Pak. j. sci. ind. res. Ser. B: biol. sci. 2025 **68B**(3) 328-334

# Antioxidant Activities and Phenolic Profile of *Senna alexandrina*

**Syed Mubashar Sabir[a]*, Maria Khalid[a], Muhammad Usman Hameed[a] and Muhammad Zubair Khan[b]**

[a]Department of Chemistry, University of Poonch, Rawalakot, Azad Kashmir, Pakistan
[b]Department of Plant Breeding and Molecular Genetics, University of Poonch, Rawalakot, Azad Kashmir, Pakistan

(received May 24, 2023; revised April 24, 2024; accepted April 30, 2024)

**Abstract.** The purpose of the present study was to evaluate and compare the antioxidant activities of *Senna alexandrina* leaves as well to determine its phenolic profile. The antioxidant activity was analyzed by *in vitro* lipid peroxidation assay, DPPH radical scavenging activity, ABTS assay, antioxidant potential assay and metal chelation assay. The extract showed remarkable scavenging activities (58% in DPPH and 68% in ABTS assay respectively), iron chelation (60%), reduction potential (68%). The extract reduced the lipid peroxidation against $FeSO_4$ (60%) and sodium nitroprusside (50%) in phospholipid homogenate extracted from egg yolk. HPLC analysis revealed the presence of quercetin, pyrogallol and gallic acid in leaves extract. On the basis of these results it is concluded that leaves extract of *S. alexandrina* are rich source of antioxidants and contain important phytochemicals.

**Keywords:** *Senna alexandrina*, DPPH activity, lipid peroxidation, HPLC analysis, metal chelation activity

## Introduction

Different oxidation processes take place in the body which results in the production of free radicals. These radicals cause damaging effect and are source of various other disorders. As these radicals are very reactive, they cause cellular spoliation. The chain reactions caused by these free radicals need to be smashed by antioxidants to avoid complications like oxidative stress and cells' degeneration etc. (Pourmorad *et al*., 2006). Antioxidants are the composites that counteract oxidation when found in restricted volume. The ultimate end of oxidation processes is to produce free radicals which are the main reason for the production of cancer cells. Antioxidants stop oxidation by different processes mainly by eliminating free radicals from the body like superoxide anion, hydrogen peroxide, nitric oxide and hydroxyl radical. Many plant species have shown the antioxidant activities due to phenolics present in extracts (Dhakad *et al*., 2024; Kausar *et al*., 2024; Sabir *et al*., 2024).

The *Senna* belongs to a large class of flowering plants belonging to legume family *Fabaceae* and the subfamily *Caesalpiniaceae.* The family consists of 260 to 350 species of *Senna* are known in farming. This plants kind of are locally known in Libya and middle east countries as Sanna Makki and its use in folk medicine is very common in these countries. The medicinal importance of *Senna* leaves are mainly due to the presence of anthraquinone glycosides, especially sennoside A and B (Najah *et al*., 2019). *Senna alexandrina* mill. or alexandrian senna, a versatile medicinal plant distinguished for its laxative effects (Leng-Peschlow, 1992), is distributed throughout the subtropical and tropical regions of the world (Pakistan, India, Saudi Arabia, Africa, Mexico) (Sãvulescu *et al*., 2018). It is also called *Cassia angustifolia* Vahl., *Cassia acutifolia* Delie., *Cassia senna* L., *Cassia lanceolata* Forssk., *Senna angustifolia* and *Senna acutifolia* (Saheed and Illoh, 2010). It is also cultivated on commercial scale in many countries like India, Sudan, Egypt, Pakistan, China and Korea. It mostly yield best and most valuable variety of the drug is a small shrub which is about 2 feet high. The stem is rigid, flat and pale green. Flowers are small and yellow (Viswanathan and Nallamuthu, 2012). It is distinguished for its laxative effects. Keeping in view the large benefits of the *Senna alexandrina* the overall aim of this study was to analyze the antioxidant activities of *S. alexandrina* and their comparison with standard ascorbic acid. The bioactive compounds of the plant were also characterized by HPLC analysis.

## Materials and methods

**Chemicals.** Gallic acid, Pyrogallol, Caffeic acid and Quercetin, $FeSO_4$, sodium nitroprusside, DPPH, ABTS, ammonium molybdate and 1, 10-phenanthroline were purchased from sigma aldrich. Analytical grade chemicals were used in the study.

*Author for correspondence; E-mail: drmubashar@upr.edu.pk

***Preparation of plant extract.*** Leaves of *S. alexandrina* were purchased from a herbal store from district Rawalakot, Azad Kahmir and identified by a botanist at department of Botany, University of Poonch, Pakistan. Leaves were grounded into fine powder form. A known amount was plant material subjected to hot boiling water (100 mL) for 15 min and filtered. The residue was extracted twice and finally the extract was concentrated in rotary evaporator at low temperature. The serial dilutions were made and the extract was used in different in *vitro* assays.

***DPPH radical scavenging activity.*** The scavenging capacity of DPPH radical by *S. alexandrina* leaves was checked using method (Valko *et al*., 2007). 0.25 mM solution of DPPH radical (0.5 mL) was added into ethanol and aqueous extract solution (1 mL) in concentration from (37.5-300 µg/mL). After shaking the mixture put it into dark for 30 min and then absorbance was checked in spectrophotometer (D-20, Spectronic, West Yorkshire, UK) at 517 nm. The ability to scavenge DPPH radical was determined as:

Scavenging (percent) = [(Ao-A1)/Ao)] × 100

Ao is absorbance of the control and A1 is absorbance of sample.

***ABTS$^{\cdot+}$ assay.*** The ABTS assay was performed by the method (Arnao *et al*., 2001). Aqueous solution of ABTS was prepared by dissolving 80 mg of ABTS in 10 mL distilled water. Then 13.2 mg of potassium per sulphate was dissolved in 10 mL of distilled and mixed with ABTS solution. The solution was kept in dark for 16 h. Afterwards, 1 mL of this solution was taken and diluted with ethanol until the absorbance reached to 0.7. Finally 0.5 mL of ABTS, 1 mL of ethanol and 100 µL of extract was reacted. After 10 min the absorbance was measured spectrophotometrically at 734 nm using ascorbic acid as a standard.

***In vitro lipid peroxidation activity.*** The ability of *S. Alexandrina* leaves to inhibit lipid peroxidation was studied following a method (Khaliq *et al*., 2015). About 1 g of egg yolk was taken, diluted to 100 mL with 100 mm Tris-HCl and its pH was adjusted to 7.4. This solution was further used as a homogenate. After that $FeSO_4$ solution (10 µM) and SNP (5 µM) were added separately to the homogenate. The tubes were incubated at 37 °C in water bath for 1 h. After that 600 µL of TBA and acetic acid were added to all tubes and incubated at 100 °C in water bath (IG-10HL). Finally 2 mL of *n*-butanol was added and tubes were centrifuged (Yongjing, 800). Finally absorbance was measured spectrophotometrically (Halo DB-20) at 532 nm.

***Metal chelating activity.*** The ability of leaves of *S. alexandrina* to chelate iron extract was studied following the method (Sabir *et al*., 2017). To the reaction mixture which contained 150 µL of freshly prepared 2mM $FeSO_4$ solution, 168 µL of 0.1M Tris-HCl solution and 218 ìL of 0.9% NaCl solution and different concentrations of extract. All the test tubes were incubated for 5 min and 13 µL of *o*-phenanthroline was added finally. After that absorbance was measured spectrophotometrically (Halo DB-20) at 510 nm.

***Antioxidant potential assay.*** The reduction potential of the *S. alexandrina* leaves was determined following phosphomolybdenum method (Prieto *et al*., 1999). The procedure basically involved the decrease in oxidation state of molybdenum from VI to V by aqueous extract. At acidic pH, a green coloured complex phosphate/Mo (V) was formed. Firstly the reagent solution was prepared by mixing 0.6 M $H_2SO_4$, 28 mm sodium phosphate and 4 mm ammonium molybdate. Then 0.1 mg/mL of the extract was added to 3 mL of the previously prepared solution. For about 90 min all the tubes were subjected to incubation at 95 °C. After that absorbance was measured spectrophotometrically at 695 nm.

***HPLC analysis of phenolics and flavonoids.*** Infusion of *S. alexandrina* were made from leaves in dried form. Filtration was done using 0.45 µm membrane filter. Qualitative as well as quantitative analysis was carried out through HPLC system (Shimadzu) set at an ambient temperature and all the functions operated in triplicate. The system was accoutered with interchanging pumps of Shimadzu LC 20 AT. A CBM 20A integrator was used to connect these pumps with a DGU 20A5 degasser. Furthermore DAD SPD-M20A detector of UV-visible range was equipped within system which ultimately ended at software LC solution 1.22 SP1. Isocractic elution system was maintained and the technique of reverse phase chromatography was used. A $C_{18}$ column was used for separation. The mobile phase was composed of 15% acetonitrile, 45% water and 40% methanol was used for the qualitative analysis of phenolics. Acetic acid (1%) also contributed to the composition of mobile phase. Before use, the mobile phase was filtered and using ultrasonic bath, degassed thoroughly. External standards were used to integrate the obtained peaks for quantitative analysis. Aqueous extract of *S. alexandrina*

was injected maintaining 10 µL volumes with a flow rate of 1 mL/min. Retention times and obtained spectra at 257 nm were compared with those of reference standards for interpretation. The compounds were identified on the basis of their retention times matched with external standards.

***Statistical analysis.*** Results were expressed as means of three determinations and significance of data was checked by ONE WAY ANOVA followed by Duncan Multiple range test (DMRT). $P < 0.05$ was considered significant in all data.

## Results and Discussion

**Scavenging activity by DPPH method.** DPPH radical is the most frequently used radical to estimate the radical scavenging ability of many plant extracts (Hatano *et al*., 1989). The colour of DPPH disappeared with the continuing addition of the extract. It was observed that the colour of control remained violet till the end of experiment, while those containing extract turned into pale to colourless. This was due to the reduction of DPPH radicals as these were neutralized by the hydroxyl group of anti oxidants. It is a well known fact that plant extracts contain phenol containing compounds and flavonoids which neutralize DPPH radicals (Ozcan and Arslan, 2011). Figure 1 show that the scavenging percentage as obtained by extract was slightly less than that of standard ascorbic acid. However, leaves extract showed significantly higher antioxidant activities. Table 1 showed that there is significant difference ($P<0.005$) among different tested concentrations for DPPH activities. The highest percentage scavenging was found 58% which is less compared to ascorbic acid (80%). Setiawan *et al*. (2015) reported the DPPH radical scavenging activity of *S. alexandrina* which showed $IC_{50}$ value of 142 µg/mL.

***Scavenging activity by ABTS$^{\cdot+}$ method.*** Blue-green coloured $ABTS^{\cdot+}$ is produced when ABTS reacts with potassium/sulphate. The colour of $ABTS^{\cdot+}$ gradually fades and ultimately it becomes colourless with the increasing concentration of antioxidants from extract (Shi *et al*., 2011). As duration of reaction between $ABTS^{\cdot+}$ and antioxidants of extract was increased, it resulted in more scavenging of $ABTS^{\cdot+}$ which resulted in increased antioxidant activity. This is probably due to the donation of hydrogen from antioxidants of *S. alexandrina*. Figure 2 shows the percent scavenging activities of extracts and ascorbic acid. The highest percentage scavenging *i.e*. 68% was shown by the concentration of extract (300 µg/mL) and it was comparable with that of ascorbic acid (72%). Table 1 showed that there is significant difference ($P<0.005$) among different tested concentrations for ABTS activities. Flavonoids and phenolic compounds present in different plants are the chief factor for their antioxidant potential (Ara *et al*., 2020).

***Lipid peroxidation activity of extract.*** The purpose of this *in vitro* experiment was to study the effects of

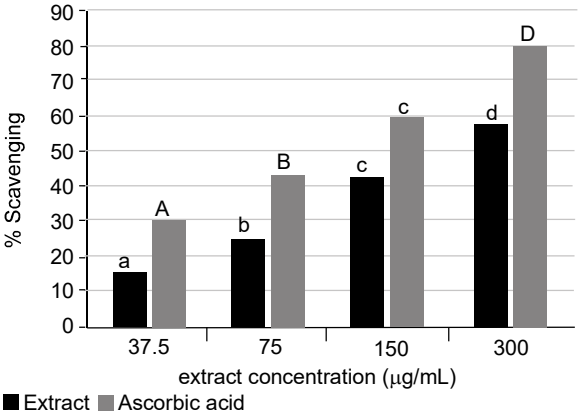


**Fig.1.** DPPH scavenging abilities of *S. alexandina* leaves extract with standard ascorbic acid. The tested concentrations which share different letters are significantly ($P<0.05$) different from each other by DMRT.

**Table 1.** Analysis of variance (ANOVA) showing the significance of various antioxidant parameters

| Sample | SS | DF | MS | F | P |
|---|---|---|---|---|---|
| DPPH radical scavenging activities | 2242.38. | 3 | 747.4 | 1993 | 0.0000 |
| ABTS radical scavenging activities of plants | 3127.38 | 3 | 1042.46 | 81 | 0.0005 |
| Metal chelation activities of plants | 1718 | 3 | 572.6 | 1145 | 0.0000 |
| Lipid peroxidation activities of plant against SNP | 2165.50 | 3 | 721.8 | 1444 | 0.0000 |
| Lipid peroxidation activities of plant against $FeSO_4$ | 2769.38 | 3 | 923.1 | 2462 | 0.0000 |
| Reducing activities of plant by phosphomolybenum assay | 3449.50 | 3 | 1149.38 | 2300 | 0.0000 |

SS = sum of squares; MS = mean of the sum of squares; Df = degrees of freedom.

*S. alexandrina* leaves extract on lipid peroxidation. In the egg yolk extracted phospholipid lipid peroxidation was induced by pro-oxidant agents *i.e*. $FeSO_4$ and SNP. It was observed that with the increase in concentration of extract, oxidation of lipids was significantly reduced. The enhanced antioxidant activity was shown against $FeSO_4$ compared to SNP. Iron results in the generation of peroxyl and alkoxyl radicals, which propagates lipid peroxidation (Zago *et al*., 2000). Figure 3 shows the corresponding percent inhibition of extract and standard ascorbic acid. Leaves extract showed 50% inhibition against lipid peroxidation in case of SNP which was significantly less than those observed against iron (60%). Table 1 showed that there is significant difference ($P<0.005$) among different tested concentrations for lipid peroxidation activities. These results are comparable to standard ascorbic acid (70%). These results are in agreement to the studies of Nayan *et al*., (2021) where the leaf powder of *S. alexandrina* prevented the lipid peroxidation *in vivo*.

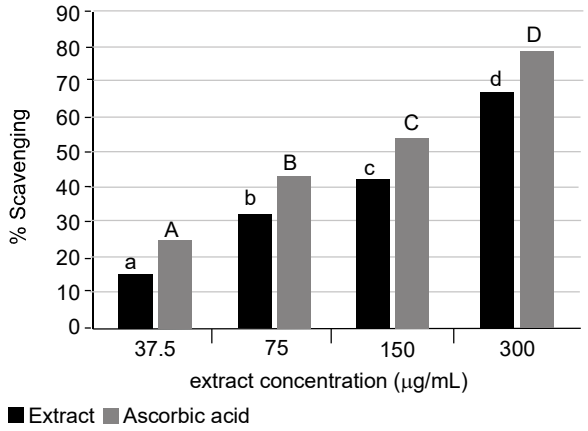


**Fig. 2.** ABTS scavenging of *S. alexandina* leaves and standard. The tested concentrations which share different letters are significantly ($P<0.05$) different from each other by DMRT.

**Metal chelating activity of extract.** When $Fe^{2+}$ reacts with 1,10-phenanthroline, a rust coloured complex is formed. Addition of extract of natural products to this complex fades its colour and it becomes colourless. This disappearance of colour is the main evidence of the chelating capacity of plant extracts (Tahseen *et al*., 2015). It was hence concluded that by increasing concentration of extract, chelating activity was also increased. At maximum concentration of extract *i.e*. 250 µg/mL, highest chelating activity was observed by leaves extract 60% and it was comparable with that of ascorbic acid *i.e*. 78% (Fig. 4). Table 1 showed that there is significant difference ($P<0.005$) among different tested concentrations for metal chelation activities.

**Reduction potential by phosphomolybdenum assay.** The reduction potential of plant extracts as evaluated by the phosphomolybdenum assay involves the

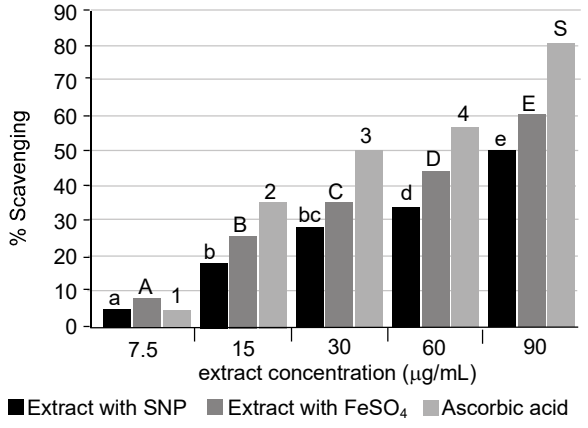


**Fig. 3.** Percentage inhibition of lipid peroxidation of *S. alexandina* leaves extract induced by iron and sodium nitroprusside (SNP). The tested concentrations which share different letters are significantly ($P<0.05$) different from each other by DMRT.

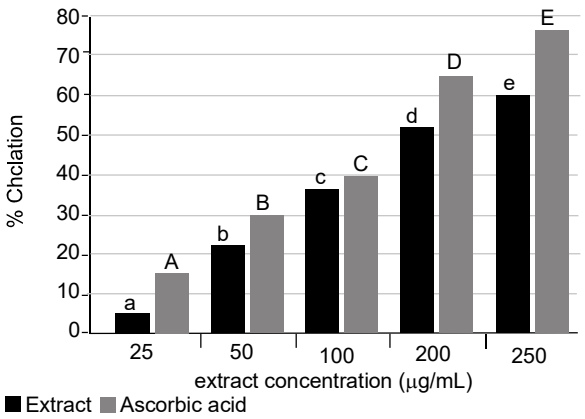


**Fig. 4.** Metal chelating ability of *S. alexandina* leaves. The tested concentrations which share different letters are significantly ($P<0.05$) different from each other by DMRT.

formation of a green coloured complex as a result of decrease in oxidation state from 6 to 5 (Ara *et al*., 2020). A direct estimate of reduction potential of *S. alexandrina* leaves extract was obtained by measuring absorbance at 695 nm. Table 1 showed that there is significant difference (P<0.005) among different tested concentrations for reducing activities. Figure 5 shows that maximum reducing ability was shown by *S. alexandrina* leaves extract *i.e*. 68% at 300 µg/mL concentration which was slightly less than ascorbic acid *i.e*. 72%. The results are in agreement to earlier studies of Eyupoglu *et al*., (2021).

***HPLC analysis*.** HPLC was used to identify the compounds responsible for the antioxidant activity of *S. alexandrina* leaves aqueous extract. A chemical profile was obtained by the aqueous extract of leaves comprising of many phenol containing compounds. By comparison quercetin, pyrogallol and gallic acid were identified in leaves extract (Fig. 6). Pyrogallol and gallic acid was identified as major compounds. The retention times of pyrogallol and gallic acid were 3.659 and 3.245 min respectively. The quercetin showed relatively minor contribution and detected at retention time of 9.270. These compounds are also well recognized for their property to inhibit lipid per oxidation and to chelate toxic metals (Sabir *et al*., 2017). Earlier studies reported luteolin, rutin, syringaldehyde, chlorogenic acid and gentisic acid (Eyupoglu *et al*., 2021) in extracts of *S. alexandrina*.

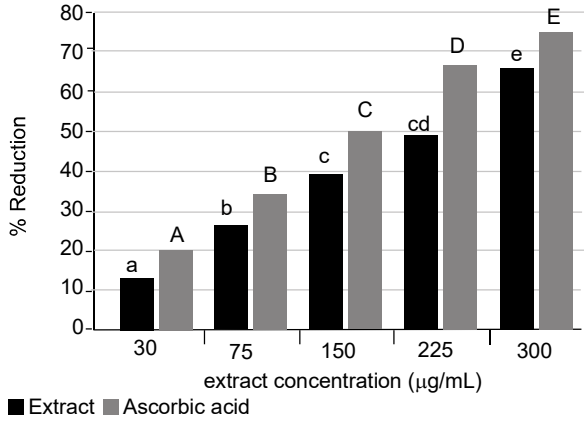


**Fig. 5.** Total antioxidant activity of *S.alexandrina* assessed by phosphomolybdenum assay. Values in figures which share different letters are significantly (P<0.05) different from each other by DMRT.

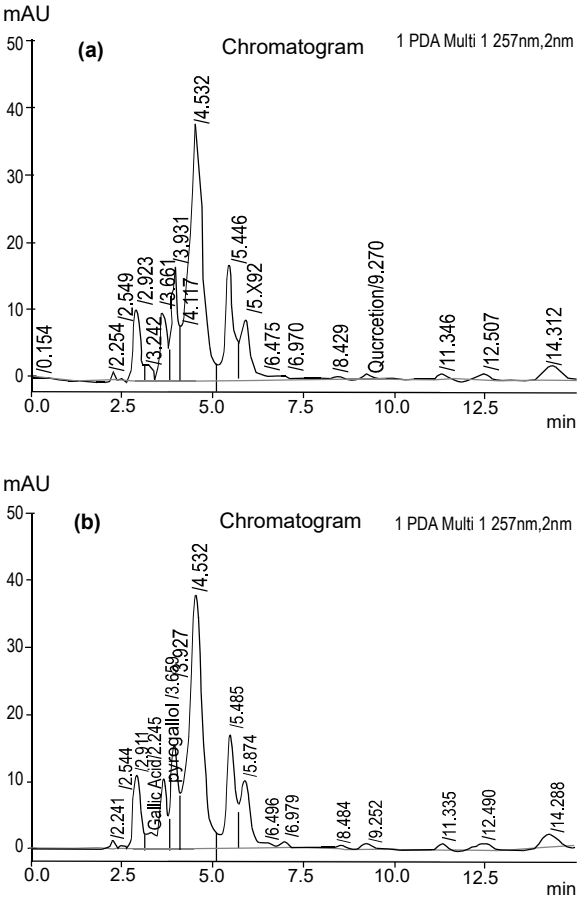


**Fig. 6.** HPLC chromatograph of leaves of *S. alexandrina* aqueous extract. **(a)** chromatogram showing the presence of quercetin **(b)** chromatogram showing the presence of gallic acid and pyrogallol.

## Conclusion

On the basis of above results it is concluded that *S. alexandrina* is potential candidate of antioxidants due to its scavenging activities against free radicals like DPPH and ABTS, metal chelation action, reduction potential and protective effects against lipid peroxidation. These results have shown that *S. alexandrina* antioxidant rich extract could be effectively utilized in food and pharmaceutical industries with additional income.

**Conflict of Interest.** The authors declare that they have no conflict of interest.

## References

Ara, I., Naqvi, S.H., Rehman, N.U., Qureshi, M.M. 2020. Comparative antioxidative and antidiabetic

activities of *Ficus carica* pulp, peel and leaf and their correlation with phytochemical contents. *Journal of Pharmaceutical Research*, **4:** 1-80.

Arnao, M.B., Cano, A., Acosta, M. 2001. The hydrophilic and lipophilic contribution on total antioxidant activity. *Food Chemistry*, **73:** 239-244.

Dhakad, A.K., Singh, K., Oberoi, H.K., Kumar, V., Shah, J.N. 2024. Proximate composition, mineral profiling and antioxidant potential in *Moringa oleifera* genotypes affected with leaf maturity stage. *South African Journal of Botany*, **168:** 227-235.

Eyupoglu, O.E., Etienne, O.K., Sinan, K.I., Mahomoodally, M.F., Jugreet, B.S. 2021. Biological properties and HPLC analyses of four medicinal plants extracts obtained at different extraction temperatures. *Journal of Food Processing and Preservation*, **46:** 2-14.

Khaliq, A., Sabir, S.M., Ahmad, S.D., Boligon, A.A., Athayde, M.L., Jabbar A., Qamar, I., Khan, A. 2015. Antioxidant activities and phenolic composition of olives (*Olea europaea*) leaves. *Journal of Applied Botany and Food Quality*, **88:** 16-21.

Kusar, S., Saddiqe, Z., Ali, F., Bashir, S., Zubairi, T. 2024. GCMS and HPLC profiling, antioxidant and anti-inflammatory activities of *Crotalaria medicaginea* Lamk. *South African Journal of Botany*, **168:** 196-208.

Leng-Peschlow, E. 1992. Senna and its rational use: classification of senna as a laxative. *Pharmacology*, **44:** 6-9.

Najah, Z., Awad M., Alshawish M. 2019. Phytochemical screening, analytical profile and green synthesis of *Senna Alexadrina* mill. *European Journal of Biomedical and Pharmaceutical Sciences*, **6:** 47-53.

Nayan, S.I., Chowdhury, F.I., Akter, N., Rahman, M.M., Selim, S., Saffoon, N., Khan, F. 2021. Leaf powder supplementation of *Senna alexandrina* ameliorates oxidative stress, inflammation and hepatic steatosis in high-fat diet-fed obese rats. *Plos One*, 1-25. https: //doi.org/10.1371/journal.pone.0250261

Özcan, M.M., Arslan, D. 2011. Antioxidant effect of essential oils of rosemary, clove and cinnamon on hazelnut and poppy oils. *Food Chemistry*, **129:** 171-174.

Pourmorad, F., Hosseinimehr, S.H., Shahabimajd, N. 2006. Antioxidant activity, phenols, flavonoids contents of selected Iranian plants. *Journal of Biotechnology*, **5:** 1142-1145.

Prieto, P., Pineda, M., Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, **269:** 337-341.

Puntel, R.L., Nogueira, C.W., Rocha, J.B.T. 2005. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *in vitro*. *Neurochemical Research*, **30:** 25-235.

Sabir, S.M., Zeb, A., Awan, N.S., Iqbal, N., Abbas, S.R. 2024. Antioxidant activities, xathine oxidase inhibitory effect and phenolic profile of wild straw berry (*Fragaria nubicola*) from Pakistan. *Latin American Journal of Pharmacy*, **43:** 475-479.

Sabir, S.M., Athayde, M.L., Boligon, A.A., Rocha, J.B.T. 2017. Antioxidant activities and phenolic profile of Baccharis trimera, a commonly used medicinal plant from Brazil. *South African Journal of Botany*, **113:** 318-123.

Saheed, S.A., Illoh, H.C. 2010. A taxonomic study of some species in Cassiinae (*Leguminosae*) using leaf epidermal characters. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **38:** 21-27.

Sãvulescu, E., Georgescu, M.I., Popa, V., Luchian, V. 2018. Morphological and anatomical properties of the *Senna Alexandrina* mill (*Cassia Angustifolia* Vahl.). *Sciendo*, 305-310. DOI: 10.2478/alife-2018-0045

Setiawan, F., Adnyanai, K, Sukandar, E.Y., Yuniarto, A., Pebritrinasari, V. 2015. Pharmacological screening for potency of antiobesity of five plants based on ethnopharmacological use. *Asian Journal of Pharmaceutical and Clinical Research*, **8:** 299-301.

Shi, Y.X., Xu, Y.K., Hu, H.B., Na, Z., Wang, W.H. 2011. Preliminary assessment of antioxidant activity of young edible leaves of seven *Ficus* species in the ethnic diet in Xishuangbanna, Southwest China. *Food Chemistry*, **128:** 889-894. DOI: 10.1016.

Tahseen, G., Nouman, A., Zahid, M., Saiqa, A. 2015. Comparative study of antioxidant, metal chelating and antiglycation activities of *Momordica charantia* flesh and pulp fractions. *Pakistan Journal of*

*Pharmaceutical Sciences*, **28:** 1217-1223.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., Tesler, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, **39:** 44-84.

Viswanathan, S., Nallamutu, T. 2012. Phytochemical screening and antimicrobial activity of leaf extracts of *Senna alexandrina* Mill. against human pathogens. *International Journal of Current Science*, **2:** 51-56.

Zago, M.P., Verstraeten, S.V., Oteiza, P.I. 2000. Zinc in the prevention of $Fe^{2+}$ initiated lipid and protein oxidation. *Biological Research*, **33:** 143-150.