Academic

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

RADICAL SCAVENGING ACTIVITIES AND NATURAL INDICATOR ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *Rosa Damascena*

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Received: 12 Oct, 2012, Revised and Accepted: 13 Nov, 2012

ABSTRACT

Rosa damascena mill L. commonly known as rose having several pharmacological properties including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, anti-diabetic and relaxant effect on tracheal chains have been reported for this plant. The present works were carried out to evaluated antioxidant activity of Aqueous and ethanolic extract of rose flower petals. The antioxidant activities were measured using free radical scavenging activity with DPPH and reducing power assay. Being both dietary and biologically active compounds, flavonoids have attracted much attention of investigators as potent species capable of affecting various biological processes in living organisms. They are colour impart polyphenol. The total flavonoids contents of aqueous extract & ethanolic extracts of *R.damascena* flower petals were found to be12.73% and 32 % respectively. The HPLC analysis of ethanolic extract revealed presence of quarcetin having RT 2.33 min. Further, an attempt had been made to evaluated the used of above extract as herbal acid-base indicator in acid base titration. Natural pigments in plants are highly colour substances and may show colours changes with variation of pH. This attempt had been made to investigate the indicator activity of these extracts and to replace the synthetic indicators.

Key words: *R.damascena,* DPPH, Reducing power, Indicator, HPLC.

INTRODUCTION

Rosa damascena mill L commonly known as Damask rose[1]. Rosaceae are well known ornamental plants and have been referred to as the king of flowers [2-3]. At present time, over 200 rose species and more than 18000 cultivars form of the plant have been identified[4]. Apart from the use of *R. damascena* as ornamental plants in parks, gardens, and houses, they are principally cultivated for using in perfume, medicine and food industry[5]. However *R. damascena* is mainly known for its perfuming effects[6]. The most therapeutic effects of *R. damascene* in ancient medicine are including treatment of abdominal and chest pain, strengthening the heart[7], treatment of menstrual bleeding and digestive problems[8], and reduction of inflammation, especially of the neck[9]. North American Indian tribes used a decoction of the root of *R. damascena* plant as a cough remedy to ease children's cough[10]. This plant is also used as a gentle laxative[11]. Rose oil heals depression, grief, nervous stress and tension. It helps in the reduction of thirst, healing old cough, special complaints of women, wound healing, and skin health.Vapour therapy of rose oil is helpful for some allergies, headaches, and migraine[12]. Several components were isolated from flowers, petals and hips (seed-pot) of *R. damascena* including terpenes, glycosides, flavonoids, and anthocyanins. This plant contains carboxylic acid, myrcene, vitamin C, kaempferol and quarcetin. Flowers also contain a bitter principle, tanning matter, fatty oil and organic acids. The essential oil of *R. damascena*, contains eighteen compounds represented more than 95% of the total oil. The identified compounds were; β-citronellol (14.5-47.5%), nonadecane (10.5-40.5%), geraniol (5.5-18%), and nerol and kaempferol were the major components of the oil. Analyses of rose absolute showed that phenyl ethylalcohol (78.38%), citrenellol (9.91%), nonadecane (4.35%) and geraniol[13].

Reactive oxygen species (ROS) are produced as a natural by product in biological process in body by normal oxygen metabolism. However, during time of stress ROS levels can increase dramatically, which can result in cell damage. This cumulates into a situation known as oxidative stress. ROS stimulate the pathogenesis of many diseases like atherosclerosis, ischemic heart disease, aging, inflammation, diabetes and immunosuppression etc.[14] Antioxidant compounds in food play an important role as a health protecting factor. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro- peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[15].Natural products are becoming the cynosure to inhibit and scavenge these reactive oxygen species[16]. The antioxidant activities of aqueous and ethanolic extract of petals were measured using free radical scavenging activity with DPPH and reducing power assay. Titrate and Titrant with indicator shows sharp and intense colour changes at the equivalence point that is at neutralization[17].An attempt had been made to evaluated the used of above extract as herbal acid - base indicator in acid base titration.

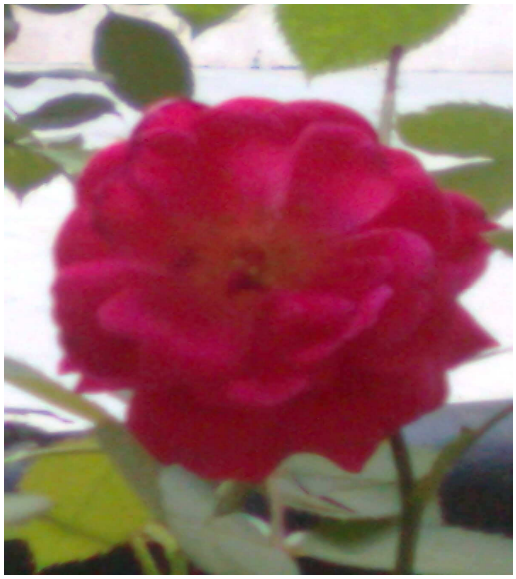


Fig. 1: Flower of *R. damascena*

MATERIAL AND METHOD

Plant material collection

The Flowers were collected from botanical garden L.N.C.P.Bhopal (M.P.) and authenticated.

Preparation of Extract

About 80gm of fresh flower petals were successively extracted with water and ethanol by soxhelation.

Phytochemicals Screening

Extracts were tested for the presence of active principles such as phytosterols, tannins, flavonoids, saponins, alkaloids, glycoside, triterpenoids and proteins. Following standard procedures were used[18].

Total Flavonoids contents

The content of total flavonoids was determined by aluminum chloride colorimetric method. The content of flavonoids was determined as quercetin equivalent. 10 mg/ml of plant extract in respective solvent (stock solution SS) was mixed with 2 ml $AlCl_3$ (2% w/v) in methanol and the solution was made up to 25ml with methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). 1ml of SS was made up to 25ml with methanolic solution of acetic acid (contrast solution CS).The absorbance of PS and CS was measured at 420nm after 30 minutes. The result expressed as % of total Flavonoids content[19].

%TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\ cm}$ x wt. of extract in gms

Antioxidant Activity

DPPH Radical Scavenging Activity: Ascorbic acid and dried extracts were weighed (25 mg each) and dissolved in 250 ml of methanol to get 500 µg/ml stock solutions separately. Lower concentrations of ascorbic acid and extracts (2, 4, 6, 8, 10 µg/ml and 2, 4, 6, 8, 10 µg/ml respectively) were prepared by serially diluting stock solutions. The stable DPPH radical was used for determination of free radical-scavenging activity of the extracts. The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of extracts were added at an equal volume (2ml) to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Radical scavenging activity was calculated by the following formula[20].

% Radical Scavenging Activity = $A_{control}$- A_{sample} /$A_{control}$ * 100

Where, $A_{control}$ = Absorbance of control

A_{sample} = Absorbance of sample

The inhibition curve was plotted for duplicate experiments and represented as % of mean inhibition.

Ferric Reducing Antioxidant Power (FRAP) Assay

5mg of each extracts were dissolved in 50 ml of methanol to get 100 µg/ml stock solutions separately. Lower concentrations of extracts (20, 40, 60, 80, 100 µg/ml) were prepared by serially diluting stock solution. Ascorbic acid was weighed (5 mg) and dissolved in 10 ml of methanol to get 500 µg/ml stock solutions Lower concentrations of ascorbic acid (20, 40, 60, 80, 100 µg/ml) were prepared by serially diluting stock solution.

Various concentrations of sample and standard solutions (1ml each), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were mixed separately and allowed to incubate at 50 º C for 30 min and 2.5 ml of 10% TCA was added to the mixtures and centrifuged for 10 min at 3000 rpm. About 2.5 ml of the supernatant was diluted with 2.5 ml water and is shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm[21].

HPLC Analysis

The HPLC analysis was performed using a LC-100, Cyberlab™, Salo Torrace, Millburry, MAO 1527, USA with LC-UV-100 UV detector. A CAPCELL (C-18) HPLC-packed column (4.6 mm I.D.X 250 mm), type MG 5 µm, number AKAD/05245 was used for the chromatographic separations. The mobile phase consisted of methanol: Acetonitrile:water (40:15:45) with 1% of acetic acid. The flow rate was 0.5 mL/min, and a column temperature of 25°C. The injection volume was 25µl, and UV detection was effected at 368 nm.

Indicator Activity of Aqueous and ethanolic extract of *R. damascene*

The intention behind this study is simply to bring in market the use of plant pigments and to increase the wealth of traditional medicinal system of India which is mostly plant based and to help farmers regarding cultivation, collection of plants as well as to industry regarding preparation of above indicators[22]. The experiment was carried out by using a same set of glass wares for all types of titrations. The reagents were not calibrated: as same aliquots were used for both titrations i.e. titration by using standard indicator and herbal extracts.10 ml of titrant and 1 drops of indicator was titrated. All the parameters for the experiment are given in Table 6. Each titration was carried five times and results were recorded. Mean and standard deviations were calculated from the results. The aqueous and ethanolic extracts were screened for its use as indicator for Acid-Base titration and the results of this screening were compared with the results obtained by using standard indicators.

RESULT AND DISCUSSION

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols *etc*. The macroscopic characteristics of *R.damascena* flower was tabulated in table 1.The successive extraction flower petals of *R.damascena* were carried out in ethanol & water. The preliminary phytochemical tests revealed the presences of alkaloids, flavonoids, phenols, carbohydrate, saponins, sterols and tannins in both the extracts. (Table 2).Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. The total flavonoids contents of aqueous extract & ethanolic extracts of *R.damascena* flower petals were found to be 12.73% and 32 % respectively (Table 3).Antioxidant activity of both the extracts were carried out by DPPH & reducing power assay by using ascorbic acid as standard. DPPH is a purple-coloured stable radical of organic nitrogen with a maximum absorbance at 517 nm and it is widely used to study radical scavenging activities of extracts and pure compounds. When the odd electron becomes paired off in the presence of a free radical scavenger to form hydrazine, the absorption reduces and the 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 samples[23]. In the present study, the IC_{50} values of ethanolic and aqueous extract were found to be 18.46 and 22.1µg/ml. The results were tabulated in table 4 and the %inhibition vs concentration graph were showed in fig.2, 3 &4. The result of Ferric reducing power assay was showed in fig.5,6 & 7.The absorbance of the sample and standard were tabulated in table 5. HPLC analysis of ethanolic extract revealed presence of quarcetin having RT 2.33 min (fig.8). The aqueous & ethanolic extracts of flower petals of *R.damascena* were screened for its use as an indicator in acid base titration and the results were compared with the results obtained by standard indicators methyl red & phenolphthalein. The results of the screening for strong acid- strong base (HCl vs NaOH & H_2SO_4 vs NaOH) were listed in Table 6 & 7.Colour changes at end point were observed after titration showed in fig.9.The screening was carried out using one molar strength of acids and alkalis (1M). The table represents mean of five titrations ± standard deviation (n = 5). For both titrations equivalence point obtained by the flower petals extracts were much closed with the equivalence point obtained by the standard indicators. This represent the usefulness of flower petals extract as an indicator in acid base titration.

Table 1: Morphological characteristics of flower of *R.damascena*

Features	Morphological characteristics
Colour	Red
Odur	Aromatic
Taste	Aromatic &characteristic
Extra of flower	Perfect,regular(actinomorphoric)(4)5 sepal.(4)5petals.stamens 1-10 to many,often in multiple of 5 around centraaal cup,ovary inferior to sub-inferior.3 to many carples, 1 to many pistils.

Table 2: Phytochemical Analysis of Aqueous & ethanolic extract of *R.damascena*

Test	Aqueous	Ethanolic
Carbohydrate		
Molish	(+)ve	(-)ve
Benedict	(+)ve	(-)ve
Starch	(+)ve	(-)ve
Hexose sugar	(-)ve	(-)ve
Tannin		
$FeCl_3$	(+)ve	(+)ve
Pot.ferric cyan- ate	(+)ve	(+)ve
Protein		
Biuret	(+)ve	(+)ve
Xanthoprotein	(+)ve	(+)ve
Amino acid		
Ninhydrin	(+)ve	(+)ve
Alkaloids		
Dragnodroff	(+)ve	(+)ve
Mayer	(+)ve	(+)ve
Steroid		
Salkowski	(+)ve	(+)ve
Libermann – Bucher	(+)ve	(+)ve
Flavonoids		
Shinoda	(+)ve	(+)ve
NaOH	(+)ve	(+)ve
Lead acetate	(+)ve	(+)ve
Coumarin	(-)ve	(-)ve
Glycosides		
Baljet	(+)ve	(+)ve
Legal	(+)ve	(+)ve
Killer-Killani	(+)ve	(+)ve
Modified Borntrager test	(+)ve	**(-)**

(+)ve = Present (-)ve Absent

Table 3: Total Flavonoids contents of Aqueous & ethanolic extract of *R.damascena*

S. No.	% Total flavonoids content	
	Aqueous extracts	Ethanolic extracts
1.	12.73	32

Table 4: 50% inhibition (IC_{50}) of Aqueous & ethanolic extract of *R.damascena* by DPPH method

S.No	Sample	IC_{50} (µg/ml)
1.	Ascorbic acid	3.17
2.	Aqeous extract	22.1
3.	Ethanolic extract	18.46

Table 5: Total reducing power of Aqueous & ethanolic extract of *R.damascena*

Concentration(µg/ml)	Absorbance		
	Ascorbic acid(standard)	Aqeous extract	Ethanolic extract
2	0.130	0.603	0.385
4	0.221	0.649	0.613
6	0.302	0.656	0.818
8	0.745	0.672	1.210
10	0.850	0.842	1.281

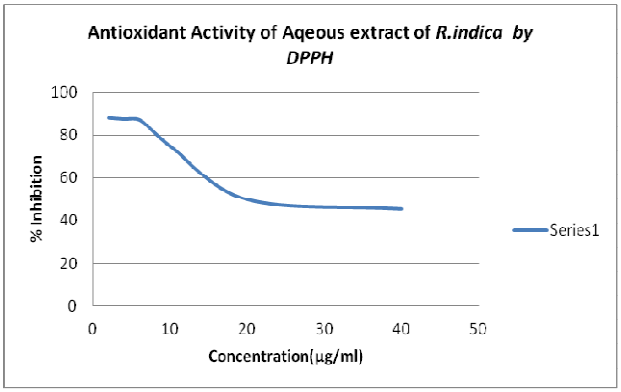


Fig. 2: Antioxidant activity of Aq. Extract of *R.damascena* by DPPH Method

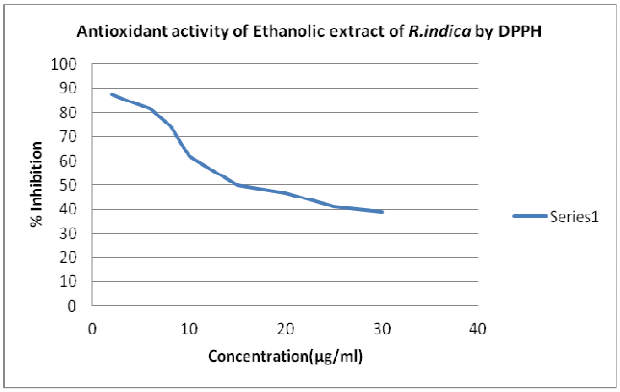


Fig. 3: Antioxidant activity of ethanolic extract of *R.damascena* by DPPH Method

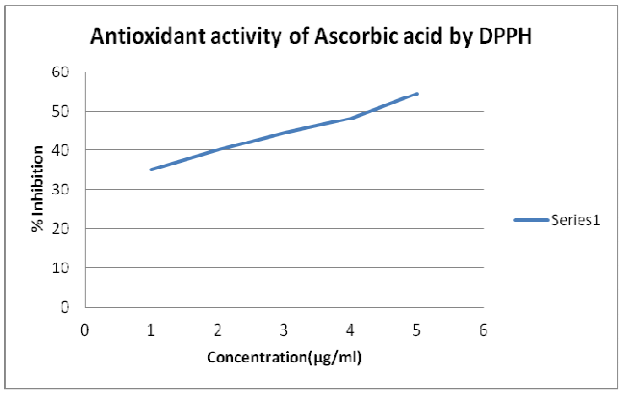


Fig. 4: Antioxidant activity of Ascorbic acid by DPPH Method

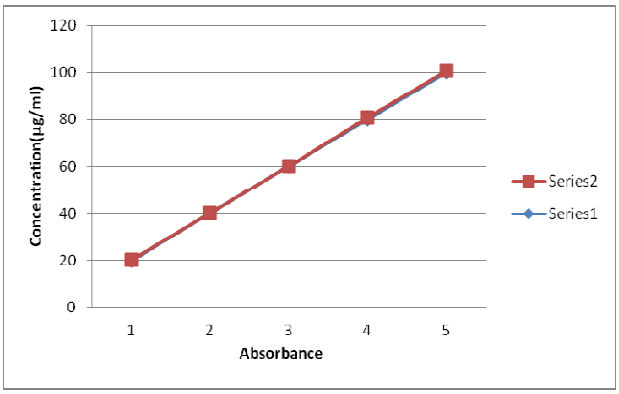


Fig. 5: Reducing Power of Ascorbic acid

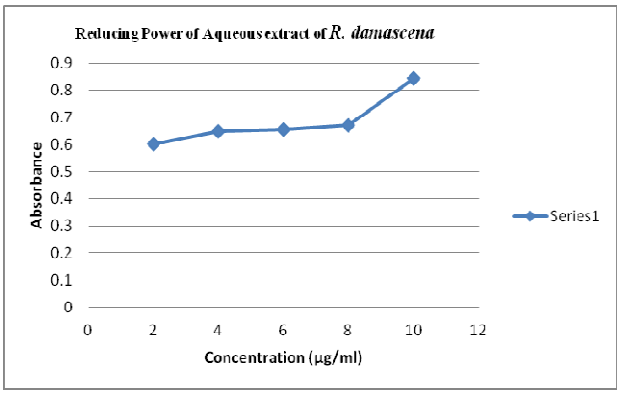


Fig. 6: Reducing Power of Aqueous extract of *R.damascena*

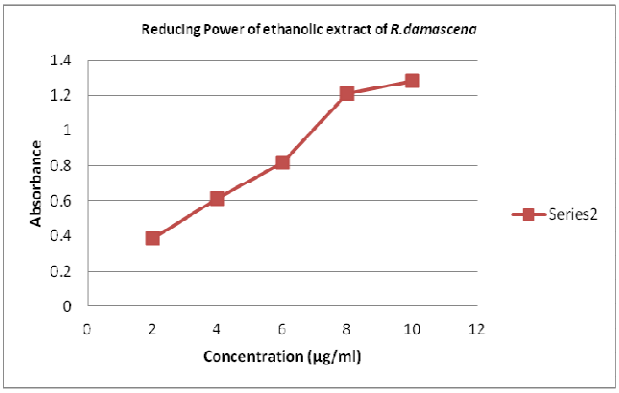


Fig. 7: Reducing Power of ethanolic extract of *R.damascena*

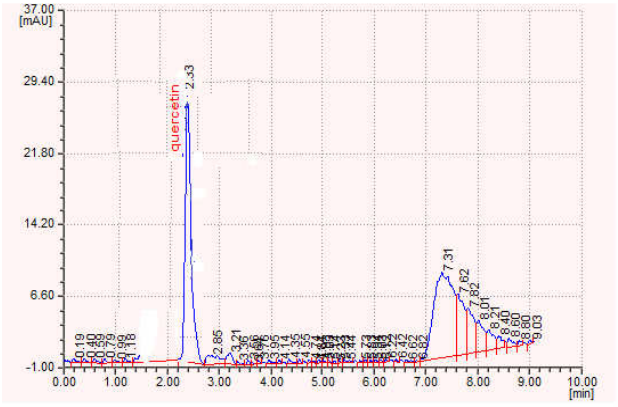


Fig. 8: HPLC Analysis of ethanolic extract of *R.damascena*

Table 6: Parameters for titration

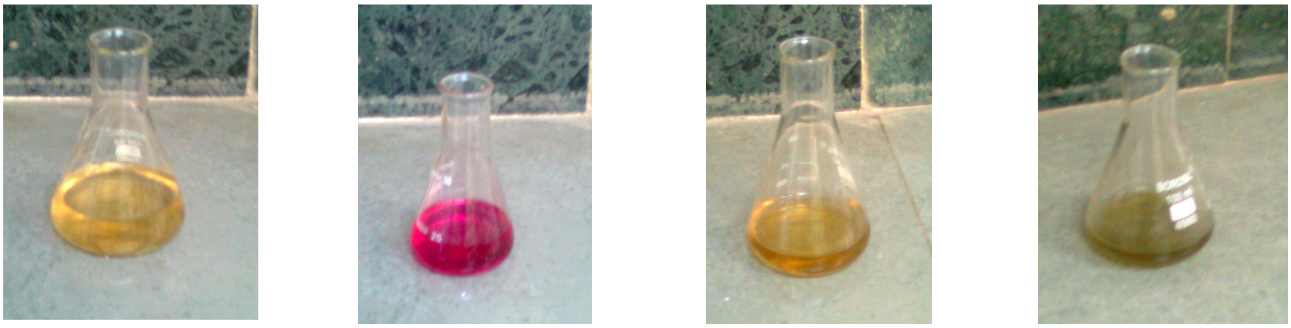
Titrant (1M)	Titrand	Indicator colour change and pH range		
		Indicators	Colour change	pH
HCl / H_2SO_4	NaOH	PH	Colourless to pink	8.2-10
HCl / H_2SO_4	NaOH	MR	Pink to yellow	4.4-6.3
HCl /H_2SO_4	NaOH	Aqu.extract	Pink to yellow	4
HCl / H_2SO_4	NaOH	EtOH extract	Red to olive green	6

PH= Phenolpthalein, MR=Methyl red, Aqu. extract= aqueous extract, EtOH= ethanolic extract

Table 7: Mean volume in(ml) at equivalence point for titrations

Strength (M)	Hydrochloric acid v/s Sodium Hydroxide				Sulphuric acid v/s Sodium Hydroxide			
	PH	MR	Aqu.extract	EtOH extract	PH	MR	Aqu.extract	EtOHextract
1	10 ± 0.19	10.5±0.20	10.1± 0.20	10.5±0.20	15.5± 0.20	11.5± 0.24	12.1± 0.24	11.5±0.18

PH= Phenolpthalein, MR=Methyl red, Aqu. extract= aqueous extract, EtOH= ethanolic extract



Methyl red **Phenolpthalein** **aqueous extract** **ethanolic extract**

Fig. 9: Colour observation of indicators after titration

CONCLUSION

The preliminary phytochemical screening of aqueous & ethanolic extracts showed presence of flavonoid. Free radical production in animal cells can either be accidental or deliberate. With the increasing acceptance of free radicals as common place and important biochemical intermediates, they have been implicated in a large number of human diseases**.** Quarcetin by acting as antioxidants exhibited several beneficial effects, such as anti-inflammatory, anti-allergic, antiviral as well as an anticancer activity. They have also been suggested to play a protective role in liver diseases, cataracts, and cardiovascular diseases. Quarcetin acting as free radical scavengers were shown to exert a protective effect in reperfusion ischemic tissue damage[24]. The scavenging activity of flavonoids present in aqueous and ethanolic extract had been determined by DPPH and reducing power assay. The HPLC analysis of ethanolic extract of *R.damascena* was also carried out for determination of quarcetin. The results obtained in acid base titrations lead us to conclude that it was due to the presence of flavonoids sharp colour changes, which occurred at end point of titrations. At the end point of it states that *R.damascena* aqueous and ethanolic extract as an indicator in all types of acid base titrations because of its economic, simple, accurate and precise.

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