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EVALUATION OF THE ANTIOXIDANT ACTIVITIES OF AQUEOUS EXTRACTS OF FRESH MADENI ROSE PETALS

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ABSTRACT
Madeni rose (*Rosa damascena* mill) is considered one of the most important economic products of Al-Madinah Al-Munawrah, Saudi Arabia. It is used as popular drink and reported to have antioxidant properties. In this research, the total phenolic content (TPC) and the total flavonoid content (TFC) of the aqueous fresh petals extracts were estimated by Folin-Ciocalteu and aluminum chloride methods, respectively. Parameters measured in different concentrations of extracts (2.5, 5, and 10 mg/ml) include 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, the reducing power, ferrous ion chelating and hydrogen peroxide (H_2O_2) scavenging activity. Qualitative analysis of the major phenolic compounds was performed using high performance liquid chromatography (HPLC). The results showed that the TPC and TFC of the extract were 388 ± 0.01µg gallic acid equivalent and 17.8 ±1.1 µg catechin equivalents in 10 mg/ml, respectively. The flavonoid content was low and started at 5 mg/ml of rose extract. The scavenging activity of DPPH, H_2O_2, and the reducing power were 78.1 ± 0.9% 73.1 ±2 .9%, and 1.3 ±0.09 compared to Trolox as a standard (94.3 ± 1% 71.2 ±1.8% and 1.3 ±0.07, respectively) at the same concentration. Iron chelating activity of 63.8 ± 3.6% at 10 mg/ml compared to EDTA as a standard (83.4 ± 4.6%). The phenolic compounds identified by HPLC were gallic acid, catechin, rutin, and quercetin. In conclusion, aqueous extracts of fresh rose petals contains a high content of phenolic and flavonoid, which contributes to its antioxidant ability to scavenge free radicals and chelate iron. Thus, the extract of fresh *Rosa damascena* mill of Al-Madinah region of Saudi Arabia is a significant source of natural antioxidant and may have beneficial effects on human health.

KEY WORDS: *Rosa damascena* Mill, antioxidant activity, total phenolic, total flavonoid and HPLC analysis.

INTRODUCTION:

Roses are members of the Rosaceae family which are used for food and medicinal purposes through history (Hummer & Janick, 2009). The genus Rosa includes about 100 species that are widely distributed in Europe, Asia, Middle East, and North America (Yilmaz & Ercisli, 2011). *Rosa damascena* mill, commonly known as Damask rose, is considered as one of the most important *Rosa* species for its beauty, flavor and distinguished fragrance (Das *et al.*, 2012). There are many therapeutic effects of *R. damascena* in ancient medicine. These include the treatment of abdominal and chest pain, strengthening the heart the treatment of menstrual bleeding and many digestive problems (Boskabady *et al*., 2011). The flower of this species was also reported to have analgesic, anti-inflammatory, antidepressant, and diuretic activity (Leenen *et al*., 2000; Dugas *et al*., 2000; Kumar *et al*., 2008; Hajhashemi *et al*., 2010). Rose oil heals depression, grief, nervous stress and tension as well as wound healing, and skin health (Pal, 2013; Tirupathi & Golla, 2016). Moreover, some studies demonstrated other rose usage such as anti-HIV (Gao *et al*., 2013), antibacterial (Basim & Basim, 2003), antitussive (Shafei *et al*., 2010), antidiabetic (Gholamhoseinian & Fallah, 2009) and as respiratory smooth muscle relaxant (Boskabady *et al*., 2006). Several components were isolated from the flower, petals and hips (seed-pot) of *R. damascena* including terpenes, glycosides, flavonoids, and anthocyanins (Kumar *et al*., 2006). In addition, it contains carboxylic acid, myrcene (Boskabady *et al*., 2011), vitamin C (Libster, 2002; Boskabady *et al*., 2011), kaempferol, quercetin (Kumar *et al*., 2009; Kwon *et al*., 2009; Himesh *et al*., 2012) and tannins (Nyeem *et al.*, 2006). The antioxidant activity of rose is attributed to their content of polyphenols. Phenolic compounds and flavonoids are known to have a wide spectrum of biochemical activities and play an important role in human health as antioxidants and free radical scavengers (Leenen *et al*., 2000). In addition, phenolic compounds have been reported to correlate with reduced risk of cancer and cardiovascular disease (Huang *et al*., 2009). Recently, It was proposed that the distillation of rose petals were supposed to be rich in polyphenol, particularly flavonols (Schieber *et al*., 2005), which have been demonstrated to exert antioxidant properties both in vivo (H.-Y. Kim *et al*., 2003) and in vitro (Wang *et al*., 2006). Madeni rose, Ward Madeni (*Rosa damascena* mill) is a type of Damask rose, which is considered one of the most important economic products of Madinah Al-Monawarah in Saudi Arabia. Fresh Madeni rose petals are suggested to have useful antioxidants properties which carries important health benefits. Phenolic compounds are responsible for the antioxidant activities associated with

R. damascena properties to scavenge free radicals, chelate metals and break radical chain reactions (Naczk & Shahidi, 2004). In this paper, six different parameters are used to evaluate the ability of fresh Madeni rose petals to act as antioxidant and free radical scavenger. The total phenolic content (Kim *et al*., 2003) and the total flavonoids content (King *et al.*, 2013) were estimated by Folin-Ciocalteu and aluminum chloride method, respectively. These methods are widely used to estimate phenolic and flavonoids content in processed of *Rosa damascena* mill (Kumar *et al*., 2009; Himesh *et al*., 2012; Antoaneta *et al.*, 2013) and edible flower tea (Ngoitaku *et al*., 2016). The evaluation of antioxidant power was performed by 1,1-diphenyl-2-picrylhydrazyl (DPPH). This is a stable free radical often used to evaluate the antioxidants activity of several natural compounds in a relatively short time compared with other methods (Satheesh *et al*., 2010; Li *et al*., 2014). Scavenging hydrogen peroxide (H_2O_2), by rose petals extracts was also measured. The ability of a fresh rose petals to chelate free iron which is considered as an important antioxidant property (Rana *et al.*, 2014) as well as the reducing power of the petals extracts (Jayanthi & Lalitha, 2011) were both estimated. Finally, the phenolic constituents of Madeni rose petals were qualitatively estimated by high performance liquid chromatography (HPLC). To our knowledge, fresh Madeni rose petals extracts have not been investigated for their phenolic constituents.

MATERIALS & METHODS

Chemicals

Fresh rose petals were purchased from local supplier, Folin-Ciocalteu's phenol reagent, ferric chloride anhydrous, 2,2-diphenyl-1bicrylhydrazyl, trichloroacetic acid (TCA), ferrous chloride, ferrozine, gallic acid, sodium nitrite, sodium hydroxide, aluminum chloride, hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA), anhydrous sodium carbonate, sodium dihydrogen orthophosphate, di-sodium hydrogen orthophosphate, phosphate buffer saline (PBS), vitamin C, potassium ferrocyanide, (+)-catechin, Trolox, Rutin hydrate and quercetin were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, formic acid, and ethanol were obtained from Merck (Darmstadt, Germany) and of HPLC grade.

Sample preparation

Fresh rose petals were prepared as traditionally consumed by Saudi people. 1 g of clean fresh rose petals were soaked in 100 ml boiling water for 10 min (10 mg/ml). The extract was filtered and two different concentrations were prepared, 2.5 and 5 mg/ml. Extracts were prepared fresh before each experiment.

Sample extraction for HPLC analysis

The method of Abdel-Hameed *et al* (2013) was used in which 900 g of fresh rose petals were extracted three times by 4 L methanol (80%). The solvet was dried under vacum using rotary evaporator. The resulting residues (98 g) were stored at 4°C in brown glass bottles for further investigation.

Total phenolic content

The amount of total phenolic content was determined by Folin-Ciocalteu method of Kim *et al*. (2003).The three concentrations of rose extract (0.5 ml) were mixed with 5 ml distilled water, 0.5 ml Folin's reagent and 1 ml of 2% sodium carbonate. After 1 h incubation in the dark, the absorbance was read at 750 nm. The concentration of total phenolic content was expressed as µg of gallic acid equivalent per gram of the extract.

Total flavonoid content

The method of King *et al*. (2013) was used to determine the total flavonoid content of fresh rose petals with minor modification. 250 µl of the extracts were added to 1.25 ml deionized water and 75 µl of 5% $NaNO_2$ (w/v). After 5 min, 150 µl of 10% $AlCl_3$ and 0.5 ml of 1 M NaOH (w/v) were added. The volume was completed to 2.5 ml with deionized water and measured at 510 nm. The concentration of total flavonoid content was expressed as µg of catechin equivalent per gram of extracts.

DPPH radical scavenging activity

The ability of fresh Madini rose petals extracts to remove reactive species by donation of a hydrogen was measured by DPPH radical scavenging test. The method was described by Bersuder *et al*. (1998). Briefly, 250 µl of extracts and Trolox at different concentrations 2.5, 5, and 10 mg/ml were added to 250 µl of 99.5% ethanol. DPPH 0.02 g was prepared in ethanol and kept in the dark. To the previous mixture, 62.5 µl of DPPH was added and the absorbance was measured at 517 nm after 1 h incubation. Radical scavenging activity was expressed as inhibition percentage and calculated as follow:

% scavenging activity = $\{(A_{control} - A_{sample})/ A_{control}\} \times 100$

Where $A_{control}$ = the absorbance of the control without sample or standard, and A_{sample} = the absorbance with samples or standards.

H_2O_2 radical scavenging activity

The H_2O_2 scavenging activity of fresh rose petals were determined according to the method of Ruch *et al*. (1989). 0.6 ml of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4) and was mixed with 1 ml rose petal extracts. The absorbance was determined spectrophotometrically at 230 nm after 10 min. The blank was only phosphate buffer without H_2O_2.Trolox was used as a standard reference. The percentage scavenging of H_2O_2 was calculated using the following equation:

% scavenged (H_2O_2) = $\{(A_{control} - A_{sample})/ A_{control}\} \times 100$

Where $A_{control}$ = the absorbance without sample or Trolox, A_{sample} = the absorbance in the presence of the sample or Trolox.

Ferrous ion chelating activity

The ferrous ion chelating activity was determined according to the procedure of Dinis *et al.* (1994). 500 µl of extracts were mixed with 50 µl of 2 mM ferrous chloride and 1.5 ml distilled water. The reaction was initiated by the addition of 5 mM Ferrozin (100 µl). The mixture was kept at room temperature for 10 min. The absorbance was measured at 562 nm. EDTA was used as a positive control. Ferrous ion chelating activity was calculated as follows:

Chelating effect % = $\{(A_{control} - A_{sample})/ A_{control}\} \times 100$

Where $A_{control}$ = the absorbance of control without sample or EDTA. A_{sample} = the absorbance in the presence of sample or EDTA.

Reducing power activity

The reducing power of fresh rose petals extracts was performed as described by Do *et al*. (2014). 1 ml of various concentrations of the extracts 2.5, 5, and 10 mg/ml) add were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1% w/v). The mixture was incubated in water bath at 50^0C for 30 min. After cooling, trichloroacetic acid (2.5 ml, 10% w/v) was added and centrifuged at 6000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and a freshly prepared ferric chloride solution (0.5 ml, 1% w/v). The presence of antioxidants causes the conversion of the ferric-ferricyanide complex to the ferrous form with blue color, which was measured at 700 nm. Trolox at concentrations of 2.5, 5, and 10 mg/ml was used as a standard.

Identification of phenolic constituents by HPLC analysis

The separation of phenolic compounds was performed according to the method of Abdel-Hameed *et al*. (2012), with some modification. The instrument used was 1100 HPLC (Agilent Technologies, California, USA) equipped with a ChemStation software, a degasser, a binary gradient pump, an autosampler, a column thermostat and a diode array detector. The column used was Pinnacle DB C_{18} (5 µm, 250 × 4.6 mm, USA). Chromatographic separation was carried out at room temperature with a flow rate 1.0 ml/min of gradient elution using two solvents: A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). Linear gradient elution was used: 95% A (5 min), 95–90% A (15 min), 90–50% A (50 min), 50–95% A (60 min), 95% A (65 min). The injection volume was 20 µl, and the detection wavelength was 280 nm.

Standard and sample preparation for HPLC analysis

Standard solutions (500 µg/ml) of gallic acid, catechin, rutin hydrate and quercetin were prepared in 50% acetonitrile/water and filtered using membrane disc (0.45 µm). A solution of 2.5 mg/ml of rose petals was prepared in HPLC grade of 50% acetonitrile /water and filtered as described above (Abdel-Hameed *et al*., 2012). Identification of phenolic compounds in the extract was achieved by comparing the retention times with that of standard compounds under the same chromatographic conditions (Çoruh & Özdoğan, 2015).

Statistical analysis

All experiments were carried out in triplicates and presented as mean ± SD of three independent incidents. Statistical analysis was performed using SPSS version 22.0 program. One-way analysis of variance (ANOVA) test followed by Tukey's test was used to measure the degree of significance for ($p < 0.05$).

RESULTS & DISCUSSION

Total phenolic content (TPC) and total flavonoid content (TFC)

The estimation of TPC of plants became an important tool to understand the plant antioxidant benefits. Table 1 shows the fresh rose petal content of phenolic compounds 388 ± 0.01 µg GAE/mg of extract which decrease with decreasing concentration to 118 ±0.01 µg GAE/mg of extract at 2.5 mg/ml. In contrast, the TFC was lower and start at the concentration of 5 mg/m with 3.1 ± 0.05 µg catechin/mg of extract and increase to 17.8 ±1.1 µg catechin/mg of extract. These results are lower than the values estimated by Takte *et al*. (2015) with dried aqueous extract of the same species and Demir *et al*. (2014) with different spices of rose hip fruits.

TABLE 1. Total phenolic and total flavonoid content of aqueous extracts of fresh Madeni rose petals

Concentration (mg/ml)	Total phenolic content (µg/mg of gallic acid)	Total flavonoid content (µg/mg of catechin)
2.5	118 ± 0.01	--
5	23 5± 0.02	3.1 ± 0.05
10	388 ± 0.01	17.8 ± 1.1

* All values are expressed as mean ± SD for three determinations

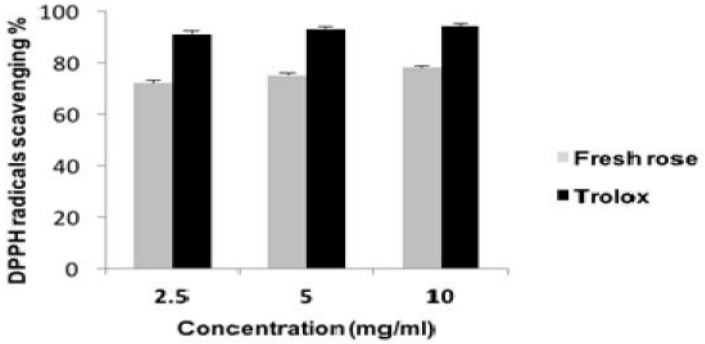

FIGURE 1: DPPH radicals scavenging activity of different concentrations of fresh Madeni rose petals and Trolox which was used as a standard. All values are expressed as mean ± SD for three determinations.

DPPH radical scavenging activity

The DPPH radical scavenging activity of rose fresh petals compared to Trolox as a well-known antioxidant are shown in Figure 1. The scavenging activity percentages were increased by increasing the concentrations of sample extract. The percentages were 71.9 ± 0.8%, 74.9 ± 0.6% and 78.1 ± 0.9%, respectively. They were significantly lower than Trolox concentrations, which were 91.1±1%, 93.3 ± 0.7% and 94.3 ± 1%, respectively ($p < 0.001$). Also, there is a significant difference between fresh petals 2.5 and 10 mg/ml ($p < 0.001$). These findings are in line with Takte *et al.* (2015), Yassa *et al*. (2015) and Li *et al*. (2014) although they used different extraction method with organic solvents.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is formed in vivo as a product of oxidizing enzymes such as superoxide dismutase. It has the ability to cross membranes and oxidize a number of compounds. H_2O_2 may reacts with Fe^{2+} and to less extent with Cu^{2+} ions to form hydroxyl radical which may cause many toxic effects (Babu *et al*., 2013).The ability of fresh Madeni rose petals to scavenge hydrogen peroxide is shown in Figure 2. It was also compared with that of Trolox as standard. Fresh Madeni rose petals extracts were capable of scavenging H_2O_2 in a concentration dependent manner. At 2.5, 5, and 10 mg/mL concentration, water extracts of rose petals exhibited 30.7 ± 3.0%, 50.2 ± 1.7%, and 73.1 ± 2.9%, respectively scavenging activity. On the other hand, Trolox showed 29.3 ± 6.4%, 44.6 ± 1.5%, and 71.2 ± 1.8%, respectively of hydrogen peroxide scavenging activity at the same concentration. These results showed that water extracts of fresh Madeni rose petals exhibited the same scavenging activity as Trolox with no significant differences between them. Fresh Madeni rose petals scavenged H_2O_2 and this may be due to the presence of phenols which could donate electrons, converting it into water as an end product of the reaction (Banerjee & Bonde, 2011).

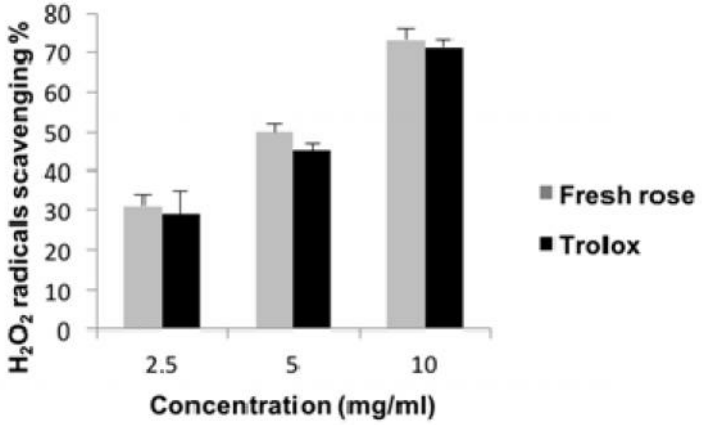

FIGURE 2: H_2O_2 radicals scavenging activity of fresh Madeni rose petals and Trolox which was used as a standard. All values are expressed as mean ± SD for three determinations.

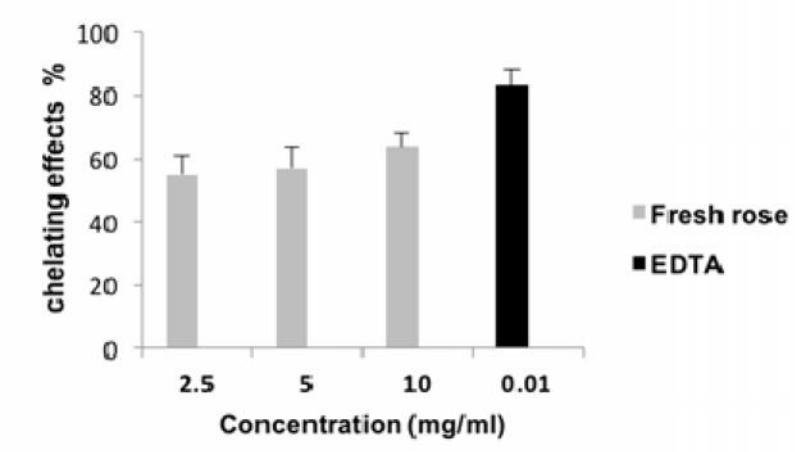

FIGURE 3: Ferrous ion chelating effects of different concentrations of fresh Madeni rose petals. EDTA at the concentration of 0.01 mg/ml was used as a standard. All values are expressed as mean ± SD for three determinations.

Ferrous ion chelating activity

Free iron plays an important role to form the free radicals (Patralekh & Mukherjee, 2010). Excessive deposition of iron in the vital organs such as liver and kidney can lead to the loss of the function of those organs. Therefore, the chelation of free iron can prevent the formation of free radicals and the resulted disorders. In this assay, all the investigated substances were capable of chelating Fe^{2+} ions. The chelating effect of EDTA and various concentrations of the extracts on ferrous ions are shown in Figure 3. EDTA showed the maximum chelating activity of 83.4 ± 4.6%. The chelating effect of fresh rose petals was concentration dependent and ranges between 63.8 ± 3.6% at 10 mg/ml and 54.5 ± 6.5% at 2.5 mg/ml concentration. These results showed higher chelating activities compared to other species of four different rose cultivars estimated by Li *et al*. (2014).

Reducing power

The reducing power of the extract may serve as a significant reflection of its antioxidant activity (Sravani & Padmaa, 2011). The reducing power of rose petals at different concentrations are shown in Figure 4 and represented as increase of absorbance, compared to Trolox as a standard. All three different concentrations showed reducing power. The reducing power was increased with the increase of the concentrations. At 2.5 mg/ml, the reducing power was 1.0 ± 0.3 and reached 1.3 ±0.09 at 10 mg/ml. As indicated by Dave (2009) that compounds with reducing power can act as electron donors and may reduce the oxidized intermediates of lipid peroxidation processes. It is obvious that the extract of the Madeni rose petals may act as primary and secondary antioxidants. Himesh *et al*. (2012) studied the antioxidant screening of *Rosa damascena* using aqueous and ethanoic extracts of different concentration and the values of absorption were somewhat similar to the present work.

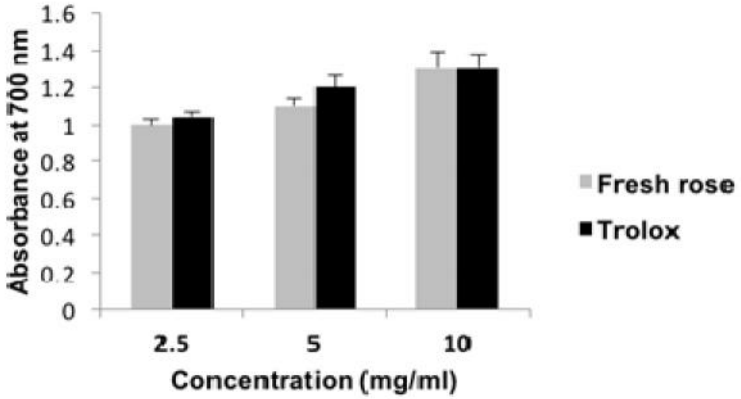

FIGURE 4: Reducing power of different concentrations of fresh Madeni rose petals and Trolox which was used as a standard. All values are expressed as mean ± SD for three determinations.

Identification of phenolic constituents by HPLC analysis

HPLC is a multipurpose technique widely used for the isolation and identification of natural products (Boligon & Athayde, 2014). HPLC analysis of methanolic extract of fresh rose petals showed the presence of four standard phenolic compounds. They were gallic acid (GA); catechin (CH), rutin hydrate (RH) and quercetin (QU) compared to standard compounds based on the retention time at the same condition. The elution order and the retention times for GA, CH, RH, and QU were 4.9, 17.9, 27.3, and 36.2 min, respectively Figures 5 and 6. Roses are considered as a rich source of phenolic compounds (Yoshida *et al*.,1993: Hvattum, 2002: Nowak & Gawlik-Dziki, 2007). RH and CH both showed beneficial effects such as antioxidant, anti-ageing and may prevent cardiovascular complications (Mandel & Youdim, 2004; Thielecke & Boschmann, 2009). On the other hand, other phenolic compounds found in the extracts such as QU and GA also might have useful effects on human health and eases oxidative stresses (Pandey & Rizvi, 2009). Kumar *et al*. (2008) reported that fresh flowers of *R. bourboniana*, *R. brunonii* and *R. damascena* were found to contain ten polyphenols; four of them were identified in the fresh Madeni rose petal extracts.

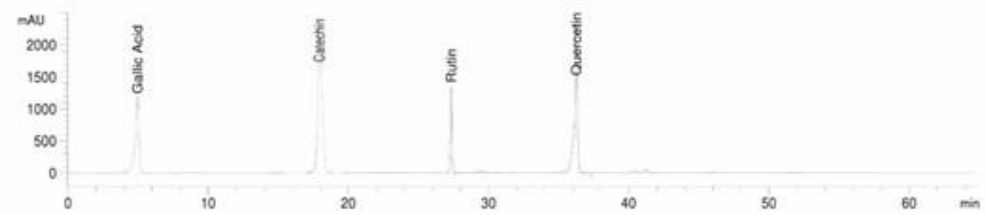

FIGURE 5: HPLC chromatogram of some standard of phenolic compounds

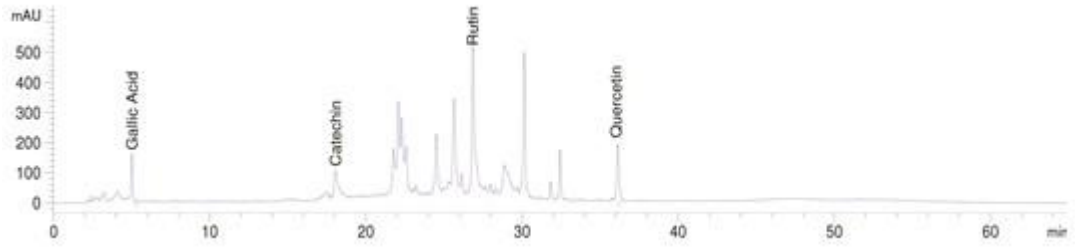

FIGURE 6: HPLC chromatogram of methanol extract from fresh Madeni rose petals

CONCLUSION

The results obtained from this study clearly indicated that fresh Madeni rose petals extracts contain phenolic and flavonoid compounds. These compounds might be helpful in reducing the risk of the different diseases associated to the oxidative stress. Thus, the addition of rose petals extracts to food, drinks cosmetic and pharmaceutical products can act as a non-caffeine source of natural antioxidants.

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