Phytochemical Screening, Proximate Analysis and Antioxidant Activity of *Dracaena reflexa* Lam. Leaves

ABHA SHUKLA*, SWATI VATS AND R. K. SHUKLA1
Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar-249 407, 1Department of Chemistry, Gurukula Kangri Vishwavidyalaya, Haridwar-249 404, India

Shukla, et al.: Phytochemical and Antioxidant Evaluation of *Dracaena reflexa* leaves

In the present study, the antioxidant activity of successive leaf extracts of *Dracaena reflexa* was investigated using the scavenging activity on 1,1-diphenyl-2-picrylhydrazyl and reducing power by ferric reducing antioxidant power assay. Methanol extract was found potent in both the assays. IC50 values of 1,1-diphenyl-2-picrylhydrazyl assay for methanol extract was 0.97 mg/ml and ferric reducing antioxidant power value for the same is 1.19. Phytochemical screening, proximate analysis and total phenolic content were also determined. Qualitative screening for phytochemical showed the presence of alkaloids, flavonoids, terpenoids, glycosides and saponins. Highest phenolic content was shown by methanol extract (49.69 mg gallic acid equivalent/g dry weight). Proximate analysis showed moisture content (3.31%), ash content (8.02%), crude fibre (1.31%), crude fat (0.97%), total protein (3.70%), total carbohydrate (86.01) and nutritive value (367.56 kcal/100 g), which would make it a potential nutraceutical. This study suggested that *Dracaena reflexa*, a potential natural free radical scavenger, which could find use as an antioxidative.

Key words: *Dracaena reflexa*, phytochemical, proximate, total phenolic content, antioxidant activity

Herbal medicine plays vital role in maintaining the health and wealth of mankind. Majority of world population use herbal medicines. The World Health Organization (WHO) reports that approximately 21,000 plants have been used for medicinal purposes[1]. Herbs have stood the test of time for their safety, efficacy, cultural acceptability and minimal side
effects[3]. Therapeutic power of some plants is mainly
due to the presence of some secondary metabolites,
which collectively are referred to as phytochemicals[3].
These phytochemicals have potential to be developed
as herbal medicines or could serve as precursors for
modern medicine.

It is now widely understood that free radicals are
involved in the pathogenesis of many diseases.
Natural defence mechanisms of the human body
prevents development of these diseases but in cases
of increased onslaught by free radicals and reactive
oxygen species bodys defence mechanisms needs to
be supplemented by external antioxidants. Synthetic
antioxidants are less favoured due to their side
effects, hence interest in natural antioxidants of herbal
origin is on the increase[4]. Plant-derived phenolic
compounds and flavonoids have been reported to be
radical scavengers and act as good antioxidant agents.

This study was performed on leaves of Dracaena
reflexa, family, Liliaceae. Certain species of Dracaena
such as D. cinnabari stem have been investigated for
in vitro lipid peroxidation[5], antioxidant activity[6],
antinflammatory activity[7], antimicrobial and
cytotoxicity[8]. Investigations on D. draco revealed
antimicrobial and antioxidant effect[6], cytotoxic
effect and presence of many phenolic compounds[9].
Another species D. cambodiana showed antitumour[10],
antioxidant[11] and antimicrobial activity[12]. Similarly
D. cochinchinensis, D. angustifolia, D. arborea and
D. vand were examined by different researchers
for medicinal potential. Present study focused on
phytochemical screening, proximate analysis, nutritive
value and antioxidant activity of leaves of D. reflexa.

Folin Ciocalteu reagent, methanol, sodium acetate and
glacial acetic acid were procured from Merck India,
Mumbai, sodium carbonate was obtained from Thomas
Baker, Mumbai, gallic acid from Hi-Media, Mumbai,
1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,4,6-tri-2-
pyridyl-1,3,5-triazine (TPTZ) were obtained from Sigma
Aldrich, St. Louis, USA, hydrochloric acid from Fischer
Scientific, Mumbai and ferric chloride hexa hydrate
from S. D. Fine Chem Ltd., Mumbai, India. All other
chemicals employed were of standard analytical grade.

Fresh leaves of D. reflexa were collected from
Dehradun district of Uttarakhand, India. The plant
material was authenticated by the Botanical Survey
of India, Dehradun. A herbarium sample (accession
No. 114095) was also preserved in the Department
of Chemistry, Kanya Gurukula Campus, Gurukula
Kangri Vishwavidyalaya, Haridwar, India for further
reference. Fresh leaves were washed with water, dried
under the shade for 15 days, crushed in a grinder to
powder form and then stored in an air tight container
for further extraction and various processes.

Proximate analysis of the powdered leaves included
estimation of moisture content, ash content, crude
fibre, crude fat and protein content[13], whereas total
carbohydrate was calculated using the Eqn., total
carbohydrate=100−(% ash+% moisture+% crude
fibre+% crude protein). Nutritive value of the leaf
was expressed in kilocalories/100 g of dry weight of
leaves, which was calculated using the formula[14],
nutritive value=(4×% protein)+(9×% crude fat)+(4×%
total carbohydrate).

One hundred and fifty grams of the dried powdered
leaves of D. reflexa were weighed, loaded and
extracted in a soxhlet apparatus using 1.5 l each of
petroleum ether, dichloromethane, methanol and water
successively in accordance to the hierarchy of polarity
of solvents. Extraction was continued for 72 h or
until the solvent coming out of the siphoning tube
was colourless[15]. Extracts were concentrated under
reduced pressure in rotary vacuum evaporator and
refrigerated for further use.

Phytochemical analysis of the extracts was performed
using standard qualitative methods[16,17]. The extracts
were analysed for the presence of compounds like
alkaloids, flavonoids, tannins, glycosides, terpenoids,
steroids, fat and oil, saponins, protein. Total phenol
content (TPC) of Dracaena reflexa leaf extract was
measured by employed the method of Singleton[18].
To 1 ml of extract (1000 μg/ml) in methanol, 10 ml
of 10% Folin Ciocalteu reagent and 8 ml of sodium
carbonate (7.5% w/v) solution was added and the
reaction volume was made up to 20 ml with distilled
water. After 2 h of incubation at room temperature,
absorbance was measured at 765 nm on a UV/Vis
spectrophotometer. Total phenolics were quantified
by calibration curve of gallic acid (25-300 μg/ml).
The phenolic content of the sample was expressed
as milligram gallic acid equivalents/gram of dry
extract (mg GAE/g), which was calculated using the
formula[19], T=C×V/M, where, T is the TPC (mg/g
of plant extract in GAE), C is the concentration of gallic
acid from the calibration curve, V is the volume of...
the extract in ml and M is the weight of the pure plant extracts.

The free radical scavenging activity of the plant extract was determined using Brand and William method with a slight modification\(^{20}\). Reaction mixture contained 1 ml of extract of different concentrations (0.1 to 4 mg/ml) and 3 ml of working DPPH in methanol (0.004%). After 30 min of incubation at room temperature in a dark place, the absorbance was measured at 517 nm against methanol as blank on a UV/Vis spectrophotometer. Methanol with DPPH solution was used as control. Percent inhibition of free radical DPPH was calculated as per the formula, 

\[
\% \text{ inhibition} = \left( \frac{\text{absorbance of the control} - \text{absorbance of sample}}{\text{absorbance of control}} \right) \times 100
\]

Results were expressed as IC\(_50\), the concentration producing 50% inhibition, which was obtained from the graph plotted between concentrations versus % inhibition.

Ferric reducing antioxidant power (FRAP) value was calculated using the method of Benzie and Strain\(^{21}\), which was based on reduction of Fe\(^{3+}\)-TPTZ to Fe\(^{2+}\)-TPTZ. The FRAP reagent was prepared mixing 300 mM acetate buffer (pH=3.6), 10 mM TPTZ and 200 mM FeCl\(_3\), 6H\(_2\)O in a ratio of 10:1:1 at 37\(^o\). The reaction mixture contained 1 ml of extract (1500 μg/ml) and 10 ml of working FRAP reagent. The mixture was incubated at 37° for 30 min. The antioxidant potential of samples was determined from standard curve plotted using ascorbic acid as standard)×100. Results were expressed in μM/ml and FRAP value of sample is calculated using the formula, FRAP=(absorbance of sample/absorbance of control)×100. Results were expressed as IC\(_50\), the concentration producing 50 % inhibition, which was obtained from the graph plotted between concentrations versus % inhibition.

Petroleum ether, dichloromethane, methanol and aqueous extracts were prepared to perform phytochemical screening, proximate analysis, total phenolic content and antioxidant activity. Results of proximate analysis are demonstrated in Table 1. The results revealed that leaves of *Dracaena reflexa* are a good source of mineral elements as they contained a high percentage of ash. Moisture content is in the range of 5-15% indicated that leaves are good for formulating because low moisture content prevented microbial growth\(^{22}\). Leaves also appeared to be a good supplement for protein, carbohydrate and fat, which are the major building blocks of nutrition\(^{23}\). Leaves also could provide high energy content as the nutritive value is much higher. Collectively proximate analysis showed that leaves of *Dracaena reflexa* have the potential to be a food supplement, energy drink and a neutraceutical.

Phytochemicals present in leaves of *D. reflexa* were shown in Table 2. Leaves of *D. reflexa* were found to be a rich source of alkaloids, flavonoids, saponins, glycosides, carbohydrate, amino acids, terpenoids, tannins, steroids, triterpenoids, fat and oil. Some of the major constituents like alkaloids contribute towards analgesic and antimicrobial activity\(^{24}\). Flavonoids and tannins are major groups which act as antioxidant. Tannins also reported to have antibacterial properties\(^{25}\). Glycosides are likely to possess cardiac activities and might be useful in treating congestive heart failure and cardiac arrhythmia\(^{26}\). Saponins are likely to demonstrate antibacterial, antiinflammatory, anticancer, and antiidiabetic activities. Terpenoids have been reported to be antibacterial in nature\(^{27}\). Presence of all these phytochemical in leaves might contribute towards the therapeutic potential of *D. reflexa*.

TPC gives a measure of to what extent phenolic compounds are present in the extract. In plants usually tannins, flavonoids, phenolic acids and phenol diterpenes contributes to TPC. Phenol group offers potent antioxidant activity due to its redox potential, which plays an important role in scavenging free radicals, in neutralizing the reactive oxygen species or act as an electron donor to peroxide radical\(^{28}\). As the *D. reflexa* leaf methanol extract showed higher TPC, it is likely to be a potential antioxidant. TPC content of the extracts are summarized in Table 3.

Plants with potent antioxidant properties are reported to exhibit freeradical scavenging activity. DPPH is a stable free radical, which gives purple colour in methanol solutions. This purple colour changes to

### TABLE 1: PROXIMATE COMPOSITION OF DRIED LEAVES OF DRACAENA REFLEXA

| Parameter         | Dracaena leaves (percentage dry weight basis) | Mean±SD  |
|-------------------|---------------------------------------------|----------|
| Moisture content  | 3.31±1.15                                   |          |
| Ash content       | 8.02±0.25                                   |          |
| Crude fibre       | 1.31±0.13                                   |          |
| Crude fat         | 0.97±0.27                                   |          |
| Total protein     | 3.70±0.39                                   |          |
| Total carbohydrate| 86.01                                       |          |
| Nutritive value*  | 367.56                                      |          |

Values are expressed as mean±SD of the three replicates. *Nutritive value is calculated in kcal/100 g dry weight of leaves. SD: Standard deviation
TABLE 2: PHYTOCHEMICAL CONSTITUENTS IN DRACAENA REFLEXA LEAF EXTRACTS

| Phytochemical constituents | Test performed | Petroleum ether | Dichloromethane | Methanol | Water |
|----------------------------|----------------|----------------|----------------|----------|-------|
| Alkaloids                  | Wagner’s test  | −              | −              | +        | +     |
|                            | Hager’s test   | −              | −              | +        | +     |
|                            | Dragendorff’s test | −         | −              | +        | +     |
|                            | Mayer’s test   | −              | −              | +        | +     |
| Flavonoids                 | Alkaline test  | −              | +              | +        | +     |
|                            | Lead acetate test | −            | −              | +        | +     |
| Carbohydrates              | Molisch’s test | −              | −              | +        | +     |
|                            | Bendict’s test | −              | −              | +        | +     |
|                            | Barfoed’s test | −              | −              | +        | +     |
|                            | Fehling’s test | −              | −              | +        | +     |
| Tannins                    | Ferric chloride test | −          | −              | +        | −     |
| Glycosides                 | Borntrager’s test | −            | +              | +        | +     |
|                            | Legal’s test   | −              | −              | +        | +     |
|                            | Keller-Killiani test | +          | +              | −        | −     |
| Terpenoids                 | Liebermann burchard test | +        | +              | −        | −     |
|                            | Salwoski test  | +              | +              | −        | −     |
|                            | Salwoski test (triterpenes) | −        | +              | +        | −     |
| Steroids                   | Liebermann burchard test | +        | +              | −        | −     |
| Fat and oil                | Saponification test | +        | +              | −        | −     |
|                            | Filter paper test | +          | +              | −        | −     |
| Saponin                    | Foam test      | −              | −              | +        | +     |
|                            | Froth test     | −              | −              | +        | +     |
| Protein                    | Ninhydrin      | −              | −              | +        | +     |
|                            | Biuret         | −              | −              | +        | +     |

+: Present, −: absent

yellow colour if the DPPH free radical is scavenged by donating either hydrogen radical or any alkyl radical. So plants secondary metabolites which have tendency to donate radical exhibit the good antioxidant power\(^{29}\). The \(D.\) reflexa leaf methanol extract showed very high free radical scavenging property as evidenced from the \(IC_{50}\) values obtained. Here a direct correlation can be demonstrated between the TPC and free radical scavenging property exhibited by the methanol extract graph of % inhibition versus concentrations showing comparison with standard BHT is demonstrated in fig. 1 and \(IC_{50}\) of different extract is listed in Table 4.

FRAP assay mainly measures the reducing potential of an antioxidant reacting with \(Fe^{3+}\) TPTZ and producing a coloured \(Fe^{2+}\) TPTZ complex. The reducing power of any compound or phytochemical is due to its ability to donate hydrogen atom or an electron to the metal atom. FRAP assay treats the antioxidants in a plant sample as a reducing agent in a colorimetric reaction. The reducing power of a plant is not only due to the presence of H atom donating ability but also due to presence of methoxy and keto groups, triterpenes and acid group\(^{30}\). FRAP values are given in Table 5.

TABLE 3: TOTAL PHENOLIC CONTENT OF LEAVES EXTRACTS OF DRACAENA REFLEXA

| Extracts          | Total phenolic content (mg GAE/gdw) | Mean±SD  |
|-------------------|------------------------------------|----------|
| Petroleum ether   | 5.37±0.43                          |          |
| Dichloromethane   | 28.25±0.67                         |          |
| Methanol          | 49.69±0.70                         |          |
| Water             | 10.98±0.80                         |          |

mg GAE/g means milligram of extract equivalent to gallic acid per gram of dry weight of extract. Values are expressed as mean±SD of the three replicates. SD: Standard deviation

TABLE 4: IC\(_{50}\) OF LEAF EXTRACTS OF DRACAENA REFLEXA IN DPPH ASSAY

| Extracts/standard | \(IC_{50}\) values in mg/ml | Mean±SD  |
|-------------------|-----------------------------|----------|
| BHT               | 0.050±0.27                  |          |
| Petroleum ether   | 1.26±0.34                   |          |
| Dichloromethane   | 1.36±0.09                   |          |
| Methanol          | 0.97±1.1                    |          |
| Water             | 2.66±0.03                   |          |

Values are expressed as mean±SD of the three replicates. BHT: Butylated hydroxytoluene, DPPH: 1,1-diphenyl-2-picrylhydrazyl, SD: standard deviation

The results obtained in this study clearly showed that leaf extracts of \(Dracaena\) reflexa possessed antioxidant activity and the results of proximate analysis support the use of the leaves as a food supplement.
TABLE 5: FRAP VALUE FOR LEAF EXTRACTS

| Extracts/standard | Ferric reducing antioxidant power (μM/ml) | FRAP value |
|-------------------|------------------------------------------|------------|
|                   | Mean±SD                                  |            |
| Ascorbic acid     | 489.75±0.45                              | 2.000      |
| Petroleum ether   | 330±0.06                                 | 1.34       |
| Dichloromethane   | 238.75±0.90                              | 0.97       |
| Methanol          | 292.75±1.2                               | 1.19       |
| Water             | 111.75±3.4                               | 0.45       |

Values are expressed as mean±SD of the three replicates. FRAP: ferric reducing antioxidant power; SD: standard deviation.

Fig. 1: DPPH radical scavenging activity of leaf extracts of *Dracaena reflexa*.

DPPH (1,1-diphenyl-2-picrylhydrazyl) assay of *Dracaena reflexa* (+••••) petroleum ether extract, (−■−) dichloromethane extract, (−▲−) methanol extract, (−×−) water extract and (−♦−) BHT.

Financial support and sponsorship:

Nil.

Conflicts of interest:

There are no conflicts of interest.

REFERENCES

1. Cathrine L, Nagarajan NP. Preliminary phytochemical analysis and antibacterial activity of leaf extracts of *Vitis leucocarya*. Int J Curr Pharm Res 2013;3:71-3.

2. Chandan P, Kumar V, Kamthan KP, Singh UB, Srivastava SK, Srivastava RB. Antioxidant and antimicrobial activity of ethanol and water extracts of *Cymbopogon jwarancusa* leaves. J Appl Pharm Sci 2011;1:68-72.

3. Janifer R, Chaurasia AP, Vajpayee PK, Murugan MP, Singh S. Antioxidant activity and phytochemical investigation on a high altitude medicinal plant *Dracocephalum heterophyllum* Benth. Pharmacogn J 2010;2:112-7.

4. Saha MR. *In vitro* free radical scavenging activity of methanol extract of the leaves of *Mimusops elengi* linn. Bangladesh J Vet Med 2008;6:197-202.

5. Machala M, Kubinová R, Horavová P, Suchý V. Chemoautocatalytic potentials of homoisoflavonoids and chalcones of *Dracaena cinnabari*: Modulations of drug-metabolizing enzymes and antioxidant activity. Phytother Res 2001;15:114-8.

6. Gupta D, Bleakley B, Gupta RK. Dragon’s blood: Botany, chemistry and therapeutic uses. J Ethnopharmacol 2008;115:361-80.

7. Ahmed A, Sobarry M, Cherrah Y, Aloua K. Anti-inflammatory and analgesic effects of ethanol extract of *Dracaena cinnabari* Balf. as an endemic plant in Yemen. Int J Pharm Biol Sci 2012;3:96-106.

8. Kumar VP, Chauhan NS, Padhi H, Rajani M. Search for antibacterial and antifungal agents from selected Indian medicinal plants 2006;107:182-8.

9. González AG, Hernández JC, León F, Padrón JI, Estévez F, Quintana J, et al. Steroidal saponins from the bark of *Dracaena draco* and their cytotoxic activities. J Nat Prod 2003;66:793-8.

10. Mei W, Dai H, Wu J, Zhuang L, Hong K. Study on the new use of antitumor of *Dracaena cambodiana*. Zhong Yao Cai 2005;28:871-3.

11. Luo Y, Wang H, Xu X, Mei W, Dai H. Antioxidant phenolic compounds of *Dracaena cambodiana*. Molecules 2010;15:8904-14.

12. Chen HQ, Zao WJ, Wang H, Shen HY, Luo Y, Dai HF, et al. Two new antimicrobial flavanones from dragon’s blood of *Dracaena cambodiana*. J Asian Nat Prod Res 2012;14:436-40.

13. AOAC. The Official Method of Analysis. 15th ed. Washington, DC: Association of Official Analytical Chemists; 1990.

14. Shukla RK, Painuly D, Porval A, Shukla A. Proximate analysis, nutritive value, total phenolic content and antioxidant activity of *Litchi chinensis* Sonn. Nat Prod Ind Indian J 2012;8:361-9.

15. Shukla A, Vats S, Shukla RK. Preliminary phytochemical screening, antibacterial and nitric oxide scavenging activities of *Reinwardtia indica* leaves extract. Int J PharmTech Res 2013;5:1670-80.

16. Evans WC. *Trease & Evans’ Pharmacognosy*. 16th ed. London: Elsevier Health Sciences; 2009.

17. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 4th ed. New Delhi: Springer; 1998.

18. Singleton VL, Orthofer R, Rosa RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin ciocalteu reagent. Methods Enzymol 1999;299:152-77.

19. Urzúa A, Rezende MC, Mascayano C, Vásquez L. A structure-activity relationship study of antitumoral flavonoids from Brazilian *Mimusops elengi* L. Phytochemistry 2010;4:118-26.

20. Williams BW, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebenswiss Technol 1995;28:25-30.

21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. Anal Biochem 1996;239:70-6.

22. Shellard EJ. *Exercises in the Evaluation of Drugs and Surgical Dressing*. 1st ed. London: Pitman Medical Publishing Co. Ltd.; 1958.

23. Anyasor GN, Aina DA, Olushola M, Aniyikaye AF. Phytochemical investigation on a high altitude medicinal plant *Dracaena reflexa* seeds. Glob J Pure Appl Sci 2009;15:373-6.

24. Malu SP, Obochi GO, Edem CA, Nyong BE. Effect of methods of extraction on phytochemical constituents and antibacterial properties of *Tetracarpidium conophorum* seeds. Glob J Pure Appl Sci 2009;15:373-6.

25. Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. J Univ Chem Technol Metall 2011;46:481-8.

26. Doss A, Parivuguna V, Vijayasanthi M, Surendran S. Antibacterial activity of leaf extracts of *Dracaena cinnabari* and *Dracaena reflexa* against some microbial pathogens. Indian J Sci Technol 2011;4:450-1.

27. Urzúa A, Rezende MC, Mascayano C, Vásquez L. A structure-activity relationship study of antitumoral flavonoids from Brazilian *Mimusops elengi* L. Phytochemistry 2010;4:118-26.

28. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 2011;5:450-1.

29. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules 2010;15:7313-52.

30. Patt DE, Hudson BJ. Natural antioxidants not exploited commercially. In: Hudson BJ, editor. *Food Antioxidants*. London: Elsevier Applied Science; 1990. p. 171-91.