*Pure Appl. Biol., 8(1): 479-488, March, 2019*
*http://dx.doi.org/10.19045/bspab.2018.700206*

**Research Article**

# GC-MS analysis of antibacterial compounds from floral part of methanolic extract of *Rosa damascene*

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**Citation**
Saima Maher, Iqra Anam, Nadra Naheed, Suad Naheed, Saleha Suleman Khan, Najeeb ullah, Shazia Iqbal and Noureen Khan. GC-MS analysis of antibacterial compounds from floral part of methanolic extract of *Rosa damascene*. Pure and Applied Biology. Vol. 8, Issue 1, pp479-488. http://dx.doi.org/10.19045/bspab.2018.700206

Received: 15/09/2018 Revised: 05/12/2018 Accepted: 07/12/2018 Online First: 12/12/2018

**Abstract**
The present study was carried out with the aim to evaluate the bioactive constituent in *Rosa damascena*, which is commonly called Demask rose, medicinal plant with biological significance and belongs to *Rosaceae*. The analyses were performed in methanolic extract with two fraction, Butanol and ethyl acetate. The analysis reveals presence of 09 bioactive compounds. Out of nine compounds, five were derivatives of methyl ester. Chemical compounds found in selected plants possess anti-oxidant potential which indicate potential against cancer disease. Plant also contains biological potential against microbes such as bacteria. Methanolic extract of plant in butanol and ethyl acetate fraction are found to possess significant potential to kill different bacterial species such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabillis*. All these bacterial species were isolated from different sources such as pus, urine, vaginal sweat and sputum. Extract of *Rosa damascena* was able to kill all above mentioned species but was not capable to destroy *Klebsiella oxytoca* isolated from urine.

**Keywords**: Bioactive compounds; GC-MS hyphenated technique; *in-vitro* Antibacterial assay; Medicinal plants; *Rosa damascena*

## Introduction

Resistance potential of bacteria against developed antibiotics given arise to the need for synthesizing new agent with anti-bacterial property as pathogenic bacteria are responsible for eruption of many diseases [1]. Bacterial strain and their resistance has forced researcher to search for new and active antimicrobial substance in nature which can be utilized as chemotherapeutic agent [2]. Many plants with medicinal characteristics can be used as antibacterial agent. One of such plant is *Rosa damascena* which belongs to *Rosaceae* family. The plant is commonly known as Demask rose [3]. The members of *Rosaceae* family are called king of flowers and have ornamental uses. *Rosa damascena* is among those species which have many

significance in different aspects such as in rose oil and rose water production among all member of same family. The extract of *Rosa damascena* provides material for different daily used products belonging to cosmetic and perfume industry [4]. Reported therapeutic biological activities in this medicinally important species are antispasmodic [5], analgesic [6], Antioxidant [7], antiradical activity [8] and anti-HIV potential [9] Isolated compounds from *Rosa damascena* have been analyzed for potential against bacterial diseases [10, 11].

Hyphenated GC-MS technique is significantly helpful for analysis of any plant extract is with purpose for identification of active components responsible for specific character of biological activities of medicinal plant. It is hyphenated techniques which is helpful for identification of each component in mixture. It is done by comparison of spectra obtained by GC-MS instrument by reference spectra available in digital library [12].

## Materials and methods

### Collection and identification of plant

Flower of *Rosa damascena* was collected in June 2017 from district Quetta, Province Balochistan, Pakistan. Plant sample was deposited in Department of botany in SBKWU. Petals of collected samples were isolated and washed with distilled water. Clean samples were shade dried followed by grinding until fine powder was obtained.

### Extract preparation/sample preparation

150 g of dried powder of petals were soaked in methanol 80% pure solvent for 3 days. After this period the mixture was filtered. The filtrate obtained was evaporated till crude gummy material was obtained. This gummy extract was fractionated with hexane (1000 ml), ethyl acetate (50 g) and butanol (80 g). The prepared samples were coded mentioned in (Table 1).

**Table 1. Plant extract with code**

| Plant extract code | Explanation |
|---|---|
| R.D But | *Rosa damascena* extract in butanol |
| R.D Ethyl acet. | *Rosa damascena* extract in ethyl acetate |

### GC-MS analysis

GC/MS was performed with model 'Agilent GC-MS triple quad 7000 GC 7890A'. Helium was utilized as carrier gas. Flow rate of carrier gas was 1.2 ml/min. Analysis was made in spilt mode. Column dimension was 30 m × 250 µm × 0.25 µm. Sample utilized during analysis was 3 µL. Temperature during analysis was 250 $^0$C (source) and 350 $^0$C (column) while oven temperature was maintained at 40 $^0$C. Total run time of analysis was 91.25 mins. Ionization technique utilized in this research was electron impact (EI) while detector used in GC-MS instruments was triple quadrapole. GC-MS library was also available for comparison of spectra with purpose of identification.

## Results and discussion

Development of new drugs is need of this time. Medicinal plants are best source for medicinal purpose. The drugs obtained from natural source have encounter less side effects. Phytochemical isolated from plants have many biological potentials. Thus, these constituent may be directly and indirectly incorporated for synthesis of medicines. The present study was made in order to evaluate bioactive component of *Rosa damascena* by using GC-MS technique. Detailed information with respect of retention time (RT), Molecular formula (MF), molecular weight (MW), peak area are given in (Table 2). The GC chromatogram is shown in (Figurer 1). All peaks obtained were integrated and then, compared with data base

for identification of compound. Data obtained from present research showed presence of 9 bioactive compounds (Table 3). All compounds were detected in hexane fraction of methanolic extract. All identified compounds belong to terpenes, phyto-sterol, tocopherol, fatty acid and methyl ester classes of organic compounds (Figure 2-10). Amongst identified phytochemical from *Rosa damascena*, α-Amyrin, phytosterol and tocopherol compounds [13-15] have anti-oxidant potential. Vitamin E has also been found to exhibit anti-oxidant potential [16]. Ester compound are capable of scavenging free radicals. Detected compound α-Amyrin possesses anti-depression potential as well [17]. Phyto-phenols are found to be health promoting substance [18]. Five methyl ester compounds have been detected during study. Many commercial applications have been associated with ester compounds [19]. Presence of above mentioned compounds belonging to some useful organic compounds classes in methanolic extract *of Rosa damascena* indicates its medicinal significance.

**Table 2. GC/MS analysis of hexane extract of *Rosa damascena***

| S# | R-T min | Compound name | Peak area | Molecular weight | Formula |
|---|---|---|---|---|---|
| 1 | 63.38 | α-Amyrin | 4.79 | 426 | $C_{30}H_{50}O$ |
| 2 | 62.60 | γ-Sitorol | 6.72 | 414 | $C_{29}H_{50}O$ |
| 3 | 59.38 | Vitamin E | 4.65 | 430 | $C_{29}H_{50}O_2$ |
| 4 | 52.00 | Docosanoic acid, methyl ester | 1.37 | 354 | $C_{23}H_{46}O_2$ |
| 5 | 23.42 | 5-Acetoxy-2,6,6-trimethyl-hept-3-enoic acid, methyl ester | 1.28 | 242 | $C_{13}H_{22}O_4$ |
| 6 | 20.91 | 3-Furanacetic acid | 9.51 | 240 | $C_{12}H_{16}O_5$ |
| 7 | 34.52 | 9,12-octadecadienoic acid (Z-Z),-methyl ester | 60.99 | 294 | $C_{19}H_{34}O_2$ |
| 8 | 29.98 | n-Hexadecanoic acid | 67.06 | 256 | $C_{16}H_{32}O_2$ |
| 9 | 28.39 | Hexadecanoic acid, methyl ester | 97.02 | 270 | $C_{17}H_{34}O_2$ |

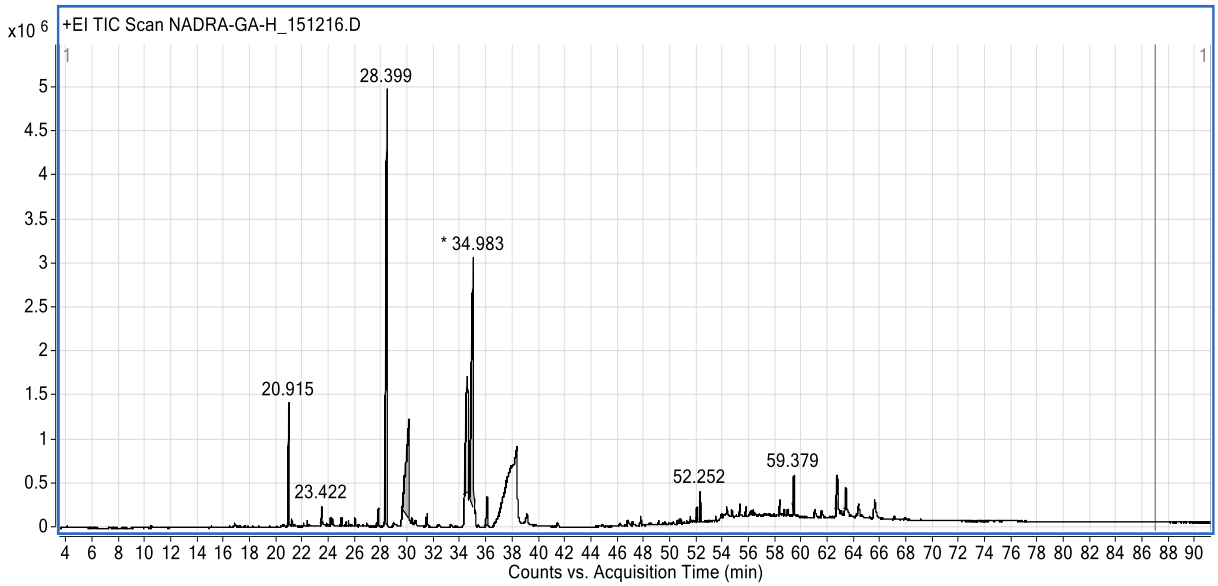


**Figure 1. GC Chromatogram of hexane extract of plant**

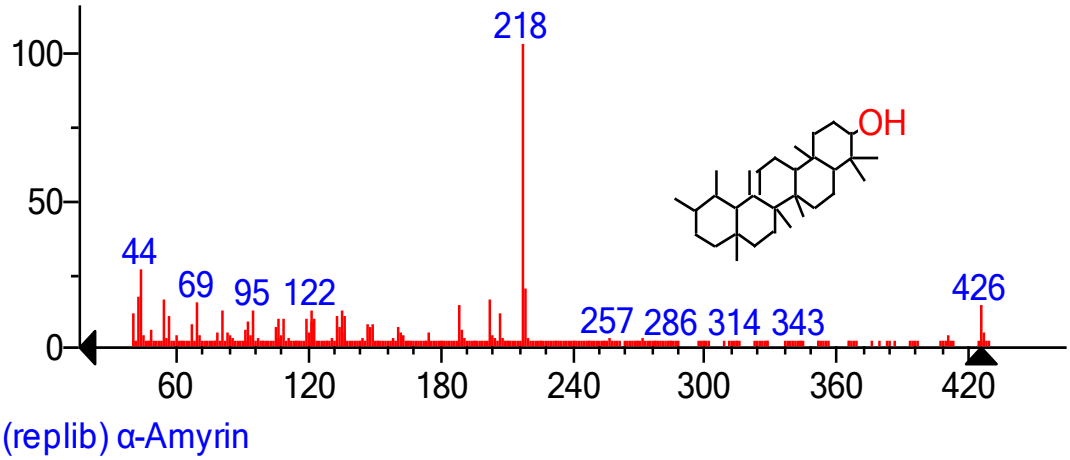


**Figure 2. Spectra of α-Amyrin**

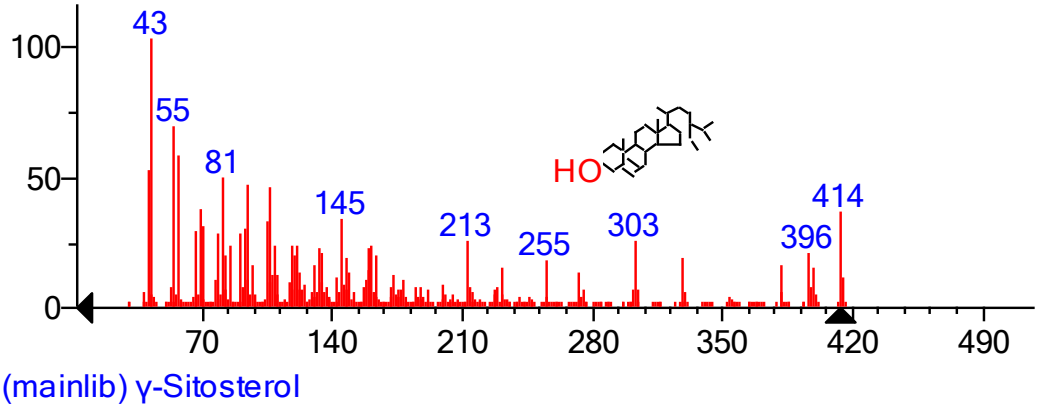


**Figure 3. Spectra of γ-Sitosterol**

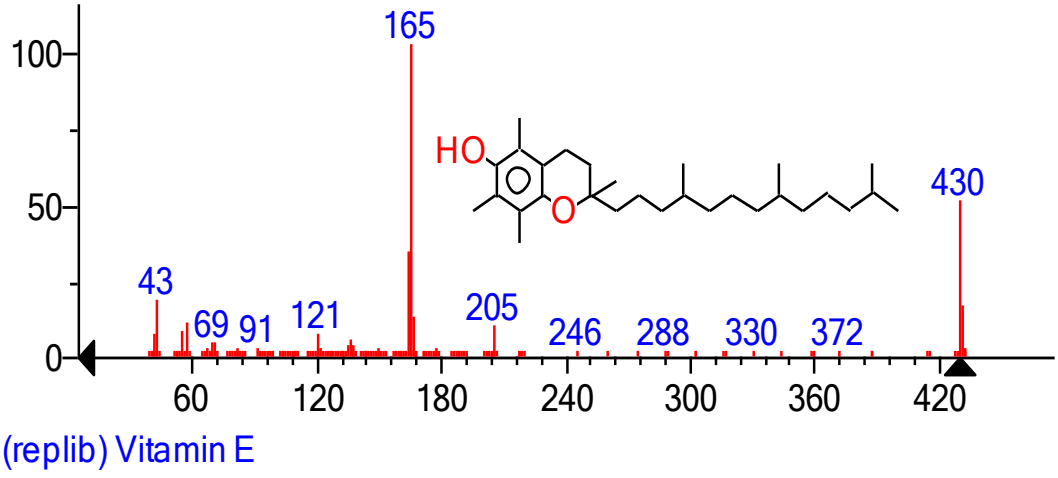


**Figure 4. Spectra of Vitamin E**

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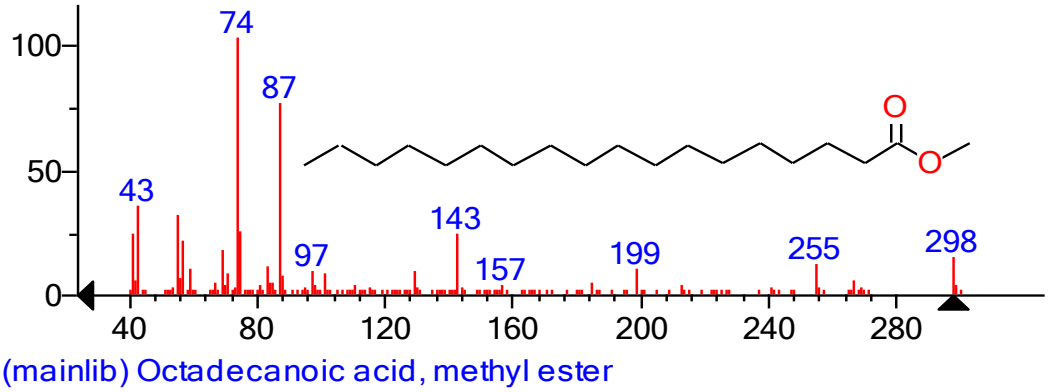


**Figure 5. Spectra of Octa Decanoic Acid Methyl ester**

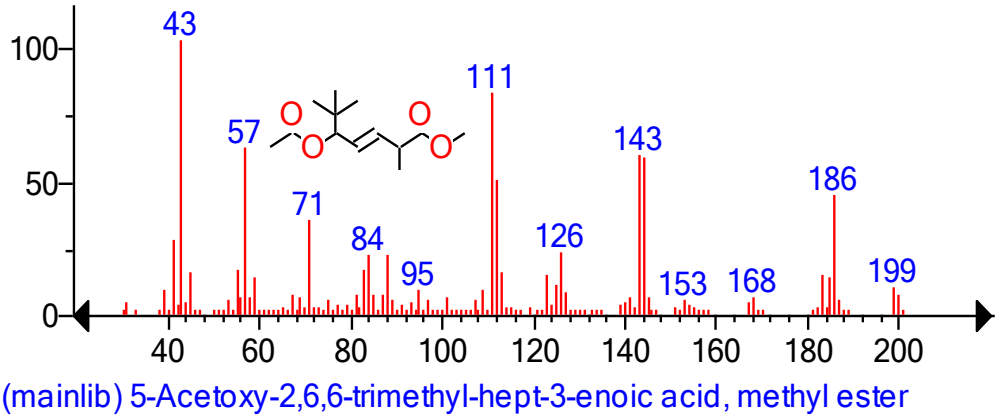


**Figure 6. Spectra of 5-Acetoxy-2,6,6-trimethyl-hept-3-enoic acid, methyl ester**

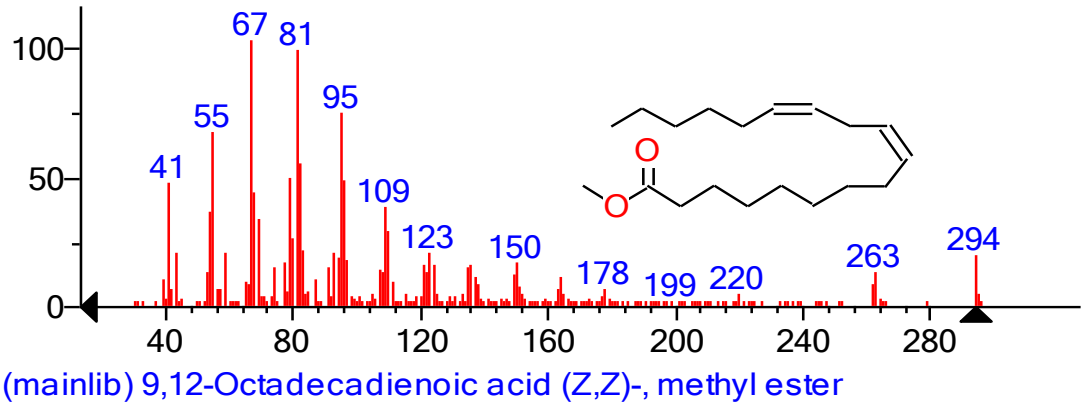


**Figure 7. Spectra of 9, 12-octadecadienoic acid (Z-Z)-methyl ester**

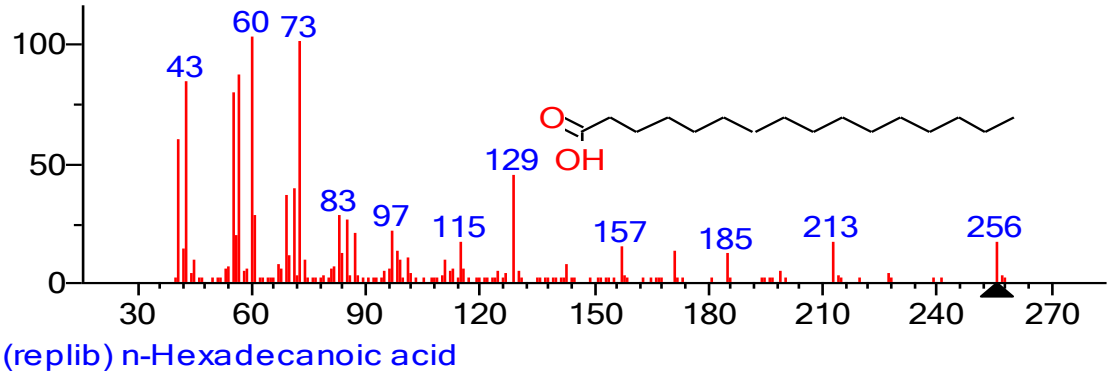


**Figure 8. Spectra of n-Hexadecanoic acid**

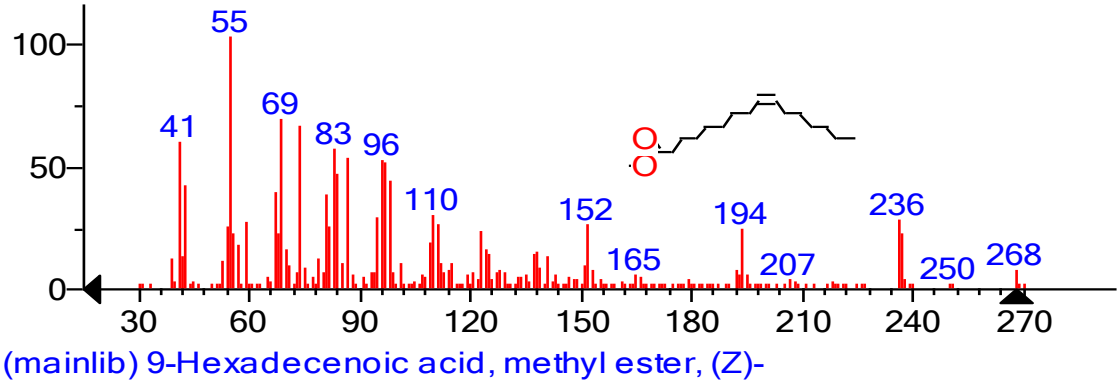


**Figure 9. Spectra of 9- Hexadecanoic acid, methyl ester**

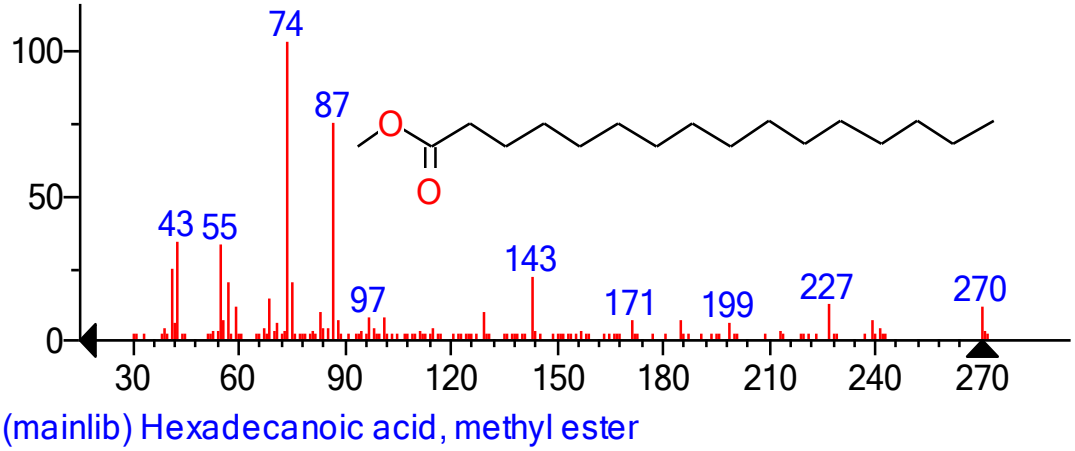


**Figure 10. Spectra of hexadecanoic acid, methyl ester**

**Antibacterial assay**

Antibacterial activity of petals extract in ethyl acetate and butanol fraction was perform at Pharmacy lab, Jinnah University for Women, Karachi. Analysis was made by well diffusion method based on protocol defined by Uddin *et al*. [1]. The analyses were made on against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus mirabillis*. These bacterial strains were isolated from pus, urine, vaginal sweat, Herpes Simplex

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Viral infection. All bacterial organisms were identified from the diagnostic Agha Khan laboratory, Karachi.

All isolated bacterial were cultured in presence of required nutrients for incubation period of 4 hours at $37^0$C. The culture obtained was transferred to ager plants separately. For analysis distilled water was used as negative control. For each cultured stain, plant extract was examined. The shrinkage in culture loop of bacterial strain reflects positive potential of plant against that bacterial strain. Potential of *Rosa damascena* against bacterial strain is mentioned in (Table 4).

**Table 3. Compound name with structure**

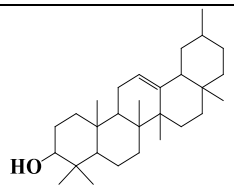
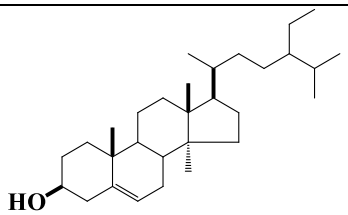
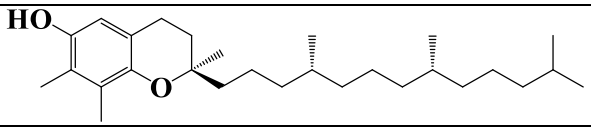
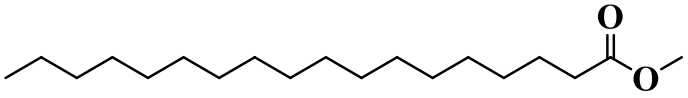
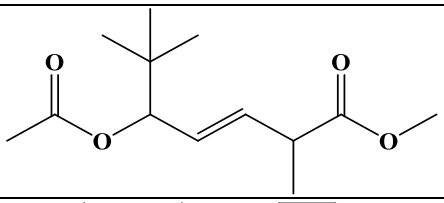
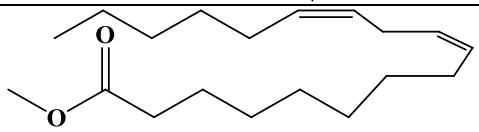
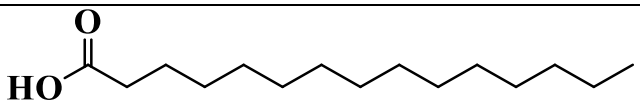
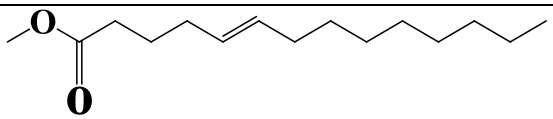
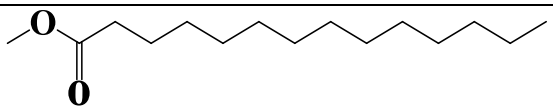
| S. No | Compound name | Structure |
|---|---|---|
| 1 | α-Amyrin |  |
| 2 | γ-Sitosterol |  |
| 3 | Vitamin E |  |
| 4 | Octa-Decanoic acid, methyl ester |  |
| 5 | 5-Acetoxy-2,6,6-trimethyl-hept-3-enoic acid, methyl ester |  |
| 6 | 9,12-octadecadienoic acid (Z-Z),-methyl ester |  |
| 7 | n-Hexadecanoic acid |  |
| 8 | 9- Hexadecanoic acid, methyl ester |  |
| 9 | Hexadecanoic acid, methyl ester |  |

**Table 4. Anti-bacterial analysis of extract obtained from petal of plant**

| Organism | Source | R.D* But. 200mg/L ZOI (mm) ± SEM) | R.D* eth. Acet. 200mg/L ZOI (mm) ± SEM |
|---|---|---|---|
| *Klebsiella pneumoniae* | Sputum | Nil | Nil |
| *Pseudomonas aeruginosa* | Pus | Nil | 19.6±2.7284 |
| *Klebsiella pneumoniae* | Urine | 18±0.577 | 19±0.5773 |
| *Klebsiella pneumoniae* | Vaginal sweat | Nil | 16.6±0.6666 |
| *Proteus mirabillis* | Urine | 10±0.5773 | Nil |
| *Escherichia coli* | Urine | 17±0.57 | 19.33±0.66 |
| *Proteus mirabillis* | Urine | Nil | 9±0.57 |
| *Klebsiella pneumoniae* | Urine | Nil | 13.66±0.33 |
| *Escherichia coli* | Herpes Simplex Viral infection | Nil | 12.33±0.33 |
| *Escherichia coli,+ proteus* | Urine | 8±0.57 | 17±1.52 |
| *Klebsiella pneumoniae* | Pus | 9±0.57 | Nil |
| *Klebsiella oxytoca* | Pus ear | Nil | 12.63±0.33 |
| *Klebsiella pneumoniae* | Pus | Nil | Nil |
| *Proteus mirabillis* | Urine | 7.33±0.33 | |
| *Escherichia coli* | Urine | 12.66±0.33 | 10.66±0.33 |
| *Klebsiella oxytoca* | Urine | Nil | Nil |
| *Pseudomonas aeruginosa* | Urine | 10.33±0.33 | Nil |
| *Klebsiella + Candida specie* | Urine | Nil | 17±0.57 |

**Rosa damascena*

Potential of *Rosa damascena* against bacterial strain is mention in (Table 4). In comparison of butanol and ethyl acetate fraction of *Rosa damascena* medicinal plant, ethyl acetate fraction was effective and showed good efficiency against maximum bacterial species. Values greater than 11 mm reflect good potential of plant against under observed bacterial species. Values in the range of 16-19 reflect best antibacterial potential. Highest activity was observed with *Pseudomonas aeruginosa*. 200 mg of ethyl acetate fraction was enough to show higher zone of inhibition which reflects plant's good potential as antibacterial agent. In butanolic fraction best activity was observed against *Klebsiella pneumoniae* and *Escherichia coli* while satisfactory activity was observed against *Proteus mirabillis, Pseudomonas aeruginosa* and *Escherichia coli.* All these were isolated from urine source. No activity was observed against bacterial isolated from all other sources. Observation on ethyl acetate fraction reveals great efficacy of plants against maximum bacterial spices except *Proteus mirabillis.* Plant was much more effective against *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with maximum zone of inhibition. Plant extract against in both fractions showed no potential to fight against *Klebsiella oxytoca* (isolated from urine), *Klebsiella pneumoniae* (isolated from sputum, pus).

## Conclusions

Medicinal plants are considered backbone for traditional treatments. *Rosa damascena* is also medicinal plant which is analyzed in present work. Research was made to find out active component by GC and to evaluate the biological potential through bacterial analysis. In the hexane extract of this plant nine active compounds were detected. Plant was observed with significance potential against bacterial strain.

**Author's contributions**

Experimental design: S Maher, Sample collection: I Anam & N Ullah, Extract preparation, S Maher, S Suleman & I Anam, Culture preparation: S Maher & I Anam, GC.MS analysis: N Naheed, Antibacterial analysis: S Naheed, GC-MS Data analysis: S Maher, I anam & S Suleman, Antibacterial data analysis: I anam, S Iqbal, N Khan & S Naheed, Wrote the paper: I Anam, N Ullah, Paper refining and checking: S Maher, I anam & S Suleman.

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