In vitro evaluation of antimicrobial and antioxidant activity of Dragon’s blood tree (*Dracaena cinnabari* Balf.f.) of Socotra Island (Yemen)

Abu-Taleb Ahmed Yehia\(^1, 2\)*, Fahad Ahmed Mohsen Alzowahi\(^1, 2\), Tukaram Angadrao Kadam\(^1\), Rafik Usman Shaikh\(^1\)

\(^1\)Department of Microbiology, School of Life Sciences, S.R.T.M. University, Nanded– 431 606, (MS), India

\(^2\)Department of Microbiology, Taiz University, Yemen

**ABSTRACT**

Objective: To evaluate the performance of preliminary phytochemical, antioxidant and antimicrobial activities of *Dracaena cinnabari* Balf.f. (*Agavaceae*) (*D. cinnabari*) resin, collected from Socotra Island (Yemen).

Methods: The powdered stem resin is extracted with different solvents by using Soxhlet apparatus and evaluated for antioxidant and antimicrobial activity against three Gram positive bacteria (*Bacillus cereus* NCIM 2106, *Staphylococcus aureus* NCIM 2127, and *Micrococcus luteus* NCIM 2169), seven Gram negative bacteria (*Escherichia coli* NCIM 2924, *Pseudomonas aeruginosa* NCIM 2863, *Salmonella typhimurium* NCIM 2501, *Klebsiella aerogenes* NCIM 2281, *Proteus morganii* NCIM 2860, *Escherichia coli* dh5α, *Escherichia coli* CSH) and three fungi (*Candida albicans* MTCC 227, *Aspergillus flavus* MTCC 873, *Aspergillus niger* MTCC 1781).

Results: The antimicrobial activity data revealed that, the selected microorganisms possess varied sensitivity response to chloroform extract followed by methanol and benzene extract of *D. cinnabari* stem resin. The samples also possess a potent DPPH, OH and SO radical scavenging activity. In addition, the preliminary phytochemical analysis demonstrated the presence of alkaloids, flavonoids, terpenoids and other organic compounds which could be reason for antimicrobial activities of *D. cinnabari* resin.

Conclusions: The present study is strengthened for the discovery and development of new antioxidant and antimicrobial drugs from *D. cinnabari* resin which encourage us to continue with pure fractions.

**KEYWORDS**

*Dracaena cinnabari*, Socotra, Yemen, Resin, Antimicrobial, Antioxidant, Phytochemical screening

1. Introduction

Plants have a boundless ability to synthesize aromatic substances mainly secondary metabolites, 12000 of which at least have been isolated, a number estimated to be less than 10% of the total\(^1\). Extracts from plants contain a multitude of biologically active compounds which have beneficial effects on health. Plant metabolism can be separated into primary and secondary products, Primary metabolites are compounds that are directly involved in the growth and development of a plant whereas secondary plant metabolites are useful for defense purposes (flavonoids, alkaloids, terpenoids, anthraquinones, tannins, phytosteroids, and related compounds). Dragon’s blood tree is a non-specific name for dark red resinous exudations from different plant species endemic to various regions around globe that belongs to four genera *Dracaena* spp. (*Agavaceae*),
Croton spp. (Euphorbiaceae), Daemonorops spp. (Palmaceae) and Pterocarpus spp. (Fabaceae) have a long history of being used as a traditional medicine the world over. Medicinal use of dragon’s blood dates back to the Ancient Greeks, Romans, Chinese and Arabs[2].

However, Dracaena cinnabari Balf. f. (D. cinnabari) belongs to Agavaceae family, which is commonly known as Damm Al-akhwain in Yemen. It is endemic to the Socotra Island, Yemen. D. cinnabari resin has traditionally been used to treat diarrhea, wounds, fevers, ulcers, hemorrhage, control bleeding, fractures, and burns[3]. In China, Daemonorops spp. and Dracaena spp. dragon’s blood resins ha been used in traditional Chinese medicine to stimulate circulation, control bleeding, treat pain, promote tissue regeneration, wounds, diarrhea, piles and assist the healing of fractures[4]. Croton spp. dragon’s blood resin is a household remedy in Latin American countries, where it is used to treat diarrhea, bone fractures, hemorrhoids, and cholera.

Within the recent years, infections have been increased to a great extent and antibiotics resistance effects have become an ever–increasing therapeutic problen[5]. Natural products of higher plant may possess a new source of antimicrobial agents with possibly novel mechanisms of action[6]. It has wide medicinal uses: haemostatic, antidiarrhetic, antiulcer, antimicrobial, antiviral, antitumor, anti–inflammatory antioxidant and wound healing[7,8]. Despite its wide uses, little research has been done to know about its true source, quality control, bioactive compounds and clinical applications.

Therefore, it is of great interest to carry out a screening of these plant parts in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. The systematic screening of them may result in the discovery of novel active compounds.

2. Materials and methods

2.1. Materials

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma–Aldrich Co. (St. Louis MO, USA), 1–10 phenanthroline, phenazine methosulphate (PMS), nitroblue tetrazolium (NBT) were obtained from S.D. Fine Chem, Mumbai. Nicotinamide Adenine Dinucleotide (NADH) was purchased from Spectrochem, Pvt. Lit. Mumbai. All the other chemicals and reagents used were of analytical reagent grade and were obtained from commercial sources.

2.2. Bacterial and fungal strains

The pathogenic bacterial strains, viz., Staphylococcus aureus ATCC 9144 (S. aureus), Klebsiella aerogenes ATCC 8329 (K. aerogenes), Micrococcus luteus ATCC 398 (M. luteus), Escherichia coli ATCC 33684 (E. coli), Pseudomonas aeruginosa NCIB 8295 (P. aeruginosa), Salmonella typhimurium ATCC 23564 (S. typhimurium) and Proteus morganii ATCC 8076 (P. morganii) were collected from NCIM– Pune, whereas Bacillus cereus NCIM 2106, E. coli dh5α, E. coli CSH and fungal strain of Candida albicans MTCC 227 (C. albicans), Aspergillus flavus MTCC 873 (A. flavus) and Aspergillus niger MTCC 1781 (A. niger) were obtained from S.R.T.M. University, Nanded.

2.3. Collection of plant material

Dragon’s blood tree (D. cinnabari) resin was collected from Socotra Island (Yemen). It has a unique and strange appearance, described as upturned, densely–packed crown having the shape of an umbrella (Figures 1 and 2). The plant samples were identified and authenticated by Environment Protection Authority of Yemen. The island is situated in the arid tropics and is subjected to intense summer monsoon activity that has created a seasonal period of isolation. Average air temperatures range from 23.5 °C in the coolest to 35 °C in the hottest months of the year. During summer, temperatures can exceed 40 °C at noon, and fall not lower than 25 °C.

2.4. Preparation of extracts

The powdered resin 600 g was extracted with different solvents depending on the polarity of the solvents. Started with non–
polar to polar (hexane, benzene, diethyl ether, chloroform, ethyl acetate, dichloromethane, acetone, ethanol and methanol) by Soxhlet apparatus for 8 h for each solvent. Extracts were filtered and concentrated under vacuum in a rotary evaporator. The extracted samples were stored at 4 °C for further use.

2.5. Phytochemical screening and total phenol content (TPC) determination

The qualitative phytochemical screening tests were performed by using standard methodologies as described earlier[9]. Total phenols were determined by Folin–Ciocalteu reagent[10]. In brief, 0.5 mL plant extract (1 mg/mL) was mixed with 0.5 mL Folin Ciocalteu reagent and 2 mL of Na₂CO₃ (7%). The mixtures were allowed to stand for 10 min in water bath and the total phenols were determined by spectrophotometrically at 760 nm. The standard curve of catechol (μg/mL) was prepared and TPC values are expressed in terms of catechol equivalent (μg/mg of dry mass), which is a common reference compound.

2.6. Antibacterial activity

The antibacterial activity was carried out by the agar well diffusion method using Muller Hinton agar (MHA) plates[11]. MHA media had been prepared and plated with bacterial cultures 10⁻⁶ CFU/mL using seeded agar technique. A cork borer (6 mm diameter) was used to punch wells in solidified medium, which was filled with 100 μL of selected solvent extracts dissolved with DMSO (10 mg/mL). The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic vancomycin (1 mg/mL, positive control) and DMSO as negative control. The plates were incubated at 37 °C for 24 h in incubator and the diameter of the zone of inhibition was measured in mm. Each sample was assayed in triplicate and the mean values were observed.

2.7. Antifungal activity

The antifungal activity was carried out by the agar well diffusion method[11] against C. albicans, A. flavus and A. niger. PDA media was prepared and poured in sterilized Petri dishes and kept for 45 min to be solidified, inoculums containing 10⁻⁶ CFU/mL of fungal spore were spread on the solid agar media. Wells were made in the potato dextrose agar plate using cork borer (6 mm). Then 100 μL of extracts were dissolved with DMSO (10 mg/mL) and placed in the wells and kept in the refrigerator at 4 °C for 30 min for uniform diffusion of the substances then incubated at 30 °C for 24 h for C. albicans and 72 h for Aspergillus sp. The diameter of the zone of inhibition was measured in millimeter (mm). Each sample was assayed in triplicate and the mean values were observed.

2.8. Determination of minimum inhibitory concentration (MIC)

The resin plant extracts (methanol, benzene and chloroform) that showed the best antibacterial activity against the most susceptible bacteria had been selected for MIC determination. The MIC was assayed by recommended method by National Committee for Clinical Laboratory Standards 2010[12]. The MHA media containing various concentration of the methanol, benzene and chloroform extracts which were dissolved in DMSO solvent (0, 50, 75, 100, 200 and 300 μg/mL) were solidified and poured onto sterile petri dishes to give sterile MHA plates with varying dilutions of the extract. Then these plates were kept in the refrigerator (4 °C) for 24 h for uniform diffusion of the extract. The plate was then dried at 37 °C for 2 h. One loopful (diameter 3 mm) of overnight grown nutrient broth culture of each test organisms (S. aureus, M. luteus and E. coli) were diluted to 10⁻⁶ CFU/mL and spread plated on MHA plate then incubated at 37 °C for 24 h.

2.8.1. Determination of zone of inhibition

For the determination of zone of inhibition pure Vancomycin was taken as positive standard antibiotic for comparison of the results. Three sets of dilutions (100, 200 and 300 μg/mL) of resin extracts were dissolved with DMSO solvent. Sterile Mueller Hinton Agar MHA plates were prepared and incubated at 37 °C for 24 h to check any sort of contamination and plated with bacterial cultures 10⁻⁶ CFU/mL using spread technique. A cork borer (6 mm diameter) was used to punch wells in solidified medium, which was filled with 100 μL of three sets of dilution. The plates were incubated at 37°C for 24 h in incubator and the diameter of the zone of inhibition was measured in millimeter (mm). Each sample was assayed in triplicate and the mean values were observed.

2.9. Antioxidant study

2.9.1. DPPH radical scavenging assay

DPPH radical scavenging assay was carried out as reported method with slight modifications[13,14]. Briefly, 1 mL of test solution (individual plant extract) was added to equal quantity of 0.1 mmol/L solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1 mmol/L) was used as reference compound. Experiment was performed in triplicate and averaged. Percent scavenging activity was calculated from control using the following equation:

Scavenging activity % = (1−Absorbance sample/Absorbance control) ×100

2.9.2. Hydroxyl (OH) radical scavenging assay

The OH radical scavenging activity was demonstrated with Fenton reaction[15]. The reaction mixture contained, 60 μL of FeCl₂ (1 mmol/L), 90 μL of 1−10 phenanthroline (1 mmol/L), 2.4 mL of phosphate buffer (0.2 mol/L, pH 7.8), 150 μL of H₂O₂ (0.17 mol/L) and 1.5 mL of individual plant extract (1 mg/mL). The
reaction was started by adding H₂O₂. After 5 min incubation at room temperature, the absorbance was recorded at 560 nm. Ascorbic acid (1 mmol/L) was used as reference compound. The OH scavenging activity percentage was calculated according to the following equation:

Scavenging activity % = (1 – Absorbance sample/Absorbance control) × 100

2.9.3. Superoxide radical (SOR) scavenging assay

The superoxide anion scavenging assay was performed by the reported method[16]. Superoxide anion radicals were generated in a non-enzymatic phenazine methosulphate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS, NADH and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In this experiment superoxide anion was generated in 3 mL of Tris HCl buffer (100 mmol/L, pH 7.4) containing 0.75 mL of NBT (306 µmol/L), 0.75 mL of NADH (936 µmol/L), and 0.3 mL of plant sample (1 mg/mL). The reaction was initiated by adding 0.75 mL of PMS (120 µmol/L) to the mixture. After 5 minutes’ incubation at room temperature the absorbance at 560 nm was measured by using spectrophotometer. Ascorbic acid (1 mmol/L) was used as reference compound. The SOR scavenging activity percentage was calculated according to the following equation:

Scavenging activity % = (1 – Absorbance sample/Absorbance control) × 100

3. Results

3.1. Phytochemical screening and TPC

The phytochemical analysis of various solvent extracts of D. cinnabari is shown in Table 1. It was observed that almost all solvent extracts of D. cinnabari resin contain the various phytochemical constituents. The glycosides were observed as a major component in all solvent extracts, while none of the extracts was found to contain saponins. However, methanol, ethanol, benzene and chloroform extracts possess all tested phytochemicals except the saponins. On the basis of antimicrobial activity results, the TPC of selected extracts (benzene, chloroform, methanol and ethanol) were carried out. The data obtained were summarized in Figure 3. It was observed that the benzene extracts possess highest TPC (97 µg/mg) followed by ethanol (89 µg/mg), methanol (89 µg/mg) and chloroform (84 µg/mg) extracts. Since TPC of benzene methanol and chloroform extracts were high, these extracts were further taken for antioxidant assay.

3.2. Antibacterial activity of selected samples

The data of antibacterial activity is summarized in Table 2 and Figure 4. The chloroform extract of D. cinnabari resin was...
observed to possess most potent antibacterial activity. The highest zone of inhibition was observed in *M. luteus* (25.0±2.1) mm by chloroform extract, followed by benzene extract (24.0±1.3) mm. Moreover, chloroform extract also inhibits *S. aureus* (21.0±2.6) mm, *P. aeruginosa* (22.0±2.8) mm, and *E. coli* (21.0±1.8) mm. It was interesting to note that the bacterial strain such as *S. aureus*, *M. luteus*, and *E. coli* were found to be susceptible towards all solvent extracts, while *K. aerogenes* and *P. morganii* were found to be resistant to crude solvent extracts and vancomycin. The rest of the extracts showed a moderate inhibitory activity to the selected bacterial strains in the range of 9–20 mm. The vancomycin which shown standard inhibition to *S. aureus* (20.0±0.5) mm, *M. luteus* (25.0±1.7) mm, *E. coli* (22.0±1.9) mm, *E. coli* (dh5α) (18.0±1.5) mm, and *E. coli* CSH (22.0±0.8) mm. There is no zone of inhibition with negative control DMSO.

### 3.3. Evaluation of MIC

The samples which showed a maximum inhibitory activity towards the most susceptible bacteria were selected for MIC. The results obtained are summarized in Table 3. Three extracts, as methanol, benzene, chloroform and bacterial strain as, *S. aureus*, *M. luteus*, *E. coli* were evaluated for MIC. The results obtained indicate that, the methanol and chloroform extracts showed a MIC at 100 µg/mL with zone of inhibition in the range of 7 to 10 mm and benzene extract at 200 µg/mL towards *S. aureus*, *M. luteus*, and *E. coli* respectively with zone of inhibition in the range of 8 to 9 mm.

### 3.4. Determination of antifungal activity

The results obtained in Table 4, shown the effective values of the extracts against the pathogenic fungal strains such as *C. albicans*, *A. flavus*, and *A. niger*. It was observed that, ethyl acetate extract possess a highest inhibitory activity towards *A. flavus* (22.0±1.8) mm, followed by *C. albicans* (18.0±1.6) mm and *A. niger* (14.0±0.9) mm. The lowest activity was reported from ethanol extract towards *C. albicans* (7.0±0.1) mm. None of the fungal strains get affected by acetone extract.

### 3.5. Antioxidant activity screening of plant samples

The antioxidant activity of the selected plant extracts were assayed by DPPH, OH and SOR scavenging assay. The results obtained were summarized in Figure 5. It was observed that, the plant extracts exhibit a promising radical scavenging activity. The results radical scavenging activities were considerable as a comparison among DPPH, OH and SOR. The highest DPPH radical scavenging activity was observed by methanol 60.76, while OH by ethanol 63.84 and SOR by chloroform 56.32. The benzene 1.29 and acetone 5.13, extracts demonstrated as poor DPPH radical scavenger.

### Table 3

| Selected plant extracts | MIC (µg/mL) | Zone of inhibition (mm) |
|-------------------------|------------|------------------------|
|                         | *S. aureus* | *M. luteus* | *E. coli* | *S. aureus* | *M. luteus* | *E. coli* |
| Methanol                | 100        | 100          | 100       | 7           | 9           | 10        |
| Benzene                 | 200        | 200          | 200       | 9           | 9           | 8         |
| Chloroform              | 100        | 100          | 100       | 9           | 8           | 9         |

**Table 4**

| Antifungal activity of selected solvent extract. | Zone of inhibition of different solvent extracts (mm) |
|-----------------------------------------------|-----------------------------------------------------|
| **Fungal Strains**                           | **Ethanol** | **Methanol** | **Benzene** | **Acetone** | **Dichloromethane** | **Chloroform** | **Diethyl ether** | **Ethyl acetate** |
| C.a.                                         | 7.0±0.1     | 8.0±1.3      | –           | –           | –                   | 14.0±0.6       | 09.0±1.8          | 18.0±1.6          |
| A.f.                                         | 8.0±0.4     | 9.0±0.8      | 12.0±2.4    | –           | 10.0±0.5           | 13.0±1.8       | 14.0±1.1          | 22.0±1.8          |
| A.n.                                         | 8.0±0.9     | 8.0±1.3      | 11.0±1.5    | –           | 09.0±1.1           | 10.0±1.9       | 12.0±2.1          | 14.0±0.9          |

C.a.: *C. albicans*, A.f.: *A. flavus*, A.n.: *A. niger*; –: No inhibition; n=3, mean±SD.
The present work was designed for evaluating the potential of D. cinnabari resin as antimicrobial and antioxidant agents in the midst of searching novel therapeutic agents. Plants are used worldwide for the treatment of diseases and novel drugs continue to be developed through research about these plants. There are more than 20000 species of higher plants used in traditional medicine and reservoirs of potential new drugs[17]. As a source of most medicinal agents in industrialized countries, it becomes familiar nowadays that the advanced drug researches, modern medicine and chemical synthesized drugs play an important role instead of traditional plants medicine in spite of the importance of plants as a source of these compounds. However, in developing countries the majority of the population cannot afford pharmaceutical drugs and use their own plant-based indigenous medicines.

The present study of antimicrobial, phytochemical and antioxidant activity of D. cinnabari resin shown noteworthy results against different pathogenic microorganisms such as, S. aureus, M. luteus, E. coli, S. typhimurium, P. aeruginosa, C. albicans and A. niger and the interesting thing clear the zone of inhibition which revealed the statistical efficiency. The antioxidant evaluation study was done by using DPPH, OH and SOR assay. It is observed that the methanol and chloroform extracts of most of the resin solvent extracts (1 mg/ml) possess potent radical scavenging ability. Whereas, the others crude solvent extracts were found to have moderate activity demonstrated by various crude solvent extracts from D. cinnabari resin sample which might due to presence of aforesaid phytochemicals. The phytochemical screening revealed the presence of different types of phytochemical constituent. Due to the existence of TPC that was observed in three extracts (methanol, benzene and chloroform), throughout the achieved results it could be conclude that TPC and the phytochemical bioactive compounds are factors for antimicrobial and antioxidant activities of D. cinnabari resin. In conclusion, previous phytochemical studies of D. cinnabari confirmed that there was an isolation of a number of flavonoids, bioflavonoid, triflavonoid, sterols and triterpenoids[18,19]. Few reports in other related literature appeared good interaction with the antioxidant activities of the ingredients of D. cinnabari against cytochrome P450 enzymes[20], with the antiviral activity of the methanolic extract against influenza and herpes simplex viruses[21], and with the antibacterial and antifungal activity of the crude extract against different microorganisms[17]. Despite its wide uses, little research has been done to know about its true source, quality control, bioactive compounds and clinical applications. Therefore, this work was carried out for a further analysis to its biological effects against some claimed effects and to explore new potentials for this commonly prescribed herbal remedy.

D. cinnabari resin revealed huge potential interaction with antimicrobial and antioxidants activities which gives us the curiosity for investigating whether the isolated purified compounds from Dragon’s blood may have better therapeutic potential efficacy as compared to crude extract or not. Since there is considerable variation in the chemical composition among various samples of Dragon’s blood, quality control/assurance needs to be established for the traditional medical trade. This work is an effort to highlight the potential uses of D. cinnabari resin and the problems related to the sources and possibilities of isolating new pharmaceutically active molecules, using traditional knowledge in our search, for new and effective drugs molecules.

Conflict of interest statement

The authors declare that, we have no conflict of interest.

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Comments

Background

Natural products of higher plant may possess a new source of antimicrobial agents with possibly novel mechanisms of action. D. cinnabari resin has traditionally been used to treat diarrhea, wounds, fevers, ulcers, hemorrhage, control bleeding, fractures, and burns. Therefore, the potential of these plants as alternative Medicine play a critical role in the reducing the antibiotic resistance phenomenon and healthcare provision.

Research frontiers

The present study is strengthened for the discovery and development of new antioxidant and antimicrobial drugs from D. cinnabari resin which encourage us to continue with
pure fractions.

**Related reports**

The phytochemical analysis of various solvent extracts of *D. cinnabari* was observed that almost all solvent extracts of *D. cinnabari* resin contain the various phytochemical constituents. The antioxidant activity of the selected plant extracts were assayed by DPPH, OH and SOR scavenging assay were observed that the plant extracts exhibit a promising radical scavenging activity.

**Innovations and breakthroughs**

*D. cinnabari cinnabari* Balf. F, blood resins has been used in traditional of Chinese medicine to stimulate circulation, control bleeding, treat pain, promote tissue regeneration, wounds, diarrhea, piles and assist the healing of fractures. The present work was designed for evaluating the potential of *D. cinnabari* resin as antimicrobial and antioxidant agents in the midst of searching novel therapeutic agents.

**Applications**

From the literature survey it has been found that the *D. cinnabari* resin revealed huge potential interaction with antimicrobial against different pathogenic microorganisms such as, *S. aureus*, *M. luteus*, *E. coli* (dh5α and CSβ), *S. typhimurium*, *P. aeruginosa*, *C. albicans* and *A. niger* and antioxidants activities which gives us the curiosity for investigating whether the isolated purified compounds from Dragon’s blood may have better therapeutic potential efficacy as compared to crude extract or not.

**Peer review**

It would be advantageous to standardize methods of extraction and work in which authors have demonstrated and highlight the potential uses of *D. cinnabari* resin with the problems related to the sources and possibilities of isolating new pharmaceutically active molecules, using traditional knowledge in our search, for new and effective drugs molecules.

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