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Pharmacognostic evaluation of *Achyranthes coynei*: Leaf



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ABSTRACT

Achyranthes coynei Sant., (Amaranthaceae) is a rare, medicinal shrub, endemic to India. The plant especially, leaves are used in treatment of various disorders by folk healers. It has been scientifically evaluated for its antimicrobial and antioxidant properties. Pharmacognostic studies have not been carried out so far in this plant. So, the present study was undertaken to evaluate pharmacognostic characters of leaf of *A. coynei*. The studies were carried out in terms of morphological, microscopic characters and physicochemical parameters using standard methods. Leaf size (15–23 × 7–10 cm) and petiole nature (pink above and green beneath) were the distinguishing morphological character observed in the present study. Transverse section of leaf and petiole showed presence of long, multicellular, warty trichomes with pointed apex and short, glandular trichomes with blunt apex on thick walled epidermis; conjoint, collateral closed, endarch vascular bundles; rosette crystals of calcium oxalate in ground tissue. Ash and extractive values, micro and macro elements and nutritive factors were estimated. Leaf powder showed fluorescence under the influence of different solvents. Preliminary phytochemical screening showed the presence of alkaloids, saponins and triterpenoids. High Performance Thin Layer Chromatographic (HPTLC) analysis yielded eight bands in leaf extract. The study forms the first report on pharmacognostic characters for *A. coynei*, which could be useful for identification and authentication of the plant.

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1. Introduction

Plants are used as medicine to maintain human health from ages [1]. Plants are also major natural sources of medicinal

compounds in current pharmacopoeias [2]. Indian Materia Medica includes about 2000 drugs of natural origin and most of them are derived from different traditional system and folklore practices [3]. However, there are large numbers of

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Table 1 – Histochemical tests.

Section treated with Reagents	Tests for	Observations		Result	
		Leaf	Petiole	Leaf	Petiole
Conc. sulphuric acid	Cellulose	Green	Green	Present	Present
Ruthenium red solution	Mucilage	– ^a	– ^a	Absent	Absent
Phlorogucinol + conc. HCl	Lignin	Magenta	Magenta	Present	Present
Iodine	Starch	Blue	Blue	Present	Present
Ferric chloride solution (10%)	Tannin	– ^a	– ^a	Absent	Absent
Conc. HCl	Calcium oxalate crystals	Dissolved	Dissolved	Present	Present

^a No results observed.

plants, which have not been mentioned in these reports, in spite of their usage in the traditional and folk medicinal systems.

Achyranthes coynei Santapau (Amaranthaceae) is one such plant, which is a profusely branching perennial shrub growing up to a height of 2.0–4.5 m. The plant is endemic to India and reported from Maharashtra and Karnataka states [4]. The plant was categorized as rare in Red data book of Indian plants [5]. This plant is locally known as *Kempu Uttarani* (Kannada) and *Lal Agada* (Marathi) and used to treat various ailments by folk healers, most of the times, similar to that of *Achyranthes aspera* [4]. Leaves are reported to have antibacterial [6] and antioxidant [7] activities.

According to Hegde et al., every plant shows unique nature in terms of its botany, chemistry and therapeutic potency and it is essential to study pharmacognostic characters of a medicinal plant, not only for its proper identification, but also to understand its structure and biology [8]. The available literature revealed that, no such studies have been carried out for *A. coynei*. Hence in the present investigation, the leaves of *A. coynei* was studied for its pharmacognostic characteristics.

2. Materials and methods

2.1. Collection of plant material

Leaves of *A. coynei* along with petiole were collected from Madhanbhavi, Belgaum district, Karnataka. The plant specimen was authenticated and herbarium was deposited at Regional Medical Research Centre, (ICMR), Belgaum, Karnataka, India for future reference (Voucher number: RMRC 784).

2.2. Chemicals, reagents and solvents

All chemicals, reagents and solvents used during the experimentation were of analytical grade.

2.3. Macroscopic and microscopic analysis

Key morphological features of the leaf were observed during macroscopic analysis using dissecting microscope (Labomed, India). Transverse section (TS) of the leaf and petiole were taken using LEICA CM (1850) cryostat. For this, fresh plant material was mounted on the specimen disk covered with tissue freezing medium (Jung). The specimen disks were kept for freezing at -18 ± 2 °C for about 30 min. Frozen plant

materials were used for sectioning at a thickness of 20 ± 2 microns. Histochemical and powder studies were carried out by using reagents and stains like iodine, concentrated sulphuric acid, concentrated hydrochloric acid, ferric chloride, Sudan III, ruthenium red and phloroglucinol with Conc. HCl (1:1) [9]. Similarly, organoleptic characters like colour, odour and taste were determined for the leaf powder [9].

2.3.1. Quantitative microscopy

The quantitative examinations such as vein islet number, vein termination number, palisade ratio and stomatal index were studied using standard methods [9].

2.3.2. Microphotography

Microphotographs of the sections and powder microscopy were taken using microscope (Olympus BX 41) at different magnifications (4×, 10× and 40×) with inbuilt analogue camera (ProgRes C3-JENOPTIK). Computer images were captured using software ProgRes[®] CapturePro 2.1.1-JENOPTIK laser optical system.

2.4. Preparation of extracts and preliminary phytochemical analysis

The powdered material was serially extracted by continuous shaking method using petroleum ether, chloroform, methanol and water. Leaf powder (5 g) was extracted with 20 mL of respective solvent on a shaker at room temperature at 120 ± 10 rpm for overnight. The same was filtered and evaporated to dryness. The extracts were stored at 4 °C for further use. These extracts were subjected for preliminary phytochemical screening as per standard pharmacognostic methods [7].

2.5. Physico-chemical and nutritive content analysis

Physico-chemical parameters of the powdered drug such as total ash, water-soluble ash and acid-insoluble ash were determined [9]. Soluble extractive values were determined as per standard procedure. The moisture content was detected by loss on drying method [9]. Determination of macro elements (phosphorus, potassium, sulphur, calcium, sodium and magnesium) and microelements (zinc, iron, manganese, and copper) were estimated using atomic absorption techniques [10,11]. Nitrogen content in the leaf powder was estimated by Kjeldahl method [10,11]. Nutritive contents viz. percent starch, total carbohydrates, reducing and non-reducing sugars were

also estimated [11,12]. Protein was calculated using the formula:

$$\% \text{ Proteins} = \% \text{ Nitrogen} \times 6.125[8].$$

2.6. Fluorescence analysis

Different reagents were used to check the fluorescence activity [12]. In fluorescence analysis 0.1 g of leaf powder was mixed with 1.5 ml of respective reagent (Table 3). The mixture was kept for a minute and the same was observed under visible light, short ultra-violet light (254 nm) and long ultra-violet light (365 nm).

2.7. High performance thin layer chromatography

Extraction method given by Tandon [13], was employed for HPTLC analysis. A CAMAG High performance thin layer chromatography (HPTLC) system was used for detection and separation. Analysis was performed on a pre-coated TLC silica gel G60 F₂₅₄ plates. Standards and sample bands (6 mm) were applied using CAMAG Automated TLC Sampler (ATS-4) equipped with 25- μ l syringe operated with settings: band length 6 mm, application rate 150 μ l/s, distance from the bottom of the plate (Y) 5 mm, distance from edge of plate (X) 5 mm, distance between bands were auto set (10 mm). The plates were developed to a distance of 75 mm with chloroform: methanol (9:1, v/v) as mobile phase in a CAMAG twin trough glass chamber previously saturated with the mobile phase at room temperature for 20 min. After the run plates were dried in air current using drier and developed using

Table 3 – Fluorescence analysis.

Treatment	Visible light	UV light at 254 nm	UV light at 365 nm
P	Green	Black	Green
P + water	Dark green	Green	Bluish green
P + methanol	Florescent green	Black	Biscuit brown
P + con. H ₂ SO ₄	Blackish green	Green	Florescent green
P + con. HCL	Bottle green	Black	Black
P + 1 N NaOH	Dark green	Black	Parrot green
P + acetic acid	Reddish green	Green	Brick red
P + nitric acid	Brick red	Black	Brown
P + formic acid	Reddish green	Green	Dark green
P – Powder.			

anisaldehyde–sulphuric acid reagent followed by heating for 5 min at 110 ± 2 °C. Visualization of various coloured bands on plate in white light was recorded using CAMAG TLC visualizer. In the present study, R_f value, band colour, intensity of colour and colour codes were used to identify and differentiate the visualized bands. The colour codes were given using Adobe Photoshop 7 software.

2.8. Data analysis

Cell dimensions were represented as RDS (radius for circle), DST (Length of line) and Maj (Length of large half axis for ellipse) in microscopy as defined in ProgRes® CapturePro 2.1.1-JENOPTIK, software. Standard deviation was calculated as mean of three replicates using Microsoft Excel (2007).

Table 2 – Physicochemical parameters and nutritive content.

Ash value (% w/w)	
Total ash	12.7
Acid insoluble ash	1.31
Water soluble ash	6.03
Moisture	10.23
Soluble extractive values (% w/w)	
Ethanol	9.61
Water	15.24
Macro elements (%)	
Nitrogen	2.82
Potassium	0.192
Phosphur	1.976
Sulphur	0.332
Calcium	2.64
Magnesium	1.02
Sodium	0.367
Micro elements ppm	
Iron	6113.13
Zinc	33.39
Copper	37.97
Manganese	171.18
Nutritive contents (%)	
Reducing sugars	11.94
Non reducing sugars	1.68
Total carbohydrates	13.62
Starch	25.89
Protein	17.272

3. Results

3.1. Morphological description

A. coynei is a profusely branching perennial shrub growing up to a height of 2–4.5 m (Fig. 1a). The plants were seen on the sides of small streams and canal bunds of the agricultural land. Leaves were simple, opposite, deciduous, showing dimorphic nature. The lower leaves were larger in size (15–23 \times 7–10 cm) and the upper leaves gradually decreases in size. Leaves were elliptic to lanceolate, acute or acuminate; sub-glabrous above, pubescent beneath especially on the midrib, primary nerves and margins. Leaf margins were entire; petiole 1–3.5 cm long, channelled, pink above, green beneath (Fig. 1b).

3.2. Anatomical description and powder microscopy

Transverse section of leaf and petiole were taken for the anatomical study. The sections stained with Phloroglucinol:Concentrated HCl (1:1) have been shown in Fig. 1.

3.2.1. Leaf

Fig. 2a, shows the transverse section of leaf passing through midrib and lateral lamina. Transverse section shows single layered, thick walled epidermal cells (size:



Fig. 1 – a: *A. coynei* habit; b: petiole; c: ventral view of leaf; d: dried leaves; e: leaf powder.

238.17–14.33 sq μm) covered by cuticle (thickness of DST: 5.557–1.621 μm). The epidermal cells were rectangular in shape with convex nature on upper and lower margins. The cells of the upper epidermis were larger in size than the lower epidermis. Long, multi-cellular warty trichomes (DST: 348.6–476.2 μm) with pointed apex were present on the epidermis. Few short, irregularly bent glandular trichomes (DST: 20.59–73.2 μm) with blunt apex were seen in the midrib region of the epidermal layer. Transverse section shows 4–6 rows of collenchyma below the upper epidermis and 2–3 layers after lower epidermis in the midrib region. The cells of collenchyma were thick walled; oval in shape, showing tiny air spaces followed by broad parenchymatous ground tissues. The cells of the ground tissue were round/oval/polygonal in nature with intercellular spaces. Conjoint, collateral closed, 5–9 vascular bundles were present in ground tissue. The vascular bundles were endarch, enclosed by sclerenchymatous sheath; phloem consists of companion cells and sieve tubes; xylem consists of spiral annular thickened vessels, tracheids, fibres and xylem parenchyma. Rosette crystals of calcium oxalate were observed in ground tissue and palisade cells of midrib and lamina (RDS: 22.69–10.21 μm). Lamina TS of the leaf shows 2–3 layers of palisade cells underneath the upper epidermis and 2–4 layer of spongy parenchyma below lower epidermis.

The peels of epidermis were analysed to observe the stomata and its nature. Both lower (Fig. 2c) and upper epidermis (Fig. 2d) show stomata (Maj: 7.241–11.37 μm) and were of similar nature. Two guard cells of stomata were surrounded by three to four wavy walled epidermal cells falling in both anisocytic and anomocytic category.

3.2.2. Petiole

Crescent shaped petiole was observed in TS (Fig. 2b), showing a layer of thick walled epidermis with trichomes as mentioned in the leaf. Thick walled, 4–5 layers of collenchymas were present beneath the epidermal layer. Various sized parenchymatous cells forms the ground tissue with rosette crystals of calcium oxalates (RDS: 30.48–17.3 μm). An arc of vascular bundles was present at the centre of the petiole and the nature is similar to that of leaf.

3.2.3. Powder microscopy

Powder of the leaf along with petiole showed fragmented pitted and spiral vessels (Fig. 2e, j), trichomes attached to the epidermal cells (Fig. 2g, h, m), palisade and spongy parenchyma cells (Fig. 2f, i), calcium oxalate crystals (Fig. 2f, k), collenchyma cells (Fig. 2l), thick walled epidermal cells and stomata (Fig. 2l, m).

3.2.4. Leaf constant

Leaf venation was reticulate with 8 ± 4 pairs of alternate lateral veins (Fig. 1c). Vein islet number is 12 ± 6 and vein termination number is 15 ± 5.5 . The stomatal index for lower epidermis is 23.63% and for upper epidermis 20.69% (Fig. 2c, d) and palisade ratio varies from 1:7 to 1:13.

3.3. Histochemical analysis

Leaf and petiole sections were treated with different reagents to know various cell components. The results are presented in Table 1. Both leaf and petiole showed the presence of calcium oxalate crystals soluble in Conc. HCl with lignin and cellulose.

3.4. Organoleptic characters

Dried leaves (Fig. 1d) and leaf powder has green colour (Fig. 1e) without any specific odour and taste.

3.5. Physicochemical parameters

The physicochemical characters such as total ash, acid soluble ash, moisture content, and extractive values in ethanol and water of dried leaf powder were calculated in terms of air dried sample. The results were presented in Table 2. Total ash plays an important role in evaluation of purity of drugs and for *A. coynei* leaf powder it was 12.7% w/w. Quantitative estimation of extractive values was represented as percent yield. Soluble extractive percent yield for leaves was higher in water (15.24% w/w) than in ethanol (9.61% w/w). The results showed greater extractive values on water extract followed by ethanol indicating the concentration of secondary metabolites. Macro elements (nitrogen, potassium, phosphor, sulphur, calcium, magnesium, sodium), microelements (iron, zinc, copper,

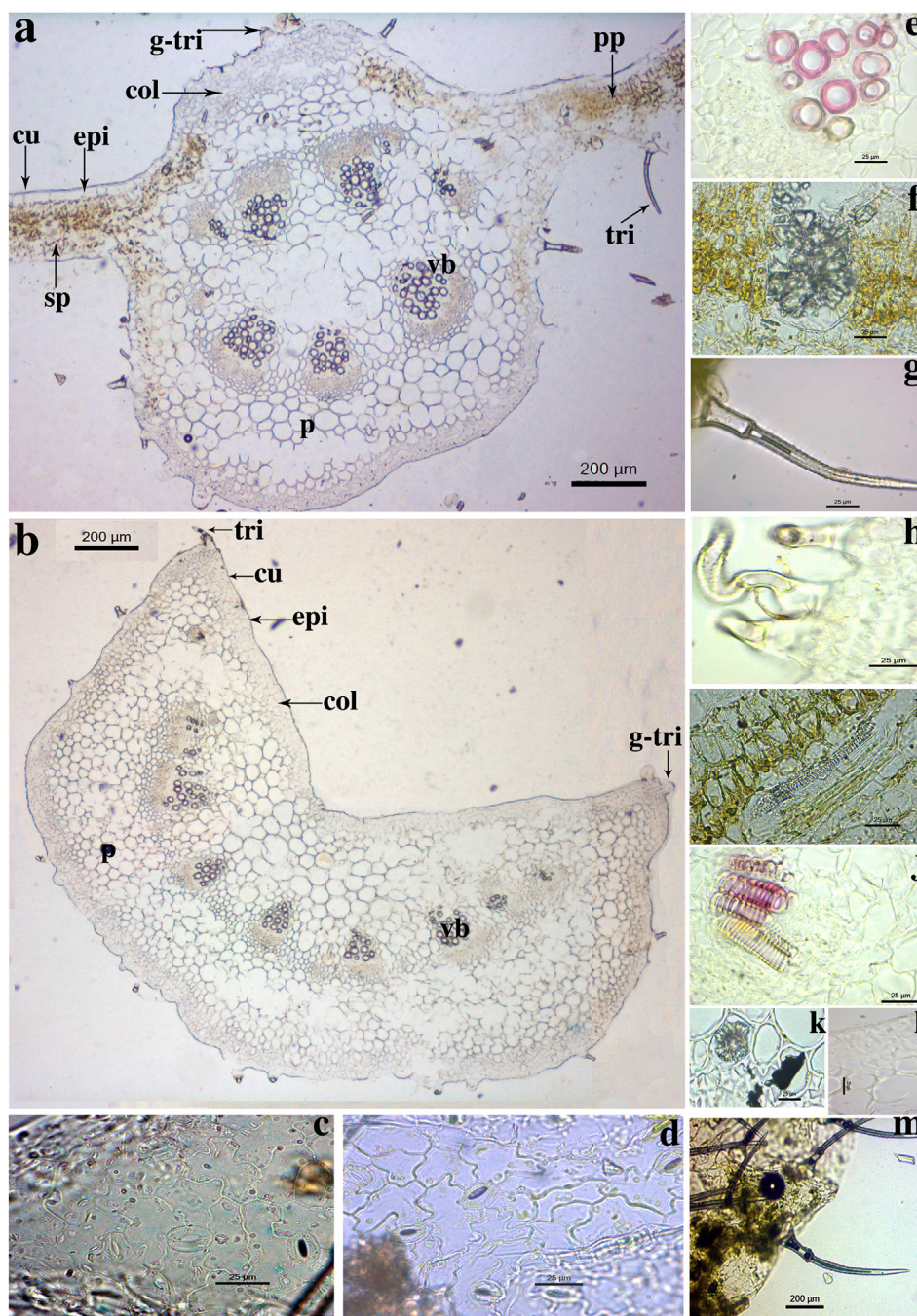


Fig. 2 – a: TS of leaf; **b:** TS of petiole; **c:** lower epidermis; **d:** upper epidermis; **e:** vascular bundles; **f:** calcium oxalate crystal; **g:** long, multi-cellular warty trichome; **h:** glandular trichome with blunt apex; **i:** palisade and spongy parenchyma cells; **j:** xylem vessels; **k:** calcium oxalate crystals; **l:** collenchyma cells; **m:** epidermis with trichoms; **col:** collenchyma cells; **cu:** cuticle; **epi:** epidermis; **g-tri:** glandular trichomes with blunt apex; **p:** parenchyma; **pp:** palisade parenchyma; **tri:** long, multi-cellular warty trichome; **vb:** vascular bundle; **sp:** spongy parenchyma.

manganese) were estimated for the leaves and were represented as percent and parts per million (ppm) respectively (Table 2). Among micro elements iron (6113.13 ppm) content was high in *A. coynei* leaves. The results indicate considerable content of moisture, fat and fibre in leaf. Nutritive contents like reducing and non-reducing sugars, carbohydrates, starch and protein were also estimated in the present study (Table 2).

3.6. Fluorescence analysis

Fluorescence analysis of leaf powder was carried out after treating with several solvents. Fluorescence was observed at 254 and 365 nm comparing its change of colour in visible light. The observations are presented in Table 3 showing the variation in colour.

Table 4 – Preliminary phytochemical analysis.

Test for	Leaf extracts					
	Aqueous	Methanol	Ethyl acetate	Chloroform	Benzene	Petroleum ether
Steroids	+ ^a	+ ^a	+ ^a	+ ^a	+ ^a	+ ^a
Triterpenoids	+ ^a	+ ^a	+ ^a	+ ^a	– ^b	– ^b
Saponins	+ ^a	+ ^a	+ ^a	– ^b	– ^b	+ ^a
Flavonides	+ ^a	+ ^a	– ^b	– ^b	– ^b	– ^b
Alkaloids	+ ^a	+ ^a	– ^b	+ ^a	– ^b	+ ^a
Tannins	– ^b	– ^b	– ^b	– ^b	– ^b	– ^b
Glycosides	+ ^a	+ ^a	+ ^a	+ ^a	– ^b	+ ^a

^a Present.
^b Absent.

3.7. Phytochemical analysis

Respective extracts were subjected to preliminary phytochemical screening and the results (Table 4) validated the presence of triterpenoids, alkaloids, glycosides, steroids, and saponins in leaves.

3.8. HPTLC analysis

Fast screening of the triterpenoids were studied using applicability of thin layer chromatography (TLC). A better separation with chloroform: methanol (9:1 v/v) was observed as determined by Tandon [13]. The results for the TLC run were captured on the visualizer and the photograph of the chromatographic plate is presented as Fig. 3. In all, 8 bands were identified after spraying of anisaldehyde–sulphuric acid reagent (Fig. 3). Colour of band, intensity of colour and R_f values for all the 8 bands were recorded (Table 5).

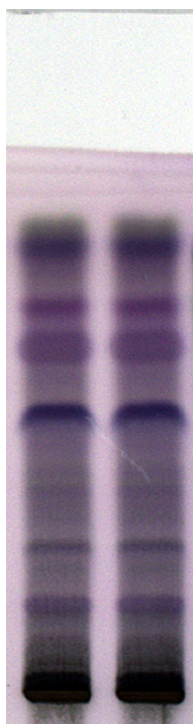


Fig. 3 – HPTLC plate image.

4. Discussion

In codified traditional medicine (like Ayurveda, Homeopathy, Sidhha) *A. aspera* is a well known drug and used to cure various disorders [13,14]. *A. coynei* and *A. aspera* belongs to the same family and genus [4]. Pai et al., reported that *A. coynei* is used by the local community for similar purposes to that of *A. aspera* because of the similarities in their morphological characters [4]. Pharmacognostical and physicochemical studies, being reliable and inexpensive, play an important role in quality control issues of the crude drug samples [15]. The macro and micro characters observed in the leaves of *A. coynei* serve as basis for the identification of right sample of the plant. Morphologically *A. coynei* was differentiated through its perennial shrubby nature and rosy-purplish coloured flowers on the inflorescence from *Achyranthes aspera* [4]. The petiole of *A. coynei* is pink above and green beneath, whereas in *A. aspera* petiole was pale green. The bigger size of leaves and typical petiole colour in *A. coynei* can be used to differentiate it from *A. aspera*. It is reported that *A. aspera* have 4–5 vascular bundles in leaf and petiole [13,14] whereas more number of vascular bundles were observed in *A. coynei*. This may be because of the larger size of leaves in *A. coynei*. However, the TS and powder study reveals similarities in *A. coynei* and *A. aspera* in terms of rosette shaped calcium oxalate crystals; long, multi cellular, warty, pointed apex and short, blunt glandular trichomes; anisocytic and anomocytic types of stomata.

Total ash, acid insoluble ash and water soluble ash parameters indicate the presence of inorganic and silica components in the sample studied [8]. The ash content of *A. coynei* lies within the range of *A. aspera* as reported in Ayurvedic

Table 5 – HPTLC analysis of *A. coynei* leaf extract.

R_f Values	Band colour and intensity ^a	Colour code ^b
0.17	Purple ²	#68566C
0.28	Dark purple ²	#62576A
0.38	Light blue ³	#736676
0.42	Light pink ³	#766B7C
0.53	Blue ¹	#382C5A
0.65	Indigo ¹	#6F5271
0.72	Violet ¹	#65456D
0.83	Dark blue ¹	#493A56

^a 1: High intensity; 2: medium intensity; 3: low intensity.

^b Codes as per Adobe Photoshop 7.

pharmacopeia [14]. The results of the water and ethanol extractive studies reveal the presence of secondary metabolite in the powder sample [8] and Upadhyia et al. reported considerable amount of total phenolic content in the leaves of *A. coynei*, which was further reported to be responsible for *in vitro* antioxidant activities [7]. Leaf powder display the florescence activity due to the presence of diverse chemical entities under the influence of ultraviolet light and different reagents.

The results of HPTLC analysis for triterpenoids from *A. coynei* leaf extract were in accordance with the reports of Tondon, wherein they have differentiated the TLC profile on the basis of R_f values. The band with R_f value 0.57 was attributed as oleanolic acid for *A. aspera* [13]. In the present study, similar observations for R_f value of 0.53 with intense blue colour band was recorded. This indicated the presence of oleanolic acid, as also reported by Upadhyia et al., using HPLC method [16].

5. Conclusion

The phytochemical studies reported in the present study need further scientific investigation to ascertain its identity up to compound level. Study on various biological activities similar to that of *A. aspera* are needed to substantiate the usage of *A. coynei* by folk healers. Pharmacognostic characters studied will be helpful in quantitative and qualitative standardization of *A. coynei*. However, detailed differential studies using molecular and chemical markers are required for *A. coynei* and *Achyranthes aspera*, for their authentication especially in their drug form.

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REFERENCES

- [1] Upadhyia V, Hegde HV, Bhat S, Hurkadale PJ, Kholkute SD, Hegde GR. Ethno medicinal plants used to treat bone fracture

- from North-Central Western Ghats of India. *J Ethnopharmacol* 2012;142:557–62.
- [2] Kingston DG. Modern natural products drug discovery and its relevance to biodiversity conservation. *J Nat Prod* 2011;74:496–511.
- [3] Narayana DBA, Katayar CK, Brindavanam NB. Original system: search, research or research. *IDMA Bull* 1998;29:413–6.
- [4] Pai SR, Upadhyia V, Hegde HV, Kholkute SD. *Achyranthes coynei* Santapau (Amaranthaceae) – an addition of endemic taxon to Flora of Karnataka, India. *J Threat Taxa* 2011;3:1875–9.
- [5] Sharma BD, Kulkarni BG. *Achyranthes coynei* Santapau, Amaranthaceae. In: Nayar MP, Sastry ARK, editors. *Red data book of Indian plants*, vol. 2. Calcutta: Botanical Survey of India; 1987. p. 8–9.
- [6] Ankad G, Upadhyia V, Pai SR, Hegde HV, Roy S. *In vitro* antimicrobial activity of *Achyranthes coynei* Sant. *Asian Pac J Trop Dis* 2013;3:930–5.
- [7] Upadhyia V, Pai SR, Ankad G, Hurkadale PJ, Hegde HV. Phenolic contents and antioxidant properties from aerial parts of *Achyranthes coynei* Sant. *Indian J Pharm Sci* 2013;75:483–6.
- [8] Hegde SV, Hegde GR, Mulgund GS, Upadhyia V. Pharmacognostic evaluation of leaf and fruit of *Capsicum frutescens* (Solanaceae). *Pharmacogn J* 2014;6:14–22.
- [9] Khandelwal KR. *Practical pharmacognosy*. 10th ed. Pune: Nirali Publication; 2003.
- [10] Thimmaiah SK. *Standard methods of biochemical analysis*. 1st ed. Calcutta: Kalyani Publisher; 1999.
- [11] Chopra SL, Kanwar JS. *Analytical agricultural chemistry*. 1st ed. Calcutta: Kalyani Publishers; 1991.
- [12] Kokoshi CJ, Kokoshi RJ, Salma FJ. Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J Pharm Sci* 1958;47:715–7.
- [13] Tandon N, editor. *Quality standards of Indian medicinal plants*, vol. 9. New Delhi: Indian Council of Medical Research; 2011.
- [14] Anonymous. *The ayurveda pharmacopeia of India part 2*, vol. 2. New Delhi: Department of AYUSH Ministry of Health and Family Welfare; 2007. p. 7–9.
- [15] Bigoniya P, Singh CS, Srivastava B. Pharmacognostical and physico-chemical standardization of *Syzygium cumini* and *Azadirachta indica* seed. *Asian Pac J Trop Biomed* 2012;S290–5.
- [16] Upadhyia V, Ankad GM, Pai SR, Hegde HV, Kholkute SD. Accumulation and trends in distribution of three triterpenoids in various parts of *Achyranthes coynei* determined using RP-UFLC analysis. *Pharma Mag* 2014;10:398–401.