



## Phytochemical and Pharmacological Investigations of Different Extracts of *Dracaena spicata* Roxb. Available in Bangladesh

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### ABSTRACT

Natural product-based drug discovery from plant sources has frequently been practiced for many years in the field of health research. The present study aimed to determine the phytochemicals, antioxidant, antibacterial, cytotoxic, and thrombolytic properties of different solvent extracts of *Dracaena spicata* Roxb. Secondary metabolites were isolated from the methanol extract of *D. spicata* using a series of chromatographic techniques and characterized by NMR spectral analysis. The antioxidant potential of all the extracts was determined using DPPH free radical scavenging assay besides determining their total phenolic and flavonoid contents. Antibacterial activity was tested using the disc diffusion method, and cytotoxicity was assessed using the brine shrimp lethality assay. The thrombolytic activity of all the extracts was also measured. Two secondary metabolites were isolated from the methanol extract and characterized as vanillic acid (1) and isovanillic acid (2). The methanol extract exhibited the highest free radical scavenging abilities ( $IC_{50}$  value =  $47.38 \pm 2.96$   $\mu$ g/mL), the highest concentrations of phenolic ( $244.73 \pm 0.25$  mg/g gallic acid equivalent) and flavonoid content ( $297.08 \pm 2.58$  mg/g quercetin equivalent) and it was also shown to be the most cytotoxic ( $LC_{50} = 38.79 \pm 2.79$   $\mu$ g/mL). The dichloromethane extract exhibited the highest inhibitory activity against *Vibrio mimicus*, producing a  $32.8 \pm 0.8$  mm zone of inhibition and was found to have the highest thrombolytic activity ( $29.01 \pm 1.25\%$ ). The bioactivities of *D. spicata* validate the traditional use of this plant as well as urge further investigations to identify more bioactive compounds.

**Keywords:** Antioxidant, antimicrobial, *Dracaena spicata*, vanillic acid, isovanillic acid

### Introduction

Low antioxidant level and related oxidative stress is the result of the accumulation of free radicals inside our bodies. It has been implicated in the pathogenesis of various fatal diseases like cancer, hypertension, hyperlipidemia, inflammation, Alzheimer's disease, etc. Natural antioxidants, particularly phenolic compounds and flavonoids found in fruits, vegetables, and medicinal plants, have garnered tremendous attention recently for their ability to boost the immune system and reduce the risk of developing these diseases. Apart from being effective, these natural phenolic compounds are also safe, economical, and structurally diverse.<sup>1</sup> However, despite the promises of these entities, very few lead compounds have progressed into clinical trials. This is particularly true in the field of cancer, immunosuppressive, and metabolism-related diseases, where nature-derived products have played a crucial role in novel drug discovery and development.<sup>2</sup>

The tribal populations of Chittagong Hill Tracts, Bangladesh, are dependent on the traditional tribal healers and on the use of various native medicinal plants for the treatment of a number of diseases for many years.<sup>3</sup>

The present study was conducted with one ethnopharmacologically important plant of Bangladesh named *Dracaena spicata* Roxb. to establish the scientific basis of its traditional use. *D. spicata* Roxb. (Family: Asparagaceae, Bangla name: Ognikundo, tribal name: Kadorateng gaas) is a shrub and widely found in the greenwoods of Chittagong and its Hill Tracts, Cox's Bazar, Andaman Islands, and some parts of Myanmar.<sup>4</sup> Many hill tribes of Bangladesh have been using this plant to treat various ailments for many years. Traditional healers of the Marma tribe of Chittagong Hill Tracts use the juice of *D. spicata* leaf for treating fever, cold and coughs.<sup>5</sup> Chakma tribe of Chittagong Hill Tracts use leaves of this plant for the treatment of measles.<sup>6</sup> Pharmacological studies have shown that different solvent extracts of *D. spicata* have mild thrombolytic and membrane-stabilizing activity<sup>4</sup> and the methanol extract of *D. spicata* has modest antimicrobial activity.<sup>7</sup>

The literature review demonstrated very few biological and chemical studies on *D. spicata* grown in Bangladesh. As a continuation of our current studies on medicinal plants native to Bangladesh,<sup>8-11</sup> we aimed to isolate the secondary metabolites and assess the antioxidant, antibacterial, cytotoxic, and thrombolytic activity of three solvent extracts of *D. spicata*.

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### Materials and Methods

#### Apparatus and Chemical Substances

2,2-Diphenyl-1-picrylhydrazyl or DPPH for free radical scavenging assay, deuterated chloroform, and deuterated methanol for sample preparation for NMR analysis were purchased from the Sigma Aldrich Co. (USA) through a local vendor. Among other materials, Folin-Ciocalteu (FC) reagent, ascorbic acid, silica gel 60 F<sub>254</sub> plates and silica gel were purchased from Merck, Germany. For the antimicrobial assay, the required nutrient agar media, standard antimicrobial discs of

kanamycin, and ketoconazole were obtained from HiMedia, India. Streptokinase was collected from Beacon Pharmaceuticals Ltd. Bangladesh. The 400 MHz NMR spectrometer from Bruker, Switzerland was used for NMR analysis. Analytical grade solvents and reagents were used in this study.

#### Plant Materials Collection and Extraction Procedure

The aerial part of *D. spicata* was collected from Pablakhali, Rangamati, Chittagong Hill Tracts (21°40'00"N 92°08'00"E), Bangladesh in January 2017. The plant sample was identified in the National Herbarium of Bangladesh, which is located at Mirpur, Dhaka, by the taxonomist, and a voucher specimen with an accession number (DACB 40632) was retained there for further reference. The aerial part of the plant was cut and divided into small pieces, dried up in the air for a few days, and ground to powder using a motorized grinder (Joypan MFM-2100, India). The powdered plant materials were divided into three parts, each having 300 g of powder, using a weighing machine (A & D Co. Ltd., FZ-300i, Japan) and submerged into petroleum ether, dichloromethane, and methanol separately using 1.5L of solvent in three air-tight containers for seven days followed by filtration through fresh cotton bed and Whatman no.1 filter paper, respectively. The amount of the filtrates was reduced with a rotary evaporator (Heidolph 60-01100-00, Germany) with reduced pressure, maintaining the temperature at 40°-50°C. The obtained crude extracts were preserved in well-closed containers at 4°C temperature for future use.

#### Isolation and Purification of Compounds

The methanol extract of *D. spicata* (2.7 g) was separated by column chromatography using silica gel (mesh 70 - 230). A gradient of petroleum ether, dichloromethane, and methanol with increasing polarity was used to elute the column, which resulted in 10 fractions (F-1 to F-10). Compound **1** was obtained from a slightly impure white mass of column fraction F-3 (petroleum ether/50–60% dichloromethane) after purifying it with various solvents. Column fractions F-5 (petroleum ether/90-100% dichloromethane) and F-6 (dichloromethane/1-5% MeOH) were mixed together and passed through a small-sized column packed with silica gel (mesh<sup>230</sup> - 400). The column was developed with the gradient of *n*-hexane, dichloromethane and methanol with increasing polarity which afforded 10 fractions (FF-1 to FF-10). Compound **2** was isolated from the column fraction FF-5 with the eluant system of *n*-hexane/25-35% dichloromethane.

#### Preliminary Phytochemical Screening

Phytoconstituents of the plant extracts were identified using different reagents following standard procedures.<sup>12</sup> For the detection of alkaloids- Mayer's reagent, Hager's reagent, Wagner's reagent, and Dragendorff's reagent were used. Carbohydrates were identified by Molisch's reagents, and flavonoids were screened by Shinod's test using concentrated hydrochloric acid; saponins were identified by their ability to produce froth in distilled water, Keller Killiani test was used for glycosides, Ferric Chloride test was performed to identify the presence of tannins and Salkowski test using chloroform followed by concentrated H<sub>2</sub>SO<sub>4</sub> was done for the detection of steroids (Table 1).

#### DPPH Free Radical Scavenging Assay

A solution (400 µg/mL) of DPPH was prepared, and 100 µL of the prepared solution was added to the solutions of standard ascorbic acid, petroleum ether extract of *D. spicata* (PDS), dichloromethane (DDS) and methanol (MDS) extracts (400 to 12.5 µg/mL). After 30 min, the UV absorbance was recorded at 517 nm using Shimadzu UV PC-1600 spectrophotometer.<sup>13</sup> The free radical scavenging potential was determined using equation 1.

$$\% \text{ of free radical scavenging} = \frac{1 - A_{\text{sample or standard}}}{A_{\text{control}}} \times 100 \% \dots \dots (1)$$

#### Phenolic Content Determination

Folin-Ciocalteu reagent was utilized to measure the phenolic content present in the assayed samples.<sup>14</sup> Briefly, 1 mL of sample solutions of PDS, DDS, MDS (200 µg/mL) and standard gallic acid (50 to 250 µg/mL) were prepared. About 5 mL of Folin-Ciocalteu reagent (volume diluted up to 10 fold) and a 7.5% w/v solution of sodium carbonate were added in each sample preparation at 25°C and after a period of 30 min, the absorbances of the samples were taken using a UV spectrophotometer at 765 nm wavelength. A calibration curve of the standard gallic acid was used to determine the total phenolic content, which was expressed as mg/g GAE or gallic acid equivalent.

#### Flavonoid Content Determination

The total flavonoid content was assayed following the colorimetric method using aluminum trichloride.<sup>15</sup> Solution of PDS, DDS, MDS (200 µg/mL) and standard quercetin solutions ranging from 50 to 250 µg/mL were prepared in which NaNO<sub>2</sub> solution (5% w/v), AlCl<sub>3</sub> (10% w/v), NaOH (2% w/v) and distilled water were added. The solutions were kept at rest for 30 min at ambient temperature and later subjected to centrifugation for 15 min at 3000 rpm. At 510 nm wavelength, the absorbance was measured using the UV spectrophotometer. The calibration curve of quercetin was built to analyze the total flavonoid content present in the samples and was measured as mg/g QE or quercetin equivalent.

#### Antibacterial Assay

Antibacterial activity of PDS, DDS, MDS (300 and 600 µg/disc) and standard kanamycin (30 µg/disc) was measured by the disc diffusion technique against thirteen Gram-positive and Gram-negative bacterial strains.<sup>16</sup> Sterile filter paper discs infused with different extracts were placed on Petri dishes containing approximately 20 mL of nutrient agar, where suspension of individual microorganisms was inoculated prior to the experiment. All the plates were kept in an incubator (BK-4266, EHRET Incubator, Germany), maintaining the temperature at 37°C, and after 24h, zones of inhibition were determined by measuring the diameter of the clear zones.

#### Brine Shrimp Lethality Bioassay

The solution of each extract was developed by dissolving 4 mg of sample of each in 60 µL DMSO and was diluted to different concentrations by serial dilution technique ranging from 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.562 µg/mL.<sup>17</sup> Vincristine sulphate was used as the reference standard. A total of 10 living nauplii (*Artemia salina*) were added in each experimental solution, and after 24h, the number of survived nauplii was counted. Lethal concentration (LC<sub>50</sub>) for each of the extracts was calculated.

#### Determination of Thrombolytic Activity

For this assay, a lyophilized streptokinase vial (15,00,000 I.U.) was used where sterile distilled water (about 5 mL) was added. From this stock solution, 100 µL (30,000 I.U.) was utilized for the in-vitro thrombolytic assay. From healthy human volunteers, whole blood was collected from which a volume of 1 mL of blood was transferred to the sterile weighed Eppendorf tubes. They were then left for 45 min at 37°C to form clots. After the formation of clots, the serum was completely removed, and to determine the clot weight, each of the Eppendorf tubes was re-weighed. After that, 100µL of aqueous solutions of different extracts (10 mg/mL) and the standard streptokinase were added to each of the Eppendorf tubes which were incubated for 90 min at 37°C temperature, again to allow clot lysis. Then, after removing the released fluid, all the Eppendorf tubes were weighed.<sup>18</sup> The extent of clot lysis was calculated using equation 2.

$$\% \text{ of clot lysis} = \frac{\text{Weight of the lysed clot}}{\text{Weight of the clot before lysis}} \times 100 \% \dots \dots (2)$$

#### Statistical Analysis

All the calculations were carried out using Microsoft Excel, 2010. All the assays were replicated thrice, and the results were denoted as mean ± standard deviation (SD).

## Results and Discussion

### NMR Data and Characterization of Compounds

Vanillic acid (**1**): (10.0 mg, brown amorphous solid); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.89 (3H, s, -OCH<sub>3</sub>), 7.59 (1H, d, *J* = 8.0 Hz, H-2), 6.87 (1H, d, *J* = 8.0 Hz, H-3), 7.53 (1H, bs, H-6). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 55.9 (-OCH<sub>3</sub>), 121.9 (C-1), 124.4 (C-2), 113.9 (C-3), 150.5 (C-4), 146.5 (C-5), 115.0 (C-6), 168.8 (-COOH).

Isovanillic acid (**2**): (8.0 mg, white amorphous solid); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 3.98 (3H, s, -OCH<sub>3</sub>), 7.60 (1H, d, *J* = 1.6 Hz, H-2), 6.99 (1H, d, *J* = 8.4 Hz, H-5), 7.72 (1H, dd (*J* = 8.4, 2.0 Hz, H-6).

The <sup>1</sup>H NMR and <sup>13</sup>C NMR data of compound **1** confirmed the presence of eight carbon atoms corresponding to three sp<sup>2</sup> methines, one carboxylic acid group, one sp<sup>3</sup> methoxy and three sp<sup>2</sup> quaternary carbon atoms. The <sup>1</sup>H NMR spectrum of compound **1** revealed signals for phenolic compound, with three aromatic proton signals at δ = 7.59, 6.87, and 7.53 ppm, identified as H-2, H-3, and H-6, respectively. Furthermore, the <sup>1</sup>H NMR spectra showed the distinctive signal at δ = 3.89 ppm for a methoxy group. The above-mentioned data and published literatures<sup>19,20</sup> confirmed compound **1** as vanillic acid.

Compound **2** exhibited phenolic compound indications in its <sup>1</sup>H NMR spectrum, with three doublets of aromatic proton signals at δ = 7.60, 6.99, and 7.72 for H-2, H-5, and H-6, respectively and a typical signal

at δ = 3.98 ppm for a methoxy group. Based on the aforementioned data and comparison with the literature,<sup>21</sup> compound **2** was identified as isovanillic acid, an isomer of vanillic acid (Figure 1).

### DPPH Free Radical Scavenging Assay

DPPH free radical scavenging assay was conducted to measure the radical scavenging potential of the various extracts of *D. spicata* and the result was compared with a standard antioxidant ascorbic acid (Table 2). The highest scavenging potential with an IC<sub>50</sub> value of 47.38 ± 2.96 µg/mL was observed for MDS, followed by DDS (IC<sub>50</sub> = 68.71 ± 3.99 µg/mL), whereas the IC<sub>50</sub> for ascorbic acid was 18.16 ± 1.79 µg/mL. The nonpolar PDS extract showed a minimum scavenging potential among all the extracts with an IC<sub>50</sub> value of 133.29 ± 2.74 µg/mL.

### Phenolic Content Determination

The total phenolic content of the extracts was measured utilizing the standard curve of gallic acid (*y* = 0.005*x* - 0.179; *R*<sup>2</sup> = 0.803). The result suggested that MDS contained the highest amounts of phenols (244.73 ± 0.25 mg/g GAE). The phenolic content observed for DDS and PDS were 224.23 ± 0.33 mg/g GAE and 205.57 ± 0.41 mg/g GAE, respectively (Table 2).

**Table 1:** Results of phytochemical screening of three extracts of *Dracaena spicata*

Extracts	Alkaloids	Carbohydrates	Flavonoids	Saponins	Steroids	Tannins	Glycosides
PDS	+	+	+	+	+	+	+
DDS	+	+	+	+	+	+	+
MDS	+	+	+	+	+	+	+

(+): Present; (-): Absent; PDS: Petroleum ether extract of *D. spicata*, DDS: Dichloromethane extract of *D. spicata*, MDS: Methanol extract of *D. spicata*

### Flavonoid Content Determination

The total flavonoid content of different extracts of *D. spicata* was estimated from the standard curve of quercetin (*y* = 0.004*x* - 0.133; *R*<sup>2</sup> = 0.920). Like phenolic content, MDS contained the highest amount of flavonoids (297.08 ± 2.58 mg/g QE) followed by DDS (274.38 ± 0.56 mg/g QE) and PDS (212 ± 0.63 mg/g QE) (Table 2).

### Antibacterial Activity

Among the three extracts, DDS was active against all the bacterial strains at both concentrations (300 and 600 µg/disc), producing a 32.8 ± 0.8 mm zone of inhibition against *Vibrio mimicus* at 600 µg/disc. PDS showed highest antibacterial activity against *Staphylococcus aureus* (28.2 ± 0.9 mm) at 600 µg/disc. The strong antibacterial activity of MDS was observed against *V. mimicus* (31.3 ± 1.5 mm) and *S. aureus* (31.3 ± 1.2 mm) at the concentration of 600 µg/disc (Table 3). However, this extract showed no zone of inhibition against *S. paratyphi*, *S. typhi*, *S. dysenteriae*, *P. aeruginosa*, and *S. boydii*.

### Brine Shrimp Lethality Bioassay

All extracts produced moderate to strong cytotoxicity against brine shrimp nauplii in a dose-dependent way. Consistent with the findings of the phenolic and flavonoid content assays, MDS produced maximum cytotoxicity with an LC<sub>50</sub> value of 38.79 ± 2.79 µg/mL followed by DDS (LC<sub>50</sub> = 47.58 ± 5.49 µg/mL) and PDS (LC<sub>50</sub> = 71.91 ± 6.63 µg/mL) (Table 4).

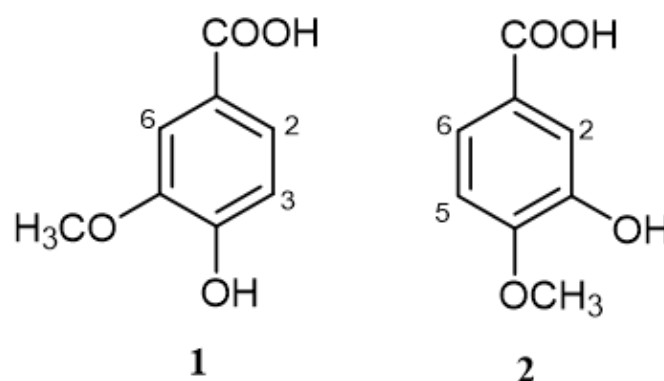
### Thrombolytic Activity

In the thrombolytic activity determination assay, the highest clot lysis potential was exhibited by the DDS (29.01 ± 1.25%) and the lowest by PDS (21.43 ± 1.17%), indicating moderate thrombolytic activity. The thrombolytic activity score of the standard streptokinase was 64.24 ± 1.78% (Table 4).

**Table 2:** Free radical scavenging potential, total phenolic and flavonoid content of three extracts of *Dracaena spicata*

Extracts	IC <sub>50</sub> value (µg/mL)	Total phenolic content (in mg/g GAE)	Total flavonoid content (in mg/g QE)
PDS	133.29 ± 2.74	205.57 ± 0.41	212 ± 0.63
DDS	68.71 ± 3.99	224.23 ± 0.33	274.38 ± 0.56
MDS	47.38 ± 2.96	244.73 ± 0.25	297.08 ± 2.58
AA	18.16 ± 1.79		

Values are expressed as mean ± SD; n = 3. PDS: Petroleum ether extract of *D. spicata*, DDS: Dichloromethane extract of *D. spicata*, MDS: Methanol extract of *D. spicata*; AA: Ascorbic acid.



**Figure 1:** Structures of compounds isolated from the plant *Dracaena spicata* Roxb.

Secondary metabolites play versatile roles in plants that range from conferring immunity to communication and signal transduction.<sup>22</sup> The research findings suggested that all the extracts of *D. spicata* Roxb. are enriched in various biologically active secondary metabolites, including polyphenols. Phenolic compounds are known for their diverse biological potential. Depending on the aromatic ring and the presence of one or more hydroxyl groups, they can be classified as simple or complex phenolic acids.<sup>23</sup> Vanillic acid and isovanillic acid are simple phenolic compounds and are synthesized in the shikimate pathway from precursors phosphoenol pyruvate and erythrose-4-phosphate.<sup>24</sup> In this study, vanillic acid and its isomer isovanillic acid has been isolated from the methanol extract of *D. spicata* using a series of chromatographic processes, and characterized by NMR spectral data analysis.

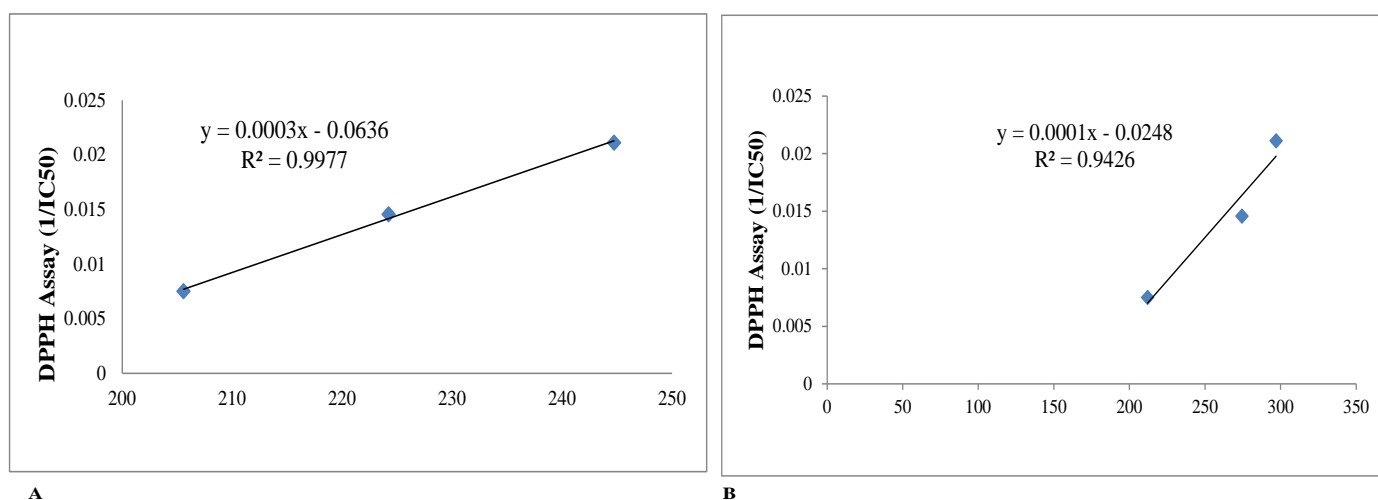
DPPH is a free radical and requires one single electron to be stable. The multiple hydroxyl groups present on polyphenols and flavonoids,

particularly on the B and C rings of their structures, enable them to donate an electron to DPPH and produce radical scavenging activity.<sup>25</sup> The propensity of antioxidant potential of a compound is determined by estimating IC<sub>50</sub> values, which is defined as the concentration of the extracts required to stabilize 50% of the DPPH.<sup>26</sup> A high correlation between the DPPH scavenging activity and the total phenol and flavonoid content is also observed in this study, indicating their possible involvement in conferring the antioxidant properties of the plant (Figure 2A., Pearson's correlation coefficient,  $r = 0.9988$  and coefficient of determination,  $R^2 = 0.9977$  and Figure 2B., Pearson's correlation coefficient,  $r = 0.9709$  and coefficient of determination,  $R^2 = 0.9426$ ). Thus, the strong radical scavenging potential of methanolic extract can be attributed to vanillic acid and isovanillic acid content as their antioxidant property has already been established.<sup>27</sup>

**Table 3:** Observation of zone of inhibition of three extracts of *Dracaena spicata*

Microorganisms	PDS		DDS		MDS		Kanamycin 30 µg/disc
	300 µg/disc	600 µg/disc	300 µg/disc	600 µg/disc	300 µg/disc	600 µg/disc	
<b>Zone of inhibition (mm)</b>							
<b>Gram-positive bacteria</b>							
<i>Bacillus cereus</i>	20.6 ± 0.5	26 ± 1.0	15.2 ± 1.0	22.5 ± 0.5	26.5 ± 0.8	27.5 ± 0.5	35.6 ± 0.7
<i>Bacillus megaterium</i>	-	8.3 ± 0.6	26.1 ± 0.3	27.8 ± 0.8	9.3 ± 0.6	11 ± 1.0	36.8 ± 0.7
<i>Bacillus subtilis</i>	8.5 ± 0.5	9.6 ± 0.6	20.8 ± 0.8	21.6 ± 0.6	10.6 ± 1.2	11.3 ± 1.2	35.5 ± 0.5
<i>Sarcina lutea</i>	7.2 ± 0.9	9.3 ± 0.6	17.2 ± 0.8	20.6 ± 0.6	7.1 ± 0.9	9.16 ± 1.2	30.3 ± 0.6
<i>Staphylococcus aureus</i>	25.5 ± 0.5	28.2 ± 0.9	28.5 ± 0.9	31.6 ± 0.6	30.7 ± 0.6	31.3 ± 1.2	37.5 ± 0.5
<b>Gram-negative bacteria</b>							
<i>Salmonella paratyphi</i>	11.6 ± 0.7	15.3 ± 0.6	30.8 ± 0.8	31.3 ± 1.1	-	-	35.5 ± 0.5
<i>Salmonella typhi</i>	20.3 ± 0.3	21.8 ± 0.8	20.6 ± 0.6	21.3 ± 0.6	-	-	35.3 ± 1.15
<i>Vibrio parahaemolyticus</i>	-	-	15.6 ± 0.6	16.6 ± 0.6	7.3 ± 0.6	8.2 ± 0.9	40.0 ± 0.0
<i>Vibrio mimicus</i>	25 ± 1.0	27.3 ± 0.6	31.3 ± 1.2	32.8 ± 0.8	29.5 ± 0.5	31.3 ± 1.5	36.5 ± 0.5
<i>Escherichia coli</i>	9.3 ± 0.6	10.7 ± 0.6	16.6 ± 1.1	20.3 ± 1.1	7.6 ± 0.6	9.2 ± 0.3	35.7 ± 0.6
<i>Shigella dysenteriae</i>	8.3 ± 0.6	11.3 ± 1.1	16.2 ± 0.3	17.3 ± 1.1	-	-	34.3 ± 0.6
<i>Pseudomonas aeruginosa</i>	11.3 ± 0.6	12.3 ± 1.5	25.8 ± 0.7	26.8 ± 0.8	-	-	28.2 ± 0.3
<i>Shigella boydii</i>	11.5 ± 0.5	13.0 ± 0.0	13.6 ± 1.1	17.3 ± 1.1	-	-	24.3 ± 1.1

Values are expressed as mean ± SD; n = 3. PDS: Petroleum ether extract of *D. spicata*, DDS: Dichloromethane extract of *D. spicata*, MDS: Methanol extract of *D. spicata*; (-) means no zone of inhibition.



**Figure 2:** A. Correlation curve of DPPH assay and phenolic content. B. Correlation curve of DPPH assay and flavonoid content.

**Table 4:** % mortality of shrimp nauplii and thrombolytic activity of three extracts of *Dracaena spicata* and standards

Extract/Standard	LC <sub>50</sub> value (µg/mL)	% Clot lysis
PDS	71.91 ± 6.63	21.43 ± 1.17
DDS	47.58 ± 5.49	29.01 ± 1.25
MDS	38.79 ± 2.79	23.16 ± 0.29
VS	1.69 ± 0.28	
SK		64.24 ± 1.78

Values are expressed as mean ± SD; n = 3. PDS: Petroleum ether extract of *D. spicata*, DDS: Dichloromethane extract of *D. spicata*, MDS: Methanol extract of *D. spicata*, VS: Vincristine sulphate, SK: Streptokinase

The dichloromethane extract of *D. spicata* exhibited strong antibacterial activity against all the bacterial strains. The highest antibacterial activity was observed against *S. aureus*, *S. paratyphi* and *V. mimicus* (30 - 32 mm). The preliminary phytochemical analysis confirmed the presence of phenolic compounds in the plant extract, which can be responsible for this potent antibacterial activity. It is scientifically established that the lipophilic nature of the phenolic compounds enables them to interact with the lipid bilayer of the cell membrane.<sup>28</sup> The aftermath of such interaction may result in an alteration of the cell membrane permeability and cell wall rigidity, culminating in irreversible damage to the cell membrane, leaking out of the cellular contents and cell death. In addition to interaction with the cell membrane, phenolic compounds can also alter the intracellular protein function to exert their antibacterial activity.<sup>29</sup> LC<sub>50</sub> ranging from 0-100 µg/mL are classified as highly toxic, and many scientific studies established the usefulness of brine shrimp lethality bioassay as an alternative approach for animal or other *in vivo* assays to determine the toxicity in humans.<sup>30</sup> Phenolic compounds may exert their anti-carcinogenic activity by inducing cell cycle arrest or modulating reactive oxygen species (ROS) or by promoting tumor suppression protein.<sup>23</sup> Besides, all the extracts were found to have alkaloids and steroids by phytochemical screening and these phytochemicals were also reported to have cytotoxicity.<sup>31</sup> So, the strong cytotoxic effect of the extracts may be related to the isolated phenolic compounds, alkaloids and steroids of the extracts, though more specific tests are required to confirm this.

The formation of a thrombus (blood clots) in the vascular system leads to circulatory blockade and the development of thrombosis, preceding fatal consequences.<sup>32</sup> Several thrombolytic agents have been used in the treatment of such disorders, showing noticeable side effects. Therefore, medicinal plant- derived secondary metabolites with thrombolytic properties confer a better safety profile over synthetic agents. The present study revealed that the extracts of *D. spicata* possessed moderate thrombolytic activity when compared to the standard streptokinase. The thrombolytic activity of streptokinase is contingent on its ability to convert plasminogen to plasmin and subsequent lysis of the clot.<sup>33</sup> Scientists have reported that antioxidants of natural origin can exert a potential action against thrombus and thrombus formation.<sup>34</sup> It was also reported that plant flavonoids negatively regulate thrombosis by interfering with platelet activation.<sup>35</sup> Even the cardiac glycosides possess better thrombolytic activity.<sup>36</sup> The presence of flavonoids and glycosides in all the extracts of *D. spicata* thus explains the thrombolytic activity of the plant, though further studies are required to know the exact mechanism of thrombolytic activity produced by flavonoids or glycosides.

## Conclusion

*Dracaena spicata* Roxb. is a potential source of polyphenol-like phytoconstituents and antioxidants of natural origin. The notable bioactivity of the plant also makes it a potential source of active metabolites that could be used as lead molecules for the drug development process. However, research should be conducted focusing on the isolation of more bioactive components of the plant responsible for its bioactivities.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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