IN-VITRO ANTHELMINTIC ACTIVITY OF SENNA UNIFLORA MILL. LEAF EXTRACTS AGAINST PHERETIMA POSTHUMA

Mrs. A. Sarala, M.Pharm, MBA.,
Associate professor, Department of pharmaceutical chemistry, Arunai college of Pharmacy,Tiruvannamalai, Tamil Nadu. E-Mail Id: asaralasop@gmail.com
DR. S.K. Senthilkumar M. Pharm, Ph.D.,
Professor, Principal &Head Department of Pharmaceutics, Arunai college of Pharmacy, Tiruvannamalai, Tamil Nadu. E-Mail Id: senthilmekala@gmail.com
Ms. J. Ayisha farhana
Arunai college of Pharmacy, Tiruvannamalai, Tamil Nadu.
E-Mail Id: mariyamjaila12@gmail.com
Mr. M. Bathri narayanan
Arunai college of Pharmacy, Tiruvannamalai, Tamil Nadu.
E-Mail Id: bathrin01@gmail.com
Mr. R. Darshan sarathy
Arunai college of Pharmacy, Tiruvannamalai, Tamil Nadu.
E-Mail Id: darshansarathy33@gmail.com
Ms. M. R. Dhanashree
Arunai college of Pharmacy, Tiruvannamalai, Tamil Nadu.
E-Mail Id: shreeramesh122@gmail.com
Ms. E. Dharani
Arunai college of Pharmacy, Tiruvannamalai, Tamil Nadu.
E-Mail Id: dharanie544@gmail.com

Corresponding Author

Mrs. A. Sarala, M.Pharma, MBA.,
Associate professor, Department of pharmaceutical chemistry, Arunai college of Pharmacy,Tiruvannamalai, Tamil Nadu. E-Mail Id: asaralasop@gmail.com

ABSTRACT:

Helminthiasis is prevalent globally but more common in developing countries. *Senna uniflora* Mill is a plant that is native to South America and common weeds available in like tropics and poultice area of Tamil Nadu, India. The leaves and roots of the plant are used in traditional medicine for laxative and purgative properties. Anthelmintics are drugs that kill or expel helminths as an anthelmintic agent. Additionally clinical trials are essential to validate its effectiveness in human populations. *Senna uniflora* Mill. is a flowering plant species belonging to the Fabaceae family. It is known for its distinct appearance, bright yellow flowers, and medicinal properties. Its leaves were extracted with Aqueous, Chloroform, and Ethanol (Maceration method) and Ethyl acetate (Soxhlation method), and the yield was found to be 11.4%, 9.69%, 10.7% and 8.19% respectively. The extracts were subjected to qualitative phytochemical tests to find out the active constituents, which showed the presence of alkaloids, steroids, carbohydrate, proteins and amino acids in Chloroform extract and alkaloids, tannins, proteins, amino acids, carbohydrates, glycosides are present in the Ethanol extract. It was revealed that they showed significant activity as compared to standard drug Albendazole. The present study provides significant results regarding anthelmintic activity of aqueous extract, ethyl acetate extract, chloroform extract, ethanol extract and standard drug. The plant's bioactive compounds, such as tannins, alkaloids and flavonoids, have been found to exhibit anti-parasitic properties, suggesting its potential use in the treatment of helminth infections. The Ethyl acetate extract being more potent comparable with Standard drug. And also aqueous, ethanol, chloroform extracts produce less activity comparatively to the ethyl acetate extract. Further exploration of its pharmacological properties and development into a viable therapeutic option could contribute significantly to the field of parasitology and public health.

KEY WORDS:

Senna uniflora Mill., *Pheretima posthuma*, Dimethyl sulfoxide, Extracts, Anthelmintic activity.

INTRODUCTION:

Anthelmintics are the drugs that either kill (vermicide) or expel (vermifuge) infesting helminths[1]. Helminthiasis is prevalent globally, but is more common in developing countries with poorer personal and environmental hygiene[2]. Helmintic infections are most common health problems in India, in developing countries they pose a large to treat to public[3]. These infections can affect most population in endemic areas with major economic and social consequences. *Senna uniflora* Mill. commonly known as "Brazilian Senna," or “one leaf senna” is a flowering plant species belonging to the Fabaceae family[4]. This plant is native to South America and is primarily found in Brazil, Mexico, central America, The Caribbean, Colombia, Venezuela, and the most of Brazil and has been introduced to India, Mauritius and reunion[5]. *S. uniflora* (Caesalpiniaceae) is common weeds available in like tropics and poultice area of Tamil Nadu, India[6,7]. Although it is somewhat weedy and invasive, it is used to out-complete the pernicious weed *Parthenium hysterophorus*[8].

where it grows in various habitats, including savannas, grasslands, and open woodlands[9,10]. *Senna uniflora* Mill. is known for its distinct appearance, bright yellow flowers, and medicinal properties. Mexico, Central America, The Caribbean, Colombia, Venezuela, and the most of Brazil and has been introduced to India, Mauritius and reunion[11,12]. *Senna uniflora* Mill. has a history of traditional medicinal used in Brazil, where it is commonly known as "Fedegoso" or "Fedegosa." Various parts of the plant, including the leaves and roots, are used in traditional medicine[14]. It is known for its purgative and laxative properties[15]. The leaves and roots are often used to treat constipation and other digestive issues[16]. The plant contains bioactive compounds such as anthraquinones, which have a laxative effect on the human body. The leaves are used as poultices for wounds[17,18]. Although it is somewhat weedy and invasive, it is used to out-complete the pernicious weed *Parthenium hysterophorus.*

Figure:1 ***(Senna uniflora Mill.)***

MATERIALS:

- Drugs : Shade dried of leaves of *Senna uniflora* Mill. and Albendazole
- Solvents : Aqueous, Chloroform, Ethanol and Ethyl acetate.
- Apparatus : Iodine flask, Soxhlet apparatus, Beaker, Funnel.

METHODS:

- Maceration process
- Soxhlation process

GEOGRAPHICAL SOURCE OF PLANTS:

This plant is native to South America and is primarily found in Brazil, Mexico, central America, The Caribbean, Colombia, Venezuela, and the most of Brazil had introduced to India, Mauritius. *S. uniflora* (Caesalpiniaceae) is common weeds available in tropics and poultice area of Tamil Nadu, India[19,20].

PLANT MATERIAL

The leaves of *Senna uniflora* Mill. were collected from the so.kilnachipattu(village), Tiruvannamalai, Tamil Nadu, India.

PREPARATION OF EXTRACT

The leaves of *Senna uniflora* Mill. were shade dried, crushed to produce coarse powder and subjected to successive extraction by Maceration solvents like aqueous, ethanol, chloroform and Soxhlation using solvent like ethyl acetate based on their polarity. The extract was double filtered by using muslin cloth and whatmann no.1 filter paper and concentrated by evaporation on a water bath.

ANIMALS

Indian adult earthworms *Pheretima posthuma* collected from moist soil and washed with normal water to remove all faecal matter and furtherly washed with distilled water were used for the anthelmintic study. The earthworms of 11cm in length and 0.3-0.4cm in width were used for all experimental protocol due to its anatomical and physiological resemblance with intestinal roundworms parasite of human beings.

DRUGS AND CHEMICALS

The following drugs and chemicals were used.

Drugs : Leaves of *Senna uniflora* Mill. and Albendazole.

Solvents: Chloroform, Aqueous, Ethyl acetate, Ethanol and Dimethyl sulfoxide (DMSO)

PREPARATION OF PLANT EXTRACTS:

MACERATION PROCEDURE

100g powdered is taken in a stoppered container. About 200ml of menstrum is added and allowed to stand for three days in a warm place with frequent shaking. The mixture of crude drug containing solvent is filtered until most of the liquid drains off. The filtrate and the washing are combined to produce 100ml of the solution. Concentrate the solutions and dried individually.

SOXHLET EXTRACTION PROCEDURE:

In this method, the finely ground crude drug 80g is placed in a porous bag or thimble made of strong filter paper, which is placed in chamber of the Soxhlet apparatus. The extracting solvent in flask is heated and its vapours condense in condenser. The condensed extractant drips into the thimble containing the crude drug, and extracts its by contact. When the level of liquid in chamber rises to the top of Siphon tube the liquid contents of chamber Siphon into flask. This process is continuous and is carried out until a drop of solvent from the Siphon tube does not leave residue when evaporated.

EVALUATION OF ANTHELMINTIC ACTIVITY

Anthelmintic activity was carried out on adult Indian earthworm (*Pherethima posthuma*) of nearly equal size, six in each group. Each extract was suspended in 3% v/v Dimethyl sulfoxide solution prepared in distilled water to obtain concentration of 20, 40, 60mg/mL. Reference standard Albendazole suspension was diluted by the same suspending agent to obtain concentration of 20, 40, 60mg/mL. Worms were placed in petri dishes containing 10mL of sample solution. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously or when dipped in warm water(50^0c). Death was included when the worms lost their motility followed with white secretions and fading away of their body colours.

RESULTS:

Table: 1 PHYTOCHEMICAL ANALYSIS

S.NO	PHYTO CONSTITUENTS	AQUEOUS	ETHANOL	CHLORO FORM	ETHYL ACETATE
1.	Alkaloids	+	+	+	+
2.	Saponins	-	+	+	-
3.	Carbohydrates	+	-	+	+
4.	Tannins	+	+	+	+
5.	Flavonoids	+	-	+	+
6.	Steroids	+	+	-	+
7.	Glycosides	-	-	-	-
8.	proteins & Amino acids	+	+	+	+
9.	Fixed oil & Fat	+	+	+	+

(PHYTOCHEMICAL CONSTITUENTS PRESENT IN VARIOUS LEAVE EXTRACTS OF
***Senna uniflora* Mill.)**

EXTRACTIVE VALUES

Table: 2

S.NO	EXTRACTS	OBTAINED YIELD(g)	PERCENTAGE YIELD (%)
1.	Aqueous	11.4	11.4
2.	Ethanol	9.69	9.69
3.	Chloroform	8.19	8.19
4.	Ethyl acetate	10.7	10.7

ANTHELMINTIC ACTIVITY OF VARIOUS LEAVE EXTRACTS OF *SENNA UNIFLORA* MILL.

Table: 3

S.NO	EXTRACTS	PARALYSIS TIME (MINS)			DEATH TIME (MINS)		
		CONCENTRATION			CONCENTRATION		
		20mg/mL	40mg/mL	60mg/mL	20mg/mL	40mg/mL	60mg/mL
1.	CONTROL (DMSO)	---	---	---	---	---	---
2.	ALBENDAZOLE	5.45	4.53	3.55	7.30	6.21	5.19
3.	AQUEOUS	15.19	12.25	9.41	28.07	26.10	22.04
4.	ETHANOL	17.29	14.53	11.15	26.51	22.13	19.15
5.	CHLOROFORM	16.05	13.25	9.52	29.21	26.41	22.14
6.	ETHYL ACETAE	9.51	7.35	6.13	14.17	13.23	9.21

Data were analysed by ANOVA followed by Dunnet's test. Values are represented as mean ±SEM,
(n=6)., *P<0.05.

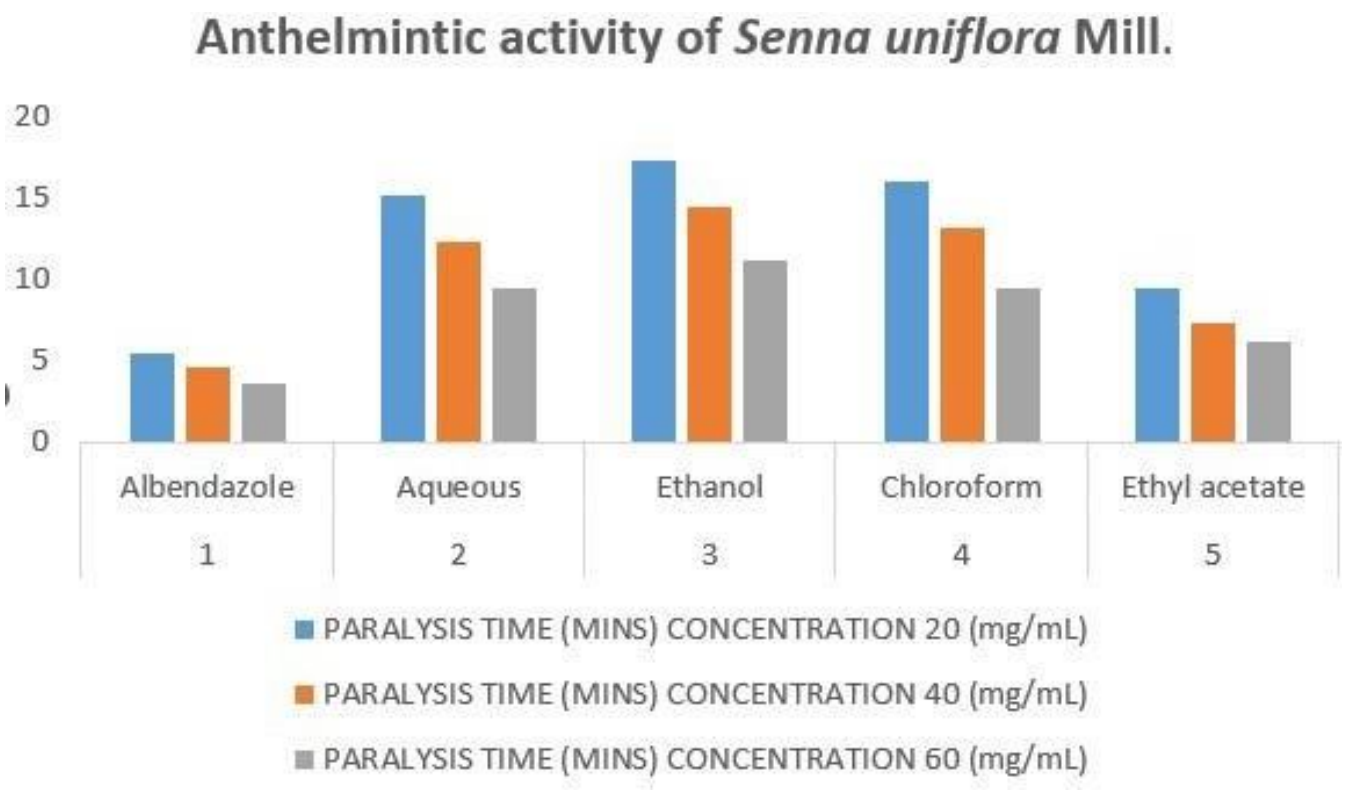

Figure: 2 (Paralysis time)

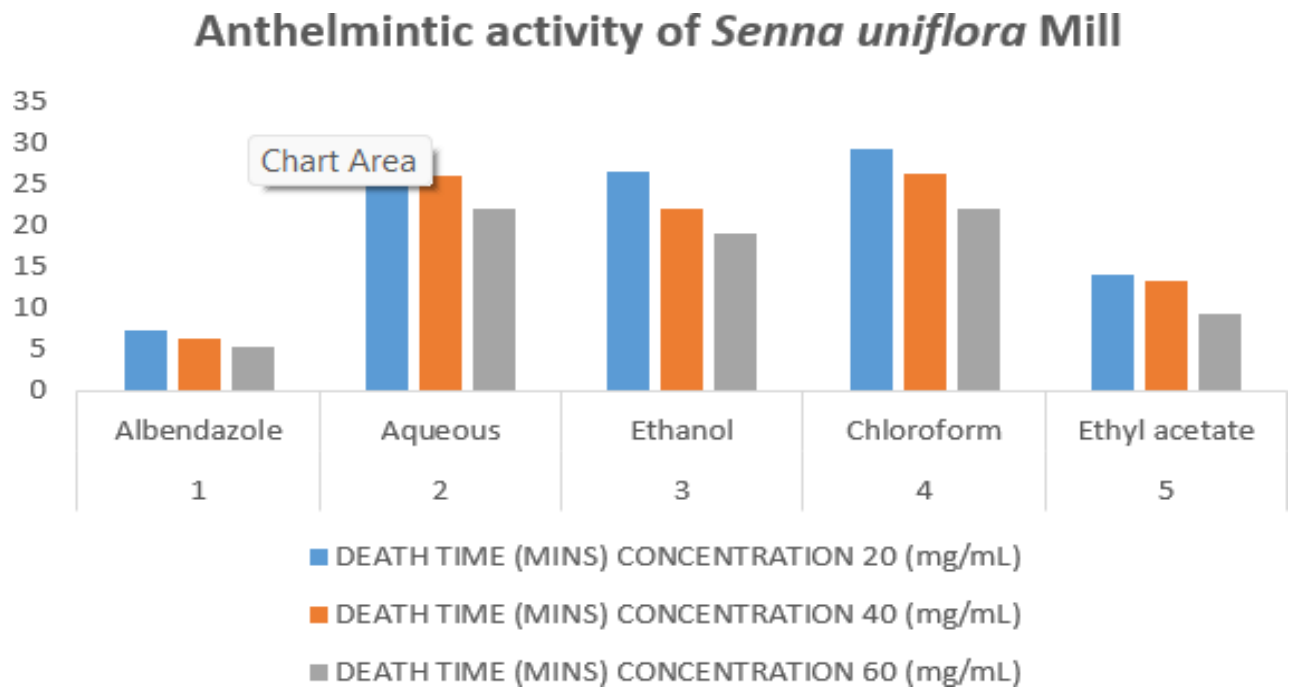

Figure: 3 (Death time)

DISCUSSION:

The leaves of *Senna uniflora* Mill. belonging to the family Fabaceae have been investigated in systematic way, covering Pharmacognostical, Preliminary phytochemical investigation and Anthelmintic activity in *Pheretima posthuma*.

PHARMACOGNOSTICAL STUDIES

Botanical information and macroscopical characters were studied. The above studies enable for the identification of the plant material for investigation and form an important aspect of drugs studies. Ethanomedical information of various parts of *Senna uniflora* Mill. were reviewed.

PRELIMINARY PHYTOCHEMICAL STUDIES

The leaves of *Senna uniflora* Mill. were extracted with aqueous, chloroform and ethanol (Maceration method) and ethyl acetate (Soxhlation method), and the extractive value was found to be 11.4%, 9.69%, 10.7% and 8.19% respectively.

The extracts obtained were subjected to qualitative phytochemical analysis to find out the active constituents, which showed the presence of alkaloids, steroids, carbohydrates, proteins and amino acids in Chloroform extract and alkaloids, carbohydrate, tannins, proteins and amino acids are present in Ethyl acetate extract and alkaloids, tannins, flavonoids, carbohydrates are present in the Ethanol extract.

PHARMACLOGICAL ACTIVITY

The results are shown in the Table No.1, 2, and 3 and Figure No. 2 and 3. The present study provides significant results regarding anthelmintic activity of aqueous, ethanol, chloroform and ethyl acetate extracts of *Senna uniflora* Mill. leaves. It was revealed that they showed significant activity as compared to standard drug Albendazole.

CONCLUSION:

From the above results, it was concluded that all the four extracts of *Senna uniflora* Mill. leaves showed significant anthelmintic activity. Relatively, Ethyl acetate extract showed more activity than Aqueous extract and Aqueous extract showed more activity than Chloroform extract and Chloroform extract showed more activity than ethanol extract.

Further it would be interesting to isolate the possible phytoconstituents which may be responsible for the Anthelmintic activity and to find out the possible mechanism of action.

The plant's bioactive compounds, such as tannins, alkaloids and flavonoids, have been found to exhibit anti-parasitic properties, making it a promising candidate for the development of anthelmintic agents. Various studies have provided evidence of the plant's efficacy in inhibiting the growth and reproduction of parasitic worms, suggesting its potential use in the treatment of helminth infections.

ACKNOWLEDGEMENT:

We would like to mention a special thanks to my co- authors, and the management, Arunai college of Pharmacy, Tiruvannamalai, helped us in this project work. And we would like thanks to my friends and family for encouragement during the study.

REFERENCES:

1. Vijay D, Ahmed AB, Kumar RA, Gnanasekaran D, Panagal M, CS R. Comparative evaluation of extracts of Senna uniflora for anti-inflammatory activity. International Journal of Pharmacy and Technology. 2010;2(2):325-32.
2. Joshi N, Papule MJ, Bhosale S. Evaluation of the Microscopy Study of Senna Uniflora Plant and Leaf Powder by Foldscope. International Journal of Pharmaceutics and Drug Analysis.;6(12):614-20.
3. Kumari JA, Prasad PR. Assessing the allelopathy and autotoxicity effects of Parthenium hysterophorus L., Senna uniflora (Mill.) HS Irwin and Barneby and Hyptis suaveolens (L.) Poit. Russian Journal of Biological Invasions. 2018 Jul;9:290-8.
4. Kothapallia LP, Papulea PP, Thomasa AB, Bhujbal SS. Evaluation of Senna Uniflora (MILL.) Leaf Extract and its Tropical Gel Formulation for Biological Activity. Int. J. Pharm. DRUG Anal. 2017;5(8):354-65.
5. Aswar M, Aswar U, Watkar B, Vyas M, Wagh A, Gujar KN. Anthelmintic activity of Ficus benghalensis. International Journal of Green Pharmacy (IJGP).2008;2(3).
6. Khadse CD, Kakde RB. In vitro anthelmintic activity of Fenugreek seeds extractagainst Pheritima posthuma. Int. J. Res. Pharm. Sci. 2010;1(3):267-9.
7. Apte AK, Khot VS, Biradar NS, Patil SB. Anthelmintic activity of Trachyspermum ammi (l) extract. International Journal of Pharmacy and Pharmaceutical Sciences ISSN-0975-1491 Vol. 2014;6.
8. Raj LA, Gadamsetty GA, Sarada NC. Comparative studies on in vitro anthelmintic activity of Gymnema sylvestre and Acalypha fruticosa Forssk. Int J Pharm Pharm Sci. 2012;4(1):107-9.
9. Anbu J, Murali A, Sathiya R, Saraswathy GR, Azamthulla M. In Vitro Anthelmintic activity of leaf ethanolic extract of Cassia alata and Typha angustifolia. SASTech-Technical Journal of RUAS. 2015;14(2):41-4.
10. Thenmozhi S, Vibha K, Dhanalakshmi M, Manjuladevi K, Diwedi S, Subasini U. Evaluation of anthelmintic activity of Vitex trifolia Linn. leaves against Pheretima posthuma. International Journalof Pharmaceutical & Biological Archives.2013;4(5):878-80.
11. Bendgude RD, Kondawar MS, Patil SB, Hirave RV. In vitro anthelmintic activity of roots of Capparis zeylanica Linn. Journal of Advanced Pharmacy Education & Research. 2011;2:154-8.

12. Raul SK, Padhy G, Dhavala M, Shravani B. Comparitive study of anthelmintic activity of Vitex negundo, Moringa oleifera, Tamarindus indica on Indian earthwormPhertima posthuma. Drug Invention Today. 2012;4(7):407-8.
13. Rabiul H, Subhasish M, Parag G. Investigation of in vitro anthelmintic activity ofCinnamomum camphor leaves. Int. J. Drug Dev. Res. 2011 Jan;3:295-300.
14. Kanthal LK, Mondal P, De S, Jana S, Aneela S, Satyavathi K. Evaluation of anthelmintic activity of carica papaya latex using pheritima posthuma. International Journal of Life Science and Pharma Research. 2012;2(1):10-2.
15. Bi S, Goyal PK. Anthelmintic effect of Natural Plant (Carica papaya) extract against the gastrointestinal nematode, Ancylostoma caninum in Mice. ISCA J. Biological Sci. 2012;1(1):2-6.
16. Douaouri NE, Djebli N. In vivo anti-inflammatory activity and chemical composition of Algerian pomegranate (Punica granatum L.). International Journal of Biosciences. 2018;12(2):76-90.
17. Jamkhande PG, Wattamwar AS, Chandak PG. In vitro anthelmintic efficacy of ethno-medicinal plant Annona reticulata L. roots against Indian earthworms (Pheretima posthuma).
18. Kumar T, Alexander A, Dewangan D, Khan J, Sharma M. Investigation of in- vitro anthelmintic activity of Bauhinia racemosa linn. Journal of Applied Pharmaceutical Science. 2011 Apr 30(Issue):73-5.
19. Joshi KC, Nanda D, Nainwal P, Saini P. In vitro anthelmintic activity of Ocimumsanctum. International Journal of Pharma Science. 2013;3(4):287-8.
20. Buchineni M, Pathapati RM, Kandati J. Anthelmintic Activity of Tulsi Leaves (Ocimum sanctum Linn.) An In vitro Comparative Study. Saudi Journal of Medical and Pharmaceutical Sciences. 2015;2:47-9.