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Pharmacognostic study and preliminary phytochemical investigation of *Andrographis paniculata* (Burm.f.) Wall. ex Nees

Shubhi Shukla [1], Rashmi Singh [1] and Manoj Tripathi [2,*]

[1] *Department of Botany, Govt. Auto. P.G. College, Satna (M. P.) India.*
[2] *Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.) India.*

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Abstract

Andrographis paniculata (family –Acanthaceae) is an annual herbaceous plant with branches that grows up to a height of 50-150 cm in moist, shaded areas. Its stem is abruptly quadrangular, heavily branched, and has a delicate texture that is readily broken. It is found in Southeast Asia, tropical and subtropical Asia, and a few other nations like Vietnam, Cambodia, and the Caribbean islands, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka and Thailand etc. It is widely used in different traditional medicinal systems or countries for medicinal purposes by the traditional practitioners, tribes or community as a folklore remedies. The present communication provides a detailed account of the pharmacognostic study carried out on various parts of the *Andrographis paniculata*. The study includes macroscopy, microscopy, powder microscopic studies, physicochemical tests, preliminary phytochemical screening, development of HPTLC (High Performance Thin Layer Chromatography) fingerprints profile and heavy metal tests. Physicochemical parameters were performed and found average values of three parts (root, stem and leaf) such as root loss on drying at 105^{0}C 4.93% w/w, total ash value 10.16% w/w, acid insoluble ash value 0.21% w/w, alcohol soluble extractive value 13.98% w/w and water soluble extractive value 25.77%w/w. Stem loss on drying at 105^{0}C 6.23% w/w, total ash value 6.88% w/w, acid insoluble ash value 0.25% w/w, alcohol soluble extractive value 18.46% w/w and water soluble extractive value 21.94%w/w and leaf loss on drying at 105^{0}C was found 6.29% w/w, total ash value 7.30% w/w, acid insoluble ash value 0.13% w/w, alcohol soluble extractive value 21.93% w/w and water soluble extractive value 38.68%w/w. HPTLC (High Performance Thin Layer Chromatography) fingerprints profile of methanolic extract was done by using mobile phase toluene: ethyl acetate (7:3). Andrographolide and Ferullic acid standard markers were applied, major spots Rf values with colour were recorded before derivatization at 254nm. Rf values are 0.25 black, leaf, stem and root with Andrographolide standard marker, and 0.20 light black color of stem, and root with Ferullic acid standard marker. Andrographolide is higher present in leaf which was range from 1.51-162 than the stem range1.46-153 and root range 1.45-1.150, while Ferulic acid was higher present in stem range from 0.91-0.96 than root 0.76-0.82 but absent in leaf. Heavy metals i.e Pb, Cd, As, & Hg were tested and found under WHO limits Established parameters can be used as standards for quality control and identification of the plant in herbal compound formulations and also preparation of a monograph of the plant.

Keywords: *Andrographis paniculata*; HPTLC fingerprinting; Andrographolide; Ferullic acid; Phyto-chemical screening; Pharmacognostic; Heavy metals

1. Introduction

People are using herbal medicines from centuries for safety, efficacy and cultural acceptability. Plants and plants derived products have utilized with varying success to cure and prevent diseases throughout history. Written records about medicinal plants date back at least 5000 years to the sumerians and ancient records are suggested earlier uses of medicinal plants. The reason for the herbal medicines popularity and acceptability is belief that all natural products are

* Corresponding author: Manoj Tripathi

safe, non-toxic, less side effects, easily availability and affordable prices and due to side effects of synthetic products. Therefore now days, there is a revival of interest with herbal based medicine due to the increasing realization of the healthcare [1, 2].

Andrographis paniculata another name is Kalmegh or "King of Bitters. The native habitats of the plant are Taiwan, Mainland China, and India. Additionally, Southeast Asia, tropical and subtropical Asia, and a few other nations like Vietnam, Cambodia, Caribbean islands, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand, and Vietnam are typical places to find it [3]**.** This plant can be found in China, America, the West Indies, and Christmas Island, spanning several phytogeographical and edaphic zones [4]. In China, India and other south-east asian nations, the medicinal herb *Andrographis paniculata* (Burm.f.) Nees family- Acanthaceae has been using for centuries to treat a wide range of chronic and infectious ailments, including fever, malaria, dysentery, diarrhoea, fevers herpes, sore throats, upper respiratory tract infections and gastrointestinal tract infections. According to the Indian Pharmacopoeia, it is a main ingredient in at least 26 Ayurvedic compound formulations. *Andrographis* is regarded in traditional Chinese medicine with a significant "cold property" that can be used to treat fevers and remove toxins from the body. It is widely used in Scandinavian nations to both prevent and treat common colds. The plant has hepatoprotective, antimicrobial, anti-inflammatory, antithrombotic, and immune-boosting qualities. The plant is used in Malaysian traditional medicine to treat hypertension and diabetes [5,6,7,8]**.** Several phyto-constituents *are containing in Andrographis paniculata* such as andrographolide, diterpenoids, neoandrographolide, and dehydroandrographolide. While andrographolide is present throughout the plant, it is primarily concentrated in the leaves. This diterpene has an unsaturated C-2 moiety that connects the lactone ring to the decalin ring system. Its pharmacological characteristics include antitumor, anticancer, antihepatotoxic, anti-HIV, hypoglycemic, and hypotensive effects. Because andrographolide is an intriguing pharmacophore with immunomodulatory and anticancer properties, it may also be explored as an anticancer chemotherapeutic drug [9]. Despite the numerous medicinal uses attributed to this plant, there are no systematic pharmacognostical studies on the several parts of this plant have so far been carried out. Hence the present work deals with the morphological, anatomical evaluation, physicochemical tests, preliminary phytochemical screening, heavy metals test, florescence study and High-Performance Thin Layer Chromatography.

2. Material and methods

2.1. Collection of samples

Whole fresh plant of *Andrographis paniculata* (Burm.f.) Wall. ex Nees was collected from the Arogyadham campus, Deendayal Research Institute in Chitrakoot, Satna (M.P.) India. Dr. Manoj Tripathi, Senior Scientist & Head (R&D Department), Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.), India, identified and verified the plant materials. Prepared the herbarium (voucher specimen, Govt./PGC/548), and placed in the unit of herbarium under department of botany, Government Autonomous Post Graduate, College, Satna (M.P.) for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of High Performance Thin Layer Chromatography fingerprint profile.

2.2. Macroscopic study

Macroscopic or organoleptic characters of *Andrographis paniculata* root stem and leaf like appearance, colour, odour and taste were evaluated [10].

2.3. Microscopic study

For transverse sections of *Andrographis paniculata* fresh root and stem were taken between the thumb and two fingers of right hand and sections were cut by using sharp razor held in the left hand, (for leaf used the potato pieces and put leaf in incision made on potato piece). Numerous sections cut and examined under microscope. The best sections were selected and transferred in to water containing watch glass, added the chloral hydrate solution. Sections were stained with phloroglucinol and hydrochloric acid (1:1) and the same was mounted in glycerin. Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 21l with Digi-eye camera using Caliper plus version 4.2 software [11].

2.4. Powder microscopic study

The shade dried root, stem and leaves of *Andrographis paniculata* were powdered and completely passed through 355 µm IS Sieve (old sieve number 44) and not less than 50% passel on through 180 µm IS Sieve (old sieve number 85) separately. Each about 2 g of powder washed thoroughly with potable water, poured out the water without loss of

material. Mounted a small portion in glycerin were used to all characters of the *Andrographis paniculata* root, stem and leaf. Small quantity of samples cleared by heating with chloral hydrate solution, wash and mounted in glycerin, treat a few mg with iodine solution and mount in glycerin, another small quantity of sample stained with sudan red solution and mounted with glycerin, all mounted slides were seen under microscope at 40 x 10x magnification of the Trinocular Research Microscope and captured the images [12]

2.5. Physico-chemical parameters

Physico-chemical tests were performed and set up the certain standards for *Andrographis paniculata* root, stem and leaf separately in order to avoid the batch-to batch variation and also to check their adulteration and quality. The study was also giving an idea regarding the nature of phyto-constituents present, quality, safety and efficacy of drugs. Physico-chemical tests of drugs powder were carried out using the methods prescribed in the Ayurvedic Pharmacopoeia of India and Quality Standards of Indian Medicinal Plants by subjecting them to various determinations like [13,14]. Physicochemical tests were includes moisture content (loss on drying at 105^0C), water soluble extractive value, alcohol soluble extractive value, total ash value and acid insoluble extractive value.

2.6. Preliminary phyto-chemical analysis

Chemical tests for screening and identification of bioactive chemical constituents present in the *Andrographis paniculata* root, leaf and stem samples such as water and alcoholic extract were used for the preliminary photochemical screening [15,16].

2.7. High Performance Thin Layer Chromatography (HPTLC) fingerprint profile

For high performance thin layer chromatography, the powdered 5 gm of each samples (root, stem and leaf) were extracted with 100 ml of methanol overnight, filtered and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of toluene*: ethyl acetate* (7: 3v/v) near ascending development was carried out in 10x10cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min. at room temperature. The length of chromatogram run was 8 cm. 20 ml of the mobile phase. Subsequent to the development, thin layer chromatography plate was dried with the help of Hot Air Oven. The peak area for samples and standard were recorded with camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with 5% Methanolic - sulphuric acid reagent) at UV light with Win cat software and R_f values noted [17, 18]

3. Results and discussion

3.1. Macroscopic characters

Figure 1 Plant

Figure 2 Root

Figure 3 Stem

Figure 4 Leaf

Andrographis paniculata root colour is brown, hard, thin and adventitious shape. Stem color is dark green fracture short, hard, woody to semiwoody, spherical, slender about 8-10mm thick, branched with swollen nodes and 4 winged projections, taste bitter and leaves color is dark green, lower surface granular, upper surface glabrous. Lower surface shows 4 to 8 pairs of lateral veins. Petiole short, winged, 4 to 7mm in length. Leaves are simple, opposite, thin, exstipulate, membranous, lanceolate, 2 to 8 cm in length and 1 to 2.5 cm in width. Entire to somewhat undulated, acute to acuminate (Figure 1-4).

3.2. Microscopic characters

Detailed Transverse Section (TS) of the *Andrographis paniculata* root shows 3-5 layers of cork cells. Secondary cortex represent by 3-12 layers of thick walled parenchymatous cells, some showing radial wall formation, tangentially elongated with sinuous walls. Seconday phloem composed of thin walled strands of sieve tubes, companion cells and phloem parenchyma. Secondary xylem composed of vessels, tracheids parenchyma and xylem fibres, all elements lignified and thick walled. Centre of wood more or less spongy and hollow in most cases. Outer woody ring remaining strongly lignified. Vessels show scalariform thickening and also simple and bordered pits, tracheids similar in thickening as the vessels. Fibres have simple pits. Mucilage present in secondary cortical cells. Minute acicular crystals present in abundance in secondary cortex and phloem region.

Transverse section of *Andrographis paniculata* of mature stem shows a layer of epidermis covered with thin cuticle and few sessile glandular trichome, interrupted at places with bigger sized cells embedded with cystolith. Cortex is narrow, the cells lying under the wing and at places in between the wings are collenchymatous cells, the remaining major cortical cells being chlorenchymatous. Endodermis is distinct. Phloem is narrow, it is traversed with few isolated thin-walled fibres and small acicular crystals of calcium oxalate. Few layers of cambium lies underneath it. Xylem is very wide, consist of few small sized isolated, scattered vessels, tracheids, fibres and parenchyma, the major elements being of fibres. Pith is parenchymatous, occasionally embedded with an acicular crystals of calcium oxalate and few simple starch grains.

And TS of *Andrographis paniculata* leaf shows upper and lower epidermis covered with thin cuticle, cells at places embedded with cystolith and stomata, the latter on lower sides only and bearing simple and glandular trichomes. Simple trichomes are 1 to 4 celled long and majority of them being located towards the margin of leaf. Glandular trichomes are with unicellular stalk and multicellular head. A layer of palisade runs under the upper epidermis, the remaining mesophyll tissue consists of 4 to 5 rows of spongy parenchyma, underneath both the epidermis of midrib lie few layers of collenchymatous tissue they being more celled in the lateral elevated region. The ground tissue of the midrib is parenchymatous and is embedded with an arc of the meristele.

3.3. Powder microscopic characters

Under microscope *Andrographis paniculata* root powder showed cork cells in surface view, group of fibres, prismatic crystals of calcium oxalate, spiral thickening and tracheids. Stem powder shows cork cells in surface view, cork cells in sectional view, prismatic crystals of calcium oxalate, tracheids, simple pitted vessels, thick walled fibres and parenchymatous cells filled with starch grains, prismatic and acicular crystals of calcium oxalate. And leaf powder shows various shape and size of glandular trichomes, epidermis of midrib in surface view, simple pitted vessels, cluster crystals of calcium oxalate, spiral thickening, simple pitted vessels, reticulate thickening, simple multicellular covering trichomes, fragments of fibres, lower epidermis in surface view showing striated cuticle with stomata, upper epidermis in surface view with stomata and palisade cell with spongy parenchyma containing a cluster crystal of calcium oxalate.

3.4. Physico-chemical analysis

The physico-chemical tests such as Loss on drying on 105^{0}C, extractive values such as water soluble extractive values, alcohol soluble extractive value, total ash value and acid insoluble ash value were performed. The results are expressed as mean (n=3) ± standard deviation in w/w. Results are given in **Table1.**

Table 1 Physico-chemical analysis of *Andrographis paniculata*

Name of parts	LOD (% w/w)	Mean (%)	Total ash (% w/w)	Mean (%)	AI ash (% w/w)	Mean (%)	ASE (% w/w)	Mean (%)	WSE (% w/w)	Mean (%)
Root	5.04	4.93	10.16	10.16	0.21	0.21	14.05	13.98	26.51	25.77
	5.56						13.10		25.50	
	4.21						14.80		25.30	
Stem	6.29	6.23	6.88	6.88	0.25	0.25	18.38	18.46	22.66	21.94
	6.22						18.15		21.00	
	6.20						18.85		22.18	
Leaf	6.30	6.29	7.30	7.30	0.13	0.13	22.66	21.93	38.27	38.68
	6.21						21.23		38.78	
	6.37						21.90		39.01	

It is observed that the leaf LOD value 6.29% is higher than the stem LOD 6.23% and root LOD 4.93%. Respectively total ash value was higher in root (10.16%) as compared to leaf (7.30%) and stem (6.88%). While, acid insoluble ash is higher in stem 0.25% than root 0.21% and leaf 0.13%. Water soluble extractives were higher than alcohol soluble extractives in root (25.77, 13.98%W/W), leaf (38.68, 21.93%W/W) and stem (21.94, 18.46%W/W). The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug.

3.5. Preliminary phyto-chemical investigation

Table 2 Preliminary phyto-chemical investigation of *Andrographis paniculata*

	Solvent	Acetone			Methanol			Ethanol			Water			Benzene			Diethyl ether			Chloroform		
S. no	Tests	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
1	Alkaloids	-	+	-	+	+	+	-	-	-	+	+	+	-	-	+	-	-	-	+	+	+
2	Carbohydrates	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	-	-	-	+	-	+
3	Proteins	+	+	-	-	+	-	+	-	-	+	+	+	-	-	-	+	-	-	+	-	+
4.	Resins	-	+	-	-	+	+	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-
5	Saponin	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	+
6	Flavonoid	-	+	-	+	+	-	+	-	+	+	-	+	-	-	-	+	-	-	-	-	-
7	Steroid	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Glycoside	-	-	+	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+
9	Tanni		+	+	+	+		+	+	+		+	+	-	-	-	+	-	-	-	+	+
10	Terpenoid	-	+	-	+	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	+	+

Preliminary phyto-chemical analysis was performed in petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water of *Andrographis paniculata* root, stem and leaf powder was carried out and outcomes are shown **in table 2** It was observed that the phytochemical higher present in aqueous extract than the other extracts. The results

indicated that the alkaloids were present in Petroleum ether, chloroform, benzene and water extracts of *Andrographis paniculata* leaf and stem while root was devoid of it. Flavonoid was present in chloroform, acetone, methanol and water extracts of *Andrographis paniculata* root while absent in leaf and stem. Saponins were present only in methanol and water extracts of *Andrographis paniculata* root. Methanol and water extracts of *Andrographis paniculata* leaf, root and stem contained carbohydrates. Phytosterols were found in petroleum ether and acetone extracts of *Andrographis paniculata* leaf. Acetone, methanol and water extracts of *Andrographis paniculata* root were found to contain tannins and phenolic compounds. Cardiac glycosides and coumarins were absent in petroleum ether, benzene, chloroform, acetone, methanol and water extract of *Andrographis paniculata* leaf, root and stem. Anthraquinone glycosides were present in chloroform, acetone, methanol and water extracts of *Andrographis paniculata* leaf. Dissimilar result of phytoconstituents in successive extracts of *Andrographis paniculata* leaf, stem and root helps in identification of plant.

3.6. HPTLC finger print profile

High performance thin layer chromatography (HPTLC) study of the methanolic extracts of *Andrographis paniculata* leaf, stem and root with Andrographolide and Ferullic acid standard marker spots applied in precoated TLC plate. Samples (leaf, stem and root) as well as standard markers (Andrographolide and Ferullic acid) were applied by spotting test solution 8 µl (each test solution leaf, stem and root) on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe. The samples, in the form of bands of length 6 mm were spotted 15 mm from the bottom, 15 mm from the left margin of the plate, and 10 mm part. And apply 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0, µl standard markers Andrographolide and Ferullic acid, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 µl on pre-coated silica-gel aluminum plate 60 F_{254} (10x20 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 µl Hamilton syringe and standard markers, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from the left margin the plate and 10 mm part.

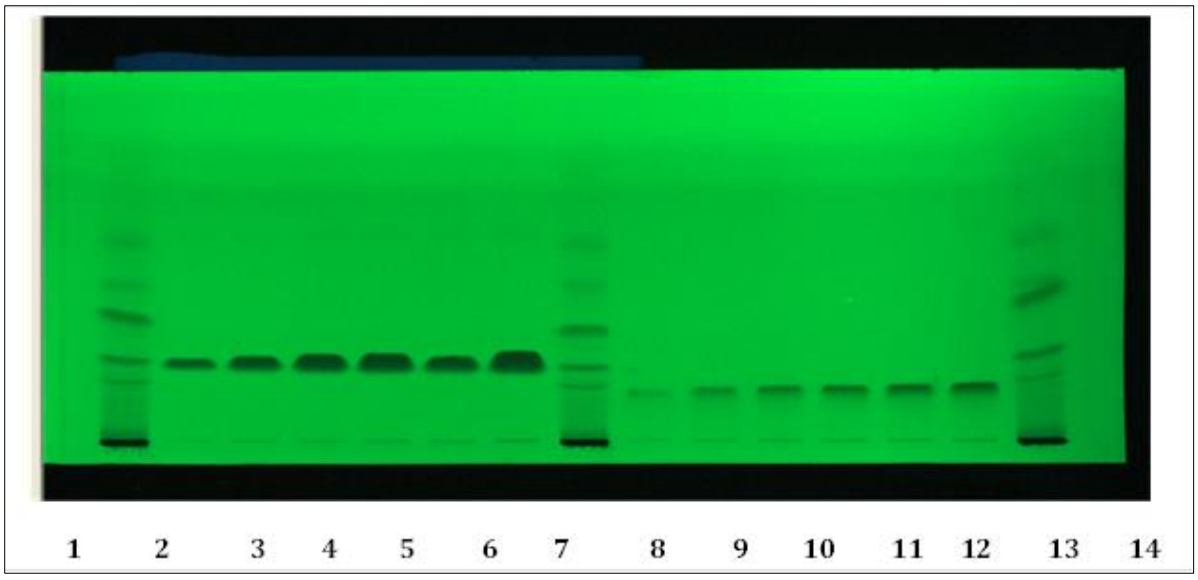

Figure 5 HPTLC Fingerprint profile of test solution of *Andrographis paniculata* (Leaf, Stem & Root) at 254nm before derivatization

Abbreviation- Track 1: test solution of *Andrographis paniculata* Leaf; Track 2-7 Andrographolide standard; Track 8: test solution of *Andrographis paniculata* stem; Track 9-14: Ferulic acid standard; Track 15: test solution of *Andrographis paniculata* root.

The plate was developed using a mobile phase consisting of *toluene: ethyl acetate* (7:5v/v). Linear ascending development was carried out in a 20x20cm twin through glass chamber equilibrated with the mobile phase. The optimized chamber saturation time for the mobile phase (20 ml) was 30 min at room temperature. The length of the chromatogram run was 85 cm. Subsequent to the development, a thin layer of chromatography plate was dried at room temperature. The peak area for samples and standards were recorded with the camera photo documentation system Camag Reprostar 3 and the plate was scanned densitometrically with the help of Scanner 4. Record the respective areas and prepare a calibration curve by plotting peak area *vs* concentration of standard markers Andrographolide and Ferullic acid. Major spots R_f values with colour were recorded before derivatization at 254nm. Major spots of R_f values before derivatization at 254nm major spots R_f values are 0.25 black, *Andrographis paniculata* leaf, stem and root with

Andrographolide standard marker, 0.20 light black *Andrographis paniculata* stem, and root with Ferullic acid standard marker. It is observed that the Andrographolide is higher present in *Andrographis paniculata* leaf range from 1.51-162 than the stem 1.46-153 and root 1.45-1.150, while Ferulic acid was present higher in *Andrographis paniculata* stem range from 0.91-0.96 than the root 0.76-0.82 but absent in leaf.

3.7. Heavy metals tests

Heavy metals detected through Atomic Absorption Spectrophotometer in *Andrographis paniculata* root, stem and leaf as per described standard method. The results obtained in ppm and ppb level and found within limits as per guideline of WHO/API for heavy metals. As per obtained results of heavy metals, it was observed that the screened metals Pb, Cd, As and Hg are detected in very low values, means samples are safe and not harmful for the health.

Table 3 Determination of heavy metals in *Andrographis paniculata*

S. No.	Parameter	*Andrographis paniculata*			Actual Conc. Unit	API Limits
		Root	Stem	Leaf		
1.	Lead (Pb)	7.5612	6.9812	7.0371	ppm	10 ppm
2.	Cadmium (Cd)	0.04561	0.4317	0.0921	ppm	0.3 ppm
3.	Arsenic (As)	4.4509	4.9213	4.6721	ppb	03 ppm
4.	Mercury (Hg)	6.0912	6.3421	6.3671	ppb	01 ppm

The macroscopic, microscopic and powder microscopic distinguished characters have been established to identify *Andrographis paniculata* different parts such as root, stem and leaf. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC finger print profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The HPTLC profile also helps to identify and isolate's important phyto-constituents. Major spots R_f values are found 0.25 black, *Andrographis paniculata* leaf, stem and root with Andrographolide standard marker, 0.20 light black *Andrographis paniculata* stem, and root with Ferullic acid standard marker. It is observed that the Andrographolide concentration is higher present in *Andrographis paniculata* leaf range from 1.51-162 than the stem 1.46-153 and root 1.45-1.150, while Ferulic acid was present higher in *Andrographis paniculata* stem range from 0.91-0.96 than the root 0.76-0.82 but absent in leaf. Heavy metal elements are found under limits as per guideline WHO. These finding could be helpful in identification and authentication of *Andrographis paniculata* various parts.

4. Conclusion

Due to the side effects of modern medicines on human health, the importance and uses of herbal medicines are increasing day by day all over the world. Because the plants have natural chemicals which do not have any adverse side effects on human health. But the herbal medicines however, suffering from lack of standardization parameters and quality control. Hence the standardization and quality control of herbal drug is very important. *Andrographis paniculata* is one of the most important plant of India and other countries and its different parts such as root, stem, leaf, flowers and seeds are used to treat different types of human ailments and diseases such as fever, malaria, dysentery, diarrhoea, fevers, herpes, sore throats, upper respiratory tract infections and gastrointestinal tract infections and preparation of ayurvedic compound formulations. Due to its wide therapeutic importance, it is worthwhile to standardize it for use as drug.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest.

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