Investigation of in-vitro Anthelmintic Potential of Fruits of some Ethnobotanically Important Trees of Punjab, Pakistan

*SHAZIA KANWAL MALIK¹, ZAHEER-UD-DIN KHAN² & FARAH KHAN³

1, 3 Department of Botany, LCW University, Lahore, Pakistan, 2 Department of Botany, GC University, Lahore, Pakistan.

ABSTRACT

The current research is the investigation of an ethnopharmacological impact i.e., anthelmintic action of fruits of three traditionally used trees, i.e. Pterospermum acerifolium Linn, Diospyros malabarica Kostel and Putranjiva roxburghii Wall. The crude extracts of fruits were obtained in polar and non-polar solvents i.e., petroleum ether, chloroform, methanol and water and evaluated for their in vitro anthelmintic activity and compared with standard medicine i.e. Leva-misole (positive control). The criterion was the apparent mortality of a worm Haemonchus contortus on the visual basis. The aqueous and chloroform fruit extracts of P.acerifolium and petroleum ether extract of D.malabarica demonstrated most noteworthy wormicidal activity (2:00hrs) against H. contortus, while different extract exhibited fluctuated level of potency (3:00 to 4:00 hrs), approximately equal to the standard drug, i.e. Leva-misole.

Key words: Anthelmintic activity, Haemonchus contortus, Putranjiva roxburghii Wall, Diospyros malabarica Kostel, Pterospermum acerifolium Linn.

INTRODUCTION

Plants have been a wealth of medications for human and animal diseases for centuries. Today, 80% of the world population depends on the natural medication. Helminthiasis is a parasitic group causing diseases in animals (Lateef et al., 2006). Use of anthelmintics result into the resistance in helminthes, against anthelmintic compounds and chemical residues. Lateef et al. (2003) has provoked the use of anthelmintic medicinal plants instead of chemicals in present clinical practices. So research in this area is necessary for the search of medicinal plants. The natural compounds of these plants and the right selection for the anthelmintic experiments may open ways for controlling parasitic diseases.

Parasitic nematodes, particularly H. contortus (Rudolphi), is among the most widely recognized reasons for anemia and weight loss in ruminants. Anthelmintic drugs are used to control these parasites. Plant based medication are generally used for the treatment of ruminants. Ademola & Eloff (2010) determined the anthelmintic activity of leaf extract and parts of Combretum molle against H. contortus. Eguale et al.(2007); Fall et al. (2008); Sujon et al. (2008); Nery et al. (2010); and Ademola & Eloff (2010) investigated anthelmintic activity of various plants like Croton zehntneri, Lippia sidoides, Eucalyptus staigeriana, Allium sativum and Ficus religiosa against all H. contortus and proved that these plants had anthelmintic activity.

The objective of the present study was to investigate the in vitro anthelmintic activity of ethnobotanically important angiosperms (P. roxburghii, D. malabarica, P. acerifolium) in comparison with the synthetic anthelmintic drug i.e. Levamisole against, H. contortus.

MATERIAL AND METHODS

Fruits of P. roxburghii, D. malabarica, and P. acerifolium were procured from GC University Botanic Garden, Lahore during March to May (2010) and validated by Dr. Zaheer-ud-Khan, GC University, Lahore.

Preparation of Plant extracts

The parts of plants including (fruit, branches, leaves and bark) were dehydrated (21°C) and were pounded to powder, 150g of dehydrated powder was extracted by soaking for 3 days in petroleum ether (40 to 60°C)/chloroform/methanol/distilled water as per Inmaculada et al. (2005). Physical characteristics of dehydrated powder are given in Table I.

Powder, 5 g was dissolved in 0.1 ml of Dimethylsulfoxide according to Rabel et al. (1994) and afterward 9.9 ml Phosphate-buffered saline was added and mixed to get homogenous solution.

In vitro anthelmintic activity (Adult motility assay)

In lab experiments were performed by using extracts on H. contortus, According to Sharma et al. (1971). The worms were picked up with forceps
from goat abomasal, washed in Phosphate-buffered saline. 10 worms were taken in petri plates and run in triplicates.

- Plant extract = 0.5 mg/ml
- Levamisole = 0.5 mg/ml (standard prescription)
- Phosphate-buffered saline + 1 % Dimethylsulfoxide solution = (control)

The movement of worms was observed in the prepared plant extracts. The worms in petri plates were soaked in extracts. Worms with high absorption of extracts became less motile and finally static. Duration of time taken by worms for complete death was determined. These worms were checked for restoration of life by keeping them in Phosphate-buffered saline (37ºC) for 30 minutes.

Data analysis

One-way ANOVA, LSD tests were used, using SPSS 13.0 (statistical software).

RESULTS AND DISCUSSION

Physicochemical examination of fruit extracts (Table 1) showed variation in colour, texture and amount of dried compounds. Proteins, Sugars, Lipids, Fiber and Vitamins, Saponins and herbal oils have different solubility rates in different solvents according to their polarity ratios. Hence they show variations in physicochemical analysis (Gulfraz et al., 2006; Wilfred et al., 2010). These studies will help us in screening of plants and plant parts of natural profitable compounds to be used in preparation of medicines.

### Table I: Yield and physical characteristics of fruit extracts of P. acerifolium, D. malabarica and P. roxburghii

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Solvent name</th>
<th>Yield (%)</th>
<th>Color</th>
<th>Physical state</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. acerifolium</td>
<td>Petroleum Ether</td>
<td>1.461</td>
<td>Olive Colour</td>
<td>Powder</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>1.492</td>
<td>Green</td>
<td>Solid mass</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>1.474</td>
<td>Maroon</td>
<td>Oily resinous</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1.470</td>
<td>Maroon</td>
<td>Solid mass</td>
</tr>
<tr>
<td>D. malabarica</td>
<td>Petroleum Ether</td>
<td>1.490</td>
<td>Yellowish green</td>
<td>Powdery mass</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>1.494</td>
<td>Olive Colour</td>
<td>Gummy resinous</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>0.955</td>
<td>Maroon</td>
<td>Resinous</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1.360</td>
<td>Maroon</td>
<td>Powdery mass</td>
</tr>
<tr>
<td>P. roxburghii</td>
<td>Petroleum Ether</td>
<td>1.417</td>
<td>Olive Colour</td>
<td>Oily resinous</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>1.470</td>
<td>Yellowish green</td>
<td>Powdery mass</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>1.448</td>
<td>Maroon</td>
<td>Resinous</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>1.447</td>
<td>Maroon</td>
<td>Gummy resinous</td>
</tr>
</tbody>
</table>

The comparative analysis (Table 1, Figs. 1-6) of in vitro anthelmintic activity of fruits of P.acerifolium, D.malabarica, and P.roxburghii, with reference to Levamisole and control revealed significantly different results. The aqueous and chloroform fruit of P.acerifolium and petroleum ether extract of D.malabarica exhibited high potency i.e. mortality of worms in 2 hr against H. contortus, while other extracts presented varied degree of potency (3:00 to 4:00 hrs) as that of Levamisole. These results showed that extracts of fruits of trees contained anthelmintic compounds in them (Amaral et al., 1998; Lateef et al., 2003; Gblolade and Adeyemi, 2008; and Maphosa et al., 2010). These
bioactive compounds may have affected the blood circulatory system as vasoconstriction was observed in worms of experimental plates with substantial decrease in reddish pink color to pale yellow that may have finally resulted in the death of worms (Plates 1 & 2). Change in the color of worms (dead) was a sign parameter that showed extracts disseminate transcutaneously into the body of the worms hence harmed the circulatory system by vasoconstriction. This may be due to the production of free radicals (Cumming et al., 1997) or may be blockage of the transmittance of electrical activity in nerves and muscle cells (Bloomquist, 1996; Bloomquist 2003), or through binding to G-protein coupled receptors called latrophilins (Weinbach & Garbus 1969; Willson et al., 2004).

Plate 1: Living H. contortus

Plate 2: Effect of fruit extract on H. contortus

Variations in the anthelmintic movement under the influence of the extracts of fruits of diverse trees, may be because of the distinction in the objectives on the parasites for activity of the compounds, presence of secondary metabolites, qualitative and/or quantitative contrasts in the dynamic standards showed in extracts. Diverse compounds/dynamic standards of extracts may exasperate the ordinary biochemical and physiological procedures prompting starvation, basic changes, neuromuscular intrusions, and different impacts on helminthes (Kohler, 2001; Mottler et al. 2006).

The result of ANOVA for anthelemintic activity of petroleum ether, chloroform, methanol and aqueous fruit extract of P. acerifolium, D. malabarica and P. roxburghii showed no significant difference at P>0.05 but significant difference was observed during time taken to kill worms (Figs 1 to 6).

Fig., 2: In vitro anthelmintic activity of Levamisole (taken as positive control). From pink color to pale yellow.
**Fig., 3:** Comparative analysis of *in vitro* anthelmintic activity of *fruit*.

**Fig., 4:** Comparative analysis of *in vitro* anthelmintic activity of *fruit*.

**Fig., 5:** Comparative analysis of *in vitro* anthelmintic activity of *fruit*.
Conclusion and Recommendations

The study not only supports the folk uses of the crude drugs but also justifies the ethnopharmacological approach in the search for novel bioactive compounds. Nevertheless, this study has exhibited in vitro anthelmintic movement of ethnobotanically important trees. Nonetheless, because of the significant variation in conditions experienced in vivo, as metabolic biotransformation, connection with food material and assimilation, the outcomes acquired by the in vitro technique couldn’t be extrapolated for in vivo action. There is henceforth required that these outcomes ought to be explored by in vivo assessment. Besides there is requirement for institutionalization of dosages and lethality.

REFERENCES


pathogenic races of \textit{Fusarium oxysporum} f. sp. Ciceris: Enzyme production in culture and purification and characterization of major isoforma as an alkaline endo-$\beta$-(1,4)-xylanase of low molecular weight,” A van Leeuw. J. Microb. \textbf{88}(1):48-59.


