別紙様式８

研　究　主　論　文　抄　録

論文題目　Green pressurized technology for extraction of bioactive compounds of henna leaves（ヘナ葉の生理活性成分の抽出のためのグリーン高圧技術）

熊本大学大学院自然科学研究科　複合新領域科学　専攻　衝撃ｴﾈﾙｷﾞｰ科学　講座
（　主任指導　佐々木　満　准教授　）

論文提出者　タイエバハレ ゾホリアン（Tayyebeh Haleh Zohourian）

主論文要旨

Natural products are a significant source of synthetic and traditional herbal medicine and are still the primary health care system. Utilization of natural bioactive compounds with pharmacological and nutraceutical properties as medicine or health supplements is gaining popularity in recent years because synthesis of such would be tedious and even more costly especially for highly complex molecular structures like polyphones. One promising species of plant that catches our interest is henna (*Lawsonia inermis*) a world renowned source of natural hair dye component called lawsone (2-hydroxy-1,4-naphthoquinone) and some polyphenolic compounds, thus efficient and safe extraction methods are sought. In this regard, the use of green and generally regarded as safe solvents such as supercritical carbon dioxide (SC-CO_2) and subcritical water for extraction of bioactive components of henna leaves are being considered. Being non-toxic and environment-friendly, SC-CO_2 extraction has been used in various applications in processing of foods and biomaterials. Alternatively, water being regarded as a generally safe and environmentally benign solvent, can be used instead. It has received increased attention as an important alternative to conventional separation method. For a more efficient extraction, the use of microwave is also being considered. Microwave assisted extraction (MAE), has been proven to be a promising technique for the extraction of phytochemicals from botanicals. This work investigates the application of the two above mentioned environmentally benign and generally regarded as safe solvents for extraction of useful compounds from powdered henna leaves focusing on

the extraction of the main compound – lawsone. In the case of subcritical water treatment, heating is performed using microwave irradiation. The effect of various parameters on extraction yield and composition of the products are also investigated.
Soxhlet extraction with methanol (MeOH) as a solvent was also carried out to determine the amount of lawsone present in the sample which was taken as a basis for calculating the recovery of lawsone in the succeeding extraction experiments.The applicability of employing SC-CO_2 to extraction of useful compounds from henna leaves including lawsone was first investigated at a temperature of 50 ^{o}C, while varying the pressure from 10 to 30 MPa. Experimental results showed that the extraction yield was low at around 1.86 to 8.15 mg/100 g sample. Henna leaves may contain minimal amount of lipid-like components that are soluble in SC-CO_2, and this could be the likely reason for the obtained low extraction yield. The amount of lawsone in the extracts was not so significant in the range of 18.7 to 26.8 mg/100g-extracts. These data correspond to recovery of 0.01 to 0.43 % at pressures of 10 to 30 MPa, respectively. Other than lawsone, GC-MS analyses have also identified some volatile and non-polar components in SC-CO_2 henna extracts.The water-soluble extracts as well as henna extract samples were analyzed by High performance liquid chromatography (HPLC) for the presence of the main components such as lawsone. Based on the results of SC-CO_2 extraction experiments, it was observed that SC-CO_2 was selective to extraction of non-polar compounds, and was not suitable for extraction of the target compound - lawsone. Instead, we explored the use of water as a solvent at elevated temperatures and pressures heated by microwave irradiation. Experiments were performed at temperature range of 100 to 200^{o}C, and extraction time of 5 to 30 min. The extracts were analyzed for the presence of lawsone using HPLC analysis. Further procedures including centrifuge and freeze drying were performed to evaluate the yield and recovery of the target compound. The result revealed the highest yield of 51.7% was obtained at 160^{o}C in 30 min and the maximum extraction yield obtained in short reaction time of 2 min at a microwave power of 700 W was 37.1%. The highest composition of lawsone close to 24% was obtained at microwave power of 600 W in irradiation time of 1 min as well. Moreover the highest recovery of lawsone (76.2%) could be obtained at 120^{o}C and extraction time of 30 min. At higher temperatures and longer treatment time, the yield and recovery decreased due most likely to the degradation of the target compounds at severe conditions. Mild hydrothermal conditions assisted with microwave heating are recommended in order to obtain high recovery of lawsone. Direct microwave heating of the polar components and solvent could lead to rapid extraction of the target compounds. This present research work also focuses on the elucidation of the effects of parameters such as temperature, time and microwave power on total polyphenolic contents (TPC) and its relationship with the antioxidant activities of the obtained henna extracts.

Therefore evaluation of the antioxidant activities of the leaf extracts was also carried out. The antioxidant activity of henna extracts and pure lawsone was determined based on DPPH radical scavenging activity. The free radical scavenging effect of the extracts was assessed by the decolorization of a methanol solution of DPPH. Briefly, 400μL aqueous solutions of 2.5 mg/mL of test compounds were added to DPPH solution. The reaction mixture was shaken vigorously and then kept at room temperature for 30 min. The absorbance of the remaining DPPH was measured at 517 nm using Shimadzu UV-1200 UV-Vis spectrophotometer. The antioxidant activities of the samples were reported in mg BHA equivalents/100 mL. The TPC of henna leaf extracts was determined spectrophotometrically using Folin-Ciocalteu's reagent. Approximately 0.02 g of dried extract dissolved in 10 mL of methanol-water mixture (4/6 v/v ratio) 0.4 mL of such solution was pipetted onto a glass tube and 2 mL of Folin-Ciocalteau reagent, 0.6 mL of sodium carbonate (7.5%w/v) were added to it and allowed to stand at room temperature for 30 min in dark environment. Gallic acid was used as standard and TPC were expressed in mg GAE/L.Results for the effect of various temperatures showed that the TPC of the extracts decreased with increasing temperatures up to 160 ^{o}C, and showed increasing trends above 160 to 200 ^{o}C. The decreasing TPC trends at temperatures up to 160 ^{o}C, might be due to degradation of some polyphenolic compounds obtained at lower temperatures. Whereas, at higher temperatures above 160 ^{o}C, extraction of other polyphenolic compounds of different molecular structures or those obtained from hydrolysis of cellulosic components was likely the reason for the increasing amount of TPC. With treatment at constant temperature and time, best values of DPPH radical scavenging activity (RSA) were obtained at mild temperature range of 100-120 ^{o}C. Controlling microwave power at short irradiation time gave better results than temperature-controlled treatment. Furthermore, comparison with the result obtained at room temperature confirmed that the use of microwave was more effective for extracting polar components that normally possess higher antioxidant activities.

Plots of DPPH/RSA-to-TPC ratios at various temperatures and reaction times showed a fair Correlation of antioxidant activity and total polyphenol contents of the extracts. These results imply that the polyphenolic compounds obtained at milder conditions possess higher antioxidant activities than those obtained at relatively higher temperatures and longer times. The same behavior was also observed on the effect of microwave power on DPPH-to-TPC ratios.

Results show that lawsone also exhibits antioxidant properties, and the values are almost half of the antioxidant activities of the extracts. The overall antioxidant activity of henna extracts at 1 min and 30 min is in the range of 7.58-13.08 mg BHA equivalents/100mL. Other research directions also include elucidation of the antioxidant properties of the extracts obtained by pulsed microwave irradiation at low temperatures.

It was suggested that due to the interaction of MW with the materials, heating cannot be simply treated as that similar to the conventional methods as there are a lot of possible mechanisms of activation of materials that might possibly occur. The quest for confirming true existence of microwave non-thermal effects motivated us to investigate separation or reaction processes carried out at controlled near ambient temperature conditions. In this study, low-temperature controlled extraction system utilizing MW irradiation in pulsed mode was developed. Its application to extraction of thermolabile bioactive compounds from henna (*Lawsonia inermis*) leaves in the temperature range of 15 to 60 °C was then investigated. In this apparatus, the temperature, time and MW power output can be monitored and automatically controlled. A MW-transparent coolant (T close to -15°C) was allowed to flow through the jacket of a 60-ml glass extractor placed inside the cavity, thus doing extraction at low temperature was practically feasible even with continuous supply of pulsed microwaves. In a typical run, sample of about 2 g and 50 ml of distilled water were mixed with a magnetic stirrer while irradiating microwaves to reach the set extraction temperature. The extracts were then analyzed of its composition, total polyphenolic contents and antioxidant activities.

As a result pulsed microwaves were shown to more effectively accelerate extraction of thermolabile components such as lawsone than conventional method, thus increasing the percentage of yield and lawsone composition as well as extraction of components with high antioxidant activity under pulsed MW significantly observed. Moreover the pulsed microwave improved the extraction rate of lawsone at low temperatures. Comparison of the temperature and MW power output profiles at 30 and 60 °C as well indicated the selectivity of pulse MW method which supply required energy for reactions even at low temperatures. Therefore, increasing the extraction rate of components at 60 °C probably is attributed to non thermal effect under pulsed MW condition.