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Pharmacognostical studies on the root of *Anacyclus pyrethrum* DC.

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World Health Organization (WHO) appreciated the importance of medicinal plants for public health care in developing nations. *Anacyclus pyrethrum* DC. roots have important role in the traditional Ayurvedic and Unani systems of holistic health and herbal medicine of the East. Especially the roots of *A. pyrethrum* are reported to have good medicinal values in traditional system of medicine. The present study highlights the pharmacognostical studies on roots including parameters such as taxonomical, macroscopic, microscopic characters, physico-chemical, ultra-violet analysis and chromatographic. The roots are brown in colour, cylindrical in shape with slightly aromatic odour and pungent taste and 7-15 cm in length, with a few hairy rootlets. Microscopical studies indicate the presence of periderm comprising three or four layers of rectangular suberised cells, canals up to 100 µm in diam., unique and characteristic secondary xylem. Powder of the root exhibited vessel elements are 190 µm long and 40 µm wide, tailed vessel elements 260 µm long and 20 µm wide, thick pieces of periderm are frequently seen, canals are several µm long and 5 µm thick and these are found to be additional features of diagnostic values. Ultra-violet and ordinary light analysis with different reagent is useful in identifying the drug in powder form. Physico-chemical evaluation gave, ash values, viz. total ash, acid insoluble ash and water soluble ash, and sulphated ash were 9.3, 7.6, 1.7, and 8.6%, respectively. Extractive values, viz. alcohol soluble extractive value, water soluble extractive and ether soluble extractive values were: 20.8, 8.8, and 3.2%, respectively. Loss on drying was 1.6%. Chromatographic studies of the alcoholic extract of root gave 10 phytoconstituents. This parameter can be utilized for quick identification of the drug and are particularly useful in the case of powdered forms and also contribute towards establishing pharmacopoeial standards.

Keywords: *Anacyclus pyrethrum*, HPTLC, Macroscopy, Microscopy, Pharmacognosy, Physico-chemical, Root, Ultra-violet analysis.

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Introduction

India is one of the largest producers of herbs and herbal products. Nature around us has provided everything of necessity of mankind. The large resources of vegetables, mineral and animal kingdom have been used continuously for the treatment of various diseases and other related problems[1]. Herbal medicines are prepared from various plant parts like leaves, stems, roots, barks and seeds which usually contain many bioactive compounds and are used primarily for treating mild or chronic ailments. Due to the increasing demand in the field of herbal medicines, it has become necessary and pertinent to probe into the area of systematic knowledge about herbal drugs. There is a need for the application of this knowledge in authentification, detailed study and practical utilization of crude drugs[2]. The knowledge gathered by generations was either documented or passed on to the posterity and this practice evolved the development and documentation of traditional medicine. Plants are also appropriated in pharmaceutical research as a major resource for new medicine and a growing body of medical literature supports the clinical efficacy of herbal treatment[3]. Today, about 40% doctors, especially in India and in China (the Mystic Orient) have reverted to increasing use of indigenous drugs and natural medicine. Steadily, a sizable section of scientists in biological, biochemical and biomedicinal discipline have embarked on research on medicinal plants, which are the staple sources of many indigenous drugs[4,5]. The World Health Organization (WHO) estimate that 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their

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primary health cares. In almost all the traditional medicines, the medicinal plants play a major role and constituents the backbone of the traditional medicine[6,7]. *Anacyclus pyrethrum* DC. is a highly medicinal plant, belongs to family Asteraceae. It is a perennial, procumbent herb, widely distributed in North Africa, elsewhere in the Mediterranean region, in the Himalayas, in North India and in Arabian countries. The plant roots contain anacycline, pellitorine, enetriyne alcohol, hyrdocarolin, inulin (c 50%), traces of volatile oil and (+) – sesamin, amides (I, II, III, IV). The plant roots are stimulant, cordial and rubifacient. A gargle of infusion is prescribed for relaxed vulva and also used for toothache, rheumatic and neuralgic affections and rhinitis. Use of the drug in patient with insulin-depended diabetes mellitus reduces the dose of insulin. It decreases the plasma glucose and serum cholesterol level after administration for 3-6 weeks. In large dose the powdered root is an irritant to the mucous membrane of the intestine causing blood stools, tetanus-like spasms and profound stupor. The root is considered tonic and is used to treat paralysis and epilepsy. An infusion of the roots is used as a cordial and stimulant and also in certain stages of fever. A decoction of the roots is useful for pharyangitis and tonsillitis. It is used in the treatment of hemiplegia and chronic opthalmia[8-12]. Detailed pharmacognostical studies of the root of *A. pyrethrum* have not been reported so far. Therefore, an attempt has been made to standardize the drug on the basis of botanical and physico-chemical parameters and also chromatographic studies are useful to identify the genuine sample.

Materials and Methods

Collection of plant material

The dried sample of roots of *A. pyrethrum* was purchased in the month of January 2011 from M.A.S. Stores, Country drugs wholesale and retail in Erode and authenticated as *A. pyrethrum* DC. by Prof. P. Jayaraman, Director, National Institute of Herbal Science, Chennai-45, (Ref. no: PARC/2011/896).

Taxonomic description, vernacular names, habit and habitat of the plant and morphological characteristics were noted from the available literature[13-28].

Macroscopical studies

Organoleptic characters

In organoleptic evaluation, appropriate parameters like taste, odour, size, shape and colour of the roots and root powder were studied[10,26].

Morphological characters

Morphological investigations of the plant root were done[26,29].

Microscopical studies

Care was taken to select healthy plant and normal organ. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5 ml + Acetic acid-5 ml + 70% Ethyl alcohol-90 ml). After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the schedule[30]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the section was 10-12 µm, dewaxing of the sections was by customary procedure[31]. The sections were stained with toluidine blue as per the methods[32]. Since toluidine blue is a polychromatic stain. The staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies, etc. wherever necessary sections were also stained with safranin and Fast-green and IKI (for starch). Glycerin mounted temporary preparation were made from macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured[33,34].

Photomicrograph

Microscopic descriptions of tissue are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon labphoto 2 microscopic units. For normal observation bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy book[35,36].

Ultra-violet analysis

Ultra-violet analysis of the powdered drug with different chemicals were observed in day light and ultra-violet light. Various solvent extracts were also subjected to day light and ultra-violet light for its florescence characteristic.

The powdered root was treated with various solvents like picric acid, acetic acid, concentrated nitric acid, concentrated sulphuric acid, concentrated hydrochloric acid, ferric chloride, aqueous KOH, alcoholic KOH, iodine solution, ammonia solution 25% v/v and observed under day light and also U.V. 254 nm, U.V. 366 nm[37-39].

Physico-chemical standards

In the physico-chemical evaluation, ash values, viz. total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values, viz. alcohol soluble extractive value, water soluble extractive and ether soluble extractive values, and loss on drying were determined as per standard procedure[26,40,41].

The ash values represent the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. The percentage w/w values were calculated with reference to the air-dried drug.

Chromatographic studies

HPTLC finger print profile

HPTLC studies were carried out on alcoholic extract using camag HPTLC system equipped with Linomat IV sample applicator, Camag TLC scanner 3 and CATS 4 software for interpretation of data. An aluminium plate (5×10 cm) precoated with silica gel 60 F_{254} (E Merk) was used as adsorbent. The plates were developed using Ethyl acetate-Methanol-Water (10 : 1.3 : 1) in a camag twin trough chamber to a distance of 8 cm each after development using Dragendorff's reagent followed by 10% ethanolic sulphuric acid as post derivatasion reagent and scanned at 254 nm.

Results

Scientific classification[13]

Kingdom : Plantae
Division : Spermatophyta
Sub division : Angiosperms
Class : Dicotyledons
Sub class : Metachlamydae
Order : Asterales
Family : Asteraceae
Tribes : Anthemideae
Genus : Anacylus
Species : Pyrethrum
Botanical name : *Anacyclus pyrethrum* DC.[14]
Synonyms : *Anthemis pyrethrum* Linn.[14], *Anacyclus depressus* Mairs[15], *A. freynii* Porta & Rigo[15], *Pyrethrum radix*[15].

Vernacular names

Following vernacular names of the plant are recorded in literature in different languages: *Aqer Qerha* (Arabic)[16], *Akarkara* (Bengali)[17], *Tagendaste* (Berber)[18], Spanish Pellitory, Pellitory (English)[14,15], *Anacycle*, *Pyrethre*, *Pyrethre d' Afrique* (French)[15], *Akarkara, Akarkaro* (Gujarati)[17,19], *Forusoon, Forsoon, Qoos, Qoobrum, Foriyum* (Greek)[16], *Akarkara* (Hindi)[10,20,21], *Akkalakari, Akkalkara* (Kannada)[17,22], *Aqer qerha, Aqerqerha* (Urdu)[21], *Akkalakaram, Akkikkaruka* (Malayalam)[22], *Akkirakaram* (Marathi)[23], *Kakra, Kalu, Kazdam, Beekhe Tarkhoon* (Persian)[16,21], *Agragrahi, Akarakarabha, Akarakarava* (Sanskrit)[17,23], *Akkarakkara, Akrapatta, Jallpattam* (Sinhalese)[24], *Akkirakaram* (Tamil)[22], *Akkalakara, Akkalakram, Akkarakaramu* (Telugu)[8,25,26].

Habit and Habitat

A perennial procumbent herb bearing alternate and pinnate leaves; segments linear; ray florets white, purplish beneath, much like chamomile in habitat and appearance, the root is brown, rough, shriveled surface, with the root bark closely adhering to the wood. They have a slight aromatic smell and persistent pungent taste. The plant is native to North Africa, distributed in Mediterranean region; it has been grown on an experimental scale at elevations of 900 m at Katra (Jammu and Kashmir), and Himalayan region from seeds imported from Algeria[10]. The roots of the plant have long been imported into India for medicinal use[10,15].

Botanical description

This is the *Anthemis pyrethrum* of Willdenow, the name of which has been changed by De Candolle, and the plant placed in a new genus on account of a difference in the structure of its seeds[27]. It is a perennial herb with numerous spreading, prostrate or ascending branched stems[24], more or less hairy in

their upper portion, nearly smooth below, and coming from the crown from a long, tapering, vertical, brown, slightly branched root. Leaves alternate, the ones at the root crown long stalked, ovate or oblong in outline, deep bipinnatisect, segments linear, acute often again 2 or 3 fid, more or less hairy or nearly glabrous. Heads terminal, large, 2.5-4 cm or more wide, with a wide disk; involucral scales in several rows, imbricated, ovate-lanceolate, varying in width, blunt or sub acute, smooth, pale green, bordered with an edge of brown; receptacle slightly convex, with large obovate rounded transparent scales beneath the flowers. Disk flowers bisexual, corolla tubular, contracted below with 5 equal triangular spreading teeth, yellow; style exerted, stigma bifid, with 2 linear branches. Ray flowers female in a single row, corolla ligulate, the limb broadly oval, trifid at the apex, white above, tinged with bright pink below[14]. The root as found in shops is simple, 7-5-10 cm long, 1-1.3 cm thick, cylindrical or tapering, sometimes terminated at the top by bristly remains of leaves and having only a few hair like rootlets, externally it has a brown, rough, shriveled surface, is compact and brittle, the fractured surface being radiate and destitute of pith which is almost obliterated, and internally radiating secondary wood occupying about 2/3 of total thickness particularly in older roots. The root is characterized with and aromatic odour and a persistence pungent taste[19,28].

Macroscopical studies

Organoleptic characters

In organoleptic evaluation, appropriate parameters like taste, odour, size, shape and colour of the roots and root powder were studied. They are brown in colour, cylindrical in shape with slightly aromatic odour and pungent taste.

Morphological characters

A perennial herb with numerous spreading, prostrate or ascending, branched stems, more or less hairy in the upper portion, nearly smooth below, and coming from the crown of the long, tapering, vertical, brown, slightly branched root. The roots are tough, cylindrical,7-15 cm in length, tapering slightly at both ends, with a few hairy rootlets and occasionally topped by bristly remains of leaves, external surface rough, brown, shriveled, bark up to 3 mm thick, not easily separable, on chewing gives tingling sensation to tongue and lips and causes excessive flow of saliva (Plate 1).

Microscopical studies

Transverse section of root

Microscopically the cortical part of the root is remarkable on account of its suberous layer, which is partly made up of sclerenchyma (thick walled cells)[19], the transverse section, magnified, present a beautiful radiate structure with many yellow or brown oleoresin glands scattered[42], several layer of tangentially slatted cork cells composed thick sub sized walls and devoid of any cell contents; some stone cells are also found in the outer bark, the development of periderm is exogenous, the cork cambium on inner side produced a few layer of parenchyma cells constituting the secondary cortex it is followed by a single layer of endodermis[17,28]. Most of the parenchymatous cells are loaded with inulin[19], in spherical granules or irregular masses, from 0.01 to 0.1 mm. in diam., which is not affected by the addition of iodine T.S.[42]; after the secondary growth, major portion of the stellar region is occupied by radiating secondary xylem in discrete strands caped with few layers of secondary phloem on outer side. The secondary wood is interrupted by broad rays. The xylem and phloem are made up of usual component, the pith is almost absent[17], but often noted in young roots. In older root about 25-30 strands of secondary xylem are noticed. Vessels are mostly in tangential bands and fibres are found in small group associated with vessels. Crystal of varying shape and size abundantly occur in the parenchyma cell of phloem xylem ray and pith region[28].

The root is thick with rough surface. It is thicker towards the top and gradually thin towards the base. The tapering basal part has thin, less distinct

Plate 1—*Anacyclus pyrethrum* root

superficial periderm comprising three or four layers of rectangular suberised cells. Inner to the periderm is a wide zone of parenchymatous cortex where the cells are variable in shape and size and random is orientation (Plate 2a). The cortex is gradually transformed in to wide secondary phloem. In the outer part of the phloem, the phloem elements are compressed and collapsed forming thin, dark tangential streaks. In the inner portion, the phloem elements are intact and are arranged radial files, wide, circular lysigenous secretory canals are wide spread in the phloem tissue (Plate 2a, 2b, 2f). The canals are up to 100 µm in diameter. The thicker upper portion has wider phloem and xylem zones. The phloem tissues are similar in the lower and upper portions of the root-stalk (Plate 2b, 2e, 2f). Secondary xylem is unique and characteristic. These are several thin radiating, long segments of xylem separated from each other by narrow canal-like parenchymatous rays. The xylem radii are narrow towards the cube and become gradually wider towards the periphery (Plate 2b, 2c, 2f, 2g). Fairly wide, thick walled, angular vessels are seen all along the radial length of the xylem segments, the vessels more in frequency and wider in the outer

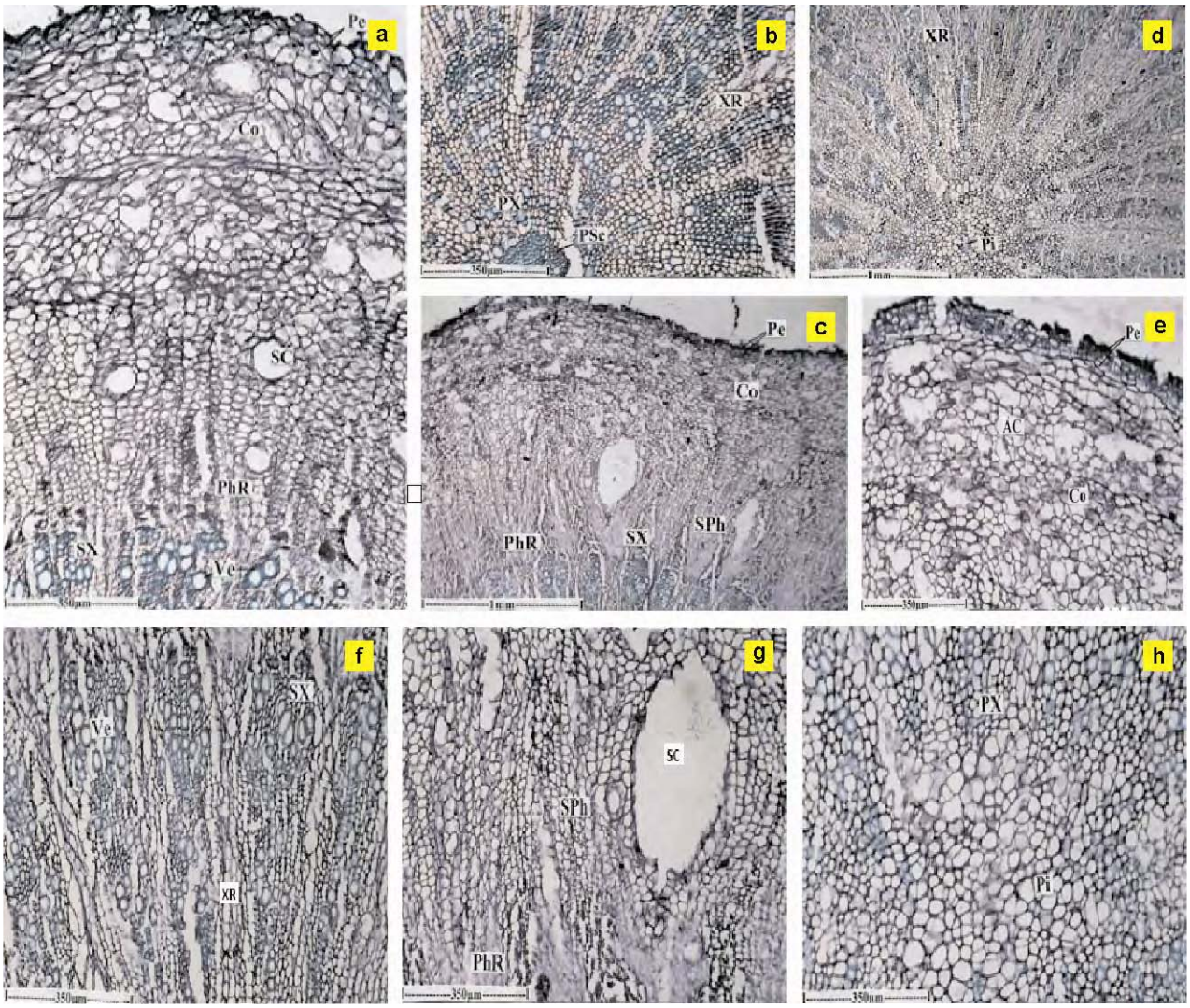


Plate 2—T. S. of *Anacyclus pyrethrum* root: a-T.S. of root – showing periderm and secondary phloem, b-T.S of root–through upper thick part: periderm and secondary phloem, c-T.S of root – pith and secondary xylem, d-T.S. of root – secondary xylem and central sclerotic pith, e-T.S of Periderm and Cortex – Enlarged, f-T.S of Secondary Phloem - Enlarged, g-T.S of Segments Secondary Xylem – Enlarged, h-T.S of Pith and Primary Xylem – Enlarged

[**Abbreviations:** Co: cortex; Pe: periderm; PhR: phloem Ray; Sc: secretory canal; Sx: secondary xylem; Ve: vessels; XR: Xylem ray; Px: Primary xylem; Psc: Pith sclerenchyma; SPh: Secondary phloem; Pi: Pith; Ac- Air- chambers; VB: Vascular Bundle; VE: Vessel element; Ta: Tail; ScT: Scalariform thickenings; Pa: Parenchyma cell].

terminal portion of the xylem segments, xylem fibres for in this ground tissues in the segments (Plate 2g, 2h). The pith fairly wide comprises parenchyma cells and sparely distributed small nests of sclerenchyma. In the lower end of the root, the piths are occupied by a solid circular cylinder of sclerenchyma (Plate 2c, 2d).

Powder microscopy

Microscopic analysis of powder and macerates preparations of the root material shows the following elements:

(i) Vessel elements (Plate 3a, 3b, 3c)
Vessels are seen in bundles or isolated vessel elements are also observed (Plate 3a, 3c). The vessels have scalariform lateral wall thickenings (Plate 3c). A few vessel elements are narrow, long and have prominent tails (Plate 3b). The vessel elements are 190 µm long and 40 µm wide. The tailed vessel elements are 260 µm long and 20 µm wide.

(ii) Secretory canals (Plate 3d)
Long, thin unbranched, non seprate secretory canals are wide spread in the powder. They are darkly stained and are either entire or broken into small units. The canals are several µm long and 5 µm thick.

(iii) Periderm tissue (Plate 3e)
Thick pieces of periderm are frequently seen in the powder. The tissue fragments consist of thin layer of parenchyma cell which are arranged in regular parallel rows.

Ultra-violet analysis

Powdered drug under ultra-violet and ordinary light when treated with different reagent emitted various colour radiations which help in identifying the drug in powder form (Table 1).

Physico-chemical standards

The physico-chemical evaluation, ash values, viz. total ash, acid insoluble ash and water soluble ash, sulphated ash, and extractive values, viz. alcohol soluble extractive value, water soluble extractive and ether soluble extractive values, and loss on drying were calculated and recorded. The ash values, viz. total ash, acid insoluble ash and water soluble ash, sulphated ash were: 9.3, 7.6, 1.7, and 8.6%,

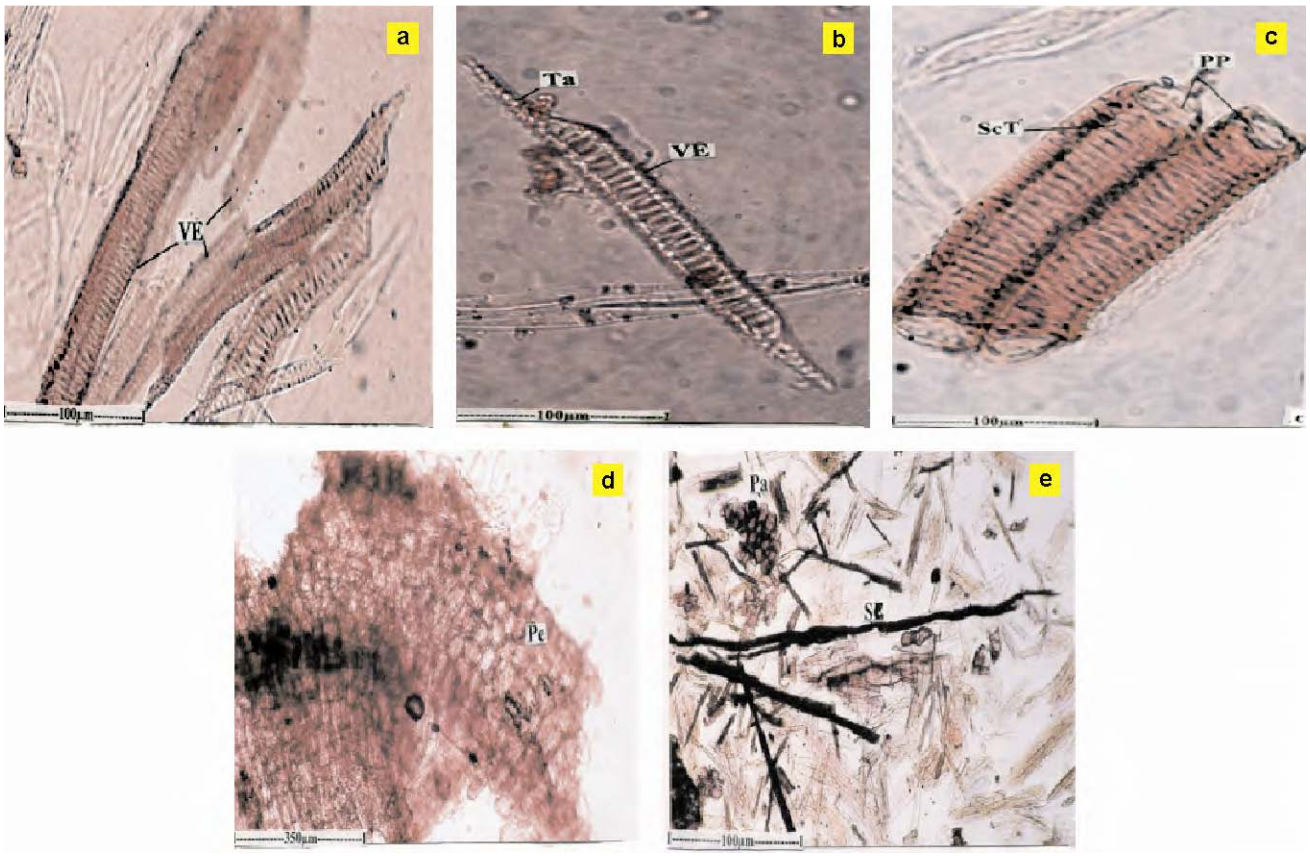


Plate 3—Powder microscopy of *Anacyclus pyrethrum* root: a-Bundle of vessels, b-A single tailed vessel element, c-Cylindrical tailless vessel element, d-Secretory canals – Isolated and scattered in the powder, e-Periderm tissue – A fragment

respectively. Extractive values, viz. alcohol soluble extractive value, water soluble extractive and ether soluble extractive values were: 20.8, 8.8, and 3.2%, respectively. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14% w/w[43]. The moisture content (Loss on drying) was 1.6%.

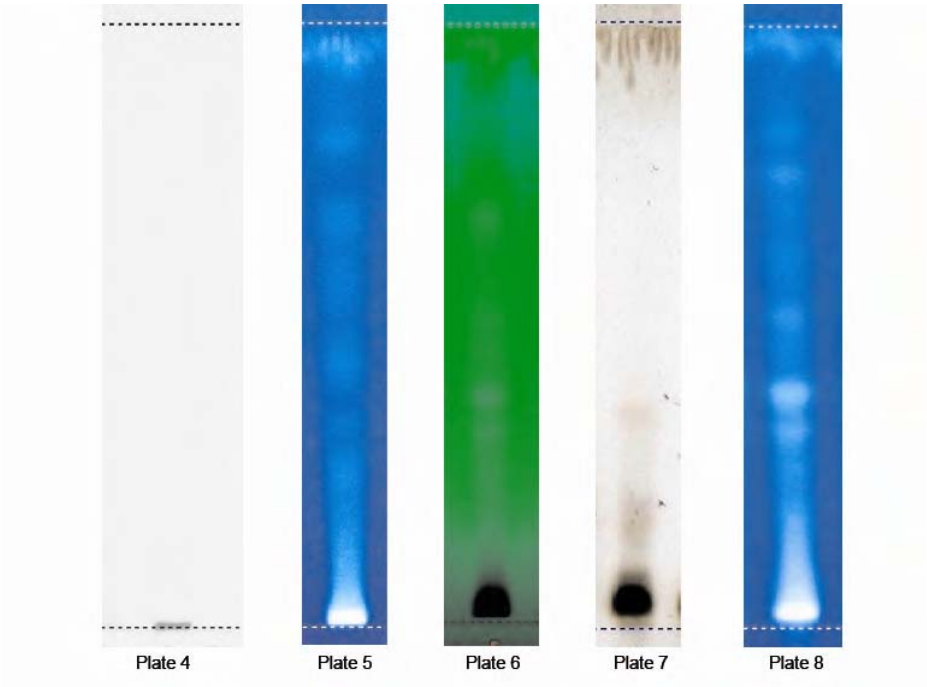
Chromatographic studies

HPTLC finger print profile

HPTLC finger print of alcoholic extract of root revealed 10 phytoconstituents having Rf values 0.03, 0.06,0.14,0.18,0.23,0.40,0.45,0.78,0.84,0.89 with a most pronounced spot of maximum area at Rf 0.18 (Plates 4-9).

Table 1—Ultra-violet analysis of the powdered *Anacyclus pyrethrum* root

S. No.	Treatment of powder	Visible light	UV light	
			Short wave (254 nm)	Long wave (366 nm)
1	Picric acid	Pale Yellow	Pale Green	Dark Green
2	Acetic acid	Pale Brown	Pale Green	Dark Green
3	Conc. Nitric acid	Pale Yellow	Pale Green	Greenish Black
4	Conc. Sulphuric acid	Black	Black	Black
5	Conc. Hydrochloric acid	Light Yellow	Light Green	Dark Green
6	Ferric chloride solution	Pale Brown	Pale Green	Dark Green
7	Aqueous KOH	Straw Yellow	Pale Green	Dark Green
8	Alcoholic KOH	Pale Yellow	Light Brown	Dark Brown
9	Iodine solution	Light Brown	Pale Brown	Dark Brown
10	Ammonia solution 25% v/v	Straw Yellow	Light Green	Dark Green



Plates 4—Chromatogram under day light before derivatization, Plate 5—Chromatogram under UV 366nm before derivatization, Plate 6—chromatogram under UV 254 before derivatization, Plate 7—chromatogram under day light after derivatization, Plate 8—chromatogram under UV 366 after derivatization

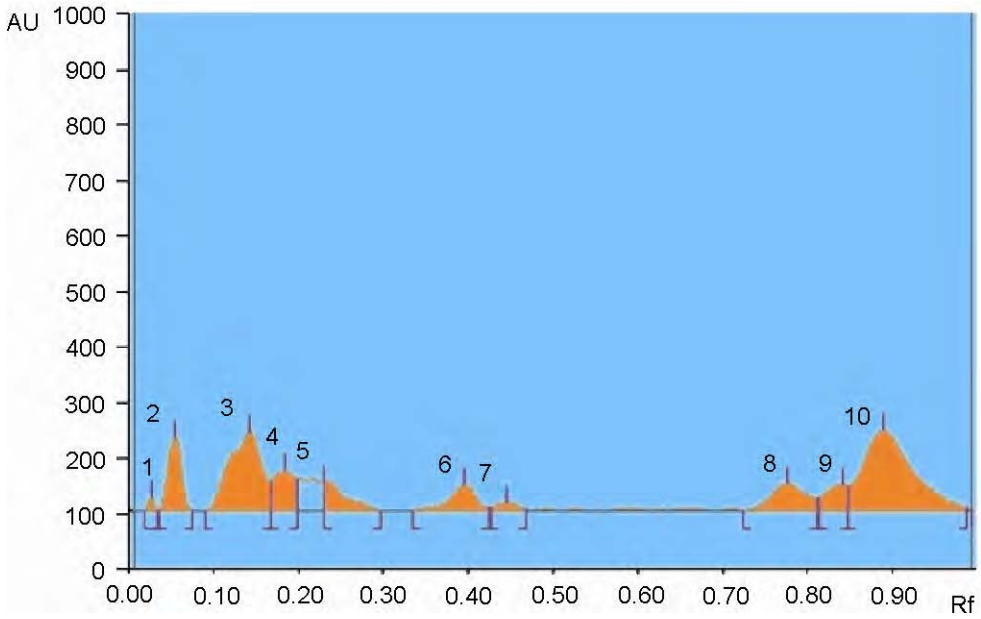


Plate 9—HPTLC profile of alcohol extract of *Anacyclus pyrethrum* root

Discussion

This is the first report of pharmacognostical studies on the root of *A. pyrethrum*. The plant is sold by the Ayurveda raw material wholesaler in the form of small fragments, mostly in dry condition. The exomorphic features of the plant with the flowers and fruits are not usually available in the crude drug, so anatomical features of the root are to be sought for establishing the genuineness of the drugs. The roots are brown in colour, cylindrical in shape with slightly aromatic odour and pungent taste. Macroscopic studies indicate presence of the root having 7-15 cm in length, with a few hairy rootlets. Microscopical studies indicate that the presence of periderm comprising three or four layers of rectangular suberised cells, canals are up to 100 µm in diam., secondary xylem is unique and characteristic. Powder of the root exhibited vessel elements are 190 µm long and 40 µm wide, tailed vessel elements are 260 µm long and 20 µm wide, thick pieces of periderm are frequently seen, canals are several µm long and 5 µm thick are additional features of diagnostic values. In ultra-violet and ordinary light analysis with different reagent is useful in identifying the drug in powder form. Physico-chemical evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The ash values, viz. total ash, acid insoluble ash and water soluble ash and sulphated ash were: 9.3, 7.6, 1.7 and 8.6%, respectively. Extractive values, viz. alcohol soluble extractive value, water soluble extractive and ether soluble extractive values were: 20.8, 8.8 and 3.2%, respectively. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14% w/w. The moisture content (Loss on drying) was 1.6%. HPTLC finger print of alcoholic extract of root revealed 10 phytoconstituents.

Conclusion

The present study concludes that the complete pharmacognostical parameters of the root of *A. pyrethrum* will provide useful information for identification and to determine the quality and purity of the plant materials. HPTLC profile for alcoholic extract helps to establish marker compound and to isolate and identify the biomarker/bioactive constituent. These observations will help in the pharmacognostical identification and standardization of the drug and also contribute towards establishing pharmacopoeial standards.

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