Biotechnology Research and Innovation (2018) 2, 1–8

Biotechnology Research & Innovation

http://www.journals.elsevier.com/biotechnology-research-and-innovation/

RESEARCH PAPER

# Phytochemical constituents of *Dracaena mahatma* leaves and their anti-bacterial, anti-oxidant and anti-inflammatory significance

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Received 19 March 2018; accepted 15 September 2018
Available online 28 September 2018

**KEYWORDS**
Ornamental plant;
*Dracaena mahatma*;
Antibacterial activity;
Gas chromatography-mass spectrometry;
Antioxidant;
Anti-inflammatory

**Abstract** The present study aims to analyze the antimicrobial, antioxidant and anti-inflammatory activity of *Dracaena mahatma* leaf extract. Qualitative phytochemical screening of leaf powder confirmed the presence of tannins, alkaloids, phenols, terpenoids, sterols, triterpenes and anthraquinone glycosides. GC-MS analysis of methanolic extract suggested the presence of 2 unknown compounds and 8 known phytochemicals, of which benzene-(1,6-hexanediylidene)tetrakis constituted a maximum percentage of the crude extract. The total phenolic content of the crude extract was found to be $32.9 \pm 0.002$ GAE/g. Crude extract demonstrated 90% antioxidant activity in DPPH assay at 100 μg/ml concentration and also demonstrated significant antioxidant activity in FRAP assay at 100 μg/ml concentration. The crude extract did not show any antagonism against fungal pathogens *Aspergillus niger* and *Aspergillus flavus*. Crude extract demonstrated significant antibacterial activity against 5 studied pathogen, with the highest antagonism against *Escherichia coli* with a zone of inhibition of 22 mm at 10 mg/well concentration.

## Introduction

*Dracaena mahatma* is an ornamental plant which remains colorful in bright light and in semi-shade. Dracaena leaf remains attractive and colorful for a longer period of time at moderate temperatures, humidity and moisture conditions. This plant can be grown well in pots as well as ground (Palanivelu, Kunjumon, Suresh, Nair, & Ramalingam, 2015). Aslam et al. reported that numerous steroidal saponins that are produced by *Dracaena draco* exhibited cytostatic activity on Leukemia HL 60 cells. The clotting process was greatly improved in mice by the extract obtained from the *Dracaena cochinchinensis*. The stem of *D. draco* contains a red resin known as Dragon's blood, which has been used as traditional

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https://doi.org/10.1016/j.biori.2018.09.002

**Figure 1** *D. mahatma* plant sample used for this study.

medicine as an ''herbal remedy'' (Aslam, Mujib, &, Sharma, 2013).

*Dendranthema grandiflorum* is a hybrid ornamental plant, mainly grown for flower farming and also used in the production of insecticidal products. The bioactive compounds isolated from this plant showed larvicidal activity against *Aedes aegypti* larvae (Spindola et al., 2016). Aslam et al. (2013) reported that several activities like antileishmanial, antimalarial, molluscicidal, fungicidal and bacteriostatic activities are showed by Spirocanozole-A an active saponin compound extracted from *Dracaena arborea* and *Dracaena mannini*.

Based on these literature surveys and based on the lack of research articles on *D. mahatma* and due to the reported bioactivity of this genera of plants, this plant was chosen as the plant of interest for this analysis. This study analyzed the antibacterial, antioxidant and anti-inflammatory activity of *D. mahatma* leaves and also analyses the phytochemical composition of the extract by the biochemical reaction and GC-MS method (Fig. 1).

### Scientific classification

Kingdom: Plantae
Clade: Angiosperms
Clade: Monocots
Order: Asparagales
Family: Asparagaceae
Subfamily: Nolinoideae
Genus: Dracaena
Species: Mahatma

## Materials and methods

### Leaf crude extract preparation

Fresh leaves of *D. mahatma* were collected in the sterile bag from VIT University, Vellore, Tamil Nadu, India. The leaves were washed thoroughly with double distilled water to remove dust and foreign particles. Then the leaves were shade dried for 3–4 days. Once it gets completely dried, the leaves were ground into a fine powder.

The leaf powder of about 10–15 g was dissolved in 200 ml of methanol in an Erlenmeyer flask. Then the flask was sealed with parafilm and placed in a shaker for 48 h at 120 rpm. After 48 h the content was filtered using a Whatman filter paper No. 1. The methanol solvent was evaporated by using a rotary vacuum evaporator. The crude extract was concentrated and further studies have been carried out using this extract. The solvent extract was done once for every 15 g of sample with 200 ml methanol, in a single flask.

### Qualitative analysis of phytochemicals

Using standard protocol, phytochemicals such as phenol, alkaloid, saponin, tannin sterol and triterpenes and anthraquinone glycoside were analyzed (Andriani et al., 2015; Sundar, Segaran, Shankar, Settu, & Ravi, 2017).

#### Water extract

To the 14 ml of distilled water, add 1 g of *D. mahatma* dried leaf powder and using mantle this content was boiled for 5 min. Then the content was filtered using Whatman No. 1 filter paper to obtain water extract

*Phenol test*. To the 1 ml of the filtrate, add few drops of $FeCl_3$, the appearance of dark green color indicates the presence of phenol.

*Saponin test*. In a test tube add 2 ml of distilled water along with the small amount of crude extract and shaken vigorously. The presence of saponin can be confirmed by the formation of stable foam.

*Tannin test*. Add few drops of 2% $FeCl_3$ to the test tube containing crude extract, the presence of tannin was confirmed by the display of blue-green color.

#### Acid extract

In the beaker, add 1 g of dried leaf powder and 5 ml of concentrated HCl, then the content was mixed gently and allow it to stand for 20 min. Using Whatman filter paper the filtrate was filtered.

*Flavonoid test*. To the 2 ml of 2% sodium hydroxide solution, add a small amount of filtrate, on the addition few drops of diluted acid, the yellow colored solution turned to colorless, this shows the presence of flavonoid.

#### Alcohol extract

Few milliliters of methanol was added to the leaf powder and allowed to stand for 30 min then the content was filtered using Whatman filter paper. The filtrate was kept on the mantel until the solution get evaporates. Finally, to resuspend, 3 ml of chloroform were added to it.

*Alkaloid test*. In a filter paper, few drops of alcohol extract were deposited, and then over the filter paper Dragendroff's reagent was splashed. The presence of alkaloid was confirmed by the appearance of reddish brown color.

*Sterol and triterpenes test*. Few drops of concentrated $H_2SO_4$ and acetic anhydride were added to the sidewise of the test tube containing 2 ml of alcoholic extract. A positive result can be confirmed by the appearance of reddish brown color.

*Anthraquinone glycoside test*. A volume of 1 ml ammonia was mixed with extract then the mixture was shaken vigorously, the positive result was indicated by the appearance of green color in the bottom and reddish color in the aqueous layer.

## Determination of total phenol content

Folin-Ciocalteu photometric method was carried out to determine total phenol content of plant extract. A small amount of methanolic plant extract was oxidized with Folin-Ciocalteu reagent. Then the saturated sodium carbonate was added to neutralize the reaction. To dilute the solution 50 ml volume of distilled water was used. Then the samples were incubated at room temperature for 90 min, the absorbance was measured at 750 nm. Gallic acid was used as a standard solution (Senguttuvan, Paulsamy, & Karthika, 2014).

## Antioxidant activity: DPPH radical scavenging assay

The DPPH radical scavenging assay is a standard procedure to determine the antioxidant activity of the leaf extract. Aliquots of methanolic extract at different concentrations (25, 50, 75, 100 mg/ml) were added to 2 ml of DPPH. Gallic acid was prepared at different concs (25, 50, 75, 100 mg/ml) and used as reference standard. A mixture of 1 ml of methanol and 2 ml of DPPH was used as the control. The reaction was carried out in triplicates and the mixture was shaken well, incubated in dark condition for 30 min after the incubation period he absorbance was measured at 517 nm against the blank containing methanol. The radical scavenging activity of DPPH was calculated using the following equation (Sahu, Kar, & Routray, 2013).

$$\text{DPPH radical scavenging effect} \quad (\%) = [A_0 - A_1/A_0] \times 100$$

where $A_0$ is the absorbance of the control; $A_1$ is the absorbance of the sample.

## Antioxidant activity: FRAP assay

Leaf extracts were taken at different concentration (25, 50, 75, 100 μg/ml) and it was mixed with phosphate buffer of 2.5 ml and potassium ferricyanide of 2.5 ml. Then, this mixture was placed in water bath at 50 °C for 20 min. Once the mixture was cooled add 2.5 ml of 10% trichloroacetic acid (TAA) and centrifugation was carried out at 3000 rpm for 10 min. 2.5 ml of the supernatant was taken carefully and to that 2.5 ml of distilled water and 0.5 ml of freshly prepared ferric chloride solution was added. Control was prepared by adding all the solution with the absence of the sample (leaf extract). Standard was prepared at different concentration (25, 50, 75, 100 μg/ml) using gallic acid. The absorbance value was taken at 700 nm. If the absorbance value of the reaction mixture increases it indicates increases in the reducing power (Jayanthi & Lalitha, 2011).

## Antibacterial activity

Agar well diffusion method was carried out to determine antibacterial activity using Muller-Hinton agar. The stock culture was prepared using nutrient broth and inoculated with bacteria *Escherichia coli* (MTCC 1687), *Proteus mirabilis* (MTCC 3310), *Bacillus cereus* (MTCC 0430), *Klebsiella pneumoniae* (MTCC 7028) and *Staphylococcus aureus* (MTCC 3160), then it was incubated at 37 °C for 24 h. Further, the bacterial culture was streaked over the surface of the Muller-Hinton agar plate. Using cork borer wells were made on the plates and these wells were filled with 100 μl leaf extract at different concentrations (25, 50 and 100 mg/ml). The crude extract was dissolved in 1% DMSO solution, to obtain the different concentrations. Ampicillin disk was used as positive control and these plates were kept for incubation at 37 °C for 24 h. After the incubation period, the zone of inhibition was measured (Abew, Sahile, & Moges, 2014). All experiments were performed in triplicates and average was considered as the final result.

## Antifungal activity

Antifungal activity of the methanolic crude extract was assayed by agar well diffusion method. Fungal pathogens such as *Aspergillus niger* (MTCC 3323) and *Aspergillus flavus* (MTCC 2799) were cultured and maintained in PDA (Himedia). To the prepared PDA media plate, lawn culture of the fungal pathogen were developed using a sterile cotton swab of fungal spores. Sterile cork borer were used to make wells and to these wells, different concentration of leaf extracts were added. The plates were incubated at 28 °C. The cultured plates were observed for the zone of inhibition after 48–72 h indicates the presence of antifungal activity (Ambikapathy, Gomathi, & Panneerselvam, 2011). All experiments were performed in triplicates and average was considered as the final result.

## GC-MS analysis of crude extracts

The crude extract was subjected to GC-MS analysis. The extracts were analyzed in a Perkin Elmer Clarus 680 equipped with mass spectrometer Clarus 600(EI) fitted with Elite-5MS capillary column (30, 0.5 mm ID, 250 μm df). The instrument has an oven with an initial temperature of 55 °C for 3 min and a ramp program 10 $min^{-1}$ to 300 °C, hold 6 min. With a constant flow rate of 1 ml/min, helium was used as a carrier gas. The source temperature and mass transfer line was set at 240 °C. Turbo version 5.4.2 software was used for the spectral analysis. An individual component was recognized with typical mass spectra from National Institute of Standards and Technology (NIST-LIB 0.5) libraries are In-built by the software of the GC-MS system (Wiley GC-MS-2007) and literature data (Papitha, Lokesh, Kaviyarasi, & Selvaraj, 2016). The crude extract dissolved in methanol, was directly injected into the GC-MS instrument.

## In vitro anti-inflammatory activity: egg albumin denaturation method

The 5 ml of test solution consists of 2.8 ml of phosphate buffered saline (PBS, pH 6.4), 0.2 ml of fresh hen's egg albumin and 2 ml of different concentrations of extract so that final concentrations becomes 100, 200, 300 μg/ml. The same amount of de-ionized distilled water used as a control. Then the solutions were incubated at (37 ± 2 °C) in a BOD incubator (Lab-line Technologies) for 15 min and the solutions heated at 70 °C for 5 min. After cooling, their absorbance

was measured at 660 nm. Acetyl salicylic Acid (Aspirin) was used as a standard at the varying concentration of 100, 200, 300 μg/ml were treated similarly for determination of absorbance. Calculating formula for the % inhibition of protein by the following equation (Ullah et al., 2014).

$$\%\text{Inhibition} = \text{Abs control} - \text{Abs test}/\text{Abs control} \times 100$$

where Abs control = absorbance of the control; Abs test = absorbance of the test sample.

### In-vitro anti-inflammatory activity: inhibition of albumin denaturation

The anti-inflammatory activity of *D. mahatma* methanolic extract was studied by using inhibition of albumin denaturation technique. 500 μl of 1% bovine serum albumin was added to different concentrations of plant extract. This mixture was kept at room temperature for 37 °C for 20 min, followed by heating at 51 °C for 20 min. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition of protein denaturation was calculated using following equation (Leelaprakash & Mohan Dass, 2011).

$$\%\text{Inhibition} = \text{Abs control} - \text{Abs test}/\text{Abs control} \times 100$$

where Abs control = absorbance of the control; Abs test = absorbance of the test sample.

## Results

### Qualitative analysis of phytochemicals

The results of the phytochemical screening indicate the presence of bioactive compounds such as tannins, phenols, alkaloids, sterols, triterpenes and anthraquinone glycosides; and absence of saponins and flavonoids. Table 1 shows the results of qualitative phytochemical analysis of *D. mahatma* leaf powder.

**Table 1** Qualitative phytochemical screening of *D. mahatma* leaf powder.

| Phytochemicals | Inference |
|---|---|
| Phenols | + |
| Tannins | + |
| Saponins | – |
| Flavonoids | – |
| Alkaloids | + |
| Sterols, triterpenes | + |
| Anthraquinone glycosides | + |

'+', positive; '–', negative.

**Table 2** Total phenolic content of methanol extract from *D. mahatma* leaves.

| Extract | Concentration (μg/ml) | Total phenolic content (mg of GAE/g of extract) |
|---|---|---|
| Methanol extract of *D. mahatma* leaves | 100 | 32.9 ± 0.002 |
| | 400 | 35.5 ± 0.0015 |

### Total phenolic content

Using the Folin-Ciocalteu's reagent, the total phenolic contents present in the methanolic extract was estimated. The values obtained for the concentration of total phenols are expressed as mg of GAE/g of extract. From the calibration curve equation ($y = 0.003x + 0.711$), the total phenolic content of the crude extract was estimated to be 35.5 ± 0.0015 Gallic acid equivalents/g at the concentration of 400 μg/ml (as shown in Table 2).

### DPPH radical scavenging activity

Methanolic extract of *D. mahatma* leaves demonstrated significant antioxidant property, in comparison to standard control ascorbic acid. A linear increase in the activity in relation to increasing concentration of the extract (25–100 μg/ml) was observed, similar to that of ascorbic acid. The methanolic extract of *D. mahatma* showed maximum free radical scavenging activity of 90% while ascorbic acid showed 93% at 100 μg/ml concentration. This signifies that the methanolic extract is a potent antioxidant agent (Fig. 2).

### FRAP assay

The reducing power of the methanolic extract of *D. mahatma* leaves was evaluated using Ferric Reducing Activity Power (FRAP) assay. The reduction of the $Fe^{3+}$/ferricyanide complex to the ferrous form is caused by the reducers present in the methanolic extract of the sample. There is a gradual increase in the absorbance (ferrous) value as the concentration increases. Increase in absorbance directly signifies the increase in ferrous formation. This suggests that the methanolic extract of *D. mahatma* leaves is a potential reducing agent and also has significant electron

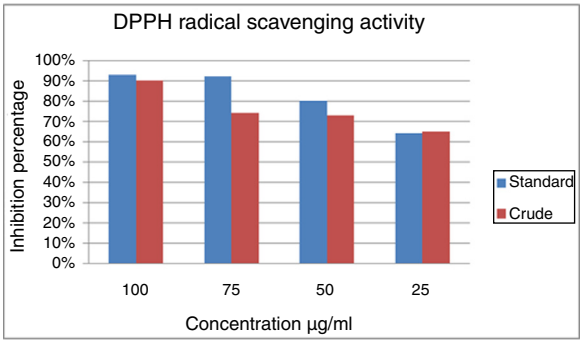



**Figure 2** DPPH free radical scavenging assay result.

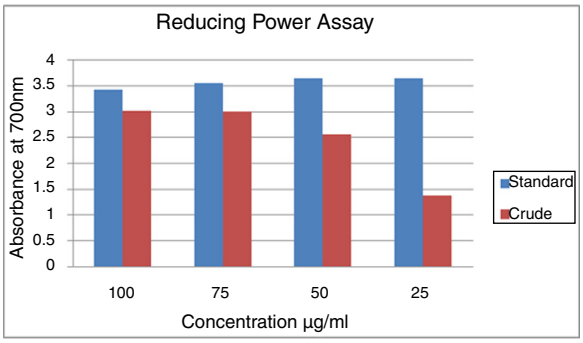



**Figure 3** FRAP assay result.

transfer potential. The result of anti-oxidant screening is graphical represented in Fig. 3.

## Antibacterial activity

The results of this study indicated that the crude solvent extracts obtained from the leaves of *D. mahatma* showed antagonism against the studied bacterial pathogens. At 10 mg/100 µl, the methanol extract had a slightly higher antibacterial activity with mean zones of inhibition of 19, 19, 18, 22 and 20 mm against *S. aureus*, *P. mirabilis*, *B. cereus*, *E. coli* and *K. pneumoniae* respectively. Among these, *E. coli* was highly susceptible to the methanolic extract with the zone of inhibition of 22 mm. Methanol extract demonstrated strong antagonism against *S. aureus*, *P. mirabilis*, *B. cereus*, *E. coli* and *K. pneumoniae* at various concentrations as tabulated in Table 3. Observed zone of inhibition against *K. pneumoniae* and *S. aureus* are shown in Fig. 4.

## Antifungal activity

Antifungal activity was determined by agar well diffusion method. The result revealed that the methanolic crude extract of *D. mahatma* showed no activity against the studied fungal pathogens (*A. niger* and *A. flavus*).

## GC-MS analysis

The extraction yielded 3 g of crude extract for every 15 g of plant sample, which is equal to 200 mg/g yield.

GC-MS analysis was done for the methanolic crude extract of *D. mahatma* to identify the chemical components present. Gas chromatogram revealed the presence of 8 known compounds and 2 unknown compounds. The identified compounds, with their reference similarity, RT, and structure are given in Table 4.

Among the 8 known compounds, propanamide, 2-hydroxy-, methanol, (methyl-onn-azoxy)-, acetate (ester) and dichloroacetaldehyde, were previously reported to have antibacterial activity in other studies.

Among the two unknown compounds, the compound at RT 23.7 min, contributed the most percentage (70.4%) of the crude extract, based on the area % analysis of GC-MS. The GC chromatogram and area percentage analysis of methanolic extract are shown in Fig. 5.

**Table 3** Zone of inhibition of methanol extract at various concentrations.

| Pathogens | Methanolic extract 2.5 mg | Methanolic extract 5 mg | Methanolic extract 10 mg | Positive control (streptomycin 10 µg) |
|---|---|---|---|---|
| *Staphylococcus aureus* (MTCC 3160) | 13 mm | 15 mm | 19 mm | 17 mm |
| *Proteus mirabilis* (MTCC 3310) | 13 mm | 14 mm | 19 mm | 24 mm |
| *Bacillus cereus* (MTCC: 0430) | 12 mm | 16 mm | 18 mm | 20 mm |
| *Escherichia coli* (MTCC: 1687) | 15 mm | 16 mm | 22 mm | 20 mm |
| *Klebsiella pneumoniae* (MTCC 7028) | 14 mm | 16 mm | 20 mm | 19 mm |

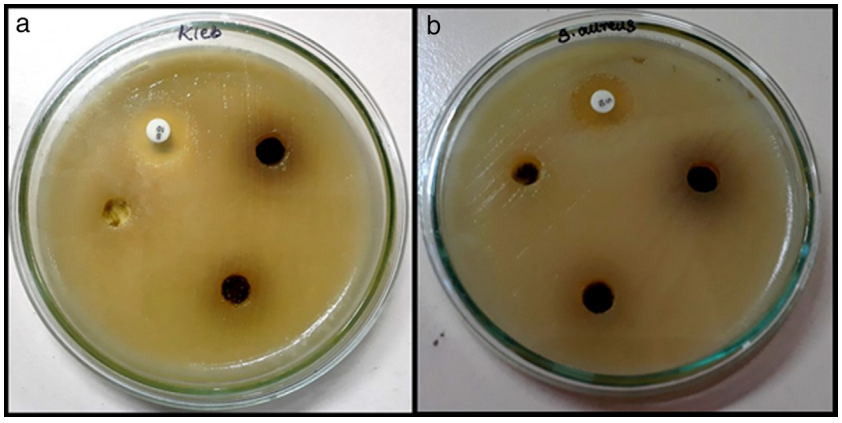
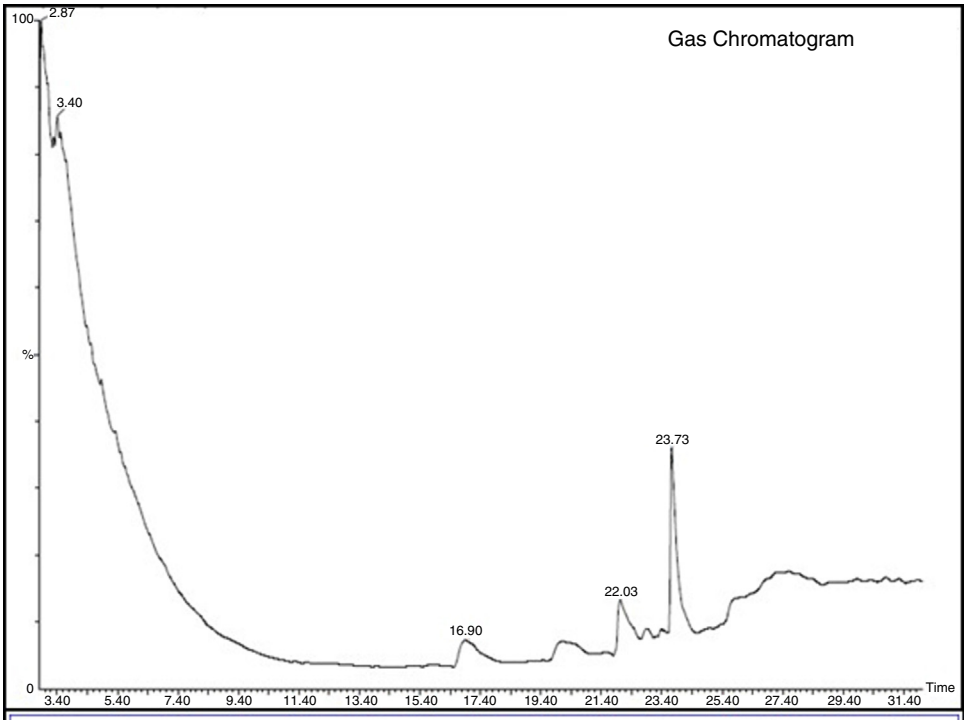
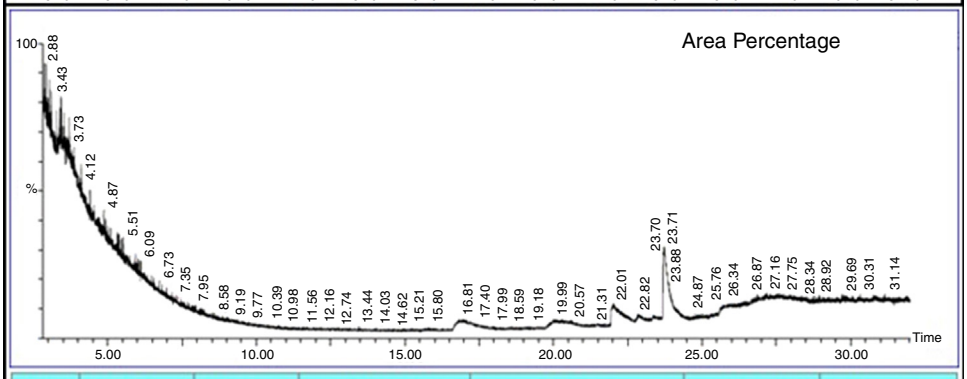



**Figure 4** Antibacterial activity of methanolic extract of *D. mahatma* leaves; (a) *K. pneumoniae*; (b) *S. aureus*.

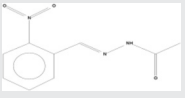
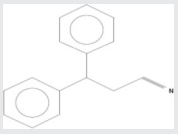



| # | RT | Scan | Height | Area | Area % | Norm % |
|---|---|---|---|---|---|---|
| 1 | 3.109 | 62 | 13,139.553 | 169,115.5 | 3.266 | 4.64 |
| 2 | 3.499 | 140 | 7,539.232 | 149,695.8 | 2.891 | 4.11 |
| 3 | 3.534 | 147 | 13,091.114 | 247,392.6 | 4.778 | 6.79 |
| 4 | 22.016 | 3842 | 5,497.414 | 966,325.4 | 18.664 | 26.51 |
| 5 | 23.722 | 4183 | 20,400.902 | 3,645,039.2 | 70.401 | 100.00 |

**Figure 5** GC-MS analysis of methanolic extract of *D. mahatma* leaves.

**Table 4** List of NIST library matches for compounds present in the crude extract.

| RT | Reverse | Forward | Compound name | Structure |
|---|---|---|---|---|
| 2.84 | 673 | 545 | Propanamide, 2-hydroxy- |  |
| 3.10 | – | – | – | Unknown |
| 3.37 | 693 | 629 | Methanol, (methyl-onn-azoxy)-, acetate (ester) |  |
| 3.50 | 675 | 375 | Dichloroacetaldehyde |  |
| 4.85 | 546 | 467 | Diisopropanol nitrosamine disodium salt |  |
| 16.88 | 504 | 365 | 1,1′-Biphenyl, 3-methyl- |  |
| 20.03 | 524 | 314 | o-Nitrobenzaldehyde acetylhydrazone |  |
| 22.90 | 568 | 370 | Benzene, 1,1′,1″,1‴-(1,6-hexanediylidene)tetrakis- |  |
| 22.01 | 666 | 302 | Benzenepropanenitrile, beta-phenyl- |  |
| 23.72 | – | – | – | Unknown |

## In vitro anti-inflammatory activity

### Egg albumin denaturation method

Egg albumin denaturation method was performed for the methanolic extracts of *D. mahatma* to determine its anti-inflammatory activity. Due to its high color intensity, the results were inconclusive thereby its anti-inflammatory activity could not be determined.

### Inhibition of albumin denaturation

The methanolic extract of *D. mahatma* was analyzed for its anti-inflammatory property by albumin denaturation methods. Maximum inhibition of 40% was observed at 100 μg/ml in its highest studied concentration. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 79% at the concentration of 100 μg/ml (Fig. 6).

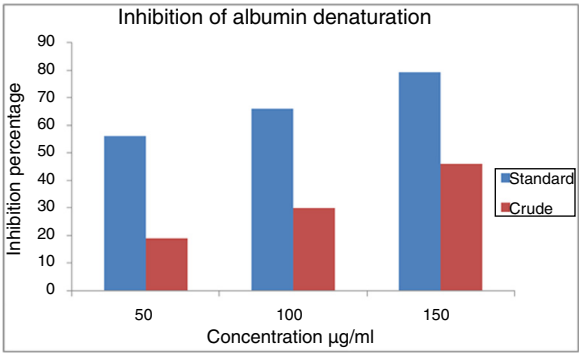



**Figure 6** Prevention of albumin denaturation assay.

## Conclusion

This is the first study on the antibacterial and anti-inflammatory activity of *D. mahatma* plant. It can be concluded from the results, that the methanolic leaf extract of *D. mahatma* showed antibacterial activity against the tested isolates (*S. aureus*, *P. mirabilis*, *B. cereus*, *E. coli* and *K. pneumoniae*). The current study on the antibacterial and anti-inflammatory activity adds consequence medicinal property to the *D. mahatma* leaves. Phytochemical analysis suggested that the leaves are rich in alkaloids, glycosides, sterols, triterpenes, phenols and tannins. GC-MS

analysis suggested the presence of potential antibacterial compounds previously reported. Also, a major constituent in the crude extract is an unknown compound that could potentially be a novel antibacterial agent. Hence, this study opens up opportunities for further study and application of *D. mahatma* leaves in antibacterial research.

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgement

The authors thank VIT University for providing all the facilities to carry out this research work.

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