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Total phenolic, *in vitro* antioxidant activity and safety assessment (Acute, sub-chronic and chronic toxicity) of industrial Taif rose water by-product in mice

El-Sayed S. Abdel-Hameed[1-3*], Salih A. Bazaid[1] and Abdel Nasser A. Sabra[4]

[1]*Natural Products Analysis Laboratory, Faculty of Science, Taif University, Saudi Arabia*
[2]*Chemistry Department, Faculty of Science, Taif University, Saudi Arabia*
[3]*Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza, Egypt*
[4]*Laboratory of Pharmacology, Theodor Bilharz Research Institute, Giza, Egypt*

ABSTRACT

The by-products or residues of agriculture industries have been taken more attention for their valuable source of natural antioxidants in recent decades. In this work, the Taif rose water by-product obtained after hydro-distillation of Taif rose (Rosa damascena trigintipetala Dieck) was investigated for its biological and phytochemical properties. The results showed that the Taif rose water byproduct had free radical scavenging activity toward artificial 1,1-diphenyl picrylhydrazyl (DPPH$^{\bullet}$) radical with SC_{50}= 23.72±0.36 µg/ml and also had high antioxidant capacity (329.53±18.75 mg ascorbic acid equivalent/g dry extract) and reducing power activity (211.31±2.79 mg ascorbic acid equivalent/g dry extract). Phenolic compounds are the major components and the antioxidant properties were attributed to them. The direct infusion ESI(-ve)-MS analyses of Taif rose water by-product showed the presence of phenolic compounds belonging to hydrolysable tannins and flavonoids. The acute, sub-chronic and chronic toxicity studies of Taif rose water by-product in mice suggested that it was safe and not toxic. From this work, it is obvious that the water by-product obtained after hydro-distillation of Taif rose could be used as a good natural inexpensive source of antioxidant polyphenolics after more phytochemical, in vitro and in vivo studies.

Keywords: Industrial Taif Rose water byproduct; Antioxidant; Phenolics; sub-chronic and chronic toxicity; ESI(-ve)-MS.

INTRODUCTION

The antioxidative phytochemicals especially phenolic compounds found in vegetables, fruits and medicinal plants have received increasing attention for their potential role in prevention of many human diseases[1]. The human can use antioxidants, either as dietary, food supplement or as a drug. Recently, the residues or by-products of agriculture industry take attention from their valuable source of natural antioxidants[2].

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[2-4].

Roses are the important ornamental plants and have been referred to as the queen of flowers. Different products of roses have long been used in perfumes, cosmetics, foods and for medicinal purposes. The physiological functions of roses may be partly attributed to their abundance of phenolics[5,6].

Taif rose (*Rosa damascena trigintipetala* Dieck); a sort of Damask rose (*Rosa damascene)*; is considered one of the most important economic products of Taif. The essential oil of Taif rose has an excellent reputation as a perfume which obtained by a hydro - distillation method. Every year during the harvest season of Taif rose (March-April), and after production of essential oil, there is a huge amount of water rose by-product found in distillation bottle which after that through away. In this work, the rose water by-product was studied for its biological and phytochemical properties. The biological investigation includes *in vitro* antioxidant activity followed by studying the safety and the side effects of by-product in experimental animals (mice) by using large doses for a long time and the effect of dose accumulation on physiological and histopathological parameters. Phytochemical investigation including estimation of total phenolic, flavonoid and flavonol compounds followed by direct infusion ESI-MS analysis.

MATERIALS AND METHODS

2.1. Chemicals

All solvents, standards and reagents were of high quality. Solvents for HPLC analysis were HPLC grade from Sigma-Aldrich Chemicals. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA).

2.2. Preparation of Taif rose water by-product extract

During the harvest season of Taif rose (March-April, 2013), and after production of essential oil in Taif rose oil factories in Taif, there are a huge amount of water rose by-product found in distillation bottle. Five liters of water by-product were filtered using filter paper Whatman No. 1, centrifuged followed by evaporation under reducing pressure by rotary evaporator. The dried extract (brown colour) weighted and stored in glass brown bottle until chemical and biological investigations.

2.3. Biological investigation

2.3.1. Antioxidant activity

Three different chemical methods were used for the evaluation of the antioxidant activity of Taif rose water byproduct; 1,1-diphenyl picrylhydrazyl scavenging activity, phosphomolybdenum method and reducing power assay. These assays were performed as described by **Abdel-Hameed *et al.*[7]**

Scavenging ability towards 1,1-diphenyl picrylhydrazyl (DPPH)˙ radical

Two ml of different concentrations of sample was 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 37 ^{o}C 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:

% DPPH radical scavenging activity = 1 - [$A_{sample}/A_{control}$] x 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.

Determination of the total antioxidant capacity by phosphormolybdenum method.

Three hundred µml of sample solution and 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 95 ^{o}C for 90 min. All samples were cooled to room temperature and 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.

Reducing power assay

Two ml of each sample and ascorbic acid in methanol (200 µg/ml) were mixed with 2 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2 ml of 1% $K_3Fe(CN)_6$ were incubated at 50 ^{o}C 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.

2.3.2.Toxicological studies:
In this part, the safety and the side effects of Taif rose water by-product in experimental animals were studied. The study including acute, sub-chronic and chronic toxicity investigations by using large doses for a long time and the effect of dose accumulation on physiological and histopathological parameters.

2.3.2.1. Animals:
Laboratory outbred Swiss albino mice (CD-1), weighing 20±2 g were used; they were obtained from the Schistosome Biology Supply Center (SBPC), Theodor Bilharz Research Institute (TBRI) Giza, Egypt.

2.3.2.2. Acute toxicity (LD_{50}):
A group of adult normal Swiss albino mice (42 mice) was used to study the acute toxicity of Taif rose water by-product extract. Mice were subdivided into seven subgroups, each group contain six mice. All subgroups were treated orally with rising doses of 500, 1000, 2000, 3000, 4000, 5000 and 6000 mg/kg of Taif rose water by-product extract. Mortality rates were recorded 24 hrs post treatment. The LD_{50} (The lethal dose that killed 50 % of the animals) was determined using computerized program "PCS" (Pharmacologic calculation system) by plotting the number of mice mortality (versus living mice) against different doses taken.

2.3.2.3. Sub-chronic toxicity:
A group of adult normal Swiss albino mice (25 mice) was used to study the sub-chronic toxicity of water by-product extract of Taif rose. Mice were subdivided into two subgroups, normal group contain 10 mice and treated group contain 15 mice. Five % of LD_{50} of water by-product extract of Taif rose were used daily for 28 days. Animals body weight were recorded before and every week during drug administration and at the end of durations. Mortality rates were recorded during the durations. Animals sacrificed 24 hrs after the end of treatment, vital organs were weighted, histopathological changes were examined, liver and kidney functions were also tested.

2.3.2.3. Chronic toxicity:
A group of adult normal Swiss albino mice (50 mice) was used to study the chronic toxicity of water by-product extract of Taif rose. Mice were subdivided into two subgroups, each group contain 25 mice. Five % of LD_{50} of water by-product extract of Taif rose was used at the beginning of experiment for two days and at third day the dose increased with 5% of LD_{50}, this was done for three months. Mortality rates were recorded during the durations. Every month, a group of mice was sacrificed, total body weight and vital organs were weighted, histopathological changes were examined, liver and kidney functions were also examined.

2.3.2.4. Parameters of assessment:
a)-Biochemical parameters
I-Liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were assayed spectrophotometrically using the commercially available kits according to the methods of **Reitman and Franke[8]; Kind and King[9].**

II-Kidney function tests: blood urea and creatinine were assayed spectrophotometrically using the commercially available kits according to the method of **Henry[10].**

b) - Histopathological studies
Specimens from the liver, kidneys, heart, lungs, intestine and spleen were collected and fixed in 10% buffered neutral formalin solution, dehydrated in gradual ethanol (70-100%), cleared in xylene, and embedded in paraffin. Five-micron thick paraffin sections were prepared and then routinely stained with hematoxylin and eosin (HE) dyes and Masson's trichrome stain for collagen fibers[11] and then examined microscopically.

2.4. Phytochemical analysis
2.4.1.Estimation of the total phenolic, flavonoid and flavonol contents
The total phenolic, flavonoid and flavonol contents of Taif rose water byproduct were measured according to the methods described by **Abdel-Hameed[12].**

The total phenolic content of plant extracts was determined using Folin-Ciocalteu's reagent (FCR). Hundred µl of 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 sample 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. Hundred µl of sample solution (1 mg/ml) was mixed with 100 µl of 2% aluminum trichloride in ethanol and a drop of acetic acid followed by dilution 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 Taif rose water byproduct 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 the plant extract solution, A_o is the absorption of the standard rutin solution, m is the weight of plant extract (mg) and m_o is the weight of rutin in the solution (mg).

The content of flavonols was determined by using quercetin as a reference compound. One ml of 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:

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

2.4.2.ESI(-ve)-MS direct infusion

The direct infusion method was performed to get full scan for the sample using electrospray-negative mode-mass spectrometry [ESI(-ve)-MS] (Waters 3100) at constant capillary voltage (3 kV) and three different cone voltage (30, 70 and ramp gradient 20-70 V), different. The analytical conditions for injection include the injection of sample (5 mg/ml) directly to the ion source by means of a syringe pump at a flow rate (20 µl/min) for 10 min. in addition to the previous parameters for capillary and cone voltage, the analytical conditions for mass spectrophotometer were; cone gas flow (50 L/h), desolvation gas flow (600 L/h), source temperature (150 ^oC), and desolvation temperature (350 ^oC). Mass spectra were scanned in the ESI negative mode in the range between m/z 50-1000. Maslynx 4.1 software was used for data analysis.

2.5. Statistical Analysis

Data in the tables are presented as mean (SEM using SPSS software version 13.0 (SPSS Inc., Chicago, IL). One-way ANOVA test followed by unpaired Student's t-test.

RESULTS AND DISCUSSION

3.1. Antioxidant activity

In spite of the strong radical scavenging activity of synthetic antioxidants, they usually have side effects, thus the interest in finding natural antioxidants without undesirable side effects has been increased greatly[7,13]. The plant phenolics characterized by its redox properties which allow them to act as hydrogen donators, metal chelating properties; hydrogen donators and singlet oxygen quenchers[12,14]. Many studies have revealed that the byproducts produced after processing vegetables and fruits in food industry still contain a huge amount of phenolic compounds[2,4].

The Taif rose water by-product produced after hydro-distillation of Taif rose was estimated for its antioxidant properties using three rapid and stable methods; 1,1-diphenyl picrylhydrazyl scavenging activity, phosphomolybdenum method and reducing power activity. These methods may serve as a significant indicator of its potent antioxidant activity. The Taif rose byproduct water extract exerted radical scavenging activity toward artificial radical $DPPH^{\cdot}$ with SC_{50}= 23.72±0.36 µg/ml and equivalent to 245.85±3.77 mg ascorbic acid equivalent/g extract. The total antioxidant capacity monitored by the phosphomolybdenum method showed value = 329.53±18.75 expressed by mg ascorbic acid equivalent/g dry extract. The by-product extract showed high reducing power activity with value 211.31±2.79 expressed by mg ascorbic acid equivalent/g dry extract. The above results encouraged the authors to complete study on experimental animals. Before complete the work on animals it must be studied its safety and possible side effects.

3.2. Toxicological studies

3.2.1. Acute toxicity

No death adult Swiss albino mice (0 % mortality) were observed 24 hrs. post treatment with rising doses of water by-product extract of Taif rose starting from 500 mg/kg up to 6000 mg/kg body weight orally. The LD_{50} >6000

mg/kg. The results of acute toxicity study revealed that the LD_{50} was > 6000 mg/kg this indicate that the extract is safe in Swiss albino mice at this dose.

3.2.2. Sub-chronic and chronic toxicity

In Sub-chronic toxicity, there were no significant changes in body weights and organ weights of mice treated with water by-product extract of Taif rose group (after 28 days) from the normal animal group. Concerning liver and kidney function tests, results showed no significant differences were recorded of water by-product extract group compared to normal animal group (Tables 1- 3). Similarly, no mortality was observed during sub-chronic toxicity period. In Chronic toxicity**,** the percentage mortality of water by-product extract of Taif rose were 8 % compared to 8 % (two mice in each group out of 25 mice) at 1st month, where the percentage mortality was 12.5% (two mice out of 16 mice) compared to 6.25% (one mouse out of 16 mice) at the 2nd month. The 3rd month showed no mortality (Table 4). No significant differences were recorded in total body weight and vital organs weight of water by-product extract during three months compared to the normal animal group during this period (Tables 5- 6). Liver (ALT, AST and ALP) and kidney (blood urea and creatinine) function tests of water by-product extract animal group did not show any significant differences in the level of these enzymes when compared to normal animal group (Table 7).

Table-1 : Total body weight of water by-product extract of Taif rose (Sub-chronic toxicity)

Total body weight(gram)					
Animal groups	**Start**	**1st week**	**2nd week**	**3rd week**	**4th week**
Normal N=10	22.84±0.87	22.75±0.89	24.84±0.98	24.35±0.89	23.58±0.88
Water by-product ext. N=15	22.71±0.55	23.05±0.61	24.57±0.70	23.98±0.79	23.92±0.81

N=number of animals in each group

Table-2 : Vital organs weight of of water by-product extract of Taif rose during 28 days (Sub- chronic toxicity)

Vital organs weight(gram)						
Animal groups	**Liver**	**Lung**	**Heart**	**Spleen**	**Kidneys**	**Intestine**
Normal N=10	1.00±0.04	0.16±0.01	0.12±0.01	0.10±0.01	0.28±0.02	1.07±0.06
Water by-product ext. N=15	1.08±0.06	0.14±0.01	0.12±0.01	0.09±0.01	0.28±0.01	1.11±0.06

N=number of animals in each group

Table-3 : Liver and kidney function tests of water by-product extract of Taif rose during 28 days (Sub chronic toxicity)

Liver and kidney function tests					
Animal groups	**ALT U/L**	**AST U/L**	**ALP IU/L**	**Urea mg/dL**	**Creatinine mg/dL**
Normal N=10	27.50±2.19	123.80±7.94	57.80±6.60	37.82±2.82	1.91±0.11
Water by-product ext. N=15	26.86±1.73	126.07±7.80	64.81±1.99	34.87±1.56	2.03±0.11

N=number of animals in each group

Table-4 : Percentage mortality of water by-product extract of Taif rose during three months

Percentage mortality			
Animal groups	**1st month**	**2nd month**	**3rd month**
Normal	2/25 (8.0%)	1/16 (6.25%)	0/8 (0%)
Water by-product ext.	2/25 (8.0%)	2/16 (12.5%)	0/7 (0%)

Table-5 : Total body weight of water byproduct extract of Taif rose during three months

Total body weight (gram)				
Animal groups	**Start**	**1st month**	**2nd month**	**3rd month**
Normal	21.69±0.85 N=25	23.29±0.91 N=23	26.30±0.99 N=15	29.61±0.72 N=8
Water by-product ext.	22.61±0.71 N=25	23.78±0.73 N=23	26.27±1.04 N=14	30.84±0.93 N=7

N=number of animals in each group

Table-6 : Total body weight and vital organs weight of water by-product extract of Taif rose during three months (chronic toxicity)

Total body weight and vital organs weight (gram)								
Times	**Animal groups**	**Total body weight**	**Liver**	**Lungs**	**Heart**	**Spleen**	**Kidneys**	**Intestine**
1st month	**Normal N=7**	21.98±0.75	1.00±0.07	0.20±0.01	0.13±0.01	0.11±0.02	0.26±0.03	1.61±0.15
	Water by-product ext. N=7	21.20±1.10	1.07±0.11	0.17±0.02	0.10±0.01	0.07±0.01	0.25±0.02	1.98±0.23
2nd month	**Normal N=7**	24.77±1.77	1.16±0.09	0.19±0.01	0.17±0.02	0.09±0.01	0.34±0.03	1.46±0.10
	Water by-product ext. N=7	24.07±1.03	1.39±0.07	0.18±0.01	0.14±0.01	0.10±0.01	0.32±0.02	2.08±0.06
3rd month	**Normal N=8**	29.61±0.72	1.54±0.06	0.23±0.01	0.18±0.01	0.13±0.01	0.46±0.02	1.51±0.03
	Water by-product ext. N=7	30.84±0.93	1.61±0.09	0.28±0.04	0.16±0.01	0.12±0.01	0.41±0.02	1.91±0.18

N=number of animals in each group

Various parameters were thoroughly studied in the sub-chronic and chronic toxicity study. The body weights and vital organ weights were found to be unaltered during the duration of sub-chronic (28 days) and chronic (three months) treatment period when compared to the normal animal group during this period. No mortality was observed during sub-chronic toxicity period. In chronic toxicity period the mortality was similar to normal animal group in the 1st and 3rd month, while in the 2nd month the mortality was increased from 6.25% to 12.5% (one mouse to two mice). These results indicate no toxic effect of the water by-product extract of Taif rose during sub-chronic toxicity period with minimal percentage mortality during 2nd month of the chronic toxicity period due to no changes in such parameters, which are often the first signs of toxicity[15].

Potential adverse effects of herbal drugs have received intense attention in recent years. Although drugs and toxins can affect most body organs, liver and kidney are of greatest importance in this regard[16-20]. The serum biochemical parameters were studied to evaluate the possible alterations in hepatic and renal functions influenced by the extracts. Serum levels of ALT, AST and ALP are among the most significant laboratory markers of liver tissue damage[17]. In this study, serum biochemical parameters related to hepatic function (ALT, AST& ALP) exhibited no significant alterations and remained within the normal range during sub-chronic and chronic toxicity period. These findings suggest that *Rosa damascena trigintipetala* Dieck infusion may be easily tolerated by hepatocytes. It is well known that almost all drugs, chemicals and xenobiotics are eliminated through renal excretion hence it was found necessary to estimate the effects of the extracts on kidney functions[21].

In this study, serum biochemical parameters related to kidney functions (blood urea and creatinine) demonstrated no significant differences with respect to normal group animals during sub-chronic and chronic toxicity period. Therefore, it can be inferred that the water by-product extract did not affect the normal hepatic and renal functions during the period of sub-chronic (28 days) and chronic (three months) toxicity study.

3.2.4. Pathological findings

3.2.4.1. Normal control (sub-chronic and chronic toxicity):

The examined organs (Liver, intestine, heart, lungs, kidneys and spleen) were normal. The liver showed normal hepatocyte and sinusoidal architectures, blue stained nuclei of varied shapes and numbers (one or sometimes two for each cell) and portal areas. Branches of portal vein, hepatic artery and bile duct were visualized in these portal areas. The kidneys showed normal glomerular tufts and renal tubules. These tubules were varied from the proximal, distal and collecting tubules that mostly lined with cuboidal epithelium. The heart showed normal pericardium, myocardium and endocardium. The coronary blood vessels and the myocytes of the myocardium were in normal pattern. The lungs revealed normal bronchi, bronchioles and alveolar spaces. The interlobular and interalveolar septa were thin and in normal pattern. The intestine revealed intact mucosa that lined with columnar epithelium and few scattered goblet cells, submucosa and lamina propria. The spleen showed normal lymphoid aggregation in the white pulp and sinusoids in the red pulp besides normal splenic capsule and trabeculae.

3.2.4.2. Sub-chronic toxicity:

The examined organs of water by-product extract of Taif rose, were normal. The liver showed a normal hepatic architecture and central vein. The kidneys revealed normal glomerular and tubular structure. The heart showed normal cardiac myocytes. The lungs showed normal bronchioles and alveoli. The spleen and intestine were normal.

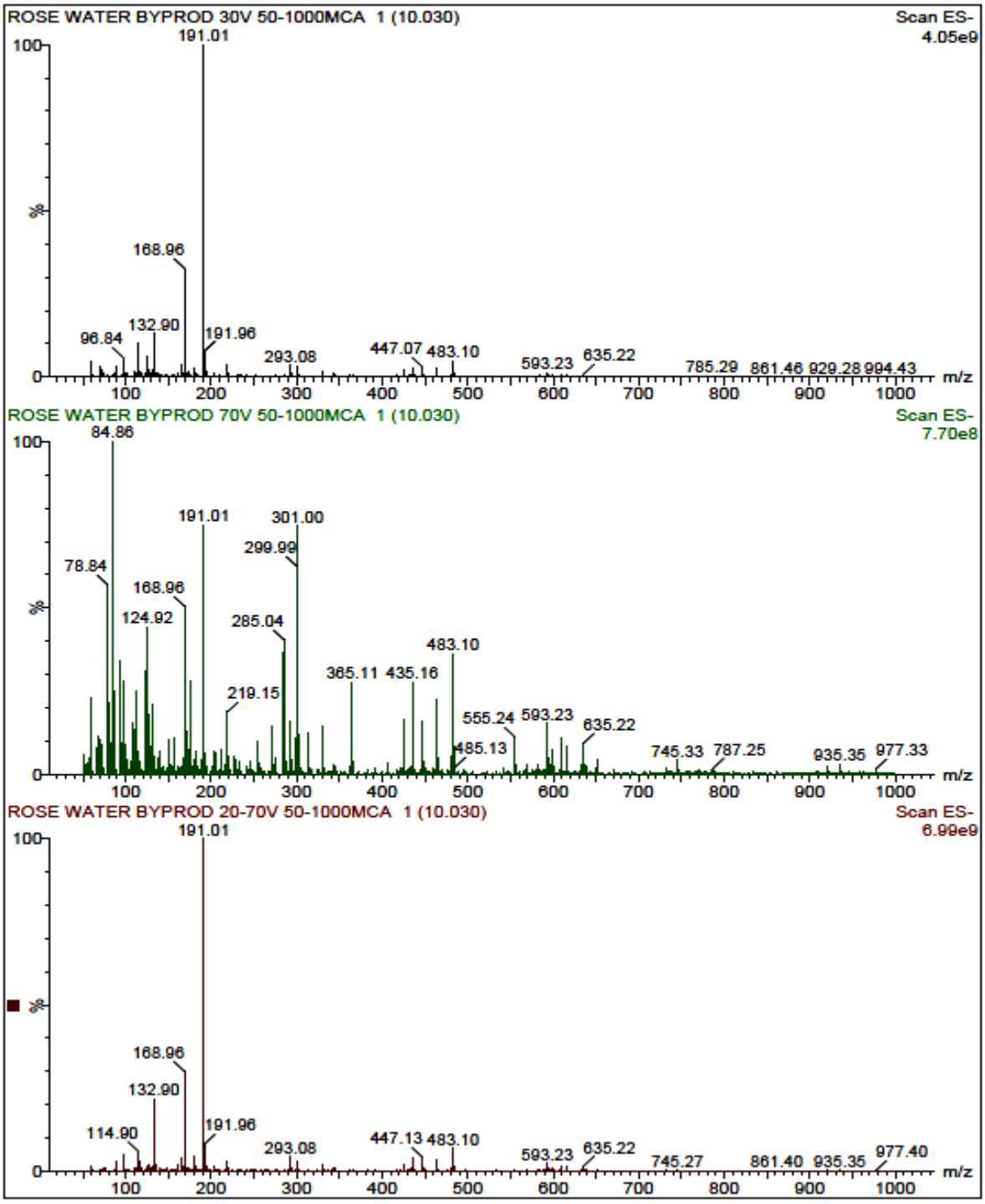

Figure-1 : ESI-(-ve)-MS full scanned spectrum from direct infusion of Taif rose industrial water by-product

3.2.4.3. Chronic toxicity:

i- 1st month

The lesions of the water byproduct extract were almost similar to those described with the sub-chronic toxicity. The liver showed mild hydropic degeneration. The kidneys revealed normal glomerular and tubular structure. The heart showed slight congestion in coronary blood vessel with mild perivascular edema. The lungs revealed normal bronchioles and alveoli. The spleen and intestine were normal.

ii- 2nd month

The liver of water byproduct showed mild hydropic degeneration and slight congestion of the hepatic blood vessels. The kidneys revealed mild vacuolations and hydropic degeneration in the tubular epithelia and normal glomeruli.

The heart showed granular eosinophilic sarcoplasm and slightly congested coronary blood vessels. The lungs revealed peribronchiolar aggregations of round cells with thickening the adjacent interalveolar septa. The spleen and intestine were normal.

iii- 3rd month

The lesions in water byproduct for three months were similar to those described with two months. Where, the liver revealed periportal hydropic degeneration and congested blood vessels. The kidneys showed mild vacuolations in the renal tubular epithelium. The heart revealed focal hyaline degeneration in the cardiac muscle fibers. The intestine showed an increase in the numbers of goblet cells and slight activation of Paneth cells. The spleen revealed sub capsular edema and normal white pulp. The lungs were normal.

3.4. Phytochemical analysis

3.4.1. Total phenolic, flavonoid and flavonol contents

The biological properties of roses were found attributed to its major contents of phenolic compounds[7,13]. The total phenolic, flavonoid and flavonol components of Taif rose water byproduct extract were estimated using the Folin-Ciocalteus assay for total phenolics, aluminum chloride method for total flavonoids and aluminium chloride/sodium acetate for total flavonols. The Taif rose water byproduct extract showed to have total phenolic contents expressed by mg gallic acid equivalent/g extract equal 48.27±1.27 whereas the total flavonoids and flavonols were found equal to 16.68±0.73 expressed by mg rutin equivalent/g extract and 8.47±0.44 expressed by mg quercetin equivalent / g extract. The result of total phenolics was similar to our previous study on 80% methanol extract of Taif rose but the total flavonoids and flavonols here were lower[13]. This difference may be attributed to that the extraction by water (highly polar extraction solvent) during the hydro-distillation of rose increase the extraction of tannins which considered the highly polar phenolic compounds.

3.4.2. ESI(-ve)-MS analysis

The full scanned mass spectrum (m/z 50-1000) of Taif rose water byproduct obtained by direct infusion into the negative ion mode ESI-MS at different cone voltages was shown in Fig. 1. It is obvious that the spectrum at cone voltage 70 V was suitable for ionization and fragmentation of the different compounds in the sample rather than using the other two-cone voltage (fixed 30 and ramp gradient 20-70 V). Two obvious peaks appeared at m/z 168.9 and 191 that were previously detected and identified in our previous work in 80% methanol extract of fresh Taif rose and other reports in some Rosa species as gallic acid and quinic acid[13,22]. The ESI(-ve)-MS full scanned mass spectrum represented many peaks at m/z: 132.90, 168.9, 191.01, 285.04, 301.00, 313.09, 331.07, 365.11, 435.16, 447.07, 463, 483.10, 555.24, 593.23, 599.12, 609, 615, 635.22, 787.25, 935.35, 977.33. The previous peaks may be attributed to gallic acid, quinic acid, flavonoid compounds, and hydrolysable tannins[1,13,22-25]. Now the Taif rose water byproduct was subjected to LC-MS analysis and detailed identification for individual compounds.

Table-7: Liver and kidney function tests of water by-product extract of Taif rose during three months (chronic toxicity)

Liver and kidney function tests						
Times	**Animal groups**	**ALT U/L**	**AST U/L**	**ALP IU/L**	**Urea mg/dL**	**Creatinine mg/dL**
1st month	**Normal N=7**	20.57±0.75	37.86±0.83	76.30±5.23	58.58±2.77	1.26±0.13
	Water by-product ext. N=7	22.40±2.02	39.40±1.03	79.66±9.23	52.80±4.03	1.08±0.19
2nd month	**Normal N=7**	38.67±0.62	120.71±7.19	101.91±10.78	41.09±2.05	1.79±0.08
	Water by-product ext. N=7	39.86±1.08	115.00±7.53	99.15±12.44	46.68±3.39	1.93±0.06
3rd month	**Normal N=8**	43.29±8.70	134.83±6.09	101.17±16.32	59.90±4.40	1.87±0.12
	Water by-product ext. N=7	53.33±3.56	141.29±5.74	111.64±14.22	53.78±2.24	2.11±0.06

N=number of animals in each group

CONCLUSION

The results of this study provide evidence that the Taif rose water by-product obtained after hydro-distillation of Taif rose showed antioxidant activity. Phenolic compounds are the major components of Taif rose water by-product and the antioxidant properties were attributed to them. Taif rose water by-product extract exhibited excellent safety profile in acute, sub-chronic and chronic toxicity studies in adult male Swiss albino mice. Furthermore, pathological examinations of the internal organs revealed no pathological abnormality. The present study establishes the reliable safety profile of water by-product extracts in adult male Swiss albino mice offering no obvious toxicity. The Taif

rose water by-product obtained after hydro-distillation of Taif rose could be used as a good inexpensive source of antioxidant polyphenolics after more phytochemical, *in vitro* and *in vivo* studies.

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