

## Analysis of Phytochemical Constituents And Antimicrobial Activities of *Wedelia Chinensis* Against Pathogens



### Zoology

**KEYWORDS :** *Wedelia chinensis*, Antimicrobial, Phytochemical

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### ABSTRACT

The present study was to evaluate the antimicrobial properties of solvent extracts from leaf and stem of *Wedelia chinensis*. The ethylacetate, acetone, methanol and hexane extract of medicinal plant were evaluated against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* by using well diffusion assay. The leaf extract showed maximum antimicrobial activity against *Bacillus subtilis* ( $16.4 \pm 0.7$ ) and lowest activity against *Escherichia coli* ( $9.4 \pm 0.6$ ) compared with ciprofloxacin. The stem extract showed maximum antimicrobial activity against *Staphylococcus aureus* ( $15.3 \pm 0.8$ ) and lowest activity against *Pseudomonas aeruginosa* ( $6.3 \pm 0.5$ ) compared with ciprofloxacin (10mcg). The antimicrobial activity could be confirmed in most species used in traditional medicine in South India. Nevertheless, traditional knowledge might provide some leads to elucidate potential candidates for future development of new antibiotic agents.

### 1. Introduction

Medicinal plant are still major parts of traditional medicinal systems in developing countries many infectious disease are known to be treated with herbal remedies throughout the history of mankind. Even today plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Sukanya *et al.*, 2009). Medicinal plants which from the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. Human infections particularly those involving microorganisms i.e. bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world (Gupta *et al.*, 2009). The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds (Edeoga *et al.*, 2005).

*Wedelia chinensis* is a perennial herb of family Asteraceae, commonly known as "Pilabhamgara" or "Bhringraj" in Hindi, *Wedelia* in Chinese and "Manjalkarisalanganni" in Tamil (Chopra *et al.*, 1956). Traditionally, the fruits, leaves and stem are used in child birth and in the treatment of bites and stings, fever and infection. The leaves are used in the treatment of kidney dysfunction, cold, wounds and amenorrhea (Parihar *et al.*, 2006). Decoction of the plant is used in menorrhagia and skin diseases (Saxena *et al.*, 1986). The aim of this study was to evaluate the Antimicrobial effect of *W.chinensis* leaf and stem extract upon the various Gram-positive and Gram-negative bacteria. Therefore, simultaneous determination of the compounds those are possibly responsible for any biological activity would facilitate decision-making process as in the selection of the plants for in-depth future investigation.

### Materials and Methods

#### Plant Collection and identification

The leaves and stem of *Wedelia chinensis* (Asteraceae) were collected from Auxilium College campus, Katpadi, Vellore. The taxonomic identification was made by Ms. Isabella Rosaline, S., M.Sc., M.Phil. (Ph.D.) Department of Botany, Auxilium College, Katpadi, Vellore.

#### Preparation of extracts

The fresh leaves and stem of *Wedelia chinensis* were thoroughly washed to remove debris and the earth remains. From these the stem were divested and chopped into bits and al-

lowed to dry for 7-14 days under shade at the room temperature. The 300g of dried leaves and stem were weighed using an electronic weighing balance and powdered by using electrical grinder. The powder was soaked in hexane (1200ml), acetone (1200ml), ethylacetate (1200ml) and methanol (1200ml). The powder soaked in solvents for three days. The extracts were filtered through a Buchner funnel with Whatmann's number 1 filter paper. Then the filtered sample was poured into a soxhlet apparatus (boiling point range 100°C). After 2 hours the filter paper was concentrated at room temperature until oily paste formed and kept at cool dry place for further use.

### Determination of Antimicrobial activity

#### Well diffusion method

The antimicrobial activity was tested against solvent ethyl acetate, acetone, methanol and hexane extracts of *Wedelia chinensis*. The inoculation of microorganism was prepared from bacterial culture (Mathew, 1983). The inoculums suspension was spread uniformly over the agar plates using spreader, for uniform distribution of bacteria. Subsequently, using a sterile borer, well of 0.5cm diameter was made in the inoculated media in addition to 0.2ml of each extract was aseptically filled into the well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24h at 37°C for room temperature. Ciprofloxacin (10 mcg) was used as control. The results were recorded by measuring the diameter of inhibition zone. Zone of inhibition surrounding the discs was measured using a transparent ruler and the diameter was recorded in mm.

#### Phytochemical Analysis

The TLC studies showed that among the four solvents (hexane, ethylacetate, acetone and methanol) used for extraction, the high polarity solvent methanol extracted higher quantity of secondary metabolites of medicinal importance viz., alkaloids, flavonoids, phenols, tannins, chlorophyll and carotenoids from the leaves and stems of *Wedelia chinensis*. Suitable mobile phases with appropriate proportion were determined for the methanol extract. The combination of ethylacetate and hexane stem extract contained the compounds namely phenols, tannins and chlorophyll. The alkaloids, flavonoids and carotenoids were absent.

#### Statistical Analysis

Data were expressed as mean  $\pm$  standard deviation. The leaf

extract showed maximum antimicrobial activity against *Bacillus subtilis* and lowest activity against *Escherichia coli* compared with ciprofloxacin. The stem extract showed maximum antimicrobial activity against *Staphylococcus aureus* and lowest activity against *Pseudomonas aeruginosa* compared with ciprofloxacin.

**Results and Discussions**

Results of inhibition zones in the well diffusion assay using solvents ethylacetate, acetone, methanol and hexane extract of *Wedelia chinensis*. The leaf extracts (Table 1) showed maximum antimicrobial activity against *Bacillus subtilis* (16.4±0.7) and lowest activity against *E.coli* (9.4± 0.6) compared with ciprofloxacin. The stem extract (Table 2) showed maximum antimicrobial activity against *S. aureus* (15.3±0.8) and lowest activity against *Pseudomonas aeruginosa* (6.3±0.5) compared with ciprofloxacin (10mcg).

In the previous study examined the *invitro* antibacterial activities of various organic solvent extracts (petroleum ether, chloroform, ethyl acetate and methanol) of *Wedelia chinensis* belonging to the Asteraceae family. The extracts were tested against fifteen (both Gram positive and Gram negative) bacteria using disc diffusion method. The susceptibility of the test

microbes varied with the types of solvents used. All the extracts showed sufficient inhibitory activity to the test strains. Among the solvents, methanol showed strong antimicrobial activity. Ethylacetate and chloroform extracts were found to have moderate effect, whereas, petroleum ether extract possessed least activity on the test bacteria. The most susceptible Gram positive bacterium was *Streptococcus faecalis* (26.27Å±0.15mm), while the most susceptible Gram negative bacterium was *Pseudomonas aeruginosa* (22.47Å±4.20mm). The Gram positive strain *Streptococcus faecalis* was more sensitive to the extracts (range 26.27-9.67mm) than the Gram negative strains (range 22.47-9mm).

**Conclusion**

In conclusion, Asteraceae plants have a definite role to play in the health care system. Antimicrobial activity of these medicinal plant, if translated into clinical practice would lead to the development of indigenous, chemical free, cost effective. The leaves are used in the treatment of kidney dysfunction, cold, wounds and amenorrhoea. Decoction of the plant is used in menorrhagia and skin diseases with continued growth of biotechnology and increasing tools for validation of the bioactive compounds, the potential is high that one day our food will serve as medicine.

**Table 1**  
Antimicrobial activity of *Wedelia chinensis* leaf extracts against microorganisms by agar well diffusion method.

S. No	Test organisms	Methanol extract	Acetone extract	Hexane extract	Ethylacetate extract	Control (Ciprofloxacin) (10mcg)
1.	<i>Bacillus subtilis</i>	13.5±0.5	10.3±0.7	16.4±1.0	14.0±0.6	17.0±0.6
2.	<i>Staphylococcus aureus</i>	14.6±0.8	13.5±1.2	13.0±0.5	14.2±0.8	18.3±0.4
3.	<i>Klebsiella pneumonia</i>	13.3±0.6	11.6±0.7	11.3±0.6	12.0±0.5	15.4±0.8
4.	<i>Pseudomonas Aeruginosa</i>	14.0±0.7	15.3±1.2	15.3±0.7	16.0± 0.4	18.5±0.5
5.	<i>Escherichia coli</i>	15.4±0.6	10.0±0.8	10.5±0.5	9.4± 0.6	15.4±0.6

Values are expressed as mean ± standard deviation of the three replicates.

**Table 2**  
Antimicrobial activity of *Wedelia chinensis* stem extracts against microorganisms by agar well diffusion method.

S. No	Test organisms	Methanol extract	Acetone extract	Hexane extract	Ethylacetate extract	Control (Ciprofloxacin) (10mcg)
1.	<i>Bacillus subtilis</i>	14.0±0.6	7.3±1.2	7.0±0.8	8.0±0.4	15.4±0.8
2.	<i>Staphylococcus aureus</i>	15.0±0.8	15.0±1.2	10.0±0.5	14.0±0.8	16.4±0.5
3.	<i>Klebsiella pneumonia</i>	14.4±0.8	7.3±0.6	12.0±0.4	15.0±0.5	16.3±0.6
4.	<i>Pseudomonas Aeruginosa</i>	7.0±0.3	8.3±0.7	6.3±0.5	14.0±0.4	15.0±0.4
5.	<i>Escherichia coli</i>	7.0±0.5	14.0±0.7	9.3± 0.5	6.5± 0.4	14.0±0.6

Values are expressed as mean ± standard deviation of the three replicates.

**Table 3**  
Phytochemicals analysis of different solvent leaf extracts of *Wedelia chinensis*.

Phytoconstituents	Leaf solvent extract			
	Hexane	Ethylacetate	Acetone	Methanol
Alkaloids	+	-	+	-
Flavonoids	-	-	-	+
Phenols	+	-	-	+
Tannins	+	-	+	+
Chlorophyll	-	+	+	-
Carotenoids	+	+	+	+

Note: + = Present - = Absent

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