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Evaluation of Anthelmintic Activity of Holoptelea Integrifolia

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Abstract: ***Holoptelea intergrifolia (Roxb) Planch (Ulmaceae) is commonly known as Indian elm, kanju. It is widely distributed throughout India in deciduous forests. In traditional system of medicine, bark and leaves are used as bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism. The present study was carried out to investigate the anthelmintic activities of different extracts of benzene, chloroform, methanol and aqueous extracts of the stem bark of Holoptelea integrifolia against adult earth worm Pheretima posthuma. The time taken for each worm for paralysis and death were determined. The results were compared with the results of standard i.e. Piperazine citrate. Methanolic and aqueous extracts both were found to possess significant anthelmintic activity in comparison to the standard drug. Both the extract showed dose dependent anthelmintic activity.***

Keywords: Holoptelea integrifolia, Anthelmintic

1. Introduction

1.1 Helminthiasis

Helminthiasis, also known as **worm infection**, is any macroparasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as helminths. There are numerous species of these parasites, which are broadly classified into tapeworms, flukes, and roundworms. They often live in the gastrointestinal tract of their hosts, but they may also burrow into other organs, where they induce physiological damage. Soil-transmitted helminthiasis and schistosomiasis are the most important helminthiases, and are among the neglected tropical diseases.[1] This group of helmianthiases have been targeted under the joint action of the world's leading pharmaceutical companies and non-governmental organizations through a project launched in 2012 called the London Declaration on Neglected Tropical Diseases, which aims to control or eradicate certain neglected tropical diseases by 2020.[2]

Helminthiasis has been found to result in poor birth outcome, poor cognitive development, poor school and work performance, poor socioeconomic development, and poverty.[3][4] Chronic illness, malnutrition, and anemia are further examples of secondary effects.[5]

Soil-transmitted helminthiases are responsible for parasitic infections in as much as a quarter of the human population worldwide.[6] One well-known example of soil-transmitted helminthiasis is ascariasis.

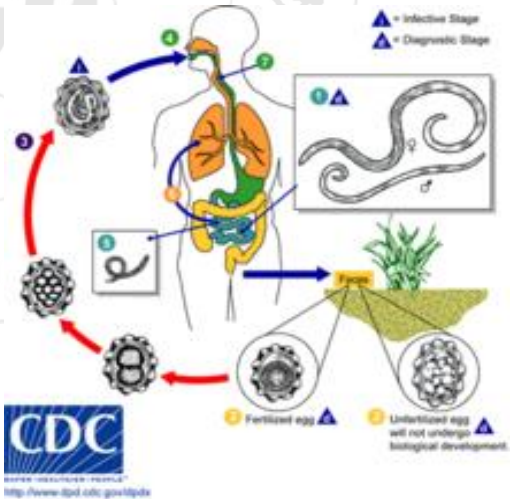
2. Signs and Symptoms

Example clinical photo: Guinea worm infection (dracunculiasis), worm coming out of the foot of an infected person. Ascaris infection: Antimesenteric splitting of the outer layers of the bowel wall due to a large amount of ascaris (South Africa)

The signs and symptoms of helminthiasis depend on a number of factors including: the site of the infestation within the body; the type of worm involved; the number of worms and their volume; the type of damage the infesting worms cause; and the immunological response of the body. Where the burden of parasites in the body is light, there may be no symptoms.

Certain worms may cause particular constellations of symptoms. For instance, taeniasis can lead to seizures due to neurocysticercosis.[7]

2.1 Helmintics causing infections:[9]



Ascaris life cycle: Adult worms in the lumen of the small intestine

1) The female produces eggs (approximately 200,000 per day) that are excreted with the feces .
2) Unfertilized eggs are harmless, but fertilized ones are infective after 18 days to several weeks.
3) Infective eggs are ingested .
4) Enter the gut.
5) Develop into larvae in the intestine, and penetrate the blood vessel to enter lungs, where they develop further.

6) After 10 to 14 days, penetrate the alveolar walls, ascend the bronchial tree to the throat, and are re-swallowed
7) Upon reaching the small intestine, they develop into adult worms.
8) It takes 2 to 3 months for one complete cycle. Adult worms can live 1 to 2 years.

2.2 Transmission

Helminths are transmitted to the final host in several ways. The most common infection is through ingestion of contaminated vegetables, drinking water, and raw or undercooked meat. Contaminated food may contain eggs of nematodes such as *Ascaris*, *Enterobius*, and *Trichuris*; cestodes such as *Taenia*, *Hymenolepis*, and *Echinococcus*; and trematodes such as *Fasciola*. Raw or undercooked meats are the major sources of *Taenia* (pork, beef and venison), *Trichinella* (pork and bear), *Diphyllobothrium* (fish), *Clonorchis* (fish), and *Paragonimus* (crustaceans). Schistosomes and nematodes such as hookworms (*Ancylostoma* and *Necator*) and *Strongyloides* can penetrate the skin directly. Finally, *Wuchereria*, *Onchocerca*, and *Dracunculus* are transmitted by mosquitoes and flies.[11] In the developing world, the use of contaminated water is a major risk factor for infection.[12] Infection can also take place through the practice of geophagy, which is not uncommon in parts of sub-Saharan Africa. Soil is eaten, for example, by children or pregnant women to counteract a real or perceived deficiency of minerals in their diet.[13]

2.3 Diagnosis

Specific helminths can be identified through microscopic examination of their eggs (ova) found in faecal samples. The number of eggs is measured in units of eggs per gram.[14] However, it does not quantify mixed infections, and in practice, is inaccurate for quantifying the eggs of schistosomes and soil-transmitted helminths.[15] Sophisticated tests such as serological assays, antigen tests, and diagnosis are also available;[14][15] however, they are time-consuming, expensive and not always reliable.[16]

2.4 Prevention

Disrupting the cycle of the worm will prevent infestation and re-infestation. Prevention of infection can largely be achieved by addressing the issues of WASH—water, sanitation and hygiene.[17][18][19] The reduction of open defecation is particularly called for,[19][20] as is stopping the use of human waste as fertilizer.[6]

Scientists are also searching for a vaccine against helminths, such as a hookworm vaccine.[21]

2.5 Treatment

2.5.1 Medications

Main article: Anthelmintic

Broad-spectrum benzimidazoles (such as albendazole and mebendazole) are the first line treatment of intestinal roundworm and tapeworm infections. Macrocyclic lactones (such as ivermectin) are effective against adult and migrating larval stages of nematodes. Praziquantel is the drug of choice for schistosomiasis, taeniasis, and most types of food-borne trematodiases. Oxamniquine is also widely used in mass deworming programmes. Pyrantel is commonly used for veterinary nematodiasis.[20]

Example of ascariasis (ascaris infection) - Difficult surgical procedure in South Africa on a gangrenous piece of bowel that had to be cut out; live ascaris worms are emerging.

If complications of helminthiasis, such as intestinal obstruction occur, emergency surgery may be required.[8][51] Patients who require non-emergency surgery, for instance for removal of worms from the biliary tree, can be pre-treated with the anthelmintic drug albendazole.[8]

2.5.2 Deaths

As many as 135,000 die annually from soil transmitted helminthiasis.[3][22]

2.5.3 Plant Profile

Nature has blessed mankind with a treasure of medicinal plants. Natural products have always remained a profile source for the discovery of new drugs and are used since Vedic period. *Holoptelea integrifolia* is a medium-sized large glaborous tree about 15-25 m in height with whitish or yellowish grey bark exfoliating in irregular flakes and possesses an offensive smell when cut freshly.It belongs to family Ulmaceae and is having 15 genera and 200 species.

Figure 2: Fruits of holoptelea integrifolia

Figure 3: Bark of holoptelea integrifola

Vernacular Names

Hindi- Chirmil, Chilbil, Chilla,
Dhamna, Kandru, Kanju, Karanji, Kumba, Kunjanali Kunj;
Gujarati- Charel;

Marathi-Papara;
Sanskrit-Chirbilva;
Tamil-Ayi [23][24]

2.5.4 Distribution

It is widely distributed all over tropical and temperate regions of Northern hemisphere including Indian Peninsula to Indo China, Burma and Srilanka. It is abundantly found in sub Himalayan hills of Assam, Bihar, Ajmere, Bundelkhand.

2.5.6 Pharmacognostic Studies

Holoptelia integrifolia is a large spreading glaborous deciduous tree about 15-18 m high having mucilaginous bark and elliptic leaves [26].

- Leaf:- Leaf is green in colour with slight aromatic odour.External margin of leaf is rough, with particulate venation acute apex and symmetrical base with curved petiole and broad alternate lamina. Broad approx 2-3 cm in size [25]. Leaves are 7.5-12.5 by 3.3-6.3 cm in size. These are elliptic, acuminate, glaborous having rounded base [26].The upper epidermis of leaf consists of small barrel shaped parenchymatous cells. Trichomes are present on both the surfaces of leaf and majority of them are present along the midrib and minimum are found along thelamina. Stomata are present on lower surface and represented by anomocytic type[25].
- Stem:-Brown in colour having agreeable smell and smooth texture. The transverse section of stem is circular and covered with many unicellular uniseriate trichomes. The outermost multilayered periderm consists of cork cambium and secondary cortex. The cork layer is interrupted at many places due to the presence of lenticels. The cortex is multilayered and consists of parenchymatous cells. The primary phloem remains as patches of crushed tissue. The secondary phloem consists of sieve tubes, companion cells, phloem, parenchyma and phloem rays. Vessels are present in broken conditions and crushed form. The xylem is represented by both primary and secondary xylem tissue. It consists of vessels and tracheids. The pith is large and remains to the central part of the stem. It consists of thin walled parenchymatous cells having many intercellular spaces. The pith regions have oil droplets.
- Flowers:-They are sessile, male and bisexual,ciliate, and obtuse. Stamens are biseriate and anthers are slightly curved, hairy, introrse.
- Ovary:- It is compressed,ovate. Stalk is elongating in fruit which is dry, winged, compressed samara, reticulate.
- Seeds are flat and exalbuminous [25].

2.6 Chemical Constituents

The plant has been reported to possess chemical constituents like terpenoids, sterols, saponins, tannins, proteins, carbohydrates and alkaloids[26], flavonoids. The phytoconstituents isolated from stem bark are holoptelin- A and holoptelin-B, 2-aminonaphthoquinone, Friedlin, epifredlin, $_{\beta}$-sitosterol,$_{\beta}$-D-glucose,$_{\beta}$-amyrin,hederagenin (heart wood), hexacosanol.1, 4-naphthalenedione has been isolated from leaves of *Holoptelea integrifolia* and is reported to possess antibacterial activity against Staphalococcus aureus.

2.7 Traditional Uses

Plant is useful in treatment of obesity, edema, and bronchitis. It has been known to be protease inhibitor. Mucilage and juice obtained from boiled bark has been reported to be useful in rheumatism, intestinal tumour when applied externally. Bark juice is applied to rheumatic swellings [6]. Bark juice is useful as oxytoxic in pregnancy. Paste of seeds and bark stem is externally useful in ringworm, eczema and cutaneous affections. Paste of stem bark is applied externally to treat inflammation of lymph gland and common fever, scabies and ringworm. Paste of bark and leaf is applied externally for treatment of leucoderma [23,24]. It is used for ornamental purposes in Pakistan [23]. Bark boiled in oil of *Pongamiaglabra* and garlic is applied externally for the treatment of eczema [13]. Bark and leaves are astringent, bitter, anthelmintic, and are used for the treatment of diabetes, skin disease, intestinal disorder, leprosy, rheumatism [28] and wound-healing in form of paste [14].It is an important pollenallergent plant of India [5, 15].

2.8 Phytochemical evaluation

Phytochemical examinations were carried out for all the extracts as per the standard methods[26].

Phytochemical evaluation*:* Phytochemical examinations were carried out for all the extracts as per the standard methods.

1) Detection of alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Formation of a yellow cream precipitate indicates the presence of Alkaloids.
b) Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.
c) Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.

2) Detection of carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic á-naphthol solution in a test tube and 2 ml of Conc. Sulphuric acid was added carefully along the sides of the test tube. Violet ring at the junction indicates the presence of Carbohydrates.
b) Benedict's test: Filtrates were treated with Benedict's reagent and heated on water bath. Orange red precipitate indicates the presence of reducing sugars.
c) Fehling's test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3) Detection of glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycoside.
b) Legal's test: Extracts were treated with sodium nitropruside in pyridine and methanolic alkali. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4) Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.
b) Foam test**:** Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5) Detection of phytosterols

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.
b) Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of phytosterols.

6) Detection of phenols

Ferric Chloride Test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

7) Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

8) Detection of flavanoids

a) Alkaline Reagent Test : Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
b) Lead acetate Test : Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

9) Detection of proteins and amino acids:

a) Xanthoproteic Test: The extracts were treated with few drops of concentrated Nitric acid solution. Formation of yellow colour indicates the presence of proteins.
b) Ninhydrin test: To the extract, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

10) Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with few drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

3. Materials and method

3.1 Pretreatment of Plant

The plant leaves was initially washed with simply water and then dried in sunlight under shade for 2-3 days until leaves became completely dry. The dried leaves were taken in mixture grinder and powder was made from that. The dried leaves powder was taken for further extraction procedure.

3.2 Pretreatment of plant

The bark of plant holoptelea integrifolia was collected, washed with water and dried in sunlight under shade for 2-3 days until they dried. The dried bark were taken in mixture grinder and powdered make from that. The dried powder was taken for extraction. The plant leaves was initially washed with simply water and then dried in sunlight under shade for 2-3 days until leaves became completely dry. The dried leaves were taken in mixture grinder and powder was made from that. The dried leaves powder. The plant leaves was initially washed with simply water and then dried in sunlight under shade for 2-3 days until leaves became completely dry. The dried leaves were taken in mixture grinder and powder was made from that. The dried leaves powder Pretreatment of Plant. The plant leaves was initially washed with simply water and then dried in sunlight under shade for 2-3 days until leaves became completely dry. The dried leaves were taken in mixture grinder and powder was made from that. The dried leaves powder was taken for further extraction prPretreatment of Plant The plant leaves was initially washed with simply water and then dried in sunlight under shade for 2-3 days until leaves became completely dry. The dried leaves were taken in mixture grinder and powder was made from that. The dried leaves powder was taken for further extraction prPretreatment of Plant. The plant leaves was initially washed with simply water and then dried in sunlight under shade for 2-3 days until leaves became completely dry. The dried leaves were taken in mixture grinder and powder was made from that. The dried leaves powder was taken for further extraction prPretreatment of Plant. The plant leaves was initially washed with simply water and then dried in sunlight under shade for 2-3 days until leaves became completely dry. The dried leaves were taken in mixture grinder and powder was made from that. The dried leaves powder was taken for further extraction pr

3.3 Preparation of extract

The crude ethanolic extract of the Holoptelea integrifolia bark was prepared according to the standard method. One hundred grams of the powdered plant material was mixed with 500mL of ethanol. Extraction is done by the maceration method and then dried on electrical hot water bath.Kept at 4^0C until required.

3.4 Anthelmintic Assay

The assay was performed on adult earthworm Pheretima posthuma due to anatomical and physiological resembalance with the intenstinal round worm parasite of human beings. Because of easy availability, earthworms have been used extensively for the preliminary in _vitro evaluation of anthelmintic compounds in_ vitro. 50 ml formulations containing four different concentrations, ethanolic extract of its various fractions (10, 25, 50,100mg/ml in distilled water) were prepared and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50^0c) Albendazole(10mg/ml) was used as reference standard.

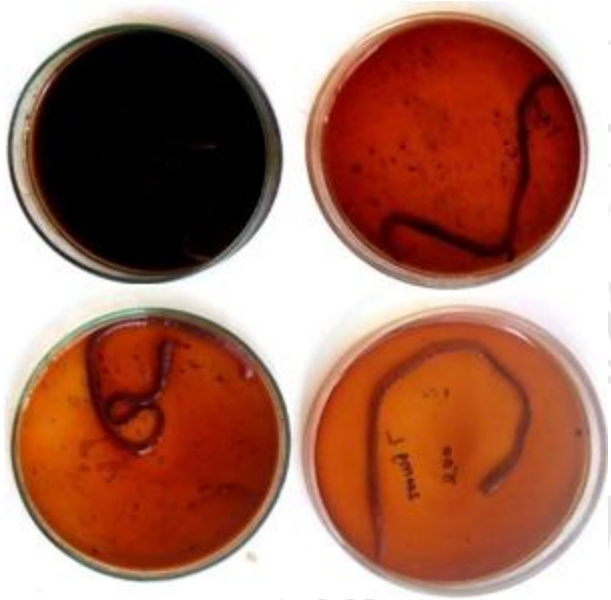


Figure 1: Test Samples

Figure 2: Std.Albendazole

4. Result and discussion

The qualitative phytochemical investigation of different extracts of H*oloptelea integrifolia* showed the presence of an array of active chemical constituents including saponins, steroids, carbohydrates, alkaloids, tannins, glycosides, flavonoids and phenols. These phytoconstituents may be responsible to show a potent anthelmintic activity in ethanolic extract.

Test substance	Concentration (mg/ml)	Paralysis time(min.)	Death time (min.)
Std.1	(50mg/ml)	16min	50min
Std.2	(100mg/ml)	7 min	14min
Test 1	(10mg/ml)	30min	140min
Test2	(25mg/ml)	23min	115min
Test 3	(50mg/ml)	15min	45min
Test 4	(100mg/ml)	6min	14min

5. Conclusion

It can be concluded that active constituents responsible for anthelmintic activity are present in the ethanolic extract of bark of *Holoptelea integrifolia.* The isolation and characterization of active principles responsible for anthelmintic activity of bark extracts of *Holoptelea integrifolia was found to be more potent than standard drug.*

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