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# Antibacterial Activity of Celapanin, a Sesquiterpene Isolated from the Leaves of *Celastrus paniculatus* Willd.

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## ABSTRACT

A sesquiterpene derivative celapanin was isolated from the acetone soluble fraction of an ethanol extract of *Celastrus paniculatus* leaves. The antibacterial activity of the crude ethanol extract and the isolated purified constituent celapanin was screened against 30 clinical strains isolated from different infectious sources which belonging to Gram-negative *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, and Gram-positive *Staphylococcus aureus*. The minimal inhibitory concentrations of the ethanol extract and the constituent celapanin were determined against AmericanTypeCellCulture and MicrobialTypeCellCulture strains. Concentrations higher than 100 µg/100 µL of the ethanol extract and 50 µg/100 µL of the constituent celapanin indicated that their effect was bacteriostatic. The agar wells loaded with celapanin and the ethanol extract exhibited a significant zone of inhibition against the clinical strains of *S. aureus* (22.18 ± 0.30 mm) isolated form the puss of old wound samples of infected patients. A moderate zone of inhibition was observed on the clinical strains of *K. pneumonia* (13.58 ± 0.22 mm) and *P. aeruginosa* (13.10 ± 0.29 mm). The antibacterial activity of celapanin was promising against Gram-positive *S. aureus*, comparative with the standard drug Ciprofloxacin (50 µg/100 µL).

**Keywords:** clinical isolates
**Abbreviations:** **ANOVA**, analysis of variance, **ATCC**, American type cell culture; **DMSO**, dimethyl sulfoxide; **FDD**, flora of Davanagere District; **$^1$H NMR**, proton nuclear magnetic resonance; **IR**, infrared; **MIC**, minimum inhibition concentration; **MTCC**, microbial type culture collection

## INTRODUCTION

In recent years there has been a rising interest in the discovery of new antimicrobial compounds, due to alarming increase in the rate of infections with multi-drug resistant microorganisms (Bassam *et al*. 2006). The increased prevalence of antibiotic resistance bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control bacterial diseases (Cowan 1999). The investigation of new bioactive compounds is of utmost important in the control of antibiotic resistant microorganisms Many investigators have evaluated the bioactivity of plant extracts and the isolated constituents against serious infectious organisms (Kausik *et al*. 2002; Parekh and Sumitra 2006).

*Celastrus paniculatus* Willd. (Celastraceae) is a woody climbing shrub, sparsely distributed in the hilly regions of India and South East Asia, up to an altitude of 1200 meters (Manjunath *et al*. 2004). In the Indian system of medicine this plant is popularly known as 'Jyotishmati' (Sanskrit). The seeds are therapeutically used to treat tranquilization, sedation, hypothermia, anxiety, beriberi and anticonvulsant activity. The decoction of seeds is also administered orally for rheumatism, gout, paralysis, leprosy, scabies, eczema; leucoderma and body ache (Kirthikar and Basu 1995). Seed oil is popularly known as Malkanguni oil, which has been used to improve memory. The ethanol extract of the oil was screened for attenuated hydrogen peroxide and glutamate induced injury in embryonic rat forebrain neural cells (Godkar *et al*. 2006). The possible mechanism in enhancing cognition by the antioxidant property of the seed was reported by Kumar and Gupta (2002). The seed oil extract was screened for antimicrobial against *Escherichia coli*, *Staphylococcus aureus* and fungi like *Candida albicans* and *Tycophytum rubrum*, and for anti-inflammatory activity (Parcha *et al*. 2003).

The phytochemical analysis of the seed extract of *C. paniculatus* revealed the presence of sterols, sesquiterpenoids of β-dihydroagarofuran and β-dihydrogenic series (Tu *et al*. 1993). The seed oil also contains alkaloids such as celastrine and paniculatin (Young and Chewn 1993). The phytochemical constituents of the leaves has not yet been evaluated.

The seed oil was previously screened for antimicrobial activity and was shown to contain celapanin as one of the major compounds. So, in the present study celapanin was isolated from the acetone fraction of ethanol extract of the leaves. The antibacterial activity of the ethanol extract and the constituent celapanin were screened against pathogenic clinical strains of *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from patients of different infectious sources.

## MATERIALS AND METHODS

### Plant material

Leaves of *Celastrus paniculatus* were collected from the Bhadra Wild Life sanctuary of the Western Ghats region of Karnataka, India. The taxonomic identity was confirmed by comparing with the authenticated specimen deposited at Kuvempu University Herbaria (voucher specimen FDD No. 140). The fresh leaves were shade-dried for four days and mechanically ground to a powder. One kg of the powdered material was refluxed with ethanol in a Soxhlet apparatus for 48 hrs in batches of 250 g each. The extract was filtered, pooled and the solvent was removed under reduced pressure at 40 ± 5°C using a rotary flash evaporator (Büchi, Flawil, Switzerland); the yield was 8.22 g.

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**Table 1** Profile of the clinical strains used for antibacterial activity.

| Clinical strains | Clinical condition | Source |
|---|---|---|
| ***S. aureus*** | | |
| Sa1 | Abscess in immunodeficiency | Wounds |
| Sa2 | Burns | Pus |
| Sa3 | Septicemia | 0ld Wounds |
| Sa4 | Food poisoning | Stool |
| Sa5 | Burns | Pus |
| Sa6 and Sa7 | Unknown | Hospital effluent |
| Sa8 | Abscess in immunodeficiency | Sputum |
| Sa9 | Otitis media | Ear swab |
| ***K. pneumonia*** | | |
| Kp1 | Pneumonia | Mucus |
| Kp2 | Gram negative Follicullitis | Stipules |
| Kp3 | Burns | Pus |
| Kp4 | UTI | Urine |
| Kp5 | Septicemia | Sputum |
| Kp6 | Cross infections in UTI | Urine |
| Kp7 | Abscess in immunodeficiency | Wounds |
| Kp8 | Upper UTI | Urine |
| Kp9 | Unknown | Hospital effluent |
| ***P. aeruginosa*** | | |
| Pa1 | Bronchitis | Wounds |
| Pa2 | Otitis media | Pus |
| Pa3 | Burns | Sputum |
| Pa4 and Pa5 | Upper UTI | Stool |
| Pa6 | Food poisoning | Hospital effluent |
| Pa7 | Cross infections in UTI | Hospital effluent |
| Pa8 | Septicemia | 0ld Wounds |
| Pa9 | Unknown | Ear swab |

The constituent celapanin was isolated from the acetone fraction of the ethanol extract. The ethanol extract was washed with acetone and allowed to settle. After 2 hrs the acetone soluble fraction was separated and the insoluble fraction was resuspended and washed again with fresh acetone two to three times. The acetone soluble fraction was chromatographed on a silica gel column using chloroform and acetone in the ratio of 6:4. The eluted fractions were collected at an interval of 5 ml each and were monitored by thin layer chromatography. The fraction recovered in higher concentration was recrystalized from acetone to get a whitish crystalline aromatic compound. The compound was tested qualitatively for its sesquiterpene nature. The structure of the compound was confirmed by IR, $^1$H NMR and mass spectral studies (Sophisticated Analytical Instrument Facility (SAIF), Luknow, India) and the data is mentioned in the results section.

## Antibacterial activity

The antibacterial activity of the crude ethanol extract and its isolated constituent celapanin was screened by the agar well diffusion method (Carron *et al*. 1987) against 27 clinical isolates of each of nine bacterial strains belonging to Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (**Table 1**). The bacterial strains used for screening antimicrobial activity were collected from different infectious statuses of patients who had not taken any antibacterial drugs for at least two weeks with the help of an authorized physician, in the district health center of Gulberga, Karnataka State, India. The clinical isolates were identified following a standard method (Cown *et al*. 1993). The bacterial suspensions were diluted in $10^{-1}$ to $10^{-8}$ phosphate buffered saline. Samples were homogenized and then loaded in six aliquots of 20 µL each onto nutrient agar plates (agar, 15 g/L, beef extract 1 g/L, peptone 5 g/L, NaCl 5 g/L, yeast extract 2 g/L; diameter 55 mm, final pH 7.0 ± 0.2)

The plates were incubated for 24 h at 37°C and counting was done on plates containing 50 to 100 colonies. The activity was screened comparatively with reference ATCC strains (*Pseudomonas aeruginosa*: ATCC-20852; *Staphylococcus aureus*: ATCC 29737) and MTCC strain (*Klebsiella pneumonia*: MTCC-618). The fluoroquinolone antibiotic Ciprofloxacin (BioChemika, ≥98.0% (HPLC) (Fluka)) was used as the standard (50 µg /100 µL of sterilized distilled water) concomitantly with the test samples.

The minimal inhibitory concentrations (MIC) of the crude ethanol extract and the constituent celapanin were determined by micro dilution techniques in nutrient broth, according to National Committee for Clinical Laboratory Standard, USA guidelines (NCCLS 1990). The inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth micro dilution procedure. The microtiter plates were incubated at 37°C and the MIC was determined after 24 h of incubation.

A sensitive radial diffusion technique was used for the assessment of antibacterial activity of the test samples. Sterilized nutrient agar medium was poured into sterilized Petri dishes. Nutrient broth containing 100 µL of 24 h-incubated cultures of the respective clinical isolates and the ATCC and MTCC strains were spread separately on the agar medium. Wells were created using a stainless steel sterilized cork borer under aseptic conditions. Ten mg of ethanolic extract and 5 mg of celapanin were each dissolved in 10 ml of 10% DMSO. One hundred µg/100 µL of the extract and 50 µg/100 µL of celapanin were loaded into corresponding wells. The standard drug Ciprofloxacin was tested at 50 µg/100 µL. The plates were incubated for 24 h at 37°C and the diameter of the zone of complete inhibition of the bacteria was measured around each well and readings were recorded in mm. The results of these experiments are expressed as mean ± SE of six replicates in each test. The data were evaluated by one-way ANOVA followed by Tukey's pair-wise Comparison Test and the results were considered significant when $p<0.05$.

## RESULTS AND DISCUSSION

The whitish crystalline needles eluted from the acetone fraction of ethanol extract of the leaves of *Celastrus paniculatus* tested for qualitative chemical analysis, showed positive in the test for sesquiterpenes (The Ayurvedic Pharmacopoeia of India 1990). The isolated constituent was characterized by IR, $^1$H NMR and MASS spectral analysis and interpretation by comparing with literature data (Wagner and Heckle 1973). In IR a spectrum absorption frequency was observed at 1740 cm$^{-1}$ due to –COO, 1590 cm$^{-1}$ due to N=C bond. The $^1$H NMR spectrum showed signals at δ 2.12 and δ 1.68 ppm for the acetyl-$CH_3$-group. Mass Spectrum showed the peak at 569 indicating the molecular weight of the compound. The melting point of this compound is 246°C, and based on the above data the compound was characterized as celapanin.

Ten different clinical strains of *Staphylococcus aureus* were isolated form the puss samples of old wounds of infected patients. The ethanol extract and the constituent celapanin were most effective in controlling the growth of these clinical strains and also the ATCC strain (**Table 2**). A significant zone of inhibition was observed in the wells loaded with the crude ethanol extract (19.90 ± 0.80) which is nearest to the value of the standard antibiotic Ciproflaxin (20.23 ± 0.49). The zone of inhibition of the constituent celapanin was higher than (22.10 ± 0.98) the value of the standard antibiotic (20.23 ± 0.49). Many researchers have attempted to verify the synergism between antimicrobial

**Table 2** Antibacterial activity of ethanolic extract and its constituent, celapanin against *Staphylococcus aureus*.

| Bacterial strains tested | Zone of inhibition (in mm) | | |
|---|---|---|---|
| ***Staphylococcus aureus*** | **Ethanolic extract** | **Celapanin** | **Reference drug Ciprofloxacin** |
| Sa-1 | 18.43 ± 0.36 | 22.18 ± 0.30 | 18.68 ± 0.26 |
| Sa-2 | 17.95 ± 0.33 | 21.45 ± 0.35 | 20.27 ± 0.26 |
| Sa-3 | 18.83 ± 0.23 | 21.00 ± 0.45 | 20.22 ± 0.23 |
| Sa-4 | 16.80 ± 0.19 | 20.85 ± 0.29 | 19.07 ± 0.20 |
| Sa-5 | 18.85 ± 0.16 | 20.20 ± 0.27 | 21.17 ± 0.26 |
| Sa-6 | 15.85 ± 0.21 | 19.18 ± 0.18 | 18.93 ± 0.30 |
| Sa-7 | 18.97 ± 0.18 | 22.10 ± 0.11 | 20.72 ± 0.22 |
| Sa-8 | 16.18 ± 0.17 | 21.07 ± 0.22 | 20.65 ± 0.17 |
| Sa-9 | 16.95 ± 0.20 | 20.68 ± 0.33 | 19.85 ± 0.15 |
| F-value | 27.1 | 10.0 | 14.0 |

Sa - *Staphylococcus aureus*
The value of each constituents consisted of ± S.D. of 06 replicates.
The F value is significantly different when $p < 0.05$%.

**Table 3** Antibacterial activity of ethanolic extract and its constituent, celapanin against *Klebsiella pneumoniae*

| Bacterial strains tested | Zone of inhibition (in mm) | | |
|---|---|---|---|
| ***Klebsiella pneumoniae*** | **Ethanolic extract** | **Celapanin** | **Reference drug Ciprofloxacin** |
| Kp-1 | 11.45 ± 0.21 | 12.25 ± 0.20 | 19.90 ± 0.13 |
| Kp-2 | 10.67 ± 0.17 | 12.34 ± 0.26 | 20.25 ± 0.28 |
| Kp-3 | 10.40 ± 0.24 | 11.33 ± 0.22 | 20.32 ± 0.30 |
| Kp-4 | 10.18 ± 0.19 | 13.26 ± 0.27 | 21.07 ± 0.43 |
| Kp-5 | 9.35 ± 0.27 | 10.35 ± 0.30 | 19.15 ± 0.24 |
| Kp-6 | 9.92 ± 0.25 | 11.23 ± 0.22 | 20.48 ± 0.25 |
| Kp-7 | 9.78 ± 0.16 | 9.40 ± 0.27 | 20.17 ± 0.23 |
| Kp-8 | 10.22 ± 0.16 | 12.24 ± 0.20 | 19.45 ± 0.30 |
| Kp-9 | 11.87 ± 0.26 | 13.58 ± 0.22 | 20.63 ± 0.26 |
| F-Value | 13.7 | 30.5 | 4.5 |

Kp - *Klebsiella pneumoniae*
The value of each constituents consisted of ± S.D. of 06 replicates.
The F value is significantly different when $p < 0.05\%$.

**Table 4** Antibacterial activity of ethanolic extract and its constituent, celapanin against *Pseudomonas aeruginosa*.

| Bacterial strains tested | Zone of inhibition (in mm) | | |
|---|---|---|---|
| ***Pseudomonas aeruginosa*** | **Ethanolic extract** | **Celapanin** | **Reference drug Ciprofloxacin** |
| Pa-1 | 11.92 ± 0.25 | 12.23 ± 0.15 | 19.73 ± 0.23 |
| Pa-2 | 09.10 ± 0.23 | 12.02 ± 0.15 | 20.60 ± 0.15 |
| Pa-3 | 10.08 ± 0.12 | 11.17 ± 0.16 | 20.82 ± 0.27 |
| Pa-4 | 08.33 ± 0.21 | 13.10 ± 0.29 | 21.47 ± 0.32 |
| Pa-5 | 11.68 ± 0.21 | 12.35 ± 0.19 | 20.28 ± 0.23 |
| Pa-6 | 08.08 ± 0.18 | 11.73 ± 0.23 | 20.67 ± 0.26 |
| Pa-7 | 08.33 ± 0.22 | 11.70 ± 0.17 | 18.80 ± 0.20 |
| Pa-8 | 10.02 ± 0.28 | 12.37 ± 0.18 | 20.02 ± 0.21 |
| Pa-9 | 09.12 ± 0.28 | 11.92 ± 0.17 | 19.22 ± 0.16 |
| F-Value | 39.3 | 7.8 | 13.0 |

Pa - *Pseudomonas aeruginosa.*
The value of each constituents consisted of ± S.D. of 06 replicates.
The F value is significantly different when $p < 0.05\%$.

drugs and some plant extracts against the pathogenic *S. aureus* (Betoni *et al*. 2006). In this study both the ethanol extract and the constituent celapanin of *C. paniculatus* were most effective in controlling the growth of the pathogenic strains of *S. aureus* isolated directly from the wounds of infected patients.

Among different clinical strains the Gram negative *Pseudomonas aeruginosa* and *Klebsiella pneumonia* isolated from the urine samples of the urinary tract of infected patients, the ethanol extract and the constituent celapanin-loaded wells showed a moderate zone of inhibition when compared to the Ciprofloxacin-loaded well. Similar to the clinical strains, the zone of inhibition of the colony of ATCC strain of *P. aeruginosa* and MTCC strain *K. pneumonia* was similar to the culture of clinical isolates (**Tables 3, 4**). The clinical strains of *P. aeruginosa* and *K. pneumonia* were resistant to multi drugs and were highly disruptive to the internal epithelial barrier and caused lethal sepsis within the intestinal tract (Rogerio *et. al*. 2004; Zaborina *et al*. 2006). Generally Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt 1971) whereas Gram-negative bacteria, having an outer phospholipidic membrane, carry structural lipopolysaccharide components. These make the cell wall impermeable to drug constituents. Most clinical isolates displayed multiple antibiotic resistances to various antibiotics clinically used against both Gram-positive and Gram-negative strains (Zaborina *et al*. 2006). The ethanolic extract of leaves and the constituent celapanin differed significantly in their activity against tested microorganisms. These differences may be attributed to fact that the cell wall in Gram-positive bacteria of a single layer, whereas the Gram-negative cell wall is multi-layered structure (Yao and Moellering 1995). In the present study the ethanol extract and the constituent celapanin of *C. paniculatus* exhibited a more significant inhibitory effect on Gram-positive strains of *S. aureus*. Gram-positive *S. aureus* causes a variety of supperative (pus-forming) infections and toxinoses in humans. It also causes superficial skin lesions such as boils and also more serious infections such as pneumonia, mastitis, phlebitis and meningitis. Reports indicated that clinical isolates from different infectious sources from hospitals showed resistance against the drug Methicillin (Bassam *et al*. 2006). The growth of inhibition was moderate in the cultures of Gram-negative strains of *P. aeruginosa* and *K. pneumonia*, which commonly infect the urinary tract (Tripathi *et. al* 1992).

In most countries popular herbal medicines are increasingly used as remedies for many infectious diseases. Plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made large contributions to human health and well-being. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to available antibiotics has led many researchers to investigate the antimicrobial activity of medicinal plants: *Allium sativum* (MIC - 5.05 mg/ml) by Benkeblia (2004); *Zingiber officinale* (MIC - 3.56 mg/ml) by Konning *et al*. (2004); *Psidium guajava* (MIC - 0.52 mg/ml) by Qadan *et al*. (2005); *Syzygium aromaticum* (MIC - 0.36 mg/ml) by Lopez *et al*. (2005). In the Indian system of medicine, *C. paniculatus* is a well known plant used to cure many infectious disorders. The antibacterial activity of the sesquiterpene celapanin was most significant against *S. aureus*, and the zone of inhibition of the colony was more than that of the standard antibiotic Ciprofloxacin. The results of this investigation are promising and show the potential of this isolated constituent (celapanin) in the treatment of infectious diseases caused by *S. aureus*.

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