

BIBECHANA

ISSN 2091-0762 (Print), 2382-5340 (Online)

Journal homepage: <http://nepjol.info/index.php/BIBECHANA>

Publisher: Department of Physics, Mahendra Morang A.M. Campus, TU, Biratnagar, Nepal

Phytochemical Screening and Biological Activities of *Oxalis corniculata* Leaves of Sindhupalchowk District, Nepal

Kamala Pandey, Shanta Pokhrel*

Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal

*Email: shantabhattacharai2014@gmail.com

Article Information:

Received: October 30, 2021

Accepted: December 27, 2021

Keywords:

Antibacterial

Antioxidant

IC₅₀

Phytochemical screening

ABSTRACT

The *Oxalis corniculata* (Oxalidaceae) is one of the most potential medicinal herbs and well known for multiple therapeutic properties (anti-inflammatory, anticancer, antioxidant, antimicrobial, analgesic and diuretic activity). This study investigated phytoconstituents, antibacterial activity and antioxidant activity of *Oxalis corniculata* leaves extract. Methanol based phytochemical screening of *Oxalis corniculata* revealed the presence of alkaloids, proteins, phenols, flavonoids, glycosides. The antibacterial activity revealed better potential inhibitory effect against gram-negative bacteria, particularly *Escherichia coli* and *Salmonella typhi* compared to *Staphylococcus aureus*, a gram-positive bacterium. However, 1% methanol extract showed highest antibacterial activity against *Escherichia coli* followed by *Staphylococcus aureus* and *Salmonella typhi*. Antioxidant activity of the methanol extract with 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging showed antioxidant activities with 3.87 µg/mL IC₅₀ value.

DOI: <https://doi.org/10.3126/bibechana.v19i1-2.46094>

This work is licensed under the Creative Commons CC BY-NC License. <https://creativecommons.org/licenses/by-nc/4.0/>

1. Introduction

Oxalis corniculata Linn. (Oxalidaceae) is one of the most useful medicinal plants having a wide spectrum of biological activity. It is commonly known as creeping wood sorrel (**Chari amilo** in Nepali) possess all the essential constituents which are

required for normal and good health of humans [1]. It is a low growing herbaceous plant dispersed in damp shady places, roadsides, lawns, and in most parts of India [2] and Nepal. The stem and bark of this plant utilize to treat malaria, snakebite, bronchitis and juice of the leaves are used as

digestive agent, antidote to reptile venom, in treating dysentery and diuretic activity [3,4]. The products of the plant include glycoside, flavonoid, protein, tannin, phenolic compounds, essential oil which are associated with medicinal and pharmacological properties of the plants. The leaves and stem of *Oxalis corniculata* contain tartaric acid, citric acid and malic acid, also rich source of essential fatty acids like palmitic, oleic, and linoleic acid [5-7]. It is commonly used as an antibacterial, antifungal, anti-inflammatory, in wound healing, and in the treatment of jaundice, antiseptic, anemia, cancer, piles and in stomach troubles [8]. Traditionally it is used in the treatment of bacterial infections and kidney stones [9]. Das *et al.*, studied the inhibitory activity of its aqueous extract and biofabricated silver nanoparticles against urinary tract infection (UTI) causing bacteria and urolithiasis. It is reported that its leaves aqueous extract effectively suppressed the growth of struvite stones and resulting the dissolution of stones. Furthermore, the inhibitory activity was further improved by its biofabricated silver nanoparticles [9]. The present paper is dealing with phytochemistry and biological activities of n-hexane and methanolic extract of *Oxalis corniculata* leaves.

2. Materials and Methods

2.1 Plants extract preparation via soxhlet extraction

Leaves samples (*Oxalis corniculata*) were collected from the Sindhupalchowk district, Nepal and washed by pure water to remove dust, soil, and other impurities. Cleaned plant material was air dried in shade for 4 weeks and ground to powder and stored in clean and dry bottle. 65 g of leaves powder was successively extracted by soxhlet method in hexane followed by methanol. The process was continued (7-8 hours) until the colourless solvent appeared from plant material in the siphon. Thus obtained extract was concentrated to get crude extract by rotary evaporator and stored in refrigerator for phytochemical screening, antibacterial and antioxidant property.

2.2 Phytochemical screening

Different crude extract, prepared from soxhlet extraction were used for phytochemical screening to

identify various bioactive chemical constituents like alkaloid, flavonoid, steroids, glycosides, and carbohydrates. The method employed is primarily based on the standard protocols [9].

2.3 Antibacterial screening

Agar well diffusion method was used to study the antibacterial activity of plant extract [10,11]. Different concentration of solution (1%, 10%, and 50%) was prepared from the plant crude extract and tested against standard gram positive microbial strains *Staphylococcus aureus* and gram negative strains *Escherichia coli* ATCC 25922 and *Salmonella typhi*. These standard strains were collected from MED MICRO Nepal Lab, Kathmandu. The 50 μ L of the working solution of the plant extract, dimethyl sulfoxide as negative control (NC) and 25 μ L (100 mg/mL) of ofloxacin as positive control (PC) were loaded into the separated well (6 mm). The plates were incubated overnight at 37 °C and examined inhibition of bacterial growth indicated by a clear zone around the wells. The size of the zone of inhibition (ZOI) was measured in mm.

2.4 Antioxidant activity

The free radical scavenging activity of methanolic extract and standard ascorbic acid solution was evaluated as their capability to react with stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical [11,12]. Its antioxidant property was determined as IC₅₀ values which was calculated following the protocol mentioned in our previous paper [11, 13].

3. Results and Discussion

3.1 Phytochemical screening

The phytoconstituents were identified by the color reaction with different reagents following the standard phytochemical screening methods [9]. The qualitative results were expressed as (+) for the presence and (-) for the absence of phytochemicals which is compiled in Table 1.

Phytochemical screening of methanol extract of *Oxalis corniculata* leaves revealed the presence of large number of bioactive secondary molecules like alkaloids, proteins, phenols, flavonoids, glycosides.

Similar results are reported by Kaur [14]. However, slightly different results were observed by other researchers [15,16]. This is due to the different environmental conditions and variation in altitude of plants. Flavonoids, alkaloids, tannins, phenols were reported in the ethanol extract [17]. Hence, the

presence of various bioactive molecules makes the potential use of *Oxalis corniculata* leaves to stop bleeding from wounds and effective in certain skin disease like warts, corns, antidote to reptile venom, dysentery and diuretic [3].

Table 1. Results of phytochemical screening of *Oxalis corniculata* leaves extract

S.No.	Phytochemicals	n-Hexane Extract	Methanol Extract
1	Alkaloids	-	+
2	Flavonoids	-	+
3	Phenols	-	+
4	Carbohydrate	-	-
5	Glycosides	-	+
6	Proteins	-	+
7	Saponins	-	-

(+) indicate presence, (-) indicate absence.

3.2 Antibacterial susceptibility assay

The antibacterial efficacy was tested on the basis of the magnitude of zone of inhibition (in mm) and the results are presented in Figure 1 and data indexed in Table 2. The results suggested that, hexane extracts of *Oxalis corniculata* leaf showed significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* with ZOI value 10 mm, 9 mm and 11 mm respectively in 50% concentration. But the crude hexane extract did not show antibacterial activity. The 1% concentration of methanol extract showed higher antibacterial efficacy against *Escherichia coli* than *Staphylococcus aureus* and *Salmonella typhi* with ZOI value 12 mm, 11 mm, and 10 mm respectively. But in 10% concentration methanolic extract showed antibacterial activity against only *Staphylococcus aureus* with ZOI value 10 mm. Therefore, 1% methanolic leaf extract is more potent

towards antibacterial activity than 10% of it. Both n-hexane and methanolic extracts showed highest zone of inhibition against gram negative bacteria *Salmonella typhi* and *Escherichia coli* (11 mm in 50% concentration of hexane extract and 12 mm in 1% concentration of methanol extract). Taley et al. reported 6-14 mm ZOI of methanol extract against *Staphylococcus aureus* and *Escherichia coli* [18] which support the present findings whereas Mukharjee et al. showed 12 to 19 mm range of inhibition zone [19]. Kathiriya et al. and Rahman et al. reported that methanol and chloroform *Oxalis corniculata* leaf extract showed higher antibacterial activity than the positive control [20, 21]. Mohan and Pandey concluded that the selected leaf extracts were showing highest antimicrobial activity with chloroform and ethanol extract [22]. Therefore, this plant can be used for curing common diseases like diarrhea, cough, fever etc.

Table 2. Antibacterial activity of *Oxalis corniculata* leaf extracts on pathogenic bacteria (ZOI in mm)

S. N.	Plant extract (Solid)	Bacteria	Extracts (100 mg/mL)		Ofloxacin (100 mg/mL)
			50%	Crude	
1.	Hexane	<i>E. coli</i>	10	0	30
		<i>S. aureus</i>	9	0	32
		<i>S. typhi</i>	11	0	26
			10%	1%	
2.	Methanol	<i>E. coli</i>	0	12	30
		<i>S. aureus</i>	10	11	32
		<i>S. typhi</i>	0	10	26

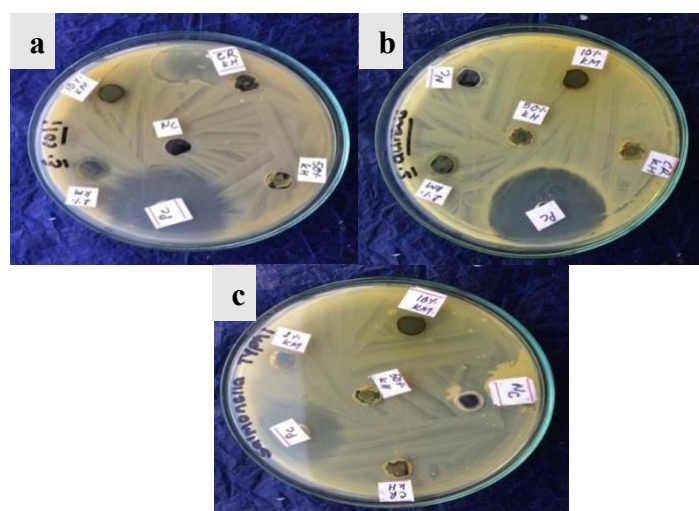


Fig. 1: Images of antibacterial activity of *Oxalis corniculata* leaf extract against (a) *E. coli*, (b) *S. aureus* and (c) *S. typhi*.

3.3 Antioxidant activity via DPPH assay

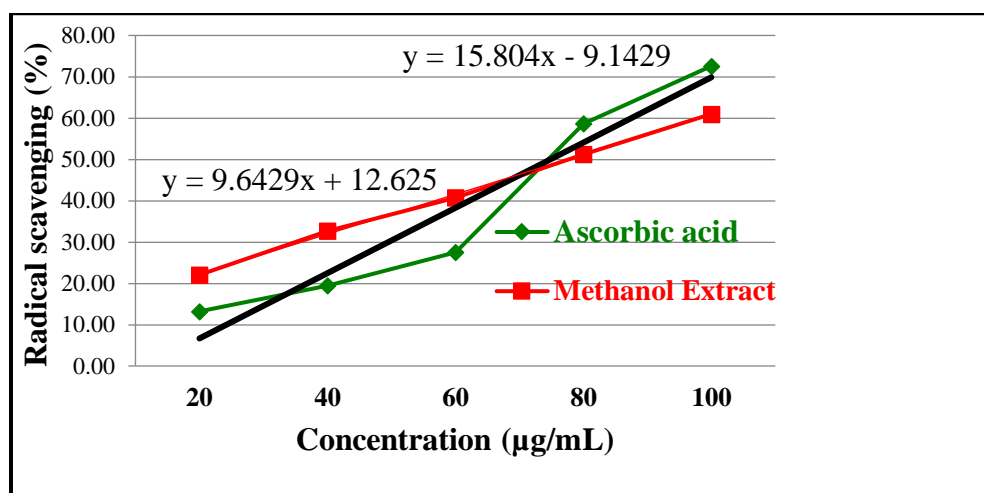
The antioxidant activity of methanol plant extract was measured by using 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH) radical scavenging assay. Antioxidants are defined as radical scavengers having strong anti-cancer activity by preventing oxidative cell damage [23], and protect the human body against free radicals and reduce the risk of disease [24]. The DPPH radical assay was performed with methanol extract of *Oxalis corniculata* leaf by using ascorbic acid as standard. The observed absorbance with different

concentration of ascorbic acid and methanol extract was plotted in the graph (Figure 2).

Table 3. Radical scavenging activities of plant with methanol extract (ME) and ascorbic acid.

Concentration (µg/mL)	20	40	60	80	100
% of radical scavenging by methanol extract	22.05	32.68	40.80	51.25	60.98
% of radical scavenging by ascorbic acid	13.21	19.38	27.50	58.66	72.59

Fig. 2: Percentage radical scavenging activity vs. concentration of ascorbic acid and methanol extract.



The Table 3 depicts the results of radical scavenging activity of *Oxalis corniculata* with methanol extract and ascorbic acid in different level of concentrations. Higher the value of IC₅₀ gives lower antioxidant potential describes their opposite relation. The IC₅₀ value of *Oxalis corniculata* leaf in methanol extract and standard ascorbic acid was found 3.87 µg/mL and 3.74 µg/mL respectively; however, Poudel et al. mentioned 4.06 µg/mL as standard IC₅₀ value of ascorbic acid for both ethanol and aqueous extract [25]. Methanol extract showed slightly less antioxidant activity than the standard ascorbic acid, which is also reported by Borah et al. [26]. This antioxidant activity is due to the existence of phenols and flavonoids in plant leaves, which may result the free radical scavenging effect [26]. Ahmed et al. also reported good antioxidant activities of methanol extract and its sub-fractions in various solvents

of *Oxalis corniculata* [27]. Tibuhwa also explored the antioxidant activities and suggested it as the source of side effect-free natural antioxidants which could be the best alternative to synthetic antioxidants in the food processing industry and also possible used in preventive medicine [28]. As antioxidants, flavonoids from these plants provide anti-inflammatory activity [29]. Therefore, *Oxalis corniculata* is used in treatment of wounds, burns and ulcer in herbal medicine [17].

Conclusion

Methanolic and n-hexane extract of *Oxalis corniculata* was successfully prepared from soxhlet extraction. The phytochemical screening of methanolic leaf extract revealed the presence of large number of bioactive secondary molecules like alkaloids, proteins, phenols, flavonoids, glycosides whereas n-hexane extract showed

absence of phytochemicals. Both extracts (n-hexane and methanol) showed highest zone of inhibition against Gram negative bacteria *Salmonella typhi* and *Escherichia coli*. And 1% methanolic leaf extract was found more potent towards antibacterial activity than 10% of it. Similarly, the methanolic extract also showed good antioxidant activities as standard ascorbic acid. Therefore, this result suggested that *Oxalis corniculata* leaves can be used as a medicinal supplement in pharmaceutical industry as natural antioxidant having antibacterial activities. Further research is needed to isolate and identify the important compounds present in the plant extracts to explore their biological activities.

Acknowledgements

The authors are grateful to Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal for providing laboratory facilities to perform needful chemical experiments. We are thankful to Med-Micro Nepal Lab, Thapathali, Kathmandu, Nepal, for providing the laboratory equipment's and platform to perform antibacterial activities.

References

- [1] A.K. Kumar, K. Das, M. Joshipura, and N. Mandal, *Oxalis corniculata* Linn. -The plant of Indian subtropics, Herbal Tech. Industry1 (2010)7-11.
- [2] B. Hemant, M.K. Singh, D. Thakur, T.K. Giri, and D.K. Tripathi, The Botany, Chemistry, Pharmacological and Therapeutic Application of *Oxalis corniculata* Linn– A Review, Int.J. Phytomedicine 3 (2011) 01-08.
- [3] P. Gupta, S.K. Bajaj, K. Chandra, K.L. Singh, and J.S. Tandon, Antiviral profile of *Oxalis Corniculata* against encephalitis causing viruses, Indian J. Exp. Biol. 43 (2005)1156-1160.
- [4] V. Hukkeri, K. Akki, R.R. Sureban, B. Gopalkrishnan, V.V. Byahatti, and S.V. Rajendra, Hepatoprotective activity of the leaves *Oxalis corniculata* Linn., Indian J. Pharm. Sci. 4 (2006) 542-543.
- [5] A. Kathiriya, K. Das, E.P. Kumar, and

K.B. Mathai. Evaluation of antitumor and antioxidant activity of *Oxalis corniculata* against Ehrlich ascites carcinoma on mice, Iran. J. Cancer Prev. 4 (2010) 157-165.

[6] M. Hioki, T.Y. Kaori, and Y. Kunijiro, Flavanoids in the leaves of *Oxalis corniculata* and sequestration of the flavanoids in the wing scales of the pale grass blue butterfly, *Pseudozizeeria maha*, J. Plant Res. 121 (2008)133-136.

[7] C.P. Khare, *Indian Medicinal Plants: An illustrated dictionary*, Springer Science & Business Media, 2008.

[8]R.A. Sharma, and A. Kumari, Phytochemistry, pharmacology and therapeutic application of *Oxalis corniculata*- Review, Int. J. of Pharm. Pharm. Sci. 6(3) (2014) 0975-1491.

[9]I. Culie, Methology for analysis of vegetable drugs, practical manuals on industrial utilization of medicinal and aromatic plant, Bucharest, Phytochemistry 63 (1982) 97-104.

[10]M. Valko, D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur, and J. Telser, Free radicals and antioxidants in normal physiological functions and human disease, Int.J. Biochem. Cell Biol. 39(1) (2007) 44-84. DOI: 10.1016/j.biocel.2006.07.001

[11]S. Pokhrel, and K. Chaulagain, Phytoconstituents and biological analysis of *Acorus calamus* rhizome of Sindhupalchowk district, Nepal, Bibechna 17 (2020) 104-109.

DOI: 10.3126/bibechna.v17i1.24674

[12]G. Funde, Phytochemicals evaluation, anticancer, antioxidant and antimicrobial activity of *Acorus calamus* different solvent extracts, J. Chem. Pharm. Res. 7 (2015) 495-504.

[13]S. Pokhrel, and P. Neupane, Phytochemical analysis, antioxidant and antibacterial efficacy of methanol and hexane extract of *Centella asiatica*, Bibechna 18(2) (2021) 18-25.

- [14]S. Kaur, G. Kaur, and J. Singh. Phytochemical screening and biological potential of methanolic extract of *Oxalis corniculata* using different parts of plants, Res. J. Chem. 7(7) (2017), 26-32.
- [15]S.M. Mohan, B. Pandey, B. Deshpande, and V. Chandrakar, Antibacterial activity of plant extract of *Oxalis corniculata*, Indian J. Sci. Res. 5(1) (2015) 37-40.
- [16]M.P. Raghavendra, S. Satish, and K.A. Raveesha, Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant, my SCIENCE, 1 (2006) 72-78.
- [17]K. Aruna., P.D.R. Rajeswari, K. Prabu, M. Ramkumar, R. Chidambaram, and S.R. Sankar, Quantitative phytochemical analysis of *Oxalis Corniculata* L. (oxalidaceae) World J. Pharm. Pharm. Sci. 3(5) 2014 711-716.
- [18]S.L. Taley, A.S. Pethe, and S.P. Rothe, Studies on antibacterial activity of some plant extracts, Int. Multidiscip. Res. J. 12 (2012) 17-18.
- [19]S. Mukherjee, H. Koley, S. Barman, S. Mitra, S. Datta, S. Ghosh, D. Paul, and P. Dhar, *Oxalis corniculata* (Oxalidaceae) leaf extract exerts *in vitro* antimicrobial and *in vivo* anticolonizing activities against *Shigella dysenteriae* 1 (NT4907) and *Shigella flexneri* 2a (2457T) in induced diarrhea in suckling Mice, J.Med.Food 16(9) 2013 801–809.
- [20]A.K. Kathiriya, K. Das, M. Joshipura, and N. Mandal, *Oxalis corniculata* Linn. the plant of Indian subtropics, Herbal Tech. Ind. 2010.
- [21]Md.-S. Rahman, Md.-M.H. Khan and Md.-A.H. M. Jamal, Anti-bacterial evaluation and minimum inhibitory concentration analysis of *Oxalis corniculata* and *Ocimum santum* against bacterial pathogen, Biotechnology 9(4) (2010) 533-536. DOI: 10.3923/biotech.2010.533.536
- [22]S.M. Mohan, and B. Pandey, Antimicrobial activity of *Oxalis corniculata* Linn, Int. J. Sci. Res. 5(7) (2016) 575-578.
- [23]D.E. Okwu, and C. Josiah, Evaluation of the chemical composition of *Bryophyllum pinnatum*, Journal of Science 6 (2006) 30-37.
- [24]H.K. Makari, N. Haraprasad, and H.B. Patil, *In vitro* antioxidant activity of the hexane and methanolic extracts of *Cordia wallichi* and *Celastrus peniculata*, The Internet Journal Aesthetic and Antiaging Medicine 1 (2008) 1-10.
- [25]S. Poudel, B. Sapkota, P. Timilsina, K. Parajuli, and A. Kaundinnyayana, Phytochemical analysis, *in vitro* antioxidant activity and *in vivo* anti-inflammatory and analgesic activities of selected medicinal plants of Nepal, Journal of Pharmacognosy and Phytochemistry 8(6) (2019) 14-25.
- [26]A. Borah, R.N.S. Yadav, and B.G. Unni, Evaluation of antioxidant activity of different solvent extracts of *Oxalis corniculata* L, Journal of Pharmaceutical Research 5(1) (2012) 91-93.
- [27]D. Ahmed, S. Zara, and H. Baig, *In vitro* analysis of antioxidant activities of *Oxalis corniculata* Linn. fractions in various solvents, African Journal of Traditional, Complementary and Alternative Medicines, 10(1) (2013) 158-165.
- [28]D.D.Tibuhwa, Antioxidant potential-ities and antiradical activities of *Oxalis corniculata* Linn from Tanzania, J. Appl. Biosci. 116 (2017) 11590-11600. DOI: 10.4314/jab.v116i1.7
- [29]D.E. Okwu, Evaluation of the chemical composition of indigeneous spices and flavouring agents, J. Sci. 7 (2004) 455-459.