Annals of Biology **38** (2) : 157-160, 2022

Impact Score : 0.32 (Scopus)

Antioxidant Potential of *Lawsonia inermis* Leaves

AMIT AND NARENDER SINGH*

Plant Tissue Culture Laboratory, Department of Botany, Kurukshetra University, Kurukshetra-136 119 (Haryana), India

**(e-mail : nsheorankukbot11@gmail.com; Mobile : 94164 12622)*

(Received : April 10, 2022; Accepted : May 24, 2022)

ABSTRACT

Lawsonia inermis (family Lythraceae) is commonly called as Heena. To determine the antioxidant activity, total phenolic and flavonoid content of petroleum ether extract, dichloromethane extract (DCM), ethanol extract and aqueous extract of henna leaves were taken. Total antioxidant evalution (phosphomolybenum method), DPPH radical scavenging assay, reducing power assay and lipid peroxidation inhibition assay were used to discover the potential of leaves as an antioxidant. In all, the evaluation carried out ethanol showed a greater potential to scavenge DPPH radical, reduce complex and to inhibit lipid peroxidation. The IC_{50} value of ethanol was far greater than that of the standard, ascorbic acid in the lipid peroxidation assay. The activity of aqueous was lesser when compared with that of ethanol but greater than petroleum and dichloromethane. The amounts of phenolics and flavonoids were present in higher amounts in ethanol followed by aqueous. Trace amounts of phenolics were detected in petroleum and dichloromethane but the amount of flavonoids were below the detection level. The study showed that the antioxidant activity and the concentrations of phenolics and flavonoids were proportionate to each other. Ethanolic extract of henna seeds were efficient antioxidants, which can be utilized for further isolation of active compounds and pharmaceutical applications.

Key words : Antioxidant, *Lawsonia inermis,* leaves

INTRODUCTION

Lawsonia inermis belongs to family Lythraceae which is commonly known as henna. It is mainly present in sub-tropical and tropical areas and is used all over the world. *L. inermis* L. is a much branched glabrous shrub or small tree (2-6 m in height), cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. The plant is reported to contain lawsone, esculetin, fraxetin, isoplumbagin, scopoletin, betulin, betulinic acid, hennadiol, lacoumarin and laxanthone. The plant has been reported to have analgesic, hypoglycemic, antimalarial, hepatoprotective, nootropic, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, anthelmintic, antifertility, tuberculostatic and anticancer properties. The phytochemical analysis of *L. inermis* disclosed the presence of carbohydrates, phenolic, flavanoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, fat, resin and tannins. It also contained 2-hydroxy-1,4-naphthoquinone (lawsone). Many alkaloids, napthoquinone derivatives, phenolics and flavonoids were isolated from different parts of *L. inermis.* Pharmacological aspect showed that *L. inermis* had antimicrobial, antifungal, antiparasitic, molluscicidal, antioxidant, hepatoprotective, analgesic, anti-inflammatory, antipyretic, wound and burn healing, immunomodulatory, antiurolithiatic, antidiabetic, hypolipidemic, antiulcer, antidiarrhoeal, antdiuretic, anticancer and many other pharmacological effects. The present study was undertaken to estimate the antioxidant potential of various extracts of leaves of this plant.

MATERIALS AND METHODS

To determine the antioxidant activity of *L. inermis* leaves extracts and phytochemicals, DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging activity showed its usefulness. To estimate the anti-oxidant activity, the amount of sample taken under observation reduced the starting DPPH conc. by 50%. One ml of the extract in methyl alcohol was combined to 0.5 ml of 0.15 milli molar DPPH solution in methyl alcohol. These were mingled strenuously and permitted to put at a temperature of 20°C and for 30 min. At 517 nano meter wavelength, the

absorbance was recorded. IC_{50} value was evaluated. Following equation was used for the evaluation of the efficacy for scavenging the 2, 2- diphenyl-1-picryl-hydrazyl-hydrateradicals :

Scavanging effectivity (%) of DPPH = [(A0-A1)/ A0] x 100

Where, A0 = Absorbance of the control reaction and

A1 = Absorbance in the presence of the sample.

Spectrophotometric protocol was utilized to determine the superoxide scavenging activity. Plant extracts of diverse concentrations were prepared by dissolving into water. During this analysis, addition of NaOH in the dimethyl sulfoxide (DMSO) super oxide radicals (O_2^-) was created. The nitroblue tetrazolium was used to reduce these free radicals at room temperature at a wavelength of 560 nm. For this, the reaction mixture made up of 0.5 ml plant extract was mingled with 1 ml dimethyl sulfoxide (DMSO alkaline). This reaction mixture was mixed with 0.2 ml Nitro blue tetrazolium dye (NBT) for upgrading the amount by 1.7 ml. The absorbance was noted down and 0.5 ml of dimethyl sulfoxide was utilized as blank. Per cent scavenging activity was calculated as :

Scavenging activity (%) = [(Ac-At)/Ac] x 100

Where, Ac = Absorbance of the control reaction, and

At = Absorbance in the presence of the sample.

Total antioxidant activity was estimated by the spectrophotometric method. Different concentrations of plant extracts were prepared by dissolving in a particular solvent. 0.1 ml of each concentration of extracts was taken in different test tubes and mixed with 1 ml of reaction mixture. Twenty-eight milli molar Na_3PO_4, 0.6 M H_2SO_4 and 4 milli molar ammonium molybdate were poured into the Eppendorf test tube. The incubation occurred at 95°C in 90 min. The absorbance of the solution of each extract was measured after it cooled to room temperature at 695 nm. 0.1 ml water was used as the blank in the place of extract. Ascorbic acid was utilized as standard. The same procedure was carried out with extracts prepared in methanol. Scavenging activity in % was evaluated as :

Scavenging activity (%) = [(Ac-At)/Ac] x 100

Where, Ac indicated the absorbance of the control reaction and At indicated the absorbance in the presence of samples alongwith the extracts.

RESULTS AND DISCUSSION

The DPPH scavenging activity of leaves extracts indicated that leaves at lower concentration showed lesser activity, whereas higher concentration of leaves recorded higher scavenging activity (Table 1). The lowest value was 25.98 and the highest value was 48.06 in case of methanolic extracts of leaves, whereas it was 24.66 and 46.29 in case of aqueous extracts of leaves. It was noticed that aqueous extracts recorded less scavenging capacity as compared to methanolic extracts.

Table 1. Percentage of DPPH scavenging activity of different leaf extracts of *L. inermi*

S. No.	Concentration (µg/ml)	MeOH leaf	Aq leaf
1.	100	25.98	24.66
2.	200	34.96	26.92
3.	300	45.30	26.57
4.	400	43.72	32.25
5.	500	47.14	45.16
6.	600	48.06	46.29

The superoxide scavenging activity was more with enhanced conc. of plant extracts (Table 2). It was found that the leaf at lower concentration (100) scavenged least superoxide, whereas leaf at higher concentration (600) scavenged maximum superoxide. The lowest value was 07.12 and the highest value was 42.41 in case of methanolic extracts of leaves, whereas it was

Table 2. Percentage of superoxide scavenging activity of different leaf extracts of *L. inermis*

S. No.	Concentration (µg/ml)	MeOH leaf	Aq leaf
1.	100	07.12	18.24
2.	200	09.35	24.63
3.	300	11.27	30.80
4.	400	19.57	34.12
5.	500	34.53	40.15
6.	600	42.41	45.58

18.24 and 45.58 in case of aqueous extracts of leaves. Aqueous extracts recorded more scavenging capacity as compared to methanolic extracts.

The per cent total antioxidant activity of leaves extracts revealed that the higher concentration of leaves supported highest per cent total antioxidant activity which decreased with the decrease in content of leaves (Table 3). Maximum activity was 81.68% and minimum was 32.98% in methanolic extracts, whereas aqueous recorded minimum value of 45.63% and maximum value of 87.49%. Aqueous extracts had higher values as compared to methanolic extracts.

Table 3. Total antioxidant activity of different leaf extracts of *L. inermis*

S. No.	Concentration (µg/ml)	MeOH leaf	Aq leaf
1.	100	32.98	45.63
2.	200	48.77	63.59
3.	300	51.31	72.57
4.	400	59.49	79.26
5.	500	73.62	81.38
6.	600	81.68	87.49

Various phytochemicals have a property to neutralize the free radicals which are produced due to their physiological reactions. These phytochemicals are known as antioxidants because they scavenge the free radicals formed in the plant body and other organism body. Free radicals are found to play a specific role in a large variety of pathological manifestations. Antioxidants work against free radicals and exert their action either by scavenging the ROS or protecting the antioxidant defense mechanisms. The DPPH purple-coloured solution bleaching method was utilized to estimate the electron donor ability of natural products. This method was based on scavenging of DPPH through the addition of a radical species or antioxidant which decolourizes the DPPH solution. The degree of colour change was proportional to the strength and potential of the antioxidants. Results of the present study noted that the plant extract consisted of chemical components which were able of donate hydrogen ion to a free radical to scavenge the potential damage.

The present investigation showed the potential of leaves to scavenge the free radicals and their total antioxidant activity. The literature also revealed that the leaves of *L. innermis* were a good source of antioxidants. It was also observed by various workers on different plants like *Panax quinquefolius* (Kitts *et al.*, 2000), *Psorospermum febrifugum, Myrianthus arboreus* and *Ceratotheca sesamoides* (Konan *et al.*, 2014), *Ephedra alata* (Jaradat *et al.*, 2015), *Terminalia glaucescens* (Olugbami *et al.*, 2015), *Leptadenia hastate* (Dluya *et al.*, 2017), *Astragalus* (Butkut *et al.*, 2018), *Feretia apodanthera* (Owolabi *et al.*, 2018), *Geranium robertianum, Asphodelus microcarpus* and *Alcea setosa* (Alhage and Elbitar, 2019), *Lasiosiphon eriocephalus* (Durgawale *et al.*, 2019) and *Anabasis aretioides* (Senhaji *et al.*, 2020).

Aqueous extracts had a higher potential of scavenging as compared to methanolic extracts as reported by the present research. The similar things were reported in *Barleria noctiflora* (Arumugam *et al.*, 2015), *Mimosa pudica* (Jagetia and Lalhmangaihi, 2018), *Calendula suffruticosa* (Ismahene *et al.*, 2018), *Mimosa pudica* (Jagetia and Lalhmangaihi, 2018), *Mussaenda macrophylla* (Lalremruati *et al.*, 2019) and *Ocimum sanctum* (Garg and Garg, 2019). Senguttuvan *et al.* (2014) investigated the number of different types of active compounds which were present in root and leaf extracts of an important medicinal herb, *Hypochaeris radicata,* and also studied their *in vitro* antioxidant potential.

CONCLUSION

The leaves of *L. inermis* exhibited different levels of antioxidant activity in all the studied extracts. The results from various free radical scavenging systems disclosed that the *L. inermis* had significant antioxidant activity and free radical scavenging activity, with effective scavenging activity against free radicals such as DPPH. Based on these data, free radical scavenging property may be one of the mechanisms by which this drug is useful as a traditional medicine. However, additional studies are needed to characterize the bioactive compounds responsible for the observed antioxidant in *L. inermis* and different antioxidant mechanisms.

REFERENCES

Alhage, J. and Elbitar, H. (2019). *In vitro* screening for antioxidant and antimicrobial properties of three lebanese medicinal plants crude

extracts. *Pharmacognosy Res.* **11** : 127-133.

Arumugam, S., Natesan, S., Ganesan, S. and Kanagarajan, S. (2015). *In vitro* screening of various extracts of *Barleria noctiflora* for their antioxidant and free radical scavenging activity. *Int. J. Pharm. Phytopharm. Res.* **5** : 41-49.

Butkut, B., Dagilyt, A., Benetis, R., Padarauskas, A., Cesevilien, J., Olšauskait, V. and Leme Dien, N. (2018). Mineral and phytochemical profiles and antioxidant activity of herbal material from two temperate *Astragalus* species. *Hindawi Bio. Med. Res. Int.* **5** : 01-11.

Dluya, T., Daniel, D. and Samuel, A. B. (2017). Studies on *in vitro* antioxidant activities of methanol extract of five African traditional plants. *Int. J. Res. Pharmacy and Biosci.* **4** : 28-36.

Durgawale, P. P., Patil, M. N., Joshi, S. A., Korabu, K. S. and Datkhile, K. D. (2019). Studies on phytoconstituents, *in vitro* antioxidant, antibacterial, antiparasitic, antimicrobial and anticancer potential of medicinal plant, *Lasiosiphon eriocephalus* Decne (Family : Thymelaeaceae). *J. Nat. Sci. Biol. Med.* **10** : 38-47.

Garg, P. and Garg, R. (2019). Phytochemical screening and quantitative estimation of total flavonoids of *Ocimum sanctum* in different solvent extracts. *The Pharma Inn. J.* **8** : 16-21.

Ismahene, S., Ratiba, S., Miguel, C. M. D. and Nuria, C. (2018). Phytochemical composition and evaluation of the antioxidant activity of the ethanolic extract of *Calendula suffruticosa* subsp. suffruticosa Vahl. *Pharmacognosy J.* **10** : 64-70.

Jagetia, G. C. and Lalhmangaihi, C. (2018). Phytochemical profiling and antioxidant activity of Lajwanti, *Mimosa pudica* Linn. *In vitro. Int. J. Plant Stud.* **1** : 1-13.

Jaradat, N., Hussen, F. and Ali, A. A. (2015). Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Decne. *J. Materials and Environ. Sci.* **6** : 1771-1778.

Kitts, D. D., Wijewickreme, A. N. and Hu, C. (2000). Antioxidant properties of a North American ginseng extract. *Mol. Cell. Biochem.* **203** : 1-10.

Konan, Y.,Witabouna, K. M., Bassirou, B. and Kagoyire, K. (2014). Antioxidant activity and total phenolic content of nine plants from Côte d'Ivoire (West Africa). *J. Appl. Pharm. Sci.* **4** : 036-041.

Lalremruati, M., Lalmuansangi, C. and Zothan, S. (2019). Free radical scavenging activity and antioxidative potential of various solvent extracts of *Mussaenda macrophylla* Wall : An *in vitro* and *ex vivo* study. *J. Appl. Pharm. Sci.* **9** : 094-102.

Olugbami, J. O., Gbadegesin, M. A. and Odunola, O. A. (2015). *In vitro* free radical scavenging and antioxidant properties of ethanol extract of *Terminalia glaucescens*. *Pharm. Res.* **7** : 49-56.

Owolabi, O. O., James, D. B. and Sani, I. (2018). Phytochemical analysis, antioxidant and anti- inflammatory potential of *Feretia apodanthera* root bark extracts. *BMC Comp. Alter. Med.* **18** : 01-12.

Senguttuvan, J., Paulsamy, S. and Karthika, K. (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. *Asian Pacific J. Tropical Biomedicine* **4** : S359-367.

Senhaji, S. , Lamchouri, F. and Toufik, H. (2020). Phytochemical content, antibacterial and antioxidant potential of endemic plant, *Anabasis aretioïdes* Coss. & Moq. (Chenopodiaceae). *BioMed Res. International* **2020** : 1-16.