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Evaluation of Ethnopharmacological Potential for Anthelmintic Activity of two common weeds, *Oxalis corymbosa* DC. and *Oxalis pes-caprae* L. of family Oxalidaceae

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Abstract
The present research was conducted to investigate the anthelmintic potential of crude extracts from the stem, leaves, and flowers of two common weeds, *Oxalis corymbosa* DC. & *O. pes-caprae* L., of family Oxalidaceae, against gastrointestinal parasitic worm (helminth), *Haemonchus contortus* in polar & non-polar solvents, i.e. distilled water, ethanol, chloroform, and n-hexane. The time taken for the paralysis and ultimately the death of the worms by the crude extracts of both plants was found reasonably well as compared to that of the standard drug, Albendazole. However, the crude extracts of stem, leaves, and flowers of *O. corymbosa* in ethanol were found to be more effective than those of *O. pes-caprae* with paralysis (17.33, 14.20, and 10.13 min, respectively) and death time (18.16, 15.10, and 9.33 min, respectively) while Albendazole recorded paralysis and death time as 59.10 and 60.06 min, respectively.

Likewise, the anthelmintic potential of stem, leaves, and flowers extracts of *O. corymbosa* in n-hexane, chloroform, and distilled water was noticeably higher such as in n-hexane, stem, leaves and flowers extracts of *O. corymbosa* had paralysis (29.20, 27.33 and 28.0, respectively) and death time (30.20, 30.16 and 30.06, respectively) while the same extracts of *O. pes-caprae* had paralysis (37.43, 36.40, and 34.06, respectively) and death time (39.10, 36.83, and 35.33, respectively). The results indicated sound anthelmintic potential of the two weeds.
Keywords: Anthelmintic activity, *Oxalis corymbosa* DC., *Oxalis pes-caprae* L., *Haemonchus contortus*

Introduction

Gastrointestinal infections caused by parasitic nematodes also known as helminths, are a significant global health concern (Sharpe *et al.*, 2018) affecting 3.5 billion people worldwide, with around 200,000 deaths yearly (Hajare *et al.*, 2021). The World Health Organization report reveals that 24% of the world's population in 2020 was influenced by parasitic infections (Goel *et al.*, 2023). The developing countries were the most susceptible and by the year 2025, 57% of the population living in those countries will be influenced by infections caused by parasites including the most vulnerable group, school-age children and pregnant women (Elmonir *et al.*, 2021).

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The barber's pole worm, *Haemonchus contortus*, is one of the helminths that live in the gastrointestinal tract of its host, take nutrients from the host and cause infection in it. This blood-feeder nematode infects both humans and animals (Hotez 2014).

In animals, this parasite primarily affects sheep and goats in warm, humid settings throughout the summer (Emery *et al*., 2016) causing acute anemia, hemorrhagic gastroenteritis, weight loss, etc. that may lead to the death of ruminants. Adult worms adhere to the mucosa of the abomasum and consume blood. Over 10,000 eggs can be laid by females each day, and the eggs are excreted by the host animal. *H. contortus* larvae molt multiple times after hatching from their eggs, resulting in an infectious L3 form for the animals. When grazing, the host consumes these larvae. After undergoing another molt, the L4 larvae and adult worms feed on blood in the animal's abomasum, which may cause anemia and edema, which may ultimately result in death (Flay *et al*., 2022). Furthermore, there have been a few cases of human infections from Iran, Brazil, Sudan, Australia, and other countries. These infections are very easily transmitted from animals to humans (Sanders *et al*., 2020).

The most widely prescribed medications for treating *H. contortus* worldwide that are still effective are anthelmintics (Calvete *et al.* 2020). The term anthelmintic refers to a drug used for the treatment of animals infected with parasitic worms. It may act locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminths or development forms that invade organs and tissues (Partridge *et al*., 2020). Despite the significant increase in infection rate and the prevalence of animal-to-human transmission, the choices are mainly limited to a handful of drugs and most of them have developed resistance against intestinal parasites. Moreover, most of the existing anthelmintics are reported to have side effects like abdominal pain, nausea, vomiting, headache, and diarrhea (Naeem *et al*., 2021). So, the need to identify, isolate, and form an effective anthelmintic to stop the prevalence of resistance cannot be overemphasized (Borges *et al*., 2020). In this regard, natural sources such as medicinal plants can play a vital role as they have elements that can be employed therapeutically to treat parasitic infections or can serve as building blocks for the creation of beneficial medications (Williams *et al*., 2014). The plants selected for the present studies are Oxalis corymbosa DC. & Oxalis pes-caprae L., belong to the family Oxalidaceae.

Oxalis corymbosa DC. is native to Central America and distributed in many parts of the world such as Africa, Asia, Australia, Europe, and Northern America. It is a perennial herb and is mostly found in cultivated grounds and open habitats. It has a bulb having translucent roots with many small and sessile bulbils around its base. It bears Rhizome and above ground stem is absent. Leaves are arranged in a basal rosette and have bifid branching cymes inflorescence. Flowers are pinkish to purple, and pedicellate and fruit are absent. Its flowering period is from December to May (Shamso *et al*., 2021). On the other hand, Oxalis pes-caprae L., an invasive species and noxious weed native to Namibia is also present in other parts of the world. It is a tufted perennial herb and is mostly found in moist and cultivated grounds, orchards, and open habitats. The plant has a Bulb-like structure and possesses contractile roots and rhizomes. The stem is green in color and the aboveground stem is absent. Leaves of the plant are arranged in a basal rosette and Inflorescence is basal, having five to eight flowers per peduncle in umbelliform cymes. Flowers are golden yellow and fruit is not seen. Its flowering period is from April to September (Shamso et al., 2021).

Traditional medicine has employed *Oxalis corymbosa* DC, also referred to as pink wood sorrel, to treat diarrhea and dysentery. Additionally, an ethnobotanical study mentions using *O. corymbosa* leaf decoction to treat diabetes. (Panda *et al*., 2016). For millennia, oxalis roots and leaves have been utilized in traditional treatments due to the plant's diuretic and antihypertensive characteristics. Raw bulbs are used to treat tapeworm and perhaps other worms as well. Additionally, it is used as a diuretic. Along with milk, fleshy subterranean runners are consumed. Golden petals are used to make yellow dye. As a result, the roots, stems, and leaves of *Oxalis pes-caprae*, also known as Bermuda buttercup, have significant medical potential and serve as analgesics, antiinflammatory agents, heat dispersers, and toxins removers (Gaspar *et al.*, 2018).

Figure 1:*Oxalis corymbosa* DC.

Figure 2: *Oxalis pes-caprae* L.

The anthelmintic activity *Oxalis corniculata* L. against *Eisenia foetida* in petroleum ether, ethyl acetate and methanol at three different concentrations was reported by Santosh *et al*. 2012. Levamisole was used as standard drug. Each extract at 100, 200, 400mg/ml showed dose-dependent anthelmintic activity. The methanol extract of the plant and extracts at concentration of 400mg/ml had significant activity as compared to the reference drug. Rudrapal *et al*. 2020 carried out the anthelmintic activity of ethyl acetate and hydro-alcoholic leaf extract of *Oxalis debilis* and found out that hydro-alcoholic extract of the leaf exhibit considerable anthelmintic activity. However, anthelmintic activity of stem and flower extract of *Oxalis corymbosa* and whole plant of *Oxalis pes-caprae* in n-hexane, chloroform, ethanol and water have not so far scientifically proved, so the present study was conducted to evaluate the comparative anthelmintic activity of *Oxalis corymbosa* and *Oxalis pes-caprae* against *Haemonchus contortus* using their extracts in different solvents.

Material & Methods

Plant Material

Oxalis corymbosa and Oxalis pes-caprae, **f**ound growing as weeds in flowering beds were collected from Botanic Garden, Government College University Lahore. The specimens were mounted on herbarium sheets and deposited in Dr. Sultan Ahmed Herbarium, Government College University Lahore, **O. corymbosa,** after voucher no. GC. Herb. Bot. 3224, and **O. pes-caprae**, GC. Herb. Bot.3224-A**.**

Preparation of Extract

The whole plants (1.5kg) were collected and the plant parts i.e. leaf, stem, and flowers of Oxalis corymbosa and Oxalis pes-caprae were manually separated, air dried at room temperature, and ground into fine powder. Extract of the plants was prepared using maceration technique in which about 25g of powdered sample of both the plants was soaked in non-polar and polar solvents i.e. n-hexane, chloroform, ethanol, and water. The mixture of plant material and solvent is kept for some time, agitated at different intervals, and filtered through a filtration material. After completion of extraction, solvents were evaporated and concentrated extract was air-dried. Then the extract was stored in air tight container and each extract of the targeted plants was used to evaluate the anthelmintic activity.

Phytochemical Analysis

Qualitative phytochemical screening of the extracts of both the plants was carried out using procedure followed by Kishor et al. (2017**)** to explore the reservoir of secondary metabolites i.e. alkaloids, glycosides, carbohydrates, proteins, terpenoids, saponins, quinones, and coumarin.

Anthelmintic Activity

Adult Motility Assay was employed to study anthelmintic activity using adults *Haemonchus contortus* adult worms obtained fromthe

Parasitology Department, University of Veterinary and Animal Sciences, Lahore following thetechnique of Chander *et al.* (2014). Eleven moving worms were placed in each petri-dishes having3ml plants extracts and 27ml Phosphate buffer. The worms were observed over maximum onehour for death and paralysis. The time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously and the time for death of the worms was recorded after ascertaining that worms neither moved after vigorous shaking nor showed any motion when dipped in warm water. Albendazole served as positive control whereas Phosphate buffer, n-hexane, chloroform, ethanol and distilled water without plant extracts served as negative control.All the experiment was performed in triplicate at room temperature 25℃-30℃.

Statistical Analysis

The time of death and paralysis of the worms were recorded after taking mean and standard deviation following Steel *et al*. 1997.

Results and Conclusion

Phytochemical Analysis

Qualitative phytochemical screening of *O. corymbosa* and *O.pes-caprae* revealed the presence of various bioactive compounds recorded in Table 1.

Table 1. Qualitative phytochemical analysis of *O. corymbosa* and *O. pes-caprae*

Plants	**Phytochemicals**	**Alkaloids**	**Glycosides**	**Carbohydrates**	**Proteins**	**Terpenoids**	**Saponins**	**Coumarin**	**Quinones**
Oxalis corymbosa	**Solvent→ / Plant Parts↓**	**n-hexane**							
	Stem	+	+	+	+	+	+	+	-
	Leaves	+	+	+	+	-	+	-	-
	Flower	+	+	+	+	+	+	+	-
Oxalis pes-caprae	Stem	+	+	+	+	+	+	+	-
	Leaves	+	+	+	+	-	+	_	-
	Flower	+	+	+	+	+	+	+	-
Plants	**Solvent→ / Plant Parts ↓**	**Chloroform**							
Oxalis corymbosa	Stem	-	+	+	+	-	+	+	-
	Leaves	-	+	+	+	-	+	+	+
	Flower	+	+	+	+	+	+	+	+
Oxalis pes-caprae	Stem	-	+	+	+	-	+	+	-

	Leaves	-	+	+	+	-	+	+	+
	Flower	+	+	+	+	+	+	+	+
Plants	**Solvent→** / **Plant Parts↓**	**Ethanol**							
Oxalis corymbosa	Stem	-	+	+	+	+	+	+	-
	Leaves	-	+	+	+	-	+	-	-
	Flower	+	-	+	+	+	-	+	+
Oxalis pes-caprae	Stem	-	+	+	+	+	+	+	-
	Leaves	-	+	+	+	-	+	-	-
	Flower	+	-	+	+	+	-	+	+
Plants	**Solvent→** / **Plant Parts↓**	**Distilled Water**							
Oxalis corymbosa	Stem	-	-	-	-	+	-	+	-
	Leaves	-	-	-	-	+	-	+	-
	Flower	+	-	+	+	+	-	+	+
Oxalis pes-caprae	Stem	-	-	-	-	+	-	+	-
	Leaves	-	-	-	-	+	-	+	-
	Flower	+	-	+	+	+	-	+	+

Note: (+) indicates the presence (-) indicates the absence

Anthelmintic Activity

The death and paralysis time of the worms were note down and recorded in table 2 and 3 for anthelmintic activity exhibited by plant parts i.e. leaves, stem and flower of *O.corymbosa* and *O.pes-caprae* in all four solvents.

Table 2: Adult motility assay of *O. corymbosa* and *O. pes-caprae* against *Haemonchus contortus*

Plants	**Solvents**	**n-hexane**		**Chloroform**		**Ethanol**		**Distilled water**	
Oxalis corymbosa	**Plant Parts**	**P (min)**	**D (min)**	**P (min)**	**D (min)**	**P (min)**	**D (min)**	**P (min)**	**D (min)**
	Flower	28.20 ± 0.20	30.06 ± 0.11	26.10 ± 0.10	28.06 ± 0.11	10.13 ± 2.10	9.33 ± 1.15	39.06 ± 0.11	40.2 ± 0.20

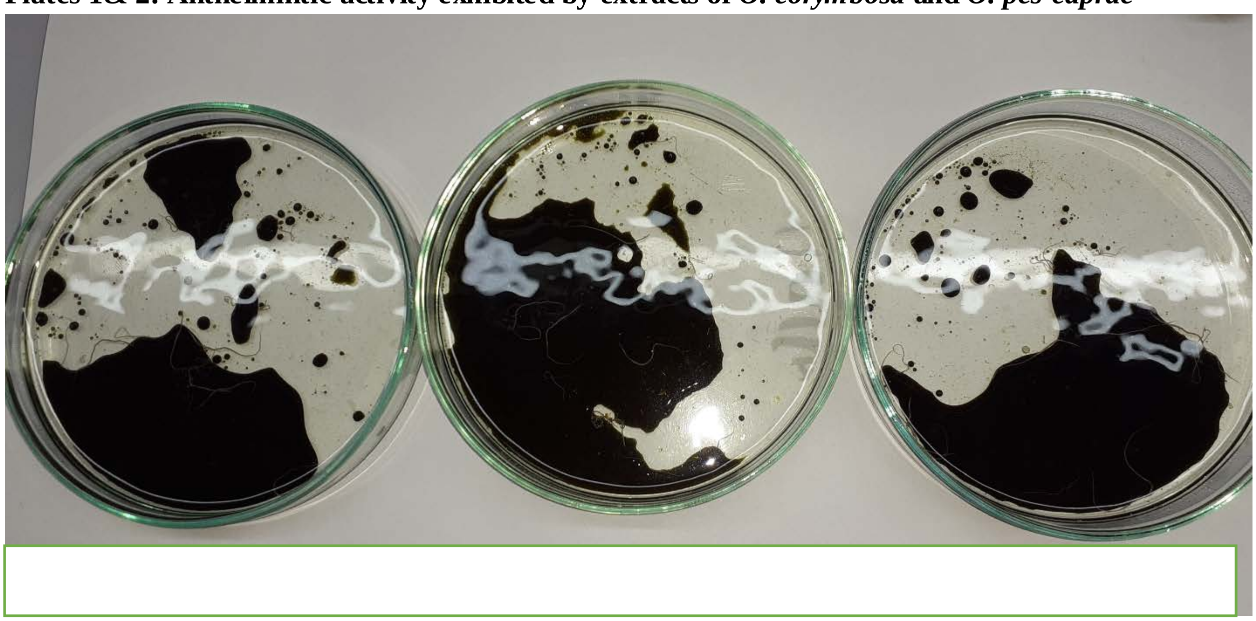
	Stem	29.20 ± 0.26	30.20 ± 0.25	27.36 ± 0.32	28.23 ± 0.25	17.33 ± 0.41	18.16 ± 0.28	47.23 ± 0.25	48.2 ± 0.26
	Leaves	27.33 ± 0.28	30.16 ± 0.28	26.56 ± 0.11	28.16 ± 0.28	14.20 ± 0.26	15.10 ± 0.10	40.73 ± 0.46	42.2 ± 0.20
Oxalis pes-caprae	Flower	34.06 ± 0.11	35.33 ± 0.35	29.30 ± 0.30	30.40 ± 0.40	10.33 ± 1.15	11.50 ± 1.32	47.06 ± 0.11	48.2 ± 0.20
	Stem	37.43 ± 0.37	39.10 ± 0.79	31.06 ± 0.11	32.23 ± 0.25	19.10 ± 0.10	20.06 ± 0.11	48.50 ± 0.50	50.06 ± 0.11
	Leaves	36.40 ± 0.36	36.83 ± 0.28	30.26 ± 0.11	31.5 ± 0.15	15.16 ± 0.27	16.40 ± 0.36	46.30 ± 0.26	47.33 ± 0.28

Note: P indicated paralysis time and D indicated death time of the worms

Table 3: Adult Motility Assay exhibited by standard drug against *Haemonchus contortus*

Standard Drug	**Paralysis time (min)**	**Death time (min)**
Albendazole	59.10 ± 0.10	60.06 ± 0.11
Negative Control		
Phosphate Buffer	-	-
n-hexane	49.13 ± 0.11	50.16 ± 0.28
Chloroform	34.17 ± 0.29	35.33 ± 0.25
Ethanol	24.13 ± 0.17	2.07 ± 0.15
Distilled water	-	-

Plates 1& 2: Anthelmintic activity exhibited by extracts of *O. corymbosa* and *O. pes-caprae*



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Anthelmintics are used to treat infections caused by parasitic worms (helminths) by starving them to death. Parasites will also perish if they become paralyzed and momentarily lose their capacity to maintain their place in the gut. Despite the widespread use of synthetic drugs in contemporary clinical settings worldwide, there is still a lot of interest in screening medicinal plants for their anthelmintic efficacy. Plants' anthelmintic properties are typically attributed to secondary metabolites such as condensed tannins or proanthocyanidins, which are also known as alkaloids, terpenoids, or polyphenols. (Chanda *et al*., 2019). In the present study, preliminary phytochemical screening of *O. corymbosa* and *O. pes-caprae* revealed the presence of alkaloids, glycosides, carbohydrates, proteins, terpenoids, saponins, coumarin, and quinones in all four solvents as shown in Table 1.

The n-hexane extract of the stems of selected plants had alkaloids, glycosides, carbohydrates, proteins, terpenoids, saponins, coumarin except quinones. The chloroform extract of the stems confirmed the presence of glycosides, carbohydrates, proteins, saponins, coumarin and absence of alkaloids, terpenoids and quinones. The ethanol extract of the stems had all the mentioned biologically active compounds excluding alkaloids and quinones while aqueous extract contained only terpenoids and coumarin. Similarly, the leaves of *O. corymbosa* and *O. pes-caprae* have reservoir of bioactive chemical compounds. The n-hexane extract of the leaves of both plants provided the phytochemicals such as alkaloids, glycosides, carbohydrates, proteins and saponins The chloroform extract confirmed the presence of glycosides, carbohydrates, proteins, saponins, coumarin, quinones and the absence of alkaloids and terpenoids. The ethanol extract had glycosides, carbohydrates, proteins, saponins while aqueous extract contained only terpenoids and coumarin excluding respective chemical compounds.

It was noticed that the n-hexane extract of flowers of both the plants contains all the listed phytochemicals except quinones. On the other hand, chloroform extract of the flowers of both plants was rich in bioactive compounds as it confirmed the presence of alkaloids, glycosides, carbohydrates, proteins, terpenoids, saponins, coumarin and quinones. However, alkaloids, carbohydrates, proteins, terpenoids, coumarin, and quinones were present and glycosides and saponins were absent in both ethanol and aqueous extract of the flowers of both the targeted weeds.

The phytochemicals found in the extracts are primarily responsible for their therapeutic qualities. Earthworm paralysis may result from alkaloids acting on the central nervous system. The presence of steroidal alkaloid oligoglycosides may have an effect by suppressing the transfer of sucrose from the stomach to the small intestine. Additionally, these alkaloid's antioxidant properties may be able to reduce the generation of nitrate, which may interfere with local homeostasis, which is necessary for the development of helminthes (Santosh*et al.*, 2012). Terpenes exhibits anthelmintic properties that harm the parasite's intestinal lining. By blocking acetylcholinesterase, saponins exhibit their anthelmintic properties and cause worm paralysis that ultimately results in death. It has been observed that they exhibit inhibitory effect against worms that parasitize animals, such as *Haemonchus contortus*. Tannins aid in the death of nematodes by preventing the worms from absorbing nutrients from the host cell or by binding to the intestinal mucosa of the parasitic worms by autolysis when the worms consume condensed tannins (Adak & Kumar, 2022).

The results revealed that the death of worms in n-hexane extract of leaves, stem and flower of *O. corymbosa* occur in 30.16, 30.20 and 30.06 minutes as compared to the negative control having 50.16 minutes death time exhibiting the anthelmintic potential of the plant. On the other hand, in chloroform extracts, death time of the worms recorded as 28.16, 28.23 and 28.06minutes respectively with respect to chloroform without plant extract having 35.33. In case of ethanol extracts, worms died in15.10, 18.16 and 9.33 minutes, respectively and when placed in ethanol that act as negative control they died in 2.07. The distilled water extracts of the plant was found more effective as compared to the control causing death of the worms in42.2, 48.2 and 40.2, respectively. Meanwhile, n-hexane extract of leaves, stemand flower of *O. pes-caprae*had death time of 35.33, 39.10 and 36.83 minutes, respectively, chloroform extracts having 30.40, 32.23 and 31.5, respectively, the ethanol extracts having 11.50, 20.06 and 16.40, respectively and the distilled water extracts having 48.2, 50.06 and 47.33, respectively depicting the anthelmintic effect of the plant that was considerable but comparatively less than that of *O. corymbosa*.

The paralysis of worms in n-hexane extract of flowers, stem and leaves of *O. corymbosa* occur in 28.20, 29.20 and 27.33 minutes compared to the negative control n-hexane having 49.13, while chloroform extracts had26.10, 27.36 and 26.56 respectively and the reading was 34.17 in chloroform acting as negative control. Moreover, paralysis of the worms in the ethanol extracts occurred in 10.13, 17.33 and 14.20, respectively in comparison to ethanol without plant extracts having 24.13. The parasitic worms showed no movement in distilled water extracts in 39.06, 47.23 and 40.73, respectively.

In contrast to the *O. corymbosa*, the n-hexane extract of flowers, stem and leaves of *O. pes-caprae* had paralysis time of 34.06, 37.43 and 36.40 minutes, respectively, chloroform extracts having 29.30, 31.06 and 30.26, respectively, the ethanol extracts having 10.33, 19.10 and 15.16, respectively and the distilled water extracts having 47.06, 48.50 and 46.30, respectively. It can be concluded that the anthelmintic effect of leaves, stem and flowers extracts of *O. corymbosa* were found reasonably well as compared to the leaves, stem and flowers of *O. pes-caprae* in all the extracts, n-hexane, chloroform, ethanol and distilled water as in table 2 with reference to the standard drug (albendazole) that caused paralysis of the worms in 59.10 and eventually their death in 60.06 minutes. Although, stems, leaves and flowers extracts of both the plants had anthelmintic effect against tested intestinal parasite in all four solvents, however, ethanol extract of *O. corymbosa* was more effective against*Haemonchus contortus* and affirmed that it could potentially be used to formulate anti-nematode drugs that can be used against intestinal parasites. To ascertain the full range of anthelmintic activity of these medicinal plants, additional studies utilizing various dosages and concentrations against various parasitic species and stages are necessary.

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