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Total Phenolics and Antioxidant Activity of Defatted Fresh Taif Rose, Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between all authors. ESA designed the study, wrote the protocol and managed the analyses of the study. SAB and MMS performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Recently, the residues or by-products of agriculture industry take attention for their valuable source of natural antioxidants. Taif rose (*Rosa damascena trigintipetala* Dieck) is considered one of the most important economic products of Taif, Saudi Arabia. In this study, the antioxidant and total phenolic contents of the defatted flowers of fresh Taif rose were investigated. The antioxidant activity was chemically determined using three methods; 1,1-diphenyl picrylhydrazyl (DPPH.) free radical scavenging activity, phosphomolybdenum method and reducing power activity. The methanol extract showed antiradical activity with SC_{50}=49.44 μg/ml. Different fractions obtained from successive fractionation of the methanol extract with organic solvents of different polarities; chloroform ($CHCl_3$), ethyl acetate (EtOAc) and *n*-butanol (n-BuOH); showed that, the EtOAc fraction had higher antiradical activity than *n*-BuOH fraction, and their respective SC_{50} were 15.62 and 36.29 μg/ml. On the other hand, $CHCl_3$ fraction had a poor antiradical activity (SC_{50}>100 μg/ml). The total phenolics, flavonoids, flavonols and RP-HPLC analysis of the methanol extract and its EtOAc and *n*-BuOH fractions were determined. The ethyl acetate fraction had the highest contents of phenolics, flavonoids

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and flavonols that correlated with the antioxidant activity. From this work, it is obvious that the by-product from concrete oil production of Taif rose could be used as a good inexpensive source of antioxidant polyphenolics.

Keywords: Taif rose; antioxidant; total phenolics; flavonoids.

1. INTRODUCTION

Free radicals are highly reactive oxygen species; superoxide (O_2.-), hydroxyl ($OH^{\cdot}$), peroxyl ($ROO^{\cdot}$), peroxinitrite ($^{\cdot}ONOO^{-}$) and nitric oxide ($NO^{\cdot}$) radicals; produced through the oxidative process within the mammalian body, biologically important materials (e.g., lipids, foods and oils) and industrially important products (e.g., rubber and lubricant) (Atta-ur-Rahman and Choudhary, 2001). Under certain conditions, the excess of free radicals and reactive oxygen species have been proposed to induce cellular damage and to be involved in several human diseases such as cancer, arteriosclerosis, inflammatory disorders as well as in ageing process. In recent years, several dietary and herbal formulations that have free radical scavenging potential have gained attention in treating such chronic diseases (Wang et al., 2005; Tiwari and Tripathi, 2007). In spite of the strong radical scavenging activity of synthetic antioxidant, they usually have side effects thus the interest in finding natural antioxidants, without undesirable side effects, has increased greatly. The antioxidative phytochemicals especially phenolic compounds found in vegetables, fruits and medicinal plants have received increasing attention for their potential role in prevention of human diseases (Cai et al., 2004). Recently, the residues or by-products of agriculture industry take attention for their valuable source of natural antioxidants (Ignat et al., 2011). Humans can use antioxidants either as dietary, food supplement or as a drug.

Roses are the important ornamental plants and have been referred to as the queen of flowers. Over 150 rose species and more than 2000 cultivars have been registered and distributed in Europe, Asia, Middle East, and North America (Cai et al., 2005). Members of the Rosaceae family have long been used in perfumes, cosmetics, foods and for medicinal purposes. The physiological functions of Rosaceae may be partly attributed to their abundance of phenolics (Ozkan et al., 2004; Liu et al., 2010).

Taif rose, (*Rosa damascena trigintipetala* Dieck), a sort of damask rose that is considered as one of the most important economic products of Taif. It's essential oil has an excellent reputation as a perfume. In this study and at a laboratory scale, the authors investigated the antioxidant activity and total phenolic contents of methanol extract and its fractions ($CHCl_3$, EtOAc and *n*-BuOH) of Taif rose by-product after obtaining rose concrete oil by extraction with n-hexane.

2. MATERIALS AND METHODS

2.1 Materials and Chemicals

All solvents and reagents were analytical grade. 1,1-diphenyl picrylhydrazyl $(DPPH)^{\bullet}$ free radical and Folin–Ciocalteu's reagent (FCR) were from Fluka Chemicals. Aluminum chloride, sodium carbonate, sodium phosphate, ammonium molybdate, rutin, quercetin, ascorbic acid, gallic acid and all solvents were from Sigma-Aldrich chemicals. The absorbance

measurements of antioxidant activity were recorded using the UV–Vis spectrophotometer Jenway 6405.

2.2 Collection and Preparation of the Plant Materials

The fresh roses were collected from a rose farm (April 2011) in Taif governorate, Kingdom of Saudi Arabia. The green parts of roses were removed and the remains were cut into small pieces.

2.3 Preparation of Methanol Extract and its Successive Fractions

The fresh cut roses (250 g) were defatted by soaking in 1500 ml n-hexane for one week at room temperature with shaking from time to time followed by filtration. This extraction was repeated four times. The *n*-hexane was removed in vacuo using rotatory evaporator. The rose residues were soaked again in a pure methanol (2000 ml) for one week at room temperature with shaking followed by filtration. This extraction was repeated four times. The methanol was removed under vacuum affording methanol extract.

Twenty grams of methanol extract was dissolved in 100 ml distilled water and then successively partitioned with chloroform, ethyl acetate and finally with *n*-butanol (3 x 120 ml solvent) affording known weight of each respective fraction. The methanol extract and its successive fractions were stored in dark bottles and become ready for investigation.

2.4 Antioxidant Activity

Three different chemical methods were used for the evaluation of the antioxidant activity of crude methanol extracts and its successive fractions; 1,1-diphenyl picrylhydrazyl scavenging activity, phosphomolybdenum method and reducing power assay. These assays were performed as described by Abdel-Hameed, 2009.

2.4.1 Scavenging ability towards 1,1-diphenyl picrylhydrazyl (DPPH)$^{\bullet}$ radical

2 ml of different concentrations of each sample were added to 2 ml solution of 0.1 mM DPPH. An equal amount of methanol and DPPH served as control. After 20 min of incubation at 37ºC in the dark, the absorbance was recorded at 517 nm. The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ DPPH radical scavenging activity (SC)} = [1- (A_{sample}/A_{control})] \times 100$$

Where A_{sample} and $A_{control}$ are absorbance of the sample and control. The SC_{50} (concentration of sample required to scavenge 50% of DPPH radicals) values were also determined.

2.4.2 Determination of the total antioxidant capacity by phosphormolybdenum method

300 µl of each sample solution or ascorbic acid (100 µg/ml) were combined with 3 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). A typical blank solution containing 3 ml of reagent solution and an appropriate volume of the same solvent was used for the sample. All tubes were capped and incubated in a boiling-water bath at 95ºC for 90 min. After the samples were cooled to room temperature, the

absorbance of the solution of each sample was measured at 695 nm against the blank using a UV/Vis spectrophotometer. The experiment was performed in triplicates. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

2.4.3 Reducing power assay

2 ml of each sample or ascorbic acid in methanol (200 µg/ml) were mixed with 2 ml of sodium phosphate buffer (0.2M, pH 6.6) and 2 ml of 1% $K_3Fe(CN)_6$ and incubated at 50ºC for 20 min. After adding 2 ml of trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant solution (2 ml) was taken out and immediately mixed with 2 ml of methanol and 0.5 ml of 0.1% ferric chloride. After incubation for 10 min, the absorbance against the blank was determined at 700 nm. Triplicates were made for each tested sample and ascorbic acid. The increase in absorbance of the reaction mixture indicates an increased reduction power. The reducing power activity was expressed as the number of equivalents of ascorbic acid.

2.5 Phytochemical Analysis

Previous phytochemical reports on Rosa species showed that the phenolic compounds constitute the major components of them (Jassbi et al., 2003; Mikanagi et al., 1995; Kumar et al., 2006 & 2009; Ercisli, 2007). In this study, the total phenolic, flavonoid and flavonol contents of methanol extract and each active fraction were measured according to the methods described by Abdel-Hameed, 2009.

The total phenolic content of plant extracts was determined using Folin-Ciocalteu's reagent (FCR). 100 µl of each sample solution (100 µg/ml) and also 100 µl of gallic acid (100 µg/ml) were mixed with 500 µl of the FCR and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined against a blank that contained all reagents without the samples or the gallic acid at the same conditions. All determinations were carried out in triplicates. The total phenolic content was expressed as the number of equivalents of gallic acid (GAE).

The flavonoids content was determined by aluminium chloride method using rutin as a reference compound. 100 µl of each sample solution (1 mg/ml) was mixed with 100 µl of 2% aluminum trichloride in ethanol and a drop of acetic acid, and then diluted with ethanol to 5 ml. The absorption at 415 nm was read after 40 min. Blank was prepared from all reagents without the samples. The absorption of the standard rutin solution (100 µg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula:

$$X = (A - m_o)/(A_o - m).$$

Where X is the flavonoid content, mg/mg plant extract in RE, A is the absorption of plant extract solution, A_o is the absorption of the standard rutin solution, m is the weight of plant extract (mg) and mo is the weight of rutin in the solution (mg).

The content of flavonols was determined by using quercetin as a reference compound. 1 ml of each sample solution (1 mg/ml) was mixed with 1 ml aluminium trichloride (20 mg/ml) and 3 ml sodium acetate (50 mg/ml). The absorbance at 440 nm was read after 2.5 h. The

absorption of the standard quercetin solution (0.5 mg/ml) in methanol was measured under the same conditions. All determinations were carried out in triplicates. The amount of flavonols in plant extracts in quercetin equivalents (QE) was calculated by the same formula used in flavonoids:

$$X = (A - m_o)/(A_o - m).$$

Where X is the flavonols content, mg/mg plant extract in QE, A is the absorption of plant extract solution, A_o is the absorption of the standard quercetin solution, m is the weight of plant extract (mg) and m_o is the weight of quercetin in the solution (mg).

2.6 RP-HPLC-UV Analysis of some Phenolics

2.6.1 Apparatus and chromatographic conditions

Reversed phase HPLC analysis was done using a system consisting of two pumps (Waters 515), UV detector (Waters 486 at 280 nm), Rheodyne injector, CBM-20A communication bus module, Waters automated gradient controller, reversed phase Symmetry C18 column (5 µm, 4.6×150 mm) from Waters and Empower software. 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 (FA) in water) and B (0.1% FA 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).

2.6.2 Preparation of standard and sample solutions

Standard stock solutions of gallic acid (500 µg/ml), catechin (400 µg/ml), quercetin-3-glucose-6-gallic acid (200 µg/ml), rutin (500 µg/ml), quercetin-3-glucose (200 µg/ml), kaempferol-3-glucose (200 µg/ml), quercetin (500 µg/ml) and apigenin (200 µg/ml) were prepared in HPLC grade of 50% acetonitrile/water and filtered using membrane disc filter (0.45 µm). First, chromatography was done separately for each standard compound using the previous analytical condition. The retention time of each compound was determined. From each individual standard stock solution, a mixed stock solution containing eight analytes were prepared and diluted to appropriate different concentrations for establishing calibration curves. For samples, stock solution (2.5 mg/ml) of the methanol, EtOAc and n-BuOH fractions were prepared in HPLC grade of 50% acetonitrile /water and filtered using membrane disc filter (0.45 µm).

2.6.3 Calibration curve and sample analysis

For quantitative analysis, six different concentrations of a mixed stock solution containing eight analytes were injected. By Empower software, a calibration curve for each compound was obtained by plotting the peak areas versus the concentration of each analyte. Chromatograms of samples obtained were analyzed using the Empower software based on comparing retention times of sample with those of the standards for qualitative analysis and calibration curve for quantitative analysis.

2.7 Statistical Analysis

All determinations in tables 1 and 2 were carried out in triplicates and the values are mean ± standard deviation. The statistical analyses were carried out using SPSS 13.0 and Microsoft Excel programs.

3. RESULTS AND DISCUSSION

Amongst fruits, vegetables and different herbs, agricultural and industrial residues (by-products) are attractive sources of inexpensive natural antioxidants. By-products, remaining after processing fruits and vegetables in the food-processing industry, still contain a huge amount of phenolic compounds. Some studies have already been done on by-products of berry skins, olive mill wastes, citrus, tomatoes, artichoke, grape, cauliflower, carrot, celery and onion which could be potential sources of antioxidants (Ignat et al., 2011; Volf and Popa, 2004; Volf et al., 2006).

One of the famous products of rose is concrete and absolute oils. The concrete oil was prepared by extraction of the fresh rose petals by n-hexane. In countries, produced concrete oil like Turkey and Belgium there is a big amount of by-product. In the present experiment and at a laboratory scale, the residues of Taif rose after concrete oil production was extracted with methanol and tested for their antioxidant and total phenolic contents. As known, the general strategy of natural products drug discovery from natural resources started with a preliminary bioassay screening of its crude extracts followed by bioassay guided fractionation, isolation and structure elucidation of the bioactive compounds. In this work, fractionation of the methanol extract was done using partition successive fractionation between aqueous methanol extract and three organic solvents with different polarities; chloroform, ethyl acetate and *n*-butanol. These fractions were estimated for their antioxidant properties and total phenolic contents.

3.1 Antioxidant Activity

It is important to select and employ a stable and rapid method to assay antioxidant activity, because the determination of many samples is time-consuming. Several methods have been developed to assay free radical scavenging capacity and total antioxidant activity of plant extracts. In this work, three methods were used; 1,1-diphenyl picrylhydrazyl scavenging activity, phosphomolybdenum method and reducing power activity.

3.1.1 Free radical scavenging activity by $DPPH^{\cdot}$

The model of scavenging of the stable DPPH radicals is a widely method to evaluate the antioxidant activity of the investigated sample in a relatively short time compared with other methods. This method depends on the reduction of purple DPPH radicals by antioxidant agents to a yellow coloured diphenyl-picrylhydrazine and the remaining DPPH radicals that show maximum absorption at 517 nm were measured. The decrease of absorbance of DPPH solution indicates an increase of the DPPH radical scavenging activity (Kumaran and Karunakaran, 2005).

The methanol extract of defatted fresh Taif rose (Table 1) exerted radical scavenging activity on $DPPH^{\cdot}$ with SC_{50}= 49.44± 0.38 μg/ml. The ethyl acetate fraction obtained from successive fractionation of methanol extract showed higher radical scavenging activity than the *n*-

butanol fraction (SC_{50}= 15.62±0.18 and 36.29±0.15 μg/ml respectively) whereas the $CHCl_3$ fraction had a poor antiradical activity (SC_{50} > 100 μg/ml).

3.1.2 Antioxidant capacity by phosphomolybdenum method

Table 1 shows that the ethyl acetate fraction has higher antioxidant capacity (498.7±8.33 mg ascorbic acid equivalent/g dry extract) than the *n*-butanol fraction (238.7±7.51 mg ascorbic acid equivalent/g dry extract) and crude methanol extract (193.3±7.51 mg ascorbic acid equivalent/g dry extract).

3.1.3 Reducing power activity

Data of reducing power activity of the methanol extract and the ethyl acetate and *n*-butanol fractions in Table 1, shows that the ethyl acetate fraction has the highest activity (555±8.75 mg equivalent to ascorbic acid /g extract) followed by the methanol extract and *n*-butanol fraction with 393±15.15 and 358±8.75 mg ascorbic acid equivalent /g extract respectively.

Table 1. Free radical scavenging activity, total antioxidant capacity and reducing power activity of methanol extract and its $CHCl_3$, EtOAc and n-BuOH successive fractions of defatted fresh Taif rose

Extract	DPPH free radical scavenging activity SC_{50} [~g/ml][a]	Total antioxidant capacity [mg ascorbic acid equivalent /g extract][b]	Reducing power activity [mg ascorbic acid equivalent /g extract][c]
MeOH	49.44± 0.38	193.3±7.51	393±15.15
$CHCl_3$	>100	NT	NT
EtOAc	15.62± 0.18	498.7±8.33	555±8.75
n-BuOH	36.29±0.15	238.7±7.51	358±8.75

Values of SC_{50}, total antioxidant capacity and reducing power activity are expressed as mean of triplicate determinations ± standard deviation.
[a] SC_{50}, Concentration in µg /ml required scavenging the DPPH radical (100 µg /ml) by 50%.
[b] Antioxidant capacity monitored by the phosphomolybdenum method expressed as mg ascorbic acid equivalent /g extract.
[c] Reducing power activity expressed as mg ascorbic acid equivalent /g extract.
[NT] not tested.

3.2 Phytochemical Analysis

Table 2 shows that the ethyl acetate fraction contains high total phenolic contents equivalent to 343.19±11.83 mg/g GAE whereas the n-butanol fraction and crude methanol extract showed the lowest content equivalent (98.62±9.04 and 53.25± 5.92 mg/g GAE respectively). Table 2 showed the total flavonoids that are the major class phenolics and their major subclass total flavonols. The ethyl acetate fraction showed the highest content (300.82±11.86 mg/g RE for flavonoids and 115.31±2.38 mg/g QE for flavonols) whereas the *n*-butanol fraction and crude methanol extract showed lower contents equivalent to 53.91±3.11 and 31.27±1.88 mg/g RE for flavonoids and 31.60±1.13 and 26.27±0.98 mg/g QE for flavonols respectively.

Table 2. Total amount of phenolic, flavonoid and flavonol compounds of methanol extract and its EtOAc and *n*-BuOH successive fractions of defatted fresh Taif rose

Extract	Total phenolics (mg gallic acid equivalent/ g extract)[a]	Total flavonoids (mg rutin equivalent/ g extract)[b]	Total flavonols (mg quercetin equivalent / g extract)[c]
MeOH	53.25± 5.92	31.27±1.88	26.27±0.98
EtOAc	343.19±11.83	300.82±11.86	115.31±2.38
n-BuOH	98.62±9.04	53.91±3.11	31.60±1.13

Values of total phenolics, flavonoids and flavonols are expressed as mean of triplicate determinations ± standard deviation

[a]Total phenolics expressed by mg gallic acid equivalent /g extract.

[b]Total flavonoids expressed by mg rutin equivalent /g extract.

[c]Total flavonols expressed by mg quercetin equivalent/g extract.

The presence of eight standard phenolic compounds; gallic acid, catechin, quercetin-3-glucose-6-gallic acid, rutin, quercetin-3-glucose, kaempferol-3-glucose, quercetin and apigenin; in methanol extract and its EtOAc and n-BuOH successive fractions were done by comparing the HPLC chromatograms of them with the HPLC chromatograms of standard compounds based on the retention time at the same conditions (Figs. 1-4). The quantity of each compound in the fractions was estimated from the calibration curve for each component in the standard mixture. Table 3 shows that four compounds were identified in the defatted methanol extract fraction: rutin (9.55±0.09 mg/g), kaempferol-3-glucose (8.19±0.22 mg/g), quercetin (7.75±0.44 mg/g) and quercetin-3-glucose (6.73±0.27 mg/g); whereas the other compounds were found in traces (gallic acid; catechin and quercetin-3-glucose-6-gallic acid), or not detected (apigenin). Six phenolic compounds were identified in the ethyl acetate fraction in which the rutin (68.86±13.27 mg/g), quercetin (65.27±8.24 mg/g) and kaempferol-3-glucose (61.95±5.50 mg/g) had the highest amount whereas quercetin-3-glucose (32.87±4.38 mg/g) was found in a moderate amount. Gallic acid and catechin were found in low amounts (10.01±0.06 and 17.78±0.08 mg/g respectively) whereas the apigenin was not detected. Three compounds were detected in the *n*-butanol fraction in low amount: rutin (14.36±0.20 mg/g), quercetin-3-glucose (18.25±2.31 mg/g) and kaempferol-3-glucose (13.57±3.01 mg/g) whereas the other compounds were not detected or found in traces. The HPLC chromatograms of the methanol extract of fresh defatted Taif rose and previous reports of other Rosa species are relatively related (Kumar et al., 2008 & 2009; Hutsebaut, 1993). Therefore, this study would encourage us to carry out more detailed phytochemical studies in the future.

From the above results the antioxidant activity of the methanol extract and its successive fractions; ethyl acetate and *n*-butanol; based on DPPH scavenging activity, phosphomolybdenum methods and reducing power activity are attributed to the presence of phenolic compounds as major components in this species and there is positive correlation between the antioxidant activity and the total phenolics, flavonoids and flavonols.

Plant phenolics constitute one of the major groups of compounds acting as a primary antioxidant or free radical terminators. Phenolic compounds such as flavonoids, phenolic acids and tannins are considered the major contributor to the antioxidant activity of vegetables, fruits or medicinal plants. The antioxidant activity of the phenolic compounds was attributed to its redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers and have also metal chelating properties (Rice-Evans et al., 1996; Heim et al., 2004; Abdel-Hameed, 2009).

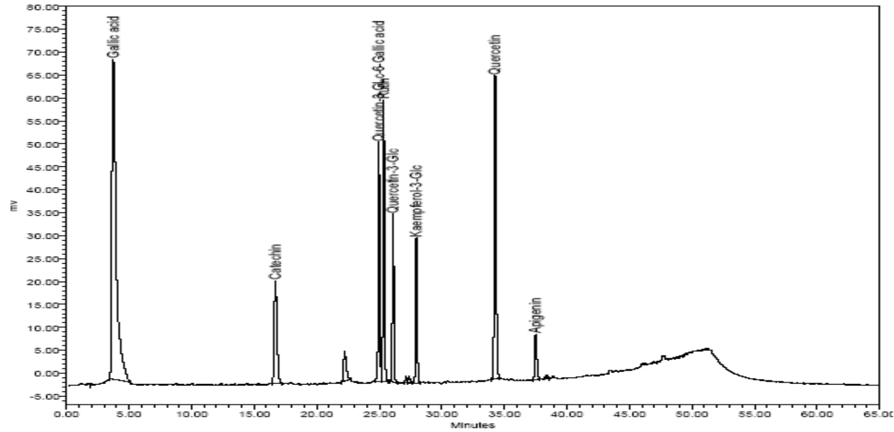

Fig. 1. HPLC chromatogram of some standard phenolic compounds

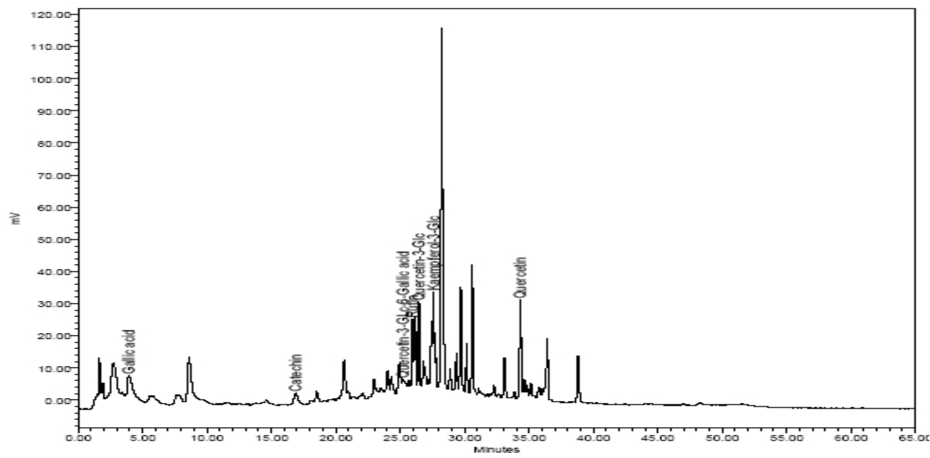

Fig. 2. HPLC chromatogram of methanol extract from defatted fresh Taif rose

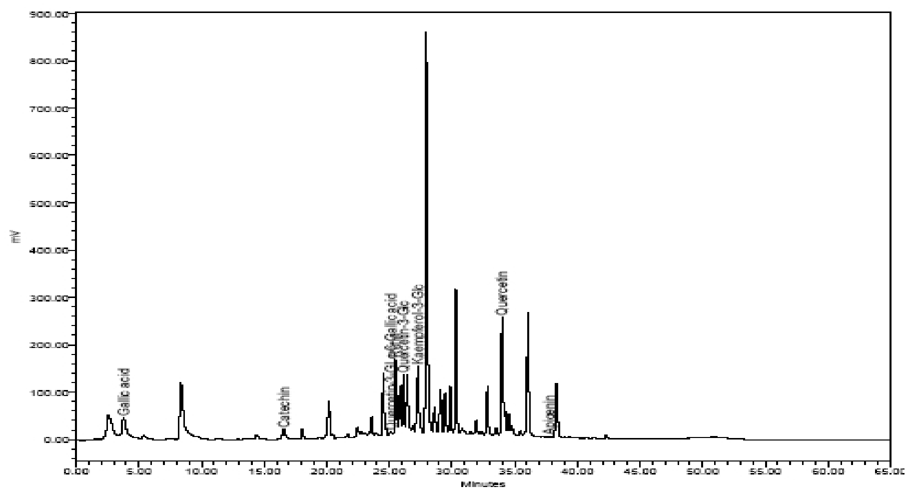

Fig. 3. HPLC chromatogram of ethyl acetate fraction

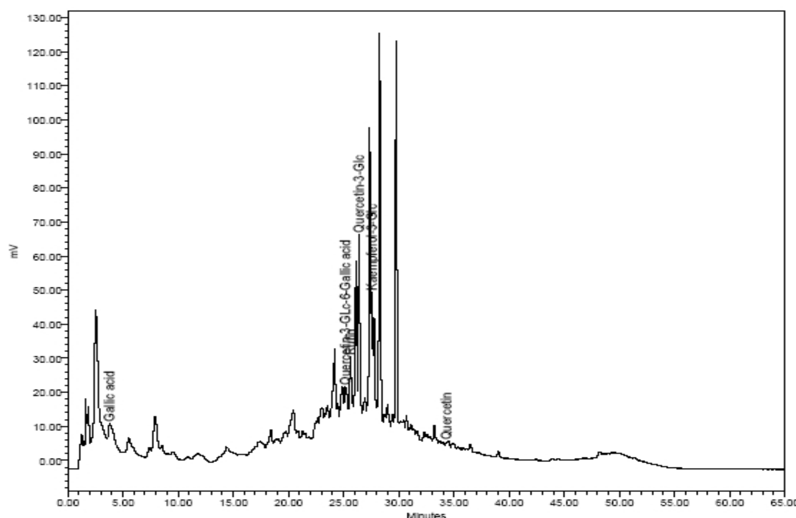

Fig. 4. HPLC chromatogram of *n*-butanol fraction

Table 3. Quantity of some phenolic compounds (mg/g extract) identified in methanol extract and its EtOAc and n-BuOH successive fractions of defatted fresh Taif rose

Phenolic compounds	MeOH extract	EtOAc fractior	*n*-BuOH fraction
Gallic acid	tr	10.01±0.06	tr
Catechin	tr	17.78±0.08	tr
Quercetin-3-glucose-6-gallic acid	tr	tr	tr
Rutin	9.55±0.09	68.86±13.27	14.36±0.20
Quercetin-3-glucose	6.73±0.27	32.87±4.38	18.25±2.31
Kaempferol-3-glucose	8.19±0.22	61.95±5.50	13.57±3.01
Quercetin	7.75±0.44	65.27±8.24	ND
Apigenin	ND	ND	ND

Values are expressed as mean of duplicate determinations ± standard deviation.
[tr] traces < 5 mg/g extract.
[ND] not detected.

4. CONCLUSION

The results of this laboratory scale study provide evidence that the residues of Taif rose obtained after concrete oil production possesses radical scavenging activity, antioxidant capacity and reducing power activity. Phenolic compounds, especially flavonoids and its subclass flavonols are the major components and the antioxidant properties were attributed to them. Therefore, the by-products from concrete oil production could be used as a good inexpensive source of antioxidant polyphenolics.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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