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Anthelmintic Activity of Selected Plants Leaf Extracts

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ABSTRACT

Medicinal plants such as *Occimum sanctum* (Tulsi), *Cassia angustifolia* (Senna), *Amaranthus viridis* (Amaranthus) is an evergreen medicinally useful plants. Medicinal plants have been a variable source of natural activity, phytochemical constituents that play an important role in the treatment of many human diseases. In the present study, leaves of *Occimum, Cassia angustifolia, Amaranthus* by their anti-helminthic activity the qualitative and phytochemical analysis. The extraction was done by using Soxahalation. Qualitative and phytochemical analysis of crude powder of *Occimum, Cassia angustifolia, Amaranthus*, leaves were carried out for various phytoconstituents. The abstractive yield was maximum in methanol extraction with Occimum, Cassia angustifolia and Amaranthus leave.

INTRODUCTION:

Anthelmintics or antihelminthics are a group of antiparasitic drugs that expel parasitic worms and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They may also be called vermifuges. They are used to treat people or animals that are infected by helminths, a condition called helminthiasis. Antiparasitics that specifically target Ascaris worms are called as ascaricides [(1).]

Traditional system of medicine continues to be widely practiced on many accounts [(2)] Population rise, inadequate supply of drugs, the prohibitive cost of treatment, side effects of several allopathic drugs and development of resistance to currently used drug for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Among these systems, Ayurveda is the most developed and widely practiced in India [(3)].

INTRODUCTION TO EARTHWORMS

Scientific classification:

Kingdom : Animalia

Class : Oligochaeta

Subclass : Oligochaeta

Families : Acnthodrilidae, Almidae [(4).]

An earthworm has a double transport system composed of coelomic fluid that moves within the fluid-filled coelom and a simple, closed blood circulatory system it has a central and a peripheral nervous system. Large numbers of chemoreceptors are concentrated near its mouth. Circumferential and longitudinal muscles on the periphery of each segment [(5)] enable the worm to move. theoretical studies have placed them, instead, in the suborder lumbricina of the order haplotaxida, but these may again soon change folk names for the earthworm including "dew-worm", "rain-worm", night crawler", and "angle- worm"[(6)].

BODY:

Depending on the species, an adult earthworm can be from 10 mm (0.39) in long and 1 mm (0.39) in wide to 3 m (9.8 ft) long and over 25 mm (0.98) in wide, but the typical *Lumbricus*

terrestris [7] grows to about 360 mm (14) in long Special ventral setae are used to anchor mating earthworms by their penetration into the bodies of their mates [8].

Fig: 01 (Earthworm)

CROSS-SECTION OF EARTHWORM:

The cross-section of the body of an earthworm (oligochaeta) shows the disposition of the more important organs the body wall consists of dermis, circular and longitudinal muscles the body cavity is divided by membranes into a series of chambers, in each of which opens the mouth of a coiled nephridium the axis of the cavity is occupied by the intestine above and below it is a longer blood vessel and below it is also the central nerve cord [9].

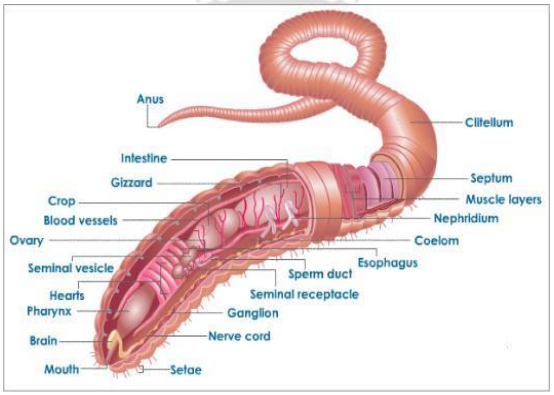


Fig: 2 (Parts of Earthworm)

Interior to the muscle layer is a fluid-filled chamber called coelom that by its pressurization provides structure to the worms bone less body [10].

HELMINTHIASIS

Fig: 3 (Helmenthiasis of Earthworm)

Helminthiasis is any macro parasitic disease of humans and animals in which a part of the body is infected with parasitic worms known as helminths. These parasites are broadly classified into tapeworms, flukes and roundworms. They often live in the gastrointestinal tract of their hosts, but may also burrow in to other organs, where they induce physiological damage [(11).]

Treatment: Albendazole in the treatment of intestinal helminthiasis in children

A single dose of 2% Albendazole suspension (400 mg in 20 ml) was administrated to 77 patients (42 males and 35 females), ages ranging between 2 to 12 years, with helminthic infections[(12)]. Ascariasis was the most prevalent infection. Patients were followed up for 3 weeks the results showed that albendazole was highly effective against *Ascaris lumbricoides*, Ancylostoma duodenal and enterobius vermicular is. Significant improvement was also observed inpatients having infections due to *Trichuris trichiura*. Albendazole was well tolerated and did not produce any significant side effects. Single dose Albendazole appears to be appropriate for mass chemotherapy to control intestinal nematode infections in highly infected communities [(13).]

PLANT PROFILES

SENNA

Scientific classification:

Kingdom : Plantae

Order : Fabales

Family : *Fabaceae*

Sub family: Caesalpinioideae

Genus : Senna

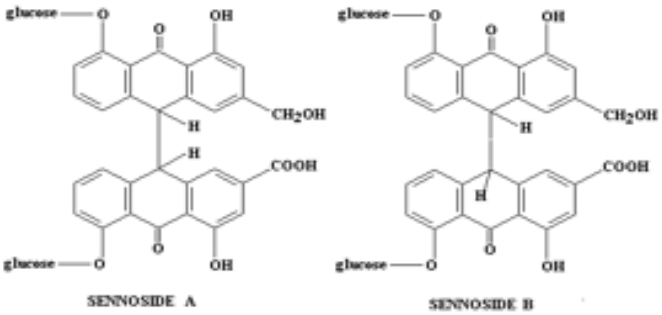
Fig-4 SENNA

Synonyms: Indian senna, senna leaves, sennafolium, cassia senna [14] Cathartocarpus, chamaefistula, diallobus, earleocassia, herpetica, isandrina palmerocassia.

Biological source: It consists of dried leaflets of **"***Cassia angustifolia* or *Cassia senna***"**.

Family: *Fabaceae*

Chemical constituents: senna contains mainly two anthraquinone glycosides called sennosides A and sennoside B (not less than 2.5%) [15].



Uses:

- ❖ Senna and its preparations are used as purgative in habitual constipation.
- ❖ Glycosides increase gastric fluid secretion and bowel motility, producing laxative action.

TULSI

Scientific classification:

Kingdom : Plantae

Order : Lamiales

Family : *Lamiaceae*

Genus : Ocimum

Binomial name: *Ocimum tenuiflorum*

Fig-05 TULSI

Synonyms **:** Sacred basil, Holy basil

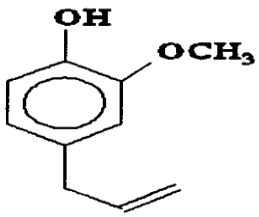
Biological source: Tulsi consists of fresh and dried leaves of **"***Ocimum sanctum Linn***"**.

Family: *Lamiaceae*

Chemical constituents:

❖ Tulsi leaves contains bright, yellow colour and pleasant volatile oil (0.1 - 0.9), 70% eugenol, carvacrol (3%), eugenol-methyl-ether (20%) and caryophyllin. Seeds contain fixed oil with good drying properties [16].

❖ The plant is also reported to contain alkaloids, glycosides, saponins, and tannins, an appreciable amount of vitamin c and traces of maleic, citric and tartaric acid.



Eugenol

Uses:

❖ The leaves are used as stimulant, aromatic, anticatarrhal, spasmolytic, and diaphoretic.

❖ The juice is used as an antiperiodic, and, and as a constituent of several preparations for skin diseases and also to cure earache.

AMARANTHUS VIRIDS

Scientific classification: **Family** : *Amaranthaceae*

Biological name: Amaranthus viridis **Genus** : Amaranthus

Kingdom : Plantae **Specie** : *A.viridis*

Order : Caryophyllales

Fig-06 AMARANTHUS VIRIDS

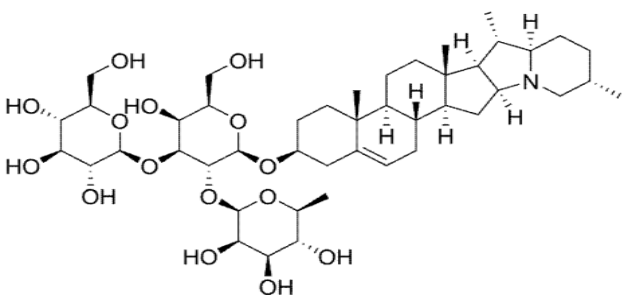
Synonyms: Green amaranth, wild amaranth, green pigweed, amaranthus caudatus, amaranthus dubius (red spinach) [17].

Biological source: it is obtained from the dried leaflets of **"***Amaranth viridis***".**

Family: *Amaranthaceae*

Chemical constituents:

- The stem and leaves contain oxalic acid.
- It is rich in minerals and contains sterols and fatty acids in the seeds.
- The total plant contains saponins



Saponin

Uses:

❖ It is used as a laxative and alexiteric, stomatic, appetizer, anti-rheumatic [18], diuretic, respiratory problems, appetite stimulatory, eye ailments and asthma.

EXPERIMENTAL PROCEDURE

SOXHLATION

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent [19].

Procedure:

Normally a solid material containing some of the desired compounds is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extraction. The soxhlet extractor is placed onto a flask containing the extraction solvent. The soxhlet is then equipped with a condenser. The solvent is heated to reflex. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid [20]. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solvent material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compounds will then dissolve in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent [21] after many cycles the desired compound is concentrated in the distillation flask. The after extraction the sample is removed, typically using a rotator evaporator, yielding the extracted compound. The non-volatile portion of the extracted solid remains in the thimble and is usually discarded.

Methanolic extract:

The marc remains after acetone extraction was dried and then it is extracted with 2 -3 liters of alcohol 95 % using soxhlet apparatus by continuous hot percolation method[22] After

extraction, it was filtered and the removal of solvent was done under pressure by a distillation process. The (10gm) extract was stored in a desiccator.

MATERIALS AND METHODS

MATERIALS AND METHOD OF TULSI

Preparation of extract

The aqueous, ethanolic and hydro alcoholic extract was prepared by cold maceration technique. All extract were screened and then evaluate for anthelmintic activity. Determinations of anthelmintic activity adult earthworms [23] (pheretima posthuma) were collected Earthworms were washed to remove adhering material. Petri dishes of equal sizes were taken cleaned and dried. Six Petri dish were arranged in a row. 20 ml of normal saline was poured in 1st petridish; 20 ml of piperazine hydrate solution containing 1mg/ml & 4mg/ml concentration were poured in 2nd and 3rd petridish respectively. 20 ml of solution of extract 2, 5, 10 mg/ml were taken in 4, 5, 6 petridish respectively (test). Six earthworms of equal sizes were introduced in each petridish and time was noted. The time taken for paralysis (motionless) and complete death of earthworm [24] were recorded, death of earthworm was confirmed by dipping it in water heated to 500c. The procedure was repeated 6 times to confirm the reading.

MATERIALS AND METHODS OF SENNA

Preparation of plant extracts

Fresh young leaves of C. angustifolia were collected, weighed and washed with de ionized water, and oven-dried at 50 °c. About 150 g of powdered leaves were extracted with 1 l of ethanol [25] (90%) in a soxhlet apparatus for 7-8 h The final crude extracts were recovered using a rotary evaporator and stored at 4 °c until further use.

Experimental design: Helminthes representative's viz. Heterakis gallinarum catatropis. Tetragona were collected from intestine of freshly slaughtered domestic fowl. Live worms were treated in vitro with three leaf extracts at various concentrations (10, 20 and 40 mg/ml, in 0.9% phosphate-buffered saline (ph 7.4). While 5, 10 and 20 mg/ml of albendazole were used as a reference drug for Gallinarum, praziquantel was tested for the other two worms. Control worms were maintained in phosphate-buffered saline with 1% dimethyl sulfoxide.

Combinations of plant extracts in ratio 1:1 were also tested on fresh worms at to observe any synergistic or additive effect.

MATERIALS AND METHOD OF AMARANTHUS:

Anthelmintic activity Water extracts of whole plant of Amaranthus spinosus Linn was evaluated for anthelmintic on adult Indian earthworms using piperazine citrate as reference standard. Aqueous extract showed anthelmintic activity in dose-dependent manner giving the shortest time of paralysis (P) and death (D) with 50 mg/ml concentration, for both the worms. The extract shows more potent activity (15 mg/ml).

RESULT AND DISCUSSION

TABLE: 1

PRELIMINARY PHYTOCHEMICAL TEST'S FOR IDENTIFICATION OF PHYTOCONSTITUENTS

PLANT NAME	TEST	RESULT
SENNA	ALKALOID TEST (Wagner's reagent)	+ve
	CARBOHYDRATE TEST (Benedict's test)	+ve
	GLYCOSIDE TEST (Keller Killiani test)	+ve
	TANNIN TEST (Test with lead acetate)	+ve
	STEROID TEST (Salkowski's test)	-ve
	TRITERPENOIDS TEST (Test with thionyl chloride)	-ve
	SAPONIN TEST (Foam test)	+ve
	FLAVONOIDS TEST (test with sulphuric acid)	+ve
	ALKALOIDS TEST (Mayer's test)	+ve

TULSI	CARBOHYDRATE TEST (Fehling's test)	-ve
	GLYCOSIDES TEST (Legal test)	+ve
	TANNINS TEST (Test with ferric chloride)	+ve
	STEROIDS TEST (Liebermann Burchard test)	+ve
	TRITERPENOIDS TEST (Test with tin &thionyl chloride)	+ve
	SAPONINS TEST (Foam test)	-ve
	FLAVONOIDS TEST (Test with NaOH)	-ve
AMARANTHUS VIRIDIS	ALKALOIDS TEST (Wagner's reagent)	+ve
	CARBOHYDRATE TEST (Benedict's test)	-ve
	GLYCOSIDE TEST (Keller Killiani test)	+ve
	TANNINS TEST (Test with lead acetate)	+ve
	STEROIDS TEST (Liebermann Burchard test)	-ve
	TRITERPENOIDS TEST (Test with tin &thionyl chloride)	-ve
	SAPONINS TEST (Foam test)	-ve
	FLAVANOIDS TEST (test with sulphuric acid)	+ve

(+ve) =present, (-ve) =absent

TABLE: 2ANTHELMINTIC ACTIVITY:

S.NO	DRUGS	TREATMENT	CONCENTRATION (mg/ml)	TIME TAKEN FOR PARALYSIS (min.)	TIME TAKEN FOR DEATH (min.)
1	*Ocimum sanctum*	Control (Normal saline)	20	–	–
		Methanol Extract	2	32±4	36±5
			10	27±5	30±5
			20	24±5	27±3
		Piperazine hydrazine	20	95±3	111±5
2	*Cassia angustifolia*	Control (Phosphate buffer solution)	20	–	–
		Methanol Extract	10	35±5	40±5
			20	30±5	27±4
			40	16±5	20±5
		Albendazole	5	–	–
			10	120±5	132±5
			20	70±4	78±5
3	*Amaranthus viridis*	Methanol Extract	60	70±5	76±5
			80	62±4	66±4
			100	32±5	36±3
		Piperazine citrate	20	105±5	112±5

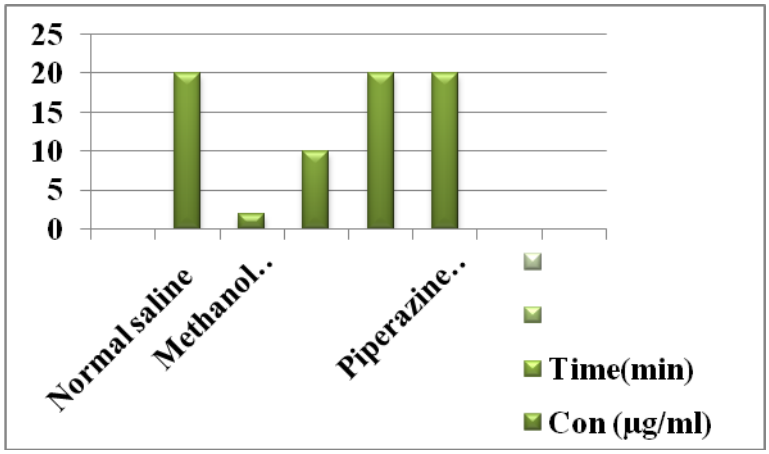


Fig-07 STANDARD GRAPH OF *OCIMUM SANCTUM*

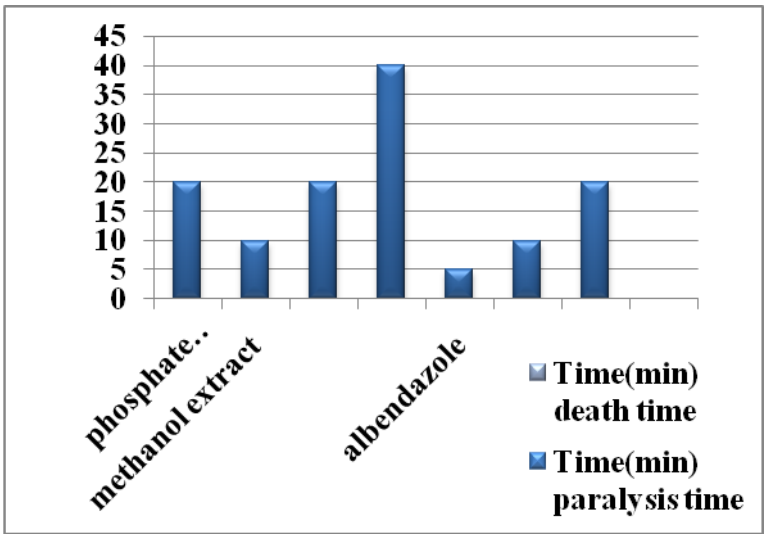


Fig-08 STANDARD GRAPH OF *CASSIA ANGUSTIFOLIA*

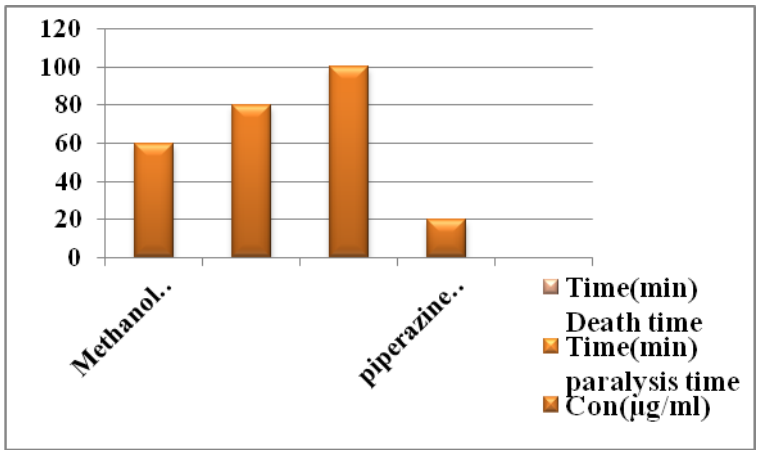


Fig-09 STANDARD GRAPH OF *AMARANTHUS VIRIDIS*

DISCUSSION

The yield of Tulsi, senna, amaranthus viridis leaf extracts were ranged from the order of increasing solvent polarity is methanol. As a result, methanol extract marked the highest percentage yield as the secondary metabolites may be extracted and highly soluble in these polar solvents.

As the medicinal plans possess copious traditional claims such as antihelminthic efficacy, various extracts of leaf were investigated for their antihelminthic potency. Adult earthworms were used in this study as their anatomy and physiology resemble those of intestinal parasitic nematodes. Based on the result of preliminary phytochemical studies antihelminthic activity may be endowed by several bioactive metabolites that were present in leaf extracts, for example, saponins, tannins, triterpenoids, gums and resins, and phenolic compounds.

Methanol extract consumed a shorter time paralyze the selected earthworms in dose-dependent manner. Methanol extracts was lethal to the earthworms which may be due to the high percentage yield of bioactive principles in these extracts in a dose-dependent manner. The results also revealed that aqueous extract was more potent than methanol extract for inhibition of earthworm motility and extermination of earthworms.

The antihelminthic efficacy studied on leaf extracts revealed that the plant may be used to eradicate nematodes from the intestines. Therefore, they have the potential to be new natural antihelminthics after carrying out in vivo research to evaluate their safety and effectiveness.

CONCLUSION

The current study is a preliminary evaluation of leaf extract of crude drugs.

1. Tulsi

2. Senna.

3. Amaranthus.

They have antihelminthic protection. Globally today helminthic infections are a serious growing issue due to the availability of limited and effective antihelminthic drugs.

As we seen from the above studies the following conclusion written as the **“all plant extraction have anti helminthic action on earthworms”**.

With the help of above mentioned results of all plants we come to conclusion that further evaluation studies on human beings are required for the development of its effective action in other disease conditions.

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