

Antibacterial Activity of Ethyl Acetate Extract of *Platycladus orientalis* against *Staphylococcus saprophyticus*

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Abstract

Fruits of *Platycladus orientalis* growing wild in Arar region, Saudi Arabia was investigated for its antibacterial activity against *Staphylococcus saprophyticus*. A bacterium associate with serious urinary tract infections (UTI) and cystitis in young human females. GC-MS analysis discloses the presence of some bioactive compounds, such as cupressene, podocarpa-8,11,13-triene, kauran-16-ol, kerruginol, glycoside compounds, cymarins and Periplocymarin which could be attributed to the antibacterial efficacy of that plant product. The extract showed remarkable antibacterial activity against *Staphylococcus saprophyticus*, the disc diffusion test recorded 16.0 ± 1.0 mm zone of inhibition, with relative percentage inhibition (RPI) of *Platycladus orientalis* extract was 66.6% compared with the chloramphenicol. The MIC and MBC values were 12.5 mg/ml and 50 mg/ml, respectively. The MBC/MIC ratio was 4, indicating that the extract has a good bacteriostatic effect. Therefore, the fruits of *Platycladus orientalis* could be used in the formulation of antibacterial drugs against *Staphylococcus saprophyticus* associated with UTI infections.

Keywords: *Platycladus orientalis*, GC-MS, antibacterial, *Staphylococcus saprophyticus*.

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(Received: 28 January 2019; accepted: 13 April 2019)

Citation: Emad Mohamed Abdallah and Eman Ramadan Elsharkawy, Antibacterial Activity of Ethyl Acetate Extract of *Platycladus orientalis* against *Staphylococcus saprophyticus*, *J Pure Appl Microbiol.*, 2019; **13**(2): 1063-1068. doi: 10.22207/JPAM.13.2.44

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INTRODUCTION

Medicinal plants have been used since times immemorial and the accumulated knowledge in herbal formulations were passed down throughout ancient civilizations until reach recent history where drug discoveries led to the isolation of numerous medications from medicinal plants such as morphine, quinine, digitoxin, cocaine, codeine and many more¹. With the turn of the twentieth century, the discovery of antibiotics has given humans victory over pathogenic microbes. The discovered antibiotics were initially natural product secretions, isolated from fungi, bacteria and environmental microorganisms. Several decades later, the molecular genetic system of microbes developed resistance against these antibiotics, which led to the need to find urgent alternatives, under the absence of any newly discovered antibiotics in the last 30 years². The growing problem of antibiotics resistance has led to raising the interest of medicinal plant investigations particularly those with ethnopharmacological heritage to uncover new antimicrobial agents³. *Platycladus orientalis* Linn. (Synonymous: *Thuja orientalis*) is belong to family Cupressaceae, it is a coniferous shrub with evergreen and scale-like leaves; It is used in traditional medicine in the treatment of bronchitis, cystitis, enuresis, psoriasis, amenorrhea, uterine carcinomas and rheumatism⁴. The essential oils obtained from *Platycladus orientalis* contains β -thujone and β -thujone which showed toxic effects on ruminant animals⁵. However, the ethanol extract of *Platycladus orientalis* is non-toxic at low concentration (≤ 0.6 mg/ml) and it suppressed the growth of human lung cancer cell line⁶. *Staphylococcus saprophyticus* is a Gram-positive coccus, a leading cause of urinary tract infections (UTI) and cystitis in young human females with virulent strains resistant to many UTI antibiotics such as fosfomycin trometamol and cefixime⁷. The present investigation is aimed to study the efficacy of ethyl acetate extract of *Platycladus orientalis* against referenced strain of *Staphylococcus saprophyticus*.

MATERIALS AND METHODS

Plant Material

The fruits of *Platycladus orientalis* were collected from Arar region, Northern area,

Saudi Arabia in March 2018. An authenticated sample was deposited in Faculty of science girl section. Fruits were cut in to small pieces, dried in incubator at 40°C for up to a week and ground to a fine powder.

Plant extraction

The air-dried powdered fruit (100 g) of *Platycladus orientalis* were subjected to successive extraction by percolation in ethyl acetate for a week. Then, the percolate was filtered and the solvent was evaporated by rotary evaporator under reduced pressure, until dryness. The extract (18.00 g) was dissolved in a suitable amount of distilled H₂O–MeOH (95:5 v/v) then the extract was kept for GC-MS analysis.

GC-MS analysis

The compounds were analyzed using a Thermo GC-Trace ultra system (Thermo Co. USA), they were separated on 30m X 0.25 mm X 0.25 μ m Elite-5MS column (Thermo Scientific GC Column). The column temperature was increased from 40°C to 220°C at a rate of 4°C/min; injector temperature is 250°C; injection volume is 1 μ l; helium carrier gas flow rate is 20ml/min; transfer temperature is 280°C. MS parameters were as follows: EI mode, with ionization voltage 70 eV, ion source temperature, 180°C; scan range, 50-600 Da. The peaks were tentatively identified based on library search using NIST and Wiley Registry 8 Edition.

Antibiotic susceptibility testing

The antibiotics susceptibility profile of *Staphylococcus saprophyticus* ATCC 43867 was performed using Kirby-Bauer disk diffusion method following the guideline of Clinical and Laboratory Standards Institute (CLSI)⁸. Briefly, an overnight culture from *Staphylococcus saprophyticus* isolate was adjusted to be equivalent to 0.5 McFarland. 100 μ l of the adjusted suspension was streaked over sterile Mueller-Hinton agar. Then, standard antibiotic rings (Mastring-STM MOH50/2 and MOH50/3) were aseptically loaded in to streaked plates and incubated for up to 18h at 37°C. The zone of inhibition around the antibiotic discs were measured and recorded as resistant or sensitive according the manufacturer's instructions that are based on the CLSI guidelines.

Antibacterial activity assay

The antibacterial activity of *Platycladus orientalis* ethyl acetate extract against *Staphylococcus saprophyticus* was initially

determined using disc diffusion method⁹, with minor modifications. The extract was re-constituted in dimethyl sulfoxide (DMSO) to get concentration 300mg/ml., DMSO has no effect on the bacterial growth. The bacterial suspension was adjusted to 0.5 McFarland standard (1×10^8 CFU/ml). 100 μ l of the adjusted suspension was spread over a sterile Mueller-Hinton Petri-dishes and left for a while. 6 mm paper discs (made from Whatman No. 1) were impregnated with about 20 μ L of the extracts and placed on the inoculated Mueller Hinton agar Petri-dish, a disc saturated with about 20 μ L Chloramphenicol (5mg/ml) was also placed on the plate to serve as control. The Petri-dish was incubated at 37°C for overnight and the diameters of the inhibition zones were measured.

Minimum inhibitory concentration (MIC)

The MIC of the extract was evaluated by tube dilution method¹⁰. The various two-folds dilutions of the extract (6.25, 12.5, 25, 50, 100 and 200 mg/ml) were made in tubes containing nutrient broth. Equal volume of the extract and nutrient broth were mixed in each test tube. 0.1ml of adjusted inoculum of *Staphylococcus saprophyticus* (1×10^8 CFU/ml) was added to each tube. All tubes were incubated for 18 hours at 37°C. Two control tubes were also maintained, one was positive control contains antibiotic instead of extract and the other one is a negative control contains bacterial inoculum, physiological saline

Table 1. GC-MS analysis of ethyl acetate fraction of fruit of *Platycladus orientalis*

No.	Compounds	Chemical structure	Percentage (%)
1	Cymarín	$C_{30}H_{44}O_9$	2.3
2	Periplocymarín	$C_{30}H_{46}O_8$	2.5
3	Cupressene	$C_{20}H_{32}$	0.23
4	Podocarpa-8,11,13-triene	$C_{17}H_{24}$	0.62
5	Keto-Dihydro-gendunín	$C_{26}H_{32}O_6$	0.23
6	Methyl abietate	$C_{21}H_{32}O_2$	0.45
7	Kauran-16-ol	$C_{20}H_{34}O$	1.4
8	Isosteviol methyl ester	$C_{21}H_{32}O_3$	1.2
9	Ferruginol	$C_{20}H_{30}O$	5.5
10	Thunbergol	$C_{20}H_{34}O$	3.2

and broth medium. MIC was considered as the lowest concentration of the extract showing no visible growth (no turbidity) when compared with the control tubes.

Minimum bactericidal concentration (MBC)

MBC was determined by sub-culturing the MIC test dilutions¹¹. Briefly, 50 μ l of the incubated test tubes (from the MIC assay) was pipetted and transferred to sterile nutrient agar plates, incubated overnight at 37°C and inspected for visible growth on the agar. The lowest concentration of MIC which exhibited no visible bacterial growth on agar medium was taken as MBC.

Relative percentage inhibition (RPI)

The relative percentage inhibition (RPI)¹² of 5mg/ml chloramphenicol (10 mg/disc) 300mg/ml *Platycladus orientalis* extract (20 μ L/disc) with respect to *Staphylococcus saprophyticus* was calculated using the following formula:

Table 2. Antibiotic profile of *Staphylococcus saprophyticus**

Sensitive	Resistant	Antibiotic
+	–	C
–	+	CAZ
+	–	IMI
+	–	CIP
+	–	PRL
+	–	T
+	–	AK
–	+	ATM
–	+	AUG
–	+	CTX
–	+	OFX
–	+	KF
+	–	TS
+	–	GM
+	–	TN
–	+	AP

* + = Present, – = Absent, C=Chloramphenicol 25 μ g, CAZ=Ceftazidime 10 μ g, IMI= Imipenem 10 μ g, CIP= Ciprofloxacin 1 μ g, PRL= Piperacillin 30 μ g, T= Tetracycline 30 μ g, AK= Amikacin 30 μ g, ATM= Aztreonam 30 μ g, AUG=Augmentin 30 μ g, CTX= Cefotaxime 5 μ g, OFX= Ofloxacin 5 μ g, KF=Cephalothin 5 μ g, TS=Cotrimoxazole 25 μ g, GM=Gentamicin 10 μ g, TN=Tobramycin 10 μ g, AP=Ampicillin 10 μ g.

$$RPI\% = \frac{100 \times (X - Y)}{Z - Y}$$

Where: (X) is the total area of inhibition of the herbal extract, (Y) is the total area of inhibition of the solvent and (Z) is total area of inhibition of the antibiotic. The total area of the inhibition zone was calculated based on the following equation:

$$\text{Area} = \pi \times r^2$$

where: (r) is the radius of the zone of inhibition, $\pi = 3.14$

RESULTS AND DISCUSSION

Medicinal herbs were the primary source of drugs since ancient times and till now. In the current investigation, the GC-MS analysis of ethyl acetate extract of *Platycladus orientalis* fruits revealed the presence of some important bioactive compounds, such as Ferruginol (5.5%), Thunbergol (3.2%), Periplocymarin (2.5%), Cymarin (2.3%),

Kauran-16-ol (1.4%), Isosteviol methyl ester (1.2%), Podocarpa-8,11,13-triene (0.62%), Methyl abietate (0.45%), Cupressene (0.23%) and Keto-Dihydro-gendunin (0.23%), (Table 1). Similar study on the fruit oil of *Platycladus orientalis* (Syn. *Thuja orientalis*) was conducted in Nigeria, the GC-MS revealed the presence of 2-carene (21.1 %), 3-carene (15.8 %), β -myrcene (8.0 %), terpinen-4-ol (7.8 %), cedrol (7.8 %). D-limonene (5.5 %), β -caryophyllene (5.2 %), γ -terpinene (4.7 %) and β -humulene (4.2 %) ¹³. Variations in the chemical constituents of the same plant species could be attributed to variations in the environmental conditions between different geographical locations.

The antibiotic profile of the investigated strain of *Staphylococcus saprophyticus* showed that it was resistant to 7 among 16 tested antibiotics, in an average of resistance equals 47.75% (Table 2).

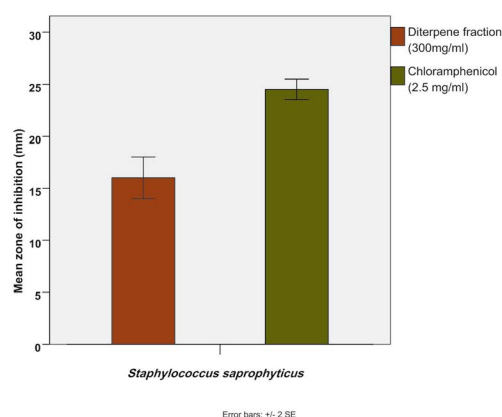


Fig. 1. Susceptibility of *Staphylococcus saprophyticus* to the extract of *Platycladus orientalis* compared with chloramphenicol



Fig. 2. Representative photo showing susceptibility of *Staphylococcus saprophyticus* to the extract and antibiotic using disc diffusion test

Table 3. Antibacterial activity of ethyl acetate fraction of fruit of *Platycladus orientalis* against *Staphylococcus saprophyticus*

Tested compound	Mean zone of inhibition (mm)
TO (300 mg/ml)	16.0±1.0
CH (2.5 mg/ml)	24.5±0.5

Legend: TO= *Platycladus orientalis* (ethyl acetate fraction), CH= chloramphenicol, mean of two replicates, diameter of the paper disc =6 mm.

Table 4. MIC and MBC value of fruits of *Platycladus orientalis* against *Staphylococcus saprophyticus*

Test	Concentration of ethyl acetate fraction of <i>Platycladus orientalis</i> (mg/ml)					
	200	100	50	25	12.5	6.25
MIC	—	—	—	—	+	+
MBC	—	—	+	+	+	+

— = No microbial growth, + = Presence of microbial growth.

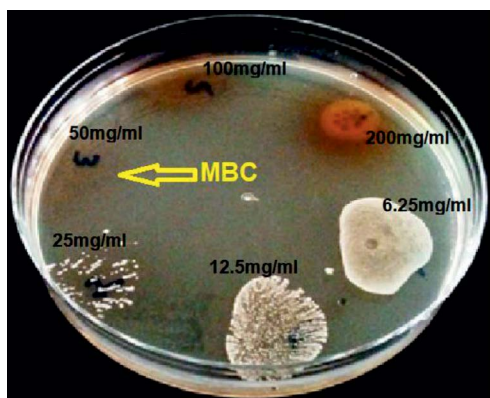


Fig. 3. Representative photo showing determination of MBC value (white spots are the bacterial growth)

This reflects the urgent need for developing new antibacterial drugs. *Staphylococcus saprophyticus* is a second most common cause of urinary tract infections, particularly in young women and it is showing growing resistance to antibiotics and requires urgent intervention to curb this serious health phenomenon¹⁴.

Result of the antibacterial activity of the ethyl acetate fraction of fruit of *Platycladus orientalis* against *Staphylococcus saprophyticus* is represented in (Table 3). Which showed that this plant extract has remarkable antibacterial activity, with mean zone of inhibition 16.0 ± 1.0 , in comparison with the standard drug (chloramphenicol) that recorded 24.5 ± 0.5 mm zone of inhibition (Fig. 1 and 2). The RPI, MIC and MBC values supported the findings of the disc diffusion test, which are tabulated in (Table 4), The Relative percentage inhibition (RPI) was calculated and it was found to be 66.6%, which means that the ethyl acetate fraction of fruit of *Platycladus orientalis* has comparable anti-bacterial efficacy against *Staphylococcus saprophyticus*. The MIC value was 12.5 mg/ml, whereas the MBC value was 50 mg/ml (Fig. 3). Moreover, the MBC/MIC ratio is 4, which means that the extract has a bacteriostatic effect on *Staphylococcus saprophyticus*. It is known that, the plant extract is considered to be bactericidal when the ratio of $MBC/MIC \leq 4$, while it is considered bacteriostatic when $MBC/MIC \geq 4$ ¹⁵. It was reported that, the essential oil from seed coats of *Platycladus orientalis* (Syn. *Thuja*

orientalis) has shown good to moderate activity against all six tested bacteria, including *Salmonella typhi* which was the most susceptible bacteria¹⁶. Also, the essential oil extracted from fresh fruits of *Thuja orientalis* (Syn. *Platycladus orientalis*) recorded remarkable antibacterial effect against all the Gram-positive and Gram negative bacteria tested with MIC values ranging between 12.8 to 25.6 mg/ml¹⁷. A recent study stated that, *Platycladus orientalis* possessed a potential antibacterial activity against hospital-and community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) and it was recommended for the control of this pathogen¹⁸. However, no data or study was conducted on the antibacterial effect of *Platycladus orientalis* on *Staphylococcus saprophyticus*, in particular, and as the best of the authors knowledge this is the first study on this issue. Accordingly, the current study recommends this plant as a potential antibacterial drug against *Staphylococcus saprophyticus*.

CONCLUSION

Based on the findings of the current investigation, Fruits of *Platycladus orientalis* could be used in the formulation of effective antibacterial drug to be used against UTI infections, in particular. However, compounds revealed by the GC-MS analysis should be isolated and studied individually and more future toxicological and pharmacological studies are recommended. Moreover, it is worthy for the pharmaceutical companies to pay great attention to the natural products and medicinal herbs, in order to innovate new effective antibacterial drugs.

ACKNOWLEDGEMENTS

None.

CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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