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Pharmacognostic studies and quality control parameters of *Euphorbia neriifolia* L.

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Abstract: In present scenario, the growing demand of herbal medicines has created a huge challenge to standardize herbal materials extensively at rapid pace. To establish quality control parameters of a locally occurring medicinal plant, *Euphorbia neriifolia* is utilized as folk medicine in traditional system of medicine. In tremendous diverse world of medicinal plants, *E. neriifolia* is one of the important plants which contains remarkable medicinal values and is generally used as an effective herbal drug in several ailments such as rheumatism, sciatica, bronchitis, leucoderma. It has been a part of various traditional systems of medicine from the time immemorial. In classical literature of Unani medicine, a number of its varieties are mentioned like danda thuhar, nadhra thuhar, chaudhara thuhar, unglia thuhar and nagfani thuhar. The drug was taken for evaluation through various scientific parameters viz. macro and microscopical studies, physico-chemical analysis and HPTLC fingerprinting. The drug was also analysed for detection of heavy metals, pesticide residues, microbial load and aflatoxins to ensure its safety and efficacy. The data presented in the present study can be helpful in developing pharmacopoeial standards of *E. neriifolia* stem.

Index Terms **- Aflatoxins, HPTLC fingerprinting, Microbial load, Pesticide residues, Physico-chemical analysis**

1. INTRODUCTION

The drug consists of fresh thorny stem pieces of *Euphorbia neriifolia* L. commonly known as 'Thuhar' belongs to Euphorbiaceae family. This plant is a large branched erect, glabrous, succulent xerophytic shrub reaching upto 6.5 m. this plant commonly found in wildly on rocky places throughout central India (Fig-A-B). It is a cactus like plant originated from South Asia and normally grows around dry, rocky and hilly areas of India, in Myanmar, Thailand and Malaysia. Besides, this is herbal drug origin plant, which has been a part of traditional healthcare in most parts of the world since thousands of years. The specific name of neriifolia means 'Leaves like an oleander'. The genus Euphorbia has more than 1500 species are widely distributed in the world ranging from annual weeds to trees. Generally, *E. neriifolia* is an herb covering full of spine and popularly known as 'Sehund' or Thuhar'. It is also called milk hedge in English. Whereas, in Unani classical literature there are many variety of thuhar is mentioned such as danda thuhar, nadhara thuhar, chaudhara thuhar, unglia thuhar, nagfhani thuhar etc.

E. neriifolia contain plenty of latex having purgetive, diuretic, antiasthamatic, expectorant properties. Due to its diverse medicinal properties it is used in cure of many disorders like ascites, polyuria, scabies, and ulcers in traditional system of medicine. Furthermore, the milky juice exudate from injured fleshy stem is commonly used in Ayurveda medicine as drastic cathartic and to relieve earache. Stem is roasted in ashes and the juice with honey and borex is given in small doses to promote expectoration of phlegm. Pulp of the stem mixed with ginger is used to prevent hydrophobia. Also, traditional use of *E. neriifolia* for curing many diseases has a long history as effectively been employed for the treatment of various ailments like hudar (rheumatism), irqunnisa (sciatica), niqrous (gout), warm-e-shobatein (bronchitis), warm-e-tihal, waja-ul-uzn (otalgia), iltehab (inflammatory conditions), zeeq-un-nafs (asthma), bars (leucoderma) etc. [1,6, 10,11,12,14,15].

Now a days, application of herbal drugs has been increased manifolds in last few decades not yet only in Asian countries but also in other parts of the world. In the early 20th century, there was limited scientific data available on herbal drugs. Later on, the standardization of plant material or crude drugs became an essential practice to check their purity and authenticity. Given this, a number of modern tools and techniques are employed for standardization of herbal drugs. Therefore, the present study was aims to develop quality parameters and evaluate the data to lay down the pharmacopoeial standards specially for Thuhar stem. Besides this, many conventional parameters such as macroscopy, microscopy, powder study, physico-chemical evaluations (i.e. water and alcohol soluble extractives, total ash, acid insoluble ash, pH values) along with HPTLC fingerprinting were carried out. The quality control parameters such as heavy metals estimation, pesticide estimation, microbial load and aflatoxins were also analysed in order to assess the quality of single drug. [17, 18]

2. MATERIALS AND METHODS

2.1 Drug procurement and authentication

The raw drug was procured from herbal garden P-block society, sanjay nagar Ghaziabad and identified by the botanist using pharmacognostical method. After authentication, some stem samples were washed thoroughly with clean water and dried under a gentle stream of air in the laboratory till no loss in weight (temperature 30 C) and powdered in an electric grinder. [2]

2.2 Pharmacognostic analysis

The dried stem were subjected to macroscopic studies as per approved format of Ayurvedic Pharmacopoeia of India and evaluated systematically .Thin transverse section were taken from stem stained with safranin and mounted in glycerine by following the microtechnique method.Microphotography was performed for the drug. [7,9]

2.3 Physico-chemical analysis

The physico-chemical parameters such as moisture content, water and ethanol extractive values, ash values, pH values were analyzed as per standard methods [5].

2.4 HPTLC fingerprinting

The drug samples (2g each) were extracted separately with 25 ml each of chloroform and ethanol by sonicating for 20 minutes and filtered. The extracts were concentrated; made up to 10ml in volumetric flasks and used for HPTLC (High Performance Thin Layer Chromatography) fingerprinting. 10 µl of each extract was applied on aluminum TLC plate pre-coated with silica gel 60 F254 (E. Merck) by employing CAMAG Linomat IV automatic sample applicator. The plate was developed up to a distance of 9cm in twin trough glass chamber (10x10), using 10ml of the solvent system Toluene: Ethyl acetate: Formic acid (9:1:0.5) as mobile phase. The plate was air-dried at room temperature and observed under UV at the wavelength 254nm and 366nm. Further the plate was dipped in 1% vanillin-sulphuric acid reagent and heated at 105o C till coloured bands appeared. The plate was finally examined under visible light [16, 17,19].

2.5 Quality control analysis

The herbal materials are being used worldwide as effective remedies since they exhibit less adverse effects. People's faith in herbal products leads to concern over their quality check. Therefore, the different quality control parameters like microbial load, heavy metals and pesticide residues were carried out to asess the quality of the drug Thuhar. Estimation of microbial load was conducted as per standard methods [1]. Heavy metal analysis was also carried out as per standard method by using Atomic Absorption Spectrophotometer (LABINDIA). Pesticide residues were analyzed using Triple Quadrupole GC-MS/MS system (Thermo) equipped with mass selective detector as per standard methods. [3,4,5]

3. RESULTS

3.1 Macroscopic characters

Stem cylindrical, succulent, glabrous, five angled branches bearing short, stipular thorns, more or less confluent in vertical or slightly spiral lines, occasionally its surface is white marbled, on indentation white latex comes out, dark green when fresh. odor disagreeable and taste astringent (Fig. 1. A-B).

Fig. 1 (A-B). Morphology of *Euphorbia neriifolia* L. A.Plant; B.Stem

3.2 Microscopic characters

Transverse section (T.S.) of stem shows a single layered epidermis composed of squarish thin walled parenchymatous cells, followed by the cortex, differentiated into two parts, outer thin walled rectangular parenchymatous cells, inner wide zone, consisting of thin walled ovoid, elongated cells having a number of rounded to oval latex cells (lactiferous cells). Below cortex, about ten layers of phloem present, containing group of fibres, xylem consists of vessels, tracheid, fibres and xylem parenchyma. Pith consists of thin walled, rounded or oval, parenchymatous cells, starch and calcium oxalate crystals absent (Fig. 2 A-F).

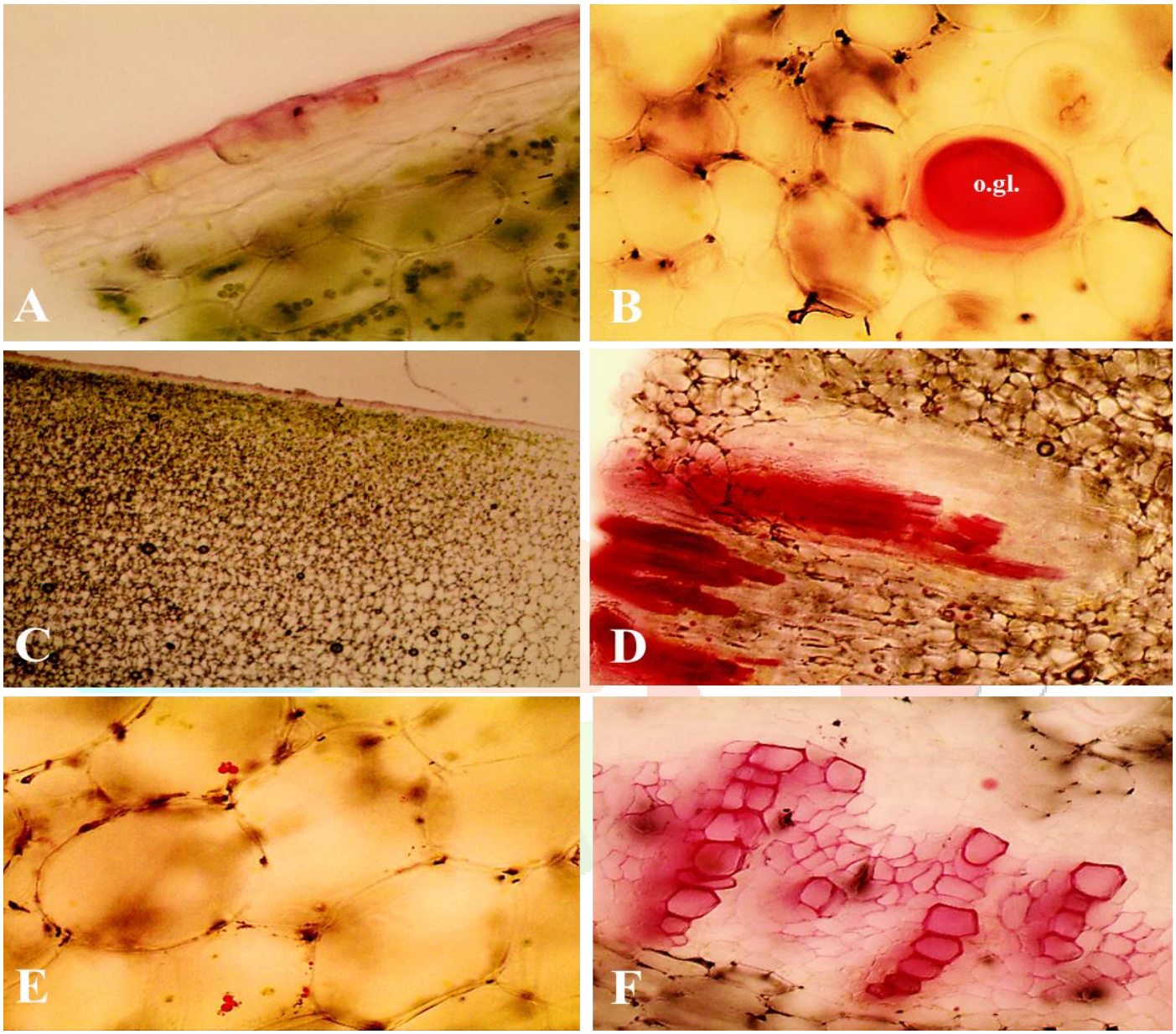
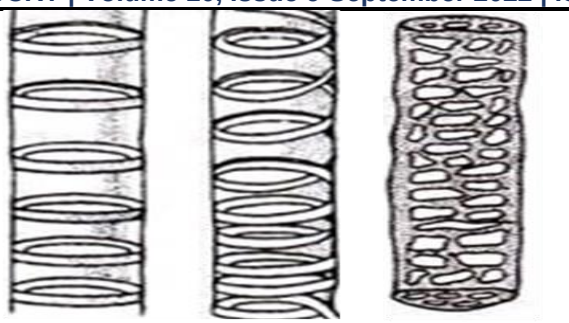


Fig. 2 (A-B). Microscopic characters of *Euphorbia neriifolia* L. (A) Epidermal cells and Chlorenchyma cells, 40x; (B) Parenchyma cells with oil glands, 40x; (C) L.S. of stem, 4x; (D) Laticiferous cells, 10x; (E) Parenchyma cells containing starch grains, 40x; (F) Vascular bundle exhibiting medullary rays, phloem cells and cambium cells, 20x.

3.3 Powder microscopy and organoleptic characters

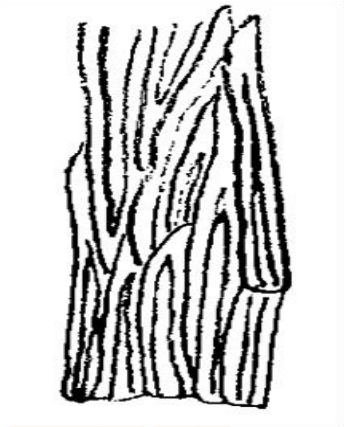
Greenish brown; odor not characteristic; taste acrid; under microscope it shows following characters- rectangular thick walled epidermal cells in surface view; fragments of vessels (annular, spiral and reticulate type); thick walled fibres; group of fibres; parenchyma cells with starch grains; lactiferous vessels embedded with latex ; thick walled stone cells ; epidermis with actinocytic type of stomata (in surface view) (Fig. 3).

Epidermal cells

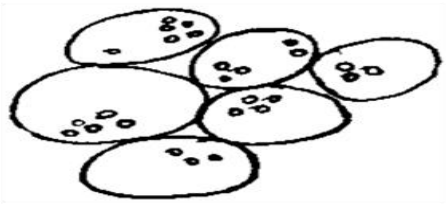


Annular Spiral Reticulate Vessels

Fibre



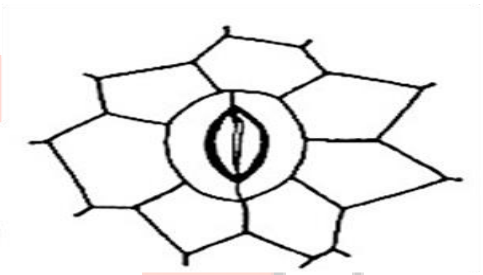
Group of fibres



Parenchyma cells with starch grains

Laticifers

Stone cells



Epidermis with stomata (in surface view)

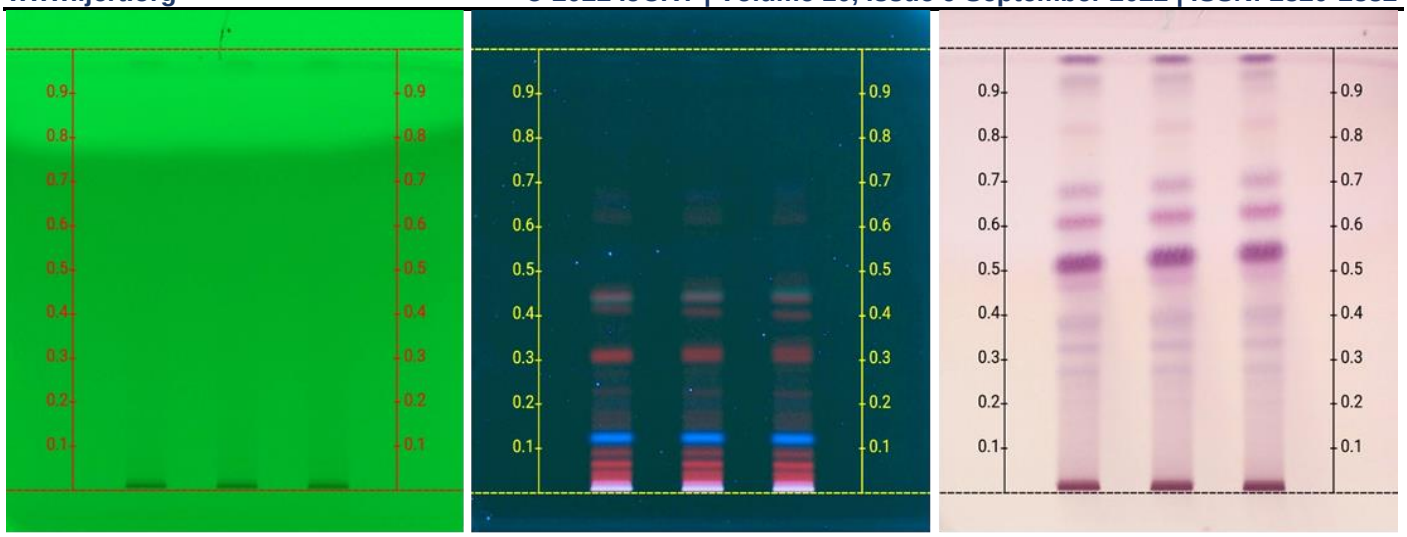
3.4 Physicochemical analysis

The physicochemical analysis such as extractive values, ash values and foreign matter were analysed (Table 1).

S.No.	Parameters	Results
1.	Ethanol extractive matter (w/w %)	6.25 – 7.12
2.	Water extractive matter (w/w %)	16.50 – 17.65
3.	Loss in weight on drying at 105^0C (w/w %)	6.55 – 7.30
4.	Total ash (w/w %)	3.20 – 3.85
5.	Acid insoluble ash (w/w %)	0.62 – 0.75
6.	pH of 1% aqueous solution	5.30 – 5.50
7.	pH of 10% aqueous solution	5.72 – 5.85

3.4 Physicochemical analysis

HPTLC fingerprinting is a sensitive and dependable technique for identification of crude drugs of plant origin. HPTLC images of both the extracts of Thuhar was observed under UV 254nm, UV 366nm and under visible light after derivatization. The three samples of Thuhar show similar colourful bands with similar Rf values. (Fig. 4 and 5).



UV 254 nm
Q

UV 366 nm
R

Visible light
(After derivatization)
S

Fig. 4 (Q-S). HPTLC fingerprint with chloroform extracts

3.5 Quality Control Parameters

3.5.1 *Microbial load*

Determination of microbial growth is essential parameter in traditional medicines. It implies whether the herbal material has spoilage micro-organisms in permissible limits. The assessment is done for evaluating the total aerobic bacterial count (TABC), total yeast and molds count (TYMC), Enterobacteriaceae members and Specific objectionable pathogens. The results of microbial load are shown in Table 1 which indicate that the drug sample is safe for internal use (Table. 2).

Table 2. Microbial load analysis

S. No.	Microbial Studies	Results
1.	Total aerobic bacterial Count (TABC)	1.2×10^3 CFU/gm
2.	Total yeast and molds count (TYMC)	2.2×10^2 CFU/gm
3.	**Enterobacteriaceae members**	
	Escherichia coli	ND
	Salmonella sp.	ND
	Shigella sp.	ND
	Klebsiella sp.	ND
4.	**Specific objectionable pathogens**	
	Pseudomonas aeruginosa	ND
	Staphylococcus aureus	ND
	Candida albicans	ND

3.5.1 *Heavy metal analysis*

The results of Heavy metal estimation are given in Table 2. A heavy metal has relatively high density or atomic weight and is toxic to human health even at low concentrations. The heavy metal content in Thuhar sample was found to be below detection limit which indicated that the drug was free from any heavy metal contamination (Table 3).

3.5.2 *Pesticide residues*

The results of pesticide residues are given in Table 4. It is very difficult nowadays to grow agriculture produce without the use of pesticides but as per WHO guidelines, the pesticide residues in herbal drugs must be in permissible limits. In order to estimate the pesticide residue, the drug was analyzed on GC-MS/MS system. The results indicated that the drug is free of pesticide residues and safe for use (Table-4).

Table 3. Heavy metals analysis

S. No.	Parameter Analyzed	Results	WHO Permissible limit (ppm)
1.	Lead	Not detected	10.00
2.	cadmium	Not detected	0.30
3.	Arsenic	Not detected	3.00
4.	Mercury	Not detected	1.00

Table 4. Pesticide residue estimation

S. No.	Name of Pesticide	Result (mg/Kg)	Permissible limit (mg/Kg)
1.	Alachlor	BLQ	0.02
2.	Aldrin (Aldrin and dieldrin combined expressed as dieldrin)	BLQ	0.05
3.	Azinophos-methyl	BLQ	1.0
4.	Bromopropylate	BLQ	3.0
5.	Chlordane (cis, tans and oxychlordane)	BLQ	0.05
6.	Chlorfenvinphos	BLQ	0.5
7.	Chlorpyrifos	BLQ	0.2
8.	Chlorpyrifos-methyl	BLQ	0.1
9.	Cypermethrin (and isomers)	BLQ	1.0
10.	DDT (all isomers, sum of p,p'-TDE (DDD) expressed as DDT)	BLQ	1.0
11.	Deltamethrin	BLQ	0.5
12.	Diazion	BLQ	0.5
13.	Dichlorvos	BLQ	1.0
14.	Dithiocarbamates (as CS2)	BLQ	2.0
15.	Endosulphan (sum of isomers & Endosulphan sulphate)	BLQ	3.0
16.	Endrin	BLQ	0.05
17.	Ethion	BLQ	2.0
18.	Fenitrothion	BLQ	0.5
19.	Fenvalerate	BLQ	1.5
20.	Fonofos	BLQ	0.05
21.	Heptachlor (sum of Heptachlor & Heptachlor epoxide)	BLQ	0.05
22.	Hexachlorobenzene	BLQ	0.1
23.	Hexachlorocyclohexane isomer (other than γ)	BLQ	0.3
24.	Lindale (γ – Hexachlorocyclohexane)	BLQ	0.6
25.	Malathion	BLQ	1.0
26.	Methidathion	BLQ	0.2
27.	Parathion	BLQ	0.5
28.	Parathion methyl	BLQ	02
29.	Permethrin	BLQ	1.0
30.	Phosalone	BLQ	0.1
31.	Piperonyl butoxide	BLQ	3.0
32.	Pirimiphos methyl	BLQ	4.0
33.	Pyrethrins (sum of isomers)	BLQ	3.0
34.	Quintozen (sum of Quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	BLQ	1.0

*BLQ-Below Limit of Quantification

3.6 Therapeutic uses

Different parts of plant are useful in cure of many diseases such as skin disease, leucoderma,eczema,anaemia, fever, bronchitis, piles, loss of appetite, ulcers, cough, colitis, whooping cough, paralysis, sciatica etc. [8, 10, 13].

4. DISCUSSION

The study sets the specific macro-microscopic, physicochemical evaluations, HPTLC protocols to establish identity of the crude drug of stem of *Euphorbia neriifolia* and also standardize the Unani, Siddha and Ayurveda formulation containing as an ingredient. The quality control parameters such as heavy metals estimation, pesticide estimation, microbial load and aflatoxins were also analysed in order to assess the quality of the formulation.

5. CONCLUSION

Currently there is limited scientific evidence for the safety, efficacy and authenticity of traditional medicines which usually does not provide standards for assertive identification. Thus, standardization is vital for evaluation of the quality of traditional medicines. *Euphorbia neriifolia* (Thuhar) was evaluated through pharmacopoeial parameters which certainly provide an assurance of quality of the drug. All the microscopical, physicochemical parameters, quality control parameters viz. heavy metals, microbial load, aflatoxins and pesticidal residue were found within permissible limits which conspicuously show that the drug *Euphorbia neriifolia* (Thuhar) in free from harmful toxins and can be used safely. Hence, the present study contains high significance to ensure authenticity and quality of the drug.

6. ACKNOWLEDGEMENT

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