International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

The In-vitro Antihelmentic Activity of Ethanolic and Aqueous Extracts of Leucasdeflexa Leaf and Embeliaschimperi Fruit Traditionally Used Medicinal Plants in Bench, Sheko and Meinitethinic Groups of SNNPR, Ethiopia

AlemuTadesseFeroche[1], Salahuddin Mohammed[2]

[1]Department of Pharmacy, Mizan Tepi University, Mizan Teferi, Ethiopia

[2]Corresponding Author: P.O.BOX 260, Mizan Teferi, Ethiopia

Abstract: ***The antihelmentic activity of ethanolic and aqueous extracts of lucasdeflexa leaf and embeliaschimperiwere tested against an earthworm, particularly ethanolic extract of Embeliaschimperi fruit showing to be better than the standard drug Albendazole, which is used in the treatment of helmentic parasite infections.***

Keywords: lucasdeflexa; antihelmentic; embeliaschimperi; earthworm

1. Introduction

Helminth infections are among the most common infections in man, affecting a large proportion of the world´s population. In developing countries they pose a large threat to public health, and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited thoseareas, and some of them can be developed in template climates (Bundy, D.A.P. and et al., 1994). The limited availability and affordability of pharmaceutical medicines mean that the world´s population depends to a great extent on traditional medical remedies, and some20,000 species of higher plants are used medicinally throughout the world.

Many well-known drugs listed in the modern pharmacopoeia have their origins in nature, including for example, quinine from the bark of Cinchona tree for the treatment of malaria, which has been followed by the subsequent development of the synthetic derivates chloroquine, a modiaquine, primaquine and mefloquine.

More recently, the wider recognition of the antimalarial activity of artemisinin from the herb *Artemisia annua* has led current research tofocus on the development of a large number of synthetic and semi-synthetic compounds, which are more active than artemisinin (S.Tagboto, S. Townson, 2001).

Thus use of traditional medicine is widespread and plants still present a large source of novel active biological compounds with different activities, including anti-inflammatory, anticancer,anti-viral, anti-bacterial and cardio-protective activities. This is the era of competition between allopathic and herbal medicines where, herbal medicinal products occupy a significant place in consumer consciousness in the developed world and are important in healthcare in most developing countries (Om Prakash et al., 2009).

There is increasing interest from the medical and scientific communities in giving them a place in evidence-based medicine and this is consolidated by a more sympathetic attitude on the part of regulatory authorities than has previously been the case. *Leucasdeflexa* leaf and *Embeliaschimperi* fruit which are widely utilized in traditional medicine systems in Bench, Sheko and Meinitethinic groups of SNNPR, Ethiopia. The leaves of *Leucasdeflexa* additionally have been used to treat stomach ache, diarrhoea, ascariasis and snakebite in Bench and Sheko ethnic groups. The fruits and roots of *Embeliaschimperi* have been used for the treatment of ascariasis and taeniasis in this area (Mirutse G., Zemede et al., 2010).

2. General Objective

To investigate *in vitro*antihelmentic activity of ethanolic and aqueous extracts of*leucasdeflexa* (leaf) and*embeliaschimperi* (fruit)

2.1 Specific objectives

- To investigate antihelmentic activities of 80 % ethanolic extracts of the two plants
- To investigate antihelmentic activities of aqueous extracts of the two plants
- To test antihelmentic activities of the solvent fractionates

3. Materials and Methods

3.1 Plant Material

The leaf of the plant *Leucasd eflexa* and the fruit of *Embeliaschimperi* were collected from TemenjaYaj, Andekel, Janchu and KobKebeles of Bench Maji Zone, SNNPR. The leaves of these medicinal plants were collected, dried, properly identified and authenticated at the National Herbarium of the Addis Ababa University.

3.2 Extraction Procedure

The leaf and fruit of the two plant parts were dried separately in shade and powdered to get a coarse powder. About a significant amount of dry coarse powder was extracted with ethanol ($40\text{-}60^0$C) by continuous hotpercolation using soxhlet apparatus (Khadse et al., 2010). The ethanol and aqueous extract were filtered and concentrated separately to a dry mass by using vacuum distillation. A deep green viscous residue will be obtained having characteristic odour. Further the extracts were evaporated to dryness.

3.3 Chemicals and Standard Drugs

The following drug and chemicals was used. Drug: Albendazole (EPHARM) and allother chemicals were of analytical grade.

3.4 Experimental Model

Adult earthworms (*Pheretimaposthuma*) were collected from moist soil and washed with normal saline to remove all fecal matter (Yalaga, Rama R. and Shiferaw Belachew, 2013). The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Nirmal, S.A. and et al., 2007).

Because of easy availability, earthworms have been widely used for the evaluation of antihelmentic compounds in vitro (Sollman T.,1918 and Dash G.K, Suresh P, Kar DM and et al.,2002).

A wooden bed with a length of 10 meters and width of 5 meters was constructed at Aklilu Lemma Institute of Pathobiology. The height from the ground was 1 meter and kept in one of the rooms of Aklilu Lemma Institute of Pathobiology. The wooden bed was kept in such a way to protect against direct sun light and wind. Three different types of waste materials were used for this procedure. These are Cow dung slurry, Horse manure and Grass clipping of wheat. Each of the waste materials were soaked in water and kept for three weeks before inoculated to the large plastic beans. The plastic bins were placed on the wooden bed and prepared the bed. At the bottom of the first bin, broken bricks were kept as the first layer, which is followed by the coarse sand as the second layer. Above these layers, cow dung slurry were placed (Yalaga Rama Rao and Shiferaw Belachew, 2013).

3.5 Antihelmentic Investigation

The anthelmentic experiment was carried as per the method described by Mathew *et al*, and Dash *et al* with minor modifications. The experiment was performed on adult earthworm due to its a natomical and physiological resemblance with the intestinal roundworm parasite of human beings (Khadse et al., 2010; Kosalgeet al., 2009). Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmentic compounds *invitro*.50ml of formulation containing five different concentrations of ethanol and aqueous extracts (2.5, 5, 10, 20 & 50mg/ml in distilled water) were prepared and four worms of approximately equal size were placed in it. Observations were made for the time taken to paralyze and/or death of individual worms up to four hours of test period. Time for paralysis was noted when no movement of any sort could be observed except when the worms are shaken vigorously (Ghosh, T., Maity, T.K. and et al.,2005). Albendazole (10mg/ml) was used as reference standard while distilled water as control (Raut et al., 2009).

3. Statistical Analysis

All the data obtained was presented as Mean± SEM and were analyzed with student-t test.

4. Results and Discussion

The earthworm *Pheretimaposthuma* (Annelida, Megascolecidae) were used for evaluating the antihelmentic activity of the leaf of *Leucasdeflexa* and fruit of *Embeliaschimperi* extracts using a reference substance for comparison. The antihelmentic activity was evaluated on the earthworm *Pheretimaposthuma* (Annelida, Megascolecidae) collected due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. The method of Mathew *et al*. was followed for antihelmentic screening. Emulsions of leaf of *Leucasdeflexa* and the fruit of *Embeliaschimperi* extracts were prepared, and further diluted to givedoses of (2.5, 5, 10, 20 & 50mg/ml emulsions.

Table 1: Antihelmintic activity of *Leucasdeflexa* leaf extract

Type of Extract	*Dose (mg/ml)*	*Time (min) taken for Paralysis of Earth worms (mean values)*	*Time (min) taken for Death of Earth worms (mean values)*
Ethanolic	2.5	189	166
	5	137	139
	10	110	130
	20	59	42
	50	27	16
Aqueous	2.5	239	242
	5	174	182
	10	153	150
	20	79	76
	50	41	46
Albendazole	2.5	186	161
	5	137	132
	10	108	121
	20	55	38
	50	24	19

Table 2: Antihelmintic activity of *Embeliaschimperi* fruit extract

Type of Extract	Dose (mg/ml)	Time (min) taken for Paralysis of Earth worms (mean values)	Time (min) taken for Death of Earth worms (mean values)
Ethanolic	2.5	158	153
	5	126	128
	10	101	98
	20	43	40
	50	16	18
Aqueous	2.5	183	157
	5	134	133
	10	113	120
	20	57	36
	50	26	16
Albendazole	2.5	186	161
	5	137	132
	10	108	121
	20	55	38
	50	24	19

Albendazole solutions of the same concentrations were prepared using distilled water, and used as reference standard. The emulsion solution was diluted to 10 ml each using physiological solution, and further poured into Petri dishes. The anihelmentic activity was determined in duplicate. Four worms of about the same size per Petri dish were used. The death and/or total paralysis time were recorded at room temperature. The death of the worm was ascertained by transferring it into a beaker containing hot water at 50ºC, which stimulated and induced movements if the worm was alive. Five independent experiments were carried out for each observation to confirm the results.

The results in Table 1 and Table 2 indicate that the aqueous and alcoholic extracts obtained from the leaves of *Leucasdeflexa* and the fruits of *Embeliaschimperi*are active against the earthworms tested.

The ethanolic extracts of the fruit of *Embeliaschimperi* at all concentrations showed a greater antihelmentic activity than the standard drug albendazole. The aqueous extracts of *Embeliaschimperi* fruits and alcoholic extracts of *Leucasdeflexa* leaves have shown good antihelmentic activity and it is comparable with the effect produced by the reference drug used.

The aqueous extract of *Leucasdeflexa* leaves has shown activity which is less than the reference drug albendazole. These findings support the use of leaves of *Leucasdeflexa* and the fruits of *Embeliaschimperi* as antihelmentic drug in the traditional medicine.

The present study reveals that the ethanolic extract was more potent than the aqueous extract even though both the extracts were endowed with antihelmentic activity.

5. Conclusion

In conclusion, plants are blessed with immense potent activities in combating different types of diseases, the requirement is to explore it the most for its active constituents and further more regarding its mode of action and structural analysis so that a better and more advanced formulation can be prepared for the mainstream administration of the drug.

The function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the faeces of man and animals. These extracts not only demonstrated this property, they also caused death of the worms, especially at high concentrations as compared with the Albendazole. Therefore this research revealed that the aqueous and alcoholic extracts obtained from the leaves of *Leucasdeflexa* and the fruits of *Embeliaschimperi* possess good antihelmintic activity. Especially the ethanolic extracts of the fruit of *Embeliaschimperi* at all concentrations showed a greater antihelmentic activity than the standard drug albendazole. Therefore further investigation must be done to explore the chemical nature of the active constituents and other pharmacological activities of the extracts.

References

[1] D.A.P. Bundy (1994). Transactions of the Royal Society of Tropical Medicine and Hygiene,**8**: 259.
[2] S. Tagboto, S.Townson (2001).Advances in Parasitology,**50**: 199-295.
[3] Om Prakash, Rajesh Kumar, Anurag Mishra,Rajiv Gupta.(2009). Artocarpusheterophyllus (Jackfruit): *An Overview. Phcog Rev.* **3(6)**: 353-358.
[4] Mirutse G., Zemede A., Zerihun W.(2010).Ethnomedicinal study of plants used by Sheko ethnic group of Ethiopia.*Journal of Ethnopharmacology.*,**132:** 75–85,124
[5] Mirutse G., Zemede A., Zerihun W.(2009). Medicinal plant knowledge of the Bench ethnic group of Ethiopia: an ethnobotanical investigation. *Journal of Ethnobiology and Ethnomedicine*,**5**:34
[6] Abebe, D. and Hagos, E. (1991). Plants as a primary source of drugs in the traditional health practices of Ethiopia. *Plant Genetic Resources of Ethiopia, Cambridge University Press, Cambridge*, pp. 01–13.
[7] Chandrashekhar D. Khadse, Rajendra B. Kakde, (2010). *In vitro* anthelmintic activity of Fenugreek seeds extract against *Pheritimaposthuma. Int. J. Res. Pharm. Sci.***1(3)**: 267-269
[8] Satish b. Kosalge, Ravindra A. Fursule, (2009). Investigation of in vitroanthelmintic activity of Thespesia lampas (cav.);*Asian J. Pharm Clin Res*.**2(2)**:69-71.
[9] Yalaga Rama Rao and ShiferawBelachew, (2013).Vermicompost Technology For The Sustainable Development of Agriculture of Bale Zone,South East Ethiopia**.** *International Indexed & Refereed Research Journal, ISSN 0975-3486,(Print)E-ISSN-2320-5482, April- May (Combind), VOL-IV * ISSUE 43-44*
[10] Nirmal, S.A., Malwadkar and Laware, R.B. (2007). Anthelmintic activity of *Pongamiaglabra*. Songklanakarin. *J. Sci. Technol.*, Vol. 29; No.3.
[11] Vidyarthi R.D. A. (1967). Textbook of Zoology. 14thed. New Delhi: S. Chand and Co.
[12] Thorn G.W, Adams R.D, Braunwald E, Isselbacher K.J, Petersdorf R.G. (1977). *Harrison's Principles of Internal Medicine. New York: Mc Graw Hill Co.*

[13] Sollman T. (1918). Anthelmintics: Their efficiency as tested on earthworms. *J. Pharmcology Exp. Ther*.**12**: 129-70.
[14] Jain ml, Jain S.R. (1972). Therapeutic utility of Ocimumbasilicum var. album. Planta Med; **22**:66-70.
[15] Dash G.K, Suresh P, Kar D.M, Ganpaty S, Panda S.B. (2002). Evaluation of Evolvulusalsinoides Linn. For antihelmentic and antimicrobial activities. *J Nat. Rem*.**2**: 182-5.
[16] Deore S.L., Kamdi K.S., Ingle V.P., Kawalkar N.G., Sawarkar P.S., Patil U.A.(2009). *In vitro* Antihelmentic activity of Cassia tora. *International J of Chem Tech Research*. Vo. 1, No.2, pp 177-179.
[17] Mathew, A.S., Patel, K.N., and Shah, B.K.(1995). *Indian J. Nat. Prod*., **14(1)**: 11.
[18] Dash, G.K., Mishra, B., Panda, A., Patro, C.P. and Ganapaty, S.(2003).*Indian J. Nat. Prod*., **19(3)**: 24.
[19] Ghosh, T., Maity, T.K., Bose, A. and Dash, G.K. (2005).Antihelmentic activity of *Bacopamonneri*. *Indian J. Nat. Prod*., **21(2)**: 16.
[20] Dipak N. Raut, Subodh C. Pal, Subhash C. Mandal. (2009). Anthelmintic potential of *DendrophthoeFalcata*Etting(L.F.) Leaf. *Int. J. Pharm. Res. Develop., Online*, 6(2).