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Comparison of total phenols content and antioxidant potential of peel extracts of local and imported lemons samples

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ABSTRACT

The design of this study was to investigate and compare the total phenols content and antioxidant activity of various crude extracts of lemon peels collected from local farmers and imported one from the local supermarket. Investigation of the total phenol content and antioxidant activity of various polarity peel extracts were performed by modifying Folin-Ciocalteu reagent (FCR) and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) bioassay. The maximum total phenol content in locally grown lemon was found in methanol extract and the minimum was obtained in butanol extract. However, the maximum concentration of total phenol content in imported lemon was also found in the methanol crude extract and the minimum was found in chloroform extract. The high antioxidant activity among the six prepared extracts of local lemon peels was found in the ethyl acetate extract and the lowest was found in chloroform extract. Similar antioxidant activity result was found in the imported lemon collected from the supermarket. Our results show that both lemon peels have the considerable concentration of total phenols content and antioxidant activity, respectively. The lemon peels are cheap and available globally and may find extensive use in medicine, food and perfume industries as vital sources of natural antioxidants.

1. Introduction

The importance of phenolic compounds has increased tremendously due to their health benefit and nutrition value. The antioxidant properties of phenols and their derivatives play a vital role in preventing and curing various life-threatening diseases such as cancer, cardiovascular diseases etc. (Irkin et al.,2011; Gonzalez et al., 2010). Several classes of phenolic compounds like phenols, cinnamic acid, rosmarinic acid, gallic acid and flavonoids, and their derivatives are the focal chemical compounds occurring widely in foods, plants and formulated herbal products. The nutritional value and antioxidant potential of fresh and processed foods completely depend on their focal bioactive compounds specially the phenolic compounds and their derivatives (Liu et al., 2013). The human diet contains different group compounds, especially phenols and their derivatives are good for human health due to their chemopreventive effects. These chemopreventive effects depend on the number and amount of phenolic compounds consumed as a diet per day (Irkin et al., 2011). The nutritive value of food and beverage products can be improved by the addition of phenolic compounds as an additive. In this study, the correlation between the total phenols content and antioxidant activity of various extracts of lemon peels collected from local farmers and the supermarket was investigated.

Lemons are the most common crop cultivated globally and grow very well in both tropical and subtropical countries. Several varieties of lemons are available globally, including Oman and all of them are belonging to the family Rutaceae (Diankov et al., 2011). The lemon is a small evergreen tree native to Asian countries (Diankov et al., 2011). The origin of lemon is still unknown; however, the literature search reveals that it first grew in Assam (India), Burma (Myanmar), and China (Oboh and Ademosun, 2012). In the 15th century, first extensive cultivation of lemons was in Geona, Europe (Anghel et al., 2014). In 1943, it was familiarized and spread tremendously to the Americas when Columbus brought lemon seeds to Hispaniola on his voyages (Anghel et al., 2014). The fruit is an oval shape with a broad, low and apical nipple. Initially, the fruit is green color and then it is gradually turning to yellow due to its maturity. The outside peel is yellow when ripe in most varieties of lemons (Fig. 1) (Manthey and Grohmann, 2001). The lemon contains different types of chemical constituents like volatile oils, flavonoids, terpenoids, and tannins (Najwa and Hossain, 2014). In addition, there are about 90% monoterpene hydrocarbons, composed mainly of limonene (70%), with lesser amounts of γ-terpinene, β-pinene, sabinene, α-pinene, and myrcene are present. A trace amount of sesquiterpenes, waxes, and coumarins are also present in the lemon juice (Najwa and Hossain, 2014; Kamaliroosta et al., 2016). It has

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Fig. 1. Lemon fruits picture.

several health benefits. Traditionally, it is widely used for the treatment of various infections, stomach problem, constipation, teeth problems, fever, bleeding, rheumatism, burns, breathing disorders, cholera and high blood pressure. It is also used for hair and skin care (Lai et al., 2013). The fresh juice of lemon is used mainly for the treatment of fever, dental and hair care, internal bleeding, rheumatism, memory loss, breathing disorders and cholera (Lai et al., 2013). The lemon juice with low pH values has used an antibacterial agent. In India, it is widely used in Siddha and Ayurveda traditional medicine systems (Ros et al., 1996). Lemon oil is also important and used widely in traditional healing systems like aromatherapy, homeopathy, message, Siddha and Ayurveda medicine (Campêlo et al., 2011). Scientists from the USA found that lemon oils do not influence the human immune system. However, they concluded that the lemon oil may change the human mood and behavior through aromatherapy and message (Najwa and Hossain, 2014; Liu et al., 2013). Most of the *in vitro* and *in vivo* biological activity works have been done globally on different varieties and different parts and species of lemons, however, still, there is no work done by the scientists on total phenols and antioxidant activity of Omani varieties lemon species (Najwa and Hossain, 2014). Therefore, the intention of our study was to investigate and compare the total phenols content and antioxidant activity of different lemon extracts from peels collected from local farmers and a supermarket (imported).

2. Material and methods

2.1. Chemicals

The reagents that were used in this experiment like hexane, ethyl acetate, chloroform, butanol, and methanol were purchased from BDH, UK. The other chemicals or reagents were of analytical grade obtained from established Companies. Anhydrous Na_2CO_3 crystals obtained from industrial Estate, Mumbai. 2,2-Diphenyl-1-pikryl-hydrazyl (DPPH), dimethyl sulphoxide (DMSO), gallic acid and Folin-Ciocalteu reagent were obtained from the Sigma-Aldrich Company, Germany.

2.2. Instruments

The absorbance of the tested samples for the quantification of total phenols and antioxidant activity was measured by a UV-Visible spectrophotometer (Shimadzu, Model 1800, Japan).

2.3. Sample collection

The matured yellow lemon fruits were collected locally from farmers and the imported samples from the local supermarket in Muscat during the month of December 2014 at around 10 am. The morphological characterization of the collected local sample was done by the local people and website. Both collected samples were carried out in the Research Lab, University of Nizwa, Sultanate of Oman for the dry and extraction process.

2.4. Sample preparation

Both the local and imported samples were initially washed with water to remove unwanted materials. The peels were separated from lemons by the knife and the separated peels were dried at sun for 5 days. The dry peels were ground into coarse powder with a kitchen blender machine. Both local and imported varieties coarse powdered samples (78.79 g and 63.8 g) were used for extraction with methanol solvent by using a Soxhlet apparatus for 72 hours (Hossain et al., 2017; Zainab and Hossain, 2016; Ros et al., 2016). After completion times, the methanol solvent was dried by a rotary evaporator at ambient temperature under reduced pressure. Both methanol crude extracts from local and imported were suspended separately in 250 ml of water and partition successively with hexane, chloroform, ethyl acetate and butanol with ascending polarity to give hexane (1.11 and 1.81 g), chloroform (0.649 and 1.22 g), ethyl acetate (0.696 and 1.35 g) and butanol (5.56 and 1.99 g) crude extracts, respectively. The fractionation process was repeated twice and combined together and evaporated the mother solvent by using a rotary evaporator under pressure. The water part was evaporated the same way for water crude extract.

2.5. Gallic acid calibration curve

The gallic acid standard curve was prepared by adding modified Folin-Ciocalteu reagent (FCR) method (Rehab and Hossain, 2017). Three milligrams of gallic acid were dissolved in 10 ml of methanol solvent. Various concentrations (200, 100, 50, 25, 12.5 μg/ml) were prepared by adding the same solvent. 200 μl of each prepared concentration sample was placed in a separate test tube and added 1.5 ml of 10% FCR solution and kept in the dark place for 5 minutes. After incubation, 1.5 ml of 6% Na_2CO_3 was added to each test tube and then the solution was mixed together. All the test tubes were kept in the dark for 90 minutes. The absorbance was measured by UV-visible spectrophotometer at the fixed wavelength 750 nm.

2.6. Total phenols content assay

The evaluation of total phenol content of different polarities lemon peels extracts at different concentration by using modified Folin-Ciocalteu reagent (FCR) method (Hossain et al., 2013; Tahiya et al., 2014). Individual crude extracts (5 mg) were dissolved separately in methanol (5 ml) to prepare a stock solution. From the stock solution, 200 μl of stock solution of both lemon samples was placed in a separate test tube and added 1.5 ml of 10% FCR solution and kept it in a dark place for 5 minutes. 1.5 ml of 6% Na_2CO_3 was added to each test tube and the solution was mixed well by hand and left for 90 minutes in the dark place. The absorbance of test samples was measured by UV-visible spectrophotometer at 750 nm. The total phenol content was determined by applying gallic acid calibration curve and expressed in mg of gallic acid equivalents/g dry crude samples.

2.7. Antioxidant activity assay

The stock solution of each polarity crude extract of lemon peels was prepared by adding three milligrams of each crude extract in methanol (10 ml). Various concentrations (300, 150, 75, 37.5 and 18.75 μg/ml) of each crude extract were prepared by dilution method. 300 μl of each crude extract stock solution and 700 μl of 3.3% DMSO were taken in a test tube and mixed well. Finally, 2.7 ml of DPPH solvent was added to each concentration test tube and shaken and kept in a dark place for 90 minutes. After completion incubation, the absorbance of each concentration crude extract was measured by using UV-visible spectroscopy at 517 nm (Asma et al., 2016: Tahiya et al., 2014). The antioxidant activity of crude extract of both local and imported lemon peels