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PHARMACOGNOSTIC STUDIES ON LEAF OF *JATROPHA GOSSYPIFOLIA* L.

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Keywords:

Jatropha gossipifolia, anatomy, phytochemicals, antioxidants, HPTLC, GC-MS.

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ABSTRACT: Since, the free radicals induced oxidative stress is posing threat present researchers are focusing on natural sources that could serve as antioxidants to combat the oxidative damage mediated diseases such as diabetes, atherosclerosis and cancer. In this context, present study was carried out to investigate the botanical characters, chemical composition and *in vitro* antioxidant potential of *Jatropha gossypifolia* L. so as to provide scientific evidences for the medicinal properties claimed on this plant. Microscopic studies were conducted on both entire leaf and leaf powder, the elemental composition of leaf powder was investigated. Extracts of *J. gossypifolia* leaf was prepared with hexane, chloroform, ethyl acetate, ethanol and water and screened for the detection of various phytochemicals and the major phytochemical compounds present were identified through HPTLC and GC-MS analysis. *In vitro* antioxidant activity of solvent extracts was evaluated through reducing power and DPPH radical scavenging assays. Phytochemical screening revealed the presence of phenols, flavones, glycosides, steroids, alkaloids and quinines in the ethanolic extract. Quantification of phytochemicals showed 3.35 mg/kg of alkaloids, 3.60 mg/kg flavonoids, 0.22 mg/kg tannins and 0.12 mg/kg glycosides. Elemental composition revealed the existence of high level of calcium (3.89%) and iron (85 ppm), which have clinical importance. Presence of alkaloids, quercetin, and fraxetin were confirmed with HPTLC analysis. Among the different solvent extracts, ethyl acetate exhibited higher antioxidant activity. Presence of phytochemicals such as p-Cymene, Fumaric acid, 12-Octadecanoic acid, 1-Heptadecanol, and Yashabushiketol were confirmed in this plant through GC-MS analysis. Botanical and chemical standards were determined for *J. gossypifolia* leaf through the present investigation and the results of in vitro antioxidant assays gives the scientific evidence for the health benefits and medicinal effects claimed on *J. gossypifolia.*

INTRODUCTION: Free radicals and related species have attracted a great deal of Researcher's attention in recent years. These radicals are mainly derived from oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS), and are generated in our body through various endogenous systems due to exposure to different physicochemical conditions or pathophysiological states [1].

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Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations. Free radical damage to protein can result in loss of enzyme activity. Damage caused to DNA, can result in mutagenesis and carcinogenesis. Nature has endowed us with protective antioxidant mechanisms - superoxide dismutase, catalase, glutathione, glutathione peroxidases and reductase, Vitamin E, Vitamin C etc., apart from many dietary components. There are epidemiological evidences correlating higher intake of components/ foods with antioxidant abilities that can lower incidence of various human morbidities or mortalities [1].

Natural products from dietary components such as Indian spices and medicinal plants are known to possess antioxidant activity. *Jatropha gossypifolia* L. is a well-known plant belonging to the family Euphorbiaceae, originated from Brazil, commonly called as "bellyache bush" is a bushy gregarious shrub, grow wildly almost throughout India and used as a therapeutic agent [2]. It is a weed, found in dry tropical regions of India and used in traditional system of medicine to cure stomach ache, venereal diseases, wounds and anaemia. The leaf decoction of this plant is used for treating wounds, sores, sprains, rash and bewitchment [3].

Traditionally various parts of the plant are used to treat intermittent fevers, carbuncles, eczema and itches. The stem sap stops bleeding and itching of cuts and scratches. The roots are employed against leprosy, as an antidote for snakebite and in urinary complaints. A decoction of the bark is used as an emmenagogue and leaves for stomach-ache, venereal disease and as blood purifier [4]. It possesses significant anticancer antimicrobial activities [5]. In the other side aqueous extract of latex, stem bark and leaf of this plant have potent molluscicidal, larvicidal and pesticidal activity [5]. *J. gossypifolia* leaves contain jatropholone, naringenin, histamine, apigenin, vitexin, isovitexin and tannins. The bark contains the alkaloid jatrophine and a lignin jatrodien is found in its stem. The latex of *J. gossypifolia* yielded two cyclic octapeptides i.e. cyclogossine A and B. The aerial parts contain lignan, gossypiline and jatrophenone [6-8].

Since, the free radicals induced oxidative stress is posing a threat, we want to focus our research on naturally derived antioxidants to combat the oxidative damage related diseases such as diabetes, atherosclerosis and cancer. In this context, present study was carried out to investigate the phytochemical compounds and antioxidant potential of different solvent extracts of a common medicinal plant, *Jatropha gossypifolia* L.

MATERIALS AND METHODS:

Plant collection: The selected plant *Jatropha gossypifolia* was collected from in and around the SASTRA University campus during Sep 2014. The plant was identified and authenticated by Dr. N. Ravichandran, Botanist, Department of CARISM, SASTRA University. The leaves were dried in shade and coarsely powdered.

Microscopic studies:

Anatomical studies: The free hand sections were taken with the help of razor blade, thin sections were selected and stained with Toluidine blue O and salient microscopic features were observed.

Powder microscopic examination:

Staining with phloroglucinol: A pinch of the powdered plant material was placed on a microscope slide and stained with 1% solution of phloroglucinol in ethanol for 1- 2 min. The phloroglucinol was drained off and few drops of Conc. HCl was added. The excess of acid was drained off and few drops of 30% glycerol was added, mounted and observed under microscope. Lignin appears in pink to cherry red colour, if present.

Staining with Iodine-Potassium Iodide (IKI):

A pinch of the powdered plant material was placed on a microscope slide and stained with IKI solution. Cover glass was placed over it and observed under a microscope. Starch grains are stained in dark blue to dark purple colour, if present.

Staining with Sudan Red:

A pinch of powdered material was placed on a microscopic slide and stained with 2-3 drops Sudan red and was allowed to stand for few minutes and was observed under microscope. If development of orange red to red colour observed, it indicates the presence of fats, fatty oils, volatile oil and resins. The slides are then irrigated with ethanol (75%) and heated gently. The presence of fats and fatty oils are confirmed by the appearance of intact orange red to red colour.

Chloral hydrate treatment:

A pinch of powdered material was placed on a microscopic slide and a small amount of chloral hydrate and few drops water were added. Then the slide was warmed over a water bath for few minutes and the specimen was mounted with 30% glycerol and observed under microscope. This method is helpful to visualize the presence of

crystals, trichomes, leaf epidermal cells and parenchyma cells with clarity.

Confirmation test for calcium oxalate crystals: A small quantity of powdered plant material was placed on microscopic slide and treated with 2N acetic acid for about 15 min. Then the acetic acid was drained off followed by treatment with 1% silver nitrate in 15% hydrogen peroxide for about 15 min at 22°C. The excess silver nitrate solution was drained and washed with distilled water. This was further counterstained with 2% Safranin for 1 - 3 min and observed under microscope. The calcium oxalate crystals, if present, appear in black colour against red background.

Elemental composition:
Potassium, Sodium and Calcium elements of *J. gossypifolia* leaf was analyzed using flame photometry and Manganese, Molybdenum, Copper, Iron and Zinc were analyzed in atomic absorption spectrometry and Carbon, Hydrogen, Nitrogen, Oxygen and Sulphur were detected using CHNSO analyzer.

Phytochemical screening:
Five grams of the powder was extracted with 50 ml of hexane, chloroform, ethyl acetate, ethanol, and water and kept for 48 h. The samples were filtered and the filtrate was collected and kept for evaporation at 70°C on water bath. Then the dried extracts were re-suspended in respective solvents in 1 mg/ml ratio and used for further experiments. The preliminary phytochemical screening was carried out in ethyl acetate extract of *Jatropha gossypifolia* by following the method of Harbone [9]. The presence of phenolic compounds was identified by taking 1 ml of extract with 5 ml alcohol and a pinch of ferric chloride. The presence of flavones was detected by adding 2 ml of extract with 1 ml of Hydrochloric acid and a pinch of Magnesium turnings and boiled for few minutes. To detect the presence of glycosides, anthrone test was carried out by adding 0.2 ml of extract with a pinch of anthrone in a watch glass, and one drop of conc. Sulphuric acid was added and warmed gently.

To know the presence of anthraquinones, Borntager's test was performed in which 1 ml of extract was macerated with ether and filtered, and then 1 ml of aqueous Ammonia was added to the filtrate. To know the presence of quinones, 0.5 ml of extract was added with 1 ml of sodium hydroxide. For confirming the presence of steroids, Leibermann-Burlard test was performed, in which, the chloroform was added with the extract and 3 ml of acetic anhydride and few drops of concentrated sulphuric acid were added. In the second test, 2 ml of Ferric chloride, acetic acid reagent and 1 ml of concentrated sulphuric acid were added to the extract. The presence of alkaloids was detected by using Dragendorff's test, in which, 0.5 ml of extract was taken with 0.2 ml of acetic acid and 1 ml of Dragendorrf's reagent and shaken well.

Quantification of phytochemicals:
The total alkaloid content of ethyl acetate extract of *J. gossypifolia* was estimated according to the method described in Indian Pharmacopoeia [10]. Total flavonoid content was determined using aluminium chloride according to the method of Zhishen *et al*. [11] using quercetin as a standard. The extract was investigated for tannins content according to the method of Rajpal [12]. The glycosides content of the extract was analyzed according to method of Sakulpanich and Gritsanapan [13].

Antioxidant activity:
The phosphomolybdate reducing power of extracts was evaluated according to the method of Prieto *et al*. [14]. An aliquot of 100 µl of extract was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a screw-capped vial. The vials were closed and incubated in a water bath at 95 C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results expressed as ascorbic acid equivalent antioxidant activity.

The DPPH radical scavenging activity was analyzed for each by following Sanchez-Moreno *et al*. [15] method. The extract (100 µl) was added to 3.9 ml of DPPH solution (0.025 g/L) and the reactants were incubated at 25°C for 30 min. Different concentrations of ferulic acid was used as a positive control and ethanol was used instead of extract in

blank. The decrease in absorbance was measured at 515 nm using a spectrophotometer. The radical scavenging activity of tested samples was calculated and expressed on percentage basis.

HPTLC study:
The experiment was performed on a pre-coated silica gel 60 F-254 (0.2 mm thickness) HPTLC plate (10 x 10 cm, Merck, Germany). Samples were applied on the plate as 7 mm bands, 15 mm apart from the edges of the plate, with a Camag Linomat V sample applicator. Mobile phase of Toluene: Ethyl acetate: Diethylamine (7:2:1) was used for detecting alkaloids and for flavonoids Toluene: Ethyl acetate: Formic acid (5:4:1) was applied. The plates were developed to a distance of 80 mm at 25 ± 5°C in a Camag twin trough glass chamber. The saturation time was 30 min and after development, plates were dried in a hot-air oven, viewed in a Camag UV chamber and the chromatograms were scanned with a Camag TLC Scanner. The Rf values and fingerprint data were recorded using WINCATS software.

GC-MS analysis:
The ethyl acetate extract was analyzed using Gas Chromatographic system coupled with Mass Spectrometry (Perkin Elmer, Model: Clarus-500). Silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness, Elite-5 MS non-polar fused) was used. Oven temperature was programmed with an increase of 6°C/min to 150°C; injector temperature was 280°C; carrier gas was helium with the flow rate of 1 ml/min. Sample (1.4 µl) was injected with split ratio of 1:10. Ionization energy 70 ev was used in the electron ionization mode; ion source temperature was set at 160-200°C, mass was scanned in the range of 40-450 amu. The resulted mass spectrum was compared with inbuilt NIST library database and fragments of various compounds present in the extracts were identified.

RESULTS:

Microscopic studies: T.S. of midrib and lamina revealed the anatomical features of *J. gossypifolia* leaf (**Fig. 1**). The powder microscopy exhibits the occurrence of calcium oxalate crystals, starch grains, trichomes, Sclereids and stone cells **(Fig. 2**).

Elemental composition:
The elemental composition of *Jatropha gossypifolia* leaves indicated the presence of high levels of potassium (3.02%), calcium (3.89%) and magnesium (3.06%) (**Table 1).**

Phytochemical screening:
Phytochemical screening revealed the presence of phenols, flavones, glycosides, steroids, alkaloids and quinines in the ethyl acetate extract of *J. gossypifolia* leaf (**Table 2**). Presence of these phytochemical constituents might be responsible for the therapeutic properties exhibited by this plant.

Phytochemical constituents:
The extractive values were calculated based on the difference between the empty weight of the vessel and extract. The extractive value for hexane extract is 0.14 g, chloroform extract 0.19 g, ethyl acetate extract 0.23 g, ethanol extract 0.48 g and water extract 0.90 g per 5 g of raw material (Dry leaf powder). Quantification of major phytochemicals showed 3.35 mg/kg of alkaloids, 3.60 mg/kg flavonoids, 0.22 mg/kg tannins and 0.12 mg/kg glycosides in ethyl acetate extract of *J. gossypifolia* (**Table 3**).

Antioxidant activity:
Based on phosphomolybdate assay and DPPH radical scavenging activity, ethyl acetate extract of *J. gossipifolia* was found to have high antioxidant activity when compared to other extracts of the present study **(Fig. 3**). Even though the extract yield of ethyl acetate extract (4.6%) was lower when compared to the yield of ethanol extract (9.6%) and water extract (18%), the high antioxidant power exhibited by the ethyl acetate extract could be due to the presence of bioactive constituents.

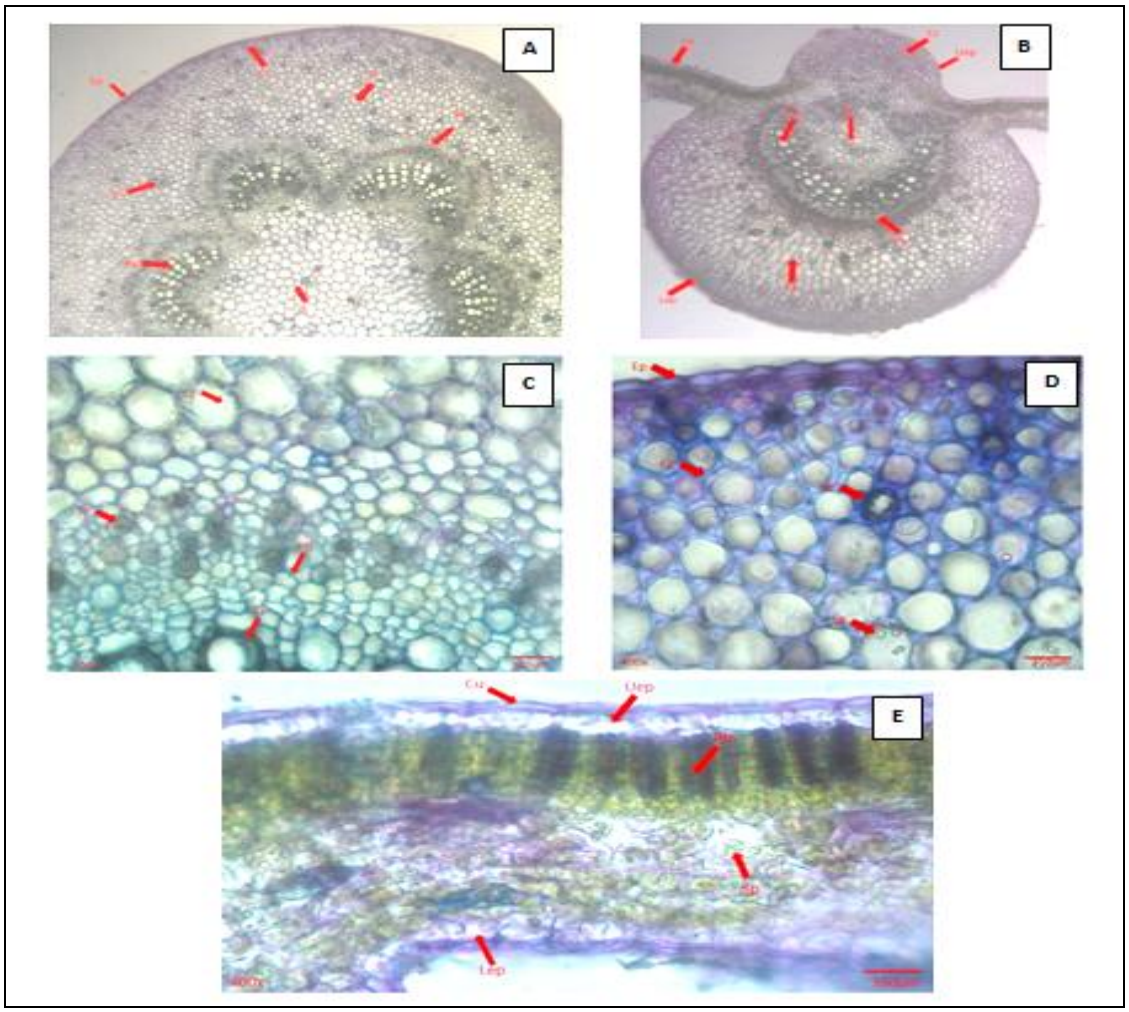
HPTLC study:
Presence of quercetin and fraxetin were confirmed in *Jatropha gossypifolia* extract using HPTLC analysis (**Fig. 4**).

GC-MS analysis:
The GC-MS analysis of ethyl acetate extract of *J. gossypifolia* revealed the presence of various phytochemical constituents (**Fig. 5**). Based on peak

area, the major compounds identified in ethyl acetate extract of *Jatropha gossypifolia* are p-Cymene, Fumaric acid, 12-Octadecanoic acid, 1-Heptadecanol, and Yashabushiketol.

DISCUSSION:

Microscopic studies: The TS of petiole revealed the presence of single layered epidermis, many cell layered cortex, vascular bundles and pith (**Fig. 1A**).



(CC- Collenchyma; Ct – Cortex; Cu – Cuticle; Dr – Druses; EP – Epidermis; La – Lamina; Lep – Lower epidermis; Ph – Phloem; Pi – Pith; PP - Palisade parenchyma; Sc – Secretory cells; Sg – Starch grains; Sp – Spongy parenchyma; Uep – Upper epidermis; Xy – Xylem).

FIG.1: MICROSCOPIC STUDIES ON *J. GOSSYPIFOLIA* LEAF (A - T.S. of petiole; B - **T.S. of leaf; C - TS of leaf midrib showing vascular bundles; D - TS of leaf midrib showing collenchyma; E - TS of lamina).**

The T. S. of leaf consists of midrib and lamina. Midrib is ovoid in shape with upper and lower epidermis (Fig. 1B). The outer wall of the epidermis shows the presence of thick cuticle. Both epidermis contains covering and glandular trichomes. Covering trichomes are uni-cellular and uni-seriate. In the case of glandular trichomes, both head and stalk are multi-cellular. Epidermis followed by collenchyma, the abaxial side shows 15-17 cell layered but adaxial side shows presence of 2-3 layered cells. In adaxial side the collenchyma is followed by several cell layered cortex, some of the cortex cells contains prismatic and druses type of calcium oxalate crystals and secretary cells. The cortex is followed by horse shoe shaped vascular bundle (**Fig. 1C**). Vascular bundle contains 6-7 cell layered phloem and some of the phloem cells contain druses type of calcium oxalate crystals and starch grains (**Fig. 1D**). The phloem cells are followed by 2-3 cell layered cambium and then xylem region. Xylem region contains xylem vessels with bicellular medullary rays. Secretary cells are present in the cortex region. Phloem cells are 3-4 layered and xylem is endarch.

The lamina shows upper and lower epidermis (**Fig. 1E**). Outer wall of the epidermal cells contain thick cuticle. Both the epidermis contain covering and glandular trichomes. Lower side shows more trichomes than upper side. Covering trichomes are unicellular, uniseriate and some of the uniseriate trichomes are collapsed with warty and smooth walls. The glandular trichomes are uni-cellular with multi-cellular heads, uni-seriate and multi-cellular stalk. The epidermis is followed by 2-3 layered longitudinally elongated palisade cells which is followed by 4-5 cell layered spongy parenchyma cells with wide intercellular spaces. The powder microscopy revealed the presence of prismatic and druses types of calcium oxalate crystals (**Fig. 2A & B**). Simple and compound starch grains are round and oval in shape (**Fig. 2C**). Covering trichomes are unicellular and uni-seriate (**Fig. 2D**). Glandular trichomes are uni-cellular and multi-cellular head with uniseriate stalk (**Fig. 2E & 2F**). Epidermal cells are wavy (**Fig. 2G).** Xylem vessels showed spiral and pitted thickening **(Fig. 2H & 2I**). Sclereids and stone cells are found with pitted thickening and the lumen is narrow (**Fig. 2J).** Some of the sclereids are seen with circular lumen (**Fig. 2K**). Lignified parenchyma cells (**Fig. 2L**) and brown contents (**Fig. 2M**) are present.

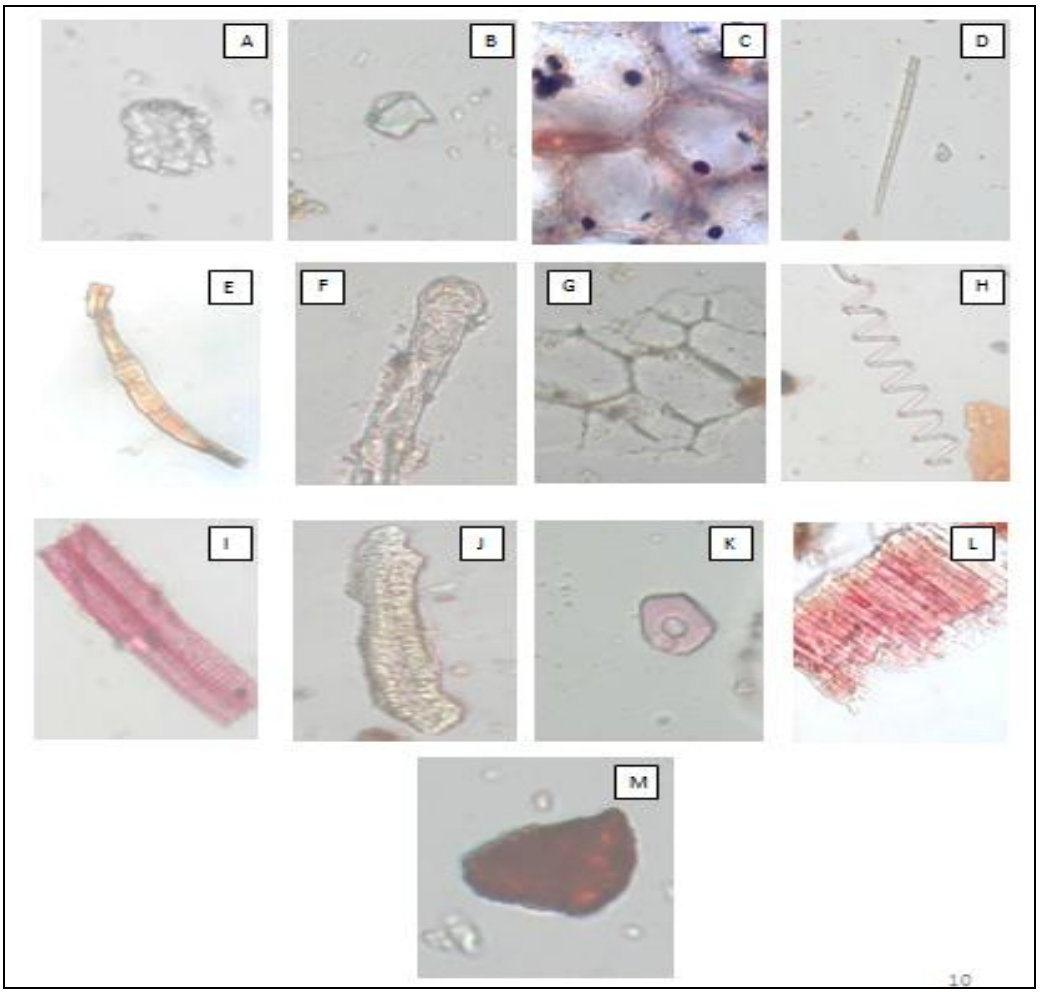


FIG.2: POWDER MICROSCOPIC CHARACTERISTICS OF *J. GOSSYPIFOLIA* LEAF (A - Druse type calcium oxalate crystal; B - Prismatic calcium oxalate crystal; C - Starch grains; D - Uniseriate trichome; E - Glandular trichome with unicellular head; F - Glandular trichomes with multicellular head; G - Epidermal cells; H - Spiral thickening; I - Xylem vessel with pitted thickening; J – Sclereids; K - Stone cells; L - Lignified parenchyma; M - Brown content).

Elemental composition:

Presence of high levels of potassium, calcium and magnesium could be useful in providing necessary micronutrients in addition to contribute medicinal effects (**Table 1**). The minor elements such as iron (85.19 ppm) and manganese (50.56 ppm) were found at higher levels in the leaves of *Jatropha gossypifolia*. Presence of iron in this leaf material could be useful in treating anaemia and other iron-deficient diseases.

TABLE 1: ESTIMATION OF MAJOR INORGANIC CONSTITUENTS OF *J. GOSSYPIFOLIA* LEAF

S. No.	Name of the element	Content
1	Moisture	2.09 %
2	Organic carbon	2.49 %
3	Nitrogen	1.21 %
4	Potassium	3.02 %
5	Sodium	0.05 %
6	Calcium	3.89 %
7	Magnesium	3.06 %
8	Zinc	2.09 ppm
9	Copper	0.40 ppm
10	Iron	85.19 ppm
11	Manganese	50.56 ppm
12	Molybdenum	0.02 ppm

Phytochemical screening:

Presence of phenolic compounds was identified by the appearance of bluish black colour when the extract was treated with alcohol and a pinch of ferric chloride. The presence of flavones was detected based on the colour change to reddish green upon adding the extract with Hydrochloric acid and a pinch of Magnesium turnings followed by boiling. Presence of glycosides was confirmed by the appearance of dark green colour while adding the extract with a pinch of anthrone and one drop of conc. Sulphuric acid followed by gentle warming. Presence of anthraquinones was identified by observation of red colour in Borntager's test. Appearance of red colour when treating the extract with sodium hydroxide indicates the presence of quinones. Further, the presence of steroids and alkaloids were confirmed by Leibermann-Burlard and Dragendorrf's tests, respectively.

TABLE 2: RESULTS OF PHYTOCHEMICAL SCREENING IN ETHYL ACETATE EXTRACT OF *J. GOSSYPIFOLIA* LEAF

S. No.	Name of the compound	Present / Absent
1	Phenolic compounds	+
2	Flavones	+
3	Glycosides	+
4	Steroids	+
5	Alkaloids	+
6	Quinones	+

The + sign indicate presence and – sign indicates absence of compound

Presence of phenols and flavones contributes significant antioxidant property. Glycosides are sugar moieties of phytochemical compounds exhibits anti-diabetic effect as reported in *Cynodon dactylon* [16]. Presence of steroids in this plant might be responsible for anti-obesity related properties while alkaloids and quinones could exhibit anti-cancer and anti-microbial properties.

Phytochemical constituents:

Presence of such high amounts of alkaloids and flavonoids (**Table 3**) contributes the medicinal effects that are exhibited *J. gossypifolia* plant

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TABLE 3: ESTIMATION OF MAJOR PHYTOCHEMICAL CONSTITUENTS PRESENT IN THE ETHYL ACETATE EXTRACT OF *J. GOSSYPIFOLIA* LEAF

S. No.	Name of the element	Content (mg/kg)
1	Total alkaloids	3.35
2	Total flavonoids	3.60
3	Tannins	0.22
4	Glycosides	0.12

Alkaloids are natural product that contain heterocyclic nitrogen atoms and are basic in nature. They possess many pharmacological activities including anti-hypertensive effects (many indole alkaloids), anti-arrhythmic effect (quinidine, spareien), anti-malarial activity (quinine), and anti-

cancer actions (dimeric indoles, vincristine, vinblastine). Some alkaloids have stimulant property such as caffeine and nicotine, morphine are used as analgesic and quinine used an antimalarial drug [17].

Flavonoids are phenolic substances found in vascular plants with over 8000 individual known compounds. Apart from their physiological roles in the plants, flavonoids are important components in the human diet, although they are generally considered as non-nutrients. Indeed, the level of intake of flavonoids from diet is considerably high as compared to that of Vitamin C (70 mg/day), Vitamin E (7-10 mg/day), and carotenoids (beta-carotene, 2-3 mg/day). Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating actions. However, interest has been devoted to the antioxidant potential of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals [18].

Antioxidant activity:

The phosphomolybdate assay revealed high reducing power of ethyl acetate extract of *J. gossipifolia* (1245 Ascorbic acid equivalent antioxidant activity / mg extract) when compared to other solvent extracts as well as reference ferulic acid (**Fig. 3A**). When the molybdenum (VI) is reduced to Mo (V) by an antioxidant, it forms a green coloured complex at acidic pH in the presence of phosphorous with the absorption maxima at 695 nm. This assay could be useful in evaluating the reducing or electron donating power of the antioxidant to Molybdenum and the intensity of PMo(V) complex is proportional to antioxidant power of the extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant property.

The evaluation of the antioxidant power by DPPH radical scavenging activity has been widely in use. DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a stable radical, methanolic solution of which is dark purple colour with maximum absorption at 515 nm**.** Antioxidants can reduce DPPH through hydrogen transfer into its non-radical form (DPPH-H) and hence the absorption disappears at 515 nm. The decrease in absorbency at 515 nm may be due to the reaction between phytochemicals and DPPH, which indicates the antioxidant power. At high concentration, ethyl acetate extract of *J. gossipifolia* revealed high antioxidant power (72.88%), which is comparable to that of reference compound ferulic acid (86%) (**Fig. 3B**).

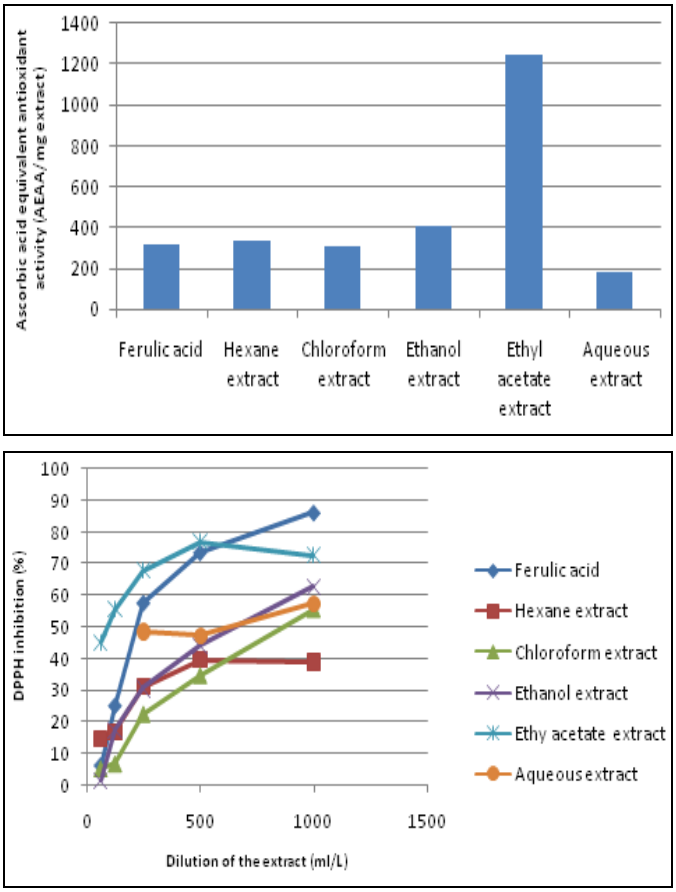

FIG.3: ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *J. GOSSYPIFOLIA* LEAF (A – Phosphomolybdate assay and B – DPPH assay)

HPTLC study:

The HPTLC analysis of *J. gossipifolia* extract shows the presence of both quercetin and fraxetin (**Fig. 4).** The compound fraxetin was also identified in a related species *Jatropha podagrica* [19]. Fraxetin was also detected in *Fraxinus rhinchophylla, Cortex fraxini* and *Lawsonia inermis* and are proven as antioxidants [20-22]. Fraxetin is an anti-hyperglecemic compound, which prevents rotenone-induced apoptosis by induction of endogenous glutathione in human neuroblastoma cells [23]. Antibacterial activity of fraxetin against *Staphylococcus aureus* was investigated by Wang *et al.* [24]

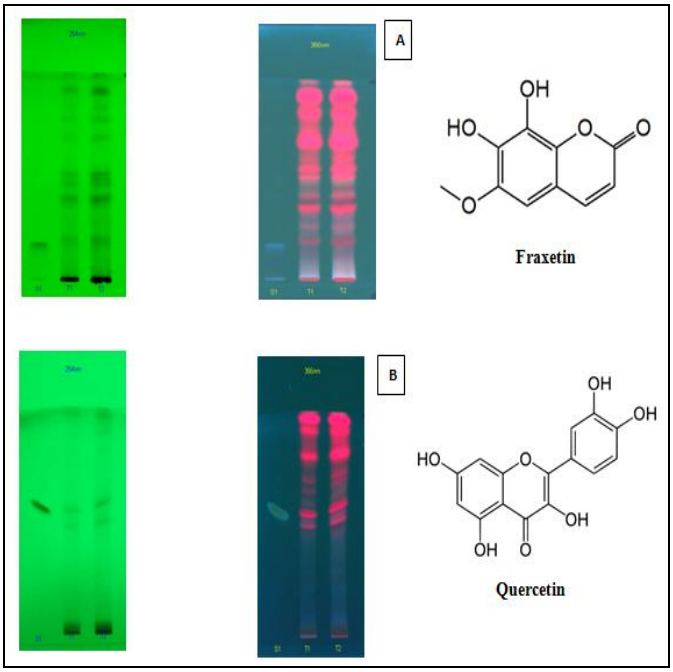

FIG.4: IDENTIFICATION OF FRAXITIN (A) AND QUERCETIN (B) IN ETHYL ACETATE EXTRACT OF *J. GOSSYPIFOLIA* LEAF THROUGH HPTLC STUDY

In agreement to our work, presence of quercetin was confirmed by HPTLC analysis of methanol extract of related species *Jatropha curcus* [25]. Quercetin is an unique flavonoid that has been extensively studied by researchers over the past 30 years. Quercetin is found in many common foods including apples, tea, onions, nuts, berries, cauliflower, cabbage and many other foods [26]. Quercetin seems to be the most powerful flavonoids for protecting the body against reactive oxygen species. Quercetin provides many health promoting benefits, including improvement of cardiovascular health, eye diseases, allergic disorders, arthritis, reducing risk of cancer [26].

GC-MS analysis:

The GC-MS analysis of *J. gossipifolia* extract exhibited the presence of various phytochemicals (**Fig. 6).** The peak corresponds to molecular mass 134 is compared to mass bank data which indicates this compound could be p-cymene, which is a monoterpene consisting of a benzene ring substituted with an isopropyl group at its para position. It is a constituent of a number of essential oils such as oil of cumin and thyme and also it is a precursor of thymol and carvacrol. Presence of p-cymene was also reported in the essential oil of related plant species *Jatropha ribifolia* root [27].

In vitro studies have demonstrated that the p-cymene can be used as an antimicrobial against Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative *Escherichia coli* [28]. Furthermore, different studies have shown that p-cymene and its derivative monoterpenes are efficient antioxidant and anticarcinogenic agents, and they are approved in the United States and Europe to preserve and to give specific flavours to foods and feeds [29]. However, p-cymene has been reported to be less effective against food-related pathogens. p-Cymene has been shown to have lipolytic properties [30]. Moreover, recent studies have found that these compounds are effective against *Leishmania* [31]. p-Cymene allows a proton leak through the phosphorylative system and changes the mitochondrial proton motive force and ATP synthesis capacity and therefore the mitochondria could be a target for p-cymene toxicity action mechanisms to pathogens [32].

The next major compound with molecular mass 116 is fumaric acid, which is the isomer of unsaturated dicarboxylic acid, maleic acid. It is reported to be an active principle responsible for the antimicrobial activity of *Aloe vera* [33] and *Fumaria indica* [34]. This compound is reported to exhibit anti-inflammatory and analgesic activities [35]. Fumaric acid has been used in the treatment of multiple sclerosis, psoriasis with immune-modulatory effect [36].

The mass spectrum of the compound 12-Octadecanoic acid methyl ester showed molecular mass of 296 in ethyl acetate extract of *Jatropha gossipifolia*. The same compound was reported in related species *Jatropha curcas* [37] and other plant species such as *Iris germanica* [38], *Rorippa indica* [39] and *Indigofera suffruticosa* [40]. This compound was reported to possess antioxidant, nematicidal, pesticidal and anti-androgenic properties [39].

1-Heptadecanol with molecular mass of 256 was detected in the *Jatropha gossipifolia* extract. The same compound was also found in *Allamanda violacea* [41]. This compound was reported to demonstrate antioxidant, hypocholesterolemic, haemolytic, nematicidal and pesticidal activities [42]. Peak identified with molecular mass of 280 indicates the presence of yashabushiketol, which is

a diarylheptanoid. Asakawa *et al.* [43] stated that yashabushiketol was isolated from buds of *Alnus sieboldiana* while this compound separated from *Alnus firma* was reported to be effective against HIV virus [44].

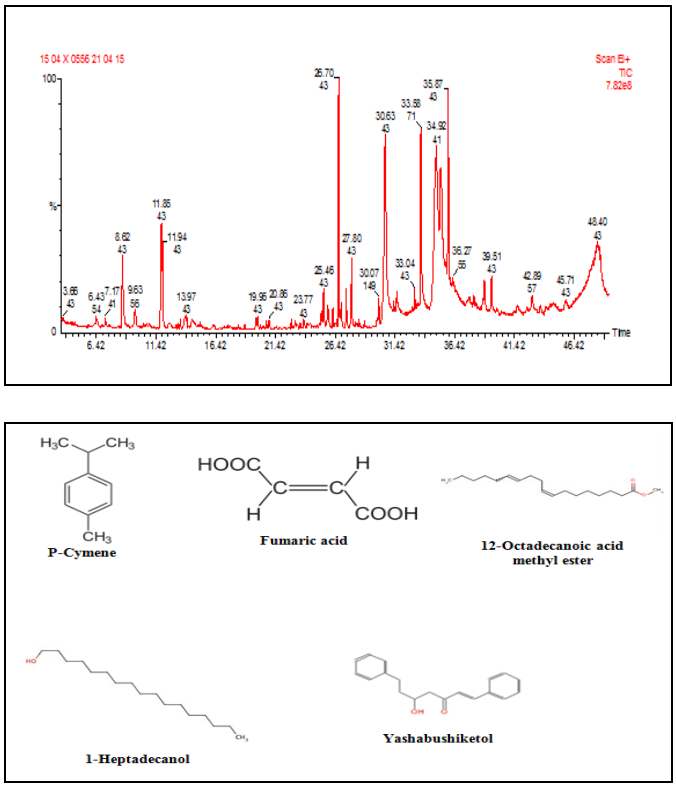

FIG.5: GC-MS FINGERPRINTING OF ETHYL ACETATE EXTRACT OF *J. GOSSYPIFOLIA*

CONCLUSION: The microscopic studies were carried out on entire leaf and leaf powder of *Jatropha gossypifolia* and salient anatomical features were recorded. The elemental composition of leaf powder revealed the presence of higher level of potassium and iron. Screening of solvent extracts exhibited the presence of various phytochemical constituents and also quantification data showed significant levels of alkaloids, flavonoids, tannins and glycosides in the ethyl acetate extract. *In vitro* antioxidant activity of various solvent extracts was compared and ethyl acetate exhibited higher antioxidant activity among the different solvent extracts. The major phytochemical compounds such as fraxetin and quercetin were identified and confirmed in the ethyl acetate extract through HPTLC analysis. GC-MS analysis revealed the presence of p-Cymene, Fumaric acid, 12-Octadecanoic acid, 1-Heptadecanol, and Yashabushiketol in the ethyl acetate extract. Hence, the present study given scientific evidence for the traditional use of *J. gossypifolia* plant in Indian traditional system of medicine for various oxidative stress mediated diseases.

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