

Phytochemical Screening, Cytotoxic and Antioxidant Activity of *Alternanthera sessilis* and *Moringa oleifera*

Ishwor Pathak^{1*}, Rabin Budhathoki¹, Nisha Yadav¹, Muna Niraula², Surya Kant Kalauni^{3*}

¹Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu, Nepal

²Department of Forest and Soil Conservation, Babarmahal, Kathmandu, Nepal

³Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal

*Email: pathakishwor14@gmail.com & skkalauni@gmail.com

(Received: 19 April, 2020, Received in revised form: 2 August, 2020, Accepted: 10 August, 2020, Available online)

Highlights

- Methanol and hexane extracts of *Moringa oleifera* and *Alternanthera sessilis* were prepared.
- Phytochemical screening, brine shrimp bioassay, and antioxidant activity test of each extract of both plants were performed.
- *Alternanthera sessilis* extracts showed high toxicity against brine shrimp larvae.
- Methanol extract of both plants showed the highest antioxidant activity.

Abstract

Methanol and hexane extracts of leaves of *Moringa oleifera* and aerial parts of *Alternanthera sessilis* were screened for the presence of different classes of phytoconstituents. Phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, terpenoids, polyphenols, glycosides, and coumarins in methanol extracts. Volatile oils, quinines, and phytosterols were absent in all extracts and saponins were present in all extracts. The biological activity of all the extracts was tested by performing brine shrimp bioassay. All the extracts except hexane extract of *Moringa oleifera* were found to be cytotoxic against brine shrimp nauplii. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was used to evaluate the antioxidant activity of the extracts. Methanol extracts of *Moringa oleifera* showed the strongest antioxidant activity with an IC_{50} value of 65.77 $\mu\text{g/mL}$. Regarding the ascorbic acid (IC_{50} value 39.53 $\mu\text{g/mL}$) as standard, methanol extracts of both plants showed high free radical scavenging activity than that of hexane extracts.

Keywords: Extract, phytochemicals, antioxidants, brine shrimp bioassay

Introduction

Plants possess a wide variety of secondary metabolites that are rich in antioxidant activities and other several therapeutic values (Harborne, 1998). Several bioactive molecules can be isolated from the plants and such molecules serve as a starting material for drug synthesis (Dhanani *et al.* 2017). Medicinal plants have been used by mankind for the treatment of various ailments for thousands of years (Siew *et al.* 2014).

Moringa oleifera, a member of moringaceae family is a native to Indian subcontinents, Africa, and Nepal (Bukar *et al.* 2010). *Moringa* trees are commonly known as “drumstick” and its Nepali name is sittalchini. It is a highly valued plant for

*Corresponding author

nutritional food and medication. People use its leaves, flowers, roasted seeds, and fresh pods as vegetables, seeds as cooking and cosmetic oils and roots for spice (Anwar *et al.* 2006). It can also be used as a livestock feed. The chemical composition of the fiber present in it affects the digestive ability of animals (Moyo *et al.* 2011). *Moringa* seeds have shown analgesic, antipyretic, hepatoprotective and wound healing activities. Its leaves are natural energy booster as it contains more than 14 amino acids, carbohydrates, proteins, vitamin A, C and E, calcium, and magnesium that the human body requires (Kumbhare *et al.* 2012), (Shahriar, 2012). A variety of biological activities of different parts of the *M. Oleifera* have been reported to date and this plant can serve a potent antioxidant supply for both human beings and animals (Suphachai, 2014), (Arora & Onsare, 2014), (Hossain *et al.* 2014).

Alternanthera sessilis (L.), commonly known as “bhiringijhar” in Nepal, belongs to the family *Amaranthaceae*. It is an aquatic plant and commonly observed in marshy areas, wetlands, streams, reservoirs, and river banks (Hossain *et al.* 2014). Geographically, it is native to Asia, Australia, and different Islands. In Nepal and Indian subcontinents, people use this plant to treat gonorrhea, low sperm count, treatment of burning sensation, diarrhea, skin diseases, fever, cuts and wounds, liver, and spleen diseases (Rahman & Gulshana, 2014), (Nayak *et al.* 2010). It contains bioactive phytoconstituents especially polyphenols, alkaloids, stigmasterol, campesterol, palmitates of sterol, etc. (Walter *et al.* 2014). Aerial parts of this plant are a good source of minerals, vitamins, pigments, and antioxidants. The therapeutic values of this plant are closely associated with the presence of these active constituents (Jalalpure *et al.* 2008), (Borah *et al.* 2011). Several previous studies reported that the *A. sessilis* found in other countries have high scavenging activities; however, any report of antioxidant activity of *A. sessilis* found in Nepal has not been reported to date (Balakrishnan *et al.* 2013), (Borah *et al.* 2011), (Murugan *et al.* 2013). An attempt has been made in this study to compare phytochemicals, antioxidant and cytotoxic activities of *A. sessilis* and *M. oleifera* found in different habitats and geographical regions of Nepal.

Materials and Methods

Plant materials

Aerial parts of *Alternanthera sessilis* and matured leaves of *Moringa oleifera* were collected from Dang and Rautahat districts of Nepal respectively, in the December month of 2018. The plants were authenticated at the Department of Botany, Amrit Campus, Kathmandu, Nepal. The collected samples were cleaned, shade dried and powdered and stored in airtight bottles.

Extraction

The dried and powdered parts of *Alternanthera sessilis* and *Moringa oleifera* were extracted separately with two different solvents hexane and methanol by using soxhlet apparatus. Accurately weighed 50 g powdered samples of each plant were filled separately in each thimble and placed in the central assembly of the soxhlet apparatus. Accurately measured 300 mL solvents (hexane and methanol) was added separately to a 500 mL round bottom flask. The extraction was done continuously 6 hours at 68 °C for hexane solvent and 64 °C for methanol solvent. The obtained liquid extracts were concentrated using a rotary evaporator (IKA RV 10 digital). The percentage yield (w/w) of the crude extracts of both plants was calculated and stored in the refrigerator at 5 °C until used for further experiment.

Phytochemical screening

Phytochemical screening is the method of finding the main class of chemical compounds present in the plant extracts. The freshly prepared crude extracts were subjected to phytochemical screening using standard procedures (Harborne, 1998). The various phytochemicals present in the extracts were identified by the color reaction with different reagents.

Biological assay

The brine shrimp lethality assay of each extract was performed to evaluate cytotoxicity by following the standard protocol (Meyer *et al.* 1982). The method determines the LC₅₀ values (Lethal concentration for 50% mortality) for the crude extracts. Extract of LC₅₀ value less than 1000 ppm is considered as potentially cytotoxic and pharmacologically active (Montanher *et al.* 2002).

The artificial seawater was prepared in a small tank to hatch the shrimps. The shrimp eggs were added to the tank containing seawater. A lamp (60 watts) was placed above the open side of the tank by adjusting the temperature at 30 °C. After 48 hours, the shrimps mature as nauplii (*Artemia salina*) and are ready for the assay. Stock solutions were prepared by dissolving 25 mg of each extract separately in DMSO in two separate test tubes. The solution thus prepared was used as a stock solution. From each

stock solution, solutions of 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.625 µg/mL and 7.81 µg/mL were prepared by serial dilution method. 2.5 mL of each concentration was transferred into test tubes, three for each concentration. Similarly, 2.5 mL of DMSO was taken in three test tubes as a blank. Labeling of test tubes was done and then they were kept for 24 hours to evaporate the solvent (DMSO). After complete evaporation of the solvent, ten matured shrimps in 5 mL artificial seawater were transferred to all test tubes containing samples. Similarly, three controlled vials were taken and ten matured nauplii were introduced in each vial. After 24 hours of illumination under a table lamp (60 watts), the numbers of survivors were counted with the help of disposable pipettes. The LC₅₀ value (lethal concentration for 50% mortality) was determined using probit regression (Finney, 1971).

Antioxidant activity test

The free radical scavenging activity or antioxidant activity of each extract was measured by using DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging assay by following the standard protocol with some modifications (Hossain *et al.* 2011), (Sharma *et al.* 2015). Different concentrations (10, 30, 50, 70, 90 & 110 µg/mL) of extracts and ascorbic acid (positive control) were prepared. From each solution, 1 mL of each solution was taken in different Eppendorf tubes. 1 mL of the 0.2 mM DPPH solution was added on each tube containing 1 mL solution. The tubes were shaken and allowed to stand at 30 °C for half an hour. The absorbance of each solution was taken on a UV-Visible spectrophotometer (UV professional double beam, Shimadzu made) at 517 nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The experiment for each extract was performed in triplicate and the percentage radical scavenging activity was calculated using the following equation (Subba & Basnet, 2014):

$$\% \text{ radical scavenging activity} = (\text{Abs.}_{\text{control}} - \text{Abs.}_{\text{sample}} / \text{Abs.}_{\text{control}}) \times 100\%$$

The sample which shows a higher percentage radical scavenging activity can act as strong antioxidants (Sharma *et al.* 2015). The graph of concentration of extract versus the percentage of free radical scavenging activity was plotted. Based on this graph, the IC₅₀ value of each extract was calculated. The IC₅₀ value is the inhibitory concentration of the sample required to scavenge 50% of the free radicals. The concentration corresponds to inhibition percentage 50 on the plot is IC₅₀. The IC₅₀ values of the samples were compared and the value closest to that of ascorbic acid is considered to have the best antioxidant property (Khalaf *et al.* 2008).

Results and Discussion

Extractive values of different extracts

The plant material (each 50 g) was successively extracted with hexane and methanol. The highest amount of extract was obtained with methanol. The results of the yield of the different extracts are shown in Table 1.

Table 1. Percentage yield of different extracts

Sample taken	Percentage yield of different extracts	
	Hexane	Methanol
Aerial parts of <i>A. sessilis</i>	4.0	6.5
Leaves of <i>M. oleifera</i>	5.5	7.0

Phytochemical screening

The phytochemical screening of methanol and hexane extracts of *M. oleifera* and *A. sessilis* (Table 2) showed the presence of different phytoconstituents. Alkaloids, flavonoids, carbohydrates, terpenoids, polyphenols, glycosides, and coumarins were present in methanol extract of both plants. Saponin was present in all extracts while volatile oils, quinines, and phytosterols were absent in all extracts. The result showed that the more polar methanol extract possesses more phytoconstituents than non-polar hexane extracts. The antioxidant and other biological properties of the plant are related to the presence of these phytochemicals in the extracts (Rice-Evans *et al.* 1996), (Kota *et al.* 2017), (Gupta *et al.* 2012).

Table 2: Results of phytochemical screening of different extracts

Phytochemicals	<i>Alternanthera sessilis</i>		<i>Moringa oleifera</i>	
	Methanol extract	Hexane extract	Methanol extract	Hexane Extract
Tannins	-	-	+++	-
Alkaloids	+	-	++	-
a) Dragendroff's test	+	-	++	-
b) Mayer's test	+	-	+	-
Flavonoids	++	+	++	-
Proteins	-	-	+	-
Carbohydrates				
a) Fehling's test	++	-	+	-
b) Molisch's test	+	-	++	-
Coumarins	+	+	++	-
Quinones	-	-	-	-
Phytosterols	-	-	-	-
Saponins	++	+	+++	+
Terpenoids	+	+	+	-
Fats and fixed oils	-	-	-	++
Volatile oils	-	-	-	-
Polyphenols	++	-	+	-
Glycosides	+	-	+	-

Key: + = Present - = Absent

Biological assay

Brine shrimp lethality activity of the plant extracts was calculated by using probit regression and shown in table 3. Crude extracts resulting in LC_{50} values of less than 1000 $\mu\text{g/ml}$ were considered significantly active and had the potential for further investigation. The methanol extract of both plants *A. sessilis* and *M. oleifera* were found to be cytotoxic against brine shrimps as shown by their LC_{50} values 255.40 $\mu\text{g/mL}$ and 380.50 $\mu\text{g/mL}$ respectively. The hexane extract of *A. sessilis* showed the LC_{50} value of 925.68 $\mu\text{g/mL}$ and *M. oleifera* showed >1000. The results revealed that the active constituents were mainly distributed in the methanol extract of both plants.

Table 3: Brine shrimp bioassay and antioxidant activity results of plant extracts and ascorbic acid

Sample taken	Extract	LC_{50} value ($\mu\text{g/mL}$)
<i>Alternanthera sessilis</i>	Methanol	255.40
	Hexane	925.68
<i>Moringa oleifera</i>	Methanol	380.50
	Hexane	>1000

Antioxidant activity test

The radical scavenging activity of different extracts and ascorbic acid (taken as standard) was evaluated using the DPPH assay. Table 4 represents the absorbance values and % radical scavenging activity of plant extracts and the standard taken at different concentrations. IC_{50} value of ascorbic acid (Figure 2) and each extract was calculated from the plot between concentration and % radical scavenging activity (Figure 1). Methanol extracts of *M. oleifera* exhibited a higher potential of radical scavenging

activity, with an IC₅₀ value of 65.77 µg/mL compared to its hexane extract (IC₅₀ value 88.90 µg/mL). Similarly, methanol extract of *A. sessilis* showed high antioxidant activity (IC₅₀ value 71.10 µg/mL) than its hexane extract (IC₅₀ value 92.54µg/mL). It was found that methanol extract of both plants possesses high antioxidant activity as their IC₅₀ values were found to be close to the IC₅₀ value of standard ascorbic acid taken (39.53µg/mL).

Table 4: Absorbance values and % of radical scavenging at different concentrations of samples

Concentration (µg/mL)	Absorbance values					% Free radical scavenging				
	<i>A. sessilis</i>		<i>M. oleifera</i>		Ascorbic acid	<i>A. sessilis</i>		<i>M. oleifera</i>		Ascorbic acid
	ME	HE	ME	HE		ME	HE	ME	HE	
0	0	0	0	0	0	0	0	0	0	0
10	0.486	0.623	0.491	0.615	0.366	30.6	11	29.9	12.1	47.7
30	0.404	0.532	0.441	0.531	0.287	42.3	24	37	24.2	59.0
50	0.347	0.488	0.375	0.466	0.237	50.5	30.3	46.3	33.5	66.1
70	0.326	0.417	0.299	0.389	0.216	53.4	40.4	57.2	44.5	69.2
90	0.314	0.332	0.263	0.335	0.179	55.1	52.6	62.5	52.2	74.5
110	0.287	0.329	0.241	0.319	0.154	59.0	53.0	65.6	54.5	78.0
IC ₅₀ value (µg/mL)						71.10	92.54	65.77	88.9	39.53

Absorbance of control (1mL MeOH + 0.5 mL DPPH) = 0.7

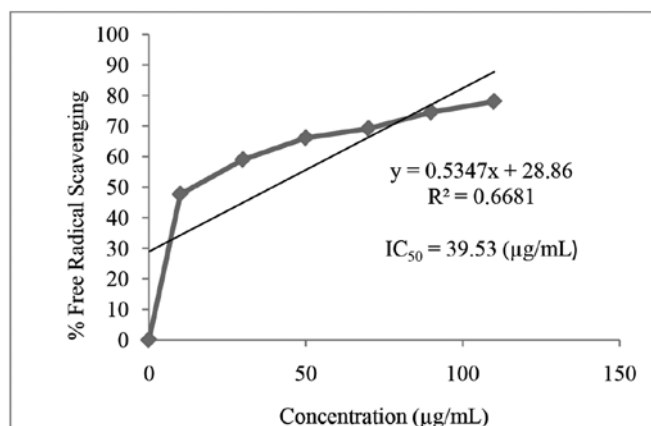
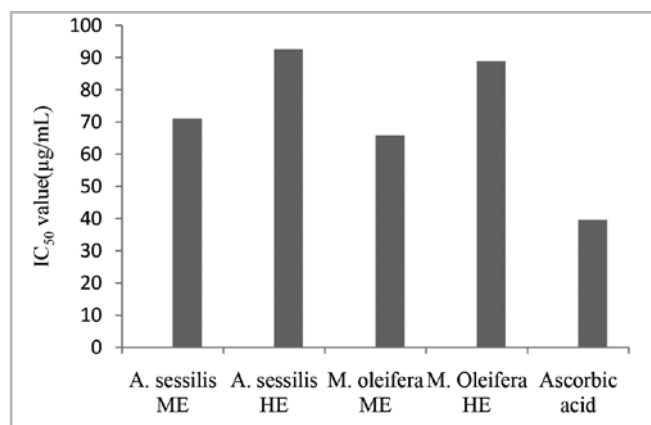


Fig 1: Percentage radical scavenging activity of ascorbic acid at different concentrations



(ME: Methanol extract, HE: Hexane extract)

Fig 2: IC₅₀ values of different extracts and ascorbic acid

Polyphenols and flavonoids found in methanol extracts of *A. sessilis* and *M. oleifera* (Table 2) are recognized as potent sources of antioxidants. Several previous studies also reported that the methanol extract of *M. oleifera* leaves contained polyphenols and had DPPH radical scavenging activity (Kumbhare, *et al.* 2012), (Shahriar, 2012), (Suphachai, 2014). The results reflect that *A. sessilis* and *M. Oleifera* can act as a very good option in the field of medicine based on the antioxidant property of natural products chemistry.

Conclusions

Phytochemical screening of methanol and hexane extracts of *M. oleifera* and *A. sessilis* chiefly showed the presence of alkaloids, flavonoids, carbohydrates, terpenoids, polyphenols, glycosides, coumarins, and saponins. More phytoconstituents were present in methanol extracts than that of in hexane extracts. Brine shrimp bioassay showed that all extracts except hexane extract of *M. oleifera* were cytotoxic against brine shrimp nauplii. Analysis of DPPH free radical scavenging of the extracts showed that both plants are highly potent in terms of antioxidant activity and the extent of antioxidant activity is following the presence of chief phytoconstituents like flavonoids and polyphenols present in the plant. Further research is recommended to explore these plants as dietary supplements as a source of antioxidants.

Acknowledgements

We would like to thank the Department of Chemistry, Amrit Campus, Tribhuvan University, Kathmandu and Department of Forest and Soil Conservation, Babarmahal, Kathmandu for providing the laboratory facility.

References

- Anwar, F., Latif, S., Ashraf, M. and Gilani, A.H. 2006. *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytotherapy Research*. **21**(1): 17-25.
- Arora, D.S. and Onsare, J.G. 2014. In vitro antimicrobial potential, biosafety and bioactive phytoconstituents of *Moringa oleifera* stem bark. *World Journal of Pharmaceutical Research*. **3**(2): 2772-2788.
- Balakrishnan, B., Ayyavoo, J., Sadayan, P. and Abimannan, A. 2013. Evaluation of antioxidant activity of *Clitoria ternatea* and *Alternanthera sessilis* plant extracts using model system for yeast cells. *African Journal of Basic & Applied Sciences*. **5**(3): 134-138. DOI: 10.5829/idosi.ajbas.2013.5.3.1135.
- Borah, A., Yadav, R.N.S. and Unni, B.G. 2011. In vitro antioxidant and free radical scavenging activity of *Alternanthera sessilis*. *International Journal of Pharmaceutical Sciences and research*. **2**(6): 1502-1506.
- Bukar, A., Uba, A. and Oyeyi, T. 2010. Antimicrobial profile of *Moringa oleifera* extracts against some food – borne microorganisms. *Bayero Journal of Pure and Applied Sciences*. **3**(1): 43-48. <https://doi.org/10.4314/bajopas.v3i1.58706>.
- Dhanani, T., Shah, S., Gajbhiye, N.A. and Kumar, S. 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*. **10**(1): 1193-1199. doi.org/10.1016/j.arabjc.2013.02.015.
- Finney, D. 1971. *Probit analysis*, third edition. Cambridge University Press, Cambridge, UK.
- Gupta, R., Mathur, M., Bajaj, V.K., Katariya, P., Yadav, S., Kamal, R. and Gupta, S. 2012. Evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes. **4**: 164-171.
- Harborne, A.J. 1998. *Phytochemical methods: A guide to modern techniques of plant analysis*. Springer Science & Business Media, UK.
- Hossain, M.A., Shah, M.D., Gnanaraj, C. and Iqbal, M. 2011. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetragium* from Sabah. *Asian Pacific Journal of Tropical Medicine*. **4**(9): 717-721. [https://doi.org/10.1016/S1995-7645\(11\)60180-6](https://doi.org/10.1016/S1995-7645(11)60180-6).

- Hossain, A.I., Faisal, M., Rahman, S., Jahan, R. and Rahmatullah, M. 2014. A preliminary evaluation of antihyperglycemic and analgesic activity of *Alternanthera sessilis* aerial parts. *BMC Complementary and Alternative Medicine*. **14**(1): 169-173.
- Jalalpure, S.S., Agrawal, N., Patil, M.B., Chimkode, R. and Tripathi, A. 2008. Antimicrobial and wound healing activities of leaves of *Alternanthera sessilis* Linn. *International Journal of Green Pharmacy*. **2**(3): 156-163.
- Khalaf, N.A., Shakya, A.K., Al-Othman, A., El-Agbar, Z. and Farah, H. 2008. Antioxidant activity of some common plants. *Turkish Journal of Biology*. **32**(1): 51-55.
- Kota, S., Govada, V.R., Anantha, R.K. and Verma, M.K. 2017. An Investigation into phytochemical constituents, antioxidant, antibacterial and anti-cataract activity of *Alternanthera sessilis*, a predominant wild leafy vegetable of South India. *Biocatalysis and Agricultural Biotechnology*. **10**: 197-203.
- Kumbhare, M., Guleha, V. and Sivakumar, T. 2012. Estimation of total phenolic content, cytotoxicity and in-vitro antioxidant activity of stem bark of *Moringa oleifera*. *Asian Pacific Journal of Tropical Disease*. **2**(2): 144-150. [https://doi.org/10.1016/S2222-1808\(12\)60033-4](https://doi.org/10.1016/S2222-1808(12)60033-4).
- Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E. and McLaughlin, J.L. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*. **45**(5): 31-34. <https://doi.org/10.1055/s-2007-971236>.
- Montanher, A.B.P., Pizzolatti, M.G. and Brighente, I.M.C. 2002. An application of the brine shrimp bioassay for general screening of brazilian medicinal plants. *ActaFarm. Bonaerense*. **21**(3): 175-8.
- Murugan, S. M., Reshma, A., Deepika, R., Balamurugan, S. and Sathishkumar, R. 2013. Antioxidant capacities of *Amaranthus tristis* and *Alternanthera sessilis*: A comparative study. *Journal of Medicinal Plants Research*. **7**(30): 2230-2235. DOI: 10.5897/JMPR2013.2567.
- Moyo, B., Masika, P.J., Hugo, A. and Muchenje, V. 2011. Nutritional characterization of *Moringa* (*Moringa oleifera* Lam.) leaves. *African Journal of Biotechnology*. **10**(60): 925-933.
- Nayak, P., Nayak, S., Kar, D. and Das, P. 2010. Pharmacological evaluation of ethanolic extracts of the plant *Alternanthera sessilis* against temperature regulation. *Journal of Pharmacy Research*. **3**(6): 1381-1383.
- Rahman, A. and Gulshana, M. 2014. Taxonomy and medicinal uses on amaranthaceae family of rajshahi, Bangladesh. *Applied Ecology and Environmental Sciences*. **2**(2): 54-59.
- Rice-Evans, C.A., Miller, N.J. and Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*. **20**(7): 933-956.
- Shahriar, M. 2012. Preliminary phytochemical screening, in-vitro antioxidant and cytotoxic activity of five different extracts of *Moringa oleifera* Leaf. *Journal of Applied Pharmaceutical Science*. **2**(5): 65-68. <https://doi.org/10.7324/JAPS.2012.2510>.
- Sharma, K.R., Kalauni, S.K. and Awale, S. 2015. Antioxidant, phytotoxic and antimicrobial activities of methanolic extract of *Bauhinia variegata* Barks. *Journal of Institute of Science and Technology*. **20**(2): 37-41. <https://doi.org/10.3126/jist.v20i2.13946>.
- Siew, Y.Y., Zareisedehizadeh, S., Seetoh, W.G., Neo, S.Y., Tan, C.H. and Koh, H.L. 2014. Ethnobotanical survey of usage of fresh medicinal plants in Singapore. *Journal of Ethnopharmacology*. **155**(3): 1450-1466. <https://doi.org/10.1016/j.jep.2014.07.024>.
- Subba, B. and Basnet, P. 2014. Antimicrobial and antioxidant activity of some indigenous plants of Nepal. *Journal of Pharmacognosy and Phytochemistry*. **3**(1): 62-67.
- Suphachai, C. 2014. Antioxidant and anticancer activities of *Moringa oleifera* leaves. *Journal of Medicinal Plants Research*. **8**(7):318-325. <https://doi.org/10.5897/JMPR2013.5353>.
- Walter, T.M., Merish, S. and Tamizhamuthu, M. 2014. Review of *Alternanthera Sessilis* with Reference to Traditional Siddha Medicine. *International Journal of Pharmacognosy and Phytochemical Research*. **6**(2): 249-254.