Pak. J. Bot., 55(3): 995-999, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-3(27)

FLAVONOID DETECTION AND ANTIOXIDANT ESTIMATION IN *ROSA DAMASCENA* MILL. GROWING IN TAIF AND AL-MADINAH CITIES, SAUDI ARABIA

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Abstract

The genus *Rosa* L. belongs to the important family Rosaceae. Some taxa in Rosaceae produce delicious and beneficial fruits and some are good source of wood. In Saudi Arabia, *Rosa* L. is represented by different species, one of which is *Rosa damascena* Mill. *R. damascena* grows in many localities in Saudi Arabia. The most popular cities in growing rose are Taif and Al-Madinah Al-Munawarah. There are some differences between the taxa growing in both localities. Some refers to the chemical components and some are regarding to their harvesting time. This work aimed to find scientific phytochemical informations about both roses. In this paper the phenolic and antioxidant compounds were investigated and recorded in taxa from both locations. Paper chromatography, thin layer chromatography and High-performance chromatography were used to detect the flavonoids compounds and to give the antioxidant estimation. Quercetin and Letulin were the main agylcons presented in Rosa. Qercetin represents the flavonol group while Letulin represents the flavones one. Some of their glycosides are common in both Taif and Al-Madinah roses such as: Quercetin-3- arabinoside, Quercetin-7-glucoside and Apigenin-7-rhamnoglucoside. On the other hand, some flavonoids are restricted to the plants from one area only. For example, Gossyptin-3-monogalactoside was observed in Al-Madinah samples, while it was undetected in Taif samples. In contrast Quercetin-3-rhamnoside, Quercetin-3-monorhamnoside and Kaempferol-3-monoglucuronide were detected in Taif samples only. Finally, the antioxidant estimation of both localities showed that the Flavonoids content in Almadinah Rose leaves were very much greater than Taif Rose leaves.

Key words: Chromatography Rosaceae, Phytochemical constituents, HPLC, PC, TLC, Phenolic compounds.

Introduction

Rose belongs to the great family Rosaceae. It is one of the most important families in dicotyledons. Rosaceae follows the order Rosales. The later has nine families, 261 genera and 7,700 species (Heywood, 2007). The top four large families of Rosales are Rosaceae, Urticaceae, Moraceae and Rhamnaceae. Rosaceae is the 19th largest family of plant kingdom because it consists of about 95 to more than one hundred genera and 2830-3100 species (Kellerhals, 2009). It is distributed throughout the world, being especially common in North America, Europe and Asia. The habit in Rosaceae has great variations. Mostly, they are herbaceous and wild, but many are grown for their fruit or as ornamentals. The major genera in Rosaceae according to Singh (2004) are Rubus (750 species), *Potentilla* (500), *Prunus* (430), *Crataegus* (240), *Cotoneaster* (230), *Sorbus* (230), *Rosa* (225), *Alchemilla* (220), *Spiraea* (100) *Pyrus* (60), *Malus* (55), *Geum* (40) and *Fragaria* (15). According to Abdel-Hameed *et al.*, (2013) the genus *Rosa* L. has more than 150 species and can be found in Europe, Asia, Middle East and North America.

Rosa damascena is one of the most important species belong to the genus *Rosa* L. Before the 16th century it was introduced from the Middle East into Western Europe and was re-introduced later in the 16th century (Rusanov *et al*., 2005).

It is economically crucial and it has a significant important as it is been used in traditional medicine (Akram *et al.*, 2019). Furthermore, Damask Rose can be an effective source of antioxidants, such as phenolics, flavonoids, carotenoids, and anthocyanins. The inhibitory activity of phenolic compounds is due to their acting as reducing agents and donors of hydrogen (Alizadeh & Fattahi, 2021).

The most famous cultivation areas for growing *R. damascena* in Iran are Kashan, Shiraz, Fars, Meshed and Azerbaijan, while Isparta, Burdur, Denizli, Afyonkarahisar are well known in Turkey and the Kazanlak valley in Bulgaria (Ginova *et al*., 2012).

In Saudi Arabia Rosaceae is represented by seven genera and nine species, *Alchemilla cryptantha Steudel* ex A Rich., *Cotoneaster nummularia* Fischer & C. Meyer, *Crataegus sinaica* Boiss., *Potentilla dentata* Forssk.,*Prunus arabica* (Oliv.) Meikle, *Prunus korshinskyi* Hand.- Mazz., *Rosa abyssinica* Lindley, *Rubus asirensis* D.F. Chamb. and *Rubus sanctus* Schreber (Thomas, 2011). Taif and Al-Madinah are the most popular areas for growing this Rose. Thus, the studying areas were Al-Madinah, which belongs to Al-Madinah region and Taif, which belongs to Makkah region in the Kingdom of Saudi Arabia.

Even though Taif Rose is very famous and popular, Al-Madinah Rose is also known for its fragrance and usefulness. According to local people, the quality and the price of Taif Rose's oil is different than Rose's oil from other places in the world. Also, the Taif Rose is used mainly for oil and water extraction, but Al-Madinah Rose is mainly used for ornamentation and drinking with tea. There is another difference, which is that the Rose of Al-Madinah city is harvested throughout the year and available in all four seasons, but less in the winter. Whereas, Taif Rose is harvested in a certain period in the spring, between March and April only (Anon., 2018). This work aimed to find scientific phytochemical information about the differences between both Roses especially

Flavonoid detection and Antioxidant estimation in *R. damascena* growing in Taif and Al-Madinah cities, Saudi Arabia regarding to the differences between both Roses in order to reserve this species and improve its quality in Saudi Arabia and the world.

Material and Methods

Study area: Al-Madinah Al-Munawarah is located in the north-western part of Saudi Arabia, to the east of the red sea. It is at longitude 39°36' east and latitude 24°28' north. This city is 620 meter (2,046 ft) above sea level (Imaratalmadinah, 2012). The climate in Al-Madinah generally is very hot, in summer, but it's moderate in autumn and spring. The winter in Al-Madinah is mild cold. Also, the length of the day is varying. The total number of hours of sunshine during the year in Almadinah is 3134 h/y. Almadinah affected by the Red Sea in terms of humidity, fog and low temperatures. The Rain is irregular and varies but, increases in spring (Tulbah, 2002).

Taif City is located in the west part of Saudi Arabia between longitude 40°42' E and latitude 20°22' N with a height of 1700 to 2500 meter above the sea level (Anon., 2013). The climate in Taif Generally, is characterized as moderated in summer and cold in winter, with low humidity and the rain full annually is about 16 milliliters. The total number of hours of sunshine during the year is 3030 h/y (Tulbah, 2002).

Sample collection: *Rosa damascena* samples were collected during flowering time (between March–April (2016) from Alhada area in Taif and Abyar Almashi in Al-Madinah. There were at least three replicates for each study. Some samples were pressed and kept in the herbarium of King Abdulaziz University-girls Section for herbarium vouchers (Yaslam 9,10). Others were prepared for Phytochemicals studies, older leaves were collected and dried in fresh air for extractions. Dried leaves were used for phytochemical studies. The samples were extracted following the techniques of Harborne (1984) and Markham (1982) as following.

Preparation of samples

For Paper Chromatography (PC) and Thin Layer Chromatography (TLC): Dried leaves of each sample were put in test tubes with 80% Methanol, heated at 80-85˚C in a block heater for about 10- 15 minutes to aid extraction and destroy the enzymes. Then, the tubes were taken out and left over night. The solvent then was decanted in a watch glass and evaporated to dryness in a fume cupboard. The Samples extracts were divided into two, one was kept for PC and the other was used to make the acid hydrolysis for TLC.

For High Performance Liquid Chromatography (HPLC): The dried leaves were powdered to fine particles using porcelain mortar. Into 15-mL screw capped glass test tube, a weight of 125 mg of leaves powder was mixed with 5 mL methanol. This mixture was vortexed for 2 min, sonicated for 45 min and filtered through 0.22 µ PTFE syringe membrane filter to a clean dry glass test tube. The clear extract was dried with nitrogen gas. This residue was mixed with 700 µL water, 200 µL 10M HCl, vortexed for 1 min, heated at 90°C for 1h, cooled, mixed with 100 µL 15M ammonia solution, vortexed for 30 sec and a volume of 10 µL was injected for HPLC-UV analysis.

Two-dimensional Paper Chromatography (PC): This was used to detect the Flavonoid glycosides. The first extract was re-dissolved in 80% MeOH and run using 2-D paper chromatography (PC) with BAW (n-butanol 4: acetic acid 1: water 5) in the first dimension and then 15% HOAc (acetic acid) in the second dimension. Rutin was spotted as a marker with each sample.

All chromatogram papers were air-dried and examined under UV at 360nm. The relative R_f value for each spot in each solvent was calculated according to the following equation:

$$R_f = \frac{\text{The distance travelled by compound on the chromatogram}}{\text{The distance travelled by solvent on the chromatogram}} \times 100$$

The R_f and color were recorded before and after fuming with ammonia NH_4OH and compared with R_f value from some references.

Thin Layer Chromatography (TLC): This step was used to identify the Flavonoid algycones, The Second Sample extract was used for the acid hydrolysis as follows: 2-3 ml of 2M hydrochloric acid (HCl) was added to the extract and heated in a boiling water bath for 30 minutes. After cooling down, it was decanted into separate funnel and extracted with an equal volume of ethyl acetate. The ethyl acetate layer was left to dry in a fume cupboard. Then it was re-dissolved in 100% MeOH and run on cellulose thin-layer chromatography (TLC) in CAW (Chloroform 10: HOAc 9: H2O 1) and in 50% HOAc. The R_f and colours under UV (365nm) were recorded to be compared with R_f from references.

High Performance Liquid Chromatography (HPLC): The major constituent of Flavonoids exist as conjugated form with sugar. All Flavonoids were converted to quercetin by hydrolysis with hydrochloric acid. The Liquid Chromatography system was operated isocratically using a mobile phase consisting of acetonitrile: water containing 0.1% acetic acid (45: 55, v/v), at a flow rate of 0.8 ml/min. Injection volume was 10 µL. Quantitation was performed at maximum wavelength of 360 nm.

The calibration System suitability solution, 50 ng/µl was injected 7 repetitive times. All separation parameters were calculated. The Relative Standard Deviation (RSD) values were calculated. The concentration of total flavanols was calculated as mg of quercetin per gram powder of leaves powder from the calibration curve. The obtained results expressed as ng/µl = µg/mL, then multiplied by 8 to get the concentration of flavanols as µg per gram plant powder. Each plant sample was extracted and analyzed three times.

Results

Each spot in Paper Chromatography (PC) and Thin Layer Chromatography (TLC) were numbered and R_f values and colors were recorded as shown in (Tables 1-3). According to the R_f values and colors obtained from TLC, two compounds were identified, which are Quercetin with R_f 12.5 in CAW and 32 in 50% Acetic Acid, by Dark color under UV and then Yellow under UV + Ammonia. Also, there was Letulin with R_f 20 in CAW and 41.9 in 50% Acetic Acid, by Dark color under UV and then Yellow under UV + Ammonia. Some compounds represented in both plants in PC were identified as: Quercetin-3- arabinoside, Quercetin-7-glucoside and Apigenin-7-rhamnoglucoside. On the other hand, some compounds were detected in one plant and not in the other. For example, Gossyptin-3-monogalactoside was observed in Almadinah samples, while it was undetected in Taif samples. In contrast Quercetin-3-rhamnoside, Quercetin-3-monorhamnoside and Kaempferol-3-monoglucuronide were detected in Taif samples only. Additionally, there are eight more unidentified spotted compounds by PC in both samples.

In HPLC Results, the intact free quercetin was detected in non-hydrolyzed samples at very low concentrations. After the hydrolyzation, the average free quercetin found in Almadinah Rose leaves was 3177.15 µg/g of plant powder and the average of Flavonoid was 168.34 µg/g, with total Flavonoid 3345.49 µg/g, while in Taif Rose leaves the free quercetin was 693. µg/g of plant powder and the average of Flavonoid was 43.51 µg/g, with total Flavonoid 736.94 µg/g. The results show that the Flavonoids in Almadinah Rose are very much greater than Taif Rose as shown in (Table 4).

Table 1. Compounds appeard in the Paper Chromatography and their Rf values in Al-Madinah samples.

#	BAW	H2O	15%Ac OH	UV Color	UV+ Ammonia
1.	17.6	20	40	Dark	Yellow
2.	33	10	5	Yellow	Yellow
3.	74	30	34	Dark	Yellow
4.	71	49	23.5	Dark	Yellow orange
5.	40	9	-	Dark	yellow
6.	33	17.6	-	Light purple	F. green
7.	10	22	-	Dark yellow	.....
8.	34	-	41	Orange	Yellow
9.	60	-	53	Dark	Yellow
10.	49	-	38	Dark purple	Yellow
11.	50	-	36	Yellow	.....
12.	23	-	40	Dark	Yellow

Table 2. Compounds appeared in the paper chromatography and their Rf values in Taif sampleas.

#	BAW	H2O	15%AcOH	UV Color	UV +Ammonia
1.	0.35	-	0.060	Dark	Yellow
2.	0.34	0.095	0.045	Yellow	Yellow
3.	0.31	0.086	-	Yellow	Bright yellow
4.	0.39	0.22	-	Bright yellow	Bright yellow
5.	0.33	0.02	-	Bright yellow	Yellow
6.	0.72	-	0.34	Dark	Yellow
7.	0.71	0.22	0.49	Dark	Orange
8.	0.34	-	0.41	Orange	Yellow
9.	0.6	-	0.53	Dark	Yellow
10.	0.49	-	0.38	Dark purple	Yellow
11.	0.5	-	0.36	Yellow	.....
12.	0.23	-	0.4	Dark	Yellow
13.	0.147	0	-	Dark	Dark
14.	0.68	0.15	-	Yellow	Yellow
15.	0.74	0.22	-	Bright green	Yellow orange
16.	0.73	0.43	-	Yellow	Orange
17.	0.60	0.69	-	Greenish yellow	Br. green yellow
18.	0.59	0.23	-	Bright yellow	Orange
19.	0.53	0.68	-	Yellow	Bright yellow
20.	0.67	0.83	-	Yellow	Orange
21.	0.37	0.39	-	Dark	Purple
22.	0.5	0.6	-	Dark orange	Orange yellow

Table 3. Total detected compounds in Al-Madinah and Taif Roses samples with their Rf values and colours.

Compound	BAW	15% Ac.Ac	H2O	UV	UV + NH3	Almadinah	Taif
				Flavonol s glycosides			
Quercetin-3-arabinoside	73	34	30	Dark	Yellow	∏	∏
Quercetin-7-Glucoside	34	5	1	Yellow	Yellow	∏	∏
Quercetin-3-Rhamnoside	71	49	22	Dark	Orange		∏
Quercetin-3-Glucuronide	49	38		Dark purple	Yellow	∏	∏
Quercetin-3-monoglucosid	50	36		Yellow	…..	∏	∏
Quercetin-3-monorhamnoside	74		22	Bright green	Yellow orange		∏
Quercetin-3-triglucoside	23	40	20	Dark	Yellow	∏	∏
Myricetin-3-monorhamnoside	68		15	Yellow	Yellow		∏
Gossypetin-3-monogalactoside	40		9	Light purple	Flor. green	∏	
Gossypetin-7-monoglucoside	33		2	Bright yellow	Yellow		∏
Kaempferol-3-monoglucuronide	53		68	Yellow	Bright yellow		∏
Kaempferol-3-Rhamnoglucoside	59		23	Bright yellow	orange		∏
				Flavones glycosides			
Apigenin-7-Rhamnoglucoside	60	53		Dark	Yellow	∏	∏
Unknown 1	71	23.5	49	Dark	Yellow orange	∏	
Unknown 2	35	60		Dark	Yellow		∏
Unknown 3	39		22	Bright yellow	Bright yellow		∏
Unknown 4	47		0	Dark	Dark		∏
Unknown 5	73		43	Yellow	Orange		∏
Unknown 6	60		69	Greenish yellow	Bright greenish yellow		∏
Unknown 7	67		83	Yellow	Orange		∏
Unknown 8	50		60	Dark orange	Yellow orange		∏

Table 4. Calculated content of quercetin in Taif Rose and Al-Madinah Rose.

Taif sample					
	Time	**Area**	**Height**	**Width**	**µg/g**
Q	5.561	785.2	91.3	0.1264	762.58
FLAV	7.678	48.9	4.2	0.1715	47.49
Q	5.569	751.1	86.9	0.1269	729.46
FLAV	7.688	47.2	4.1	0.1712	45.84
Q	5.567	605.7	68	0.1319	588.25
FLAV	7.686	38.3	3.3	0.1729	37.20
				Aver Q	693.43
				Aver FL	43.51
				Total FL	**736.94**
Almadinah sample					
	Time	**Area**	**Height**	**Width**	**µg/g**
Q	5.571	2348.9	293.1	0.1215	2281.23
FLAV	7.693	129.3	12	0.1613	125.57
Q	5.573	3093.2	390.6	0.1184	3004.08
FLAV	7.702	160.5	15.1	0.1581	155.88
Q	5.574	4372.1	539	0.1206	4246.14
FLAV	7.709	230.2	20.4	0.1677	223.57
				Aver Q	3177.15
				Aver FL	168.34
				Total FL	**3345.49**
				Q Ratio M/T	**4.58**
				FL Ratio M/T	**3.87**
				Total, Ratio M/T	**4.54**

Discussion

In this study, phytochemical analysis has been applied to compare between *R. damascena* growing in Al-Madinah and Taif cities in Saudi Arabia. The results showed some considerable differences between both

plants. As far as we know, there are no previous records of Flavonoids contents of the leaves of *R. damascena*. Most of the previous studies focused on petals and essential oil contents (Mawarni *et al*., 2019). Consequently, the current study is the first record of chemical compounds in the leaves of *R. damascena* in Saudi Arabia.

Flavonoid glycosides obtained in the current research were analyzed and compared with literature data. They were generally varied between Al-Madinah Rose and Taif Rose. The differences are in the type and the amount of Flavonoid compounds. This variation could be referring to the differences in localities and environment. According to Mirali *et al.*, (2012), who studied the essential oil component from *R. damascena* species growing in different regions of Syria, they suggested that, the variation in the essential oil percentage is due to the environmental conditions in each area. Furthermore, the differences between Taif and Al-Madinah Roses could be due to some genetic differences, which is in agreement with a study concluded that, the variation in essential oil content of five varieties of *R. damascena* grown in Himalaya is due to genetic variation between them (Kumar *et al.*, 2014). In addition, a study conducted on twenty-four Damascene roses from different regions in western and eastern Azerbaijan, showed that there were some rose taxa are recommended for use in cosmetics, perfumes, sanitary, and food industries. On the other hand, another rose taxa grown in different areas have high amount of phenols and flavonoid with the high antioxidant activity. These later taxa are recommended for nutraceutical and pharmacological uses (Alizadeh & Fattahi, 2021).

The results found that both Roses from Al-Madinah and Taif cities are important economically and have important flavonoids compounds. The total Flavonoids quantity content in Al-Madinah Rose is very much greater than Taif Rose. Consequently, Al-Madinah Rose could be considered as a good source of Antioxidant.

Conclusion

In conclusion, the results clearly confirm that, there are differences as well as similarities between Al-Madinah and Taif Roses. There are some variations in chemicals compounds and the quantity of compounds like Quercetin, which is higher in Al-Madinah Rose than Taif Rose. So the mentioned phytochemical differences between both location plants might be related to the genetic diversity between them, in addition to the different environmental conditions between Al-Madinah and Taif.

Overall, the current study expresses that Al-Madinah Rose and Taif Rose could be layering from two different plants. Since both Roses are not recorded as a flora of Saudi Arabia and it is known that, Taif Rose comes from *R. damascena* from Turkey, the original plant for Al-Madinah Rose as far as we know is not known yet

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(Received for publication 25 June 2021)