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## Research Article

### ANTIBACTERIAL ACTIVITY OF FLOWER HEADS OF *WEDELIA TRILOBATA* (L.) A. S. HITCHC

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#### ABSTRACT

Present study is conducted to analyse the antibacterial activity of flower heads of *Wedelia trilobata* against pathogenic bacterial strains and to analyse phytochemical background of various extracts. Fresh flower heads collected from Kerala, India. Air dried flower heads were extracted successively in petroleum ether, chloroform, acetone, ethanol and water. Preliminary antibacterial activity was analysed by disc-diffusion method and further confirmed by MIC and MBC. Preliminary detection of phytochemicals was done. Among the ten bacterial strains nine showed considerable inhibition of growth towards acetone extract. Acetone extract selected for detailed antibacterial evaluation tests like minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). The MIC and MBC of floral extracts tested against one of the most sensitive strains, *Streptococcus haemolyticus*. MIC and MBC values were 25 mg/ml and 50 mg/ml respectively. Preliminary phytochemical evaluation revealed the presence of flavonoids, phenolics and terpenoids in active acetone extract. The present investigation showed the effectiveness of crude extract of this plant against tested bacterial strains. The presence of potential phytochemicals like phenolics, flavonoids and terpenoids in active acetone extract might be one of the reasons for its antibacterial property.

**Keywords:** *Wedelia trilobata*; antibacterial activity; disc diffusion; phytochemicals.

#### INTRODUCTION

*Wedelia trilobata* (L.) A.S. Hitchc, belongs to the family Asteraceae, its common name is Singapore Daisy, synonym is *Silphium trilobatum* L. The plant is herb, introduced as garden plant, now runs wild and observed as weed. Plant is distributed in all districts of Kerala, India. The plant is a native of Tropical America<sup>1</sup>. Antioxidant and antibacterial activities of methanolic extract of flowers of *W. trilobata* were reported<sup>2</sup>. Essential oils isolated from *W. trilobata* showed antioxidant activity<sup>3</sup>. Ethanolic extract of *W. trilobata* leaves exhibited antioxidant activity, also indicated its potential in wound healing<sup>4,5</sup>. Bioactive compounds (ent-kaura-9(11), 16-dien-19-oic acid) from ethanolic extracts of *W. trilobata* leaves that could influence wound healing<sup>6</sup>. Anthelmintic activity of the various extracts obtained from the leaves of *W. trilobata* showed significant and dose dependant activity compared to standard drug albendazole, amongst them petroleum ether extract showed better activity<sup>7</sup>. Crude extracts from *W. trilobata* showed antibacterial activity against gram-positive and gram-negative bacteria. None of the tested extracts showed biological activity against fungi<sup>8</sup>. Wedelolides A and B: Novel Sesquiterpene  $\delta$ -Lactones, (9R)-Eudesman-9, 12-olides, from isolated from *W. trilobata*<sup>9</sup>. Aqueous extracts of root, stem, leaf and the whole plant of *W. trilobata* showed allopathic potential on rice<sup>10</sup>. The present investigation was carried out to analyse antibacterial activity and phytochemical evaluation of flower heads of *W. trilobata*.

#### MATERIALS AND METHODS

##### Collection of Plant Materials

Fresh specimens (flower heads) of *Wedelia trilobata* (L.) A. S. Hitchc, (Asteraceae) collected from Pala of Kottayam district Kerala, India in month of December 2013. The plant was authenticated by Department of Botany, St. Thomas College, Palai, Kerala, India, where a voucher specimen (RS- 5876) was deposited.

##### Preparation of Extract

Air dried flower heads were powdered well and used for preparing extracts. 50 g of powdered material extracted in petroleum ether, chloroform, acetone, ethanol and water in the gradation of increasing polarity by keeping the powder in each solvent for 24 hours<sup>11</sup>. The extraction provided yield of 0.5 %, 1.5 %, 2.5 %, 2.3 % and 1.2 % in petroleum ether, chloroform, acetone, ethanol and water respectively.

##### Bacterial Strains

Test organisms include *Vibrio parahaemolyticus*, *Salmonella typhi*, *Bacillus cereus*, *Salmonella paratyphi*, *Streptococcus haemolyticus*, *Proteus vulgaris*, *Serratia marcescens*, *Proteus rettgeri*, *Staphylococcus aureus* and *Escherichia coli*. The species that were not purchased, but originally isolated from clinical materials collected from patients and identified using standard biochemical tests. The bacterial strains were maintained on nutrient agar slants at 4°C in the refrigerator to maintain the stock culture.

**Antibacterial Test (Disc-diffusion Method)**

The disc-diffusion method as described by Bauer *et al.*<sup>12</sup> was used to determine the growth inhibition of bacteria in plant extract. Sterile liquid Muller Hinton agar media was transferred into sterile petridish and after solidification; the bacteria were swabbed with a sterile swab under aseptic conditions. Sterile discs prepared using Whatman no. 1 filter paper, of 5mm diameter were used in the study. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 500 mg/ml. 10 µL of the solution was loaded per disc to attain a concentration of 5 mg/disc. The discs (including control) were used after drying them in an incubator at 50°C to remove any trace of solvent. Discs were introduced to the medium. The plates were incubated overnight at appropriate incubation temperatures. Microbial growth was conducted in more than three replicates and average inhibitory zone diameter along with standard deviation was recorded. The diameter of the inhibitory zone around each disc was measured. Also sterile disc impregnated with solvents alone were used as control. For each organism average inhibitory zone diameter was determined.

**Confirmation of Antibacterial Activity of Active Extract**

Antibacterial activity of active extracts was confirmed by determining Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC). The solidified extract was dissolved in DMSO to make sample concentration of 100 mg/ml

**Minimum Inhibitory Concentration (MIC)**

The MIC of the extracts was performed by incorporating various amounts (100–0.78 mg/ml) of the extract into sets of test tubes with the culture media. 50 µL of the bacterial broth culture was added into each of the test tubes. The bacterial cultures containing the plant extracts were incubated at 37°C for 24 hours. Test tube containing only the growth medium and each of the organisms was also incubated under the same conditions as positive controls. The minimum inhibitory concentration was expressed as the lowest concentration of the extracts that did not permit any visible growth when compared to that of the control tubes<sup>13</sup>.

**Minimum Bactericidal Concentration (MBC)**

Samples from the tubes in previous studies, which did not show any visible growth after a period of incubation, were sub cultured onto a freshly prepared nutrient medium. The minimum bactericidal concentration was taken as the lowest concentration of the extract that did not yield a single colony<sup>14</sup>.

**Preliminary Phytochemical Analysis of the Extract**

The crude samples were subjected to preliminary phytochemical screening for the presence of alkaloids, phenolics, Terpenoids, flavonoids using the method of Harborne<sup>15</sup>.

**RESULTS**

Antibacterial activity and phytochemical analysis of *Wedelia trilobata* (L.) A.S. Hitchc were conducted using

petroleum ether, chloroform, acetone, ethanol and water as extracting solvents in the gradation of increasing polarity. Out of ten bacterial strains tested, *Serratia marcescens* did not show any kind of susceptibility to any of extracts. Nine pathogenic strains showed sensitivity towards petroleum ether and acetone extracts (Table 1). Chloroform extract showed antimicrobial activity towards four pathogenic strains such as *Vibrio parahaemolyticus*, *Streptococcus haemolyticus*, *Escherichia coli* and *Staphylococcus aureus*. Maximum antibacterial activity observed in acetone extract. The extract was highly effective against *Streptococcus haemolyticus*. Ethanol extract showed antibacterial activity towards five bacterial strains such as *Vibrio parahaemolyticus*, *Streptococcus haemolyticus*, *Proteus rettgeri*, *Proteus vulgaris* and *Staphylococcus aureus*. *Streptococcus haemolyticus* and *Staphylococcus aureus* showed susceptibility towards water extract of flower heads of the plant. While all other bacteria showed resistance towards various extracts. The Acetone extract was selected for confirmatory antibacterial evaluation tests like Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) towards *Streptococcus haemolyticus*. The observed MIC and MBC values towards *Streptococcus haemolyticus* were 25 mg/ml and 50 mg/ml respectively. Preliminary phytochemical evaluation revealed the presence of alkaloids, flavonoids, phenolics and terpenoids. Alkaloids were detected in chloroform and ethanol extracts (Table 2). Flavonoids were found in petroleum ether, chloroform, acetone and ethanol extracts; while water extract was free of alkaloids, flavonoids and terpenoids. Terpenoids were detected in chloroform and acetone extracts.

**DISCUSSION**

In this work, antibacterial activity of flower head extract of *W. trilobata* demonstrated against a wide range of bacterial strains. The highest antibacterial activity is being observed against *Streptococcus haemolyticus*. In recent years multi drug resistant bacteria make therapeutic problems. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects. But side effects are often associated with synthetic antimicrobial compounds. Pharmaceutical companies are now looking for other natural alternative<sup>16</sup>. Plants are important source for the development of potential new chemotherapeutic drugs. The broad spectrum of antibacterial activities of the plant extract, possibly due to the identified alkaloids, flavonoids, terpenoids etc. and confirm its use as a health remedy in folklore medicine<sup>17</sup>. The antibacterial activity of various plant extracts may be due to the presence of various active principles like alkaloids, terpenoids etc. The bioactivity of plant extracts is attributed to phytochemical constituents for example, alkaloids isolated and characterized from *Alstonia rupestris* with cytotoxic, antibacterial and antifungal activities<sup>18</sup>. Alkaloids isolated from plant are commonly found to have antibacterial and antioxidant properties<sup>19</sup>. Terpenoids isolated from plants have antibacterial properties<sup>20</sup>. Alkaloids, phenols, and flavonoids were observed in various extracts of flower heads of *W. trilobata*.

**Table 1: Antibacterial activity of flower heads of *Wedelia trilobata* towards various bacterial strains**

Bacterial strains	Zone of inhibition diameter (mm)				
	Extracts used (Concentration - 5 mg/disc)				
	Petroleum ether	Chloroform	Acetone	Ethanol	Water
<i>Vibrio parahaemolyticus</i>	11 ± 0.2	10 ± 0.26	14 ± 0.04	13 ± 0.53	---
<i>Salmonella paratyphi</i>	10 ± 0.32	---	13 ± 0.71	---	---
<i>Bacillus cereus</i>	9 ± 0.5	---	10 ± 0.04	---	---
<i>Salmonella typhi</i>	10 ± 0.05	---	11 ± 0.20	---	---
<i>Streptococcus haemolyticus</i>	20 ± 0.42	26 ± 0.08	24 ± 0.41	22 ± 0.05	22 ± 0.28
<i>Proteus vulgaris</i>	9 ± 0.22	---	13 ± 0.13	10 ± 0.07	---
<i>Serratia marcescens</i>	---	---	---	---	---
<i>Proteus rettgeri</i>	13 ± 0.12	---	14 ± 0.49	8 ± 0.63	---
<i>Staphylococcus aureus</i>	11 ± 0.57	17 ± 0.31	16 ± 0.27	9 ± 0.09	8 ± 0.06
<i>Escherichia coli</i>	13 ± 0.08	9 ± 0.22	12 ± 0.09	---	---

Values : Mean ± Standard Deviation (n = 4)

**Table 2: Phytochemical constituents of flower heads of *Wedelia trilobata***

Extract used	Alkaloids	Phenolics	Flavonoids	Terpenoids
Petroleum ether	-	+	+	-
Chloroform	+	-	+	+
Acetone	-	+	+	+
Ethanol	+	+	+	-
Water	-	+	-	-

Values : + present, - absent

Active acetone extract showed the presence of phenolics, flavonoids and terpenoids. The antibacterial activity of the active acetone extract of the plant might be due to the presence of these phytochemicals. The present result of antibacterial activity of flower heads of the plant as herbal medicine supported the earlier investigation of antimicrobial activity of the plant reported by Taddei and Rosas-Romero<sup>7</sup>. Similar result of antibacterial activity was reported from the plant extract by Govindappa *et al*<sup>21</sup>. The present study revealed that acetone extract possessed wide range of antibacterial activity towards gram positive and gram negative bacteria. Therefore acetone extract can be explored for isolating and characterising antibacterial principles involved in antibacterial activity.

## CONCLUSION

Flower heads of *Wedelia trilobata* was evaluated for its antibacterial threshold and phytochemical contents in various extracts prepared in the gradation of increase in polarity towards pathogenic bacteria. The flower heads of the plant exhibited antibacterial activity in acetone extract. The acetone extract of the plant showed maximum level of activity towards *Streptococcus haemolyticus*. *Streptococcus haemolyticus* showed maximum level of susceptibility towards various extracts. The presence of phenolics, flavonoids and terpenoids in active acetone extract; acetone extract exhibited minimum inhibitory concentration as 25 mg/ml and minimum bactericidal concentration as 50 mg/ml towards *Streptococcus haemolyticus*.

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