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Antibacterial and Antifungal Activity of Roots of *Wattakaka volubilis*

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Abstract: *Wattakaka volubilis* is a tall woody climber belonging to the family Asclepiadaceae. Antibacterial and antifungal screening was carried out using both tube dilution and well diffusion method for the first time exclusively on ethanolic extract of the roots of the plant which was tested against six bacterial species viz *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium, Staphylococcus aureus, Klebsiella species* and two antifungal species viz *Aspergillus niger, Claviceps purpurea.* The extract was effective against all the bacterial and fungal species tested. Increase in extract concentration showed appreciable decline in viable count of the micro-organism indicating significant bioactivity.

Key words: Wattakaka Volubilis · Asclepiadaceae · Antibacterial · Antifungal

INTRODUCTION

Wattakaka volubilis (Linn.f.) Stapf., (Syn. *Dregea volubilis* (L.f.) Benth. ex Hook.f., *Marsdenia volubilis* Cooke) (Family: *Asclepiadaceae*) is a tall woody climber, with densely lenticellate and pustular branches, leaves opposite, broadly ovate or suborbicular, cordate, acuminate, flowers bright yellowish-green, in lateral, drooping, umbellate, cymes, follicle usually 2, broadly lanceolate covered with brown, mealy, ferruginous tomentum, turgid, c. 2cm long; seeds yellowish brown, broadly ovate or broad elliptic, winged, comose [1]. It is found distributed in Bengal, Assam [2, 3], Konkan, Maharashtra, Deccan [3], Bangalore, Mysore, Sri Lanka [4] and districts of Madras [5]. Many phytoconstituents like steroids, steroidal glycosides, sugars, triterprnoids, flavonoids, phenolic compounds and some alkaloids are found to be present in the plant [1]. The leaves are much employed as an application for boils and abscesses. The roots and tender stalks are considered emetic and expectorant [1, 2]. It is also used in eye diseases and snake bites [6].

MATERIAL AND METHODS

Collection of Plant Material: The roots of *Wattakaka volubilis* were collected from Gomantak Ayurvedic Mahavidyalaya and Research Centre, Shiroda-Goa by Dr. S.K. Das during November. It was authenticated by Prof. G.I. Hukkeri, Associate Professor, Department of Botany, Dhempe College of Arts and Science, Miramar- Goa, India.

Preparation of Ethanolic Extract: The roots were dried in shade. The dried roots (500g) were powdered and exhaustively extracted by maceration with ethanol for three days. After three days, the ethanolic layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off using rotary flash evaporator and the concentrate was evaporated to a syrupy consistency and then evaporated to dryness (10g).

Preliminary Phytochemical Analysis: Qualitative analysis was carried out of the crude ethanolic extract which revealed the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, steroids, phenolic compounds, glycosides and starch.

Isolation of Phytoconstituents from Ethanol Soluble Fraction: Chromatographic elution's led to the isolation of 8 plant constituents namely 9, 12-octadecadienoic acid; β-sitosterol; drevogenin A; 1, 2-benzenedicarboxylic acid diisooctyl ester; 5, 7-dihydroxy-6, 8-dimethoxy flavone; quinic acid, N-[4-Bromo-n-Butyl]-2-Piperidinone and digitoxose [7].

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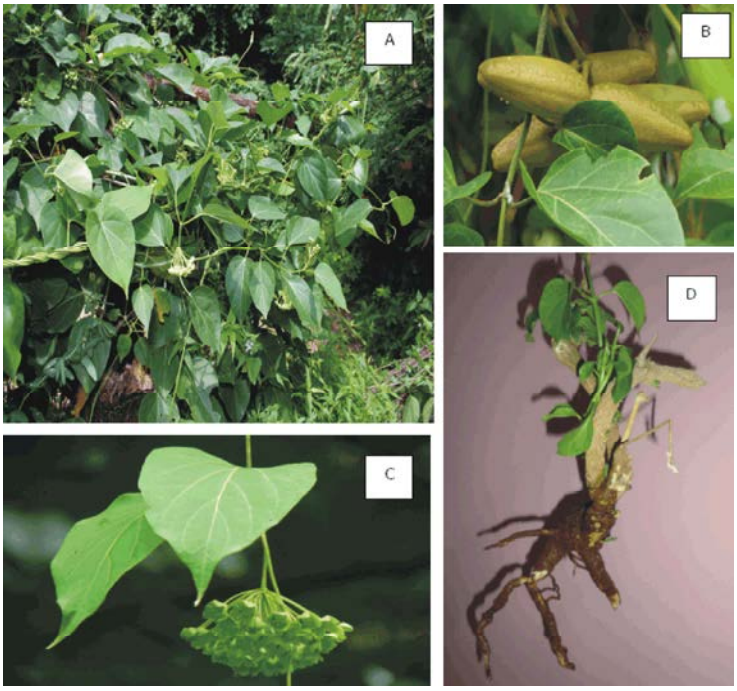


A- The entire plant
B- Fruits
C- Leaves
D- The whole plant along with roots that were used in the study

Microbial Strains: Six bacterial and two fungal strains were used for their susceptibility to *Wattakaka volubilis*: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium* ATCC 23564, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 19429, *Klebsiella species, Aspergillus niger* ATCC 10864 and *Claviceps purpurea* NCIM 1046. The following strains were procured from National Chemical Laboratory (NCL)-Pune.

Determination of Antimicrobial Activity: The antibacterial and antifungal activities were performed by using well diffusion method and tube dilution method. Minimum Inhibitory Concentration (MIC) was performed by two-fold dilution of extract in respective medium under sterile condition. The suspension prepared was verified by streaking on specific medium for colony identification and purification. Appropriate controls were maintained.

Mueller-Hinton agar and Sabourauds Dextrose agar was used for antimicrobial activity testing by well diffusion method of bacteria and fungi respectively. 100µl of the suspension was transferred into containing agar using a micropipette. The plates were then allowed to solidify at room temperature to form the layer. Using a sterile cork borer, wells were bored at the center of each plate. While boring the next plate the borer was dipped in alcohol and flamed to avoid cross contamination. Test solution was introduced into the well by using sterile micropipettes. The inoculated plates were kept in the refrigerator at 2-8°C for 10-15 mins for the diffusion of the test solution. The plates were then incubated for 24 hrs in an incubator at 37°C for bacterial strains and 7 days for fungal strains [8].

Mueller-Hinton broth and Sabourauds dextrose broth was used for tube dilution method. Concentrations of the extract ranging from 1000-31.25 µg/ml were prepared by successive dilution of the broth. 100 µl of culture suspension was transferred into each test tube. All the tubes were incubated for 24 hrs for bacterial strains and 7 days for fungal strains at 35°C on a shaker [9].

The plates were checked visually and diameter of zone of inhibition was measured in mm. Clear zones around the well indicated significant activity. The bioassay was repeated thrice to determine effectiveness of procedure. The MIC was determined by measuring the optical density (O.D.) using Elico colorimeter filter no. 60 and MIC results were further reinforced by determining viable count by pour plate method.

RESULTS

The present study revealed that the ethanolic extract of *Wattakaka volubilis* possesses potential antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella species* and antifungal activity against *Aspergillus niger* and *Claviceps purpurea*. The MIC ranged from 500-1000 µg/ml.

DISCUSSION

From the literature survey if was found that the ethanolic extract of the leaves possesses antibacterial [10] and aqueous, petroleum ether, methanol and ethyl acetate extract of whole plant of the plant possesses antibacterial and antifungal activity [11]. The phytoconstituents β- sitosterol [12]; 1, 2-benzenedicarboxylic acid diisooctyl ester (di-octyl phthalate) [13]; 2-phenyl-5, 7-dihydroxy-6, 8-dimethoxy flavone [14]; quinic acid [15] and N-[4-Bromo-n-Butyl]-2-Piperidinone [16] have been reported to possess antimicrobial properties.

The stem and leaves contain a pigment taraxrol, a triterpenoid, Kaempferol, a glycoside of kaempferol and saponins. From the stems, leaves and bark drevogenin A, drevogenin P, D-cymarose and L-oleandrose have been separated. The seeds contain a number of pregnane glycoside like drevoside A,B,C, and D. The roots contain traces of an alkaloid and a glucoside that lowered the carotid blood pressure when administered intravenously and had mild stimulant action on organs having autonomic nerve supply [1].

The plant is also known to possess anti-inflammatory, analgesic, antipyretic [17], anthelmintic [18], anti-oxidant [10, 19], antidiabetic, antihyperlipidaemic [19], anti-diarrheal [20], anti-tumor [21] and hepatoprotective property [22].

In the present study the ethanolic extract of the roots showed significant antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella species* and antifungal activity against *Aspergillus niger* and *Claviceps purpurea* (Tables 1,2). In the MIC studies negligible growth was

Table 1: Effect of ethanolic extract of *Wattakaka volubilis* by well diffusion method

	Diameter of Zone of Inhibition for Bacteria in mm					
sample	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*	*Salmonella typhimurium*	*Klebsiella species*
Extract	14	22	13	15	25	14
Streptomycin	34	50	40	39	44	34

Concentration of the extract is 25mg/ml
Solvent control did not exhibit any zones

Table 2: Effect of ethanolic extract of *Wattakaka volubilis* by tube dilution method on fungi

	Tube Dilution Method											
	Aspergillus niger						*Claviceps purpurea*					
Concentration in µg/ml	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25
Extract	-	-	-	-	+	+	-	-	-	+	+	+

Concentration of extract is 2mg/ml
- Negative indicates no growth
+ Positive indicates growth

Table 3: Effect of ethanolic extract of *Wattakaka volubilis* by tube dilution method on bacteria

Concentration µg/ml	*Escherichia coli*	*Salmonella typhimurium*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*	*Klebsiella species*
+ve	0.91	1.00	0.99	1.03	0.70	0.82
31.25	0.69	0.64	0.87	0.65	0.58	0.76
62.5	0.54	0.47	0.43	0.52	0.32	0.60
125	0.40	0.29	0.34	0.42	0.20	0.52
250	0.16	0.11	0.12	0.23	0.13	0.44
500	0.13	0.11	0.11	0.12	0.11	0.17
1000	-	-	-	-	-	-

Concentration of extract is 2mg/ml
Negative control O.D – 0.01
- Indicates no growth

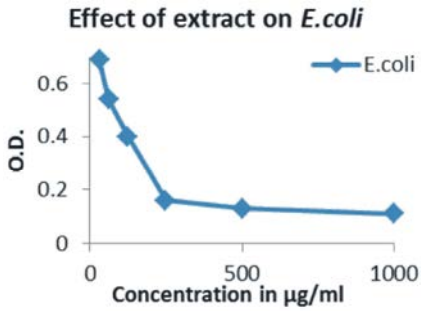
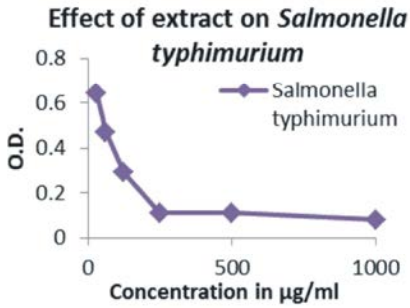


Fig. 1:



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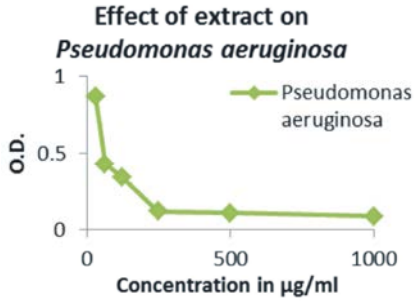

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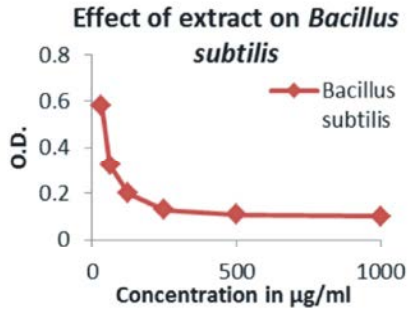
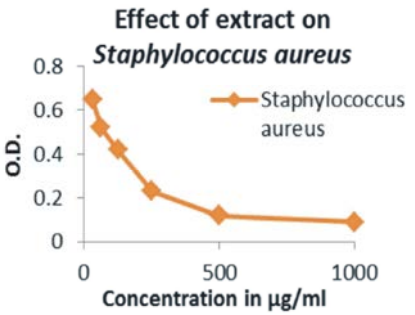


Fig. 4:

observed at 500 µg/ml concentration and significantly no growth at 1000 µg/ml concentration of the extract. The O.D. results indicated that as the concentration of the extract increases viable count decreases so the MIC ranges from 500-1000 µg/ml (Table 3, Figures 1-6).



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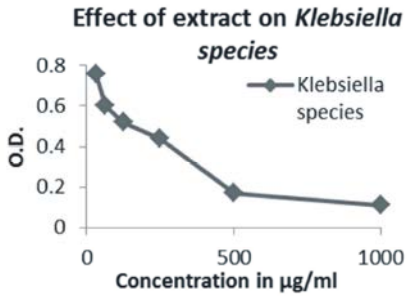

Fig. 6:

It is interesting to know that cultures like *Staphylococcus aureus* and *Pseudomonas aeruginosa* were inhibited by the extract are often the causative agents for boils and abscesses [23]. This plant has been traditional medicine for the treatment of boils and abscesses [1].

The antibacterial and antifungal activity has been carried out for the first time from the ethanolic extract of the roots of *Wattakaka volubilis* and it shows a broad spectrum of antimicrobial activity.

CONCLUSION

The present study confirmed that the roots of the plant *Wattakaka volubilis* possess significant antibacterial and antifungal activity. The phytochemical investigation led to the isolation of a fatty acid 9, 12-octadecadienoic acid; a steroid β-sitosterol; a triterpenoid aglycone drevogenin A; aromatic ester 1, 2-benzenedicarboxylic acid diisooctyl ester (di-octyl phthalate); a flavonoid 5, 7-dihydroxy-6, 8-dimethoxy flavone; a phenolic compound quinic acid, an alkaloid N-[4-Bromo-n-Butyl]-2-Piperidinone and a desoxy sugar digitoxose. The antimicrobial activity may be attributed to the presence of these bioactive constituents and also due to synergistic effect of other components.

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