Dhaka Univ. J. Biol. Sci. **34**(1): 75-83, 2025 (January) DOI: https://doi.org/10.3329/dujbs.v34i1.79861

BIOCHEMICAL POTENTIALS AND STABILITY OF *OXALIS CORNICULATA* L. LEAF EXTRACTS

Md. Miraj Kobad Chowdhury[1*], Md. Ashraful Islam[1], Farjana Yasmin[2] And Md. Ayub Ryhan[1]

[1]*Department of Genetic Engineering and Biotechnology, University of Dhaka, Dhaka-1000, Bangladesh*

[2]*Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Sicence and Technology University, Santosh, Tangail-1902, Bangladesh.*

Keywords: antioxidant, flavonoids, free radical scavenging, *Oxalis corniculata* L., phenolics

Abstract

Biochemical activity and its stability of *Oxalis corniculata* L. leaf extracts in methanol, cyclohexane and water were investigated in this study. Preliminary phytochemical screening of the total phenolic and flavonoid contents for the methanolic extract were estimated as 18.63 ± 0.55 mg gallic acid equivalent and 6.46 ± 0.85 mg quercetin equivalents per gram of fresh leaf respectively. Maximum flavonoid content was observed in cyclohexane extract and the phenolics of aqueous extract were relatively stable as compared to the other extracts. The estimated total antioxidant capacity and the total reducing power of methanolic extract were the highest among the extracts, and were 12.33 ± 0.54 mg and 3.79 ± 0.37 mg ascorbic acid equivalent per gram of fresh leaf respectively. The IC_{50} values of DPPH free radical scavenging activity and hydrogen peroxide scavenging activity of the methanolic extract of fresh leaf were 10.14 ± 0.04 mg and 11.1 ± 0.01 mg, respectively. Such activities were absent or negligible in other extracts. Thin layer chromatographic studies detected the presence of at least two compounds with potent antioxidant activity. However, the phenolic and flavonoid contents, and all biochemical activities of the extracts were reduced after six months of preservation at -20°C. These data indicated that freshly prepared methanolic extract of *O. corniculata* could be the most potent, and such fresh preparation should be used to attain the desirable biochemical activities.

Introduction

A number of therapeutically important compounds have been isolated from plants and these compounds have been applied to cure a number of diseases(1). Most of the plants studied are herbal plants and they belong mainly to phanerogamae clade and few to cryptogamae(2). However, pharmacological studies involved with most of these plants are

* Author for correspondence: miraj@du.ac.bd

not properly studied till today[3]. As different parts like seeds, leaves, barks, roots or rhizomes of plant interact differently with the environment and its contents; they individually produce varieties of secondary metabolites[4]. These secondary metabolites exert multiple physiological roles in plants, animals and microorganisms[5]. As plant contains numerous chemical compounds, different solvents yield different extracts with varying degree of biological activities[6].

The creeping woodsorrel, scientifically known as *Oxalis corniculata* L., is a herbaceous plant of cosmopolitan distribution[7]. It is a perennial herb that is distributed worldwide. It is regarded as weed in gardens, agricultural fields and lawns and common in damp and shady places, roadsides, and pastures. The leaves of *O. corniculata* are quite edible with a tangy taste[8]. Such tangy taste is mostly due to the presence of vitamin C, oxalate, tartaric acid and other mild acids. So, a low dose of *O. corniculata* is safe and infusions can be made using this plant[9]. Ethonomedical report suggests that the leaves of this plant possess several biological activities and fresh leaves are used by different tribes as vegetable as well as medicine[10]. Thereby, the leaves of this plant might be a good source of potentially bioactive compounds[8,11]. Bioactive alkaloids, phenolics, tarpenoids, flavones, saponins and low molecular weight compounds are already isolated from *O. corniculata* leaves[8,11]. These compounds include but not limited to apigenin, acacetin, botulin, ethyl gallate, swertisin, isovitexin, syringic acid, 5-hydroxy-3′,4′,6,7-tetramethoxyflavone, *β*-sitosterol, 4-hydroxybenzoic acid, sertisin and isoorientin[12]. Some studies on this plant suggested that the leaf extract can be used as antimicrobial, liver tonic, and antinematode agent. Leaf extract of this plant also shows some degree of antioxidant activity[11,13] and DPPH free-radical scavenging power[14]. In Zairean pharmacopoeia, it is used as antivenom[15] and others also suggested antifungal and insecticidal activities of *O. corniculata* extracts[9,16]. The leaves are considered as a skin healing agent, specifically for warts and scabies[15]. A previous study from Bangladesh also reported antioxidant, free radical scavenging and Fe^{3+}-reducing activities of methanolic extract and its sub-fractions of *O. corniculata* leaf[17]. Similar studies were reported from other countries of Indian subcontinent[18]. Such activities could reduce the oxidative stress generated from multiple metabolic processes. However, the stability of such activities is still in question as the compounds present in the extracts are often unstable due to oxidation or polymerization[19]. On top of this, none of these reports can be translatable to fresh weigh leaves. Since people can consumes fresh leaves of *O. corniculata*, it would be interesting to know the equivalents of *O. corniculata* leaves for their biochemical activities.

In this study, fresh and frozen methanolic, aqueous and cyclohexane extracts of *O. corniculata* leaves were investigated for different biochemical potentialities. We found that fresh methanolic leaf extract exerted the best biochemical activities though such potentials decreased if the extracts were preserved by freezing.

Materials and Methods

Fresh leaves of *O. corniculata* were collected during early rainy season. 500 gram of fresh leaves were blended with 100 ml cold water and filtered to produce aqueous extract. For methanolic and cyclohexane extractions, 700 gram of fresh leaves were dried in an oven at 60°C for 24 hours and immediately extracted with solvent as plant material to solvent ratio of 1:5. Solvent was evaporated using rotary evaporator and amount of extracts and extract yields were determined. The pH of all these extracts was adjusted to pH 7.0 using concentrated NaOH solution. Half of the extracts were preserved at -20°C for six months. 10 µg of freeze-dried extract or 10 µl crude aqueous extract in 500 µl solvent were used for all the experiments mentioned below except DPPH free-radical scavenging activity and thin-layer chromatography (TLC). All experiments were done in triplicate and data were analyzed using Microsoft Excel.

The phenolic content was determined using Folin-ciocalteu reagent with gallic acid as standard[20]. For this, extracts were treated with 2.5 ml of Folin-ciocalteu reagent for 10 minutes and further incubated with 2.5 ml of 7% sodium carbonate for 2 hours. Then the absorbance was measured at 765 nm using spectrophotometer.

The flavonoid content was determined using aluminium chloride with quercetin as standard[21]. For this, extracts were treated with 100 µl 10% $AlCl_3$, 100 µl 1 M potassium acetate, and 2.8 ml distilled water. After half an hour, absorbance was recorded at 415 nm.

The total antioxidant capacity was determined by phospomolybdate assay using ascorbic acid as standard[22]. For this, extracts were treated with 6 ml reagent (0.6 M H_2SO_4, 4 mM ammonium molybdate, 28 mM NaH_2PO_4) for 90 minutes and absorbance was measured at 695 nm.

Total reducing power was determined as iron reducing capacity using ascorbic acid as standard[21]. For this, extracts were treated with 2 ml phosphate buffer (pH 6.6) and 2 ml 1% $K_3Fe(CN)_6$ and incubated for 40 minutes. After that, 2 ml of 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 minutes. 2.5 ml distilled water and 0.4 ml of 1% $FeCl_3$ was added to each 2 ml supernatant to quantitate the formation of ferric-ferrous complex at 700 nm.

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay was done by adding 10 µg or 10 µl crude aqueous extracts in 2 ml methanol with 2 ml 0.1 M DPPH solution in methanol. After an hour, the absorbance was measured at 517 nm and % inhibition of DPPH was calculated as % inhibition = 100 x (Absorbance in blank – Absorbance of sample)/Absorbance of blank[20].

H_2O_2 scavenging activity was determined by adding 10 µg or 10 µl extracts in 3.4 ml methanol and 0.6 ml 43 mM H_2O_2. This solution was incubated for 30 minutes and absorbance was noted at 230 nm using UV-spectrophotometer. H_2O_2 scavenging activity as calculated as % scavenged = 100 x (Absorbance in blank – Absorbance of sample)/ Absorbance of blank[21].

The thin-layer chromatography (TLC) was done using commercially available aluminium TLC plates. Combination of chloroform and ethanol was used as the solvent system. Briefly, samples were spotted on a dried plate and placed on solvent. After the completion of chromatography, the plates were developed with iodine or by spraying with 0.1 M DPPH solution[23].

Results and Discussion

Crude plant extract was prepared as discussed in the materials and method section. 13.15% (w/w) of methanolic, 1.53% (w/w) of cyclohexane and 13.63% (v/w) aqueous extract per gram of fresh *O. corniculata* leaf were obtained (Table 1). The methanolic crude extract was green in color. On the contrary, the cyclohexane and fresh leaf aqueous extracts were dark green and light green in color respectively. Lower yield of cyclohexane extract could be due to the comparatively lower abundance of non-polar compounds in leaf. Color of the extracts could be associated with phytopigment contents in respective extracts[24].

Table 1. Physical properties of the crude *O. corniculata* extracts

Types of Extract	% Yield	Properties of extract
Methanolic extract	13.15	Green in color, pungent taste
Cyclohexane extract	1.53	Dark green in color, good aroma
Aqueous extract	13.63	Light green in color, good taste and aroma

To estimate the phenolic content, we used gallic acid as a standard phenolic. Our study showed that the total phenolic content of methanol, cyclohexane and aqueous extracts were 18.63 ± 0.55 mg, 16.56 ± 1.28 mg and 0.35 ± 0.12 mg gallic acid equivalent respectively per gram of fresh leaf based on the gallic acid standard curve. Such finding showed a significant decrease of phenolics in aqueous extract as compared to methanolic and cycloxane extract (Table 2). However, keeping the extract for six months at -20 °C reduced the phenolic content in methanolic and cyclohexane extracts (10.59 ± 0.20 and 14.53 ± 0.89 mg gallic acid equivalent per gram of leaf respectively), but not in aqueous extract (0.36 ± 0.09 mg gallic acid equivalent per gram of leaf). Possibly, the phenolics in aqueous extract, although very low in amount, could be more stable as compared to the phenolics in other extracts[25]. The total flavonoids contents was measured based on quercetin standard curve and the flavonoids contents of methanolic, cyclohexane and aqueous extracts were 6.46 ± 0.85 mg, 15.72 ± 0.089 mg and 2.06 ± 0.01 mg quercetin equivalent per gram of fresh leaf respectively (Table 2). However, preservation of extracts at -20°C for six months reduced the flavonoids content in all extracts (1.32 ± 0.02 mg, 2.53 ± 0.10 mg and 0.29 ± 0.03 mg quercetin equivalent per gram of fresh leaf respectively for methanolic, cyclohexane, and aqueous extracts). This data indicated that most of the *O. corniculata* leaf flavonoids could be unstable[26].

Table 2. Chemical composition of *O. corniculata* extracts

Extract	Total Phenolics*		Total Flavonoids#	
	Fresh	Preserved	Fresh	Preserved
Methanolic	18.63 ± 0.55	10.59 ± 0.20	6.46 ± 0.85	1.32 ± 0.02
Cyclohexane	16.56 ± 1.28	14.53 ± 0.89	15.72 ± 0.089	2.53 ± 0.10
Aqueous	0.35 ± 0.12	0.36 ± 0.09	2.06 ± 0.01	0.29 ± 0.03

*equivalent to mg gallic acid ± standard deviation (n = 3) per gram of fresh leaf.
#equivalent to mg quercetin ± standard deviation (n = 3) per gram of fresh leaf.

Ascorbic acid was used as a standard to assess all the antioxidant potentials of *O. corniculata* leaves in this study and such potentials was estimated as per gram of fresh leaf. The total antioxidant capacity of methanolic, cyclohexane and aqueous extracts were 12.33 ± 0.54 mg, 2.81 ± 0.43 mg and 0.71 ± 0.02 mg ascorbic acid equivalent per gram of fresh leaf respectively (Table 3). However, the total antioxidant capacities of these extracts were 3.78 ± 0.25 mg, 0.78 ± 0.01 mg, and 0.19 ± 0.03 mg ascorbic acid equivalent per gram of fresh leaf respectively after preservation. This data suggested that the total antioxidant capacity of these extracts decreased over time and very unstable due to oxidation[27]. The total reducing power of methanolic, cyclohexane and aqueous extracts were 3.79 ± 0.37 mg, 0.29 ± 0.06 mg and 0.27 ± 0.05 mg ascorbic acid equivalent per gram of fresh leaf respectively (Table 3). After preservation for six months at -20°C, the reducing power of methanolic extract was reduced to 1.34 ± 0.14 mg ascorbic acid equivalent per gram of fresh leaf and no reducing power was detected in other extracts. This data clearly indicated that the reducing power of these extracts, especially the cyclohexane and aqueous extracts, were very ineffective and could be volatile[28]. IC_{50} of DPPH free radical scavenging activities of fresh leaves methanolic and aqueous extracts were 10.14 ± 0.04 mg, and 129.55 ± 1.88 mg, respectively (Table 3). The cyclohexane fraction failed to show any DPPH free radical scavenging activity, supporting the poor antioxidant power of such extract. Moreover, a 75% reduction of DPPH free radical scavenging activity in methanolic extract and complete reduction of the same in aqueous extract were observed after six months of preservation. IC_{50} of the hydrogen peroxide (H_2O_2) scavenging activity of methanolic extract and aqueous extract of fresh leaves were about 11.1 ± 0.01 mg and 174.3 ± 4.12 mg, respectively (Table 3). After six months, the H_2O_2 scavenging activity of methanolic extract was reduced to about 51%. Cyclohexane extract never showed any peroxide scavenging activity. All these data indicated that the methanolic extract could be the best for *O. corniculata* since it possess better chemical compositions and biochemical activities. Reduction of biochemical potentials and chemical contents in these extracts after preservation could be associated with compound instability and oxidation. In order to develop neutraceuticals, a freshly prepared extract must be used since the potency of extract become unstable after six months, and the prepared extracts should not be used after six months even though refrigerated. Finally, we concluded that consumption of one gram of fresh *O. corniculata* leaf would provide 0.35 ± 0.12 mg gallic acid equivalent phenolics and 2.06 ± 0.01 mg quercetin equivalent flavonoids, and such compounds would exert antioxidant potentials equivalent to maximum 710.0 ± 2.0 μg equivalence of ascorbic acid (vitamin C).

Table 3. Biochemical activities of *O. corniculata* extracts

Extract	Total antioxidant capacity[$]		Total reducing power[$]		DPPH free radical scavanging IC_{50}[£]		H_2O_2 scavenging power IC_{50}[£]	
	Fresh	Preserved	Fresh	Preserved	Fresh	Preserved	Fresh	Preserved
Methanolic	12.33 ± 0.54	3.78 ± 0.25	3.79 ± 0.37	1.34 ± 0.14	10.14 ± 0.04	76.34 ± 0.34	11.1 ± 0.01	56.9 ± 0.56
Cyclohexane	2.81 ± 0.43	0.78 ± 0.01	0.29 ± 0.06	-	-	-	-	-
Aqueous	0.71 ± 0.02	0.19 ± 0.03	0.27 ± 0.05	-	129.55 ± 1.88	-	174.3 ± 4.12	-

[$]equivalent to mg ascorbic acid ± standard deviation (n = 3) per gram of fresh leaf.
[£]mg fresh leaf ± (n = 3) standard deviation.

To further estimate the bioactive compounds in *O. corniculata* extracts, we performed thin layer chromatography (TLC) to detect the presence of any alkaloid. TLC of *O. corniculata* methanolic extract using solvent system chloroform:ethanol (8:2) showed 2 spots of R_f values 0.42 and 0.38, and with solvent system of chloroform:ethanol (2:8) showed 2 spots with R_f values of 0.32 and 0.20 (Fig. 1) as detected iodine that forms iodine-unsaturated compound intermediate. Antioxidant activities of these spots were observed as indicated by color change of DPPH solution (data not shown) due to antioxidant effect of compounds enriched in the methanolic extract of *O. corniculata*[23]. These compounds will be isolated, purified and identified using ^{1}H NMR spectroscopy in future studies.

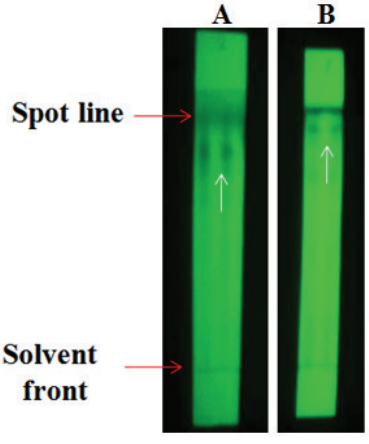

Fig. 1. TLC fingerprints of *O. corniculata* methanolic extract. (A) with solvent system chloroform:ethanol 8:2, (B) with solvent system chloroform:ethanol 2:8. White arrows indicate bands.

Conclusion

From the above studies, it could be suggested that methanolic leaf extract of *O. corniculata* should be a good source of bioactive compounds. The higher biochemical activities of methanolic extract compared to the aqueous and cyclohexane extracts indicated

that the active compounds were mostly semi-polar and unstable in water. One possibility could be that these compounds are readily oxidized in aqueous environment. Thereby, it is concluded that the fresh methanolic extract could be used to develop nutraceuticals[29] but the stability of such nutraceuticals remained questionable. Further strategies would be needed to stabilize the extract for such application.

Acknowledgement

We thank the Advanced Biotechnology Research Laboratory of the Department of Genetic Engineering and Biotechnology, University of Dhaka for supporting this research with necessary facilities. We also thank Professor Dr. M Aftab Uddin for his critical review and suggestions with our experiments.

Author Contributions

MMKC developed the original idea, provided grants and designed experiments. MMKC, MAI and FY performed all the experiments. MAI, FY and MAR did the literature review. MMKC, MAI and MAR wrote the article. All authors read, critically reviewed and agreed to publish this research.

Funding

This research was funded by a grant from the Biotechnology Research Centre of the University of Dhaka, Dhaka-1000, Bangladesh.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Sofowora A, E Ogunbodede and A Onayade 2013. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. **10**:210-229.
2. Mbuni YM, S Wang, BN Mwangi, NJ Mbari, PM Musili, NO Walter, G Hu, Y Zhou and Q Wang 2020. Medicinal Plants and Their Traditional Uses in Local Communities around Cherangani Hills, Western Kenya. Plants. **9**:331.
3. Mushwan K, SB Uddin, AZ Siddiki and MO Faruque 2024. Molecular identification of eight ethnomedicinal plants used by the indigenous communities of Bangladesh through the partial genome sequencing of three plastid markers. Gen Resour Crop Evol. **71**:2117-2134.
4. Al-Khayri JM, R Rashmi, V Toppo, PB Chole, A Banadka, WN Sudheer, P Nagella, WF Shehata, MQ Al-Mssallem, FM Alessa, MI Almaghasla and AA Rezk 2023. Plant secondary metabolites: the weapons for biotic stress management. Metabolites. **13**:716.
5. Erb M and DJ Kliebenstein 2020. Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. Plant Physiol. **184**:39-52.

6. Sasidharan S, Y Chen, D Saravanan, KM Sundram and L Yoga Latha 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. **8**:1-10.

7. Groom QJ, J Van der Straeten and I Hoste 2019. The origin of *Oxalis corniculata* L. PeerJ. **7**:e6384.

8. Sarfraz I, A Rasul, G Hussain, MA Shah, B Nageen, F Jabeen, Z Selamoglu, I Ucak, M Asrar and S Adem 2022. A review on phyto-pharmacology of *Oxalis corniculata*. Comb Chem High Throughput Screen. **25**:1181-1186.

9. Rashmi V and P Sahu 2020. A review on biochemical and medicinal properties of *Oxalis corniculata* Linn. J Interdis Cycle Res. **12**:177-182.

10. Golbarg H and MJ Mehdipour Moghaddam 2021. Antibacterial potency of medicinal plants including *Artemisia annua* and *Oxalis corniculata* against multi-drug Resistance E. coil. Biomed Res Int. **2021**:9981915.

11. Debnath A and A Das 2024. Isolation of bioactive compounds from low-cost agricultural resources and its utilization in daily life. Access Microbiol. **6**:000660.

12. Adiveppa N, K Mosin, R Patil Yuvaraj and P Poonam 2023. From traditional knowledge to modern medicine: *Oxalis corniculata* as a hepatoprotective and antibacterial remedy. J Ayur Integ Med Sci. **8**:134-142.

13. Ahmed D, S Zara and H Baig 2012. In vitro analysis of antioxidant activities of *Oxalis corniculata* Linn. fractions in various solvents. Afr J Tradit Complement Altern Med. **10**:158-165.

14. Hossain S, Islam J, Ahmed F, Hossain M, Siddiki M, and Hossen M 2015. Free radical scavenging activity of six medicinal plants of Bangladesh: a potential source of natural antioxidant. J Appl Pharma. **7**(1):96-104 .

15. Li PJ-k 2017. Some notes on animals and plants for Proto-Austronesian speakers. Language and Linguistics. **18**:254-268.

16. Kusamba C 1987. Antivenomous Plants Used in the Zairean Pharmacopoeia. Afr Stud Mono. **7**:21-35.

17. Alam MB, MS Hossain, NS Chowdhury, M Mazumder, ME Haque and AI Anwarul Islam 2011. *In vitro* and *in vivo* antioxidant and toxicity evaluation of different fractions of *Oxalis corniculata* Linn. J. Pharmacol. Toxicol. **6**:337-348.

18. Reddy K, S Kumar, S Lakshmi and S Angothu 2010. Antioxidant properties of methanolic extract of *Oxalis corniculata*. Int J Phytopharmacol. **1**:43-46.

19. Taghvaei M and SM Jafari 2015. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. J Food Sci Technol. **52**:1272-1282.

20. Yu L, S Haley, J Perret, M Harris, J Wilson and M Qian 2002. Free radical scavenging properties of wheat extracts. J Agric Food Chem. **50**:1619-1624.

21. Aryal S, MK Baniya, K Danekhu, P Kunwar, R Gurung and N Koirala 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants. **8**:96.

22. Uddin M, K Mitra and J Hoque 2015. Comparative bio-active compounds determination and *in vitro* antioxidant properties of newly developed soy mixed wheat flour and traditional wheat flour. Int J Food Proper. **19**:2113-2126.

23. Akar Z, M Kucuk and H Dogan 2017. A new colorimetric DPPH(*) scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. J Enzyme Inhib Med Chem. **32**:640-647.

24. Lu X, W Li, Q Wang, J Wang and S Qin 2023. Progress on the extraction, separation, biological activity and delivery of natural plant pigments. Molecules. **28**:5364.

25. Z eng L, M Ma, C Li and L Luo 2017. Stability of tea polyphenols solution with different pH at different temperatures. Int J Food Proper. **20**:1-18.

26. Kumar S and Pandey AK 2013. Chemistry and biological activities of flavonoids: an overview. ScientificWorldJournal. **2013**:162750.

27. Flieger J, W Flieger, J Baj and R Maciejewski 2021. Antioxidants: classification, natural sources, activity/capacity measurements, and usefulness for the synthesis of nanoparticles. Materials . **14**:4135.

28. Bhandari DP, DK Poudel, P Satyal, K Khadayat, S Dhami, D Aryal, P Chaudhary, A Ghimire and N Parajuli 2021. Volatile compounds and antioxidant and antimicrobial activities of selected citrus essential oils originated from Nepal. Molecules. **26**:6683.

29. Rana S and S Bhushan 2016. Apple phenolics as nutraceuticals: assessment, analysis and application. J Food Sci Technol. **53**:1727-1738.

(Manuscript received on 11 July, 2024; accepted on 18 December, 2024)