ppm	Zn ppm	Mn ppm	Cu ppm
JN-L	567.92	17.57	80.77	7.62
JN-R	753.88	8.88	0.76	8.61
JGO-L	1985.3	10.55	78.62	7.73
JGO-R	472.94	27.51	-	6.03

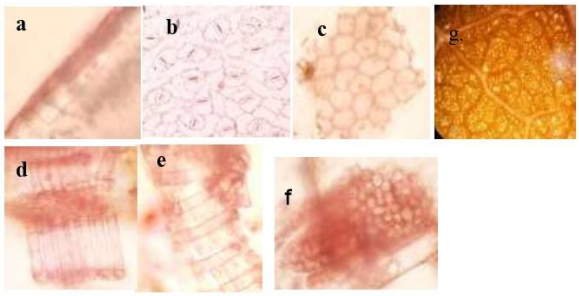
JGL-L	538.41	14.87	102.82	9.11
JGL-R	125.8	5.56	16.77	8.26

Tab. 5: Analytical parameters of *Jatropha* species.

Parameter	*J. gossypifolia*	*J. glandulifera*	*J. nana*
Palisade ratio	1:4	1:4	1:3
Stomatal index	3-4	3-5	4-6
Vein-islet number mm^2	3-7	3-6	4-8
Vein-termination number	14-29	16-27	15-32

J. nana Dalz. ex Gibs.
aerial parts of plant

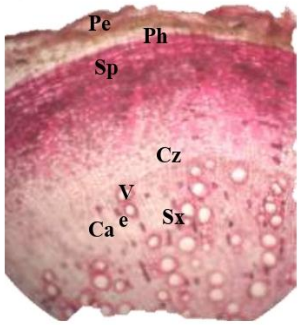




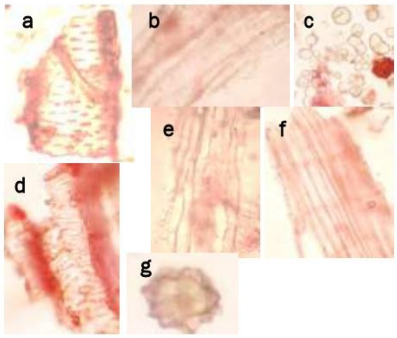
T.S. of Leaves: *Cu-Cuticle, Ue*-Upper epidermi,s *Pa*-Palisade *St*- Spongy *Hy- Co*-Hypodermal Collenchyma *Ca*-Calcium oxalate crystals *Xt*-Xylem, *Mt*-Mesophyll *Ph*-Phloem *Pat*-Parenchyma *La*-Laticeferous glands *Le*-Lower epidermis, Ty-Tyloses

a- Upper epidermis Tyloses, b - Paracytic Stomata c - Epidermal cells, d– Vessels, e– Annular vessels f- Upper epidermis with underlying palisade cells, g. Vein islets

J. nana Dalz. ex Gibs.
Underground part of Plant



T.S. of Root *:Pe*-Periderm, *Ph*-Phelloderm, *Ve*-Vessel, *Sp*-Secondary Phloem, *Sx*-Secondary xylem, *Cz*-Cambial zone,

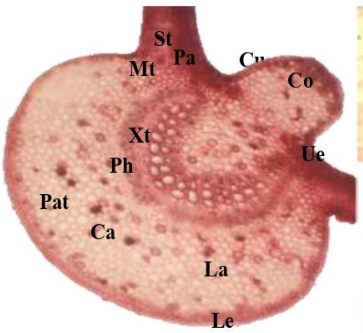


a- Scalariform vessels, b- Fibers of cork, c- Starch grains, d- e- f- Cork tissue in surface view, g- Crystal of calcium oxalate

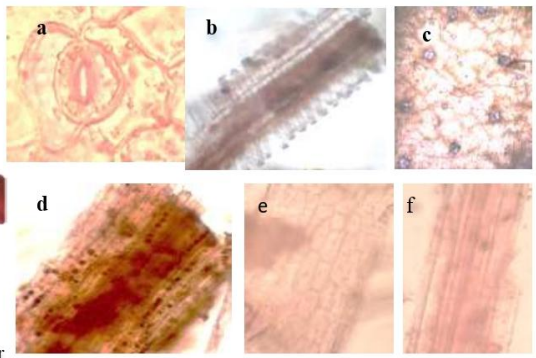
Magnification 100x

Fig. 1: Morphological, anatomical and pharmacognostic characters of *J. nana* leaves and root

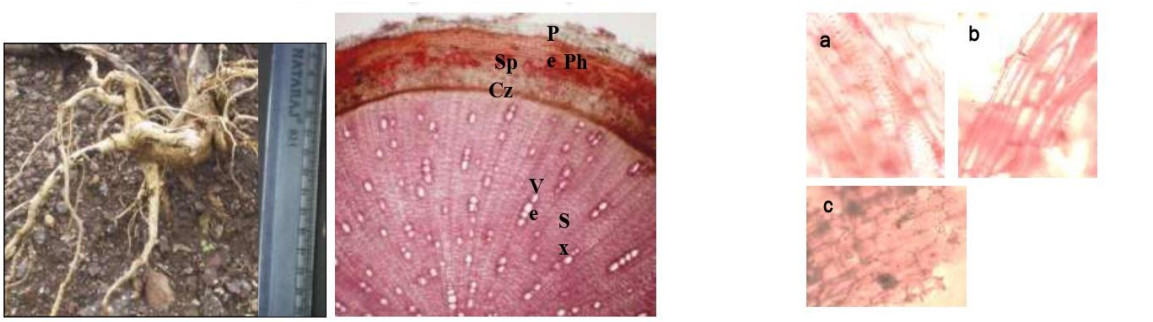
Jatropha gossypiifolia L.
aerial parts of plant



T.S. of Leaves: *Cu-Cuticle, Ue*-Upper epidermi,s *Pa*-Palisade *St*- Spongy *Hy-Co*-Hypodermal Collenchyma *Ca*-Calcium oxalate crystals *Xt*-Xylem, *Mt*-Mesophyll *Ph*-Phloem *Pat*-Parenchyma *La*-Laticeferous glands *Le*-Lower epidermis



Leaf- a- Stomata b- Annular vessels b- Phloem with abundant crystals of Ca oxalate C & C1- Vein islet with Calcium oxalate crystals e- Epidermal cells f- Fibers



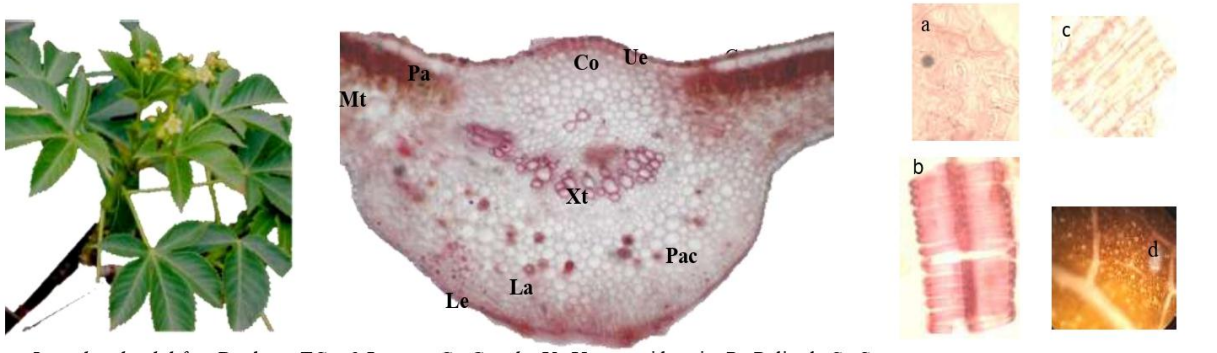
Jatropha gossypiifolia L.
Underground part of Plant

T.S. of Root :*Pe*-Periderm, *Ph*-Phelloderm, *Ve*-Vessel, *Sp*-Secondary Phloem, *Sx*-Secondary xylem, *Cz*-Cambial zone,

a- Scalariform vessels, b- Phloem fibers, c- Cork cells in surface view

Magnification 100x

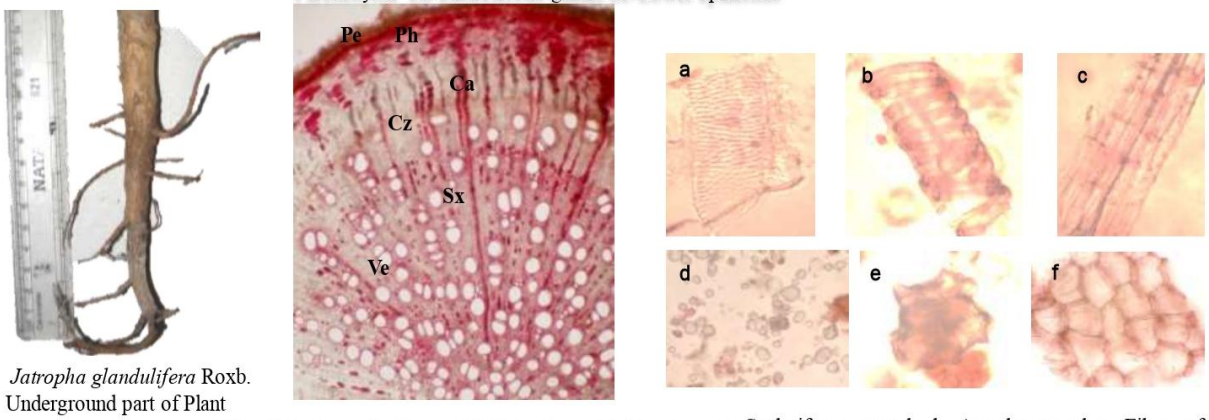
Fig. 2: Morphological, anatomical and pharmacognostic characters of *J. gossypifolia* leaves and root



Jatropha glandulifera Roxb.
aerial parts of plant

T.S. of Leaves: *Cu*-*Cuticle*, *Ue*-Upper epidermi,s *Pa*-Palisade *St*- Spongy *Hy*- *Co*-Collenchyma *Xt*-Xylem, *Mt*-Mesophyll *Ph*-Phloem *Pac*-Parenchyma *La*-Laticeferous glands *Le*-Lower epidermis

a- Stomata, b- Annular vessel c- Fibers, d-Vein islets



Jatropha glandulifera Roxb.
Underground part of Plant

T.S. of Root :*Pe*-Periderm, *Ph*-Phelloderm, *Ve*-Vessel, *Sp*-Secondary Phloem, *Sx*-Secondary xylem, *Cz*-Cambial zone, *Ca*-Calcium oxalate crystals

a- Scalariform vessels, b- Annular vessel, c- Fibers of cork, d- Starch grains, e- Crystal of Ca oxalate f- Cork tissue in surface view

Magnification 100x

Fig. 3: Morphological, anatomical and pharmacognostic characters of *J. glandulifera* leaves and root

DISCUSSION

The powder of matured roots showed presence of starch grains in JGO and JN but not in in JGL. In the secondary structure, a thick periderm formed by suber and phelloderm strata was detectable. Secondary xylem vessels are 1-4 cells in length with wider lumen in JGL and JN while in JGO these are 1-5 cells in length with narrow lumen. Microscopic investigation of leaf powder showed the presence of calcium oxalate crystals in two species JGL and JGO, while these are absent in JN. In all three *Jatropha* species stomata are paracytic and the leaves are amphistomatic. These species are also evaluated for stomatal number, stomatal index, vein-islet number and palisade ratio. Tyloses are found in only JN leaves while these were absent in JGO and JGL. JN was reported for scalariform and reticulate vessels in roots while JGL was for scalariform and annular vessels and JGO for only scalariform vessels. Laticifers, crystals of calcium oxalate in various sizes, vessels with annular and scalariform secondary wall thickenings, and stomatal

number, palisade ratio, vein-islet number are some of the essential diagnostic characteristics that are effective in verifying the legitimacy of the drug sample. Although the exact purpose of calcium oxalate crystals in plant tissues is not yet fully understood, evidence from a number of studies suggests that they may act as a reservoir to ensure calcium supply for metabolic processes when its absorption and translocation are hampered by environmental stresses like salinity or heavy metals (Hunsche *et al.,* 2010). Numerous laticifers for the transfer of latex were present in the leaves, a characteristic of Euphorbiaceae, and they were spread throughout the parenchyma (Metcalfe and Chalk 1960; Sallykutte 2016).

The total percentage of ash values, acid insoluble ash, water soluble ash and percentage yield of extractives in different solvents, phytochemical screening and HPTLC are constant features of a part of the plant which may constitute individual drug. These reports would be extremely important for determining the authenticity of the drug sample. The leaves and roots of JN, JGO, and JGL were subjected to pharmacognostic and phytochemical analyses in the current study. The taxonomic characteristics of the taxon, macro- and microscopical characteristics, and diagnostic characteristics of the portion employed are all included in the pharmacognostical research. It was discovered that the physical constants and phytochemical characteristics were helpful in assessing the pharmacopoeial standards (Mishra 2020**)**. The qualitative phytochemical examination provided insightful data on the many phytoconstituents contained in the extracts, enabling future researchers in selecting the best extract for further study on isolating the active principle. The development of fingerprints of a particular plant species is an important parameter in the standardization. So in the present investigation an attempt has been made to develop HPTLC fingerprints for *Jatropha* species and to present the comparison of HPTLC patterns in various solvent systems this will aid in the detection of adulteration in the samples. Understanding the promotion of health and lowering the risk of disease is crucial where crude fibre plays a significant role. A wealth of scientific evidence demonstrates that adequate dietary fiber intake has a number of health benefits, including maintenance of healthy laxation and the reduced risk of cardiovascular disease and cancer. (Madhu *et al.,* 2017). The main components of crude fibre are cellulose (60-80%) and lignin (4-6%), along with some mineral stuff. These fibres help to treat or prevent constipation, haemorrhoids, diverticulosis, coronary heart disease, and some types of cancer. It also has laxative properties. (55 Howe *et al.*, 1992, 56 Pera 2007). It is also helpful in analyzing different foods and food items to look for adulteration, quality, and quantity, which draws attention to the potential of *Jatropha* species.

Micronutrients are essential for maintaining tissue function and metabolism. The micronutrient analysis of leaves and roots with respect to Fe, Zn, Mn and Cu by AAS showed that JGO-L (1985.3 ppm) has higher level of Fe followed by JN-R (753.9 ppm), while Zn content is high in JGO-R (27.51 ppm) followed by JN-L (17.57 ppm) and JGL-L (14.87 ppm**).** The Mn content is high in JGL-L (102.8 ppm), followed by JN-L (80.8 ppm) and JGO-L (78.6 ppm), while Cu content is high (9.1 ppm) in JGL-L than JN-R (8.6 ppm). The Mn is absent in JGO-R.

This investigation offers proof of the economically significant species viz. *J. nana, J.gossypifolia,* and *J.glandulifera* species of which *J. nana* is ephemeral restricted to western ghats only while remaining two are adapted to wastelands and desert regions and exhibit distinctive anatomical features. With regard to its abundance, wild occurrence, and simple ways of propagation, there is a wide range of potential applications for human health issues.

CONCLUSIONS

The adulteration of raw materials is the main issue facing the herbal medicine industry. The current research is an essential step in the prevention of adulteration by way of the creation of various biomarkers (Phytochemical, Pharmacognostic and anatomical), and this can be utilized as a means of identifying the illicit drug from three *Jatropha* species.

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