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Pharmacognostic Standardization of the phytochemicals in *Andrographis paniculata* leaves and *Phyllanthus emblica* fruit for evaluation of Anthelmintic activity

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Abstract

The purpose of this study was to assess the anthelmintic activity of *Phyllanthus emblica* and *Andrographis paniculata* using qualitative phytochemical analysis and Pharmacognostical standards. To determine their efficacy, these plants aqueous and ethanolic extracts were tested against the (Earthworm) *Pheretima posthuma*. *Phyllanthus emblica* and *Andrographis paniculata* were purchased from the local Indian market. Ethanol and distilled water were used as solvents to create the extracts for anthelmintic screening from dried leaves and fruits. First, using established procedures, the phytochemical makeup of both plants was examined. After that, the anthelmintic activity was assessed against *Pheretima posthuma* (Earthworm), which was procured from a nearby vermicomposting farm, Lucknow. Glycosides, alkaloids, flavonoids, saponins, fixed oils, and tannins were detected by phytochemical screening in *Andrographis paniculata* and *Phyllanthus emblica*. With paralysis and death durations of 12 ± 0.4 and 28 ± 0.2 minutes, respectively, the aqueous extract (50 mg/mL) showed the maximum effectiveness in the anthelmintic investigation. Albendazole (20 mg/mL), a common medication, showed death and paralysis time of 14 ± 0.1 and 7 ± 0.7 minutes, respectively. The study verified the anthelmintic potential of *Phyllanthus emblica* and *Andrographis paniculata* against *Pheretima posthuma*. According to the research, these plants have strong anthelmintic qualities and might be used as substitute therapies for worm infestations.

Keywords: Phyllanthus emblica, Andrographis paniculata, phytochemical analysis.

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1. Introduction

For generations, Andrographis paniculata has been a vital component of traditional Asian medicine. Known for its "blood-purifying" qualities, A. paniculata, also called Chirayetah in Urdu and Kalmegh in Hindi, is an annual plant that grows one to three feet tall and is used extensively in Ayurveda and Unani therapy. It is known as the "King of Bitters" or "Creat" in English. There are flavonoids, lactones, and diterpenes in Andrographis paniculata. Although they have been separated from the leaves, flavonoids are mostly found in the root. The plant's alkanes, ketones, and aldehydes are found in its aerial sections [2]. At first, the lactone andrographolide was thought to be the bitter ingredient in the leaves. Nevertheless, more investigation showed that the leaves contain two bitter substances: kalmeghin and andrographolide. Furthermore, six ent-labdane-type diterpenoids, two diterpene glucosides, and four diterpene dimers (bis-andrographolides A, B, C, and D) have been extracted from the aerial parts [3].

In addition, two novel flavonoid glycosides, a new diterpenoid (andrographic acid), and 12 new flavonoids and 14 diterpenoids have been identified from the aerial portions. From the aerial portions, two novel ent-labdane diterpenoid glycosides were recently identified [4]. The antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, and adaptogenic properties of Andrographis paniculata are well-known. It is classified as an emmenagogue, diuretic, aperient, anti-inflammatory, emollient, astringent, carminative,

antihelmintic, and antipyretic in the Unani system [5]. It can be used to cure scabies, leprosy, gonorrhea, boils, skin eruptions, and both seasonal and chronic fevers because of its "blood purifying" qualities. Infants are given juice or an infusion of fresh leaves to help with loss of appetite, irregular bowel movements, and griping [6]. The herb is referred to as Lanhelian, Yijianxi, or Chuanxinlian in China. It is used to eliminate "pathogenic heat" from the blood and is said to be chilly and bitter [7]. It also has detoxifying, anti-inflammatory, antipyretic, and detumescent qualities. The herb is used to cure snake bites, carbuncles, sores, coughing up thick sputum, diarrhea, dysentery, and pharyngolaryngitis. Herpes zoster, chickenpox, mumps, neurodermatitis, eczema, burns and other ailments have all been successfully treated with various preparations of the herb, both infectious and non-infectious [8].

Figure 1: *Andrographis paniculata*

Figure 2: *Phyllanthus emblica*

2. Materials and methods

2.1 Collection and extraction of plants parts

The fresh leaves and fruits, were collected from the local store and venders in the month of October [9]. ***Andrographis paniculata*** and ***Phyllanthus emblica*** was collected and purchased from the local market. The taxonomical identification of the plant was done at Centre of Advanced Study in Botany Institute of sciences Banaras Hindu University, Varanasi, India. The collected fresh leaves of ***Andrographis paniculata***, Fruits of ***Phyllanthus emblica*** dried in hot air oven at 40ºC to avoid degradation of phyto constituents [10].

After drying, plant materials were coarsely powdered with grinding mill and kept in well-closed container **Figure 3** and **Figure 4.** About 30 g powder of each specimen, respectively were defatted with petroleum ether (60—80ºC) in a Soxhlet apparatus followed by extraction with ethanol and distilled water [11]. The collected extracts were further concentrated on rotary evaporator and were kept in a vacuum dryer until used.

Figure 3: Dried leaves of Andrographis *paniculata* crude plant extract

Figure 4: Dried fruits of *Phyllanthus emblica* crude plant extract

2.2 Phytochemical analysis of extracts

Using established methods, the extract residues from each plant were examined for the presence of phyto-constituents, including proteins, reducing sugars, alkaloids, tannins, flavonoids, saponins, glycosides, resins, triterpenes, and glycosides [12].

2.3 Pharmacognostical evaluation of the plant materials

2.3.1 Morphological studies:

The organoleptic properties of the plant were assessed by looking at its colour, odour, taste, size, shape, and distinctive morphological features, such as texture. As part of the quantitative microscopic analysis, the stomatal number and stomatal index were measured on fresh plant leaves [13].

2.3.2 Microscopical studies

Water was used to soften the leaves of ***Andrographis paniculata*** and Fruits of ***Phyllanthus emblica***. General observations were made using bright-field

microscopy, whereas crystals, starch grains, and lignified cells were examined using polarized light [14].

2.3.3 Powder analysis

A few drops of chloral hydrate solution **were added to the** powdered plant material **placed on a slide, then** covered with a cover slip **and** gently heated over a Bunsen burner**, taking care to** avoid vigorous boiling [15]**. Once the** clearing process was complete**, the slide was examined under a** microscope**. A** drop of glycerol **was then added to the slide to** prevent crystallization **upon cooling [16].**

2.4 Experimental animals

Anti-Helmintic activity the anti-Helmintic activity was evaluated on adult Indian earthworm, ***'Pheretima posthuma'*** as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings (**Figure 7**).

Figure 7: Adult Indian earthworm (*Pheretima posthuma*)

2.5 Anti-helmintic screening

All the earthworms were washed into normal saline solution before they are released in to Petri dishes. Indian adult earthworms (***Pheretima posthuma***) with a length of 6–8 cm were employed for the anthelmintic activity investigation [17]. There were twenty-two groups of earthworms, each consisting of six earthworms. Sterile distilled water was used to dissolve ethanol and aqueous extracts of ***Andrographis paniculata*** and ***Phyllanthus emblica*** at concentrations of 10, 20, 30, 40, and 50 mg/ml [18]. The final volume was adjusted to 50 ml. Prior to the trials, the extracts and the common medication Albendazole (20 mg/ml) were both newly made. Separate Petri plates were filled with the standard solution and the various extract concentrations [19]. A standard saline solution was used to rinse all of the earthworms before they were put into the Petri plates. Observation was made for time taken to paralyze (paralysis was said to occur when earthworms didn't revive in normal saline) and death (death was concluded when earthworms lost their motility and followed with their body colour fading away) [20]. All the results were expressed as a mean ± SEM of six earthworms in each group.

Figure 8: Aqueous Extract of Indian adult earthworms (*Pheretima posthuma*), *Andrographis paniculata* and *Phyllanthus emblica* at various concentrations

Figure 9: Ethanolic Extract of Indian adult earthworms (*Pheretima posthuma*), *Andrographis paniculata* and *Phyllanthus emblica* at various concentrations

3. Results and discussion

The methanolic extract of ***Andrographis paniculata*** and ***Phyllanthus emblica*** was brownish black and brownish in colour and solid in consistency with an extractability of 5.65% and 5.44%. The aqueous extract of ***Andrographis paniculata*** and ***Phyllanthus emblica*** it was firm in substance, extractable and dark brown in colour and with an extractability of 4.55% and 4.87%. **(Table 1)**

Table 1: Extractability and physical properties of *Andrographis paniculata* and *Phyllanthus emblica*

S. No	Physical Properties	*Andrographis paniculata*		*Phyllanthus emblica*	
		Ethanolic Extract	**Aqueous Extract**	**Ethanolic Extract**	**Aqueous Extract**
1	Colour	Brownish black	Dark brown	Brownish	Dark brown
2	Consistency	Solid	Solid	Solid	Solid
3	Extractability	5.65%	4.55%	5.44%	4.87%

3.1 Phytochemical analysis of extracts

In phytochemical analysis, ethanolic and aqueous extracts of ***Andrographis paniculata*** and ***Phyllanthus emblica,*** **(Figure 10)** it verified that reducing sugars, tannins, saponins, and fixed oils were present as common elements. Alkaloids were also included as an additional component in the ethanolic extract. **(Table 2)**

Figure 10: Phytochemical Analysis, Ethanolic and Aqueous Extracts Of *Andrographis Paniculata* and *Phyllanthus Emblica*

Table 2: Phytochemical analysis of *Andrographis paniculata* and *Phyllanthus emblica* extracts

S. No	Active Principle	Test Applied	*Andrographis paniculata*		*Phyllanthus emblica*	
			Ethanolic	**Aqueous**	**Ethanolic**	**Aqueous**
1	Alkaloids	a) Dragendorff test b) Wagner's test	**(-)** **(-)**	**(-)** **(-)**	**(+)** **(+)**	**(-)** **(-)**
2	Reducing sugars	Benedict's test	**(+)**	**(+)**	**(+)**	**(+)**
3	Glycosides	Benedict's test	**(-)**	**(-)**	**(-)**	**(+)**
4	Tannins	a)Lead acetate test b)Ferric chloride test	**(+)** **(-)**	**(+)** **(+)**	**(+)** **(+)**	**(+)** **(+)**
5	Resins	Hydroalcoholic extract solution in distilled water	**(-)**	**(-)**	**(-)**	**(-)**
6	Saponins	Foam test	**(+)**	**(+)**	**(+)**	**(+)**
7	Sterols	Salkowski test	**(-)**	**(-)**	**(-)**	**(-)**
8	Fixed oils	Filter paper test	**(+)**	**(+)**	**(+)**	**(+)**
9	Proteins	Biuret test	**(-)**	**(-)**	**(-)**	**(-)**
10	Anthroquinones	Bontrager's test	**(-)**	**(-)**	**(-)**	**(-)**
11	Flavonoids	Addition of diluted NaOH solution and diluted HCl	**(-)**	**(-)**	**(+)**	**(-)**

3.2 Pharmacognostical evaluation of *Andrographis paniculata* morphological studies:

The leaf has thick lamina and prominent midrib. The lamina is uniformly smooth and even. The midrib has short conical adoxial hump and a wide hemispherical abaxial part. The midrib measures 500 µm both in vertical and horizontal planes. The adaxial hump has thin layer of epidermis and a mass of collenchyma cells

3.2.1 Microscopical studies

T.S. of leaf passing through mid-rib shows laterally extended lamina region through mid-rib shows centrally located vascular bundle. Single layer upper epidermal cells are interrupted by few cystoliths and sessile glandular head trichrome, lower epidermis single layered cells smaller than upper epidermis.

T.S. through mid-rib shows upper epidermis followed by3-4 layer of collenchymatous cells, gives mechanical support to the centrally situated vascular bundle which consist of xylem and phloem covered by ground tissue from both sides, lower epidermal cells smaller and often intercepted by cystoliths.

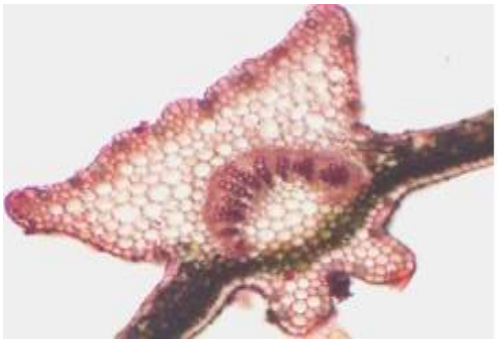

Figure 11: Midrib

Figure 13: T.S. Lamina

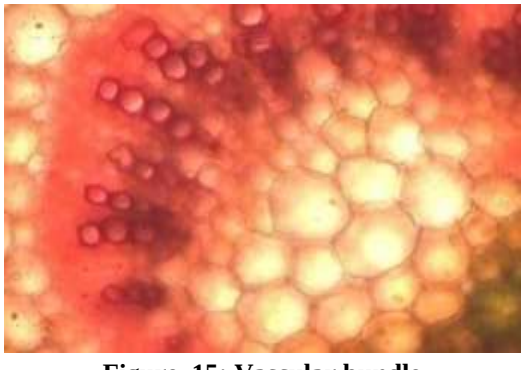

Figure 15: Vascular bundle

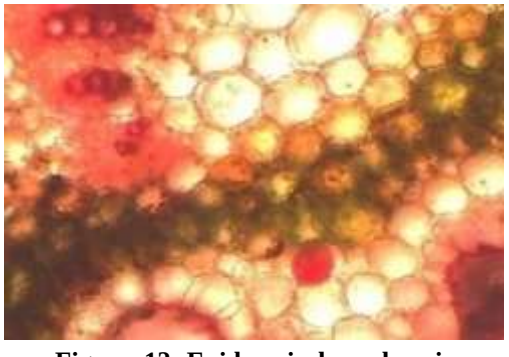

Figure 12: Epidermis, hypodermis

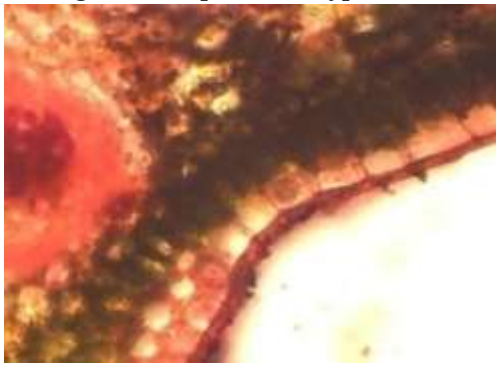

Figure 14: Xylem and phloem

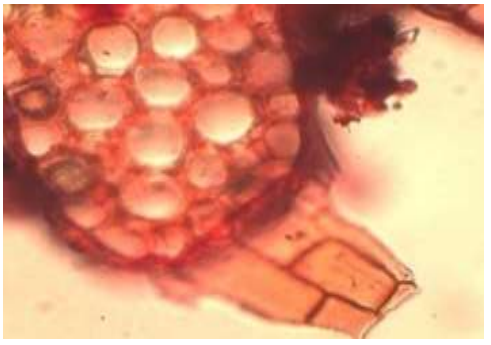

Figure 16: Glandular trichrome

3.2.2 Powder analysis

The leaf powder is dark green in colour with an unpleasant Odour and intensely bitter in taste. On microscopically examination the powder showed numerous multi cellular (1- 5 celled) covering trichrome which are, sometimes broken and rarely collapsed. Diacytic or caryophyllaceous type of stomata with irregularly shaped epidermal cells are seen. The stomata occur on the lower epidermis; the common wall of the subsidiary cells is at right angles to the long axis of the stomata. Some of the stomata have more than one set of subsidiary cells; the guard cells are either elliptic or circular.

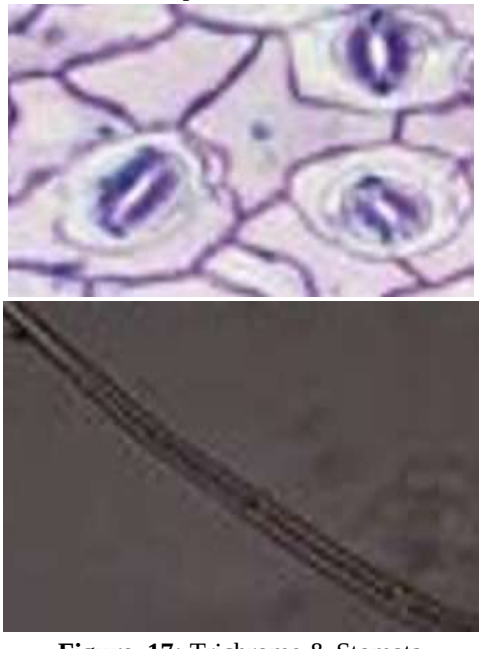

Figure 17: Trichrome & Stomata

3.3 Pharmacognostical evaluation of *Phyllanthus emblica*

3.3.1 Morphological studies

The fresh fruit is globular, fleshly, obscurely six lobed. The surface is smooth and pale or yellowish green in colour. The fruits contain a juicy pulp enclosing an obtusely, obvate, triangular, 3 celled nuts, containing two seed in each cell. The organoleptic characters shows that fruit of wild variety is strong in Astringent taste with pale yellow colour and characteristic Odour, whereas cultivated has less astringent taste, greenish colour and sour smell.

3.3.2 Microscopical studies

Epicarp

Epidermis: The outermost part of the epicarp consist single layer of epidermis covered externally with a cuticle, epidermal cells are tabular in shape and appear in surface view as polygonal.

Hypodermis: The inner part of the epicarp just below the epidermis, is made up of 2-4 layers of Hypodermis of tangentially elongated cells, thick- walled, smaller in dimension than epidermal cells.

Mesocarp: Inner to the epicarp, mesocarp forms bulk of fruit, consisting of thin-walled parenchymatous cells with intercellular spaces, peripheral 6-9 layers smaller, ovoid or tangentially elongated while rest of cells larger in size, isodiametric and radially elongated; several collateral fibrovascular bundles scattered throughout mesocarp consisting of xylem and phloem; xylem composed of tracheal elements, fiber tracheids and xylem fibers; tracheal elements show reticular scalariform and spiral thickenings.

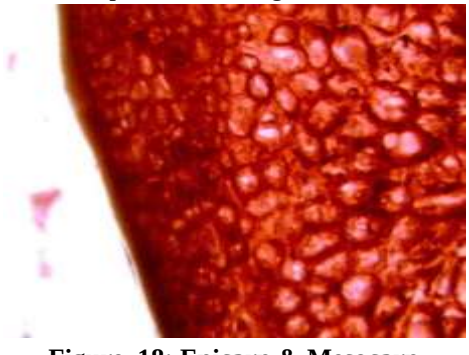

Figure 18: Epicarp & Mesocarp

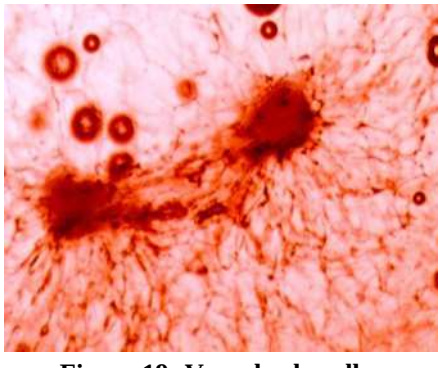

Figure 19: Vascular bundles

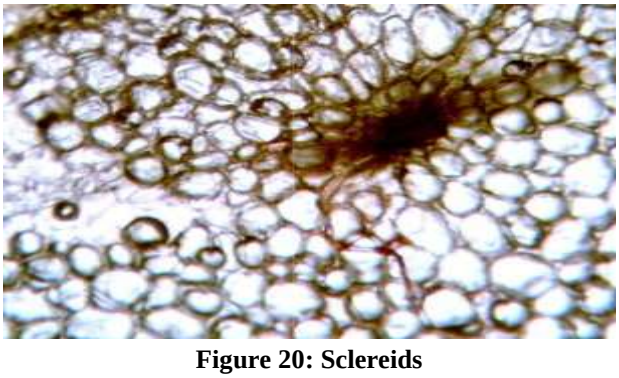

Figure 20: Sclereids

3.3.3 Powder analysis

Powder of both varieties of Amalaki shows fragments of uniformly thickened epidermal cells, mesocarp cells, sclereids, fibres, stone cells, prismatic crystals of silica and oil globules but the wild variety shows more fibres, silica crystals, stone cells and sclereids than cultivated variety.

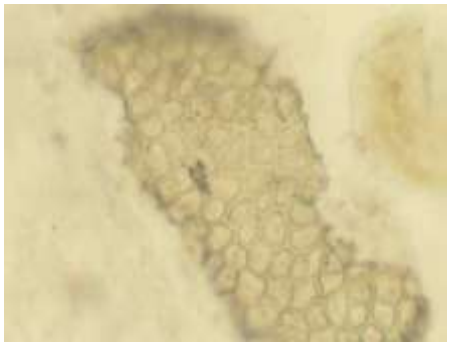

Figure 21: Fragment of Epicrap

Figure 22: Fibre

Figure 22: Stone cell

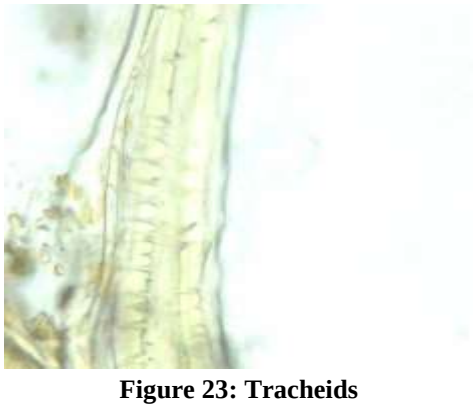

Figure 23: Tracheids

3.4 Anti-helmintic screening

Anti-Helmintic Screening was done using, control solution (Distilled water), Control Solution (Albendazole-20 mg/ml) and aqueous extracts ***Andrographis paniculata*** and ***Phyllanthus emblica*** at concentrations of 10, 20, 30, 40 and 50 mg/ml. Observation was made for time taken to paralyze (paralysis was said to occur when earthworms didn't revive in normal saline) and death (death was concluded when earthworms lost their motility and followed with their body colour fading away). All the results were expressed as a mean ± SEM of six earthworms in each group.

Figure 24: Effect of *Andrographis paniculata* and *Phyllanthus emblica* extracts in Aqueous medium

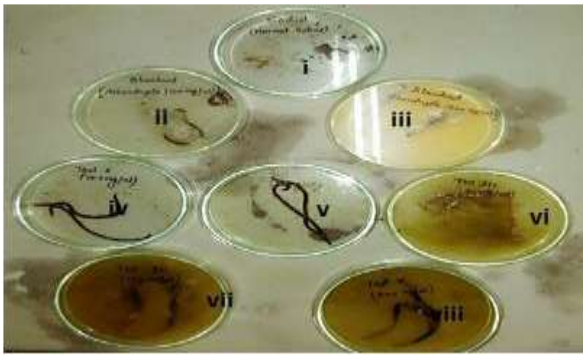

Figure 25: Effect of ***Andrographis paniculata*** and ***Phyllanthus emblica*** extracts in Ethanolic medium, Distilled Water (Control) and Standard Albendazole (20 mg/ml)

Table 3: Anti-Helmintic Activity of Distilled Water (Control) and Standard Albendazole (20 mg/ml) against Earthworm

Treatment	Time taken by earthworms for	
	Paralysis (min) mean ± SEM	Death (min) mean ± SEM
Distilled Water (Control)	00	00
Standard Albendazole (20 mg/ml)	07 ± 0.7	14 ± 0.1

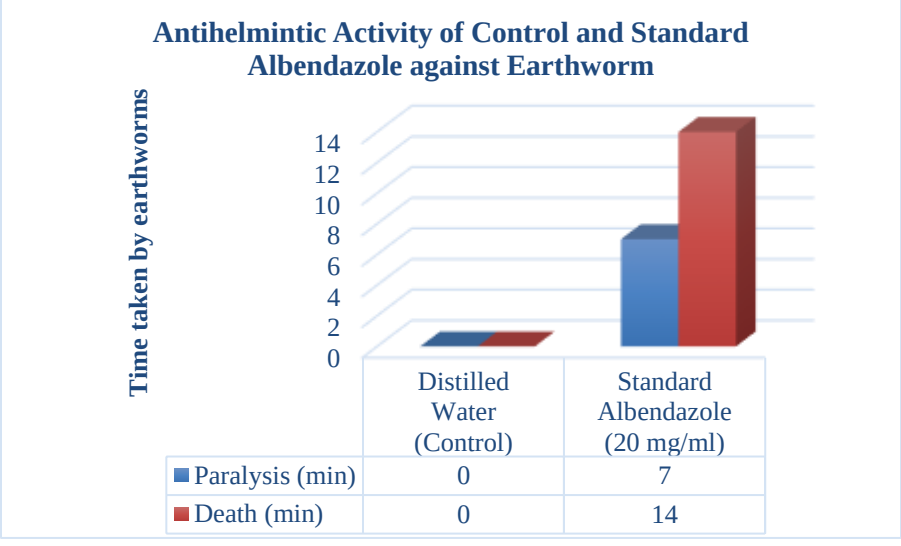

Figure 26: Anti-Helmintic Activity of Distilled Water (Control) and Standard Albendazole (20 mg/ml) against Earthworm

Table 4.: Anti-Helmintic Activity of Aqueous Extract - *Andrographis paniculata* against Earthworm

Treatment	Time taken by earthworms for	
	Paralysis (min) mean ± SEM	Death (min) mean ± SEM
Aqueous Extract of *Andrographis paniculata*		
10 mg/ml	108 ± 0.5	128 ± 0.7
20 mg/ml	101 ± 0.1	114 ± 0.1
30 mg/ml	94 ± 0.5	108 ± 0.8
40 mg/ml	65 ± 0.2	69 ± 0.5
50 mg/ml	54 ± 0.3	57 ± 0.9

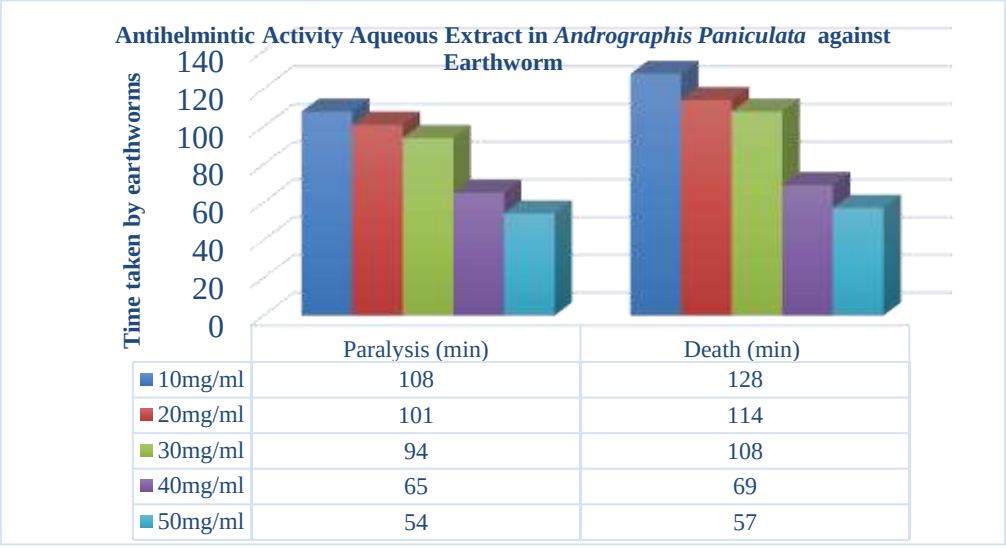

Figure 27: Anti-Helmintic Activity of Aqueous Extract - *Andrographis paniculata* against Earthworm

Table 5: Anti-Helmintic Activity of Ethanolic Extract - *Andrographis paniculata* against Earthworm

Treatment	Time taken by earthworms for	
	Paralysis (min) mean ± SEM	Death (min) mean ± SEM
Ethanolic Extract of *Andrographis paniculata*		
10 mg/ml	143 ± 0.1	155 ± 0.2
20 mg/ml	121 ± 0.3	144 ± 0.4
30 mg/ml	107 ± 0.4	124 ± 0.1
40 mg/ml	99 ± 0.9	109 ± 0.3
50 mg/ml	87 ± 0.1	98 ± 0.5

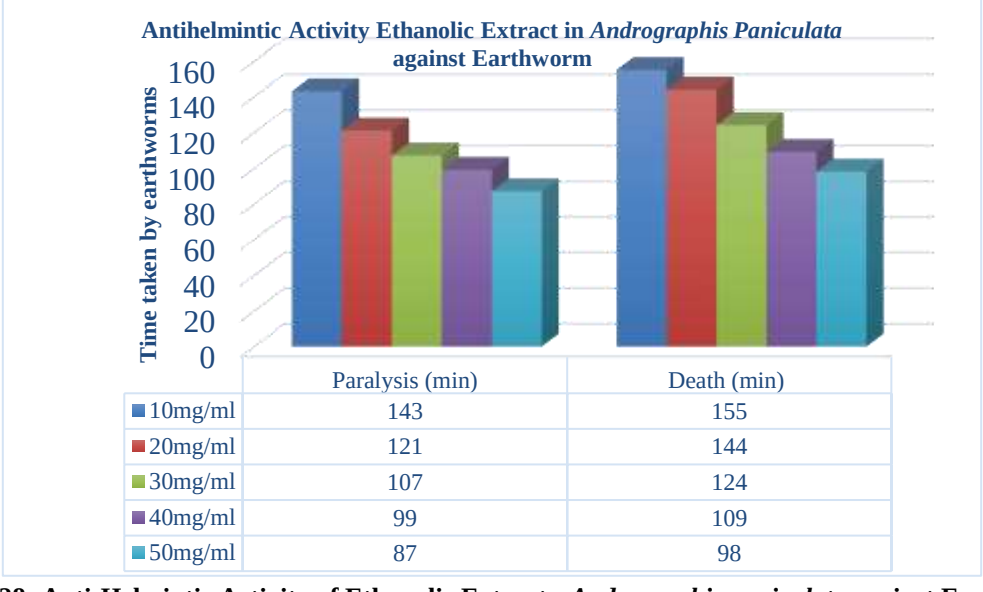

Figure 28: Anti-Helmintic Activity of Ethanolic Extract - *Andrographis paniculata* against Earthworm

Table 6: Anti-Helmintic Activity of Aqueous Extract - *Phyllanthus emblica* against Earthworm

Treatment	Time taken by earthworms for	
	Paralysis (min) mean ± SEM	Death (min) mean ± SEM
Aqueous Extract of *Phyllanthus emblica*		
10 mg/ml	101 ± 0.1	111 ± 0.4
20 mg/ml	99 ± 0.3	107 ± 0.2
30 mg/ml	87 ± 0.7	98 ± 0.6
40 mg/ml	55 ± 0.5	77 ± 0.3
50 mg/ml	34 ± 0.7	61 ± 0.1

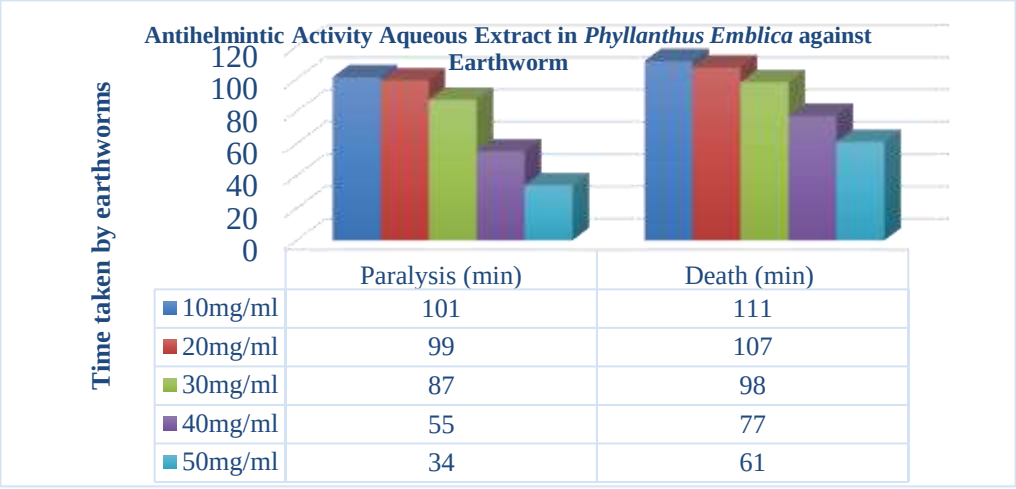

Figure 29: Anti-Helmintic Activity of Aqueous Extract - *Phyllanthus emblica* against Earthworm

Table 7: Anti-Helmintic Activity of Ethanolic Extract - *Phyllanthus emblica* against Earthworm

Treatment	Time taken by earthworms for	
	Paralysis (min) mean ± SEM	Death (min) mean ± SEM
Ethanolic Extract of *Phyllanthus emblica*		
10 mg/ml	123 ± 0.1	147 ± 0.2
20 mg/ml	111 ± 0.3	134 ± 0.4
30 mg/ml	101 ± 0.4	114 ± 0.1
40 mg/ml	91 ± 0.9	102 ± 0.3
50 mg/ml	82 ± 0.1	92 ± 0.5

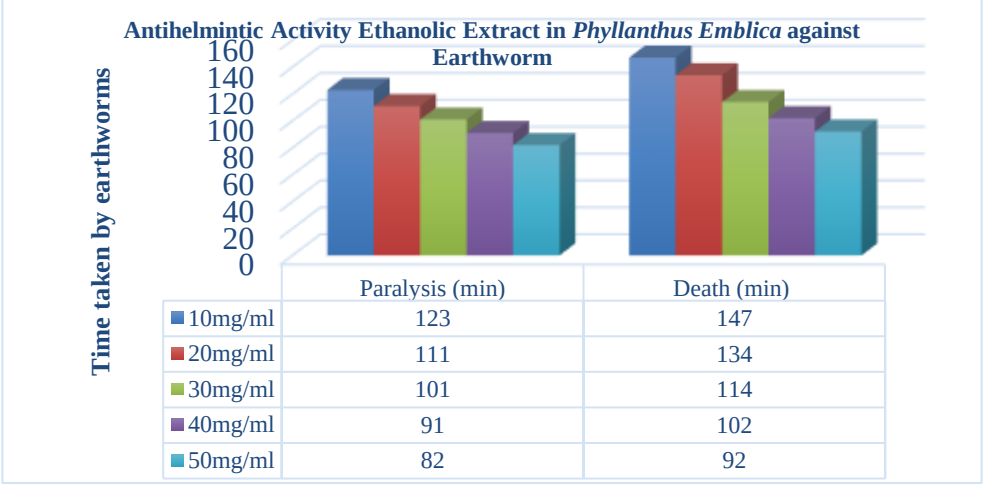

Figure 30: Anti-Helmintic Activity of Ethanolic Extract **- *Phyllanthus emblica*** against Earthworm

Table 8: Anti-Helmintic Activity of Aqueous Extract - *Andrographis paniculata* and *Phyllanthus emblica* (Both together) against Earthworm

Treatment	Time taken by earthworms for	
	Paralysis (min) mean ± SEM	Death (min) mean ± SEM
Aqueous Extract of *Andrographis paniculata* and *Phyllanthus emblica* (Both together)		
10 mg/ml	104 ± 0.7	118 ± 0.5
20 mg/ml	87 ± 0.1	100 ± 0.1
30 mg/ml	46 ± 0.8	64 ± 0.9
40 mg/ml	24 ± 0.6	42 ± 0.3
50 mg/ml	12 ± 0.4	28 ± 0.2

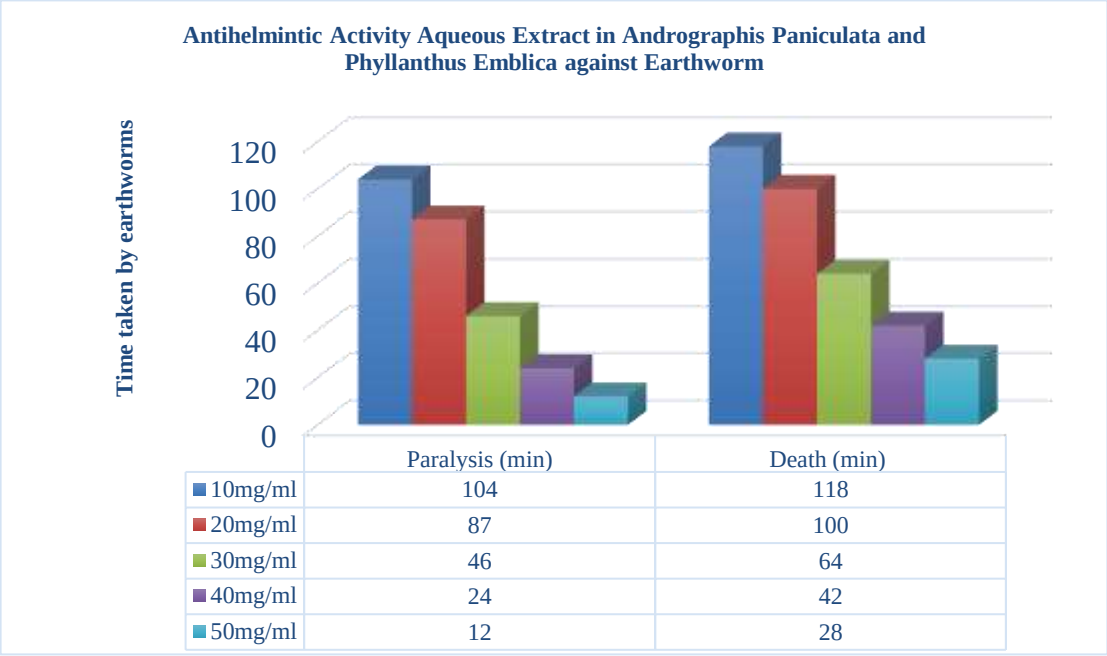

Figure 31: Anti-Helmintic Activity of Aqueous Extract - *Andrographis paniculata* and *Phyllanthus emblica* (Both together) against Earthworm

Table 9: Anti-Helmintic Activity of Ethanolic Extract - *Andrographis paniculata* and *Phyllanthus emblica* (Both together) against Earthworm

Treatment	Time taken by earthworms for	
	Paralysis (min) mean ± SEM	Death (min) mean ± SEM
Ethanolic Extract of *Andrographis paniculata* and *Phyllanthus emblica* (Both together)		
10 mg/ml	118 ± 0.4	131 ± 0.7
20 mg/ml	95 ± 0.8	114 ± 0.2
30 mg/ml	67 ± 0.3	89 ± 0.5
40 mg/ml	48 ± 0.1	64 ± 0.6
50 mg/ml	30 ± 0.2	38 ± 0.8

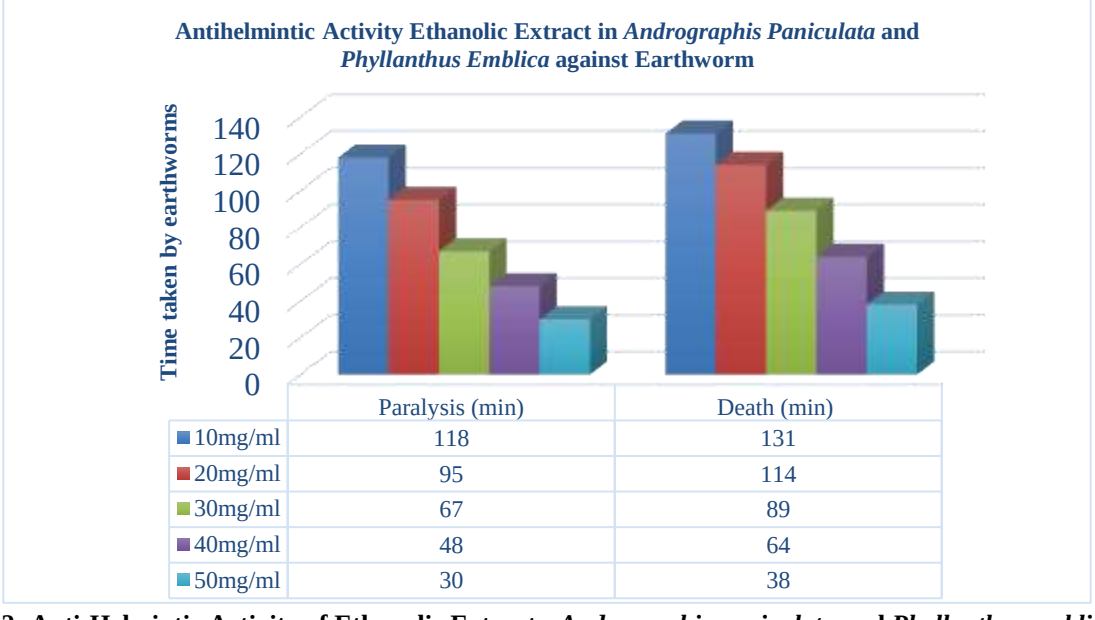

Figure 32: Anti-Helmintic Activity of Ethanolic Extract - *Andrographis paniculata* and *Phyllanthus emblica* (Both together) against Earthworm

4. Conclusion

Using *Pheretima posthuma*, the anthelmintic qualities of the aqueous and ethanolic extracts of *Andrographis paniculata* and *Phyllanthus emblica* were assessed in relation to the common medication Albendazole. These extract potential as antihelmintic agents is demonstrated by their capacity to paralyze and kill worms. With paralysis and death durations of 12 ± 0.4 and 28 ± 0.2 minutes, respectively, the aqueous extracts of *Andrographis paniculata* and *Phyllanthus emblica* were shown to be most efficacious at a concentration of 50 mg/ml. strategy that shows promise is screening medicinal herbs for anthelmintic action. The water extract's wormicidal action against earthworms indicates that it may be useful in treating human parasite infections. To determine the active ingredient causing this anthelmintic activity and assess its pharmacological characteristics, more research is required.

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