Evaluation of Anthelmintic, Antimicrobial, and Antioxidant Activities of Ethyl Acetate and Methanol Extracts of *Oxalis corniculata* (L.)

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Abstract

Traditional medicine encompasses a wealth of knowledge and practices rooted in the cultural heritage of indigenous communities, playing a vital role in maintaining health and treating various ailments. *Oxalis corniculata* (L.), commonly known as creeping wood sorrel, is a widely distributed medicinal plant recognized for its rich content of bioactive compounds, including alkaloids, flavonoids, and organic acids. This study aimed to evaluate the anthelmintic, antibacterial, and antioxidant properties of O. corniculata extracts. Methanolic and ethyl acetate extracts were tested, with the methanolic extract demonstrating significant anthelmintic activity at a concentration of 50 mg/mL. Antibacterial evaluation revealed that the ethyl acetate extract exhibited inhibition zones of 4 mm against Salmonella typhi and 8 ± 0.29 mm against Staphylococcus aureus. The methanol extract showed zones of 2 mm against Escherichia coli, 4 mm against S. typhi, and 7 mm against S. aureus. Antioxidant activity was also notable, with the methanol extract showing 65.26% and the ethyl acetate extract showing 55.40% activity compared to the standard (88.97%). The results indicate that O. corniculata possesses significant pharmacological potential and warrants further phytochemical investigation and in vivo validation for its therapeutic applications.

Keywords: *Oxalis corniculata*, Anthelmintic, Antimicrobial, Antioxidant, DPPH

1. Introduction

Traditional medicine refers to the knowledge, skills, and practices rooted in the theories, beliefs, and experiences of various indigenous cultures, used to maintain health and to prevent, diagnose, treat, or improve physical and mental illnesses [1]. Herbs, deeply embedded in a wealth of traditions shaped by theories, beliefs, and lived experiences across diverse civilizations and historical periods, are extensively utilized often in intricate and culturally symbolic ways for maintaining health through the prevention, diagnosis, enhancement, and treatment of various illnesses [2]. The global utilization of herbal medicines, phytonutrients, and nutraceuticals is experiencing a rapid surge, as an increasing number of individuals turn to these bioactive compounds for the management and therapeutic intervention of diverse health conditions across varying national healthcare systems [3]. Around four billion people, or 80% of the world's population, live in developing countries where traditional medicine, especially the use of herbs, is an important part of their culture. For many of them, herbal medicines are their main source of healthcare [4–6].

Helminthiasis is a parasitic infectious disease affecting humans and other animals, characterized by the colonization of the gastrointestinal tract and, in some cases, other organs by helminths, leading to significant physiological and pathological alterations [7]. In developing regions, helminthic infections significantly impact public health, contributing to malnutrition, anemia, eosinophilia, and pneumonia. Anthelmintics, acting as vermicidal or vermifugal agents, target helminths primarily in the gastrointestinal tract, though some inhabit or migrate through tissues. Pathogenicity arises from nutrient depletion, blood loss, organ damage, obstruction, and toxin secretion [8]. "Although helminthiasis is seldom fatal, it remains a significant contributor to chronic morbidity, often leading to long-term health complications such as fatigue, impaired cognitive development, reduced physical growth, and weakened immune function [9].

Globally, microbial spoilage affects all food categories, leading to significant food waste, even in developed nations. Microbial activity is responsible for up to 40% of annual food losses, driven by enzymatic degradation, microbial growth, and biochemical changes in food [10].

Plant extracts have gained significant attention for their antioxidant properties, which play a crucial role in neutralizing reactive oxygen species and preventing oxidative stress. These bioactive compounds, including polyphenols, flavonoids, and carotenoids, exhibit potent free

radical scavenging activity. The antioxidant potential of plant extracts has been widely explored for their therapeutic applications in mitigating chronic diseases linked to oxidative damage [11].

Oxalis corniculata (L.), commonly referred to as creeping wood sorrel, is a globally distributed plant known for its diverse medicinal and nutritional benefits. It is rich in bioactive compounds, such as alkaloids, flavonoids, and organic acids, which contribute to its therapeutic efficacy. Traditionally, the plant has been utilized in folk medicine to address conditions like inflammation, digestive issues, and skin ailments [12]. Recent research has underscored its potent antioxidant, antimicrobial, and anthelmintic properties, indicating its potential for use in contemporary therapeutic applications.

2. Materials and methods

2.1. Collection and preparation of extract

The whole plant of *Oxalis corniculata* (OC) was collected from Hatgaon, Bargarh, Odisha, India, and authenticated by the Botanical Survey of India, Kolkata (Voucher number: CNH/Tech.II/2024/17). The plant parts were shade-dried at room temperature, then ground into a coarse powder and sieved for uniformity. Approximately 70g of the powdered material was extracted successively with petroleum ether, ethyl acetate, and methanol using a Soxhlet apparatus. The extraction continued based on solvent boiling points, and the concentrated extracts were evaporated using a rotary evaporator and stored in a desiccator at a cool temperature.

2.2. Chemicals used

Petroleum ether, ethyl acetate, and methanol (Merck), DMSO (Merck), Piperazine citrate (Torque Pharmaceuticals Pvt. Ltd.), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) (Sigma Aldrich), Ascorbic acid (Sigma Aldrich), Ciprofloxacin (Cipla Pvt. Ltd.).

2.3. Evaluation of anthelmintic activity

The anthelmintic activity was evaluated following the method of Dash et al. [7], with slight modifications. *Eisenia fetida* earthworms were selected due to their anatomical and physiological resemblance to human intestinal parasites. The worms were rinsed with normal saline prior to testing. Plant extracts were dissolved in 2% DMSO to prepare concentrations of 12.5, 25, and 50 mg/mL. The standard drug (Piperazine citrate) was also dissolved in 2%

DMSO to obtain a 15 mg/mL solution. Earthworms were distributed into control, standard, and test groups (six worms per petri dish). Paralysis and death times were recorded, with death confirmed when the worms showed no movement upon vigorous shaking or pricking with a syringe.

2.4. Evaluation antioxidant activity

The DPPH assay was conducted following the procedure described by Hussen and Endalew et al., with necessary modifications [13]. The free radical scavenging property of *Oxalis corniculata* were investigated by DPPH assay. Ascorbic acid was used as standard. The percentage inhibition was calculated by using following formula:

$$\% \text{ of Inhibition} = \frac{A - B}{A} X100$$

Where, A is the absorbance of control, B is the absorbance of sample at different concentration.

2.5. Evaluation of antimicrobial activity

The antibacterial activity of the plant extracts was assessed using the agar disk diffusion method. Standard Gram-positive and Gram-negative bacteria, including Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, and Staphylococcus aureus, were used. Sterile paper disks were impregnated with plant extract solutions (50 µL, 100 µL, 150 µL, and 200 µL in respective solvents) and placed on the surface of nutrient agar plates. Ciprofloxacin (25 µL, 50 µL, 75 µL, and 100 µL) served as the standard reference drug. Plates were incubated at 35–37°C for 18–24 hours for bacterial strains and 24–48 hours for yeast strains. Antimicrobial activity was evaluated by measuring the diameter (in mm) of the zone of inhibition around the disks using vernier calipers [14].

2.6. Statistical data

Statistical analysis was performed using Microsoft Excel and GraphPad Prism version 10. The data were analyzed for significance, and results were expressed as mean ± standard deviation (SD).

3. Results and discussion

3.1. Anthelmintic activity of *Oxalis corniculata*

All the extract of OC showed paralysis as well as death of worms at all the concentrations of the sample. It was observed that the methanolic extract and ethyl acetate extract of OC were

showed potent anthelmintic activity at the concentration of 50 mg/ml, when it compared with the standard drug (piperazine citrate 15 mg/ml) in a dose dependent manner (Table 1, and Fig. 1).

3.2. Antimicrobial activity of *Oxalis corniculata*

The ethyl acetate and methanol extracts of *Oxalis corniculata* demonstrated notable antimicrobial activity at a concentration of 200 mg/mL. The ethyl acetate extract exhibited a zone of inhibition measuring 4 ± 0.32 mm against *Salmonella typhi* and 8 ± 0.29 mm against *Staphylococcus aureus*. Similarly, the methanol extract showed inhibition zones of 2 ± 0.29 mm against *Escherichia coli*, 4 ± 0.32 mm against *S. typhi*, and 7 ± 0.29 mm against *S. aureus* (Table 2 and Fig 2).

3.3. antioxidant activity

DPPH assay method has been broadly used to study of different antioxidant substances. In the DPPH assay Antioxidant was capable to reduce the stable radical DPPH. DPPH is widely used to evaluate free radical scavenging activity of antioxidants. By this method the antiradical power of an antioxidant can be determine by decrease in the absorbance of DPPH· at 517 nm. The result showed the plant OC has significant antioxidant activity of ethyl acetate (55.40 ± 0.43), and methanolic extract (65.26 ± 0.36) as dose dependent manner as compare to standard (88.97 ± 0.47) (Table 3 and Fig. 3).

Table 1: Anthelmintic activity ethyl acetate and methanol extract of OC

Treatments	**Concentrations (mg/mL)**	**Time taken for paralysis (Mean ± SD) min**	**Time taken for death (Mean ± SD) min**
Control (2% DMSO)	-	-	-
Standard (Piperazine Citrate)	15	50.76 ± 0.40*	66.93 ± 0.20*
Ethyl acetate	12.5	77 ± 0.20*	109.66 ± 0.35*
	25	64.03 ± 0.35*	91.73 ± 0.37*
	50	53.30 ± 0.36*	88.80 ± 0.26*
Methanol extract	12.5	71 ± 0.27*	93.66 ± 0.31*
	25	62.03 ± 0.29*	86.23 ± 0.35*
	50	48.30 ± 0.36*	78.80 ± 0.26*

Values are expressed as Mean ± Standard deviation (SD). Statistical significance and standard error were found out by one-way ANOVA. Significance level *P = ≤ 0.05

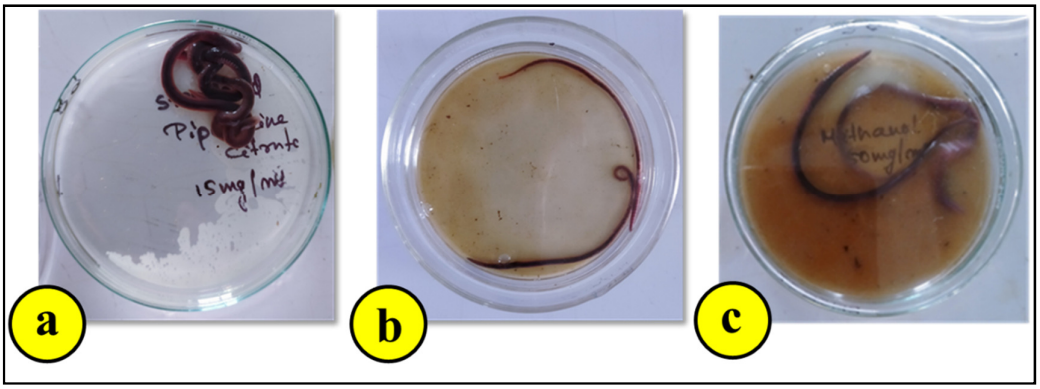

Fig 1: Anthelmintic activity of (a) piperazine citrate, (b) ethyl acetate, and (c) methanol extract of *Oxalis corniculata*

Table 2: Antibacterial activity of *Oxalis corniculata*

Treatment	Dose	Zone of inhibition (mm)			
		E. coli	**S. typhi**	**P. aeruginosa**	**S. aureus**
Normal Control	DMSO 2%	-	-	-	-
Positive Standard (Ciprofloxacin + microorganism)	25 µg/ml	04 ± 0.34	20 ± 0.13	04 ± 0.38	08 ± 0.19
	50 µg/ml	08 ± 0.14	20 ± 0.36	07 ± 0.29	09 ± 0.14
	75 µg/ml	11± 0.29	21 ± 0.32	07 ± 0.51	13 ± 0.22
	100 µg/ml	16 ± 0.11	22 ± 0.18	09 ± 0.23	15 ± 0.16
Ethyl acetate extract	50mg/ml	-	-	-	-
	100 mg/ml	-	-	-	-
	150 mg/ml	-	-	-	3 ± 0.24
	200 mg/ml	-	4± 0.32	-	8 ± 0.29
Methanol extract	50mg/ml	-	-	-	-
	100 mg/ml	-	-	-	-
	150 mg/ml	-	-	-	5 ± 0.49
	200 mg/ml	2 ± 0.29	4± 0.32	-	7 ± 0.29
Values are expressed as Mean ± Standard deviation (SD)					

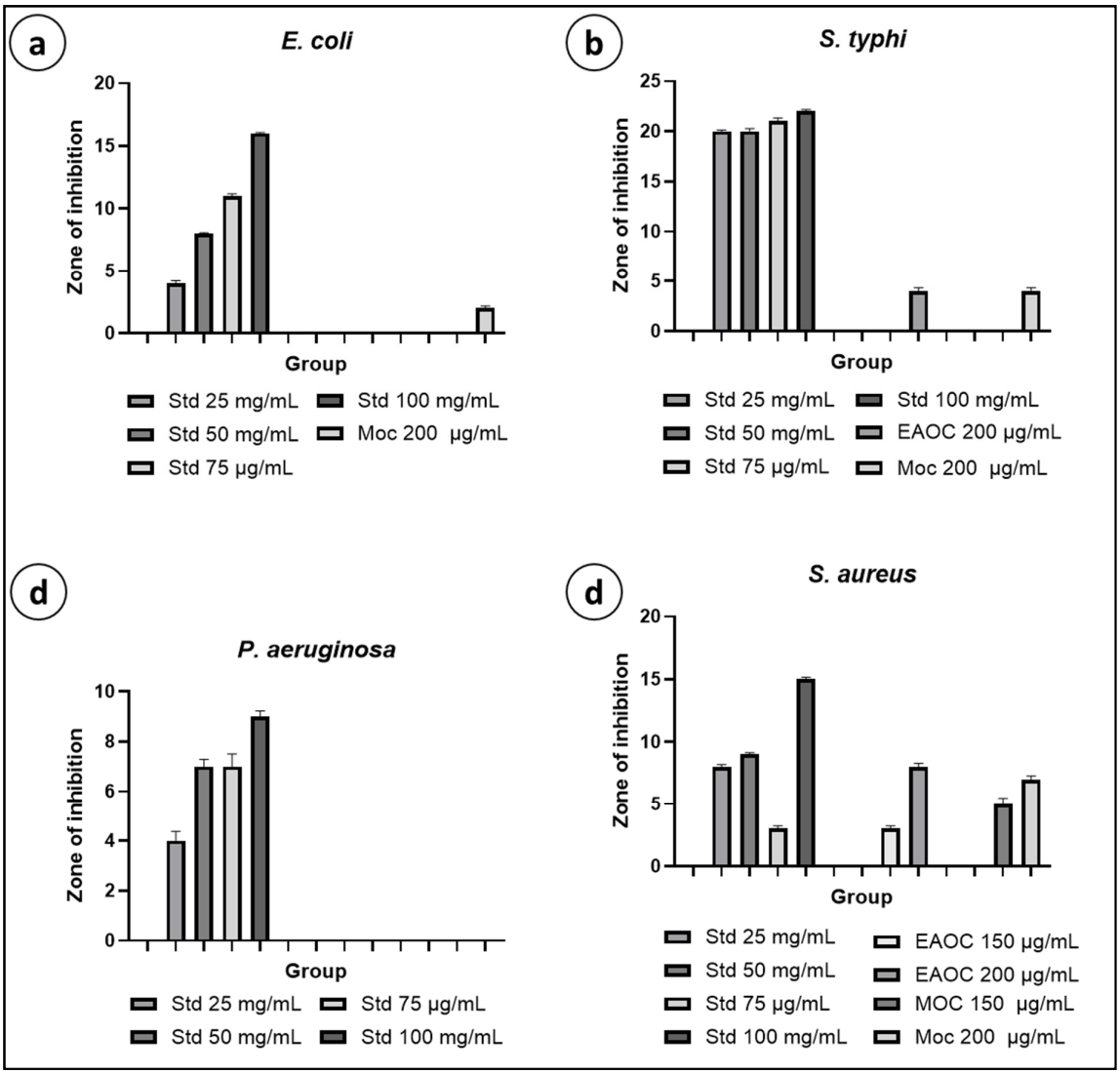

Fig. 2: Antimicrobial activity of *Oxalis corniculata*, a, b, c, and d represent zone of inhibition of standard and test against E. coli, S. typhi, P. aeruginosa, and S. aureus.

Table 3: DPPH antioxidant activity of *Oxalis corniculata*

Concentration	**% Radical Scavenging activity**		
	Ascorbic acid	**EAOC**	**MOC**
20	48.12 ± 0.25	38.03 ± 0.14	42.72 ± 0.37
40	68.08 ± 0.35	41.31 ± 0.71	46.01 ± 0.41
60	76.76 ± 0.17	48.12 ± 0.26	56.34 ± 0.28
80	81.69 ± 0.31	54.69 ± 0.34	61.74 ± 0.21
100	88.97 ± 0.47	55.40 ± 0.43	65.26 ± 0.36
IC50	**12.32 ± 0.16**	**70.34 ± 0.19**	**45.48 ± 0.21**

Values are expressed as Mean ± Standard deviation (SD)

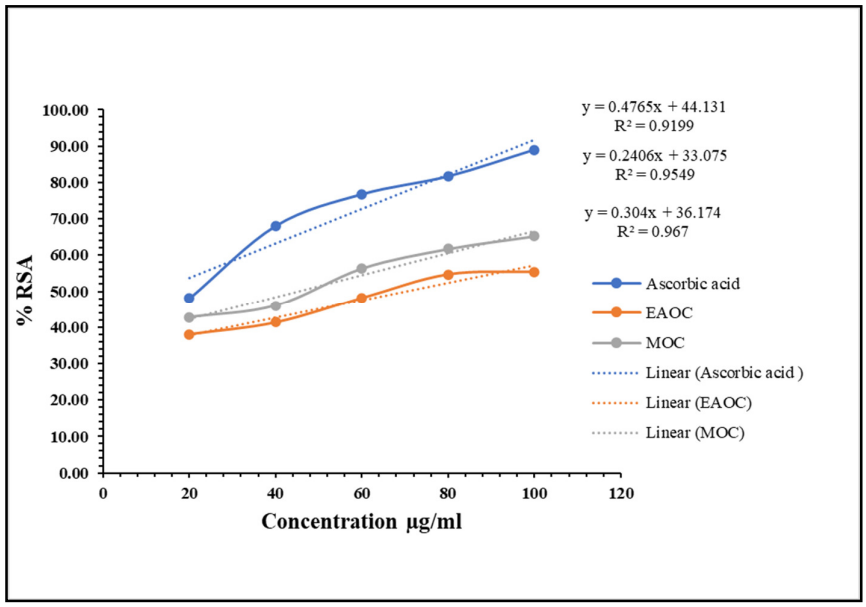

Fig. 3: Determination of DPPH radical scavenging activity of Ascorbic acid, ethyl extract of *Oxalis corniculata* (EAOC), and methanol extract of *Oxalis corniculata* (MOC)

4. Conclusion

In conclusion, *Oxalis corniculata* demonstrated notable anthelmintic, antibacterial, and antioxidant activities in a dose-dependent manner. These findings support its potential as a source of bioactive compounds. Further investigations, including detailed phytochemical analysis and both in vitro and in vivo studies, are recommended to explore and validate its therapeutic potential.

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