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RESEARCH ARTICLE

Extraction, phytochemical screening and antibacterial activity *Wattakaka volubilis* (Linn F) Stapf

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ABSTRACT:
The antibacterial activity of *Wattakaka volubilis* and the compounds responsible for this activity were screened. During the preliminary phytochemical analysis, the aqueous extract of *Wattakaka volubilis* was selected for the presence of carbohydrates, phenolic compounds, fats, proteins, saponins, alkaloids, tannins, phytosterols and flavonoids. Antibacterial activity of *Wattakaka volubilis* was tested with various solvent viz. methanol, acetone, chloroform and acetonitrile against various bacterial pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *E.coli*, *S.marcescens*, *P.mirabilis* and *K.pneumoniae*. of the selected various extract, the extract using acetone showed amaximum zone of inhibition. The acetone extract of *Wattakaka volubilis* contain an antimicrobial effective compound which was confirmed by GC-MS hence, this can be used as apotential antibacterial source for various infective pathogens.

KEYWORDS: Antimicrobial activity, *Wattakaka volubilis*, Antibiotic sensitivity test, GC-MS

INTRODUCTION:
Plants with effective bioactive compounds are exemplified as both medicinal and toxic with the beneficial or adverse consequence may depend on the quantitydevoured[1]. The food and fodder with bioactive compounds with less distinct effects, the intakes are usually regarded as beneficial because they have abioactive compound that has medical properties[2].

Nowadays multiple drug resistance (MRD) has emerged due to the unsystematic use of commercial antimicrobial medication commonly used in the treatment of disease. Along with this issue, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity and allergic reactions[(2-4)]. This situation forced scientists to search for new antibacterial substances.

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Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world[(5-8)].

Medicinal plants are the crucial source of drugs in many countries as drugs for various chemotherapeutic uses. There are about 3000 plant species with medicinal value in the traditional Indian medicine. The use of plant natural compounds used as alternative sources of medicine continues to play major roles in the general wellness of people all over the world. They are grouped as alkaloids, glycosides, corticosteroids, coumarin, flavonoids, and essential oils. Over 50% of all modern clinical drugs are of natural origin and play an important role in the development of drugs. Many herbs have been used for treating disease caused by microorganisms such

as typhoid, cholera, diarrhea and bacterial enteritis. Moreover, the huge economy is invested in the imports of medicines especially antibiotics from other parts of the world[9-13].

Wattakaka volubilis:
The plant *Wattakaka volubilis* Linn (Family-Asclepiadaceae), is a traditional medicinal plant used to treat various diseases in Indian traditional system of medicine. The plant material used in folk medicine for the treatment of various diseases such as antifungal, antibacterial, hypoglycemic, anti-inflammatory, analgesic and anti-lipid peroxidative. [14-18].

MATERIALS AND METHODS:

Plant material:
The whole plant of *Wattakaka volubilis* was collected from, Trichy district, Tamilnadu (India). The collected material was washed thoroughly, shade dried and powdered coarsely[16].

Preparation of Extracts:
The leaves powder was crushed to small piece using mortar and pestle and powdered in an electric grinder. Dried and powdered plant material was extracted using with methanol, successively with chloroform, Acetonitrile, and Acetone and centrifuged at 3000 rpm for 20 minutes and the supernatant was collected separated. The obtained extracts were evaporated in vacuum results in semi-solidresidues. It was stored at 4^{o}C in a tight container until used. When needed, the residual extract was dissolved in the solvent and serially diluted in water in and used for the study. The extract also was subjected to qualitative and quantitative chemical tests for the identification of various phytoconstituents[14,17,21,24].

Phytochemical screening
Generally, plants have bioactive compounds like alkaloids, terpenoids, saponins, tannins, flavonoids, phenols, glycosides etc., which plays a major role in themedicinal property.

Flavonoids, Alkaloids, Tannins, Terpenoids, Phenols showed positive results confirming the presence of compounds[22].

Alkaloids:
Mayer's Test:
Solution A: 1.358g of mercuric chloride was dissolved in 60ml of distilled water.
Solution B: 5g of potassium iodide was dissolved in 10ml of distilled water.
Solution A and B were mixed and made up to 100ml with distilled water.0.5ml of Mayer's reagent (potassium mercuric iodide) was added to the filtrate of *Wattakaka volubilis*. The presence of alkaloids was confirmed by the formation of a cream precipitate.

Wagner's Test
1.27 gm of iodine and 2mg of potassium iodide were dissolved in 5 ml of water and made upto 100ml with distilled water.0.5 ml of Wagner's reagent (aqueous solution of iodine and potassium iodide) was added to 1ml of *Wattakaka volubilis* filtrate. The presence of alkaloids was confirmed by the formation of a reddish white precipitate.

Hager's Test (Saturated aqueous solution of picric acid)
To 1ml of the filtrate of *Wattakaka volubilis*, 0.5 ml of Hager's reagent was added. The presence of alkaloid was confirmed by the formation of a yellow precipitate.

Test for glycosides:
A portion of the filtrate of *Wattakaka volubilis* was hydrolyzed by boiling with dilute HCl in a water bath for 30 minutes cooled and filtered. The hydrolysates were subjected to Legal's, Borntrager's and modified anthraquinone test.

Legal's Test:
To each 1ml of the hydrolysates of *Wattakaka volubilis*, 1ml of pyridine and a few drops of sodium nitroprusside solution were added and then it was made to alkaline condition with sodium hydroxide solution. The appearance of pink colour showed the presence of glycosides.

Borntrager's Test:
1ml of each extract of *Wattakaka volubilis* were shaken gently with an equal volume of chloroform and then the upper chloroform layer was separated. To this, an equal quantity of dilute ammonium solution was added. If ammonia layer acquires pink colour it showed the presence of O-anthraquinone glycosides.

Modified anthraquinone test:
To 1ml of the hydrolysates, an equal volume of 5% ferric chloride solution and dilute hydrochloric acid were added and heated to boiling water bath for 5 min, cooled and shaken gently with benzene. Benzene layer separated and equal volume ammonia was added to the benzene layer. Formation of pink colour indicated the presence of C-anthraquinone glycosides.

Phenol:
To the filtrate of 1ml extract, two drops of ferric chloride solution were added. Formation of blue colour indicated the absence of phenols.

Phenol:
To the filtrate of 1ml extract, 0.5 ml of 10% lead acetate was added. Appearance of white precipitate indicated the presence of phenol

Test for flavonoids:
To the filtrate of 1ml extract, magnesium bits and 2 drops of concentrated hydrochloric acid were added and gently heated. Formation of pink colour indicated the presence of flavonoids.

To the filtrate of extract, 0.5ml of amyl alcohol was added followed by the addition of 3 drops of sodium acetate and 2 drops of ferric chloride. Formation of blood red colour indicated the presence of flavonoids.

Test for triterpenoids:
To 1ml of extract, 2 drops of thionyl chloride were added. Formation of pink colour indicated the presence of triterpenoids.

Test for Curcumin:
To the filtrate of 1ml extract, 1ml of 2N sodium hydroxide was added. Formation of yellow colour indicated the presence of Curcumin. Further 1ml of 5N hydrochloric acid was added. If colourless solution formed at the upper layer, it shows the absence of Curcumin.

Test for saponins:
Foam test: The extract was diluted with 1ml of water separately and shaken well. Stable froth formation indicated the absence of saponins.

Test for tannins:
To the filtrate of 1ml extract, 0.5 ml of 10% lead acetate was added. The appearance of white precipitate indicated the presence of tannins.

Test for carbohydrates:
To the filtrate of 1ml extract, 2drops of concentrated Sulphuric acid was added slowly on the sides of the test tube and then 0.5 ml of α-naphthol solution was added. Formation of the purple ring at the junction of two layers showed the presence of carbohydrates.

Test for reducing sugars:
To an aliquot of extract, 2ml of diluted Hydrochloric acid was added and boiled for 10 min and it was neutralized with diluted sodium hydroxide then cooled for 5 min, after cooling equal volume of Fehling's A and B solutions were added and boiled in a water bath.

Formation of reddish brown precipitate indicated the presence of reducing sugars.

Test for phytosterol:
The extract was refluxed with the solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted with distilled water and extracted with ether.

The ether layer was evaporated and the residue was tested for Libermann Burchard test.

Libermann Burchard test:
10mg of the extract was dissolved in 3 ml of acetic anhydride. To this solution, 2 drops of concentrated sulphuric acid were added slowly along the sides of the test tube. The appearance of bluish green colour showed the absence of phytosterol.

Salkowski Test:
1ml of the filtrate was mixed with 1ml of chloroform separately. To this solution 1ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of red colour indicated the presence of sterols.

Antimicrobial activity
The four different concentrations of the extracts were tested for antimicrobial activity using disc diffusion assay according to the method of Bauer et al. (1966)[25]. The test microorganisms used in this study *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Serratia marcescens, Proteus mirabilis* and *Klebsiella pneumoniae***.**

Phytochemical screening:

Table 1 shows the preliminary phytochemical analysis of the Methanol extract of *Wattakaka volubilis*.

Name of the test	Inference	Result
Test for alkaloids		
Mayer's Test	Formation of a cream precipitate	Absence of alkaloids
Wagner's test	Formation of reddish brown precipitate	Presence of alkaloids
Test for phenol	Absence of blue colour	Presence of phenol
Test for flavonoids	Absence of blood red colour	Presence of flavonoids
Test for flavanols	Formation of white precipitate	Presence of flavanols
Test for Coumarins	Formation of Colourless solution	Absence of coumarins
Test for saponins	Stable froth formation	Absence of saponins
Test for tannins	Appearance of white precipitate	Presence of tannins
Test for carbohydrates	Absence of purple ring	Absence of carbohydrates
Test for reducing sugar	Formation of reddish brown precipitate	Presence of reducing sugar
LibermannBurchard test	Appearance of bluish green	Absence of phytosterol
Salkowski test	Formation of red colour	Presence of sterol
Test for aminoacidsNinhydrin test	Purple colour	Absence of aminoacids

RESULT AND DISCUSSION:

Ethyl acetate extracts report the presence of phenol, flavonoids, terpenoids and steroids. Acetone extract reports the presence of phenol, flavonoids, quinones and steroids and in the case of n-hexane extracts phenols, flavonoids and quinones show their presence. The findings are also in line with previous findings and reported literature.

Preliminary phytochemical analysis of methanol extract of *Wattakaka volubilis* showed the presence of flavonoids, flavonols, carbohydrates, tannins, reducing sugar, sterol, presence of proteins and the absences of alkaloids, phenol, coumarins, saponins, phytosterol and amino acids

Antimicrobial activity results:

Table 2 showing the effect of various extract on bacteria using Agar diffusion method

Microorganisms	Solvents			
	Chloroform	Methanol	Acetone	Acetonitrile
E. coli	No zone	+	+	No zone
B. subtilis	+	+	No zone	+
S. marcescens	No zone	+	No zone	+
Proteus mirabilis	*No zone*	+	No zone	No zone
S.aureus	+	+	+	+
Klebsiella pneumoniae	No zone	No zone	No zone	No zone

E.coli did not respond to the fraction of chloroform and Acetonitrile, But *Staphylococcusaureus* showed complete response against all the extracts of *Wattakaka volubilis.* The other organism like *S. marcescens* and *Proteusmirabilis* showed varied reactions against the extracts. *K.pneumoniae* showed no response towards any of the solvent extracts.

TLC results:

TLC analysis also suggests the presence of different kinds of phytochemicals in leaves extract. Table 3 reports the Rf values for various extracts and Figure1 shows photographs of the studied TLC slides. TLC of plant extract in ethyl acetate and hexane reports three spots for various phytochemicals. The reported spots are separated with enough space and having various Rf values showing the presence of at least three phytochemicals in ethyl acetate and hexane solvent extracts. Extracts in, ethanol and n-hexane, chloroform and Acetonitrile report only one to two spot. It is prominent and uniquely identified.

$$\text{Rf value} = \frac{\text{Distance from start to the center of sample spot}}{\text{Distance from sample to solvent front}}$$

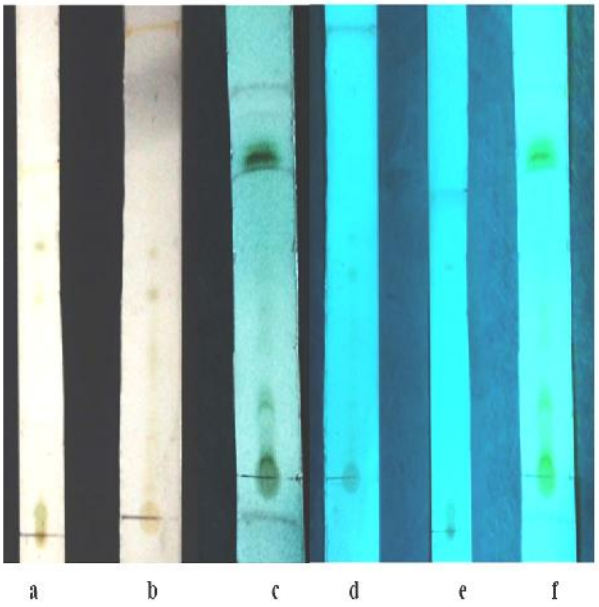

Figure -1 TLC plates showing the band formed

a, b, c are the pigment separated from Methanol, Acetone and chloroform absorbed under normal light

d, e, f are the pigment separated from Methanol, Acetone and chloroform absorbed under UV light

Table 3 showing the Rf values of various extract on TLC

Plant Extract	Rf value
	Ethyl acetate and hexane (7:3)
Methanol	0.85
	1.10
Acetone	0.62
	0.65
	0.70
Chloroform	0.54
Acetonitrile	0.83

When a new compound to be discovered, the qualitative phytochemical analysis is a very important step as it gives information about the presence of any particular primary or secondary metabolite in the extracts of the plant which is having a clinical significance. In any case, if any significant bioactive natural product is present, it is necessary to separate that compound from the mixture of compounds by using the suitable chromatographic technique.

The different phytochemicals tests performed on the extracts of *Wattakaka volubilis* leaves show the presence of phenols, flavonoids, quinones, tannins, steroids and cardiac glycosides in ethanol extract, the presence of phenols, flavonoids, quinones, saponin, tannins, terpenoids and cardiac glycosides in Methanol extract.

FT-IR

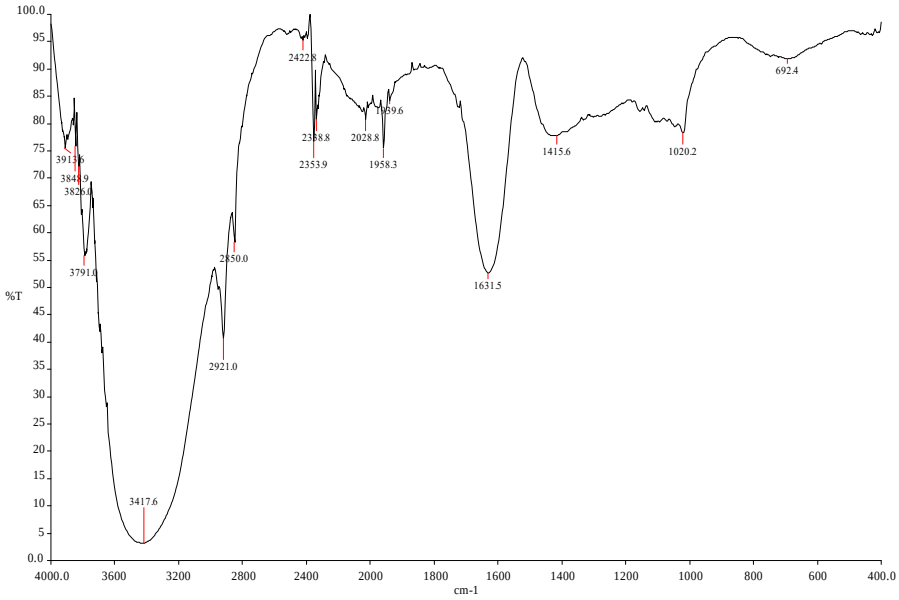

Figure 5 FT – IR spectrum

GC-MS

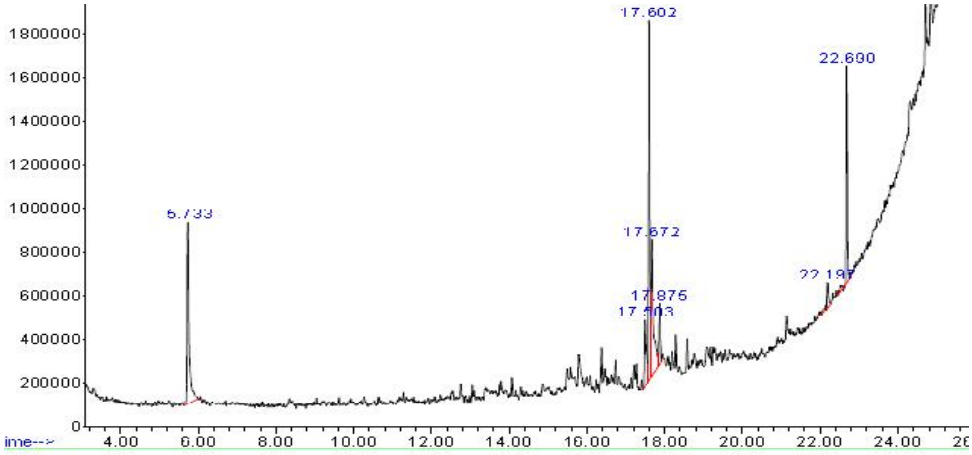

Figure 6 showing the GC result of Acetone purified fraction

The present study carried out on the *W.volubilis* revealed the presence of medicinal active constituents. In GC-MS analysis, 21 bioactive phytochemical components were identified in the ethanolic extract of *W. volubilis*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. Diethyl phthalate ($C_{12}H_{14}O_4$) with RT 17.668 has peak area 22.693, Bis(2-ethylhexyl) phthalate, Diisooctyl phthalate with RT 14.93 has peak area 17.58 and propane 1, 1, 3-trimethoxy ($C_9H_20O_3$) with RT 6.84 has peak area 10.20, the results were present. Essawi et al., 2000 found in 26 bioactive phytochemical compounds were identified in the acetone extract of *Wattakaka volubilis*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula.

CONCLUSION:

The result of in vitro antibacterial screening showed that 4 leaf extracts from *W.volubilis* had different ranges of antibacterial activities. It mainly influenced both the Gram-positive bacteria and Gram-negative bacteria, the acetone extracted samples showed the highest inhibitory effect against *Staphylococcus aureus* (100%).

The phytochemical compounds were screened by GC-MS method. The acetone leaf extracts presented 32 bioactive compounds. The identification of

phytochemical compounds is based on the peak area, molecular weight and molecular formula. Diethyl phthalate $C_{12}H_{14}O_4$ with RT 17.668 has peak area 22.693, Bis(2-ethylhexyl) phthalate, Diisooctyl phthalate with RT 14.93 has peak area 17.58 and propane 1, 1, 3-trimethoxy ($C_9H_{20}O_3$) with RT 6.84 has peak area 10.20, the results were present.

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