Phytochemical evaluations and Anthelmintic potential of aerial parts of *Senecio laetus*

[1]Nazia Banday, [2]Prof. Z.A.Bhat
[1]PhD Scholar, [2]Head of Department (Prof.)
[1,2] Department of Pharmaceutical Sciences, University of Kashmir, Srinagar, Jammu and Kashmir, India

Abstract*:** Anthelmintics from the natural sources may play a key role in the treatment helminthiasis. Owing to its natural origin and lesser side effects, it is getting popularized in developing and developed countries. Researches are being carried out now-a-days on large scale to discover the herbal alternatives for various allopathic medicines. Anthelmintic drugs are one such example for which herbal alternatives are being searched. In view of this, an attempt has been made to study the anthelmintic activity in the aerial parts of the plant under investigation, **"Senecio laetus***"**. Since, there is no previous literature available about the anthelmintic activity of the *Senecio laetus*, the present investigation serves as the first report.

Key words: Antihelmintics, herbal, Senecio laetus

I. Introduction

Helminthiasis is one of the most common parasitic diseases leading to severe morbidity by affecting population in endemic areas with major economic and social consequences (Tagbota and Townson, 2001). It is world wide spread disease of all ages especially in third world countries, affecting a large proportion of the world's population (Gnaneswari et al., 2013).

In developing countries, they pose a large threat to public health and contribute towards prevalence of *malnutrition*, *anaemia*, *eosinophilia*, and *pneumonia*. They harm the host by depriving them of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of morbidity. Although the majority of infections due to worms are generally limited to tropical regions, they can also occur to travellers who visiting such areas (Bundy, 1994).

Helminthiasis is a disease in which a part of the host body is infested with parasitic worms such as *nematode*, *cestode* or *trematode*. Typically, the worms reside in the *gastrointestinal tract* but may also burrow into the *liver* and other organs like *lungs, skin, and eyes*. Infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation (Idika et al., 2012). Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Parasitic diseases cause severe morbidity, including *filariasis* (a cause of *elephantiasis*), *onchocerciasis* (*river blindness*), and *schistosomiasis* (Lukhoba et al., 2006)

As per **WHO**, synthetic drugs are frequently used in the treatment of helminthiasis in human beings, but these synthetic drugs are out of reach of millions of people and have many side effects.

Anthelmintic from the natural sources may play a key role in the treatment helminthiasis. Owing to its natural origin and lesser side effects, it is getting popularized in developing and developed countries. Researches are

being carried out now-a-days on large scale to discover the herbal alternatives for various allopathic medicines. Anthelmintic drugs are one such example for which herbal alternatives are being searched. Because of fewer side effects, the importance of herbal drugs as a remedy has tremendously increased in the recent years. Consequently, the need for the herbal formulation for helminthiasis has been felt. In view of this, an attempt has been made to study the anthelmintic activity in the aerial parts of the plant under investigation, **"***Senecio laetus***"**.

II. Objective

- Thorough literature survey of the plant under investigation so as to avoid any duplication of research work.
- Collection, identification and authentication of plant.
- Preliminary Phytochemical studies.
- Preparation of alcoholic extract and fractionations using different solvents of variable polarities.
- To perform in-vitro anthelmintic activity using earthworms.

III. Materials and Methods

i) Plant material collection, identification and authentication

Senecio laetus was collected from Lar, Ganderbal, Kashmir, at an altitude of 1650 m in the month of July, 2017.

The plant was identified and authenticated by Prof. Akhtar. H. Malik, Curator, Centre for Biodiversity and Taxonomy (CBT), Department of Botany, University of Kashmir, Srinagar under voucher specimen no. **2605–(KASH).** A sample specimen of collected material was deposited in herbarium for future reference.

ii) Preparation of extracts

The aerial parts of the plant material were air dried and powdered. The powdered plant material was then passed through sieve no. 40 and subjected to hot extraction with soxhlet extraction unit using ethanol as solvent. The plant extract was filtered and then evaporated to dryness using rotary evaporator at 40 ºC and preserved in desiccators for further use. The ethanolic extract of the plant material was then subjected to fractionation using various solvents viz. hexane, chloroform, ethyl acetate and butanol. Fractions obtained were concentrated using rotary evaporator and stored in desiccators.

iii) Phytochemical Screening

A stock concentration of 1 % (w/v) of each extract was prepared using ethanol as solvent. These extracts were tested for the presence of active phytochemicals viz: alkaloids, carbohydrates, proteins, amino acids, tannins, flavonoids, saponins, phenolics, cardiac glycosides, anthroquinone glycosides, triterpenoids, phytosterols, fixed oils & fats and gums & mucilage following standard methods (Harborne, 1998; Kokate, 2005) as described below:

A: Test for Alkaloids

Approximately 50 mg of extract was dissolved in 5 mL of distilled water. Further 2 M hydrochloric acid was added until an acid reaction occurred and filtered. The filtrate was tested for the presence of alkaloids as detailed below

1. Dragendroff's Test: To 2 mL of the filtrate was added 1 mL of Dragendroff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the test as positive.

2. Mayer's Test: To 1 mL of test solution or filtrate was added a drop or two of the Mayer's reagent along the sides of the test tube. A white or a creamy precipitate confirmed the test as positive.

3. Hager's Test: To 1 mL of test solution or filtrate, a drop or two of Hager's reagent was added. The formation of yellow precipitate indicated the test as positive.

4. Wagner Test: Two drops of Wagner's reagent was added to 1 mL of the test solution along the side of the test tube. The formation of yellow or brown precipitate confirmed the test as positive for alkaloids.

B: Test for Carbohydrates

1. Molisch's test: To 1 mL test solution added few drops of 1 % alpha-napthol and 2-3 mL concentrated sulfuric acid along the side of test tube. The reddish violet or purple ring formed at the junction of two liquids confirmed the presence of carbohydrates.

2. Fehling's test: Dissolved 2 mg dry extract in 1 mL of distilled water and added 1mL of Fehling's (A+B) solution, shake and heated on a water bath for 10 min. The brick red precipitate formed confirmed the presence of carbohydrates.

C: Test for Proteins

Biuret test: To 2 mL of the test solution added 5 drops of 1 % copper sulphate solution and 2 mL of 10 % NaOH and mixed thoroughly. Formation of purple or violet color confirmed the presence of proteins.

D: Test for Amino Acids

1. Millon's test: Added 5 drops of millons reagent to 1 mL test solution and heated on a water bath for 10 min, cooled and added 1 % sodium nitrite solution. Appearance of red color confirmed the presence of amino acids.

2. Ninhydrin test: To 1 mL of sample, 5 drops of ninhydrin reagent were added and heated for 2 min in a boiling water bath. Purple color indicated the presence of amino acids.

3. Xanthoproteic test: To 3 mL of sample, 1 mL of conc. Nitric acid was added and heated for 3 min. Then 0.5 mL of NaOH was added to it. Reddish orange color indicated the presence of aromatic acids.

E: Test for Tannins

Ferric chloride Test: Added a few drops of 5 % ferric chloride solution to 2 mL of the test solution. Formation of blue color indicated the presence of hydrolysable tannins.

F: Test for Flavonoids

1. Shinoda test: A few magnesium turnings and 5 drops of concentrated hydrochloric acid was added drop wise to 1 mL of test solution. A pink, scarlet, crimson red or green to blue color appeared after few min confirmed the test.

2. Alkaline reagent test: Addition of 5 drops of 5 % sodium hydroxide to 1 mL of the test solution results an increase in the intensity of the yellow color which became colorless on addition of a few drops of 2 M hydrochloric acid which indicated the presence of flavonoids.

3. Lead acetate test: A few drops of 10 % lead acetate added to 1 mL of the test solution resulted in the formation of yellow precipitate confirmed the presence of flavonoids.

G: Test for Saponins

1. Foam Test: 5 mL of the test solution taken in a test tube was shaken well for five min. Formation of stable foam confirmed the test.

2. Olive oil test: Added few drops of olive oil to 2 mL of the test solution and shaken. The formation of a soluble emulsion confirmed the test.

H: Test for Phenolics

Ferric chloride test: To 2 mL of extract, 5 % ferric chloride solution was added. Deep blue black color indicated the presence of phenolics.

I: Test for Cardiac Glycosides

Keller -Killiani test: Added 0.4 mL of glacial acetic acid and few drops of 5% ferric chloride solution to a little dry extract. Further 0.5 mL concentrated sulfuric acid was added along the side of the test tube carefully. The formation of blue color in acetic acid layer confirmed the test.

J: Test for Anthraquinone Glycosides

Hydroxyanthraquinone Test: To 1 mL extract added few drops of 10 % potassium hydroxide solution. The formation of red color confirmed the test positive.

K: Test for Triterpenoids

Salkowski Test: Approximately 2 mg of dry extract was shaken with 1 mL of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids.

L: Test for Phytosterols

Liebermann-Burchard Test: The extract (2 mg) was dissolved in 2 mL of acetic anhydride, heated to boiling, cooled and then 1 mL of concentrated sulfuric acid was added along the side of the test tube. A brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols.

M: Test for Fats and Fixed Oils

To 5 drops of the sample were added 1 mL of 1 % copper sulphate solution and a few drops of 10 % sodium hydroxide. The formation of a clear blue solution confirmed the presence of fats.

N: Test for Gum and Mucilage

About 10 mL of the extract was slowly added to 25 mL of absolute alcohol under constant stirring. Precipitation indicates the presence of gum and mucilage.

iv) In Vitro Anthelmintic activity

Indian earthworm, *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil. The earthworms of 6-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol. They were washed with tap water for the removal of the adhering dirt. The anthelminthic assay was carried as per the method of *Ajayieoba et al.* (Ajaiyeoba et al., 2001) with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Suresh et al., 2011; Vidyarthi et al., 1967; Chatterjee et al., 2011).

Pheretima posthuma worms are easily available and used as a suitable model for screening of anthelminthic drug. The earthworms were divided into fourteen groups containing six earthworms (same size) in each group. All the earthworms were released into 20 mL of formulation as follows:

Group I: Received Hexane extract at a dose of 25 mg/mL

Group II: Received Hexane extract at a dose of 50 mg/mL

Group III: Received Chloroform extract at a dose of 25 mg/mL

Group IV: Received Chloroform extract at a dose of 50 mg/mL

Group V: Received Ethyl acetate extract at a dose of 25 mg/mL

Group VI: Received Ethyl acetate extract at a dose of 50 mg/mL

Group VII: Received Butanol extract at a dose of 25 mg/mL

Group VIII: Received Butanol extract at a dose of 50 mg/mL

Group IX: Received Chloroform + Ethyl acetate extract at a dose of 25 mg/mL

Group X: Received Aqueous extract at a dose of 25 mg/mL

Group XI: Received Aqueous extract at a dose of 50 mg/mL

Group XII: Received Albendazole extract at a dose of 25 mg/mL

Group XIII: Received Albendazole extract at a dose of 50 mg/mL

Group XIV: Received Saline water which served as a control

Both the test solutions and standard drug solution were freshly prepared and '***time for paralysis'*** was noted when no movement of any sort could be observed except when the worms were vigorously shaken. The '***time for death'*** of worms was recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50 °C followed with fading of their body color.

IV. Results and Discussion

A) Extractive value: The percentage yield main extract (ethanolic) was found to be 22% approximately using soxhlet extraction method. Among fractions highest yield obtained was that of butanol (16.23%) followed by ethyl acetate (8.76%), hexane (4.05 %) and chloroform (1.08%)

Table 1: Percentage yield of ethanolic extract of aerial parts of *Senecio laetus*

S. No.	Type of extract	Color	Consistency	Dried extract (g)	% Yield
1	Ethanolic	Dark Green	Greasy	42	22.105

Table 2: Percentage yield of fractions of ethanolic extract of aerial parts of *Senecio laetus*

S. No.	Type of fraction	Color	Consistency	Dried extract (g)	% Yield
1	Hexane	Dark Green	Greasy	1.4	4.05
2	Chloroform	Brownish	Sticky	0.623	1.8
3	Ethyl acetate	Brownish	Sticky	3.03	8.76
4	Butanol	Dark Brown	Sticky	5.88	16.23

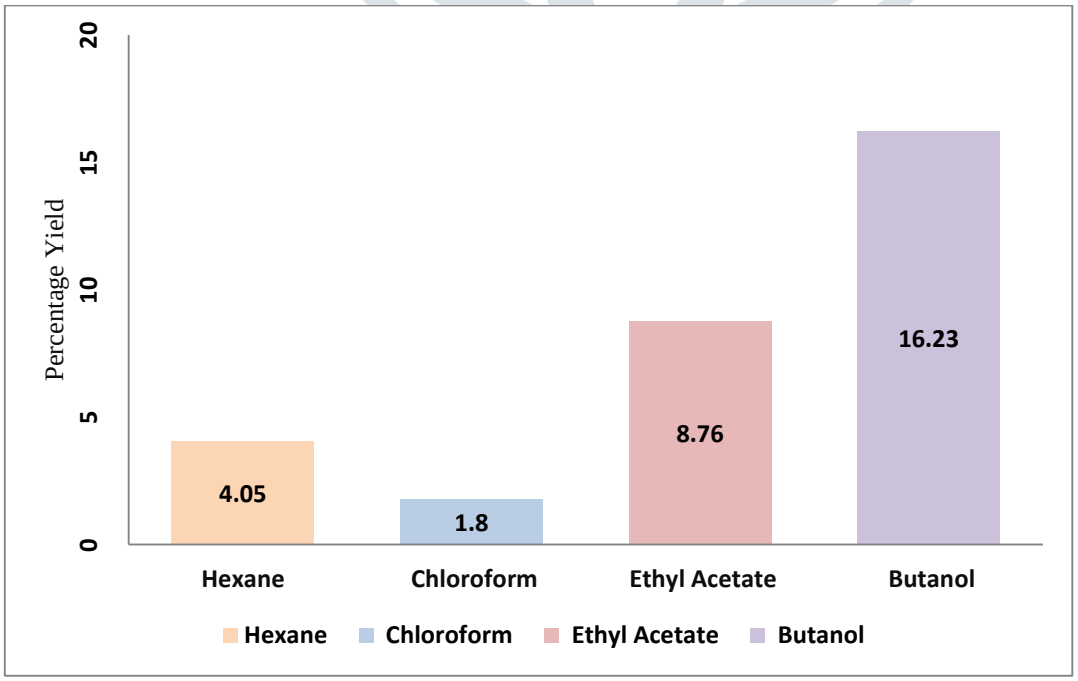

Figure1: Graphical representation of extractive values of fractions of *Senecio laetus*

B) **Phytochemical evaluation:** Phytochemical evaluation of the main ethanolic extract shows the presence of alkaloids, carbohydrates, tannins, flavanoids, saponins, phenolics, anthraquinone glycosides, triterpenoids, phytosterols, fixed oils and fats. Amino acids, cardiac glycosides, gums and mucilages are absent.

Table 3: Phytochemical analysis of ethanolic extract of aerial parts of *Senecio laetus*

S. No.	Phytochemical Test	Plant extract (Ethanolic)
1	**Alkaloids**	
	Dragendroff's Test	+
	Mayer's Test	+
	Hager's Test	+
	Wagner's Test	+
2	**Carbohydrates**	
	Molish's Test	+
	Fehling's test (reducing sugars)	+
3	**Proteins**	
	Biuret Test	–
4	**Amino acids**	
	Millon's test	–
	Ninhydrin test	–
	Xanthoproteic test	–
5	**Tannins**	
	Ferric chloride test	+

6	**Flavonoids**	
	Shinoda Test	+
	Alkaline reagent test	+
	Lead acetate test	+
7	**Saponins**	
	Foam Test	+
	Olive oil test	+
8	**Phenolics**	
	$FeCl_3$ Test	+
9	**Cardiac glycosides**	
	Keller-Killian Test	–
10	**Anthraquinone glycosides**	
	Borntranger's test	+
11	**Triterpenoids**	
	Salkowski Test	+
12	**Phytosterols**	
	Libermann Burchard Test	+
13	**Fixed oils and fat**	+
14	**Gum and mucilage**	–

Symbols denote: + Positive; − Negative

C) **In Vitro Anthelmintic activity:** From the observations made all the fractions of the ethanolic extract of aerial parts of Senecio laetus were found to exhibit a potent and dose dependent

anthelmintic activity against earthworms when compared to the standard drug. The paralysis and death time for ethyl acetate extract at dose 50 mg/mL were recorded to be 0.35 ± 0.02 min and 5.15 ± 0.40 min respectively. For chloroform extract at dose 50 mg/mL paralysis and death time were recorded to be 0.50 ± 0.03 min and 2.5 ± 0.04 min respectively. The reference drug Albendazole at 50 mg/mL dose exhibited the same at 12.09 ± 0.11 min and 19.09 ± 0.10 min respectively. Among all extracts the aqueous extract showed least anthelmintic activity with the paralysis and death time at dose 50 mg/mL being 5.05 ± 0.45 min and 70 ± 3.03 min respectively.

Table 4: Anthelmintic potential of various extracts of aerial parts of *Senecio laetus*

S. No.	Extract Type	Conc.(mg/mL)	Paralysis Time (min)	Death Time (min)
1	**Hexane**	25	1.5 ± 0.05	19 ± 0.10
		50	1 ± 0.03	10 ± 0.08
2	**Chloroform**	25	1.4 ± 0.06	4.55 ± 0.06
		50	0.5 ± 0.03	2.5 ± 0.04
3	**Ethyl acetate**	25	1.14 ± 0.04	9 ± 0.08
		50	0.35 ± 0.02	5.15 ± 0.40
4	**Butanol**	25	2 ± 0.04	53 ± 1.10
		50	0.56 ± 0.02	31 ± 0.30
5	**Chloroform + Ethyl acetate**	25	0.2 ± 0.02	3.45 ± 0.05
6	**Albendazole**	25	27.5 ± 0.26	33.05 ± 0.18
		50	12.09 ± 0.11	19.09 ± 0.10

7	**Aqueous**	25	10 ± 1.02	155 ± 4.40
		50	5.05 ± 0.45	70 ± 3.03

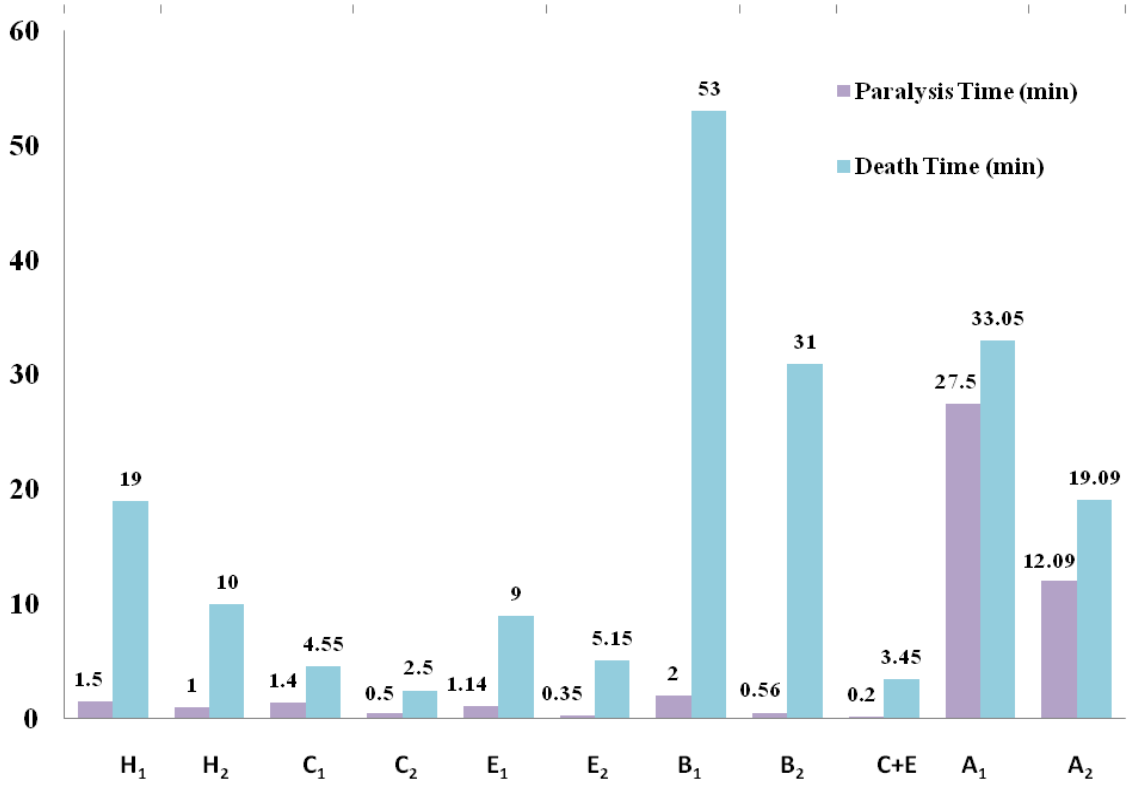

Figure 2: Anthelmintic potential of various fractions of *Senecio laetus*

H_1: Hexane fraction (25 mg/mL); **H_2:** Hexane fraction (50 mg/mL); **C_1:** Chloroform fraction (25 mg/mL);**C_2:** Chloroform fraction (50 mg/mL); **E_1:** Ethylacetate fraction (25 mg/mL) ; **E_2:** Ethylacetate fraction (50 mg/mL); **B_1:** Butanol fraction (25 mg/mL); **B_2:** Butanol fraction (50 mg/mL); **C+E:** Chloroform fraction (25 mg/mL) + Ethylacetate fraction (25 mg/mL) ; **A_1:** Albendazole (25 mg/mL); **A_2:** Albendazole (50 mg/mL).

NOTE: Due to greater variation in time for death and time for paralysis, the aqueous extract readings are neglected in the graph.

V) Discussion

Preliminary phytochemical analysis showed the presence of alkaloid, carbohydrate, phenolic, triterpenoid, flavonoid, saponin and tannin like phytoconstituents in the ethanolic extract of Senecio laetus. Some of these phytoconstituents may be responsible to show a potent anthelmintic activity.

The least time taken for paralysis by earthworms was exhibited by the combination of ethyl acetate (25

mg/mL) and chloroform (25 mg/mL) fractions (20 seconds), followed by ethyl acetate (50 mg/mL) fraction (35 seconds).

The earthworms were more sensitive to the chloroform fraction at concentration 50 mg/mL as compared to other fractions and the reference drug, Albendazole (50 mg/mL).

The chloroform fraction not only demonstrated the property of paralysis, they also caused death of the worms especially at 50 mg/mL in the least time i.e., 2 minutes 50 seconds.

The potent anthelmintic nature of various extracts may be due to the presence of alkaloids, saponins and tannins in them.

The alkaloids have ability to intercalate with DNA synthesis of parasites (Shaibani et al., 2009). Also alkaloids suppress the transfer of sucrose from stomach to small intestine, diminishing the support of glucose to the helminths, and act on CNS causing paralysis

Saponins possess the property of vacuolization and disintegration of teguments of parasitic worms.

Tannins increase the supply of digestible proteins by animals via forming protein complexes in rumen, interfere with energy generation by uncoupling oxidative phosphorylation, cause a decrease in gastrointestinal metabolism which leads to paralysis and death of helminths (Tiwari et al., 2011).Tannins may also induce physiological changes in the gut of the host resulting in secretion of mucous and chemicals harmful to the parasite (Bachaya et al., 2009).

VI) Conclusion

Our results demonstrate that *Senecio laetus* exhibits significant anthelmintic activity. The potent anthelmintic nature of various extracts may be due to the presence of alkaloids, saponins and tannins in them.

Consequently, our results suggested that these plant extracts can be utilized as an effective source of antihelmintics. Further studies have to follow up the improved new methodology in evaluating the anthelmintic activity with potential role in worm infections.

Since, there is no previous literature available about the anthelmintic activity of the *Senecio laetus*, the present investigation serves as the first report.

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