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Pharmacognostic Features, Preliminary Phytochemical Screening and *in vitro* Antioxidant Studies of *Calotropis gigantean*

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ABSTRACT

Calotropis gigantea is a common plant found numerously in Pakistan and well known for its folklore uses it belongs to *Apocynaceae* family. The current research work based on its identification and for the purification techniques of crude plant drug for its uses in modern medical sciences. This study includes pharmacognostic features evaluation, preliminary and phytochemical studies, microscopic evaluation and powder microscopy. In this study transverse section of the different parts of the plants shows arrangements of fragment and cells. Fluorescence analysis is used for the color identification of plant and in this study fluorescence analysis is performed with different chemical and solution and results are observations are noted in short and long wavelength. For the identification of presence of saponins, alkaloids, tannins and flavonoids phytochemical tests were performed for the evaluation of crude drug powder. Fourier-transform infrared spectroscopy is used for the functional group identification of plant parts which would be used for the identification and characterization of plant parts. Physio chemical parameters such as total ash value, Water soluble Ash, Swelling Index, Foaming Index and moisture contents were performed for the physical evaluation of crude powder. Anti-oxidant *in-vitro* studies have been performed for the evaluation of any anti-oxidant property which make it a brief description about its pharmacological effects regarding rancidity and provide a description about its further usage in any of anti-oxidant formulation.

Keywords: Apocynaceae, Phytochemical studies, *C.gigantea*, FTIR, DPPH.

INTRODUCTION

Plants are the immense power of nature. Mother Nature provide us with various kind of plants and trees which are not only complete our basic need of food and other basic necessities but also provide medicines. As with the evaluation of mankind world also proceed with medical science and technology but the basic origin of drug is remained form our mother nature [1,2]. As with the passage of time medical industry evaluate as well as allopathic but now scientist understand the effectiveness of plants and move towards plant origin. So as a result there is need to increase the identification and evaluation techniques and this research work based upon on this evaluation [3].

This research work include phytochemical and physiochemical evaluation which disclose its effectiveness for its folklore uses as well as for the medicinal properties of the plant. Histological studies, fluorescence analysis, proximate analysis and phytochemical analysis. Microscopic evaluation of *C.gigantea* powder was subjected for the diagnostic characteristics of individual stem and leaves *Calotropis gigantea* is widely distributed in south Asia and North Africa. It is widely distributed in Thailand, India, Pakistan, Nepal, China, Srilanka, Cambodia Malaysia and Indonesia. It also found in Somalia and tropical Africa [4].

This plant is drought resistant and salt tolerant and distributed mostly in sandy soils. It quickly get be grown anywhere by spreading its seeds by animals or wind. The folk uses of *Calotropis gigantea* are very important leaves of the plant *C.gigantea* were used for the treatment of swellings, paralysis in Ayurveda. Other folklore uses include Wound Healing Activity, Cytotoxic Activity, Anti-diarrheal Activity, Antioxidant activity [5].

Some of the validated folkloric uses of *C.gigantea* are analgesic, antibacterial, and anti-pyretic, anti-convulsant, contraceptive, anti-ulcer and wound healing. One of the major folklore uses is that it was used as an antidote for snake poisoning and it was also used as

antifungal due to its pharmacological background [6].

Despite of its vast uses and pharmacological actions it did not possess any handful information about pharmacognostic features, morphology and physiochemical properties. Therefore this study includes comprehensive information about its pharmacognostic features which are used for the authencity and identification of plants which is the necessary tool in any research and development project. This is the baseline which is set for any development that material should be pure and adulterant free. This study provide the major identification pattern for the new developers [7].

MATERIALS AND METHODS

Plant material

The plant was collected from Botanical Garden of Government College University, Lahore and was authenticated by Sir Zaheer Department of Botany, Government College University, and Lahore Pakistan, based on authenticity established by Gamble, Benthum and Hooker. A specimen of plant was deposited in herbarium of Government College University, Lahore under Voucher Specimen No: GC. Herb. Bot. Aerial parts were separated and all parts were dried under shade and then preserved in amber colored glass bottles.

Chemicals, reagents and instruments

Different chemicals were used in this study such as analytical grade acetonitrile, methanol, acetic acid, n-Hexane, saffranin, lead acetate, sulphuric acid, hydrochloric acid, ammonia solution, fast green, Million's reagent, Molisch's reagent, Quercetin, chloral hydrate Ascorbic Acid and ethyl acetate, sodium hydroxide, ferric chloride and iodine were used in this study.

In this study different instruments were used which are named as UV-Vis spectrophotometer (Shimadzu), Muffle Furnace, Ultraviolet Lamp, Electron Microscope, and Fourier-transform infrared spectroscopy (FTIR (Shimadzu) were used.

Extractive values of plant parts in different solvents

For the determination of Extractive values 5 g of weighed powder of Plant was extracted with analytical grade Methanol by the process of cold maceration [8].

Organoleptic evaluations

Organoleptic evaluations of plants were noticed by observing the parameters such as shape, texture, odor, taste as well as their appearance Different Organoleptic evaluations of fresh and dry state of parts of plant such as leaf and stem were observed and are determined according to the protocols [9,10].

Powder study

Powder study was performed with chloral hydrate. Slides were made of stem and leaves and were observed under microscope according to the specific protocols and photographs were taken with digital camera [11,12].

Transverse section cutting

Fresh leaf and stem were preserved in formalin: acetic acid: 70% alcohol (5:5:90) for 24 hours transverse sections were made by commonly used blade. Sections were stained with safranin and fast green and observed under microscope and photographs were taken [11,12].

Fluorescence analysis

Fluorescence analysis was carried out by using different chemicals and reagents and viewed under ultraviolet light of different wavelength as well as with daylight. Different colors were noted [10].

Phytochemical analysis

Phytochemical tests are performed on the methanolic extract of the plant and the determination of Alkaloids, Glycosides, Tannins; saponins [8].

Physicochemical analysis

Powdered samples stem and leaves of *C.gigantea* were subjected to physicochemical analysis as their extractive values and with total ash, foaming index, moisture content, water soluble ash, and acid insoluble ash study [9,10].

Phytochemical screening by FTIR

Fourier Transform Infrared Spectrophotometer commonly abbreviated as FTIR is the powerful and the most beneficial tool for the identification of chemical bonds which are more prescribed as functional groups which are in the compound or the sample which has to be identified [10,11]. *In vitro* Antioxidant activity by DPPH free radical scavenging method.

Antioxidant activity of plant extracts (methanol, n-hexane and chloroform) was determined according to method which is described in the reference is used with slight modifications [11].

RESULTS AND DISCUSSION

Extractive values

Different solvents were used to calculate % age yield of the roots of the plant including water, chloroform and n-hexane. The extractive values and % age yield of parts of *C.gigantea* (Figure 1).

	Solvents			
Plants/Part	**Chloroform**	**Methanol**	**n-Hexane**	**Water**
C.gigantea				
• **Stem**	19.53%	31.01%	6.41%	2.95%
• **Leaves**	28.94	40.66%	12.02%	11.90%

Figure 1: The extractive values and % age yield of parts of *C.gigantea*.

Organoleptic evaluation

Different Organoleptic evaluations of fresh and dry state of plant parts were observed (Figure 2).

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C.gigantea
Organoleptic Evaluation

Features	*C.gigantea* stem		*C.gigantea* leaf	
Stage	**Fresh**	**Dry**	**Fresh**	**Dry**
Taste	Tasteless	Tasteless	Bitter	Bitter
Texture	Rough	Rough	Smooth	Brittle
shape	Branched	Branched	Alternate With Leaf Stalks	As fresh
odor	Odorless	Odorless	Odorless	Odorless
color	Light Brown	Light Brown	Light Green	Fainted Green

Figure 2: Determination of different organoleptic evaluations of fresh and dry state of plant parts.

Microscopic evaluation

Microscopic evaluation of *C.gigantea* Stem: Slides were prepared with chloral hydrate and observed under electron microscope and photographs were taken from digital camera (Figure 3).

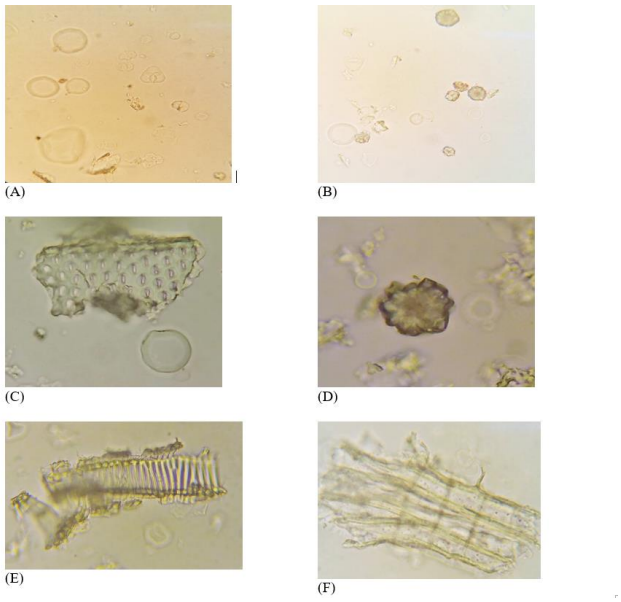

Figure 3: Microscopic Evaluation of *C.gigantea* stem (A) Starch granules, (B) Micro rosette crystals of calcium oxalate, (C) Broken fragment of bordered pitted vessel, (D) A single micro rosette crystal, (E) Vessel with reticulate thickenings, (F) Group of fibers.

Powder microscopy of *C.gigantea* leaf: Slides were prepared with chloral hydrate and observed under electron microscope and photographs were taken from digital camera (Figure 4).

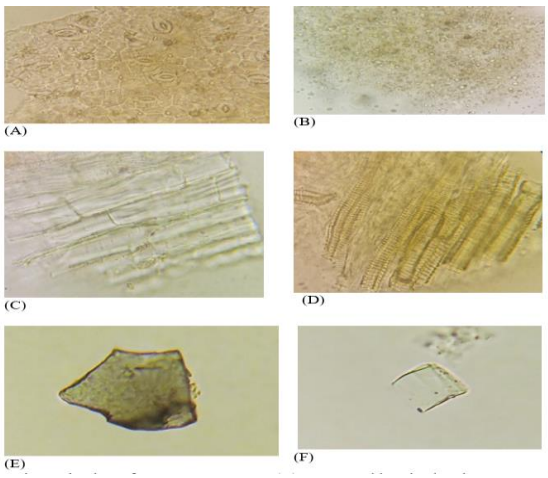

Figure 4: Microscopic Evaluation of *C.gigantea* leaves (A) Lower epidermis showing stomata (B) Oil globules, (C) Epidermal cells (D) Vessels, (E) Phytolith, (F) Prism crystal of calcium oxalate.

Section cutting of *C.gigantea* stem: Section cutting was performed with microtome and stained with fast green. The slides were observed under microscope for the identification and tissues and cells (Figure 5).

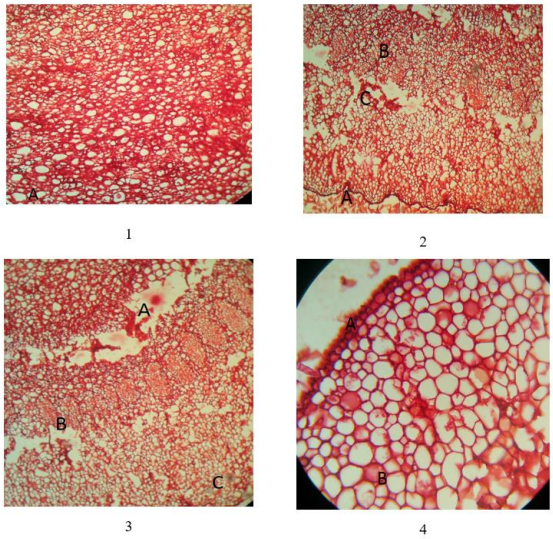

Figure 5: 1) A) epidermis, 2) A) epidermis, B) collenchyma, C) parenchyma, 3) A) intercellular spaces medullary rays, 4) B) parenchyma.

Transverse section cutting of *C.gigantea* leaf: Section cutting was performed with microtome and stained with fast green. The slides were observed under microscope for the identification and tissues and cells (Figure 6).

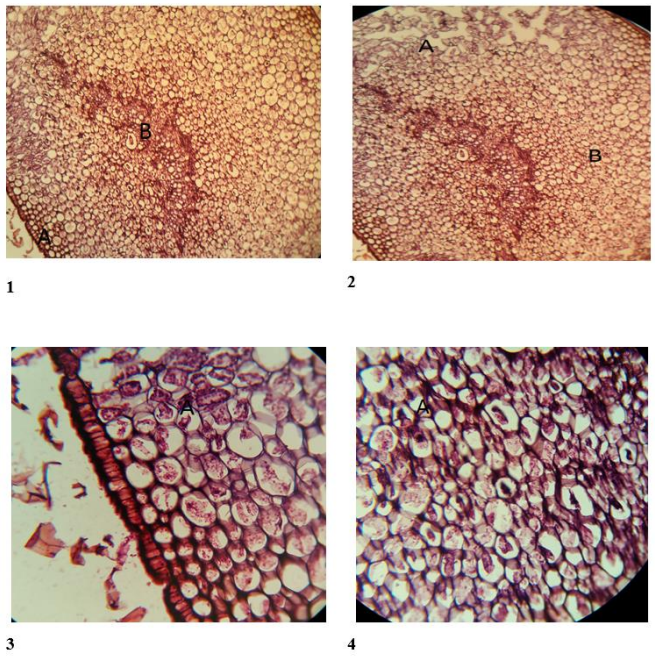

Figure 6: 1) A) epidermis, B) fibers,2) A) Inter cellular spaces) collenchyma cells, 3) A) epidermis 4) A) oil glands.

Fluorescence analysis: The powdered plant material was treated with

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different reagents and after the change in color for each sample was observed for change in color under day light and short wavelength 254 and long wave length 366 UV light respectively (Figures 7 and 8).

Fluorescence Analysis of *C.gigantea* stem

Sr. no	Reagent's used	Visible range	Short wavelength	Long wavelength
1	Powder +FeCl3	Yellow color	orange brown	greenish black
2	Powder+HCl	Black	Orange brown	Green
3	Powder+Conc HNO3	Orange	Dark Orange	Red
4	Powder + NaOH	Yellow	Orange brown	Light Brown
5	Powder+Conc H2SO4	Black	Grey	Brown Red
6	Powder+Bromine water	Light Off white	Brown	Sea green
7	Powder+Chloroform	Off white	Orange Red	Green
8	Powder+Methanol	Yellow	Brown	Grey
9	Powder+Acetic Acid	Yellowish	Brown Red	Sea Green
10	Powder+Iodine	Brown red	Orange	Black
11	Powder+NH3	Yellow	Orange	Green
12	Powder+water	Cream color	Orange	Sea green

Figure 7: Fluorescence Analysis of *C.gigantea* stem.

Fluorescence Analysis of *C.gigantea* Leaves

Sr. no	Reagent's used	Visible range	Short wavelength	Long wavelength
1	Powder +FeCl3	Dark Green	Bick Red	Brown
2	Powder+HCl	Green	Orange Red	Brown
3	Powder+Conc HNO3	Yellow	Pink	Grey Green
4	Powder + NaOH	Green	Orange	Pale green
6	Powder+Conc H2SO4	Black	Brick red	Pale green
7	Powder+Bromine water	Green	Orange	Grey
8	Powder+Chloroform	Green	Orange	Dark red
9	Powder+Methanol	Fresh green	Brown	Blood red
10	Powder+Acetic Acid	Green	Orange Brown	Red
11	Powder+Iodine	Greenish Brown	Orange	Dark Brown
12	Powder+NH3	Green	Orange brown	Green
13	Powder+water	Green	Red	Light brown

Figure 8: Fluorescence Analysis of *C.gigantea* Leaves.

Phytochemical analysis of *C.gigantea*: Phytochemical tests are performed on the methanolic extract of the plant and the determination of Alkaloids, Glycosides, Tannins, saponins (Figure 9).

Phytochemical Constituents of *C.gigantea*

Sr.No	Phytochemical constituents	Tests Performed	Results Stem	Results Leaves
1.	Alkaloids	1. Mayer's Reagent	Present	Present
		2. Wagner's Reagent	Present	Present
		3. Hager's Reagent	Present	Present
2.	Carbohydrates	1. Benedict's Tests	Absent	Absent
		2. Fehling's Test	Absent	Absent
3.	Phenols	1. Ferric Chloride test	Absent	Present
4.	Flavonoids	2. Lead Acetate Test	Absent	Present
5.	Tannins	Gelatin Test	Present	Absent
6.	Lipids	Spot Test	Absent	Absent
7	Saponins	Foam Test	Absent	Absent

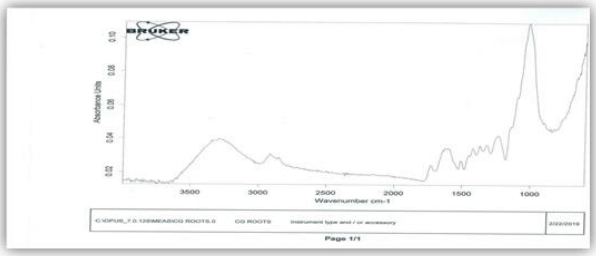
Figure 9: Phytochemical constituents of *C.gigantea.*

Physiochemical analysis: Physiochemical analysis or proximate analysis including Total ash, water soluble ash, acid insoluble ash, swelling index, Moisture contents, Foaming index of *C.gigantea* roots and leaves (Figure 10).

Sr.No	Physico-chemical Parameters	*C.gigantea* Stem	*C.gigantea* Leaves
1	Total Ash(less than 13%)	4.59%	8.59%
2	Water soluble Ash(less than 10%)	1.38%	1.94%
3	Acid insoluble Ash (0.5-5.5%)	1.42%	2.06%
4	Swelling Index(up to 5ml)	1ml	1.5ml
5	Moisture Contents (8-14%)	2.48%	2.79%

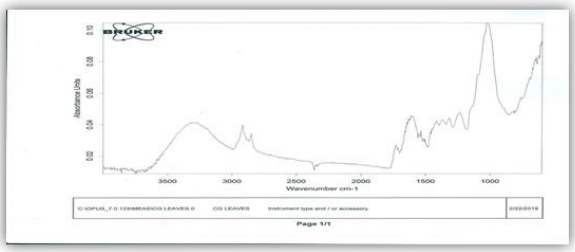
Figure 10: Physico-chemical Parameters.

FTIR (Fourier Transform Infrared Spectrophotometer): Technique of FTIR has been used for the determination of functional groups and for the specific bonds of the plant powder (Graphs 1 and 2).



Graph 1: FTIR scans of *C.gigantea* stem.

The peak is at 3200 and broad, and the peak at 1000 is sharp and strong.



Graph 2: FTIR scan of *C.gigantea* leaves.

The major peak is at 3450 and have a broad spectrum and the peak which is at 1000 nm is sharp and strong.

Antioxidant activity

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In vitro Antioxidant activity by DPPH free radical scavenging method. Antioxidant activity of plant extracts (methanol, n-hexane and chloroform) was determined according to method which is described in the reference is used with slight modifications (Figures 11-15 and Graphs 3 and 4).

Antioxidant study of Ascorbic Acid	
Concentrations (µg/ml)	**Methanol**
200	0.198±0.001
400	0.178±0.001
600	0.129±0.001
800	0.109±0.001

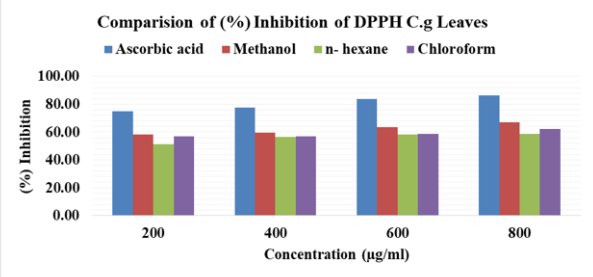
Figure 11: Antioxidant study of ascorbic acid.

Antioxidant Activity of *C.gigantea* leaves			
Concentrations(µg/ml)	**n-Hexane**	**Chloroform**	**Methanol**
200	0.386±0.002	0.341±0.001	0.330±0.001
400	0.342±0.001	0.339±0.002	0.319±0.004
600	0.331±0.002	0.326±0.001	0.326±0.001
800	0.326±0.001	0.297±0.001	0.261±0.001

Figure 12: Antioxidant study of *C.gigantea* leaves in (µg/ml).

Concentration(µg/ml)	**200**	**400**	**600**	**800**
Ascorbic acid	74.97	77.46	83.71	86.24
Methanol	58.18	59.62	63.50	66.96
n- hexane	51.13	56.66	58.05	58.73
Chloroform	56.83	57.13	58.73	62.40

Figure 13: (%) Inhibition of *C.gigantea* leaves.



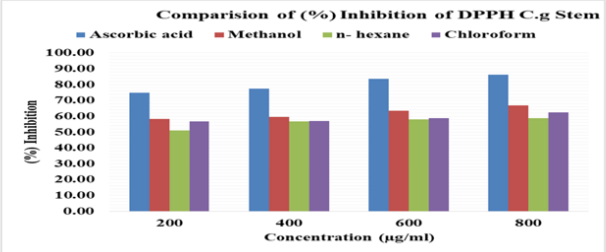
Graph 3: Antioxidant study of *C.gigantea* stem.

Antioxidant study of *C.gigantea* Stem			
Concentrations	**n-Hexane**	**Chloroform**	**Methanol**
200	0.386±0.002	0.347±0.001	0.357±0.001
400	0.345±0.003	0.337±0.001	0.346±0.001
600	0.326±0.002	0.316 ± 0.002	0.334±0.002
800	0.321±0.001	0.308 ± 0.005	0.316±0.001

Figure 14: Antioxidant study of *C.gigantea* stem in (µg/ml).

Concentration (µg/ml)	**200**	**400**	**600**	**800**
Ascorbic acid	74.97	77.46	83.71	86.24
Methanol	54.93	69.11	57.67	59.95
n- hexane	51.13	56.37	58.73	59.40
Chloroform	56.07	57.38	60.00	60.97

Figure 15: (%) Inhibition of *C.gigantea* stem.



Graph 4: Comparison of (%) inhibition of DPPH C.g stem.

CONCLUSION

Estimation of extractive values is a necessary tool for the identification of solvent in which constituents of plant show its maximum solubility. Phytochemical profiles of every plant are different so phytochemicals are dissolved in different solvents. For the identification of best possible solvent extractive values are performed. Physicochemical studies and phytochemical studies are necessary in any early identification of plant material and in the identification of any adulterant. Powder microscopy and transverse sections are the key tests for the evaluation of structures and fragments present in plant parts which are helpful for the new researchers for the new manufacturing of pesticides as well as for the botany research. Fluorescence analysis is also another evaluation technique which is based on colors. By the help of different chemicals and reagents and observation under different wavelengths different colors were observed which provide the basic tool in any research work. As well as another important test in the identification of plant material is Fourier Transform Infrared Spectrophotometer test in which fictional groups could be identified and will be helpful in any of the early studies and then in further evaluation of plant as well as their part. One of the other tests which is performed in this study is in-vitro studies Antioxidant activity by DPPH free radical scavenging method which would be helpful in the new pharmacological studies and According to this study these plant parts are used as natural anti-oxidants in different formulations of drugs and will be derived new dosage forms with natural ingredients.

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