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Development of quality control parameters of ingredients of a folk remedy for piles and their comparative chemo profiling with homeopathic drugs#

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This study presents comparative TLC of ethanol extract of plants, *Euphorbia hirta* L. (Euphorbiaceae) and *Gomphrena celosioides* Mart. (Amaranthaceae), and formulation (*E. hirta* + *G. celosioides*, 4:1) was compared with some of the available homeopathic drugs for piles. Comparative chemo profiling showed that major components, present in homeopathic formulations of piles, were also found in both these plants and their folk remedial combination. Microscopically, *E. hirta* can be identified due to the presence of various types of simple, multicellular, uniseriate, warty and smooth trichomes and laticifers in traverse section (TS) of root, stem and leaves and *G. celosioides* due to the presence of long multicellular simple trichomes, anomalous secondary growth and pith in TS of root.

Keywords: Ethnobotany, *Euphorbia hirta, Gomphrena celosioides,* Homeopathic drug, Pharmacognosy, Piles

Introduction

Folk remedial combination for piles contains aqueous extract of two plant species [*Euphorbia hirta* L (Euphorbiaceae) and *Gomphrena celosioides* Mart. (Amaranthaceae), 4: 1][1]. *E. hirta* (Dudhi), is an annual decumbent-ascending or erect herb used in indigenous system of medicine for treatment of bowel complaints, cough, worms, dysentery, colic, bronchitis, asthma, and warts[2]. Whole plant is used in cuts and wounds, skin diseases, scabies, eczema, and boils[3]. Plant showed antiprotozoal, hypoglycaemic, and anticancer activities[4]. Main active constituent (±-amyrin) is considered as analgesic, antiemetic, anti-inflammatory, anti-tumor, anti-ulcer, cytotoxic, gastroprotective, hepatoprotective, and insectifuge, whereas, other active constituent (caffeic acid) is reported as an anti-HIV agent[5]. *G. celosioides*, an annual decumbent ascending herb up to 30 cm tall, branches clothed with shaggy white hairs, is used as antimalarial against *Plasmodium falsiparum* in traditional system of medicine in Ghana[6]. Alcohol extract of *G. celosioides* is reported to be diuretic[7] and antimicrobial[8].

Piles (hemorrhoids) are a common problem in India. Some homeopathic drugs are available for piles treatment at early stage, but for very sore and congested piles with ulceration or profuse bleeding, there is no alternative medicine. In India, crude combination of *E. hirta* and *G. celosioides* is found to be very effective in folk treatment of piles[1]. Medicinal importance of this folk remedial combination and probability of its overwhelming exploitation in future may lead to adulteration and substitution.

This study presents detailed pharmacognostical study of whole plants of *E. hirta* and *G. celosioides*. Besides, HPTLC of ethanol extract of plants and their folk remedies were compared with some homeopathic drugs (Aesculus, Aloes, Arnica, Hamamelis, Nuxvomica, Lycopodium, Pulsatilla) available in market for treatment of piles.

Materials and Methods

Whole plant of *E. hirta* and *G. celosioides* were collected during September-October 2006 from surrounding areas of Lucknow (Uttar Pradesh), and Nainital (Uttarakhand), properly identified and compared with flora of district Garhwal, North West Himalaya[9]. Herbarium specimens were deposited in departmental herbarium, CDRI, Lucknow, vide voucher specimen number KRA 23833 and 23834 respectively. For microscopic studies, transverse section (TS) were prepared and stained[10]. Leaves were boiled separately with saturated chloral hydrate solution for surface studies

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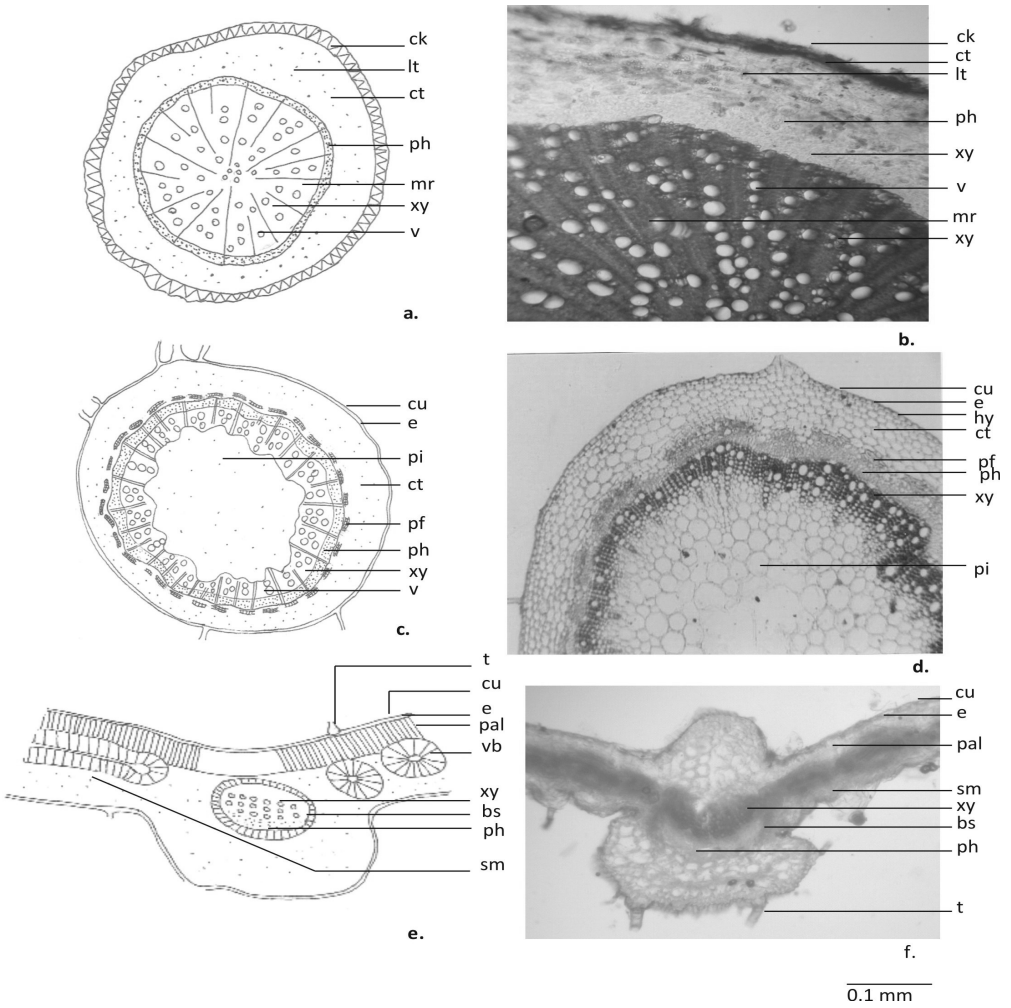

Fig. 1—Microscopy of. *Euphorbia hirta*: a. TS Root (Diagrammatic), b. TS Root, c.TS Stem (Diagrammatic), d. TS Stem, e.TS leaf (Diagrammatic), f. TS leaf [bs, bundle sheath; ck, cork; ct, cortex; cu, cuticle; e, epidermis; hy, hypodermis; lt, laticifer, mr, medullary ray; pal, palisade layer; pf, pericyclic fibre; ph, phloem; pi, pith; sm, spongy mesophyll; t, trichome; v, vessel; vb, vascular bundle; xy, xylem]

and quantitative microscopical observation of leaf. Physiochemical values (total ash, acid insoluble ash, water, and alcohol soluble extractives) were calculated as per Indian pharmacopoeia[11]. Powdered materials (5 g) were extracted with ethanol on a water bath for 10 min, consecutively three times. Extract was filtered through Whatman filter paper 1, combined, concentrated, and dried. Known quantity of extract was dissolved in ethanol for HPTLC. Mother tinctures of homeopathic drugs (Aesculus, Aloes, Arnica, Hamamelis, Nuxvomica, Lycopodium, Pulsatilla) were procured from market. Plant extracts (10 µl each) and homeopathic drugs (5 µl each) were applied on HPTLC plate through CAMAG Linomat 5 Applicator. Plates were developed in various solvent systems, both non polar and polar, and best solvent system was taken for further studies. Plates were dipped in anisaldehyde sulphuric acid reagent by using CAMAG Chromatogram Immersion Device III and heated at 110°C for 10 min. Developed chromatograms were scanned through CAMAG TLC Scanner 3 using WINCATS software version 1.3.2 and spectrum was recorded to identify and match components present in extract and homeopathic drugs. Photograph was taken with Camag Reprostar 3.

Results

Microscopy

Euphorbia hirta

Root

Diagrammatic TS of root is irregularly circular in outline and shows a wide cortex, a narrow portion of phloem and a wide xylem (Fig. 1a). Detailed TS shows

outer 7 to 8 layers of cork, outermost 3 to 4 layers are obliterated. Cortex 140 to 160 µm broad, parenchymatous, traversed with laticifers. Phloem is very narrow (30-35 µm) and consists of sieve tubes, companion cells, and parenchyma. Major portion of root is occupied by xylem, which consists of vessels, tracheids, fibres, and xylem parenchyma. Xylem vessels are mostly solitary or in a group of 2 to 3. Medullary rays are parenchymatous and uni to biseriate (Fig. 1b).

Stem

Diagrammatically, TS of stem is almost circular in outline and covered with trichomes. It consists of cortex, groups of pericyclic fibres, a ring of phloem, and xylem encircling pith (Fig. 1c). TS stem shows single layered epidermis covered with cuticle and interrupted by stomata and trichomes. Trichomes are simple, multicellular, and uniseriate. Some trichomes are warty and jointed. Cortex is collenchymatous with slight thickening at angles, 150-180 µm broad, and cells somewhat circular and laterally compressed with intercellular spaces. Pericycle is in form of groups of fibres followed by well-developed phloem. Phloem (55-70 µm broad) is made up of sieve tubes, companion cells, and phloem parenchyma. Laticifers are abundant in this region. Xylem is also well developed, 130-150 µm broad and consists of vessels, fibres, tracheids, and parenchyma. Vessels are mostly arranged in radial rows. Pith is parenchymatous; cells towards centre are larger with intercellular spaces (Fig.1d).

Leaf

Diagrammatic TS of leaf passing through midrib is deeply and broadly ridged on lower side as compared to upper side with a centrally located meristele and lamina is dorsiventral (Fig. 1e). Detailed TS of leaf passing through midrib region shows single layered upper and lower epidermis covered with thin cuticle interrupted by simple, multicellular trichomes. Upper epidermal cells are polygonal and show paracytic to anomocytic type of stomata, while lower cells are sinuous and contain anomocytic type of stomata. Cells of epidermal cells adjacent to veins are somewhat rectangular and arranged in rows. Centrally located meristele is collateral and surrounded by parenchymatous bundle sheath. Remaining mesophyll tissue is parenchymatous. TS of lamina show 2-3 layers of palisade cells interrupted with collateral vascular bundles. Each vascular bundle surrounded by very large bulbous parenchymatous cells (Fig. 1f). Stomatal numbers of upper surface is 20-26 and of lower 32-36, stomatal indices of upper surface 10.2-11 and of lower is 14-14.5; trichome numbers of upper surface 16-20 and of lower is 20-24. Palisade ratio is 3-4, and vein islet and vein termination numbers are 12-14 and 22-24 respectively.

Powder

Powder shows fragments of upper and lower epidermal cells in surface view, uni to multicellular simple and warty trichomes from both surfaces of leaf; branched and unbranched laticifer, veins of leaf in surface view with adjacent parenchyma, epidermal cells of petal in surface view; fragments of fibers and vessels (Fig. 2).

Gomphrena celosioides

Root

Diagrammatic TS of root is irregularly oval to circular in shape and consists of pericyclic fibre, anomalous vascular bundles, and central pith (Fig. 3a). Detailed TS shows 7-10 layers of cork, outer most 2-3 layers are obliterated. Cortex is well developed and made up of parenchymatous cells. Pericyclic fibres are present in patches. Anomalous secondary growth is observed, vascular bundles are arranged in an irregular fashion. Outer most rings of vascular bundles shows a continuous band of phloem and groups of xylem elements just below phloem. Middle portion of vascular region shows scattered collateral vascular bundles followed by a ring of closely arranged collateral vascular bundles. Innermost layer consists of continuous ring of xylem elements. Medullary rays are uni to multiseriate. Pith is well developed and parenchymatous. Microspheroidal crystals are scattered throughout the section (Fig. 3b)

Stem

Diagrammatically, TS of stem is slightly wavy and laterally flattened in outline covered with trichomes, stellar region made up of endodermis, a ring of pericycle, narrow zone of phloem, and xylem. Major portion is occupied by central pith (Fig. 3c). TS stem shows single layered epidermis covered with thick cuticle and interrupted by stomata and trichomes (simple, uni to multiseriate). Hypodermis 2-4 layered, 80-90 µm broad, collenchymatous, followed by 3-6 layered parenchymatous cortex. Cells of cortex are oval to circular with intercellular spaces, laticifers and rosette crystals of calcium oxalate observed in this region. Cells of innermost layer of cortex are radially elongated, narrow and compactly arranged. Endodermis is well

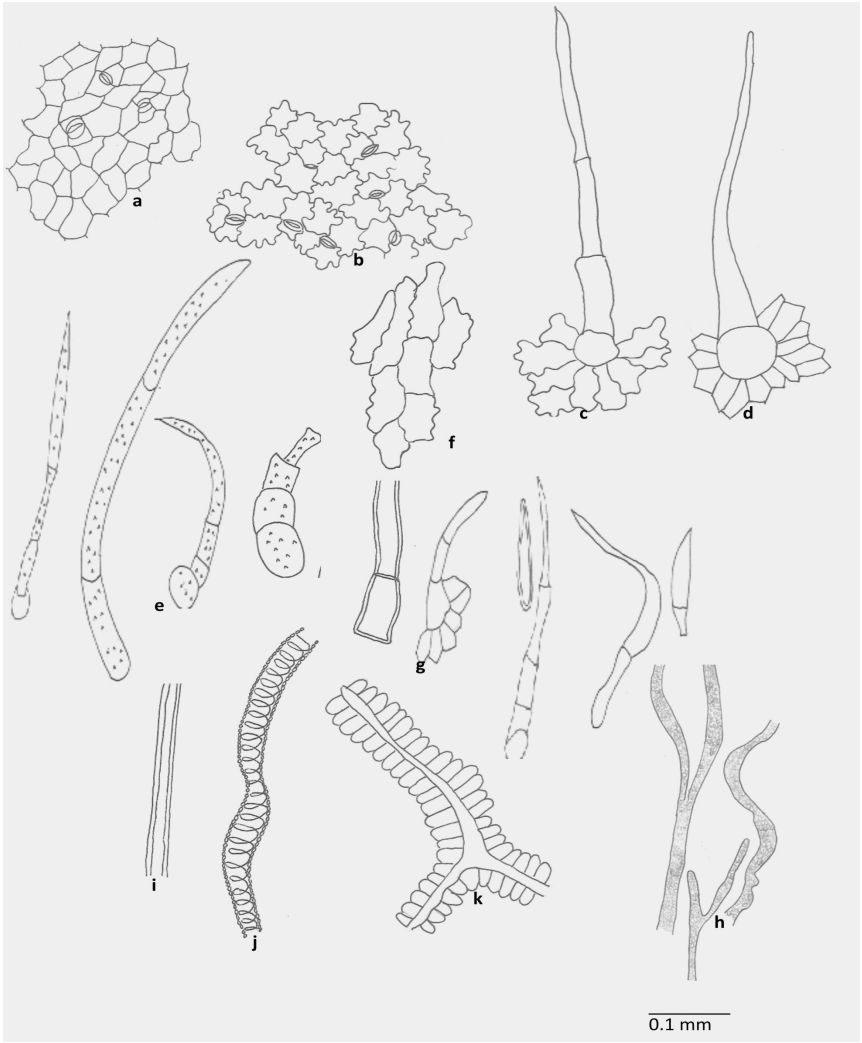

Fig. 2—Powder microscopy of *Euphorbia hirta*: a) upper epidermal cells of leaf in surface view; b) lower epidermal cells of leaf in surface view; c) trichome from the lower surface of leaf; d) trichome from the upper surface of leaf; e) warty trichome from leaf; f) epidermal cells of petal in surface view; g) simple trichome from surface of leaf; h) laticiferous vessels; i) fibre; j) vessel; k) leaf vein with adjacent elongated parenchyma

developed, followed by 1 or 2 layers of sclerenchymatous pericycle. Phloem is well-developed, 55-70 µm broad and consists of sieve tube, companion cells, and parenchyma. Cambium is distinct, 1-2 layered; xylem zone is also as narrow as phloem, 55-70 µm broad and made up of xylem vessels, fibres, tracheids, and xylem parenchyma. Pith is parenchymatous. Cells adjacent to xylem are smaller. Laticifers and rosette of calcium oxalate are also abundant in this region (Fig. 3d).

Leaf

Diagrammatic TS of leaf passing through midrib is undulated on lower side and slightly biconvex on upper side with three centrally located vascular bundles. Laminar vascular bundle being fan shaped (Fig. 3e). Detailed TS of leaf passing through midrib shows single layered epidermis of upper and lower surfaces and covered with thin cuticle. Trichomes are simple, uniseriate and multicellular, and attached to both surfaces. Epidermal cells of upper surface are somewhat angular and contain anomocytic and anisocytic type of stomata. Epidermis of lower surface is slightly wavy and consists of similar type of stomata as that of upper surface. Mesophyll tissue of midrib region consists of 3-5 layers of collenchyma on both sides, followed by parenchymatous cells embedded with rosettes of calcium oxalate at places. Intercellular spaces are prominent in mesophyll tissue. Three vascular bundles

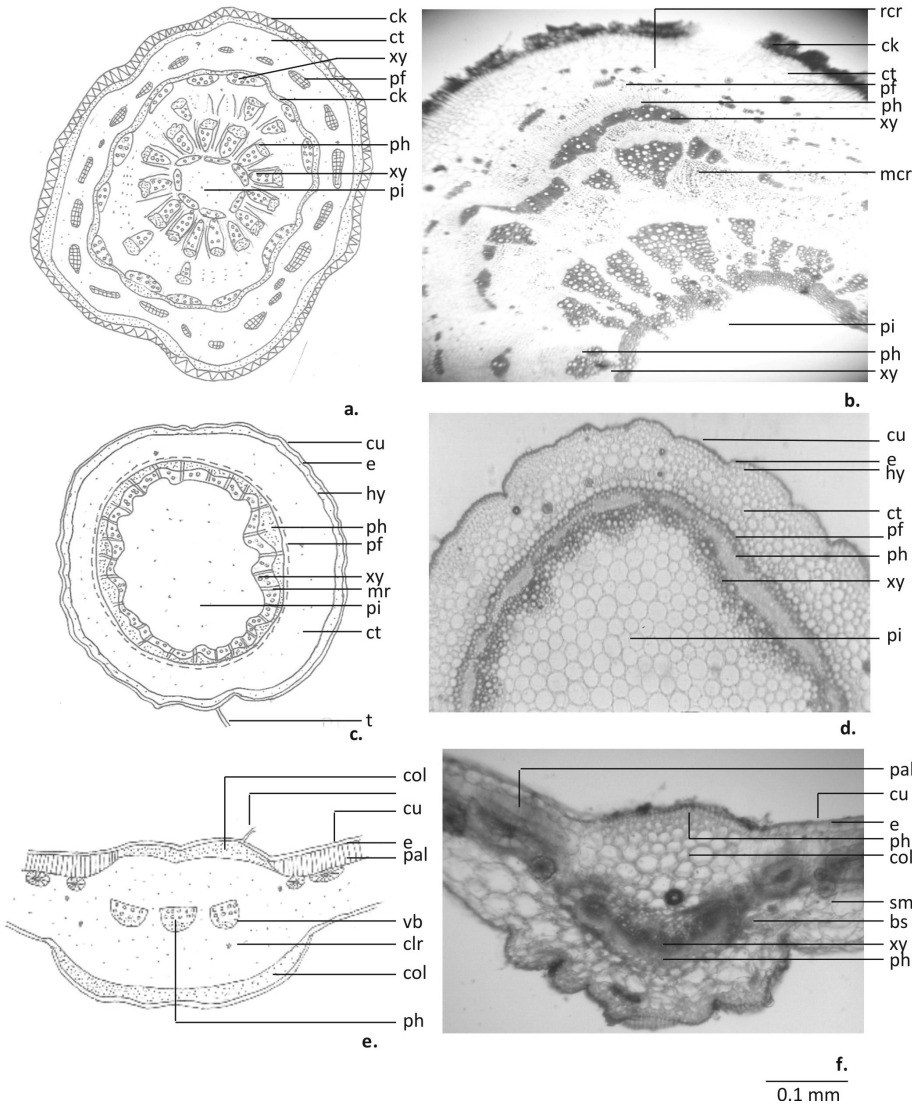

Fig. 3—Microscopy of *Gomphrena celosioides*: a) TS root (diagrammatic), b) TS root, c) TS stem (diagrammatic), d) TS stem, e) TS leaf (diagrammatic), f) TS leaf [bs, bundle sheath; ck, cork; clr, cluster crystals of calcium oxalate;col, collenchyma; ct, cortex; cu, cuticle; e, epidermis; hy, hypodermis; mcr, micro rosette crystals of calcium oxalate; mr, medullary ray; pal, palisade layer; pf, pericyclic fibre; ph, phloem; pi, pith; rcr, rosette crystals of calcium oxalate; sm, spongy mesophyll; t, trichome; vb, vascular bundle; xy, xylem]

are centrally located and arranged in a ring. Each vascular bundle is collateral. Xylem and phloem are well developed.

TS lamina shows 1-2 layered palisade cells followed by 3-6 layered spongy mesophyll, traversed with collateral vascular bundle, each of which surrounded by large parenchymatous bundle sheath (Fig. 3f). Stomatal numbers of upper and lower surfaces are 28-32 and 40-48 respectively; stomatal indices 7.2-7.5 of upper and 10.3-10.8 of lower surface; palisade ratio, vein islet, and vein termination numbers are 3-5, 13-14, and 18-20 respectively, trichome number of upper surface is 10-11 and that of lower is 11-12.

Powder

Powder shows upper and lower epidermal cells with paracytic and anomocytic type of stomata, large columnar and slightly wavy parenchymatous cells, simple uni to multicellular trichomes; rosette and prismatic crystals of calcium oxalate, fibres, vessels with annular and reticulate secondary wall thickening (Fig. 4).

Physico-phytochemical Studies

Ash (total, acid insoluble, water-soluble), alcohol soluble, and water-soluble extractives are presented in Table 1. HPTLC fingerprint profiles of *E. hirta,*

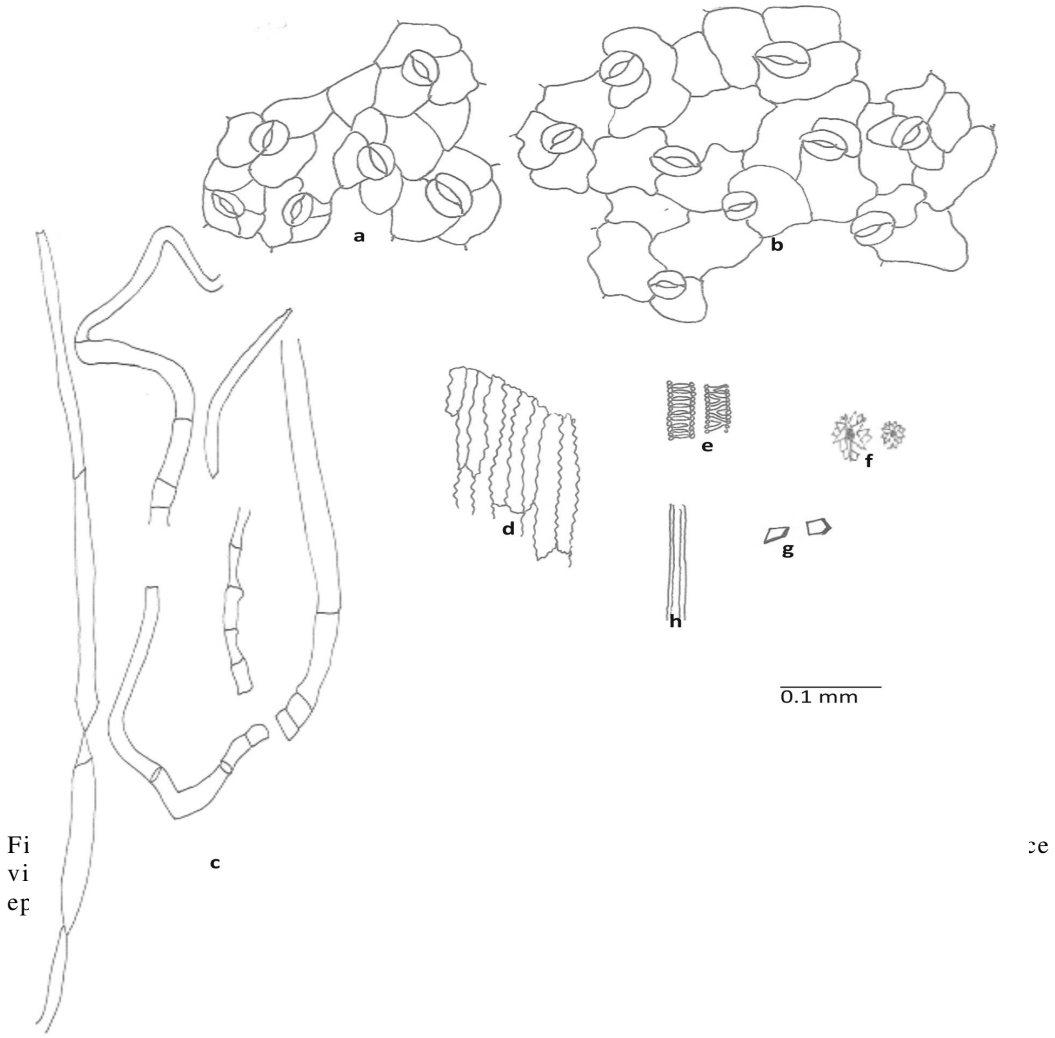

Fig. 4—Powder microscopy of *Gomphrena celosioides*: a) upper epidermal cells in surface view; b) lower epidermal cells in surface view; c) trichome from the surface of leaf; d) epidermal cells of petal in surface view; e) rosette crystals of calcium oxalate; f) prismatic crystals of calcium oxalate; g) vessels; h) fibre

Table 1—Ash and extractive values of *Euphorbia hirta* and *Gomphrena celosiodes* collected from Nainital and Lucknow

Parameters	Nainital		Lucknow	
	E. hirta[a]	G. celosiodes[a]	*E. hirta*[a]	*G. celosiodes*[a]
Total ash,%	10.76 ± 0.418	11.12 ± 0.294	10.03 ± 0.273	9.66 ± 0.140
Acid insoluble ash,%	1.34 ± 0.124	1.02 ± 0.081	1.57 ± 0.094	1.77 ± 0.124
Alcohol soluble extract, %	11.56 ± 0.056	12.68 ± 0.200	10.515 ± 0.251	9.07 ± 0.502
Water soluble extract, %	17.95 ± 0.157	20.98 ± 0.421	16.71 ± 0.372	17.37 ± 0.128

[a] Mean value of six readings ± SD

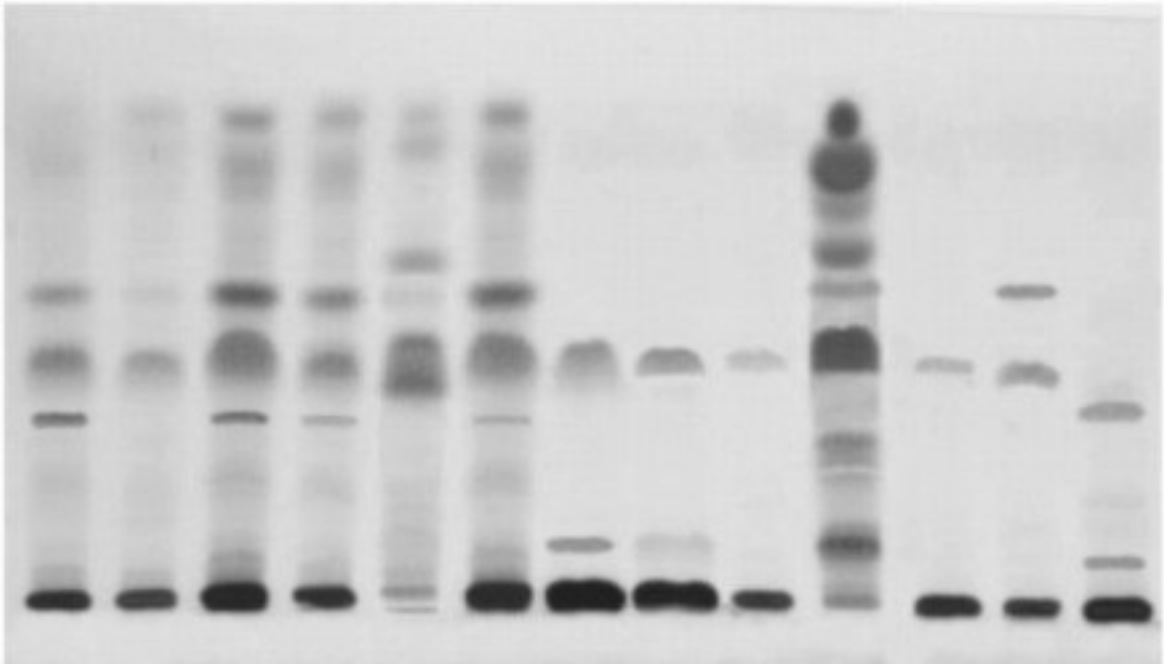

Fig. 5—HPTLC finger print profile of ethanol plant extract, their formulations and homeopathic drugs [AL, Aloe; AR, Arnica; AS, Asculin; EL, *Euphorbia hirta* Lucknow sample; EU, *E. hirta* Nainital sample; GL, *Gomphrena celosioides* Lucknow sample; GU, *G. celosioides* Nainital sample; HA, Hamamelis; LY, Lycopodium; ML, Mixture Lucknow sample; MU, Mixture Nainital sample; NU, Nuxvomica; PU, Pulsatilla]

G. celosioides, their formulation, and commercially available homeopathic drugs for piles have been developed (Fig. 5).

Discussion

Traditional system of medicine is a viable option for prevention, treatment and remedy of chronic diseases[12]. Crude combination of *E. hirta* and *G. celosioides* is reported effective in chronic conditions of piles[1]. Detailed microscopical studies showed that *E. hirta* can be identified due to presence of various type of simple, multicellualar, uniseriate, warty, and smooth trichomes at the surface of stem and leaf, sinuous epidermal cells on lower surface of leaf, laticifers in TS of root, stem, and leaves. *G. celosioides* can be identified due to presence of long multicellular simple trichomes, anomalous secondary growth, and presence of pith in TS root, rosette and prismatic crystals of calcium oxalate. Under physico-chemical parameters, no significant variation observed. Botanical and physico-chemical parameters can be used as quality control markers for ingredients of very effective and ethnobotanically reported herbal remedy for piles.

HPTLC methods are reliable and convenient, and may be used in identification of crude drugs and for assay and qualitative analysis of chemical constituents and content of formulation or batch-to-batch consistency of herbal products. Folk combination of *E. hirta* and *G. celosioides* showed close resemblance with homeopathic drug Lycopodium, with additional similarities with other homeopathic drugs to some extent. Components at Rf 0.42, 0.53, 0.75 and 0.83 present in ethanol extract of both plants and their folk remedy were also present in one or another homeopathic drug (Table 2). Blue colour spot at Rf 0.42 was observed in all samples and homeopathic drugs except Pulsatilla. Similarly, purple spot at Rf 0.53 was present in all plant extract, their folk remedial combination, *Nux vomica*, and *Lycopodium*. However, two purple spots at Rf 0.75 and 0.83 present in *E. hirta, G. celosioides,* their folk combination also observed only in Lycopodium. Blue spot at Rf 0.22 present in *E. hirta*, folk remedial combination resembled with Lycopodium and Pulsatilla and at Rf 0.32 only with Pulsatilla.

Medicinal value of any plant drug depends on the nature of active principle[13]. Folk herbal remedies contain secondary metabolites with pharmacological activities, which could be transformed into modern therapeutic agent. Resemblance of chemical constituents either

Table 2—Comparative Rf values of plant extracts, their formulations, and homeopathic drugs.

S No.	Rf Value	Colour of band	EL	GL	ML	EU	GU	MU	AS	HA	AL	LY	CO	AR	NU	PU
1	0.11	Blue	-	-	-	-	-	-	+	+	-	+	-	-	-	+
2	0.22	Blue	+	-	+	+	-	+	-	-	-	+	-	-	-	+
3	0.27	Greenish blue	-	-	-	-	+	-	-	-	-	-	-	-	-	-
4	0.28	Blue	-	-	-	-	-	-	-	-	-	+	-	-	-	-
5	0.32	Blue	+	-	+	+	-	+	-	-	-	-	-	-	-	+
6	0.38	Ink Blue	-	-	-	-	+	-	-	-	-	-	-	-	-	-
7	0.42	Purplish blue	+	+	+	+	+	+	+	+	-	+	-	-	+	-
8	0.53	Purple	+	+	+	+	-	+	-	-	-	+	-	-	+	-
9	0.58	Brown	+	+	+	+	+	+	-	-	-	-	-	-	-	-
10	0.70	Brown	-	-	+	+	-	+	-	-	-	-	-	-	-	-
11	0.75	Purple	+	+	+	+	-	+	-	-	-	+	-	-	-	-
12	0.83	Purple	+	+	+	+	+	+	-	-	-	+	-	-	-	-

[AL, Aloe; AR, Arnica; AS, Asculin; EL, *Euphorbia hirta* Lucknow sample; EU, *Euphorbia hirta* Nainital sample; GL, *Gomphrena celosioides* Lucknow sample; GU, *Gomphrena celosioides* Nainital sample; HA, Hamamelis; LY, Lycopodium; ML, Mixture Lucknow sample; MU, Mixture Nainital sample; NU, Nuxvomica; PU, Pulsatilla]

individually or in a combination of *E. hirta* and *G. celosioides* with constituents present in one or the other homeopathic drugs of piles, verify the knowledge of this patrimony and revealed scope for isolation of novel molecules for modern therapeutic value of piles.

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