Evaluation of antibacterial and antifungal efficacy of *Wedelia chinensis* leaf extracts

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ABSTRACT

The main aim of this study is to determine the antibacterial and antifungal activities of ethanol and hexane extracts of *Wedelia chinensis* leaf against both pathogenic bacteria and fungi by agar disc diffusion method. Both the extract showed significant antibacterial activities against four gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*) and four gram-negative (*Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*) bacteria. The antifungal assay was done against four fungi (*Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Alternaria alternata*). For all the cases it showed significant result. Thus this plant can be used as phytomedicine to treat the bacterial and fungal diseases.

**Keywords:** *Wedelia chinensis*; ethanolic and hexane extract; antibacterial; antifungal.

INTRODUCTION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases [1]. Human infections particularly those involving microorganisms i.e., bacteria, fungi, viruses, nematodes cause serious damages in tropical and subtropical countries of the world [2]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [3,4]. Medicinal plants are the richest source for strong antimicrobials. 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 [5].

*Wedelia chinensis* is a perennial herb of family Asteraceae, commonly known as “Pilabhamgara” or “Bhringraj” in Hindi, *Wedelia* in Chinese and “Manjalkarisalanganni” in Tamil [6]. 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 [7]. Decoction of the plant is used in menorrhagia and skin diseases [8,9]. The plant has also found its use in inflammations, helminthic diseases and liver disorders [10]. The plant is scientifically reported to possess antioxidant property which indicates its usefulness in reducing anxiety and stress in emotional conditions [11].

The aim of this study was to evaluate the bactericidal and antifungal effect of *W. chinensis* leaf extract upon the various gram-positive, gram-negative bacteria and fungi.
EXPERIMENTAL SECTION

Collection of plant material
The plants were collected surroundings of Chennai, India. The fresh leaves were rinsed with distilled water, shade dried and powdered using electric grinder. The fine leaf powder was transferred into sterile, air-tight container and stored for future use.

Preparation of extracts
500 gm of dried powder of *W. chinensis* was packed in round bottom flask extraction using 750ml of ethanol and hexane. The extraction was conducted for a period of 24 h. At the end of the extraction the solvent was concentrated under reduced pressure and the crude extract was stored in refrigerator.

Test organism
The test microorganisms used for the investigation, four gram-positive bacteria were *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus* and four gram-negative bacteria were *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, the four fungal strains were *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans*, *Alternaria alternata*. The pure cultures were maintained in nutrient agar (for bacteria) and potato dextrose agar (for fungi) slant at 4°C.

Inoculums preparation
Ten ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria and fungi were added into the tube and vortex was done. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be in $10^8$ ml$^{-1}$ and then plated out as inoculums [12].

Evaluation of bactericidal effects
The bactericidal effect of the extracts was evaluated with the modified Kirby-Bauer disc diffusion method [13]. Using a sterilized swab, aliquots from each tube were spread on dishes with Muller-Hinton agar (Hi-Media), disc with plant extract was plated and incubated at 37°C for 24 hours. As negative control, discs were soaked with each solvent and used. Each sample was used in triplicate for the determination of antibacterial activity. The diameter of zone of inhibition was measured in mm.

Evaluation of fungicidal effects
The antifungal activity of the extract was tested by disc diffusion method [14,15] against the three pathogenic fungi. Then each fungal culture was swabbed on PDA plate and the plant extract discs were plated. Blank disc impregnated with both solvent followed by drying off was used as negative control. The activity was determined after 72 h of incubation at room temperature (37°C). The diameter of zone of inhibition produced by the extract was measured. Each sample was used in triplicate for the determination of antifungal activity.

RESULTS AND DISCUSSION

The antibacterial and antifungal activity of ethanolic and hexane extract of *Wedelia chinensis* leaf has been evaluated in vitro against four gram-positive, gram-negative bacterial and four fungal species. The ethanolic extract showed significant result than hexane extract against all the test samples. This high potential activity indicates that the presence of broad spectrum antimicrobial compounds in the polar extract than the non-polar extract.

Antibacterial screening
The antibacterial activity of ethanolic and hexane extract against gram-positive is represented in Figure 1. The result showed that among the gram-positive bacteria, ethanolic extract revealed highest activity against *M. luteus* of 25mm where minimum activity against *B. cereus* of 20mm. The hexane extract also showed highest activity against *M. luteus* with clear zone of 20mm and minimum against *B. subtilis* (17mm).
Figure 1. Antibacterial effect of *W. chinensis* against gram-positive bacteria

Figure 2 shows the bactericidal effect against gram-negative bacteria. The ethanolic plant extract showed highest clear zone of 23mm against *E. coli* and lowest against *K. pneumonia* of 17mm of zone of inhibition. The hexane extract was showed maximum bactericidal effect against *S.typhimurium* and *K. pneumonia* of 17mm and minimum effect against *P.aeruginosa* of 14mm. Thus both the solvent extract showed same result against *K. pneumonia*.

Antifungal screening
The ethanolic extract of *W.chinensis* possesses strong antifungal properties. This extract was effective against all four fungal species. The plant showed highest inhibition against *C.albicans* of 24mm, while almost similar result exhibited against *A.flavus* and *A.niger* of 20mm and 21mm respectively. The fungicidal effect was moderate against *A.alternata* of 22mm among the tested fungi. But in case of hexane extract the zone of inhibition was maximum of
15mm against *A. niger* and *C. albicans*. The minimum antifungal activity was observed against *A. alternata* of 10mm by the hexane extract.

![Figure 3. Antifungal effect of *W. chinensis* against different fungal strain](image)

In conclusion, the present work confirms the ethanolic extract of *Wedelia chinensis* leaf showed strong activity against gram-positive, gram-negative bacteria and fungi than the hexane extract. The observations justify the ethanobotanical approach in studying the bioactive compound responsible for it.

**REFERENCES**