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Research Article

ANTIBACTERIAL ACTIVITY OF VULGAROL A EXTRACTED FROM THE LEAVES OF SYZYGIUM CUMINI

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ABSTRACT

Objectives: Extraction, purification and identification of antimicrobial compounds from leaves of *Syzygium cumini*.
Methods: The antibacterial activity of crude extract of *Syzygium cumini* leaves were investigated against pathogenic Gram positive and Gram negative bacteria. Petroleum ether extract was used for the separation of compound using thin layer chromatography and separated compound identified by using Gas Chromatography-Mass Spectroscopy.
Results:Extracts prepared in various organic solvents such as chloroform, methanol, petroleum ether, acetone and ethanol showing antibacterial activity against *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, but significant activity found with petroleum ether extract. The purified compound from petroleum ether extract showing antibacterial activity was identified as diterpenoids i.e. Vulgarol A by Gas Chromatography-Mass Spectroscopy. Vulgarol A shows more potential antibacterial activity against Gram negative organisms such as *P. aeruginosa*.
Conclusion:This study concludes that the extracted compound Vulgarol A from *Syzygium cumini* leaves could be used as an active pharmaceutical ingredient to control infectious diseases caused by *P. aeruginosa*.

Keywords: Syzygium cumini, diterpenoids, P. aeruginosa, GC-MS

INTRODUCTION

The genus *Syzygium* is one of the genera of family Myrtaceae. Plants of this family are well known for the presence of medicinal compounds. Some of the parts of the *Syzygium cumini* are being used medicinally and it has long tradition in an alternative medicine especially as an antidiabetic plant [1]. From all over the world, the fruits of *Syzygium cumini* have been used for a wide variety of ailments, including cough, diabetes, dysentery, inflammation and ringworm [2]. Similarly, different parts of *Syzygium cumini* were also reported for its antioxidant, anti-inflammatory, neuropsycho-pharmacological, antimicrobial, antibacterial, anti-HIV, antileshmanial and antifungal activities [3].

With increase of bacterial resistance to antibiotics, there is considerable interest to investigate the antimicrobial effects of different extracts against a range of bacteria. Hence, efforts are being made to develop other classes of safe and natural antimicrobials useful to control infectious diseases [4]. Traditional medicines based mostly on medicinal plants have been used for the treatment of various diseases by mankind for centuries. Plants are also well-known to be the rich source of biologically active compounds. The use of natural products in disease prevention and control as well as in drug development has received increased attention in recent timing; about 25% of globally prescribed drugs are obtained from plants [5]. Therefore, one approach being used for the discovery of antibacterial agents from natural sources is based on the evaluation of traditional plant extracts. In the present study we first time report the antibacterial activity of Vulgarol A from the leaf extract of *S. cumini*.

Materials and methods

Microorganisms

The cultures of *Salmonella typhimurium* NCIM 2501, *Staphylococcus aureus* NCIM 2654 and *Pseudomonas aeruginosa* NCIM 5032 used in this study were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, Maharashtra, India.

Preparation of extracts

Leaves of *Syzygium cumini* were collected from the campus of Shivaji University, Kolhapur. Leaves were washed and dried in the oven at 40 °C. The dried leaves were then crushed into fine powder and then used for further study. Extracts were prepared by addition of 0.5gm dried powder into the 10ml of various organic solvents such as ethanol, methanol, acetone, chloroform and petroleum ether kept at room temperature for 24 hrs. The mixture was filtered using Whatman filter paper No.1. Filtrate was collected and stored at 4 °C for further use.

Phytochemicals analysis

The presence of phytochemical constituents such as alkaloids, steroids, flavonoids, saponins, terpenoids and tannins from leaf extracts of various organic solvents were carried out according to standard methods described by Siddiqui and Ali (1997) [6].

Separations of compounds by TLC

The extract showing the antibacterial activity was further analyzed for the number of components present by using thin layer chromatography. In that the extract was loaded on the TLC plate of silica gel. The plate was kept into tank containing solvent mixture of chloroform and acetic acid as 9:1 ratio for the plate development. The separated compounds were visualized by using iodine vapors and visualized compounds separated from the plate and solubilized into the solvent. The separated compound was then used for further characterization.

GC-MS analysis of purified compound

The purified compound was analyzed by using QP- 2010 gas chromatography coupled with mass spectroscopy (Shimadzu). The ionization voltage was 70ev. Gas chromatography was conducted by temperature programming made with Rtx-5Ms column (60 m×250 µm i.d. × 0.25 µm film thickness). Carrier gas was He (1 ml^{-1}) and the chromatographic conditions were as follows; initial oven temperature was maintained at 80 °C for 5 min and subsequently programmed from 80 °C to 150 °C at a rate of 3 °C min^{-1} and at a rate of 10 °C min^{-1} from120 to 280 °C where it was held for another 10 min. Injector Temperature: 250 °C; Mass range: 40–650 amu; Solvent delay: 4 min.; electron impact at 70ev. The compounds were identified by comparison of retention time and fragmentation

pattern, as well as with mass spectra in NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu).

Antibacterial activity

The plant extract prepared in various solvents and compound purified by TLC were tested for the antibacterial activity against pathogenic bacteria such as *P. aeruginosa*, *S. typhimurium* and *S. aureus*. Antibacterial activity was carried out by agar well diffusion method according to the method described by Perez et al. (1990) [7]. In each well 100 µl of extract was added and organic solvents were used as negative control. The antibacterial activity of each extract expressed in terms of diameter of zone of inhibition (in cm) produced by respective extract and purified compound at the end of incubation period.

Results and discussion

Syzygium cumini is a good resource of bioactive compounds due to its content of various phytochemicals. However, most of the literature shows that the compounds of *S. cumini* are antidiabetic in nature. In support of this our study focused on the extraction and characterization of Vulgarol-A a antibacterial compound from the leaf extract of *S. cumini* which have never been investigated before.

Phytochemicals of extracts

The leaf extract of *S. cumini* was evaluated for the presence or absence of diverse phytochemicals. The leaves of *S. cumini* are rich in alkaloids, flavonoids, tannins, saponins, steroids and terpenoids, as shown in Table-1. It was observed that alkaloids and flavonoids are extracted in chloroform, methanol, acetone, ethanol and petroleum ether similarly as reported in earlier study [8]. However, we have found terpenoids extracted in petroleum ether in contrast to Kathirvel and Sujatha (2012) reported absence of terpenoids in petroleum ether extract [8].

Table 1: Phytochemical constituent of *S. cumini* leaves extracts in various solvents

Phytochemical constituents	Chloroform extract	Methanol extract	Petroleum ether extract	Acetone extract	Ethanol extract
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	+	-	+	+
Tannins	+	+	-	+	-
Terpenoids	-	+	+	-	+
Steroids	+	+	-	+	+

'+' indicates presence, '-' indicates absence

Antibacterial activity of leaf extract

Extracts prepared in solvents were tested for the antibacterial activity against Gram positive (S. aureus) and Gram negative (S. typhimurium and P. aeruginosa). All extracts showing antibacterial activity against tested organisms are shown in Table 2. Among all extracts petroleum ether extract shows higher zone of inhibition, so it was selected for the further studies. Before this Gowri and Vasantha (2010) have reported antimicrobial activity of S. cumini leaves in methanol extract due to presence of tannin and other phenolic compounds [9].

Table 2: Antibacterial activity of *S. cumini* leaves extracts

Test organism	diameter of zone of inhibition (in cm)				
	Chloroform	Methanol	Petroleum ether	Acetone	Ethanol
S. typhimurium	1.3 ± 0.10	1.8 ± 0.10	2.0 ± 0.16	1.3 ± 0.05	1.4 ± 0.14
P. aeruginosa	1.4 ± 0.15	1.8 ± 0.15	2.2 ± 0.15	1.1 ± 0.06	1.4 ± 0.09
S. aureus	1.1 ± 0.05	1.6 ± 0.12	2.0 ± 0.10	1.3 ±0.04	1.1 ± 0.06

Each value represents the mean ± standard error values

Separation of antibacterial compound

Active antibacterial compound from the petroleum ether extract was separated by the TLC, as shown in fig-1. Three compounds were observed on the TLC plate and labeled as compound 1, 2 and 3. These are then separated and tested for the antibacterial activity against Gram positive (*S. aureus*) and Gram negative (*P. aeruginosa,* and *S. typhimurium*) organisms. As shown in fig 2, among these three compounds, compound-2 shows antibacterial activity against the *S. aureus* and *P. aeruginosa*. The zone of inhibition was higher against Gram negative organisms as compared to Gram positive organism (Fig. 2).

The identification of antibacterial compound was carried out using GCMS. Gas chromatogram of purified compound-2 shows peak area 99.99% at the retention time of 22.99 mins and mass spectrum gives 308 molecular mass (Fig-3a). The data obtained from GCMS analysis, compound was identified as diterpenoids i.e. Vulgarol A (Fig-3b). Vulgarol A has been reported so far from the plants such as *Centaura sessilis* [10], *Marrubium vulgare* [11], *Otostegia fruticosa* [12].

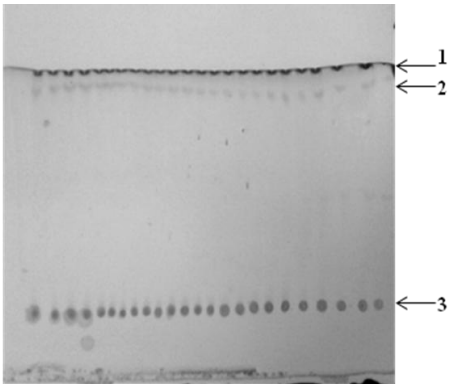

Figure1: Separation compounds from petroleum extract by TLC. Extract was loaded on silica gel TLC plate and plate was developed by chloroform and acetic acid as 9:1 ratio, spot located in iodine chamber.

Identification of purified antibacterial compound

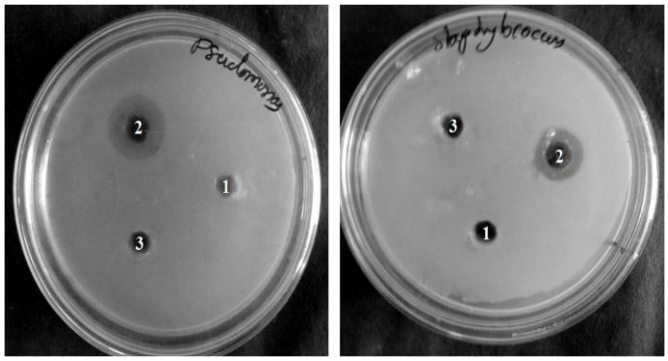

Figure2: Antibacterial activity of compounds separated on TLC plate. The antibacterial activity of compounds -1, 2, and 3 were tested against P. aeruginosa and S. aureus by agar well diffusion method.

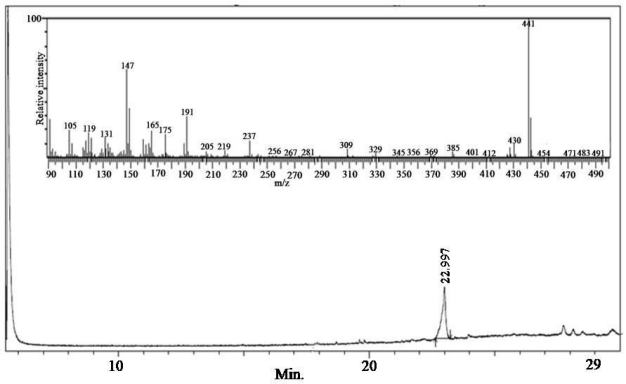

Figure3a: GC-MS chromatogram of purified compound-2.

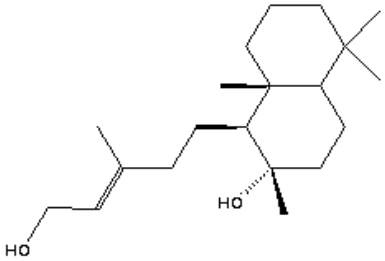

Figure3b: Structure of Vulgarol A identified by GC-MS analysis.

CONCLUSION

In this study we first time report the presence of Vulgarol A from the leaves of *S. cumini*. The compound Vulgarol A shows antibacterial activity against Gram positive and Gram negative pathogenic organisms. So Vulgarol A could be used as an active pharmaceutical ingredient in the treatment of diseases which are caused due to antibiotic resistant bacteria like S. auerus.

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