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Pharmaco-chemical characterization of *Wattakaka volubilis* (L.f.) Stapf - an antidiabetic ethnomedicinal plant

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Abstract: *Wattakaka volubilis* (L.f.) Stapf an important ethnomedicinal plant belonging to family Asclepiadaceae and it is commonly known as in tamil "Kurinjan". The plant part like root, leaf and stem are used as cure skin diseases, diabetes, cough, jaundice, poisonous bites, ulcer, and fever. The present study aimed to evaluate the Physico-chemical Constant (Ash and Extractive values), Fluorescence analysis and antibacterial activity of chloroform, ethanol and petroleum ether leaf extracts of *Wattakaka volubilis* against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) used with antibiotic tetracycline. The results of the study could be helpful to inventory of new drug to diabetes.

Keywords: Anti-diabetes, *Wattakaka volubilis.* Medicinal Plant, Antibacterial activity, *Palliyars*

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1.Introduction

Plants, owing to its medicinal value have continued to play a dominant role in the maintenance of human health since ancient times (Baker, 2005). Plants have been long provided mankind with source of medicinal agents, natural products, once serving as the source of all drugs (Balandrin *et al.,* 1993). Dependence on plants as the source of medicine is prevalent in developing countries where traditional medicine plays a major role in healthcare (Farnsworth, 1994). The rural population of a country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost. Herbal therapy although still an unwritten science is well established in more cultures and tradition and has become a way of life in almost 80% of the people in rural areas, especially those in Asia (Banquar, 1993).

Traditional medicine (TM) occupies an important place in the health care systems of developing countries. The World Health Organization (WHO) estimates that more than 80% of health care needs in these countries are met through traditional health care practices. The people in developing countries depend on TM, because it is cheaper and more accessible than Orthodox Medicine (OM) (Sofowora, 1993; Luoga *et al.,* 2000; World Health Organisation, 2002).

Plants still continue to be almost the exclusive source of drugs for a majority of the world's population (Hamburger and Hostettman, 1991. Medicinal plants have been widely claimed useful and found effective in the treatment of diabetes mellitus in various traditional applications. The ethnomedicinal plant *Wattakaka volubilis* have been reported traditionally for the treatment of diabetes (Maruthupandian *et al*., 2011).

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Considering these facts, the present study focused on the following objectives; Physicochemcial constants (ie, ash value and extractive value) and Fluorescent analyses of *Wattakaka volubilis* (L.f.) Stapf. Preliminary phytochemical screening of *Wattakaka volubilis* (L.f.) Stapf. using different solvents, and Screening of antimicrobial activity against ethanol, chloroform and petroleum ether leaf extracts of *Wattakaka volubilis* (L.f.) Stapf.

2. Materials and Methods

2.1. Collection and processing

The fresh plant materials were collected from Sirumalai hills, Dindigul district, Tamil Nadu. The leaves were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

2.2. Preparation of extracts for Phytochemical screening.

2.2.1. Extraction

Freshly collected plant material were dried in shade, and then coarsely powdered in a blender. The coarse powder (100g) was extracted successively with benzene, chloroform and methanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts (Chloroform, Ethanol and Petroleum ether) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures (Brinda *et al*., 1981; Lala, 1993). All the three extracts of the plant samples were used for Antimicrobial activity studies.

2.3. Physicochemical constant and fluorescence analyses

The Physico-Chemical parameters and fluorescence analyses were carried out as per the standard procedures (Lala, 1993). In the present study, the whole plant powder was treated with 1N aqueous sodium hydroxide and 1N alcoholic sodium hydroxide, acids like 1N hydrochloric acid, 50% sulphuric acid, nitric acid, acetic acid, Ferric chloride and nitric acid with ammonia. These extracts were subjected to fluorescence analysis in visible/daylight and UV light (254nm & 365nm).

The ash and extractive values were determined by following methods (Trockenbrodt, 1990 and Anonymous, 1996).

2.4. ANTIBACTERIAL ASSAY

2.4.1. Collection of microorganisms

Stock cultures of bacteria such as *Staphylococus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* were obtained from Research Laboratory, Department of Microbiology, Periyar University, Salem, Tamil Nadu.

2.4.2. Preparation of media

The growth media employed in the present study included Nutrient agar and Nutrient broth. The medium was adjusted to pH 7.4 and sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes.

2.4.3. Sub culturing of microorganisms

The pure culture of microorganism was maintained on nutrient agar slants by frequent sub culturing. The culture was stored at 4^0C.

2.4.4. Preparation of inoculum

Each organism was recovered for testing by sub culturing on fresh media. A loopful inoculum of each bacterium was suspended in 5ml of nutrient broth and incubated overnight at 37^0C. These overnight cultures were used as inoculum.

2.4.5. Antimicrobial activity

Antimicrobial activity was demonstrated by modification of the method described by Barry and Thornsberry, (1985). 0.1 ml of the diluted microbial culture was spread on sterile nutrient agar plate. The pre-soaked and dried discs of 6mm diameter of What man No.1 filter paper were then placed on the seeded plates and gently pressed down to ensure contact. At the same time standard antibiotic of Tetracycline (30µg/ disc) was used as reference or positive control. Respective solvents without plant extracts served as negative control. The plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extract saturated

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discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone around the discs were measured and recorded as the difference in diameter between the disc (6mm) and growth free zone.

3. Results and Discussion

3.1. Physicochemical constants (Ash and Extractive values)

The result of the ash and extractive values of *Wattakaka volubilis* are reveals in table 1 and 2. The total ash content of the powdered leaves of *Wattakaka volubilis* is 8.41% respectively. The extractive value of ethanol is more than that in other solvents investigated in the present study. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs (African pharmacopoeia, 1986). Equally important in the evaluation of crude drugs is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign organic matter such as metallic salts and/or silica (Musa *et al.,* 2006). The above said ash values are indicative of the impurities present in the drug. Since the ash values are constant for a given drug, these values are also one of the diagnostic parameters of the drug. The results of various types of ash and extractive values may provide a basis to identify the quality and purity of the drug.

3.2. Fluorescent analysis

The results of fluorescent analysis of *Wattakaka volubilis* are shown in table 3. The leaves of *Wattakaka volubilis* shows the characteristic fluorescent green colour treated with 1N HCl and Nitric acid under long UV light and Acetic acid under short UV light. Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples (Pimenta *et al*., 2006).The powdered leaves of *Wattakaka volubilis* produce dark green under day light short UV and long UV radiation.

3.3. Phytochemical analysis

The results of preliminary phytochemical screening of leaves of *Wattakaka volubilis* are presented in table 3. The chloroform extracts of *Wattakaka volubilis* shows the presence of coumarin, protein, steroid, sugar and tannin. The petroleum ether extracts of leaves exhibited alkaloid, protein, steroid, sugar and tannin. The ethanol extracts of leaves represent alkaloid, flavonoid, phenol, protein, saponin, steroid, sugar and tannin.

Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Different chemical compounds such as alkaloids, coumarin, flavonoid, phenol, protein, saponin, steroid, sugar and tannin were detected in leaves of *Wattakaka volubilis* extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound.

To understand the nature of the fluorescence emission from these crude preparations under different conditions, the preliminary phytochemical analysis of these crude preparations was compared. The comparative analysis clearly showed a correlation between a compound present in it and their fluorescent behaviour under different conditions. The major bioactive compounds present in these crude preparations are the coumarin, flavones, tannins, alkaloids and saponin. Coumarin especially hydroxyl amino acid derivatives like o-coumaric acid appears yellowish green in alkaline condition under short UV radiation. Flavones which are light yellow in aqueous condition under UV light turns to bright yellow under alkaline conditions. Similarly the phytosterols when treated with 50% H_2SO_4 show green fluorescence under UV light. Terpenoids especially sapogenins exhibit yellow green fluorescence under short UV light (Horborne, 1976). Quinine, aconitin, berberin and emetin show specific colour of fluorescence (Aconitin - light blue; berberin - light yellow; emetin - orange). Fixed oils and fats fluoresce least, waxes more strongly and mineral salts most of all (Evans, 1996). Haydon (1975) studied the photophysical characters of coumarins.

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Table -1: The total Ash values of the powdered leaves of *Wattakaka volubilis*

Sl. No.	Type of ash	Ash value (%)
1	Total ash value of powder	8.41 ± 0.13
2	Water soluble ash	3.14 ± 0.26
3	Acid insoluble ash	4.01 ± 0.06
4	Sulphated ash	9.82 ± 0.10

Table –2: The Extractive values of the powdered leaves of *Wattakaka volubilis*

Sl. No.	Name of the extract	Extractive values (%)
1	Petroleum ether	7.81 ± 0.11
2	Benzene	10.16 ± 0.27
3	Chloroform	12.14 ± 0.19
4	Acetone	10.36 ± 0.27
5	Methanol	12.17 ± 0.15
6	Ethanol	14.41 ± 0.31
7	Water	13.14 ± 0.27

Table 2. Fluorescence analysis of powdered in leaves of *Wattakaka volubilis*

S. No.	Experiment	Visible/day light	UV light	
			254 nm (SWL)	365 nm (LWL)
1	Powder as such	Light green	Dark green	Light green
2	Powder + 1N NaOH (aqueous)	Light green	Dark green	Light green
3	Powder + 1N NaOH (alcohol)	Dark green	Dark green	Light green
4	Powder + 1N HCl	Dark green	Dark green	Fluorescence green
5	Powder + 50% H_2SO_4	Dark green	Dark green	Light green
6	Powder + Nitric acid	Brown	Light green	Fluorescence green
7	Powder + Acetic acid	Fluores-cence green	Light green	Pink
8	Powder + Ferricchloride	Dark green	Dark green	Dark green
9	Powder +HNO_3 +NH_3	Dark green	Light green	Light green

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Table 3. Preliminary phytochemical screening of powdered leaves of *Wattakaka volubilis*

S. No.	Phytochemicals	Chloroform	Ethanol	Petroleum ether
1	Alkaloid	-	+	+
2	Anthroquinoune	-	-	-
3	Coumarin	+	-	-
4	Flavonoid	-	+	-
5	Phenol	-	+	-
6	Protein	+	+	+
7	Saponin	-	+	-
8	Steroid	+	+	+
9	Sugar	+	+	+
10	Tannin	+	+	+

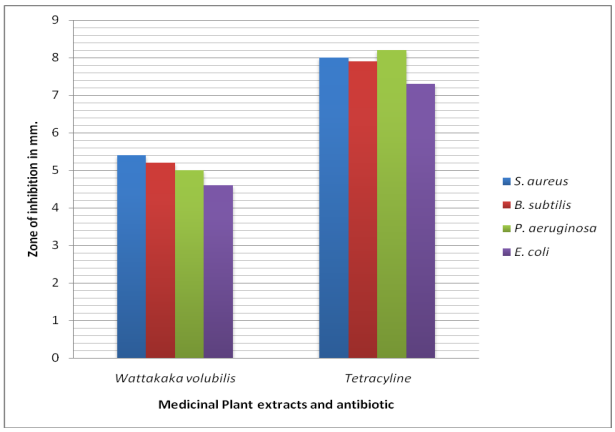

Fig.1: Antibacterial activity of chloroform extract of *Wattakaka volubilis*

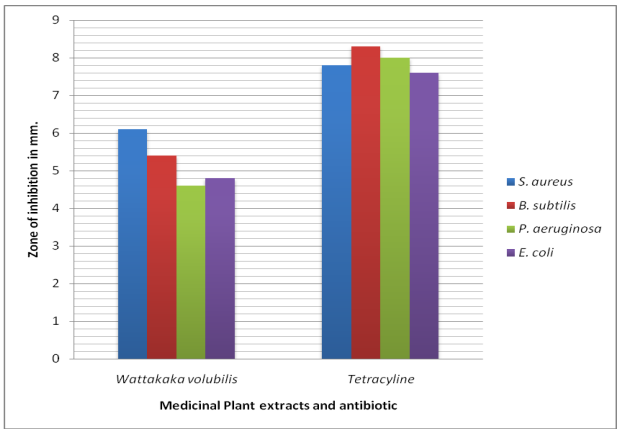

Fig.2. Antibacterial activity of ethanol extract of *Wattakaka volubilis*

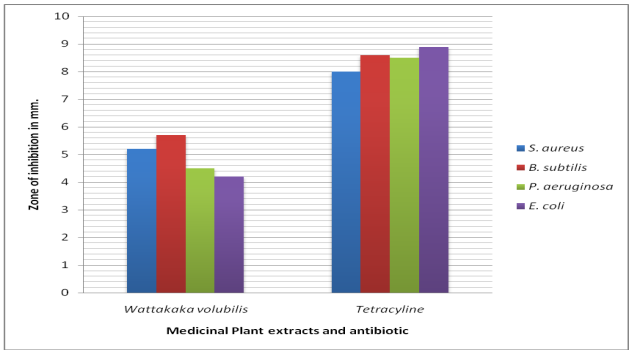

Fig.3. Antibacterial activity of petroleum ether extract of *Wattakaka volubilis*

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Hydroxy methyl coumarin fluoresced in the 420 - 440nm when observed in different solvents with increasing polarity (Chaltopudhyay, 2006). The fluorescence analysis of the crude drugs of *Wattakaka volubilis* exhibited clear fluorescence behaviour at different radiations which can be taken as standard fluorescence pattern.

4. Antibacterial activity

4.1. Antibacterial activity of chloroform extracts

Chloroform extracts of leaf powder of *Wattakaka volubilis* (Fig. 1). Considerable antibacterial activity was observed against *Staphylococcus aureus* (5.4mm) followed by *Bacillus subtilis* (5.2 mm)

4.2. Antibacterial activity of ethanol extracts

Considerable antibacterial activity was observed *Staphylococcus aureus* (6.1mm) followed by *Bacillus subtilis* (5.4mm) in ethanol extracts of leaf powder of *Wattakaka volubilis* (Fig.2).

4.3. Antibacterial activity of petroleum ether extracts

Petroleum ether extracts of leaf powder of *Wattakaka volubilis* (Fig.3). Considerable antibacterial activity was observed *Bacillus subtilis* (7mm) followed by *Staphylococcus aureus* (5.2mm).

Among the bacterial organisms tested, the chloroform and ethanol extracts of *Wattakaka volubilis* showed higher inhibitory activity with *Staphylococcus aureus* and *Bacillus subtilis.* The three extracts of above said plants inhibited the growth of this organisms compared with tetracycline as positive control. The results obtained are encouraging as the chloroform and ethanol extracts have shown considerable antibacterial activity against tested organisms. The antibacterial activities of the in this plants may be attributed to the presence of bioactive principles such as phenols, steroids, alkaloids and flavonoid. The screening and scientific evaluation of plant extracts against microbes may provide new antimicrobial substances. Also, plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic materials (Iwu *et al*., 1999). Despite the reported use of the tested plants and their products for medicinal purposes, there have been only few reports related to the antibiotic response and the phytochemical analysis of the reported plants.

Recent evidence from the pharmaceutical companies shows that for some complex diseases, natural products still represent an extremely valuable source for the production of new chemical entities, since they represent privileged structures selected by evolutionary mechanisms over a period of millions of years.

5. Conclusion

The present study selected ethnomedicinal plants were based on their ethnobotanical uses of *Palliyar* tribals in Western Ghats, Tamil Nadu for the treatment of diabetes. The study reveals that the leaf extracts of *Wattakaka volubilis* are stronger extraction capacity of ethanol could have been produced number of active constituents responsible for many biological activities. Chemical and phytochemical analysis depicted the presence of many phytochemicals. The plant extract can be used safely for longer duration as a cheap source of active therapeutics for alleviation of commonly occurring ailments by the poor and under privileged people of India.

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