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Evaluation of Phytochemical and Antioxidative properties of *Rosa kordesii* Petal Extracts

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Abstract: Plant extracts have been reported to have contributed to the increase of nutritional values which are important to the human health. Investigations were carried out to evaluate the phytochemicals content and antioxidant activity of *Rosa kordesii* petal extracts in different solvents. Methanol, water and n-hexane were used as solvent. Under an observational analytic study revealed the presences of alkaloids, flavonoids, phenols, carbohydrate, resins, saponins, steroids, tannins, anthracenosides and coumerins as phytochemicals in the extracts. The order of phenolic contents was as follows: aqueous extract; 461.93±3.24, methanolic extract; 349.59±3.62; and n-hexane extract; 42.42±1.71mg GAE/g residue whereas total flavonoid contents were found to be 78.11±2.00, 168.22±2.29 and 45±2.00 mg of catechin/g of extract, respectively. Antioxidant activity of each extract was carried out by total antioxidant capacity test, DPPH, ABTS and ferric reducing antioxidant power (FRAP) assay by using ascorbic acid as standard. Methanolic extract was found to be the most effective in all assays, except total antioxidant capacity test with IC_{50} of 21.93µg/ml (DPPH Assay) and 10.38µg/ml (ABTS Assay). The antioxidant activities were positively associated with the total phenolic and flavonoid contents of the extracts. Further study on different solvent extracts of *Rosa kordesii* would be carried out to elucidate the active principles for its outmost activity.

Keywords: *Rose, Petal, Phytochemical, Phenolic, Flavonoid, Antioxidant Activity.*

I. Introduction

Rose flower is known as an astringent, stomachic and have been used as a food ingredient as well as teas, cakes, and also providing suitable flavor to foods. It could be played a remarkable role by the addition nutritional values and their protection exerting its antioxidative properties. Reactive oxygen species (ROS) such as superoxide (O2-) and nitric oxide (NO) [1] are produced as a natural by-product during the normal oxygen metabolism. Highly reactive free radicals and oxygen species are also present in biological systems from a wide variety of sources [2]. However, when ROS are over produced, redox-active transition metal ions such as iron (II) or copper (II) can cause severe oxidative stress [3]. During oxidative stress nucleic acids, proteins, lipids or DNA may be oxidized and can initiate degenerative disease. ROS stimulate the pathogenesis of many diseases like atherosclerosis, ischemic heart disease, aging, inflammation, diabetes and immunosuppression etc. Antioxidant compounds in food have its ability to trap free radicals. 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 [2]. Natural products are becoming the cynosure to inhibit and scavenge these reactive oxygen species [4].

Rose is one of the most familiar and popular flowers in the world [5]. It is a woody perennial of the genus Rosa, within the family Rosaceae [6]. Rosa is a wide genus which includes 200 different species and more than 18000 cultivars [7] that are widely distributed in Europe, Asia, the Middle East and North America. These deciduous shrubs are widely grown in gardens for their flowers and fruits [8]. The rose flower is known as an astringent, stomachic, and is used traditionally as an agent for activating blood circulation to relieve blood stasis, aiding menstrual regulation, and counteracting toxin. It is also well known as a folk remedy for treating mastitis and diabetes mellitus. Its dry buds are used in the preparation of rose tea which is widely regarded as a dietary and cosmetic product [5]. In China, rose has been used as a medicinal plant and food [9]. Rose petals have been used as a food ingredient in teas, cakes, and flavor extracts [10]. Several groups have investigated the chemical components in the flowers, fruits, leaves, roots, and galls of Rosa taxa. It has been shown that rose is a fairly good source of aromatics, phenolics, terpenoids, fatty acid derivatives, sugars, and other polar compounds [11]. Phenolics possess a wide spectrum of biochemical activities, such as antioxidants, free-radical scavengers [12,13], anticancer [14], antimutagenic [15] and anti-inflammatory [16], however the antioxidative properties remain the core topic of investigation in recent years. Crude extracts of the plant parts rich in phenolics are increasingly of interest in the field of nutrition, health and medicine, because they retard oxidative degradation

of lipids and thereby improve the quality and nutritive value of foods [17]. Studies on rose also showed the antioxidant [8,18], antibacterial [19], anti-obesity [20], and anti-diabetic [21] activity. Antioxidant activity was found to be influenced by genotype in Rosa taxa [8]. However, very limited research work has been done to evaluate nutritional and antioxidative properties of *Rosa kordesii* petal extracts. Hence, this study was undertaken to investigate the nutritional and antioxidant activity of *Rosa kordesii* petal extract in different solvents.

II. Materials and Methods

The petals of *Rosa kordesii* were collected from the local area of Rajshahi, Bangladesh and authenticated by a seasoned Botanist, Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extracts

The petals were washed and cut into pieces and was air-dried for two weeks after which it was grinded. The fine powder was taken to the Department of Biochemistry and Molecular Biology for further processing into a coarse powder form. Three types of solvent were used for extract preparation. After Soxhlet extraction, the extracts were subjected to solvent evaporation by rotatory evaporator. The dried materials were tested for different phytochemicals like Alkaloids, Carbohydrate, Flavonoids, Glycosides, Triterpenoids, Resins, Saponins, Steroids andTanins by standard methods [22, 23]. The extracts were also used to analyze antioxidant properties.

A. Phytochemical screening

1. Test for Alkaloids

(a) Dragendorff's test

To 2 mg of the extract 5 ml of distilled water was added, 2M hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

(b) Hager's test

To 2 mg of the extract taken in a test tube, a few drops of Hager's reagent were added. Formation of yellow ppt confirms the presence of alkaloids.

c) Wagner's test

2 mg of extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent was added. A yellow or brown ppt. indicates the presence of alkaloids.

(d) Mayer's test

To a few drops of the Mayer's reagent, 2 mg of extract was added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

2. Carbohydrates

(a) Anthrone test

2 mg of extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2ml of anthrone reagent solution was added. Formation of green or blue colour indicates the presence of carbohydrates.

(b) Benedict's test

2 mg of extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 5 ml of Benedict's solution was added and boiled for 5 minutes. Formation of brick red colour ppt indicates the presence of carbohydrates.

(c) Fehling's test

2 mg of extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 1 ml mixture of equal parts of Fehling's solution A and B were added and boiled for few minutes. Formation of red or brick red colour precipitate indicates the presence of reducing sugar.

(d) Molisch's test

2 mg of extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2 drops of freshly prepared 20% alcoholic solution of α- naphthol was added. 2 ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red violet ring appear, indicating the presence of carbohydrates which disappear on the addition of excess of alkali.

3. Flavonoids
(a) Shinoda's test
2 mg of extract was dissolved in 5ml of ethanol and to this 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicates the presence of flavonoids.
4. Glycosides
(a) Molisch's test
2 mg of extract was shaken with 10ml of water, filtered and the filtrate was concentrated. To this 2-3 drops of Molisch's reagent was added, mixed and 2ml of concentrated sulfuric acid was added carefully through the side of the test tube. Reddish violet ring appear, indicating the presence of glycosides.

5. Triterpenoids
Liebermann - Burchard's test 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a pink colour indicates the presence of triterpenoids.

6. Resins
1 ml of extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicates the presence of resins.

7. Saponins
In a test tube containing about 5 ml of an extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicates the presence of saponins.

8. Steroids
(a) Liebermann-Burchard's test
2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicates the presence of steroids.

(b) Salkowski reaction
2 mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

9. Tannins
To 1-2 ml of the extract, few drops of 5% w/v $FeCl_3$ solution was added. A green colour indicated the presence of gallotannins, while brown colour indicates the presence of pseudotannins.

10. Test for Anthracenosides
4 ml of extract was concentrated to 2 ml, then ammonia solution (25%, 1-2ml) was added by shaking, a cherish red color of the alkaline solution indicates the presence of emodol (a glycine's of anthracenosides) in an oxidized form (Borntragers reaction).

B. Determination of Total Phenolic Content
Total phenolic contents in each extract were measured by the Folin-Ciocalteu method of Meda et al. [24] with a slight modification. Briefly, 100 µL of extract solution was mixed with 2.0 mL of 2% sodium carbonate (Na_2CO_3) and 100 µL of 50% Folin-Ciocalteu´s reagent. After incubation at room temperature for 5 min, the absorbance of the reaction mixtures were measured at 750 nm by using a spectrophotometer. For each extract, three replicate assays were performed.
The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/gm of extract).

C. Determination of Total Flavonoid Content
Total flavonoid contents were determined according to the method of Kim et al. [25]. 250 µL of extracts were prepared in 1.25 mL of distilled water and 75 µL of 5% $NaNO_2$. After 6 min, 150 µL of 10% $AlCl_3$ was added,

After 5 min, 500 µL of 1 M NaOH was added to the mixture. Absorbance of the mixture was determined at 510 nm. Results were expressed as catechin equivalents (mg CTE/g of extract).

D. Determination of Antioxidant Activity

1. Determination of Total Antioxidant Capacity

The total antioxidant capacity of different extract was evaluated by the phosphomolybdenum method according to the procedure described by Prieto et al. [26]. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer (against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (20, 50, 100, 150 and 200µg/ml) with methanol.

2. Ferric Reducing Antioxidant Power (FRAP) Assay

The assay was performed according to the modified method described by Soni et al. [2]. 0.25 mL samples/standard solution at different concentrations, 0.625 mL of potassium buffer (0.2 M) and0.625 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution were added into the test tubes. The reaction mixture was incubated for 20minutes at 50°C to complete the reaction. Then 0.625 mL of 10% TCA solution was added into the test tubes. The total mixture was centrifuged at 3000 rpm for 10minutes. After which, 1.8 mL supernatant was with-drawn from the test tubes and was mixed with 1.8 mL of distilled water and 0.36 mL of 0.1% FeCl3 solution. The absorbance of the solution was measured at 700 nm using a spectrophotometer against blank. A typical blank solution contained the same solution mixture without plant extracts/standard was also incubated under the same condition, and the absorbance of the blank solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increase reducing capacity.

3. DPPH Radical Scavenging Activity

DPPH assay was carried out according to the method described by Soni et al. [2].

The 0.1 mM solution of DPPH in methanol 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. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Radical Scavenging Activity} = (A_{control} - A_{sample} / A_{control}) \times 100$$

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

Then percentage DPPH radical scavenging activity was plotted against concentration, and from the graph IC_{50} was calculated.

4. ABTS Radical Scavenging Activities

ABTS assay was performed according to the method reported by Re et al. [27] with some modifications. The ABTS radical cation was obtained by adding 7 mM ABTS to 2.45 mM potassium persulphate solution, and the mixture was left to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with ethanol to obtain an absorbance of 1.0 at 734 nm. Diluted ABTS radical cation solution (1 mL) was added to 20 µL of sample fractions or Trolox standard solution. The absorbance was measured at 734 nm after 30 minutes.

The ABTS●+ radical scavenging activity of the samples was expressed as

$$\% \text{ of inhibition} = [(A_{control} - A_{sample})/A_{control})] \times 100$$

Where $A_{control}$ is the absorbance of the blank control (ABTS●+ solution without test sample) and A_{sample} is the absorbance of the test sample. Then percentage ABTS radical scavenging activity was plotted against concentration, and from the graph IC_{50} was calculated.

III. Result and Discussion

The preliminary phytochemical tests revealed the presence of flavonoids, carbohydrate, Glycosides, saponins, steroids, tannins, anthracenosides and coumerins in all three types of extract which are presented in Table-1. Thus, the preliminary screening tests may be useful in the detection of the bioactive compounds.

Total Phenolic

Plant phenolics have been shown to inhibit the formation of superoxide anion radicals generated by various enzymes [28]. The order of phenolic contents was as follows: aqueous extract; 461.93±3.24, methanolic extract; 349.59±3.62; and n-hexane extract; 42.42±1.71mg GAE/g residue (Table-2). The results showed that aqueous extract was higher in phenolic content compared to other extracts (Table 2).

Total Flavonoid

Flavonoids as the most common group of phenolic compounds are also responsible for antioxidant activity [10]. The total flavonoid contents of methanolic, aqueous & n-hexane extracts of *R. kordesii* flower petals were found to be 168.22±2.29, 78.11±2.00 and 45±2.00 catechin equivalents/gm of extract respectively (Table-2). The highest flavonoid content was observed in methanolic extract. Both phenolic and flavonoid content were significantly different among three groups ($p<0.05$).

Antioxidant activity of each extract was carried out by total antioxidant capacity test, DPPH, ABTS and ferric reducing antioxidant power (FRAP) assay by using ascorbic acid as standard.

Total Antioxidant Capacity

The total antioxidant test (TAC) is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity) [29]. The result of total antioxidant capacity (TAC) is shown in table 3 and presented in Figure 2.The total antioxidant capacity was in the order of the n-hexane extract > methanol extract > aqueous extract and all extracts at all concentrations exhibited quite low activities than the control.

Reducing power assay

Ferric-ferricyanide complex is reduced to the ferrous form due to the presence of antioxidants in the plant extracts. Therefore, the concentration of Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm [29]. The absorbance of the extracts and standard were tabulated in table 4 and the results are presented in figure 3. Methanol extract showed the highest activity than other extracts and more than half of the standard's activity and there was no significant difference between methanolic and aqueous extract while n-hexane extract exhibited quite low activities than standards and other extracts.

DPPH Scavenging Activity

DPPH• (2, 2-diphenyl-1-picrylhydrazyl) is a stable free radical, due to the delocalization of the spare electron on the whole molecule. Thus, DPPH• does not dimerize, as happens with most free radicals. The delocalization on the DPPH• molecule determines the occurrence of a purple colour, with an absorption band with a maximum around 520nm [30]. 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 decolorized 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 [2]**.** In the present study, the IC_{50} values of Ascorbic acid, methanolic, aqueous and n-hexane extract were found to be 25.26, 21.93, 22.16 and 483.5µg/ml and there was no significant difference among ascorbic acid, methanolic and aqueous extract but different with n-hexane extract ($p<0.05$). The methanolic extract contained relatively higher amounts of flavonoid which may be responsible for higher DPPH radical scavenging activity compared to other extracts. The results were tabulated in table-5 and the %inhibition vs concentration graph were showed in fig.4,5,6 and 7.

ABTS Radical Scavenging Activity

The ABTS radical is commonly used to measure the radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plant extracts [31].

The antioxidant activity was expressed as an inhibitory concentration at 50% and the IC_{50} values of the extracts and standard are tabulated in table 5 and presented in figure 8,9,10 and 11. Methanolic extract showed the most effective ABTS cation radical scavenging activity, followed by aqueous and n-hexane extract. The methanolic extract contained relatively higher amounts of flavonoid which may be responsible for higher ABTS radical scavenging activity compared to other extracts. However, when compared with standard i.e. ascorbic acid (6.89 µg/ml), the n-hexane extract shows abnormally less radical scavenging activity.

Table -1: Phytochemical screening of *Rosa kordesii* petal extracts

Name of extract	Methanolic Extract	Aqueous Extract	n-hexane Extract
Alkaloids	—	—	+ve
Carbohydrates	+ve	+ve	+ve
Flavonoids	+ve	+ve	+ve
Glycosides	+ve	+ve	+ve
Triterpenoids	—	—	—
Resins	—	—	—
Saponins	+ve	+ve	+ve
Steroids	+ve	+ve	+ve
Tannins	+	+	—

Table-2: Phytochemical content of three different extracts of *Rosa kordesii* petal

Extract	Total Phenol (mg of gallic acid equivalent(GAE)/gm of extract)	Total Flavonoid (mg of catechin equivalents(CE)/gm of extract)
Methanol Extract	349.59±3.62	168.22±2.29
Water Extract	461.93±3.24	78.11±2.00
N- Hexane Extract	42.42±1.71	45±2.00

**Results are expressed as mean ± standard deviation.*

Table-3: Total antioxidant capacity test

Concentration (mg/ml)	Absorbance			
	Ascorbic acid (Standard)	Methanolic extract	Aqueous extract	n-hexane extract
20	0.252	0.019	0.01	0.023
50	0.527	0.051	0.02	0.069
100	1.216	0.112	0.042	0.172
150	1.97	0.238	0.082	0.367
200	2.789	0.451	0.151	0.626

Table-4: Ferric Reducing Antioxidant Power (FRAP) Assay

Concentration (mg/ml)	Absorbance			
	Ascorbic acid (Standard)	Methanolic extract	Aqueous extract	n-hexane extract
10	1.088	0.523	0.451	0.039
20	1.573	0.841	0.699	0.094
40	2.35	1.356	1.026	0.208
80	3.79	2.015	1.99	0.33
100	4.837	2.56	2.145	0.505

Table-5: DPPH and ABTS Radical Scavenging Activities of Methanolic, Aqueous and n-hexane extract of *Rosa kordesii* petal

Standard/Extract	IC_{50} (µg/ml)	
	DPPH Assay	ABTS Assay
Ascorbic acid	25.26	6.89
Methanolic extract	21.93	10.38
Aqueous extract	22.16	24.53
n-hexane extract	483.5	360.53

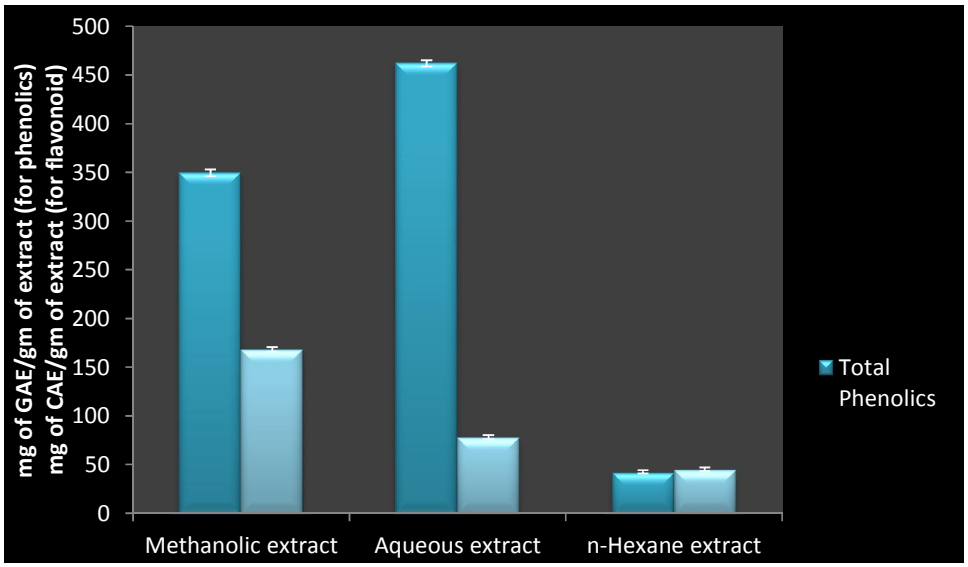

Fig. 1: Total phenolic and flavonoid contents of different *R. kordesii* petal extracts

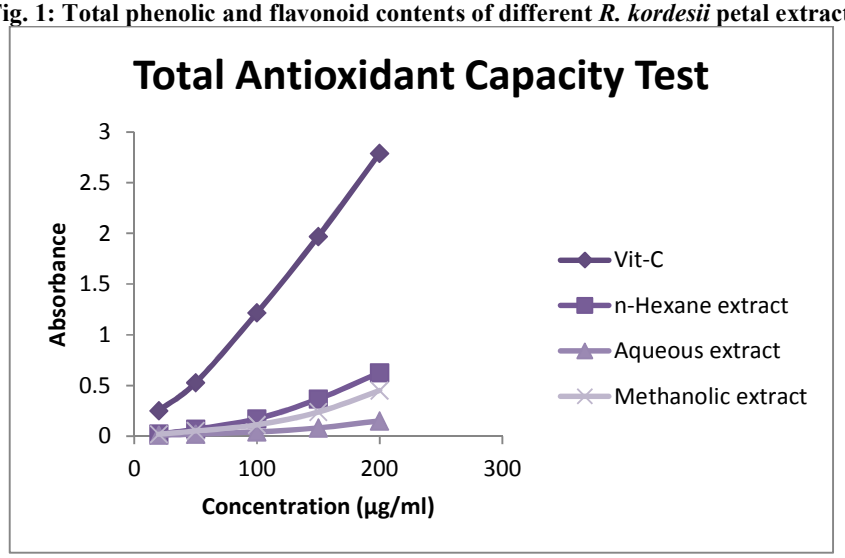

Fig. 2: Total Antioxidant Capacity test of different *R. kordesii* petal extracts

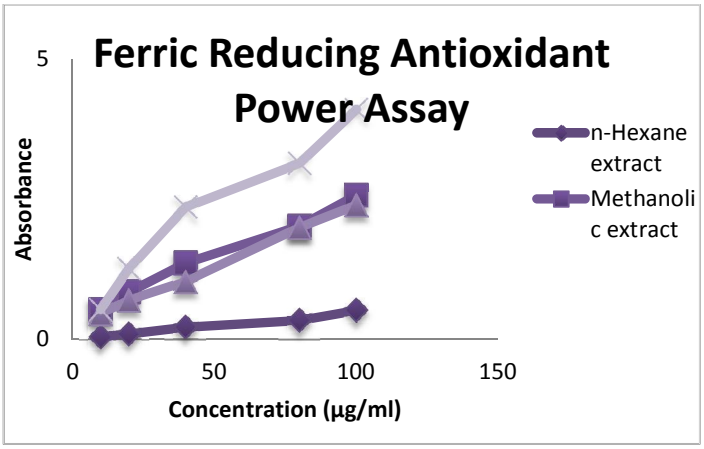

Fig. 3: Ferric Reducing Power Assay of different *R. kordesii* petal extracts

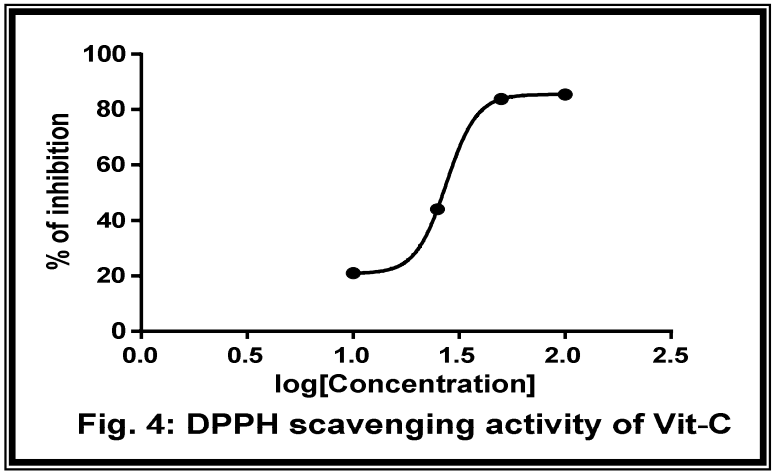

Fig. 4: DPPH scavenging activity of Vit-C

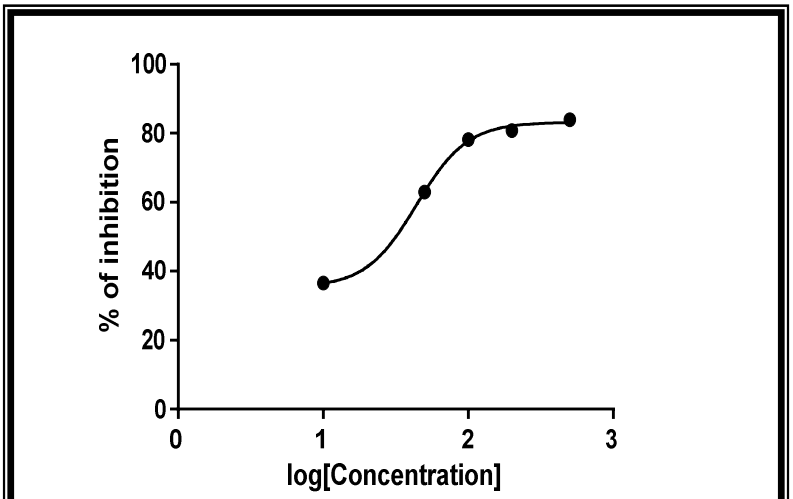

Fig. 5: DPPH scavenging activity of Methanolic extract

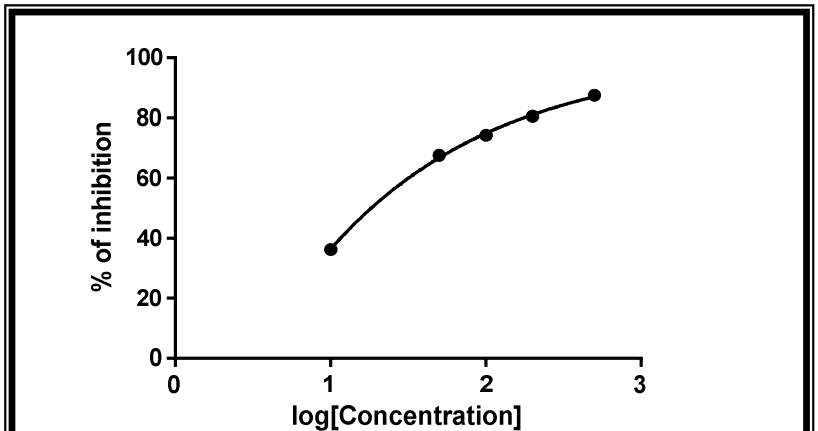

Fig. 6: DPPH scavenging activity of Aqueous extract

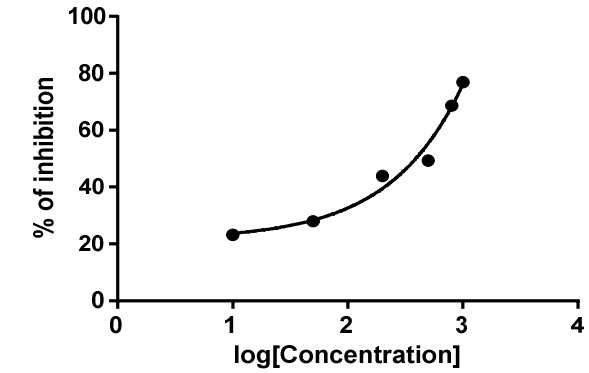

Fig. 7: DPPH scavenging activity of n-hexane extract

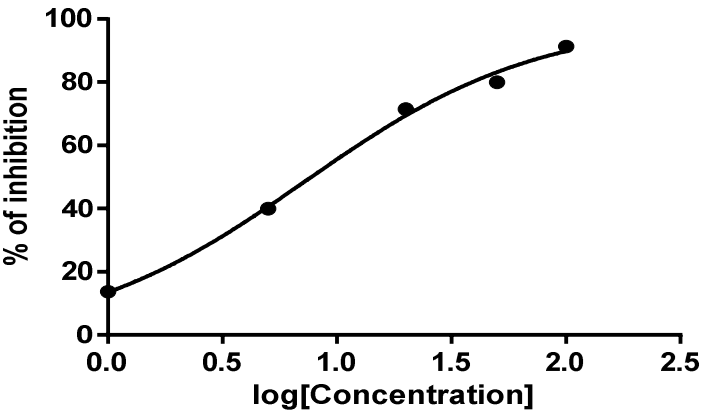

Fig. 8: ABTS scavenging activity of ViTc

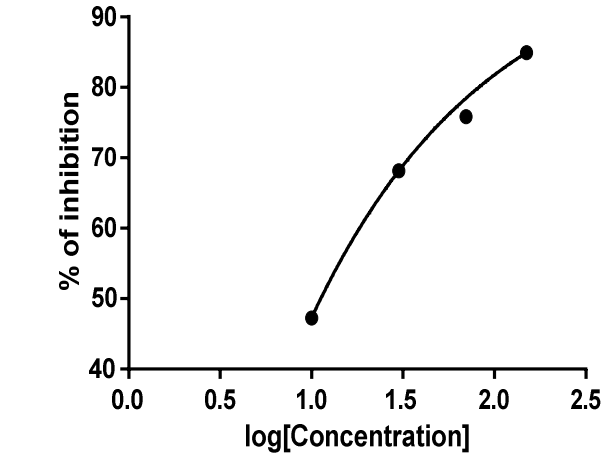

Fig. 9: ABTS scavenging activitv of Methanolic extract

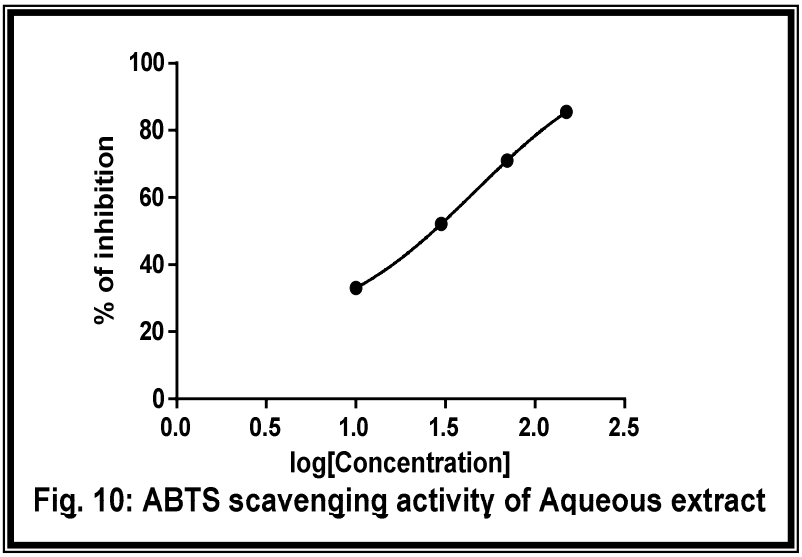

Fig. 10: ABTS scavenging activity of Aqueous extract

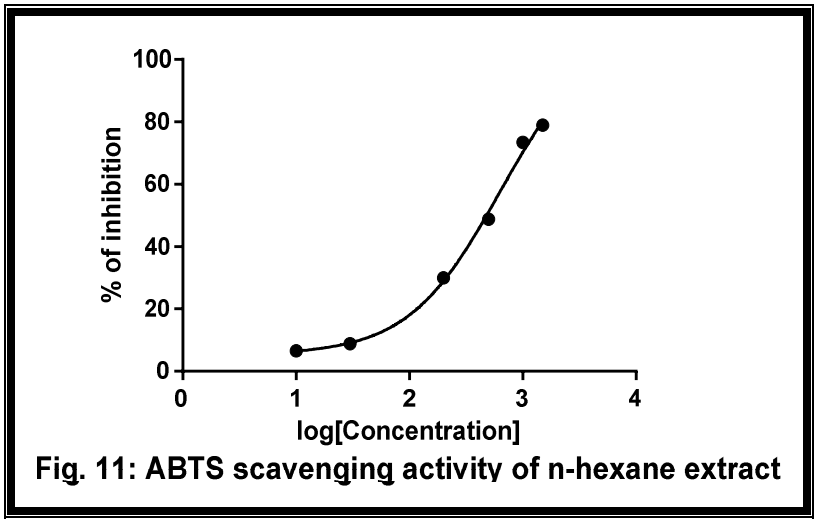

Fig. 11: ABTS scavenging activity of n-hexane extract

IV. Conclusion

From the above results, it can be concluded that *Rosa kordesii* petals are rich in free radical scavenging molecules like terpenoids, flavonoids, saponins, tannins and reducing sugar. The extracts also reveals that all the fractions of the hydroethanolic extract of *Rosa kordesii* petals implicated strong antioxidant activity, reducing power activity, ABTS scavenging activity and free radical scavenging activity along with the presence of phenolic, flavonoid compounds. High antioxidant components and activities were observed in methanolic extract of *R. kordesii* petal as compared to other extracts. However, our study suggests that it can be used as a potential source of natural antioxidant and nutrient.

Acknowledgements

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Reference

[1] Lee JJ, Lee CW, Cho YH, Park SM, Lee BC, Hyeong BP. Tinged autumnal leaves of maple and cherry trees as potential antioxidant sources. In Antiaging: Physiology to formulation 1st edition. Illinois, USA, Allured Publishing Corporation; 2006:11.

[2] Soni H, Sahu N, Sihghai AK, Malik J. Radical Scavenging Activities and Natural Indicator Activity of Aqueous and Ethanolic Extract of Rosa Damascena, International Journal of Pharmacy and Pharmaceutical Sciences 2012,Vol 4, Suppl 5.

[3] Tamsyn SAT, Pauline H, Declan PN. Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complementary and Alternative Medicine2009; 9:27, doi:10.1186/1472-6882-9-27.

[4] Alang G, KaurR, Singh A, Budhlakoti P, Singh A. Free radical scavenging activity of methanolic root extract of the plant Crotolariaburhialinn. Pharmacology online 2010; 1: 78-85.

[5] Youwei Z, Yonghong P. Changes in antioxidant activity in Rosa rugosa flowers at different stages of development. New Zealand Journal of Crop and Horticultural Science 2007, Vol. 35: 397-4010014-0671/07/3504-0397.

[6] Lin L, Hyeonmi H, Jeehye S, Younghwa K, Heon-Sang JL. Antioxidant Activities of Methanolic Extracts from Four Different Rose Cultivars. Encyclopedia of Rose Science," Elsevier, Academic Press, San Diego, CA. 2003 p. 707-716.

[7] Antoaneta G, Kiril M, Violeta K. Antioxidant Capacity of Petals and Leaves from Different Rose (Rosa damascena Mill.) Plantations in Bulgaria. Int J Pure App Biosci 2013; 1(2): 38-43.

[8] Suzan OY, Sezai E. Antibacterial and antioxidant activity of fruits of some rose species from Turkey 2011, Vol. 16, No. 4.

[9] Xuemin Z. Supplement to the compendium of material medica. Beijing, China Press of Traditional Chinese Medicine.

[10] Lin L, Hyeonmi H, Jeehye S, Younghwa K, Heon-Sang JJL. Antioxidant Activities of Methanolic Extracts from Four Different Rose Cultivars. Journal of Food and Nutrition Research 2014, Vol. 2, No. 2, 69-73.

[11] Hashidoko Y. The phytochemistry of Rosa rugosa. Phytochemistry1996;43:535-549.

[12] Leenen R, Roodenburg AJC, Tijburg LBM,Wiseman SA. A single dose of tea with or without milk increases plasma antioxidant activity in humans. European Journal of Clinical Nutrition 2000;54(1):87-92.

[13] Ng TB, Liu F, Wang ZT. Antioxidative activity of natural products from plants. Life Sciences 2000;66(8):709-23.

[14] Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. Medicinal Research Reviews 2003;23(4):519-34.

[15] Miyazawa M, Okuno Y, Nakamura SI, Kosaka H. Antimutagenic activity of flavonoids from Pogostemoncablin. J Agric Food Chem 2000;48(3):642-7.

[16] Crespo ME, lvez J, Cruz T, Ocete MA, Zarzuelo A. Anti-inflammatory activity of diosmin and hesperidin in rat colitis induced by TNBS. Planta Medica 1999;65(7):651-3.

[17] Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999;47(10):3954-62.

[18] Ewa Jab lR, Marta ZK, Janusz K. Antioxidant capacity, ascorbic acid and phenolics content in wild edible fruits. Journal of Fruit and Ornamental Plant Research 2009;17/(2):115-120.

[19] Marufa S, Kamal KD, Mrityunjoy A. Estimation of microbiological propagation and antimicrobial traits of the frequently accessible flowers. Stamford Journal of Microbiology2014, Vol. 4, Issue 1, p. 19-23.

[20] Akifumi N, Norihisa N, Yoichi M, Nobuhito S. Rosehip Extract Inhibits Lipid Accumulation in White Adipose Tissue by Suppressing the Expression of Peroxisome Proliferator-activated Receptor Gamma. PrevNutr Food Sci 2013;18(2):85-91.

[21] Orhan N, Aslan M, Hosbas S, Deliorman O. Antidiabetic effect and antioxidant potential of Rosa canina fruits. Phcog Mag 2009;5:309–315.

[22] Johonsen DA. Plant microtechnique, New York 1940,182.

[23] Chandrashekar K, Santanu S, Prasanna K. Phytochemical studies of aerial parts of the plant Leucas lavandulaefolia Der PharmaChemica 2010;2(5):434-437.

[24] Meda A, Lamien CE, Romito J, Nacoulma OG. Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry 2005;91. 571-577.

[25] Kim DO, Chun OK, Kim KJ, Moon HY, Lee CY. Quantification of polyphenolics and their antioxidant capacity in fresh plums. Journal of Agricultural and Food Chemistry 2003;51, 6509-6515.

[26] Prieto P, Pineda M, Anguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. Anal Biochem 1999;269, 337-341.

[27] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M. Rice-Evans C. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Radical Biology & Medicine 1999;26,1231-1237.

[28] Taubert D, Breitenbach T, Lazar A, Censarek P, Harlfinger S, Berkels R, Klaus W, Roesen R. Reaction rate constants of superoxide scavenging by plant antioxidants. Free Radical Biology & Medicine 2003;35.1599-1607.

[29] Rajurkar NS. Evaluation of phytochemicals, antioxidant activity and elemental content of Adiantum capillus veneris leaves. J Chem Pharm Res 2012; 4(1):365-374.

[30] Pisoschi AM, Negulescu GP. Methods for Total Antioxidant Activity Determination. A Review. Biochem & Anal Biochem 2011;1:106. doi:10.4172/2161-1009.1000106.

[31] Netzel M, Strass G, Bitsch I, Könitz R, Christmann M. and Bitsch R. Effect of grape processing on selected antioxidant phenolics in red wine. Journal of Food Engineering, 56. 223-228. 2003.