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Evaluation of Antimicrobial Activity of Leaf Extracts of *Wattakaka Volubilis* (L.) Stapf

A. Ramachandran[1*], M. Senthilkumar [2] and D. Vinothkumar[3]
(*[1]Director in Centre for Climate Change and Adaptation, Anna University, Chennai, Tamil Nadu, India)
([2]Guest lecturer, PG and Research Dept of Botany, Government Arts College, Dharmapuri, Tamil Nadu, India)
([3]Assistant Professor, Dept of Biotech, Adiyamaan Engineering College, Hosur)

ABSTRACT
Leaf samples of *Wattakaka volubilis* were used to examine their antimicrobial potential against some human pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* fungi strains *Aspergillus niger*, and *Candida albicans* growth inhibition was observed in different volumetric concentrations of this extracts and it was effective against all the bacterial and fungal species tested. Increase in extract concentration showed appreciable decline in viable count of micro-organism indication significant bioactivity.

***Keywords*:** *Wattakaka volubilis*, Medicinal plants, Antimicrobial activity, Asclepiadaceae

I. Introduction

Medicinal plants are a valuable natural resource and regarded as potentially safe drugs. They have been playing an important role in alleviating human sufferings by contributing herbal medicines in the primary health care systems of rural and remote hilly areas where more than 70% of population depends on folklore and traditional system of medicines. Medicinal plants have been tested for biological and antimicrobial activity Atta, *et al*, 1998; Glombitza, *et al*, 1994; Vats, *et al.*, 2002 [1,2,3]. *W. volubilis*, which is commonly known as 'Perukurunchan' in Tamil, is an important medicinal tall woody climber belongs to the family Asclepiadaceae. The family includes 320 genera and 1800 species. In India, the family is represented by 35 genera and 234 species, nearly 172 are endemic. Leaves cordiform, 8x6cm, cordiform, base truncate to rounded, apex shortly acuminate, penninerved, lowest 1or2 pairs arising from base; petiole to 2.5cm, 2-glandular near lamina. Umbel axillary, 25-flowered; peduncle 2.5cm; bracts concave, orbicular, 0.7mm across; pedicel to 2cm. Calyx-lobes lanceolate, imbricate, 3mm, coriaceous, puberulous without, ciliate, subacute, glandular. Corolla greenish 1.5cm across, rotate; lobes ovate, overlapping to right in bud, 7x4mm, thick-herbaceous, ciliate, subacute to obtuse. Pollinial bags 0.5mm, subsessile; receptacle 0.2mm. corona single, staminal, fleshy, radiating from center. Ovaries globose, 1.5mm; style 1.5mm. Follicles oblong, 9x2.5cm, gradually narrowed and apically blunt; seeds obovoid, flattened, 7x4mm; coma soft, dull white. It is popularly used as antidiabetic and eats various diseases in Indian traditional system of medicine. Particularly the plant material used in folk medicine for diabetes and gesic and inflammatory activity. The root is applied to snake bites and given to women to cure head ache after child birth and the leaves are applied to boils and abscesses to promote suppuration. It is emetic diaphoretic and diuretic Agarval, 1986 [4]. The ethanobotanical data of *W. volubilis* plant prompted an investigation into its antimicrobial activity. Therefore, the present study was planned to find out the antimicrobial potential of *W. volubilis* leaves for their efficacy against various human pathogenic fungal and bacterial strains.

II. Materials and Methods

2. 1.Plant materials

The plants used in this study were collected from their natural habitats from the Cauvery river banks, Tiruchirappalli district in Tamil Nadu, South India. The plants were shade-dried at ambient temperature (31°C) and the dried materials were crushed into fine powder using an electric blender.

2. 2.Solvent extract

Fifty grams of dried powdered materials (Leaves) were soaked separately in 300 ml each of the solvent viz (Ethanol, Chloroform and aqueous) in a soxhlet apparatus for 72 hrs. The extracts were evaporated under vacuum and the residues were separately dissolved in the same solvent.

2.3. Medium for microbial growth

The nutrient Agar and Rose Bengal Agar medium were prepared and sterilized for bacteria and fungi respectively. Two fungal species namely *Aspergillus niger*, and *Candida albicans* and three bacterial species such as *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* were obtained

from Eumic Analytic Lab Tiruchirappalli, Tamil Nadu. Both fungal and bacterial strains were used in agar well diffusion methods and the respective temperature at 37^0C for 48 to 72 hrs for mother culture. The different leaf extracts were taken separately at various concentrations of 25, 50, 75 and 100 µl. They were kept under incubation. After incubation the plates were observed for the zone formation and the length (mm) of the zone was measured and tabulated.

III. Results and Discussion

The present results was studied the antimicrobial activity of ethanol, chloroform and aqueous extract of *W.volubilis* species from leaves sample against microorganisms using agar well diffusion method are presented (Table 1-3 and Fig 1-3). In Chloroform extract was active against bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*) and fungi (*Aspergillus niger*, and *Candida albicans*) strains. As a general rule, plant extracts are considered active against both fungi and bacteria when the zone of inhibition is > 6mm or in the category of moderate growth inhibition or more Groove and Randall, 1955 [5]. In this plant, a maximum inhibition zone was found to be 15.2mm in the concentration of 100µl, against *E. coli*, the moderate inhibition zone was found to be 13.3mm in the same concentration100µl, against *S. aureus* and the minimum inhibition zone 9.2mm was found in concentration of 25µl, against *B. subtilis* were isolated human pathogenic bacteria. In fungal human pathogen, a maximum inhibition zone was recorded 12.6mm in the concentration of 75µl, against *C. albicans* and a minimum inhibition was found to be 8.5mm in the concentration of 25µl, against *A. niger*.

In Ethanol extract, a maximum inhibition zone was found to be 15.3mm in the concentration of 100µl, against *S. aureus*, the moderate inhibition zone 14.5 mm was found in the same concentrations 100µl, against *E. coli* and the minimum inhibition zone 10.3mm was found in concentration of 25µl, against *B. subtilis* were isolated human pathogenic bacteria. In fungal pathogen, a maximum inhibition zone was recorded 15.3mm in the concentration of 100µl, against *A. niger* and a minimum inhibition was found to be 9.3mm in the concentration of 25 µl, against *C. albicans*. In Aqueous extract, a maximum inhibition zone was found to be 14.5mm in the concentration of 100µl, against *S. aureus*, the moderate inhibition zone 13.5 mm was found in same 100µl concentrations, against *E. coli*. The minimum inhibition zone 9.8mm was found in concentration of 25µl, against *B. subtilis* were isolated human pathogenic bacteria. In fungal pathogen, a maximum inhibition zone was recorded 12.5mm in the concentration of 100µl, against *C. albicans* and a minimum inhibition was found to be 8.3mm in the concentration of 25µl against *A. niger*. The diameter of the inhibition zone for each sample against each microorganism was found to be either less than or greater than or equal to that of the standard antibiotic (Ofloxacin, 10mg/disc) used in the assay. The former showed high degree of inhibition against *Staphylococcus aureus* (11.1mm) whereas moderate antibacterial activity was associated with *Bacillus subtilis* (8.0mm) and *Eschrichia coli* (7.2mm). Desta 1993[6]; Irobi *et al*., 1994[7]; Vikas Dhingra *et al*., 1999[8]; Gould and Booker 2000[9] and Madamome and Afolayan 2003[10]. Purohit *et al*., 1995 [11], Amphawan *et al*., 1995 [12], Aida Protillo *et al*., 2001[13] who established that aqueous and methanol extracts of many plants inhibited the growth of several fungal and bacterial strains.

Table 1 Bioactivity of *W. volubilis* leaf chloroform extracts against bacterial and fungal isolates

ORGANISM	Zone of inhibition (mm) at volume of leaf sample loaded (µl)				
	1 (25 µl)	2(50 µl)	3(75 µl)	4(100 µl)	Control
E. coli	11.3	12.5	13.3	15.2	30
Staphylococcus aureus	10.5	11.2	12.5	13.3	30
Bacillus subtilis	9.2	10.3	12.3	12.5	33
A.niger	8.5	9.3	10.2	10.4	23
Candida albicans	9.5	10.2	12.6	11.2	35

Fig 1 Leaf Chloroform extract of *W. volubilis* against bacterial isolates

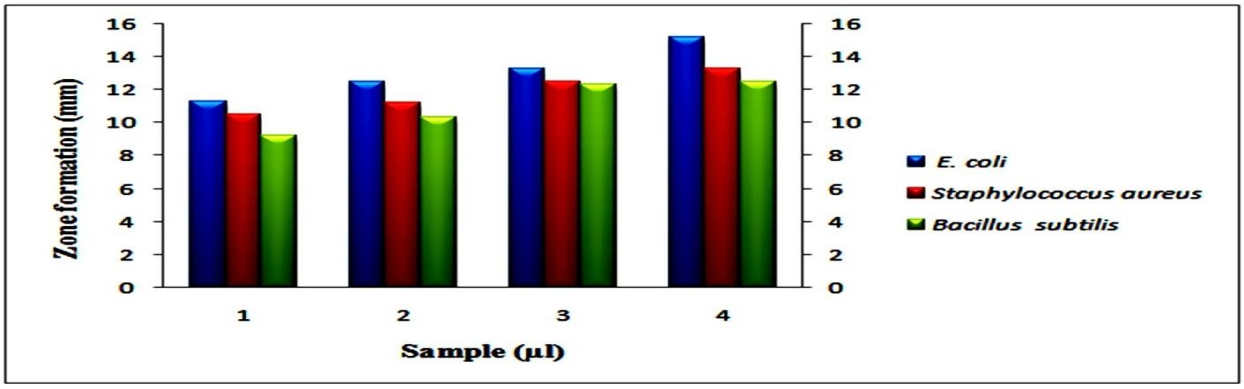


Fig 1a: Leaf Chloroform extracts of *W. volubilis* fungal isolates

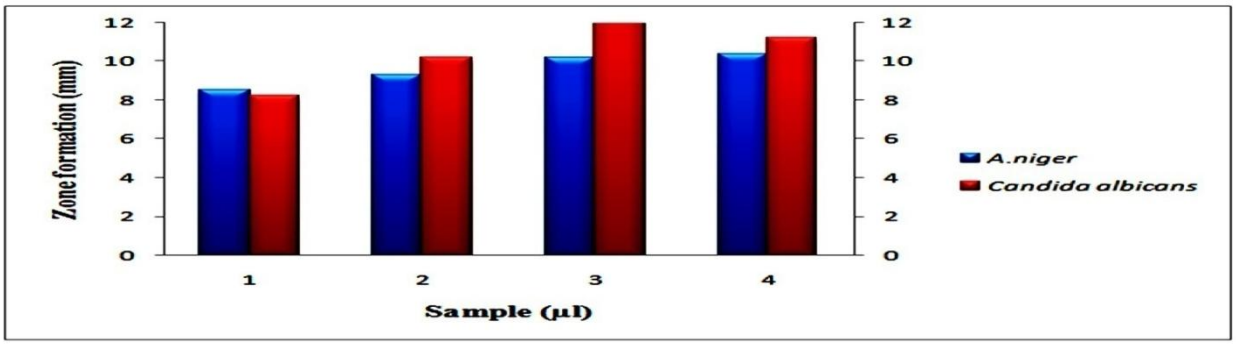


Table 2: Bioactivity of *W. volubilis* leaf ethanol extracts against bacterial and fungal isolates

Organisms	Zone of inhibition (mm) at volume of leaf sample loaded (µl)				
	1(25 µl)	2(50 µl)	3(75 µl)	4(100 µl)	Control
E. coli	12.3	12.5	13.3	14.2	30
Staphylococcus aureus	11.2	11.3	12.4	15.3	30
Bacillus subtilis	10.3	12.5	11.5	14.5	30
A.niger	12.5	13.6	14.2	15.3	20
Candida albicans	9.3	12.5	13.8	14.6	35

Fig 2: Leaf ethanol extract of *W. volubilis* against bacterial isolates

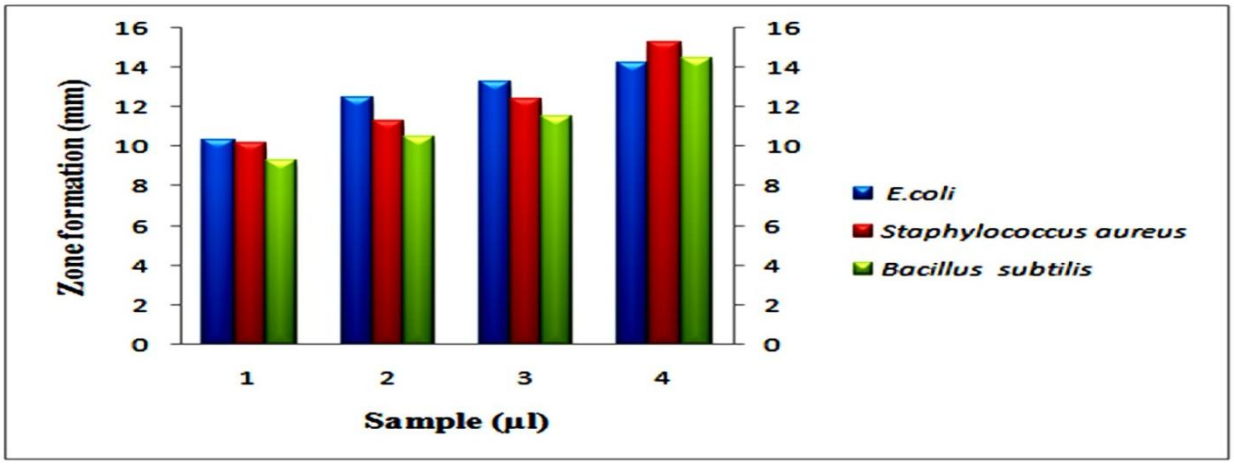


Fig 2a: Leaf ethanol extract of *W. volubilis* against fungal isolate

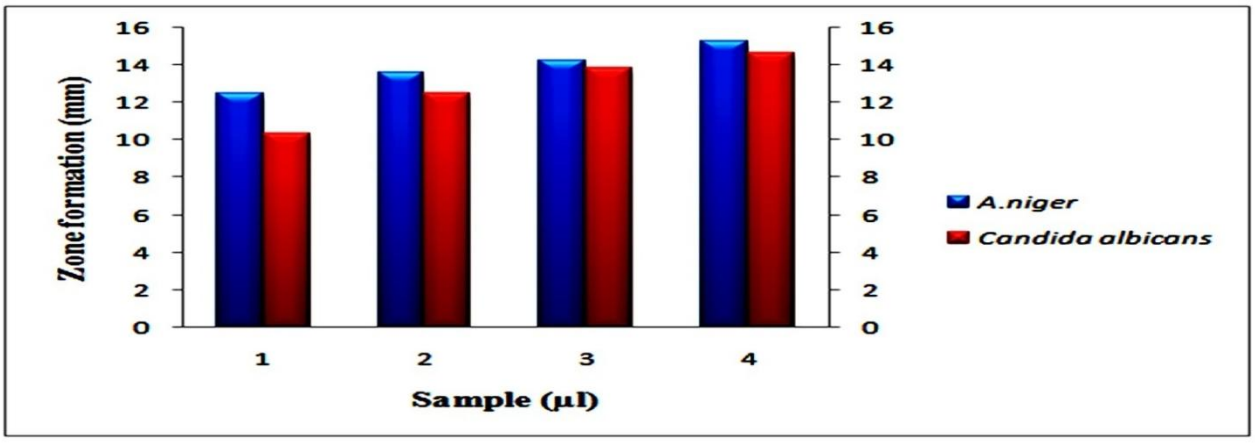


Table 3 Bioactivity of *W. volubilis* leaf aqueous extracts against bacterial and fungal isolates

Organisms	Zone of inhibition (mm) at volume of leaf sample loaded (µl)				
	1 (25 µl)	2(50 µl)	3(75 µl)	4(100 µl	Control
E. coli	12.5	11.5	12.5	13.5	30
Staphylococcus aureus	11.5	10.5	13.5	14.5	30
Bacillus subtilis	9.8	12	10.5	12.5	30
A.niger	9.5	10.5	8.3	11.5	20
Candida albicans	10	8.5	9.5	12.5	35

Fig 3: Leaf aqueous extract of *W. volubilis* against bacterial isolates

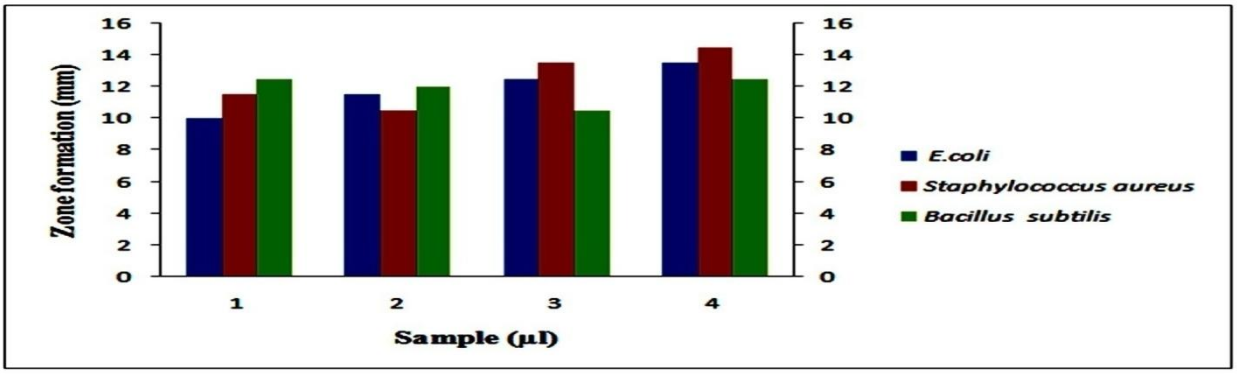
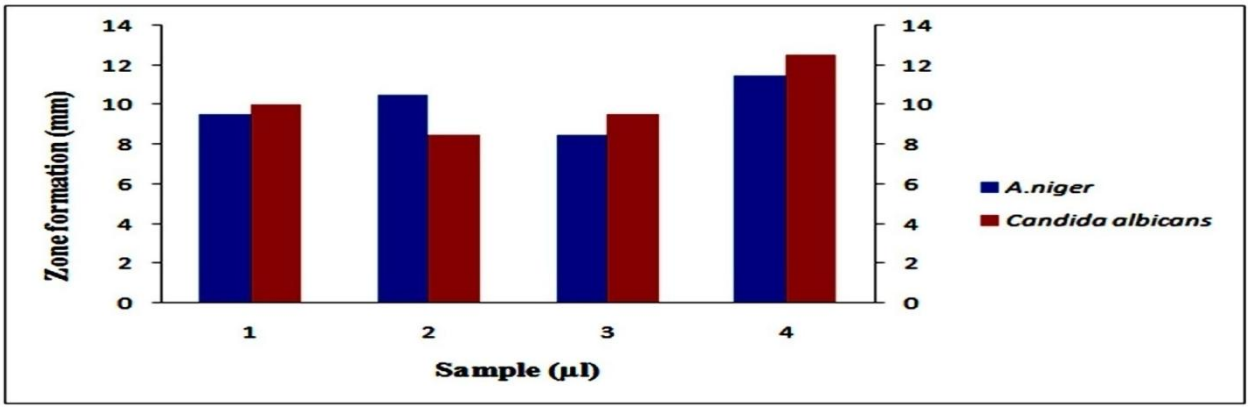


Fig 3a: Leaf aqueous extract of *W. volubilis* against fungal isolates



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