

Proximate Analysis and Antibacterial activity of *Dracaena reflexa* Leaves

Km.Preeti*¹, Arvind Kumar²

^{1,2}S.D. College of Pharmacy and Vocational Studies, Muzaffarnagar - 251001, Uttar Pradesh, India

ABSTRACT

Ayurveda is the traditional form of health care known since ancient times. *Dracaena reflexa* is a plant of the Asparagus family with several valuable phytochemical constituents used to treat various diseases. In particular, its leaves were widely used in the traditional system of medicine. The present study was aimed to evaluate the pharmacognostical, phytochemical, and antibacterial activity of *D. reflexa* L. leaves. Preliminary qualitative phytochemical screening of *D. reflexa* leaves extracts showed the presence of alkaloids, flavonoids, terpenoids, saponins, amino acids, tannins, steroids, glycosides, carbohydrates and fats. Moreover, analysis of total phenolic content suggested that *D. reflexa* leaves could be a potential source of natural, free radical scavengers that could find use as an antioxidative. The antibacterial activity of *D. reflexa* leaves extracts was analysed against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aureus* by disc diffusion method. The results showed that the methanol, petroleum ether, dichloromethane extract of *D. reflexa* leaves exhibited significant inhibition of *P. aureus* than *S. aureus* and *E. coli*. Conclusively, the findings of this study suggested the excellent medicinal activity of different extracts of *D. reflexa* leaves and describe the popularity of this plant in inhabitants medicine as a antibacterial activity.

Keywords: *Dracaena reflexa*, traditional medicine, flavonoids, antioxidant, ornamental plant, antibacterial.

INTRODUCTION

Mostly medicinal halophyte plants are salt-tolerant herbal medicines and forbs and were continual. There is a need for systematic study of plants that have properties as traditional system of medicine for scientifically validate their pharmacological or biological activity. Considering the recent success of herbal medicines in clinical use, there is a necessity to encourage industrial, academic and government laboratories occupy in research field on the invention and growth of plant-derived drugs. Multidisciplinary research on plants can lead to the identification of new drug candidates and prototype biologically active molecules. The World Health Organization (WHO) defines standards remedy as the "various well being execution, reach, understanding and faith absorbing plant, animal and/ or mineral general remedies, physical analysis, laboring and effort claim extremely or in union to continue health, also to therapy, identify, or block infection" (1). It is observable that there is a demand to validate the available information by conducting the organized research so that it can be further used as an effective therapeutic means for treatment of various ailments. Traditional medicine utilizes biological resources and the traditional indigenous knowledge of plant groups, the last being conveyed verbally from generation to generation (2).

This is nearby linked to the maintenance of biodiversity and related to intellectual property rights of indigenous people. As per the report of the WHO, herbal medicine plays important role in maintaining the health and wealth of mankind. Therapeutic properties of certain medicinal plants have been shown mainly due to the presence of multiple secondary metabolites with diverse functional group (3). Naturally derived drugs that are routinely used are of herbal origin either prepared from the plant extracts or chemically synthesized to mimic a natural plant compound. From the earliest times, herbal medicines have been valued for their pain-killing and curing capacities (4). According to the WHO, 80% of the rural people are depend on medicinal plant as initial health care system. The medicinal value of these plants lies in some chemical constituents that exhibit a clearly physiological action on the human body. The most vitat chemical constituents of medicinal plants are alkaloids, tannins, flavonoids, phenol compounds, etc. If the plant standardize all the parameter of proximate composition, then it is rather safe to be used as dietary supplement or as an herbal medicine (5).

WHO survey indicated the world's populations rely on non-conventional medicine, primary herbal sources, in their first healthcare, especially the case in growing countries where the value of consulting a western style doctor and the cost of medication are other side the means of most people. present study was performed on leaves of *Dracaena reflexa*, family, *Asparagaceae*. *D. reflexa* consists of about 40 species and narrated it as well genus of about 150 species. The genus was first explained by Linnaeus in 1767. Certain species of *Dracaena* include *D. fragrans*, *D. surculosa*, *D. draco*, *D. marginata*, *D. arborea*, *D. goldieana*, *D. sanderiana*, *D. deremensis*, *D. reflexa* and *D. mannii*. *Dracaenas* are shrubs or trees and are partition into two large groups according to their growth habits - tree *Dracaenas* and shrubby *Dracaenas*. The genus of *Dracaena* includes more than 50 species. Some species of *Dracaena* such as *D. cinnabari* stem have been examined for *in vitro* lipid per oxidation, antioxidant activity, anti-inflammatory activity, antimicrobial activity and anticancer activity. One more species *D. cambodiana* showed antitumor, antioxidant activity and antimicrobial activity. Similarly *D. cochichinensis*, *D. angustifolia*, *D. arborea* and *D.*

vand were investigated by different researchers for medicinal potential. Existing study concentrated on phytochemical screening, proximate analysis with nutritive value and antioxidant activity of plant of *D. reflexa* (6,7,8).

MATERIAL & METHODS

Collection of Plant

Fresh leaves of *D. reflexa* were collected from an Indian nursery in Roorkee, Haridwar, Uttarakhand, India and authenticated by Dr. Anupam Srivastav, Patanjali Herbal Department (PRFH/18), Patanjali Research Foundation Herbarium, Haridwar Uttarakhand.

Preparation of Leaves Extracts

Collected *D. reflexa* fresh leaves were washed with clean water, air-dried in shade, and dried parts prepared in the form of coarse powder form with the help of a blender machine. The total weight of the dried powdered leaves was 500 g which was extracted in soxhlet apparatus using solvent 1.5 L each of PE (Petroleum ether), DCM (dichloromethane), MeOH (methanol) and Water successively according to the polarity of the solvents. The extraction was done for 72 h until the coming out of the siphoning tube become transparent. After the complete extraction, solvent residues were concentrated under decrease pressure in a rotary vacuum evaporator and the dried extracts were stored at 2-4°C for further analysis.

Proximate Analysis of dried powder of leaves of *D. reflexa*

Proximate analysis of the powdered *D. reflexa* leaves was carried out to estimate the moisture content, ash content and extractive value of crude fibers. (Khandelwal et al book)

Moisture Content

The weight of the empathy crucible is 69.87 g by weighing balance. Taken 0.50 g sample of powder drug. The weight of the crucible with the sample is 70.37 g before drying. Place the crucible on hot air oven for 15 min at 100 to 105°C. Then cool the crucible in desiccator. Weight the empathy crucible with drug sample is 70.34 g after drying then calculated its percentage of moisture content.

Ash Value

Take a silica crucible place it in a muffle furnace for 15 min. then take out the silica crucible from the muffle furnace then cooled the crucible in desiccators. Then weight the empathy silica crucible is 35.93 g again tare the weighing balance and weight of the sample drug is 2 g. Place the weighing sample in muffle furnace with the temperature of 450-600°C. After then take out the silica crucible from the muffle furnace and cooled in desiccators. The weight of the silica crucible with ash drug is 36.08 g then calculated the percentage of ash value.

Extractive value

Take 4 g sample of the crude drug was transferred in a conical flask and add distilled water as solvent then well sake frequently for 6 h and kept one side for 18 h. Completely procedure for 24 h. Then 62.46 g weight of empathy china disc. Then the prepared solution was filtered by filter paper and the filtrate is transferred in 250 ml of measuring cylinder. The filtrate is transferred in a china dish on a water bath to dry out the water after evaporation place the compound in a hot air oven at 105°C the china disc was takeout from a hot air oven and cooled by desiccators. Then 62.71 g weight of china disc with drug sample after drying to water and calculated its percentage of extractive value (10).

Crude fiber content

Take 200 ml of 0.128 M sulfuric acid transfer in a conical flask. Take 2 g sample of the drug is transferred in conical flask mixed with acid solution and agitated with boiled for 30 min on a hot plate. Then the sample is filtered by clothing (remove- acid residue). Then take 200 ml of 0.313 M NaOH solution was transferred in a conical flask. Washed the filtrate then mixed well with agitated on the hot plate and again filter the sample to drain NaOH then washed with hot water to remove NaOH residue. Then observed the fiber and collected the filtrate in a clean and dried crucible till to filtrate is left and place the crucible on a hot plate to remove excess water. now place the crucible in a hot air oven for 2 h. Then take out the crucible from the oven and cool in desiccators. Then weigh the dried fiber with crucible 22.1333 g the crucible is covered with lids. Then place the inside crucible in a muffle furnace at 550°C for 2 hrs. Remove the crucible from the muffle furnace and cool in desiccators for 20 min. after 4 h collected to the ash fiber and weight, the ash fiber is 2.0816 g then calculated the percentage of crude fiber.

Preliminary Phytochemical Screening of different *D. reflexa* extracts

Preliminary phytochemical analysis of different extracts was performed to analyze the the presence of proteins, glycosides, tannins, carbohydrates, flavonoids, and saponins, in all three sub-fractions of *D. reflexa* leaves extracts (11).

Anti-bacterial study

Antibacterial activity of the extracts was performed against gram-negative and gram-positive bacteria through disc diffusion method. Measured the test samples with a specific amount then dissolved in a fixed volume of solvent (methanol) and applied with sanitary discs and well dried to vaporize the residual solvent (12). In this study kanamycin (30 µg/disc) disc was used as the standard in this method; antibiotics diffuse from a limited source by the nutrient agar gel and generate a concentration gradient. Dried and sterilized (free from microorganisms) filter paper discs (6 mm diameter) inclusive the test samples of known amounts are settled on nutrient agar medium equally seeded with the test microorganisms. Among the study standard antibiotic discs and

blank discs were used for negative control. These plates are set at minimum temperature (4°C) for 24 hrs to allow highest diffusion of the test sample to the surrounding media (Shukla *et al.*, 2015). The plates are then overturned and incubated at 37°C for 24 h for maximum growth of the bacteria.

The test sample having antibacterial property inhibit bacterial growth in the media surrounding the discs by that yield a clear, separated area defined as a zone of inhibition. The antibacterial activity of the test sample and after then measured the diameter of the zone of inhibition reveals in millimeter (Rukaiyat *et al.*, 2015). The present study was performed by crude extracts, show fractions, such as some pure compounds, tested for antibacterial activity by disc diffusion method. Solvent (methanol) was used to dissolve the chemical compounds.

Test Organisms

The strains of bacteria used for the demonstration were collected from the microbiology laboratory. Gram-negative and gram positive bacterial strains (*Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas auroginosa*) were taken for the test.

Disc Diffusion

Three discs were prepared 1. The sample discs, 2. the standard antibiotic discs, and 3. the control discs were settled slowly on the before marked zones on the agar plates pre-infused with microorganism. The agar plates were stored in a refrigerator at 4°C for 24 h upside down to permit sufficient diffusion of the materials for the discs from the surrounding agar medium. The plates were then reversed and kept in an incubator at 37°C for 24 h. The potency of antibacterial agents is measured by observed their activity to prevent the growth of the microbes around the Petri discs which revealed a clear zone of inhibition. After incubation, the antibacterial activities were measured the diameter of the zones of inhibition in mm by vernier caliper. The discs were placed in such a way that they should not closer than 15 mm for the lead of the plate and distant enough apart to prohibit overlapping the zones of inhibition.



RESULTS

Figure 1: *Dracaena reflexa* plant

Organoleptic Evaluation

Table 1: Characteristics of *Dracaena reflexa*

Colour	<u>Solid green and Yellow and Cream</u>
Odor	<u>Extreamly fragrant</u>
Taste	<u>Slightly Peppery</u>
Size	<u>5-20 cm long, 1.5-5 cm broad and 4- 5 m height.</u>
Shape	<u>Lanceolate</u>
Cultivation Status	<u>Ornamental, Wild</u>

Proximate Analysis

Table 2. Proximate Parameter of Dracaena leaves

Parameter	Percentage (%)
Moisture content	6
Ash value	7.5
Extractive value	25
Crude fiber	2.46

Phytochemical Screening:

Table 3. Phytochemical screening of all three sub-fractions of HA extract of Dracaena reflexa Lam. leaves.

S. No.	Test	Dichloromethane	Methanol	Water	Petroleum ether
1	Carbohydrate	-	+	+	-
	Carbohydrate test (Fehling's test)	-	+	+	-
	Carbohydrate test (Benedict's test)	-	+	+	-
2	Test for proteins (Biuret test)	-	-	+	-
	Test for proteins (Xanthoprotein test)	-	-	+	-
3	Test for alkaloids (Dragendroff's test)	-	+	+	-
	Test for alkaloids (Mayer's test)	-	+	+	-
	Test for alkaloids (Hager's test)	-	+	+	-
	Test for alkaloids (Wagner's test)	-	+	+	-
4	Test for cardiac glycosides (Legal's test)	-	+	+	-
	Test for cardiac glycosides (Keller-killani test)	+	-	-	+
	Test for anthraquinone glycosides (Borntrager's test)	+	+	-	-
5	Test for saponin glycosides (Foam test)	-	+	+	-
	Test for saponin glycosides (Haemolytic test)	-	+	+	-
6	Test for flavonoids (Alkaline test)	+	+	+	-
7	Test for steroid (Liebermann Burchard test)	+	-	-	+
8	Test for terpenoids (Salvoski test)	+	-	-	+
9	Test for terpenoids (Liebermann Burchard test)	+	-	-	+
	Test for Tannins (Ferric chloride test)	-	-	+	-

+ Or - means presence or absence of that phytoconstituent in that sub-fraction of HA extract of Dracaena reflexa L. leaves.

Table 4. Antibacterial test result in mm

Microorganism	PE (400 µg/disk)	DCM (400 µg/ disk)	MeOH (400 µg/disk)	Kanamycin (30 µg/mL)
<i>Staphylococcus aureus</i>	13	11	9	38
<i>Escherichia coli</i>	10	2	11	35
<i>Pseudomonas aureaus</i>	23	29	32	36



Figure 2: Zone of inhibition for different bacterial strains

DISCUSSION

The large use of natural plant medication and their therapeutic action is very primitive same as human civilization. In now day's years, demand and supply for medicinal plant products are increasing in India and all over the world for one or other factor and research on them is one of the main areas globally. Although the probable use of the plant as the origin of the development of new drugs or medicines is still lacking behind. Thousands of remedial plant species are available, but only a short fraction has been detected phytochemically and appropriately studied in the precondition of their pharmacological properties. The literature survey apparent that *Dracaena reflexa L.* is still uninvestigated to substantiate its ethnomedicinal claim; very short research work is reported on this plant. In-vitro evaluation of the medicinal plants for biological activity is the first step for achieving the target for developing eco-friendly administrate of diseases of humans through the discovery of new bio-molecules of plant origin. Macroscopically study, proximate parameters, phytochemical analysis, and biological activity (anti-bacterial activity) were done on three sub-fractions of methanol, dichloromethane, petroleum ether and aqueous extract of *Dracaenareflexa L.* *Dracaena reflexa* have been used as a medicinal plant and also used as a traditional medicine for the multipal diseases like malaria, poisoning, dysentery, and diarrhea, hemostatic and to be useful as an antipyretic, and dysmenorrhoeal. *Dracaena reflexa* helps to clean formaldehyde and, this plant also effective air cleaner also called as best plant air cleaner plant (for removing xylene and trichloroethylene). Plants are a vital source of effectively helpful structures for the growth of new chemotherapeutic agents. The primary step for this goal is the antibacterial activity assay. Some reports are available on the antioxidant, antibacterial, properties of plants. Certain of these examination have used in identifying the active principle is responsible for as activities in growing drugs for medication use in human beings. *Dracaenareflexa* extractives exhibited moderate to great antibacterial activity. The test samples of *D. reflexa* exhibited a zone of inhibition ranging from 9.0 to 32.0mm against the test organisms. In the present study, the petroleum ether extracts of *Dracaenareflexa* showed the activity against *Pseudomonas aureaus* having the highest zone of inhibition (23.0mm), *Staphylococcus aureaus* having the zone of inhibition (13.0 mm), *E. coli* having the zone of inhibition (10.0 mm). The petroleum ether extract was subsequently fractioned and monitored through bioassay essential to the separation of active fraction through further analysis. The DCM extract fraction of *D. reflexa* exhibited a zone of inhibition ranging from moderate to good against the test organisms. The highest (29.0mm) zone of inhibition was demonstrated against *Pseudomonas aureaus*. But there was the very lowest (2.0mm) zone of inhibition found against *E. coli*. A good zone of inhibition was exhibited against *Staphylococcus aureaus* (11.0mm).

The highest zone of inhibition was demonstrated by the MeOH soluble fraction against *Pseudomonas aureaus* (32.0mm) and the lowest result was found against *Staphylococcus aureaus* (9.0mm), *E. coli* having the zone of inhibition (11.0mm). Narender and Naveena (2017) have investigated for pharmacological properties of *Dracaena reflexa*. The improvement of medicinal uses of *Dracaena reflexa* and *Dracaena angustifolia*. The root and leaves of *Dracaena reflexa* and *Dracaena Angustifolia* are selected for antibacterial and antioxidant activity and found similar results.

CONCLUSION

The existing time study was carried out to explore the anti-microbial activity of *Dracaena reflexa*. From the outcome of my study, it can be achieved that, using in vitro experiments established that different solvent extracts of *Dracaena reflexa* inhibit bacterial growth. The antibacterial activity of the plant extracts was evaluated against potentially bacterial pathogenic by using the disc diffusion method at several concentrations of the extracts of *Dracaena reflexa* by understanding the most effective activity. There are certain established research reports about the phytochemical and pharmacological properties of this plant. In conclusion from our compiled data, this is only an initiatory study but the plant can be further screened against several diseases to discover its unexplored effect and potency of source for biologically vital drug candidates.

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