Pharmacogn J. 2017; 9(5):696-705
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

Pharmacognostical and Physico-chemical Standardization of *Euphorbia neriifolia* Leaves

Prashant Y. Mali* and Shital S. Panchal

ABSTRACT

Objective: To investigate pharmacognostical and physico-chemical standardization of *Euphorbia neriifolia* leaves. **Materials and Methods:** Fresh and dried leaves with powder samples of *E. neriifolia* were examined macroscopically and microscopically. As per Ayurvedic Pharmacopeia of India and World Health Organization guidelines on quality control methods for medicinal plants materials suggested parameters were determined for standardization of *E. neriifolia* leaves. Physico-chemical, primary phytochemical, fluorescence and quantitative screenings along with primary HPTLC fingerprinting assessment were performed. **Results:** Macroscopic examination demonstrated that fresh leaf of *E. neriifolia* has dark green in colour, herbaceous odour with characteristic taste. Dried leafs are grey brownish in colour, characteristic odour with broken crumpled and papery fracture. Microscopy of leaf showed the single layered thick rectangular or tubular adaxial epidermal cells. Mesophyll tissue was differentiated into two or three layered adaxial zones of radially elongated palisade cells and wider abaxial spongy mesophyll cells revealed the differentiated dorsiventral lamina. Mid-rib composed of epidermis, collenchymas and spongy parenchyma cells. Physico-chemical parameters like, foreign matter was found to be 0.46%. Total ash, acid insoluble ash and water soluble ash was found 6.33%, 1.23% and 6% respectively. Loss on drying was found to be 4.69%. Swelling and foaming index was found 11.7 ml and 333 ml respectively. Quantitative screening suggested that the leaf powder has indicated alkaloid and saponin estimation as 0.26% and 3.67% respectively. The HPTLC fingerprinting of EN6 extract fraction was showed the Rf values at 254 nm with their respective UV-visible spectrum wavelengths scanned in between 200-400 nm. They are 0.01 (265 nm), 0.05 (369 nm), 0.09 (263 nm, 264 nm), 0.18 (400 nm), 0.20 (279 nm), 0.31 (400 nm), 0.44 (378 nm), 0.45 (382 nm), 0.54 (377 nm), 0.55 (383 nm), 0.62 (400 nm), etc. at different concentrations of sample application. The HPTLC plate was also scanned at 366 nm and 540 nm. **Conclusion:** The present investigation is an additional standardization research in support with previous reports and will be helpful for qualitative and quantitative standardization of herbal formulations containing *E. neriifolia*. Further investigations are going on this extract fraction in reference to identification, quantification and validation of HPTLC methods using various standard marker compounds along with exploration of its pharmacological activities.

Key words: *Euphorbia neriifolia*, Pharmacognostical, Physico-chemical, Macroscopic, HPTLC.

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History

- Submission Date: 31-05-2017;
- Review completed: 18-06-2017;
- Accepted Date: 28-06-2017

DOI : 10.5530/pj.2017.5.110

Article Available online
http://www.phcogj.com/v9/i5

INTRODUCTION

Euphorbia neriifolia Linn. Sp. Pl. (451.1753) belonging to the family Euphorbiaceae which consisting about 05 sub-families, 49 tribes, 317 genera, 800 species and one of the most important families of flowering plants of immense importance.[1,2] Investigations has shown that some Euphorbiaceae has really potent therapeutic plants and their extracts have been patented as contemporary drugs.[3,4] About half dozen species of Euphorbia genus are under the name of snuk and its synonyms. The latex of *E. nerrifolia* is an active ingredient of many Ayurvedic formulations like Abhaya lavana, Avittoladi bhasma, Citrakadi taila, Jatyadi varti, Snuhidugdhadi varti, Snuhi ghrta, Jalodarari ras, etc. *E. neriifolia* has been traditionally indicated in Vatavyadhi, Gulma, Udara, Sula, Sotha, Arsas, Kusta and Medoroga.[5,6] *E. neriifolia* have to possess anaesthetic,[7] analgesic,[8,9] anti-anxiety / anti-convulsant / anti-psychotic,[10] anti-arthritis, [11] anti-carcinogenic / renal carcinogenesis / hepatocarcinogenesis,[12-15] antidiabetic,[16] anti-inflammatory,[8,9,17,18] antioxidant,[19,20] cytotoxicity,[21,22] death-receptor expression enhancing activity,[23] immunomodulatory,[24,25] etc. properties. It is also reported to have neriifolin-S,[26] neriifolin,[27] neriifoliene,[28] euphol,[29] neriifolione and cycloartenol,[30] nerifoliol,[31] lectin,[32] euphonerins A–G and 3-O-acetyl-8-O-tigloylingol,[33] taraxerol,[34] quercetin and rutin,[35] and antiquorin,[36] etc. phyto-compounds.

India has a glorious tradition of usage of therapeutic preparations from natural herbs which have protecting and healing properties with negligible side effects.[37] Due to emerging interest 80% of world's population is

Cite this article : Mali PY and Panchal SS. Pharmacognostical and Physico-chemical Standardization of *Euphorbia neriifolia* Leaves. Pharmacog J. 2017;9(5):696-705.

adopting traditional medicine. Government of India has initiated several attempts to explore ethanopharmacology and traditional uses of plant for evaluation of their therapeutic potential as well as help to generate data to put these botanicals in international market of public healthcare domain. [38,39] A noticeably small amount of profitable phytochemical entities have entered on facts based therapeutics but hard works are still required to recognize bioactive molecules in herbal drugs.[40] The present investigation provides outline of up-to-date information on pharmacognostical and physico-chemical standardization of *E. neriifolia* leaves.

MATERIALS AND METHODS

Instruments and chemicals

Leica photographic microscope (Leica Microsystems, Mumbai, DM-3000), U. V. chamber (Dolphin, Mumbai), Afcoset digital balance (Afcoset Balances, Mumbai, E-R-180A), analytical grade toluene, ethyl acetate, n-butanol, formic acid (98-100%), acetone (Merck, Mumbai), chloroform, methanol, petroleum ether (40-60°C) (Sd Fine-Chem Ltd, Mumbai), etc. were used for the study.

Authentification of *E. neriifolia* plant and its leaves

Fresh, well grown *E. neriifolia* plant and its leaves were collected from local region of Bhopal, Madhya Pradesh, India in August-September month of 2012 and was authenticated by taxonomist, Dr. Vijay V. Bhadane, Department of Botany, Pratap College, Amalner-425401, Maharashtra, India. Voucher specimen (No. PCA/Bot-P1637) was deposited in the same. The photograph and herbarium of *E. neriifolia* as shown in (Figure 1)

Macroscopic evaluation

Macroscopic evaluation of fresh and dried *E. neriifolia* leaves were studied as per the method of *Brain* and *Turner*.[41] The colour, odour, taste, condition, fracture, texture, size and shape parameters were considered for the evaluation.

Microscopic evaluation

Microscopic evaluation of fresh leaf was performed according to the procedure mentioned in the Ayurvedic Pharmacopoeia of India.[6] The vascular bundle, stomata types, calcium oxalates, xylem, mesophyll and epidermal cells, trichomes, starch grains, etc. were studied. The dried powder of leaf was cleared with sodium hydroxide and mounted in glycerin medium after staining. The various staining reagents such as toluidine blue, safranin, fast green and iodine were used to study calcium oxalates crystals, starch grains, stomata, trichomes, epidermal cells, parenchyma, xylem parenchyma and lignified xylem fibers. The photographs of different magnifications were taken by Leica photographic microscope.

Extraction and fractionation of *E. neriifolia* leaves

Freshly collected *E. neriifolia* leaves were dried in shade. Course powder of dried leaves (300gm) was extracted successively using petroleum ether, toluene, chloroform (Non-polar), ethyl acetate, *n*-butanol and distilled water (Polar) solvents by maceration process of extraction for the period of seven days for solvent with occasional stirring. The macerate mixtures were filtered each two times with muslin cloth separately and thereafter extract fractions obtained were concentrated, dried and designated as EN1 (Petroleum ether), EN2 (Toluene), EN3 (Chloroform), EN4 (Ethyl acetate), EN5 (n-butanol) and EN6 (Aqueous). The percentage yields and consistencies of extract fractions were recorded.

Physico-chemical analysis

The percentage of foreign matter,[42] total ash, acid insoluble ash, water soluble ash,[6] loss on drying,[43] swelling[6] and foaming index[42] were examined.

Primary phytochemical screening

Primary phytochemical screening of extract fractions of *E. neriifolia* leaves were performed to identify the presence of class of phytoconstituents like, alkaloids, amino acids, carbohydrates, flavonoids, glycosides, proteins, saponin, steroids, tannins and phenols. [43,44]

Fluorescence analysis

Dried powder of *E. neriifolia* leaves was studied for fluorescence study as such and besides after treating independently with water, 1 N of HCl, HNO_3, H_2SO_4, NaOH, $KMnO_4$, KOH, alcoholic NaOH and KOH and ammonia using normal and ultra-violet light (254 nm). Colour effect of petroleum ether, toluene, chloroform, ethyl acetate, *n*-butanol and distilled water extract fractions were also observed using normal and ultra-violet light (254 nm).[45,46]

Quantitative assessment of phytoconstituents Alkaloid determination

Alkaloid content was studied with reference to procedure mentioned by *Harbone*.[47]

Saponin determination

Saponin content was determined with reference to procedure mentioned by *Obdoni* and *Ochuko*.[48]

Primary HPTLC fingerprinting analysis

Based on the percentage yield and presence of phyto-constitutents in the different extract fractions, EN6 extract fraction was selective for studied by HPTLC technique for normal phase separation of components.

Preparation of solution of EN6 extract fraction

An accurately weighted 500 mg of EN6 extract fraction and dissolved into a 5 ml of methanol in volumetric flask respectively. It was then sonicated for 20 min. The solution was kept a side for 30 min to settle down the aliquots. The aliquots of stock solution of EN6 extract fraction was transferred to 10 ml volumetric flask and volume of was adjusted with methanol to get the final concentration 100 µg/µl of extract fraction. Further this solution was used for identification of extracted phytocompounds.

Mobile phase used for primary HPTLC studies

Toluene: Chloroform: Ethanol (4:4:2)

Chromatography and HPTLC fingerprinting analysis

A chromatographic study was performed on prewashed and preactivated 20.0 x 10.0 cm aluminum Lichrosphere HPTLC plates pre-coated with silica gel 60 F_{254} of 0.2 mm thickness layer (Merck KGaA, Darmstadt, Germany). Spots of extract fraction EN6 was applied at 2, 4, 6, 8, 10 and 15 µl at application position 8.00 mm with band length 8.00 mm by a CAMAG Linomat-V automatic TLC sample spotter (Camag Muttenz, Switzerland) equipped with a 100 µl syringe (Hamilton) under a continuous drying stream of nitrogen gas at a constant application speed of 150 nl/s. The linear ascending development with the above mobile phase in a 20.0 x 10.0 cm twin trough glass chamber (CAMAG) previously saturated with mobile phase for 15 min at room temperature (25± 2 °C) and relative humidity 40%. The development distance was 80.00 mm (development time 20 min with filter paper) and 20 ml mobile phase was used. After development, the plate was dried with a stream of hot air and densitometric scanning was performed at 254 nm in absorption-reflectance mode and 366 nm in absorption-fluorescence mode by using a CAMAG TLC scanner 3 and CAMAG visualizer with automatic digital camera linked to win-CATS software (Version 1.4.6).

The slit dimension of scanner was set at 6.00 × 0.45 mm (Micro) with 100 µm / step data resolution and 20 mm/s scanning speed. Latter, the plate was dipped in anisaldehyde sulfuric acid derivatising reagent and plate was dried with a stream of hot air, scanned at 540 nm in absorption-reflectance mode with the same software system. The volume of sample applied, color of the resolved band, peak number, R_f value, peak height and peak area were noted. The UV-visible spectra's were also scanned at 254 nm under D_2 lamp with 200-400 nm start and end wavelengths.

RESULTS

Macroscopic evaluation of *E. neriifolia* leaves

The plant is herbaceous. The fresh leaves are dark green in colour with herbaceous odour, characteristics taste, rough fracture, leathery texture with size approximately 2-7 cm wide and 8-20 cm long. The dried leaves are grey brownish in colour with characteristic odour, tasteless, broken crumpled and papery fracture, thin and papery texture with size approximately 1.5 to 6 cm wide and 15-20 cm long. Both fresh and dried leaves are cuneate shape, base is acute and apex is sub-acute. Margins are entire even, smooth throughout with pointed and acute tip. Venation is pinnate, anastomosing near the margin. Veinlets are prominent at lower surface. Lamina is entire and flat with carks. The macroscopic photographs of *E. neriifolia* leaves are shown in (Figure 2).

Microscopic evaluation

T. S. of *E. neriifolia* leaf showed the single layered thick rectangular or tubular adaxial epidermal cells. Single to double layered abaxial epidermis with circular to rectangular epidermal cells. Mesophyll tissue was differentiated into two or three layered adaxial zones of radially elongated palisade cells and wider abaxial spongy mesophyll cells revealed the differentiated dorsiventral lamina. The spongy mesophyll had wide air chambers and partition filaments formed by lobed and interconnected 6-9 layered spongy parenchyma cells. Midrib region slightly rose on the adaxial side whereas broadly semi-circular on the adaxial side. Mid-rib composed of epidermis, collenchymas and spongy parenchyma cells. In certain regions of epidermis, five celled uncreated trichomes with blunted tip and glandular trichomes with bi-celled head were embedded. Vascular bundles are prominent towards the ventral side and covered with endodermis. The vascular bundles consist of lignified xylem and non-lignified phloem. Mesophyll cells were called idioblasts. Spongy parenchyma showed the presence of starch granules. The spongy mesophyll had wide air-chambers. *E. neriifolia* leaf surface showed the anomocytic type of stomata that were covered with guard cells surrounded by 2-3 subsidiary cells followed by polygonal epidermal layers. Adaxial surface contains more stomata in comparison to abaxial surface of leaf. Leaf surface also indicated presence of glandular as well as unicellular blunted trichomes. The microscopic photographs of *E. neriifolia* leaves are shown in (Figure 3).

The microscopic examination of powder of *E. neriifolia* leaves has indicated the presence of numerous idioblastic, rosette, square, prismatic and acicular shaped calcium oxalate crystals and starch grains both simple and compound, well arranged annular vessels, anomocytic stomata, unicerrate multicellular trichome, epidermal cells, spongy parenchyma, xylem parenchyma, starch grains, lignified xylem fibres and oxalate crystals. Microscopic photographs of powder of *E. neriifolia* leaves are shown in (Figure 4).

Extraction and fractionation

The scheme for extraction and fractionation of *E. neriifolia* leaves are shown in (Figure 5). The percentage yields and consistencies of all the extract fractions are shown in (Table 1).

Figure 1: Photograph and herbarium of *E. neriifolia* Linn. (Source: Local region of Bhopal, Madhya Pradesh, India; Aug. 2012)

Physico-chemical analysis

The foreign matter was found 0.46%. Total ash, acid insoluble ash and water soluble ash was found 6.33%, 1.23% and 6% respectively. The moisture content or loss on drying was found to be 4.69%. Swelling and foaming index was found 11.7 ml and 333 ml respectively.

Primary phytochemical screening

The results for primary phytochemical screening of various extract fractions of *E. neriifolia* leaves are shown in (Table 2).

Fluorescence analysis of extract fractions and powder of *E. neriifolia* leaves

The results of fluorescence analysis of extract fractions of leaves as well as powder of *E. neriifolia* are shown in (Table 3) and (Table 4) respectively.

Figure 2: Macroscopic photographs of *E. neriifolia* leaves

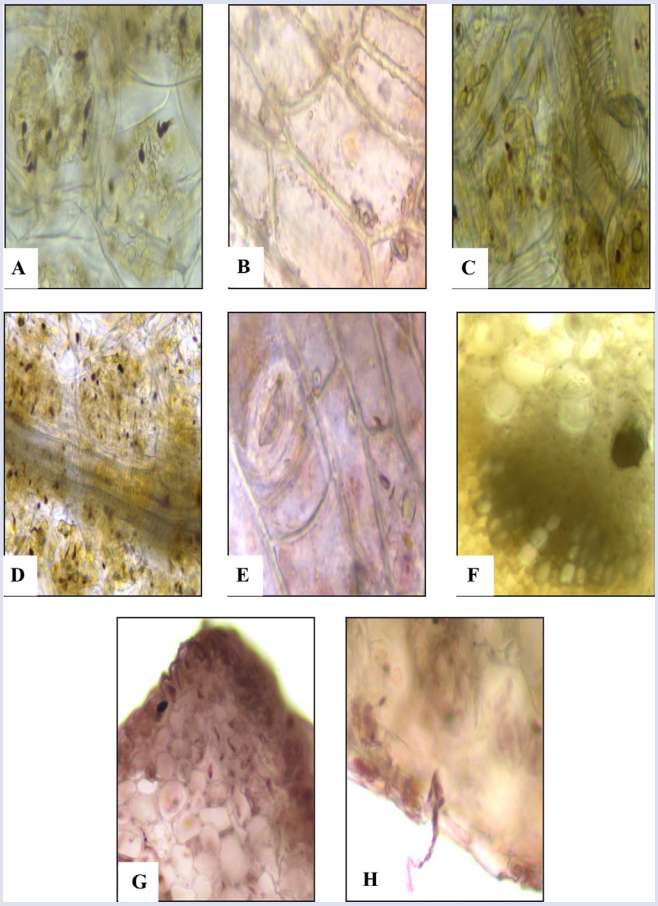


Figure 3: Microscopic photographs of *E. neriifolia* leaves. A: Idioblastic calcium oxalate crystals with simple starch granules; B: Rectagular or tubular adaxial epidermal cells; C: Mesophyll cells and annular thickening; D: Annular xylem tracheids; E: Anisocytic stomata; F: Vascular bundle; G: Lower surface of leaf; H: Trichome

Quantitative assessment of phytoconstituents

Quantitative assessment of phytoconstituents in leaf powder indicated alkaloid and saponin estimation as 0.26% and 3.67% respectively.

Primary HPTLC fingerprinting analysis

The HPTLC fingerprinting of EN6 extract fraction was showed the R_f values at 254 nm with their respective UV-visible spectrum wavelengths scanned in between 200-400 nm. They are 0.01 (265 nm), 0.05 (369 nm), 0.09 (263 nm, 264 nm), 0.18 (400 nm), 0.20 (279 nm), 0.31 (400 nm), 0.44 (378 nm), 0.45 (382 nm), 0.54 (377 nm), 0.55 (383 nm), 0.62 (400 nm), 0.63 (400 nm) and 0.71 (397 nm) at different concentrations of sample application. The HPTLC plate was also scanned at 366 nm and it was showed the different peaks with respective their R_f values 0.05, 0.12, 0.19, 0.27, 0.32, 0.34, 0.33, 0.41, 0.44, 0.45, 0.54, 0.55, 0.70 and 0.77. At 540 nm, the peaks having R_f values like, 0.01, 0.08, 0.09, 0.14, 0.16, 0.17, 0.18, 0.19, 0.24, 0.31, 0.38, 0.48, 0.57, 0.63, 0.64, 0.72 and 0.74 were found. The scanned HPTLC white normal plate, at 254 and 366 nm are shown in (Figure 6) and the volume of sample applied, color of the resolved band, peak number, R_f value, peak height and peak area at 254 nm (Table-5), 366 nm and 540 nm (Table-6).

DISCUSSION AND CONCLUSION

In spite of the accessibility of hyphenated critical techniques, identification and assessment of plant drugs by pharmacognostical and physico-chemical consideration is still more reliable, precise and economical. According to WHO, macroscopic and microscopic evaluation of plant is the first step towards establishing its identity and purity. It must be performed prior to any other tests to be carried out. [43] In line with above statement, we have performed the macroscopic and microscopic studies of fresh and dried leaf along with the powder of *E. neriifolia*. The results of macroscopic studies will be helpful for identifying it from the substitutes or adulterants. Microscopic evaluation of drug helps to categorize the ordered cellular organization of drug substance by their known histological features like epidermal cells, mesophyll cells, vascular bundles, stomata, trichomes, starch grains, etc. The use of a variety of reagents or stains helps to differentiate cellular structure depending on

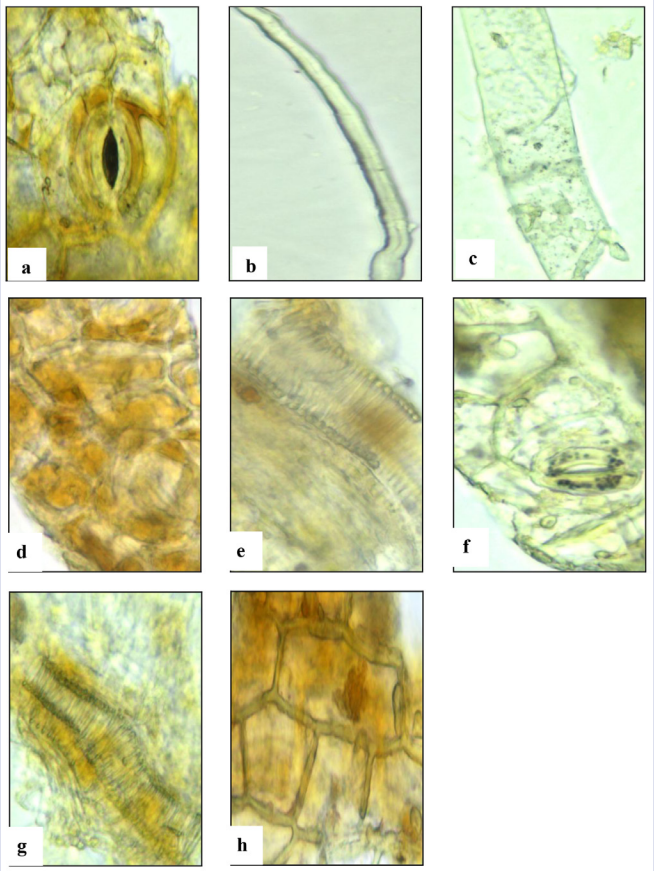


Figure 4: Microscopic photographs of powder of *E. neriifolia* leaves. a: Anisocytic stomata; b: Trichome; c: Starch grains; d: Lignified xylem fibre; e: Annular xylem tracheids; f: Acicular calcium oxalate in a parenchyma cells; g: Annular vessels; h: Epidermal cells

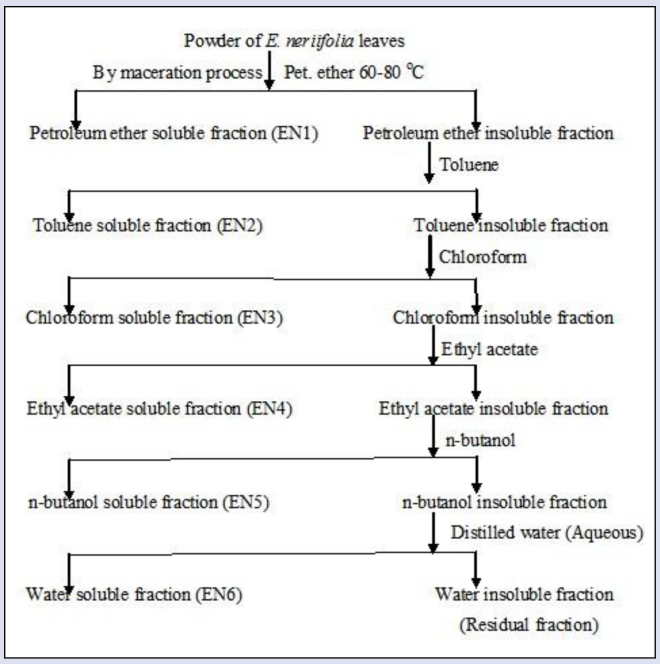


Figure 5: Extraction and fractionation scheme of *E. neriifolia* leaves

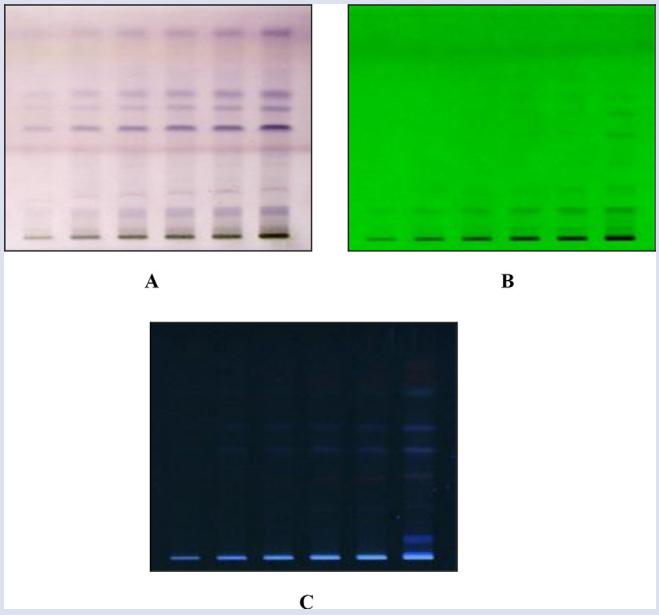


Figure 6: Scanned HPTLC developed plates. A: Scanned HPTLC normal white plate; B: Scanned HPTLC plate at 254nm; C: Scanned HPTLC plate at 366 nm

their chemical character. The results suggest that powder of *E. neriifolia* leaves have higher aqueous extractive value followed by petroleum ether, ethyl acetate, toluene, chloroform and n-butanol. The physico-chemical parameters help in judging the purity and quality of the drug. Foreign matter was present in trace amount due to first hand procurement of plant material from non-polluted location. Ash values are useful to identify the occurrence of any siliceous infectivity and water soluble salts. They are the chief quantitative standards values and useful in shaping accuracy as well as purity of drugs.[48] The results of total ash, acid insoluble ash and water soluble ash were 6.33%, 1.23% and 6%, , respectively. Low level of total ashes is indication of little amount of carbonates, phosphates and silica in the selected plant leaves. There is a chance of presence of non-physiological substances in the crude drugs so total ash value is not always reliable. Therefore, the authentication of acid insoluble ash was also studied. It showed ash in leaves. Loss on drying was found to be 4.69% which signify the significant amount of mucilage in leaves. Percent active chemical constituent is usually expressed on air-dried basis in crude drugs. Moisture content of drug was studied for making the solutions of exact strength. It should be minimizing for preventing the decay of crude drug because of microbial infectivity or chemical alteration. Swelling and foaming index was found in the range of 11.7 ml and 333 due to presence of mucilage in the leaves. Results of primary phytochemical study showed that the presence of a variety of phytocompounds in the leaves which are known to have a choice of therapeutic value for curing disease ailments. EN6, the aqueous extract fraction has shown the presence of amino acids, alkaloids, flavonoids, carbohydrates, proteins, glycosides, saponin, tannins, steroids and phenols. Previous research reports suggested that saponins, flavonoids, tannins, alkaloids and phenols have anti-inflammatory activities whereas flavonoids, glycosides, alkaloids and tannins have hypoglycemic effects.[49,50] Results of fluorescence analysis of extracts of *E. neriifolia* leaves showed its characteristic fluorescent colours in tested inorganic and organic chemicals. The fluorescent phenomenon of drug powder determines the purity and quality of plant material. Fluorescence features exhibited by many chemical constituents which indicate fluorescence at visible in day light. U.V

Table 1: Percentage yields and consistencies of extracts of *E. neriifolia* leaves

Parameters	Extracts					
	Pet. ether	Toluene	Chloroform	Ethyl acetate	n-butanol	Aqueous
% yields (Codes, w/w)	7.6 % (EN1)	1.19 % (EN2)	0.56 % (EN3)	2.36 % (EN4)	0.26 % (EN5)	8.5 % (EN6)
Consistencies	Crystals	Crystals	Viscous	Viscous	Viscous	Viscous

Table 2: Preliminary phytochemical studies of extracts of *E. neriifolia* leaves

Tests	EN1	EN2	EN3	EN4	EN5	EN6
Alkaloids						
Dragendorff's test	-	+	+	-	+	+
Wagner's test	+	+	-	+	-	+
Amino acids						
Ninhydrin test	-	-	-	-	+	+
Test for cysteine	-	-	-	+	-	+
Carbohydrates						
Molisch's test	+	+	-	-	+	+
Fehling's test	-	-	+	-	-	+
Iodine test	-	-	-	+	-	-
Flavonoids						
Test with conc. H_2SO_4 & Mg (A)	+	+	-	+	-	+
Test with aq. NaOH (B)	-	-	-	-	+	+
Test with conc. H_2SO_4 (C)	-	-	+	-	+	-
Shinoda test	+	+	-	+	-	+
Pew's test	-	+	-	-	-	+
Glycosides						
Legal's test	+	-	+	-	-	+
Killer –Killani test	-	+	-	+	+	+
Proteins						
Biuret test	-	-	+	+	-	+
Million's test	-	-	-	-	+	+
Precipitation test	-	-	-	+	-	+
Saponin						
Foam test	+	+	+	-	+	+
Molisch's test	+	+	-	+	-	+
Steroids						
Salkowski test	+	+	-	-	+	+
Libermann-Burchard test	-	-	+	-	-	+
Tannins & phenols						
Phenol test	+	+	+	-	-	+
Test with 5% of $FeCl_3$ (A)	-	-	-	+	+	+
Test with lead acetate (B)	+	+	+	+	-	-
Test with acetic acid (C)	-	-	-	-	+	+
Test with dilute iodine (D)	-	-	+	+	-	+
Test with dilute $KMnO_4$ (E)	-	-	-	-	-	+

Where, EN1- Petroleum ether; EN2-Toluene; EN3-Chloroform; EN4-Ethyl acetate; EN5-n-butanol; EN6-Aqueous; "+" = Presence of constituents; "-" = Absence of constituents

Table 3: Colour reactions of extracts of *E. neriifolia* leaves in normal and U.V. light

Observation Under	Extracts					
	EN1	EN2	EN3	EN4	EN5	EN6
Normal / Day Light	Gold	Olive	Peru	Yellowish gray	Burly wood	Dark slate gray
U.V. Light (254 nm)	Yellow	Golden rod	Olive drab	Olive	Yellow green	Yellowish dark slate gray

Where, EN1- Petroleum ether; EN2-Toluene; EN3-Chloroform; EN4-Ethyl acetate; EN5-n-butanol; EN6-Aqueous

Table 4: Fluorescence analysis of powder of *E. neriifolia* leaves in normal & U.V. light

Powder + Solvents	Observation	
	Normal / Day Light	U.V. Light (254 nm)
Dry powder	Grayish brown	Grayish brown
Powder + water	Light yellow	Yellowish green
Powder + HCl	Faint yellow	Pale green
Powder + HNO_3	Golden	Pale golden
Powder + H_2SO_4	Pale golden	Pale golden
Powder + NaOH	Yellowish brown	Grayish brown
Powder + $KMnO_4$	Dark brown	Reddish brown
Powder + KOH	Pale brown	Grayish green
Powder + Alc. NaOH	Faint yellow	Yellowish brown
Powder + Alc. KOH	Pale brown	Yellowish green
Powder + Ammonia	Pale yellow	Yellowish green

Table 5: HPTLC fingerprinting and spectrum scan of EN6 extract fraction at 254 nm

Volume applied	Color of band	Peak No.	R_f value	Peak height	Peak area	Spectrum scan (200-400 nm)
2 µl	Dark green	1	0.10	29.0	519.9	--
		2	0.19	13.9	290.8	
4 µl	Light green	1	0.01	13.4	136.9	--
		2	0.10	52.0	987.2	
		3	0.18	18.7	492.9	
		4	0.46	13.6	278.7	
		5	0.55	14.6	314.3	
6 µl	Yellowish green	1	0.01	21.4	210.5	--
		2	0.10	71.6	1437.6	
		3	0.19	29.0	663.7	
		4	0.46	15.8	338.9	
		5	0.55	17.9	411.2	
		6	0.63	13.5	248.0	
8 µl	Yellow	1	0.01	30.3	309.1	--
		2	0.10	89.6	1774.2	
		3	0.18	34.4	817.7	
		4	0.31	10.4	154.2	
		5	0.45	22.1	387.3	
		6	0.55	22.5	520.8	
		7	0.63	15.6	270.0	
10 µl	Light orange	1	0.01	34.6	365.9	265
		2	0.09	104.0	2092.0	263
		3	0.18	41.1	906.7	400
		4	0.31	11.0	205.9	400
		5	0.45	27.8	569.0	382
		6	0.55	26.4	564.0	383
		7	0.63	19.2	356.8	400
15 µl	Orange	1	0.01	42.5	441.6	265
		2	0.05	12.1	164.0	369
		3	0.09	125.2	2663.6	264
		4	0.20	55.7	1244.3	279
		5	0.31	21.6	412.9	400
		6	0.44	79.2	1627.6	378
		7	0.54	73.0	1615.1	377
		8	0.62	28.5	512.4	400
		9	0.71	11.1	278.7	397

Table 6: HPTLC fingerprinting of EN6 extract fraction at 366 nm and 540 nm

Volume applied	Color of band	Scanned at 366 nm				Color of band	Scanned at 540 nm			
		Peak No.	R_f value	Peak height	Peak area		Peak No.	R_f value	Peak height	Peak area
2 µl	Dark green	1	0.34	15.2	97.1	Dark green	1	0.09	20.8	654.7
		2	0.45	105.9	1054.7		2	0.14	22.5	310.5
							3	0.38	68.5	3032.0
							4	0.48	88.8	2488.3
							5	0.57	37.3	1133.9
							6	0.64	34.9	1132.1
4 µl	Light green	1	0.45	6.9	173.1	Light green	1	0.08	36.8	1144.9
		2	0.55	6.1	106.1		2	0.16	36.7	673.2
							3	0.38	70.8	3169.1
							4	0.48	136.4	3262.5
							5	0.57	53.7	1367.8
							6	0.64	57.4	1749.9
6 µl	Yellowish green	1	0.45	10.3	244.6	Yellowish green	1	0.01	12.8	120.0
		2	0.55	8.3	179.7		2	0.08	52.7	1612.5
		3	0.77	6.0	40.8		3	0.17	46.3	757.0
							4	0.38	72.3	3037.2
							5	0.48	173.1	3953.9
							6	0.57	68.4	1753.1
							7	0.64	75.6	2218.6
8 µl	Yellow	1	0.05	6.8	35.0	Yellow	1	0.01	12.3	142.9
		2	0.45	14.7	362.0		2	0.09	63.7	1951.0
		3	0.55	11.5	250.1		3	0.18	50.6	788.0
							4	0.31	11.8	201.1
							5	0.38	77.9	3373.7
							6	0.48	201.6	4692.7
							7	0.57	81.7	2078.7
							8	0.64	91.3	2626.0
10 µl	Light orange	1	0.32	5.9	109.7	Light orange	1	0.01	11.1	150.3
		2	0.45	19.0	479.8		2	0.09	78.7	2312.7
		3	0.55	15.2	319.5		3	0.18	63.2	918.2
							4	0.31	13.0	248.0
							5	0.38	84.3	3423.8
							6	0.48	222.6	5108.5
							7	0.57	94.7	2289.2
							8	0.64	104.3	3039.7
							9	0.74	12.1	403.7
15 µl	Orange	1	0.05	78.8	1682.0	Orange	1	0.08	102.9	3023.1
		2	0.12	7.9	39.1		2	0.19	58.0	985.1
		3	0.19	8.0	108.7		3	0.24	13.6	237.5
		4	0.27	7.2	36.7		4	0.31	19.2	341.4
		5	0.33	19.9	358.7		5	0.38	97.7	4245.0
		6	0.41	12.8	155.8		6	0.48	268.8	6342.9
		7	0.44	40.6	855.7		7	0.57	125.4	3671.6
		8	0.54	39.1	779.4		8	0.63	131.7	3974.1
		9	0.70	21.0	561.6		9	0.72	17.5	342.9
							10	0.74	18.0	303.9

light shows fluorescence in various herbal drugs. As per literature report, alkaloid such as berberine is not visible in day light. It is due to decomposition of drugs due to fluorescent derivatives after treating with various chemical reagents.[51] Results of quantitative estimation of phytoconstituents indicates that *E. neriifolia* leaves have more percentage of saponin content (3.67%) than alkaloid content (0.26%). Hence, quantitative and fluorescence standard of powder provides valuable information to substantiate and authenticate the phytomedicine. Primary HPTLC fingerprinting analysis results showed there is a presence of huge number of phytoconstituents with respect to their Rf values and UV-visible spectra's (200-400 nm) scanned at 254 nm, 366 nm and after derivatization at 540 nm. These phytoconstituents may includes, flavonoids, terpenes, steroidal saponins, alkaloids, glycosides, tannins and phenolic acids, etc. with promising biological actions which can be utilized to expand prospective drugs. Based on the results, there is a recommendation to co-relate these phytoconstituents present in the EN6 extract fraction with various standard marker compounds for its identification, confirmation and validation by analytical research. Similarly, for other chemical constituent's isolation technique are required to be developed. Based on reported biological activities *E. neriifolia* extracts and isolates can be explored for their therapeutic potential by use of modern assay methods.[52]

The present investigation is an additional standardization research in support with previous reports and will be helpful for qualitative and quantitative standardization of herbal formulations containing *E. neriifolia* as *E. neriifolia* leaves might be utilized as a potential therapeutic agent. Further investigations are going on this extract fraction in reference to identification, quantification and validation of HPTLC methods using various standard marker compounds along with exploration of its pharmacological activities.

ACKNOWLEDGEMENT

Authors are grateful to Scientist, Botany, Vindhya Herbal Testing and Research Laboratory, MFP-PARC, Barkheda Pathani, Bhopal and Anchrom Test Lab Pvt. Ltd., Mumbai for availing the necessary facilities to perform this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

ABBREVIATION USED

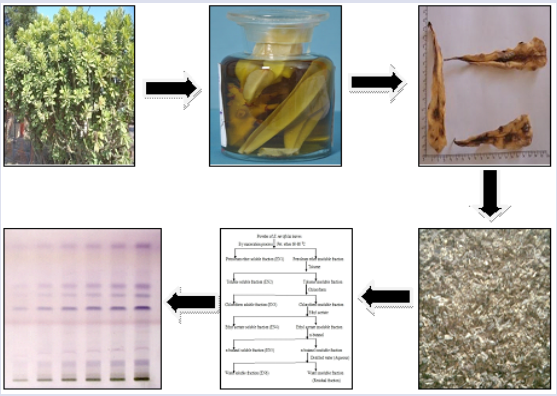
E. neriifolia: *Euphorbia neriifolia* Linn.; **HPTLC:** High performance thin layer chromatography; **NaOH:** Sodium hydroxide; **KMnO4:** Potassium permagnet; **H2SO4:** Suphuric acid; **KOH:** Pottasium hydroxide.

REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed., Vol. III. Allahabad: Lalit Mohan Basu; 2006. p. 2201-2204.
2. Webster GL. Classification of the Euphorbiaceae. Ann Mo Bot Gard. 1994; 81: 03-32.
3. Mwine JT, Van Damme P. Why do Euphorbiaceae tick as medicinal plants? A review of Euphorbiaceae family and its medicinal features. Journal of Medicinal Plants Research. 2011;5(5):652-62.
4. Pickard WF. Laticifers and secretory ducts: two other tube systems in plants. New Phytologist. 2008;177(4):877-88.
5. Chunekar KC. Illustrated Dravyaguna Vijnana. 2nd ed., Vol. II. Varanasi: Chaukhambha Orientalia; 2005. p. 924-925.
6. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. I, 1st Ed., Controller of Publications, Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homoeopathy, Government of India. New Delhi: National Institute of Science Communication (CSIR); 2001.
7. Ng AS. Diterpenes from *Euphorbia neriifolia*. Phytochemistry. 1990;29(2):662-4.
8. Gaur K, Rana AC, Nema RK, Kori ML, Sharma CS. Anti-inflammatory and analgesic activity of hydro-alcoholic leaves extract of *Euphorbia neriifolia* Linn. Asian journal of pharmaceutical and clinical research. 2009;2(1):26-9.
9. Bigoniya P, Rana AC. Pharmacological screening of *Euphorbia neriifolia* leaf hydroalcoholic extract. Journal of Applied Pharmaceutical Science. 2010;1(2):1-7.
10. Bigoniya P, Rana AC. Psychopharmacological profile of hydro-alcoholic extract of *Euphorbia neriifolia* leaves in mice and rats.
11. Ilyas M, Parveen M, Hasan MHM, Omer AB. A novel triterpene (Neriifolione): A potent anti-inflammatory and antiarthritic agent from *Euphorbia neriifolia*. Hamdard Medicus. 2003;(2):97-102.
12. Bigoniya P, Rana AC. Protective effect of *Euphorbia neriifolia* saponin fraction on CCl4-induced acute hepatotoxicity. Afri J Biotech. 2010; 9(42):7148-56.
13. Sharma V, Janmeda P, Paliwal R, Singh L, Sharma V, Sharma S. Anticarcinogenic potential of *E. neriifolia* leaves against n-nitrosodiethylamine-induced nephrotoxicity in mice. Biochem Cell Arch. 2011;11(2):393-8.
14. Sharma V, Janmeda P. Chemopreventive role of *Euphorbia neriifolia* (Linn) and its isolated flavonoid against n-nitrosodiethylamine-induced renal histopathological damage in male mice. Toxicol Int. 2013;20:101-7.
15. Janmeda P, Sharma V, Singh L, Paliwal R, Sharma S, *et al.* Chemopreventive effect of hydroethanolic extract of *Euphorbia neriifolia* leaves against DENA-induced renal carcinogenesis in mice. Asian Pac J Cancer Prev. 2011;12(3):677-83.
16. Mansuri MI, Patel VM. Anti-diabetic Potential of *Euphorbia neriifolia* Linn. Alloxan Induced Diabetic Rats. Journal of Pharmacy Research. 2012;5(5):2571-3.
17. Bigoniya P, Shukla A, Singh CS. Dermal irritation and sensitization study of *Euphorbia neriifolia* latex and its anti-inflammatory efficacy. Int J Phytomed. 2010; 2: 240-254.
18. Hasan M, Ganeshpurkar A, Bansal D, Dubey N. Protective effect of *Euphorbia neriifolia* extract on experimentally induced thrombosis in murine model. Nigerian Journal of Experimental and Clinical Biosciences. 2014;2(2):86-9
19. Pracheta J, Sharma V, Paliwal R, Sharma SP. *In-vitro* free radical scavenging and antioxidant potential of ethanolic extract of *Euphorbia neriifolia* Linn. Int J Pharm Pharmacet Sci. 2011;3(1):238-42.
20. Bigoniya P, Rana AC. Subacute effect of *Euphorbia neriifolia* Linn. on hematological, biochemical and antioxidant enzyme parameters of rat. Academic Journal of Plant Sciences. 2009;2(4):252-9.
21. Babar RS, Kataware UP, Mali NN, Patil SB, Naikwade NS. *In-vitro* cytotoxicity activity of *Euphorbia hirta*, *Euphorbia tirucalli* and *Euphorbia neriifolia* extract against B16F10 melanoma cell line. Inventi Impact: Ethnopharmacology. 2012;3(3). http://inventi.in/journal/article/impact/3/3174/ethnopharmacology/pi [Last assessed on 2016 Dec 12].
22. Patil SB, Magdum CS. Determination of LC_{50} values of extracts of *Euphorbia hirta* Linn and *Euphorbia neriifolia* Linn. Using brine shrimp lethality assay. Asian J Res Pharm Sci. 2011;1(2):42-3.
23. Kazufumi T, Takafumi N, Tahmina H, Takashi O, Midori AA, Takashi K, Thaworn K. Masami I. Cycloartane triterpenes and ingol diterpenes isolated from *Euphorbia neriifolia* in a screening program for death-receptor expression-enhancing activity. Planta Med. 2012;78:1370–1377.
24. Bigoniya P, Rana AC. Immunomodulatory activity of *Euphorbia neriifolia* leaf hydro-alcoholic extract in rats. Indian Drugs. 2008;45(2):90-7.
25. Gaur K, Rana AC, Chauhan LS, Sharma CS, Nema RK, Kori ML. Investigation of immunomodulatory potential of *Euphorbia neriifolia* Linn. Against betamethasone induced immunosuppression. Int. J. Pharm. Phytochem. Res. 2009;1:8-11.
26. Yadav RP, Patel AK, Jagannadham MV. Neriifolin S, a dimeric serine protease from *Euphorbia neriifolia* Linn.: Purification and biochemical characterisation. Food chemistry. 2012;132(3):1296-304.
27. Yadav RP, Patel AK, Jagannadham MV. Purification and biochemical characterization of a chymotrypsin-like serine protease from *Euphorbia neriifolia* Linn. Process Chem. 2011;46:1654-62.
28. Mallavadhani UV, Satyanarayana KV, Mahapatra A, Sudhakar AV. A new tetracyclic triterpene from the latex of *Euphorbia nerifolia*. Natural product research. 2004; 18(1):33-7.
29. Mallavadhani UV, Satyanarayana KVS, Mahapatra A, Sudhakar AVS, Narasimhan K, *et al.* Development of Diagnostic Microscopic and Chemical Markers of Some *Euphorbia* Latexes. J Integrative Plant Biology. 2006;48(9):1115–21.
30. Ilyas M, Parveen M, Amin KM. Neriifolione, a triterpene from *Euphorbia neriifolia*. Phytochemistry. 1998;48(3):561-3.
31. Roa DN, Row LR. The crystalline principles of Euphorbiaceae. Part III. Curr Sci. 1965;34(14):432.
32. Seshagirirao K, Prasad MN. Purification and partial characterization of a lectin from *Euphorbia neriifolia* latex. Biochemistry and molecular biology international. 1995;35(6):1199-204.
33. Toume K, Nakazawa T, Hoque T, Ohtsuki T, Arai MA, *et al.* Cycloartane triterpenes and ingol diterpenes isolated from *Euphorbia neriifolia* in a screening program for death-receptor expression-enhancing activity. Planta medica. 2012;78(12):1370-7.
34. Anjaneyulu V, Row LR. The crystalline principles of Euphorbiaceae. Part IV. Curr Sci. 1965;21:608-9.
35. Sharma V, Janmeda P. Extraction, isolation and identification of flavonoid from *Euphorbia neriifolia* leaves. Arabian Journal of Chemistry. 2014. dx.doi.org/10.1016/j.arabjc.2014.08.019. [Last assessed on 2017 April 11].
36. Sharma V, Janmeda P. Chromatography fingerprinting profile studies on the

flavonoids of *Euphorbia neriifolia* (Linn.) leaves. Int J Drug Dev Res. 2013,5(1):286-96.
37. Suresh Kumar SV, Mishra SH. Hepatoprotective effect of extracts from *Pergularia daemia* Forsk. J Ethanopharmacol. 2006; 107:164-8.
38. Mukherjee PK, Rai S, Kumar V, Mukherjee K, Hylands PJ, *et al.* Plants of Indian origin in drug discovery. Expert Opin Drug Discov. 2007;2(5):633-57.
39. World Health Organization. Research guidelines for evaluating the safety and efficacy of herbal medicine*s*. Geneva: World Health Organization; 1993. Available from: http://apps.who.int/medicinedocs/en/d/Jh2946e/ [Accessed on 25th October, 2013]
40. Makhija IK, Richard L, Kirti SP, Saleemullah K, Jessy M, et al. *Sphaeranthus indicus:* A review of its chemical, pharmacological and ethnomedicinal properties. International Journal of Pharmacology. 2011;7(2):171-9.
41. Brain KR, Turner TD. The practical evaluation of phytopharmaceuticals. Bristol: Wright-Scientechnica; 1975.
42. World Health Organization. Quality control methods for medicinal plant material. Geneva; 1998.
43. Khandelwal KR. Practical Pharmacognosy: Techniques and experiments. 19th ed., Pune: Nirali Prakashan; 2008.
44. Kokate CK. Practical Pharmacognosy. 4th ed., New Delhi: Vallabh Prakashan; 2007.
45. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Pharm Sci. 1949; 38(6):324-31.
46. Kokoski CJ, Kokoski RJ, Slama FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. Journal of the American Pharmaceutical Association. 1958;47(10):715-7.
47. Harbone JB, Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis, 3rd ed., London: Chapman and Hall; 1998.
48. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global Journal of pure and applied sciences. 2002;8(2):203-8.
49. Orhan I, Küpeli E, Sener B, Yesilada E. Appraisal of anti-inflammatory potential of the clubmoss, *Lycopodium clavatum* L. Journal of ethnopharmacology. 2007;109(1):146-50.
50. Sharma B, Balomajumder C, Roy P. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. Food and chemical toxicology. 2008 ;46(7):2376-83.
51. Mukherjee PK. Quality control of herbal drugs: an approach to evaluation of botanicals. New Delhi: Business Horizons Publication. 2002.
52. Mali PY, Panchal SS, *Euphorbia neriifolia* L.: Review on botany, ethnomedicinal uses, phytochemistry and biological activities. Asian Pacific Journal of Tropical Medicine. 2017;10(5): 430-438.

GRAPHICAL ABSTRACT



HIGHLIGHTS OF PAPER

- Pharmacognostical and physico-chemical standardization of *E. neriifolia* leaves
- Extraction and fractionation of *E. neriifolia* leaves with different solvents
- Primary phytochemical, fluorescence and quantitative screenings of *E. neriifolia*
- Primary HPTLC fingerprinting assessment of EN6 aqueous extract fraction of *E. neriifolia*

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Cite this article : Mali PY and Panchal SS. Pharmacognostical and Physico-chemical Standardization of *Euphorbia neriifolia* Leaves. Pharmacog J. 2017;9(5):696-705.