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Research Article

Isolation, Purification And Identification Of An Antimicrobial Compound From The Ethanol Seed Extract Of *Syzygium cumini*

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Abstract

This study focused on the isolation of a potential antimicrobial molecule from the seed of *Syzygium cumini*. The dry powdered seed were extracted with ethanol showed promising antimicrobial activity against the human pathogenic yeast, *Candida albicans*. The purification of this bioactive agent was done using RP-HPLC. The dried form of the active fraction was identified as 1-Monolinoleoylglycerol trimethylsilyl ether which was confirmed based on the results using FT-IR, ^{1}H NMR, ^{13}C NMR, GC and MS/MS analysis. From the overall observations, 1-Monolinoleoylglycerol trimethylsilyl ether is a promising bioactive molecule against the human pathogen *Candida albicans* which can be isolated at its purest form from the *Syzygium cumini* using the extract procedure identified in this present study.

Keywords: *Syzygium cumini;* Antimicrobial; seed extract; Ethanol extraction; *Candida albicans*

INTRODUCTION

The genus Syzygium is one of the genera of the myrtle family Myrtaceae, the genus comprises about 1,100 species, and has a native range that extends from Africa and Madagascar through southern Asia east through the Pacific. Its highest levels of diversity occur from Malaysia to northeastern Australia, where many species are very poorly known and many more have not been described taxonomically [1]. Plants of this family are known to be rich in volatile oils which are reported for their uses in medicine and many fruits of the family have a rich history of uses both as edibles and as traditional medicines in divergent ethnobotanical practices throughout the tropical and subtropical countries [2] . Some of the edible species of *Syzygium* are planted throughout the tropics worldwide. *Syzygium cumini* (L.) Skeels is one of the best known species and it is very often cultivated. In India, the plant is available throughout the plains from the Himalayas to southern India. It is commonly known as jambolan, black plum, jamun, java plum, Indian blackberry, etc.[3] It is called as Naval in Tamil Language.

S. cumini is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar and seed extract has lowered blood pressure by 34.6% and this action is attributed to the ellagic acid content[4]. The seeds have been reported to be rich in flavonoids, a well-known antioxidant, which accounts for the scavenging of free radicals and protective effect on antioxidant enzymes[5] and also found to have high total phenolics with significant antioxidant activity[6] and are fairly rich in protein and calcium. Java plums are rich in sugar, protein, calcium, mineral salts, vitamins C which fortifies the beneficial effects of vitamin C, anthocyanins and flavonoids[7].

Leaves have been used in traditional medicine as a remedy for diabetes mellitus in many countries[8]. The seeds are also used to strengthen the teeth and gums, to treat leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy, constipation and to inhibit blood discharges in the faeces[9]. Extract of seed, which is traditionally used in diabetes, has a hypoglycemic action and

antioxidant property in alloxan diabetic rats possibly due to tannins[10]. The folkloric use of this species to treat infectious diseases stimulated the investigation of this present antimicrobial study against a pathogenic *Candida albicans* isolated from infectious patients.

MATERIALS AND METHODS

Collection and preparation of plant material

The fruit of the plant, *S. cumini* were collected locally from Thanjavur, Tamil Nadu, India. Collected plant samples were washed using distilled water to remove undesirable materials and excess of water was drained off. The seeds were separated from fruit using sterile knife and were shade dried for few days. The shade dried seeds were powdered separately by grinding machine and about 5g of powdered seed was taken into clean flat-bottomed glass container and soaked with 200 ml of ethanol (95%) solvent. The containers with its contents were sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The obtained filtrates were rotary evaporated and this dried content was used for further characterization studies.

Antimicrobial activity of the ethanol seed extract

The antimicrobial activity of the ethanol seed extract was studied on Muller-Hinton agar (MHA) plates as per the earlier procedure followed by Mani *et al*[11]. The human yeast pathogens used was *Candida albicans* which was obtained from Sharmila Institute of Medicinal Products Research Academy (SIMPRA), Scientific and Industrial research organization, Thanjavur – 613007, Tamil Nadu, India. This strain was cultured overnight on yeast malt broth at 30°C and MHA plates were swab cultured with 100 µl of this strains and the wells were impregnated with 50 µl of the crude extract dissolved in phosphate buffer (pH 7). After incubation for 24 h at 30°C, the plates were examined for zone diameter of inhibition using an antibiotic zone scale.

Purification of antimicrobial compound

For the purification to a single antimicrobial molecule, one more solvent extraction procedure was carried out. The dry powder was extracted three times with different polarity based solvents like ethyl acetate, chloroform, hexane, dichloromethane and water and tested for its antimicrobial activity using the above described antimicrobial method. Ethyl acetate extracted solvent showed maximum activity which was evaporated under vacuum and used for purification studies. This extract was dissolved in 5 ml of methanol, filtered through a 0.2 µm syringe filter and separated by Reverse Phase (RP)-HPLC. RP-HPLC was performed using a Waters 600 HPLC system (Waters, USA) equipped with an Xterra Prep RP18 OBD column (Waters, USA; 5 µl, 18 × 100 mm) held at 40°C. The solvent system consisted of distilled water (solvent A) and acetonitrile (solvent B). The compounds were eluted at a flow rate of 4 ml/min with a linear gradient from the mixture A:B (100:0, vol/vol) to A:B (0:100, vol/vol) in 12 min. The absorbances of the eluted fractions were measured at 210 nm. All the collected fractions were dried and stored at - 20°C for further studies. All the extracted individual fractions were studied for antimicrobial activity and fraction showing activity was rotary evaporated and used for further chemical characterization studies.

Identification of antimicrobial compound

Study on functional groups

Functional group of this antimicrobial compound was investigated using Fourier transform infrared spectroscopy (FTIR) absorption spectrum which was recorded on an IR affinity-1 FTIR system (Shimadzu, Japan) at a spectral resolution of 4 $cm^{-1}$with an average of 10scans in the wavenumber range of 400–4000 cm^{-1}

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H & ^{13}C NMR spectrum of the purified bioactive fraction was recorded on a Bruker AV600NMR spectrometer (Germany) using deuterated $CDCl_3$ as the solvent. Chemical shifts were expressed in parts per million (ppm) downfield from an internal standard of tetramethylsilane (TMS).

Gas Chromatography Mass Spectroscopy (GC-MS)

The identification of the antimicrobial compound was also studied using GC-MS on a Thermo Trace GC Ultra coupled with Polaris Q MS and TriPlus auto-sampler using a DB-5 (0.25 mm × 30 m × 0.22 µm) column in which helium was used as carrier gas. The temperature was set between

50°C to 250°C at a rate of 10°C min^{-1}. The initial temperature was held for 2 min and final temperature of 250°C was held for 10 min. The GC flow rate was 1 ml min^{-1} and the total run time was 32 min. MS was performed at scan mode between 0 – 600 m/z with an Ion trap EI+.

RESULT AND DISCUSSION

The ethanol seed extract from the *Syzygium cumini* was tested for its antimicrobial agents and it showed promising activity against the human pathogenic yeast, *Candida albicans* (Fig. 1). The crude seed extract undergone purification of the antimicrobial molecule which was primarily extracted with different polarity based solvents.

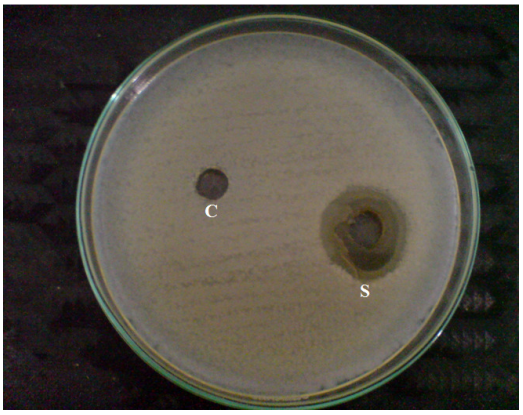

Fig. 1: Crude ethanol seed extract showing antimicrobial activity against human pathogen, *Candida albicans*. C and S letters in the petri plate representing control and seed extract sample

Ethyl acetate extracted compounds showed maximum activity and this was purified using RP-HPLC (Fig. 2). There were eight fractions isolated, in which sixth fraction eluted at the retention time of 4.730 min alone showed antimicrobial activity against this human pathogen. This fraction was rotary evaporated and lyophilized for further structural characterization.

The purified antimicrobial compound was primarily identified based on its functional groups using FT-IR spectrum (Fig. 3). Alkane ($(CH_2)n$) group were observed at the wavelengths of 3229, 3196, 3106, 2954, 2925, 2871, 2854, 1403, 1385 and 1136 cm^{-1} in which 1462 cm^{-1} representing the terminal alkane (CH_3) group. Moreover, alkene (CH=CH) functional group were also predicted at wavelength of 779, 720 and 667 cm^{-1}. The most significant trimethyl silane ($Si – (CH_3)_3$) group was depicted at 648, 597 and 540 cm^{-1}. Similarly, ester group was observed at 1296, 1237 and 1057 cm^{-1} and ether group was predicted at 1654, 1631 and 1552 cm^{-1}. All the above functional groups showed the possibilities of a 1-Monolin oleoylglycerol trimethylsilyl ether compound as shown in this spectrum.

NMR spectroscopy of the purified compound also representing the functional groups of 1-Monolinoleoylglycerol trimethylsilyl ether compound as shown in the figures 4 and 5. The chemical shifts of the ^1H NMR spectrum ranges between 0.0074-0.0085 showed the presence of protons in functional group of trimethyl silane ($Si – (CH_3)_3$). Similarly, protons in the chemical shifts ranged between 0.8821-0.9674 showed the existence of terminal alkanes (CH_3) whereas 1.0296-2.0667 predicting the aliphatic alkane ($(CH_2)n$ and 2.5191-3.4590 depicting the hydrogen atom of the alkene (CH=CH) group. Moreover, chemical shifts ranges from 2.0854-2.4994 and 3.5240-4.7165 revealed the proton presence of the ester (COO- CH_2) and Ether (R-O-CH) groups.

Similar to the ^1H NMR spectrum, ^{13}C NMR spectrum of this purified fraction showed the existence of same functional groups viz., carbon atom of the trimethyl silane ($Si – (CH_3)_3$) was observed between 0.6371-2.7671 whereas carbon atom of the terminal alkane (CH_3) ranged within 8.9337 – 33.8128, aliphatic alkane in the regions of 15.2971-25.7334 and alkene (CH=CH) carbon atom between 216.6923-219.8199. Similarly, carbon atom of the ester and ether groups was observed within the chemical shifts of 161.6764-168.1045 and 62.9180-69.4560. This proved that the chemical compounds that it could be 1-Monolinoleoylglycerol trimethylsilyl ether.

Moreover, mass spectral analysis of purified compound was illustrated in the figure 6. It exhibited a single molecular peak ion at the retention time of 22.63 min with the molecular mass of 1-Monolinoleoylglycerol trimethylsilyl ether with 513.8 MW and methylated 1-Monolinoleoylglycerol trimethylsilyl ether with 498.8 MW. Further, the MS/MS spectrum of this chemical compound showed exact sequential pattern of 1-Monolinoleoylglycerol trimethylsilyl ether viz., 73.1, 147.2, 221.3, 295.5, 369.6, 443.7, 517.8, 592.0, 666.1, 682.2, 31.1, 105.2, 179.3, 253.5, 327.6, 401.7, 475.9, 550.0, 624.1 and 682.2. This molecular pattern of 1-Monolinoleoylglycerol trimethylsilyl ether was confirmed with the NIST database for the molecular identification of unknown chemical compounds.

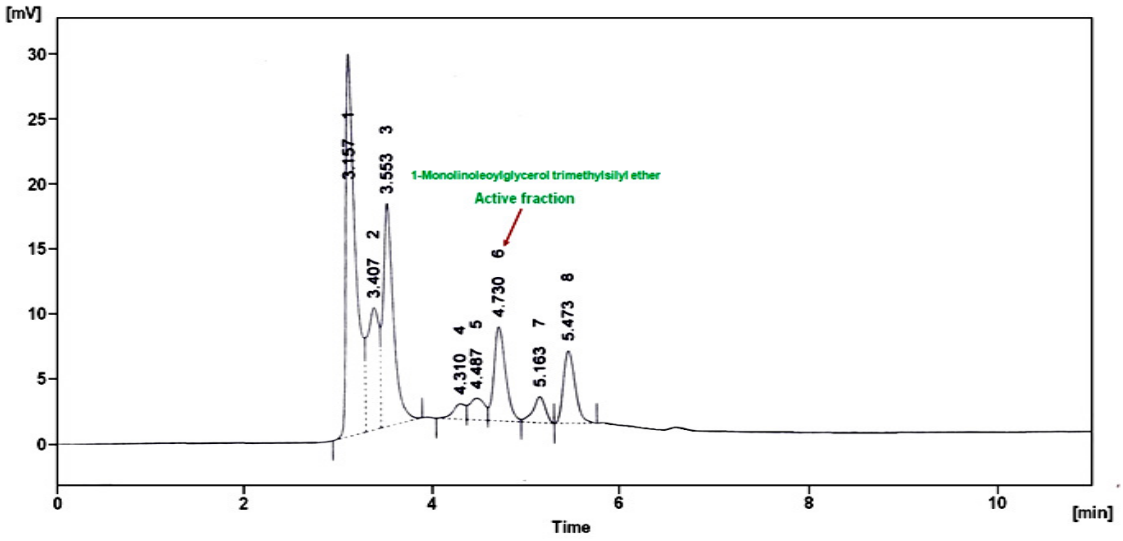

Fig. 2: HPLC chromatogram of the crude ethanol seed extract of *S. cumini*

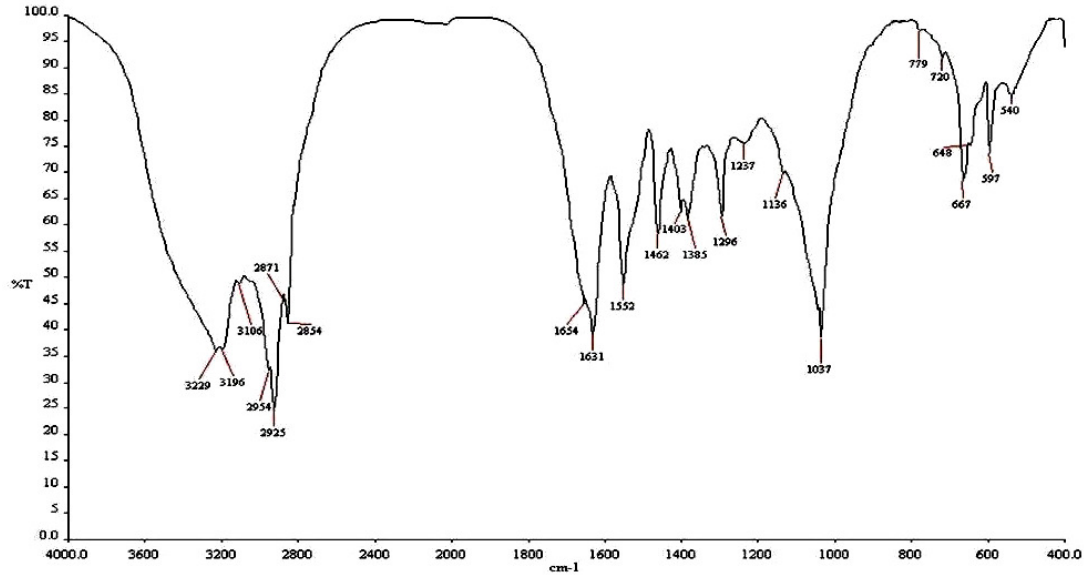

Fig. 3: FT-IR spectrum of the purified bioactive 1-Monolinoleoylglycerol trimethylsilyl ether from seed of *S. cumini*

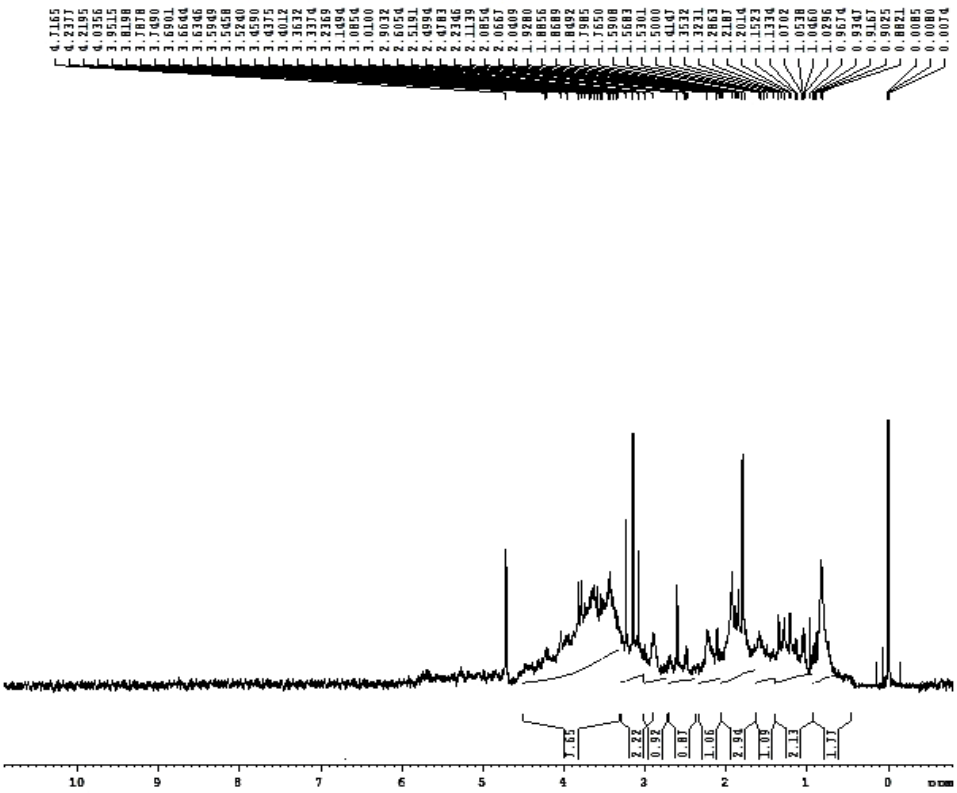

Fig. 4: ^{1}H spectrum of the purified bioactive 1-Monolinoleoylglycerol trimethylsilyl ether from seed of *S. cumini*

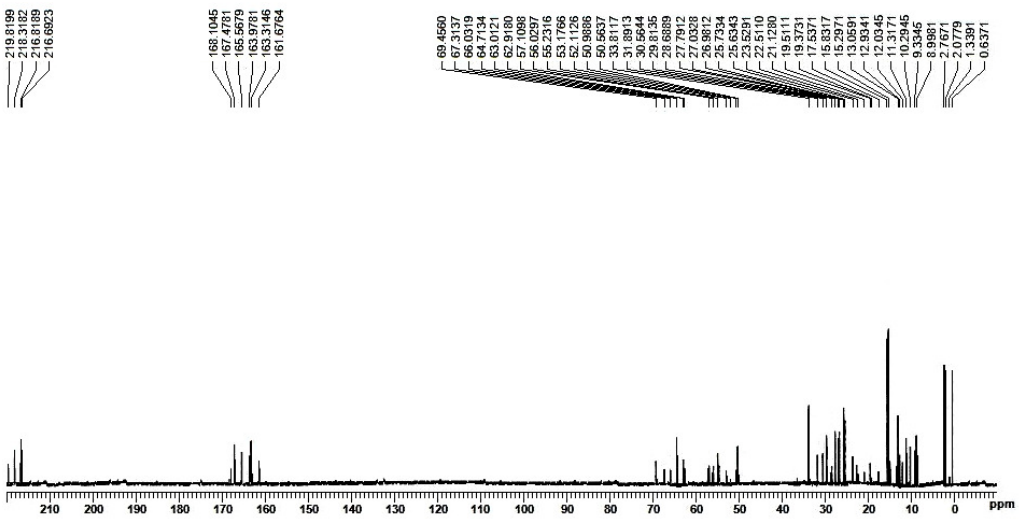

Fig. 5: ^{13}C spectrum of the purified bioactive 1-Monolinoleoylglycerol trimethylsilyl ether from seed of *S. cumini*

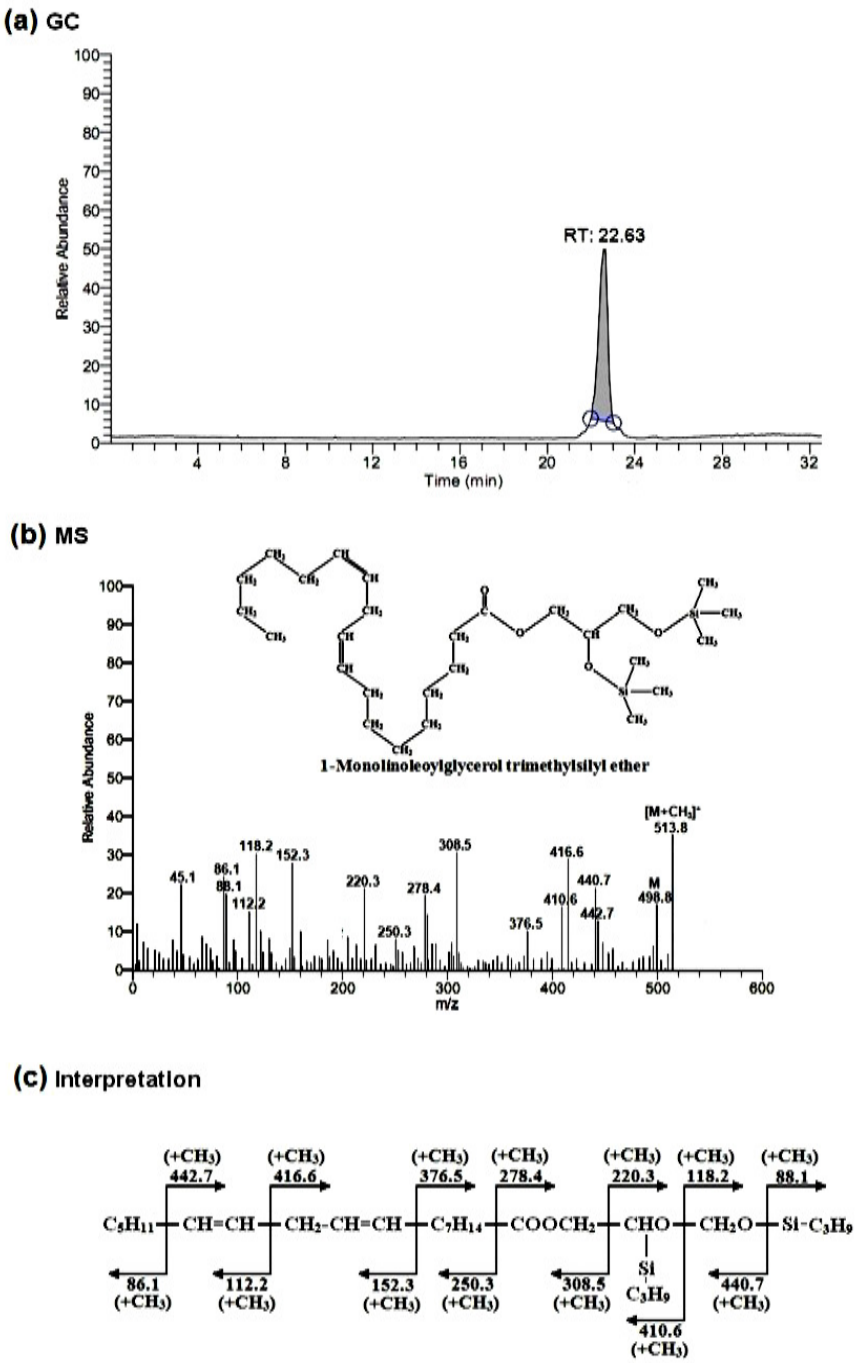

Fig. 6: (a) Gas Chromatography, (b) Mass spectrum and (C) molecular weight interpretations of the purified bioactive 1-Monolinoleoylglycerol trimethylsilyl ether from seed of *S. cumini*

From all together results, the antimicrobial compound isolated from the crude seed extract was purified up to a single bioactive molecule and its structure was identified as 1-Monolinoleoylglycerol trimethylsilyl ether. Likewise, Chattopadhyay *et al*[12] observed antimicrobial activity on the methanol and aqueous crude extracts of the seed of *Syzygium* species. Similar to the present information on chemical compounds, the seed of the *S. cumini* was earlier found to be rich sources for acylated flavonol glycosides [13], quercetin, myricetin, myricitin[14],

myricetin 3-O-4-acetyl-L-rhamnopyranoside, triterpenoids, esterase [15], galloyl carboxylase and tannin[16]. The present investigation revealed that the ethanolic seed extract of *Syzygium cumini* proved to be a potential source of this antimicrobial compound against the human yeast pathogen, *Candida albicans*, which can be made at its purest form using this procedure developed in this study.

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