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Phytochemicals and biological activities of *Ruta chalepensis* L. growing in Tunisia

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ABSTRACT

In this paper, the chemical composition of different solvent extracts from *Ruta chalepensis* L. was determined and their antioxidant and antimicrobial activities were evaluated. Indeed, the total phenol, flavonoids, ortho-diphenols, tannin and flavonols contents of these extracts were quantified, with a total phenolic content varying from 120.57 to 178.00 mg Gallic acid equivalents per gram of dry extract. The chemical analysis of the composition of these *R. chalepensis* L. extracts has shown a high content of coumarins and alkaloid compounds. Besides, their antioxidant activities were examined using various in vitro assays: DPPH radical scavenging, β-carotene bleaching method, reducing power and quantification of the total antioxidant capacity. The obtained results have confirmed that the ethanol extract exhibits the most potent antioxidant effect, followed by methanol and methanol/water (1/1) extracts. Furthermore, the ethanol, methanol, methanol/water (1/1) and ethyl acetate extracts were found to have an antibacterial activity against gram positive and gram negative strains.

1. Introduction

The quality of food rich in fat can be affected by oxidation reducing its nutritional content and promoting the development of food deterioration. In fact, lipid oxidation occurs during the processing, heat treatment of raw materials and further storage of final products. Along with free radical scavengers, antioxidants are proven to be very effective in the prevention of pathologies such as cancer, heart diseases, biological damage in living tissues, and neurodegenerative diseases, in which reactive oxygen species (ROS) or free radicals are implicated (Middleton, Kandaswami, & Theoharides, 2000). At present, many synthetic antioxidants have been largely used in food industries for the inhibition of the oxidation of food rancidity and shelf-life prolongation. The most generally used antioxidants are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are added to fatty foods to prevent oxidative deterioration (Löliger, 1991). Nevertheless, since recent research works have revealed the drawbacks of these synthetic antioxidants' application that may lead to toxic effects such as carcinogen and liver damage (Barlow & Schlatter, 2010), stronger restrictions have been placed on their application.

Microbial contamination, along with oxidation, is another key factor of food deterioration and food-borne diseases in the third world and developing countries, and even in the developed ones (Sokmen et al., 2004). It is eminent that food materials contain plentiful nutritional requirements of microorganisms. The consumption of foods contaminated with some microorganisms whose subsistence and growth may lead to spoilage, poisoning and quality deterioration of food products, represents a serious health risk to humans (Celiktas et al., 2007).

Therefore, great interest has naturally developed in the existing products, together with antioxidant and antimicrobial activities, to inhibit oxidation and microbial growth in foods while maintaining quality, freshness, and safety (Jin & Zhang, 2008). Compared to the available preservatives, using natural products is based on their availability, fewer side effects, or toxicity as well as their better biodegradability (Kalemba & Kunicka, 2003). Among the most important natural antioxidant sources we can mention the medicinal plants, whose antioxidant and antimicrobial activities have been investigated by numerous studies. Such plants contain a high level of polyphenols, whose compounds are well known as radical scavengers, metal chelators, reducing agents and hydrogen donors.

Abbreviations: TAC, total antioxidant capacity; DPPH, 1.1-diphenyl-2-picrylhydrazyl; GAEs, gallic acid equivalents; ROS, reactive oxygen species; RNS, reactive nitrogen species; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene

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Ruta chalepensis L. (Rutaceae) is a small shrub that is widely distributed in the Mediterranean region, usually growing on rocky slopes (Iauk et al., 2004). This plant is commonly used as traditional medicinal plant. It is reported to be protective against various disorders such as rheumatism, fever, mental disorders, dropsy, neuralgia, menstrual problems, convulsion and other bleeding and nervous disorders (Iauk et al., 2004). Besides being characterized with its emmenagogue, abortifacient, antihelminitic and spammolitic effects (Di Stasi et al., 2002), *R. chalepensis* L. has anti-oxidant, anti-cancer and anti-inflammatory properties (Khlifi et al., 2013). As regards the phytochemical composition of *R. chalepensis* extracts, Hnatyszyn et al. (1974) have reported that its leaves and young stems contain alkaloids, flavonoids, phenols, amino acids, furocoumarins and saponins. However, and to the best of our knowledge, there is relatively scarce information about the content of phenolic compounds and antioxidant activities of methanolic, ethanolic, methanol/water (1/1), ethyl acetate and aqueous *R. chalepensis* extracts.

Thus, the present study aims to evaluate the antioxidant properties and the in vitro antimicrobial activities of different solvent extracts of *R. chalepensis* L. and to determine the chemical composition of these extracts by LC–MS analysis.

2. Materials and methods

2.1. Plant material

The aerial parts (stem and leaves) of *R. chalepensis* L., (*R. chalepensis* L.) commonly known as Fijel were collected in March 2011 in Chebba region (Mahdia, Tunisia, latitude 35.23° and longitude 11.11°). The taxonomic identification of plant material was confirmed by Prof. Mohamed Chaieb (Department of Botany, Faculty of Sciences, University of Sfax, Tunisia), according to the Flora of Tunisia (Chaieb & Boukhris, 1998). The vouchers specimen with reference MK11 was deposited at the herbarium of the department of botany in the cited institute.

2.2. Preparation of the extracts

The air-dried aerial parts of *R. chalepensis* L. (40 g) were extracted by maceration in 300 ml of different solvents, including hexane, ethyl acetate (EtOAc), methanol (MeOH), ethanol (EtOH), MeOH/water (1/1) and water at room temperature for 48 h. Then, the obtained solution was filtered, the solvents (hexane, EtOAc, MeOH, EtOH, and MeOH/water) were removed with a rotary evaporator, and the water extract was freeze-dried. The dried sample of each extract was weighed and the yield of soluble constituents was determined. These extracts were then stored at a temperature of 4 °C until use.

2.3. Determination of total phenolic content (TPC)

The total phenolic content (TPC) in the different extracts of *R. chalepensis* was determined calorimetrically using the Folin-Ciocalteu method (Singleton & Rossi, 1965). Briefly, a 50 μl aliquot of *R. chalepensis* extracts was assayed with 250 μl of Folin reagent and 500 μl of sodium carbonate (Na_2CO_3) (20%, w/v). The mixture was vortexed and diluted with water to a final volume of 5 ml. After incubation for 30 min at room temperature, the absorbance was read at 727 nm using a spectrophotometer. The analysis was performed in triplicate and the total phenolic content was expressed in milligrams equivalents of gallic acid (GAEs) per gram of each extract, using a calibration curve of a freshly prepared gallic acid solution. For the gallic acid, the curve absorbance versus concentration is described by the following equation $y=0.001x+0.014$ ($R^2=0.999$)

2.4. Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) was measured by the colorimetric assay developed by Zhishen et al. (1999). One-milliliter aliquots of the appropriately diluted sample or standard solutions of catechin were added to a 10 ml volumetric flask containing 4 ml of doubly distilled (dd) H_2O. At zero time, 300 μl of 5% $NaNO_2$ was added to the flask. After 5 min, 300 μl of 10% $AlCl_3$ was added. At 6 min, 2 ml of 1 M NaOH were added to the mixture. Immediately, the reaction solution was adjusted to 10 ml by adding 2.4 ml of ddH_2O. Soon after vortexing, the absorbance of the mixture was determined at 510 nm using a spectrophotometer against prepared water blank. The analysis was performed in triplicate and the total flavonoids in these extracts were expressed as catechin equivalents (CEs) per g extract, using a calibration curve of a freshly prepared catechin solution. For the catechin, the curve absorbance versus concentration is described by the equation $y=0.0049x$ ($R^2=0.998$).

2.5. Determination of ortho-diphenol content (ODC)

The concentration of ortho-diphenol content (ODC) in the different extracts of *R. chalepensis* L. was determined by the method of Mateos et al. (2001). One milliliter of 5% (w/v) sodium molybdate in 50% ethanol was added to the aliquots of the methanolic extract (4 ml). The contents were mixed for 1 min and the absorbance was measured after 15 min at 370 nm against a blank reagent prepared by adding 1 ml of 50% ethanol to the aliquot of the extract instead of sodium molybdate solution. The ortho-diphenols content was expressed as mg of caffeic acid equivalents (CAEs)/g of the extract, using a calibration curve of a freshly caffeic acid solution. For the caffeic acid, the curve absorbance versus concentration is described by the following equation: $y=0.021x-0.017$ ($R^2=0.999$).

2.6. Determination of total tannin content (TTC)

The total tannin content (TTC) was performed using the method described by Hagerman and Butler (1978). Three milliliters of 4% methanol vanillin solution and 1.5 ml of H_2SO_4 (9 N in methanol) were added to 50 μl of suitably diluted sample. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm against solvent as a blank. All samples were analyzed in triplicates. The total tannins content was expressed in milligrams equivalents of catechin (CEs) per gram of each extract. For the catechin, the curve absorbance versus concentration is described by the equation that follows: $y=1.458x$ ($R^2=0.988$).

2.7. Determination of total flavonols content (TFl)

The total flavonols content (TFl) in the plant extract was estimated using the method of Yermakov, Arasimov, and Yarosh (1987). The rutin calibration curve was prepared by mixing 1 ml of various concentrations (0.5, 0.25, 0.125, 0.062, 0.031 mg/ml) of rutin solutions with 1 ml (20 mg/ml) aluminum tri-chloride and 3 ml (50 mg/ml) sodium acetate. The absorbance at 440 nm was read after 2 h. The same procedure was used for 1 ml of the different extracts of *R. chalepensis* L. instead of rutin solution. All determinations were carried out in triplicate. The flavonols content was expressed as mg of rutin equivalents (REs)/g of the extract and calculated using a standard curve obtained from various concentrations of rutin, for which the curve absorbance versus concentration is described by the following equation: $y=0.002x+0.009$ ($R^2=0.998$).