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Potential anthelmintic activity of *Pelargonium endlicherianum* Fenzl.

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ABSTRACT

Ethnopharmacological relevance: The decoction prepared from the roots of *Pelargonium endlicherianum* Fenzl. has been used for the treatment of gastrointestinal parasitism in small ruminants in Turkish Folk Medicine.
Aim of the study: The aim of the present study is to investigate *in vitro* anthelmintic activity of the extracts prepared from the roots of *Pelargonium endlicherianum* Fenzl. (Geraniaceae).
Materials and methods: So as to determine the potential anthelmintic effect of the roots of the plant, *n*-hexane, ethyl acetate (EtOAc) and methanol (MeOH) extracts were successively prepared. *In vitro* test methods were used for the determination of the anthelmintic effect of the extracts on eggs, larvae and adults of *Haemonchus contortus*. The extracts were prepared in three increasing concentrations by using Phosphate Buffered Saline (PBS) for egg hatch, larval development assay and adult motility inhibition assay. PBS was used as negative control, levamisole (in PBS) was used as a reference.
Results: The extracts exerted significant anthelmintic activity on three lifecycle stages of *Haemonchus contortus* when compared to the negative control group ($P < 0.05$). The activity was proportional to the concentrations of the plant extracts for egg hatching and the first stage larvae but not for the adult worms. Moreover, the results have shown that the MeOH extract was found to have higher ovicidal and larvicidal effects than the other extracts.
Conclusions: Results of the present research have revealed that MeOH extract obtained from *P. endlicherianum* demonstrated *in vitro* anthelmintic effect against the eggs, the first stage larvae and the adult stage of *H. contortus*. These results confirmed the folkloric use of the plant. It was suggested that the tannin content of the plant could be responsible for the anthelmintic activity.

1. Introduction

Gastrointestinal nematode parasitism in the livestock is highly prevalent and causes a huge economic loss resulting in the mortality of young animals as well as reduction in the yield of animal products (Fabiyi, 1987; Krecek and Waller, 2006; Sevimli, 2013). *Haemonchus contortus* which is the most dominant, highly pathogenic and prolific parasite, mainly causes gastrointestinal parasitism in the livestock (Jacquiet et al., 1995). Besides modern anthelmintic drugs, the control of these parasitic infections could be also provided by the use of natural sources. For centuries, plants have been used as anthelmintics by many indigenous cultures. Moreover, for the treatment of many parasitic diseases, natural sources are still an important option due to their low cost and easy accessibility, especially in many developing countries (Gazzinelli et al., 2012; Stangeland et al., 2008; Tanner et al., 2011). According to the mentioned traditional information, scientific studies regarding the evaluation of the anthelmintic potential of traditional medicinal plants should be conducted in order to identify the active constituents (Githiori et al., 2005).

Pelargonium genus (Geraniaceae) comprises about 750 species which are growing wild in temperate and subtropical climates. Many species of this genus have been reported to be used for the treatment of rheumatism and intestinal diseases such as diarrhea, dysentery and to be used as antispasmodic, analgesic, antipyretic, diuretic and anthelmintic (Lis-Balchin, 2002; Mabberley, 1997; Mabona et al., 2013). Biological activity studies on these species revealed different medicinal features including antioxidant, (Koutelidakis et al., 2009; Petlevski et al., 2013) antimicrobial (Kolodziej and Kiderlen, 2007; Lalli et al., 2008), antituberculosis (Kolodziej et al., 2003; Mativandlela, 2005), immunomodulator (Conrad et al., 2007; Kolodziej et al., 2005; Trun et al., 2007) and antiviral (Michaelis et al., 2011; Schnitzler et al., 2008;) activities. *Pelargonium* species include many important secondary

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metabolites such as coumarins, phenolic acids, flavonoids, tannins and their derivatives (Kolodziej, 2007).

In Turkish Folk Medicine, the decoction prepared from the roots and fresh flowers of *Pelargonium endlicherianum* Fenzl., known as "solucanotu" by local people in Turkey, has been used for the treatment of gastrointestinal parasitism in small ruminants (Bozan et al., 1999; Sezik et al., 2001). According to the literature survey, no scientific study has been reported on the anthelmintic activity of *P. endlicherianum*. Therefore, the aim of this study is to assess the *in vitro* anthelminthic effect of *P. endlicherianum* in order to verify the ethnobotanical use of the plant in scientific platform.

2. Materials and methods

2.1. Plant material

Roots of *Pelargonium endlicherianum* Fenzl. were collected from Kaledibi Village, Iskenderun, Hatay in Turkey, in May 2014. A voucher specimen (24520) authenticated by Prof. Dr. Hayri Duman (Department of Biology, Faculty of Science, Gazi University) is deposited at the Herbarium of Faculty of Science, Gazi University, Ankara, Turkey.

2.2. Preparation of the plant extracts

An amount of 500 g of shade dried and powdered plant material was subjected to successive solvent extractions with *n*-hexane, ethyl acetate (EtOAc) and methanol (MeOH) at room temperature for 48 h (5 × 10 L). After filtration, the extracts were evaporated by using a rotary evaporator (Buchi, Switzerland) at 40 °C. Yields of each extract were 10.13% for *n*-hexane, 15.27% for EtOAc and 27.08% for MeOH.

2.3. Biological activity assays

Egg hatching assay (EHA), larval development assay (LDA) and adult motility inhibition tests were employed for the determination of the anthelmintic effect of the extracts.

2.3.1. Collection of adult parasites and egg recovery technique

The adult *Haemonchus contortus* were picked manually with forceps from the abomasa of naturally infected sheep after their slaughter at the local abattoir. Adult worms were then washed and put into phosphate buffered saline (PBS). Female parasites separated from males (Soulsby, 1982) were used for egg collection and/or adult motility inhibition test (Jabbar et al., 2006). Female adult *Haemonchus contortus* were crushed using pestle and mortar. After liberation, the eggs were cultured for 8 days at 25 °C in a glass jar filled with autoclaved sheep faeces.

2.3.2. Egg hatching assay (EHA)

Egg hatching assay was performed according to the procedure described by Coles et al. (2006). Approximately 100 eggs were added per tube, each of which contained 1 ml of PBS and 1 ml of increasing concentrations of plant extracts prepared in PBS, levamisole at 0.125 g/ml as reference and negative control (PBS). The covered tubes were incubated at 27 °C for 48 h. Two drops of Lugol's iodine solution were added after incubation. By using a dissecting microscope, the hatched larvae and unhatched eggs were counted. The experiment was conducted in three replicates.

2.3.3. Larval development assay (LDA)

Larval development assay was performed according to the procedure described by Ademola et al. (2005a) with some modifications. Approximately 500 μl of egg suspension including about 100 eggs was added in each tube along with nutritive medium (Hubert and Kerboeuf, 1992). For the development of the first stage larvae, the covered tubes were put in an incubator at 27 °C for 48 h. An amount of 350 μl of the extracts were added. 7 days later, the third stage larvae (L_3) were collected and counted by separating the larvae as alive third stage larvae and dead larvae. The experiment was conducted in three replicates.

2.3.3.1. Determination of the 50% lethal concentration (LC_{50}). The LC_{50} was determined by the regression between the larval development parameter expressed in probit and concentration of the extracts (mg/ml). The larval development parameter was given by the relation below (Ademola et al., 2007):

Number of Living L_3/ Total number of nematodes in wells (plant extract).

Number of Living L_3/ Total number of nematodes in control tube (water).

2.3.4. Effect on adult parasites

Approximately ten active parasites were added in Petri dishes (35 × 10 mm) containing extracts at different concentrations in PBS, or only PBS for the negative control group. Levamisole (0.5 mg/ml) prepared in PBS was used as a positive control. Each concentration was tested in three replicates at room temperature (25–30 °C) (Bachaya et al., 2009). The inhibition of motility of the worms due to the applied treatments, demonstrated the anthelmintic activity. The motility was recorded at 0, 1, 2, 3 and 6 h intervals. In order to observe the revival of motility, the treated worms were put in the lukewarm fresh PBS for 30 min (Adama et al., 2009).

2.4. Data analysis

The 50% inhibitory concentration (IC_{50}) and the 50% larvicidal concentration (LC_{50}) were determined from the linear regression curve. Comparisons of different mortality rates were made using the Chi-square test and the results were considered to be significant at $P < 0.05$ by the method of Probits using the SPSS program.

3. Results and discussion

When the reference drug levamisole was applied, none of the eggs hatched. The extracts caused complete lyses of eggs. The mean inhibition values were found to be remarkably increased ($P < 0.05$) with the increase of the extract concentrations. No alive larvae (L_1) was found in the tubes with 400 and 800 μg/ml concentrations of the extracts. The MeOH extract showed significant and dose-dependent ovicidal effect at all doses tested (0.5, 1, 2 mg/ml). The ED_{50} values were represented in Table 1.

As presented in Table 2, extracts killed the infective stage larvae (L_3) in a concentration-dependent manner. The LC_{50} value of *n*-hexane extract was 0.823 mg/ml, while those of EtOAc and MeOH extracts were 0.791 mg/ml and 0.680 mg/ml, respectively with a statistically significant difference ($P < 0.05$).

As shown in Table 3, the MeOH extract displayed anthelmintic effect against *Haemonchus contortus* by causing paralysis. All of the worms treated with levamisole were dead at the end of 6 h, whereas, none of the worms were dead or paralyzed in PBS.

One of the most essential and prevalent parasitic diseases is helminthiasis which causes production losses in the livestock. For the treatment of helminths in animals, various anthelmintic agents are used. Yet, the development of resistance against anthelmintics in helminths have been considered as a big problem in animal health care systems (Akhtar et al., 2000). Despite the widespread use of synthetic anthelmintics in clinical practices, screening medicinal plants attracts attention in the scientific field as many of them have been proven to be rich sources of chemicals possessing anthelmintic effect (Lewis and Elwin Lewis, 1977; Satyavati et al., 1976). According to ethnobotanical data, a number of medicinal plants have been utilized for the treatment of parasitic infections (Sezik et al., 2001; Teklehaymanot and Giday, 2007; Tolossa et al., 2013). In the present study, anthelmintic activity of

Table 1
ED_{50} values of the extracts against *H. contortus* eggs after 48 h.

Material	Dose (mg/ml)	ED_{50} (LCL – UCL)[a](mg/ml)
***n*-Hexane extract**	0.5	15.21 (3.92–56.84)
	1	16.14 (4.23–60.37)
	2	13.26 (2.87–47.11)
EtOAc extract	0.5	12.42 (3.72–49.64)
	1	13.91 (4.02–51.03)
	2	11.25 (3.96–39.47)
MeOH extract	0.5	0.05 (0.036–0.68)
	1	0.03 (0.029–0.70)
	2	0.04 (0.041–0.53)
Levamisole	0.5	0.03 (0.017–0.42)

LCL, lower confidence limit; UCL, upper confidence limit.

[a] Values at 95% confidence intervals.