

COMPARATIVE STUDY OF ANTHELMINTIC ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF BARK OF *HOLOPTELEA INTEGRIFOLIA*

Sarabjot Kaur², Bimlesh Kumar^{1*}, Shakti Puri³, Prashant Tiwari⁴, Kalyani Divakar⁵

^{1,2,3,4} Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India.

⁵ HOD, Department of Pharmacy, Acharya & BM Reddy College of Pharmacy, Soldevanhalli, Bangalore, Karnataka, India

ABSTRACT

The aim of the present study was to evaluate the anthelmintic potential of ethanolic and aqueous extract of bark of *Holoptelea integrifolia* using *Eisenia foetida*. Various concentrations (10, 25, 50 and 100 mg/ml) of ethanolic and aqueous extract were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine Citrate (10 mg/ml) was included as standard. The results indicated that the ethanolic and aqueous extract significantly demonstrated paralysis and also caused death of worms especially at higher concentration as compared to standard references. In conclusion, the use of bark of *Holoptelea integrifolia* as an anthelmintic have been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

Key Words: *Holoptelea integrifolia*, Anthelmintic, *Eisenia foetida*, Piperazine citrate.

Introduction

The World Health Organization revealed that over two billion people are suffering from parasitic worm infections [1]. Helminthiasis or infections with parasitic worms are pathogenic for human beings. Immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminths or development forms that invade organs and tissues. Most of the existing anthelmintics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhoea [2]. The disease is highly prevalent particularly in third world countries due to poor healthcare management practices [3]. Anthelmintics

from the natural sources may play a key role in the treatment of these parasite infections [4]. Increasing problems of development of resistance in helminths against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity [5].

Holoptelea integrifolia (Ulmaceae) is also known as Kanju, Banchilla, Chilbil, Begana (Hindi), Rajain, Arjan (Punjabi), Chirabilva (Sanskrit). It is mainly distributed in Sub- Himalayas, Ajmere, Bihar, Assam, Burma, and sometimes found in peninsula, Ceylon-Cochin-China [6]. Until today; some recent explorations have been reported on this plant in which antiviral activity, antioxidant, antimicrobial & wound healing activity and antiemetic activity is important. Ethnomedically, the leaves and stem bark of this plant were used by local people for skin diseases; obesity, cancer, and leaves decoction were used in the management of diarrhoea [7]. Piperazine introduced into human medicine about 1950 and shortly thereafter

For Correspondence:

E-mail: bimlesh.12474@lpu.co.in

Telephone: +919872260354

into veterinary medicine, relaxes the large intestinal roundworms (ascarids) and pinworms (oxyurids) of man and domesticated animals so that they are eliminated with the faeces [8]. However, anthelmintic activity of *Holoptelea integrifolia* has not so far been scientifically proved. The present study was, therefore, carried out to assess the anthelmintic activity of *Holoptelea integrifolia* against *Eisenia foetida*.

MATERIALS AND METHODS

Plant collection and authentication

The stem bark of *Holoptelea integrifolia* was collected from Tamilnadu and identified and authenticated by regional research institute, Bangalore, India. The voucher specimen no. was RRI/BNG/SMP/Drug Authentication/2009-10/55. The material was dried in shade, ground finally in powder in an electric grinder and stored in cellophane bags at 4°C until use.

Worms Collection and authentication

Eisenia foetida was collected from the water logged areas of the soil and identified and authenticated at the Ujjwal Ujala Vermiculture Group, Vill. Ibban Kalan, Amritsar and its Ref. No. is UUG/13/0102/32.

Preparation of Extract

Aqueous extract preparation:

The crude aqueous extract (AQEHI) of the *Holoptelea integrifolia* bark was prepared according to the standard method. One hundred grams of the powdered plant material was mixed with 500 mL of distilled water in a 1 L flask and boiled for 1.5 h. It was allowed to cool and then filtered using whatman no.1 filter paper. The filtrate was then concentrated in a rotary evaporator and the extract stored at 4°C until required. The extract yield (% w/w) from the plant material was recorded.

Ethanolic extract preparation.

The powder bark material of *Holoptelea integrifolia* was placed in a thimble and extracted with 90% ethanol in a Soxhlet apparatus for 8-12 h. Solvents were removed at temperature below 50°C in an oven. The

residue (extract) of respective plant material was stored at 4°C until used. The extract (ETEHI) yield (% w/w) from the plant material was recorded.

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

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.

d) **Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

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 α -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.

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 ammoniacal layer indicates the presence of anthranol glycosides.

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.

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.

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) **Liebermann 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.

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.

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.

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.

Detection of proteins and aminoacids

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.

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 [9].

Anthelmintic Assay

The assay was performed on adult earthworm, *Eisenia foetida* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Because of easy availability, earthworms have been used extensively for the preliminary *in vitro* evaluation of anthelmintic

compounds *in vitro* [10]. 50 ml formulations containing four different concentrations, each of aqueous and ethanolic extract of its various fractions (10, 25, 50 and 100 mg/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 °C). Piperazine citrate (10 mg/ml) was used as reference standard .

Statistical analysis

Data were analyzed using one way factorial ANOVA tests followed by Dunnett's *t* -tests on each group. P values under 0.01 were considered highly significant (shown as **).

RESULT AND DISCUSSION

The qualitative phytochemical investigation of different extracts of *Holoptelea 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 AQEHI and ETEHI (Table 1). The data revealed that AQEHI & ETEHI showed significant anthelmintic activity at 10, 25, 50, 100 mg/ml concentrations. Results are comparable with standard drug Piperazine citrate (10 mg/ml). Comparison of AQEHI and ETEHI revealed the more potent anthelmintic activity of ETEHI w.r.t. AQEHI. The paralysis and death time at each dose was very prominent in ETEHI w.r.t. AQEHI (Table 2).

Table 1: Preliminary phytochemical screening of different extracts of *Holoptelea integrifolia*

Tests	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Phenols and Tannins	+	+
Carbohydrates	+	+
Saponins	+	+
Flavonoids	+	+
Steroids	+	+
Glycosides	+	+
Triterpenoids	+	+
Proteins and amino acids	+	+

(+) = Present, (-) = Absent

Table 2: Evaluation of anthelmintic activity

Test substance	Concentration (mg/ml)	<i>Eisenia foetida</i>	
		P (min)	D (min)
Piperazine Citrate	10	2.95 ± 0.09	7.10 ± 0.11
AQEHI	10	107.33 ± 0.91 **	289.33 ± 3.41**
	25	88.03 ± 1.71 **	179 ± 2.47**
	50	26.21 ± 0.20 **	60.45 ± 0.53**
	100	9.18 ± 0.25 **	20.36 ± 0.27**
ETEHI	10	28.26 ± 0.62 **	141.15 ± 2.69**
	25	22.31 ± 0.77 **	117.35 ± 0.66**
	50	15.98 ± 0.45 **	47.80 ± 2.43**
	100	6.26 ± 0.08 **	16.73 ± 0.21**

Where, P = time taken for paralysis of worms, D = Time taken for death of worms. Values are expressed as mean \pm SEM. Values were found out by using ONE way ANOVA followed by Dunnett's *t*-test. ** values are significantly different from control at (P<0.01)

Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis and this action may lead to the death of the worm [11]. All anthelmintics essentially kill worms by either starving them to death or paralyzing them. Because worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs. Any disruption in this process results in energy depletion. Parasites will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut [12].

The preliminary phytochemical investigation of the extracts revealed the presence of tannins. Tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that tannins contained in the extracts of *Holoptelea integrifolia* produced similar effects. In another study, polyphenols from bryophytes were shown to have anthelmintic activity. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and causes death. [13].

The phytochemical screening of the extracts also revealed the presence of saponins. Recent research addressed that the main biologic activity ascribed to saponins was their membrane permeabilizing property and pore formation which is similar with two

conventional anthelmintic drugs such as praziquantel and toltrazuril. That is, they would affect the permeability of the cell membrane of the parasites and cause vacuolization and disintegration of monogenea teguments [14].

Piperazine citrate being a heterocyclic ring possessing alkaloid, blocks the intake of acetylcholine from the host organism. Likewise the extracts also contain alkaloids which may possess the same type of pharmacological action and expels the worms by peristaltic movement of intestine. The present work proved the usage of this plant extracts in treatment of helminthiasis [15].

CONCLUSION

It can be concluded that active constituents responsible for anthelmintic activity are present in the aqueous and ethanolic extracts of bark of *Holoptelea integrifolia*. Further work will emphasize the isolation and characterization of active principles responsible for anthelmintic activity of bark extracts of *Holoptelea integrifolia*.

ACKNOWLEDGEMENT

The authors are thankful to Dr. Monica Gulati, Dean, Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara (Punjab) for providing necessary facilities and cooperation during this research work.

REFERENCES

- 1) Mulla WA, Thorat VS, Patil RV, Burade KB. Anthelmintic activity of leaves of *Alocasia indica* Linn. *International Journal of PharmTech Research* 2010; 2(1): 26-30.
- 2) Devi K, Indumathy S, Rathinambal V, Uma S, Kavimani S, Balu V. Anthelmintic Activity of *Asta Churna*. *International Journal of Health Research* 2009; 2(1): 101-103.
- 3) Lateef M, Iqbal Z, Khan MN, Akhtar MS, Jabbar A. Anthelmintic Activity of *Adhatoda vesica* roots.

- International Journal of Agriculture & Biology* 2003; 5(1): 86-90.
- 4) Aswar M, Aswar U, Watkar B, Vyas M, Wagh A, Gujar KN. Anthelmintic activity of *Ficus benghalensis*. *International Journal of Green Pharmacy* 2008; 2: 170-172.
- 5) Iqbal Z, Lateef M, Ashraf M, Jabbar A. Anthelmintic activity of *Artemisia brevifolia* in sheep. *Journal of Ethnopharmacology* 2004; 93: 265–268.
- 6) Acharya D. Monkey's favorite seasonal fruit: *Holoptelea integrifolia*. *American chronicle* 2008: 1-5.
- 7) Sharma S, Lakshmi KS, Rajesh T. Evaluation of antidiarrhoeal potentials of ethanolic extract of leaves of *Holoptelea integrifolia* in mice model. *International Journal of PharmTech Research* 2009; 1(3): 832-836.
- 9) Dwivedi A, Dwivedi S, Sitoke AK, Patel R, Jhade D. Anthelmintic Activity of a Polyherbal preparation. *Ethnobotanical Leaflets* 2009; 13: 259-262.
- 10) Roopashree TS, Raman Dang, Shobha Rani RH, Narendra C. Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *International Journal of Applied Research in Natural Products* 2008; 1(3): 20-28.
- 11) Sangeetha J, Soundarya K, Santhosh K, Sindhura C. Evaluation of In-vitro Anthelmintic Property of *Passiflora edulis* Linn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 2010; 1(3): 715-718.
- 12) Chandrashekhara CH, Latha KP, Vagdevi HM, Vaidya VP. Anthelmintic activity of the crude extracts of *Ficus racemosa*. *International Journal of Green Pharmacy* 2008: 100-103.
- 13) [Susan schoenian](#). *Understanding anthelmintics. Small ruminant info sheet* 2010.
- 14) Patel J, Kumar GS, Qureshi MS, Jena PK. Anthelmintic activity of ethanolic extract of whole plant of *Eupatorium odoratum*. *International Journal of Phytomedicine* 2010; 2: 127-132.
- 15) Wang GX, Han J, Zhao LW, Jiang DX, Liu YT, Liu XL. Anthelmintic activity of steroidal saponins from *Paris polyphylla*. *Phytomedicine* 2010; 17: 1102-1105.
- 16) Elayaraja A, Vijayalakshmi M, Garikapati D. Anthelmintic screening of various crude extracts of *Phyllanthus simplex* Retz. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 2010; 1(4): 425-428.

Author Information: Mr. Bimlesh Kumar is working as Assistant Professor at the Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India

Article History:-----

Date of Submission: 23-10-10

Date of Acceptance: 12-12-10

Conflict of Interest: NIL

Source of Support: NONE