

Pharmacological evaluation of root and leaf extracts of *Dracaena reflexa* var. *angustifolia*

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ABSTRACT

Plants were being used in the treatment of diseases for centuries. In this work, *Dracaena reflexa* var. *angustifolia*, a traditionally significant plant has been studied for its potential medicinal properties. The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. Medicinal plants represent a rich source of antibacterial agents. There is a necessity to explore their uses and to ascertain their therapeutic properties. The present study aims to open new avenues for the improvement of medicinal uses of *D. reflexa* var. *angustifolia* leaves and roots are selected area for antibacterial and antioxidant activity. The methanolic extract of root and aqueous extract of leaves of *D. reflexa* var. *angustifolia*, *Asparagaceae* were assessed for its antibacterial and antioxidant activities. The antibacterial activity against *Staphylococcus aureus*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Lacto bacillus* organisms by agar cup plate method and aqueous leaf extract (ALE) exhibited an excellent antibacterial activity than methanolic root extract (MRE). The antioxidant activity of MRE was performed on isolated frog heart by H₂O₂ induced oxidative stress method. In the present investigation the induction of cardiac arrest was observed at 15th, 25th, and 28 min H₂O₂, ascorbic acid and MRE which shows the MRE possess a good antioxidant activity. ALE exhibited an excellent antibacterial activity than MRE. These findings suggest the excellent medicinal bioactivity of methanolic extract of root and aqueous extract of leaves of *D. reflexa* var. *angustifolia*, *Asparagaceae* and explain the popularity of this plant in the folk medicine as a remedy for bacterial disorders. The traditional system was proved to be more effective when compared to synthetic drugs due to a reduction in side effects.

Keywords: 2,2-diphenyl-1-picrylhydrazyl method, antibacterial activity, antioxidant, *Dracaena reflexa*, free radicals, hydrogen peroxide scavenging assay, reducing power

Introduction

Plants serve as therapeutic agents as well as important raw materials for the manufacturing of traditional and modern medicines as well as in food industries.^[1] Many drugs commonly used today are of herbal origin. Some are made from plant extracts, and others are synthesized to mimic a natural plant compound. From the earliest times, herbs have been prized for their pain-relieving and healing abilities, and today developing countries still rely largely on the curative properties of plants.^[2,3] According to the World Health Organization, 80% of the people living in rural areas depend on medicinal herbs as primary health-care system. 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. If the plant standardize all the parameter of proximate composition, then it is quite safe to be used as dietary supplement or as an herbal drug.^[4-6]

Dracaena consists of about 40 species and described it as a genus of about 150 species. The genus was first described by Linnaeus in 1767. Some species of *Dracaena* include *Dracaena fragrans*, *Dracaena surculosa*, *Dracaena draco*, *Dracaena marginata*, *Dracaena arborea*, *Dracaena goldieana*, *Dracaena sanderiana*, *Dracaena deremensis*, *D. reflexa*, and *Dracaena mannii*. *Dracaenas* are either shrubs or trees and are divided into two broad groups based on their growth habits - tree *Dracaenas* and shrubby *Dracaenas*. Tree *Dracaenas* include *Dracaena americana* (Central American dragon tree), *D. draco* (Canary Islands draco tree), *D. marginata*, and *D. mannii* while shrubby *Dracaenas* include *Dracaena aletiformis*, *Dracaena bicolor*, *Dracaena cincta*, and *Dracaena concinna*. *Dracaenas* are used as ornamentals, medicinal plants, in photo engraving, in research, as hedge plants, colorants, etc. In Europe

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and Canada, they are cultivated and sold as ornamentals. *Dracaena* is a tender evergreen shrub or small native to Madagascar, Mauritius and other nearby islands of Indian Ocean. It is widely grown as an ornamental plant and house plant, valued for its richly colored, evergreen leaves and thick, and irregular stems. The rich green lance-shaped arching leaves spiral the upright stems from base to tip. Mature plants may bear large clusters of greenish-white flowers. Grow this plant as a multi-stemmed shrub or as a small tree pruned to a short trunk. It is also called as song of India plant, pleomele and reflexed *Dracaena* is a species from the *dracaena* genus which has become a popular house plant. This plant is sometimes classified in *Agavaceae* and sometimes put in its own family called *Dracaenaceae*. *Dracaena* is Latin for a she-dragon; *reflexa* is from reflexus, bent backward and *angustifolia* from angustus, narrow and folium a leaf. While it may reach a height of 4-5 m, rarely 6 m in ideal protected locations. *D. reflexa* is usually much smaller, especially when grown as a house plant. It is slow-growing and upright in habit, tending to an oval shape with an open crown. The lanceolate leaves are simple, spirally arranged, 5-20 cm long and 1.5-5 cm broad at the base, with a parallel venation and entire margin; they grow in tight whorls and are a uniform dark green. It is commonly grown in tropical and subtropical climates throughout the world. *D. reflexa* is an upright, multi-stemmed, evergreen shrub or small tree reaching 8-20 ft tall. Plants usually have numerous stems emerging from the ground level. It is slow growing with an irregular habit becoming oval shaped with an open crown. Stems are flexible and little-branched. At times an errant stem may grow far above the others. Older leaves are typically lighter green with creamy margins. Leaves are glossy leathery texture, and they are flexible. The leaf margin is entire or smooth. The leaves spiral upward on the stem and are alternately and compactly arranged.^[7-9]

Traditional medicine practitioners of Madagascar have long believed *D. reflexa* to cure malarial symptoms, poisoning, dysentery, diarrhea, dysmenorrhea and to be useful as an antipyretic, and hemostatic agent. The leaves and bark are mixed with parts of a number of other native plants and mixed into herbal teas. Its effectiveness in any such treatment remains unproven. It is one of the plants used in the NASA Clean Air Study and has shown to help to remove formaldehyde. It is an effective air cleaner and is said to be among the best plants for removing xylene and trichloroethylene.^[10-13]

Plant Profile (Figure 1)

Kingdom: Plantae
 Subkingdom: Tracheobionta
 Super division: Spermatophyta
 Division: Magnoliophyta
 Class: Liliopsida
 Subclass: Liliidae
 Order: Liliales
 Family: *Agavaceae*
 Genus: *Dracaena*
 Species: *D. reflexa*



Figure 1: *Dracaena reflexa* plant

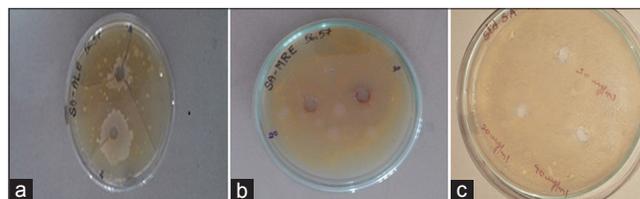


Figure 2: The zone of inhibition of *Staphylococcus aureus*. (a) Aqueous leaf extract, (b) methanolic root extract, (c) standard

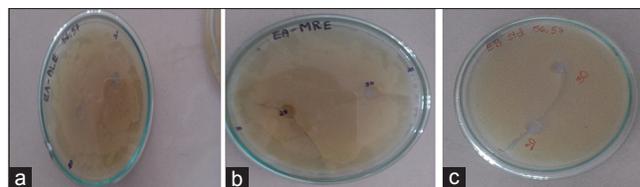


Figure 3: The zone of inhibition of *Enterobacter aerogenes*. (a) Aqueous leaf extract, (b) methanolic root extract, (c) standard

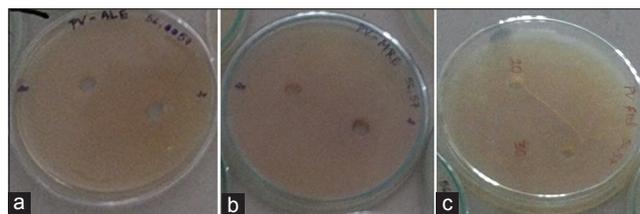


Figure 4: The zone of inhibition of *Proteus vulgaris*. (a) Aqueous leaf extract, (b) methanolic root extract, (c) standard

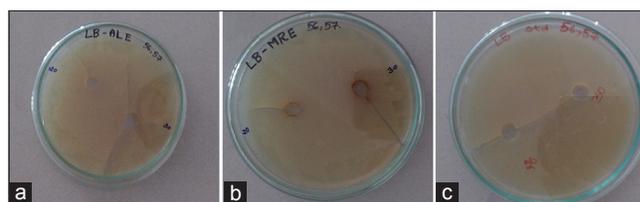


Figure 5: The zone of inhibition of *Lacto bacillus*. (a) Aqueous leaf extract, (b) methanolic root extract, (c) standard



Figure 6: The effect of H₂O₂-induced oxidative stress on isolated Frog's heart preparation

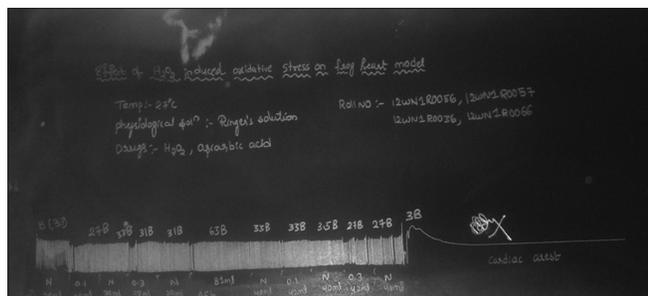


Figure 7: The effect of ascorbic acid on isolated frog heart preparation

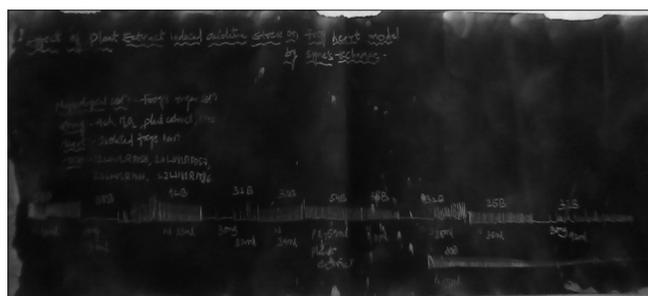


Figure 8: The effect of methanolic root extract on isolated frog heart preparation

Materials and Methods

Collection of plant

The leaves and roots of *D. reflexa* var. *angustifolia* were collected in fresh condition from Padma nursery, Siddipet, Telangana, India.

Extraction of *D. reflexa* roots

Procedure for methanolic extract paste

The roots were collected from plant and air dried under shade then ground into a uniform powder using a mechanical grinder and stored at room temperature. The powder was packed tightly into Soxhlet extractor. The powder was extracted with 250 ml of methanol for 72 h at temperature not exceeding the boiling point of the solvent. Then, the solvent was distilled off, and the extract was concentrated. The residue was kept aside, and the extraction values of the extract were calculated. Then, the extract was screened for antibacterial and antioxidant activities.

Extraction of *D. reflexa* leaves

Procedure for aqueous base extracts paste^[14,15]

The leaves were collected from plant and air dried under shade then ground into a uniform powder using a mechanical grinder and stored at room temperature. The aqueous base layer of leaves was collected by following the Flow Chart 1, then the solvent was distilled off, and the extract was concentrated. The residue was kept aside, and the extraction values of the extract were calculated. Then, the extract was screened for antibacterial activity.

Pharmacological screening

Antibacterial activity

The following microorganisms were used to study the antibacterial activity.

1. *Staphylococcus aureus*: Gram +ve bacteria
2. *Enterobacter aerogenes*: Gram -ve bacteria
3. *Proteus vulgaris*: Gram -ve bacteria
4. *Lacto bacillus*: Gram +ve bacteria

Agar medium

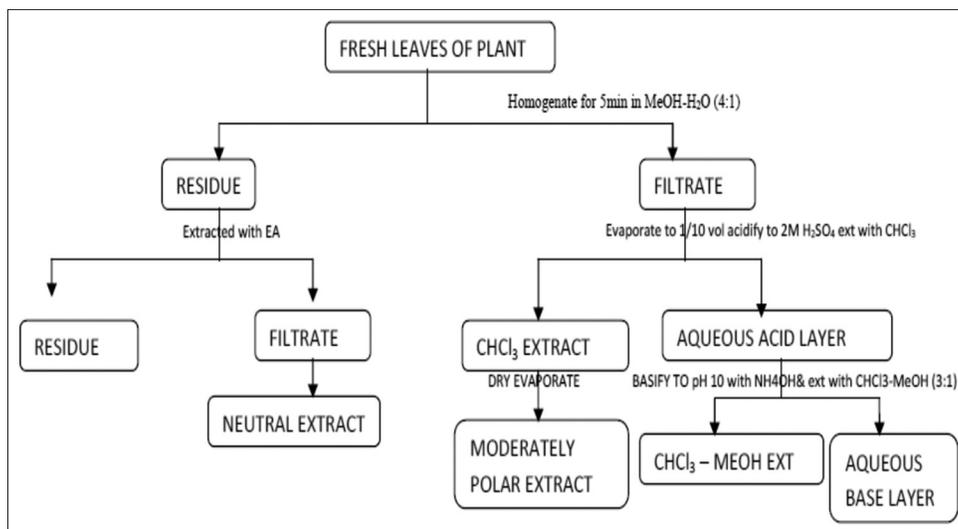
The media was prepared by dissolving the specified quantities of the dehydrated medium in purified water by heating on a water bath in a conical flask. The conical flask was sterilized by autoclaving at 121°C for 15 min. The contents of the conical flask were poured aseptically into sterile Petri dish and allowed to solidify.

- a. Preparation of plant extract: 50 mg of root methanolic and aqueous leaves extracts were weighed accurately and dissolved in 50 ml of DMSO. Further, the solutions were diluted to required quantities [Table 1].
- b. Preparation of standard solution: 20 mg/ml solution of standard solution was prepared by taking streptomycin antibiotic.
- c. Antibacterial activity of methanolic extract of roots and aqueous leaf extract: The antibacterial activity of the extracts was determined by agar cup plate method. Nutrient agar medium was used for the test. Under aseptic conditions in the laminar air flow chamber, nutrient agar medium was dispensed into pre sterilized Petri dishes to yield a uniform depth of 4 mm the media was allowed to solidify. The test microorganisms were seeded into media containing Petri dishes by spread plate method (100 U/L) with 24 h cultures of bacteria. The plates were kept for pre diffusion for 15 min before use. Wells were then punched with a sterile cork borer (6 mm) diameter, and 50 µl of the extracts (20 mg/ml, 30 mg/ml in DMSO) were placed into each well. A negative control was maintained using 50 µl DMSO in a well, and 50 µl of standard antibiotic (streptomycin at 20 mg/ml) was the positive control. Duplicates were maintained for each extract. Finally, the plates were incubated for 18-24 h at 37°C. The diameter of the zone of inhibition (mean of duplicates ± standard deviation) was indicated by clear area which was divide of growth of microbes was measured.

Antioxidant activity

The antioxidant activity was performed on isolated frog heart by H₂O₂ induced oxidative stress method.

- a. Preparation of Ringer's solution:



Flow Chart 1: Extraction process of *Dracaena reflexa* leaves

Preparation of Ringer's solution shown in Table 2.

- Preparation of extract: 100 mg of root methanolic extract and 1.0 g CMC were weighed accurately and dissolved in 100 ml of distilled water.
- Preparation of control solution: 10 ml of H_2O_2 was diluted to 100 ml with distilled water.
- Preparation of standard solution: 1.0 g of ascorbic acid was dissolved in 100 ml of distilled water.
- Isolation of frog heart using Syme's technique:
Isolation of frog heart was done by standard procedure. The Indian frog (*Rana tigrina*) was used in this experiment. The frog was stunned, and abdomen was cut and opened. The pectoral girdle was cut using a bone cutter and removed the pericardium carefully. The Syme's cannula was connected to the reservoir containing frog Ringer's solution and introduced immediately into the sinus venosus of the heart. The connecting blood vessels were cut, and heart was isolated from the animal and mounted on to a stand. Heart was connected to the Starling lever and adjusted for recording the responses of the heart. The level of frog Ringer solution in the Syme's cannula was maintained by fixing a glass tube into the cork fixed to the reservoir (Marriott's bottle) tightly. The heart was allowed to stabilize and when the heart rate and cardiac output were taken, the recordings were made on a slowly rotating drum, to which a soothed kymograph paper was affixed.^[8]

Procedure

Effect of plant extraction on H_2O_2 -induced oxidative stress

To induce oxidative stress on isolated frog heart, 1 mM of H_2O_2 in ringer solution was used. Influence of plant methanol extract on oxidative stress was studied by perfusing frog ringer solution containing plant extract and H_2O_2 solution to the isolated frog heart preparation. The parameters studied include force of contraction, heart rate, and cardiac output (n=6). Then, time taken for induction of cardiac arrest was noted by continuous perfusion of frog ringer

Table 1: Preparation of agar medium

Ingredients	Quantity
Purified water	1000 ml
Agar	20 g
pH - 7.2±0.2	

Table 2: Preparation of Ringer's solution

Ingredients	Quantity (g)
NaCl	30
$C_6H_{12}O_6$	10
KCl	0.7
$CaCl_2$	0.6
$NaHCO_3$	1.0
pH - 7.3±0.2	

solution containing plant extract and is compared with that of control (H_2O_2) and standard ascorbic acid (3 mM).

Results and Discussion

The plant extracts were assayed for preliminary phytochemical analysis antibacterial and antioxidant activity. In this study, the extracts significantly possess antibacterial activity.

Antioxidant activity of *D. reflexa*

The increased demand for medicinal plants has created an ecological crisis for medicinal herbs growing in the wild raising alarm about their rate of extinction. The methanolic root extract (MRE) and ALE were subjected for antibacterial activity, and MRE was subjected for antioxidant activity and results were investigated.

The results of antibacterial screening by agar cup plate method indicates that the ALE is showed highest antibacterial activity than the MRE of root against all the bacteria's, i.e., *S. aureus*, *E. aerogenes*, *P. vulgaris*, and *L.*

Table 3: Antibacterial activity of leaf and root extracts of *Dracaena reflexa*

Name of organism	Concentration (mg/ml)	Zone of inhibition		
		Methanolic root extract (mm)	Aqueous leaf extract (mm)	Standard streptomycin (mm)
<i>Staphylococcus aureus</i>	20	10	12	10
	30	11	13	11
<i>Enterobacter aerogenes</i>	20	11	15	11
	30	12	17	11
<i>Proteus vulgaris</i>	20	12	15	10
	30	13	16	11
<i>Lacto bacillus</i>	20	10	14	10
	30	11	16	10

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Table 4: H₂O₂-induced oxidative stress on isolated frog's heart preparation

Dose	Heart rate (beat/min)	Cardiac output (ml)
Normal	16	59
10 ng/ml	17	24
30 ng/ml	15	21
H ₂ O ₂	42	83
Normal	25	29

Table 5: The effect of ascorbic acid on isolated frog's heart preparation

Dose	Heart rate (beats/min)	Cardiac output (ml)
Normal	35	33
10 ng/ml	32	27
30 ng/ml	38	31
Ascorbic acid	81	63
Normal	41	27

Table 6: The antioxidant activity of plant extract on isolated frog's heart preparation

Dose	Heart rate (beat/min)	Cardiac output (ml)
Normal	58	40
10 ng/ml	38	30
30 ng/ml	32	32
Plant extract	54	59
Normal	30	55

Table 7: Protective effect of plant extract on cardiac arrest

Sample	Cardiac arrest (min)
H ₂ O ₂	15
Ascorbic acid	25
<i>Dracaena reflexa</i>	28

bacillus. The maximum zone of inhibition was found in *S. aureus* and next *E. aerogenes* and *L. bacillus*. In the case of *P. vulgaris*, the ALE showed less antibacterial activity. Standard antibiotic Streptomycin was effective against all organisms and showed zone of inhibition of 10-12 mm (Figures 2-5)¹⁶⁻²⁰. The MRE showed an equivalent antibacterial activity by comparing with standard inhibition except in the case of *E. aerogenes*.

The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties due to the presence of flavonoids. The antioxidant activity of *D. reflexa* is proved by H₂O₂-induced antioxidant activity. Oxidative stress induced by hydrogen peroxide (H₂O₂) may contribute to the pathogenesis of ischemic-reperfusion injury in the heart. For the purpose of investigating directly the injury potential of H₂O₂ on heart muscle, a cellular model of H₂O₂-induced myocardial oxidative stress was developed using monolayer rat cardiomyocyte cultures it was reported that an oxidative burden established by hydrogen peroxide overload may elicit post-ischemic myocardial damage. In this study, we induced oxidative stress on isolated frog heart by perfusing frog ringer solution containing H₂O₂. When ringer solution containing H₂O₂ perfused to heart preparation, the muscarinic actions of acetylcholine were not observed much indicating the oxidative stress on frog heart induced by H₂O₂; this might be due to desensitization of receptors. The cardiac arrest was produced at 15 min. This result supports the frog heart model for induction of oxidative by H₂O₂. In the presence of MRE of plant the cardiac arrest is prolonged, i.e., found at 28 min, i.e., heart was protected longer period with plant extract, against H₂O₂-induced oxidative stress when compared with the control (Table 5 and 6). The standard ascorbic acid showed the cardiac arrest at 25 min (Figures 6-8 and Table 7)²¹⁻²⁴.

Conclusion

The traditional system was proved to be more effective when compared to synthetic drugs due to a reduction in side effects, adverse effects and even a little increase in dose was not that dangerous when compared to the synthetic drugs. It was even found to be more economical and safer in all means the plant *D. reflexa* var. *angustifolia* and evaluated its medicinal property and proved its antibacterial and antioxidant nature for replacing the synthetic drug formulations.

In view of the nature of the plant, more research work can be done on humans so that a drug with multifarious effects will be available in the future market. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. Further, studies will be conducted in this plant to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

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