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Research paper

Invitro Anthelmintic Activity of *Senna occidentalis*

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ABSTRACT

Senna occidentalis is traditional medicinal plant belonging to the family Fabaceae. The present study was aimed at evaluating the in vitro anthelmintic activity of the ethanolic leaf extract of senna against the Indian earthworm *Pheretima posthuma*. There are three concentrations of the extract (10mg/ml, 20mg/ml and 30mg/ml) were tested, and the results were expressed in terms of the time required for paralysis and the time to death of the worms. The Albendazole was used as the standard reference drug. In this study, ethanolic leaf extract of *S. occidentalis* demonstrated notable anthelmintic activity, with significant effects at higher concentrations, showing improved efficacy relative to the Albendazole drug. *Senna* is traditionally used in the herbal medicine for the management of fever, constipation, skin disease and inflammatory conditions. Its pharmacological activities are attributed to a range of secondary metabolites, including anthraquinones, tannins, alkaloids, saponins and phenolic compounds, which contribute to its anthelmintic properties.

INTRODUCTION

Gastrointestinal nematodes in ruminants are among the most significant causes of animal disease worldwide, particularly in temperate and tropical regions. ^[1] Growing resistance of these nematodes to commercially available anthelmintic activity drugs, along with concerns about drug resistance of these nematodes to commercially

available anthelmintic drugs, along with risks to consumer health ^[2] has motivated the search for alternative control strategies. In this context, senna leaves and their secondary metabolites represents a promising option for parasite management. ^{[3][4]} Gastrointestinal tract (GIT) helminths have developed resistance to currently available anthelmintic drugs, creating a major challenge in the treatment of helminthic diseases. ^[5]

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Anthelmintic drugs are used to expel or kill intestinal worms,^[6] which cause anaemia, eosinophilia, economic losses, nausea, vomiting blood loss, decreased nutrient absorption, body aches, organ damage and intestinal or lymphatic obstruction through the release of toxins.^{[7][8]} The world health organisation (WHO) has also estimated that 80% of the population in developing countries relies on traditional medicine primarily plant based remedies for their primary health needs.^[9]

Senna occidentalis



Figure 1: Senna

Plants belonging to the Fabaceae family have extensively been investigated because of their high medicinal and economic uses. The antibacterial and antimalarial activities of the leaves and root-bark of *Senna occidentalis* have been reported.^[10] The plant is also known by Name vernacular names such as Septic wood, Coffee senna, Coffee wood, Mogdad coffee, Negro coffee. Senna coffee, Stephanie coffee. Stinking coffee or Styptic weed. The plant is locally called Buna Chukunda in Odisha, India.^[11] The species was formally placed in the genus Cassia and thus was formally called *Cassia occidentalis*.^[12] The plant is reported to be poisonous to cuttlefish when consumed. The plant contains anthraquinones, the roots contain emodin and the seeds contain Chrysin (1,8-dihydroxy-3-methyl-9-

anthrone) and N-methylmorphine.^[13] The leaves of *Senna occidentalis* Linn from data revealed in research "Nutritional and Anti-nutritional Analyses of *Senna occidentalis* Linn"^[14] showed that it contains protein, carbohydrate, fibres, lipids, vitamins, moisture, caloric value and low levels of anti-nutrients of the leaves whose levels could be reduced on processing before consumption. Thus, conclusion was made; that the plant can contribute significantly to the nutrient requirements of man and may ameliorate some nutrition related illnesses. Similarly, in the Review on Nutritional Potential of the plant arrived out by.^[15] An attempt was made to collect all possible ethno-botanical and nutritional potential of *Senna occidentalis* with reference to its food and medicinal applications. A baseline survey was conducted between 2011-2015, and information about *S. occidentalis* was collected through semi-structured interviews and discussion with the local healers, elderly and experienced people. Additionally, all available literature on *S. occidentalis* was reviewed and studied through an online search engine Scopus and Google Scholar. Literature collection was done from 1965 to 2015 and all the information were compiled and presented. The research suggests a huge nutritional potential of this plant, it suggests that raw seeds might have some toxicological side effects, but after proper processing, identification and removal of the harmful properties of weeds, they may be utilized to prepare a good, nourishing coffee. The research suggests that *S. Occidentalis* should be further exploited in the future in source of useful phytochemicals and nutritional compounds for the nutritional industry. A study carried out on the Similarly, in the study phytochemical, antimicrobial and antioxidant activities of the hexane, ethyl acetate and methanol crude extracts of *Senna occidentalis* leaves extracts revealed the presence of tannins, alkaloids, reducing sugar, phenols, anthraquinones, resins, saponins and

glycosides. The antimicrobial screening was carried out using the following organisms *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifera*. The results obtained showed that *Senna occidentalis* leaf extracts have interesting pharmacological active compounds with great radical scavenging and antimicrobial effects and as such could be used in ethnomedicine for treatment of some infections and ailments,^[16] carried study on the in-vitro antioxidant activity of the plant. Their study was based on the measurement of the scavenging ability test substances towards the stable DPPH radical. The research concluded that the plant has antioxidant property.^[17] The phytochemical screening, determination of bioactive constituent of *Senna Occidentalis* methanolic leaf extract using Gas chromatography-mass spectrometer (GC-MS) was carried out.^[18] The phytochemical study revealed the presence of tannins, alkaloids, glycoside, flavonoids, steroids, saponins, anthraquinones and phlobatannins while cardiac glycoside was not detected. GC-MS chromatography showed nine peaks. A total of 31 compounds were identified when the mass spectra of the constituents was taken. The first compounds identified with less retention time (15.929%) were Hexadecenoic acid, octadecanoic acid and pentadecanoic acid, while decanoic acid, decyl ester, other, octadecyl vinyl, oleic acid, hexyl ester, stearic acid, octadecyl ester and decyl fluoride took the longest retention time (20.600s) for identification. Thus, the research concluded that the presence of those compounds in the plant extract may at lousa bo responsible for one of the pharmacological properties of *S. Occidentalis* and thus could be of considerable interest to the development of now drug. As seen from the literatures reviewed above, more research

has been done on the leaves, roots and seed, with little attention to the stem of *Senna Occidentalis*.

Pheretima posthuma

Scientific classification

Kingdom: Animalia

Phylum: Annelida

Clade: Pleistoannelida

Clade: Sedentaria

Class: Clitellata

Order: Opisthopora

Family: Megascolecidae



Figure 2: *Pheretima posthuma*

Genus: *Pheretima*

Species: *Postuma*

Distribution: Commonly found in moist soils across the globe.

Habit and Habitat: They are active at night and live in damp, humus-rich soil in places like lawns and gardens. When the weather is dry, they dig deeper into the soil to stay moist. They act as herbivores and macro-decomposers and are an important food source for many birds. They also help aerate the soil and improve its fertility.^[19]

Anthelmintic Activity

Anthelmintics are a group of drugs used to treat various infections in humans and animals that are caused by parasitic worms.^[20]

Secondary metabolites that exhibit anthelmintic activity

Allelochemicals are produced by all plants^[21] and primary metabolites serve as precursors for various secondary metabolites.^[22]

- Alkaloids: Alkaloids have shown anthelmintic activity by targeting acetylcholine receptors and suppressing glucose uptake, ultimately causing helminths to die from starvation.^[23]
- Flavonoids: Flavonoids show activity by blocking phosphorylation reactions, thereby inhibiting energy production in parasitic worms and ultimately causing death.^[24]
- Tannins: Tannins, a water-soluble group of polyphenolic compounds, exhibit anthelmintic activity by disturbing nutrient absorption in nematodes, thereby larvae, nutrients from host cell.^[24] In addition, when condensed tannins are ingested by larvae, they bind to the intestinal mucosa of the parasitic worms, leading to cellular damage and autolysis.^[25]
- Phenols: Phenols show anthelmintic activity by changing the phosphatase enzyme in the helminth's tegument.^[26]

- Saponins: Saponins exert their anthelmintic effect by inhibiting acetylcholinesterase, which induces paralysis in the worms and results in their subsequent death.^[27]
- Glucosides: Glycosides disrupt the transport of sodium and potassium ions in helminths, ultimately leading to their death.^[28]

MATERIALS AND METHODS

Collection of plant material

Senna occidentalis leaves were collected in the month of March from the herbal garden of Ayurvedic Medical College Ghataprabha, Karnataka, India. The plant was authenticated by Dr. J.K Sharma, Principal of Ayurvedic Medical College Ghataprabha. The fresh leaves were collected, removed all earthy matter, washed, shade dried and powdered by Pulveriser.



Figure 3: Dried Leaves



Figure 4: Dried leaf powder

Collection of worms

Indian earthworm's *Pheritima posthuma* were collected from Patil Organic Manure and identified and washed with water to remove all kinds of dirt from them.

Chemicals and drugs used

Ethanol, Normal saline, Albendazole

Preparation of plant extract



The leaves of plant were dried under shade and crushed in pulverizer and powdered. The powdered plant extracted with ethanol in Soxhlet apparatus for 72 hours after completion of the

extraction, the extracts were cooled at room temperature and filtered and evaporated to dryness using rotary evaporator.



Figure 5: Soxhlet extraction



Figure 6: Extract

Preliminary phytochemical screening

The ethanolic extract was subjected to qualitative identification of phytoconstituents like carbohydrates, proteins, amino acids, glycosides, flavonoids, sterols etc. Phytochemical screening was carried according to the standard procedures.

Phytochemical screening

The phytochemical test for various phytochemicals presents in the extract was carried out using standard methods as described below^[29].

- Test for alkaloid
0.2g of the molten extract was mixed with little amount of HCl and then Wagners reagent. Formation of a white precipitate indicates the presence of alkaloid.
- Test for Flavonoid
0.2 g of the molten extract was mixed with 1ml of 2% ammonium chloride and the exposed to light. Yellow precipitate indicates the presence of Flavonoid.
- Test for Anthraquinone

A few drops magnesium acetate was added to 0.2 g of the molten extract. Pink colour formation indicates the presence of anthraquinone.

- Test for Quinone
0.2 g of the molten extract was treated with few drops of conc. sulphuric acid or aqueous sodium hydroxide solution. Red colour formation indicates the presence of quinone.
- Test for Phenols
0.2 g of molten extract was mixed with ferric chloride solution. A green or dirty precipitate indicates the presence of phenol.
- Test for Phlobatannins
0.2 g of the molten extract was mixed 2% Hydrochloride Solution. Appearance of red precipitate indicated the presence of phlobatannin.
- Test for Tannins
0.2 g of the molten extract was mixed with few drops of ferric chloride solution. A blue-black, green or blue-green precipitate indicates the presence of tannin.
- Test for Saponins

0.2 g of extract was shaken 5ml of distilled water in a test tube. Frothing which persists on warming indicates the presence of saponin.

- Test for Xanthoproteins

0.2 g of extract was mixed with few drops of concentrated nitric acid and then few drops of ammonia. A red precipitate indicates the presence of xanthoproteins.

- Test for Steroids (Salkowski's test)

0.2g of the extract was mixed with 3ml of chloroform and 2ml of concentrated sulphuric. A red colour appearance indicates the presence of steroid.

- Test for Cardiac-active glycoside (Keller-killani test)

0.2g of the extract dissolved in 2ml of glacial acetic acid containing one few drops of ferric chloride solution followed by the addition of few drops of concentrated sulphuric acid. Brown ring at the interface confirmed the presence of cardiac-active glycoside.

- Test for Carbohydrate

0.2g of extract was mixed with few drops of concentrated sulphuric acid then heat. Black colouration indicates the presence of carbohydrate.

- Test for Protein

0.2g of extract was mixed with few drops of biuret reagent. A pink colouration indicates the presence of protein.

- Test for Fixed oil (Spot test)

A small quantity of extract was pressed between two filter papers. Appearance of grease spot will indicate the presence of fixed oil.

Preparation of concentrations

The ethanolic extract of *Senna occidentalis* was made into Three different concentrations such 10 mg/ml, 20 mg/ml, 30 mg/ml, by dissolving in distilled water.



Figure 7: Different concentrations of extract

Evaluation of Anthelmintic activity

The anthelmintic activity was carried according to standard method [30-32]. Adult Indian Earthworm *Pheretima posthuma* has anatomical and physiological resemblance to the earthworm Intestinal roundworm parasites of human beings. Indian earthworms were placed in a Petri dish containing different concentrations (10 mg/ml, 20 mg/ml, 30 mg/ml) of ethanolic extract of *Senna occidentalis*. Each Petri dish contains earthworms and observed for time of paralysis as well as time death. Time of paralysis recorded when no movement of any sort could be observed, except when the worm was shaken vigorously as well as time of death was recorded after ascertaining that worm neither moved when shaken. Finally, the test results were observed that as high concentration, the activity is more.

Thin layer chromatography (TLC)

To prepare TLC plate, begin by selecting a clean dry plate coated with the thin layer of absorbent such as silica gel. Using the soft pencil lightly draw straight line about 1-1.5 cm from the bottom edge of the plate; this will serve as the origin where samples are spotted. mark small, even the spaced points along this line to indicate where each sample will be applied. By using Mobile phase ethyl acetate: ethanol: water (9:9:7) and use a fine capillary tube to gently spot small amounts on to the marked points, allowed spot to dry. once the sample are applied, place the plate in a developing

chamber that contains a shallow there of the chosen solvent system, ensuring the solvent level sits below the pencil line. Close the chamber allow it to saturate with solvent evaporate, and let the solvent rise up the plate by capillary action until it nears the top finally remove the plate immediately mark the solvent front with a pencil and allow the plate to dry after trying spray the ninhydrin solution. determine the Rf value of extract

RESULTS

Phytochemical screening test shows the presence of different secondary metabolites like, alkaloids, quinone, tannins, phenols, saponins, phlobotannins, steroids, cardiac glycosides, xanthoprotein, carbohydrates, fixed oil.

Sl No	Phytochemicals	Ece
1	Alkaloids	+
2	Flavonoids	-
3	Quinone	+
4	Tannin	+
5	Phenols	+
6	Saponin	+
7	Anthraquinone	-
8	Phlobatannin	+
9	Steroids	+
10	Cardiac glycoside	+
11	Xanthoprotein	+
12	Carbohydrates	+
13	Protein	-
14	Fixed Oil	+

+ Present, - Absent, ECE: Ethanol Crude Extract

Table No. 1: Phytochemical screening



Figure 6: Phytochemical test

Anthelmintic Activity

Table No. 2: Anthelmintic activity of ethanolic extract of Senna leaves and Albendazole

Drugs	Concentrations mg/ml	Paralysis(minutes)	Death(minute)
Ethanol	10	01:03	01:50
	20	00:40	01:02
	30	00:19	00:49
Albendazole	10	02:50	03:20
	20	01:30	01:46
	30	00:45	01:10

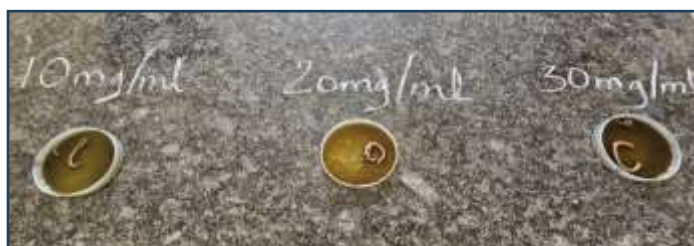


Figure 7: Anthelmintic activity

Thin layer chromatography

R_f value of Senna leaf = 0.306



Figure 8: TLC of senna leaf

CONCLUSION

It can be concluded that ethanolic leaf extract of *Senna occidentalis* produces better anthelmintic activity against Indian earth worm *Pheretima posthuma*. The anthelmintic property of the *Senna occidentalis* has shown the dose dependent action and it shows more efficient activity than albendazole.

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