



ORIGINAL ARTICLE

Documentation of bioactive principles of the exudate gel (EG) from the stem of *Caralluma retropiciens* (Ehrenb) and *in vitro* antibacterial activity – Part A

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Abstract *Caralluma retropiciens* (Ehrenb) is a desert plant widely distributed in the hilly semi-desert regions of southern part of Saudi Arabia. The exudate gel (EG) from the stem of plant is occasionally used for wound healing by the people of the southern part of Saudi Arabia. This study investigated the phytochemical composition, FT-IR, GC-MS spectral analysis and *in vitro* antibacterial activity of the EG from the stem of *C. retropiciens* (Ehrenb). The plant *C. retropiciens* (Ehrenb.) was collected from the hills of Rijal Almaa, a heritage village of Saudi Arabia. The EG was isolated from the stem of *C. retropiciens* (Ehrenb.). Physical parameters such as viscosity and zeta potential (ZP) were determined. Phytochemical analysis, FT-IR and GC-MS spectroscopy analysis were performed to determine the bio active constituents. The antibacterial activity of the isolated gel was performed by *in vitro* agar well diffusion technique. The study demonstrated that the viscosity and ZP of EG influenced the efficacy of antibacterial spectral properties. The FT-IR spectroscopy of the EG showed various functional groups at 3278.29, 2951.16, 2840.44, 2527.55,

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2161.67, 1647.40, 1450.06, 1406.7, 1286, 1108.34, 5536.63 cm^{-1} . Various pharmaceutically important chemical compounds were identified using GC–MS analysis. The bioactive compounds are “Sorbic Acid”, “Rhodopsin”, “1-Heptatriacotanol”, “Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans”, “Cholestan-3-ol, 2-methylene-, (3 α ,5 α)”, “Benzoic acid”, “3-pentyl-, methyl ester trans”, “Hexanoic acid, 2-ethyl-, oxybis (2,1-ethanedioxy-2,1-ethanedioyl) ester”, etc. The antibacterial effect of the EG showed a wide spectrum of activity against the screened human pathogenic bacteria. The results demonstrate the bioactive principles of EG from *C. retrospiciens* (Ehrenb.) exerts the antibacterial properties *in vitro*.

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1. Introduction

Medicinal plants are important and prominent sources of bioactive substances. The importance of medicinal plants as therapeutic agents has been known to mankind since early human civilization. Studies have revealed that several plants are used in traditional health care worldwide as agents for treating chronic diseases (Alghamdi et al., 2018; Roumita et al., 2018; Zamawe et al., 2018; Rukshana et al., 2017). The usage of medicinal plants is common in Saudi Arabia where it has been estimated that the usage of herbal medicine to treat various ailments accounts for about 8–76% (Aati et al., 2019; Mushabab et al., 2018; Alrowais and Alyousefi, 2017). Traditional systems of medicine are involved in natural remedies using various herbs. Indeed, several researchers have demonstrated that herbs are classical resources for many potential novel drug molecules (Hosseinkhani et al. 2017). Plants like Aloe vera has been used in folk medicine for its

wound healing properties for more than 4000 years (Moriyama et al. 2016). The World Health Organization (WHO) recognizes that the usage of herbal medicines and phytonutrients have rapidly expanded worldwide (WHO, 2004) and in fact approximately 25% of drugs prescribed worldwide originated from plants (Rates, 2001). Therefore, several research efforts in the past 4 decades have been focused on plants/herbs to scientifically document their efficacies as pharmaceutical agents for treating various ailments.

C. retrospiciens (Ehrenb.) is the synonym of *Desmidorchis retrospiciens* (Ehrenb.) belong to the Apocynaceae family and widely distributed succulent taxon found in the dry regions of the world (Nasser et al., 2017; Sharawy et al., 2015). The plant *C. retrospiciens* (Ehrenb.) is mainly distributed in the semi-desert regions of southern Saudi Arabia and parts of Africa. Traditionally, the people of Saudi Arabia have been using the EG from *C. retrospiciens* (Ehrenb.) for wound healing. However, there is no scientific evidence for



Fig. 1 The study area was Rijal Alma region, Asir province which is located 45 km away from Abha city in western side that coordinates N 2015840.65 and E 211634.87. The Region Rijal Alma is a mountainous area bordered to the east by Al-Souda Center, and from the North Muhayil of Asir province.

the wound-healing properties of this plant or its antimicrobial activity. Consequently, the present study was aimed at investigating the antibacterial properties of the stem EG of *C. retrospiciens* (Ehrenb.).

2. Materials and methods

2.1. Study area, plant collection, and identification

The study area was Rijal Almaa region located in Asir province in the Southern region of Saudi Arabia (Fig. 1). The region of Rijal Almaa is located 45 km west of the city of Abha, with coordinates N 2015840.65 and E 211634.87. It is a mountainous area bordered on the East by Al-Souda Center, on the North by Muhayil, Asir province, and on the South by Al-Darb, Jizan province. *C. retrospiciens* (Ehrenb.) (Fig. 2A) was collected from the hilly regions of Rijal Almaa during

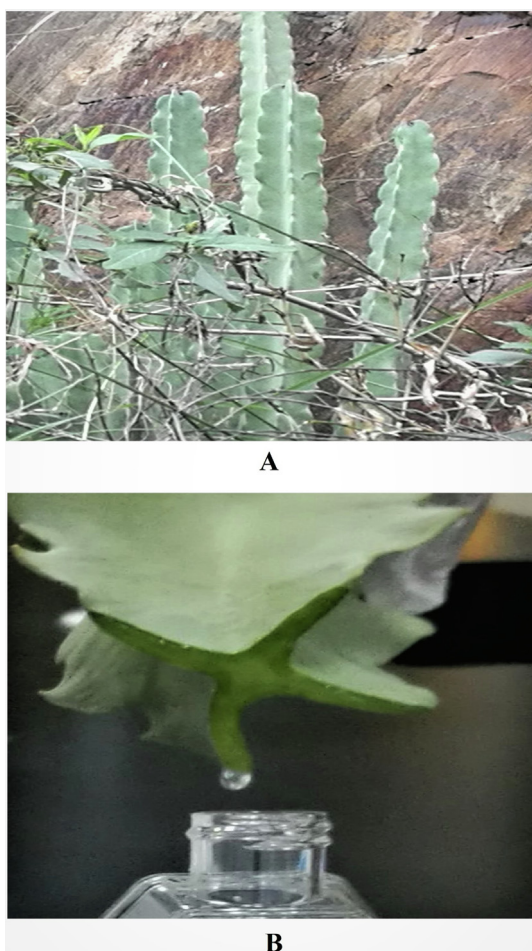


Fig. 2 Photographic representation of the plant *Caralluma retrospiciens* (Ehrenb.) in the hilly region of Rijal Alma, Saudi Arabia. (A) Plant at collecting spot with shrubs on the rocks was robust, densely branched, cactus-like, succulent which was plucked out from the rock soil, the stem grown erect 300–400 cm tall, 2–5 cm width of angular fleshy stems, smooth surface, pale green in colour (B) The Collection of exudate gel (EG) from the stem by cutting with sterile blade and collected in a sterile plastic container by dropwise. The EG was highly viscous, pale yellow in colour and homogenous in texture by visual appearance.

November 2018 by simply plucking it from the soil. The stem of the plants was used for this research work. The plant materials were packed in perforated polyethylene bags and transported to the laboratory. The stem part was thoroughly washed with normal water, and thereafter with distilled water, to remove impurities, after which it was air-dried. The plant was identified by an herbarium curator and a voucher specimen (reference number = JAZUH 1623) was deposited in the herbarium of Jazan University.

2.2. Collection and Physical characterization of EG

2.2.1. Collection

The exudate from *C. retrospiciens* (Ehrenb.) (Fig. 2B) was collected from the stem of the plant by cutting with a sterile blade and holding the stem in a slanting position. The exudate was characterized physically as gel and designated as exudate gel (EG). The sample was collected in a 50 mL sterile screw-cap glass reagent bottle and immediately stored in refrigerator at 2–8° C. EG was utilized for spectral characterization and antibacterial studies.

2.2.2. Organoleptic characteristics

The organoleptic characteristics were determined from physical appearance, colour, texture, and homogeneity. Homogeneity was determined by visual inspection after EG was put in a clean glass beaker. The EG was observed visually for appearance and the presence of any aggregates.

2.2.3. Determination of viscosity

The rheological properties of the EG samples were determined by using Brookfield digital viscometer (Model LVDV-E, USA). The viscosity of the samples was determined using spindle S63. 25 ml of EG was placed in a clean, kept the sample holder and allowed to settle for 5 min. Then the viscosity was measured at a rotating speed of 30 rpm at room temperature.

2.2.4. Determination of zeta potential

The zeta potential (ZP) of EG was determined and the 100% v/v EG was subjected to ZP analysis followed by EG was diluted at predetermined concentration 50, 10, 5, 2.5, 1.25% v/v with Millipore water. The ZP analysis was performed by using Zetasizer Nano ZS, Malvern Instruments, UK (Moni et al., 2018).

2.3. Identification of phytochemical compounds

2.3.1. Preliminary phytochemical analysis

The EG was subjected to phytochemical analysis through standard protocols for the identification of carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, steroids and saponins (Trease and Evans, 1989; Harbone, 1998; Kokate, 2000).

2.3.2. Fourier transform infrared spectroscopy studies (FT-IR Studies)

The EG was subjected to FT-IR studies for functional group analysis. The sample was placed in silicon tube and the spectra were recorded in transmission mode in the spectral region range of 4000 to 400 cm^{-1} .

2.3.3. Gas chromatography–mass spectrometry (GC–MS) analysis

The GC-MS analysis was performed using Thermo Scientific GC-MS equipped with AS 3000 autosampler, trace ultra GC and ISQ detector. The sample was diluted in methanol in a volume ratio of 1:10. The mass spectra were interpreted using xcalibur software and the fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using NIST, MAINLIB and REPLIB built-in libraries. The percentages of constituents were measured based on peak areas.

2.4. Assessment of antibacterial activity of EG

2.4.1. Bacterial strains used and standardization of bacterial cultures

The bacterial strains used in the study were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Twenty-four-hour cultures were prepared and standardized using gradient dilution from 10^{-1} to 10^{-7} with nutrient broth. The viability of bacterial culture was determined by assessing the colony-forming unit in 1 ml (CFU/ml).

2.4.2. Determination of minimum inhibitory concentration

The minimum inhibitory concentration was determined using the broth tube dilution method. Predetermined concentrations of EG 50, 10, 5, 2.5 and 1.25% (v/v) were prepared by diluting the sample 1 in Millipore water. The minimum inhibitory concentration was determined based on the visibility of bacterial growth in the broth.

2.4.3. Preparation of samples for antibacterial studies

Based on MIC study, two different concentrations of the EG samples were used to determine the potential antibacterial effect against the screened human pathogenic bacteria.

Sample 1

The collected EG was not diluted in Millipore water since the focus was to demonstrate the direct effect of the EG against human pathogenic bacteria. Therefore, the concentration of each sample was considered as 100% (v/v).

Sample 2

A 50% (v/v) of EG sample was prepared by dissolving in an equal volume of Millipore water since the exudate was highly viscous.

2.4.4. Determination of antibacterial susceptibility

The antibacterial susceptibility test was performed according to the method of [Moni et al. 2018](#). Muller Hinton (MH) agar plates were prepared for evaluating the antibacterial study.

The bacterial cultures were sub-cultured from the stock culture, and after 24 h incubation, the cultures were subjected to antibacterial studies. A spread plate technique was used for growing the organisms. Agar well diffusion technique was employed for the samples and standard ciprofloxacin at the concentration of 50 mcg/ml ([Cappuccino and Sherman, 2014](#)). The inoculation was done by dipping a sterile cotton swab into the standardized (CFU/ml) individual cultures and streaking on MH agar plates by rotating the petri dish to distribute the culture evenly. The inoculation procedure was repeated twice for better growth of the culture. The plates were allowed to dry for about 10 min before the administration of samples. The agar well diffusion technique was performed by punching holes on inoculated MH agar plates using standard sterile stainless-steel borer. The diameter of the well was 10 mm, the samples and standard were placed in separate wells of individual plates. The plates were incubated at 37 °C for 24 h and the antibacterial spectrum was assessed through the appearance of inhibitory zones after 24 h of incubation. The spectrum of activity is directly proportional to the diameter of the zones of inhibition. The diameter of the zone was measured and recorded.

3. Results and discussion

3.1. Physical characteristics

The colour of EG was pale yellow, highly viscous and homogeneous, with a uniform smooth texture. Physicochemical characteristics such as viscosity grade, surface ZP studies are essential for assessing the diffusion properties of gels into the cell. The ZP analysis showed the physical characterization of the EG. The dilution factor also influenced the zeta potential. The ZP also changed as a function of viscosity i.e. it decreased with decrease in viscosity ([Table 1](#)). The purpose of this study was to demonstrate the actual antibacterial effect of the EG against various human pathogenic bacterial organisms. The study showed that the spectrum of activity was increased when the gel was diluted to 50% v/v in Millipore water when compared to 100% undiluted EG. The antibacterial effect is directly proportional to the diffusion property of the EG. The viscosity of the gel was also influenced by the dilution factor, and it declined from 100% to 1.25%, and the consistency of decline in viscosity was seen in the gel. The R^2 value of EG was 0.9707 and confirm the linearity in the viscosities of the EG. The viscosity study showed the pseudoplastic behaviour of the gel. Similar results have been observed on formulated antibacterial creams and gels by [Mei et al. \(2016\)](#). On the other hand, the ZP of the EG was observed in the range

Table 1 Physical properties of the exudate gel (EG) from the stem of *Caralluma retrospiciens* (Ehrenb.).

| Concentration (% v/v) | Physical parameters | | | | |
|-----------------------|---------------------|---------|-------------|-------------------|-----------------------|
| | Physical appearance | Texture | Homogeneity | Viscosity (mpos)* | Zeta potential (mV)** |
| 100 | Opaque | Smooth | Yes | 649 | – |
| 50 | Opaque | Smooth | Yes | 356 | – |
| 10 | Less Opaque | Smooth | Yes | 187.5 | – 4.97 |
| 5 | Transparent | Smooth | Yes | 142 | – 3.07 |
| 2.5 | Transparent | Smooth | Yes | 53 | – 2.27 |
| 1.25 | Transparent | Smooth | Yes | 37 | – 0.715 |

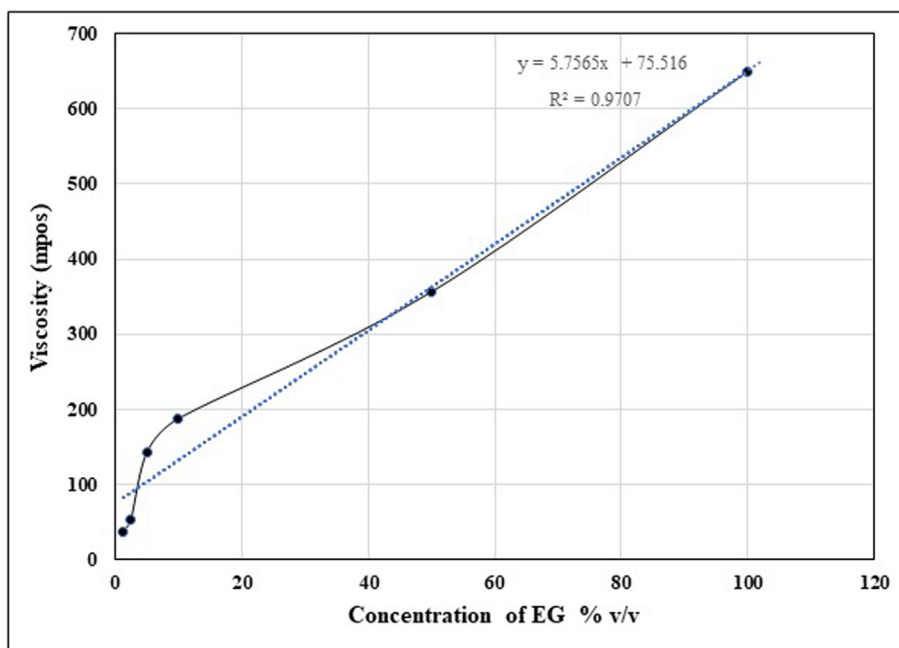


Fig. 3 Viscosity curve of sample analyte the exudate gel (EG) from the stems of *Caralluma retrospiciens* (Ehrenb.)

between -4.97 to -0.715 mV. Studies have suggested that ZP value less than -10 mV is associated with good stability due to the presence of high repulsion between particles of the gel which prevent particle aggregation (Mora-Huertas et al., 2010). Therefore, these results confirm that the EG was highly unstable. However, the ZP of sample 1 and sample 2 of EG could not be determined due to high viscosity (Table 1). The viscosities of the EG was linearly influenced by dilution factor (Fig. 3), and had a pseudoplastic behaviour as expected. Therefore, the gels complied with the non-Newtonian system.

3.2. Phytochemical analysis

The present study revealed that the EG contained various phytoconstituents such as carbohydrates, alkaloids, flavonoids,

tannins, steroids, and saponins. An earlier report on the phytochemical analysis of *Caralluma fimbriata* depicted various compounds such as carbohydrates, tannins, fats, amino acids, proteins, sterols, saponins, gum and mucilage (Packialakshmi and Naziya, 2014). In contrast, the present study revealed the absence of amino acids and proteins in the EG of *C. retrospiciens* (Ehrenb.). Kiranmayee et al., 2015 studied the phytochemical composition of *Caralluma attenuata*, and reported the presence of saponins, steroids, flavonoids, and glycosides. However, the report also showed the absence of alkaloids and cardiac glycosides in *Caralluma attenuata*. An earlier report showed that a new pregnane glycoside derivative retrospinoside (1) was isolated from the aerial parts of *Caralluma retrospiciens* (Ehrenb.) and chemically characterised (Elsebai & Ietidal, 2015).

Table 2 FT-IR finger print studies and functional groups of the exudate gel (EG) from the stem of *Caralluma retrospiciens* (Ehrenb.).

| Wave number (cm^{-1}) | Intensity | Functional groups | Phytocompounds |
|----------------------------------|-----------|--|---|
| 3278.29 | Strong | O-H str. (hydroxyl) | Glycosides, Flavonoids, Saponins, hexose sugars |
| 2951.16 | Strong | CH_2 str. (Asymmetrical) | Aliphatic compounds |
| 2840.44 | Medium | CH_2 str. (Symmetrical) | Steroids, Saponins, Flavonoids |
| 2523 | Strong | O-H str (Carboxylic acid) | Aliphatic compounds |
| 2161.67 | Strong | $\text{N}=\text{N}=\text{N}$ str (azide) | Steroids, Saponins, Flavonoids |
| 1647.40 | Medium | $\text{C}=\text{O}$ str | Glycosides, Flavonoids |
| 1450.06 | Medium | $\text{C}=\text{C}$ str (Olefinic) | Flavonoids |
| 1406.7 | Medium | CH_2 bend (Alkane) | Steroidal glycosides, Flavonoids |
| 1286 | Weak | $\text{S}=\text{O}$ (Sulfonyl chloride) | Aliphatic compounds |
| 1108.34 | Strong | C-O Stretching (phenols) | Steroids, Saponins, Flavonoids |
| 556.63 | Weak | C-O-C Str (Ether, Ester) | Tannins |
| | | S-S (disulfides) | Cutin |
| | | | Tannins |
| | | | Cutin |
| | | | Glycosides |
| | | | Glycosides |

3.2.1. FT-IR analysis

The chemical groups in the EG were determined using FT-IR spectra by recording in the fingerprint region of 400–4000 cm^{-1} (Table 2). The FT-IR spectroscopy showed the presence of many peaks at various fingerprint regions for EG. Spectral analysis of EG showed unique peaks that confirmed the presence of functional groups and their respective compounds (Fig. 4A). The FT-IR spectral studies of EG showed the bell-shaped curve at 3278.29 cm^{-1} showed the existence of various functional groups such as hydroxy (OH) group with stretching frequency representing the presence of glycosides, flavonoids and saponins.

The frequencies at 2951.16 and 2840.44 cm^{-1} represents the aliphatic compounds with CH_2 stretching vibrations and depicting the presence of steroids, tannins, and saponins. Steroids and saponins were the most abundant compounds. The finger print region at 2527.55, 2161.67, 1647.40,

1450.06 cm^{-1} exhibited the presence of glycosides, flavonoids, steroidal glycosides and saponins. The frequencies at 1406.7 and 1286 cm^{-1} displayed S=O (Sulfonyl chloride) and C-O (Phenols) indicated the presence of tannins and cutin. Earlier report suggested that the FT-IR spectrum showed the presence of alkyne (C-H), methylene (C-H), isocyanate ($-\text{N}=\text{C}=\text{O}$), organic nitrates, aliphatic nitro compounds, ammonium ion and aromatic nitro groups revealed the presence of phenolic compounds, flavonoids, saponins, steroids, terpenoids, proteins, quinones, coumarins and phytosterols (Asha et al., 2014; Showkat and Surender, 2015; Kavipriya and Chandran, 2018).

3.2.2. GC-MS analysis

The GC-MS analysis of the EG showed the presence of phytochemical compounds. The GC-MS chromatogram exhibited various peaks for the compounds, as shown in

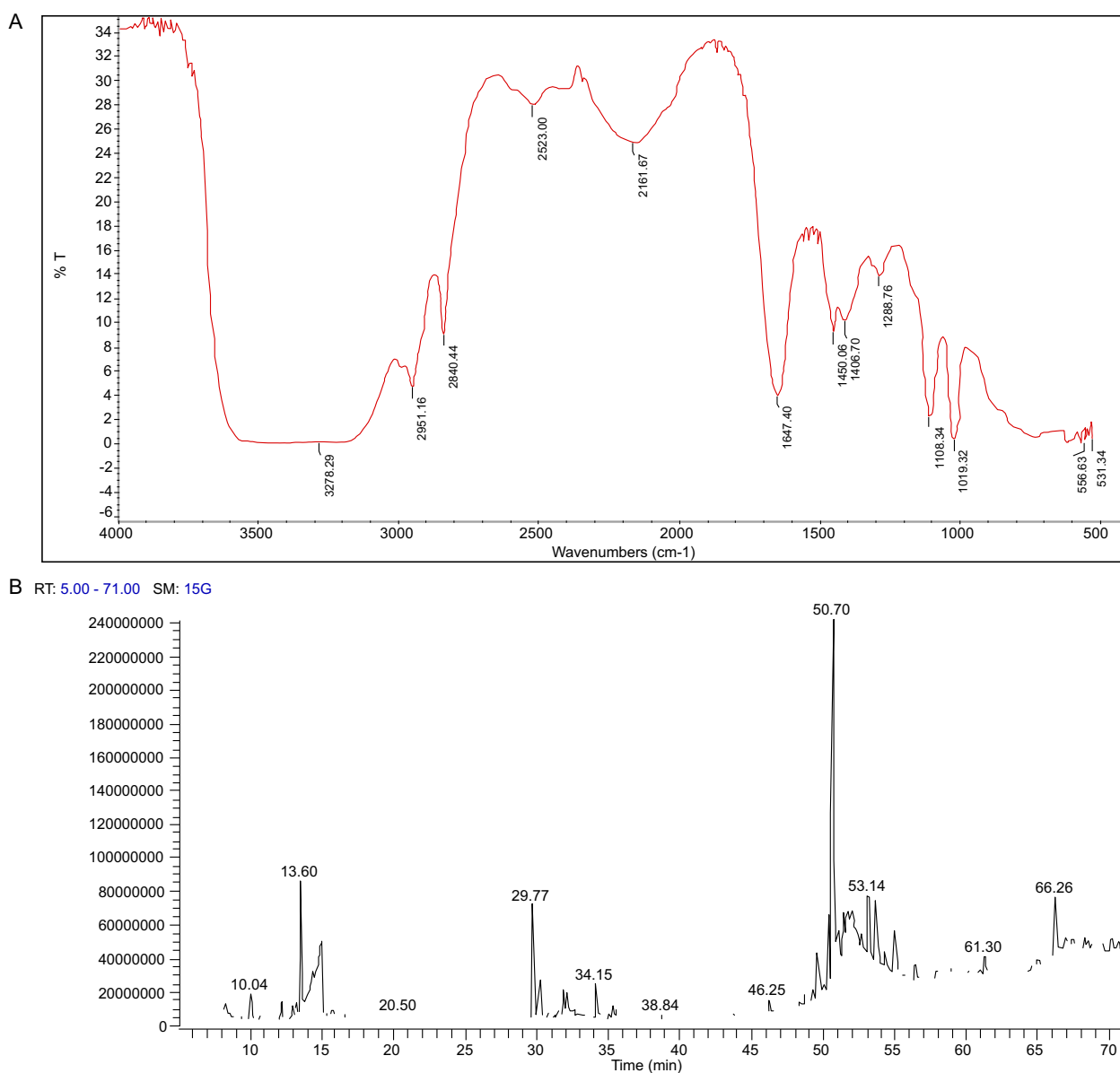


Fig. 4 Spectral characterization of the exudate gel (EG) of *Caralluma retropiciens* (Ehrenb.) (A) Finger print region of EG at 400–4000 cm^{-1} (B) GC-MS Chromatogram of the EG of *Caralluma retropiciens* (Ehrenb.) showing various peaks.

Table 3 GC-MS detection of bioactive compounds of the exudate gel (EG) from the stem of *Caralluma retropiciens* (Ehrenb.).

| Sample ID | Bioactive compound | Molecular formula | Retention time (Minutes) | Molecular weight |
|-----------|--|---|--------------------------|------------------|
| 1 | Sorbic acid | C ₆ H ₈ O ₂ | 10.67 | 112 |
| 2 | Rhodopsin | C ₄₀ H ₅₈ O | 48.10 | 554 |
| 3 | 1-Heptatriacontanol | C ₃₇ H ₇₆ O | 48.56 | 536 |
| 4 | Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans | C ₁₉ H ₃₆ O ₃ | 48.72 | 312 |
| 5 | Cholestan-3-ol, 2-methylene-, (3 α ,5 α) | C ₂₈ H ₄₈ O | 39.44 | 400 |
| 6 | Hexanoic acid, 2-ethyl-, oxybis(2,1-ethanedioxy-2,1-ethanedioyl) ester | C ₂₄ H ₄₆ O ₇ | 35.08 | 446 |
| 7 | 2-Octadecenoic acid, methyl ester | C ₁₉ H ₃₆ O ₂ | 30.72 | 296 |
| 8 | Uridine, 2'-deoxy-3-methyl-3',5'-di-O-methyl | C ₁₂ H ₁₈ N ₂ O ₅ | 30.16 | 270 |
| 9 | 3,4-Altrosan | C ₆ H ₁₀ O ₅ | 24.35 | 162 |
| 10 | α -D-Glucopyranose, 1,6-anhydro | C ₆ H ₁₀ O ₅ | 23.66 | 162 |
| 11 | D-Allose | C ₆ H ₁₂ O ₆ | 23.66 | 180 |
| 12 | Glucosamine, N-acetyl-N-benzoyl | C ₁₅ H ₁₉ NO ₇ | 25.60 | 325 |
| 13 | D-Ribo-hexose, 2,6-dideoxy-3-O-methyl | C ₇ H ₁₄ O ₄ | 15.74 | 162 |
| 14 | Benzoic acid | C ₇ H ₆ O ₂ | 14.09 | 122 |
| 15 | Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl-, ethyl ester | C ₈ H ₁₁ FN ₂ O ₃ | 11.21 | 202 |

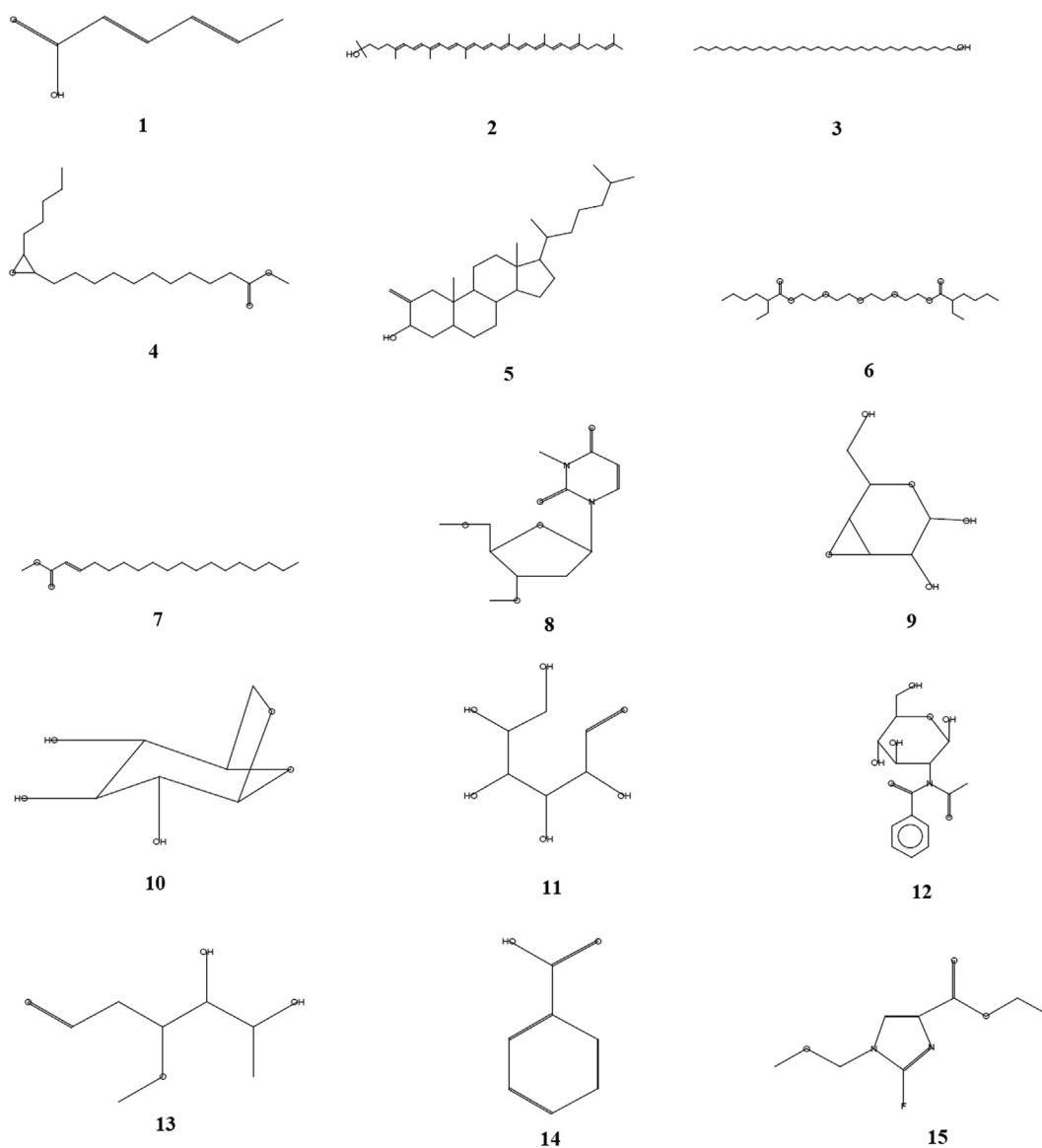
**Fig. 5** The structure of bioactive compounds of the exudate gel (EG) from the stem of *Caralluma retropiciens* (Ehrenb.) through GC-MS analysis.

Fig. 4B. The results indicated the presence of various chemical compounds such as unsaturated fatty acids, phenolic compounds, steroidal compounds, glycosides, saponins, and imidazole derivatives. GC–MS study revealed the presence of many bioactive constituents, molecular formula, retention time and their molecular weights (Table 3). The structure of bioactive constituents is represented in Fig. 5. The analysis showed the presence of various pharmaceutically important compounds in the EG. Similar results have been reported in *Caralluma geniculata* by Asha et al. (2014). The GC–MS study showed the presence of steroids, saponins, and flavonoids in EG. Kalimuthu et al. (2013) reported the presence of pharmaceutically important compounds in *Caralluma diffusa*. Presence of sorbic acid was observed in the EG in the current study. Earlier studies have shown that sorbic acid as potassium sorbate and sodium sorbate can be used as food preservatives and also it was shown that it has antimicrobial properties (Dániel et al., 2020). Rhodopsin is a photopigment very rarely present in plants (Ruiz-Gonzalez and Marin, 2004). The pigment was identified in the EG of *C. retrospiciens* (Ehrenb.) which was not reported in any *Caralluma* species. 1-Heptatriacotanol was identified in the EG and similar compound was observed in *Aloe aspera* (Dey et al., 2017). Earlier study suggested that the ethanolic extract of *Trilepisium madagascariense* DC contained 1-Heptatriacotanol as one of the constituents and was also reported to have antibacterial properties (Olufunmiso et al., 2018).

Oxirane undecanoic acid, 3-pentyl-, methyl ester, trans was present in EG. The compound was reported earlier in methanolic leaf extract of *Frankenia aucheri* Jaub et Spach

(Huda Jasim, 2018). Cholestan-3-ol, 2-methylene-, (3á,5à), a steroidal molecule, was detected in EG. Several studies have suggested that the molecule exhibits many pharmacological and biological activities such as antimicrobial, anticancer, antiarthritic, anti-inflammatory, anti-asthmatic, and hepatoprotective activities (Al-Rubaye et al., 2017; Sindhu and Neelamegam, 2015). In this study Hexanoic acid, 2-ethyl-, oxybis(2,1-ethanedioxy-2,1-ethanedioyl) ester, 2-Octadecenoic acid, methyl ester, Uridine, 2'-deoxy-3-methyl-1-3',5'-di-O-methyl, D-Ribo-Hexose, 2,6-dideoxy-3-O-methyl, a á-D-Glucopyranose, 1,6-anhydro, Glucosamine, N-acetyl-N-benzoyl and D-Allose were observed in EG of *C. retrospiciens*. 3,4-Altrosan was detected in the EG with unique retention time. Jadhav et al. (2014) reported the 3,4-Altrosan exhibited fungicidal and bacteriostatic properties. Benzoic acid is a well-known compound that has been identified in this study. The compound was reported for its antibacterial effect (Arokiyaraj et al., 2018; Eun-Soo Park, 2001). Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl-, ethyl ester also have been detected in the EG of *C. retrospiciens* (Ehrenb.) in this study. The compound is a well known for the antibacterial activity (Haider Mashkoor Hussein et al., 2016).

3.3. Antibacterial activity screening study

The MIC varied with the screened organisms (Table 4). The results indicate that the inhibitory concentration was highly influenced by the viscosity. The MICs of the EG were found to be 50% (v/v), respectively. Initially, sample 1, a 100% gel was used in the determination of antibacterial effect since the

Table 4 The minimum inhibitory concentration of the exudate gel (EG) from the stem of *Caralluma retrospiciens* (Ehrenb.).

| Bacterial organisms | Concentration of 24 h culture CFU /mL | Minimum inhibitory concentration of exudate gel (EG) (% v/v) | | | | | |
|-------------------------------|---------------------------------------|--|----|----|---|-----|------|
| | | 100 | 50 | 10 | 5 | 2.5 | 1.25 |
| <i>Bacillus subtilis</i> | 2×10^{-4} | – | – | + | + | + | + |
| <i>Staphylococcus aureus</i> | 2×10^{-5} | – | – | + | + | + | + |
| <i>Streptococcus pyogenes</i> | 2×10^{-4} | – | – | + | + | + | + |
| <i>Escherichia coli</i> | 2×10^{-6} | – | – | + | + | – | + |
| <i>Pseudomonas aeruginosa</i> | 2×10^{-3} | – | – | + | + | + | + |
| <i>Klebsiella pneumoniae</i> | 2×10^{-3} | – | – | + | + | + | + |

(+) Presence of bacterial growth; (–) No bacterial growth.

Table 5 Antibacterial effect of the exudate gel (EG) *Caralluma retrospiciens* (Ehrenb.) against selected human pathogenic bacteria.

| Organisms | Concentration CFU#/mL | Zone of inhibition (mm) of EG Analyte | | |
|-------------------------------|-----------------------|---------------------------------------|----------------------------|--------------------------|
| | | EG (100%) ^S | EG (50%) ^{SS} | Ciprofloxacin (50 µg/ml) |
| <i>Bacillus subtilis</i> | 2×10^{-4} | 17.8 ± 1.7 | 18.83 ± 0.87 ^{ns} | 25.2 ± 1.2 |
| <i>Staphylococcus aureus</i> | 2×10^{-5} | 16.5 ± 1.04 | 20.8 ± 1.2 ^{**} | 24.6 ± 1.2 |
| <i>Streptococcus pyogenes</i> | 2×10^{-4} | 16.6 ± 1 | 20.3 ± 1.2 ^{**} | 24.16 ± 0.4 |
| <i>Escherichia coli</i> | 2×10^{-6} | 20.6 ± 1 | 21.8 ± 0.75 ^{ns} | 26 ± 2.1 |
| <i>Pseudomonas aeruginosa</i> | 2×10^{-3} | 20.3 ± 1.2 | 22.3 ± 0.2 ^{ns} | 26.5 ± 1.04 |
| <i>Klebsiella pneumoniae</i> | 2×10^{-3} | 22.16 ± 1.16 | 24.5 ± 0.83 ^{ns} | 28.8 ± 1.3 |

Each value is the mean of 6 batches with standard deviation. The values of EG (Both 100% and 50%) were significantly lesser than standard ciprofloxacin at $p < 0.001$. **Extremely significant when compared to EG (100%) at $p < 0.01$. ns is non-significant when compared to EG (100%) at p greater than 0.05; ^S EG was employed in the screening directly without dilution, designated as 100% v/v gel, ^{SS} EG was employed in the screening by diluting with equal volume of Millipore water.

focus was to demonstrate their antibacterial effects against the tested bacteria. However, from the MIC study, the concentrations of the EG were fixed and their efficacies were affected. From the results, it is obvious that the viscosities of the EG influenced the antibacterial effect since 50% (v/v) of EG produced better antibacterial activities than lower concentrations. In general, the spectrum of activity against Gram-negative bacteria was wider than that against Gram-positive bacteria (Table 5). Besides, the spectrum of antibacterial activities in EG was found to be promising. Muhammad Imran Khan et al. (2018) demonstrated the antibacterial activity of saponin fractions from green tea. An earlier report showed that saponins exerted an inhibitory effect on Gram-positive bacteria but not on Gram-negative bacteria (Soetan et al. 2006). In contrast, the present study demonstrated that *C. retrospiciens* (Ehrenb.) exudate showed antibacterial activity against both Gram-positive and Gram-negative bacteria, however the antibacterial effect was more against Gram-negative bacteria than Gram-positive bacteria.

4. Conclusion

The present study has documented scientific evidence of the antibacterial properties of the exudate gel (EG) from the stem of *C. retrospiciens* (Ehrenb.). The results showed the presence of various bioactive compounds that might be beneficial in the development of new drugs for treating various diseases in future. Furthermore, the EG was showing good spectrum of activity against screened Gram-positive and Gram-negative bacteria. However, further studies are needed, especially *in vivo* investigations, to confirm the antibacterial activity of EG.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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