Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 11, 2014

Original Article

PROXIMATE COMPOSITION, NUTRITIVE VALUE AND EVALUATION OF ANTIOXIDANT POTENTIAL OF STEM OF *DRACAENA REFLEXA* LAM

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Received: 01 Oct 2014 Revised and Accepted: 31 Oct 2014

ABSTRACT

Objective: The stem of *Dracaena reflexa* was analyzed for preliminary phytochemical screening, proximate composition, nutritive value, total phenolic content and antioxidant potential.

Methods: Preliminary phytochemical screening, proximate analysis, nutritive value were evaluated using standard methods. Total phenols were calculated by folin ciocalteu method. The antioxidant activity was performed by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay.

Results: Phytochemical screening revealed the presence of various medicinal active phytoconstituent like alkaloids, flavonoids, glycosides, tannins etc. Total phenolic content was highest for dichloromethane extract (73.48mGAE/g dry weight). Proximate analysis showed content of ash (8.015%), moisture (3.31%), fat (0.97%), fiber (1.31%), protein (3.70%), carbohydrate (86.01%) and nutritive value (367.56 Kcal/100grams of stems). In DPPH assay highest activities shown by dichloromethane extract with IC_{50} values of 0.46 mg/ml and also have FRAP value 2.071.

Conclusion: Overall results revealed that *Dracaena reflexa* stem exhibits excellent nutritive value along with potent antioxidant activity and can be used in medicine as well as in food supplements to prevent the oxidation and rancidity of food.

Keywords: *Dracaena reflexa,* Phytochemical, Proximate composition, Nutritive value, Total phenol, Antioxidant potential.

INTRODUCTION

Nature has been a source of medicinal agents since time immemorial. Plants play a significant role in providing primary health care. They serve as therapeutic agents as well as important raw materials for the manufacturing of traditional and modern medicines as well as in food industries. The revival of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safer and more dependable than the costly synthetic drugs mainly of which have adverse side [1].

The medicinal value of these plants lies in some chemical constituents that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, phenolic compounds etc [2]. Also Proximate and nutrient analyses of plant and vegetables gives the idea of their nutritional significance. If the plant standardise all the parameter of proximate composition then it is quite safe to be use as dietry supplement or as herbal drug [3].

In recent years there is an increasing interest in finding antioxidant phytochemical because they inhibit the propagation of few diseases like atherosclerosis, diabetes, arthritis, cancer, Alzheimer, ageing, neurogenerative diseases, etc [4]. The free radicals are also produced by oxidation of lipids of foods is responsible for the formation of off-flavors and undesirable chemical compounds which may be harmful to health so antioxidants are used by the food industry to delay the oxidation process. Antioxidants act by donating H-atom or by donating electrons to radical oxygenated species. Radicals obtained from antioxidants with molecular structure such as phenol, are stable species and will stop the oxidation chain reactions [5]. Many studies had revealed that phenolic content in plants is mainly responsible to their antioxidant activities. Higher the total phenolic content greater is the antioxidant power. So in this study we coorelate phenolic content and antioxidant potential. In the present study, evaluation of phytochemical screening, proximate composition nutritive value, total phenolic as well as the antioxidant power of *Dracaena reflexa* has been done. Despite of known that wide range of research were carried out on other species of Dracaena genus such as *D. cinnabari* stem was evaluated for in vitro lipid peroxidation [6] antioxidant activity [7], anti-inflammatory activity [8], antimicrobial and cytotoxicity [9]. Many scientific investigations are also recorded on *D. draco,* which includes antimicrobial and antioxidant effect, cytotoxic effects and many phenolic compounds were also isolated from *D. drac*o [10]. Another specie known as *D. cambodiana* were also evaluated for antitumor, [11] antioxidant [12]and antimicrobial activity [13]. respectively. Similarly *D. cochichinensis*, *D. angustifolia, D. arborea*, *D. vand* was examined by different researchers for their medical importance.

Above studies prove the importance of the genus Dracaena but there is no report on antioxidant activity from *Dracaena reflexa*. The present study gives a clue towards the medicinal importance of this plant.

MATERIALS AND METHODS

Chemicals

Folic Ciocalteu reagent (Merck), Sodium carbonate (Thomas Baker), Gallic acid (Hi-Media), 1,1-diphenyl-2-picrylhydrazyl DPPH (Sigma Aldrich), Methanol (Merck), Ascorbic acid (Rankem, India), 2,4,6-tri-2-pyridyl-1,3,5-triazine TPTZ (Sigma Aldrich), Sodium acetate (Merck), Glacial acetic acid GAA (Merck), Hydrochloric acid HCl (Fischer Scientific), Ferric Chloride Hexhydrate (SDFCL, Mumbai, India). All other chemicals employed were of standard analytical grade.

Plant material

Fresh stems of *D. reflexa* were collected from Maheshwari nursery, Dehradun, Uttarakhand, India in the month of March, 2012. Authenticated by Botanical Survey of India (B. S. I) Dehradun. A herbarium (accession no- 114095) was also procured in the Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar, (India) for future reference. The fresh stems were washed with water and then dried under the shade for about 15 days and then crushed with the help of grinder to powder form then stored in airtight container for further extraction and various processes.

Proximate composition

Proximate analysis of the powdered stem includes estimation of moisture content, ash content, crude fiber, crude fat, protein content [14], whereas total carbohydrate was calculated by the following equation-

Total carbohydrate = 100 - (% Ash+ % Moisture + % Crude fibre+ % Crude protein)

Nutritive value

Nutritive value of stem was expressed in Kilocalories/ 100 gram of dry weight of stems and calculated by using the given formula [15]

Nutritive value = (4× % Protein) + (9× % Crude fat) + (4× % Total carbohydrate)

Preparation of extracts

150 grams of the dried powdered stem of *D. reflexa* were weighed, loaded and extracted by soxhlet apparatus using 1.3 liters each of petroleum ether, dichloromethane, methanol and water respectively in increasing order of polarity. Extraction was continued for about 72 hours or until the solvent coming out of the siphoning tube become colorless [16]. Extracts were concentrated under reduced pressure with the help of rotary vacuum evaporator and refrigerated for further use.

Phytochemical screening

Phytochemical analysis for various phytoconstituent of the extracts was undertaken using standard qualitative methods [17,18]. The extracts were screened for the presence of biologically active compounds like alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids, fat and oil, saponins, protein etc.

Total phenolic content

The total phenolic content of obtaining extracts of stems of *D. reflexa* was determined by using Folin Ciocalteu reagent [19] with some little modifications. Phenolic content of the sample reduces the molybdenum metal and changes the yellow color to prussian blue. The intensity of blue color is directly proportional to the phenolic content. The extracts were diluted with methanol to form a concentration of 1000 µg/ml and also standard solution of Gallic acid is made of concentration range of 25µg/ml to 300 µg /ml for plotting the calibration curve. 1 ml of extracts was added to 10 ml of 10 % Folin Ciocalteu reagent. Reaction to this mixture, add 8 ml of 7.5 % sodium carbonate after 8 minutes. Further, total volume is made up to 20 ml by adding distilled water.

The complete reaction mixture was incubated for about 2 hours in the dark and at room temperature of about 25°C±2. The same reaction mixture was set up with gallic acid standard dilutions and also with blank where methanol is taken in place of extract. After incubation, the absorbance was measured at 765 nm with UV-VIS spectrophotometer (Systronic, Visible Spectro-105). Calculations were made using a calibration curve of gallic acid. The total phenolic content of extracts was expressed as mg gallic acid equivalents (GAE) / gram of dry mass by following equation [20].

$T = C \times V/M$

Where, T= Total phenolic content mg/gm of plant extract in GAE,

C= Concentration of Gallic acid from the calibration curve,

V = Volume of the extract in ml,

M =Weight of the plant extracts.

DPPH free radical scavenging assay

The free radical scavenging power of all the obtained extracts of stem of *D. reflexa* were evaluated by stable DPPH free radical scavenging assay with little modification [21]. DPPH is a free radical of violet color. The antioxidants in the sample scavenge the free radical and turn it into yellow in color. The change in color from violet to yellow is proportional to radical scavenging activity of the sample.

A working solution of 0.004% DPPH was freshly prepared in methanol. 1 ml of sample or standard dilutions of different concentrations (0.5 to 3.5 mg/ml) added to 3 ml of working solution of DPPH. After 30 minutes incubation in dark at room temperature 25°C±2. The absorbance of the reaction mixture was taken at 517 nm with UV-VIS Spectrophotometer (Systronic VIS-105) which was compared with the corresponding absorbance of a same dilution range of standard 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT). 1 ml of methanol with 3 ml of working DPPH solution serves as control. The % radical scavenging activity or % inhibition was calculated by-

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} of all the extracts and standard BHT were calculated by graphical method by plotting % inhibition versus concentrations. IC_{50} is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system. Results were expressed in terms of IC_{50}.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed on the given extracts by the method with the little modification [22]. The stock solution includes 300 mM acetate buffer (pH-3.6), 10 mM TPTZ solution (in 40 mM HCl) and 20 mM $FeCl_3.6H_2O$ solution. The working FRAP reagent was freshly prepared by mixing of above reagents in a ratio of 10:1:1 (v/v) respectively and then warmed at 37°C±2 before using it. Antioxidant potential was determined by a reaction mixture which consists of 1 ml of extract (1500µg/ml) and 10 ml of working FRAP reagent. This reaction mixture is kept for 30 minutes incubation in BOD incubator at 37°C±2. Absorbance was taken in U. V spectrophotometer at 593 nm. The ascorbic acid standard solution was tested in a similar way. The standard curve was plotted between (100µM-600 µM) ascorbic acid. 1 ml of methanol in 10 ml of working FRAP reagent act as control and working FRAP reagent serves as blank. Calculations were made by calibration curve. Results were expressed in µM/ml and FRAP value of the sample is calculated by [15]

$$\text{FRAP Value} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

Statistical analysis

The experimental results are expressed as mean ± standard deviation of triplicate measurement and the results are processed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Petroleum ether, dichloromethane, methanol and aqueous extracts were prepared to examine phytochemical screening, proximate analysis, nutritive value, total phenolic content and antioxidant potential.

Extractive yield

The yield of different extracts obtained was measured and reported in Table 1. The highest yield of solid residue was obtained of aqueous extract.

Table 1: Extractive yield of different extracts *Dracaena reflexa* stem.

Extracts	Appearance	Consistency	% Yield (w/w)
Petroleum ether	Yellow	Waxy	0.707
Dichloromethane	Dark Green	Amorphous powder	0.761
Methanol	Brown	Sticky semi solid	8.09
Water	Brown	Crystalline solid	12.09

Phytochemical screening

The preliminary phytochemical screening revealed the presence of medicinally active phytoconstituents. The phytochemicals of the stem extracts of *D. reflexa* are summarized in Table.2. Almost all active phytochemicals found present. Out of which alkaloids are one of the largest group of phytochemical which led to the invention of pain killer and also responsible for antiprotozoal, cytotoxic and antimicrobial properties[23].

Flavonoids and tannins are the constituents mainly consists of phenolic group and these can act as primary antioxidants and antioxidant activity is related to many biological activities such as antibacterial, anti-inflammatory, antiallergic, anticancer, antineoplastic activity and for the treatment of intestinal disorder[24]. Glycosides were also found present and these are important class which are useful against congestive heart failure, cardiac arrhythmia and other heart related diseases[25]. Other class of glycosides are steroids and terpenoids showed analgesic and anti-inflammatory activities. Saponins which are a major class of phytochemicals, can act as anticancer, antidiabetic, hypocholestromic, antibacterial, etc [26].

From above discussion, we can interpret that the presence of these phytochemicals in successive extracts shows medicinal importance of stem of *D. reflexa*.

Table 2: Phytochemical constituents in *Dracaena reflexa* stem extracts.

Phyto constituents	Test performed	Extracts			
		Petroleum ether	Dichloro methane	Methanol	Water
Alkaloids	Wagner's test	-	-	-	-
	Hager's test	-	-	+	+
	Dragendroff'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	+	+	-	-
	Legals test	+	-	-	-
	Keller Killiani test	_	+	-	-
Terpenoids	Liebermann burchard test	-	-	-	-
	Salwoski test	+	+	+	-
	Salwoski test (Triterpenes)	+	-	-	-
Steroids	Liebermann burchard test	-	-	-	-
Fat and Oil	Saponification test	+	-	-	-
	Filterpaper test	+	-	-	-
Saponin	Foam test	-	-	+	+
	Froth test	-	-	+	+
Protein	Ninhydrin	-	-	-	+
	Biuret	-	-	-	-

"+ = present, - = absent"

Proximate composition

Results of proximate analysis of *D. reflexa* are shown in Table.3. The proximate analysis revealed that moisture content is within the limit of 6-15%. Low moisture content would hinder the growth of microorganisms and lifespan of stored samples would be higher. Ash content was found to be 8.015%, which is a reflection of the good amount of mineral elements are present in samples. [27] *D. reflexa* stem contains low fiber content.

Proteins are also found in good proportion and they are important and act as enzyme, hormones and antibodies. Protein also helps in the formation of bones, hair and the outer layer of skin.[28] Crude fat value is very low and it contributes less energy than 30 calories and thus prevent obesity and other related diseases. A diet of fat providing 1-2% energy is sufficient for a human being. [29] High amount of carbohydrates is essential for maintenance of life in plant and animals and also provide raw material for many industries[30].

Table 3: Proximate composition of dried stem of *Dracaena reflexa*.

Parameter	*Dracaena* stem (% dry weight basis)
Moisture content	3.31±1.15
Ash content	8.02±0.25
Crude fiber	1.31±0.13
Crude fat	0.97±0.27
Total protein	3.70±0.39
Total carbohydrate	86.01±0.18
Nutritive value	367.56*

nutritive value is calculated in Kcal/100gm dry weight of stem

Table 4: Total phenolic content of stem extracts of *Dracaena reflexa*

Extracts	Total phenolic content (mgGAE/gmdw)
Petroleum ether	11.43± 0.39
Dichloromethane	73.48± 0.42
Methanol	38.25± 0.92
Water	3.71 ± 0.18

Total Phenolic Content (TPC)

Total phenolic content have been recognized as antioxidant agents who acts as free radical oxidation terminator and have been known to show medicinal activity as well as for exhibiting physiological functions and contribute to human health.[31] The total phenolic content of different extracts was determined by the Folin Ciocalteu method. The TPC was expressed as milligram GAE / gram dry weight using the standard curve equation y= 0.0044x + 0.0187, R^2 = 0.9989 where y is the absorbance at 765 nm and x is the total phenolic content in 1000µg/ml of the extracts. TPC is found higher in dichloromethane extract. The results of TPC are shown in Table.4.

Table 5: IC_{50} of stem extracts of *Dracaena reflexa*

Extracts/ Standard	IC_{50} Values in mg/ml
BHT	0.050 ± 0.27
Petroleum ether	ND*
Dichloromethane	0.460±0.17
Methanol	0.825±0.92
Water	ND*

*Not determined within the taken dilution range.

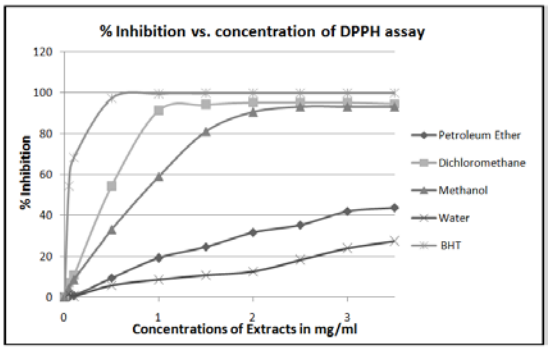

Fig. 2: DPPH radical scavenging activity of stem extracts of *Dracaena reflexa.*

DPPH assay

Hydrogen donor capacities of polyphenols for DPPH radical were found proportional to the number of hydroxyl group and the amount of inactivated DPPH radical directly related to the antioxidant potential of the sample.[32] The above statement justify the results as TPC is found higher in dichloromethane extracts of stem and also the IC_{50} is lower for the same extract. Lower the value of IC_{50}, the higher is the free radical scavenging power. Figure.2 shows the % inhibition of DPPH has a linear relationship with the concentrations of extracts. Results are expressed in terms of IC_{50} and compared with the standard BHT in Table 5.

Table 6: FRAP value for stem extracts of *Dracaena reflexa*

Extracts/ Standard	Ferric reducing antioxidant power (µM/ml)	FRAP Value
Ascorbic Acid	489.75 ± 0.45	2.000
Petroleum ether	170.75± 0.56	0.697
Dichloromethane	507.25± 0.29	2.071
Methanol	288.5± 0.93	1.178
Water	73.25± 0.76	0.299

Frap assay

The FRAP activity of extracts may be due to the presence of phenolic hydroxyl or methoxy group, flavones hydroxyl, ketone groups, free carboxylic groups and other structural features like triterpenes and their derivatives.[33] Frap assay, particularly helps in assessing the antioxidant behavior of extracts in which those phytoconstituents are present which acts by reducing ion or by donating an electron and not by radical quenching mechanism. The results were expressed as µM/ml using the standard curve equation y = 0.004x-0.137, R^2 = 0.998, where y is the absorbance at 593 nm and x is the ferric reducing ability in 1500 µg/ml of extracts in µM/ml. Results and frap value are demonstrated in Table.6.

CONCLUSION

D. reflexa stem reveals a good food supplement acquiring all the content in appropriate amount also it shows potent antioxidant potential in terms of free radical scavenger as well as an electron donator in reduction of metal and it is known that antioxidant properties of various extracts and natural product gained interest both research and food industry, because of their possible role as natural additives to replace synthetic antioxidants. Preliminary phytochemical analysis indicates various important chemical constituents such as flavonoids, tannins, and saponins etc. which are responsible for antioxidant activity. Those extracts in which phenolic content is found to be higher, shows good antioxidant potential. So further detailed studies on stem of *D. reflexa* is needed and also research should be carried out to evaluate the in vivo potential of extracts, its fraction in various animal models and isolation and identification of antioxidant principle.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGMENT

The authors are thankful to Department of chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand, India for providing all necessary facilities.

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