

In vitro Antioxidant and Free Radical Scavenging Activity of Extracts of *Rosa damascena* Flower Petals.

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ABSTRACT

Introduction: *Rosa damascena* mill L., well-known as Rose, has numerous pharmacological properties including antioxidant, antitussive, relaxant effect on tracheal chains, hypnotic, antibacterial and anti-HIV.

Objective: The objective of the present study was to evaluate antioxidant and free radical scavenging activity of various extracts of Rose flower petals.

Methods: Chloroform, acetone, ethanol, and aqueous extracts of fresh and dried Rose petals were prepared and evaluated for presence of flavonoids and phenolics. Ethanol and acetone extract showed high phenolic and flavonoid content and were selected for various *in vitro* free radical scavenging assays using DPPH scavenging, ABTS, peroxy nitrite radicals, xanthine oxidase inhibition (XOI), superoxide scavenging and Lipid peroxidation assay.

Results: All the extracts of fresh and dried Rose petals were found to contain higher amount of total phenolics expressed as mg of GAE/g of dry extract and total flavonoids expressed as mg of RE/g of dry extract. The amount of both these constituents was highest in ethanol extract followed by acetone extract, aqueous extract and least in chloroform extract. The ethanol extract of fresh and dried petals showed lowest IC₅₀ values than the acetone extract when evaluated by DPPH, ABTS, peroxy nitrite inhibition, xanthine oxidase inhibition, superoxide scavenging and lipid peroxidation inhibition methods indicating good free radical scavenging activity.

Conclusion: The present study has demonstrated that ethanol extract of fresh Rose flower petals exhibit potent antioxidant and free radical scavenging activities.

Keywords: *Rosa damascena*, total phenolics, flavonoids, rutin, DPPH, ABTS.

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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}. Reactive oxygen species (ROS) include free radicals such as $\cdot\text{O}_2^-$ (superoxide anion), $\cdot\text{OH}$ (hydroxyl radical), H_2O_2 (hydrogen peroxide) and $\cdot\text{O}_2$ (singlet oxygen) which cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes^{3,4}. The tissue injury caused by ROS include 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 stress⁵.

Rosa genus, belonging to the Rosaceae family, includes 200 species and more than 18,000 cultivars. One of the most important *Rosa* species is *Rosa damascena* Mill. This plant is called damask rose because it was originally brought to Europe from Damascus⁶. 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. Vapour

therapy of rose oil is helpful for several allergies, migraine and headaches. Numerous components were isolated from petals, flowers and hips (seed-pot) of *R. damascena* including flavonoids, anthocyanins, glycosides, and terpenes. Plant shows presence of kaempferol, carboxylic acid, quercetin, myrcene, and vitamin C. Flowers show presence of fatty oil, bitter principle, organic acids and tanning matter⁷. The essential oil of Rose contains more than 95% of total oil represented by eighteen compounds. β -citronellol (14.5-47.5%), geraniol (5.5-18%), nerol, kaempferol and nonadecane (10.5-40.5%) are the identified oils. In addition, citrenellol (9.91%), phenyl ethyl alcohol (78.38%), geraniol and nonadecane (4.35%) are also found to be present in Rose oil⁸.

MATERIALS AND METHODS

Procurement of plant material

Fresh petals of Rose flower were procured from local market at Dadar, Mumbai, India and were authenticated at Agarkar Research Institute, Pune, India.

Chemicals and instruments

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Gallic acid, 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS), Allopurinol, Bovine Brain Extract (BBE), Bovine Serum Albumin (BSA), were procured from Sigma Chemical Co. (USA). Xanthine oxidase, Nitroblue tetrazolium chloride (NBT) and xanthine were procured from Himedia Ltd. Mumbai, India. Folin-Ciocalteu solution, Pyrogallol Red, potassium dihydrogen phosphate and thiobarbituric acid, dipotassium hydrogen phosphate, trichloroacetic acid, anhydrous

sodium carbonate, ascorbic acid, butylated hydroxytoluene (BHT) potassium persulfate, ferrous ascorbate, ethylene diamine tetra acetic acid (EDTA), were purchased from S.D.Fine Chemicals, Mumbai, India. All other chemicals and solvents used were of analytical grade.

The instruments used for the study were UV spectrophotometer, (Jasco, V-630), laboratory centrifuge (Remi motors, R4C) and digital pH meter (Equip-tronics, EQ-610).

Preparation of extracts

Fresh petals of Rose were immediately used for extraction. Some of the fresh petals were dried, stored in air-tight container and then used for extraction. The extraction of plant material was carried out by Soxhlet extraction method using various solvents such as chloroform, acetone, methanol and water. All extracts were filtered individually and further evaporated to get dry extracts. The extractive values were determined. After drying, crude extracts were stored in desiccator till further use.

Phytochemicals screening

Extracts were qualitatively evaluated for the presence or absence of active principles such as phytosterols, tannins, flavonoids, saponins, alkaloids, glycoside, triterpenoids and proteins.

Quantification of total phenolics – by Folin-Ciocalteu method

The total phenolic content in various extracts of the plants were measured using Folin-Ciocalteu reagent⁹. 1 ml of extract solution was added to 0.5 ml of Folin Ciocalteu reagent and 5 ml of distilled water. The mixture was incubated at room temperature for 10 min. Then 1.5 ml of anhydrous sodium carbonate solution (10% w/v) was added and the final volume was

made upto 10 ml. The final mixture was allowed to stand at room temperature for 2 h with intermittent shaking. Then the absorbance of the dark blue colour that developed was measured at 725 nm using UV-Vis spectrophotometer. Gallic acid was used as standard for preparing the calibration curve (10 µg/ml - 100 µg/ml). The concentration of total phenolic compounds in the extracts was calculated using the following linear equation based on the calibration curve: $y = 0.010x + 0.01$ with $R^2 = 0.994$. The total phenolic content in the plant extract was expressed as mg of gallic acid equivalent per g of dry weight (mg GAE/g) of extract. All the tests were carried out in triplicate and the results are expressed as mean \pm SD.

Quantification of total flavonoids

The total flavonoid content of all the extracts was determined by aluminium chloride colorimetric method⁹. 0.5 ml of sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% NaNO₂ solution. After 6 min of incubation, 0.15 ml of 10% AlCl₃ solution was added and then allowed to stand for 6 min, followed by adding 2 ml of 4% NaOH solution to the mixture. Immediately water was added to the sample to make the final volume to 5 ml, the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance was determined at wavelength 510 nm. The total flavonoid content was expressed in mg of rutin equivalent per g of extract (mg RE/g) of extract.

In vitro antioxidant studies

The ethanol and acetone extracts of fresh and dried Rose flower petals with high phenolic and flavonoid content were further evaluated for their antioxidant and radical scavenging potential

DPPH (1, 1, diphenyl 2-picryl hydrazyl) assay

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined^{10,11}. A 0.1 mM solution of DPPH in methanol was prepared. 1 ml of the extract solution in various concentration ranges was added to 3 ml of the DPPH solution. The decrease in absorbance was determined at 517 nm after 30 min. The percentage scavenging activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. A blank is the absorbance of the control reaction (containing all reagents except the test compound). The % scavenging activity and IC_{50} value of extracts were calculated for the various concentrations. Ascorbic acid was used as standard antioxidant for comparison. All the tests were carried out in triplicate and the results were expressed as mean \pm SD.

Inhibition of pyrogallol red bleach by peroxyntirite

Peroxyntirite (ONOO-) was prepared by reacting 2 M H_2O_2 in 2 M HNO_3 with 2 M $NaNO_2$, followed by stabilization of the product with 4 M NaOH. The solution was frozen at $-70^\circ C$. Peroxyntirite concentration was determined spectrophotometrically at 302 nm ($\epsilon=1670 M^{-1} cm^{-1}$) and dilutions in 1M NaOH were made in order to achieve 200 μM solution. All the extract solutions were prepared by dissolving in methanol. 100 μM Pyrogallol red solution was prepared in 100 mM phosphate buffer, pH 7.4. 1 ml of extract solution was added to 2 ml of 100 μM pyrogallol red solution. 0.5 ml of 200 μM peroxyntirite solution was added to the mixture and immediately vortexed. After 15 min the absorbance was measured using UV-vis spectrophotometer at 540 nm.

The % inhibition of pyrogallol red bleaching was determined using the formula $[(A_1 - A_2)/A_1] \times 100$, where A_1 is the absorbance in presence of antioxidants and A_2 is the absorbance in absence of antioxidants. The IC_{50} values of extracts yielding 50% inhibition of pyrogallol red bleaching were estimated. Ascorbic acid was used as standard antioxidant for comparison. All the tests were carried out in triplicate and the results were expressed as mean \pm SD¹²⁻¹⁴.

ABTS assay

The antioxidant activities of all the extracts in the reaction with the stable $ABTS^{\bullet+}$ radical cation were determined^{15,16,17}. The reaction between ABTS and potassium persulfate generates the blue/ green $ABTS^{\bullet+}$ chromophore, which can be reduced by an antioxidant, thereby resulting in decrease of absorbance at 734 nm. ABTS was dissolved in water to a 7 mM concentration. $ABTS^{\bullet+}$ radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12–16 hr. The $ABTS^{\bullet+}$ solution was diluted with a phosphate buffer (2 mM, PH 7.4) to achieve an absorbance of 0.8 ± 0.014 at 734 nm. Extract solutions were mixed with $ABTS^{\bullet+}$ solution, and the absorbance was read after 1 min using UV-vis spectrophotometer at 734 nm. Phosphate buffer solution was used as a blank. The % radical-scavenging activity of the samples was determined using the formula $[(A_{control} - A_{test})/ A_{control}] \times 100$, where $A_{control}$ is the absorbance of the control ($ABTS^{\bullet+}$ solution without test sample) and A_{test} is the absorbance of the test sample ($ABTS^{\bullet+}$ solution with extract). The IC_{50} values scavenging 50% of $ABTS^{\bullet+}$ were estimated. Ascorbic acid and trolox were used as standard antioxidants for comparison. All the tests were carried out in

triplicate and the results were expressed as mean \pm SD.

Xanthine oxidase assay

The inhibitory effect on xanthine oxidase was measured spectrophotometrically at 295 nm^{18,19}. The reaction mixture consisted of 50 mM sodium phosphate buffer (pH 7.5), sample solution dissolved in distilled water or DMSO, freshly prepared enzyme solution (0.2 U/ml of xanthine oxidase in phosphate buffer) and distilled water. The assay mixture was pre-incubated at 37°C for 15 min. Then substrate solution (0.15 mM of xanthine) was added into the mixture. The mixture was incubated at 37°C for 30 min. Next, the reaction was stopped with the addition of 0.5 M HCl. The absorbance was measured using UV-VIS spectrophotometer against a blank prepared in the same way but the enzyme solution was replaced with the phosphate buffer. Another reaction mixture was prepared (control) having water/DMSO instead of test compounds in order to have maximum uric acid formation. Thus, XO activity was calculated using the following equation in which α is the activity of XO without test extract and β is the activity of XO with test extract. % XO inhibition = $(1 - \beta/\alpha) \times 100$

Superoxide scavenging assay

Superoxide was generated by xanthine oxidase system²⁰⁻²². The reaction mixture consisted of sodium phosphate buffer (pH 7.4), 3 mM Xanthine, 3 mM EDTA, 0.15%BSA, 15 mM Nitroblue tetrazolium chloride, sample solution The reaction mixture was incubated at 25°C for 10 min. Then reaction was initiated by adding 1.5 U/ml of xanthine oxidase and incubated at 25°C for 20 min. After 20 min the absorbance was measured at 560 nm using UV-Vis spectrophotometer. The inhibition rate was calculated by measuring

the amount of the formazan which was reduced from NBT by superoxide using the following equation where, A1- Absorbance of control and A2- Absorbance in presence of test compounds: % Inhibition= $[A1 - A2/A1 \times 100]$

Ascorbate iron induced lipid peroxidation

10 mg of bovine brain extract was mixed with phosphate buffer pH 7.4 and sonicated in an ice bath until a milk-like suspension was obtained. The lipid suspension was mixed with 1 mM FeCl₃ and extract. The peroxidation was initiated by adding 1 mM ascorbate. The mixture was incubated at 37°C for 60 min. After incubation, 10% trichloroacetic acid was added and centrifuged at 1800 rpm for 10 minutes. After centrifugation, 1ml of supernatant was collected and mixed with 1ml of 0.67% thiobarbituric acid (TBA). The mixture was vortexed and heated in boiling water bath at 100°C for 20 min and then rapidly cooled and the extent of inhibition of peroxidation was estimated from the absorbance of the upper organic layer at 532nm. A tube containing all the reaction mixture except the plant extract was used as control. Blank was phosphate buffer. The percent inhibition was calculated with the formula Percent inhibition (%) = $(\text{Abs of control} - \text{Abs of sample}) \times 100 / (\text{Abs of control})$ ²³.

RESULTS

Extractive values

The extractive values of fresh rose petals were found to be higher as compared to dried rose petals. The extractive values for fresh and dried petals of Rose flower were highest for aqueous extract (32.68, 27.90% w/w respectively) followed by ethanol extract (19.21, 15.62 % w/w respectively), acetone extract (12.94, 8.65% w/w respectively) and chloroform extract (4.56, 1.10 % w/w respectively).

Quantitation of total phenolics and total flavonoids

Ethanol extract and acetone extract of the fresh petals of Rose were found to contain higher amount of total phenolics (147.1, 129.73 mg GAE/g of extract) and total flavonoids (161.36, 141.5 mg RE/g of extract) as compared to other extracts (Figure.1). Ethanol and acetone extract of dried Rose petals were found to contain high amount of total phenolics (77.37, 66.58 mg GAE/g of extract) and total flavonoids (116.92, 114.97 mg RE/g of extract) as compared to other extracts. Therefore, these extracts were selected for various *in-vitro* antioxidant assay methods.

In vitro antioxidant assays

DPPH scavenging

Both ethanol and acetone extracts of fresh and dried rose petals were effective in reducing the stable radical DPPH to the yellow colored diphenylpicrylhydrazine, indicating that these extracts were able to scavenge DPPH radical (Table.1 and Table.2). Standard ascorbic acid exhibited maximum free radical scavenging activity with $IC_{50} = 13.24 \pm 0.48 \mu\text{g/ml}$. Ethanol extract of Rose fresh petals ($IC_{50} = 19.72 \pm 0.20 \mu\text{g/ml}$) demonstrated a stronger DPPH radical scavenging activity than acetone extract ($IC_{50} = 25.75 \pm 1.49 \mu\text{g/ml}$). Dried petals ethanol extract ($IC_{50} = 21.97 \pm 0.05 \mu\text{g/ml}$) showed good DPPH scavenging as compared to acetone extract ($IC_{50} = 29.97 \pm 1.66 \mu\text{g/ml}$).

Peroxynitrite inhibition

Pyrogallol Red is a dye which gets bleached by peroxynitrite radical. All the rose petal extracts moderately inhibited bleaching of peroxynitrite as compared to standard ascorbic acid (Table.1 & Table.2). Ethanol and acetone extracts of fresh Rose petal, ethanol and acetone extract of dried

Rose petal extracts exhibited the activity at higher IC_{50} values from 92.02 ± 0.86 , 94.09 ± 2.41 , 96.14 ± 0.73 and $104.81 \pm 3.40 \mu\text{g/ml}$ respectively as compared to standard ascorbic acid ($IC_{50} = 49.41 \pm 0.27 \mu\text{g/ml}$)

ABTS radical scavenging assay

Ethanol extracts and acetone extracts of fresh and dried Rose petals exhibited ABTS scavenging activity at higher IC_{50} values ranging from 325.36 ± 4.53 , 416.75 ± 2.78 , 554.77 ± 4.79 and $631.00 \pm 9.36 \mu\text{g/ml}$ respectively as compared to standard ascorbic acid $19.03 \pm 0.08 \mu\text{g/ml}$ and trolox $9.34 \pm 0.08 \mu\text{g/ml}$ (Table.1 and Table.2).

Xanthine oxidase inhibition assay

Allopurinol is a potent inhibitor of xanthine oxidase. It inhibited the enzyme at IC_{50} value of 3.38 ± 0.16 . Ethanol and acetone extracts of fresh Rose petals inhibited the enzyme at higher IC_{50} values of 91.59 ± 1.22 to $115.85 \pm 1.46 \mu\text{g/ml}$ respectively. Also, ethanol and acetone extracts of dried Rose petals inhibited the enzyme at still higher IC_{50} values of 129.32 ± 0.80 to $157.92 \pm 0.80 \mu\text{g/ml}$ respectively (Table.1 and Table.2).

Superoxide scavenging assay

Superoxide radical scavenging activity was shown by both ethanol and acetone extract of fresh and dried Rose petals. Allopurinol is a potent scavenger of superoxide anions with IC_{50} value of $6.00 \pm 0.23 \mu\text{g/ml}$. The superoxide scavenging effect for fresh rose petals was found to be greater for ethanol extract ($IC_{50} 696.59 \pm 1.44 \mu\text{g/ml}$) as compared to acetone extract ($IC_{50} 716.46 \pm 3.82 \mu\text{g/ml}$). Also the scavenging effect for dried rose petals was found to be greater for ethanol extract ($IC_{50} 823.85 \pm 7.64 \mu\text{g/ml}$) followed by acetone extract ($IC_{50} 888.32 \pm 2.56 \mu\text{g/ml}$) (Table.1 and Table.2).

Lipid peroxidation

Rose fresh petals ethanol and acetone extracts inhibited lipid peroxidation at higher IC_{50} values ranging from 110.42 ± 8.05 to 142.32 ± 2.75 $\mu\text{g/ml}$ as compared to butylated hydroxyl toluene 33.11 ± 1.44 $\mu\text{g/ml}$. Also, Rose dried petals ethanol and acetone extracts inhibited lipid peroxidation at still higher IC_{50} values ranging from 134.42 ± 4.00 to 167.94 ± 4.01 $\mu\text{g/ml}$ (Table.1 and Table.2).

DISCUSSION

Plant cells contain a wide variety of phytoconstituents with antioxidant activity. The healing properties of medicinal plants are mainly due to the presence of various secondary metabolites such as phenols, flavonoids, glycosides, saponins, sterols, alkaloids, etc. Polyphenolic compounds such as flavonoids and tannins, nitrogen compounds such as alkaloids, amino acids and amines), lignans, carotenoids, terpenes possess antioxidative activity by suppressing the initiation or propagation of the chain reactions. The preliminary phytochemical tests revealed the presence of carbohydrates, flavonoids, phenols, carbohydrates, saponins, sterols and tannins in both the Rose fresh petals and dried petals extracts. The preliminary screening tests are thus useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

In order to evaluate antioxidant potential of a plant, it is desirable to subject it to a series of tests that evaluate the range of activities such as scavenging of the reactive oxygen species, inhibition of membrane lipid peroxidase and enzyme inhibition. Plant extracts rich in antioxidants serve as source of nutraceuticals that alleviate the oxidative stress and therefore prevent or slow down the degenerative diseases²⁴.

This study reports the various *in vitro* antioxidant and free radical scavenging activities of the extracts of Rose petals which may be associated with its health benefits. Rose petal extracts showed antioxidant activities, however, the magnitude of antioxidant potency varied with the type of extracts. This could be due to the difference in concentrations and type of antioxidant compounds present in these extracts. The broad range of antioxidant activity of the extracts indicates the potential of the Rose flower petals as a source of natural antioxidants. It can have potential application to reduce oxidative stress with consequent health benefits.

DPPH solution is decolourised from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. IC_{50} (concentration required to obtain a 50% antioxidant capacity or is the concentration of substrate that brings about 50% loss of the DPPH) is typically employed to express the antioxidant activity and to compare the antioxidant capacity of various samples²⁵. Both the ethanol and acetone extract of fresh and dried Rose petals exhibited good activity comparable to standard ascorbic acid.

Pyrogallol red is a dye. Peroxynitrite radical bleaches this dye and the intensity of dark red colour decreases. Antioxidants scavenge the peroxynitrite radical thereby preventing the bleaching of pyrogallol red and retaining its colour intensity. In this assay, the increase in absorbance is proportional to inhibition of pyrogallol red bleach by peroxynitrite²⁶. Extracts of fresh as well as dried Rose petals were found to have appreciable peroxynitrite scavenging activity.

$ABTS^{\cdot+}$ radical is a chromophore having blue colour. The selected extracts were able to scavenge the radical cation

effectively at higher IC_{50} values as compared to standards.

Xanthine oxidase is a flavoprotein, which catalyses the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid. Studies have shown that xanthine oxidase inhibitors may be useful for the treatment of hepatic disease and gout, which is caused by the generation of uric acid and superoxide anion radical. Xanthine oxidase-derived superoxide anion has been linked to various degenerative and metabolic disorders. Superoxide radicals are generated during the normal physiological process mainly in mitochondria. Although superoxide anion is by itself a weak oxidant, it gives rise to the powerful and dangerous hydroxyl radicals as well as singlet oxygen both of which contribute to the oxidative stress. Therefore superoxide radical scavenging by antioxidants has physiological implications. Allopurinol is a potent inhibitor of xanthine oxidase. However chronic treatment with allopurinol has its own side effects. Hence, natural antioxidants which inhibit this enzyme and also have superoxide scavenging activity would be beneficial in preventing various complications of oxidative stress related disorders. Rose flower petals were found to have moderate xanthine oxidase inhibitory activity as well as superoxide scavenging activity though at higher IC_{50} values as compared to allopurinol. But the safety of this drug along with its antioxidant potential will be a better therapeutic approach^{27,28}.

Lipid peroxidation in biological systems has long been thought to be a toxicological phenomenon that can lead to various pathological consequences. Fe^{2+} -ascorbate is well known to stimulate lipid peroxidation *in vivo* as well as *in vitro*. Malondialdehyde is very reactive and takes part in cross-linking with DNA and proteins, and also damages liver cells. Rose flower

petals extracts exhibited a better antioxidant action against peroxidation of Bovine brain extract, suggesting better cell membrane protection against lipid peroxidation. An increase in oxidative stress is widely accepted as one of the main factors involving in the development of peroxyl radicals, all of which may play a role in DNA damage, glycation, and protein modification reactions, and in lipid oxidative modification in diabetes.

CONCLUSIONS

In conclusion, the present study has demonstrated that ethanol extract of fresh Rose flower petals exhibit potent antioxidant and free radical scavenging activities. The activity could be derived from compounds such as flavonoids and phenolics which are polar phytoconstituents. These phytoconstituents are well known antioxidants and free radical scavengers. These antioxidant activities can be at least partly linked to the therapeutic benefits of the certain traditional claims of Rose flower petals.

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Conflict of interest

The authors hereby declare no conflict of interest for the research work.

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Table-1: IC₅₀ values of Rose fresh petal extracts for various free radical scavenging activity

| Extract | IC ₅₀ values of extracts (µg/ml) Rose fresh petals | | | | | |
|---------------|---|---------------|-------------|------------------|-----------------------|--------------------|
| | DPPH | Peroxynitrite | ABTS | Xanthine oxidase | Superoxide Scavenging | Lipid Peroxidation |
| Ethanol | 19.72±0.20 | 92.02± 0.86 | 325.56±4.53 | 91.59±1.22 | 696.59±1.44 | 110.42±8.05 |
| Acetone | 25.75±1.49 | 94.09±2.41 | 416.75±2.78 | 115.85±1.46 | 716.46±3.82 | 142.32±2.75 |
| Ascorbic Acid | 13.24±0.48 | 49.41±0.27 | 19.03±0.08 | - | - | - |
| Trolox | - | - | 9.34±0.08 | - | - | - |
| Allopurinol | - | - | - | 3.38 ± 0.16 | 6.00±0.23 | - |
| BHT | - | - | - | - | - | 33.11±1.44 |

Table- 2: IC₅₀ values of Rose dried petal extracts for various free radical scavenging activity

| Extract | IC ₅₀ values of extracts (µg/ml) Rose dried petals | | | | | |
|---------------|---|---------------|-------------|------------------|-----------------------|--------------------|
| | DPPH | Peroxynitrite | ABTS | Xanthine oxidase | Superoxide Scavenging | Lipid Peroxidation |
| Ethanol | 21.97±0.05 | 96.14±0.73 | 554.77±4.79 | 129.32±0.80 | 823.85±7.64 | 134.42±4.00 |
| Acetone | 29.97±1.66 | 104.81±3.40 | 631.00±9.36 | 157.92±1.30 | 888.32±2.56 | 167.94±4.01 |
| Ascorbic Acid | 13.24±0.48 | 49.41±0.27 | 19.03±0.08 | - | - | - |
| Trolox | - | - | 9.34±0.08 | - | - | - |
| Allopurinol | - | - | - | 3.38 ± 0.16 | 6.00±0.23 | - |
| BHT | - | - | - | - | - | 33.11±1.44 |

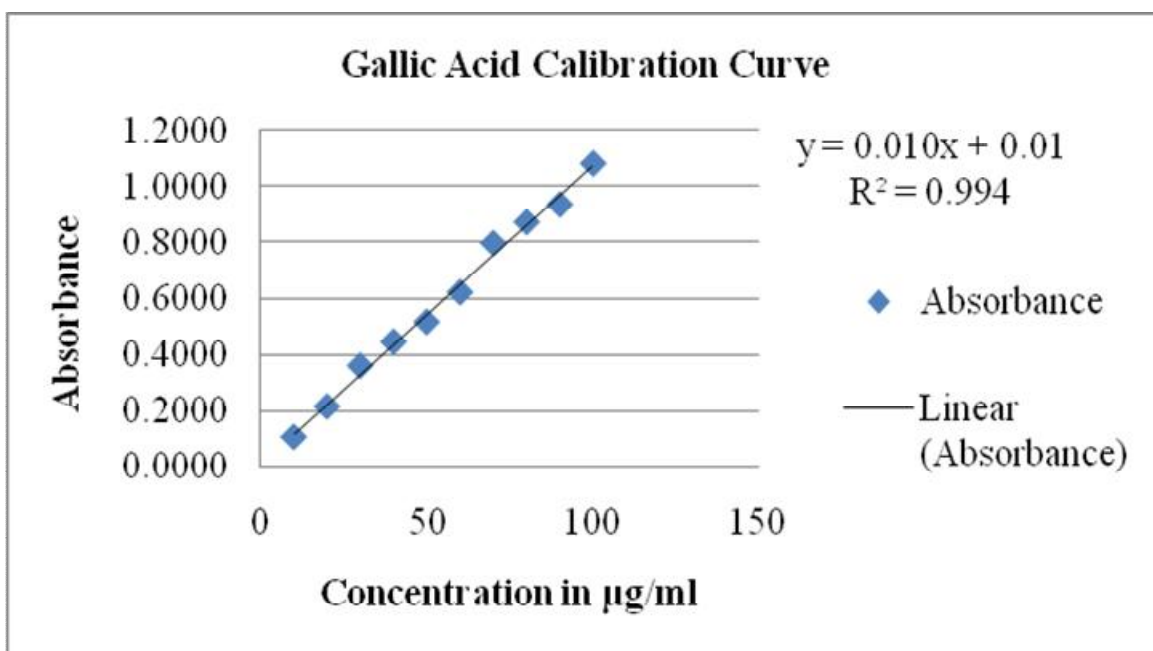


Fig. 1: Calibration Curve for Standard Gallic Acid

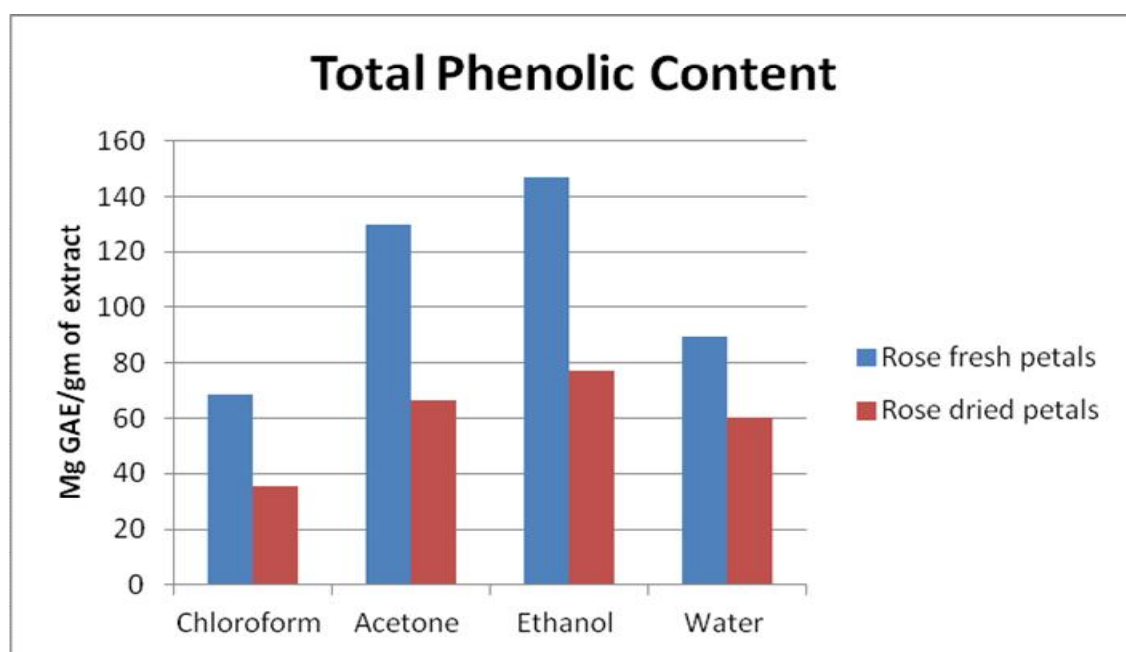


Fig. 2: Gallic Acid Equivalent of Rose fresh petals and dried petals

Values are presented as mean \pm SD ($n = 3$).

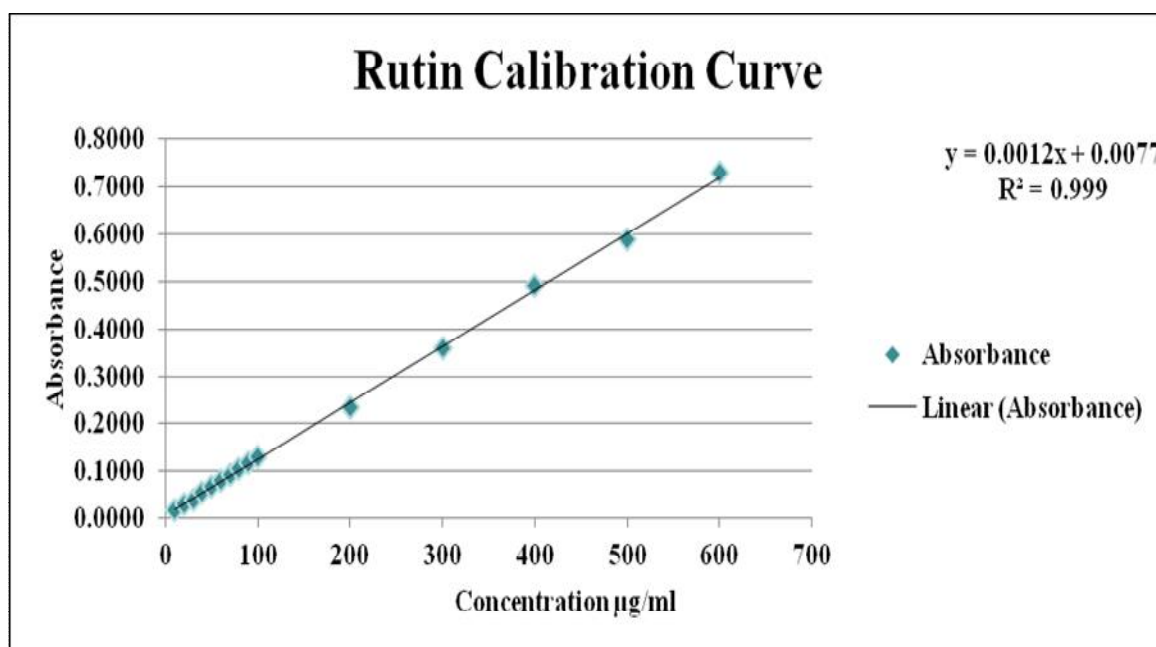


Fig. 3: Calibration Curve for Standard Rutin

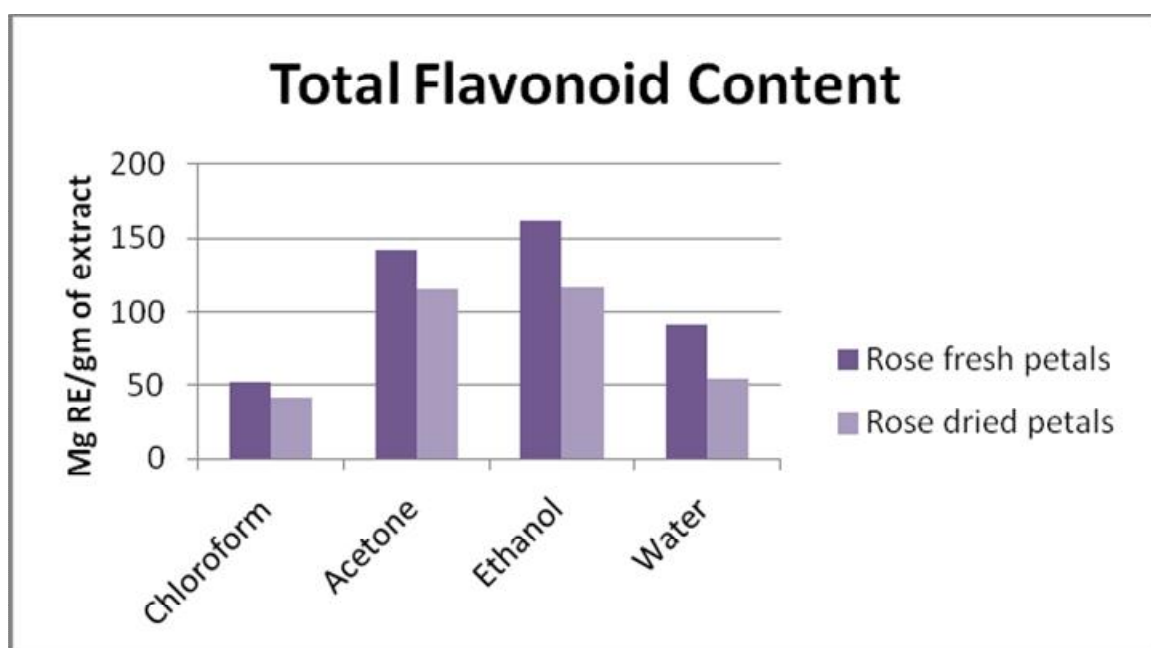


Fig. 4: Rutin Equivalent of Rose fresh petals and dried petals

Values are presented as mean \pm SD ($n = 3$).