



Phytochemical screening, FT-IR analysis and antimicrobial activity of *Wattakaka volubilis*

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Abstract

The present study was evaluated the phytochemical analysis and antibacterial activity of *Wattakaka volubilis* leaf extract. The *Wattakaka volubilis* is medicinal plant used in the treatment of various diseases (Wound, diabetics, rheumatic and diarrhea). The ethanol, methanol, petroleum ether and DMSO extract from the leaf of *Wattakaka volubilis* were screened on their phytochemicals analysis. Among these Phytochemicals were alkaloids, anthroquinone, caumarin, flavanoids, phenols, saponins, steroids, tannins and terpenoids for present in the following extracts ethanol, methanol. The petroleum ether and DMSO only present in several phytochemicals like alkaloid, anthroquinone, terpenoids and tannins. *Wattakaka volubilis* against the antibacterial like *E. coli*, *B. pumilus*, *Enterobacter aerogens*, and *Pseudomonas aeruginosa*. The zone of inhibition of *Wattkaka volubilis* leaf extract against the bacteria was maximum inhibition *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Bacillus pumilis*. The least zone of inhibition was recorded against *E.coli*. The characterization of the plant extract and the functional groups was analyzed in Fourier Transform Infrared Spectroscopy (FTIR). The absorbance bands analysis were observed in the region of 4000-400cm⁻¹ are 23 compounds derived. The compounds represented the ranges from 3905.21 to 436.41 peaks were shown. This study which is the primary report on the phytochemicals analysis and antibacterial properties of *Wattakaka volubilis* supports its traditional uses in the treatment of infectious and non- infectious diseases.

Keywords: *Wattakaka volubilis*; Phenols; Ethanol; DMSO; *Bacillus Pumilus*; Bacteria; Tannin Etc.

1. Introduction

India has a rich culture of medicinal herbs which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani and Siddha medicines but only very few have been studied chemically and pharmacologically for their potential medicinal values (Guptha *et al.*, 2005). Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems. In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs as they are generally non toxic and World Health Organization has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs. Plant derivatives with hypoglycaemic properties have been used in folk medicine and traditional healing systems around the world (Yeh, *et al.*, 2003) from very ancient time. Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem to people (Ravi, *et al.*, 2005).

The root is applied to snake bites and given to women to cure headache after child birth and the leaves are applied to boils and abscesses to promote suppuration. It is emetic diaphoretic and diuretic (Agarwal, 1986). Traditional; healers of Kerala use its leaves to treat inflammatory and painful conditions (Kirtukar and

Basu, 2003). *Wattakaka volubilis* (Linn.f.) Stap f (Family - Asclepidaceae) is a large climber with green flowers in drooping umbels, with smooth bark and ash colored, leaves rounded at the base. A large twining shrub older branches ash colored. It is found in India and South East Asia. The plant is tonic, aphrodisiac, anti-pyretic and astringent to the bowels; good for dyspepsia and inflammations; cures piles, tumours, leucoderma, asthma and urinary discharges. It is also used in colds, eye diseases and sneezing. *W. volubilis* Leaf paste is removed along with pepper to treat dyspepsia (Pandikumar, *et al.*, 2007). Bark paste, mixed with hot milk is utilized internally for treating urinary troubles (Silija *et al.*, 2008), and leaf powder is taken orally along with cow's milk have antidiabetic activity (Ayyanar *et al.*, 2008)

The literature survey revealed that among the various saponins obtained from the stem and flower of *W. volubilis*, two compounds are active against Ehrlich's as cites carcinoma (Yoshimura, *et al.*, 1983 and Sahu, *et al.*, 2002). Since *W. volubilis* has been reported to possess medicinal effects, the anti-inflammatory, analgesic and anti-pyretic activities exhibited by the constituents present in the dried root ethanol extract. Pharmacological activity studies reported earlier on these plants are *W. volubilis* Antifungal, antibacterial (Vijayal, 2008), hypoglycemic (Jasmine and Daisy, 2004), anti-inflammatory, analgesic and anti-lipid peroxidative (Divya, 2009), protection against selenite induced cataract in rat lens (Biju, *et al.*, 2007), in vitro anti-leishmanial and anti-tumour (Molisha, *et al.*, 2009), hepatotoxicity prevention of proteolysis in

rat lens (Biju, *et al.*, 2007), apoptosis inducing potential (Subbiah, 2005).

The importance of medicinal plants in traditional health care practices, providing clues to new areas of drug research and biodiversity conservation is now well recognized. Inflammation is a complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells and irritants. It is the protective attempt by the organism to remove the injurious stimuli as well as initiate healing process for the tissue and considered to be the major cause of rheumatoid arthritis. Drugs currently used for management of pain and inflammatory conditions present toxic side effects in chronic administration. Therefore, attempts are being taken to study promising plants which may lead to develop newer or safer drugs (Fayyaz, *et al.*, 1994).

2. Materials and methods

2.1. Collection of plant material

Fresh healthy leaves of *Wattakaka volubilis* (L.fil.) Stapf were collected from Thanjavur District, Tamilnadu, India, during December 2015. The Flora of Tamil Nadu Carnatic (Matthew, 1983) was used for identification and authentication of the plants. Collected leaf of *Wattakaka volubilis* washed thoroughly in running tap water, rinsed in distilled water, stored in sterile polythene bags and used for further studies.

2.2. Preparation of plant extracts

About two grams leaves of *Wattakaka volubilis* were taken in 10ml of different organic solvents, (Ethanol, Methanol, Dimethyl Sulphoxide (DMSO) and Petroleum ether) and crushed with a use of sterile mortar and pestle (Essawi and Srour, 2000). Then the solvent extracts were filtered through Whatman No.1 filter paper and series of sterile filter membranes (2-20; 0.45; 0.2 μ m). These prepared leaf extracts were stored in sterile glass bottles at 4°C for further use.

2.3. Chemicals

All the chemicals used for this work, were purchased from Hi-Media, Mumbai, India.

2.4. Qualitative phytochemical analysis

The phytochemical analysis was carried out from ethanol leaf extract of *Wattakaka volubilis* (Linn.) with standard methods (Harbone, 1993). It was done to assess the qualitative chemical composition of crude extracts using commonly employed, precipitation and colorations reaction to identify the major natural chemical groups.

Fourier Transform - Infrared Spectroscopy (FT-IR) Analysis

The methanolic extract of *Wattakaka volubilis* samples were taken in the form of fine powder (Saifuddin *et al.*, 2009) and were filtered with sieves of 0.071 and 0.500 mm mesh size. The FT-IR spectra were recorded in mid IR region 4000-400 cm^{-1} at the resolution of 4 cm^{-1} using a sophisticated computer controlled FT-IR Perkin Elmer spectrometer with He-Ne laser as reference. Air back ground spectrum was recorded before each sample.

2.5. Procurement of test organisms

Three Gram negative bacteria (*Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *E.coli*) and gram positive bacteria (*Bacillus pumilus*) were procured from Doctors Diagnostic Centre Trichy, Tamilnadu, India. Bacterial cultures were maintained on nutrient agar slants maintenance at 4°C for further experiments. Determination of antimicrobial activity

2.6. Preparation of culture Inoculums

The stock cultures of bacteria (*Enterobacter aerogenes*, *Bacillus pumilus*, *Pseudomonas aerogenosa* and *E.coli*) used in this study was maintained on nutrient agar slants at 4°C. Inoculums was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient broth and was incubated at 37°C \pm 2°C for 24 to 48 hours.

2.7. Agar well-diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity (Perez *et al.*, 1990). Nutrient agar plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old broth culture of respective bacteria. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About 100 μ l of different solvent leaf extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were incubated in an upright position at 37°C \pm 2°C for 24 h for bacterial pathogens.

3. Results

3.1. Preliminary phytochemical screening of whole plant of *W.volubilis*

The plant parts of *Wattakaka volubilis* leaf screening of qualitative phytochemical analysis with different solvents were used such as ethanol, methanol, petroleum ether and DMSO. Among the four solvent the ethanol was maximum extraction of phytochemicals such as alkaloids, anthroquinones, coumarin, flavonoids, phenols, saponins, steriods, tannins and terpenoids were recorded. The methanol extracts of the plant which analysed such as alkaloids, anthroquine, coumarin, flavonoids, phenols where as in the solvent of petroleum ether was alkaloid only presented. The DMSO solvents with *Wattakaka volubilis* plant extract was only three compounds such as alkaloids, anthroquinones and tannins were recorded. However, the solvent was one of the major roles for extract were analysed. The results are tabulated in Table 1.

Table: 1 Preliminary Phytochemical Screening of Leaves of *Wattakaka Volubilis*

| No. | Bioactive Compounds | Ethanol | Methanol | Petroleum ether | DMSO |
|-----|---------------------|---------|----------|-----------------|------|
| 1. | Alkaloids | + | + | + | + |
| 2. | Anthroquinones | + | + | + | + |
| 3. | Coumarin | + | + | - | - |
| 4. | Flavonoids | + | + | - | - |
| 5. | Phenols | + | + | - | - |
| 6. | Saponins | + | + | - | - |
| 7. | Steroids | + | - | - | - |
| 8. | Tannins | + | + | - | + |
| 9. | Terpenoids | + | + | + | - |

3.2. FTIR analysis

The characterization of the plant extract and the functional groups was analyzed in Fourier Transform Infrared Spectroscopy (FT-IR). The absorbance spectra of soluble plant extract was shown in figure 1. The absorbance bands analysis were observed in the region of 4000-400 cm^{-1} are 23 compounds derived. The compounds represented the ranges from 3905.21 to 436.41 peaks were shown Table 2 and Fig 1.

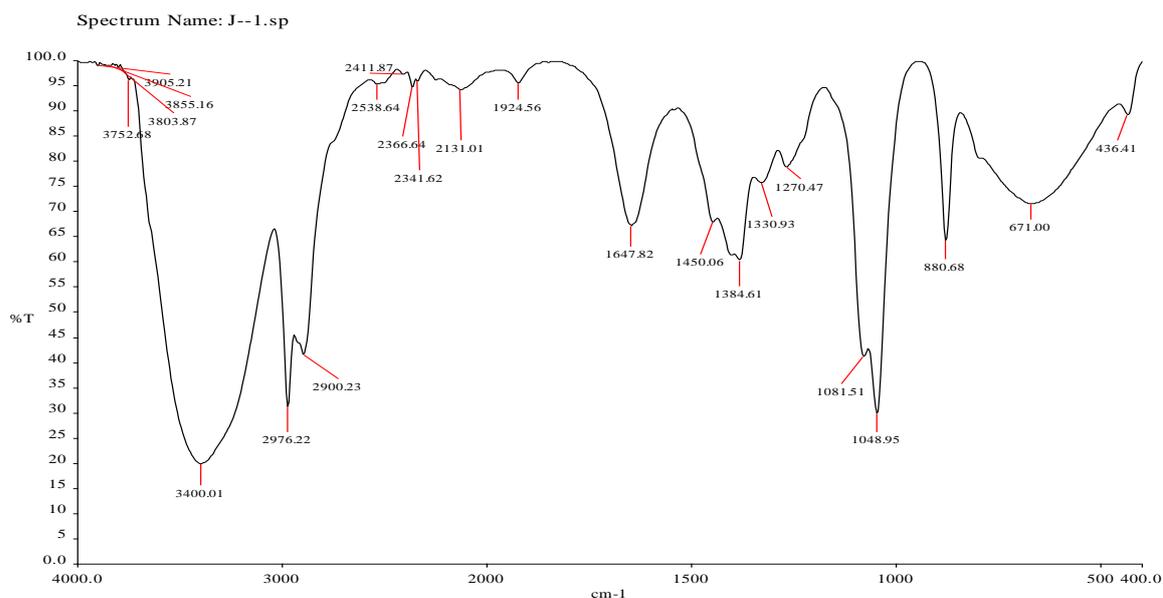


Fig. 1: FT-IR Analysis of Ethanol Extract of *Wattakka Volubilis* Leaf.

Table: 2 FTIR Analysis for Ethanol Extracts of *Wattakaka Volubilis* Leaf

| S. No | Peak area | Bond | Functional group |
|-------|-----------|-----------------------|--|
| 1 | 3905.21 | O-H stretch | Free hydroxyl alcohols phenols |
| 2 | 3855.16 | C-H stretch | Alkynes |
| 3 | 3803.87 | C H Stretch | Alkynes |
| 4 | 3752.68 | O-H stretch, | free hydroxyl alcohols, phenols |
| 5 | 3400.01 | N-H stretch | 1°, 2° amines, amides |
| 6 | 2976.22 | C-H stretch | Alkanes |
| 7 | 2900.23 | C-H bonds | Alkyl C-H Stretch |
| 8 | 2411.87 | H-C=O: C-H stretch | Aldehydes |
| 9 | 2538.64 | H-C=O: C-H stretch | Aldehydes |
| 10 | 2366.64 | H-C=O: C-H stretch | Aldehydes |
| 11 | 2341.62 | C C Stretch | Alkynes: |
| 12 | 2131.01 | -C≡C- stretch | Alkynes |
| 13 | 1924.56 | N-H Bend | Amines |
| 14 | 1647.82 | -C=C- stretch | Alkanes |
| 15 | 1450.06 | C-H bend | Alkanes |
| 16 | 1384.61 | N-O stretch | Nitrate |
| 17 | 1330.93 | C-N stretch | aromatic amines |
| 18 | 1270.47 | N-O symmetric stretch | nitro compounds |
| 19 | 1081.51 | C-O stretch | alcohols, carboxylic acids, esters, ethers |
| 20 | 1048.95 | C-N stretch | aliphatic amines |
| 21 | 880.68 | C-H "oop" | Aromatics |
| 22 | 671.00 | C-Br stretch | Alkylhalides |
| 23 | 436.41 | C-Br stretch | Alkylhalides |

3.3. Antibacterial activity of *Wattakaka volubilis*

In the present investigation the effect of antimicrobial activity of *Wattakaka volubilis* against some clinically important bacterial strains were observed. The ethanolic leaf extract of *W. volubilis* has showed the highest zone of inhibition against *Bacillus pumilus*, *Enterobacter aerogens*, *E.coli* and *Pseudomonas aeruginosa* with 13, 15, 2 and 20 mm in diameter respectively. The zone of inhibition was observed for *W.volubilis* against *Bacillus pumilus* and *E.coli* was 10 and 8 mm respectively. Whereas, no zone of inhibition in *Enterobacter aerogens* and *Pseudomonas aeruginosa* in the methanolic extract. DMSO and Petroleum ether No zone of inhibition was observed from the effect of *W.volubilis* against the tested bacterial pathogens. The results were recorded in Table 3.

Table: 3 Antibacterial Activities of *Wattakaka Volubilis* against Some Bacterial Pathogens with Different Solvents

| Bacteria | Zone of inhibition (mm) | | | |
|-------------------------------|-------------------------|----------|------|-----------------|
| | Ethanol | Methanol | DMSO | Petroleum ether |
| <i>Bacillus pumilus</i> | 13 | 10 | - | - |
| <i>Enterobacter aerogens</i> | 15 | - | - | - |
| <i>Escherichia coli</i> | 2 | 8 | - | - |
| <i>Pseudomonas aeruginosa</i> | 20 | - | - | - |

4. Discussion

Antimicrobial activity of *Wattakaka volubilis* were done against some bacterial pathogens with different solvents. The leaf extract of *Wattakaka volubilis* showed the maximum zone of inhibition in the ethanol solvent when compared to other solvents such as methanol, DMSO and petroleum ether. No zone formation of DMSO and petroleum ether from the plant extract (Table 1) may be due to the solvent nature and extraction of plant samples. The leaf extracts of *Wattakaka volubilis* (20 mm) showed maximum zone of inhibition against *Pseudomonas aeruginosa* when compared to other bacteria. Methanolic extract of *Wattakaka volubilis* showed maximum antibacterial activity against *Bacillus pumilus* (10mm) and *E.coli* (8mm). There is no zone of inhibition was observed against *Enterobacter aerogens* and *Pseudomonas aeruginosa*. No zone of inhibition was observed in petroleum ether extract against four tested bacteria. The ethanobotanical approach assumes that the popular uses of plants can offer strong clues to the biological activities of the plants. The results of the present study reveals the fact that the organic solvent extracts of ethanol, methanol was exhibited greater antimicrobial activities because the antimicrobial principles may be either polar or non polar nature.

Patil, (2009) reported the antifungal activity of ethanolic extract of the *Leucasaspera* leaves including anti-diarrhoeal, and antimicrobial, activities. Rana (1997) evaluated antifungal activity of essential oils isolated from the leaves of Bael using spore germination assay. The oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested was observed at 500ppm. They proposed that essential oil from bael leaves may interfere with the Ca⁺⁺ - dip colonic acid metabolism pathway and possibly inhibit the spore formation.

Pitre and Srivastava, (1987), demonstrate the antifungal activity of ethanolic root extract against *Aspergillus fumigatus*. Sastry and Rao (1994) reported that, some of the bacterial strains (*Proteus vulgaris*, *Micrococcus luteus* and *Bacillus megaterium*) did not respond to solvents extracts, whereas the purified fractions showed broad spectrum activity against multiple strains. This might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract. The variation of antibacterial activity of our extracts might be due to distribution of antimicrobial substances, which varied from species to species as suggested by Lustigman and Brown (1991). Similar observations were made by Vlachos et al. (1997) who found that fractionation of crude extracts tested enhanced their activity against both Gram negative as well as the resistant gram positive pathogens.

Earlier antimicrobial studies using essential oils from *Wattakaka volubilis* leaves have demonstrated that terpenoid constituents in the oil are in vivo antifungal components by Balakumar et al., (2011). Tannins, eugenol, and cuminaldehyde contribute to the antibacterial activity of the fruit extract, particularly against *Escherichia coli*. In the present study, the phytochemical screening revealed the presence of alkaloid, anthraquinone, coumarin, flavonoid, phenol, saponin, steroid, tannin and terpenoid in the methanol and ethanol extracts of *Wattakaka volubilis* plant leaf, except steroids absence in methanol extract. In the DMSO extract alkaloids, anthraquinones and tannins are presence and others are absence, where as in Petroleum ether extract it contains alkaloids. Green plants represent a reservoir of effective chemicals. Therefore, the reason to believe that the activity of *Wattakaka volubilis* could be attributed to the presence of phytochemicals as done by the preliminary qualitative phytochemical analysis.

Hulin et al. (1998) reported plants are the major role in all traditional system of food and medicine. Plants contain rich source of variety of natural products, secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids found in vitro to have antimicrobial properties. Bires et al. (2011) reported that most of these properties are believed to be due to presence of bioactive alkaloids in Bael, antibacterial phytoconstituents such as tannins, saponins, flavonoids, alkaloids, anthocyanin, glycosides, terpenoids, triterpenoids, glycoside and phenols qualitatively isolated from *Leucasaspera* leaves which displayed potent antibacterial activity against *Bacillus anthracis*, *Escherichia coli*, and *Klebsiella pneumoniae*.

Wangensteen (2004) reported that leaves of *Leucasaspera* had more phenolic content than the seeds. All the extracts were studied for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Of these, *Leucasaspera* methanolic extract showed zone of inhibition of 4.5mm and 6mm at concentration of 250µg/ml and 500µg/ml respectively against *Klebsiella pneumonia* while *Coriander sativum* diethyl ether extract showed zone of inhibition of 2.5mm and 3.5mm at a concentration of 250µg/ml and 500µg/ml respectively against *Staphylococcus aureus*. Hence coriander has strong antibacterial activity. Research suggests that the volatile oils found in the leaves of the coriander plant may have antimicrobial properties against food borne pathogen Plant steroids are known to be important for their cardiogenic activities, possess insecticidal and antimicrobial properties. Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, anti-allergic, antimutagenic and anti-inflammatory (Arts and Hollman, 2005).

Ragavendran et al. (2011) screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aervalanata* analyzing the ethanolic extracts of *Wattakaka volubilis* by FTIR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens.

From the result obtained from the present investigation it is confirmed that the therapeutic potency of *Wattakaka volubilis* leaves, stem and flower were used as traditional medicinal values. In addition to these results it is a good basis for selection of the plant for all the investigation. The plant extract possess certain constitutions with antibacterial and antifungal properties caused by pathogens. The most active extract can be subjected to isolation of the therapeutic antimicrobials and carryout further pharmacological evolution. The results of our investigation confirmed the rational process for the medicinal uses of important of the plant.

5. Conclusion

In the present investigation antibacterial activity of *Wattakaka volubilis* leaves tested against some clinically important bacteria like *Bacillus pumilus*, *Enterobacter aerogenes*, *E.coli* and *Pseudomonas aeruginosa*. Four different solvents were used viz., Ethanol, methanol, DMSO and petroleum ether. All the four genera showed resistance against DMSO and petroleum ether solvents, while in ethanol, zone of inhibition were observed in all the four. Preliminary phytochemical screening showed the presence of alkaloids, anthraquinones, coumarin, flavonoid, phenols, saponins, steroid, tannins and terpenoid in ethanol extract. Except steroids all the above phytochemicals were observed in methanolic extract. In DMSO solvent extract only anthraquinones and tannins were recorded. In petroleum ether extract only alkaloids were observed. The characterization of the plant extract and the functional groups was analyzed in Fourier Transform Infrared Spectroscopy (FTIR). The absorbance bands analysis were observed in the region of 4000-400cm⁻¹ are 23 compounds derived. The compounds represented the ranges from 3905.21 to 436.41 peaks were shown. Based on the above research work, it can be concluded that *Wattakaka volubilis* had good source for herbal drug and used to control pathogens.

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