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# Phytochemical screening and *in vitro* antibacterial activity of ethanol and aqueous extracts of *Dregea volubilis* leaves

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**ABSTRACT**

The study was carried out to evaluate the qualitative analysis of phytochemicals and *in vitro* antibacterial activity of *Dregea volubilis* (L. f.) Benth. *ex* Hook. f. [Syn. *Wattakaka volubilis* (L. f.) Stapf., *Marsdenia volubilis* (L. f.) Cooke and *Schollia volubilis* (L. f.) Jacq. *ex* Steud.] (Family: Asclepiadaceae) leaf from its ethanol (continuous hot percolation by ethanol in soxlet apparatus) and aqueous (cold maceration in distilled water for 72 hours) extract. Dried ethanol extract was subjected to preliminary phytochemical screening for the presence of different phytoconstituents and was found to have saponins, coumarins and phytosterols. Ethanol and aqueous leaf extract (ELE and ALE respectively) were subjected to antibacterial screening by disc diffusion method against three gram positive and six gram negative bacteria. ELE was found to be active whereas ALE to be inactive.

**Key words:** ALE, antibiotic, antibacterial, Disc diffusion, *Dregea volubilis,* ELE, *Marsdenia volubilis, Schollia voubilis, Wattakaka volubilis.*

## INTRODUCTION

The spread of multi-drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases[1]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to screening of several medicinal plants for their potential antimicrobial activity[2]. Hence, newer herbal antibacterial compounds from plants and their semi-synthetic derivatives to overcome the resistance are under investigation[3].

Alcoholic and hydro-alcoholic extracts of *Dregea volubilis* were reported to have anti-inflammatory, analgesic, anti-hyperlipidemic and hypoglycaemic activity in alloxan-induced diabetic rats[4, 5, 6]. A HPTLC method for quantification of Aeridin (2, 7-dihydroxy-1, 3-dimethoxy-9, 10-dihydrophenanthropyran), responsible for anti-inflammatory activity in the plant was developed and standardized[7]. Recently, an unusual novel triterpenoid ether, multiflor-7-ene-12, 13-ether and a new multiflor -7-ene-12$\alpha$-ol were also isolated and characterized[8]. Three novel polyoxypregnane glycosides, volubiloside A, B and C (1–3), were isolated from the flowers of *Dregea volubilis*[9].

The aim of the present study is to evaluate the anti-bacterial activity of *Dregea volubilis* leaf against various bacterial strains.

## MATERIAL AND METHODS

### Plant material

*Dregea volubilis* leaves were collected from Veravaram, East Godavari district, Andhra Pradesh, India. The plant was authenticated by scientist P. V. Prasanna in Botanical Survey of India, Deccan Regional Centre, Hyderabad and incorporated in the herbarium with voucher specimen number assigned as 000834. The leaves were washed with fresh water and dried under shade at room temperature. The leaves were powdered and stored. 60 gm of powdered drug was extracted separately

with ethanol and water by continuous hot percolation in soxlet apparatus and cold maceration for 3 days respectively. Both the extracts were filtered and evaporated using a rotary evaporator. Dried extracts (ELE 12.98 %w/w and ALE 41.65 % w/w) were stored at $20^0$C until used.

**Phytochemical screening**

Dried ethanol extract was subjected to screen for the presence of different phytoconstituents like amino acids, protein, lipid, anthraquinones, flavonoids, coumarins, saponins, cyanogenetic & cardioactive glycosides, tannins, catechin, phytosterols, alkaloids, gum and mucilage.

**Test organisms**

The test gram positive and negative organisms used were *Staphylococcus aureus* ATCC BAA 1026, *Staphylococcus warneri ATCC* 27836 & *Bacillus subtilis* ATCC 11774 and gram negative bacteria *Escherichia coli* ATCC 10536, *Acinetobacter baumannii* ATCC BAA 1794, *Pseudomonas putida* ATCC 700007**,** *Pseudomonas aeruginosa* ATCC 10662, *Proteus mirabilis ATCC* 14153 & *Klebsiella pneumoniae* ATCC 33495. All strains were collected from Microbes Speciality Culture Lab., above Dhanwantari blood bank, Near China Anjaneyaswamy Temple, Danavaipeta, Rajahmundry, East Godavari District- 533 103, Andhra Pradesh, India.

**Determination of antibacterial activity**

The test strains were suspended in nutrient broth (Human Diagnostic and Surgichem, Kolkata) and incubated for 24 hours at 37°C[10]. Each extract was dissolved in its respective solvent to get a concentration of 1 gm/ml as stock solution. Stock solutions were sterilised using filtration sterilisation technique (Cellulose nitrate membrane filter, 0.45 µm, Whatman International Ltd., Maidstone, England) and kept separately for use. Sterile Whatman no. 1 filter paper discs of 6 mm diameter were loaded with 5 and 10 µl of each stock solution[10, 11]. All paper discs were allowed to evaporate before inoculation[12]. ELE and ALE loaded discs of both concentrations were inoculated separately to nutrient agar (Qualigens Fine Chemicals, Mumbai) of each plate previously swabbed with same bacterial cell suspension using sterile cotton swab. Positive control for every plate was performed by loading 10 µl of antibiotic solution (1 mg/ml) per disc [Ampicillin for *E. Coli, A. baumanii, P. aeruginosa, P. putida* & *P. mirabilis* Streptomycin for *K. Pneumoniae* & *B. Subtilis* and Amikacin for *S. aureus* & *S. warneri*][13, 14]. Negative controls were served by loading 10 µl of corresponding solvent (ethanol and sterile distilled water) for every plate under each group of extract. Each organism for both ALE & ELE under a separate method without negative control disc was screened in a plate having discs of standard antibiotic (10 µg/disc) and three different concentrations of extract (5, 7.5 and 10 mg/disc) after drying. Plates were incubated overnight (18 hours) at 37°C. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of disc)[12].

## RESULTS

Ethanol extract was found to have saponins, coumarins and phytosterols. Inhibition zone is expressed separately for both the methods with and without negative control in Table 1 and Table 2 respectively. Some plates showing the inhibition zones are presented in Figure 1, 2 & 3 and Figure 4, 5 &6 for the methods with and without negative control respectively.

## DISCUSSION

Ethanol leaf extract showed significant antibacterial activity against both gram positive and negative strains whereas aqueous leaf extract was found to be inactive. In the method without negative control, the highest inhibition zone was 18 mm against *P. putida* followed by 17 mm against *P. aeruginosa* responsible for pneumonia, septic shock, urinary tract infection, gastrointestinal infection, skin & soft tissue infection especially in burns etc. and 15 mm against *S. aureus* responsible for staphylococcal scalded skin syndrome, furuncles, carbuncles, septic arthritis, atopic dermatitis, endocarditis, toxic shock syndrome, pneumonia, food poisoning & mastitis in cow. In the method with negative control, the highest inhibition zone was 17 mm against *P. putida* followed by 15 mm against *P. auruginosa* and 14 mm against *A. baumannii* responsible for pneumonia and severe

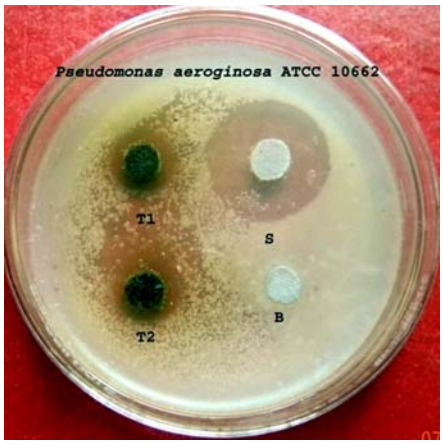


**Fig. 1: S=ampicillin 10 µg/disc, T1=ELE 5 mg/disc, T2=ELE 10 mg/disc and B= blank ethanolic disc**

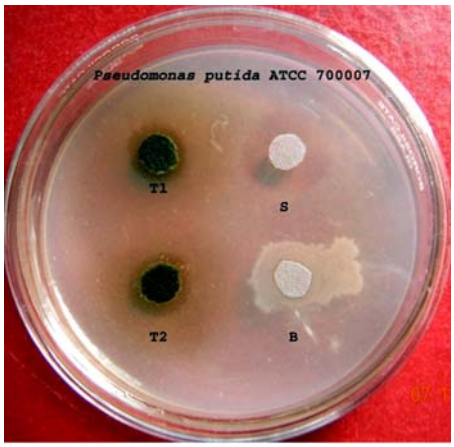


**Fig. 2: S=ampicillin 10 µg/disc, T1=ELE 5 mg/disc, T2=ELE 10 mg/disc and B= blank ethanolic disc**

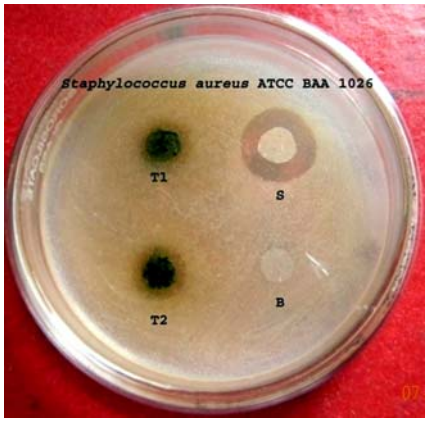


**Fig. 3: S= amikacin 10 µg/disc, T1=ELE 5 mg/disc, T2=ELE 7.5 mg/disc and T3= 10 mg/disc**

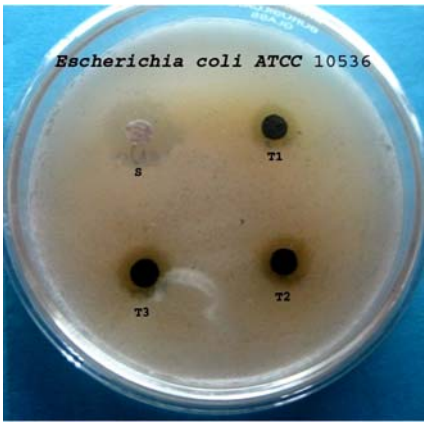


**Fig. 4: S= ampicillin 10 µg/disc, T1=ELE 5 mg/disc, T2= 7.5 mg/disc and T3=ELE 10 mg/disc**

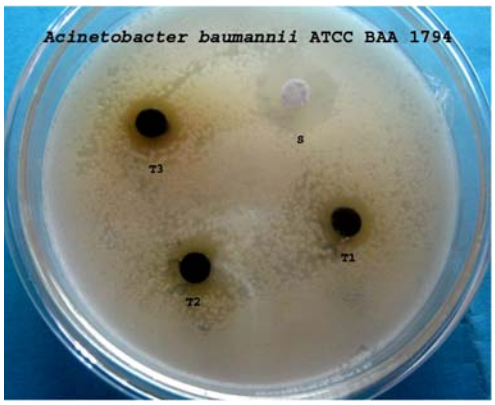


**Fig. 5: S= ampicillin 10 µg/disc, T1=ELE 5 mg/disc, T2= 7.5 mg/disc and T3=ELE 10 mg/disc**

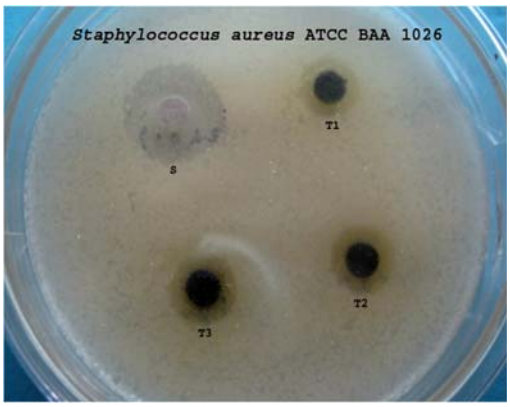


**Fig. 6: S= amikacin 10 µg/disc, T1=ELE 5 mg/disc, T2= 7.5 mg/disc and T3=ELE 10 mg/disc**

**Table 1: Inhibition zone in method with negative control**

| Bacteria | Inhibition zone (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Positive control | | ELE | | ALE | | Negative |
| | ELE | ALE | T1 | T2 | T1 | T2 | control |
| *S. aureus* | 19 | 18 | 12 | 13 | NI | NI | NI |
| *S. warneri* | 19 | 20 | 11 | 12 | NI | NI | NI |
| *B. subtilis* | 21 | 22 | 11 | 12 | NI | NI | NI |
| *E. coli* | 20 | 20 | 12 | 13 | NI | NI | NI |
| *A. Baumannii* | 19 | 20 | 13 | 14 | NI | NI | NI |
| *P. aeruginosa* | 25 | 24 | 14 | 15 | NI | NI | NI |
| *P. putida* | 19 | 20 | 16 | 17 | NI | NI | NI |
| *K. pneumoniae* | 20 | 21 | NI | NI | NI | NI | NI |
| *P. mirabilis* | 22 | 21 | 12 | 13 | NI | NI | NI |

NI: no inhibition, T1: 5 mg/disc of ELE or ALE, T2: 10 mg/disc of ELE or ALE.

**Table 1: Inhibition zone in method without negative control**

| Bacteria | Inhibition zone (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Positive control | | ELE | | | ALE | | |
| | ELE | ALE | T1 | T2 | T3 | T1 | T2 | T3 |
| *S. aureus* | 18 | 19 | 13 | 14 | 15 | NI | NI | NI |
| *S. warneri* | 19 | 19 | 11 | 13 | 14 | NI | NI | NI |
| *B. subtilis* | 21 | 20 | 11 | 12 | 13 | NI | NI | NI |
| *E. coli* | 20 | 19 | 12 | 13 | 14 | NI | NI | NI |
| *A. Baumannii* | 19 | 18 | 13 | 14 | 14 | NI | NI | NI |
| *P. aeruginosa* | 20 | 21 | 14 | 15 | 17 | NI | NI | NI |
| *P. putida* | 20 | 21 | 16 | 17 | 18 | NI | NI | NI |
| *K. pneumoniae* | 20 | 20 | NI | NI | NI | NI | NI | NI |
| *P. mirabilis* | 21 | 22 | 12 | 13 | 14 | NI | NI | NI |

NI: no inhibition, T1: 5 mg/disc of ELE or ALE, T2: 10 mg/disc of ELE or ALE.

life threatening infections such as necrotizing fasciitis.

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