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Antioxidant, Antibacterial Activities and GC-MS Analysis of Fresh Rose Petals Aqueous Extract of *Rosa damascena* Mill L.

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ABSTRACT

A rose (*Rosa damascena*) is a woody perennial plant of the genus Rosa within the family Rosaceae. The leaves of the plant are alternate to each other on the stem. Best known for its ornamental values, most of the rose plants are deciduous except a few from the South East Asia that are evergreen. The aggregate fruit of the rose is a pot-like structure containing the seeds in it called the rose hip. The sharp objects along the stem of a rose plant are outgrowths of the epidermis called as prickles. The flowers to prepare a drink which acts as an energy stimulant, blood tonic and also works in case of digestive irregularities. The extract of rose plant especially act as an antidepressant, antibacterial, antifungal, antiseptic, antiinflammatory, digestive stimulant, kidney tonic and menstrual regulator. The maximum DPPH˙ radical and superoxide ($O_2^{\cdot-}$) radical scavenging activities of fresh rose petals aqueous extract were 52.84±0.20% and 89.36±0.31% at 120 µg/mL concentration. The IC_{50} values of DPPH˙ radical and superoxide ($O_2^{\cdot-}$) radical scavenging activities were 113.55 µg/mL and 40.62 µg/mL concentration respectively. The maximum Mo^{6+} reduction and Fe^{3+} reduction of fresh rose petals aqueous extract were 82.52±0.13% and 81.54±0.42% at 120 µg/mL concentration and the RC_{50} values of Mo^{6+} reduction and Fe^{3+} reduction were 46.67 µg/mL 32.25 µg/mL concentration respectively. The fresh aqueous extract of *Rosa damascena* possessed active molecules such as E,E-6,8-Tridecadien-2-ol, acetate, 8-Carbethoxy-1-methyl-1,4,5,6,7,8-hexahydropyrrolo [2,3-b]azepin-4-one-3-carboxylic acid and 9-Octadecynoic acid, methyl ester exhibiting antioxidant, antimicrobial activities.

Keywords: *Rosa damascena*, Antioxidant, Free radical, DPPH˙, Antibacterial activity, GC-MS.

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INTRODUCTION

Free radicals are known to be the major cause of various chronic and degenerative diseases, including diabetes mellitus, inflammation, stroke, cancer, coronary heart disease, and aging [1,2,3]. Reactive oxygen species (ROS) include free radicals such as .O_2- (superoxide anion), .OH (hydroxyl radical), H_2O_2 (hydrogen peroxide) and .O_2 (singlet oxygen) which cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes[4,5]. The tissue injury caused by ROS includes protein damage, DNA damage and oxidation of important enzymes in the human cells. The consequences of these events may lead to the occurrence of various oxidative stress related diseases. Recently, plants, natural foods and food derived antioxidants such as vitamins and phenolic phytochemicals have received growing attention, since they play an important role as preventive agents against damage caused due to oxidative stress[6].

The essential oil of *Rosa damascena* is known for its fine perfumery applications and the use in cosmetic preparations. The use of *Rosa damascena* is increasing steadily worldwide for its medicinal properties health promoting benefits. Recently, the antioxidant, antibacterial, anti-HIV and activities of its essential oil have been demonstrated. This plant is also used as a gentle laxative. Rose oil is used for healing nervous stress, tension, grief and depression. It helps in the reduction of thirst, curing old cough, treating special complaints of women, wound healing, and improving skin health[7,8].

Rosa damascena mill L, commonly known as Damask rose, is one of the most important species of Rosaceae family[9] (Figure 1). Rosaceae are well- known ornamental plants and

have been referred to as the king of flowers[10]. Rose species is a perennial bushy shrub reaching approximately 1 to 2 meters in height with large, showy and colorful flowers. The leaves are imparipinnate and compound with 5-7 leaflets. Apart from the use of rose species as ornamental plants in parks, gardens, and houses, they are principally cultivated for using in perfume, medicine and food industry[11]. Compounds from flowers, petals and hips (seed-pot) of this plant have been studied in a variety of *in vivo* and *in vitro* studies. The most therapeutic effects of rose species in ancient medicine are including treatment of abdominal and chest pain, strengthening the heart, treatment of menstrual bleeding and digestive problems, and reduction of inflammation, especially of the neck.

Taxonomic classification of *Rosa damascena*

Kingdom: Plantae

Subkingdom: Viridiplantae

Infrakingdom: Streptophyta

Superdivision: Embryophyta

Division: Tracheophyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Superorder: Rosanae

Order: Rosales

Family: Rosaceae

Genus: Rosa L

Species: *Rosa damascena* Mill. (pro sp.) – damask rose

Figure.1: Habitat of *Rosa damascena*

MATERIALS AND METHODS

Collection of rose petals and preparation of extract

The rose flowers were collected from the commercial flower market, Chennai, Tamilnadu, India. About 5 g of torn pieces of rose petals was weighed and soaked in 50 mL of distilled water and boiled in cookware. The supernatant was filtered and condensed in a hot plate at 50°C, which yields pinkish viscous extract.

Determination of total phenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds[12] with slight modifications. One hundred µL of fresh rose petals aqueous extract (1 mg/mL) was mixed with 900 µL of methanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of Na_2CO_3 (20% w/v) was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured using UV-Vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Determination of total flavonoids

The total flavonoid content of fresh rose petals aqueous extract was determined using aluminium chloride colorimetric method with slight modification as described[13]. 500 µL of fresh rose petals aqueous extract (1 mg/mL) was mixed with 500 µL of methanol, 0.5 mL of 5% (w/v) sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.5 mL of 10% (w/v) aluminium chloride solution was added and incubated for further 5 min at room temperature followed by addition of 100 µL of 1 M NaOH solution. The total volume was made up to 2 mL with distilled water. The absorbance was measured at 510 nm using UV-Vis spectrophotometer. The total flavonoid content was expressed in terms of quercetin equivalent (µg/mg of extract), which is a common reference compound.

In vitro antioxidant activities

DPPH$^{\cdot}$ radical scavenging activity

The antioxidant activity of fresh rose petals aqueous extract was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical[14]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of fresh rose petals extract. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm and the IC_{50} was calculated. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH}^{\cdot} \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Superoxide radical scavenging activity was carried out by the modified method[15]. The reaction mixture contains different concentrations (20-120 µg/mL) of fresh rose petals aqueous extract with 50 mM of phosphate buffer (pH 7.6), 1.5 mM of riboflavin, 12 mM of EDTA and 50 mM of NBT, added in that sequence. The reaction was started by illuminating the reaction mixture for 15 min. Immediately after illumination, the absorbance was measured using UV-Vis spectrophotometer at 590 nm and the IC_{50} was calculated. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of superoxide } (O_2^{\cdot-}) \text{ radical inhibition} = \left[\frac{\text{Control} - \text{Sample}}{\text{Control}}\right] \times 100$$

Phosphomolybdenum reduction activity

The antioxidant capacity of the fresh rose petals aqueous extract was assessed as described[16]. The fresh rose petals aqueous extract with concentrations ranging (20-120 µg/mL) was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 95^{o}C for 90 min. The absorbance of the coloured complex was measured using UV-Vis spectrophotometer at 695 nm and the RC_{50} was calculated. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated using the following formula:

$$\% \text{ of Phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

Ferric (Fe^{3+}) reducing power activity

The reducing power of fresh rose petals aqueous extract was determined by slightly modified method[17]. One mL of fresh rose petals aqueous extract of different concentrations (20-120 µg/mL) was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1 % (w/v) potassium ferricyanide [$K_3Fe(CN)_6$] . The mixtures were then incubated at 50°C for 20 min. One mL of 10% (w/v) trichloroacetic acid was added to each mixture. Then to the 1 mL mixture of 0.1% (w/v) Ferric chloride was added and the absorbance was measured using UV-Vis spectrophotometer at 700 nm and the RC_{50} was calculated. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated using the following formula:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}}\right] \times 100$$

Antibacterial activity

Microbial strains

The microorganisms of Gram positive strains such as *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus* as well as Gram negative strains such as *Escherichia coli*, *Proteus vulgaris* and *Shigella flexneri* were used for the evaluation of antibacterial activity.

Reference and control

Tetracycline was chosen as the standard reference. The controls consist of solidifying agar onto which was solvent, and the test compounds were soluble in it.

Aseptic conditions

The aseptic chamber which consist of a wooden box (1.3m x 1.6m x 0.6m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from lamp).

Nutrient broth agar medium

Nutrient broth agar medium was prepared according to the standard methods (peptone-5 g, yeast-3 g, NaCl-5 g, distilled water- 1000 mL, agar-20 g) and was suspended in 200 mL of distilled water in a 500 mL conical flask, stirred, boiled to dissolve and then autoclaved at 15 lbs and at 121˚C for 15 minutes. The hot medium was poured in sterile petriplates which were kept in the aseptic laminar chamber. The medium was allowed to solidify for 15 min.

Agar well diffusion method

Antibacterial activity of fresh rose petals aqueous extract was carried out using agar well diffusion method[18]. The solidified nutrient agar in the petriplates was inoculated by dispensing the inoculum using sterilized cotton swabs which is previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The fresh rose petals aqueous extract was loaded into each well containing 250, 375 and 500 µg/mL concentrations. All the plates with extract loaded wells were incubated at 37ºC for 24 hours and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well.

Tetracycline (40 µg) was used as positive control, which is a broad spectrum polyketide antibiotic.

Thin layer chromatography

Thin layer chromatography (TLC) was carried out for the fresh rose petals aqueous extract in Merck TLC aluminium sheets, silica gel 60 F_{254} (20 x 20 cm), preloaded plates. The aqueous extract was spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed in a mixture of suitable solvent system. The spots were visualized with UV light at 254 nm. The R_f values of the coloured spots were recorded. The ratio in which distinct bands appeared was optimized and R_f values were calculated[19].

Calculation of R_f value:

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Gas chromatography–Mass Spectrometry (GC–MS) analysis

For GC-MS analysis, the fresh rose petals aqueous extract was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units[20].

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

RESULTS AND DISCUSSION

Determination of total phenols and flavonoids

Flavonoids and phenolics acids are the most important bioactive natural product of secondary metabolites and act as an antioxidant and anti-aging substances, capable of scavenging free radicals and reducing the risk of cancer[21]. Oxidative stress is a harmful condition that occurs when there is an excess of ROS and decrease in antioxidant levels and cause tissue damage which leads to different diseases. Flavonoids and phenolic compounds are well known for their antioxidant activity that protect humans against the damaging effects of free radicals in addition an imbalance between antioxidants and free radicals results in oxidative stress, will lead to cellular damage. Phenolic hydroxyl groups are good hydrogen donors, which are hydrogen-donating antioxidants can react with reactive oxygen species and reactive nitrogen species which breaks down the generation of new radicals in a termination reaction. Phenolic structures often have the potential to interact strongly with proteins, due to their hydrophobic benzenoid rings and hydrogen-bonding potential of the phenolic hydroxyl groups. Phenolic compounds have the ability to act as antioxidants also by virtue of their capacity to inhibit some enzymes involved in radical generation, such as various cytochrome P450 isoforms, lipoxygenases, cyclooxygenase and xanthine oxidase[22]. The total phenol content was 110.065±1.26 µg/mg of GAE and the total flavonoid content was 176.002±1.021 µg/mg of QE in the extract (Table 1). These results provide a comprehensive profile of the antioxidant activity of fresh rose petals aqueous extract with respect to their phenols and flavonoids content.

Table 1: Quantitative estimations of fresh aqueous extract of *Rosa damascena*

S. No	Phytochemicals	Amount (µg/mg)
1	Phenols	110.065±1.26 GAE
2	Flavonoids	176.002±1.021 QE

In vitro antioxidant activities

DPPH$^{\cdot}$ radical scavenging activity

DPPH radical scavenging assay is a decolorization assay that will measure the capacity of antioxidants to scavenge DPPH$^{\cdot}$ radicals by measuring absorbance at 517 nm[23]. The ability of fresh rose petals aqueous extract to scavenge free radicals was evaluated by using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH$^{\cdot}$ radical scavenging activity was 52.84±0.20% at 120 µg/mL concentration (Table 2 and Graph 1). Fresh rose petal extract was demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC_{50} was found to be 113.55 µg/mL concentration and was compared with standard (Ascorbic acid, IC_{50} = 11.98 µg/mL concentration).

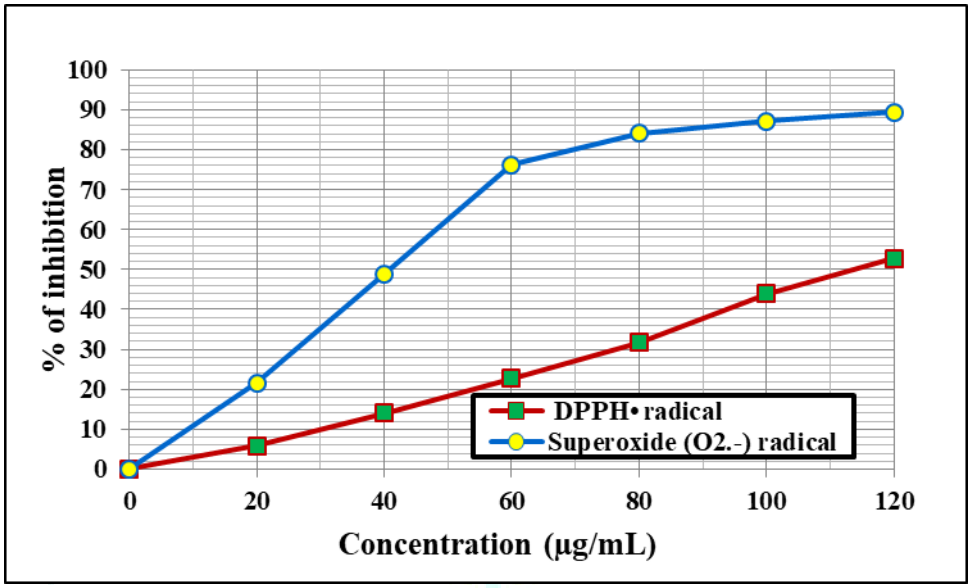
Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Superoxide anion is also very harmful to cellular components and their effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Flavonoids are effective antioxidants, mainly because they scavenge superoxide anions. Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT^{2+}) to blue formazan, which is measured at 590 nm in UV-Vis spectrophotometer. Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture[24]. The maximum superoxide ($O_2^{\cdot-}$) radical scavenging activity of fresh rose petals aqueous extract of *Rosa damascena* was 89.36±0.31% at 120 µg/mL concentration (Table 2 and Graph 1) and the IC_{50} was 40.62 µg/mL concentration. It was compared with the standard of ascorbic acid (IC_{50} = 9.65 µg/mL concentration).

Table 2: DPPH˙ radical and superoxide ($O_2^{\cdot-}$) radical scavenging activities of fresh aqueous extract of *Rosa damascena*

S. No	Concentration (µg/mL)	Fresh aqueous rose petals extract	
		% of inhibition*	
		DPPH˙ radical	Superoxide ($O_2^{\cdot-}$) radical
1	20	5.83±0.17	21.56±0.42
2	40	14.11±0.32	48.91±0.44
3	60	22.70±0.25	76.17±0.24
4	80	31.78±0.15	84.74±0.21
5	100	43.96±0.10	87.13±0.15
6	120	52.84±0.20	89.36±0.31

(*Average of duplicates)



Graph 1: DPPH˙ radical and superoxide ($O_2^{\cdot-}$) radical scavenging activities of fresh aqueous extract of *Rosa damascena*

Phosphomolybdenum reduction activity

The total antioxidant activity of fresh rose petals aqueous extract was measured by phophomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm[25]. The maximum phosphomolybdenum reduction was 82.52±0.13% at 120 µg/mL concentration (Table 3 and Graph 2) and the RC_{50} was 46.67 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50} = 6.34 µg/mL concentration). PM assay is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant and molybdenum ligand. It involves in thermally generating auto-oxidation during prolonged incubation period at higher temperature.

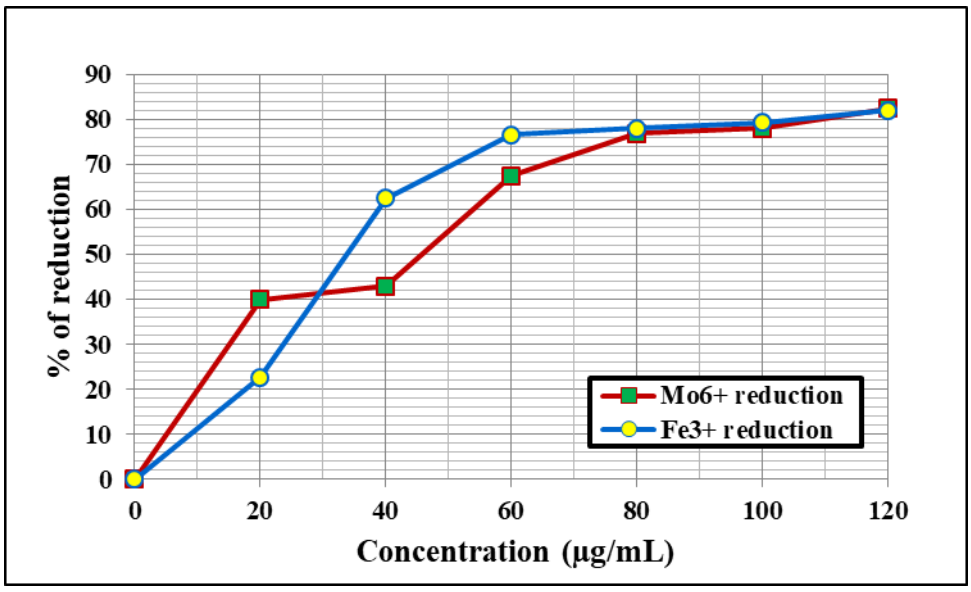
Ferric (Fe^{3+}) reducing power activity

The reducing power activity was carried out by the reduction of Fe^{3+} to Fe^{2+} by the fresh aqueous extract of *Rosa damascena* and the subsequent formation of ferro-ferric complex. The reduction ability increases with increase in concentration of the extract[26]. The maximum Fe^{3+} reduction was 81.54±0.42% at 120 µg/mL concentration (Table 3 and Graph 2) and the RC_{50} was 32.25 µg/mL concentration. It was compared with the standard ascorbic acid (RC_{50} = 7.72 µg/mL concentration). Also in this assay, higher absorbance of the reaction mixture indicates higher reduction potential. The reducing capacity of aqueous extract poses as a significant indicator of its potential antioxidant activity. The reducing capacity of the extract was performed using Fe^{3+} to Fe^{2+} reduction assay as the yellow colour changes to green or blue colour depending on the concentration of antioxidants. The antioxidants such as phenolic acids sand flavonoids were present, considerable amount in fresh rose petal extract of *Rosa damascena* and showed the reducing capacity in a concentration dependant manner.

Table 3: Phosphomolybdenum reduction activity and Ferric (Fe^{3+}) reducing power activities of fresh aqueous extract of *Rosa damascena*

S. N.	Concentration (µg/mL)	Fresh rose petals extract	
		% of reduction*	
		Mo^{6+} reduction	Fe^{3+} reduction
1	20	40.01± 0. 31	22.66±0.33
2	40	42.96±0.14	62.49±0.20
3	60	67.53±0.28	76.54±0.44
4	80	76.90±0.37	78.03±0.24
5	100	77.99±0.39	79.31±0.48
6	120	82.52±0.13	81.54±0.42

(*Average of duplicates)



Graph 2: Phosphomolybdenum reduction activity and Ferric (Fe^{3+}) reducing power activities of fresh aqueous extract of *Rosa damascena*

Antibacterial Activity

Antibacterial activity of fresh rose petals aqueous extract of *Rosa damascena* was carried out against microorganism including Gram-positive bacteria (*Bacillus subtilis*, *Micrococcus luteus, Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli, Shigella flexneri*). The antibacterial sensitivity of the crude extract and their potency was assessed quantitatively by measuring the diameter of clear zone in cultures in petriplates. *Shigella flexneri* showed maximum zone of inhibition of 20 mm at 500 µg/mL concentration (Table 4 and Figure 2). The antibacterial activity may be due to the presence of secondary metabolites such as phenolic compounds, terpenoids, tannin and alkaloids that adversely affect the growth of microbes.

Table 4: Antibacterial activity of fresh aqueous extract of *Rosa damascena*

S. No	Organisms	Zone of inhibition (mm)			Standard (Tetracycline) 40 µg
		250 µg	375 µg	500 µg	
1	*Micrococcus luteus*	14	18	19	18
2	*Staphylococcus aureus*	16	18	19	17
3	*Bacillus subtilis*	15	16	18	27
4	*Escherichia coli*	14	17	18	34
5	*Shigella flexneri*	17	18	20	18
6	*Proteus vulgaris*	14	15	16	17

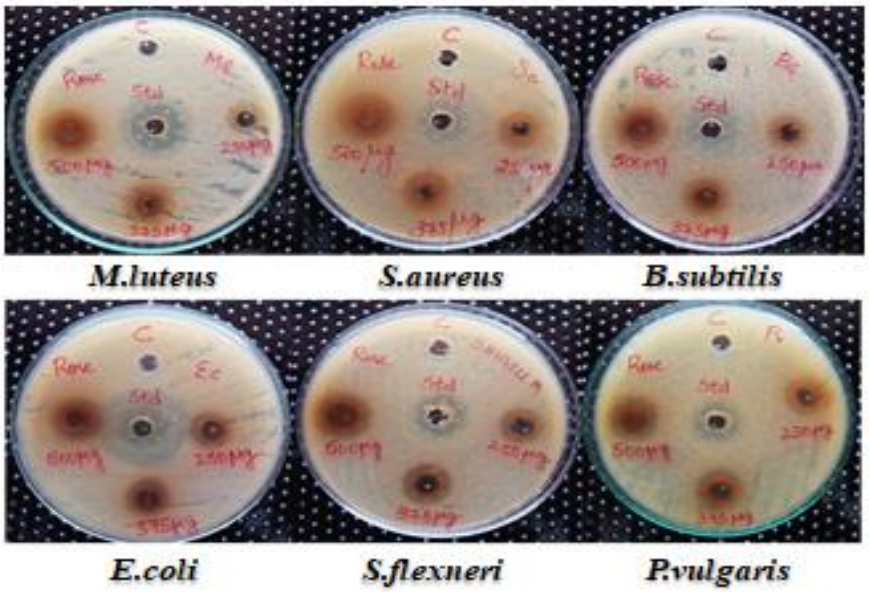


Figure.2: Antibacterial activity of fresh aqueous extract of *Rosa damascena*

Thin Layer Chromatography

Thin layer chromatography analysis for the fresh aqueous extract of *Rosa damascena* was carried out in the solvent system of Acetone : Ethyl acetate with the ratio of 1.5:0.5.

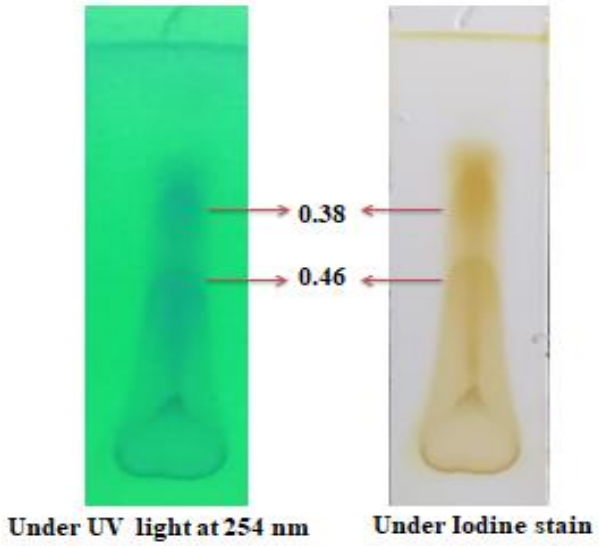


Figure.3: Active Compounds separated by Thin Layer Chromatography

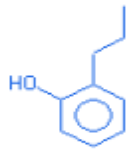
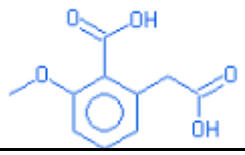
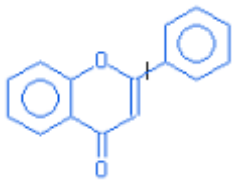
The separated compounds in TLC were showed in Figure 3. The separated compounds by TLC analysis and retention factor were calculated based on the solvent front.

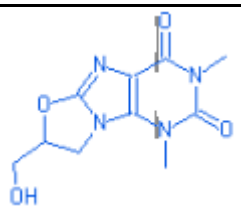
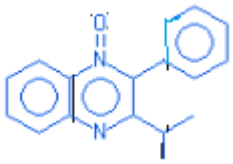
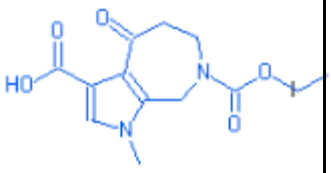
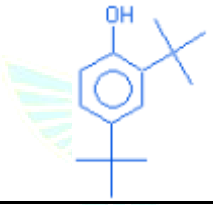
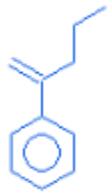
One of the oldest fields of TLC application is for the identification of plants; the TLC fingerprints of medicinal plants and extracts were implemented in the most pharmacopoeias. Among the chromatographic methods recommended in the pharmacopoeias for the analysis of medicinal plants, extracts, tinctures, essential oils, and other plant products, the proportion of TLC is: 100% in Romanian Pharmacopoeia, 84% in Italian Pharmacopoeia, 82% in European Pharmacopoeia, and 79% in Hungarian Pharmacopoeia[27,28].

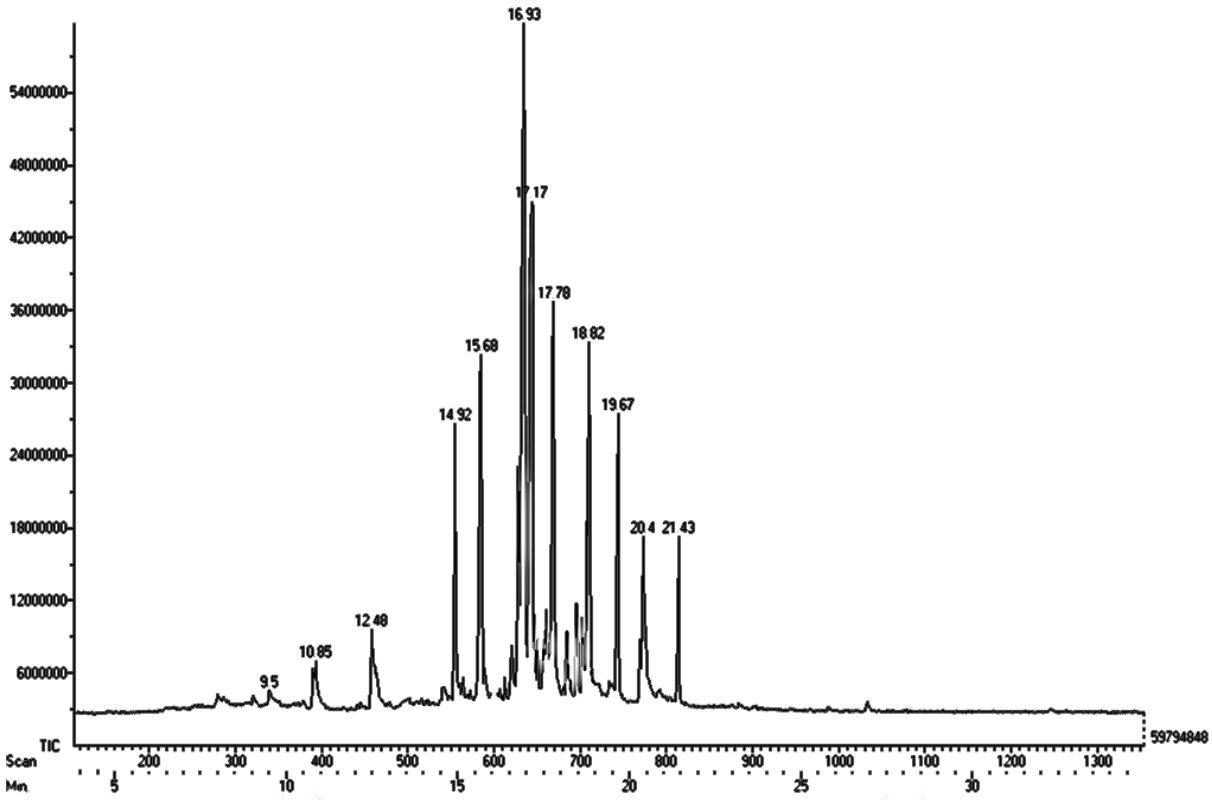
Gas chromatography–Mass Spectrometry (GC–MS) analysis

GC-MS analysis of fresh aqueous extract of *Rosa damascena* was showed in Table 5. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of nonpolar components and volatile essential oil, fatty acids, lipids and alkaloids. The active principles with their Retention time (RT), Molecular formula and Molecular weight (MW) were recorded (Graph 3). The active compounds present in the fresh aqueous extract of *Rosa damascena* exhibit antitubercular activity, antiviral activity, antimicrobial activity, etc. (Table 6).

Table 5: GCMS analysis of fresh aqueous extract of *Rosa damascena*

S. No	COMPOUND NAME	RT	COMPOUND STRUCTURE	MOLECULAR WEIGHT (g/moL)	MOLECULAR FORMULA
1	Phenol,2-propyl-	9.5		136	$C_9H_{12}O$
2	Benzeneacetic acid, 2-carboxy-3-methoxy-	14.92		210.95	$C_{10}H_{10}O_5$
3	Flavone	15.68		222	$C_{15}H_{10}O_2$
4	7-Methox-2,2,4,8-tetramethyltricyclo[5.3.1.0(4,11)]undecane	16.93		235.81	$C_{16}H_{28}O$
5	E,E-6,8-Tridecadien-2-ol, acetate	17.17		238.87	$C_{15}H_{26}O_2$

6	Oxazolo [3,2-E] Xanthine,2,3-dihydro-2-hydroxymethyl-5,7-dimethyl-	17.78		251.99	$C_{10}H_{12}N_4O_4$
7	Quinoxaline,2-isopropyl-3-phenyl-4-oxide	18.82		263.90	$C_{17}H_{16}N_2O$
8	8-Carbethoxy-1-methyl-1,4,5,6,7,8-hexahydropyrrolo [2,3-b]azepin-4-one-3-carboxylic acid	19.67		279.95	$C_{15}H_{21}N_3O_2$
9	9-Octadecynoic acid, methyl ester	20.4		294	$C_{19}H_{34}O_2$
10	Phenol,2,4-bis(1,1-dimethylethyl)-	12.48		205.90	$C_{14}H_{22}O$
11	1-Tricosene	21.43		322	$C_{23}H_{46}$
12	Benzene,(1-methylenebutyl)-	10.85		146.32	$C_{11}H_{14}$



Graph 3: GCMS chromatogram of fresh aqueous extract of *Rosa damascena*

Table 6: Biological activity of fresh aqueous extract of *Rosa damascena*

S.No	Compound Name	Pharmacological activity[29,30]
1	Phenol,2-propyl-	Antifungal, Antimicrobial and Antioxidant
2	Flavone	Relevance of plant defense mode of action is highly possible by flavonoids Formation of oxygen radicals can be prevented by flavonoids thereby inhibiting the enzyme activity
3	Quinoxaline,2-isopropyl-3-phenyl-4-oxide	Antimicrobial activity Antitubercular activity Antiviral activity Antiprotozoan activity Chronic and metabolic disease bioactivity Chronic inflammation Anti glutameric activity

CONCLUSION

Antioxidants are substances that significantly delay or prevent the oxidation of an oxidisable substrate when present in low concentrations. Plants are potential sources of invaluable antioxidants. The results of the present study indicate that fresh aqueous extract of *Rosa damascena* has significant antioxidant and antibacterial activities to reduce harmful effect of radicals and microbial infections. The results of the present study provide promising hope to use *Rosa damascena* as an antioxidant and antibacterial agent. The interest in research concerning the compounds from plants and their biological activity has significantly increased in the last few years as a result of the constantly increasing popularity of phytotherapy. As a consequence, most of the pharmacopoeias throughout the world are revising their monographs on medicinal plants, including monographs for plant extracts. Diets rich in fruits and vegetables are associated with a reduced risk of diseases associated with oxidative stress, such as coronary heart disease, some cancers, and neurodegenerative disease and also to identify those natural components from fruits and vegetables which have been consumed daily that contribute to good health in human system.

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