Evaluation of Anthelmintic Activity of Holoptelea Integifolia

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Abstract: Holoptelea integifolia (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 integifolia 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 integifolia, 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 helminthiases 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][8] Chronic illness, malnutrition, and anemia are further examples of secondary effects.[9]

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 ascarsis (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.[11]

2.1 Helminthics 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 and beef), Trichinella (pork and bear), Diphyllolothrium (fish), Clonorchis (fish), and Paragonimus (crustaceans).

Schistosomes and nematodes such as hookworms (Anclylostoma and Necator) and Strongyloides can penetrate the skin directly. Finally, Wuchereria, Onchocerca, and Dracunculus are transmitted by mosquitoes and flies. In the developing world, the use of contaminated water is a major risk factor for infection. 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.

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. However, it does not quantify mixed infections, and in practice, is inaccurate for quantifying the eggs of schistosomes and soil-transmitted helminths. Sophisticated tests such as serological assays, antigen tests, and diagnosis are also available, however, they are time-consuming, expensive and not always reliable.

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. The reduction of open defecation is particularly called for, as stopping the use of human waste as fertilizer.

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

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. Oxamnique is also widely used in mass deworming programmes. Pyrantel is commonly used for veterinary nematodiasis.

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. 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.

2.5.2 Deaths

As many as 135,000 die annually from soil transmitted helminthiasis.

2.5.3 Plant Profile

*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.
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 Sri Lanka. It is abundantly found in sub Himalayan hills of Assam, Bihar, Ajmere, Bundelkhand.

2.5.6 Pharmacognostic Studies

*Holoptelia integrifolia* is a large spreading glabrous 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 petiolo 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, glabrous 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 the lamina. 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 inner 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]. The phytoconstituents isolated from stem bark are holoptelin- A and holoptelin-B, 2-amonomoquinone, Friedelin, epifriedelin, \( \beta \)-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. Muclage 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 [10]. 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 *Pongamia glabra* and garlic is applied externally for the treatment of eczema [12]. 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 pollen allergen plant of India [15].

2.8 Phytochemical evaluation

Phytochemical examinations were carried out for all the extracts as per the standard methods [29].

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

1) Detection of alkaloids

Extrats 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) Dragendorff’s test: Filtrates were treated with Dragendorff’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 \( \alpha \)-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
Extrac
1) 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. 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. 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 powder was taken for further extraction pre

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. 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 pre

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°C until required.
3.4 Anthelmintic Assay

The assay was performed on adult earthworm Pheretima posthuma due to anatomical and physiological resemblance with the intestinal 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 asserting that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C) Albendazole(10mg/ml) was used as reference standard.

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.

References


